INSECT MOLTING HORMONES:

SOME STUDIES ON HYDROXYLATED STEROIDS

a thesis presented by

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To

The Peaceful Application of Science

and

The Social Responsibility of the Scientist.
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The chemistry of insect moulting hormones is reviewed in four parts, with particular emphasis on the developments that have taken place between 1966 and September, 1970.

The first part contains a general introduction to the field. The second deals with the occurrence of ecdysone and its analogues and the structural variations so far found in nature. Rather than giving an exhaustive treatment of structure determination only pertinent features are highlighted. Previous synthetic approaches recorded in the literature are the subject of the third section. Finally, there is a section devoted to recent biogenetic advances, the mode of action of ecdysones, physiological properties other than moulting activity and an attempted correlation of structure with moulting activity.

The author's own work falls into two related parts.

The first section describes the recently published, shortest known synthetic route to ecdysone, and is exemplified by the preparation of several model compounds, as well as the required isoecdysone and ecdysone. Some observations are made on the relation of structural features to the ratio of epimeric alcohols produced by Grignard addition to 22-aldehydes.

The remaining work arose from the \textit{cis}-hydroxylation
step employed in the ecdysone synthesis, and centres upon the electrophilic addition to the 22- double bond in the ergosterol side-chain. The isolation of a 22-iodo-23-acetoxy-derivative from a control cis-hydroxylation of 1-ergosta-7,22-dien-6-one led to the preparation of a wide variety of 22- and 23- oxygenated derivatives. The majority of the work described in this section was directed towards the determination of the absolute configurations of these compounds, their relative stereochemistry being well-defined. The results are of three-fold interest: (a) because the mode of electrophilic addition to the ergosterol side-chain is defined; (b) because the basis for a route to 24βF-methyl-25-deoxy-analogues of ecdysone is laid; and (c) because a potential source of biologically important derivatives of known stereochemistry is obtained, for use in biosynthetic studies on the ergosterol side-chain.
The following abbreviations have been used:-

b.pt. boiling point

C.d. circular dichroism

I.r. infrared

M.pt. melting point

N.m. nanometre

N.m.r. nuclear magnetic resonance

O.r.d. optical rotatory dispersion

P.L.C. preparative layer chromatography

P.p.m. parts per million

T.L.C. thin layer chromatography

U.v. ultra-violet

Dma dimethylacetamide

Dmf dimethylformamide

DmsO dimethylsulphoxide

Ether diethyl ether

Lah lithium aluminium hydride

Nbs N-bromosuccinimide

Petrol petroleum ether, fraction, b.pt. 60-80°C

THF tetrahydrofuran.
INSECT MOURTING HORMONES.

Section I - Introduction:

Until the mid-1930's, it was widely believed that insects had no hormonal requirements, and it was only after the pioneering studies of Sir Vincent Wigglesworth\(^1\) that the existence of insect hormones was firmly established. That their principal function was the regulation of post-embryonic development was shown by Karlson\(^2\) and others. The earlier literature has received close attention in a number of texts\(^2,3\), and it is with the numerous recent advances that this review will be primarily concerned. Developments have in fact been so rapid of late that, coupled with the complex stereochemistry of the compounds involved, some confusion has arisen, resulting for example in the isolation of a single compound under as many as six different names. The reviews of Berkoff\(^4(a)\) and of Wiechert\(^4(b)\) deal briefly with the more recent literature. A fuller treatment as late as August, 1968, is given by Feakins\(^5\).

Three main hormone types are recognised:

(1) Brain hormone or adenotropic hormone.
(2) Juvenile hormone or Neotenin.
(3) Moulting hormone or ecdysone (and analogues).

Because insects have exoskeletons, the increases in size from "first instar larva" to "imaginal adult" necessitate a series of moults, during which the restricting exoskeleton is shed and a new one formed, tanned and hardened. The
hormonal control of this process was established by a series of biological transplantation experiments⁶,⁷, and can be schematically represented as shown below. (Figure 1).

![Diagram of hormonal control](image)

BH = Brain Hormone
JH = Juvenile Hormone
MH = Moulting Hormone

Brain hormone from the neurosecretory cells stimulates the prothoracic gland to produce moulting hormone, which induces moulting in the larva or pupa. The larval character of the moult is guaranteed only by simultaneous secretion of juvenile hormone by the corpora allata. When the concentration of juvenile hormone is sufficiently low, a pupal or imaginal moult results.

Of brain hormone, relatively little is known. Some workers⁸ maintain that it is cholesterol, others that it is a protein⁹, and still others that it is a polypeptide¹⁰.
Neither a complete understanding of its mode of action nor
the elucidation of its structure can be achieved until a
generally acceptable bioassay for brain hormone activity
has been developed.

Topical application of juvenile hormone causes insects
to die without completing their development. This powerful
insecticidal potential has generated much interest, because:
insects could hardly develop resistance to their own hormones.
Some imaginative and highly demanding work has resulted. In
particular, the 100,000-fold purification of male silkmoth
extracts, the microgram scale degradative work and the
synthesis of the racemates of four of the sixteen possible
stereoisomers, enabled Roller and his colleagues\textsuperscript{11} to
deduce that juvenile hormone is methyl trans-trans-cis-
10-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate (1).

\[
\begin{align*}
\text{COOMe} \\
\text{(1)}
\end{align*}
\]

Several stereospecific syntheses of racemic juvenile
hormone have ensued\textsuperscript{12,13,14,15}. Determination of the absolute
configuration of the natural hormone and resolution of the
synthetic hormone have yet to be reported.

In the case of moulting hormone, the development of a
sensitive bioassay was the first step on the long road to ultimate characterisation. The "Calliphora test" bioassay of Fraenkel was used by Butenandt and Karlson in a modified form to obtain the first crystalline sample of ecdysone in 1954. (See Section II). The bioassay involves the ligaturing of the blow fly larva. The moulting hormone is thus unable to diffuse from the anterior to the posterior part, and only the former undergoes moulting. Injection of an extract solution into the posterior part is followed by puparium formation, if the extract has moulting hormone activity. The test can be readily adapted for quantitative comparisons of activity. Use of Musca larva is reported to increase the sensitivity of the test by a factor of five. An alternative technique known as "Chilo dipping" has also been described, which makes use of topical application of hormone solutions.

Originally, interest in moulting-hormone active compounds had centred on their potential use as sophisticated selective insecticides by the inducement of premature moulting. Several synthetic efforts to render them readily available have been reported in the literature (see Section III). Two factors have increased the world-wide interest in these compounds. Firstly, the surprising isolation of ecdysone analogues (initially the ponasterones) from plants in 'large' quantities. This resulted in a wave of publications, which has raised the number of ecdysones known from the six zoo-ecdysones, isolated in small yield and with great difficulty from insects and crustacea to the present total of thirty-nine zoo- and phyto-ecdysones (see Section II). Secondly, the ecdysones have been shown to
exhibit a number of properties other than moulting-hormone activity (see Section IV). The discovery that ecdysone stimulates and initiates DNA and RNA synthesis\textsuperscript{24,25,26,27,28} and that several 20-hydroxy analogues have potent anabolic effects on protein synthesis in mice\textsuperscript{29,30} have carried investigations beyond the bounds of entymology, and are of general importance. A fuller discussion is given in section IV.
14.

Section II - Ecdysone and its Analogues.

(1) **Ecdysone.** (Also known as α-ecdysone). In 1954, Butenandt and Karlson succeeded in isolating 25 mg. of the hormone responsible for moulting in insects, in crystalline form from 500 kg. of the pupae of the silkworm, Bombyx mori L. The steroid structure of ecdysone was recognised nine years later. Chemical methods led to the partial structure (2).

![Chemical Structure of Ecdysone](image)

The 14α-hydroxy-7-en-6-one system was the only possible explanation for the behaviour of ecdysone under mild acid conditions, after which the u.v. absorption maximum changes from 244 nm. to two maxima at 290 and 250 nm. (see Figure 2).

![Chemical Structure Transformation](image)

**FIGURE 2.**
Periodate oxidation had shown the presence of a vicinal diol system, which, on biogenetic grounds was assigned to the 2,3 or 3,4 positions. N.M.R. had placed a fourth hydroxyl group at the 25- position, and the fifth could only be assigned to an unknown side-chain site. It remained for Huber and Hoppe\textsuperscript{32}, using a new non-heavy atom diffuse scattering X-ray technique, to completely elucidate the structure as 2β,3β,14α,22R,25-pentahydroxy-5β-cholest-7-en-6-one (3)(a). Confirmation of this structure by unambiguous synthesis was announced in 1966 almost simultaneously by two groups, and other syntheses have followed (see Section III).

Other animal sources of ecdysone have been the adult Moroccan locust (\textit{Dociostaurus moroccanus} Thunberg)\textsuperscript{33} and the tobacco hornworm (\textit{Manduca sexta} Johannson)\textsuperscript{34}. Plant sources include the common fern (\textit{Polypodium vulgare} L.)\textsuperscript{35} and the dead leaves of common bracken (\textit{Pteridium aquilinum} Kuhn)\textsuperscript{36},
In all the cases cited above, ecdysone and crustecdysone have both been found. In the fern *Cheilanthes tenuifolia* (Burm. F.) Swartz, ecdysone, but not crustecdysone, has been isolated.

(2) **Crustecdysone.** Originally isolated from the same source as ecdysone, *Bombyx mori* L., in 1956 by Karlson and named β-ecdysone, it has been independently isolated from the same source under the names 20-hydroxyecdysone and ecdysterone. It has also been found with ecdysone and in *Calliphora stygia* and the marine crab, *Callinectes sapidus*. A major development in hormone research was the isolation of 2mg. of the pure hormone from one ton of the crayfish, *Jasus lalandei* by Hampshire et al. The name crustecdysone used by this group is probably the most generally accepted, though ecdysterone is also much used.

![Chemical structure of crustecdysone](image)

\[2\beta, 3\beta, 14\alpha, 20R, 22R, 25\text{-hexahydroxy-5\beta-cholest-7-en-6-one}.\]
It is the hormone responsible for crustacean ecdysis (moulting), but is highly active in the "Calliphora" bioassay, being more than five times as potent as ecdysone. It has been synthesised by three groups\textsuperscript{42,43,44} (see Section III) which has confirmed the proposed structure (4a)\textsuperscript{41}. It was known from u.v. and i.r. spectral data and from biological activity that crustecdysone had a structure closely related to that of ecdysone, and the mass spectrum showed it to contain an extra hydroxyl group. The location of this at C-20 could be deduced from the n.m.r. spectrum, in which the 21-methyl group occurs as a singlet at 1.56 p.p.m. in contrast to the doublet at 1.28 p.p.m. found for ecdysone. The 18-methyl signal has also shifted 0.48 p.p.m. downfield. Further, the mass spectrum showed, after initial loss of the C-25 hydroxyl (18 mass units), the expected C-20, C-22 bond fission occurring without hydrogen transfer as the fragments of m/e 363 (5), 91 (6) and 88 (7) shown below.

![Diagram](5)

![Diagram](6)

![Diagram](7)
The assignment of the stereochemistry as $20R,22R$ was made by analogy to the biogenetic C-20 hydroxylation of cholesterol, which occurs with retention of configuration$^{45}$, and by analogy to ecdysone. Synthetic confirmation of the stereochemistry has been carried out.

Crustecdysone has also been widely found in plants. Jizba et al.$^{46}$ has reported an extremely high yield of 1% from the rhizomes of *Polypodium Vulgare* L., originally using the name *polypodine A* for the hormone. Other genera in which crustecdysone have been found include *Podocarpus*,$^{47}$ *Pteridium*,$^{36}$ *Achyranthes*,$^{48,49}$ *Morus*,$^{50}$ *Vitex*,$^{51}$ *Onoclea*, *Lastrea*, *Matteuccia*,$^{52}$ *Blechnum*,$^{53}$ *Cyathula*,$^{54}$ *Ajuga*,$^{55}$ *Trillum* and *Stachyurus*.$^{56}$ A great deal of this work has been carried out by Takemoto and his colleagues as part of a programme to screen 2000 species of Japanese plants. It is to these workers that crustecdysone owes yet another synonym, *isoinokosterone*.$^{48}$

Using crustecdysone as a model, Galbraith and Horn$^{57}$ report the development of a simple micro method of measuring the rate of acetylation of hydroxyl groups in steroids in order to determine their steric environment. Much work has been carried out on the anabolic properties of crustecdysone (see Section IV for discussion), and its ready availability from plant sources have led to its use as a starting material for synthetic work in the field of insect-moulting hormones,$^{58,59,60,61}$ (see Section III).
2-Deoxycrustecdysone. Horn et al. recently reported the successful isolation of 200μg of this compound \((8)(a)\) from an awe-inspiring 3 tons of crayfish offal. It was thought at first to be ecdysone, since it was less polar than crustecdysone, and had peaks the required 16 mass units less in the mass spectrum. However it was found to be somewhat more polar than ecdysone, and the n.m.r. and mass spectra indicated that it was a position in the tetracycle from which the oxygen was missing, most probably from the C-2 or C-3 positions.

![Molecular structure](image)

\[(8) \begin{align*} (a) & \quad R = H, R' = OH \\ (b) & \quad R = OH, R' = H \end{align*}\]

It is well-known that the rates of acetylation of axial and equatorial hydroxyl groups are different. The rates of acetylation of the hormone and suitable models showed that the hydroxyl group was axial, and hence at the \(3\beta\)-position. This is also the most likely biogenetic assignment. The compound has very recently been isolated \(62\) from Blechnum minus with ecdysone and the latest analogue,
2-deoxyecdysone, in large quantities.

(4) 20,26-Dihydroxyecdysone. The structure (9) was proposed for a compound isolated from the tobacco hornworm (Manduca sexta Johannson)\(^2\). The hormone was more polar than crustecdysone, indicating an additional hydroxyl group. The location of this at C-26 (27) could be deduced by the downfield shift of the C-26 (27) protons in the n.m.r. spectrum and from the fragment ion (CH\(_2\)OH\(^+\)) at m/e 31 in the mass spectrum, characteristic of such a 1,2 diol. The n.m.r. spectrum of the model compound (10) confirmed the assignment.

The less polar 5α-epimer was also isolated from the same insect, but it seems likely that this is an artefact, since the stabilities of the 5α- and 5β-epimers of this type are of the same order.
The stereochemistry of the C-20 and C-22 hydroxyls has not been defined, but it would not seem unreasonable should it prove to be as in crustecdysone. From recent experiments, it seems likely that crustecdysone is metabolised to 20,26-dihydroxyecdysone in Calliphora, but has yet to be isolated from it.63.

(5) Callinecdysone A During their quantitative investigations into the moulting hormones present in three stages of moult of the female marine crab Callinectes sapidus, Horn et al.23 reported that callinecdysone A (11) was the major hormone present in the early and late premoult stages. Its spectral properties were identical to those of the phytecdysone inokosterone, previously described by Takemoto et al.48, and known to be a mixture of the C-25 isomers. Callinecdysone A is therefore one of the inokosterone isomers or also a mixture.

(6) Callinecdysone B The major hormone present immediately after moulting in the female marine crab (see above) is crustecdysone; the minor one is callinecdysone B (12)(a). It has been shown to have identical spectra to makisterone A65(12)(b) also known as pondecdysone D66). It is probable that callinecdys B is the C-24 epimer of makisterone A, since the C-28 marine
zoosterols, and phytosterols which are likely to be the respective precursors, are epimeric at that position.

The isolation of callinecdysone B is the only report of a 24 alkyl-ecdysone being detected in arthropods.

**Phytoecdysones.**

A new dimension in natural product chemistry was created by the discovery of moulting hormone substances in plants. In addition to the isolation of ecdysone and crustecdysone from plant sources, thirty-two new analogues have been reported since 1966. To date, they are all steroids, structurally related to ecdysone, most of them having an identical steroid nucleus with variations in the hydroxylation pattern and alkylation of the side-chain. Within certain limits these variations can occur without loss of moulting hormone activity.
R: alkyl, hydroxyl or hydroxyalkyl.

**FIGURE 3.**

The structures are generally deduced by a telling application of chemical degradation and mass spectroscopy in particular, aided by O.R.D., and n.m.r., i.r. and u.v. spectroscopy.

The trivial names of these analogues have been as exotic and varied as the plants themselves. Where it has been shown that a single compound has been isolated from different sources under several names, the alternative names have been included. The plant families in which moulting hormones have been found include Podocarpaceae, Taxaceae, Polypodiceae, Amaranthaceae, Verbenaceae, Moroaceae and Blechnaceae. Galbraith and Horn\(^47\) have suggested that plants may elaborate moulting-hormone steroids primarily for defensive purposes to interfere with the growth processes of insect predators: their source, *Podocarpus elatus*, was known to be particularly resistant to
insect attack. This hypothesis is a point of contention and will be discussed in Section IV.

The phytoecdysones are considered under four headings:
(a) Variations of Side-Chain Hydroxylation only.
(b) Variations of Side-Chain Hydroxylation and Alkylation.
(c) Variations in the Tetracycle.
(d) Other variations.

(a) Variations in the Side-Chain Hydroxylation Pattern:
(7)(i) Ponasterone A - The isolation of this compound (13(a); R = H) (together with Ponasterones B, C, and D) by Nakanishi et al. from Podocarpus nakaii HAY, was the first reported instance of the isolation of insect moulting hormone active substances from a plant. (A yield of about 0.2% was obtained.)

Periodate oxidation showed the presence of two vicinal diol systems, and a fifth hydroxyl group became apparent
from the infrared spectrum of the diacetonide. Treatment of 2-acetoxy ponasterone A with sodium periodate, followed by removal of the acetate by mild alkaline hydrolysis, gave the methyl ketone (14) which had been independently prepared during the synthesis of ecdysone\textsuperscript{67} and also from crustecdysone\textsuperscript{61}.

\[
(14)
\]

\[
(15)
\]

The recent isolation of ponasterone A by Huppi and Siddall\textsuperscript{68} as the 25-deoxy-hydrogenolysis by-product of their synthesis of crustecdysone has confirmed the 20\textsubscript{R}, 22\textsubscript{R}-dihydroxy nature of the stereochemistry.

Although not previously detected in \textit{Calliphora}, ponasterone A and inokosterone were both found in \textit{Calliphora stygia} after feeding with tritium labelled 25-deoxyecdysone\textsuperscript{63}. The latter is not however a normal major precursor of
crustecdysone in this insect, and ponasterone A has yet to be found as a naturally occurring zootecdysone. Its isolation from the plants *Blechnum amabile* and *niponicum* and *Podocarpus macrophyllus* R.Br. has been reported. Ponasterone A is one of the C-20 hydroxylated ecdysones which have been examined for protein anabolism activity in mice.

(7)(ii) Ponasteroside A from *Pteridium aquilinum* var. *latiusculum* (13b); *R* = C$_6$H$_{11}$O$_5$ i.e. (15), originally called Warabisterone, was the first glycoside of an insect moulting hormone to be isolated. Enzymatic hydrolysis gave glucose and ponasterone A. The position of the glycoside link and recognition of its β nature were deduced by detailed analysis of the n.m.r. spectrum of ponasteroside A hexacetate and by comparison with that of stigmastanyl-β-D-glucoside tetracetate.

(8) Vitocosterone E. The examination of *Vitex megapotamica* (Spreng.) Moldenke by Rimpler and Schulz has proved a fruitful source of insect moulting hormones. In addition to the known compounds (crustecdysone, inokosterone, pterosterone and polypodine B; see under these names), Rimpler has isolated vitocosterone E, which has proved to be 25-acetoxyecdysone. (4b) see page 16. The acetate group showed its presence in the i.r., mass and n.m.r. spectra in an expected manner, and the down-field movement of the C-26...
and C-27 methyl groups, in conjunction with the formation of a diacetonide, pointed to its position at C-25. Vitocosterone E tri-acetate and crustecdysone\textsuperscript{2,3,22,25} tetracetate were shown to be identical. It exhibits 1/14th the activity of ecdysone in the \textit{Calliphora} test.

(9) \textbf{Inokosterone} The root of \textit{Achyranthes fauriei} have long been used in Japanese folk medicine. Takemoto \textit{et al.}\textsuperscript{48} have obtained inokosterone (16) from several species of \textit{Achyranthes}, and established the structure as an epimeric mixture of the 2\(\beta\),3\(\beta\),14\(\alpha\),20\(R\),22\(R\),26 hexahydroxy 25\(R\) and S, 5\(\beta\)-cholest-7-en-6-ones.

\begin{equation}
\text{(16)}
\end{equation}

The racemic nature of inokosterone followed from the degradative work\textsuperscript{64}. Periodate oxidation of the 2,26-diacetate gave after base treatment the same product (14) obtained similarly from 2-acetoxyponasterone. A, together with a side-chain fragment (17). Chromic acid oxidation and methylation with diazomethane of (17) gave (\(\pm\)) methyl 4-methyl-5-acetoxyvalerate (18). At no time in the reaction sequence had racemisation at C-25 been possible.
Comparison of the n.m.r. spectra of the tetracetates of inokosterone and crustecdysone showed that each had the same C-20, C-22 stereochemistry.

Other plant sources of inokosterone have been *Vitex megapotamica* (Spreng.) moldenke and *Achyranthes rubrofusca* Wight. It occurs as a pseudo zoö-ecdysone with ponasterone A and crustecdysone as metabolites when 25-deoxyecdysone is fed to *Calliphora stygia*, and as a natural zoö-ecdysone should it prove identical to callinecdysone A.

The hormone exhibits high moultng hormone activity in insects and shrimps and stimulates protein anabolism in mice.

**Pterosterone** The stereochemistry of this moultng substance has not yet been completely defined. Found in *Lastrea thelypteris* BORY and in *Onoclea sensibilis* LINNE, the structure (19) has been proposed for it. The pregnenolone (14) has been obtained as described previously, which verifies the structure of the tetracycle, and trans-4-methylpent-2enal, (iso-hexenal) (20) as the other periodate oxidation product after dehydration, gives an indication of the location of a
hydroxyl group at C-24. Pterosterone has been isolated by Rimpler from *Vitex megapotamica*. The possibility that pterosterone is a stereoisomer of crustecdysone has not been ruled out.

Shidasterone Shidasterone (21), isolated from *Blechnum amabile* and *B. niponicum*, has been shown conclusively to be a side-chain stereoisomer of crustecdysone. Under conditions in which crustecdysone gives the $2\beta,3\beta,22R$-triacetate, shidasterone affords only the $2\beta,3\beta$-diacetate however. Direct comparison with a synthetic sample of the moulting-inactive $22$-epi-crustecdysone has eliminated this possibility.

Shidasterone in fact exhibits high moulting hormone activity and protein anabolism stimulation. Tentatively therefore, it seems that shidasterone may be $20$-epi-crustecdysone (21), with the $20S, 22R$, stereochemistry, though its high moulting hormone activity mitigates against this.
Stachysterones C and D, crustecdysone and four new compounds, stachysterones A, B, C and D, were isolated from the bark of Stachyurus praecox Sieb. and Zucc. Only the structures of C and D have as yet been elucidated, and each exhibits a novel variation.

The spectral data of C and D indicated that both contained the 2β,3β,14α,20-tetrahydroxy-5β-7-en-6-one partial structure found in most ecdysones. Stachysterone C (22) contains the additional 24-ene. The n.m.r. spectrum (in deuteropyridine) showed two olefinic methyl groups at 1.60 and 1.66 ppm, and an olefinic proton adjacent to a methylene group at 5.51 ppm. A new intense fragment at m/e 69 (23) appeared in the mass spectrum, and the fragments due to C-20, 22 bond fission at m/e 99 and 81 are two mass-units lower than the corresponding peaks in ponasterone A which contains the saturated 20,22-dihydroxycholestane side-chain.

The novel feature of Stachysterone D (24) was the tetrahydrofuran ring in the side-chain, which was shown to be unsaturated or cyclic by the C-20/C-22 fission peaks at m/e 99 and 81. The cyclic ether structure gives rise
to the oxonium ion illustrated (25)\textsuperscript{79} by loss of a β-hydrogen.

The n.m.r. spectrum confirms the structure by the chemical shift and half-band-width of the 22- proton at 3.95 p.p.m. (and at 3.85 p.p.m., $W_1/2 = 12$Hz in the diacetate). The 21- protons have also shifted and cannot be distinguished from those at C-26 and C-27 (1.06, 1.20 and 1.37 p.p.m.).
Stachysterone C shows insect moulting hormone activity comparable to other phytoecdysones, but stachysterone D at its most potent appears to be extremely weakly active.

(b) Variation of Side-Chain Hydroxylation and Alkylation.

(14) Cyasterone  The routine screening of the roots of Cyathula capitata Moquin-Tanton by Takemoto et al. led to the isolation of the first insect moulting substance not based on the cholesterol skeleton. The new C\textsubscript{29} hormone was named cyasterone (26) and has since been found in Ajuga incisa together with crustecdysone, ajugasterone A (see under polypodine B) and ajugasterone B (see below).

![cyasterone molecule diagram](26)

Spectral data showed that the steroid nucleus was the usual one, and the preparation of derivatives showed that there were two β-glycol systems. In addition, a γ-lactone group was indicated from the i.r. spectrum. Periodate oxidation gave, after silica gel treatment of the crude product, the known enal (27), which could also be obtained from crustecdysone, and the unknown aldehyde (28), which was fully characterised.
The proposed structure was thus confirmed. Although the side-chain stereochemistry has not been categorically defined, subsequent publications$^{82,83}$ have indicated that cyasterone contains the 20R,22R-dihydroxy system. That the 24-substituent has the same orientation as the 24-ethyl group of stigmasterol (29) has also been intimated$^{82}$. Cyasterone has been shown to be anabolically active and to induce moulting in Musca domestica and Sarcophaga to the same extent as crustecdysone$^{80b}$. The A-norprogesterone derivative (27) extends the usefulness of 20-hydroxyecdysones, since it is a potential source of starting materials for studies directed towards anti-androgenic agents$^{84}$. 
Cyathula capitata has also been the source of the four related novel C₂₉ insect-moulting hormones named above and of sengosterone (see under part (c) of this section).

The partial structures of amasterones A and B and of inokosterone from C-1 to C-22 were concluded to be identical by comparison of their spectral data. The tetraacetates were also compared. The isomeric relationship between the amasterones was deduced from the similarity of the mass spectral fragmentation patterns and those of their tetraacetates, which were virtually identical, except for minor differences in relative intensities of certain peaks. A careful analysis of the n.m.r. spectra followed by decoupling experiments enabled the step-wise deduction of the side-chain structures, (30) and (31).

Both compounds revealed high moulting hormone activity in the Sarcophaga test. It has been postulated that they
may be biosynthesised from the hypothetical common precursor, \(2\beta, 3\beta, 14\alpha, 20R, 22R\)-pentahydroxy-\(5\beta\)-stigmaster-7-en-6-one (32) by enzymatic hydroxylation at an end of the side-chain (C-26 or C-29).

Cyasterone (26), capitasterone (33a) and precyasterone (33b) are thought to be metabolism products of amarasterone A in *Cyathula capitata*.

Capitasterone\(^85\) has the \(\delta\)-lactone structure (33a), which was deduced in a similar manner to that described for cyasterone. Precyasterone\(^83\) is 28-hydroxycapitasterone (33b), assigned on the basis of its spectral similarity and that of its 2,3,28-triacetate with those of capitasterone and its 2,3-diacetate.

The close relationship of the three compounds has been demonstrated by the conversion of precyasterone to cyasterone by alkaline hydrolysis, followed by acidification.
(19), (20), (21) and (22) Makisterones A, B, C and D Crustecdysone, ponasterone A and four new phytoecdysones, makisterones A, B, C, and D, were obtained from the leaves of *Podocarpus macrophyllus D. Don.* Makisterones C and D have the C-29 carbon skeleton shown in structures (34) and (35), and are isomeric with each other and with amarasterones A and B.

Podecdysone A and lemmasterone isolated by Horn et al. (from *Podocarpus elatus* R.Br) and by Takemoto et al. (from *Lemmaphyllum microphyllum*) respectively, have both been demonstrated to be identical to makisterone C.
Normal β-glycol cleavage of the side-chain in the mass spectrum gave rise to strong peaks at m/e 145 and 127, showing that two extra carbon atoms were present in the side-chain. The fragment at m/e 84 \((36)\) indicated that an ethyl group was present at C-24.

![Diagram](36)

Structure \((34)\) was proposed for makisterone C on the basis of these results, supported by n.m.r. data and decoupling experiments. High insect moulting hormone and protein anabolism activity have been reported\(^{37(a)}\).

The mass spectrum of makisterone D indicated that it was isomeric with C, and three secondary methyl groups were apparent in the n.m.r. spectrum. Structure \((35)\) followed from this.

Makisterones A and B were the first C\(_{28}\) analogues of ecdysone to be isolated, and have the isomeric structures \((37)\) and \((38)\).

Makisterone A and podecdysone D\(^{65}\) are identical and probably epimeric at C-24 with callinecdysone B\(^{23}\). The additional secondary methyl group was shown to be at C-24 and not C-23 by decoupling experiments, and confirmed by the
appearance of the new fragment (39) at m/e 70 in the mass spectrum.

\[
\text{(39)}
\]

Mass spectral studies and n.m.r. decoupling experiments defined the side-chain of makisterone B as depicted in (38). Its n.m.r. spectrum was very similar to that of inokosterone (16).

Ajugasterone B The first recorded example of an additional side-chain double bond in an insect-moulting hormone was the C_{29} compound, ajugasterone B (40), a minor constituent of Ajuga incisa. Stachyysterone C, containing a 24-ene was subsequently reported.

C-20, C-22 bond fission produces in the mass spectrum the frequently encountered base peaks at m/e 363, 345 and 327 (sequential loss of water) and the side-chain fragments at m/e 143, 125 and 107, two mass units lower than the same fragments in makisterones C and D. The n.m.r. spectrum shows
two extra olefinic protons, a hydroxy methyl group (C-26) attached to a double bond and a methyl triplet (C-29). The absence of 26- and 27- methyl singlets leads unambiguously to (40). Ajugasterone B has comparable activity to other ecdysones in the Chilo dipping assay.

(C) Additional Variations in the Steroid Nucleus.

(24) Polypodine B (58-hydroxyecdysterone or Ajugasterone A)

One of the most common nuclear variations is exhibited by polypodine B (41). This heptahydroxy steroid was isolated by Jizba et al.89 and later by Heinrich and Hoffmeister90 from the rhizomes of Polypodium vulgare L. It has since been reported in Vitex megapotamica70 and in Ajuga incisa81, and closely resembles crustecdysone.

Unlike 20,26-dihydroxyecdysone (9)22, the seventh hydroxyl group was located in the tetracycle fragment after C-20, C-22 bond fission in the mass spectrum91. N.m.r. studies revealed that it was tertiary, which limits possible sites to C-5, C-9 or C-17. The carbonyl group had an infrared
absorption peak at 1687 cm\(^{-1}\) in contrast to 1655 cm\(^{-1}\) for crustecdysone. This suggested that the hydroxyl was influencing this moiety in the molecule. The rapid uptake of two equivalents of periodate was followed by the slow uptake of a further equivalent, eliminating C-9 as a possibility. C-17 was excluded since no acetic acid, expected from a 1,2,3-triol, could be detected in the reaction products. A combination of deuteriation and circular dichroism studies confirmed this. Polypodine B (41) is four times more active than synthetic ecdysone as a moulting hormone\(^9\).

Two other 5β-hydroxy compounds have been reported; ponasterone C and sengasterone.

(25) Ponasterone C (42) The structure of ponasterone C\(^{19}\) was
originally deduced to be stereoisomeric with pterosterone (19). The side-chain was shown to be the same by periodate cleavage to trans-isohexenal in both cases. Infrared hydrogen bonding studies and the n.m.r. data of the 2,3-diacetates led to the 2α,3α-glycol system being proposed for ponasterone C (43). However a re-examination of the structure was undertaken for two reasons. Firstly the 2α,3α-diol system proposed for ponasterones B and C was atypical for the ecdysones, and secondly the optical and mass spectral data of ponasterone C and polypodine B were found to be very similar.

The mass spectrum showed base peaks at m/e 379, 361, 343 and 325 after cleavage of the 20R,22R-diol system, in contrast to the more usual peaks at 363, 345 and 327. This is due to the 5β-hydroxyl group, the β-orientation being well-supported by n.m.r. data, decoupling experiments and the 'negative' chirality adopted by the C-2 and C-3 hydroxy groups as shown by the circular dichroism data for ponasterone C and polypodine B benzoates. (Partial structure 44).
(26) **Sengosterone** (45) One of many related active compounds from *Cyathula capitata*, sengosterone\(^9\) has the 5\(\beta\)-hydroxy cyasterone structure depicted below (45).

Sengosterone shows the similarities to and differences from cyasterone, crustecdysone and polypodine \(B\) that would be expected from the proposed structure.
(27) **Ajugasterone C**  The structure (46) of this non-crystalline compound extracted from several species of *Ajuga* has been corroborated by the recently developed dibenzoate chirality rule (an extension of the benzoate sector rule). Ajugasterone C-20,22-acetonide-2,3,11-tribenzoate was the first example of the rule being extended to determine the spatial disposition of non-adjacent hydroxy-groups, and confirmed the structure (46) deduced by n.m.r. decoupling experiments as 11α-hydroxyponasterone A.

![Diagram of Ajugasterone C](image)

Noteworthy is the very large $\Delta \varepsilon$ value of -30 of the 237 nm Cotton effect due to the left-hand screws of the 3β- and 2β- benzoates and 2β- and 11α-benzoates. Also in the n.m.r. of ajugasterone C tetra-acetate the 9-\(H\) at 3.39 p.p.m. is coupled to the 7-\(H\) and 11-\(H\), whereas in ponasterone A triacetate it is coupled to 7-\(H\), 11α-\(H\) and 11β-\(H\). Furthermore, ajugasterone C can
be dehydrated by heating in Alumina-benzene to a product absorbing in the u.v. at 298nm.; the 7,9(11)-dien-6-one chromophore has a calculated value of 303nm. This example of an extra ring C substituent is so far unique.

(28) Ponasterone B With the revision of the structure of ponasterone C93, ponasterone B became the only ecdysone to be assigned the 2α,3α-diol system. This conclusion was reached because the arrangements of hydroxy groups satisfying the observed criteria (including the left-handed chirality of the 2,3-dibenzoate) were 2β,3β,14α,20R,22R-and 2α,3α,14α,20R,22R-pentahydroxy-5β-cholest-7-en-6-one. The former alternative represents ponasterone A. It follows that ponasterone B must be the latter (48). It exhibits only 1/30th the activity of ecdysone in the Calliphora test.

(29) Podecdysone B The structure (49) has been deduced for this
compound, one of six active compounds isolated from *Podocarpus elatus* R. Br. Instead of the 14α-hydroxy-7-en-6-one common to the ecdysones, this analogue is the 8(9),14(15)-dien-6-one shown below.

If podecdysone B were an artefact formed by dehydration of crustecdysone during work up, it would be accompanied by 7,14-dien-6-one compounds. This was not the case. However other batches of bark have failed to yield the same compound, and the situation is not altogether clear. Structural proof was afforded by conversion of the known methyl ketone to (50) and (51) by refluxing with 0.5N ethanolic hydrochloric acid. Periodate oxidation of podecdysone B 2β-acetate, followed by mild alkaline hydrolysis, also gave (50).
In the Calliphora bioassay, the hormone exhibits about one-fifth of the activity of crustecdysone. This relatively high biological activity is surprising, because of the absence of the 7-en-6-one grouping. Its activity may be due however to in vivo isomerisation, a point emphasised by the inactivity of cheilanthones A and B (see below).

(30) and (31) Cheilanthones A (52a) and B (52b) The fern Cheilanthes tenuifolia (Burm. F.) Swartz \(^{37}\) has been the source of the novel 7,8-dihydroecdysone (52a) and 25-deoxy-7,8-dihydroecdysone (52b).

(52) (a) \( R = OH \)
(b) \( R = H \)
Neither compound exhibits any strong u.v. absorption, and the i.r. carbonyl absorption is at 1684 cm\(^{-1}\). The mass spectral fragmentation patterns afford concrete evidence of the structures. The biological inactivity of cheilanthones A and B in the Calliphora bioassay is of interest in conjunction with the activity of podecdysone B. It seems that Calliphora are unable to introduce the 7-double bond into cheilanthone A. It is likely that in the biosynthesis of ecdysone in Calliphora the 7-double bond is introduced before the introduction of all the hydroxyl groups. In the biosynthesis of ecdysone in Cheilanthes, the \(\Delta^7\)-double bond may be introduced at a later stage.

(32) 2-Deoxycdysone It has recently been found\(^6^2\) that this compound and 2-deoxycrustecdysone occur naturally in Blechnum minus ferns. That these compounds are as active as their parent compounds in the Calliphora test is of great importance and it seems probable that the biosynthesis of ecdysones proceeds via the 2-deoxy compounds (see Section IV). The structure has been shown categorically to be as in (3b) by reasoning analogous to that for 2-deoxycrustecdysone. It does not react with periodic acid under conditions normally used to cleave vicinal diols.

(d) Other Variations.

(33) Rubrosterone (53) A departure from the previously isolated phytoecdysones came with the characterisation of rubrosterone, a C\(_{19}\) astiocholane (5\(\beta\)-androstane) derivative obtained from Achyranthes rubrofusca Wight\(^4^9\) and fauriei\(^9^9\). The structure
(53) has been confirmed by numerous syntheses\textsuperscript{58,59,100,101,102} (see Section III), and it is a suggested metabolite of crustecdysone and inokosterone with which it is found. Presumably an analogous pathway (Figure 4) to the metabolism of cholesterol to dehydroepiandrosterone is followed.
The structurally significant points were the band at 1741 cm\(^{-1}\) in the i.r. spectrum, characteristic of a cyclopentanone carbonyl group, and the presence of two methyl groups only in the n.m.r. spectrum. Examination of the u.v. properties after acid treatment showed that the carbonyl group was located at C-17, and not C-15 or C-16, and that rubrosterone must be represented as in (53).

Neither the pregn-7-en-6,20-dione (14) nor rubrosterone exhibit moulting hormone activity, but both show anabolism enhancement in mouse liver\(^{58,59}\). Rubrosterone is the first example of the isolation from plants of a substance based on the aetiocholane skeleton\(^{49}\).

(34), (35) and (36) Podecysones C, E and F\(^{66}\); (37) and (38) Stachysterones A and B\(^{78}\) and (39) Ponasterone D\(^{19}\). The isolation of these six insect-moulting hormones has been reported from various sources, but their structures are as yet unknown.

A variety of purely synthetic derivatives has been described, some of which are capable of exerting hormonomimetic effects\(^{103}\), albeit weakly in many cases. The relationship between structure and activity has been clarified by reference to these synthetic compounds and the naturally occurring ecdysones, and will be more fully discussed in Section IV. Suffice it to say here that structure and stereochemistry are very critical, and high activity seems to be associated with the presence of a 3\(\beta\)-OH, a 22\(R\)-OH and a 14\(\alpha\)-OH. An increase in activity is observable in combination with a 20\(R\)-OH and a 5\(\beta\)-OH.
Examples of synthetic structural variations that have been bioassayed include epimerisation at C-5 and C-22 in ecdysone (3) and crustecdysone (4a), some less-hydroxylated compounds, and several derivatives of the C$_{21}$ 5β-pregnane (14) and of rubrosterone.
Section III - Insect Moulting Hormone Synthesis.

The discussion of synthetic work falls readily into three parts: the published routes to ecdysone; the syntheses of 20-hydroxylated analogues; and the work involving rubrosterone and other compounds. As might be expected from such challenging problems, several novel procedures have resulted to meet the synthetic demands of the molecules.

(a) The Synthetic Approaches to Ecdysone (3).

Four groups\textsuperscript{104,105,106,107} have now reported the successful synthesis of ecdysone by various routes. The shortest one, recently published by Barton et al.\textsuperscript{107}, will be the subject of another part of this thesis (see Discussion). It is proposed to discuss the Syntex Route\textsuperscript{104} in full, and then illustrate the points of difference which arise in the other work.

All the syntheses concentrate upon the introduction of four structural features in particular.

1. Ecdysone contains a 2\(\beta\),3\(\beta\)-diol system, which can be readily made from a 2-ene.

2. The A/B ring system is cis-fused, whereas A/B trans-fusion is normally more stable. There is however a 6-ketone group, and so the C-5 position can be epimerised. From theoretical considerations, it was considered\textsuperscript{105(a)} that 1,3-non-bonded interaction between the 2\(\beta\)-hydroxyl and the 19-methyl would enhance the stability of the 5\(\beta\)-isomer (see Figure 5). This has been verified practically\textsuperscript{62,104,105}. The 5\(\beta\)-isomer
becomes more heavily favoured (3:1) if the bulk of the 2β,3β-constituents is increased e.g. for the 2β,3β-isopropylidene group\textsuperscript{104(a)}.

(3) The structure requires a 14α-hydroxyl group, γ to an αβ-unsaturated ketone.

(4) A specific hydroxylation pattern of the side-chain must be elaborated.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{The Syntex Route}
\end{figure}

The first successful synthesis of ecdysone was published by this group in 1966\textsuperscript{104(a),67}, closely followed by the first of two much shorter routes from the Schering-Hofmann-La Roche team\textsuperscript{105(b)} (Schemes II and III). Soon afterwards, Syntex published a modification\textsuperscript{104(b)}, which improved their synthesis without reducing the number of stages involved (see Scheme I).

As a starting material, bis-norcholenic acid (54), obtained in five steps from a cholenic acid precursor, was
SCHEME I: THE SYNTAX EODYSONE SYNTHESIS (104b).

(54) → (57) → (58) → (59) + 5α-EPIMER

(62) + 22R-Epimer → (63) → (65)

(60) R = OMe
(61) R = H
chosen. Acetylation, methylation and Fieser trans-hydroxylation of the 5-ene gave (55). Selective oxidation with N-bromosuccinimide, hydrolysis of the 3β-acetate, tosylation and elimination with lithium carbonate gave the 2-ene-6-one (56). Cis-hydroxylation, using a modification of the Woodward-Prevost conditions, and acetylation gave the 2β,3β-diacetate, and introduction of the 7-ene was by α-face bromination and dehydrobromination to (57). Conversion to the 2β,3β,5α-triacetate by acid-catalysed acetylation followed by treatment with selenium dioxide in dioxan resulted in the required 14α-hydroxyl function. Removal of the 5α-acetate with complete retention of configuration was effected by chromous chloride. Base-catalysed epimerisation of (58) at C-5 gave the 5β-epimer (59) as major product with simultaneous loss of the acetate groups to complete the nuclear requirements of ecdysone.

The diol system was reprotected as the acetonide (60) and the remaining problem was the side-chain elaboration. Reduction with lithium aluminium triteritary butoxide hydride gave the 6β,22-diol. The allylic 6-ketone was re-established with manganese dioxide, and dimethyl sulphoxide/dicyclohexylcarbodiimide oxidation, catalysed by trifluoroacetic acid gave the 22-aldehyde, (61). The first approach, employing the lithium salt of 2-methyl-4-sulphoxyphenylbutan-2-ol tetrahydropyranyl ether (64) was abandoned, since the resulting 22-ketone allowed epimerisation at C-20, necessitating the separation of the four C-20, C-22 epimers of ecdysone.

In the modified synthesis, the 22-aldehyde is reacted
with the lithium salt of (65), 2-methylbut-3-yn-2-ol tetrahydropyranyl ether, made from acetone and acetylene, with the alcohol group protected.

The products (62) were separated and the 22S-epimer catalytically reduced to (63) (22R-OH). Removal of the protecting groups with acid furnished ecdysone.

Hofmann-Schering-La Roche Syntheses.

The first of the Hofmann-Schering-La Roche syntheses is shown in Scheme II, and also starts from a bis-norcholeenic acid derivative (66). In this case, the ketone group was introduced by perchloric acid opening of the 5,6-epoxide. The 3-ketone allowed a somewhat different approach to the 2β,3β-diol system. α-face bromination, selective reduction to the bromohydrin and acetylation gave (68), a bromoacetate amenable to the Woodward-Prevost conditions.

The rest of the synthesis closely resembles that described previously (but used later) in the modified Syntex scheme with minor variations in the order, e.g. the selenium dioxide treatment was the final step.
SCHEME II: Hofmann-Scherinz-La Roche First Synthesis

1. Peracetic acid
2. HClO₄
3. Br₂
4. LiAl(Bu⁺O)₃H
5. Acetylation
6. AgOAc/ACOH + H₂O
7. Br₂/ACOH
8. Li₂CO₃/DMF
9. LiI/Lutidine
10. Carbonyldimidazole
11. LiAl(0Bu⁺)₃H
12. MgBr₂
13. H₂/Pt
14. OH⁻

(75) SeO₂ → ECDYSONE (3c)
The second synthesis from this group is shown in Scheme III\textsuperscript{105(d)}. Two points are noteworthy.

The starting material was ergosteryl acetate, (76) which was transformed by the known chromic acid oxidation to a 5\alpha-hydroxy-7-en-6-one. By removal of the hydroxyl group with chromous chloride as in Scheme I, the required 7-en-6-one system (77) was successfully introduced by a different method.

More important was the finding that in the presence of the 14\alpha-hydroxy group, treatment of the 22-aldehyde (83) with the magnesium Grignard shown gave only the "wrong" 22R-epimer. In its absence, a mixture of 22R- and the required 22S-epimers was produced from the 5\alpha-epimer (80). A similar result was reported by Feakins\textsuperscript{5} for the 5\alpha-epimer of (83) and confirmed by the author's own work (see Discussion).

![Chemical structure](image)

**(83)**

The Synthesis of Mori et al.

The Japanese sequence of Mori et al.\textsuperscript{106} contains several original contributions, and has resulted in important side-chain configurational correlations and assignments (see Discussion).
Scheme III: Hofmann-Schering-La Roche Second Synthesis^105(a).
The side-chain was introduced in a step-wise manner. Early in the synthesis, the stigmasterol derivative (84) was ozonolysed, and the 22-aldehyde treated with ethynyl magnesium bromide, and with methyl magnesium bromide and carbon dioxide to give the acetylenic acid (86). Reduction and laetone-formation enabled the 22-epimers of (87) to be readily separated. At this point, the correct epimer had to be chosen for conversion to ecdysone. Reference to the literature on 22-hydroxycholesterols led Mori and his colleagues to initially choose the one in fact which gave iso-ecdysone, since in the earlier literature a wrong assignment was made.

The work which ensued correlated each epimer of (87) with 3β,22R- or 22S-dihydroxycholestane, the 22R-epimer of which was obtained from naturally occurring 22-hydroxycholesterol, (see below)
Since the absolute configurations of ecdysone and isoecdysone were known, it followed that the absolute configurations of all these compounds were as shown, and the previous literature assignments based on Gram's rule proven wrong.

Side-chain introduction was completed in the last stage of the synthesis by treatment of the lactone carbonyl with methyl magnesium bromide at 0°C to give ecdysone.

Two other approaches of the Japanese groups were different. Autoxidation of the 3-ketone (90) with potassium t-butoxide in butanol to a mixture of isomeric diosphenols (95), and sodium borohydride reduction of this furnished a mixture of 2,3-diols, from which the 2β,3β-diol was isolated in "satisfactory" yield. Finally, the 14α-hydroxyl group was introduced by a new technique, used independently by Barton et al., involving enol-acetylation of the 7-en-6-one system, and epoxidation to give the 14α-hydroxy-7-en-5-one directly. This reaction has analogy in other parts of the steroid nucleus.

(b) The Synthesis of C-20 Hydroxylated Hormones.

The main aim of the syntheses of crustecdysone was to
find an approach which would define the stereochemistry at C-20 and C-22. The three groups mentioned in section (a) have all reported successful syntheses\textsuperscript{42,43,44}, the same chronological order of publication being maintained. Syntex have also prepared ponasterone A\textsuperscript{68} by a slight change in their synthetic pathway, thus proving that the C-20, C-22 stereochemistry was the same as in crustecdysone.

Starting with an intermediate from their ecdysone synthesis, (Scheme I, (57)) \((2\beta,3\beta\text{-diacetoxy}-5\alpha\text{-hydroxy-20-methoxycarbonyl-20S-pregn-7-en-6-one})\), the Syntex workers\textsuperscript{42} used an analogous series of reactions (omitting selenium dioxide oxidation) to make the 22-aldehyde, (Scheme V; (96)). C-20 hydroxylation was carried out by selective enol-acetylation of the aldehyde function and epoxidation to give an inseparable mixture of epoxides (97). The hydroxy aldehydes from hydrolysis allowed separation (98 a and b), and the stereochemistry of each epimer was assigned by n.m.r. spectral comparison with 20-hydroxy pregnanes of known absolute stereochemistry. The required 20R-epimer was protected as the tetrahydropyranyl ether, the 14\alpha-hydroxyl group inserted as before, and the aldehyde function of (99) selectively alkylated to give the 22R-alcohol (100) in 55\% yield, indicating a high degree of stereospecificity. The 20S, 22R-epimer of crustecdysone was prepared from the 20S-hydroxy aldehyde.

Inversion of configuration at C-5, catalytic reduction of the 5\beta-isomer and removal of the protecting groups with dilute acid gave crustecdysone (4a). Removal of the protecting groups prior to reduction\textsuperscript{68} resulted in partial hydrogenolysis of the 25-hydroxyl function, enabling both crustecdysone and
SCHEME V: SYNTEx CRUSTEDYSONE 42 and PONASTERONE A 68 SYNTHESIS.

CHO

COOME

(1) AcOH/H⁺

(2) CrCl₂

(3) OH⁻

(4) Acetone/TsOH

(5) LiAl(Du₄O)₂H

(6) LAH

(7) CrO₃/pyridine

(8) DMSO/Et₃N-N=N-CS-Et

(9) Ac₂O/NaOAc

(10) m-Cl perbenzoic acid

(11) 1% KHCO₃/MeOH

(12) TsOH

(13) SeO₂

(14) Cl₂Mg

(15) aq. K₂CO₃/MeOH

Separate

(16) Catalytic Hydrogenation

(17) N/20 HCl in aq. THF

CRUSTEDYSONE

(4a)

PONASTERONE A

(13a)

(96)

(97)

(98a)

(98b)

(57)

(98)

(99)

(100)

(101)
ponasterone A (13a) to be isolated.

The Hofmann group\(^4\) adopted a somewhat different approach. A starting material was chosen which already contained a 20-hydroxyl group: \((20\text{S})\)-20\(\beta\)-hydroxy-5\(\alpha\)-pregna-3,6-dione (Scheme VI,\(^{102}\)), protected as the nitrate ester, was converted to the 7\(\alpha\)-bromo-2\(\beta\),3\(\beta\)-diacetate (103) by the same sequence used in their ecdysone synthesis. Reductive removal of the nitrate group, dehydrobromination and oxidation produced 2\(\beta\),3\(\beta\)-diacetoxy-5\(\alpha\)-pregn-7-en-6,22-dione (104). The 20-ketone was selectively alkylated by reaction with (107), \(4\text{-}(\text{tetrahydropyranyloxy})-4\text{-methyl-1-pentynyl magnesium bromide, to give the C-20 epimeric alcohols (105 a and b).}

\[
\begin{align*}
\text{BrMg} & \quad \equiv \\
\text{O} & \quad \equiv \\
\text{H} & \quad \equiv \\
\end{align*}
\]

(107)

Cram's rule\(^{115}\) was invoked to assign the 20(R)-configuration to the major product. Removal of the pyranyl ether and reaction of the triple bond with mercuric acetate and boron trifluoride etherate gave the 22-ketone, (106). It merely remained to complete the synthesis by the usual methods with the additional selective reduction of the 22-ketone function with LiAl\((\text{Bu}^+\text{O})_3\)\(\text{H}\). Crustecdysone was separated from the mixture of 22-epimers.
SCHÉME VI

THE HOFMANN CRUSTECYDSONE SYNTHESIS.

(1) Nitrate ester formation
(2) Br₂
(3) LiAl(Bu₄)₃H
(4) Acetylation
(5) AgOAc/A₅OH/H₂O
(6) Br₂/A₅OH
(7) Reductive removal of nitrate
(8) -HBr
(9) Oxidation

(105a) + (105b)

20R-OH  20S-OH

(11) N/2OHCl/MeOH
(12) H₅(OAc)₂/BF₃·Et₂O/MeOH

CRUSTECYDSONE

(4a)
(5α-epimer + 5α- and 5β-22 Epicrustecydsonone).

(106a)

(13) SeO₂/dioxan
(14) K₂CO₃/aq. MeOH
(15) Selective Reduction (LiAl(Bu₄)₃H)
The choice of the right epimer of (105) serves to justify the application of Cram's rule to such a 20-ketone, and the literature contains other evidence to support this. However, care in applying Cram's rule to other positions of the steroid side-chain or to the same position in different circumstances is required, as evinced by the ecdysone synthesis of Mori et al., the bromoacetate work carried out by Barton, Poyser and Sammes (see Discussion) and other examples.

The approach of the Japanese group is somewhat analogous to that developed by the German workers. The selection of (20R)-20-hydroxy-pregn-5-en-3β-ol, protected as the benzoate, was followed by elaboration to a 20-ketone containing the required tetracyclic features (see Scheme VII). Vinyl magnesium bromide gave the 20R-alcohol almost stereoselectively and ozonolysis furnished the 22-aldehyde, which with the bromomagnesium salt of (65) gave the 22R-alcohol stereospecifically. The remaining stages were completed in the established manner.

(c) Synthetic Approaches to Rubrosterone and Other Related Compounds.

(1) Rubrosterone (53) Shortly after the publication of its structure, two independent syntheses of rubrosterone were reported, and three others have followed. The first due to the Hofmann group contained no new
SCHEME VII.
THE JAPANESE CRUSTEOIDYSONE SYNTHESIS\textsuperscript{44}.

1. \( \text{B}_2\text{H}_6 \text{ and } \text{NaOH}/\text{H}_2\text{O}_2 \)
2. Jones' oxidation
3. \( \text{NaBH}_4 \)
4. \( \text{CrO}_3\)-pyridine
5. Autoxidation with \( \text{Bu}^+\text{OK} \text{ and } \text{Bu}^+\text{OH} \)
6. \( \text{NaBH}_4 \text{ and } \text{KOH}/\text{EtOH} \)
7. \( \text{AcO} \), \( \text{H}_2\text{O} \)
8. Acetone/phosphomolybdic acid
9. Phosphoric acid
10. \( \text{Ac}_2\text{O} \)
11. \( \text{Br}_2/\text{AcOH} \)
12. \( \text{Li}_2\text{CO}_3/\text{DMP} \)
13. \( \text{H}^+/\text{Ac}_2\text{O} \)
14. Monoperphthalic acid
15. \( 5\% \text{ KOH in } 90\% \text{ MeOH} \)
16. Acetone/\( \text{H}^+ \)
17. Oxidation
18. \( \text{CH}_2=\text{CHMgBr} \)
19. Removal of protecting groups
20. Ozonolysis
21. \( \text{BrKg} \)
22. Reduction
23. Removal of THP ether

**CRUSTEOIDYSONE** (4a)
synthetic features whatsoever. Their model for the ecdysone synthesis was the starting point, 17β-acetoxy-5α-androstane-3,6-dione (113), and a pathway analogous to that used in their ecdysone synthesis resulted in the normal tetracyclic features (114). Sequential removal of the acetates was accompanied by equilibration at C-5. The diol system was reprotected as the acetonide (115), the 17-ketone produced by oxidation, and the diol function re-generated by refluxing with aqueous ethanol to give rubrosterone (53).

Takemoto et al.58,59 have succeeded in accomplishing
the synthesis by two routes from crustecdysone, made possible by the ready availability of the latter by synthesis and in plants. The first route employs the periodate oxidation product of 2β-acetoxy crustecdysone, after mild hydrolysis (14). Baeyer-Villager oxidation with pertrifluoroacetic acid gave (114b) from which rubrosterone was made as in the Hofmann synthesis.

More interesting was their three stage synthesis from crustecdysone. The tetracetate (116) was produced in 42% yield by loss of the 20-hydroxyl when crustecdysone was heated in acetic anhydride with sodium acetate at 140°C for 30 minutes. Ozonolysis in chloroform, and removal of the acetates with aqueous methanolic potassium carbonate followed by separation of the required compound completed the scheme.

From a metabolism product of cholesterol, dehydroepiandrosterone (117), Shibata and Mori arrived in five steps at 3β,17β-dihydroxy-5α-androstan-6-one (118) closely resembling
the starting material (113) of the Hofmann synthesis. Rubrosterone was elaborated from this by a known procedure.\textsuperscript{106}

\begin{center}
\begin{tabular}{c}
\includegraphics[width=\textwidth]{rubrosterone_dihydro.jpg}
\end{tabular}
\end{center}

The most interesting approach to the synthesis of rubrosterone has recently been described. Termed the i-steroid method, it has been independently used in the ecdysone synthesis of Barton et al.\textsuperscript{107} (see Discussion). $3\alpha,5\alpha$-cycloandrostan-6,17-dione (Scheme VIII,120) is prepared by tosylation of $3\beta$-hydroxyandrost-5-en-17-one (119), solvolysis and Jones oxidation. Treatment with bromine in acetic acid gave the $3\beta,5\alpha$-dibromo compound (121), which gave the $3\beta,7\alpha$-dibromo derivative on acid-catalysed rearrangement. Protection of the 17-ketone as the ethylene-dioxy compound (122) allowed double dehydrobromination to the $2,7$-diene (123). Standard procedures completed the sequence.

(2) Non-Natural Ecdysones. As already indicated in the summary of approaches to rubrosterone\textsuperscript{58,59}, the ready availability of certain 20-hydroxy ecdysones has led to their use as synthetic substrates. Elimination of oxygen functions or structural modifications are now possible, instead of the introduction of these into less polar compounds. These developments began with
SCHEME VIII.

(1) Tosylation
(2) Pot. Acetate in aqueous acetone
(3) Jones' oxidation

(4) Br₂/AcOH

(5) H⁺

(6) OH

(7) Li₂CO₃/DMA

(8) Cis-hydroxylation
(9) Acetylation

(10) SeO₂/dioxan
(11) HClO₄/THF
(12) 0.03% aq. MeOH·K₂CO₃

RUBROSTERONE
the isolation of the by-product (14) from the Syntex ecdysone synthesis\textsuperscript{67}, which was also derivable from 20-hydroxy-ecdysones\textsuperscript{60}. A method for the protection of the tertiary hydroxyl groups where necessary as silyl ethers was already known\textsuperscript{119}, and so the elaboration of non-natural analogues became feasible. This may be exemplified by the work of Galbraith, Horn and Middleton\textsuperscript{61}.

The $2\beta,3\beta$-acetonide of (14) when treated with bis-trimethylsilylacetamide in DMF at $78^\circ$C gave the silyl ether (124). Selective alkylation of the 20-ketone at $0^\circ$C in THF with the Grignard from 5-chloro-2-methyl-2-tetrahydropyranyloxy-pentane (125) and a small amount of ethylene dibromide gave, after removal of the protecting groups, 22-deoxycrustecdysone (126) i.e. $20S$-hydroxy-22-deoxycdysone, in 25\% yield. Without using the silyl ether, a 0–1\% yield of the same compound was obtained.
That the 20S-hydroxyl would be produced was as predicted by Cram's rule, as in the Hofmann crustecdysone synthesis\textsuperscript{43}. Several examples of additions to 20-ketones giving this result were known\textsuperscript{120,121}.

The Australian group\textsuperscript{122} has also synthesised 2-deoxy-3-epicrustecdysone (8b) by LiAl(But\textsuperscript{+}0\textsubscript{3})\textsubscript{2}H and LAH reductions of crustecdysone-2\textbeta-tosylate. Reoxidation of the allylic alcohol after protection as the 20,22-borate ester, and chromatographic removal of the protecting group, gave the required compound probably by a mechanism, such as in Figure 6, which avoids \(\alpha\)-face approach, required by the normal \(S_{N2}\) mechanism.

\textbf{FIGURE 6}

Other derivatives have resulted from model work during other syntheses. These include (127a), (127b) and (128), which differ from ecdysone by 3, 2 and 1 hydroxyls respectively\textsuperscript{103b}. (128) arises from the Hofmann ecdysone synthesis omitting the selenium dioxide step. (127) a and b were made by partial or total introduction of the tetracyclic features of ecdysone.
into a model with the cholesterol side-chain. Barton et al.\textsuperscript{107} have reported isolation of several ecdysone intermediates or by products (129) and (130). (129) has been separately synthesised from isoamyl magnesium bromide and 20-formyl-2β,3β, 14α-trihydroxy-5α,20S-pregn-7-en-6-one, and has been taken through to 2β,3β,14α,22S-tetrahydroxy-5β-cholest-7-en-6-one.
(25-deoxyisoecdysone). The model work of Barton et al.\textsuperscript{107,117} has also led to the preparation of several ecdysone analogues of the type (131), (132), (133) and (134) (see Discussion) by functionalisation of the ergosterol-type side-chain.

\begin{align*}
&\text{(131) (a)} \quad \text{(b)} \\
&\text{(132) (a)} \quad \text{(b)} \\
&\text{(133) (a)} \quad \text{(b)} \\
&\text{(134) (a)} \quad \text{(b)} \\
\end{align*}

\text{(131-4) (a) } R = H, R' = OH.
\text{(b) } R = OH, R' = H.
The other epimeric by-products (e.g. 5α-, 20S- and 22S- epimers) of the syntheses of ecdysones have in effect been dealt with in the relevant sections and will not be discussed further. Certain synthetic 5α-cholestane and 24β-methyl-5α-cholestane derivatives exhibit ecdysone-antagonistic effects and are dealt with in Section IV. Their preparation was via i-ketone intermediates, which were ring-opened with a halide nucleophile, followed by dehydrohalogenation, and then further elaboration of the 2-ene double bond.
Section IV - General Discussion of Other Aspects.

Origin of the hormones

The discovery of moulting hormone substances in plants\(^{19}\) led to speculation as to whether the hormones were synthesised within the insect body or ingested. Related to this alternative was the hypothesis\(^{47}\) that plants may elaborate ecdysone-like steroids for defensive purposes to interfere with the growth processes of insect predators, prompted by the discovery that *Podocarpus elatus*, a particularly insect-resistant species, was a rich source of crustecdysone. Two results seem to negate the "external source" theory. Firstly no moulting is observed\(^{124}\) when the desert locust (*Schistocerca gregaria*) is fed the bracken *Pteridium aquilinum*, which contains moulting hormones, although extracts are active by injection. Furthermore, certain insects that prey on larvae suffer no ill-effects despite the large amounts of hormones that the larvae contain. Support\(^{125}\) and rejection\(^{30}\) of the ingestion theory have been expressed.

Secondly it now appears that ecdysone-like steroids may play a role in the reproductive processes of plants, a suggestion derived from structural comparison with the plant sex hormone, antheridiol\(^{126}\) (135). Their defensive properties, if any, thus appear coincidental or secondarily contrived. Arguments have been presented\(^{127}\) that steroid hormones evolved in Precambrian times, over 600 million years ago.
Some studies directed towards an understanding of the biosynthetic origins of ecdysones have been carried out.

The initial work of Karlson and Hoffmeister, extended by Horn et al., has shown by administering tritium-labelled cholesterol and 7-dehydrocholesterol to Calliphora larvae that both are precursors of ecdysone and crustecdysone, a finding consistent with the insect's dietary requirements. Since conversion to the 7-dehydro-compound has been established elsewhere, dehydrogenation of cholesterol is probably the first biosynthetic step towards ecdysones. It has been demonstrated that tritium-labelled ecdysone is rapidly metabolised to crustecdysone only, whereas 25-deoxyecdysone gives, in addition, ponasterone A and inokosterone, neither of which occur naturally in Calliphora. 25-deoxyecdysone is not a normal crustecdysone precursor therefore. Surprisingly, no labelled ecdysone was detectable from this, perhaps because hydroxylation at C-25 is slower than at C-20, so that ponasterone A is produced more rapidly.
than ecdysone, but is more slowly converted to crustecdysone. C-25 and C-26 hydroxylation rates of ponasterone A are probably the same, since inokosterone and crustecdysone were found in equal concentrations\(^{131}\).

In tobacco hornworm\(^{133}\), labelled 5β-chole-st-7-en-2β,3β, 14α-triol-6-one gave rise to ecdysone and crustecdysone, showing that side-chain hydroxylation may be brought about after elaboration of the nuclear features.

In Calliphora, 22-deoxyecdysone seems unlikely to be a precursor, because no 22-deoxycrustecdysone was detectable\(^{40}\). The latter has been shown to have low biological activity\(^{61(a)}\), and it follows that side-chain hydroxylation at C-22 and C-25 of precursor sterols precedes elaboration of the tetracycle. Similarly, Calliphora is unable to introduce a 7-double bond in cheilanthone A, and it is likely that the 7-double bond is introduced before all the hydroxy-groups have been established. In Cheilanthes\(^{37}\), the 7-ene introduction may take place at a later stage. The discovery\(^{62}\) that 2-deoxyecdysone and 2-deoxy-crustecdysone occur naturally in plants and are as active in the Calliphora test as the parent compounds, indicates that the biosynthesis of ecdysones probably proceeds through 2-deoxy intermediates\(^{134,135,136,137,138}\).

Labelling studies in various plants have shown that 2-\(^{14}C\)-mevalonic acid is incorporated into crustecdysone and ponasterone A, and that 4-\(^{14}C\)-cholesterol is incorporated
into ecdysone, crustecdysone, ponasterone A and 5β-hydroxy-
crustecdysone. The incorporation of a \( \text{26}^{14}\text{C} \) \text{ label into}
crustecdysone shows that no side-chain degradation is involved\(^{138}\). Labelled precursors which have failed to be incorporated include
4\( ^{14}\text{C} \) \text{-cholest-4-en-3-one}^{134} (known to be involved in 5-ene
to 5β-H- transformation in animals where there is no \( \Delta_7 \)-ene
present), 26\( ^{14}\text{C} \) \text{-25-hydroxycholesterol}^{138} and the 4\( ^{14}\text{C} \) \text{-5α,}
6α- and 5β,6β-epoxy-cholesterols.

A superficial sequence for the biogenesis of ecdysones
in insects may therefore be as in Figure 7, and in plants
as in Figure 8. Further work is required before a fuller
knowledge can be obtained.

![Figure 7](image_url)

![Figure 8](image_url)

It has been suggested that \( \text{C-20, C-22 hydroxylation}
occurs, as in cholesterol metabolism, prior to oxidation to a
20-ketopregnane (14). Although this has not so far been found.
with crustecdysone\textsuperscript{60}, the other catabolism product, 4-methyl-4-hydroxy-pentanoic acid (136), was isolated\textsuperscript{139} in the form of dimethyl butyrolactone (137). C-20,22-bond scission may not however be a major metabolic pathway.

\[
\begin{align*}
\text{HOOC-} & \quad \text{OH} \\
(136) & \quad (137)
\end{align*}
\]

No scheme has yet been proposed for the biogenesis of 24-alkylated ecdysones, though certain postulates have been made. Amarasterones A and B\textsuperscript{82} may be biosynthesised from (32), a hypothetical common precursor, which is stigmasterol derived. It would appear that Amarasterone A is probably further metabolised to its descendants capitasterone, precyasterone and cyasterone, from which sengosterone\textsuperscript{94} is produced.

The 24-methyl ecdysones are presumably biosynthesised from the marine and zoo-sterols of corresponding methyl orientation.

One point is clear. Providing the non-incorporated substrates reached the active sites, the enzymes involved in the biogeneses are fairly specific in their substrates, and slight variations in structure render them inactive, or vastly reduce their potency. This seems quite reasonable in the light of our present knowledge of enzyme action modes.
Mode of Action

Moulting hormone is not only responsible for the skin-shedding part of metamorphosis, but also for tanning and hardening of the cuticle\textsuperscript{23}, and for puparium formation and shape\textsuperscript{140}, and dosage effects have been observed on the normality of the emerging adult\textsuperscript{19}.

Although new criteria have allowed the differentiation of the moulting cycle into 21 stages\textsuperscript{141}, it is sufficient for the present purposes to consider that events leading up to the moulting may be sequentially triggered partly by different ecdysones and partly by a rising hormone titre. This was exemplified by Horn et al.\textsuperscript{23}, who found that early premoult crabs contained a small amount of callinecdysone A, that late premoult contained a larger titre with some crustecdysone and that "soft-shell" crabs had a very high titre of crustecdysone and some callinecdysone B, presumably to aid shell hardening.

Shaaya and Karlson\textsuperscript{142} found in Calliphora a rapid increase in the titre at the beginning of puparium formation. Recently it has been shown that the moulting hormone present in prepupae is crustecdysone\textsuperscript{40}, to which ecdysone is quickly metabolised\textsuperscript{131}. After puparium formation, there is a rapid decrease in the moulting hormone titre attributed\textsuperscript{143} to the appearance of enzymes which catabolise the hormone.

Other Physiological Properties of Ecdysones.

The recent literature abounds with reports of investigations into the extensive and dramatic physiological
actions attributed to moulting hormones. The most exciting is the ability to induce protein synthesis. 15 minutes after injecting $2 \times 10^{-6}$ M ecdysone into the midge, Chironomus tentans, "puffing" of the giant salivary gland chromosomes occurs at the sites of messenger RNA synthesis. The small dosage and rapid effect suggest a primary reaction. It is possible that ecdysone interacts directly with DNA, but Karlson prefers the view that ecdysone combines with chromosomal protein. Emmerich has shown incorporation of labelled ecdysone all over chromosomes and nucleolus, and Clever has proposed the scheme in Figure 9.

A molecular basis of learning and memory is now attributed to quantitative changes in RNA and protein synthesis. However, Carlisle and Ellis report that on injecting prothoracic gland extracts into locusts, the time devoted to marching was reduced (a non-learned response), whilst no apparent effect on social aggregation (a learned activity) was observed.

Kroeger has suggested that ecdysone controls the $\text{Na}^+;\text{K}^+$ ion ratio which in turn controls gene activity.
Ecdysone shows certain enzyme effects. Dopa (β-(3,4-dihydroxyphenyl)-L-alanine) decarboxylase, an important mammalian enzyme, is responsible for the production of N-acetyldopamine, a sclerotising agent. Ecdysone increases the activity of this enzyme in Calliphora⁸ and seems to repress 5-hydroxytryptophan decarboxylase¹⁴⁹. Increased activity of tyrosine-α-ketoglutarate transaminase¹⁵⁰ and glutamic decarboxylase¹⁵¹ have been reported.

Kobayashi¹⁵² has shown that in Bombyx mori ecdysone causes labelled glucose to be preferentially converted to the disaccharide trehalose at the expense of the polysaccharide glycogen.

Some *in vivo* experiments on mice indicate¹⁵³ regression and inhibition of sarcoma 180 tumours by ecdysone, but reports¹⁵⁴ of lack of effect on other tumour cells have also been published.

The discovery²⁹,³⁰ that 20-hydroxyecdysones (crustecdysone, ponasterone A, pterosterone, shidasterone, makisterone C) are as potent as the anabolic steroid 4-chlorotestosterone in stimulating protein synthesis in mice, has produced a wave of publications²⁹,⁷⁴,¹⁵⁵. 5β-hydroxyecdysterone has been reported to be less active, and ecdysone inactive, whereas cyasterone has the greatest stimulatory effect. Rubrosterone and 2β,3β,14α-trihydroxy-5β-pregn-7-en-6,20-dione are also also active⁵⁸,⁵⁹. Oral feeding of young rats however showed no pharmacological activity whatsoever¹⁵⁶. Labelling experiments to demonstrate
the increased capacity for incorporation of leucine, uridine, and thymidine into protein and RNA have been carried out in various arthropods\textsuperscript{157,158,159} and mammals\textsuperscript{160}.

The screening of ecdysones for other properties will continue and can be expected to produce further interest in these compounds.

**Correlation of Structure to Mode of Activity in the Calliphora test**

No complete correlation has yet been made, though the Prague group\textsuperscript{123} and Horn and his co-workers\textsuperscript{122} are carrying out an in-depth exploration of structural requirements and variations.

That it is not sufficient for an active molecule to possess merely the right tetracyclic features is seen from the inactivity of Rubrosterone in the Calliphora test\textsuperscript{49}. However the synthetic analogues (127(a),(b)) and (128) have activities 1/80th, 1/50th and 1/15th that of ecdysone\textsuperscript{103b}, showing that the 14α-hydroxyl group must be present or insertable for high activity, and that whilst there is a side-chain, at least some moulting effect will be produced.

Providing there are 22\textsuperscript{R}-and/or 20\textsuperscript{R}-hydroxyl groups the side-chain can be varied as to alkylation, hydroxylation and unsaturation within certain limits without loss of activity. Changes in the stereochemistry at C-22 (isoecdysone, 22-epicrustecdysone) or C-20 (the epimeric intermediates (106 (a) and (b) of the Hofmann crustecdysone synthesis\textsuperscript{43}) or variations such as
furan formation (stachysterone D\textsuperscript{78}) or saturation at C-22 (22-deoxycrustecdysone with 1/50th ecdysone activity\textsuperscript{61}) render the molecule inactive.

Similarly a shortening of the side-chain or certain nuclear changes reduce (podecdysone B\textsuperscript{98}) or stop (the cheilanthones\textsuperscript{37}) activity. However, insertion of a hydroxyl into the nucleus, either retains potency (ajugasterone C\textsuperscript{55}) or increases it (polypodine B\textsuperscript{91}, sengosterone etc.). Presumably activity depends on the potentiality of the molecule to be converted \textit{in vivo} to active ecdysones. The recent work of Horn and his colleagues\textsuperscript{62,122} has shown that whereas 2-deoxyecdysones are as active as the parent compounds, 2-deoxy-3-epicrustecdysone is only 1/3 as active as crustecdysone, and ponasterone B (2\textalpha,3\textalpha-diol) is only 1/30th as active as ponasterone A (2\textbeta,3\textbeta-diol). It therefore seems very likely that the 3\textbeta-hydroxyl group is necessary for high biological activity and that the 2\textbeta-hydroxyl group of the majority, whilst important for the stabilisation of the 5\textbeta-isomer, is not essential for biological activity.

The relevance of this last observation is only apparent in the light of the complete change of action of these compounds on inversion of the A/B ring linkage.
Certain ecdysone-antagonistic effects (inhibition of cuticle-hardening and sclerotisation) are encountered with a number of $2\beta,3\beta$-dihydroxy-6-oxo-5α-steroids of the pregnane (138) and cholestane (139) series. Correspondingly substituted steroids of the androstane skeleton (140) exhibit a toxic action towards the final larval instar of *Pyrrhocoris Cysterus* L., and the 17-ketone (140a) is reported to have a sterilising effect on the common house fly. These effects seem related to the $3\beta$-hydroxy-6-one system.

\[ R \]

(138) (a) $R + R' = 0$

(b) $R = \text{OH}, R' = \text{H}$

(139) (a) $R = \text{H}$

(b) $R = \text{OH}$

(c) $R = \text{H}$

(140) (a) $R + R' = 0$

(b) $R = \text{OH}, R' = \text{H}$
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DISCUSSION.

Part I — The Synthesis of Ecdysone and Related Compounds

When this work began in 1965\textsuperscript{3}, only a fraction of our present knowledge of ecdysones had been attained. Ecdysone was the only known moulting hormone and the climax of many years' effort had just been realised in the X-ray determination of its structure by Huber and Hoppe\textsuperscript{2}. Subsequently, several syntheses were reported\textsuperscript{3,4,5,6}.

The synthetic efforts which are described below have led to the shortest and most recently published route\textsuperscript{1}.

After some deliberation, the readily available sterol, ergosterol (1) was chosen as the starting point, and the reasons for this choice are apparent from a structural comparison with ecdysone (2).

\begin{center}
\includegraphics[width=0.5\textwidth]{ester.png}
\end{center}

\textsuperscript{3} D.H.R. Barton, P.G. Feakins and P.G. Sammes were joined by the present author in October, 1967.
Feature by feature, the suitability of ergosterol can be seen, and this has also been recognised by another group\(^5\). It possesses (a) a 3β-hydroxy-group which, by a number of routes, can give rise to the required 2β,3β-diol function of ecdysone; (b) the 5,7-diene system, which can act as a precursor for the 14α-hydroxy-7-en-6-one system; and (c) the side-chain 22-double bond, from which the ecdysone side-chain can be elaborated via an oxidative cleavage. Moreover, once the 2β,3β-diol and the 6-ketone groups have been introduced, base-catalysed equilibration at C-5 allows the cis-fused A/B ring system to be produced, a phenomenon which has been closely studied by Jones et al.\(^7\) and emphasised by others\(^4,8\).

The full details of the development of the early stages of this work into reproducible routines have been described previously\(^1,9\). A brief summary is given below, together with the author's own attempts to improve or adapt the route.
The first synthetic sequence to be considered, was the known oxidation\textsuperscript{10} of the diene system with chromium trioxide, as shown in Figure 1. The Hofmann group\textsuperscript{5} have subsequently used this method.

![Diagram of synthetic sequence]

**FIGURE 1.**

It was decided to reject this in favour of a more novel approach, avoiding the introduction of the unwanted 5\(\alpha\)-hydroxyl group. The solvolysis of 5-en-3\(\beta\)-tosylates is known to furnish \(\beta\)-alcohols. In this case, ergosteryl tosylate (3) gave an unstable allylic \(\beta\)-alcohol (4), which was oxidised without purification using active manganese
ergosterol \textsuperscript{11} in chloroform to the cyclopropyl ketone (5) in 65\% overall yield. The published procedure\textsuperscript{12,13} was modified\textsuperscript{1} to allow the reaction sequence (Figure 2) to be carried out on a large scale.

\begin{center}
\textbf{FIGURE 2.}
\end{center}

Attempts were made to find a one-step rearrangement oxidation. Cholesteryl tosylate (7) when heated with sodium
acetate in dimethyl sulphoxide for four hours at 80-90°C is reported to give i-cholestanone (8) in 51% yield. When similar conditions were used in the present system, only the triene (6) (in 85% yield) was obtained, because of the allylic nature of the i-alcohol. Treatment of the tosylate with manganese dioxide under rearrangement conditions stopped at the i-alcohol stage, anhydrous conditions being necessary for the manganese dioxide to remain finely divided and active.

No i-ergosta-7,22-dien-6-one was produced however when a basic sample of manganese dioxide was used in dry benzene, the triene (6) again resulting.

The use of dry DMF with pyridine N-oxide on the tosylate (Figure 3) or dry DMSO with pyridine N-sulphur trioxide and triethylamine on the i-alcohol or the tosylate gave none of the required product, under conditions in which cinnamyl and benzyl alcohols were oxidised. Other "activators", well-known in the literature were considered, but no improvement on the original method could be obtained. An extensive n.m.r. study of the Doering method did not add to the findings of Torssel, though the optimum procedure was deduceable.
Isomerisation and Cis-Hydroxylation.

In order to introduce the 2β,3β-dihydroxy function, it was necessary to isomerise the cyclopropyl ketone (5) to the 2-ene. Similar transformations had been achieved\(^\text{17}\) by a two-step process of acid catalysed ring-opening with a suitable nucleophile, to give the 3β-halo compound for example, followed by elimination to the 2-ene. However, it was believed that a one-step process might be feasible, and there was precedent for this. Butenandt\(^\text{18}\) had reported that 1-androsta-6,17-dione (9) afforded the isomer (10) when heated in quinoline.

Figure 4 shows a plausible mechanism for a cyclopropyl 7-en-6-one.
Treatment of a model, i-cholestan-6-one, with a variety of bases gave only recovered starting material, presumably due to preferred enolisation towards position C-7. Effectively the same result was obtained with i-ergosta-7,22-dien-6-one (5), since it was found that base-treatment (e.g. sodium hydroxide/MeOH) caused equilibration between the 7(8)-(5) and 8(14)-(12) double bond isomers. A characteristic downfield shift of the 18-methyl group was seen in the $^1$H n.m.r. With the C-14 position blocked by a hydroxyl-group, equilibration occurred to the 8(9)-isomer (13), for example with 14α-hydroxy-i-cholest-7-en-6-one.

Attention was turned to the use of buffered acid systems, the mechanism shown in Figure 5 being anticipated.
Opening of the cyclopropyl ring to the 3β-chloro-compound was achieved with pyridine hydrochloride in pyridine, and using pyridine tosylate in pyridine a complex mixture containing the 2-ene was obtained. That the ring-opening had to be carried out prior to introduction of the 14α-hydroxyl became evident when buffered-acid treatment caused dehydration of compounds containing the 14α-hydroxy-7-ene-6-one system.

The key experiment proved to be the treatment of the cyclopropyl ketone (5) with tosic acid in dry benzene, which resulted, after initial 8(14)-isomerisation, in a mixture of products, (14) (75% yield) and (15), in which the toluene p-sulphonate anion had acted as a weak nucleophile upon the incipient carbonium ion. Examples of this are known in the literature. In the mass spectrum of the epimeric mixture of 3β-toluene-p-sulphonates, ready loss of toluene p-sulphonic acid was observed, suggesting that heating the tosylates, or even better, the reaction mixture, could well lead to the Δ2-compound.
After many experiments, the procedure now employed was established, in which the i-ketone (5) was heated for 1.5 hours under nitrogen at 160°C in sulpholan containing toluene p-sulphonic acid (5% w/w of the cyclopropyl ketone).

Experiments were later performed in which the use of lithium perchlorate in dry benzene and sulpholan, and silver perchlorate in nitromethane was investigated, but the standard conditions were found to be the best of those employed.

Careful chromatography allowed the separation of the 14α- (11) and 14β- (16) isomers of the 2,7,22-triene mixture, the former of which was later to be described by the German workers.

Equilibrium experiments by Feakins showed that whereas in the cyclopropyl compound (5) the conjugated to non-conjugated ketone ratio is 6:4, after ring-opening the conjugated ketone is preferred by a 10:1 ratio. Addition of a proton at C-14 must therefore occur after ring-opening, a process taking place from either the α- or β-face. Prior to the publication of various aspects of ecdysone work, no mention had been made in the literature of the double-bond isomerisation of 7-en-6-one steroids.
Using a modified Woodward-Prevost procedure\textsuperscript{21} (Figure 6), the crude mixture of 2-ene compounds was \textit{cis}-hydroxylated, and the C-14 epimeric monoacetate diols (17) were isolated. An axial position for the acetate group and an equatorial one for the hydroxyl was assigned from the width at the half-height of the signals due to the protons geminal to these functions\textsuperscript{22} in the n.m.r. spectrum. Separation of the epimers was possible after acetylation, but a technique was sought for introduction of the 14α-hydroxyl group from both isomers.

An alternative process for the \textit{cis}-hydroxylation was conceived via an oxymercurial intermediate, in the hope of improving upon the 15\% overall yield of the monoacetate diols
from the i-ketone (5). Using cholest-2-ene as a model, formation of (19(a)) by the literature methods was found to occur in 40% yield over several days, and the more useful (19(b)) formed even more slowly, and was never obtained pure. If the collapse of (19(b)) to the acetoxonium ion (18) could have been induced the same intermediate as in the Woodward-Prevost reaction would have resulted, allowing a novel cis-hydroxylation procedure: 19(b) —→ 18 (Figure 6).

However, this aspect was abandoned, because of the yields and slow reaction time involved in the first step.

The yield in the cis-hydroxylation step was unsatisfactory, and a control experiment was undertaken to determine whether or not the more hindered trans-double bond at C-22 was being oxidised as well as the cis-2-ene. Treatment of i-ergosta-7,22-dien-6-ene (5) under identical conditions gave one major product, the iodoacetate (20). Little of the cyclopropyl ketone remained after half an hour, though reaction was slower than with the 2,7,22-trien-6-ones (11 and 16), and maximum yields of the iodoacetate (53%) were obtained with a reaction.
time of 3 hours. After prolonged stirring (several days), the iodoacetate gradually gave an inseparable mixture of hydroxy acetates(21). Amongst the many byproducts of the cis-hydroxylation of the 2-enes (11) and (16) were compounds, some of which gave positive Beilstein halogen tests, resulting from attack on both the 2- and 22-double bonds.

These results are analogous to those of Barton et al. on a similar system using more vigorous conditions (Figure 7).
The structure of the iodoacetate (20) was consistent with its spectral data. The molecular ion occurred at m/e 580 in the mass spectrum. In the n.m.r. spectrum, the protons, α to the acetate and to the iodide occurred as doublets at 4.5τ and 5.75τ respectively. The determination of the side-chain stereochemistry will be discussed in Part II of this section, and sprang from a study of the reactivity of the 22-double bond. Interest in this was three-fold: firstly, to discover the stereochemistry of the electrophilic attack on the 22-double bond; secondly, as a possible route, by dehalogenation, to 24βF-methyl-25-deoxyecdysones, (this is fully dealt with in Part II of the Discussion); and finally, as a potential source of biosynthetically useful 22- and 23- derivatives.

Introduction of the 14α-Hydroxy Group.

Fieser and Huang26 had shown that a hydroxy group could be introduced into the γ-position of an αβ-unsaturated ketone (22(a)) by enol-acetylation and peroxycacid oxidation to the alcohol (22(b)).

Other examples are also known 27.
Treatment\(^9\) of the monoacetate diols (17) with acetic anhydride in carbon tetrachloride containing a trace of perchloric acid\(^{28}\) catalyst resulted in the formation of a single compound, the dienol acetate (23). The \(^1H\) n.m.r. spectrum showed the expected downfield shift of the 18-methyl signal, and the vinylic proton at C-7 appeared as a broadened doublet by allylic coupling to the 5α-proton. The chromophore was found to absorb at 254 nm in the u.v. spectrum, which is consistent with the heteroannular system shown (23) and not with either the 5,7- or 6,8(9)-isomers, which are both homoannular\(^{29}\).

![Chemical Structure](image)

No evidence was found for epimerisation about C-5. The pure 14β-epimer of (17) gave the same enol acetate as the crude epimeric mixture.

Introduction of the 14α-hydroxy-function was brought\(^9\) about smoothly and in high yield using monoperphthalic acid in ether. The 18-methyl signal showed a downfield shift in the n.m.r. spectrum, in contrast to an upward shift expected for a 14β-hydroxyl group\(^{30}\). That this was the same product
as that which would be obtained using selenium dioxide on the 7-en-6-one, (a reagent known to result in 14\(\alpha\)-hydroxylation)\(^{31}\), was shown by a series of model reactions on the cyclopropyl ketone (5). Mori and his colleagues\(^{19}\) have since reported a similar technique employed in their synthesis of ecdysone. Providing only a small (20\%) excess of monoperphthalic acid was used, oxidation was confined mainly to the tetra-substituted \(\Delta8(14)\)-double bond.

![Chemical Structure](image)

This hydroxy-ketone (24) was synthesised independently by the German workers\(^5\).

When following the epoxidation step by T.L.C. one of the constituents of the crude enol-acetylation mixture was observed to remain unaffected. This component had a similar polarity to the principal enol-acetate which precluded isolation. However after the per-acid treatment of the mixture (during which the enol-acetate reacts to give the required product), this component was isolated and shown to be 2\(\beta\),3\(\beta\)-diacetoxy-5\(\alpha\),14\(\beta\)-ergosta-7,22-dien-6-one (25), one of the two C-14-epimers separated by Peakins\(^9\) after acetylation of the monoacetate diols (17). This result appears to indicate that the 14\(\alpha\)-H-epimer of (25) is preferentially enol-acetylated. Prolonged
reaction time causes both epimers to be completely consumed.

Side-Chain Modification.

The remaining problem was the elaboration of the required side-chain by ozonolysis to the 20-formyl derivative and reaction with a suitable Grignard reagent. Both 3β-ergosta-7,22-dien-6-one (5) and 2β,3β-diacetoxy-14α-hydroxy-5α-ergosta-7,22-dien-6-one (24) gave the corresponding 20S-formylpregn-7-en-6-ones (26) and (27) in very good yields.

The ozonolysis was carried out under the conditions developed by Slomp and Johnson\textsuperscript{32}, in which the substrate is ozonolysed at -70°C in 1% pyridine/methylene dichloride to give \textit{in situ} reduction, with T.L.C. control of the reaction. The aldehydes were purified by P.L.C. and used immediately.

The \textsuperscript{1}H n.m.r. spectrum of the model aldehyde (26) showed no evidence of epimerisation about position 20 at the reaction temperature. However on heating in the presence of a little pyridine, a mixture was formed, as seen by the splitting of the low-field aldehyde proton signal and of the methyl signals.
Feakins, in the first approach to the side-chain synthesis, had prepared 4-bromo-2-methyl-butan-2-ol-tetrahydropyranyl ether (28), but this compound had resisted all attempts to make the corresponding Grignard reagent. The acetylenic ether (29) was therefore used instead.

Horn and his co-workers have since reported no difficulty in preparing and using the magnesium salts of 5-chloro-2-methylpentan-2-ol tetrahydropyranyl ether (30) and of (31) in their synthesis of 22-deoxycrustecdysone (32), a method of exchange with ethyl magnesium bromide being employed.
There should be no intrinsic reason therefore why (28) should not similarly form a Grignard salt.

Addition to the 20-ketone (33) (in the presence of the bulky 14α-trimethylsilylether group) gave only the required 20S-alcohol, the product predicted by Cram's rule.

Feakins\(^9\) found that the bromomagnesium salt of (29), when reacted with the aldehyde (27) gave only one major adduct (34(a)) and a very small amount of a less polar material (presumably (34(b))). This is again a Cram's rule addition.
Only (34(a)) was obtained in sufficient quantity to allow further manipulation and was taken through the reaction sequence to be described shortly to 22-isoecdysone (35), identical with an authentic sample.\(^3\)

Similar stereospecificity of attack has also been observed\(^5\) for the reaction of the same Grignard reagent with the 5β-epimer of (27) to give the 5β-epimers of (34(a)) and (34(b)) in the ratio 4:3:1. However, the same workers\(^5\) obtained mainly the opposite (required) 22S-epimer in violation of Cram's rule, when using the aldehyde (36), which contains no 14α-hydroxyl group. Although not specified, the ratio of the two epimers calculated from their results is in the order of 7:4. It is most surprising that the absence of a 14α-hydroxyl group should render the adoption of the eclipsed conformation required by Cram's rule so difficult.
In contrast to the results with the magnesium salts, the lithium salt of the acetylenic ether (29) gives rise to both epimers with aldehydes containing a 14α-hydroxy group, as demonstrated by the Syntex group\(^3\) on the aldehyde (37), and later confirmed by the present author using the aldehyde (27) in the ecdysone synthesis\(^1\).

The ratio of (34a) to (34b) in this case was 6:5, the "Cram product" (34a) being the major isomer. Had this not been so, an alteration in the order and mode of introduction of the 14α-hydroxy group would have been necessary.

The different results obtained using the bromomagnesium Grignard salt on (27) and (36) are very intriguing, even more so than the epimer ratio differences resulting from the bromomagnesium and lithium salts used in the presence of a 14α-hydroxy group. In the latter case, both salts give the same major product, and the effect is one of an exaggeration of a trend, rather than a new phenomenon. It is probable that the
lithium salt is more reactive and is therefore less sensitive to steric inhibitions imposed by the aldehyde substrate.

In the former case, a long-range electrostatic interaction has been invoked, since molecular models show that the 14α- and 22-iso-hydroxy groups are in fact a considerable distance apart (ca. 7 Å). However three examples from crustecdysone syntheses serve to show that the situation is by no means simple. In the Syntex synthesis, the chloromagnesium salt of (29) was reacted with the 14α-hydroxy, 20R-tetrahydropyranyloxyaldehyde (38) and only the required 20R, 22R-product (39) (55% yield) was isolated, indicating a high degree of stereospecificity.

The very bulky 20-substituent must be the more dominant influence in this instance. However the same reaction
carried out on the 20S-aldehyde (40) gave a mixture of 20S, 22S- and 20S, 22R- products, indicating that at least two competing influences are acting.

The situation seems to be different for 20-oxo-pregnanes, and the above observations are not general. With the 20-ketone (41), used in the German synthesis\textsuperscript{36}, the bromomagnesium salt of the homologous acetylenic ether (31) gave a mixture of epimers in a favourable ratio of 4:1 for the required 20R-compound.

The Japanese synthesis\textsuperscript{37} confirms both the Syntex and the German findings. Vinyl magnesium bromide with the 20-ketone (42) gave the 20R-epimer almost exclusively, in concordance with the Prelog-Cram rule. The 22-aldehyde product of ozonolysis, a compound very similar to the Syntex substrate (38), showed the same stereospecific control, yielding only the required
The effect of the presence of a 14α-hydroxyl group on Grignard additions to 20-ketones appears to be one of increase in stereospecificity of attack. The acetylenic Cram-product (20R for the two crustecdysone cases and 20S for 22-deoxycrustecdysone) is produced predominantly.

Before tackling the vital ecdysone synthesis therefore, it was decided to carry out some similar Grignard additions to 22-aldehydes, which, whilst resulting in analogous and potentially interesting products, would also shed some light on the factors controlling the ratio of the resulting epimeric alcohols. Four reactions were examined (including the ecdysone synthesis). Obviously a much more exhaustive programme would have been necessary to reach a precise and analytical conclusion, but within the self-imposed limitations certain trends became apparent.

The first model reaction was that between isoamyl magnesium bromide and (20S)-20-formyl-3α,5α-cyclopregn-7-en-6-one (26). Addition of the Grignard salt, using 5 molar equivalents excess, was carried out in dry ether at room temperature for five minutes, followed by refluxing for a similar period.
Multiple-elution P.L.C. enabled the two required epimeric alcohols (43 a and b) to be isolated. Both were crystalline compounds and were fully characterised. By an u.v. comparison of yields, the ratio of the products was shown to be 6.3:1 in favour of the more polar epimer, a degree of stereospecificity which had not been anticipated. The more polar compound was assigned the 22S-configuration on two counts: (a) by analogy of its polarity with those of ecdysone and isoecdysone; and (b) by comparison with the two alcohols obtained by LAH reduction (and manganese dioxide oxidation) of 1-cholest-7-en-6,22-dione (44), also (43 a and b), produced in a ratio 3:1. The

![Chemical structure](image)

(43)(a) $R^1 = H$, $R^2 = OH$
(b) $R^1 = OH$, $R^2 = H$. 

assignment of the LAH reduction products followed by analogy with the 22-hydroxy-cholestan-3β-ols and similar compounds, the absolute stereochemistry of which is categorically known from the work of Caspi and his colleagues and Mori et al.
These LAH reductions of 22-ketones are "anti-Cram" reactions. For example the major product from the LAH reduction of (44) is the same as that from the isoamyl magnesium bromide addition to (26), which could not be the case were both reactions consistent with Cram's rule.

Cram's rule\(^{40}\) states that in kinetically controlled addition reactions (other than catalytic hydrogenation) of a carbonyl compound IMSCCOR (e.g. Grignard addition, hydride reduction), the predominantly formed stereoisomer may be predicted as follows: The asymmetric carbon is so rotated that the carbonyl group is flanked by the two smaller groups, M (medium) and S (small), attached to the asymmetric carbon, with the large group L eclipsing R. The reagent will then approach from the side of the smaller group S, as shown in Figure 8. This model does not apply if S, M or L is capable of complexing with the organometallic (e.g. OH, NH\(_2\)).

That the 22-ketone (44) should be less likely to adopt a hindered conformation than the 22-aldehyde (26) is not surprising, an alkyl group occupying much more space than a hydrogen atom. Furthermore the stereospecificity in the case of
the encroaching hydride ion is much less (3:1) than the isoamyl magnesium bromide (6.3:1), presumably because the smaller nucleophile is not so sensitive to the steric environment it approaches.

The stereoselectivity in the case of the cyclopropylaldehyde (26) was much greater than expected from the published ecdysone work. Far from getting similar results to those obtained by the German workers\(^5\) with (36) (no 14α-OH), the preponderant epimer had the same configuration as their major product from an aldehyde containing a 14α-OH group (5β-Δ-(27)). It would appear therefore that the cyclopropyl group is exerting a long-range conformational effect of greater significance than the presence of a 14α-substituent, especially since the isoamyl Grignard should be less restricted by steric factors than the more bulky acetylenic ether Grignard.

A further point from this experiment was that the difference in susceptibility of the 6-ketone and 22-aldehyde to isoamyl magnesium bromide was not as great as that observed in the ecdysone work. Byproducts of the type (45) were formed, in small but significant amounts.
To draw any unequivocal conclusions from the above results, it would be necessary to examine under identical conditions the product ratios observed for the reaction between isoamyl magnesium bromide and the aldehydes (46), (47) and (48). This would immediately define the influences exerted by each moiety of (26) both separately and in concert, and would make an interesting starting point for future work.

The reaction between isoamylmagnesium bromide and the 20 S-formyl compound (27) was carried out under several conditions. When a ten-fold excess of Grignard reagent was added at 0°C to the aldehyde in tetrahydrofuran, the main product was a compound whose spectral data showed it to be (49), due to elimination of the tertiary-hydroxy group from the required adduct. The infra-red spectrum had a carbonyl
absorption at $1720\text{cm}^{-1}$ and the molecular ion was at $514\text{ m/e}$ in the mass spectrum.

Using a smaller excess of isoamyl magnesium bromide (7 molar equivalents) at $-30^\circ\text{C}$ (cardice/carbon tetrachloride), only one 22–alcohol (50) was isolated and fully characterised, a 60% yield being obtained allowing for the recovery of unreacted aldehyde. A smaller amount of a less polar compound, which resisted complete characterisation, but which was almost certainly the 22–R–epimer, was also isolated.

The configurations of (49) and (50) were shown to be 22–S–alcohols by the identity of the crystalline compound (50) with the hydrogenolysis byproduct isolated by Feakins$^9$ during his preparation of isoecdysone. The i.r. spectrum showed bands at 3500, 1730, 1670 and $1250\text{cm}^{-1}$, revealing all the necessary structural features. The chromophore absorption was
at 240.5 nm in the u.v. spectrum as required and the mass spectrum showed ready loss of 18 units from the molecular ion at m/e 532. The fragmentation pattern and n.m.r. spectrum were fully consistent with the assigned structure.

Assuming that the uncharacterised, less polar compound was the 22-R-epimer, a product ratio of about 3:1 was achieved. This addition was therefore less stereospecific than that observed with the analogous cyclopropyl aldehyde, (26). An increase in preference for the 22S- over the 22-R- epimer in the presence of a 14α-hydroxy group would have been predicted from the available literature data. The contribution of the cyclopropyl group to a long range conformational effect therefore must be very large.

It would be interesting to examine the same reaction with (20-S)-20-formyl-i-pregn-7-en-14α-ol-6-one, (51). For this compound the ratio of the 22S- to 22-R-epimer would be predicted to be considerably greater than the 6.3:1 found for (26). Furthermore the compound (51) should be easily obtained by reductive ozonolysis of i-ergosta-7,22-dien-14α-ol-6-one (52), itself prepared by selenium dioxide oxidation of the corresponding cyclopropyl ketone, (5). This would again form a suitable basis for future studies.

When the 14,22S-diol (50) was refluxed under nitrogen for 3 hours with 0.3% potassium carbonate solution in 90% aqueous methanol two compounds were isolated. These proved to be the expected (22S)-2β,3β,14α,22-tetrahydroxy-5β-cholest-7-en-6-one and its 5α-epimer i.e. 25-deoxy-5β- and 25-deoxy-5α-isoecdysone.

At this juncture, it was decided to return a little more
towards the main objective, the completion of the ecdysone synthesis.

The acetylenic alcohol (53), prepared by the Grignard reaction between acetone and acetylene, readily formed the tetrahydropyranyl ether (29) when treated with dihydropyran in the presence of a little tosic acid. The lithium derivative, used in the side-chain elaboration, was made by an exchange reaction between the ether and freshly standardised methyl lithium carried out in the absence of moisture and air.

\[
\begin{align*}
\text{(53)}
\end{align*}
\]

Several experiments were required to establish optimum conditions of reaction with the cyclopropyl aldehyde (26). At first using an excess of five equivalents of the lithium salt at room temperature, the two major products were less polar than expected. Both compounds, (54 (a) and (b)), isolated in approximately equal amounts, were non-crystalline, and were not completely defined. All the spectral data indicated that they were the products due to additional attack at the 6-ketone position followed by dehydration (as observed in the
case of isoamyl magnesium bromide). The i.r. spectrum showed bands due to the alcohol and the ether groups only, and the chromophore absorbed at 258 and 276 nm in its u.v. spectrum.

Because of extreme ease of fragmentation, the molecular ion (m/e 644) was hardly discernible, but loss of four fragments of 85, 84, 84 and 83 mass units could be seen. These represent the sequential loss of fragments derived from two pyranyl ether and two acetylenic alcohol units, verifying the proposed structure. In a second experiment, the allylic 6α-hydroxy precursors of (54 a and b) were also isolated, and showed a tendency to dehydrate.

Dropwise addition of less than two equivalents of the lithium salt of (29) in dry ether to the aldehyde (26) in dry THF stirred under nitrogen, gave a white non-crystalline solid in 50% yield, which ran as one spot after six elutions on T.L.C. Its spectral data indicated that it had the required structure (55), and at first it was assumed to be the 22S-epimer by analogy to the results of the German workers.\(^5\)
Successful reduction of the side-chain in 75% yield was brought about by catalytic hydrogenation using 5% palladium-charcoal in purified ethyl acetate containing a few drops of triethylamine. The product was again non-crystalline and apparently a single compound (56).

Removal of the protecting group with N/20 methanolic hydrochloric acid gave a product, which could be separated into two crystalline epimeric products, the structures of which proved to be (57 a and b), establishing that (55) and (56) were both epimeric mixtures. The product ratio of the more polar (22S) to less polar (22R) epimer appeared to be approximately 6:4 by T.L.C., and their complete characterisation confirms the structures of the partially characterised precursors. The assignment of orientation was by polarity analogy with ecdysone and iso-ecdysone and by product ratio analogy with the work published on ecdysone concerning Grignard additions of the lithium salt of the acetylenic ether (29).
In the above experiment, the ratio of the 22-alcohols seems to be the same as that obtained using the same Grignard reagent on an aldehyde containing a 14α-hydroxy group. The German workers found that the ratio was reversed when the bromomagnesium salt was used on an aldehyde which had not been hydroxylated at C-14. To obtain a parallel result to this with the bromomagnesium salt of (29) on (26) would be most intriguing. Arguments can be presented from our present knowledge both for and against this probability.

The additions to the C-22-carbonyl group may be summarised as follows:—

1. In the presence of a 20R-hydroxy group, the product is exclusively the 22βF-epimer.
2. In contrast, the 20S- and 14α-hydroxy groups appear to be competing influences, since a mixture of 20S, 22S- and 20S, 22R- alcohols result in this case.
3. For (20-S-)-20-formyl-3a,5α-cyclopregn-7-en-6-one, (26), the high ratio in favour of the 22αF-epimer indicate that the eclipsed "Cram" conformation is readily adopted and a favourable long-range conformational effect is in operation. This follows
from (a) the smaller ratio in favour of the "Cram" product resulting from isoamyl magnesium bromide on (20-\( \hat{S} \)-)-20-formyl-2\( \beta \),3\( \beta \)-diacetoxy-14\( \alpha \)-hydroxy-5\( \alpha \)-pregn-7-en-6-one (27) and from the bromomagnesium salt of the acetylenic ether (29) on the 5\( \beta \)-epimer of (27); and (b) the preference for the "anti-Cram" epimer in the case of (20-\( S \)-)-20-formyl-2\( \beta \),3\( \beta \)-diacetoxy-5\( \alpha \),14\( \beta \)-pregn-7-en-6-one (36). In the latter case, it appears that (36) is unable to assume the required eclipsed conformation, although the only structural difference between (36) and (26) is the presence of a 2\( \beta \),3\( \beta \)-diacetate system in the one and a 3\( \alpha \),5\( \kappa \)-cyclopropyl group in the other. (27) differs from (36) in the presence of a 14\( \alpha \)-hydroxy group, and is able to adopt the eclipsed conformer sufficiently to favour the 22\( \alpha \)-epimer.

An extension of these studies as outlined in the preceding pages would clarify considerably the above speculations. Further it is of interest that lithium salts of acetylenic ethers are much less sensitive to conformational arrangement of the aldehyde, and the product ratios are more nearly equal.

It would seem that in most cases, the 22-aldehydes can reasonably assume the eclipsed conformation required by Cram's rule (i.e. not the ground state). This is not the case for the LAH reduction of 22-ketones, where presumably the interactions resulting in the required conformation are so great that it cannot be taken up by the ketone, and reduction occurs in a more stable conformation giving the observed "anti-Cram" major product.
It must be noted that factors other than steric control and conformation in the substrate will come into play in these Grignard additions, for example reaction conditions, nature of nucleophile, concentration, temperature, etc., and the above predictions serve only as a tentative guide.

The remaining work in this section was involved with the preparation of ecdysone and isoecdysone.1

The dropwise treatment of the aldehyde (27) in dry THF with the freshly prepared lithium salt of (29) (four equivalents) for ten minutes at -30°C under nitrogen smoothly gave, after work up, a product, which could be separated by P.L.C. (three elutions) into four major polar components. The two less polar compounds corresponded to the required 22S- and 22R-adducts, and were isolated in a 6:5 ratio. The more polar 22R-component was a crystalline solid, identical to that isolated by Feakins1,9 using the bromomagnesium salt of (29), and was hydrogenated and the ether protecting group removed as described above. As well as the required tetrahydro-derivative (58), a slightly less polar compound was also isolated, which was the 7,23-cis-diene (59).

Heating (58) for three hours under nitrogen in 0.3% potassium carbonate in refluxing 90% methanol resulted in a simultaneous removal of the acetate groups and equilibration about the 5-position. The 5α- and 5β- epimers of isoecdysone (35) were separated by T.L.C. in a ratio of 6:4 in favour of
the more polar 5β-compound. Both samples were identical to authentic specimens\textsuperscript{34} and equilibration of either gave the same equilibrium products in the same ratio as observed with authentic samples.

Reduction of the triple bond of the non-crystalline less polar compound followed by removal of the tetrahydro-pyranyl ether group afforded the crystalline diacetate (60). Equilibration and hydrolysis again afforded two products. The more polar isomer corresponded in chromatographic properties to an authentic sample\textsuperscript{34} of ecdysone (2), and the less polar one to 5α-ecdysone.

Spectral data on the authentic and synthetic ecdysone were in good agreement, except in the case of its mass spectrum. In addition to the expected fragmentation at m/e 446, 428, 348 and 330, the spectrum also showed small peaks at m/e 462, 444 and 426, resulting from traces of 23-dehydro-
material, a contaminant arising from incomplete reduction. Repeated hydrogenation removed the impurity to yield a sample identical in all respects with the authentic material.

As mentioned above, two more polar fractions were also isolated from the Grignard reaction. These were due to loss of the acetate groups, by attack of excess Grignard reagent. These components were taken through the usual sequence to give more of the ecdysones and isoecdysones.

This completed the initial aim, which was to synthesise the insect-moulting hormone, ecdysone. The resulting twelve stage synthesis is the shortest recorded route.
SCHEME I

SYNTHESIS OF ECYDSONE.
Part II - Side-Chain Derivatives and Their Constitution.

The isolation of the rather stable iodoacetate (20) by application of the modified cis-hydroxylation procedure to \( \text{i-ergosta-7,22-dien-6-one (5) } \) was a result sufficiently unexpected to prompt us to investigate more closely electrophilic addition to this 22-double bond. The \( \beta_F \)-methyl groups at the tertiary C-20 and C-24 positions give the double bond a fairly symmetrical environment, and the other substituents at these positions, the steroid nucleus and the isopropyl group, respectively, are both sterically "large". That a single iodoacetate (both spectroscopically and by twelve elutions on TLC) should be isolable however points to a high degree of stereospecificity and the encroaching electrophile finds one approach far preferable to all others. It was envisaged that the stereochemistry of the iodoacetate could be determined. This would immediately define the mode of addition of this particular iodoacetylation, and would for the first time indicate the stereochemistry of the major products of all such kinetically-controlled electrophilic additions to the ergosterol side-chain double bond. Several instances of this kind of derivative have previously appeared in the literature (for example (60) and (61)), but the stereochemistry has not been previously illucidated. Furthermore, the possibility of using the side-chain iodoacetate as an intermediate in the preparation of \( 24r\beta_F \)-methyl-25-deoxyecdysones could be assessed once its structure was defined.
Lastly, the occurrence of ergosterol in nature is of biosynthetic interest, and addition to the double bond results in a potential source of biologically important derivatives of known stereochemistry.

The absolute configuration of ergosterol itself is well-known. As originally suggested by Ruzicka et al.\textsuperscript{43} and verified by Carlisle and Crowfoot\textsuperscript{44} by complete X-ray analysis of cholesteryl iodide, the C-17 side-chain of most naturally occurring steroids has the \( \beta \)-configuration, and ergosterol is no exception. A series of infra-red spectroscopic studies\textsuperscript{45} has shown that the 22-double bond has the \underline{\text{trans}}-configuration. Absolute configurational work by several groups\textsuperscript{46,47} has shown that the methyl group at C-24 has the \( \beta_F \)- or \( R \)-orientation, in contrast to campesterol (62). Oxidation of the latter yields (+) 5,6-dimethylheptan-2-one (63a) under conditions where ergosterol gives (−) 5,6-dimethylheptan-2-one (63b). The studies of Kishida and his co-workers\textsuperscript{48} and of Romeo et al.\textsuperscript{49} confirmed these results.
Incontrovertible X-ray determinations have not been carried out on ergosterol itself, but the closely related derivative, calciferol, (64) was successfully examined\(^5\) as its 4-iodo-5-nitrobenzoate. A representation (65) of the three-dimensional structure is given below, and the stereochemical points enumerated above are shown to be justified. The 20-\(\beta\)- (or \(S\))- orientation\(^5\) of the 21-methyl groups was also demonstrated, and the side-chain was shown to adopt a fully staggered, zigzag conformation in the crystal.
The necessity to determine absolute configuration has led to the development of a variety of methods in the literature. Of prime importance is X-ray crystallography\textsuperscript{52}. Until recently, it was essential to be able to form a "heavy atom" derivative of suitable crystalline shape and size. Improvements and advances, both in machinery and technique, are now allowing the old anomalous dispersion method to be extended to suitable crystals of certain parent compounds, as exemplified by Huber and Hoppe\textsuperscript{2} in the case of ecdysone.

In the present instance, the iodoacetate (20) could only be obtained in a micro-crystalline form, although many attempts were made to grow crystals from various solvents. It was therefore decided to make the corresponding bromoacetate.

Treatment of the cyclopropylketone (5) with N-bromo-succinimide in aqueous tetrahydrofuran under nitrogen at 0\textdegree C for several hours\textsuperscript{53} enabled a crude bromohydrin product to be isolated. Multiple-elution P.L.C. was accompanied by some
decomposition. Two impure components were isolated however, which were unstable and could not be completely characterised. Both were obtained as crystalline solids, which tended to be discoloured by loss of bromine, but showed one proton α to bromine and one α to hydroxyl in the n.m.r. spectrum. The minor, more polar component seemed to consist of more than one compound.

Both components were acetylated with dry pyridine and acetic anhydride in this first preparation. In subsequent work, it was found to be no advantage to separate the intermediate bromohydrins, and acetylation with acetic anhydride was carried out on the crude product from the NBS treatment. Three bromoacetates were eventually characterised. The major (least polar) bromoacetate (referred to henceforth as B.A.III) was readily isolated in 37% yield by P.I.C. Separation of the second major and the minor (most polar) bromoacetates (B.A. II and B.A.I respectively) was achieved by further T.I.C. purification. Yields were 21% and 9% respectively. The three compounds were spectroscopically almost identical, the data being consistent with structures of the type (66). The three identical mass spectra showed the expected doublet molecular ions at 532 and 534.

The major bromoacetate (B.A.III) crystallised as beautiful, long needles, which were, however, unsuitable for X-ray work because of "twinning". Eventually one crystal was obtained of sufficient quality and the initial X-ray work was carried out upon it to give the unit cell dimensions
(66) (a) $R_1 = H$, $R_2 = OAc$, $R_3 = Br$, $R_4 = H$.
(b) $R_1 = OAc$, $R_2 = H$, $R_3 = H$, $R_4 = Br$.
(c) $R_1 = H$, $R_2 = Br$, $R_3 = OAc$, $R_4 = H$.
(d) $R_1 = Br$, $R_2 = H$, $R_3 = H$, $R_4 = OAc$.

(6 x 10 x 18^2 \text{Å}^3), the number of molecules per unit cell (2) and the probable space group (P \text{121}). Unfortunately, the crystal tended to decompose under X-ray bombardment, and preliminary studies indicated that the side-chain was vibrating too loosely in the crystal lattice to render the determination practicable.

It had been considered reasonable on mechanistic grounds to assume that the unique iodoacetate and the major bromoacetate contained the halogen and acetate groups on the same atoms in each case and with the same orientations. This was later justified chemically by converting each to the same acetate, alcohol and ketone. The smaller halogen should obviously find approach from more than one direction easier than the larger one, a major and a minor bromonium ion resulting, the opening of which explains the multiplicity of products. However, that
there is a preferred direction of addition is borne out by there being one product obtained in much higher yield than any other. Furthermore, the opening of the halonium ion intermediates by the acetate group must also take place predominantly at one of the carbon atoms. If this were not so, two products in approximately equal abundance would result. From a purely stereochemical standpoint, eight isomers are possible for (66), four arising from trans-addition (those shown) and four from cis-addition. It has been assumed that only the four trans-isomers are possible products of the type of electrophilic additions considered in this section. There is a wealth of supporting evidence for this in the literature\textsuperscript{54}, and no further mention of the cis-adducts will be made. Despite repeating the preparation of the bromoacetates many times, only three of the four possible trans-isomers were isolated. The $R_f$ of the fourth bromoacetate was known approximately from an independent experiment (equilibration of B.A.I by heating), but attempts to isolate it directly failed. Several components occurred at about the required polarity, but the only component isolated (and thought most likely to be B.A.IV) proved to be a bromoketone (resulting from NBS oxidation of a bromohydrin).

Lack of stereoselectivity during electrophilic addition to double bonds has been exemplified in the literature\textsuperscript{55} by specific attack being followed by partial equilibration via intermediates (e.g. 67, 68) similar to those proposed for the formation of the original product. This is especially so for additions to cyclohexenes, in which the initial diaxial compound rearranges to give the more stable diequatorial one.
It is probable that this is not the case here because only the one iodoacetate is isolated, there being no evidence of a second equilibration product.

Although progress could not be made by the X-ray examination of B.A.III, well-formed and large crystals of the corresponding alcohol-III (after debromination and removal of the acetate) were obtained. The same alcohol was derived from the iodoacetate by deiodination and removal of the acetate. It is hoped that the X-ray examination of this alcohol will confirm the deductions made from the studies described in the succeeding pages. The preliminary results are as follows:

- Dimensions of the unit cell = $7.5 \times 8 \times 19 \text{ A.}$
- Molecules per unit cell = 2.
- Probable space group = $P2_1$.

The crystals are monoclinic.

* These crystals are at present under examination by Dr. S. Neidle of Queen Mary College, London.
Other methods for the determination of the absolute stereochemistry of these derivatives were explored and involved further chemical transformations.

Firstly, the very least which could be achieved chemically should be the pinpointing of the exact position of oxygenation. This proved to be more troublesome than had first been supposed, but was eventually accomplished. Secondly, chemical methods should give us an intimate knowledge of the relative configurations of the compounds under consideration. One absolute configurational determination then results in the definition of the stereochemistry of a whole range of related compounds.

A list of some of the available methods for absolute configurational determination is given below.

1. (a) X-ray crystallography.
   (b) Electron diffraction.
   (c) Microwave spectroscopy.
   (d) Vibrational spectroscopy.

2. Conversion to or definite relation to a compound of known absolute configuration or possibly to a centre of known absolute configuration within the same molecule.

3. Empirical Methods:
   (a) Molecular rotational differences.
   (b) Partial resolution by the methods of Prelog and Horeau.
   (c) (i) Optical rotatory dispersion and circular dichroism studies, especially the Cotton effect.
   (ii) The olefin octant rule, the benzoate sector rule and the recently developed benzoate chirality rule.
(d) Product prediction rules e.g. Cram's and Prelog's rules.
(e) Examination of models, and stereochemical considerations.

4. Other techniques limited in applicability.
(a) Kinetics - rates of esterification and hydrolysis.
(b) N.m.r. studies - use of chemical shifts, coupling constants and decoupling experiments, etc., which will give, for example, indirect estimates of dihedral angles.

Methods 1 and 2, when carried to a successful conclusion, give definite, incontrovertible proof of structure. Methods 3 and 4 are all somewhat limited in applicability and have varying degrees of theoretical support. The techniques listed under 3 are only useful within the bounds set for them by experimental observation, and modifications and new limitations arise as situations occur in which they break down. Examples have already been quoted in Part I for the collapse of Cram's rule, and also in the review in the discussion of the ecdysone synthesis of Mori et al. These techniques are used to predict configurations, but by their very nature, are dependent on conformation. In the case of axial or equatorial isomers of cyclic systems, the favoured conformations are usually well known and fairly fixed. In such circumstances, molecular rotation differences have been very useful. Insufficient data has not allowed their extension to the steroid side-chain, the conformation of which is much more variable from compound to compound. The same limitations at present apply to 3(c), though this is a fast developing method. 3(b), (d) and (e) have been applied to the steroid side-chain in certain circumstances,
and the partial resolution methods seem to be the most dependable. As the following discussion develops, these methods will be more fully assessed and their application attempted.

It is well-known that the treatment of a halohydrin or haloacetate with base results in the formation of an epoxide by trans-elimination. In order to investigate this relationship in the present system, i-ergosta-7,22-dien-6-one (5) was first treated overnight with excess monoperphthalic acid. Two separable 22-epoxides (69 and 70) were isolated in good yield, the less polar compound (designated 69) being more abundant by a ratio of 3:2.
Using the conditions of Akhtar and Barton\textsuperscript{68}, the less polar bromohydrin fraction was refluxed with potassium acetate in methanol and found to cyclise to a single epoxide, identical in all respects to the more polar epoxide from direct oxidation of the double-bond. The other bromohydrin fraction gave mainly the same product, together with a small amount of the less polar epoxide. The bromoacetates III and II and the iodoacetate all cyclised to the more polar epoxide only, using potassium carbonate. In contrast, bromoacetate I gave only the less polar epoxide.

That the two major bromoacetates should give rise to the minor epoxide is perfectly consistent with the mechanism of bromohydrin and oxide formation. The major bromonium and major oxonium ions would both be formed from the least hindered side of the double-bond. The trans-opening of the bromonium ion at either carbon atom by base would result in two bromohydrins. Cyclisation of these would give the epoxide less favoured by direct oxidation (Figure 9).

As mentioned previously, the trans-diaxial products of electrophilic addition to cyclohexenes isomerise readily to the trans-diequatorial compounds. It was found that B.A. III and B.A. II when heated for a short time at a temperature just below their melting points in sealed capillaries each equilibrated to 50:50 mixtures of the two. Similarly, B.A.I gave rise to a new compound, less polar than any of the three bromoacetates already isolated, which was presumably the
elusive fourth bromoacetate. Owing to the small amount of B.A. I available and the accompanying extensive thermal decomposition, this method could not be used to prepare bromoacetate-IV in quantities sufficient for characterisation.

An alternative approach to this compound employing the opening of the less-polar epoxide with 45% hydrogen bromide in glacial acetic acid was abandoned, since the cyclopropyl group in ring A also underwent ring-opening.

The thermal equilibration of the iodoacetate (20) gave rise to a new, more polar isomer, as would be expected if it were a single compound analogous to the major bromoacetate-III. The mass spectrum of the new compound proved it to be isomeric with the original iodoacetate, and therefore of analogous configuration to B.A.II.
The two epoxides described above can be used as sources of the potentially useful 22- and 23- oxygenated products. It has recently been reported that isomerisation of epoxides to ketones can be brought about by stirring at 80°C for three hours in dimethylsulphoxide with sodium iodide and n-propyl iodide. The more polar epoxide was unaffected by these conditions. The less-polar compound was slightly more reactive, but after forty-eight hours reaction was still incomplete, and the reaction mixture contained many products, including those resulting from double-bond isomerisation.

A further possibility was the reductive trans-cleavage of the two epoxides to give the four corresponding alcohols. Treatment of the less polar epoxide with an excess of lithium aluminium hydride in dry ether at room temperature gave initially a slightly more polar product which showed no response to u.v. light on T.I.C. Mild acid work up resulted in the isolation of a crystalline non-polar product, which had u.v. and n.m.r. spectra very similar to the triene byproduct (6) isolated previously. The 6,8(14)-diene system had the same absorption at 259.5nm. in each, and the protons at C-6 and C-7 appeared as doublets at 3.83τ and 4.78τ. The epoxide ring had been unaffected by these conditions and could be seen as a broad, two-proton signal at 7.3τ, and the total structure is as shown in (71).

The intermediate allylic alcohol (72) could be isolated by aqueous work-up of the reaction mixture. The configuration of the alcohol group followed from investigations which will be described shortly.
The two analogous compounds from the more polar epoxide were also prepared (73 and 74) and fully characterised. A comparison of specific and molecular rotations is shown in Table 1.
<table>
<thead>
<tr>
<th>SYSTEM</th>
<th>MORE POLAR EPOXIDE-I</th>
<th>LESS POLAR EPOXIDE-II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$[\alpha]_D$</td>
<td>$[\alpha]_{DxM}/100$</td>
</tr>
<tr>
<td>i-ergosta-7-en-6-one</td>
<td>(+)</td>
<td>40.1</td>
</tr>
<tr>
<td>i-ergosta-7-en-6α-ol</td>
<td>(+)</td>
<td>88.5</td>
</tr>
<tr>
<td>i-ergosta-6,8(14)-diene</td>
<td>(+)</td>
<td>173.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SYSTEM</th>
<th>22(23)-EPOXIDE-I</th>
<th>22 or (23)-EPOXIDE-II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$[\alpha]_D$</td>
<td>$[\alpha]_{DxM}/100$</td>
</tr>
<tr>
<td>i-ergostan-6-one</td>
<td>(-)</td>
<td>17.7</td>
</tr>
<tr>
<td>6-oxa-B-Homo-i-ergosta-</td>
<td>(-)</td>
<td>43.7</td>
</tr>
</tbody>
</table>

i-ergosta-7-en-6-one: (+) (−); i-ergosta-7-en-6α-ol: (+) (−); i-ergosta-6,8(14)-diene: (+) (−); 22(23)-EPOXIDE-I: 22 or (23)-EPOXIDE-II.
i-ergosta-7-en-6-one; $[\alpha]_D = +75.1^\circ$; $[\alpha]_{DxM/100} = (+)297.4^\circ$

i-ergosta-7-en-6β-ol; $[\alpha]_D = +101.6^\circ$; $[\alpha]_{DxM/100} = (+)404.4^\circ$

i-ergosta-6,8(14)-diene; $[\alpha]_D = +146.4^\circ$; $[\alpha]_{DxM/100} = (+)556.3^\circ$. 

(Prepared as described later).

$[\alpha]_D = (+)33^\circ$ (c 1; CHCl₃) for i-ergostan-6-one and $[\alpha]_{DxM/100} = (+)131.3$.

No obvious trends are apparent from this table, despite the fact that two sets of three compounds with identical side-chains (for example the set in the first column) have been prepared, differing only in the tetracyclic nucleus. Conformational effects of the nucleus on the side-chain are therefore in play, and this does not augur well for the application of the method of molecular rotation differences to the steroid side-chain.

In this preliminary epoxide work, it was also decided to prepare the 22-epoxides of i-ergosta-22-en-6-one (75). This served two purposes: firstly, the labile 7β-double bond was removed and equilibration problems in further investigations avoided; and secondly, the effect of a small change in the tetracycle upon the side-chain conformation could be assessed. The literature method of lithium and liquid ammonia reduction of (5) was used, and gave (75) in high yield. A byproduct isolated in 6% yield proved to be 5α-ergosta-22-en-6-one (76) arising from the reductive opening of the 3α,5-cyclopropyl group. (76) had not been reported by the earlier workers and was therefore characterised in full.
Excess monoperphthalic acid oxidation of (75) gave the required epoxides, the less polar one (30% yield) again being preferred by a ratio of about 6:5. Unlike the reaction of the 7-en-6-one compound (5), which goes smoothly to the required products, the epoxides in this case where accompanied by two more polar byproducts, each isolated in about 15% yield. Both were 22-epoxides as evinced by the i.r. and n.m.r. spectra, the former of which also exhibited strong absorption bands at 1735 cm\(^{-1}\), characteristic of lactone carbonyls. These compounds were the Baeyer-Villager products (77 and 78), arising by the mechanism shown in Figure 10. The alternative B-Homo-6-one-7-oxa- structures can be discounted, since there was no evidence in the n.m.r. of the required methylene signal in the region 5.8\(\tau\) (see also Table 1).

Meanwhile, a second series of reactions had been carried out, directed towards locating unambiguously the position of oxygenation, either in the iodoacetate or the major bromoacetate.
The most obvious approach was to prepare the corresponding ketone (at C-22 or C-23) by dehalogenation, removal of the acetate and mild oxidation of the resulting alcohol. Bromoacetate-II should, on similar treatment, give rise to a different ketone, with the carbonyl group located at the
alternative position. The two ketones should exhibit characteristic fragmentation patterns in their mass spectra and, failing this, the Beckmann rearrangement of the corresponding oxime tosylates should also specify the original position of oxygenation.

Successful dehalogenation also opens up a number of other possibilities. Ester formation from the parent alcohols allows us to test the applicability of molecular rotation differences to the side-chain, the C.D. and O.R.D. curves of the benzoates can be measured and compared, and partial resolution methods can also be applied.

Shortly after establishing the structure of the iodoacetate, dehalogenation experiments were attempted. Reduction with zinc dust in glacial acetic acid caused elimination to give the parent olefin (5) in excellent yield. Catalytic hydrogenation using 10% palladium on charcoal in ethyl acetate, and 10% palladium on calcium carbonate in ethanol and in benzene left the iodoacetate unaffected. Prolonged reaction times resulted in the isolation in small yield of a compound with no absorption in its u.v. spectrum and a carbonyl absorption at 1710 cm\(^{-1}\) in the i.r. spectrum. By analogy with the isolation of i-ergostan-6-one as a byproduct in the reduction of i-ergosta-7,22-dien-6-one (5) under similar conditions\(^7\), it seems probable that this compound was i-ergostan-6-one-22,23-iodoacetate.

In recent years, interest has grown in the use of chromous (II) species to bring about the reduction of various haloalkane derivatives and organic monohalides. Chromous
salts (e.g. acetate, chloride, and perchlorate) with the latter class of compound result in the replacement of the halide by hydrogen with the more reactive α-haloketones, and allylic and benzylic halides only. Kochi and his colleagues have extended this reaction to include even primary alkyl chlorides at room temperature by using certain complexes (e.g. ethylenediaminechromium II and ethanolamine-chromium II). The reactivity of halides is in the order t-alkyl > secondary alkyl > primary alkyl and iodide > bromide > chloride.

In the case of β-substituted halides, there are two competing reactions. Under similar conditions to those applied above, reductive elimination occurs to give the olefin (see equation 1). Again the mildest and most general conditions are obtained by use of the potent chromous (II) complexes. Barton and his co-workers have found that, in the presence of active hydrogen donors, the reduction of β-bromohydrins by chromous (II) acetate affords the alcohol, (see equation 2). In this manner, the elements of HY can be added to an olefin as shown in Figure 11.

Barton et al. have thus successfully reduced 9α-bromo-11β-hydroxy progesterone (79) at room temperature under nitrogen using chromous acetate in DMSO containing n-butane thiol or hypophosphorous acid to give 11β-hydroxyprogesterone (80) in high yield. Other 9α-bromo compounds have also been successfully reduced.
Solid chromous acetate was therefore prepared by the literature method\textsuperscript{80} using a system devised in this laboratory\textsuperscript{81}
which did not require a glove box and facilitated quick and easy drying of the product. The compound was relatively stable in air when dry, but for storage purposes it had to be sealed in ampoules under nitrogen.

After some initial difficulty, Barton, Danks and Bolton had brought about the debromination of 6β-hydroxy-5α-bromocholestan-3β-yl acetate (81a) by addition of the powdered steroid to chromous acetate, DMSO and n-butane thiol stirred under nitrogen at 40°C for one hour.

In order to prove the viability of this technique, a sample of (81a) was prepared by the acid-catalysed treatment of cholesteryl acetate with N-bromoacetamide in dioxan. The required tertiary debromination to (82) occurred with retention of configuration in 83% yield under the conditions outlined above.

Acetylation of (81a) gave 3β,6β-diacetoxy-5α-bromocholestan (81b), which also underwent debromination by this method to give (83) in 84% yield. The diacetate (83) was independently synthesised by acetylation of (82) (see Scheme II). The two samples were identical and had melting points in good agreement with the literature value.

Attempts to reduce the iodoacetate (20), under the same conditions and with minor variations, failed to give any of the required acetate. Changes were made in the order of addition of reactants, in inert solvents present (since the
iodoacetate was not very soluble in DMSO), in reaction time and temperature, but without effect. A polar byproduct, which appeared to be the hydroxyacetate hydrolysis product (21), was the only new compound isolated at 40°C. At higher
temperatures (60-80°C), the less polar elimination product, (5), was formed in about 40% yield. Similar results were obtained with the major bromoacetate-III, as would be expected from the order of reactivity. Unreacted starting material was isolated in all cases, (M.Pt., mixed M.Pt. and n.m.r.).

The failure to dehalogenate either haloacetate was somewhat surprising, since controls on steroids such as (81b) gave successful reduction. A comparison of these models with the side-chain adducts showed two differences. The model compounds contained tertiary halide atoms held rigidly on the α-face of the steroid nucleus, i.e. in a sterically unhindered environment. The haloacetates however possessed secondary halide atoms in restricted surroundings.

The likelihood that a question of solubility was the main factor involved was lessened by applying the method of Barton et al.82 to the crude mixture of side-chain bromohydrins. The olefin (5) was isolated in 25% yield and proved to be identical to an authentic sample.

Preparation of an unhindered secondary bromohydrin model was then undertaken. Treatment of cholest-2-ene with N-bromosuccinimide in aqueous tetrahydrofuran gave 3α-bromo-2β-hydroxy-5α-cholestane in 49% yield, the spectral data of which was concordant with the published data86.

After 20 hours under standard conditions, unreacted starting material was isolated (30%) with three new products in 20%, 20% and 10% yield. These were respectively and in decreasing order of polarity, cholest-2-ene, 2β,3β-epoxy-5α-
cholestane and a compound which appeared to be the required 2β-hydroxy-5α-cholestane. The latter could not be obtained crystalline, but had an n.m.r. spectrum consistent with this structure.

It appears from these results that even unhindered secondary bromines are resistant to the reagent, and for a short time attention was turned from this method to another possibility.

An alternative route to the 22- and 23-ketones was the Jones' oxidation of the bromohydrins to the corresponding bromoketones, followed by zinc dust and acetic acid reduction. The problem in this case was not to accomplish the necessary steps, but to guarantee the stereoisomeric purity and the origins of the intermediate bromoketones. This arose because it was found impossible to obtain the bromohydrins pure, and oxidation of the crude material resulted in mixtures of products, which were exceedingly difficult to separate. Two bromoketones were eventually characterised, the major, marginally less polar one, probably corresponding to bromoacetate-III and the other to bromoacetate-II. The resulting ketones from these and from other bromoketone fractions were very similar in melting points, rotations, i.r., u.v. and n.m.r. spectra and the mass spectra surprisingly showed only minor differences. It was thus decided that the best method to pursue the preparation of the two ketones was to return to the dehalogenation experiments described previously since several possibilities remained untried.
Kuivila and his colleagues have described the preparation of tri-n-alkyl tin hydrides by LAH reduction of the chlorides and have used them in simple dehalogenation procedures, either neat or in solution. Attempts to apply this reagent to the dehalogenation of the iodoacetate both neat and in a variety of solvents (benzene, ether, chloroform, bromobenzene and anhydrous benzene) appeared to leave the iodoacetate unaffected. The recovered starting material gave a positive Beilstein test and seemed to be unchanged. A hydroxyacetate component was also isolated, but proved to be a more polar byproduct than that obtained from the chromous acetate reductions.

The chromous acetate reagent was next tried in the presence of ethanolamine and an active hydrogen donor, using the material recovered from the above experiment. T.L.C. indicated that no reaction had occurred after one hour, and so several further manipulations (e.g. addition of aqueous DMF and of ethylene diamine) were carried out. After several days, no change could be discerned by T.L.C. The major component, of similar Rf to the iodoacetate, was isolated as a crystalline solid from methanol, M.Pt. 147-148°C (cf. Iodoacetate, M.Pt. 157-9°C). The i.r. and u.v. spectra were very similar to those of the starting material, but the Beilstein halogen test was negative. Furthermore, the n.m.r. spectrum had lost the doublets at 5.75 and 4.5 due to protons α to iodide and to acetate, and instead, exhibited a broad signal at 4.9 due to the proton α to the residual acetate. Microanalysis confirmed that the required compound (84a) had been obtained.
Attempts were then made to repeat this result in a reproducible manner. The similarity of the two compounds rendered useless the normal methods for following reactions, n.m.r. being the only reliable technique. Using pure starting material, all efforts to effect the dehalogenation with complexed chromous (II) acetate failed. After twenty-four hours exposure to the reagent, crystals, which were mainly the starting material could be obtained from the component of the right Rf, as shown by microanalysis and mixed M.Pt. However, the n.m.r. spectrum of the mother liquor residues showed protons in both kind of acetate environment and two types of acetate methyl, and so reaction was proceeding to some degree as required. The success of the first attempt was never repeated, partial reaction being the best that could be achieved.

Although other possibilities were still open (for example, the use of the chromous perchlorate/ethylenediamine complex), the dehalogenation of secondary halohydrins and haloesters with chromous (II) ion and active hydrogen donor seemed to be ineffective or impracticable. All examples of such dehalogenations in the literature are of tertiary halohydrins. Fortunately, a close examination of the above results supplied the answer to the problem.

In the uniquely successful chromous experiment, the iodoacetate used was that recovered from the tri-n-butyl tin hydride work. From the irreproducability of this result, it seemed probable that the dehalogenation may have occurred partially, if not completely, during the first experiment.
The physical and spectral similarities of product and starting material suggest that it was possible for such a successful reaction to have taken place without necessarily being detected. A sample of tri-n-butyl tin hydride was therefore freshly prepared, and stirred with the iodoacetate under nitrogen in anhydrous tetrahydrofuran overnight. After removal of excess reagent by P.L.C. the required acetate (84a) was isolated as a crystalline solid in 32% yield. Subsequent, larger scale reductions raised the yield to 82%. The product was identical to that obtained previously in all respects. Similar treatment of the major bromoacetate also gave rise to the same compound (M.Pt., mixed M.Pt. and spectral data).

![Chemical Structure](image)

(84) (a) R = Ac  (d) R = \[
\text{I}
\]
(b) R = H  (e) R = Ts
(c) R = Bz

Hydrolytic removal of the acetate group by base was accompanied by a number of byproducts, but LAH reduction to the diol, followed by manganese dioxide oxidation to restore the unsaturated ketone function, gave the corresponding alcohol (84b) quantitatively. The same alcohol was derived from the
major bromoacetate and the iodoacetate. The intermediate diol (85) was also characterised, and the corresponding 6,8(14)-diene (86a) made by mild acid treatment as described for the epoxides. The acetate (86b) and benzoate (86c) of (86a) were prepared for possible application of the molecular rotation difference method for determination of side-chain stereochemistry.

The orientation at C-6 in (85) was established by a comparison of i-ergosta-7,22-dien-6β-ol (4), (prepared by solvolysis of ergosteryl 3β-tosylate) with the LAH reduction product of i-ergosta-7,22-dien-6-one. The stereochemistry of (4) and the mechanism for its production are well known. Attempts to crystallise it from methanol gave the triene (6). The T.L.C. properties of the LAH reduction product showed that it was a different, less polar alcohol accompanied by a
small amount of (4). The widths at the half-heights of the signals due to the protons α to the hydroxy groups in the n.m.r. spectra were compared. The 6β-alcohol from solvolysis had a broad signal, \( W_{1/2} = 20 \text{ Hz.} \), whereas the other had \( W_{1/2} = 10 \text{ Hz.} \). Examination of models of the two epimers indicated that the dihedral angle between the 6 and 7 protons was much greater in the 6β-ol case. This verifies that the major LAH reduction product was the 6α-ol. Mechanistically, the reducing agent would be expected to approach from the less hindered side. This is normally the α-face, but the 6-position is sufficiently distant from the 18β- and 19β-methyl groups for these to lose their dominance. The steric effects associated with the groups attached to C-5 must therefore be the deciding factors. The LAH reduction product is the one predicted by Cram's rule, which would be so if this were the case. Furthermore, the two methods would be expected to give rise to different epimers. In the solvolysis, the attacking specie is the hydroxy group, and in the reduction, it is the hydride-donor. Both would approach from the same face, producing the observed epimer in each case. The literature assignments for the epimeric i-cholestanols, based on rates of acetylation, order of chromatographic elution, molecular rotational analogies\(^{90,91}\) (see Table 2), n.m.r. spectroscopy\(^{91}\), and partial resolution\(^{63c}\), are in complete agreement with the above results.

The alcohol (84b) proved to be the source of several useful compounds and is itself being examined by X-ray crystallography. The benzoate (84c) and the p-iodobenzoate (84d) were prepared primarily to determine the molecular
rotations. Oxidation with Jones' reagent gave the side-chain ketone in good yield. In the mass spectrum, the molecular ion occurred at m/e 410 (100%) as required. The possible fragmentations arising from α-cleavage or McLafferty rearrangement for the C-22 and C-23 ketones are shown in Figure 12.

FIGURE 12.

The ketone derived from the major bromoacetate (identical to that from the iodoacetate) exhibited fragmentation which indicated that it was the 23-ketone (87) rather than the 22-ketone (88), i.e. m/e 339 (33%) and 71 (60%), 311 (16%) and 99 (27%), and 296 (58%). However a fragment ion at m/e 297 (110%), as well as a very small peak at 325, makes the evidence less conclusive.
### TABLE 2.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\theta_{D}6\alpha^\circ$</th>
<th>$\theta_{D}6\beta^\circ$</th>
<th>$\Delta \theta_{D}^\circ$ ($\theta_{D}6\alpha - \theta_{D}6\beta$)</th>
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</thead>
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<td>6-hydroxy-i-cholestane</td>
<td>+293</td>
<td>+193</td>
<td>+100</td>
</tr>
<tr>
<td>6-acetoxy-i-cholestane</td>
<td>+419</td>
<td>+205</td>
<td>+214</td>
</tr>
<tr>
<td>6-p-nitrobenzoyloxy-i-cholestane</td>
<td>+370</td>
<td>+300</td>
<td>+70</td>
</tr>
<tr>
<td>6-acetoxy-17-ethylenedioxy-i-androstan</td>
<td>+258</td>
<td>+69</td>
<td>+189</td>
</tr>
<tr>
<td>6-amino-i-cholestane</td>
<td>+237</td>
<td>+134</td>
<td>+103</td>
</tr>
</tbody>
</table>

Debromination of bromoacetate-II as before gave a different acetate (-II) (89a) (solid, but not crystalline), which yielded a different, crystalline alcohol (-II) (89b) after LAH reduction and manganese dioxide oxidation. Again the didial intermediate (90), the 6,8(14)-diene alcohol, acetate and benzoate (91a, b and c) and the 7-en-6-one benzoate (89c) were prepared.
Jones' oxidation of alcohol-II furnished a ketone, which was very similar in most respects to (87), e.g., i.r., u.v., n.m.r., rotation, T.L.C. properties, M.Pt. However, mixed melting point with (87) resulted in a large depression, and the mass spectrum contained distinct differences from that of the 23-ketone. Fragmentation of the molecular ion (m/e 410; 100%) was consistent with its assignment as the 22-ketone (88).
α-cleavage was observed in ions at m/e 325 (12%) and 85 (38%),
297 (35%) and 113 (52%), and McLafferty rearrangement by
the fragment at m/e 341 (50%).

Because of a shortage of material, the minor bromoacetate-
I was only used to prepare the acetate, alcohol and benzoate of
the 7-en-6-one system as described for the other isomers.
Again the acetate was crystallised with difficulty, and the
bromoacetate seemed to be more resistant to dehalogenation
than in the other cases. Longer reaction time and several
T.L.C. purifications were required to give a crystalline
product. Attempts were made to obtain pure samples of acetates
I and II by acetylation of the corresponding alcohols, but
with no more success than the preparations from the bromoacetates
Only three bromoacetates had been isolated in the
original experiments, and one of these in small quantity.
Methods were therefore sought which would allow epimerisation
of compounds related to the two major bromoacetates. In this
way, the one would give rise to compounds related to bromoacetate-
I, and the other provide a pathway to bromoacetate-IV derivatives

Chang and Bickenstaff have reported that cholestan-
3β-ol can be converted to the 3α-ol by heating a solution
of the tosylate in 5% aqueous DMF at 78°C, and hydrolysing the
resulting 3α-formate on an alumina column. Attempts were
made to prepare the tosylate from the major alcohol-III.
Initially, intractable mixtures were produced, but eventually,
under controlled conditions of purity and with prolonged
reaction times at and below room temperature, a major
component, less polar than the starting material, was isolated. Its i.r. spectrum showed none of the absorptions characteristic of a tosylate, and the n.m.r. spectrum contained only an additional olefinic proton, seen as a triplet at 4.89\tau, and an olefinic methyl signal at 8.48\tau. The mass spectrum showed the product to be isomeric with 1-ergosta-7,22-dien-6-one. These observations are explicable in terms of tosylate formation followed by elimination towards the tertiary position. In other words, the product would be the 23-ene if the hydroxy group was at C-23, and the 20-ene if at C-22. A definite chemical means of establishing the position of oxygenation is obtained. The olefin was therefore ozonolysed under the normal reductive conditions\textsuperscript{32}. A single compound was produced, which had an aldehyde proton seen as a lowfield triplet in the n.m.r. spectrum. The molecular ion was at m/e 340 in the mass spectrum, and an accurate mass measurement verified the structure as the 23-aldehyde (92). This was therefore derived from a 23-alcohol, via the 23-olefin (93).
A further reaction sequence involving the side-chain ketone also confirmed the site of oxygenation in the major bromoacetate as C-23. This employed the Beckmann rearrangement of the oxime tosylate of the side-chain carbonyl. Two preliminary oxime formations were carried out to discover if the required adduct could be prepared preferentially. When i-ergosta-22-en-6-one (75) was heated for ten minutes at reflux in ethanol/water with hydroxylamine hydrochloride and sodium acetate, the 6-oxime was isolated in 85% yield. To achieve the same result (94a) with i-ergosta-7,22-dien-6-one (5) under the same conditions, the reaction time had to be extended to five days. It seemed likely that the required preferential oxime formation would therefore be possible.

However no reaction occurred between i-ergosta-7-en-6, 23-dione (87) and hydroxylamine after thirty minutes at reflux temperature as above. Similarly using one equivalent of hydroxylamine hydrochloride in dry pyridine overnight, there was no reaction. This reluctance to form the oxime was consistent with the findings of Tsuda and Hayatsu that 22-oxo-cholesterol was very sterically hindered and did not react with normal ketone reagents. The additional 24-methyl group in (87) should make the steric congestion even greater.

However, a vast excess of hydroxylamine hydrochloride in dry pyridine for five days gave the 6,23-dioxime (95) in good yield. A small amount of a less polar monoxime was also isolated. The i.r. spectrum of this compound showed the presence of the side-chain carbonyl (1710cm⁻¹) and the 6-oxime.
The preferential oxime formation was unexpectedly with the ring-B ketone.

Exposure to freshly crystallised tosyl chloride in dry pyridine for 16 hours gave the oxime tosylate (94b), as a fairly stable, crystalline solid from benzene/petroleum ether. The Beckmann rearrangement was brought about slowly on an alumina column and gave 6-aza-1-B-homoergosta-8,23-dien-7-one (96) in an analogous reaction to that reported recently for 6-hydroxyimino-1-cholestan.
The structure was verified by the i.r. carbonyl absorption (1675 and 1632 cm\(^{-1}\)), the u.v. absorption (at 224 nm, due to the \(\alpha\beta\)-unsaturated lactam) and the n.m.r. and mass spectra.

From the tosylation of the dioxime (95), a compound was isolated which exhibited the characteristics of an oxime tosylate and of a secondary amide, instead of those of the expected dioxime ditosylate. The ring-B N-ester was known to be stable under the conditions of preparation, and it transpired that the oxime tosylate of the side-chain had undergone a Beckmann rearrangement under the tosylation conditions. The position of the amide nitrogen should be defined by the mass spectral fragmentation, see Figure 13.

Owing to rapid elimination of p-toluenesulphonic acid, the molecular ion cannot be seen in the mass spectrum, the peak of highest m/e being at 440 (100%). A fragment at m/e
352 indicates that the nitrogen is present as a 24-aza- group, and the carbonyl as a 23-ketone (97). Confirmation of this came from the product of the Beckmann rearrangement, (carried out with difficulty as before), which proved to be the amide lactam (98). All the characteristic fragmentation of such a 25-aza-24-one amide can be seen in the mass spectrum (see Figure 13).

The location of the side-chain carbonyl of 1-ergosta-7-en-6,23-dione (87) has therefore been conclusively proved in a variety of ways. The opening of the major bromonium ion must therefore take place mainly by attack at position C-23. The C-23 atom is the furthest point of approach from the tetracyclic nucleus for the nucleophile, and it is not surprising that this less-hindered position should be favoured.

Before making any predictions as to the absolute stereochemistry of the three bromo-acetates one more relationship remained to be established: was the position of
oxygenation of the minor bromoacetate—I, the same as that of the major bromoacetate or that of bromoacetate—II? This could have been accomplished by oxidation of alcohol—I, to the ketone, but the 6,22- and 6,23-diones were so similar (except for some mass spectral differences) that an alternative method was adopted. The LAH reductions of the two ketones, followed by manganese dioxide oxidation, were carried out to identify the products and determine the epimer ratios.

Two alcohols were produced in this way from the 6,23-dione (87), in about equal quantities. The more polar epimer was identical in all respects to the alcohol obtained from bromoacetate—III. The other compound was less polar than any of the alcohols previously synthesised, and could only be the alcohol corresponding to the unisolated bromoacetate—IV. Complete consistency between the proposed structure and the observed spectral data was obtained. The isolation of alcohol—IV enabled the acetate and benzoate to be prepared, thus allowing the rotation tables 5 and 6 (shown later) to be compiled. To complete these tables, the three parent compounds were synthesised. Catalytic hydrogenation of i-ergosta-7,22-dien-6-one over 5% palladium on charcoal gave the known i-ergosta-7-en-6-one. Treatment of this with LAH in sodium-dried ether furnished the second parent, i-ergosta-7-en-6α-ol, from which i-ergosta-6,8(14)-diene was produced by mild acid-induced elimination.

The reduction of (87) was repeated at −20°C to try to impose sufficient restriction upon the system to discover which alcohol was formed preferentially. A ratio of 6:5 in
favour of alcohol—IV was found after restoration of the αβ-
unsaturated ketone group.

Similar treatment at room temperature of the 22-ketone
obtained from alcohol—II was much more stereospecific. The
two products corresponded exactly to alcohols—I and —II, and
the former was favoured by a ratio of 7:1. The minor bromo-
acetate therefore was oxygenated at C-22, and the above
reaction provided a useful supplementary source of its
derivatives.

Both the 22- and 23-carbonyls are vicinal to asymmetric
centres, the situation for which Cram's rule is used to
predict the predominant product of electrophilic attack.
Application of Cram's rule to the reduction of the two ketones
(87 and 88) results in the predictions shown in Figure 14.

Without considering our knowledge of the relationships
between these four alcohols, the predictions seem reasonable
enough. The higher stereoselectivity in the case of the
22-ketone is consistent with the bulky nature of the steroid
nucleus, compared to that of the isopropyl group attached to
C-24. However, the large group (L) must adopt an eclipsed
conformation in Cram's view of the reaction, and the
discriminating factor with regard to the side of approach is
the difference between the sizes of the small (S) and medium
(M) groups. These are hydrogen and methyl respectively in
both cases. The influence of the large group, which results
in the difference of stereoselectivity of reduction, must
therefore be its ability to allow the necessary reaction
MAJOR REDUCTION PRODUCT OF 22-KETONE AS PREDICTED BY CRAM'S RULE.

MAJOR REDUCTION PRODUCT OF 23-KETONE AS PREDICTED BY CRAM'S RULE.

FIGURE 14.

conformation to be assumed.

At first sight, the less reliable prediction would be
for products of the 23-ketone reduction, where the epimer ratio is only a little greater than 1:1. Certainly one of these reductions is an exception to Cram's rule, because according to this, alcohols I, II, III and IV should have the structures A, B, C and D depicted in Figure 14. At the same time, B and C must have hydroxy groups of like configuration, because they are derivable from bromoacetates II and III, which give rise to the same epoxide on treatment with base and to each other when equilibrated thermally. A similar demand is placed upon A and D by analogous relationships. The experimental evidence for these correlations is unambiguous, and so it must be the empirical Cram's rule that is invalid for one of the cases.

Examination of a model of i-ergosta-7,22-dien-6-one (5) shows that the least hindered, fully staggered conformation of the side-chain is very similar to that found in the crystal of calciferol ((64) and (65); see pages 141/2). The A_1^,3 strain due to non-bonded interaction is minimised in this conformation, shown diagrammatically in Figure 15. Looking from above, hydrogens 1, 2, 3 and 4 are approximately co-planar and parallel, as are hydrogens 5 and 6. The 21-, 26- and 27-methyl groups obstruct the approach of electrophiles from "above", and the preferred direction of approach is from the opposite side to these methyls. Having proved conclusively from the observed products that cleavage of the bromonium intermediate takes place by nucleophilic attack at C-23, the absolute configurations of bromoacetates-I, -II, -III and -IV would be predicted to be as shown in structures (99), (100), (101) and (102) respectively.
H₁, H₂, H₃ and H⁴ are approximately parallel and co-planar. H₅ and H₆ are almost parallel and co-planar similarly.

Diagrammatic View from Above of Ergosterol Side-Chain in Least-Hindered Conformation.

FIGURE 15.

As can be seen by comparison with Figure 14, (101) and (102) only are consistent with the Cram's rule predictions, giving rise to (C) and (D). The indication is that the
the preponderance of alcohol-I from the reduction of the 22-ketone by a 7:1 ratio is in conflict with the major alcohol expected from Cram's rule. As hinted previously, this must be due to the reluctance or even the impossibility for the large steroid nucleus to assume the eclipsed conformation required for the Cram product.

Reference to the literature revealed that the configurations of the 22-hydroxycholesterols had been the subject of much discussion. The LAH reduction of 22-oxocholestan-3β-ol was initially reported by Fieser and Huang to give only one of the two possible 3β,22-diols. It was assumed by Klyne and Stokes to be the Cram product and assigned the 22R- (or 22βF-) configuration. Hayatsu later found that sodium borohydride reduction of 22-oxocholest-5-en-3β-yl acetate gave both 3β,22-diols in a ratio of 3:1 in favour of the more polar alcohol. The assignment of the 22R- configuration to the major product was confirmed by Tsuda and Hayatsu using Prelog's asymmetric
synthesis method\textsuperscript{62}. Similarly, reduction of 2-oxocholesteryl-3β-methyl ether gave almost equal quantities of the 22-ols. Treatment of the phenylglyoxyllic esters of these with methyl magnesium iodide, followed by hydrolysis of the atrolactic acid ester, afforded dextrorotatory atrolactic acid from the ether corresponding to the major reduction product isolated by Hayatsu\textsuperscript{97}. The other ester yielded the laevorotatory material (see equation 3). Stabursvik\textsuperscript{98} isolated a cholest-5-en-3β,22-diol from Narthecium ossifragum Huds, which on catalytic hydrogenation gave a diol different from that obtained by Fieser and Huang\textsuperscript{26}. It was therefore assigned the 22S- (or 22α\textsubscript{H}-) configuration.

For ten years these results went unchallenged. Recently, however, two groups of workers have independently shown that these literature assignments were erroneous. Caspi et al.\textsuperscript{38} wished to prepare samples of cholesterols stereospecifically labelled with a tritium atom at C-22 for biosynthetic studies. They therefore used Horeau's method\textsuperscript{63} of partial resolution to determine the stereochemistry of the sodium borohydride reduction products of 22-oxocholest-5-en-3β-yl benzoate, the more polar product predominating by a ratio of 3:1. Hydrolysis of the excess reagent, after ester formation of the major product with racemic α-phenylbutyric acid anhydride, gave the laevorotatory acid, showing that the major alcohol had 22S-configuration. The diacetate and dibenzoate of this
compound were identical to those obtained from the alcohol previously assigned as 22R by Prelog's method. Caspi and his colleagues38 therefore repeated the work of Tsuda and Hayatsu94, and determined the configurations of the hydroxy-methyl ethers by Horean's and by Prelog's procedures. The methods both indicated the 22S-configuration for the less polar epimer, and the diacetate from this was the same as the diacetate obtained by them from the 22S-hydroxybenzoate previously examined. Similarly, the results with the other epimer were self-consistent and established its 22R-configuration. The compound assigned to be the 3β,22R- (or 22βR-) diol was found to be identical to that isolated by Stabursvik98.

While this new evidence was in the press, Mori et al.99 had recourse to the published work on 22-hydroxycholesterols in order to choose which of their epimeric intermediates (103a and b) could be converted to ecdysone. (103a) was transformed chemically to a 3β,22-dihydroxy-5α-cholestane different from that obtained from naturally occurring 22-hydroxycholest-5-en-3β-ol, at that time believed to be the 22S-epimer. (103a) was therefore selected, but resulted in isoecdysone after being taken through the reaction sequence. Ecdysone was isolated from (103b). Since the absolute configuration of ecdysone is know from X-ray crystallography, the structures of (103a) and (103b) must be as shown. The absolute configuration of the 22-hydroxycholesterols have therefore been unambiguously defined by relation to ecdysone. The naturally occurring compound is 3β,22R-dihydroxycholest-5-ene. The hydride reductions of 22-oxocholestan-3β-ol and 22-oxocholest-5-en-3β-yl acetate and benzoate yield as major products the more
polar 22S-epimers by ratios of 3:1, contrary to Cram's rule in each case.

This precedent for the anti-Cram product to be favoured in the reduction of 22-ketones adds considerable weight to the structures predicted for the bromoacetates and related compounds from the combination of established relationships and consideration of models. If the anti-Cram product results in the 22-oxo-i-ergosta-7-en-6-one case, then both sets of predictions are coincident. There is further evidence to augment this.

In the case of i-ergosta-7-en-6,22-dione (88), the LAH reduction proceeds in a ratio of 7:1 to the more polar epimer, which has every indication (except Cram's rule) of being the 22S-alcohol. The reduction of i-cholest-7-en-6,22-dione (44) was also examined. Treatment with LAH followed by manganese dioxide oxidation to restore ring B again gave two alcohols (43a and b) in a 3:1 ratio in favour of the more polar epimer, as found for the 22-oxocholestane derivatives. Table 3 gives
a list of molecular rotations of various similar pairs of epimeric 22-alcohols. The 22R-epimer seems invariably to have the more positive rotation, and the indication is that the major, more polar product has again the anti-Cram 22-configuration. Furthermore, the same pair of alcohols can be synthesised (see Part I) by the reaction between isoamyl magnesium bromide and (205)-20-formyl-i-pregn-7-en-6-one (26). The more polar epimer is again the major product by a ratio of 6.3:1. For both these reactions to result in the same major product, one of them must be proceeding in an anti-Cram manner. In one case, the aldehyde carbonyl is being alkylated and in the other the hydride-donor is attacking the ketone carbonyl. Obviously, it is the former of these which can most easily adopt the required eclipsed conformation (104a), since the large steroid tetracycle is aligned with the hydrogen atom rather than with the isopentyl group. This emphasises the anti-Cram reduction of the 22-ketone. The

Grignard reaction also allows us to make analogies with the Rf's and molecular rotations of the products of other such reactions with 22-aldehydes, e.g. the 2β,3β-diacetoxy-14α,22-dihydroxy-5β-cholest-7-en-6-ones and ecdysone and
<table>
<thead>
<tr>
<th>Compound</th>
<th>22R</th>
<th>22S</th>
<th>Δ</th>
<th>22R-S</th>
</tr>
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<tr>
<td></td>
<td>[α]_D</td>
<td>[α]_D xM/100</td>
<td>ΔOH (parent)</td>
<td>[α]_D</td>
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<tr>
<td></td>
<td>18</td>
<td>72.7</td>
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<td>3β,22γ-dihydroxy-cholest-5-enes</td>
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<td>cholesterol</td>
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<td>3β-benzolyloxy-22γ-hydroxy-cholest-5-enes</td>
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<td></td>
<td>83</td>
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<td>143</td>
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(Parent: [α]_D = +146.4, [α]_D xM/100 = +556°.)
### TABLE 3 continued.

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<th>Compound</th>
<th>$\Delta$D</th>
<th>$\Delta$D$_{DM}$/100</th>
<th>$\Delta$OH (parent)</th>
<th>$\Delta$D</th>
<th>$\Delta$D$_{DM}$/100</th>
<th>$\Delta$OH (parent)</th>
<th>$\Delta$ 22R-S</th>
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<td><strong>2β,3β,14α,22β,25-penta-hydroxy-5β-cholesterol-7-en-6-ones</strong></td>
<td>(+)</td>
<td>(+)</td>
<td>-</td>
<td>(-)</td>
<td>3''</td>
<td>14</td>
<td>302</td>
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<tr>
<td><strong>22β,25-dihydroxy-i-ergosta-7-en-6-ones</strong></td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td><strong>22β,25-dihydroxy-5α-cholestan-3,6-diones</strong></td>
<td>90.5</td>
<td>387</td>
<td>9</td>
<td>30.2</td>
<td>9</td>
<td>38.9</td>
<td>69.1</td>
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<td><strong>But EXCEPTIONAL:</strong></td>
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<td><strong>22β-hydroxy-3,6-dioxo-homo-5α-cholan-25-oic acid</strong></td>
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<td>+</td>
<td>+</td>
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<tr>
<td><strong>25→22 lactones</strong></td>
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</tbody>
</table>
isoecdysone. (See Part I). By this means alone, the more polar alcohol would be assigned the 22S- and the less polar the 22-R-configuration. In all these cases, the more polar epimer of 22-S-orientation has the more negative (or less positive) molecular rotation. (See Table 3). That these rotational observations are not general to all the side-chain positions can be seen by examination of the results for C-23 shown in Table 4. Much less is known about this position, but the more positive rotation seems to be associated with the 23S-epimer. Stokes and Bergman have suggested that the steroid (or triterpene) nucleus and part of the side-chain can be regarded as being similar to an ethyl group for the purposes of considering the rotary contributions of chiral centres in the side-chain. On this basis, Entwistle and Pratt have correlated the molecular rotations of the 23-hydroxylanosterols (naturally occurring and from 23-keto-lanosterol) with those of R(-)- and S(+)-2-methylpent-2-en-4-ol. (The required 2-methylhex-2-en-4-ol has never been resolved). The present work supports these results, and, for the first time, puts assignments of C-23 epimers on a firm foundation, by unambiguous correlation with the absolute determinations at C-22. The hydride reduction of a 24R-methyl-23-ketone is, by this means, predicted to give rise preferentially to the Cram product, the less polar 23S-epimer. This was precisely the case observed by Entwistle and Pratt for lanost-8,24-dien-3,23-dione, the major product predominating by a 6:5 ratio (as found for i-ergosta-7-en-6,23-dione).

**Confirmation by other means:**

As an empirical method for the determination of
<table>
<thead>
<tr>
<th>Compound</th>
<th>$[\alpha]_D$</th>
<th>$[\alpha]_{DxM/100}$</th>
<th>$\Delta$(OH) parent</th>
<th>$[\alpha]_D$</th>
<th>$[\alpha]_{DxM/100}$</th>
<th>$\Delta$(OH) parent</th>
<th>$\Delta$ 23R-23S</th>
</tr>
</thead>
<tbody>
<tr>
<td>23-hydroxy-i-ergosta-7-en-6-ones</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>54.3</td>
<td>224</td>
<td>73</td>
<td>57.5</td>
<td>237</td>
<td>60</td>
<td>13</td>
</tr>
<tr>
<td>23-hydroxy-i-ergosta-6,8(14)-dienes</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>146.6</td>
<td>581</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i-ergosta-6,8(14)-diene) $[\alpha]<em>D = 146.4$, $[\alpha]</em>{DxM/100} = +556$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3β,23-hydroxy-lanosta-8(9),24-dienes</td>
<td>+</td>
<td>+</td>
<td>-13</td>
<td>+</td>
<td>+</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>53</td>
<td>234</td>
<td>-13</td>
<td>73</td>
<td>322</td>
<td>+75</td>
<td>88</td>
</tr>
<tr>
<td>(Lanosterol; $[\alpha]<em>D = +58$, $[\alpha]</em>{DxM/100} = +247$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Methylpent-2-en-4-one</td>
<td>-4.01</td>
<td>-4.01</td>
<td>+4.01</td>
<td>+4.01</td>
<td>+4.01</td>
<td>+4.01</td>
<td>8.02</td>
</tr>
</tbody>
</table>
absolute configuration, molecular rotation differences have been very useful in the past. In particular, the method has proved of great value in structural problems in the steroid field, largely owing to the work of Barton and his colleagues. The principle involved is that the rotational contributions of individual groups in compounds of multiple oxygen function are independent of one another providing that highly unsaturated groups are absent and that the functional groups are separated in the molecule by a certain number of carbon atoms. The influence of other contributions felt by any one contributing group is known as "vicinal action".

Some of the types of molecular rotation differences which have been used are listed below.

(1) $\Delta OH = [M]_D C^\ominus OH - [M]_D C^\ominus H$
(2) $\Delta OA = [M]_D C^\ominus OA - [M]_D C^\ominus H$
(3) $\Delta OB = [M]_D C^\ominus OB - [M]_D C^\ominus H$
(4) $\Delta_1 = [M]_D C^\ominus OA - [M]_D C^\ominus OH$
(5) $\Delta_2 = [M]_D C^\ominus OB - [M]_D C^\ominus OH$
(6) $\Delta_3 = [M]_D C = O - [M]_D C^\ominus OH$

The earlier steroid workers restricted their attention mainly to sites in the tetracyclic nucleus, where the conformation was more fixed and the known information more abundant and reliable. Attempts to extend the method have been made but only in a limited way. The wrong predictions made by Klyne and Stokes (though on the basis of Cram's rule and not molecular rotational differences) are cautionary in themselves.
Tables 1, 3, 4, 5 and 6 have been drawn up to examine what inferences can be made from the present work with regard to the various $\Delta$'s listed above. Unfortunately, although many derivatives of the positions C-22 and C-23 have been prepared, sufficient comparisons from other systems are not available. The most useful and reliable parameter seems to be the difference in rotations between the two epimeric alcohols, $\Delta_{R-S}$ (Tables 3 and 4). For a certain side-chain position, its sign seems constant (positive and large for C-22 and negative for C-23). The other factors nearly all show enough variations in sign and magnitude to render them unreliable. Table 1 contains the rotations of the $22R, 23R$- and $22S, 23S$-epoxides of several ergostane derivatives. Changes in the nucleus cause variations in $(\sigma_d(\text{Epoxide}) - \sigma_d(\text{parent}))$ and $\Delta_{R-S}$. This must be due to long distance "vicinal action" or conformational effects as observed by Barton and Cox\textsuperscript{104} for conjugated dienes and $\alpha\beta$-unsaturated ketone groups.

Klyne and Stokes\textsuperscript{61} have proposed general rules regarding the molecular rotation contributions of hydroxyl groups and derived functions in alicyclic compounds. For the side-chain positions, they have, where possible tried to compare $\Delta$'s with the corresponding values for similar simple carbinols. For the asymmetric centre $C^\Phi$ (figure 16):

1. If $R$ and $R'$ are both CH$_2$, the rotation contribution of $C^\Phi$ will be small and attempts to predict its sign are unwise.

2. If $R$ is -CH$_2$-C and $R'$ is -CH or -C-C the rotation contribution of $C^\Phi$ will be negative and appreciable.
(3) If $R$ is $-\text{CH}$ or $-\text{C}-\text{C}$ and $R'$ is $-\text{CH}_2-\text{C}$, the rotation contribution of $C^\pm$ will be positive and appreciable.

![Diagram]

FIGURE 16.

The only parameters (see Tables 5 and 6, Compound 1) which have consistent alternating signs for the C-22 and C-23 derivatives of i-ergosta-7-en-6-one are $\Delta \text{OBz}$ and $\Delta^2$. Figure 17 shows the relation of the determined structures to these signs. The same sign is obtained for $\Delta^2$ in the case of 22S-benzoyloxy-i-ergosta-7-en-6-one and 22S-benzoyloxy-i-cholesta-7-en-6-one (benzoate of 43a). For C-22 Klyne and Stokes' rules seem to hold, but the signs are reversed for C-23. However, for the i-ergosta-6,8(14)-diene system, both the 22R- and 23R-benzoates examined fail to give signs consistent with the rules. In fact, the molecular rotational contributions of groups in the side-chain appear to be very sensitive to long range "vicinal" or conformational effects of the tetracyclic nucleus.

Conformation of the configurational assignments was also sought from the circular dichroism and optical rotatory dispersion curves of the four 7-en-6-one benzoates-I, -II, -III and -IV (84c and 89c) and of the two 6,8(14)-diene 22R- and 23R-benzoates (86c and 91c). The former all have the same
Opposite to Klyne and Stokes' rules.

\[
\begin{align*}
\text{BENZOATE-I} \\
(\text{R}=\text{CH}_3)
\end{align*}
\]

and 22(S)-Benzoyloxy-i-cholest-7-en-6-one.

\[
\begin{align*}
\text{BENZOATE-II} \\
(\text{R}=\text{H})
\end{align*}
\]

\[\Delta\text{OBz} \} \text{ large and positive} \]
\[\Delta_2 \} \text{ positive.}\]

\[\text{BENZOATE-IV} \]

\[\Delta\text{OBz} \} \text{ large and negative} \]
\[\Delta_2 \} \text{ negative.}\]

\[\text{BENZOATE-III} \]

\[\Delta\text{OBz} \} \text{ large and positive} \]
\[\Delta_2 \} \text{ positive.}\]

\[\text{BENZOATE-II} \]

\[\Delta\text{OBz} \} \text{ large and positive} \]
\[\Delta_2 \} \text{ positive.}\]

\[\text{BENZOATE-III} \]

\[\Delta\text{OBz} \} \text{ large and positive} \]
\[\Delta_2 \} \text{ positive.}\]

\[\begin{align*}
\text{FIGURE 17.}
\end{align*}\]
shaped c.d. curves, and the only detectable difference is that of the magnitude of the Cotton effect at 230-5nm. Both the benzoates of R-configuration have negative $\Delta\varepsilon$ of greater magnitude than their S-epimers and of similar magnitude to each other. The S-epimers also have Cotton effects of the same order of negativity.

The two R-benzoates in the 6,8(14)-diene series have c.d. curves of a different shape from those in the 7-en-6-one series, but similar to each other. They exhibit only a small negative effect at 223nm ($\Delta\varepsilon$ -6 to -7) and a large, positive effect at 259nm ($\Delta\varepsilon$ +14 to +17). Unfortunately, the two S-benzoates were not available for comparison purposes. The sign of the maximum effect is however obviously no indication of configuration, since it changes merely by altering the chromophore. It is possible that the magnitude may give some indication, the R-epimers of the 7-en-6-one series having distinctly greater, negative effects at ca. 233nm.

Little further information could be gleaned from the o.r.d. curves, analogous similarities of shape, sign changes and orders of magnitude being observed in the various cases.

Because of these results, any attempt to apply the octant rules, the benzoate sector rule or the chirality rules\textsuperscript{56,65,66,67,109} must also fail. The benzoate chromophore is too distant from the chromophore of the nuclues for the interactions to differ from epimer to epimer. The four 22- and 23- benzoate epimers all lie in the same octant.
## TABLE 5A.

<table>
<thead>
<tr>
<th>Compound</th>
<th>PARENT</th>
<th>22R $[\alpha]_{D}^{{\text{Me}}/100}$</th>
<th>KETONE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OH</td>
<td>$\Delta OH$</td>
<td>OAc</td>
</tr>
<tr>
<td>22-hydroxy-5a-cholestan-3p-ol</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>22-hydroxy-5a-cholestan-3p-ol benzoate</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>22-hydroxy-5a-cholestan-3p-ol methyl ether</td>
<td>-</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td>22-hydroxy-5a-cholestan-3p-ol &amp; 17-ene</td>
<td>-</td>
<td>-</td>
<td>74</td>
</tr>
<tr>
<td>22-hydroxy-5a-cholestan-3p-ol &amp; 17-ene</td>
<td>-</td>
<td>-</td>
<td>168</td>
</tr>
<tr>
<td>22-hydroxy-5a-cholestan-3p-ol &amp; 17-ene</td>
<td>+</td>
<td>399</td>
<td></td>
</tr>
</tbody>
</table>
### TABLE 5B.

<table>
<thead>
<tr>
<th>Compound</th>
<th>22S $[\alpha]_{D}^{25}/100$</th>
<th>ketone $\Delta_3$</th>
<th>22R-S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OH</td>
<td>$\Delta OH$</td>
<td>CAc</td>
</tr>
<tr>
<td>22-hydroxy-i-ergosta-7-en-6-one</td>
<td>297</td>
<td>198</td>
<td>99</td>
</tr>
<tr>
<td>22-hydroxy-5$\alpha$-cholestan-3$\beta$-ol</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>22-hydroxy-cholestan-5-en-3$\beta$-ol</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>22-hydroxy-cholestan-5-en-3$\beta$-yl benzoate</td>
<td>153</td>
<td>217</td>
<td>64</td>
</tr>
<tr>
<td>22-hydroxy-cholestan-5-en-3$\beta$-yl methyl ether</td>
<td>74</td>
<td>142</td>
<td>68</td>
</tr>
<tr>
<td>22-hydroxy-i-cholestan-7-en-6-one</td>
<td>325</td>
<td>449</td>
<td>124</td>
</tr>
<tr>
<td>Compound</td>
<td>( \text{PARENT} )</td>
<td>( \text{OH} )</td>
<td>( \Delta \text{OH} )</td>
</tr>
<tr>
<td>----------</td>
<td>-----------------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>23-hydroxy-i-ergosta-7-en-6-one</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>23-hydroxy-i-ergosta-6,8(14)-diene</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>23-hydroxy-i-ergosta-7-en-6α-ol</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Although confirmation from these other empirical methods was not in itself forthcoming, the configurational assignments which have been made for the iodoacetate (20), the bromoacetates and their derivatives and the various epoxides are viewed with confidence. Final X-ray determination of configuration is awaited, but the evidence discussed in the foregoing is self-consistent and in agreement with the known facts concerning the C-22 and C-23 positions of the steroid side-chains of cholesterol, ergosterol and lanosterol.

From this work, the major product of electrophilic addition to the 22-double bond of the ergosterol-type side-chain is predicted as having 22R-, 23S-configuration in the case where two groups are added. These derive from the major cyclic 22R-23R-intermediate. The major epoxide product has this configuration.
<table>
<thead>
<tr>
<th>Compound</th>
<th>PARENT</th>
<th>23S $\alpha$ D$_{x}$M/100</th>
<th>KETONE</th>
<th>23R-S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OH</td>
<td>ΔOH</td>
<td>OAc</td>
<td>ΔAOAc</td>
</tr>
<tr>
<td>23-hydroxy-1-ergosta-7-en-6-one</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>297</td>
<td>237</td>
<td>60</td>
<td>259</td>
</tr>
</tbody>
</table>
By analogy to the present work, the dibromo-compounds (60, 61 and many others) must be the $22\text{R}, 23\text{S}$-dibromides, since they are the major products of kinetically-controlled addition.

The anti-Cram nature of LAH reduction of 22-ketones of the cholesterol and ergosterol side-chains has been confirmed. 23-Ketones would similarly be predicted to give the Cram-products.

The C-22 and C-23 hydroxy compounds are now of known configuration. The derivatives of bromoacetate-II can be selected for conversion to 25-deoxy-24S-methyl analogues of (22R)-ecdysone. Similarly, for biosynthetic work, the required hydroxy compound can be selected and the nucleus converted as necessary or the bromoacetylation can be carried out on compounds already containing the right nucleus.

Extension of this work to $24\alpha\text{P}$-methyl-22-ene- and to other 24-alkylated-22-ene-steroids, and to cholest-22-ene is an attractive proposition, and would throw considerable light on the preferred products of attack in the various steric environments of the 22-double bond.
Physical Techniques:— The following general procedures were used in experiments described in this section. Where any deviation from these have been made, the details are given in the relevant places.

M. Pts. were determined on a Kofler block and recorded without correction. Rotations were measured in a 10cm. cell (0.5ml.) at the sodium D-line wavelength on a Perkin-Elmer 141 polarimeter. I.r. spectra were taken on a Unicam S.P. 200 spectrometer with sodium-chloride plates. Nujol mulls were used for crystalline or solid samples, and liquid films for oils. U.v. spectra were run in ethanol solution, using a Unicam S.P. 700 spectrometer. An A.E.I. high resolution M.S. 9 instrument was employed for the determination of mass spectra by the direct inlet technique. N.m.r. spectra were measured in deuteriochloroform solutions with tetramethylsilane as an internal standard. A Varian A.60 or H.A. 100 spectrometer was used, according to the quantity of material available. The following abbreviations apply to n.m.r. data:

\[
\begin{align*}
\text{Hz} & = \text{Hertz.} \\
p.p.m. & = \text{parts per million.} \\
s & = \text{singlet} \\
d & = \text{doublet} \\
\text{dd} & = \text{double doublet} \\
t & = \text{triplet} \\
q & = \text{quartet} \\
m & = \text{multiplet} \\
b & = \text{broad.}
\end{align*}
\]
Wherever possible, reactions were followed by T.I.C. on 0.1mm. plates of Merck silica gel, GF$_{254}$. P.L.C. was carried out on 0.01x20x20, 0.05x20x60 or 0.1x20x60 cm$^2$. plates of the same material. T.L.C. plates were normally run in acetone/petrol, the percentage of acetone being specified in the appropriate place, and were developed with phosphomolybdic$^{105}$ or dilute sulphuric acid spray.

Solvents were dried, where necessary, using standard techniques. Organic solvent extracts of aqueous solutions were dried with anhydrous sodium sulphate, which was removed by filtration after twenty minutes.
3α,5α-cycloergosta-7,22-dien-6-one (5)\(^{1,12,13}\) - Ergosterol (1) (30g.) dried by azeotropic distillation with sodium-dried benzene. After removal of the solvent, the residue was dissolved in dry pyridine (150ml.) and treated with recrystallised toluene-p-sulphonyl chloride (36g.) at room temperature overnight. The reaction mixture was poured into ice-cold, aqueous potassium hydrogen carbonate solution (4% w/v; 3l.) and the crude toluene-p-sulphonate (3) collected, washed with ice-cold water, and immediately added to a refluxing solution of potassium hydrogen carbonate (18.0g.) in acetone (6l.) and water (1.5l.). After 5 minutes, solvent was removed by distillation until the volume had been reduced by approximately half. The resulting turbid solution (ca. 3.5-4 l.) was cooled to 0\(^\circ\)C and the solid cyclopropyl alcohol (4) collected and taken up in chloroform (300ml.). This solution was washed with water and dried, and the volume adjusted to 750ml. with more chloroform. Finely divided active manganese dioxide\(^{11}\) (180g.) was added and the mixture stirred at room temperature overnight under nitrogen. The oxidant was removed by filtration through Kieselguhr and the filtrate evaporated under reduced pressure to yield the crude ketone (5) (27.6g.). Crystallisation from ether gave the pure compound as plates, m.pt. 165-6\(^\circ\)C (lit.,\(^{13}\) 166\(^\circ\)C), \(\nu_{\text{max.}}\) 1650cm\(^{-1}\), \(\lambda_{\text{max.}}\) 249nm. (ε 12,000), in yields varying from 57 to 64%.

The mother liquors, after evaporation, were chromatographed over alumina (Grade V). Initially, 3α,5α-cycloergosta-6,8(14), 22-triene (6) was obtained as plates, m.pt. 100-102\(^\circ\)C (from acetone) (lit.\(^{106}\), 101\(^\circ\)C), but continued elution gave rise to more ketone (5).
Oxidation experiments on ergosta-5,7,22-trien-3β-yl tosylate (3) and i-ergosta-7,22-dien-6β-ol (4).

(a) Dimethyl sulphoxide oxidation. The crude tosylate (3) (300mg.) was heated with dry DMSO (10ml.) and sodium acetate (300mg.) at 80-90°C for 4 hours according to the published procedure. The product was not the required i-ketone (5), but the triene (6) in 85% yield.

(b) Attempted combination of the normal solvolysis and oxidation stages. - The tosylate (3) (250mg.) was dissolved in acetone/water (4:1; 50ml.) and active manganese dioxide (5g.) added. The reaction mixture was stirred in the presence of a 0.5% sodium hydrogen carbonate buffer solution. No i-ergosta-7,22-dien-6-one (5) was formed, reaction ceasing after the formation of the i-alcohol (4).

(c) The crude tosylate (3) (100mg.) in sodium-dried benzene (5ml.) was stirred with a basic sample of active manganese dioxide (1.2g.; normally washed until neutral during preparation). The main product was the cyclopropyl triene (6).

(d) Using DMF and pyridine N-oxide. - The crude tosylate (3) (100mg.) was dissolved in dry DMF (5ml.; distilled from calcium hydride) and stirred with pyridine N-oxide (500mg.). The triene (6) and the i-alcohol (4) were the only products. Attempts to dry the pyridine N-oxide thoroughly were made (by refluxing with dry benzene or dry toluene), but the reaction followed the same course.

(e) With DMSO/pyridine N-sulphur trioxide/triethylamine (a) Pyridine N-sulphur trioxide (1.2m. moles, 210mg.; prepared by the literature procedures) in dry DMSO (1ml.) was added to a mixture of i-ergosta-7,22-dien-6β-ol (4) (0.4m. moles, 160mg.), DMSO (1ml.) and triethylamine (50m. moles, 500mg.) with stirring at room temperature. No oxidation to the i-ketone (5) was
observed, although this method is specifically recommended$^{16(a)}$ for the oxidation of allylic alcohols. Treatment of cinnamyl alcohol under analogous conditions gave cinnamaldehyde quantitatively, identified by T.L.C. comparison with authentic material and by isolation of the 2,4-dinitrophenylhydrazone, m.pt. 253-6°C ($^{107}$, 255°C).

Other activators which were employed, instead of pyridine N-sulphur trioxide, included toluene-p-sulphonyl chloride, mesyl chloride, iodine pentoxide, sulphuryl chloride, phosphorus trichloride, and benzoyl chloride. No improvement on the standard manganese dioxide oxidation method was obtained.

When the procedure$^{16(a)}$ was followed by n.m.r. spectroscopy, the order of addition of reactants was found to be critical. If the literature order was observed, oxidation of cinnamyl alcohol could be obtained (appearance of aldehyde proton doublet at 0.50τ and disappearance of methylene doublet at 6.93τ). If the alcohol was added to pyridine N-sulphur trioxide in DMSO, followed by addition of triethylamine, no aldehyde formation occurred. The alcohol appeared to form a sulphate, shown by a downfield shift of 25Hz. for the methylene signal. Addition of triethylamine to pyridine N-sulphur trioxide in DMSO resulted in the production of free pyridine, and the formation of a highly labile singlet peak, very concentration dependent, probably due to the triethylammonium ion.
5α,14β-ergosta-2,7,22-trien-6-one (11) and (16) \(^1\) - The cyclopropyl ketone (5) (5g.) and toluene-p-sulphonic acid (250mg.) in dry, freshly distilled sulfolane (30ml.) were heated at 160°C for 1.5 hours with stirring under nitrogen. The resulting green solution was cooled, poured into water, and extracted with 20% benzene/petrol (3x50ml.). The combined extracts were filtered through alumina (grade V, 60g.) and elution completed with 20% benzene/petrol (150ml.). Evaporation afforded a crude mixture of 2-ene products, as an oil (4.3g.), normally used without further purification in the cis-hydroxylation procedure. Feakins \(^9\) carried out a chromatographic separation of the mixture on alumina (grade V; 450g.) and showed it to consist of the 14β-isomer (16)\(^1\), the 14α-isomer (11)\(^1\) and 5α-ergosta-2,8(14),22-triene.

Alternative direct ring-opening experiments.

(a) with lithium perchlorate in benzene. - i-ergosta-7,22-dien-6-one (5) (100mg.) in dry benzene/dry dioxan (6:4; 10ml.) was refluxed for twenty-four hours with dry lithium perchlorate (150mg.). Isomerisation of the 7-ene to a 6:4 equilibrium mixture with the 8(14)-ene compound was the only observed change.

(b) with silver perchlorate in nitromethane. - The ketone (3) (100mg.) and dry silver perchlorate (150mg.) were dissolved in dry nitromethane (10ml.) and refluxed. After initial isomerisation as before, the reaction resulted within one hour in a mixture of more polar decomposition products, none of the required trienes being produced.

(c) with lithium perchlorate in sulfolane. - The ketone (3) (100mg.) and lithium perchlorate (150mg.) were suspended in
dry, freshly distilled sulpholan (10ml.) and heated at 160°C under nitrogen. The results were compared with those obtained using toluene-p-sulphonic acid. Isomerisation was followed by the required ring-opening, but the reaction proceeded much more slowly than under the standard conditions. Use of lithium perchlorate with a little toluene-p-sulphonic acid (5mg.) gave a fairly clean ring-opening after four hours, but was not significantly better than the usual procedure.

2β-acetoxy-3β-hydroxy-5α,14β-ergosta-7,22-dien-6-ones (17) -
The crude mixture of 2-ene products (see above; 4.3g.) was stirred at room temperature under nitrogen in glacial acetic acid (100ml.) with silver acetate (4.0g.). Powdered iodine (2.9g.) was added in portions over a period of fifteen minutes. After a further fifteen minutes, water (0.5ml.) was added and stirring continued overnight. The silver iodide was filtered off, and the residue washed well with chloroform. The filtrate was diluted with more chloroform (250ml.) and washed with water (2x200ml.), saturated aqueous sodium hydrogen carbonate solution (2x100ml.) and with water (2x200ml.). The dried organic layer was evaporated under reduced pressure, and the residue chromatographed over alumina (grade V; 200g.). Elution with 20% benzene/petrol (500ml.) removed non-polar impurities. Further elution with ether and eluates collected in 25ml. fractions gave a mixture of epimeric monoacetate diols (17) (887mg.), m.pt. (needles from ether) 181-7°C, νmax. 3500, 1718, 1665 and 1620cm.⁻¹, λmax. 240nm. (ε 11,500). (Found: C, 76.3; H, 9.6. C₃₀H₄₆O₄ requires C, 76.55; H, 9.85%).
Model oxymercurylation experiments as a first step in a novel cis-hydroxylation method.

(a) 2β-Methoxy-3α-acetoxymercuri-5α-cholestane (19a)\textsuperscript{23}. - 5α-cholest-2-ene (75mg.) and mercuric acetate (65mg.) (equimolar proportions) were shaken for several days in methanol (10ml.). The resulting solution was filtered of any insoluble material, evaporated under reduced pressure, and the residue extracted with ether. Evaporation gave a white solid, (50mg.; 40% yield), m.pt. 57-60°C, $v_{\text{max}}$. 1580, 1220 and 1090cm.$^{-1}$, (no acetate stretches observable in the i.r. spectrum). The n.m.r. spectrum however showed signals at τ 6.7 (1H,s) (–OH), 7.95 (3H,s) (–OAc), 9.07, 9.16 and 9.33.

(b) 2β-Acetoxy-3α-acetoxymercuri-5α-cholestane (19b)\textsuperscript{23}. - Cholest-2-ene (76.3mg.) and mercury acetate (67.8mg.) were shaken together for some time in glacial acetic acid (10ml.) or in chloroform/acetic acid (1:1; 10ml.). After two weeks, the solution was diluted with chloroform, washed acid-free with water, the organic layer dried and evaporated at reduced pressure. A crude white solid was obtained, which contained some of the required product. $v_{\text{max}}$. = 1725 and 1240cm.$^{-1}$ (–OAc) and 1580cm.$^{-1}$. The n.m.r. showed no vinyl protons.

(c) 2β-Hydroxy-3α-acetoxymercuri-5α-cholestane (19c)\textsuperscript{24}. - According to the method of Brown et.al., prolonged reaction times as used above are unnecessary and even deleterious. Mercuric acetate (160mg., 0.5m.moles) was added to a small flask, followed by water (0.5ml.) and then tetrahydrofuran (0.5ml.). A yellow precipitate was produced. Finally, cholest-2-ene (185mg., 0.5m.moles) was added and the solution
stirred.

In the experiments of Brown and Geoghegan (in which the oxymercurials were not isolated), the yellow precipitate disappeared normally within a matter of minutes after olefin addition, reaction being complete after a further ten times this period. In the present case, the yellow precipitate showed no signs of disappearing even over several days, and these experiments were abandoned.

Control cis-hydroxylation of i-ergosta-7,22-dien-6-one (5).21

The ketone (5) (500mg.) was treated as described above for 5α,14β-ergosta-2,7,22-trien-6-ones, using 1/8 x the quantities of reagents. The more hindered trans-double bond at C-22 was oxidised under these conditions, but more slowly than the cis-2-double bond. After 24 hours, some starting material was recovered from the crude product by P.L.C., but the major component was (22R)(23S)-23-acetoxy-22-iodo-i-ergosta-7-en-6-one (20) (75mg.) as a microcrystalline white solid, m.pt. 157-159.5°C (from benzene/petrol), [α]$_D$ + 53° (c 0.517), v$_{max}$. 1730, 1645 and 1230cm.$^{-1}$, λ$_{max}$. 243.5nm. (ε 14,200), τ 4.2 (1H, dd) (C-7 vinyl proton), 4.5 (1H, d, J 10Hz), (proton α to acetate), 5.75 (1H, d, J 10Hz) (proton α to iodide), 7.93 (3H, s) (-OAc), 9.0, 9.05 and 9.25; m/e 580(M$^+$), 453(M$^+$-I), 393(M$^+$-I-AcOH), 267(M$^+$-side-chain). (Found: C, 62.0; H 7.8; I, 21.6. C$_{30}$H$_{45}$IO$_3$ requires C, 62.1; H, 7.8; I, 21.9%). Yield for these conditions was 12%.

Optimum yields of the iodoacetate (53%) were obtained
by omitting the addition of water, and by reducing reaction
time to 3 hours. Although stable to the reaction conditions
for upto 24 hours, prolonged exposure to the cis-hydroxylation
procedure for several days resulted in a gradual conversion
of the hindered iodoacetate to an inseparable mixture of
hydroxyacetates (21).

2β,3β-Diacetoxy-14α-hydroxy-5α-ergosta-7,22-dien-6-one (24)\(^1\). -
The epimeric mixture of monoacetate diols (17) (700mg.) in
carbon tetrachloride (47.5ml.), acetic anhydride (2.5ml.),
and perchloric acid (60% aqueous; 0.005ml.) was left at room
temperature for 3 hours. The solution was then washed with
water, saturated aqueous sodium hydrogen carbonate, and
finally more water, dried, and evaporated in vacuo. The oily
residue was purified by P.L.C. in 16% acetone/petrol to give
the enol acetate (23) (520mg.) as a non-crystalline solid,
\(ν_{\text{max.}}\) 1740 cm.\(^{-1}\), \(λ_{\text{max.}}\) 254 nm. (ε 11,000), \(τ\) 4.10 (1H, d, \(J\) 2.5Hz).

The enol-acetate (500mg.) in ether (6.5ml.) containing
monoperphthalic acid (33mg. per ml., 1.2 equivalents; stand-
ardised iodometrically immediately before use) was left for
24 hours at room temperature. Work-up in the normal manner
afforded, by P.L.C. in 16% acetone/petrol, the required
hydroxy-ketone (24) (330mg.), as needles, m.pt. (from methanol)
220-223°C (lit.\(^5\), m.pt. 226-7°C), [\(α\)]\(_D\) + 66° (ε 0.36),
\(ν_{\text{max.}}\) 3347, 1733, and 1655 cm.\(^{-1}\), \(λ_{\text{max.}}\) 241 nm. (ε 14,000), \(τ\)
4.09 (1H, d, \(J\) 2.5Hz), 4.75 (3H, m), 5.17 (1H, m). (Found:
C, 72.7; H, 9.0. C\(_{32}\)H\(_{48}\)O\(_6\) requires C, 72.7; H, 9.1%). The
overall yield from the monoacetate diols was 42%.
2β,3β-Diacetoxy-5α,14β-ergosta-7,22-dien-6-one (25)\(^1\). During the epoxidation step described above, T.L.C. showed that all the enol-acetate (23) had not reacted. Isolation of the unreacted material and further treatment with monoperphthalic acid gave rise to more of the hydroxy-ketone (24). One component of the recovered enol-acetate (marginally more polar) was still unaffected and proved to be the 14β-epimer (25) (50mg.), m.pt. (from methanol) 161-4°C (lit.\(^9\), 153-6°C), \(\nu_{\text{max.}}\) 1735, 1670 and 1250cm\(^{-1}\), \(\lambda_{\text{max.}}\) 246nm. (ε 11,000), \(\tau\) 4.21 (1H, d, \(J\) 2.5Hz) (C-7 vinyl proton), 4.76 (3H, m), 5.23 (1H, b) (proton α to 3β-OAc), 7.96 (3H, s), 8.03 (3H, s), 9.02, 9.13, 9.20 and 9.39; m/e 512(M\(^+\)), \([\alpha]_D + 32^\circ\) (c 0.047). (Found: C, 74.7; H, 9.1. \(C_{32}H_{48}O_5\) requires C, 74.7; H, 9.4%).

(20S)-3α,5α-Cyclo-20-formylpregn-7-en-6-one (26)\(^1,32\). The cyclopropyl ketone (5) (520mg.) in methylene dichloride (20ml.) and pyridine (0.2ml.) was ozonised at -70°C, the extent of reaction being controlled by T.L.C. The solution was allowed to warm to room temperature in the presence of water, and washed with 0.1N hydrochloric acid and water. The separated organic layer was dried and evaporated under reduced pressure to give an oil (465mg.). Filtration through alumina (grade V) with benzene as eluant afforded the unstable aldehyde (26) (402mg.), m.pt. (from methanol) 138-141°C, \([\alpha]_D + 91^\circ\) (c 0.44; CHCl\(_3\)), \(\nu_{\text{max.}}\) 1725 and 1650cm\(^{-1}\), \(\lambda_{\text{max.}}\) 244nm. (ε 13,900), \(\tau\) 4.28 (1H, t, \(J\) ca. 2Hz), 0.29 (1H, d, \(J\) 3.0Hz). (Found: C, 80.3; H 8.8%; M\(^+\), 326. \(C_{22}H_{30}O_2\) requires C, 80.9; H 9.25%; M, 326). Average yields were in the region of 93%.

The n.m.r. signal due to the aldehyde proton remained as a doublet under the conditions used above. On heating in
the presence of a little pyridine, epimerisation about position 20 was observed as splitting of this signal and of the methyl signals.

\[(20S)-2\beta,3\beta\text{-Diacetoxy}-20\text{-formyl}-14\alpha\text{-hydroxy}-5\alpha\text{-pregn-7-en-6-one} \]

\[(27)^1\]. - The hydroxy-ketone \((24)\) (400mg.) in methylene dichloride (100ml.) and pyridine (1.0ml.) was ozonolysed at \(-70^\circ\text{C}\) as previously described. After the usual work-up, the crude aldehyde was purified by P.L.C. (in 25\% acetone/petrol) to give the pure aldehyde \((27)\) (305mg.) as colourless needles, m.p. 234-6\(^\circ\text{C}\) (from ether), \([\alpha]_D + 49^\circ\) (c 0.41), \(\nu_{\text{max}}\), 3475, 1730, 1715 and 1670cm.\(^{-1}\), \(\lambda_{\text{max}}\) 244nm. (c 12,400), \(\tau\) 0.40 (1H, d, J 5Hz), 4.07 (1H, d, J 2.5Hz), 7.92 (3H, s), and 7.97 (3H, s). (Found: C, 67.7; H 8.2\%; M\(^+\), 460. C\(_{26}\)H\(_{36}\)O\(_7\) requires C, 67.8; H, 7.9\%; M, 460). Yields of 87.5\% were typical.

\[(22S)\text{-and (22R)-22-hydroxy-3\alpha,5\alpha\text{-cyclocholest-7-en-6-one}} \]

\[(43a \text{ and } b)\]. - Magnesium turnings (125mg.; 5 equivalents) in a dry flask, fitted with a tap-funnel and a reflux condenser, were stirred with a solution of isoamyl bromide (1.05g.; an excess) in sodium-dried ether (5ml.). A crystal of iodine was added to catalyse the reaction. The clear grey solution was added to the pure, freshly prepared cyclopropyl aldehyde \((26)\) (300mg.) in sodium-dried ether (20ml.) at room temperature. A white complex was immediately formed. After 5 minutes, the mixture was refluxed for a further 5 minutes. Ice-cold, saturated aqueous ammonium chloride was added to decompose the complex. The layers were separated, the aqueous layer extracted twice with ether, and the ethereal solutions combined, washed with water and dried. Evaporation under reduced pressure gave an
oil, which when evaporated on an oil pump gave a crude, white solid (380mg.). Multiple-elution P.L.C. (10% acetone/petrol) allowed the epimeric products to be separated. An u.v. comparison showed that the more polar 22S-alcohol (43a) (190mg.) predominated by a ratio of 6.3:1. It crystallised as chunks and needles (from ethyl acetate/petrol) m.pt. 186.5-189.5°C, \[ \left[ \alpha \right]_{D}^{28} + 82^\circ (c 0.698), \nu_{\text{max.}} 3600, 1650, \text{and} 1620\text{cm}^{-1}, \]
\[ \lambda_{\text{max.}} 247\text{nm.} \ (c 11,300), \tau 4.23 \ (1H, t), 6.35 \ (1H, b), 8.87 \ (3H, s), 9.05 \ (3H, d), 9.15 \ (3H, d) \text{and} 9.30 \ (3H, s). \] (Found: C, 81.2; H, 10.6%; M⁺, 398. C\textsubscript{27}H\textsubscript{42}O\textsubscript{2} requires C, 81.4; H, 10.6%; M, 398).

The minor, less polar 22R-epimer (43b) (30mg.), also crystallised as chunks and needles (from ethyl acetate/petrol), m.pt. 170-173°C, \[ \left[ \alpha \right]_{D}^{26} + 100^\circ \ (c 0.229), \nu_{\text{max.}} 3400, 1635 \text{and} 1618\text{cm}^{-1}, \lambda_{\text{max.}} 247\text{nm.} \ (c 11,700), \tau 4.28 \ (1H, dd) \ (C-7 \text{ vinyl proton}), 6.45 \ (1H, b), 8.94 \ (3H, s), 9.05 \ (3H, d), 9.11 \ (3H, d), 9.15 \ (3H, d) \text{and} 9.34 \ (3H, s). \] (Found: C, 81.5; H, 10.4%; M⁺, 398. C\textsubscript{27}H\textsubscript{42}O\textsubscript{2} requires C, 81.4; H, 10.6%; M, 398).

The two epimers were produced from the aldehyde in yields of 51.8 and 8.2% respectively.

Small amounts of less polar byproducts (45) were also isolated. The major and least polar component, of these was presumably the 22S-epimer of (45), and was a non-crystalline solid, \[ \nu_{\text{max.}} 3450\text{cm}^{-1}, \lambda_{\text{max.}} 265 \text{and} 259\text{nm.}, \tau 4.03 \ (1H, s) \ (C-7 \text{ proton}), 6.3 \ (1H, b), 9.07, 9.16 \text{and} 9.25.

3\alpha,5\alpha-Cyclocholest-7-en-6,22-dione (44)⁹. - The epimeric mixture of 22-alcohols (43) (760mg.) (from a repeat of the preceding experiment on double the scale) was dissolved in
acetone (10m1.) and oxidised with Jones' reagent in the usual way. After addition of methanol to destroy excess oxidant, water was added and the mixture extracted with ether. The combined extracts were washed with water, dried and evaporated to give the crude diketone (400mg.). Crystallisation (from methanol) gave the pure compound as needles, m.pt. 158-90°C; \( \nu_{\text{max}} \) 1702, 1644 and 1619cm.\(^{-1}\); \( \lambda_{\text{max}} \) 246nm. (e 13,300), \( [\alpha]_{D}^{26} + 115^\circ (c 0.229), \tau 4.23 (1H,t), 8.85 (3H,d), 8.92 (3H,s), 9.10 (3H,d), 9.30 (3H,s). \) (Found: C, 81.5; H, 9.9. \( C_{27}H_{40}O_2 \) requires C, 81.8; H, 10.2%). With respect to the aldehyde, the yield was 54.7%.

LAH reduction and manganese dioxide oxidation of \( \text{i-cholest-7-en-6,22-dione (44)}. \ - The dione (44) (33mg.) was dissolved in sodium-dried ether (6m1.) and stirred for 1 hour at room temperature with LAH (81mg.). Excess LAH was destroyed by addition of wet ether, followed by sufficient water to cause the metal hydroxides to gel and allow the decantation of the ethereal solution. The aqueous gel was washed twice with ether, the ether portions combined, washed with water, dried and evaporated, to give the 6\( \alpha,22\_\varepsilon \)-diols as a white foam (30mg.).

The crude mixture of diols was dissolved in analar chloroform, and stirred overnight under nitrogen with active manganese dioxide (330mg.). Removal of the oxidant by filtration through Kieselguhr, evaporation at reduced pressure and multiple elution preparative T.L.C. (in 10% acetone/petrol) enabled the 22\( S \)– and 22\( R \)-alcohols (43a and b) to be separated in a ratio of 3:1 by u.v. comparison. The two compounds were identical in every respect to the two epimeric products of the reaction between the aldehyde (26) and isoamyl magnesium
bromide (see above).

Reaction between isoamyl magnesium bromide and \((20S)-2\beta,3\beta\)-diacetoxy-20-formyl-14\(\alpha\)-hydroxy-5\(\alpha\)-pregn-7-en-6-one\) \((27)\).

(a) \((22S)-2\beta,3\beta\)-Diacetoxy-22-hydroxy-5\(\alpha\)-cholest-8(9), 14-dien-6-one\) \((49)\). - The aldehyde \((27)\) \((200\text{mg.})\), freshly prepared and purified, was dissolved in anhydrous, freshly distilled THF \((5\text{ml.})\) at 0\(^\circ\)C and to it was added rapidly with magnetic stirring a solution of isoamyl magnesium bromide in THF (made from magnesium turnings \((110\text{mg.}; 10\text{ equivalents})\) and isoamyl bromide \((1.0\text{g.}; \text{an excess})\) in anhydrous, distilled THF \((5\text{ml.})\)). After twenty minutes, the reaction was worked up, as described for the preceding Grignard reaction, and multiple elution P.I.C. allowed the isolation of three components. The two minor products were not identified completely, but were probably the epimeric 22-ols described below. The major component proved to be the dehydration product \((49)\) \((70\text{mg.})\) of the major required alcohol \((50)\). It crystallised from ethyl acetate/petrol, m.pt. 180-4\(^\circ\)C, \([\alpha]_D^{22} + 49^\circ (c 0.514), \nu_{\text{max.}} 3550, 1730, 1720, 1260\) and 1235cm.\(^{-1}\), \(\lambda_{\text{max.}} 245\text{nm.} (c 14,000)\) and 251\text{nm.} (c 13,900), \(\tau 4.60\) (1H,t) \((\text{C-15 olefinic proton})\), 4.70 (1H,b) (proton \(\alpha\) to 2\(\beta\)-acetate), 5.20 (1H, very \(b\)), (proton \(\alpha\) to 3\(\beta\)-acetate), 6.35 (1H,b) (proton \(\alpha\) to hydroxyl), 7.93 (3H,s), 8.00 (3H,s), 8.74, 9.04, 9.13 and 9.19, m/e 514 (\(M^+\)), 496 (\(M^+-H_2O\)), 454 (\(M^+-\text{AcOH}\)), 394 (\(M^+-2\text{AcOH}\)), 384 (\(M^+-\text{side-chain}\)) (Found: C, 71.6; H, 8.7; C\(_{31}H_{46}O_6\) requires C, 72.3; H, 9.0\%).

The product was obtained in 31.3\% yield.
(b) (22\text{S})-2\beta,3\beta-Diacetoxy-14\alpha,22-dihydroxy-5\alpha-cholest-7-en-6-one (50) and its 22\text{R}-epimer. - Isoamyl magnesium bromide (7 equivalents) (made from magnesium turnings (113mg.) and isoamyl bromide (0.693g.) in sodium-dried ether (7.5ml.)) was added to the aldehyde (27) (210mg.; freshly prepared) in anhydrous, distilled THF (7.5ml.) at -30°C. After 12 minutes, the reaction was worked up in the usual manner. Isolation of the major, most polar component by multiple-elution P.L.C. (in 30% acetone/petrol) afforded the diol (50) (80mg.), as needles (from ethyl acetate/petrol), m.pt. 243-245°C, $[\alpha]_D^{25} + 61^\circ$ (c 0.459), $\nu_{\text{max}}$ 3500, 1730, 1725, 1675, 1250 and 1240 cm$^{-1}$, $\lambda_{\text{max}}$ 241nm. (ε 14.400), τ 4.15 (1H, d, J 2.5Hz), 4.8 (1H, m), 5.3 (1H, b), 6.4 (1H, b), 7.99 (3H, s), 8.05 (3H, s), 8.78, 9.04, 9.11, 9.17 (3H, s) and 9.35 (3H, s), m/e 532 (M$^+$, small), 514 (M$^+$-H$_2$O), 496 (M$^+$-2H$_2$O), 472 (M$^+$-AcOH), 384 (M$^+$-H$_2$O -side-chain) (Found: C, 70.0; H 8.9. C$_{31}$H$_{48}$O$_7$ requires C, 69.9; H, 9.1%). This compound (50) was identical (mixed m.pt. and spectra) with a component isolated by Feakins$^9$ as a hydrogenolysis by-product during catalytic hydrogenation of (22\text{R})-2\beta,3\beta-diacetoxy-14\alpha,22-dihydroxy-25-tetrahydropyran-2-yloxy)-5\alpha-cholest-7-en-23-yne-6-one (34a). This was also converted to isoecdysone (35), verifying the 22\text{S}-configuration of (49) and (50).

A component, slightly less polar than (50), proved to be unreacted aldehyde (27) (mixed m.pt. and spectra). Allowing for this recovery, (50) was obtained in 58% yield.

A third, less polar compound was also isolated (30mg.) (22% yield with respect to reacted aldehyde). It resisted complete characterisation, but had most of the spectral
features associated with the 22R-epimer of (50). It was a non-crystalline solid, $\nu_{\text{max.}}$ 3500, 1740, 1730, 1680, 1250 and 1240 cm$^{-1}$, $\lambda_{\text{max.}}$ 242 nm., $\tau$ 4.17 (1H, d), 4.65 (1H, b), 5.2 (1H, very b), 6.4 (1H, broad), 7.92 (3H, s), 7.97 (3H, s), 8.75, 8.92, 9.05, 9.13 and 9.31, m/e 532, 514, 496, 472, and 384. (M$^+$, 532. C$_{31}$H$_{48}$O$_7$ requires M, 532).

(c) (22S)-2b,3b,14a,22-Tetrahydroxy-5b-cholest-7-en-6-one (or 25-deoxy-5b-isoecdysone) and the 5a-epimer. - The diol (50) (30mg.) in methanol (5ml.) containing aqueous potassium carbonate (0.3%; 0.5ml.) was heated to reflux under nitrogen for 3 hours, then diluted with saturated sodium chloride solution and extracted with ethyl acetate (3x20ml.). The dried organic layer was concentrated under reduced pressure and the residue purified by preparative T.L.C. (8% methanol/chloroform; 2 elutions). The main components were isolated. The major more polar compound was the 5β-isomer which gave platelets (10mg.) (a yield of 40%), m.p. 154-7°C (from methanol/water), $[\alpha]_D^{27} + 41^\circ$ (c 0.216), $\nu_{\text{max.}}$ 3400(b) and 1648 cm$^{-1}$, $\lambda_{\text{max.}}$ 241 nm. ($\epsilon$ 10,000), m/e 448 (M$^+$, small, 430 (M$^+$-H$_2$O), 412 (M$^+$-2H$_2$O), and 300 (M$^+$-H$_2$O - side-chain). C.d. curve: $\Delta \epsilon$ +1.52 (223nm.), -3.27 (252nm.), and +1.08 (326nm.). O.r.d. curve: $[\phi]$ + 13,550 (238nm.) and -7,350 (267nm.) (Found: M$^+$-18;430. C$_{27}$H$_{44}$O$_5$-H$_2$O requires M-18;430).
The corresponding, less polar 5α-epimer (i.e. 25-deoxy-5α-isoecdysone) was a non-crystalline solid, (3mg.) (a yield of 12%), \( v_{\text{max.}} \) 3420 and 1650 cm\(^{-1}\), \( \lambda_{\text{max.}} \) 241 nm, m/e 448 (M\(^+\)), 430 (M\(^+\)-H\(_2\)O), 412 (M\(^+\)-2H\(_2\)O), and 300 (M\(^+\)-H\(_2\)O-side-chain). (Found: M\(^+\)-18, 430. \( \text{C}_{27}\text{H}_{44}\text{O}_{5}-\text{H}_{2}\text{O} \) requires M-18, 430).

2-Methylbut-3-yn-2-yl-Tetrahydropyranyl ether (29)\(^{22} \). - 2-methylbut-3-yn-2-ol (53) (8.4g.) and dihydropyran (9.2g.) were treated with toluene-p-sulphonic acid (4mg.) overnight at room temperature. Excess of dihydropyran was removed under a stream of nitrogen and the product immediately distilled under reduced pressure. The main fraction, b.p.t. 57°C/3.5 mm Hg (13.0g.), was the ether (29), \( N_{D}^{24} \) 1.4459, \( v_{\text{max.}} \) 3320, 1240, 1160, 1130, 1080 and 1040 cm\(^{-1}\), \( \tau \) 4.97 (1H, t) (proton \( \alpha \) to both ether linkages), 6.23 (1H, t), 6.55 (1H, t) (two protons \( \alpha \) to pyran ether oxygen), 7.68 (1H, s) (acetylenic proton), 8.4 (6H, b), 8.52 (3H, s) and 8.56 (3H, s). The yield was 77.4%.

**Estimation of methyl lithium.** - The ethereal lithium methyl solution (2ml.) was transferred via a syringe into water (ca. 20ml.), phenolphthalein (2 drops) added and titrated against N/10 hydrochloric acid (30.2ml. required).

A second aliquot (2ml.) was added via a syringe to dried methylene dichloride (20ml.). After shaking well, water and phenolphthalein were added, and a second titration carried out (11.4ml. required).
Methyl lithium (2ml.) is therefore equivalent to 18.8ml. N hydrochloric acid, i.e. it is 0.94N.

(22S)- and (22R)-22-hydroxy-6-(3'-methylbut-1-yl)-3-yl tetrahydropyranyl ether)-25-(tetrahydroxypan-2'-yloxyl)-3a,5a-cyclocholest-6,8(14)-dien-23-yl. (54a and b). - In a first attempt to prepare the acetylenic alcohols (55), the freshly redistilled THP ether (29) (1.5g.) in sodium-dried ether (15ml.) was stirred under nitrogen and ethereal lithium methyl (1.1M; 7.5ml.) introduced by injection through a serum cap. After 30 minutes, the solution (6 equivalents) was added to the pure aldehyde (26) (426mg.) in dry, freshly distilled THF (30ml.). After 20 minutes at room temperature, the reaction was worked-up in the normal manner, and the residue separated by P.L.C. (8% acetone/petrol; 3 elutions). The more polar, 22S-product (54a) (90mg.) (a yield of 11%) was a non-crystalline solid, \( \nu_{\text{max.}} \) 3450, 1615, 1250, 1220, 1160, 1130, 1080, 1040 and 1000cm.\(^{-1}\), \( \lambda_{\text{max.}} \) 256nm. (\( \epsilon \) 20,800) and 276nm. (\( \epsilon \) 12,500), \( \tau \) 4.02 (1H,bs), 5.0 (1H,dt) and 5.5 (1H,dt) (protons \( \alpha \) to both ether linkages), 6.16 (2H,b) and 6.53 (2H,b) (methylene \( \alpha \) to the pyranyl ether oxygens) 8.53 (12H,m) (methyls \( \alpha \) to the protected alcohols), 8.4, 8.87, 8.97, 9.07, 9.23 and 9.35, m/e 644 (M\(^+\), very small), 559, 475, 391 and 308 (loss of two acetylenic alcohol and two dihydroxyran units).

The less polar 22R-alcohol was also a non-crystalline solid (54b) (90mg.) (a yield of 11%), \( \nu_{\text{max.}} \) as for (54a), \( \lambda_{\text{max.}} \) 258nm. (\( \epsilon \) 28,300) and 277nm. (\( \epsilon \) 14,700), \( \tau \) 4.03 (1H,bs), 5.0 (1H,t), 5.48 (1H,t), 6.16 (2H,b), 6.56 (2H,b), 8.52 (6H,s), 8.54 (6H,s), 8.4 (12H,m), 8.75, 8.83, 8.90, 9.10, 9.23 and 9.37, m/e 644 (M\(^+\), very small), 559, 475, 391 and 308 (loss of two acetylenic alcohol and two dihydroxyran units).
22 \( \xi \) -Hydroxy-25-(tetrahydropyran-2-yloxy)-3\( \alpha \),5\( \alpha \)-cyclocholest-7-en-23-\( \nu \)-6-ones (55). Since in the first experiment the bis-adducts were obtained, the aldehyde (26) (640mg.) in dry, redistilled THF (30ml.) was stirred at room temperature and treated with the lithium salt of the acetylenic ether (29) (less than 2 equivalents) (made from the ether (0.8g.) in dry ether (12ml.) and methyl lithium (1.1M; 3.6ml.) by stirring for 0.5 hours) for 15 minutes. The usual work-up gave rise to a product which was purified by P.L.C. (10% acetone/petrol; 6 elutions), to give a non-crystalline white solid, which was an inseparable mixture of 22-epimers (55) (488mg.), \( \nu_{\text{max.}} \) 3450, 1650, 1260, 1170, 1135, 1080 and 1040 cm\(^{-1} \), \( \lambda_{\text{max.}} \) 241.5 nm., \( \tau \) 4.22 (1H, dd), 4.73 (1H, m), 4.97 (1H, bt), 5.53 (1H, t), 6.16 (1H, b), 6.47 (1H, b), 8.48 (3H, s), 8.52 (3H, s), 8.61, 8.91, 9.30 and 9.36, m/e 494 (M\(^+ \)), 410 (M\(^+ \) - dihydropyran), 326 (410-acetylenic alcohol), and 297 (M\(^+ \) - side-chain from C-20) (Found: C, 75.6; H, 9.3. \( \text{C}_{32}\text{H}_{46}\text{O}_{4}\cdot\text{H}_{2}\text{O} \) requires C, 75.0; H, 9.4%). The yield with regard to aldehyde was 50.3%.

22 \( \xi \) -Hydroxy-25-(tetrahydropyran-2-yloxy)-3\( \alpha \),5\( \alpha \)-cyclocholest-7-en-6-ones (56). In a typical experiment, the THP ether (55) (60mg.) was hydrogenated over prereduced 5% palladium-charcoal (20mg.) in purified, dry ethyl acetate (12.5ml.) containing triethylamine (2 drops) until the uptake of hydrogen ceased (ca. 5ml. in 3 hours). The catalyst was filtered off, and the solvent removed under reduced pressure. Preparative T.L.C. of the residue (54mg.) gave an inseparable mixture of 22-epimers (56) (45mg.) as a non-crystalline solid, \( \nu_{\text{max.}} \) 3470, 1648, and ether bands at 1200-1000 cm\(^{-1} \), \( \tau \) 4.21
(1H,dd) (C-7 vinylic proton), 5.28 (1H,dt) (proton α to both ether linkages), 6.16 (1H,b) and 6.42 (1H,b) (protons α pyran ether linkage), 8.74 (3H,s), 8.75 (3H,s), 8.91, 8.97, 9.08 and 9.31 (3H,s), m/e 498 (M⁺; only a trace), 414 (M⁺-dihydropyran), 298 (M⁺-side-chain from C-20) and 243. (55) was isolated in 75% yield.

(22S)-and (22R)-22,25-Dihydroxy-3α,5α-cyclocholest-7-en-6-one (57a and b). - The ether mixture (56) (40mg.) was dissolved in N/20 methanolic hydrochloric acid (5ml.) (made by adding concentrated hydrochloric acid (11.5M; 0.1ml.) to dry methanol (23ml.)). After 10 minutes, the reaction was quenched with saturated aqueous sodium chloride, extracted with ether (3x20ml.), the combined extracts washed with water, dried and evaporated. The residue was separated with difficulty by preparative T.L.C. (25% acetone/petrol; 4 elutions). The major, more polar component was the 22S-epimer (57a) (15mg.), which crystallised as needles, m.pt. 190-5°C (from ethyl acetate/petrol), [α]D27 + 82°(c 0.425), νmax. 3500, 3370, and 1638cm.⁻¹, λmax. 247nm. (c 11,900), τ 4.28 (1H, t, J 2.5Hz), 6.4 (1H,b), 8.80 (6H,s) (C-26 and C-27 methyl protons), 8.96, 9.06, 9.12 and 9.37 (3H,s) (C-18 methyl protons), m/e 414 (M⁺, small), 396 (M⁺-H₂O), 381 (396-Me), 327 (M⁺-side-chain from C-22), 298 (M⁺-side-chain from C-20) (Found: C, 77.9; H, 10.5. C₂₇H₄₂O₃ requires C, 78.2; H, 10.2%). The 22S-epimer was obtained in 45% yield.

The less polar component was the 22R-epimer (57b) (10mg.), which after stringent purification crystallised as needles, m.pt. 188.5-191°C (from ethyl acetate), [α]D21 + 90.5°(c 0.178), νmax. 3460, 3390, 1645 and 1615cm.⁻¹, λmax. 245.5nm. (c 12,500),
The 22R-epimer was produced in 30% yield.

(22R)-and (22S)-2β,3β-Diacetoxy-14α,22-dihydroxy-25-(tetrahydro-
pyran-2-yloxy)-5α-cholesten-23-yn-6-ones (34a and b). The aldehyde (27) (380mg.) in dry THF (25m1.) was treated dropwise at -30°C with the lithium salt of the acetylenic ether (29) (less than four equivalents) (preparation from (29) (1.3g.) and methyl lithium (1.67M; 1.9m1.) in dry ether (10m1.) under nitrogen). After 10 minutes, saturated aqueous ammonium chloride was added to the yellow solution, which was ether extracted (3x50m1.). The combined extracts were washed, dried and evaporated. The residue was separated into its components by P.L.C. (20% acetone/petrol; 3 elutions). In a typical experiment, there were two major components (34a and b), with some additional less polar material (discarded), and two more polar components (2β,3β-dihydroxy-(34) and a monoacetate diol of (34)).

The major 22R-product (third most polar; more polar than aldehyde (27)) (34a) (105mg.) was the same compound as that isolated by Feakins using the bromomagnesium salt of (29) on aldehyde (27) (mixed m.pt. and spectra). It crystallised (from ether) as needles, m.pt. 224-6°C, $[\alpha]_D^{24} + 71° (c 0.329), v_{max.} 3470, 1730, 1720, 1665, 1625, 1265, 1250 and 1200-1000cm.\textsuperscript{-1}, \lambda_{max.} 240nm. (e 14,600), \tau 4.14 (1H, d, J 2Hz) (C-7 vinylic proton), 4.80 (1H, m) (proton α 2β-acetate), 5.01 (1H, t) (proton α both ether linkages), 5.3 (1H, b) (proton α 3β-acetate)
5.55 (1H,d) (proton α 22-01), 6.18 (1H,b) and 6.58 (1H,b) (protons α pyran ether only), 8.01 (3H,s) and 8.06 (3H,s) (two acetate methyls), 8.53 (3H,s) and 8.57 (3H,s) (C-26 and C-27 methyls), 8.92, 8.98, 9.05 (3H,s) (C-19 methyl) and 9.36 (3H,s) (C-18 methyl), m/e 628 (M⁺, weak), 527 (M⁺-H₂O-dihydropyran), 512, 491 and 384 (M⁺-side-chain) (Found: C, 68.8; H, 8.1. C_{36}H_{52}O_{6} requires C, 68.8; H, 8.3%). The yield of (34a) was 20.2%.

The less polar of the two major products (less polar than aldehyde (27)) was the 22S-epimer (34b) (75mg.) (precursor of ecdysone). It was obtained as a non-crystalline foam, [α]$_D^{21}$ = 14° (c 0.236), ν$_{max}$. 3450, 1740, 1720, 1680, 1250 and 1200-1000cm.$^{-1}$, λ$_{max}$. 240.5nm. (ε 9,000), m/e 527, 512, 491 and 384 (M⁺-side-chain) (as for (34a)). (14.5% yield).

The most polar fraction was a non-crystalline mixture of the epimeric 22-0ls (75mg.), in which both acetate groups had been lost by attack of excess Grignard reagent during the reaction. The spectral data confirmed the structure as the 25-(tetrahydropyran-2-yloxy)-2β,3β,14α,22δ-tetrahydroxy-5α-cholest-7-en-23-yn-6-ones, ν$_{max}$. 3450, 1660 and 1200-1000cm.$^{-1}$, ν 4.17 (1H,d), 4.96 (2H,m) (1 proton α both ether linkages and one other), 5.54 (2H,b), 6.1 (b) and 6.5 (b) (methylene α pyran ether + others), 8.51 (3H,s) and 8.54 (3H,s) (C-26 and C-27 methyls), 8.76, 9.01 and 9.34.

The second most polar component was also a non-crystalline epimeric mixture (40mg.) of (34a and b) from which one acetate
had been lost by Grignard attack. The (34) monoacetate diol structure was confirmed by the spectral data, \( \nu_{\text{max}} \): 3450, 1720, 1670, 1265 and 1200-1000 cm\(^{-1} \), \( \tau \): 4.18 (1H,d), 4.96 (2H,m), 5.55 (1H,m), 6.14 (b) and 6.48 (b), 7.87 (3H,s), 8.50 (3H,s), 8.53 (3H,s), 8.76 and 9.42.

Using six equivalents of the lithium salt of (29), as in the first attempts to prepare (34a and b), a control experiment was carried out, in which 3\( \beta \)-acetoxyl-cholest-5-ene (70mg.) was stirred with the Grignard reagent in dry THF (5ml.) for ten minutes. Work-up was in the usual way. A T.L.C. examination of the residue showed that approximately 75\% of the cholesteryl acetate had been converted to cholest-5-en-3\( \beta \)-ol.

(22S)-2\( \beta \),3\( \beta \)-Diaceotyloxy-14\( \alpha \),22,25-trihydroxy-5\( \alpha \)-cholest-7-en-6-one (58). - The acetylenic diol (34a) (96mg.) was hydrogenated in ethyl acetate (16ml.) containing triethylamine (0.1ml.) over 5\% palladium-charcoal (30mg.). After 4 hours (hydrogen uptake 8.4ml. at S.T.P.) the mixture was filtered, and the solution evaporated. The residue (90mg.) was treated with methanolic hydrochloric acid (0.05N; 5ml.) at room temperature for 10 minutes, poured into saturated aqueous sodium hydrogen carbonate and extracted with ether (3x20ml.). The crude product was purified by P.L.C. (30\% acetone/petrol; 3 elutions) to give the triol (58) (50mg.) as needles (from ether/petrol), m.pt. 241-243\(^\circ \), \([\alpha]_D^{24} + 56\(^\circ\) (c 0.305), \( \nu_{\text{max}} \): 3480, 3400, 1730, 1720, 1645 and 1245 cm\(^{-1} \), \( \lambda_{\text{max}} \): 240nm. (c 10,800), \( \tau \): 4.10 (1H, d, J 2Hz), 4.73 (1H,m), 5.15 (1H,m), 7.94 (3H,s), 8.00 (3H,s), 8.77 (6H,s), 9.01 (3H,s), 9.09 (3H,d), and 9.22 (3H,s), m/e 548 (M\(^+\), weak), 530 (M\(^+\)-H\(_2\)O), 512 (M\(^+\)-2H\(_2\)O), 470 (530-AcOH),
432 (M⁺-side-chain from C-20), 414 (432-H₂O), 384 (M⁺-side-chain) (Found: C, 67.6; H, 8.6. C₃₁H₄₈O₈ requires C, 67.9; H, 8.8%). The desired product was obtained in a yield of 59.7%.

(22R)-2β,3β-Diacetoxy-14,22,25-trihydroxy-5α-cholest-7, cis-23-dien-6-one (59)¹. In an initial experiment to synthesise (58), the acetylenic diol (34a) (50mg.) was hydrogenated as already described over prereduced 5% palladium-charcoal (20mg.) in ethyl acetate (10ml.). After the uptake of 1 mol. of hydrogen (2ml. at S.T.P.), the mixture was filtered, the filtrate evaporated, and the tetrahydropyranyl group removed with methanolic hydrochloric acid (0.05N; 2.5ml.) as before. Purification by P.L.C. gave the hydrogenolysis product (50) (5mg.), the tetrahydrogenated compound (58) (10mg.) and a material of intermediate polarity, which proved to be the cis-23-dehydro-compound (59) (15mg.). Crystallisation from methanol or ether gave needles, m.pt. 226-231°C, [α]D²8 +60° (c 0.359), νmax. 3450, 1725, 1720, 1665, 1265 and 1240 cm⁻¹, λmax. 241nm. (ε 10,300), m/e 528 (M⁺-H₂O), 510 (M⁺-2H₂O), 492 (M⁺-3H₂O), 384 (M⁺-side-chain), 115 and 97. (Found: C, 63.7; H, 8.5. C₃₁H₄₄O₈·2H₂O requires C, 63.9; H, 8.6%). The 23-dehydro-compound was isolated in 34.5% yield.

(22S)-2β,3β,14α,22,25-Pentahydroxy-5α-cholest-7-en-6-one (35) (22-Isomexylsone) and the 5α-epimer. The diacetate (58) (45mg.) in methanol (6ml.) containing aqueous potassium carbonate (0.3%; 0.6ml.) was heated to reflux for 3 hours, diluted with saturated sodium chloride solution and extracted with ethyl acetate (3x20ml.). The dried organic layer was concentrated under reduced pressure and the residue purified
by P.L.C. (10% methanol/chloroform; 3 elutions). Two components were isolated in a ratio of 6:4 in favour of the more polar compound. This proved to be the 5β-epimer of 22-isoecdysone (35), which crystallised from water as needles (20mg.), m.pt. 241-4°C, v<sub>max</sub>. 3450-3200 and 1642 cm<sup>-1</sup>, λ<sub>max</sub>. 243.5 nm (ε 10,500), 446 (M<sup>+</sup>-H<sub>2</sub>O), 428 (M<sup>+</sup>-2H<sub>2</sub>O), 410 (M<sup>+</sup>-3H<sub>2</sub>O), 330 (446-side-chain from C-20), 99 and 81. (Found: C, 67.4; H, 9.7. C<sub>27</sub>H<sub>44</sub>O<sub>6</sub>·2H<sub>2</sub>O requires C, 67.2; H, 9.6%). Its properties were found to be identical with those of an authentic sample of 22-isoecdysone (T.L.C. in a variety of solvent systems, mass spectra and c.d. curves). C.d. curve: Δε +2.49 (1.99 nm.), +2.28 (223 nm.), -3.62 (249 nm.) and +1.76 (327 nm.). The product yield was 52.5%.

The less polar isomer was the 5α-epimer of 22-isoecdysone (13mg.), which crystallised as platelets (from methanol/water), m.pt. 243-5°C, v<sub>max</sub>. 3500-3400 and 1655 cm<sup>-1</sup>, m/e 446 (M<sup>+</sup>-H<sub>2</sub>O), 428 (M<sup>+</sup>-2H<sub>2</sub>O), 410 (M<sup>+</sup>-3H<sub>2</sub>O), 330 (446-side-chain from C-20), 99 and 81. (Found: C, 65.0; H, 9.6. C<sub>27</sub>H<sub>44</sub>O<sub>6</sub>·2H<sub>2</sub>O requires C, 64.8; H, 9.66%). Its properties were in fact identical with those of an authentic sample of 5α,22-isoecdysone. It was obtained in 35% yield.

Both (35) and the 5α-epimer, when exposed to equilibration conditions, gave the 5α- and 5β-epimers in the same ratio as did the authentic samples.

The overall yields based on ergosterol were:
- 5β-isoecdysone 0.26%
- 5α-isoecdysone 0.17%
(22R)-2β,3β-Diacetoxy-14α,22,25-trihydroxy-5α-cholest-7-en-6-one (60). - The THP ether (34b) (75mg.) was reduced as before to give the tetrahydrogenated product (65mg.), τ 4.2 (1H, d), 4.38-4.76, 5.14, 5.32, 5.76, 6.12 and 6.5, 7.98 (3H, s), 8.05 (3H, s), 8.78 (6H, s) (upfield movement from (34b)), 8.83, 8.93 and 9.02. Removal of the THP protecting group as previously described gave the crystalline diacetate (60) (27mg.), as needles (from methanol), m.p. 213–6°C, [α]_D^23 + 35° (c 0.200), ν_max. 3420, 1735, 1720, 1675 and 1250cm.⁻¹, λ_max. 241mm. (c 9,000), m/e 548 (M⁺, weak), 530 (M⁺-H₂O), 512 (M⁺-2H₂O), 99 and 81, with a small peak at m/e 528 corresponding to a trace of (M⁺-H₂O) of the 23-dehydro-product. (Found: C, 65.1; H, 9.5%; M⁺-2H₂O, 512. C₃₁H₄₈₀₈.H₂O requires C, 65.7; H, 8.9%; M-2H₂O. 512). The diacetate (60) was isolated in 41.3% yield.

(22R)-2β,3β,14α,22,25-Pentahydroxy-5β-cholest-7-en-6-one (2) (5β-ecdysone) and the 5α-epimer. - As described before, the diacetate (60) (27mg.) in methanol (8ml.) was hydrolysed and equilibrated with mild base. P.L.C. (10% methanol/chloroform; 3 elutions) afforded two compounds. The more polar (7mg.) had T.L.C. properties identical with those of an authentic sample of ecdysone. However, its mass spectrum showed, besides the expected peaks at m/e 446 (M⁺-H₂O), 428 (M⁺-2H₂O), 99 and 81, small peaks at m/e 462, 444 and 426, which disappeared when the material was rehydrogenated in methanol containing a trace of triethylamine. The product (7mg.) was then identical to the authentic sample of 5β-ecdysone, m.p. (from water) 236-9°C (decomp.), (mixed m.p., i.r. spectra, and c.d. curves). A yield of 30.4% was obtained.
C.d. curve: Δε +1.90 (196 nm.), +1.21 (227 nm.), −5.34 (256 nm.) and +1.21 (338 nm.).

The less polar material (3 mg.) from the equilibration step was identical with an authentic sample of 5α-ecdysone (T.I.C. properties in various solvent systems and mass spectral fragmentation pattern (m/e 464 (M⁺, weak), 446 (M⁺-H₂O), 428 (M⁺-2H₂O), 99 and 81). It was obtained in a yield of 13%.

Equilibration of either of the synthetic samples of 5α- or 5β-ecdysone with base gave the same mixture as did the authentic, reference sample.

The overall yields based on ergosterol were:
5β-ecdysone 0.074%
5α-ecdysone 0.032%.

The most polar products of the Grignard reaction (25-(tetrahydropyran-2-ylxyloxy)-2β,3β,14α,22β-tetrahydroxy-5α-cholest-7-en-23-yn-6-one) (75 mg.), contained no acetate functions. Hydrogenation as usual followed by mild acid removal of the protecting group furnished a mixture, which served as a direct, supplementary source of 5α-ecdysone and 5α,22-isoecdysone.

The monoacetate diol byproduct was taken through the usual sequence to yield the 5α- and 5β-epimers of ecdysone and 22-isoecdysone.
(22R), (23S)-23-Acetoxy-22-bromo-3,5α-cycloergosta-7-en-6-one (66c); (22S), (23R)-22-Acetoxy-23-bromo-3α,5α-cycloergosta-7-en-6-one (66b); and (22R), (23S)-22-Acetoxy-23-bromo-3α,5α-cycloergosta-7-en-6-one (66a)\[53,107.\]

- N-bromosuccinimide (500mg.; recrystallised) was added in portions over 10 minutes to a magnetically stirred solution of the cyclopropyl ketone (5) (1.0g.) in THF/water (4:1; 100ml.) at 0°C under nitrogen. After two hours, THF was removed under reduced pressure below 40°C, the reaction mixture poured into ice-water (150ml.) and extracted with benzene (3x50ml.). The combined extracts were dried and evaporated under reduced pressure below 40°C.

The crude product was dissolved in dry pyridine (10ml.) and acetic anhydride (5ml.) and left overnight. Work-up was by pouring into ice-water, benzene extraction (3x50ml.) and the combined extracts washed pyridine-free with dilute hydrochloric acid, acid-free with water and dried. Evaporation under reduced pressure gave an oily residue, which was separated by P.L.C. (5% acetone/petrol; 5 elutions). The major, least polar bromoacetate product (66c) (500mg.) was obtained pure directly, and crystallised as long needles (from methanol), m.pt. 166-9°C, \([\alpha]_D^{24} + 42^\circ (c 0.487, \text{ chloroform}), v_{max.} 1735, 1650 and 1230cm.^{-1}, \lambda_{max.} 246.5nm. (c 12,000), \tau 4.25 (1H,t) (C-7 vinylic proton), 4.63 (1H,dd, J 11Hz) (proton α acetate), 5.88 (1H,dd, J 11Hz) (proton α iodide), 7.98 (3H,s) (acetate methyl), 8.76, 8.93, 9.01, 9.08, 9.15 and 9.32, m/e 534 and 532 (M\(^+\)), 519 and 517 (M\(^+\)-Me), 394 (M\(^+\)-Br/OAc), 393 (M\(^+\)-H/Br/OAc), 392 (M\(^+\)-HBr/AcOH), 269 (M\(^+\)-side-chain) and 267. (Found: C, 67.7; H, 8.2. C\(_{30}\)H\(_{45}\)O\(_3\)Br requires C, 67.5; H, 8.4%). The yield was 37%.
Further P.L.C. purification of the more polar material allowed bromoacetate-II (66b) (280mg.) to be isolated, and was crystallised as needles (from methanol), m.pt. 146.5-148.5°C, $[\alpha]_D + 43^\circ$ (c 0.396, chloroform), $\nu_{\text{max.}}$ 1725, 1650, 1620 and 1240 cm.$^{-1}$, $\nu_{\text{max.}}$ 246.5nm. (c 11,600), $\tau$ 4.10 (1H, t), 4.7 (1H, dd, J 12Hz), 5.92 (1H, dd, J 12Hz), 7.99 (3H, s), 8.76, 8.93, 8.98, 9.01, 9.05, 9.10 and 9.20, m/e 534 and 532, 519 and 517, 394, 393, 392, 269 and 267. (Found: C, 67.4; H 8.3. C$_{30}$H$_{45}$O$_3$Br requires C, 67.5; H, 8.4%). The yield was 21%. 

The most polar bromoacetate (66a) (60mg.) required further preparative T.L.C. purification to allow crystallisation as microplatelets (from methanol), m.pt. 152-3°C, $[\alpha]_D^{25} + 36^\circ$ (c 0.098, chloroform), $\nu_{\text{max.}}$ 1725, 1650, 1620 and 1240 cm.$^{-1}$, $\nu_{\text{max.}}$ 246nm. (c 11,700), $\tau$ 4.28 (1H, t), 4.80 (1H, dd, J 10Hz), 5.77 (1H, dd, J 10Hz), 7.96 (3H, s), 8.78, 8.94, 9.06, 9.11 and 9.31, m/e 534 and 532, 519 and 517, 394, 393, 392, 269 and 267 (Found: C, 67.6; H, 8.6. C$_{30}$H$_{45}$O$_3$Br requires C, 67.5; H, 8.4%). The yield of (66a) was 9%. 

(22R),(23R)-22,23-Epoxy-3α,5α-cycloergosta-7-en-6-one (69) and (22S),(23S)-22,23-Epoxy-3α,5α-cycloergosta-7-en-6-one (70). The cyclopropyl ketone (5) (400mg.) was dissolved in the minimum amount of ether (20ml.) and an ethereal solution of monoperphthalic acid (6ml.; 3 equivalents; 100mg. per ml.) added. After allowing to stand overnight, work-up in the usual manner gave a crude product which could be separated into three components by P.L.C. (5% acetone/petrol; 4 elutions). The least polar component was recovered starting material (36mg.) (m.pt., mixed m.pt. and i.r. spectrum). The major less polar product was the (22R),(23R)-epoxide (69) (153mg.),
which crystallised from methanol as flat, white plates, m.p.t. 158-160°C, \([\alpha]_D^{27} = +74^\circ (c 0.453)\), \(\nu_{\text{max.}}\) 1650, 1625, 970 and 925-900 cm\(^{-1}\), \(\lambda_{\text{max.}}\) 244 nm. \((c 13,100)\), \(\tau 4.22\) (1H, t), 7.25 (2H, m), 8.92, 9.03, 9.15, 9.25 and 9.35 \((\text{Found: } C, 82.0; H, 10.4. \text{C}_{28}\text{H}_{42}\text{O}_2 \text{requires } C, 81.9; H, 10.3%)\). The product was obtained in a yield of 41%.

The more polar epoxide (70) (95mg.) also crystallised from methanol as rhomboid plates, m.p.t. 179-182°C, \([\alpha]_D^{24} = +40^\circ \) \((c 0.809, \text{ chloroform})\), \(\nu_{\text{max.}}\) 1655, 1625, 970, and 920-900 cm\(^{-1}\), \(\lambda_{\text{max.}}\) 244 nm. \((c 13,000)\), \(\tau 4.2\) (1H, t), 7.45 (2H, m), 8.92, 9.01, 9.13, 9.24 and 9.35 \((\text{Found: } C, 81.7; H, 10.3. \text{C}_{28}\text{H}_{42}\text{O}_2 \text{requires } C, 81.9; H, 10.3%)\). The most polar component was obtained in a 25% yield.

Correlation of Epoxides (69) and (70) with the bromohydrins, bromoacetates and the iodoacetate\(^{68}\) - The general procedure was to heat a methanolic solution of the bromohydrin or haloacetate under reflux under nitrogen with a mild base, and follow the reaction by T.L.C.

(a) The less polar bromohydrin fraction (2mg.) (prepared by purification of the crude bromohydrin product from NBS and the cyclopropyl ketone (5)) was treated as noted above with potassium acetate (2mg.) in methanol (2ml.). T.L.C. showed that the only epoxide product was (70), the more polar isomer.

(b) The more polar bromohydrin fraction (impure) under similar conditions gave mainly (70), with a little of the less polar epoxide (69).
(c) The iodoacetate (20) was resistant to potassium acetate treatment, but using potassium carbonate in an analogous procedure, only the more polar epoxide (70) was produced.

(d) Bromoacetate-III (5mg.) (66c) heated under reflux with potassium carbonate (0.3%) in methanol/water (9:1; 5ml.) under nitrogen. No change in Rf was observed by T.L.C., but isolation of the product showed it to be solely (70) (m.pt., mixed m.pt.).

(e) Identical treatment of bromoacetate-II (5mg.) (66b) also gave the more polar epoxide.

(f) Bromoacetate-I (2mg.) (66a) was treated as described above and gave rise only to the less polar epoxide (69).

**Equilibration of bromoacetates-III, -II, and -I and the iodoacetate.** - The general procedure was to immerse the haloacetate (1mg.), sealed in a capillary tube, in a bath, preheated to a constant temperature 5-10° below the melting point of the haloacetate, for 10 minutes.

(a) and (b) Bromoacetates-III (66c) and -II (66b) both gave mixtures containing equal quantities (by T.L.C.) of each other on undergoing this treatment. Extensive decomposition was also observed.

(c) Bromoacetate-I (66a) on the otherhand gave rise to an equilibrium mixture containing a new compound, less polar than the three known bromoacetates, which could only be the elusive bromoacetate-IV (66d). There was also extensive decomposition, which precluded isolation.
(d) (22R)(23S)-23-acetoxy-22-iodo-3α,5α-cycloergosta-7-en-6-one

Equilibration of the iodoacetate (20) under these conditions afforded a new, more polar material, as expected for the 23S-acetoxy-22R-iodo-compound (20). The iodoacetate (20) (40mg.) was equilibrated by heating under nitrogen at 145-150°C for 30 minutes. The most polar component, slightly more polar than (20), was isolated, to give the 22S-acetoxy-23R-iodo-compound (5mg.). m/e 580(M+), 520, 453, 394, 393, 392, 341, 297, 269, 267 (Found: M+ 580; requires M+ 580); microplatelets (from ether/petrol), m.pt. 133-143°C, max. 247nm. (ε 11,000), [α] D +42° (c 0.106, chloroform). The yield was 12.5%.

Attempted preparation of 23S-acetoxy, 22S-bromo-3α,5α-cycloergosta-7-en-6-one (66d). - The less polar epoxide (69) (60mg.) was dissolved in glacial acetic acid (2ml.) and hydrobromic acid (45% in glacial acetic; 25mg.) added until T.L.C. showed reaction to be complete. After stirring for several hours, the reaction mixture was evaporated under reduced pressure and acetylated with pyridine/acetic anhydride. The usual work-up gave a crude product, which was a complex mixture with little or no material at the Rf expected for bromoacetate-IV. This route was abandoned, since it appeared that the cyclopropyl ring was undergoing attack in addition to the side-chain epoxide.

Attempted Isomerisation of the Epoxides (69) and (70). - The more polar epoxide (70) (100mg.) in DMSO (3.5ml.) was stirred magnetically and sodium iodide (184mg.) and n-propyl iodide (208mg.) were added. After 3 hours at 80°C, the brown reaction mixture was poured into water (25ml.) and extracted with benzene (3x25ml.). The extracts were washed with sodium thiosulphate solution and with water, dried and evaporated under reduced pressure. T.L.C. showed that the starting material had been unaffected by the reaction conditions.
Under similar conditions, the less polar epoxide (69) was slightly more reactive, but after 48 hours reaction was still incomplete and the reaction mixture contained many products, including those due to double bond isomerisation. Again these experiments were abandoned.

(22R), (23R)-22,23-epoxy-3α,5α-cycloergosta-7-en-6α-ol (72) and (22R), (23R)-22,23-epoxy-3α,5α-cycloergosta-6,8,(14)-diene (71).

The less polar epoxide (69) (200mg.) was stirred in dry ether (25ml.) with LAH (65mg.; 3 equivalents). After 45 minutes, the excess LAH was destroyed with ethyl acetate, and saturated ammonium chloride solution added. The aqueous layer was extracted with ether (2x20ml.), the extracts combined with the original ether layer, washed with water, dried and evaporated. The residue was crystallisable from methanol, but was purified by P.L.C. (20% acetone/petrol) as a precaution. The allylic alcohol (72) (170mg.) crystallised as large chunks (from methanol), m.pt. 159-161°C, [α] D 28 + 73° (c 0.471), v max. 3475, 1240 and 910 cm. -1, τ 4.77 (1H,m) (C-7 vinylic proton), 5.63 (1H,m) (C-6 allylic proton), 7.33 (2H,m) (protons α epoxide), 7.60 (1H,b), 8.98, 9.04, 9.09, 9.13 and 9.42 (3H,s) (C-18 methyl) (Found: C, 81.6; H, 10.7. C 28H 44 O 2 requires C, 81.5; H, 10.75%). The 6α-ol was obtained in an 85% yield.

The alcohol (72) (85mg.) in ether (10ml.) was treated with glacial acetic acid (2ml.) for 15 minutes. The reaction mixture was then washed with water, dried and evaporated. P.L.C. of the crude residue (5% acetone/petrol) gave the diene (71) (55mg.) as needles (from chloroform/methanol),
m.p.t. 104-6°C, $[\alpha]_{D}^{26}$ +162° (c 0.657; chloroform), $\nu_{\text{max.}}$ 925 and 910cm. $^{-1}$, $\lambda_{\text{max.}}$ 259.5nm. (c 22,500) and 251.5nm. (c 21,000), $\tau$ 3.83 (1H, "d", $J$ 10Hz) (C-7 vinyl proton), 4.78 (1H, $d$, $J$ 10Hz) (C-6 allylic proton), 7.3 (2H, m), 8.85, 8.95, 9.02, 9.10, 9.20 and 9.35 (Found: C, 85.4; H, 10.7. C$_{28}$H$_{42}$O requires C, 85.2; H, 10.7%). The yield with respect to the cyclopropyl ketone (5) was 55%.

(22S),(23S)-22,23-Epoxy-3α,5α-cycloergosta-7-en-6β-ol (74) and (22S),(23S)-22,23-epoxy-3α,5α-cycloergosta-6,8(14)-diene (73).

The more polar epoxide (70) (200mg.) was reduced as described above and worked up with ethyl acetate and saturated aqueous ammonium chloride. P.L.C. of the crude residue from evaporation (20% acetone/petrol) gave the allylic alcohol (74) (165mg.) as chunks (from methanol), m.p.t. 166-170°C, $[\alpha]_{D}^{26}$ +89° (c 0.352), $\nu_{\text{max.}}$ 3400 and 920cm. $^{-1}$, $\tau$ 4.82 (1H,dd), 5.7 (1H,b), 7.55 (2H,m), 8.94, 9.01, 9.06, 9.12 and 9.14 (Found: C, 81.6; H, 10.7. C$_{28}$H$_{44}$O$_2$ requires C, 81.5; H, 10.75%). The yield was 82%.

Mild acid treatment of the alcohol (74) (85mg.) as described previously resulted, after P.L.C. (5% acetone/petrol), in the isolation of the diene (73) (70mg.) as needles (from chloroform/methanol), m.p.t. 133-6°C, $[\alpha]_{D}^{27}$ +174°(c 0.895, chloroform), $\nu_{\text{max.}}$ 1610, 925 and 920cm. $^{-1}$, $\lambda_{\text{max.}}$ 260nm. (c 17,000) and 252nm. (c 16,100), $\tau$ 3.88 (1H; $d$, $J$ 11Hz), 4.83 (1H, $d$, $J$ 11Hz), 7.6 (2H,m), 8.98, 9.04, 9.07, 9.12, 9.23 and 9.40 (Found: C, 85.2; H, 10.6. C$_{28}$H$_{42}$O requires C, 85.2; H, 10.7%). The yield was 70%.
3α,5α-cycloergosta-22-en-6-one (75)\textsuperscript{70} and 5α-ergosta-22-en-6-one (76). - The literature method\textsuperscript{70} was used to prepare (75). The cyclopropyl ketone (5) (1.72g.) in dry ether (60ml.) was added to lithium metal (0.1g.) in liquid ammonia (35ml.) with vigorous stirring. After 2 minutes, saturated ammonium chloride solution was added to destroy excess lithium. The layers were separated, and the aqueous layer extracted with ether (2x40ml.). The ether portions were combined, washed with saturated aqueous sodium chloride, dried (over potassium carbonate) and the solvent removed. Chromatography of the product on alumina (grade III, 110g.) and elution with benzene/petrol mixtures (increasing from 5-15\%) allowed the isolation of the required compound (75) as the major product (1.42g.). Crystallisation from methanol gave needles, m.pt. 114-7°C (lit.\textsuperscript{70}, 108-110°C), \(\nu_{\text{max}}\), 1690 cm.\(^{-1}\), \(\tau\) 4.83 (2H,m), 8.93, 9.00, 9.03, 9.13, 9.22, 9.26 and 9.33. (75) was obtained in 82\% of the theoretical yield.

A slightly less polar byproduct, not previously reported, was also isolated. This proved to be 5α-ergosta-22-en-6-one (76) (100mg.), obtained as platelets (from methanol) m.pt. 116.5-119°C, \([\alpha]\)\textsubscript{D}\textsuperscript{29} \(-35°\) (c 0.540), \(\nu_{\text{max}}\), 1710 cm.\(^{-1}\), \(\tau\) 4.83 (2H,m); 8.94, 9.05, 9.13, 9.20, 9.27 and 9.32 (Found: C, 84.5; H, 11.6. \(\text{C}_{28}\text{H}_{46}\text{O}\) requires C, 84.35; H, 11.6%). The yield was 6\%.

(22R),(23R)-22,23-Epoxy-3α,5α-cycloergosta-6-one; (22S),(23S)-22,23-epoxy-3α,5α-cycloergosta-6-one; (22R),(23R)-23,24-epoxy-6-oxa-B-homo-3α,5α-cycloergosta-7-one (77); and (22S),(23S)-23,24-epoxy-6-oxa-B-homo-3α,5α-cycloergosta-7-one (78). - The 7,8-dihydrocyclopropyl ketone (75) (500mg.) in
ether (10ml.) and an ethereal solution of monoperphthalic acid (excess; 7ml.; 100mg. per ml.) were left for 24 hours, and worked up as described previously. P.L.C. (3% acetone/petrol; 3 elutions) enabled the residue to be separated into five components, the least polar of which was recovered starting material (75) (50mg.) (m.pt. and mixed m.pt.).

The major product was the required less polar epoxide (138mg.), which crystallised as needles (from methanol), m.pt. 128-130°C, \([\alpha]_D^{29} + 27^\circ(c 0.661), v_{\text{max.}} 1690 and 915\text{cm.}^{-1},\tau 7.35 (2H,m), 8.87, 9.00, 9.07, 9.10 and 9.30 (\text{Found: } C, 81.6; H, 10.6. \text{C}_{28}H_{44}O_2 \text{ requires } C, 81.5; H, 10.75\%). A 29.7% yield was obtained.

The second major product was the required more polar epoxide (120mg.), which crystallised as chunks (from methanol), m.pt. 151-5°C, \([\alpha]_D^{26} - 18^\circ(c 0.497), v_{\text{max.}} 1685 and 920\text{cm.}^{-1},\tau 7.38 (2H,m), 9.00, 9.10 and 9.29 (\text{Found: } C, 81.5; H, 10.6. \text{C}_{28}H_{44}O_2 \text{ requires } C, 81.5; H, 10.75\%). The yield was 25.6%.

Of the two byproducts, the less polar one was the lactone epoxide (77) (70mg.), obtained as chunks (from methanol), m.pt. 145-7°C, \([\alpha]_D^{25} + 20^\circ(c 1.003, \text{chloroform}), v_{\text{max.}} 1740, 1260 and 922\text{cm.}^{-1},\tau 7.4 (2H,m), 8.54, 8.76, 8.93, 8.98, 9.03, 9.07, 9.10, 9.14 and 9.25 (\text{Found: } C, 78.3; H, 10.3. \text{C}_{28}H_{44}O_3 \text{ requires } C, 78.45; H, 10.35\%). The yield was 14.4%.

The more polar byproduct was the lactone epoxide (78) (70mg.), giving needles (from methanol), m.pt. 195.5-197.5°C, \([\alpha]_D^{26} - 44^\circ(c 0.629, \text{chloroform}), \tau 7.45, (2H,m), 8.54, 8.73, 8.92, 9.00, 9.10 and 9.26 (\text{Found: } C, 78.2; H, 10.2. \text{C}_{28}H_{44}O_3 \text{ requires } C, 78.45; H, 10.35\%). Again the yield was 14.4%.
Attempted dehalogenation of 23S-acetoxy-22R-iodo-3α,5α-cycloergosta-7-en-6-one (20). - (a) The iodoacetate (20) (50mg.) was dissolved in glacial acetic acid (5ml.) and zinc dust (100mg.) added. After 2 hours the reaction mixture was filtered, diluted with chloroform, washed acid-free with water, dried and evaporated under reduced pressure. Preparative T.L.C. (10% acetone/petrol) gave the elimination product, the cyclopropyl ketone (5) (31mg.) (m.p., mixed m.p., i.r. and n.m.r. spectra), in 91% yield.

(b) Three attempts were made to use catalytic hydrogenation by the normal procedure, in which the iodoacetate (80-100mg.) in the solvent (10-15ml.) was shaken with the catalyst (25-90mg.) in an atmosphere of hydrogen. The usual work-up was used in each case.

(i) 10% palladium charcoal in purified ethyl acetate. - Mainly unchanged starting material was recovered together with a non-crystalline product which appeared to be 7,8-dihydro-(20) (v_{max}. 1710cm.^{-1}).

(ii) 10% palladium-calcium carbonate in dry ethanol. - In addition to the products obtained in (i), the cyclopropyl ketone (5) was also produced.

(iii) 10% palladium-calcium carbonate in dry benzene. - The results were as for (i).

Since the Δ^7-double bond was being hydrogenated before attack on the iodine, these experiments were abandoned.

Chromous (II) Acetate^{80,81}. - The literature procedure was
used. Zinc turnings (17g.), chromic (III) chloride (10g.) in degassed water (12ml.) and concentrated hydrochloric acid (23ml.) in a separating funnel were maintained for 20 minutes under an atmosphere of carbon dioxide. The solution was run off through a glass wool plug into a solution of hydrated sodium acetate (27.5g.) in degassed water (36ml.) under carbon dioxide in another separating funnel. The precipitated chromous (II) acetate was rapidly filtered through a sintered-glass funnel under a stream of nitrogen. After washing with degassed water, degassed ethanol and finally with ether, the brick-red solid (5g.) was blown dry with nitrogen, crushed and sealed in ampoules under nitrogen.

3β-Acetoxy-5α-bromo-6β-hydroxycholestane (81a). - Cholest-5-en-3β-yl acetate (2g.) in dioxan (16ml.; redistilled from sodium wire) and aqueous perchloric acid (0.28M; 1ml.) was stirred vigorously in a dark flask at room temperature. N-bromoacetamide (1g.) was added in portions over 30 minutes, and stirred for a further 30 minutes. The reaction mixture was then cooled to 0°C, and water (8ml.) and aqueous sodium sulphite (10%; 12ml.) added. The product was extracted with ether (3x30ml.), the extracts combined, washed with water, dried and evaporated. Crystallisation from ether/light petrol (40-60°C) gave the bromohydrin (81a) (1.1g.), m.pt. 169-170°C (lit. 168, 172-4°C), \([\alpha]_D^{25} - 35^\circ (c 1.006, \text{chloroform})\) \(v_{\text{max.}}\) 3480, 1710 and 1280 cm\(^{-1}\). The product was obtained in 45% yield.
5α-Bromo-3β,6β-diacetoxycholestanate (81b). - The bromohydrin (81a) (1.0g.) was suspended in acetic anhydride (10g.) and dry benzene (20ml.) added to effect dissolution. Toluene p-sulphonic acid (3mg.) was used to catalyse the reaction, and the mixture was left at room temperature for 48 hours. The usual work-up produced a white solid, which gave the pure bromoacetate (81b) (1.03g.), as needles (from methanol), m.pt. 97-101°C (lit. 89-91°C), $[\alpha]_D^{21} = 63^\circ$ (c 0.598, chloroform), $\nu_{\text{max.}}$ 1740, 1240, 1225 and 750 cm.$^{-1}$, $\tau$ 2.66 (1H, b) (?), 4.3 (1H, very b) (proton $\alpha$ 3β-acetate), 4.6$\tau$ (1H, b, W 1/2 10Hz) (proton $\alpha$ 6β-acetate), 7.89 (3H, s) and 7.97 (3H, s) (acetate methyls), 8.70, 9.11, 9.18 and 9.30. A 95% yield was obtained.

Debromination of a model bromohydrin.

3β-Acetoxy-6β-hydroxy-5α-cholestanate (82). - Chromous acetate (2g.) was stirred under nitrogen and degassed, dry DMSO (24ml.) and n-butane thiol (3ml.) added. The finely powdered bromohydrin (81a) (1.02g.) was added in portions over 30 minutes at 40-45°C. The reaction mixture was stirred for a further 30 minutes after the last addition, poured into hydrochloric acid (1N; 180ml.) and extracted with ether (3x50ml.). The combined extracts were washed acid-free with water, dried and evaporated. Final evacuation at the oil pump gave a crystalline solid. The required product (82) (725mg.) was obtained as platelets (from benzene/petrol), m.pt. 158-159°C, $[\alpha]_D^{24} = -6^\circ$ (c 0.467, chloroform), (lit. 82, m.pt. 156-7°C, $[\alpha]_D - 6^\circ$ (chloroform)), $\nu_{\text{max.}}$ 3560, 1718 and 1258 cm.$^{-1}$, $\tau$ 5.23 (1H, very b), 6.14 (1H, b), 7.95 (3H, s),
8.94, 9.08, 9.16 and 9.28. The product was obtained in 85.6% yield.

Debromination of a model bromoacetate.

3β,6β-Diacetoxy-5α-cholestane (83). - The procedure was precisely as described for the preceding experiment, using however the finely powdered bromoacetate (81b) (1.1g.). Crystallisation of the crude solid product gave the required diacetate (83) (797mg.) as platelets (from methanol), m.pt. 137-8°C (lit. 85, 138°C), $\left[\alpha\right]_{D}^{24} = 21^\circ$ (c 0.600, chloroform), v max. 1735 and 1255cm.⁻¹, τ 5.03 (1H, b) (proton α 6β-acetate), 5.25 (1H, very b) (proton α 3β-acetate), 7.97 (3H, s), 8.00 (3H, s), 8.99, 9.11, 9.20 and 9.31. A yield of 88.6% was obtained.

The diacetate (83) was also synthesised by pyridine/acetic anhydride acetylation of the hydroxy-acetate (82) in the normal manner. The product was identical to that obtained by the debromination (m.pt., mixed m.pt.), except that the rotation was markedly different ($\left[\alpha\right]_{D}^{24} = 62^\circ$ (c 0.747, chloroform)). The yield by acetylation was 82%.

Attempted dehalogenation of the iodoacetate, bromoacetate-III and bromohydrins.

(1) The above procedure was carried out using the various substrates. In the case of the iodoacetate (20) and the bromoacetate-III (66c), starting material was recovered in high yield (90%) (m.pt., mixed m.pt., and n.m.r. spectrum). Similarly, the crude 22,23-bromohydrin mixture (added dropwise in dry, degassed THF) was mainly unaffected by these conditions, though some cyclopropyl ketone (5) was isolated,
arising from the alternative elimination process.

(ii) When a reaction temperature of 60-80°C was used, the bromoacetate-III (200mg.) gave rise to unreacted starting material (70mg.) and the cyclopropyl ketone (5) (90mg.) (a yield of 45%) (m.pt., mixed m.pt., spectra).

(iii) Experiments were also carried out on the iodoacetate (20), added in a solution of DMSO with and without an inert solvent (dioxan; THF), in which alternative active hydrogen donors were used (hypophosphorous acid; n-hexane thiol). Extended reaction times (24 hours or longer at room temperature) resulted in the isolation of the cyclopropyl ketone (5), recovered starting material and a new product, which proved to be a hydroxyacetate (one of the isomers of (21)), obtained as small needles (from methanol), m.pt. 197-200°C, $\left[a\right]_D^{28} + 160^\circ$ (c 0.225), $\nu_{\text{max}}$ 3400, 1725, 1640 and 1250 cm$^{-1}$, $\lambda_{\text{max}}$ 240 nm. (e 11,500), $\tau$ 4.05 (1H,dd), 4.86 (1H,b), 7.33 (1H,b), 7.97 (5H,s), 8.74, 8.92, 9.03, 9.13, 9.24 and 9.27, m/e 470 (M$^+$), 452 (M$^+$-H$_2$O), 442 (M$^+$-CO). A yield of about 10% was obtained.

Preparation and attempted dehalogenation of a model secondary bromohydrin.

(a) 3α-Bromo-2β-hydroxy-5α-cholestane$^{53}$. - 5α-Cholest-2-ene (500mg.) in THF/water (5:2; 35ml.) was treated with N-bromo-succinimide (300mg.) under nitrogen at 0°C as previously described for another compound (5). The crude product (760mg.) from the normal work-up was separated into its components by P.L.C.(5% acetone/petrol). The required bromohydrin (310mg.) gave crystals (from methanol), m.pt. 134-137°C (lit. $^{86}$, 135-136°C), $\nu_{\text{max}}$ 3450 cm$^{-1}$, $\tau$ 5.75 (2H,m) (protons α hydroxyl and α bromine), 8.98, 9.08, 9.17 and 9.34. A yield of 49%
was obtained.

(b) Several attempts were made to debrominate 3α-bromo-2β-hydroxy-5α-cholestane, using the general procedure, in which the bromohydrin (100mg.) in DMSO (1ml.) and dioxan (1ml.) was added at room temperature to a stirred solution of chromous acetate (350mg.) in DMSO (4ml.) containing an active hydrogen donor (e.g. hypophosphorous acid (0.25ml.)). After 24 hours, the normal work-up and P.L.C. allowed the isolation of three products, together with recovered starting material (30mg.). The least polar product (16mg.) was 5α-cholest-2-ene, which crystallised as needles (from acetone/ether), m.pt. 74-75°C (lit.108, 75-76°C) (a yield of 20%). The next least polar component, also produced in 20% yield, proved to be 2β,3β-epoxy-5α-cholestane (17mg.), obtained as needles (from methanol), m.pt. 85-87.5°C (lit.87, 89-91°C). The most polar product (slightly more polar than the original bromohydrin) was a non-crystalline material, which was probably impure 2β-hydroxy-5α-cholestane (8mg.) (a yield of 10%), τ 6.05 (1H,b), 8.77, 9.04, 9.10, 9.22 and 9.35.

(22R)-22-Bromo-3α,5α-cycloergosta-7-en-6,23-dione and (23R)-23-bromo-3α,5α-cycloergosta-7-en-6,22-dione. - In a series of experiments, the 22,23-bromohydrins of i-ergosta-7,22-dien-6-one (5) were used either as crude mixtures or after attempted purification. A slight excess of Jones' reagent (prepared by dissolving chromium trioxide (2.67g.) in concentrated sulphuric acid (2.3ml.) and making up the solution to 10ml. with water) was added to the bromohydrin substrate in 5% chloroform/acetone. P.L.C. and T.L.C. purifications were then carried out to isolate the bromoketones. The best results were obtained from oxidation of purified bromohydrin fractions, because of
the similarity of the Rfs of the bromoketone products.

The major bromoketone isolated from these experiments was the 22R-bromo-23-ketone, chunks (from acetone/methanol), m.pt. 197-201°C, $[\alpha]_D^{24} + 124^\circ$ (c 0.218, chloroform), $\nu_{\text{max.}}$ 1710 and 1650 cm.$^{-1}$, $\lambda_{\text{max.}}$ 242 nm. ($\epsilon$ 13.100), $\tau$ 4.28 (1H, t), 5.40 (1H, d, J 1.5 Hz), 7.30 (1H, m), 8.94, 9.03, 9.08, 9.16 and 9.28, m/e 490 and 488 ($M^+$), 409 ($M^+\text{-Br}$), 341, 325, 311, 297 ($M^+\text{-side-chain from C-20}$), 269 ($M^+\text{-side-chain}$) (Found: C, 68.6; H, 8.5. $C_{28}H_{41}O_2Br$ requires C, 68.7; H, 8.4%). The yield was 17%.

A slightly more polar product also obtained from these experiments was the 23R-bromo-22-ketone, which gave microcrystals (from benzene/methanol), m.pt. 182-5°C, $[\alpha]_D^{29} + 37^\circ$ (c 0.306, chloroform), $\nu_{\text{max.}}$ 1710 and 1655 cm.$^{-1}$, $\lambda_{\text{max.}}$ 246.5 nm. ($\epsilon$ 12,700), $\tau$ 4.26 (1H, t), 5.38 (1H, d, J 1.5 Hz), 7.32 (1H, m), 8.76, 8.93, 9.01, 9.06, 9.13 and 9.24, m/e 490 and 488, 409, 339, 325, 311, 297 and 269 (Found: C, 68.5; H, 8.4. $C_{28}H_{41}O_2Br$ requires C, 68.7; H, 8.4%). The yield was ca. 10%.

Other bromoketones were isolated but resisted purification.

**Debromination of bromoketones.** - Treatment of bromoketones of the type described above with zinc dust in glacial acetic acid for 2 hours at room temperature in the normal manner gave debrominated ketones, but it was found, at this stage, impossible to obtain the two possible ketones sufficiently pure to distinguish between them.
Tri-n-butyl tin hydride. - LAH (0.468g.; 12m.moles) and tri-n-butyl tin chloride (9.75g.; 30m.moles) were added to dry ether (45ml.) at 0°C. The mixture was stirred for 15 minutes at 0°C and at room temperature for 3 hours. Water (30ml.) was slowly added at 0°C, the ether layer separated, washed with ice-water (2x30ml.), dried (over magnesium sulphate) and the ether removed by distillation. The hydride was then distilled as rapidly as possible at oil-pump pressure, and the fraction, (5.2g.), b.pt. 75-80°C/0.4mm.Hg, collected. The liquid had $v_{\text{max}}$ 1800 and 1470cm$^{-1}$, $\tau$ 4.76 (1H,s), 8.6 (18H,m) and 9.07 (9H,t). The yield was 60%.

Preliminary dehalogenation experiments with tri-n-butyl tin hydride. - Following the literature procedure, the hydride (slight (5%) excess) was added to the iodoacetate (50mg.) (1) without solvent; (2) in benzene (1ml.); (3) in ether (3ml.); (4) in chloroform (0.5ml.); and (5) in bromobenzene (1ml.). An additional experiment used dry benzene (1ml.) under nitrogen for 60 hours. T.L.C. indicated in all cases that the iodoacetate was unaffected. Recovered starting material (87%) gave a positive Beilstein halogen test. A non-crystalline hydroxyacetate was also isolated (in 5% yield), but was more polar than that described previously. ($v_{\text{max}}$, 3500, 1725, 1645 and 1245cm$^{-1}$, $\tau$ 4.24 (1H,t), 5.0 (1H,b), 6.25 (1H,b), 7.97 (3H,s), 8.74, 8.92, 9.03, 9.10 and 9.35).

(23R)-23-Acetoxy-3α,5α-cycloergosta-7-en-6-one (84a). - The dehalogenated, required product (84a) was first obtained by an irreproducible process. The iodoacetate (20) (150mg.; recovered from the preceding work) in DMSO (5ml.) and THF (1ml.) was stirred under nitrogen and n-hexane thiol (1ml.),
ethanolamine (0.1ml.) and chromous acetate (500mg.) added. After 1 hour, T.L.C. indicated that no change had occurred (only later were the inadequacies of T.L.C. in this reaction fully appreciated). Subsequently, water (0.5ml.) was added and stirring continued overnight. Aqueous DMF (5ml.) and ethylene diamine (0.1ml.) were also added. After several days, the usual work-up afforded an oil, which slowly solidified. Crystallisation (from methanol) gave the required acetate (84a) (39mg.) (a yield of 33%), as needles, m.pt. 147-150°C, \([\alpha]_{D}^{25} 48^\circ (c 0.374), \nu_{\text{max}} 1720, 1655, 1620 \text{ and } 1255 \text{cm}^{\text{-1}}, \lambda_{\text{max}} 246 \text{nm.} (\epsilon 15,400), \tau 4.23 (1H, t), 4.93 (1H, b), 7.96 (3H, s), 8.91, 9.01, 9.12, 9.23 \text{ and } 9.32. \) (Found; C, 79.0; H, 10.0. C\(_{30}\)H\(_{46}\)O\(_{3}\) requires C, 79.2; H, 10.2%).

Attempts were made to repeat this success, using n.m.r. spectroscopy to follow the reaction, since product and starting material had identical R\(_f\)s. Repetition of the procedure in full or in part using crystalline samples of the iodoacetate, gave, at best, products containing two kinds of acetate and two kinds of acetate environment for protons, \((\tau 7.96 \text{ and } 7.99, \text{ and } 4.5 \text{ and } 4.9)\). It began to seem probable that the dehalogenation had been brought about by the experiment prior to complexed chromous ion treatment.

Bromoacetate-III (66c) (55mg.) in dry, redistilled THF (10ml.) under nitrogen was stirred at room temperature for 24 hours with a freshly prepared sample of tri-n-butyl tin hydride (65mg.). The reaction mixture was diluted with ether, washed with water (3x25ml.), dried and evaporated. Crystallisation from methanol gave the acetate (84a) (15mg.) as needles, m.pt. 149-150°C (mixed m.pt. with the sample
isolated previously 147-150°C), \([\alpha]_{D}^{25} + 66^\circ (0.466, \text{chloroform})\), 
\(\nu_{\text{max}}\) 1718, 1655 and 1256cm.\(^{-1}\), \(\lambda_{\text{max}}\) 246.5nm. (c 14,100),
\(\tau\) 4.28 (1H,t), 4.98 (1H,b), 8.00 (3H,s), 8.72, 8.80, 9.04, 9.10, 9.16 and 9.36, m/e 454 (M\(^+\)), 394 (M\(^+\)-AcOH), 341, 325, 311, 297, 296, 269, 267, 243, and 71. (Found: C, 79.0; H, 10.0. \(C_{30}H_{46}O_{3}\) requires C, 79.2; H, 10.2%). This experiment gave a 32\% yield, but on a larger scale (1.542g.),
the product (1.08g.) was obtained in an 82.3\% yield.

Similar treatment of the iodoacetate gave an identical acetate (84a) in equally good yield.

(23\text{R})-3\alpha,5\alpha-cycloergosta-7-en-6\alpha,23-diol (85); (23\text{R})-23-
hydroxy-3\alpha,5\alpha-cycloergosta-7-en-6-one (84b); and (23\text{R})-23-
hydroxy-3\alpha,5\alpha-cycloergosta-6,8(14)-diene (86a).

(a) Removal of the acetate from (84a) by mild alkaline hydrolysis (methanolic potassium carbonate or hydroxide at reflux temperature) gave rise to several products, and a more satisfactory, alternative approach was used.

(b) As previously described for the reduction of \(\text{-cholesterol-7-en-6,22-dione (44)}\), a two-stage process of LAH reduction in dry ether, followed by manganese dioxide oxidation in chloroform, was employed.

Treatment of the acetate (84a) (99mg.) with LAH (36mg.) in dry ether (5ml.) resulted in sequential reduction, first of the 6-ketone, and then of the acetate function. Work-up in the normal manner gave the diol (85) (90mg.), which gave microcrystals (from methanol), m.pt. 165-169°C, \([\alpha]_{D}^{20} + 112.5^\circ\) (c 0.115, chloroform), \(\nu_{\text{max}}\) 3370cm.\(^{-1}\), \(\tau\) 4.83 (1H,m, low J), 5.79 (1H,d, low J), 6.35 (1H, very b), 9.02, 9.05, 9.12, 9.21
250.

and 9.40, m/e 414 (M⁺), 396 (M⁺-H₂O), 271 (M⁺-side-chain) and 253 (396-side-chain). (Found: C, 80.9; H, 11.3%; M⁺, 414. C₂₈H₄₆O₂ requires C, 81.1; H, 11.2%; M, 414). The yield was quantitative.

The diol (85) (30mg.) in chloroform (10ml.) was stirred for 5 hours under nitrogen with active manganese dioxide (250mg.). Crystallisation of the residue obtained by filtration and evaporation gave the alcohol (84b) (25mg.), as chunks (from methanol), m.pt. 188-192°C, [α]D₂₀ + 54° (c 0.313, chloroform), νmax. 3420, 1640 and 1618cm⁻¹, λmax. 247nm. (c 12,300), τ 4.26 (1H, t), 6.34 (1H, b), 8.93, 8.98, 9.04, 9.12, 9.20 and 9.30, m/e 412 (M⁺), 397, 394, 341 (M⁺-side-chain from C-23), 297 (M⁺-side-chain from C-20), 269 (M⁺-side-chain), 149 (269-ring A). (Found: C, 81.2; H, 10.7. C₂₈H₄₄O₂ requires C, 81.5; H, 10.75%). The alcohol (84b) was obtained in 83% yield.

The diol (85) (40mg.) in ether (5ml) was stirred for ten minutes with glacial acetic acid (1ml.), water (0.5ml.), and hydrochloric acid (6N; 0.1ml.). The mixture was washed acid-free with water, dried and evaporated. Trituration with petrol gave the diene (86a) (30mg.), as microcrystals, m.pt. 95-98°C, [α]D³₀ + 147° (c 0.389, chloroform), νmax. 3500cm⁻¹, λmax. 261.5nm. (c 20,300) and 255nm. (c 19,700), τ 3.91 (1H,d, J 10Hz) (C-7 proton), 4.87 (1H,d, J 10Hz) (C-6 proton), 6.36 (1H,b), 9.00, 9.06, 9.09, 9.13 and 9.25, m/e 396 (M⁺), 381, 363, 265, 253 (M⁺-side-chain), and 199 (253-C₄H₆). (Found: C, 84.7; H, 11.0. C₂₈H₄₄O₂ requires C, 84.8; H, 11.2%). A yield of 78% was obtained.
(23R)-23-Acetoxy-3α,5α-cycloergosta-6,8(14)-diene (86b). - The diene (86a) (15mg.) was acetylated in the usual way to give the diene acetate (86b) (15mg.) as needles (from methanol), m.pt. 131-5°C, $[\alpha]_D^{25}$ + 122° (c 0.218, chloroform), $\nu_{\text{max.}}$ 1726 and 1246cm.\(^{-1}\), $\lambda_{\text{max.}}$ 261.5nm. (ε 21,700) and 255nm. (ε 20,700), ν 3.90 (1H,d, J 10Hz), 4.84 (1H,d, J 10Hz), 4.98 (1H,b) (proton α acetate); 7.97 (3H,s), 8.99, 9.04, 9.11, 9.14 and 9.21, m/e 438 (M\(^+\)), 423, 363, 253 (M\(^+\)-side-chain), and 199 (253-C\(_4\)H\(_6\)). (Found: C, 81.9; H, 10.7. C\(_{30}\)H\(_{46}\)O requires C, 82.1; H, 10.6%). The yield was 91%.

(23R)-23-Benzoyloxy-3α,5α-cycloergosta-6,8(14)-diene (86c). - This benzoate (86c) was first isolated as a byproduct in an attempted benzoylation of the diol (85). A better preparation was by the treatment of the diene (86a) (20mg.) with freshly distilled benzoyl chloride (28mg.; 4 equivalents) in dry pyridine (3ml.) overnight. The reaction mixture was poured into water, extracted with ether (2x20ml.), the combined extracts washed with hydrochloric acid (1N; 3ml.), saturated sodium bicarbonate solution and water, dried and evaporated. T.L.C. purification (3% acetone/petrol) gave the benzoate (86c) (20mg.) as needles (from acetone), m.pt. 137-139°C, $[\alpha]_D^{24}$ + 147° (c 0.307, chloroform), $\nu_{\text{max.}}$ 1710, 1610, 1278 and 720cm.\(^{-1}\), $\lambda_{\text{max.}}$ 261.5nm. (ε 25,200), 256.5nm. (ε 24,000) and 231.5nm. (ε 21,700), ν 1.99 (2H,m), 2.45 (3H,m), 3.90 (1H, d, J 9Hz), 4.65 (1H,b), 4.85 (1H,d, J 9Hz), 8.94, 9.04, 9.06, 9.10, 9.18 and 9.24 (3H,s), m/e 500(M\(^+\)), 485, 378 (M\(^+\)-BzOH), 363, 253, 199, 105 and 77 (Found: C, 83.75; H, 9.7%; M\(^+\), 500. C\(_{35}\)H\(_{48}\)O\(_2\) requires C, 83.9; H, 9.7%; M, 500).
C.d. curve: $\Delta$e $-7.38$ (203nm.), $-5.0$ (215nm.), $-6.91$ (225nm.), and $+16.96$ (250nm.). O.r.d. curve: $[\theta]$ $-69,100$ (238nm.) and $+44,700$ (277nm.). The yield obtained was 80%.

Comparison of $3\alpha,5\alpha$-cycloergosta-7,22-dien-6$\beta$-ol (4) with $3\alpha,5\alpha$-cycloergosta-7,22-dien-6$\alpha$-ol. - In order to establish the configuration of the 6-ol resulting from LAH reduction of the 6-ketone, a sample of the 6$\beta$-ol (4) was prepared by solvolysis in the usual way, and compared with the product obtained by LAH reduction of the cyclopropyl ketone (5) (itself resulting from manganese dioxide oxidation of the 6$\beta$-ol).

The 6$\beta$-ol was the more polar compound, (crystallisation from methanol giving the triene (6)), $\tau$ 4.52 (1H,m), 4.76 (2H,m), 6.59 (1H,b, $W_{1/2}$ 20Hz), 8.92, 9.03, 9.13, 9.23 and 9.37. The width at the half-height of the $\tau$ 6.59 signal confirmed the $\alpha$-face configuration of the C-6 proton (dihedral angle with the C-7 proton approaching 90°).

The LAH reduction product contained the 6$\beta$-ol (5%), but was mainly the less polar 6$\alpha$-ol, $v_{\text{max}}$, 3340cm.$^{-1}$, $\tau$ 4.63 (3H,m), 5.49 (1H,b, $W_{1/2}$ 10Hz), 8.83, 8.93, 9.04, 9.13 and 9.32, m/e 396 (M$^+$), 378 (M$^+$-H$_2$O), 363 (378-Me), 337 (363-C$_2$H$_2$), and 253 (378-side-chain). The width of the half-height of the $\tau$ 5.49 signal confirms the $\beta$-face configuration of the C-6 proton (dihedral angle with the C-7 proton smaller than for the 6$\alpha$-H). There is ample supporting evidence from other systems for these assignments in the literature$^{63(c)}$, 90, 91.
(23R)-23-Benzoyloxy-3α,5α-cycloergosta-7-en-6-one (84c). -

The alcohol-III (84b) (22mg.) was benzyolated by the standard technique to give after T.L.C. purification (6% acetone/petrol) the benzoate (84c) (18mg.), as chunks (from methanol), m.p.t. 186-188°C, [α]D20 + 59° (0.212, chloroform), νmax. 1710, 1650, 1610, 1280 and 720 cm⁻¹, λmax. 233 nm. (ε 19,500) and 247 nm. (ε 13,500). τ 2.04 (2H,m), 2.62 (3H,m), 4.32 (1H,t), 4.71 (1H,b), 8.98, 9.02, 9.06, 9.08, 9.13, 9.19 and 9.44, m/e 516 (M⁺), 501, 394 (M⁺-BzOH), 379, 296 (M⁺-side-chain from C-20), 268 (M⁺-side-chain), 243, 105 and 77. (Found: C, 81.3; H, 9.45. C₃₅H₄₈O₃ requires C, 81.35; H, 9.4%). The benzoate (84c) was isolated in 70% yield.

C.d. curve: Δε +9.37 (204nm.), −19.46 (233nm.), and +2.28 (319nm.). O.r.d. curve: [α] +91,500 (212nm.), −29,100 (252nm.) and −10,400 (298nm.).

(23R)-23-p-Iodobenzoyloxy-3α,5α-cycloergosta-7-en-6-one (84d). -

The alcohol (84b) (50mg.) in dry pyridine (3ml.) was treated with p-iodobenzoyl chloride (136mg.; more than 4 equivalents) (prepared by heating under reflux p-iodobenzoic acid (4g.) with thionyl chloride (12.5ml.) in dry benzene (15ml.) containing a trace of DMF). Reaction was incomplete even after several days, and the normal work-up gave recovered starting alcohol (25mg.) and the p-iodobenzoate (84d) (10mg.) as chunks (from benzene/petrol), m.p.t. 199-201°C [α]D25 + 52° (c 0.193, chloroform), νmax. 1712, 1648, 1617, 1585 and 1275 cm⁻¹, λmax. 255 nm. (ε 27,800) (absorption of αβ-unsaturated ketone completely masked), τ 2.31 (4H,d, J 3Hz), 4.32 (1H,t), 4.72 (1H,b), 8.79, 8.98, 9.05, 9.11, 9.16, 9.21 and 9.46, m/e 642 (M⁺), 627, 614, 394 (M⁺-p-IbOH), 378, 247 and 231 (Found: C, 65.45; H, 7.4. C₃₅H₄₇O₃ requires C, 65.4; H, 7.3%). The yield was 25%.
3α,5α-cycloergosta-7-en-6,23-dione (87). - Jones' oxidation of the alcohol (84b) (410mg.) in 5% chloroform/acetone (50ml.) under the normal conditions gave a crude product (400mg.), from which the pure diketone (87) (320mg.) could be crystallised as platelets (from methanol), m.p.t. 177-180°C, [α]D20 + 52° (c 0.086; chloroform), νmax. 1705 and 1655 cm.⁻¹, λmax. 246 nm. (ε 12,700), τ 4.28 (1H, t), 8.78, 8.94, 9.00, 9.08, 9.14, 9.20 and 9.30, m/e 410 (M⁺), 395, 339 (M⁺-side-chain from C-23), 311 (M⁺-side-chain from C-22), 297, 296 (M⁺-side-chain from C-20 (McLafferty)), 99 and 71 (Found: C, 81.8; H, 10.4%. C28H42O2 requires, 81.9; H, 10.3%). The yield was 78%.

(22R)-22-Acetoxy-3α,5α-cycloergosta-7-en-6-one (89a). - Bromoacetate-II (66b) (511mg.) was debrominated with tri-n-butyl tin hydride as described for bromoacetate-III (66c). The required acetate (89a) (363mg.) was originally obtained as an oily solid by P.L.C. Subsequently, repeated purification gave chunks (from petrol), m.p.t. 100-110°C, [α]D22 + 62° (c 0.203, chloroform), νmax. 1730, 1650 and 1250 cm.⁻¹, λmax. 246 nm. (ε 13,300), τ 4.14 (1H, t), 4.93 (1H, b), 7.93 (3H, s), 8.90, 8.98, 9.06, 9.16 and 9.31, m/e 454 (M⁺), 439, 394, 315, 313 and 311. (Found: C, 79.0; H, 10.0%. C30H46O3 requires C, 79.2; H, 10.2%). The yield was 83%. The acetate (89a) was not obtained in any better crystalline form, even by reacetylation of the alcohol (89b) (see below).

(22R)-3α,5α-Cycloergosta-7-en-6α,22-diol (90); (22R)-22-hydroxy-3α,5α-cycloergosta-7-en-6-one (89b); and (22R)-22-hydroxy-3α,5α-cycloergosta-6,8(14)-diene (91a). - LAH reduction in dry ether of the acetate (89a) (360mg.) gave the 6α,22R-diol (89b) (300mg.) as chunks or broken needles (from methanol), m.p.t. 93-96°C,
\[ [\alpha]_{D}^{25} + 117^\circ (c 0.257, \text{chloroform}), v_{\text{max.}} 3380\text{cm}^{-1}, \tau 4.85\]  
(1H,dd, J 2Hz), 5.70 (1H,b), 6.32 (1H,bm), 9.07, 9.15, 9.21, 9.28 and 9.42, m/e 414 (M\(^+\)), 399, 396, 381, 355, 253 and 161 (Found: C, 80.9; H, 11.0%; M\(^+\), 414. \(C_{28}H_{46}O_2\) requires C, 81.1; H, 11.2%; M, 414). The product was isolated in 94% yield.

The diol (90) (250mg.) was oxidised by the usual procedure to give a product (240mg.) from which the alcohol (89b) (220mg.) was crystallised as needles (from methanol), m.pt. 219-222\(^\circ\)C, \[ [\alpha]_{D}^{24} + 86^\circ (c 0.191, \text{chloroform}), v_{\text{max.}} 3390, 1635 and 1618\text{cm}^{-1}, 245.5\text{nm} (\varepsilon 13,000), \tau 4.30 (1H,t), 6.31 (1H,b), 8.79, 8.96, 9.10, 9.16, 9.22 and 9.34, m/e 412 (M\(^+\)), 397, 394, 379, 298, 283, and 243. (Found: C, 81.3; H, 10.8%; M\(^+\), 414. \(C_{28}H_{44}O_2\) requires C, 81.5; H, 10.75%; M, 414). The yield was 89%.

The acetate (89a) (120mg.) was converted by the usual two-step reduction/acid catalysed elimination procedure to the diene (91a) (90mg.), which crystallised as needles (from acetone), m.pt. 156-158.5\(^\circ\)C, \[ [\alpha]_{D}^{25} + 104^\circ (c 0.620, \text{chloroform}), v_{\text{max.}} 3550\text{cm}^{-1}, \lambda_{\text{max.}} 262\text{nm.} (\varepsilon 20,400), 254.5\text{nm.} (\varepsilon 19,100) and 274\text{nm.} (\varepsilon 13,300), \tau 3.92 (1H,d, J 10Hz), 4.85 (1H,d, J 10Hz), 6.25 (1H,b), 9.05, 9.10, 9.15, 9.24 and 9.30, m/e 396 (M\(^+\)), 381, 363, 311 (M\(^+\)-side-chain from O-22), 293, 281 (M\(^+\)-side-chain from O-20), 253, and 199. (Found: C, 83.26; H, 11.0. \(C_{28}H_{44}O_{1/2}H_2O\) requires C, 82.94; H, 11.1%). The diene (91a) was obtained in 86% yield.

(22R)-22-Acetoxy-3\(\alpha\),5\(\alpha\)-cycloergosta-6,8(14)-diene (91b). Using the general conditions of acetylation, the diene (91a)
(20mg.) was converted to the acetate (91b) (18mg.), which crystallised as needles (from methanol), m.pt. 86-90°C, $[\alpha]_D^{27} + 130^\circ$ (c 0.254, chloroform), $v_{\text{max.}}$ 1735 and 1240cm.$^{-1}$, $\lambda_{\text{max.}}$ 261nm. (€ 23,100), 253nm. (€ 22,000) and 274nm. (€ 14,100), $\tau$ 3.94 (1H,d, J 10Hz), 4.87 (1H,d, J 10Hz), 5.05 (1H,b), 8.04 (3H,s), 8.79, 9.06, 9.13, 9.15, 9.22, 9.26 and 9.32, m/e 438 (M$^+$), 423, 378, 363, 290, 253 and 199. (Found: C, 82.0; H, 10.4. C$_{30}$H$_{46}$O$_2$ requires C, 82.1; H, 10.6%). The yield was 82%.

(22R)-22-Benzoyloxy-3α,5α-cycloergosta-6,8(14)-diene (91c). - The diene (91a) (20mg.) under the usual conditions gave the benzoate (91c) (19mg.) as needles (from acetone/methanol), m.pt. 186-80°C, $[\alpha]_D^{27} + 118^\circ$ (c 0.266, chloroform), $v_{\text{max.}}$ 1708, 1280 and 720cm.$^{-1}$, $\lambda_{\text{max.}}$ 261.5nm. (€ 27,000), 253nm. (25,500), 232nm. (€ 19,700) and 274nm. (€ 16,600), $\tau$ 2.02 (2H,m), 2.58 (3H,m), 3.91 (1H,d, J 10Hz), 4.84 (1H,d, J 10Hz), 4.86 (1H, b), 8.78, 8.96, 9.03, 9.11, 9.15, and 9.23, m/e 500(M$^+$), 485, 378, 363, 293, 253, and 199. (Found: C, 83.6; H, 9.6. C$_{35}$H$_{48}$O$_2$ requires C, 83.9; H, 9.7%). The yield was 76%.

C.d. curve: $\Delta \varepsilon$ -7.5 (205nm.), -5.0 (215nm.), -5.98 (223nm.) and +14.8 (259nm.). O.r.d. curve: $[\phi]$ -46,900 (240nm.) and +35,100 (279nm.).

(22R)-22-Benzoyloxy-3α,5α-cycloergosta-7-en-6-one (89c). - The alcohol (89b) (50mg.) was benzoylated to give after T.L.C. purification (3% acetone/petrol) the benzoate (89c) (39mg.) as fine needles (from methanol), m.pt. 206-208.5°C, $[\alpha]_D^{20} + 35^\circ$ (c 0.204, chloroform), $v_{\text{max.}}$ 1710, 1652, 1285 and 720cm.$^{-1}$, $\lambda_{\text{max.}}$ 233nm. (€ 23,500) and 247nm. (€ 15,400) (partially
257.
masked by benzoate absorption), \( \tau \) 2.00 (2H, m), 2.67 (3H, m),
4.24 (1H, t), 4.83 (1H, b), 8.93, 9.02, 9.11, 9.19, 9.23, and
9.32, m/e 516 (M\(^{+}\)), 501, 394, 347, 268, 243, 105 and 77.
(Found: C, 81.3; H, 9.5. \( C_{35}H_{48}O_{3} \) requires C, 81.4; H, 9.4%).
The yield of (89c) was 63%.
C.d. curve: \( \Delta \varepsilon \) +6.54 (205nm.), -24.13 (236nm.) and +2.86° (315nm.).
O.r.d. curve: [\( \gamma \)] +65,100 (201nm.), +54,760 (215nm.), -20,720
(253nm.), -5,920 (298nm.) and +2,960(352nm.).

3α,5α-cycloergosta-7-en-6,22-dione (88). - Jones' oxidation of
the alcohol (89b) (80mg.) in acetone (35ml.) gave, by
crystallisation of the crude product, the dione (88) (70mg.),
as platelets (from methanol), m.pt. 182-184.5°C, [\( \alpha \)]\(_D\) 20 + 52°
(c 0.172, chloroform), \( \nu_{\text{max}} \) 1715 and 1650cm.\(^{-1}\), \( \lambda_{\text{max}} \) 246nm.
(\( \varepsilon \) 12,800), \( \tau \) 4.28 (1H, t), 8.87, 8.93, 9.11, 9.15, 9.22 and
9.32, m/e 410 (M\(^{+}\)), 395 (M\(^{+}\)-Me), 341 (M\(^{+}\)-side-chain from C-23
(McLafferty)), 325 (M\(^{+}\)-side-chain from C-22 (\( \alpha \)-cleavage)),
297 (M\(^{+}\)-side-chain from C-20 (\( \alpha \)-cleavage)), 269, 243, 113
and 85. (Found: C, 81.75; H, 10.4. \( C_{26}H_{42}O_{2} \) requires C, 81.9;
H, 10.3%). The yield of the diketone (88) was 88%.

(22S)-22-acetoxy-3α;5α-cycloergosta-7-en-6-one (also depicted
by 89a). - By the usual debromination procedure with extended
reaction times of up to 48 hours, the bromoacetate-I (66a)
(50mg.) gave the acetate (89a) (10mg.), after two T.L.C.
purifications, as an oily solid. Crystallisation from methanol
with seeding gave needles, m.pt. 150-155°C, [\( \alpha \)]\(_D\) 27 + 38.5° (c
0.273, chloroform), \( \nu_{\text{max}} \) 1723, 1650, 1625 and 1243cm.\(^{-1}\),
\( \lambda_{\text{max}} \) 247nm. (\( \varepsilon \) 11,000), \( \tau \) 4.27 (1H, t), 4.94 (1H, b), 8.00 (3H, s),
8.93, 8.98, 9.04, 9.16, 9.20, 9.34, 9.42 and 9.52, m/e 454 (M\(^{+}\)),
439, 394, 379, 269 (M\(^{+}\)-side-chain), 268 and 267. (Found:
C, 77.7; H, 9.9. C$_{30}$H$_{46}$O$_3$.1/2H$_2$O requires C, 77.7; H, 10.2%. (This analysis was consistently obtained, and the alternative explanation is that the bromoacetates with bromine at C-22 are particularly difficult to debrominate, the low analysis resulting from persistent traces of the parent bromoacetate (C, 67.5; H, 8.4%). The yield of the acetate (89a) was 23%. (Low yields from the small quantity of bromoacetate-1 (66a) available made work with derivatives of this isomer doubly difficult).

Attempts were made to reacetylate samples of the alcohol (89b), but the acetate (89a) obtained in this way was of no better quality than that derived by debromination of (66a).

(22S)-22-Hydroxy-3α,5α-cycloergosta-7-en-6-one (also depicted by 89b). - LAH reduction of the acetate (89a) (20mg.), followed by manganese dioxide oxidation for four hours, gave the alcohol (89b) (12mg.) as chunks (from methanol), m.pt. 204-8°C, $[α]_D^{28} + 31^0$ (c 0.263, chloroform), (this is lower than the rotation of a sample of the alcohol obtained from the 6,22-dione (88), $[α]_D^{25} + 48^0$ (c 0.180, chloroform) (see later)), $ν_{max}$. 3420 and 1640cm.$^{-1}$, $λ_{max.}$ 247.5nm. (ε 12,800) and 301nm. (ε 840) (trace of dienone impurity), $τ$. 4.26 (1H, t), 6.29 (1H, b), 8.93, 9.03, 9.10, 9.16, 9.22 and 9.33, m/e 412 (M$^+$), 410 (dienone, very small), 397, 394, 327 (M$^+$-side-chain from C-22), 298 (M$^+$-side-chain from C-20), 269 and 243 (Found: C, 81.3; H, 10.8. C$_{28}$H$_{44}$O$_2$ requires C, 81.5; H, 10.75%). The yield was 67%. 


It was found that the dienone impurity arose during the manganese dioxide oxidation of the 6α,22S-diol, reaction overnight in chloroform giving as much as 50% impurity. Restriction of the reaction time to several hours avoided most of the formation of the highly laevorotating dienone.

(22S)-22-benzoyloxy-3α,5α-cycloergosta-7-en-6-one (also depicted as (89c). - The alcohol (89b) (25mg.) reacted very slowly under the normal benzyolation conditions to give the benzoate (89c) (6mg.) as a non-crystalline solid, [α]_D$^29 + 69°$ (c 0.141, chloroform), $\nu_{max.}$ 1715, 1658, 1275 and 718 cm$^{-1}$, $\lambda_{max.}$ 231.5 nm (ε 21,900) and 247.5 nm (ε 13,600) (partially masked by benzoate absorption), τ 2.00 (2H,m), 2.58 (3H,m), 4.28 (1H,t), 4.68 (1H,b), 8.76, 8.85, 8.94, 9.16, 9.32 and 9.42, m/e 516 (M$^+$), 501, 394, 379, 347, 284, 268, 256 and 243. (Found: M$^+$, 516. C$_{35}$H$_{46}$O$_3$ requires M, 516). The yield of the benzoate (89c) was 19%.

C.d. curve: Δε +9.9 (202 nm.), -11.95 (227 nm.), and +24.12 (316 nm.).

O.r.d. curve: [β] +44,000 (215 nm.), 37,600 (240 nm.), -27,000 (250 nm.) and -44,000 (254 nm.).

3α,5α-cycloergosta-7,23-dien-6-one (93). - The alcohol-III (84b) (50mg.) in dry pyridine (3ml.) was treated with freshly recrystallised p-toluene sulphonyl chloride (120mg.; 5 equivalents) for 48 hours in an attempt to prepare the tosylate. Work-up was by pouring into water (30ml.), and extraction with ether (2x25ml.). The combined ether extracts were washed with water, dried, and evaporated to give a residue, which solidified at the oil pump. T.L.C. purification gave as major product the less polar diene (93) (15mg.), as needles (from methanol), m.pt. 143-7° C, [α]_D$^{30} + 89°$ (c 0.145),
\[\text{v}_{\text{max.}} 1650\text{cm}^{-1}, \lambda_{\text{max.}} 247.5\text{nm.} (\epsilon 13.100), \tau 4.26 (1\text{H},t), 4.89 (1\text{H},m) \text{ (C-23 vinylic proton)}, 8.48 (3\text{H},s) \text{ (olefinic methyl at C-24)}, 8.93, 9.00, 9.06, \text{ and } 9.33 (3\text{H},s), m/e 394 (M^+), 379, 366, 311 (M^+\text{-side-chain from C-23}), 297 (M^+\text{-side-chain from C-20}) \text{ and } 269 (M^+\text{-side-chain}) \text{ (Found: C, 83.1; H, 10.75%; M^+, 394). C}_{28}\text{H}_{42}O\cdot 1/2\text{H}_2\text{O requires C, 83.3; H, 10.7%; M, 394). The yield was 31%.}

\[\text{24\text{-Nor}-23\text{-oxo-5\alpha-cyclo}-20\alpha\text{-}chol-7\text{-en}-6\text{-one (92).} \text{ Using the conditions described previously, the diene (93)} \text{ (8mg.) was ozonised in 1\% pyridine/methylene dichloride (2ml.) at } -70^\circ\text{C, the reaction time being controlled by T.L.C. The usual work-up gave a sticky gum, from which, by T.L.C. purification (15\% acetone/petrol), the aldehyde (92) (5mg.) was obtained as plates (from ether), m.p.t. 133-7^\circ\text{C, } [\alpha]_D^{30} + 76^\circ (c 0.204), \text{ v}_{\text{max.}} 1718 \text{ and } 1658\text{cm}^{-1}, \lambda_{\text{max.}} 246\text{nm.} (\epsilon 11,500), \tau 0.30 (1\text{H},t, \text{ low J}) \text{ (aldehyde proton)}, 4.27 (1\text{H},t), 8.77, 8.93, \text{ and } 9.29, \text{ m/e 340 (M^+), 325 (M^+\text{-Me}), 312 (M^+\text{-CO}), 311, 269 (M^+\text{-side-chain)}, 175 \text{ and } 159 \text{ (Found: M^+, 340). C}_{23}\text{H}_{32}O_2 \text{ requires M, 340). A 74\% yield was obtained.}

\[\text{6-Hydroximino-3\alpha,5\alpha-cycloercesssta-22-one.} \text{ - Hydroxylamine hydrochloride (45mg.) and sodium acetate (45mg.) were dissolved in water (1.5ml.), and ethanol (4ml.) added. The precipitated sodium chloride was filtered off, and the solution added to the 7,8\text{-dihydrocyclopropyl ketone (75) (125mg.) in ethanol (3ml.)}. \text{ After heating the reaction mixture under reflux for ten minutes, the solid product was collected (110mg.) and crystallised from ethanol to give the oxime (91mg.) as chunks,}
m.pt. 165-168°C, [α]$_D^{28}$ -7° (c 0.473), ν$_{max}$ 3270, 3150, and 1650 cm.$^{-1}$, τ 2.26 (1H, b) (D$_2$O exchangeable), 4.83 (2H, m), 6.75 (1H, b), 8.94, 9.06, 9.10, 9.13, 9.22 and 9.31. (Found: C, 81.3; H, 11.1; N, 3.1. C$_{26}$H$_{45}$NO requires C, 81.7; H, 11.0; N, 3.4%). A yield of 70% was obtained.

6-Hydroxyimino-3α,5α-cycloergosta-7,22-diene (94a). - Using identical conditions to those described above, a reaction time of 5 days was required to complete oxime formation in the case of the cyclopropylketone (5) (125mg.). On cooling, a precipitate formed, which was collected (85mg.) and crystallised from ethanol to give the oxime (94a) (40mg.) as needles, m.pt. 201-6°C, [α]$_D^{27}$ -16° (c 0.495), ν$_{max}$ 3280, 3210, 1635, and 1620 cm.$^{-1}$, λ$_{max}$ 239nm. (ε 10,100) and 266nm. (ε 7,500), τ 2.30 (1H, b), 4.22 (1H, b), 4.83 (2H, m), 8.93, 9.06, 9.17, 9.24, 9.33 and 9.36. (Found: C, 81.9; H, 10.3; N, 3.3. C$_{28}$H$_{43}$NO requires C, 82.1; H, 10.6; N, 3.4%). The yield was 31%.

6,23-Dihydroxyimino-3α,5α-cycloergosta-7-ene (95). - Using the above conditions for 30 minutes, the dione (87) was unaffected. Similarly, it was resistant to treatment overnight with hydroxylamine hydrochloride (1mole) in dry pyridine. However, when the dione (87) (107mg.) in dry pyridine (3ml.) was treated with hydroxylamine hydrochloride (500mg.; more than 20 moles) for several days, work-up by evaporation of the solvent at the oil pump, solution of the residue in THF, dilution with benzene, washing with water, drying and evaporating gave a white solid, from which the dioxime (95) (80mg.) could be obtained as microcrystals (from THF/methanol), m.pt. 234-6°C, [α]$_D^{29}$ -23° (c 0.351, dry dioxan),
From a preliminary experiment, T.L.C. separation also allowed the isolation of a less polar compound in poor yield. This proved surprisingly to be 6-hydroxyimino-3α,5α-cycloergosta-7-en-23-one, vmax. 3300, 3240, 1710, 1645, 1638, 975, 950 and 935 cm⁻¹, m/e 425 (e), 409, 394, and 241. The compound was however not completely characterised.

6-Hydroxyimino-3α,5α-cycloergosta-7,22-dionyl toluene p-sulphonate (94b). - As a model experiment, the oxime (94a) (350mg.) in dry pyridine (5ml.) was treated with freshly recrystallised toluene p-sulphonyl chloride (340mg.; 2 moles) in a stoppered vessel for 16 hours. The reaction mixture was poured into water, the solid collected, washed with water and dried in vacuo to give the oxime toluene p-sulphonate (94b) (435mg.). A sample was purified by T.L.C. (8% acetone/petrol) and crystallised as rectangular platelets (from benzene/petrol), m.pt. 151-4°C (with decomposition), [α]D²⁴⁺ -20° (c 0.548, dry benzene), vmax. 1622, 1600, 1195, 1177, and 675 cm⁻¹, λmax. 250.5nm. (ε 22,200) and 230.5nm. (ε 21,600), τ 2.24 (2H,d, J 8Hz), 2.80 (2H,m, J 9Hz), 3.78 (1H,dd) (α-7-vinylic proton), 4.85 (2H,m) (side-chain olefinic protons), 7.63 (3H,s) (aromatic methyl protons), 8.96, 9.03, 9.06, 9.12, 9.21 and 9.41, m/e (M⁺ not observable), 547, 409 (elimination of tosylate), 393, 378, 268, 253, 241, 172 and 91 (Found: C, 74.5; H, 8.6;
263.

N, 2.4; S, 5.8. C_{35}H_{49}NO_{3}S requires C, 74.6; H, 8.8; N, 2.5; S, 5.7%). The yield was 90%.

6-Aza-3α,5α-cyclo-B-homoergosta-8,23-dien-7-one (96). - The oxime tosylate (94b) (120mg.) was added, in the minimum volume of dry benzene (1ml.), to an alumina column (grade I; 10g.) according to the literature procedure. After 15 minutes, the column was eluted with petrol, benzene, 30% chloroform/benzene, and chloroform. The first eluates contained unreacted tosylate (94b) (30mg.), followed by the crude product of Beckmann rearrangement, which crystallised to give the lactam (96) (60mg.), as needles (from methanol), m.pt. 192-195°C, $[α]_D^{23} + 160$° (c 0.517, chloroform), $ν_{max}$. 3300-3200, 1675 and 1632 cm.⁻¹, $λ_{max}$. 224 nm. (c 15,100), $τ$. 4.10 (1H, dd) (C-8 vinyl proton), 4.47 (1H, s) (lactam-NOH), 4.96 (2H, m), 8.92, 8.98, 9.04, 9.06, 9.14, 9.21 and 9.39, m/e 409 (M⁺), 394, 366, 284, and 149. (The lack of any strong fragmentation indicated that the Beckmann rearrangement in ring B would not interfere with the mass spectral investigation of the structure of the side-chain Beckmann rearrangement product). (Found: C, 81.9; H, 10.5; N, 3.3. C_{28}H_{43}NO requires C, 82.1; H, 10.6; N, 3.4%). The yield was 92% with regard to reacted oxime tosylate.

24-Aza-6-hydroximino-3α,5α-cycloergosta-7-en-23-one toluene p-sulphonate (97). - Tosylation of the dioxime (95) (60mg.) under identical conditions to those described above gave a product (isolated by filtration or by ether extraction) in which the side-chain oxime tosylate had undergone Beckmann rearrangement. The resulting amide oxime toluene p-sulphonate (97) (72mg.) could not be recrystallised, but was a solid,
consisting of broken platelets, m.pt. 98-102°C, $[^{29}]D +4^\circ$
(c 0.221), $\nu_{\text{max}}$ 3430, 3340, 1647, 1605, 1550, 1196, 1182
and 675 cm.$^{-1}$, $\lambda_{\text{max}}$ 230.5 nm. (e 18,800) and 250.5 nm. (e 18,600),
$\tau$ 2.24 (2H, d, J 8 Hz), 2.77 (2H, m, J 8 Hz), 3.78 (1H, dd), 4.66
(1H, very b), 6.16 (1H, b), 6.9 (1H, very b), 7.62 (3H, s), 8.76, 8.92, 8.98, 9.09, 9.12
and 9.38 (3H, s), m/e (no M$^+$ observable), 440 (M$^+$-tosylate), 425, 352 (typical amide
fragmentation for the proposed structure), 312 (440-side-chain from C-20), 172, 155, 149
and 91 (Found: M$^+$-154, 440.
C$_{28}$H$_{44}$O$_2$N$_2$ requires M$^+$, 440 i.e. C$_{35}$H$_{50}$N$_2$O$_4$ - C$_7$H$_6$O$_2$S). The
yield was 90%.

6,25-Diaza-3α,5α-cyclo-B-homoergosta-8-en-7,24-dione (98). -
The amide oxime tosylate (97) (50mg.) was rearranged on
alumina as described above$^95$, Elution was with benzene, 30-50%
chloroform/benzene, and chloroform. After recovering starting
material (30mg.), the product was eluted, evaporation giving
the amide lactam (98) (10mg.), as a solid, consisting of
chunks and platelets, m.pt. 123-127°C, $[^{31}]D +140^\circ$ (c 0.284),
$\nu_{\text{max}}$ 3330, 1670, 1650, 1625, and 1555 cm.$^{-1}$, $\lambda_{\text{max}}$ 223 nm
(e 13,500), $\tau$ 4.15 (1H, dd), 4.48 (1H, s), 4.75 (1H, b), 6.2
(1H, b), 6.9 (1H, b), 8.93, 9.90, 9.09 and 9.39, m/e 440 (M$^+$),
425, 397, 354 (typical amide fragmentation for proposed structure)
325, 312, 284, 258 and 244. (Found: M$^+$, 440. C$_{28}$H$_{44}$O$_2$N$_2$
requires M, 440). The yield was 73%.

LAH reduction (and manganese dioxide oxidation) of 3α,5α-
cycloergosta-7-en-6,23-dione (87). - (a) By the usual reduction/
oxidation technique, the dione (87) (250mg.) gave an epimeric
mixture of the 23-alcohols (84b), which was separated by
T.I.C. (7% acetone/petrol; 5 elutions). The more polar epimer (70mg.) was identical in all respects (m.pt., mixed m.pt., spectra) to the 23R-alcohol (84b) derived from bromoacetate-III (66c). The yield was 28%.

The less polar component was the 23S-alcohol (also depicted by 84b) (70mg.), obtained as needles (from methanol), m.pt. 178-9°C, $[\alpha]_D^{25} + 57.5^\circ$ (c 0.200, chloroform), $\nu_{\text{max}}$ 3395 and 1635cm$^{-1}$, $\lambda_{\text{max}}$ 247nm. ($\varepsilon$ 13.400), $\tau$ 4.27 (1H,t), 6.44 (1H,b), 8.76, 8.93, 9.07, 9.13, 9.18, 9.26 and 9.32, m/e 412 (M$^+$), 397, 394, 379, 384, 341 (M$^+$-side-chain from C-23), 311, 297 (M$^+$-side-chain from C-20 (McLafferty)) and 269.

(Found: C, 81.3; H, 10.5%; M$^+$, 412. C$_{26}$H$_{44}$O$_2$ requires C, 81.5; H, 10.75; M, 412). The yield was again 70%, though the less polar epimer had appeared (by T.I.C.) to predominate.

(b) In order to arrive at an estimate of the epimer ratio, the LAH reduction of the dione (10mg.) was repeated at -20°C, at which temperature a greater degree of stereospecificity should be imposed. T.I.C. separation of the products showed a predominance of the less polar alcohol (corresponding to the non-isolated bromoacetate-IV) of 6:5 (by weight). By T.I.C. estimation, the ratio appeared to be nearer 7:3.

(23S)-23-Acetoxy-3a,5a-cycloergosta-7-en-6-one (84a). - The 23S-alcohol (84b) (15mg.) was subjected to the usual acetylation conditions to give the 23S-acetate (84a) (15mg.) as a microcrystalline solid (from methanol), m.pt. 86-89°C, $[\alpha]_D^{31} + 57^\circ$ (c 0.307, chloroform), $\nu_{\text{max}}$ 1730, 1650, 1625 and 1248cm$^{-1}$, $\lambda_{\text{max}}$ 247nm. ($\varepsilon$ 12,400), $\tau$ 4.27 (1H,t), 5.03 (1H,b), 8.03 (3H,s), 8.77, 8.94, 9.04, 9.11, 9.16, 9.23 and 9.35, m/e
(23S)-23-Benzoyloxy-3α,5α-cycloergosta-7-en-6-one (84c). - The 23S-alcohol (84b) (28mg.) gave by the normal method the 23S-benzoate (84c) (15mg.) after T.L.C. purification (8% acetone/petrol; 2 elutions). The product was obtained as chunks (from methanol), m.pt. 168-170°C, \([\alpha]_D^{22} + 37^\circ\) (c 0.253; chloroform), \(\nu_{max}\) 1710, 1650, 1620, 1290 and 730cm.\(^{-1}\), \(\lambda_{max}\) 234nm. (\(e 20,500\)) and 249nm. (\(e 13,800\)) (partially masked by benzoate absorption), \(\tau 1.98\) (2H,m), 2.57 (3H,m), 4.26 (1H,t), 4.71 (1H,b), 8.76, 8.95, 9.08, 9.13, 9.36 and 9.42, m/e 516 (M\(^+\)), 394, 379, 296 (M\(^+\)-side-chain from C-20 (McJafferty)), 277, 267, 243, 149, and 105 (Found: C, 81.3; H, 9.15%; M\(^+\), 516. C\(_{35}H_{48}O_3\) requires C, 81.35; H, 9.4%; M, 516). The yield was 43%. C.d. curve: \(\Delta e +11.10\) (204nm) -14.30 (234nm.) and +2.52 (316nm.). O.r.d. curve: \([\beta]\) +34,800 (204nm.), -26,400 (256nm.) and -9,900 (295nm.), 3α,5α-Cycloergosta-7-en-6-one\(^70\). - An adaptation of the literature procedure\(^70\) was used for the preparation of the system parent compound. The cyclopropyl ketone (5) (400mg.) in ethyl acetate (dried; 50ml.) containing triethylamine (0.5ml.) was hydrogenated over 5% palladium-charcoal catalyst (220mg.) for 4 hours, when the uptake of hydrogen had ceased. Filtration through kieselguhr and evaporation gave an off-white solid, from which the 22,23-dihydrocyclopropyl ketone (320mg.) was isolated as white microcrystals (from methanol), m.pt. 144-147°C, (lit.\(^70\), 142-4°C), \([\alpha]_D^{23} + 75^\circ\) (c 0.819, chloroform) (lit.\(^70\), +77°), \(\nu_{max}\) 1653 and 1625cm.\(^{-1}\), \(\lambda_{max}\) 247.5nm. (c 13,100), \(\tau 4.20\) (1H,t), 8.90, 9.01, 9.08, 9.14, 9.24 and 9.31. The yield was 80%.
3α,5α-Cycloergosta-7-en-6α-ol and 3α,5α-cycloergosta-6,8(14)-diene. - i-ergosta-7-en-6-one (200mg.) was reduced with LAH (100mg.) in dry ether (25ml.) in the usual way to give the parent, i-ergosta-7-en-6α-ol (155mg.) as a non-crystalline solid, [α]D23 + 102° (c 0.485, chloroform), νmax. 3325cm.⁻¹, τ 4.84 (1H,dd), 5.71 (1H, W1/2.6Hz), 9.07, 9.14, 9.21, 9.28 and 9.44 (3H,s), m/e 398 (M⁺), 380, 365, 340, 271, and 253 (Found: M⁺, 398. C28H46O requires M, 398). The yield was 77%.

Mild acid treatment of the allylic alcohol (80mg.) in the usual manner gave the parent, i-ergosta-6,8(14)-dien (61mg.) as platelets (from acetone), m.pt. 74-78°C [α]D53  146° (c 0.780, chloroform), νmax. no strong absorption, λmax. 261.5nm. (ε 19,300), 253nm. (ε 17,600) and 275nm. (ε 11,700), τ 3.92 (1H,d, J 10Hz), 4.90 (1H,d, J 10Hz), 9.06, 9.08, 9.13, 9.22, 9.25 and 9.28, m/e 380 (M⁺), 365, 339 (365-C2H₂), 253, and 199 (Found: C, 88.3; H, 11.5. C28H44 requires C, 88.35; H, 11.65%). The yield was 80%.

LAH reduction (and manganese dioxide oxidation) of 3α,5α-cycloergosta-7-en-6,22-dione (88). - The general reduction/oxygenation sequence was used to convert the dione (88) (50mg.) to a mixture of the epimeric 22-alcohols (89b). T.L.C. purification (8% acetone/petrol; 6 elutions) enabled the products to be separated. The minor, less polar epimer (5mg.) was the 22R-alcohol (5mg.) identical in all respects (m.p.t., mixed m.p.t., spectra) to the sample isolated from bromoacetate-II (66b). The yield was 10%.
The more polar, major component was the 22S-alcohol (35mg.), identical to the alcohol isolated from bromoacetate-I (66a) (m.pt., mixed m.pt., and spectra). The yield was 70%, and the product ratio, by weight, was 7:1.

(22S)-22-Hydroxy-3α,5α-cyclocholest-7-en-6-one. - The 22S-alcohol (43a) (100mg.) was benzoylated in the usual way to give the 22S-benzoate (65mg.) as a solid, consisting of chunks, m.pt. 66-68°C, $[\alpha]_D^{28} + 89.5^\circ$ (c 0.240), $\nu_{\text{max.}}$ 1710, 1655, 1275 and 715 cm$^{-1}$, $\lambda_{\text{max.}}$ 231.5nm. (e 11,800), $\tau$ 2.02 (2H, m), 2.60 (3H, m), 4.32 (1H, t), 4.86 (1H, m), 8.87, 8.97, 9.13, 9.19 and 9.33 (3H, s), m/e 502 (M$^+$), 487, 380, 365, 268, 243 and 105. (Found: M$^+$, 502. C$_{34}$H$_{46}$O$_3$ requires M, 502). The yield was 52%.
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