SYNTHETIC STUDIES ON INSECT MOULTING HORMONES

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TO JOY

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ABSTRACT

A review of the chemistry of insect moulting hormones is presented. This review is in three sections. Section one is a brief introduction to the biology and biochemistry of the insect moulting hormone ecdysone.

Section two deals with the occurrence and structural determination of compounds which have been shown to have moulting hormone activity.

The final section deals with the synthetic efforts on moulting hormones that have appeared in the literature.

The discussion of the author's own work is in two sections. The first section describes the efforts which led to the shortest synthesis yet known, (from the readily available sterol ergosterol), to substances with insect moulting hormone activity. This was exemplified by the preparations of 22-iso-ecdysone. Included in the route is a new method of ring-opening 3α, 5α-cyclo-6-keto steroids directly to a 2-ene derivative. A novel method of introducing a 14α-hydroxy group into a 7-en-6-one steroid is outlined, as is a hitherto unknown method of simultaneously introducing 7-ene and 14α-hydroxy functions into a 6-keto steroid.

Section two deals with efforts directed at introduction of the C-20 hydroxy group, into a suitable precursor made available from the earlier work, in order that a route may become available to the C-20 hydroxylated insect moulting substances.

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P A R T I

REVIEW: THE CHEMISTRY OF INSECT MOULTING HORMONES

SECTION I: Introduction

It is only thirty years ago that the existence of insect hormones was firmly established when the early work of Kopec was repeated by Bounhiol and Kuhn and further confirmed by the indpendent work of Wigglesworth. Later work by Karlson and others showed that insect hormones were primarily involved in the regulation of postembryonic development. (For a review of the literature up to 1966 see reference [1]). This development takes place through several instars, (the larval state of the insect), the change from one instar to the next involves moulting of the old chitinous exoskeleton and subsequent formation of a new one.

By a series of biological transplantation experiments (for early reviews see references [2,3]) the sequence outlined in Fig. 1 was established. The adenotropic or brain hormone stimulates the prothorax to produce the moulting hormone ecdysone, which in turn induces moulting in the larvae or pupae. Simultaneously, (in the case of a larval moult), the corpora allata secretes the juvenile hormone which guarantees the larval character of the moult. In the absence of the juvenile hormone the larvae undergoes a pupal or 'imaginal' moult.

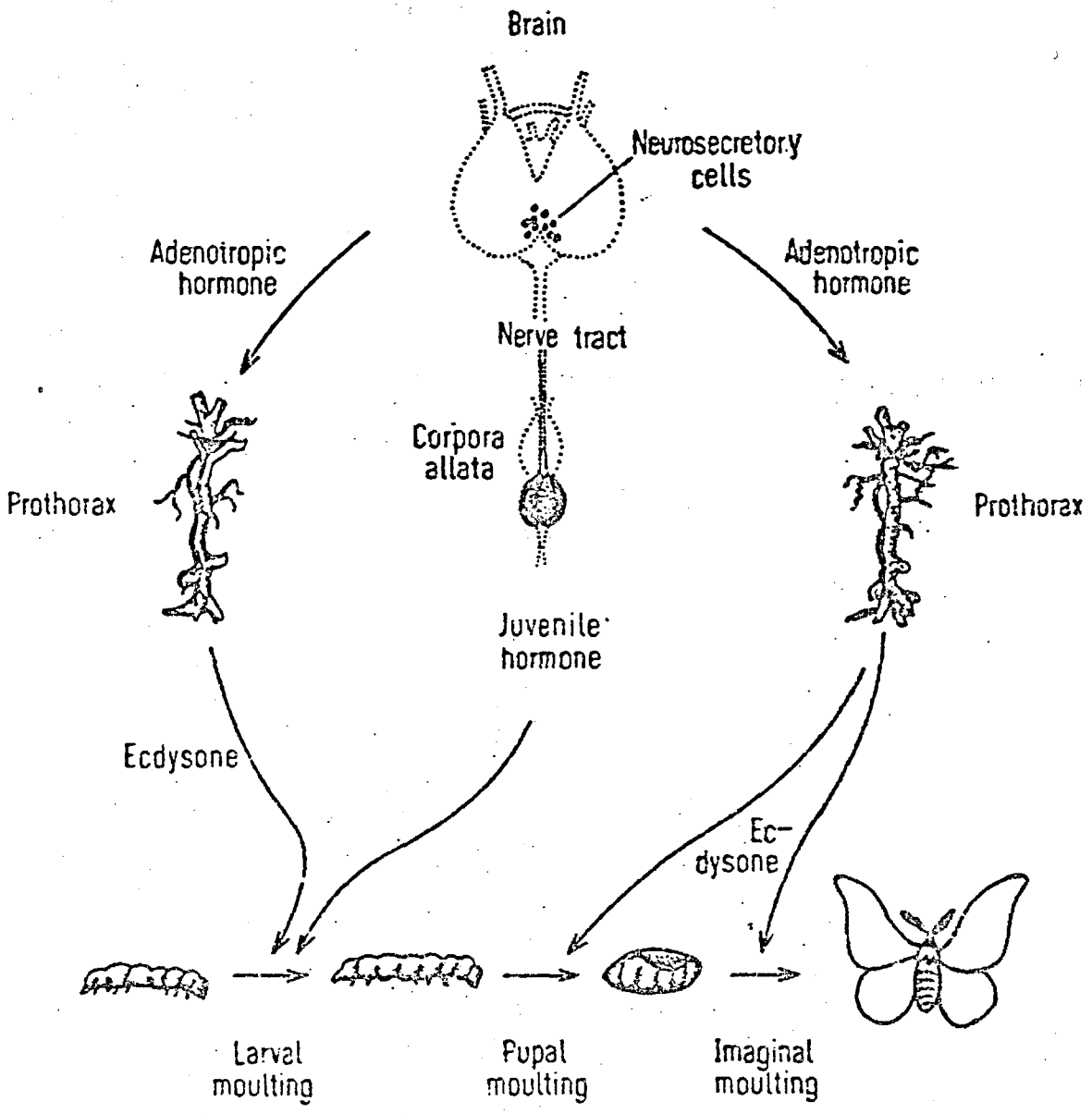

Brain
Neurosecretory cells
Adenotropic hormone
Adenotropic hormone
Nerve tract
Corpora allata
Prothorax
Prothorax
Juvenile hormone
Ecdysone
Ec-dysone
Larval moulting
Pupal moulting
Imaginal moulting

Fig. 1.

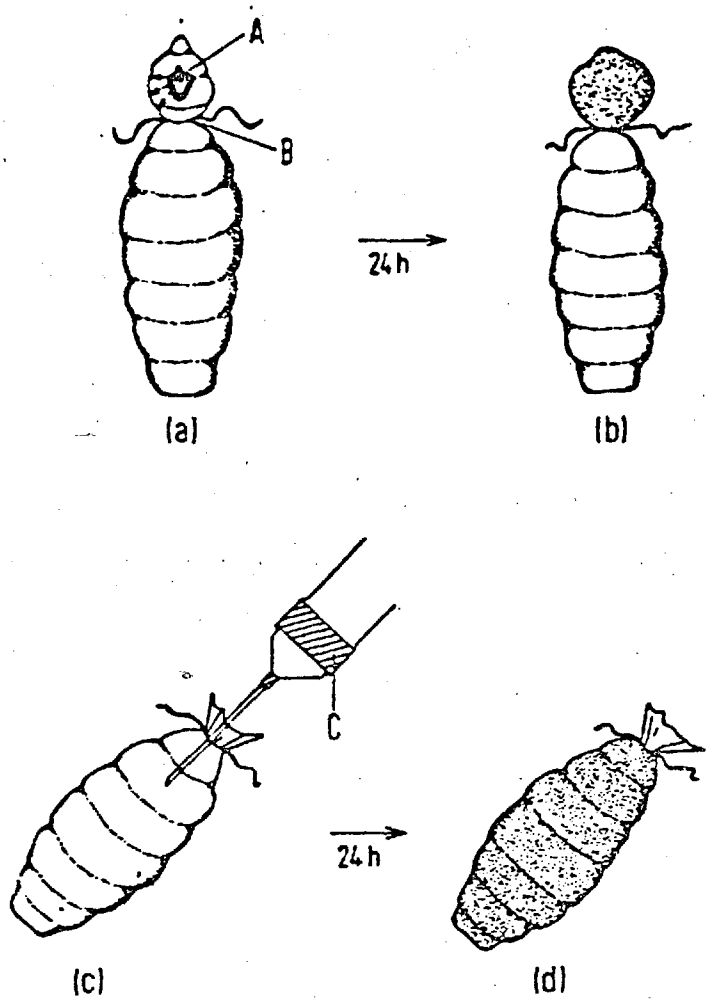
It was not until the "Calliphora test" bioassay of Fraenkel was available that it was practical to attempt an isolation of the pure moulting hormone. Using a modified Calliphora bioassay the first cyrstalline sample of ecdysone was obtained by Karlson[4] in 1954.

The bioassay used is of some interest, and is outlined diagrammatically in Fig. 2. The larvae are ligatured (a) hence the hormone produced in the anterior portion is unable to diffuse into the posterior portion and only the anterior part forms a puparium (b). After injection of the hormone (c) the hind part is induced to puparium formation (d).

The structural determination of the moulting hormone will be discussed in detail below. It is of some interest, however, to note some of the biochemical results which have been reported. Using tritium labelled cholesterol Karlson and Hoffmeister[5] managed to demonstrate that ecdysone was derived from cholesterol.

The mechanism of action of ecdysone has been studied and the conclusions reached are that the hormone is responsible for stimulating the direct production of messenger R.N.A. and hence protein synthesis. (Recent reviews give detailed discussions on this work [6, 7]).

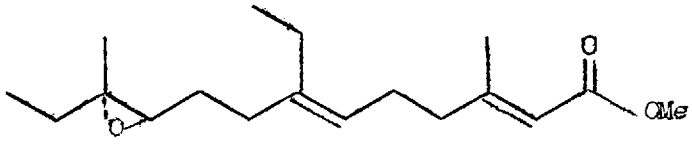
Finally, in this brief introduction, it should be noted that the structure of the juvenile hormone has recently been shown to be methyl - 7-ethyl-3,11,-dimethyl-10-epoxy-2, 6-tridecanoate (1) by the elegant work of Roller et al.[8]



Bioassay for ecdysone on the blowfly *Calliphora erythrocephala* ('*Calliphora* test').

A = endocrine gland
B = ligature
C = syringe with hormone solution.

Fig. 2.



(1)

SECTION II: Occurrence and Structural Determinations

Since the publication of the structure of ecdysone[9] there has been a growing interest in the sphere of steroidal insect hormones. This interest is undoubtedly due to the potential industrial applications of such compounds as insecticides, and, as shown more recently, many possess potent anabolic activity [10]. Additional screening of these compounds could well lead to the discovery of further physiological properties. Further, the isolation of relatively large amounts of steroidal compounds which showed insect moulting hormone activity from the leaves of Podocarpus nakaii HAY by Nakanishi[11], has added further impetus to this field. Subsequently a large number of plants have been screened for these compounds by various groups, resulting in the isolation of many different, but related compounds.

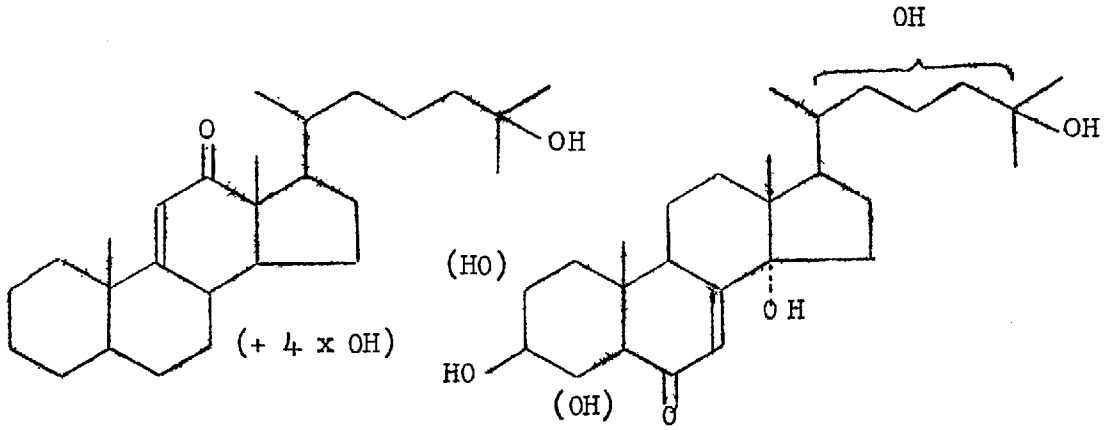
Several other active compounds of the same type have been isolated in small amounts from various insect sources.

These compounds will be briefly reviewed in two groups,

those isolated from insect sources, or Zooecdysones, and those isolated from plant sources, or Phytoecdysones.

Zooecdysones.

a) Ecdysone (4). This was the first steroidal insect moulting hormone to be isolated in crystalline form, achieved by Butenandt and Karlson[4] in 1954, from the pupae of the silkworm Bombyx mori L. Structural work was hampered by the small amounts of material available and chemical methods failed[12] to give the full correct structure. A tentative partial structure (2) was initially proposed and was later modified to the correct partial structure (3).



(2) (3)

A key reaction in the determination of this partial structure was the behaviour of ecdysone under mild acid conditions. Two compounds were isolated having their ultraviolet absorption maximum at 250 mμ and 290 mμ respectively. This result could only be explained by positioning the α,β-unsaturated ketone

moiety as a 7-en -6-one system with a hydroxyl group at position 14. Hence dehydration would result in two compounds being formed as indicated in Fig. 3.

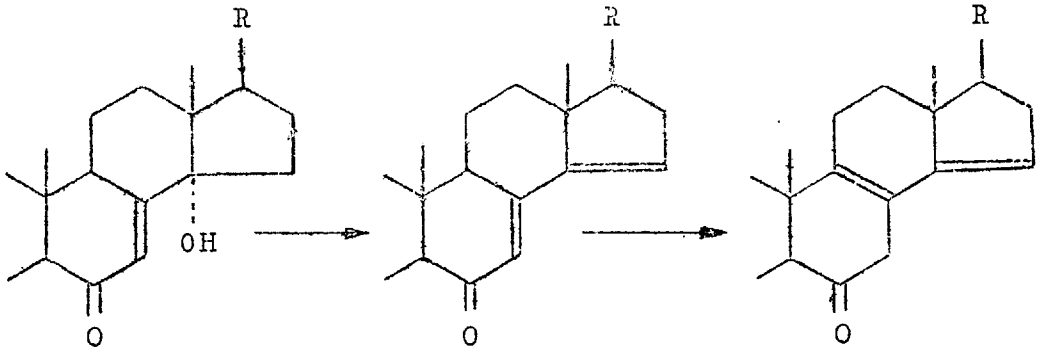
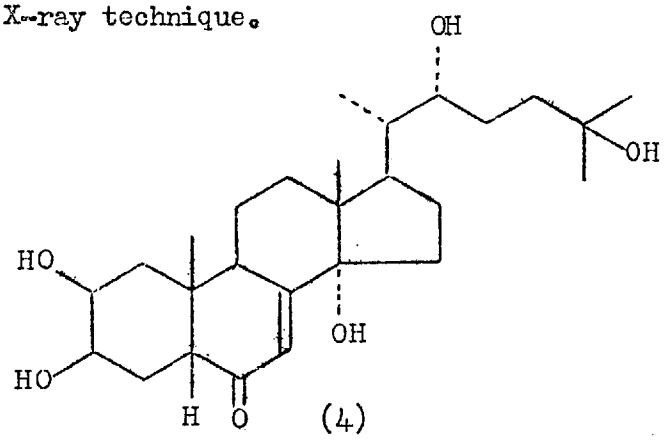


Fig. 3.

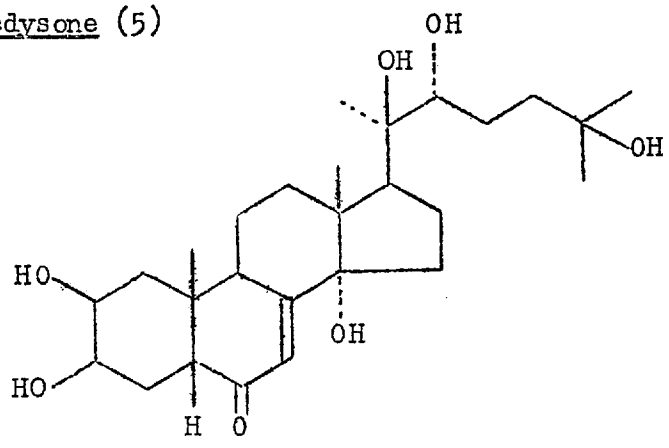
A 1, 2 - glycol system was evident from periodate oxidation studies, and was tentatively assigned as a 2,3 - glycol or 3,4 - glycol on biogenetic grounds. The remaining hydroxyl was thought to be located on the side chain (along with the 25 hydroxyl group indicated by n.m.r. studies). The full structure was finally elucidated as 2β, 3β, 14α, (22R), 25, pentahydroxy 5β, cholest -7-en -6-one (4) by Huber and Hoppe[13] using a new diffuse scattering X-ray technique.



(4)

Subsequently the hormone has been isolated from the adult Moroccan locust (Dociosturus moroccanus Thunberg),[14] and also the tobacco hornworm (Manduca sexta Johannson)[15].

b) Crustecdysone (5)



(5)

This compound was first isolated from Bombyx mori. L in 1956 by Karlson[3] and named β-ecdysone.

Some confusion arose in the literature when the same compound was isolated from B. mori L. independently by two other groups who named it as 20-hydroxy-ecdysone[16] and ecdysterone[17]. Careful comparative studies have shown these compounds are in fact identical[18] and are also the same as an active compound isolated from the crayfish (Jasus lalandei) by Hampshire et al.[19] which they named Crustecdysone.

The compound has also been isolated from D. moraccanus Thunberg,[14] M. sexta Johannson[15] and Antherea pernyi[20].

The structure of the compound was determined by a combination of chemical and physical methods.[19] The mass spectrum contained the parent ion peak at m/e 480 showing that crustecdysone contained

one more hydroxyl group than ecdysone. As the compound had similar biological activity to ecdysone it was assumed that the two structures were closely related. This was confirmed by the ultraviolet and infrared spectroscopic data which indicated the presence of an α,β-unsaturated carbonyl chromophore. Furthermore, after brief treatment with acid, the ultraviolet spectrum altered to give the characteristic maxima at 248 and 295 m μ which had been observed for ecdysone after similar treatment.

The n.m.r. spectrum of both ecdysone and crustecdysone were shown to be similar, but differences arose in the position of the signals due to the 18 and 21-methyl groups. The 18-methyl signal was shifted downfield by 0.48 p.p.m. and the 21-methyl signal occurred as a singlet at 1.56 p.p.m. as compared to a doublet at 1.28 p.p.m. which suggested the additional hydroxyl group may have been located at C-20. Studies on suitable model compounds tended to confirm this assignment.

Further, the mass spectrum of crustecdysone showed strong peaks at m/e 81 and 99, both of which occur in the spectrum of ecdysone and indicate that C-20, C-22 bond fission of the side chain has taken place. The strong peak at m/e 363 confirmed that this bond fission had occurred, and as expected from the cleavage of a vicinal diol, no hydrogen transfer had taken place as is seen in the spectrum of ecdysone. The fragmentation is summarised in Fig. 4.

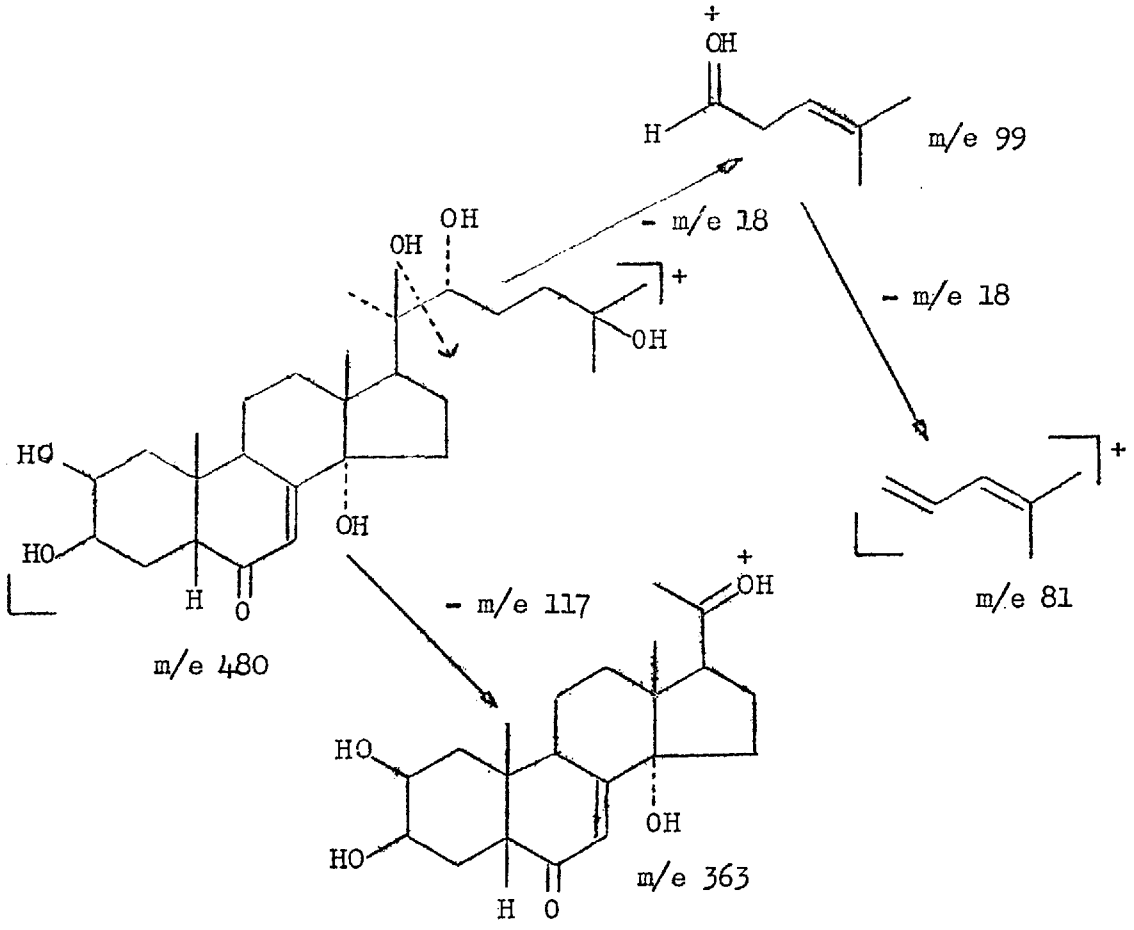
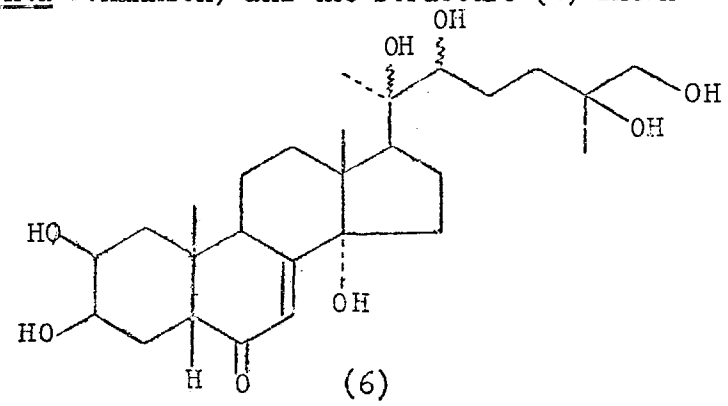


Fig. 4.

On biogenetic grounds the stereochemistry at C-20 was designated as 20R since the biogenetic C-20 hydroxylation of cholesterol is known to take place with retention of configuration[21] and, furthermore, by analogy with ecdysone, the 20R, 22R, stereochemistry is suggested. Final proof of the structure came by synthesis of the hormone,[22, 23] which is discussed further below.

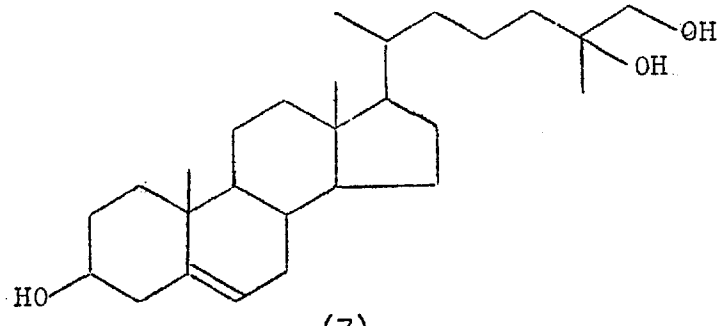
c) 20,26-Dihydroxyecdysone (6)

This hormone was isolated from the tobacco hornworm (Manduca sexta Johannson) and the structure (6) shown was proposed.[24]



(6)

The presence of an additional hydroxyl group was first suggested by the T.L.C. behaviour of the compounds when compared with crustecdysone. The ultraviolet spectrum of the new compound, both before and after treatment with acid, showed the characteristic behaviour of ecdysone and hence the presence of both the 14-hydroxyl and 7-en -6-one moieties. The n.m.r. spectrum agrees well with that of crustecdysone with the exception that the signal due to the C-26 (27) protons was moved down field indicating the additional hydroxyl group was located at C-26 (27). Furthermore, the position of the C-26 (27) protons in the model compound (7) agreed well with the position found for these protons in the new compound.



(7)

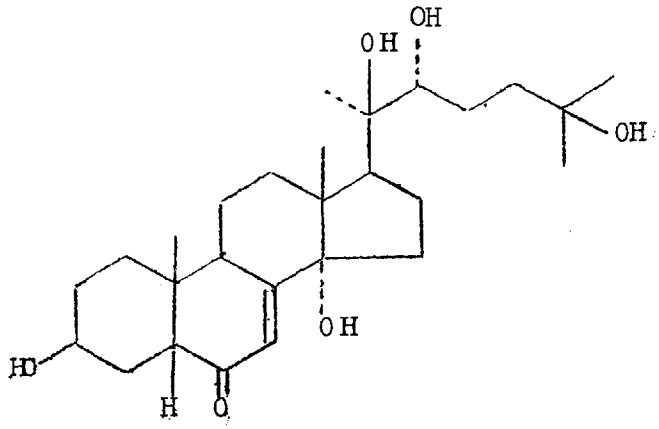
Finally, the mass spectral data supported the structure and showed the same C-20, C-22 bond cleavage, without rearrangement, that occurs in crustecdysone. The fragmentation pattern also confirmed the location of the additional hydroxyl group as being in the side chain. An intense peak at m/e 31 due to the ion CH_2OH^+ was also observed. This peak is characteristic of a 1, 2-glycol and further supported the assigned structure.

It should be noted that a less polar isomer of 20, 26-dihydroxyecdysone was also isolated from the same insect and it was tentatively formulated as the 5α isomer. It is probable that the compound is an artefact as it is well known that the stabilities of the 5α and 5β epimers of this type are of the same order.

d) 2-Desoxycrustecdysone (8)

When first isolated from the crayfish (Jasus lalandei) this moulting hormone,[25] which was less polar than crustecdysone, was thought to be ecdysone. However, closer investigation showed it to be somewhat more polar. The structure was shown to be 2-desoxy-crustecdysone (8) by the usual combination of chemical and physical data.

The mass spectrum was particularly helpful in showing the desoxy nature of the tetracycle. The spectrum was similar to that of crustecdysone except that the major peaks were at 16 mass units lower in the desoxy compound. The appearance of peaks at m/e 99 and 81 and the loss of a fragment mass 117 from the parent ion showed that



(8)

the side chain was the same in both compounds (cf. Fig. 4). The n.m.r. spectrum confirmed this result, and showed that the position of the missing oxygen was most probably at C-2 or C-3. Acetylation of the material showed the presence of two readily acetylated hydroxyl groups one of which is at C-22. As the remaining hydroxyl group is in the tetracycle, and the rate of acetylation would be different depending on the orientation (axial or equitorial), a study of the rates of acetylation of this hydroxyl would decide between the C-2 and C-3 positions. In fact, when the rates of acetylation of the hormone and various suitable models was determined, the results showed that the compound, together with those models with 3 β axial hydroxyl groups had similar pseudo-first order rate constant and hence the position of the hydroxyl was assigned to the C-3 β,(axial) position showing the compound was a 2-deoxycrustecdysone. This assignment was also the most probable on biogenetic grounds.

PHYTOECDYSONES

a) Ecdysone (4)

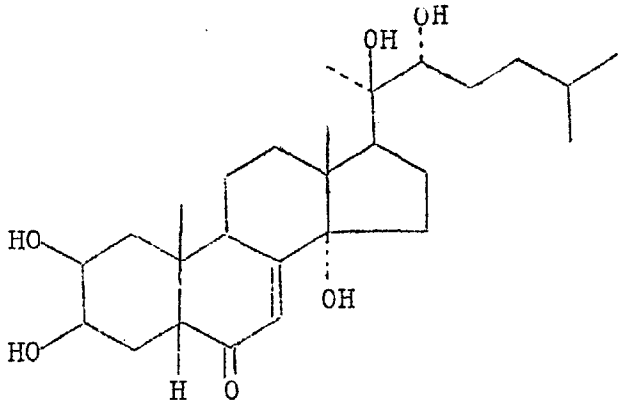
The hormone has been isolated from two plants. Hoffmeister et. al. extracted the compound from the common fern (Polypodium vulgare L.)[26] and by the usual methods showed it to be identical with the material extracted from insect sources. Kaplanis et. al. isolated ecdysone from the dead leaves of common bracken [27] (Pteridium aquilinum (L) KUHN) and also established the identity of the material by comparison with authentic ecdysone.

b) Crustecdysone (5)

This compound has been found to occur widely in plants, some of which contain considerable quantities, e.g. a figure of about 1% has been claimed by Jizba et. al. in Polypodium Vulgare L. These authors originally called the material Polypodine A until its identity was known. Other plants which have been shown to contain the hormone are: Podocarpus elatus R.Br.,[29], Pteridium aquilinum (L) KUHN[27], Achyranthis Radix[30], Achyranthis Fauriel[31], (N.B. the compound isolated from this species was originally called iso inokosterone until shown to be identical with crustecdysone)[30], Morus sp., [32] Vitex megapotamica (SPRENG)[33], Onoclea sensibilis LINNE, Lastrea thelypteris BORY, and Matteuccia struthiopteris[34] among many others. In all cases the structure of the isolated hormone was shown to be the same as crustecdysone.

c) Ponasterone A (9)

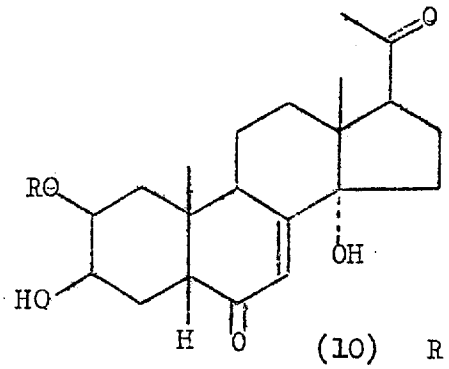
Ponasterone A was first isolated by Nakanishi et. al.[11] from the dried leaves of Podocarpus nakaii HAY. and was, together with crustecdysone, the first reported instance of the isolation of a substance with insect moulting hormone activity from a plant.



(9)

The molecular formula was established by microanalysis and from mass spectral data as $C_{27}H_{40}O_6$. Its infrared spectrum showed the presence of hydroxyl groups and an α,β-unsaturated carbonyl moiety. The preparation of various derivatives showed the presence of four hydroxyl groups, but a fifth was shown to be present by the appearance of hydroxyl absorption in the infrared spectrum of the diacetonide. Two α-glycol systems were shown to be present by the consumption of 1.9 moles of periodate by the compound. This experiment also established the nature of the side chain, (by isolation and characterisation of iso-hexanal as the 2, 4-dinitrophenyl-hydrazone), which was confirmed by the mass spectral data (see p.11). N.m.r. data suggested the nature of the tetracycle, which was further confirmed by ultraviolet spectral data on the compound after acid treatment (c.f. ecdysone p. 8). Final proof of the structure was obtained in two ways. Firstly the 2-acetoxyl derivitive when oxidised by sodium periodate[35]

gave a methyl ketone (10, R = Ac), which on mild alkaline hydrolysis yielded the known methyl ketone (10, R = H) which had been independently synthesised.[36]



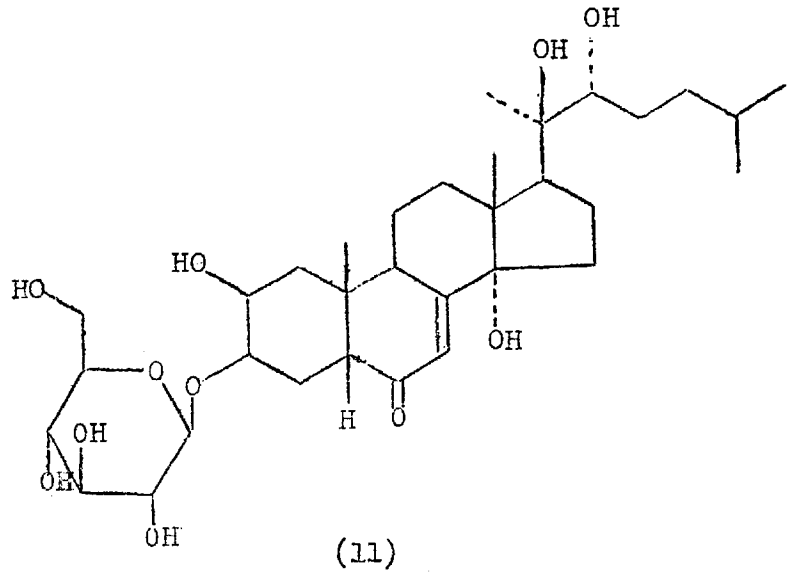
(10) R = H
R = Ac

Finally the stereochemistry of the side chain was established as 20R, 22R, by synthesis[37] (see below).

d) Ponasteroside A (11)

Recently the first glycoside of an insect moulting hormone was isolated from Pteridium aquilinum var. latiusculum.[38] This compound was named warabisterone, but was subsequently shown to be a glucoside of ponasterone A (9) by enzymatic hydrolysis to yield glucose and ponasterone A and hence the name was changed to ponasteroside A [39] (11).

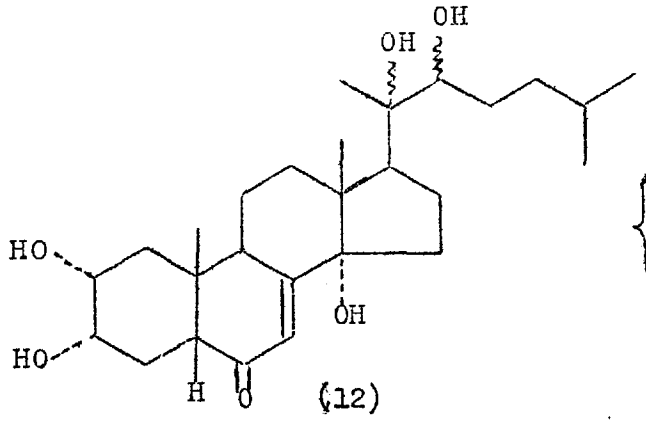
The position of the glycoside link and its β nature were deduced by detailed analysis of the n.m.r. spectrum of the hexacetate of ponasteroside A and by comparison with the n.m.r.



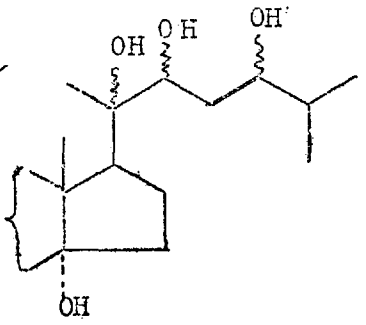
(11)

spectrum of stigmastanyl -β-D-glucoside tetraacetate.

e) Ponasterones B (12), and C (13)



(12)



(13)

Both of these compounds were isolated, along with ponasterone D, in small quantities from Podocarpus nakaii HAY.[11]

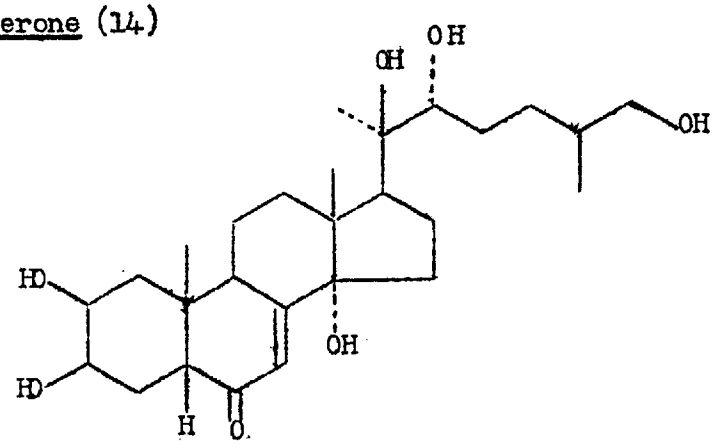
The presence of the 2, 3-cis glycol and 7-en -6-one moieties in both of these compounds were indicated by detailed analysis of the n.m.r. data obtained from suitably acetylated derivatives. The presence of the 14α-hydroxyl group was shown by the methods already discussed. The evidence for the side chain structure in

ponasterone C (13) was obtained by the isolation of trans-iso-hexanal as the 2, 4 - dinitrophenylhydrazone after periodate oxidation, dehydration having occurred after cleavage of the α-glycol linkage.

In the case of ponasterone B (12) the close similarity of the mass spectrum with that of ponasterone A indicated that the two compounds were merely configurational isomers. This was confirmed by the isolation of iso-hexanal after periodate oxidation of ponasterone B (12).

The A/B cis ring fusion was indicated by O.R.D. studies, the curves obtained from both ponasterones B and C were very similar to those obtained from other A/B cis steroidal insect hormones. Finally the 2α, 3α cis glycol function was established by infrared hydrogen bonding studies together with the n.m.r. data obtained from the acetylated 2α, 3α,- derivatives[40]. The absolute stereochemistry at C-20 and C-22 has not been firmly established but is likely to be the same as that of ponasterone A, i.e. 20 R, 22 R since in each the chemical shifts of the C-18 methyl and C-21 methyl groups are similar.

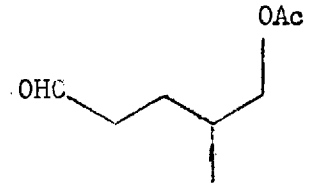
f) Inokosterone (14)



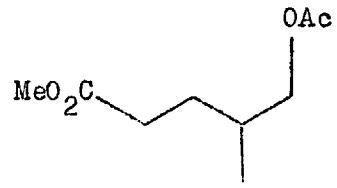
(14)

This hormone has been isolated from Achyranthes fauriei Léveillé et Vaniot by Takemoto et. al.[30] The structure was established by the usual methods. The absolute stereochemistry was established as follows.[41] Acetylation of inokosterone yielded the 2,22-diacetate which on periodate oxidation gave the known methyl ketone (10, R = Ac).

Hydrolysis then gave the methyl ketone (10, R = H)[35] which had been independently synthesised.[36] Hence the stereochemistry of the tetracycle was proved. The other periodate oxidation product was the acetoxy aldehyde (15), which on oxidation with chromic acid, followed by methylation with diazomethane gave methyl γ-methyl-δ-acetoxyvalerate (16) as a racemic mixture.



(15)

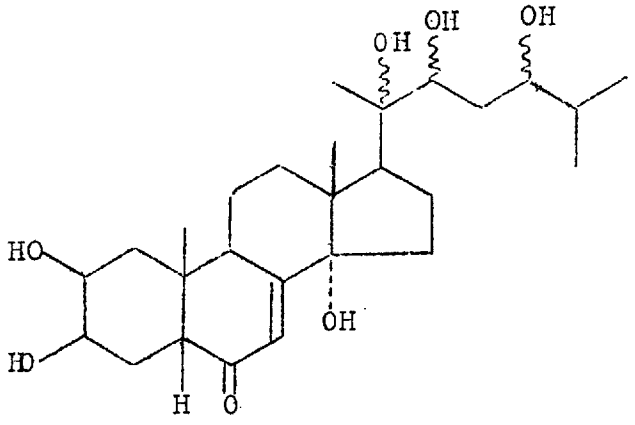


(16)

As there had been no opportunity of racemization at C_{25} during the reaction sequence the evidence suggested that inokosterone was a mixture of C-25 epimers. The absolute stereochemistry at C-20 and C-22 was shown by comparison of the n.m.r. data of inokosterone tetraacetate with that of crustecdysone tetraacetate. The chemical shifts of the C-18, C-21 and C-22 protons were very similar and hence as the stereochemistry of crustecdysone had been previously established by synthesis[22, 23], the stereochemistry of inokosterone was deduced. The inokosterone isolated had therefore been shown to

be an epimeric mixture of 2β, 3β, 14α (20 R), (22 R), 26-hexahydroxy (25 R and S), 5 β, cholest-7-one-6-ones.

g) Pterosterone (17)



(17)

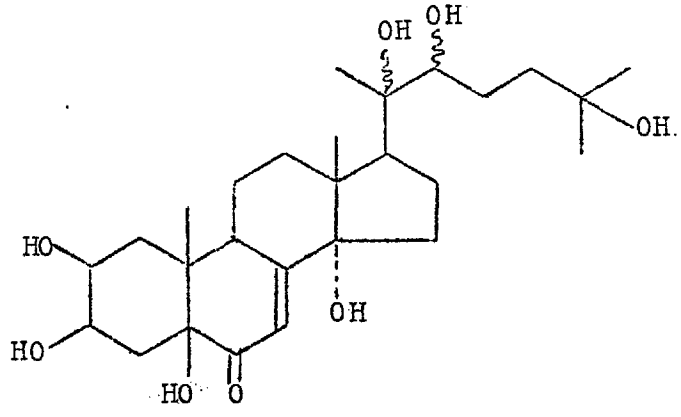
This moulting substance has been isolated from Lastrea thelypteris BORY. and Onoclea sensibilis LINNÉ[42]. It was shown to be a stereoisomer of ponasterone C (13) by periodate oxidation to give trans, iso-hexenal, identified as the 2, 4-dinitrophenylhydrazone. The nature of the tetracycle was established by methods already described. The absolute stereochemistry of the side chain is, as yet, unestablished.

h) Polypodine B. (18)

Jizba et. al.[43] isolated this active compound, together with crustecdysone, from the rhizomes of Polypodium vulgare L. as the monohydrate, and established that the molecule contained an additional hydroxyl group somewhere in the tetracycle by a combination of mass spectral and n.m.r. studies.[44] Furthermore, the n.m.r. studies revealed that the additional hydroxyl group was tertiary and hence

could only be located at C-5, 9, or 17.

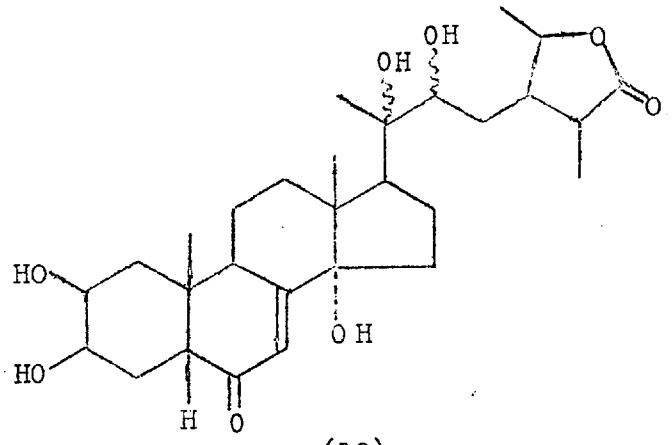
The shift to longer wave length of the carbonyl peak (by 32 cm^{-1}) in the infrared spectrum of polypodine B, as compared to crusteodysone, suggested the additional hydroxyl group was located next to this moiety in the molecule. Periodate oxidation showed the rapid uptake of two equivalents of oxidant with a slower uptake of a further equivalent. Thus C-9 can be excluded as the location of the additional hydroxyl group. No acetic acid was detected in the reaction products and hence C-17 can also be excluded. The remaining position, i.e. C-5, was confirmed as the location of the additional group by a combination of deuteration and circular dichroism studies. The structure could therefore be represented by (18).



(18)

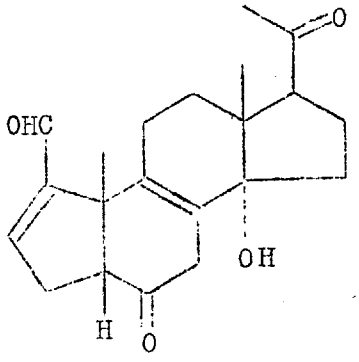
i) Cyasterone (19)

This novel compound was the first insect moulting substance isolated which was not based on the cholesterol skeleton. It was isolated by Takemoto et. al. from the roots of Cyathula capitata Moquin-Tanton.[45]

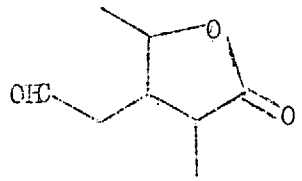


(19)

The normal nature of the tetracycle was deduced by a combination of physical data as described above, together with the preparation of derivitives of the α-glycol systems present. The infrared spectrum indicated the presence of a γ-lactone moeity in the molecule, which had to be located in the side chain, together with the two additional carbon atoms. Pericdate treatment revealed the nature of the side chain and further confirmed the structure of the tetracycle by production, (after silica gel treatment of the crude products), the known enal (20), (also isolated by an analogous treatment of crustecdysone[45]), and the unknown aldehyde (21) whose structure was fully established by conventional physical methods.

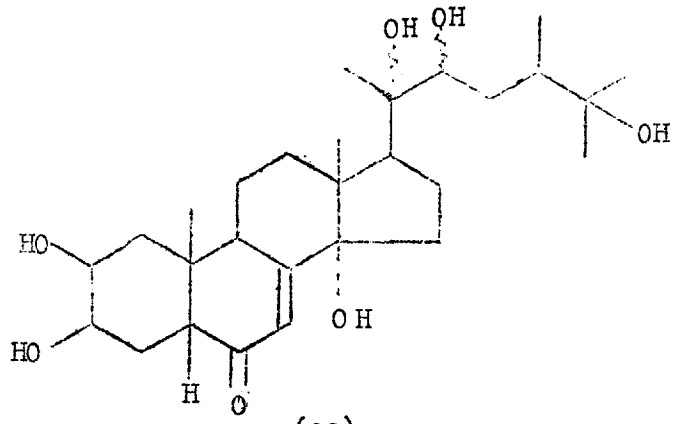


(20)



(21)

j) Makisterone A (22)



(22)

This compound, the first C-28 insect moulting substance, was isolated by Nakanishi et. al.[46] from the leaves of Podocarpus macrophyllus D. DON. together with a number of other active compounds (see below). The usual nature of the tetracycle was deduced by methods already described above.

The additional sec-methyl group, which was detected in the n.m.r. spectrum, was shown to be attached to C-24 and not C-23 by decoupling experiments.

This assignment was confirmed by the appearance in the mass spectrum of makisterone A of the base peak at m/e 70 due to a cleavage of the type shown in Fig. 5.

Peaks at m/e 131, 113, and 95, due to the normal cleavage of the side chain glycol system, also confirmed the position of the additional methyl groups as being in the side chain. The side chain stereochemistry was of course undefined as a result of these experiments.

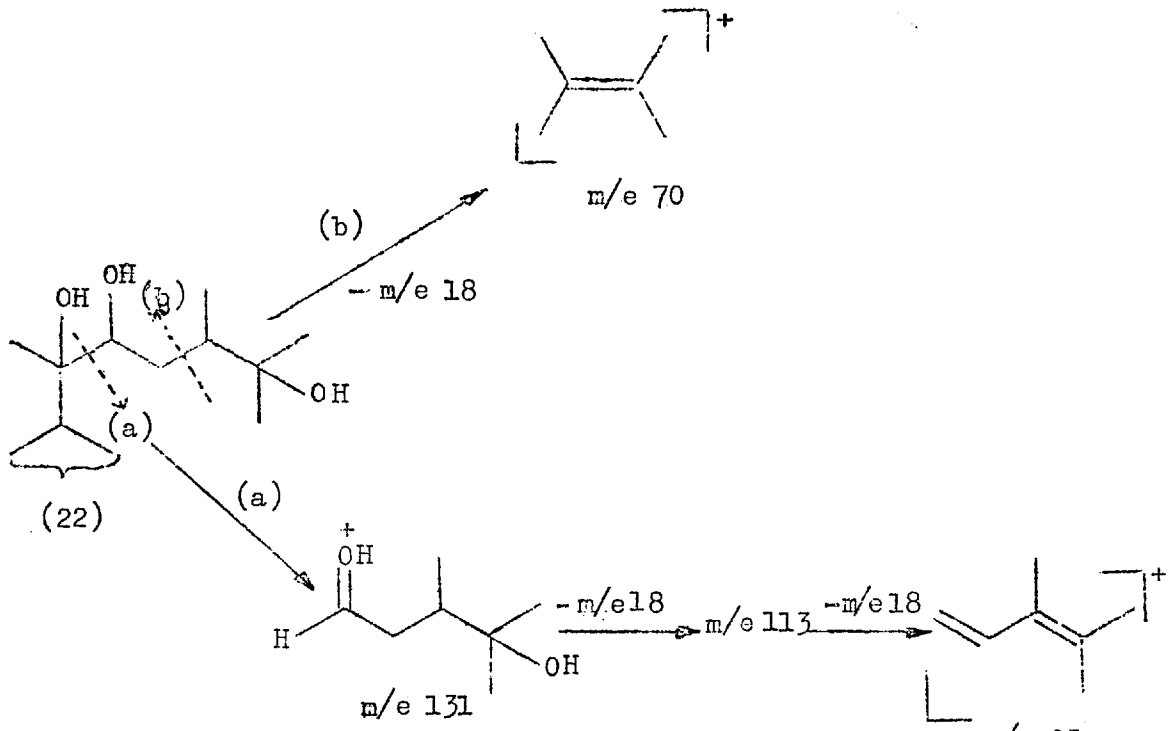


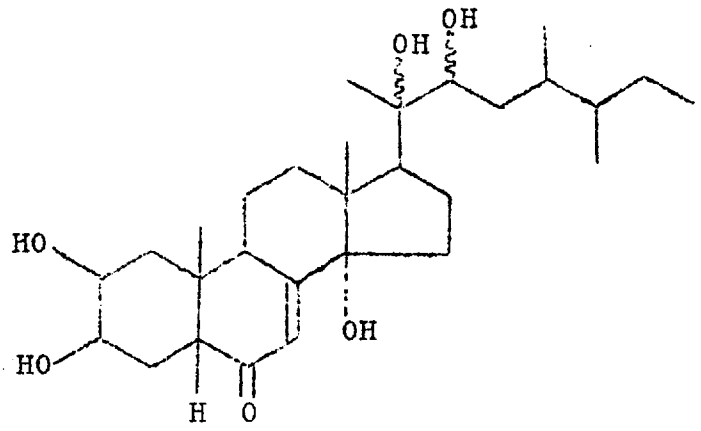
Fig. 5.

k) Makisterones B (23), C (24) and D (25).

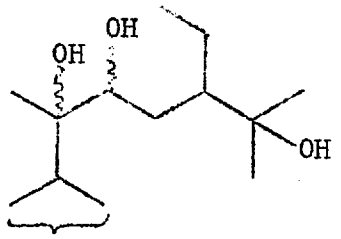
These related compounds were all isolated, together with makisterone A, from Podocarpus macrophyllus D. DON [46].

The structures of the tetracycle in all three compounds were shown to be identical by methods already described above.

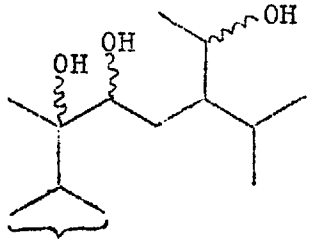
Makisterone B (23) was shown to be a side chain isomer of makisterone A (22) by mass spectral studies, and the C-26 position of the hydroxyl group was deduced by n.m.r. decoupling experiments together with the similarity of the spectrum to that of inokosterone (14). Thus the total structure can be represented by (23).



(23)



(24)



(25)

The mass spectrum of makisterone C (24) showed it to be a C-29 steroid by the occurrence of strong peaks in the spectrum at m/e 145 and 127 due to normal α-glycol cleavage of the side chain. (see Fig. 6).

In addition the appearance of a strong peak at m/e 84 indicated the position of the ethyl group was at C-24 (Fig. 6). This information, together with n.m.r. data, supported by decoupling experiments, led to structure (24) being proposed for makisterone C.

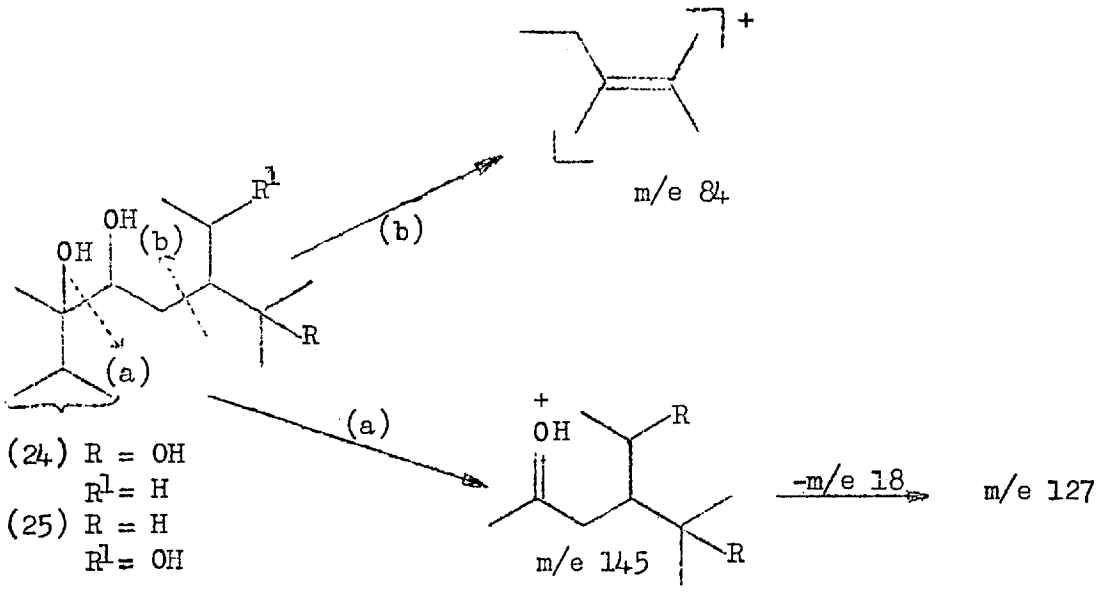
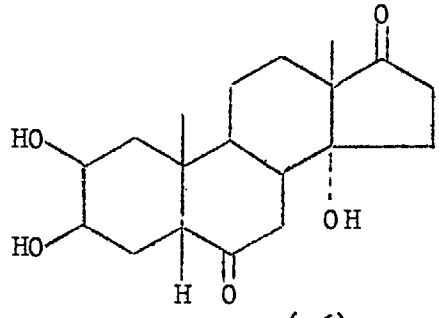


Fig. 6.

The mass spectrum of makisterone D (25) again showed strong peaks at m/e 145 and 127 (see Fig. 6) indicating that the side chain was isomeric with that of makisterone C (24). N.m.r. data showed the presence of three secondary methyl groups and hence the structure of makisterone D could only be represented by (25).[47]

Two other compounds, Podecdysone A and lemmasterone, were isolated by Horn et. al., (from Podocarpus elatus, R. Br.),[48] and Takenoto et. al., (from Lammaphyllum microphyllum),[49] respectively, and both shown by these authors to be identical with makisterone C (24).

1) Rubrosterone (26)



(26)

This C-19 steroid was isolated by Takemoto et. al. from Achyranthes rubrofusca WHITE, and has been suggested as a possible metabolite of the other insect moulting substances in plants via an analogous pathway to the metabolism of cholesterol to dehydroepiandrosterone.[50]

The structure was deduced by methods analogous to those used for the other insect moulting substances. The C-19 nature of the molecule was shown by mass spectroscopic data and microanalysis. The infrared spectrum revealed a band at 1741 cm^{-1} characteristic of a cyclopentanone carbonyl group. The n.m.r. spectrum showed only two methyl signals, confirming the absence of the normal side chain. The location of the additional carbonyl groups at C-17 followed from an examination of the ultraviolet spectrum of the acid treated compound. The two normal maxima at 241 and 295 mμ were the only bands observed showing the additional carbonyl group could not be located at C-15 or C-16. The above observations led to the structure indicated for rubrosterone (26).

The above review of naturally occurring steroids, which have insect moulting hormone activity, represents a complete literature survey to the end of August 1968.

SECTION III. Synthetic approaches to insect moulting hormones.

The publication of the structure of ecdysone[13] and the subsequent discovery of many closely related compounds naturally led to attempts at syntheses of this type of compound. Several successful attempts have been reported in the literature in recent years. It is proposed to review this work in three parts. Firstly, the work published to date on the syntheses of ecdysone. Secondly, that work that has appeared on the syntheses of the C-20 hydroxylated hormones, and finally, the synthesis of rubrosterone.

The synthesis of ecdysone.

To date three groups have succeeded in the synthesis of ecdysone by several routes. As many of the steps in these syntheses are common to each other, the synthesis of the various moieties in the molecule will be considered individually. An example of a synthesis from each group will be summarised at the end of the section.

Introduction of the 2β, 3β-diol system

Several methods have been used by the three groups of workers for the introduction of the 2β, 3β-diol function. A method common to the Syntex[51] and Hoffmann/Schering groups[52, 53] was the well known Woodward/Prevost cis- hydroxylation procedure which employs silver acetate and iodine in wet acetic acid as the reagents. Both groups had arrived at a 2-ene-6-oxo intermediate by elimination of a 3β-tosylate or mesylate. The reaction gives a 2β-acetoxy-3β-hydroxy derivative which can then be conveniently protected by acetylation or hydrolysed to the cis-glycol. An illustration of this is shown in Fig. 7.[51]

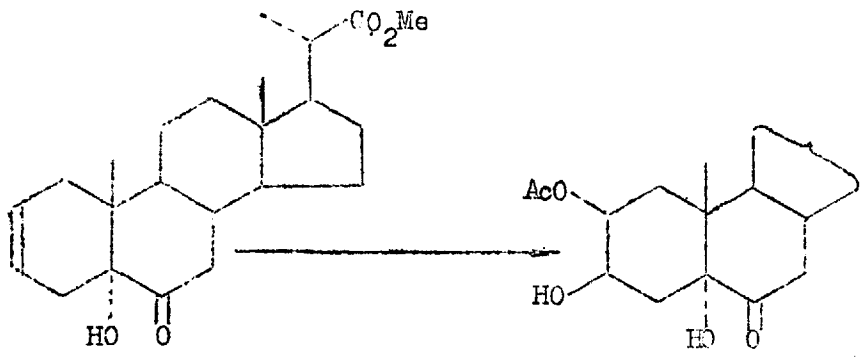
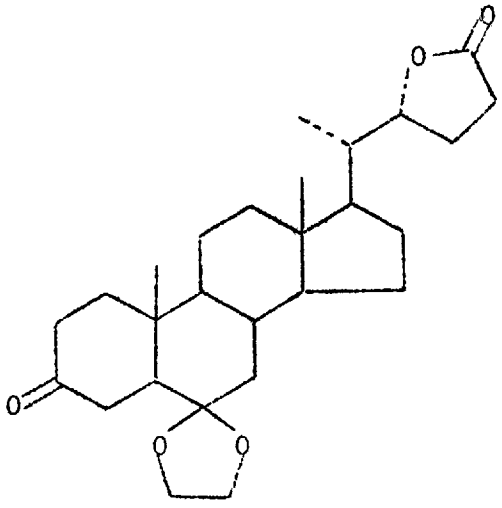
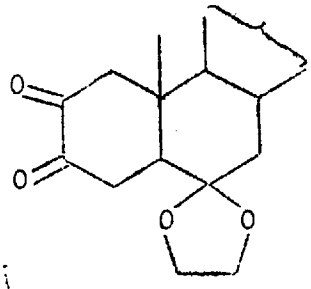


Fig. 7.

The Japanese group developed a further novel method of introducing this moiety[54, 55]. Autoxidation of the 3-ketone (27) with potassium t-butoxide in t-butanol led to a mixture of isomeric diosphenols (28), (shown for convenience as the 2,3-dione), which was reduced with sodium boro-hydride in methanol to a mixture of the various possible 2, 3-diols from which the 2β, 3β-diol was isolated in "satisfactory" yields [55].

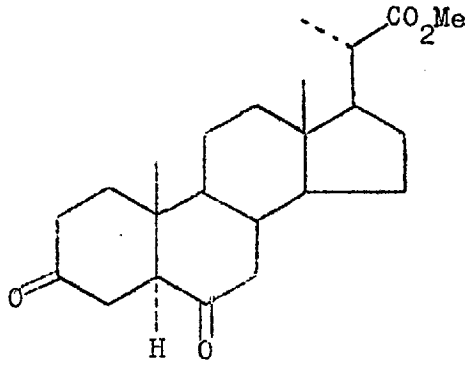


(27)

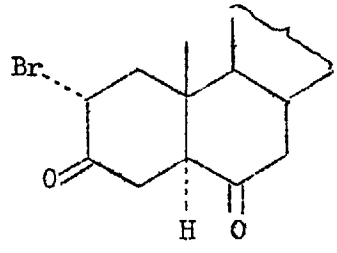


(28)

Yet another method was used by the Hoffmann group. The method involved α-face bromination at the 2 position of a 3, 6-dione (29) to give the α-bromoketone (30).

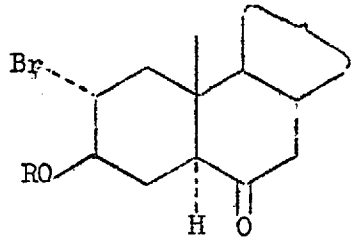


(29)

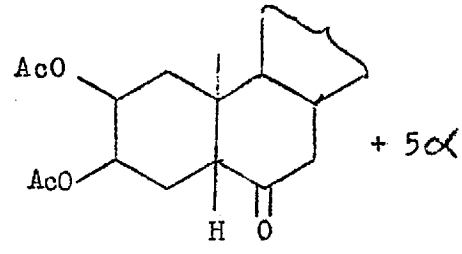


(30)

Selective reduction of the 3-oxo function gave the bromohydrin (31), R=H, which on treatment, (after acetylation (31), R=Ac), with silver acetate in acetic acid gave a mixture of C-5 epimers of the required 2β, 3β-diacetates (32)[52, 56].



(31) R = H
R = Ac



(32)

Introduction of the 7-en -6-one and 14α-hydroxy moieties

Both the Syntex and Hoffmann groups introduced the 6-oxo function by functionalisation of a 5-ene double bond. The Hoffmann group made the 5, 6-epoxide, followed by perchloric acid treatment to open the epoxide to the required 6-ketone [52,56]. (See Fig. 6)

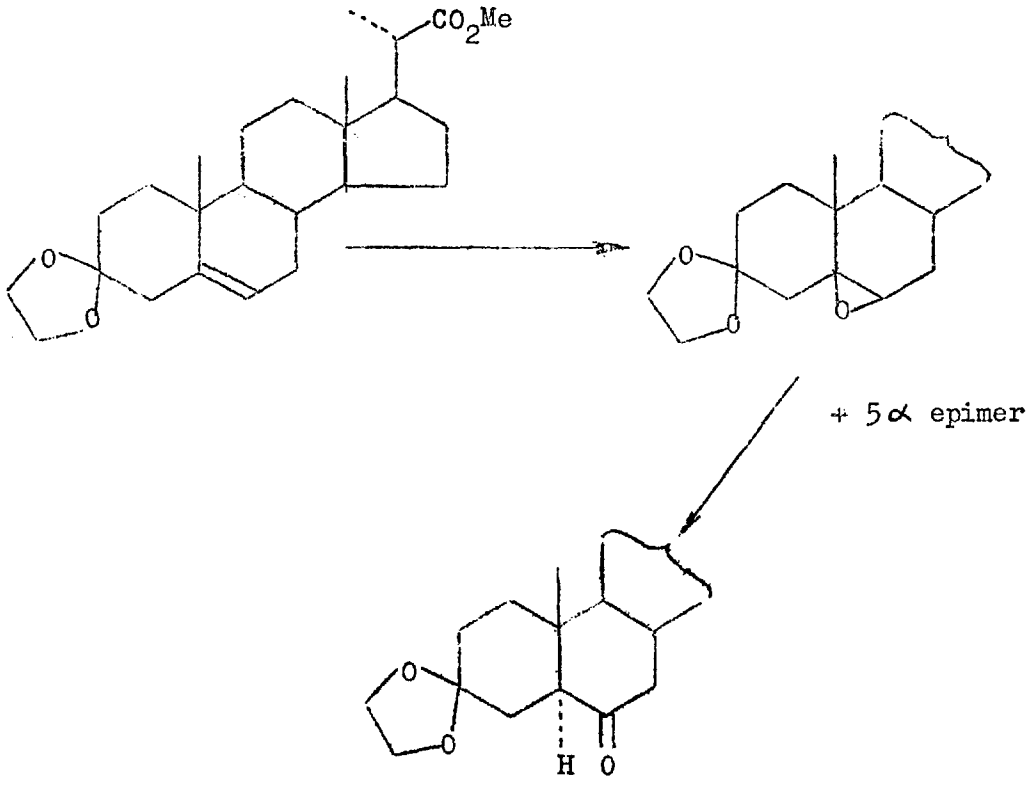
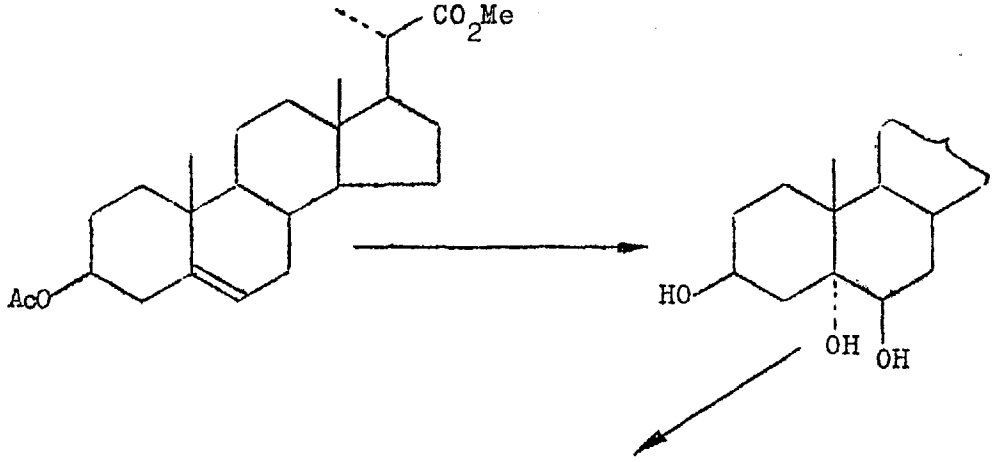


Fig. 8.

On the other hand, the Syntex group carried out a Fieser trans hydroxylation to give a 5 , 6 β-glycol which on oxidation with N-bromosuccinimide gave the required 6-ketone[51]. (See Fig. 9).



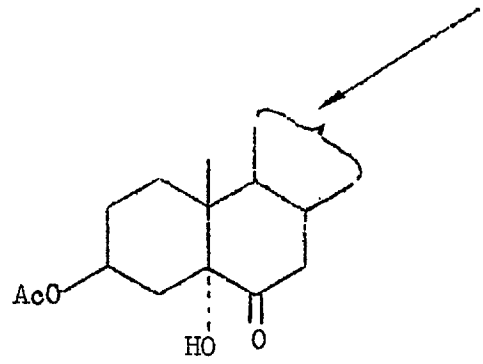
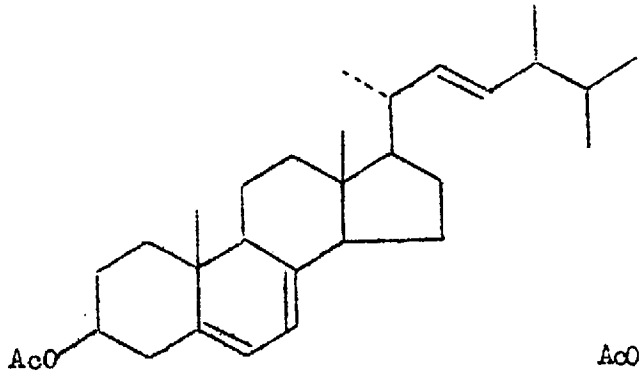
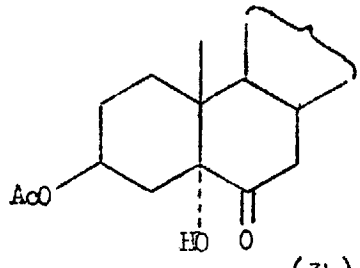


Fig. 9.

The Hoffmann group also utilised the known oxidation, with chromic acid, of the 5, 7-diene system in ergosterol acetate (33) to introduce the 6-oxo function directly into the molecule (34).



(33)



(34)

The Japanese group chose as their starting material[55] stigmast-22-en-3,6-dione and hence had no need to introduce a 6-oxo function.

All three groups used the same method for introduction of a 7-ene double bond. The method involves α-bromination of a 6-oxo steroid at the 7 position, followed by elimination of HBr under basic conditions e.g. with lithium carbonate in dimethylformamide. This sequence can be illustrated by an example from the Syntex synthesis[51]. (see Fig. 10).

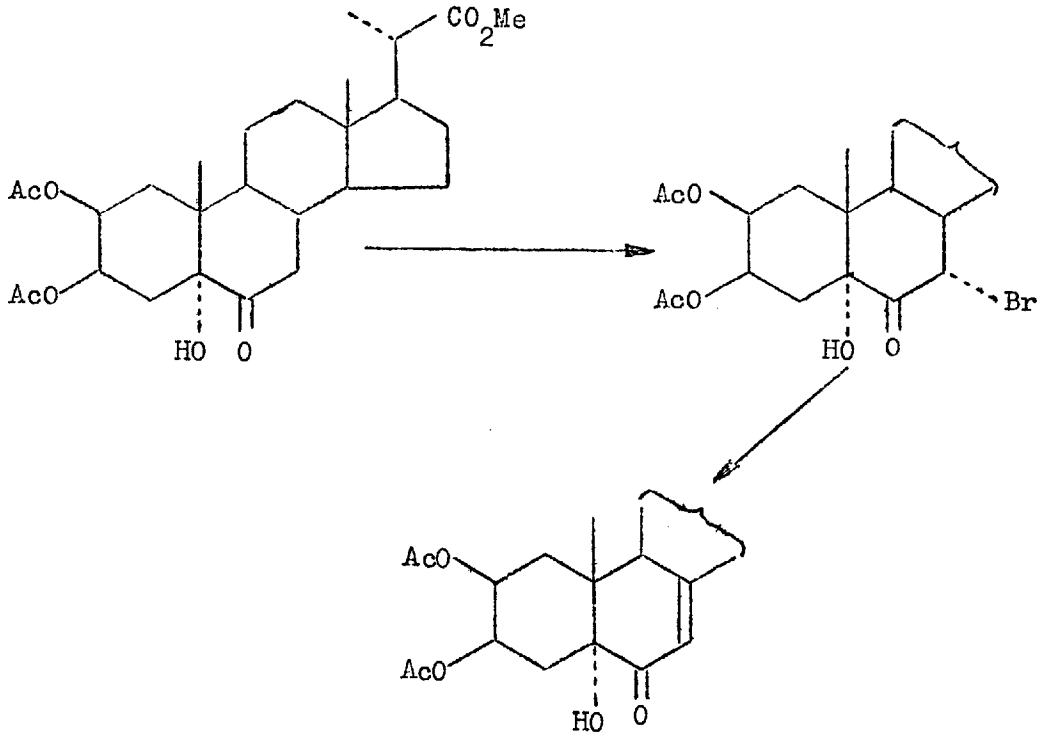
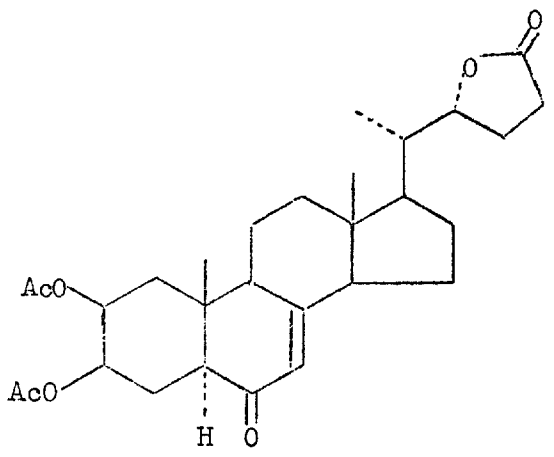


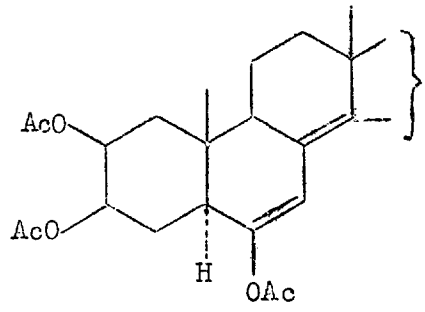
Fig.10.

The 14α-hydroxyl group was readily introduced by both the Syntex and Hoffmann groups, by the known allylic oxidation of a 7-ene system with selenium dioxide in dioxan[57].

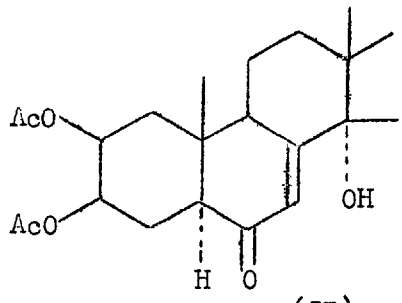
A more novel method of introducing the 14α-hydroxyl group was employed by the Japanese group. Enol acetylation of the 7-en-6-one moiety in (35) with acetic anhydride in ethyl acetate (perchloric acid catalysed) gave the dienol acetate (36), which on oxidation with monoperphthalic acid gave the required 14α-hydroxylated compound (37)[55].



(35)



(36)



(37)

This reaction has analogy in other parts of the steroid nucleus[58] but it would appear to be the first published report of its use to introduce a 14α-hydroxyl group.

Introduction of the A/B cis-fused system.

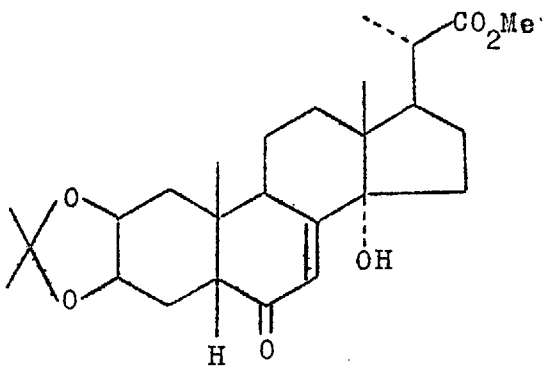
The achievement of the A/B cis-fused ring system presented no problems in any of the syntheses of ecdysone. The only requirements in order to obtain this system are that the 2, 3-cis-glycol and 6-oxo functions are present. When this is the case a simple acid or base catalysed equilibration of the A/B trans system, leads to a predominance of the required, more stable, A/B cis isomer. The reason for this is that the formation of the A/B cis fused ring system relieves a strong 1:3 diaxial interaction between

the 2 β-hydroxyl group and the 19-methyl group. The equilibrium lies, in the case of the 2β, 3β-diols or diacetates, just on the side of the 5β isomer (ca. 3:2)[51, 52, 53] but when a more bulky group, e.g. isopropylidene, is attached to the 2β, 3β-diol then the equilibrium lies even further in favour of the 5β isomer (ca. 3:1)[51]

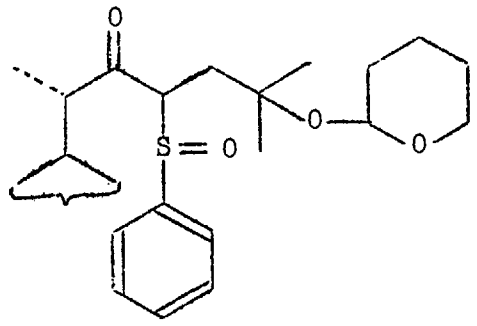
Introduction of the side chain

All the methods used for the introduction of the side chain and hence the 22 and 25- hydroxyl groups, depend on the Grignard alkylation of a suitable 22-oxygen function. All suffer from lack of stereoselectivity and lead to mixtures of C-22 epimers. The original Syntex synthesis, in fact, leads to all four possible C-20, C-22 epimers.[59]

Reaction of the ester (38) with the lithium salt of 2-methyl-4-sulphoxyphenyl butan-2-ol, tetrahydropyranyl ether, leads to the ketone (39).

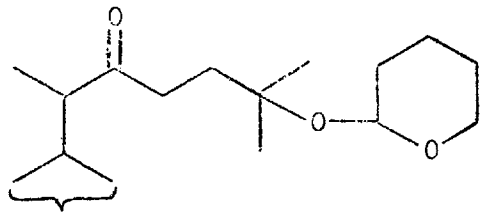


(38)



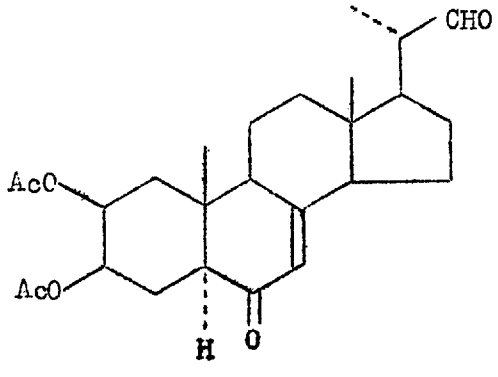
(39)

Hydrogenolysis of the sulphinyl group with aluminium amalgam then led to the 22-ketone (40), which after reduction of the ketone gave a mixture of all four possible C-20, C-22 isomeric alcohols.

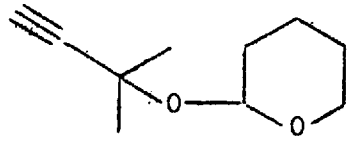


(40)

An alternative route used by both the Syntex[60] and Hoffman groups[52,53,56] was the reaction of a 22-aldehyde with the lithium or magnesium salt of 3-methylbut-1-yne-3-ol, tetrahydropyranyl ether, to give directly a mixture of the C-22 epimeric alcohols. The reaction can be illustrated by the following example from the work of the Hoffmann group.

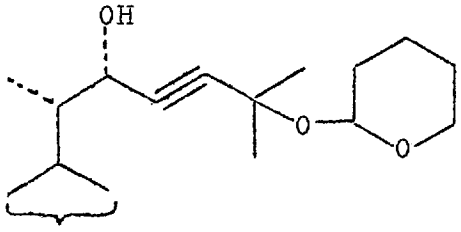


(41)

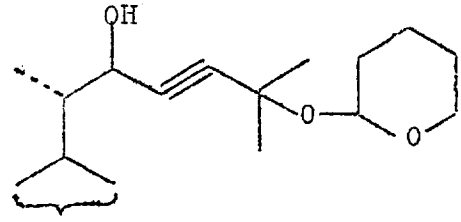


(42)

The aldehyde (41) was reacted with a Grignard solution, (prepared by the addition of the acetylenic tetrahydropyranyl ether (42) to a solution of ethyl magnesium bromide), to give a mixture of the C-22 epimers (43) and (44).



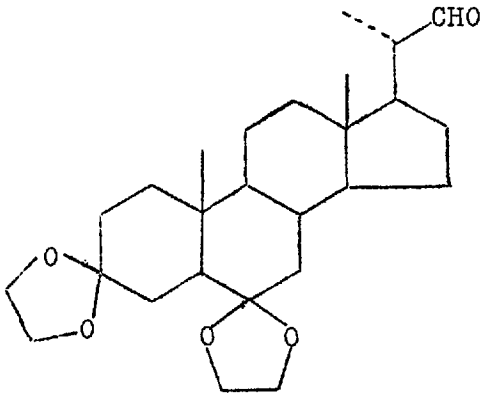
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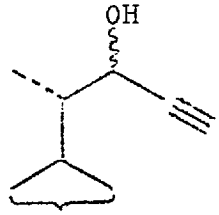
(44)

Reduction of the triple bond, and removal of the tetrahydropyranyl group with dilute acid, gave the desired side chain.

The Japanese group developed a more novel approach to a side chain synthesis.[55] The aldehyde (45) was reacted with ethynylmagnesium bromide to give the ethynyl compound (46) as a

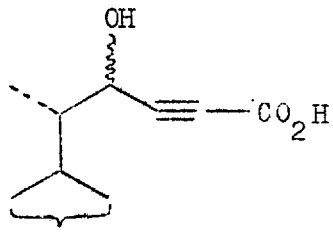


(45)

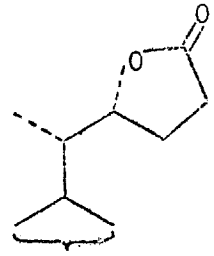


(46)

mixture of C-22 isomers, which on further treatment with methylmagnesium bromide and carbon dioxide, gave a mixture of unsaturated acids (47). The mixture of acids was then catalytically reduced to yield the lactone (48), which was readily separated from the other epimer at a later stage in the synthesis.

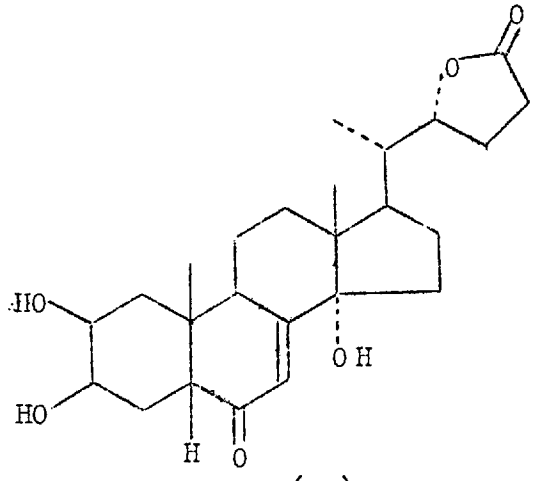


(47)

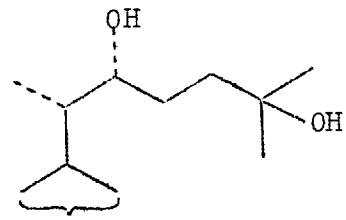


(48)

The completion of the side chain synthesis was carried out as the last step in the sequence by the reaction of the lactone (49), with excess methylmagnesium bromide at $0^{\circ}C$, to give ecdysone (4).



(49)



(4)

Summary. Three complete syntheses are outlined in the following Schemes I, II and III. The sequences were chosen to illustrate the points made in the above discussion. The shortest synthesis of ecdysone published to date is due to the Hoffmann group and utilises ergosterol as the starting material and involves 15 stages[53].

The synthesis of C-20 hydroxylated hormones

The uncertainty as to the stereochemistry at C-20 and C-22 of crustecdysone and other C-20 hydroxylated steroids led to the need for a synthetic approach which could define the stereochemistry at these points and two groups have succeeded in carrying out syntheses of crustecdysone[22, 23]. In addition one of them (Syntex) isolated ponasterone A by a change in the sequence of their synthetic pathway thus allowing the assignment of the absolute stereochemistry at C-20 and C-22 in both these compounds[22, 37].

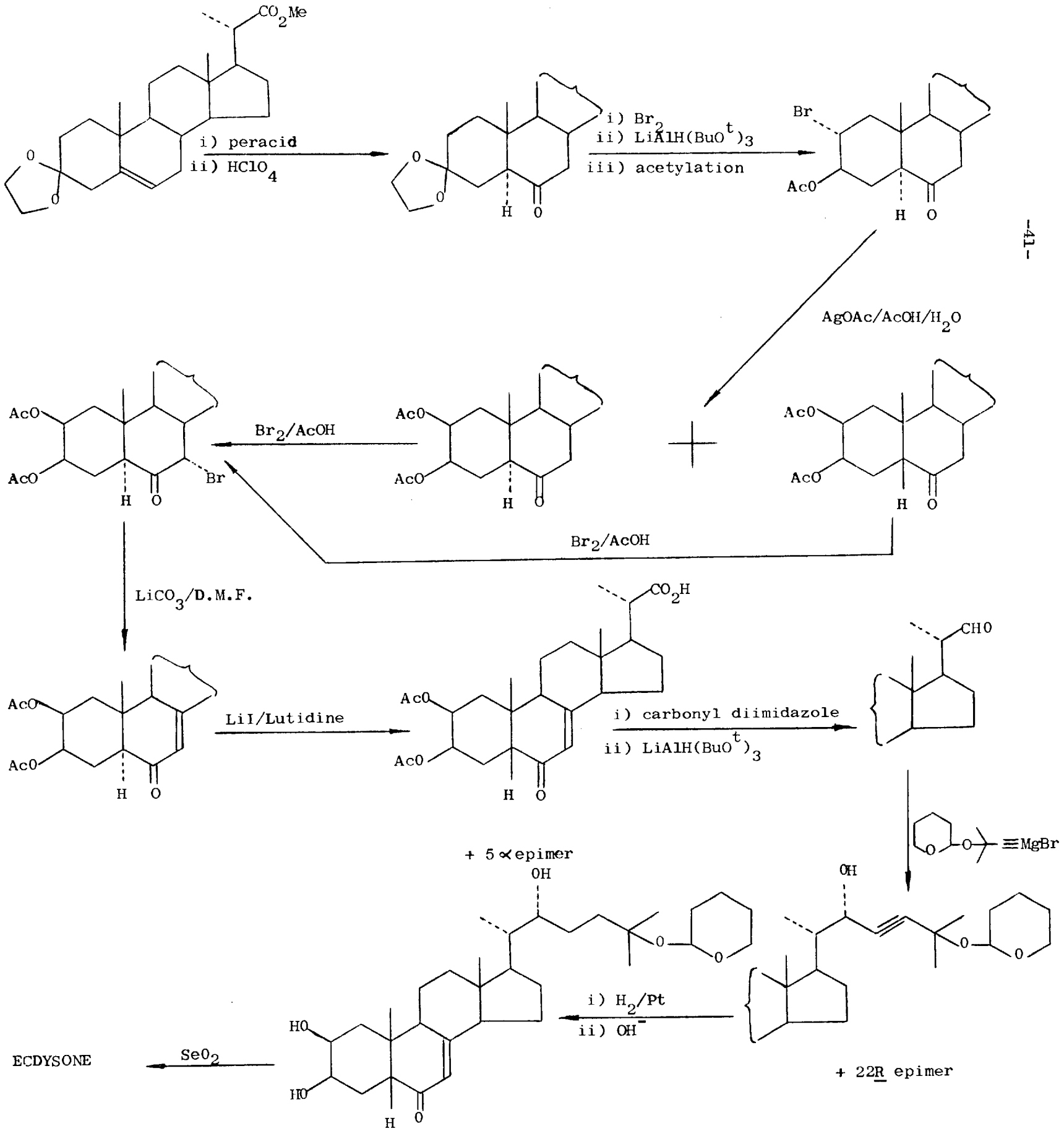
As both groups approach the key problem, i.e. the introduction of the C-20, C-22 diol system, in a different manner each will be considered separately.

The Syntex Synthesis.[22, 37]

The synthesis started with an intermediate made available during their preparation of ecdysone (see Scheme II, p. 42) namely, (20S)-2β, 3β-diacetoxy-5α-hydroxy-20-methoxy-carbonylpregn-7-en-6-one (50), which was converted into the aldehyde (51) by a series of reactions analogous to those used in their ecdysone synthesis.

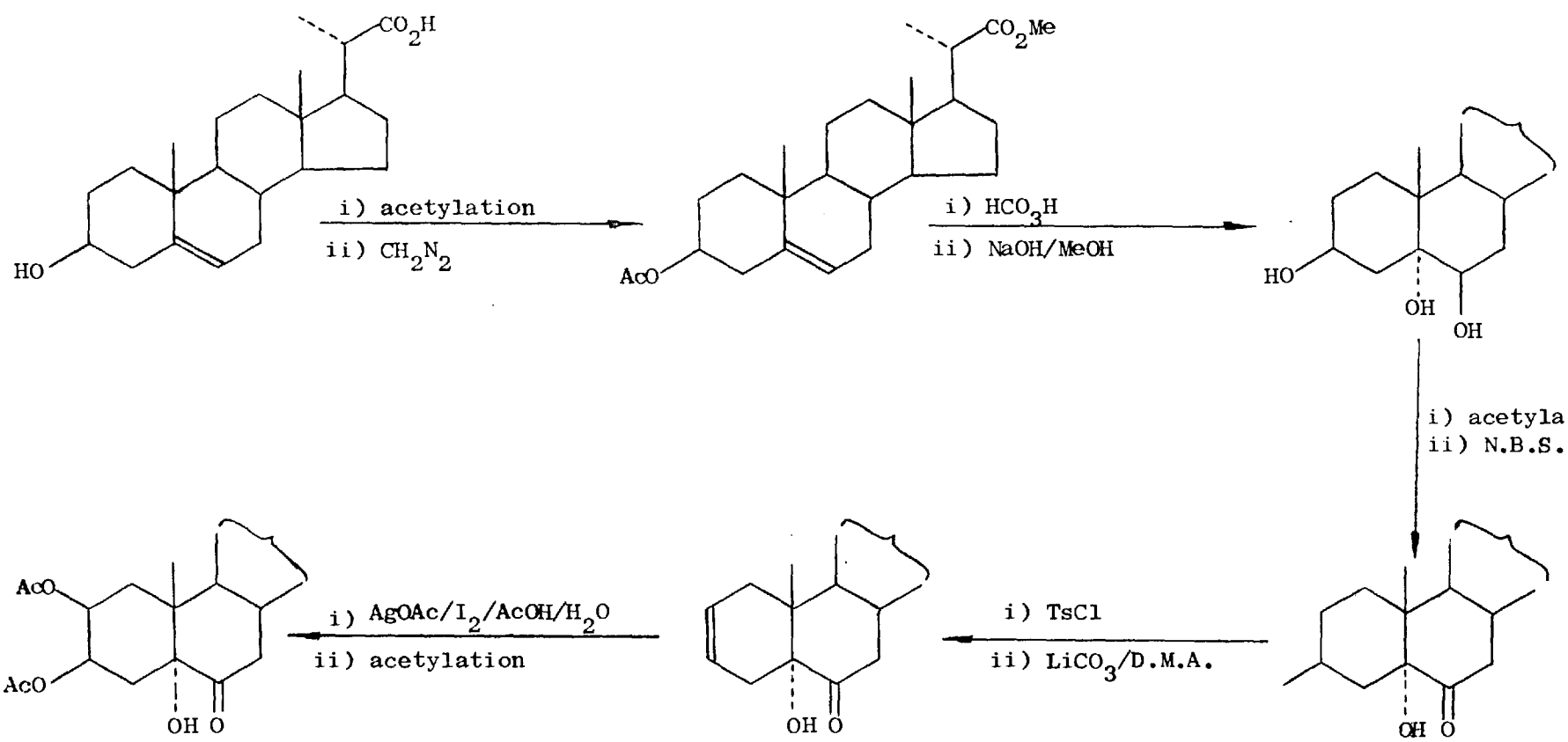
S C H E M E I

(HOFFMAN/SCHERING GROUP)



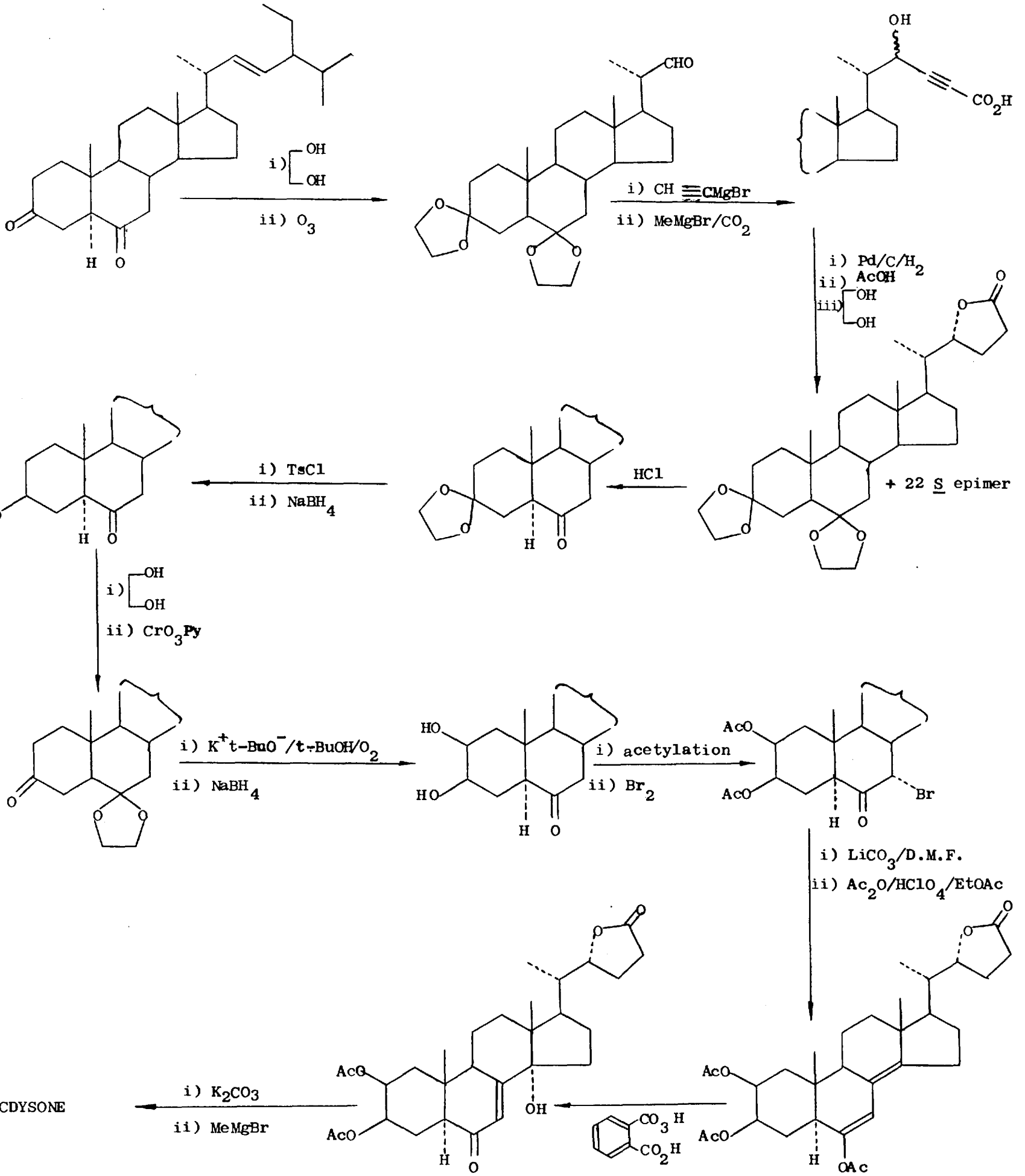
S C H E M E I I

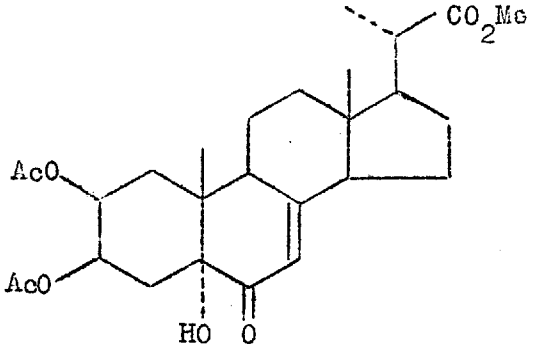
(SYNTEX GROUP)



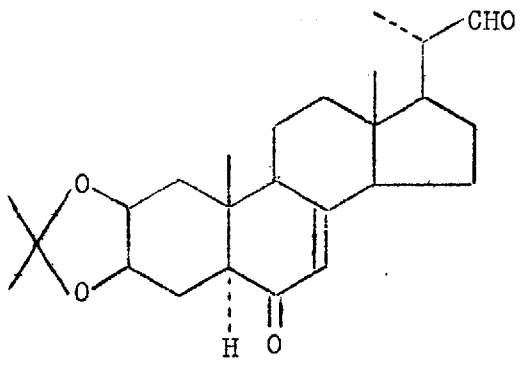
S C H E M E I I I

(JAPANESE GROUP)



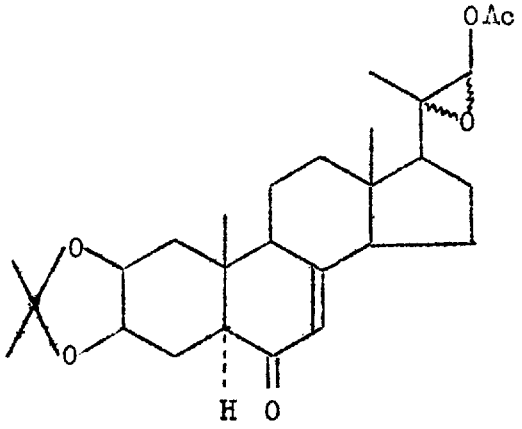


(50)

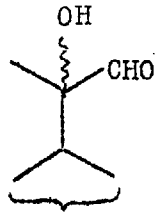


(51)

Introduction of oxygen at C-20 was effected by selective enolacetylation of the aldehyde function, followed by non stereoselective epoxidation of the enol acetate, to give an inseparable mixture of epoxides (52). Hydrolysis of these epoxides led to a mixture of separable hydroxy aldehydes (53).

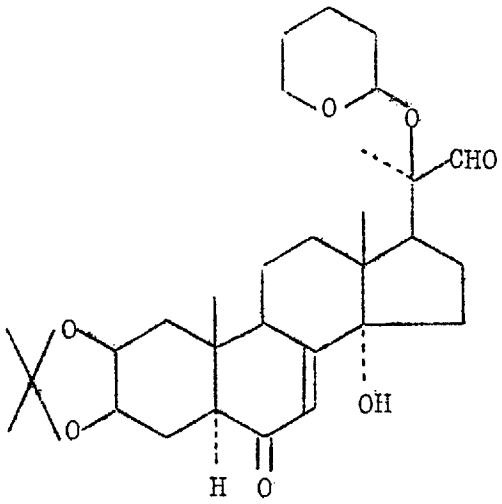


(52)

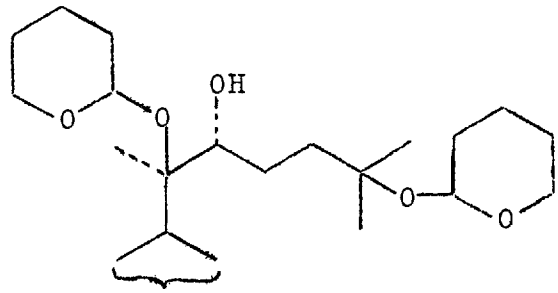


(53)

The stereochemistry of each epimer at C-20, was assigned at this point by comparison of their n.m.r. spectra with the n.m.r. spectra of 20-hydroxypregnanes of known absolute stereochemistry. After protection of the hydroxyl group of the required 20 R epimer, (as the tetrahydropyranyl ether), and introduction of the 14α-hydroxyl group with selenium dioxide, the resulting keto aldehyde (54), was selectively alkylated to yield, stereospecifically, the 22R-alcohol (55) (c.f. ecdysone synthesis above).

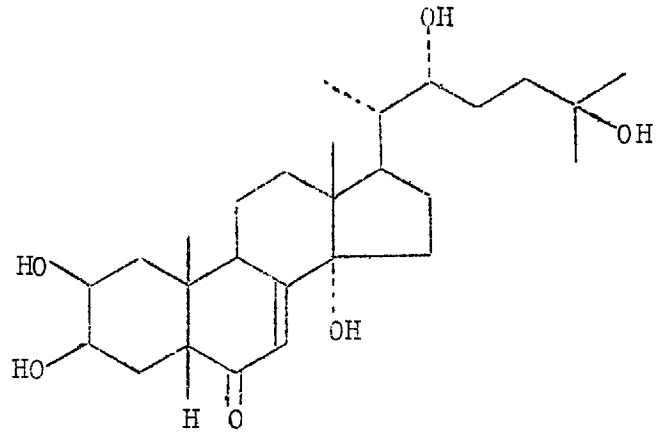


(54)



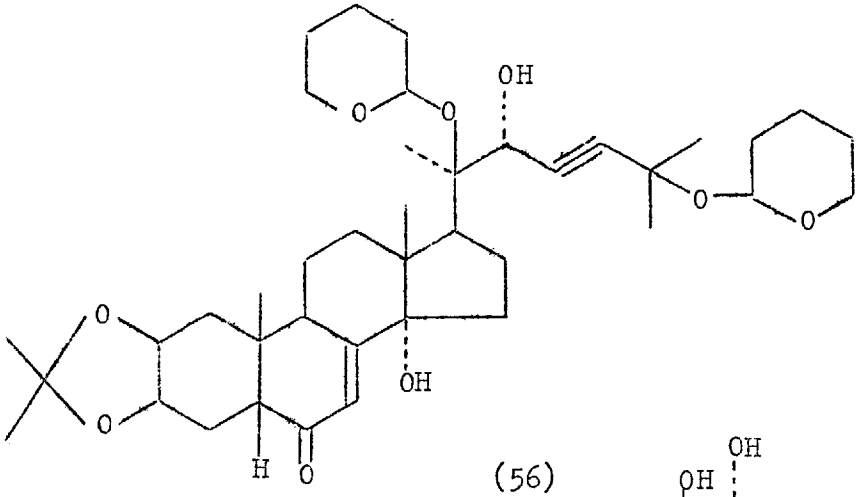
(55)

Inversion of configuration at C_5 to the desired A/B cis fused system, was carried out in the usual way with dilute base. After separation of the required 5β isomer and catalytic reduction of the triple bond, the synthesis was completed by removal of the protecting groups with dilute acid to yield crustecdysone (5).

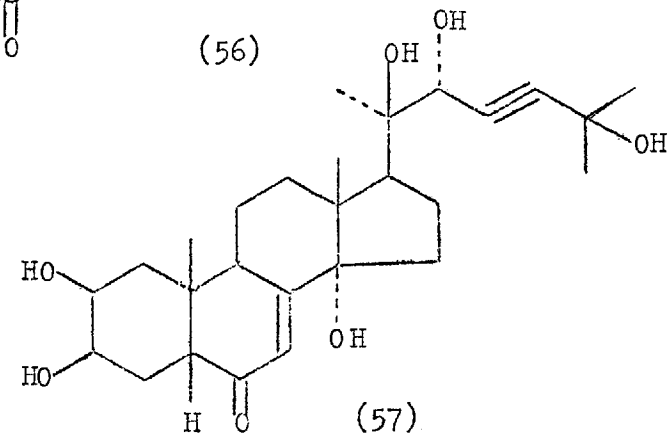


(5)

Alternatively, removal of the protecting groups from (56) prior to hydrogenation, gave the hexahydroxy acetylene (57), which, after hydrogenation, gave crustecdysone and ponasterone A (9) as a hydrogenolysis product.



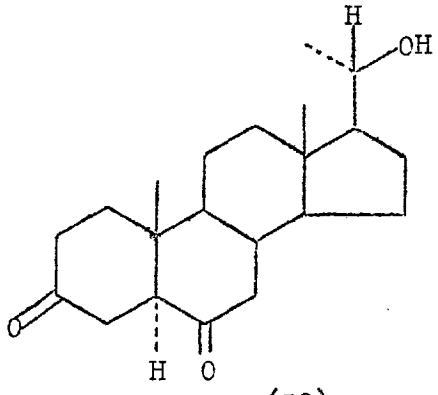
(56)



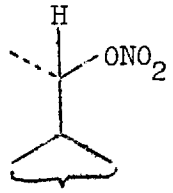
(57)

The Hoffmann synthesis[23].

The synthesis by this group started with 20β-hydroxy-5α-pregna -3, 6-dione

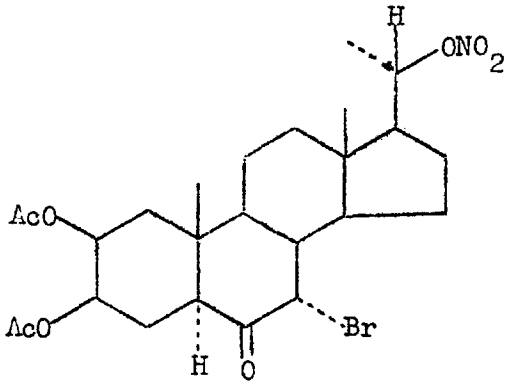


(58)

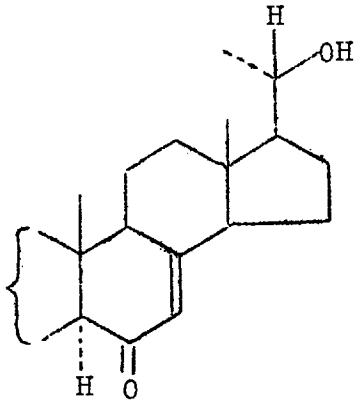


(59)

After conversion of the 20-hydroxyl to the nitrate ester (59), a series of reactions analogous to those used in their synthesis of ecdysone (see scheme I, p 41) led to the bromonitrate ester (60).



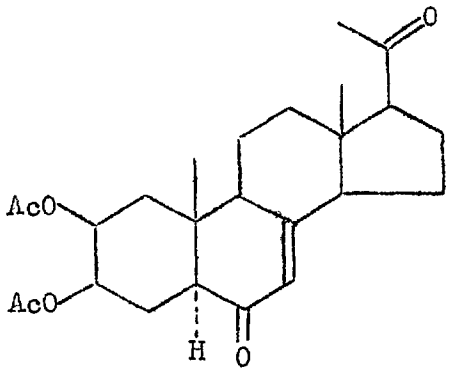
(60)



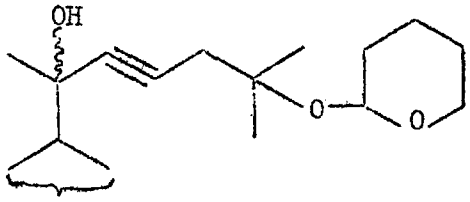
(61)

Reductive removal of the nitrate group followed by dehydrobromination then gave the α,β-unsaturated ketone (61), which was oxidised to the 6,20-dione (62).

Selective alkylation of the 20-keto group with [4-(tetra hydropyranyloxy)-4-methyl-1-pentynyl] magnesium bromide then gave a mixture of C-20 epimeric alcohols (63).

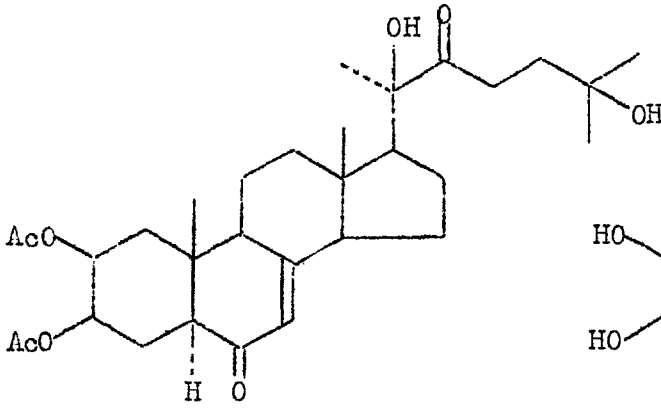


(62)

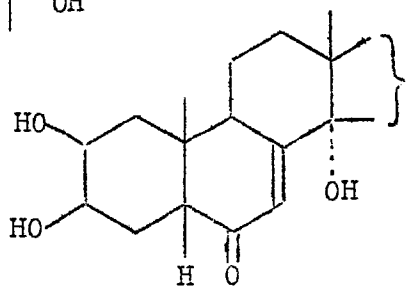


(63)

The major product was assigned the 20R configuration by the application of Cram's rule (which predicted the reaction should give the 20R isomer). Removal of the pyranyl ether protecting groups, followed by reaction with mercuric acetate and boron trifluoride etherate gave the 22-ketone (64). The 14α-hydroxyl group was then introduced in the usual way by allylic oxidation with selenium dioxide.



(64)



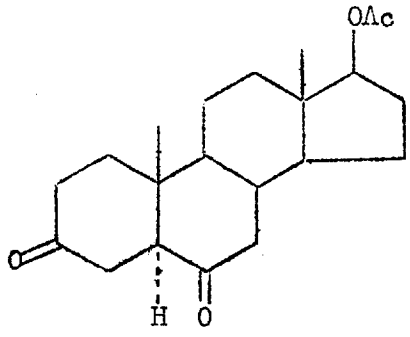
(65)

The synthesis was completed by removal of the acetoxyl groups, with simultaneous inversion at C_5, to give the 5β-pentanol (65), and then selective reduction of the 22-keto group yielded a mixture of crustecdysone and 22-iso-crustecdysone.

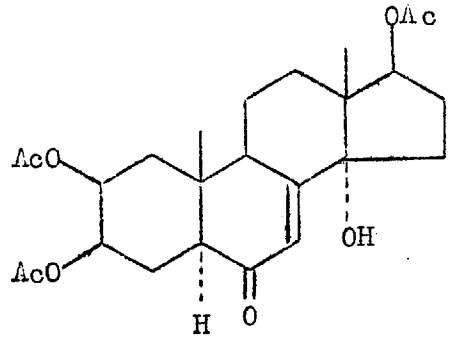
The Synthesis of Rubrosterone (26)

Shortly after the publication of the structure of rubrosterone[50], two independent syntheses appeared in the literature.

The first due to the Hoffmann group merits little attention as no new synthetic features are present. Starting from 17β, acetoxy-5α-androstan-3, 6-dione (66), the sequence proceeded by a pathway analogous to that used in their synthesis of ecydsone and crustecdysone to arrive at the α,β-unsaturated ketone (67).

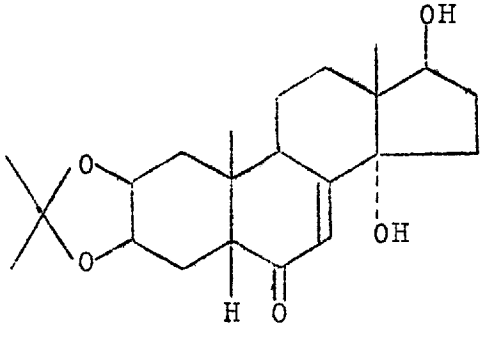


(66)

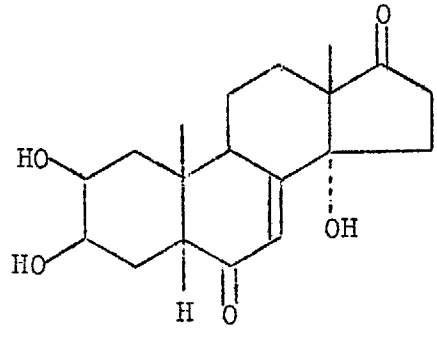


(67)

After sequential removal of the acetoxy groups (with simultaneous equilibration at C_5), reprotection of the 2β, 3β-diol function as the acetonide (68), oxidation of the 17β-hydroxyl group to the required 20-ketone, the synthesis was completed by removal of the acetonide group to yield rubrosterone (26).

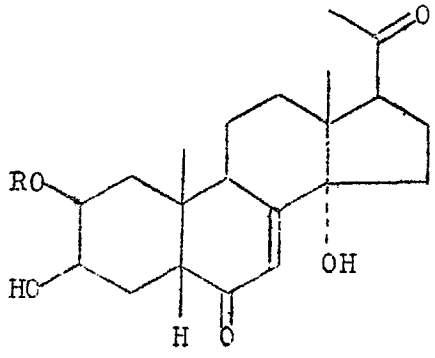


(68)

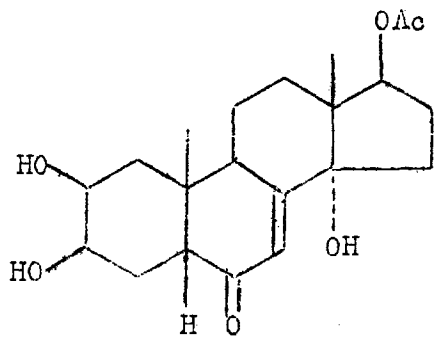


(26)

The other published synthesis of rubrosterone by Takemoto et. al.[62] also merits little attention and starts from a degradation product of crustecdysone i.e. 2β-acetoxy-3β, 14α-dihydroxy-5β-pregn-7 ene-6, 20,-dione.(10) R = Ac.



(10) R = Ac
R = H



(69)

After hydrolysis to the triol (10) R = H, a Baeyer-Villiger oxidation with pertrifluoroacetic acid gave the acetate (69). The sequence was completed to give rubrosterone in an identical manner to that described above in the Hoffmann synthesis.

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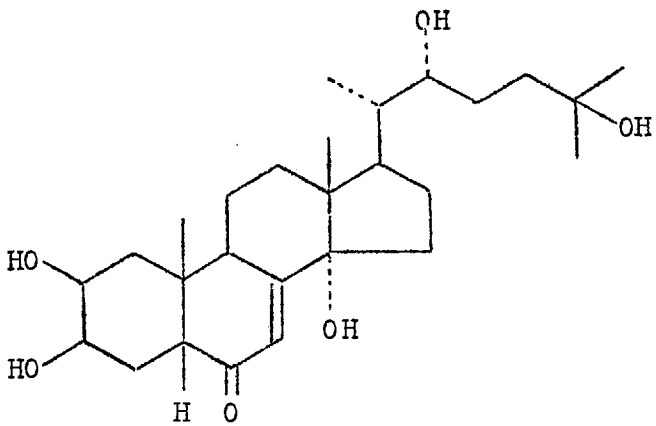
P A R T I I

SECTION I. The synthetic approach to Ecdysone

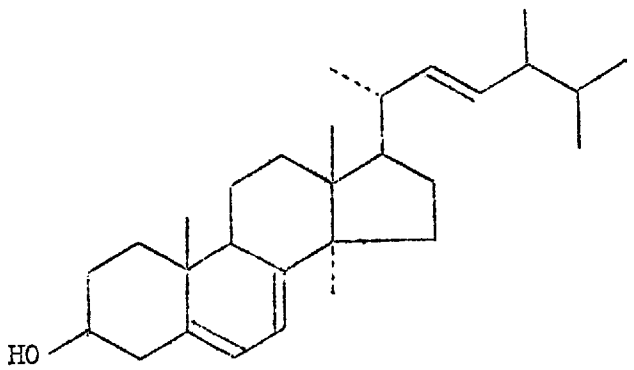
a) Introduction

When this synthetic work on insect moulting hormones was started very little of the chemistry of these compounds had appeared in the literature. In fact, only ecdysone (1) had been isolated. Its constitution had been fully determined by the X-ray work of Huber and Hoppe[1]. (Chemical methods had failed because of the small amounts of material available).

Our first objective was to decide upon a suitable starting material. After some deliberation it was decided to use the readily available sterol, ergosterol (2). The reasons for this choice can readily be seen if we compare the two structures (1) and (2).



(1)



(2)

As can be clearly seen, the positions of the functional groups in ergosterol (2), coincide closely with those that appear in ecdysone (1). If each feature is examined individually the reason for this choice of starting material becomes even more apparent.

The conversion of the 3β-alcohol moiety in ergosterol, into the 2β, 3β-diol system occurring in ecdysone, is clearly possible by a number of routes,and will be discussed in detail below.

Furthermore, there are many instances of the conversion of 5-ene steroids into the corresponding 6-keto derivatives, and of course, the requirements in ecdysone of a 7-ene double bond are fulfilled without further oxidation being necessary. Methods are also well known whereby the allylic alcohol group at C-14 can be introduced into such a system.

This leaves the problem of achieving the required side chain, and the A/B cis ring junction. The obvious route to the side chain, was of course, by oxidative cleavage of the 22-ene double bond in ergosterol to give a 22-oxygenated derivative amenable to further manipulations.

It was predicted that the required A/B cis ring junction could be achieved by equilibration of a suitable 2β, 3β-diol, 6-keto derivative. This prediction was later given foundation, when the work of Jones et. al.,[2] on the equilibration of 3β substituted 6-keto steroids, appeared in the literature.

The approach to the synthesis can be divided into three parts, the synthesis of the A/B ring system, the introduction of the 14α-hydroxyl group, and finally, the methods investigated in order to achieve the necessary side chain.

b) Synthesis of the A/B ring system

Starting from ergosterol (2) there were several possible courses to the required A/B ring system.

The diene system in ring B could have been oxidised directly by the known procedure[3] to a 6-ketone, (after suitable protection of the 3β-alcohol group). Then, by sequential hydrolysis, formation of the 3β-tosylate or mesylate, followed by elimination , a 2-en-6-one substrate could be arrived at (see Fig. 1). This is amenable to further manipulation.

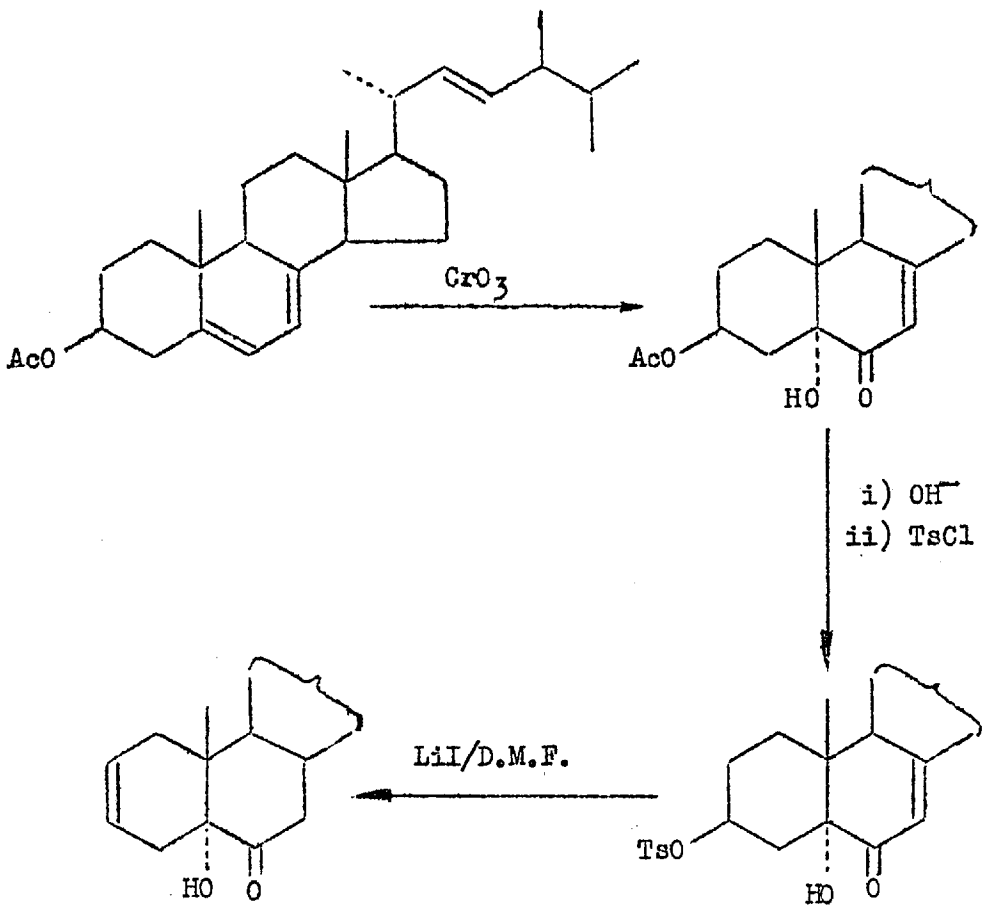


Fig. 1.

This procedure was the one used in a subsequent synthesis of ecdysone (1)[4]. However, in the present work a more novel approach was adopted. By the known procedure outlined in Fig. 2, 3 α, 5 α,-cyclo-ergosta-7,22.-dien-6-one (3) was prepared in about 65% yield, (based on ergosterol). As large amounts of this material were required the published procedure had to be modified to allow preparation on an increased scale (see experimental, Part III).

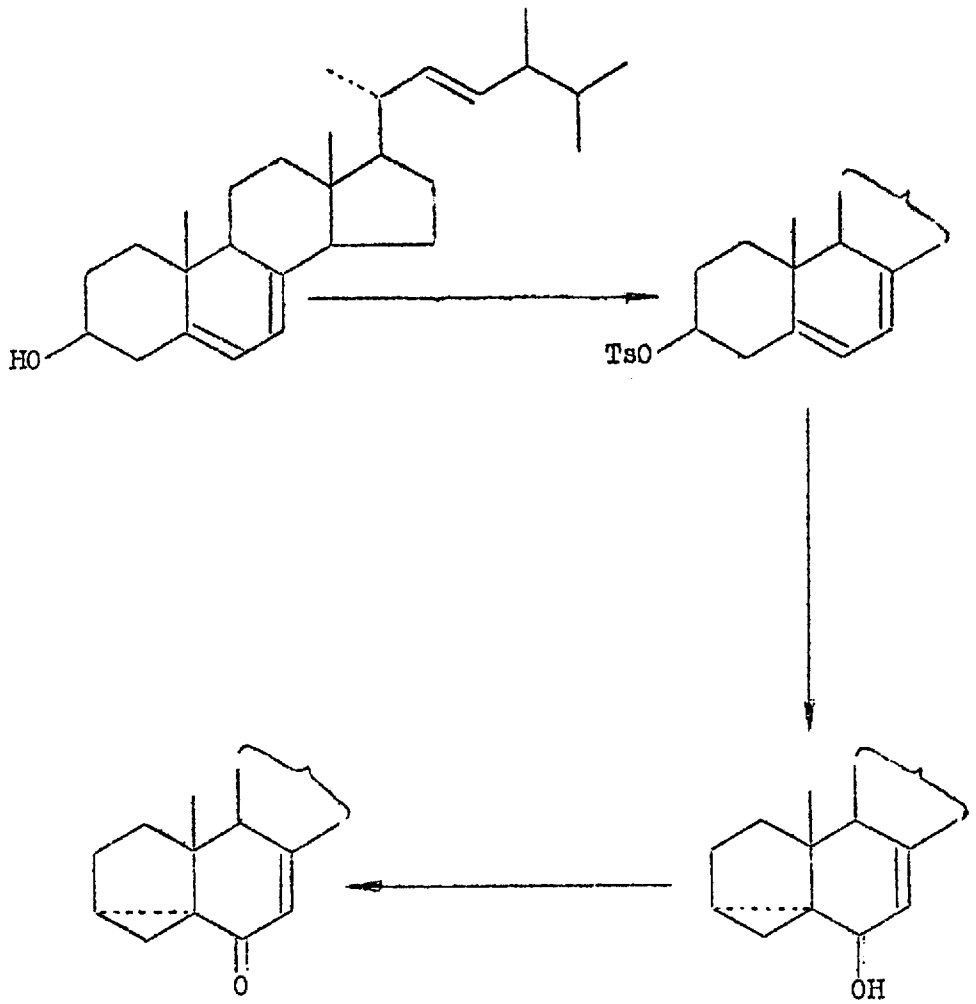
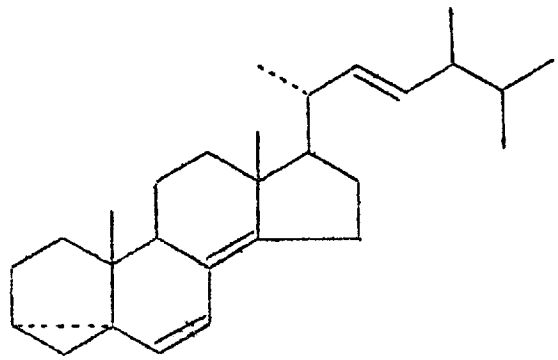


Fig. 2.

The known cyclopropyl-diene (4) was isolated as a by-product of this reaction.

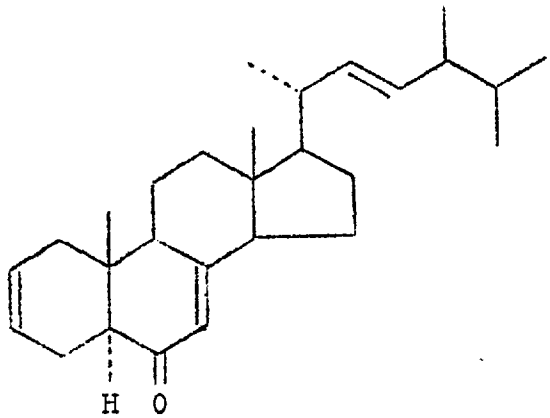


(4)

The reasons for preparation of the cyclo-steroid (3) were twofold. First, it was hoped to achieve direct opening of the 3 α, 5 α-cyclopropyl ring to the required 2-ene compound (5) (see below), and secondly, if this approach failed, it was possible to arrive at the same 2-ene system (5) by the known cyclopropyl ring opening reaction with a suitable nucleophile[5] e.g. I^- or Br^-, followed by dehydrohalogenation under basic conditions.

The second approach has been used by Hora et. al.[6] in their subsequent syntheses of potentially active analogues of ecdysones.

However, the first course was adopted as it was considered possible to open the cyclopropyl ring directly to the 2-ene system (5) using basic conditions. A plausible mechanism can be written for this reaction (See Fig. 3).



(5)

Furthermore, a careful search of the literature revealed that the same reaction had already been observed by Butenandt et. al.[7] in the

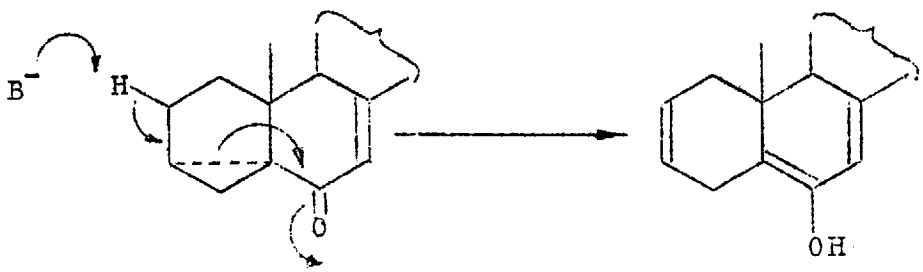
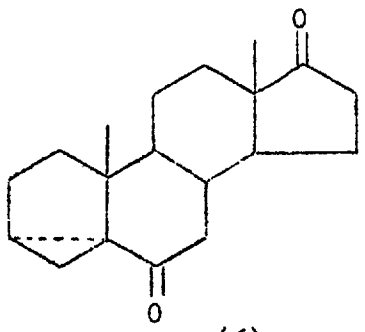
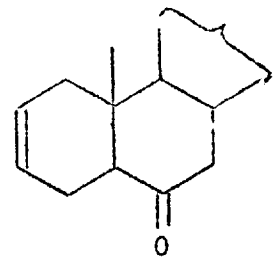


Fig. 3.

case of the androsterone cyclo-steroid (6).



(6)

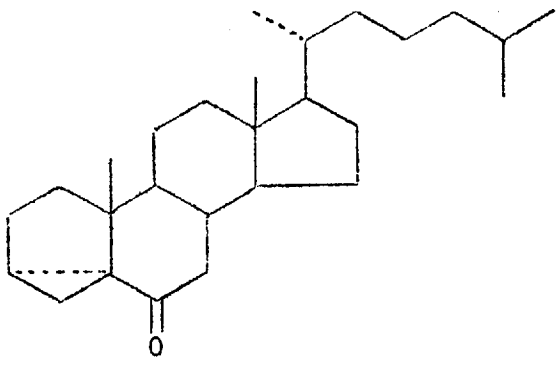


(7)

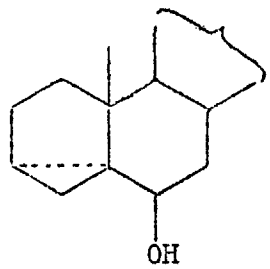
In this early work, the 2-ene steroid (7) was obtained directly by treatment of the cyclopropyl compound (6) with quinoline for several hours. It was decided to repeat this work and 3 α, 5 α-cyclo-cholest-6-ene (8) was chosen as a suitable model compound for these experiments.

The preparation of this compound was carried out by the known sequence[8] to the cyclopropyl alcohol (9). Oxidation of this alcohol (9) with Jones reagent gave the required ketone (8) in excellent yield.

However, under the conditions employed by Butenandt, no reaction was observed in the case of the ketone (8). Even extended reaction times of several days failed to give any of the desired product and only the unchanged ketone (8) could be recovered from the reaction mixture. A variety of other bases, e.g. potassium



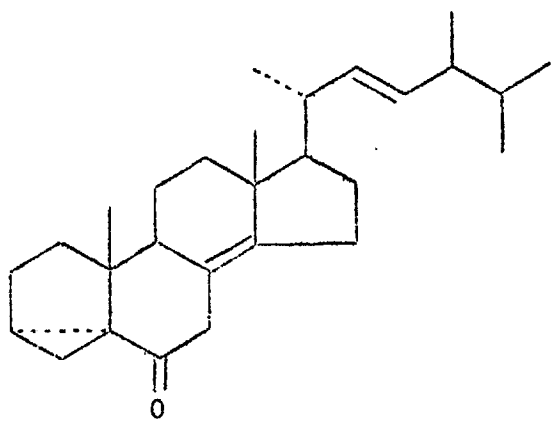
(8)



(9)

hydroxide, sodium hydride, also failed to react. Potassium t-butoxide reacted to give a complex mixture in which no olefinic protons could be detected by n.m.r. examination of the reaction product. The reason for the failure of the cyclopropyl rihg to open under these basic conditions may have been that the prefered direction of enolisation in the ketone (8) was to position 7. Hence, once this enolisation had occurred, the driving force for the reaction was removed (see Fig. 3). On the basis of this argument, the reaction was attempted on the corresponding ketone in the ergosterol series (3), in which the 7 position is blocked by a double bond.

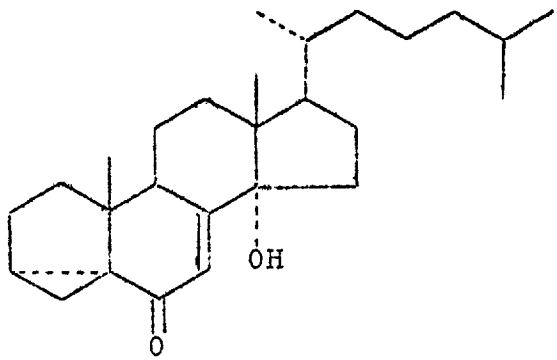
Treatment of this ketone (3), with sodium hydroxide in methanol, gave a new compound, but even after prolonged treatment there was always some of the starting ketone left in the reaction mixture. Infrared spectroscopic examination of the reaction mixture showed the new compound had carbonyl absorption at 1675 cm^{-1}. The starting ketone (3) had its carbonyl absorption at 1650 cm^{-1} and the magnetude of the shift observed (25 cm^{-1}), was that expected if the cyclopropyl conjugation had been removed. However, ultraviolet spectroscopic examination of the isolated product indicated the absence of the required α,β-unsaturated ketone chromophore. On the basis of these results, the isomeric structure (10) was assigned to the product.



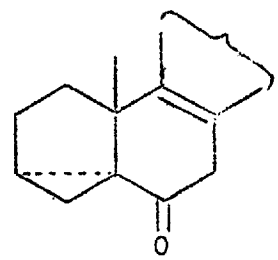
(10)

(The 8(14)-position of the double bond is favoured on steric grounds). This isomerisation of the 7-ene double bond to the 8(14)-position, under basic conditions, had left the 7 position free for enolisation to take place, once again removing the driving force for the required ring opening reaction.

From other model work (see later), some 14 α-hydroxy-3 α, 5 α-cyclo-cholest-7en-6-one (11) was available and so it was decided to try some ring opening experiments, under basic conditions on this compound.



(11)



(12)

In compound (11) the 14 position is blocked with a hydroxyl group, and hence, no isomerisation of the 7-ene double bond can take place to the 8(14)-position.

Treatment of the ketone (11) with a number of bases, e.g. sodium hydroxide, lithium N, N-dimethylamide and lithium N-phenyl-N-methylamide, only gave the isomerised compound (12). The structure of this compound (12) was assigned from the spectroscopic data obtained. The ultraviolet spectrum showed no chromophore due to a α,β-unsaturated ketone, and the infrared spectrum showed a shift in the carbonyl peak absorption to shorter wavelength.

Sodamide, under anhydrous conditions, and collidine failed to react with the ketone (11).

As a result of these experiments, the attempted base opening of the cyclopropyl ring was abandoned, and our attention was turned to the use of buffered acid systems in order to attempt an opening of the cyclopropyl ring directly to the required 2-ene system.

It is well known that 3α, 5α-cyclo steroids are ring opened with suitable nucleophiles to the corresponding 3β substituted compounds, and that these may be eliminated to the required 2-ene system.[7] It was reasoned that in a buffered acid system, the intermediate carbonium ion that must be formed, could be induced to lose a proton to give the required system (see Fig. 4).

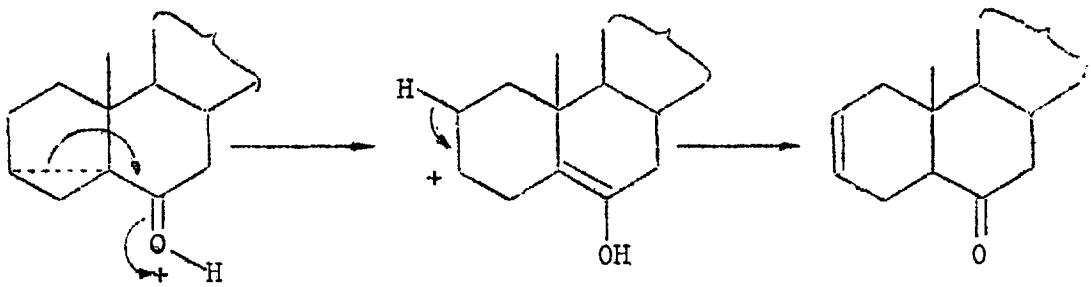
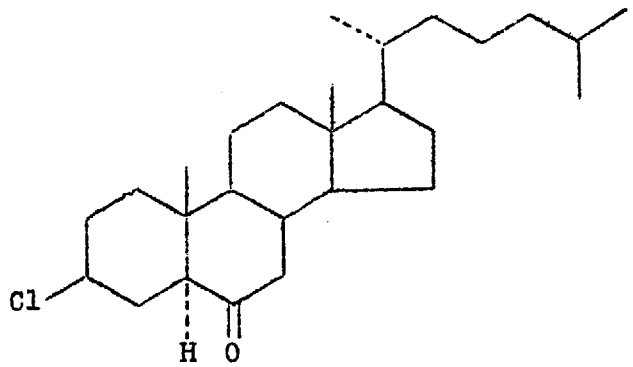


Fig. 4.

The initial experiment was carried out on the model cyclopropyl compound in the cholestrol series (8), which, when treated with pyridine hydrochloride in pyridine, smoothly gave the 3β-chloro compound (13).



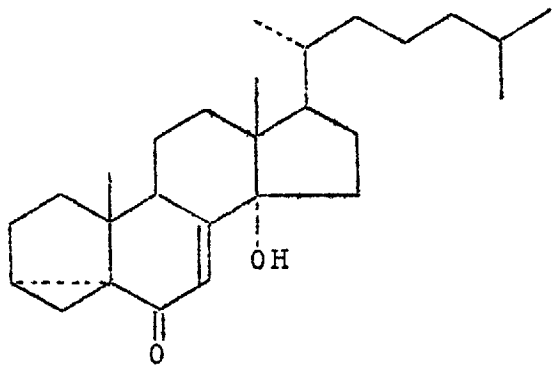
(13)

This encouraging result, prompted a repeat of the experiment but using pyridine tosylate in pyridine, (tosylate anions being much poorer nucleophiles than chloride ions).

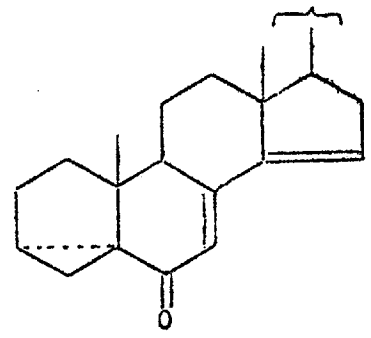
The result was a complex mixture, but n.m.r. examination of this mixture indicated the presence of vinyl protons, and the infrared spectrum had a new carbonyl band at 1710 cm^{-1} indicating the

cyclopropyl ring had indeed opened. T.L.C. comparison of the reaction mixture with an authentic sample of the 2-ene, (prepared by the method of Pataki et. al.)[9] indicated that the required 2-ene compound was present.

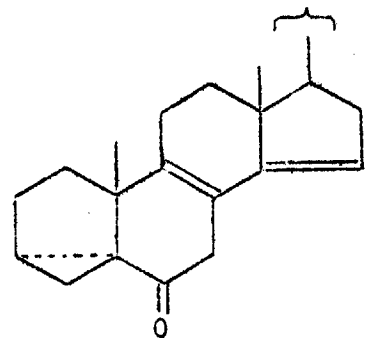
As a method for simultaneously introducing the 7-ene and 14 α-hydroxyl groups into suitable models had already been devised (see p. 84), (it was possible that this alternate approach may become necessary), use was made of the 14-hydroxylated compound (11).



(11)

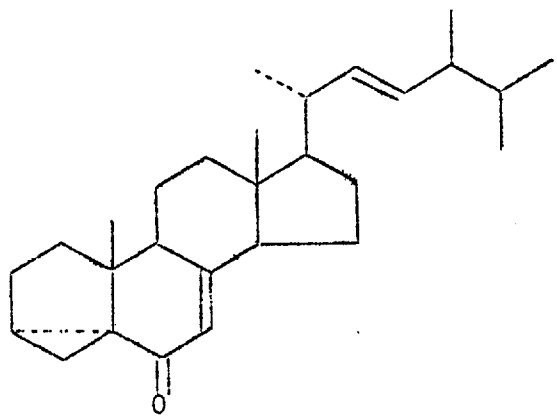


(14)



(15)

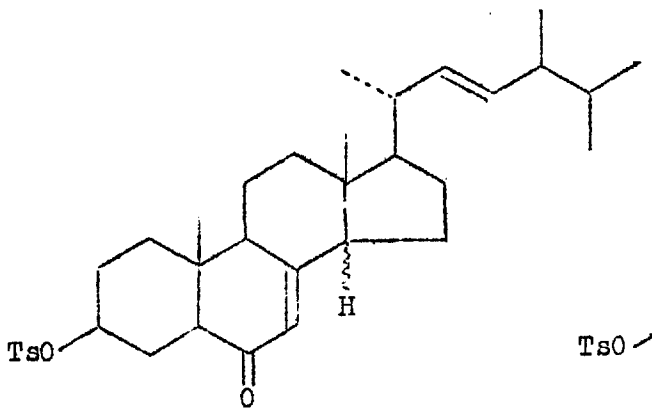
However, all buffered acid treatments tried, e.g. pyridine tosylate in pyridine, resulted in dehydration of the 14 α-hydroxyl groups to give the two expected dehydration products[10] (14) and (15) (see below). A somewhat surprising result was obtained when the alcohol (11) was treated with tosyl acid in acetic acid at room temperature. Only the more unstable dienome (14) was obtained, none of the rearranged diene (15) being formed. Dehydration even occurred when the compound (11) was treated with lithium iodide in pyridine. Thus, it was obvious from these results, that any ring opening reaction, using acidic conditions, had to be carried out on a non 14-hydroxylated intermediate and attention was refocused on the main series cyclopropyl ketone (3).



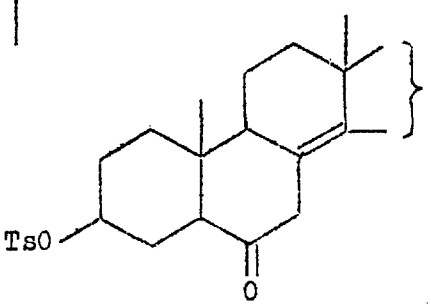
(3)

Treatment of the ketone (3) with pyridine tosylate in pyridine, in the same way as described above for the model cholesterol compound, only resulted in the same isomerisation of the 7-ene double bond that had been observed in the previous base treatment.

The treatment of the ketone (3) with tosyl acid in dried benzene, proved to be the key experiment in solving the ring opening problem. This treatment, first gave the isomerised product (10), but on prolonged reaction yielded two new compounds. The major product was separated, in 75% yield, by chromatography, and shown to be the 3β-tosylate (16). The other, minor, component was not isolated but was presumably the corresponding isomerised material (17).



(16)



(17)

The nature of the tosylate (16) was fully established from infrared, n.m.r., mass spectral and microanalytical results. However, it was later shown (see p. 78) that the crystalline material obtained was probably a mixture of C-14 epimers.

The mads spectral studies were particularly revealing in that they suggested the solution to the ring opening problem. Examination of the mass spectrum indicated that the molecule underwent an initial loss of tosyl acid to give the required 2-ene compound (5). This then underwent, as expected, a retro Diels-Alder fragmentation to give butadiene, strong peaks at m/e 54 and m/e 340 being present in the spectrum (see Fig. 5).

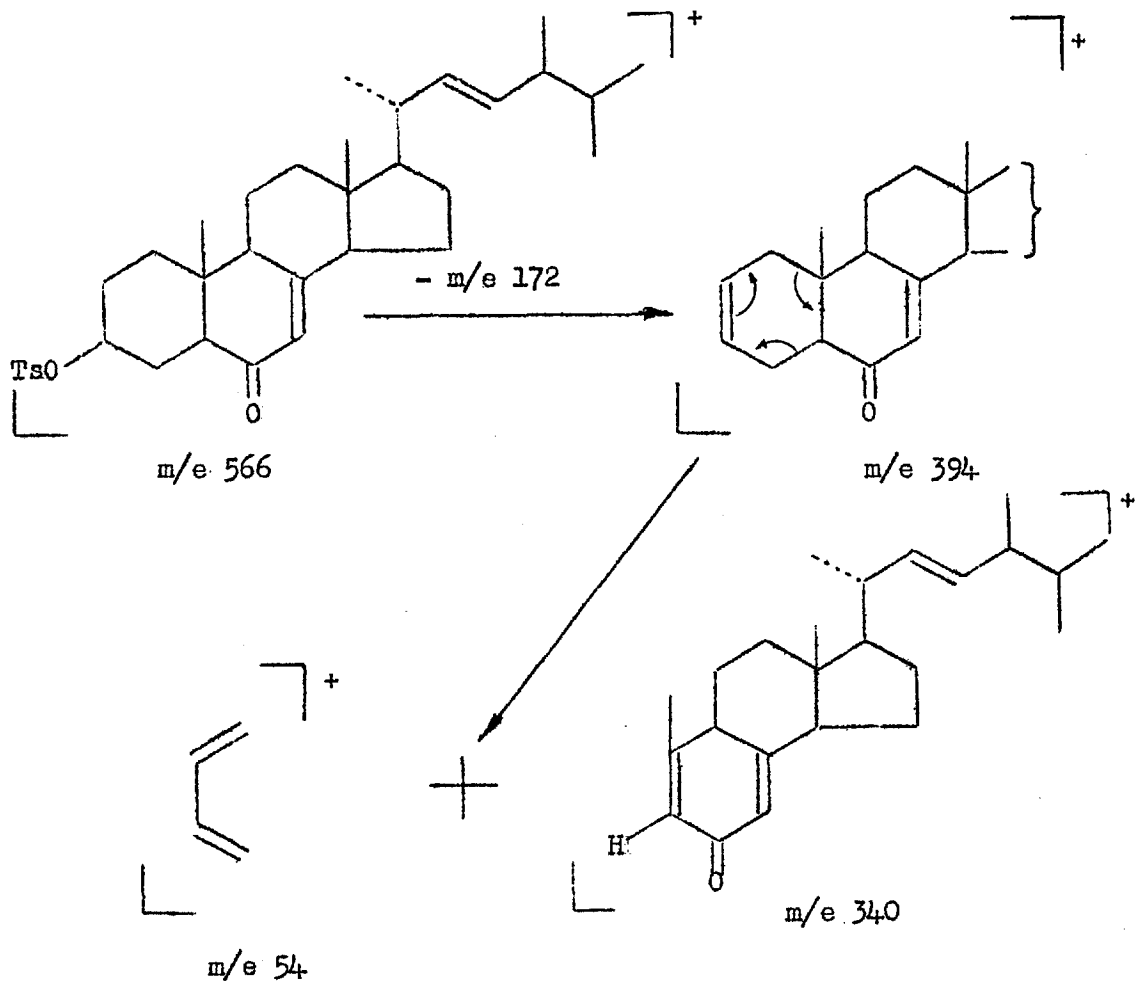


Fig. 5.

Formation of the tosylate (16) was somewhat surprising as it was an example of p-toluene-sulphonic acid acting as a nucleophilic reagent, even though it is well known that tosyl anions are notoriously poor nucleophiles.

The loss of tosyl acid observed in the mass spectrum suggested that either simply heating the tosylate (16), or the cyclopropyl ketone (3) with tosyl acid, at a high temperature, could well lead to the required 2-ene ring opened product.

In the event, when both these experiments were carried out, at a temperature of 220°C in the asbsence of a solvent, the resulting, similar, reaction mixtures both showed additional vinyl protons in their n.m.r. spectrums. Both also showed major spots on T.L.C. at an R_F value identical to that of a sample of 5 α,14 α - ergost-2, 7,22-trien-6-one (5), which had prepared by an alternate route (see below).

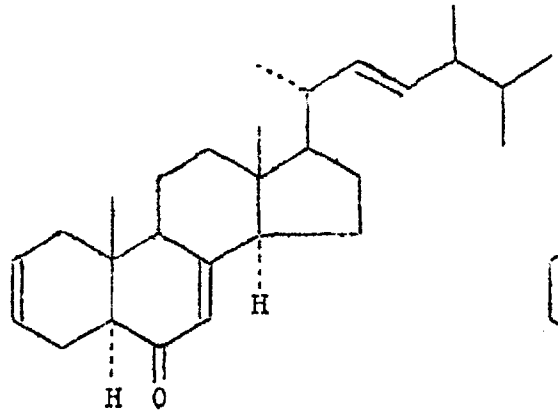
Since the procedures described above produced rather complex mixtures, further experiments were conducted in solutions of various solvents. Tosyl acid in dimethylacetamide reacted slowly at 140° to give a cleaner reaction mixture, but use of sulpholane as a solvent, containing tosyl acid (5 percent w/w of the cyclopropyl ketone used), gave an even cleaner reaction product. An examination of this crude reaction product indicated that isomerisation of the required 2,7-dien-6-one into the 8,(14)-ene and the isomeric 14β compound had occurred (see below). Since it was rather difficult to separate this crude product into its individual components, it was used

directly for the cis hydroxylation procedure, carried out as the next stage in the sequence (see p. 81).

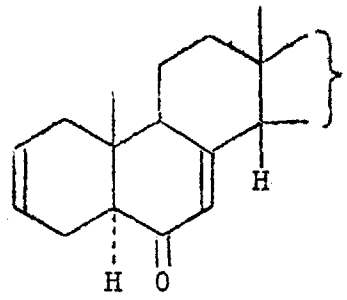
As an alternative to tosyl acid, perchloric acid was employed in benzene/dioxane solutions, as a catalyst for the ring opening procedure. Although n.m.r. and T.L.C. indicated the required ring opening had occurred, the yield of the desired 2-ene product was much lower than when the tosyl acid procedure was used. Hence no further work was carried out in this direction. In theory, perhloric acid should have been a superior catalyst as perchloric ions are even poorer nucleophiles than tosylate anions.

An alterantive method of arriving at the required 2-ene system was to eliminate the 3β tosylate (16) under basic conditions. When this reaction was carried out, using lithium carbonate in dimethylformamide, and the reaction product carefully chromatographed, two isomeric 2-ene compounds were isolated. One was the required 2-ene (5) and the other was the 14β-epimer (18).

Both compounds had very similar mass spectra, (as would be expected), showing ready loss of butadiene (c.f. Fig. 5), and similar infrared specta. Their n.m.r. spectra were most useful in assignment of structure. The signal due to the 7-proton in the 14 α-epimer could not be seen as it occurred under the 2-ene protons at 5.70 p.p.m.



(5)



(18)

But the signal due to the 7-proton in the 14β compound occurred as a doublet at 5.80 p.p.m., allylic coupling now only occurring with the C-9 proton. Since the dihedral angle between the C-7 and C-14 protons is now close to zero, no coupling occurs with the 14β proton. (N.B. The 14 ∝ compound (5) had by this time been independently synthesised by Korb et. al. in their work on ecdysone. The constants reported were in good agreement with those found for this compound).[11]

The isolation of both the 14 ∝ and 14β epimeric 2-ene compounds from the elimination reaction on the tosylate (16), suggested that the crystalline tosylate isolated (see above) was in fact a mixture of C-14 epimers. Hence both tosylates must have been formed during

the ring opening reaction. This result can be explained if the extent of equilibration between the cyclopropyl 7-en- -one (3) and the isomeric 8,(14)-en-6-one (10) is compared with the same equilibrium in the case of a ring opened compound e.g. the 2-ene. Considering that the equilibrium, in the case of the cyclopropyl compounds, lies in favour of the non conjugated ketone (10), and that in the case of the ring opened compounds it lies in favour of the conjugated ketone. Ring opening then requires isomerisation of the 8,(14)-ene to the 7-ene position, which, in turn, requires addition of a proton at C-14, a process that can take place from either the α or β face. (see Fig. 6).

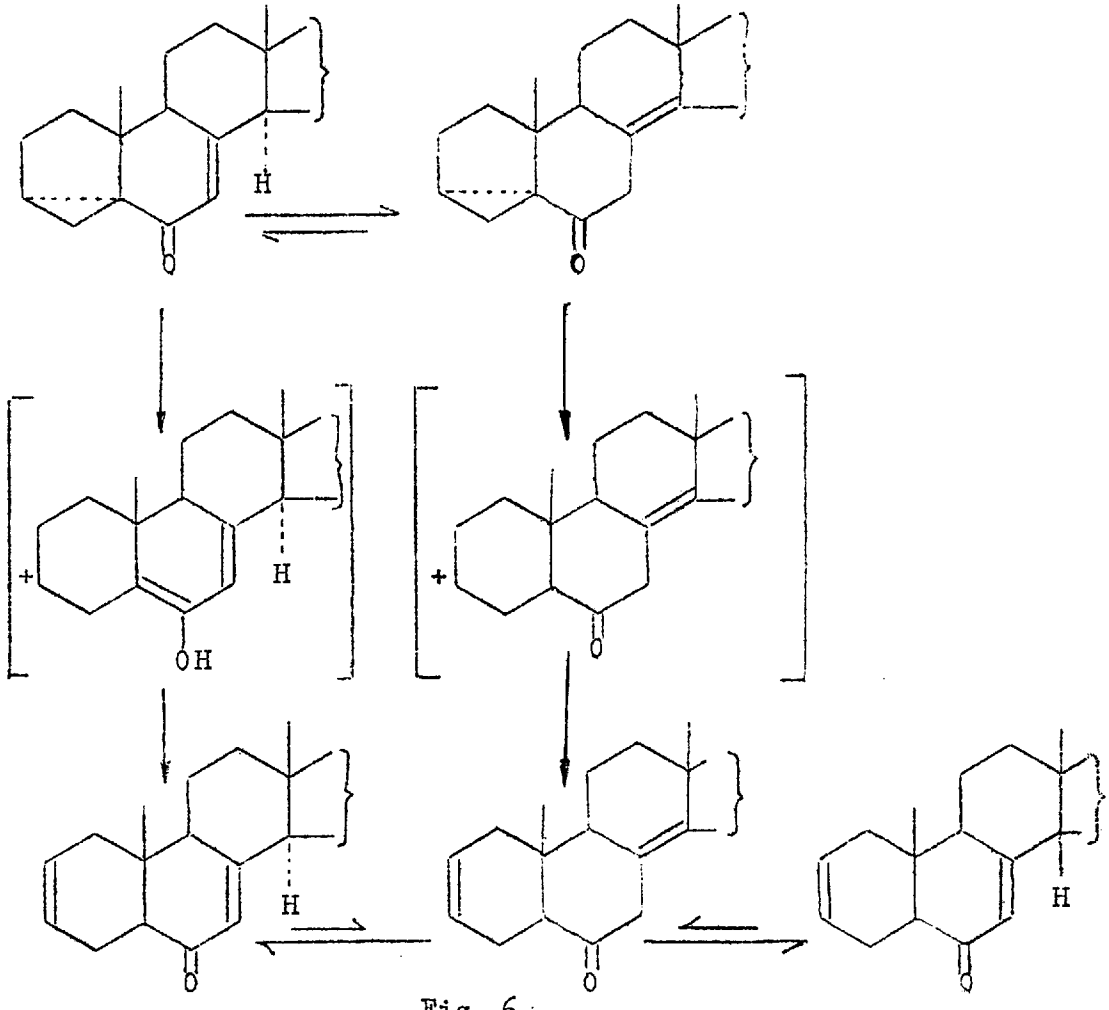


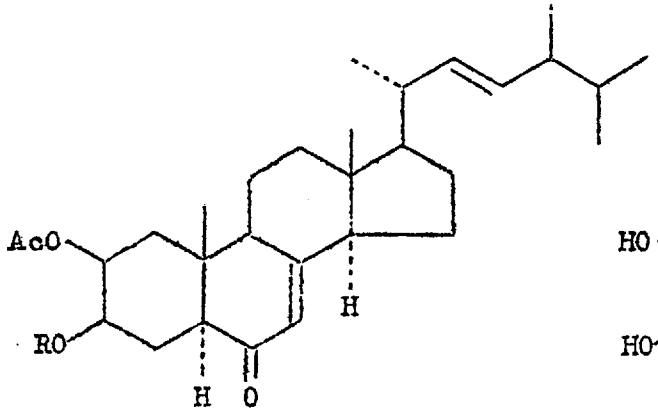
Fig. 6.

To confirm this hypothesis the extent of the acid catalysed equilibrium in the cyclopropyl and 2-ene cases were determined by ultraviolet spectroscopy. In the cyclopropyl case the ratio of conjugated (3) to unconjugated (10) isomer at equilibrium was 6:4. In the 2-ene case, the equilibrium ratio was much more in favour of the conjungated ketone, being 10:1. This difference is probably due to the strain induced in ring B by the cyclopropyl ring, the isomerisation of the 7-ene double bond to the 8 (14) position releaving this strain. The occurrence of both 14 α and 14β epimers can therefore be explained as above.

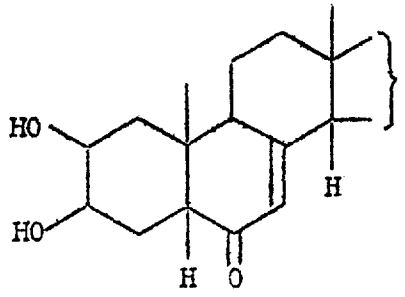
It is interesting to note that up to the time that this work was done, no mention of the isomerisation of the double bond in 7-en-6-one steroids, had appeared in the literature.

Since then several instances have appeared,[4,6,12] and all are connected with synthetic work that has been carried out on ecdysone. For example, Furlenmeier, et. al.[4], isolated the 14β compound (19) from the reaction mixture obtained after the 14 α compound (20), R=H, had been treated with base. In addition the non conjugated ketone (21) was isolated.

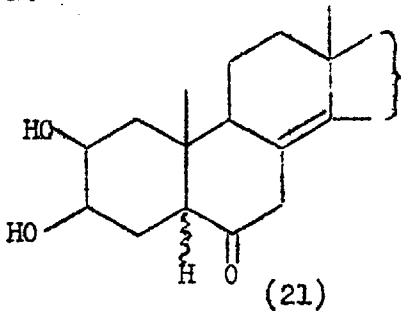
As the key problem of opening the cyclopropyl ring had now been solved, the next stage in the synthesis was investigated, i.e. the introduction of the 2β, 3β-diol moiety.



(20) R = H
R = Ac

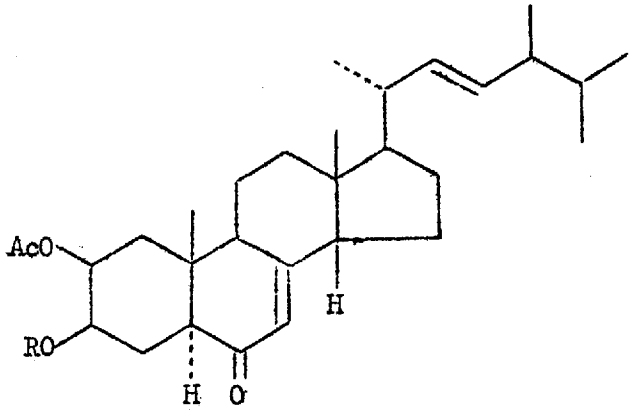


(19)



(21)

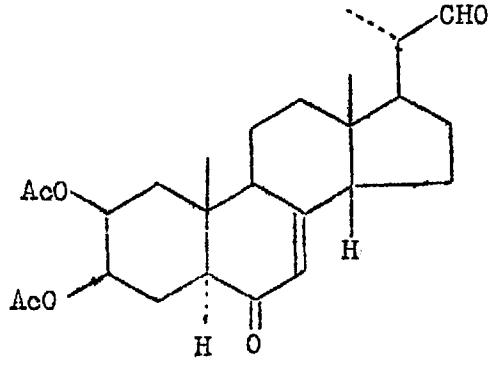
The crude mixture of 2-ene compounds obtained after opening of the cyclopropyl ring was <u>cis</u> hydroxylated using a modified Woodwood/Prevost[13] procedure to give, after chromatography, a mixture of epimeric monoacetate diols (20) R = H, and (22) R = H.



(22) R = H
R = Ac

After acetylation of the mixture to the corresponding diacetates (20), R = Ac, and (22), R = Ac, the two C-14 epimers were separated by preparative layer chromotography (P.L.C.) using the successive elution technique. The structure of the 14β epimer was again deduced from its n.m.r. spectrum, the C-7 proton appearing as a doublet. The n.m.r. spectrum of the 14 α epimer showed the C-7 proton as the expected triplet. The other spectroscopic data and melting point of this compound were in excellent agreement with those reported in the literature for this compound by Furlenmeier et. al. [4]

A further instance of the isolation of a 14β compound from these reaction sequences was the preparation of the aldehyde (24).



(24)

The mother liquor residues, obtained after the initial crystallisation of the epimeric diacetates (20), R = Ac and (22) R = Ac, were used in an exploratory ozonolysis experiment, and the

reaction product, after initial purification by P.L.C., yielded the crystalline aldehyde (23). Once again, the n.m.r. spectrum showed the C-7 proton as a doublet and so revealed the 14β nature of the product.

Since, at this stage, it was possible to readily obtain a mixture of the 14 α, (20), R = H and 14β, (22), R = H epimers, and if desired, with some difficulty the pure separated diacetates, (20), R = Ac and (22), R = Ac, attention was concentrated on the problem of the introduction of a 14 α-hydroxyl group. At this point no attempt was made to achieve the desired A/B cis fused system, since the publication of the work of Siddall et. al.[14] had confirmed the predictions made earlier (see p. 61). This group had demonstrated that provided the 2β, 3β-diol and 6-keto functions were present, a mild base equilibration of a 5 α steroid would lead to the A/B cis fused system thus relieving the severe 1:3 diaxial interaction present between the 2β-alcohol and 19-methyl groups.

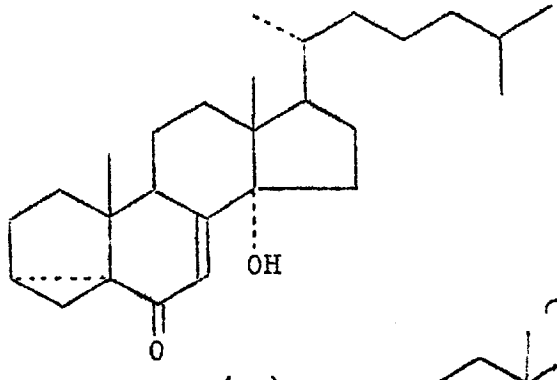
C) Introduction of the 14 α-hydroxyl group.

i) Selenium dioxide oxidations

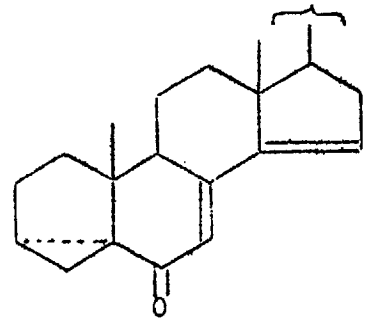
The allylic oxidation of 7-ene steroids with selenium dioxide is known to yield 14 α hydroxylated derivatives[15]. Selenium dioxide is also known to dehydrogenate steroidal ketones to give the corresponding α, β-unsaturated ketons[16,17]. With this information at hand, it was considered likely that both the 14 α-hydroxy and 7-ene moieties could be introduced into a 6-keto steroid simultaneously.

The oxidation of 3 α, 5 α -cyclo-cholest -6-one (8) with selenium dioxide in t-butanol, in the presence of catalytic amounts of pyridine,[18] was attempted. The reaction proceeded slowly and was only partly complete after several days.

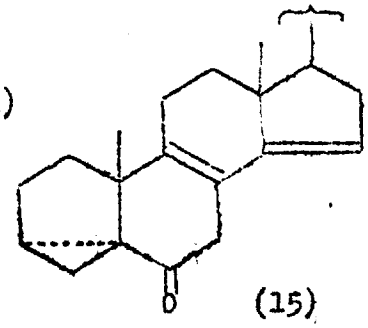
Examination of the reaction mixture showed the presence of three compounds, each of which had the correct ultraviolet spectrum for a α,β-unsaturated ketone of the type required. One of them showed no hydroxyl absorption in its infrared spectrum. Both the other compounds showed the required hydroxyl and α,β-unsaturated carbonyl absorption in their infrared spectra. The major product was shown to be the required 14 α -hydroxylated, α,β-unsaturated ketone (11) by dehydration with dilute mineral acid to give the conjugated dienone (14) and the diene (15), (identified from their ultraviolet spectra). A similar experiment had indicated the presence of the same moiety in ecdysone.[10]



(11)



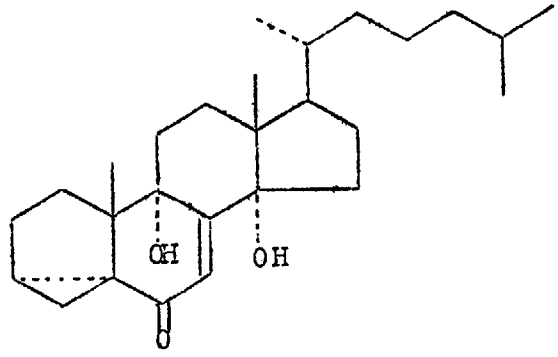
(14)



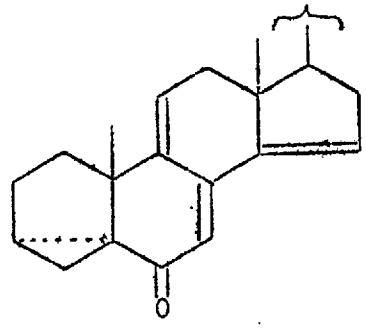
(15)

The other hydroxylated product was shown to have an additional hydroxyl group somewhere in the molecule from mass spectral and microanalytical data.

Dehydration of this compound resulted in only one compound being isolated, its ultraviolet absorption maxima appeared at 309 mμ. Further the signal due to the C-7 proton in the n.m.r. spectrum of the hydroxylated product occurred as a sharp singlet at 6.05 p.p.m. Taking this data as a whole the only structure which can be written for this material is 9 α, 14 α -dihydroxy-3 α, 5 α -cyclo-cholest-7-en-6-one (24). The dehydration product would, therefore, have structure (25) and it would be the only dehydration product expected from the diol (24).



(24)

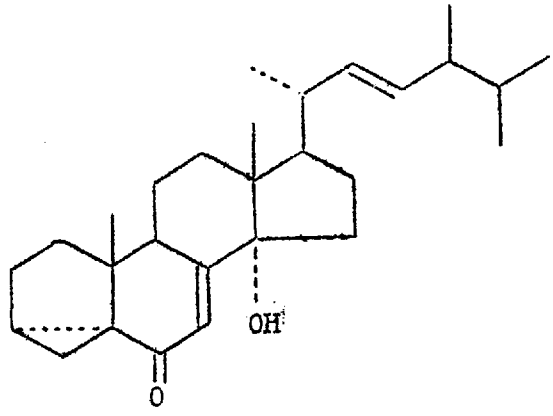


(25)

Further evidence for the nature of the diol (24) was provided by the reoxidation, under the same conditions, of the alcohol (11), which smoothly gave the diol (24). The reaction time was shortened,

to a few hours, and the yield of the alcohol (11) greatly improved, by increasing the amount of pyridine in the reaction mixture to about 25% by volume. Unfortunately, this approach to the problem had to be abandoned when it was found that the cyclopropyl ring could not be opened, to the required 2-ene system, without dehydration of the 14 α -hydroxyl group occurring (see above). However, it appears to be the first reported instance of the simultaneous introduction of the double bond and the allylic alcohol by the reaction of selenium dioxide on a ketone.

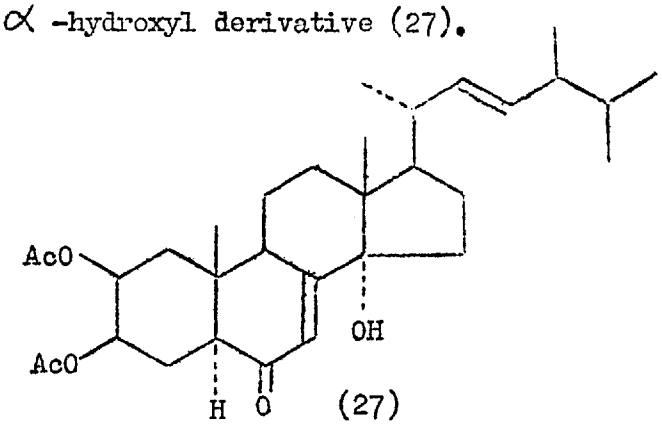
The allylic oxidation of the model compound, 3 α, 5 α -cyclo-ergosta-7 en-6-one (3), was also carried out, using the modified conditions outlined above, to yield the 14 α -hydroxy compound (26) in excellent yield.



(26)

The purpose of this reaction was to confirm that the main series α, β-unsaturated ketone (20), R=Ac, could be oxidised to

the required 14 α -hydroxyl derivative (27).



(27)

Because the major product of the cis hydroxylation procedure (see above) was a mixture of C-14 epimers (20), R = H and (22), R = H, attempts could have made at this point at a selenium dioxide oxidation of the acetylated mixture in order to ascertain if both compounds would give the same 14 α -hydroxylated derivative (27).

However, it was preferred to approach this problem in a more novel manner and this will now be fully discussed.

ii) Introduction of the 14 α-hydroxyl via an enol-acetate intermediate

Fieser et. al.[19] had shown it was possible to introduce an hydroxyl group in the γ-position of an α,β-unsaturated ketone. Their sequence is outlined in Fig. 7.

It was considered that this reaction could be adapted for use in the present case. This would solve the problem of having a mixture of C-14 epimers, as such a reaction sequence would remove the asymmetric centre at C-14, and subsequent oxidation would give only one alcohol.

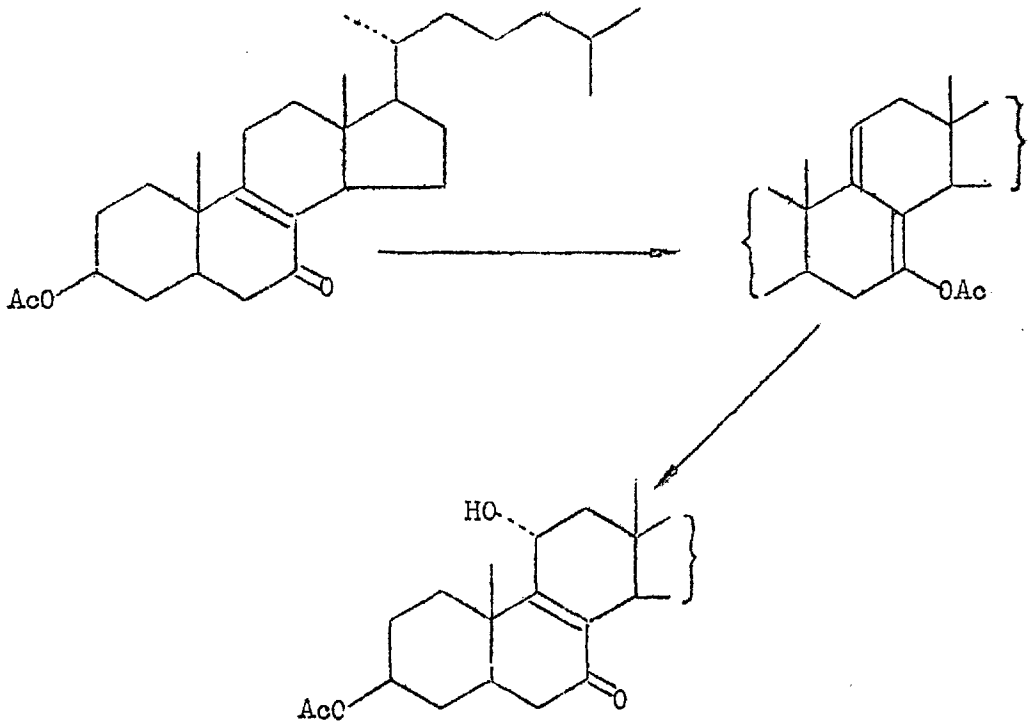
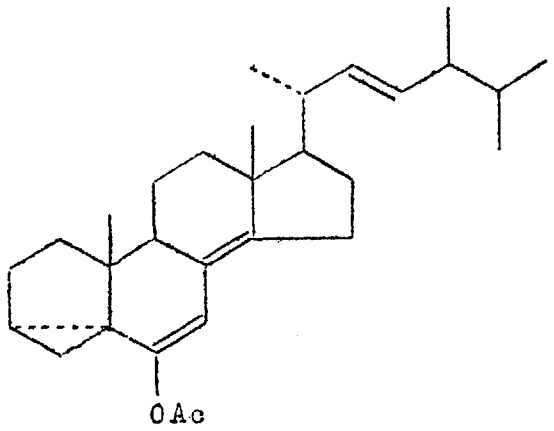


Fig. 7.

Initial experiments were carried out using the cyclopropyl compound (3) as a suitable model.

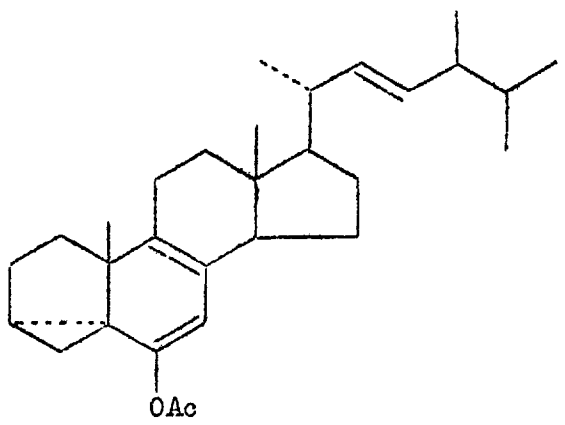
Enol acetylation was smoothly achieved using the conditions of Barton et. al.[20] to give the dienol acetate (28) in good yield.

The n.m.r. spectrum of this compound contained the signal due to the C-7 proton as the expected sharp singlet.



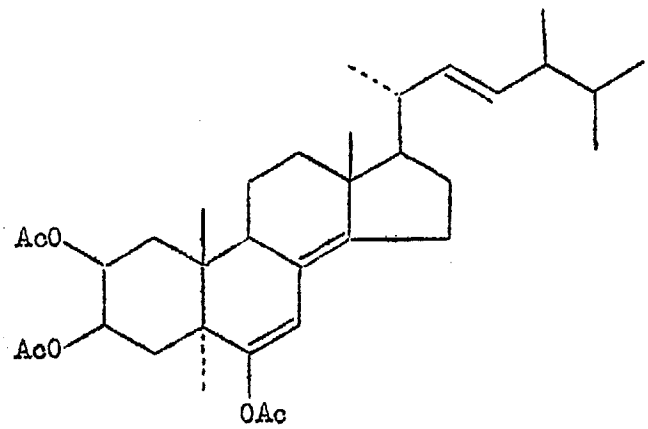
(28)

The position of the 8 (14) double bond was at first somewhat uncertain as the ultraviolet spectrum of the product (28), had its absorption maxima at 263 mμ. This value lies between those calculated for the exocyclic diene (28) (252 mμ) and the homocyclic diene (29) (274 mμ).



(29)

However, when the reaction was repeated in the main series, firstly on the pure 14β isomer (22), R=Ac, and then on the mixture of C-14 epimeric diacetates (20), R=Ac and (22), R=Ac, the same dienol acetate (30) was smoothly obtained. Furthermore, the ultraviolet spectrum of the product (30) now had its absorption maximum at 254 mμ. This result confirmed the position of the 8 (14) double bond and, in addition, also confirmed that the products of ring opening are epimeric about C-14, and not C-9 which was the only other possibility.



(30)

Up to this point the stereochemsitry at C-5. in the ring opened products, had only been assumed to be α . This experiment allowed confirmation of this assumption. The n.m.r. spectrum of the enol acetate (30) showed a signal at 5.90 p.p.m., due to the C-7 proton, as a clear doublet, (which was due to allylic coupling with the proton at C-5). A study of molecular models showed that if a 5β configuration had been present, the dihedral angle between

C-7 and C-5 would have been almost zero and hence little coupling would have been observed. In the case of the 5 α epimer, the dihedral angle is close to 90°, hence the observed doublet nature of the signal.

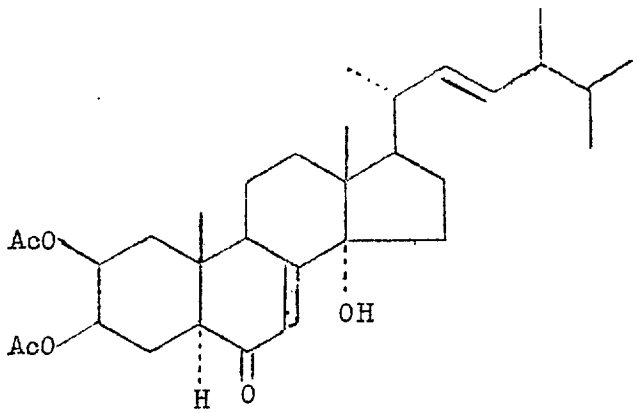
In the normal way the enol acetylation peaction is carried out on the epimeric mixture of monoacetate diols (20), R = H and (22), R=H, acetylation of the 3β-hydroxyl group taking place under the conditions employed.

The only problem remaining nowwas that of obtaining selectivity during the oxidation of the 8 (14) double bond with peracid. It was believed that this could be achieved since the 8 (14) double bond was the only tetra-substituted double bond in the molecule, (peracids are known to preferentially oxidise this type of double bond).

When the model enol acetate (29) was oxidised with 1:2 equivalents of monoperphthalic acid, a high degree of selectivity was observed, and, the expected 14α-hydroxylated compound (26) was formed in good yield.

This material was identical, in every respect, to that obtained by the selenium dioxide oxidation of the cyclopropyl ketone (3). (see p. 86).

The main series enol acetate (30) reacted in an analogous manner to yield the 14 α-hydroxy diacetate (27), which by this time had been independently synthesised, and the physical data of this material was in excellent agreement[4] with that obtained for the diacetate (27).



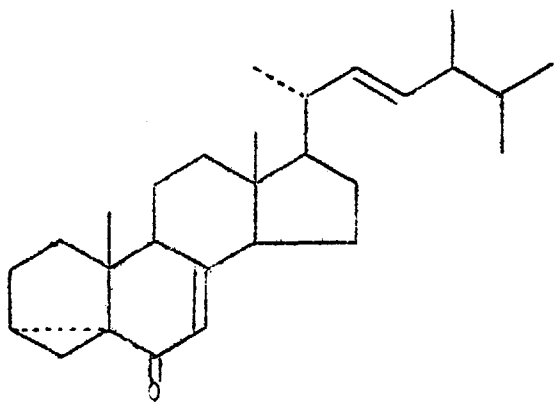
(27)

At this point the synthesis had arrived at a compound which had all the necessary tetracyclic features apart from the A/B cis fused ring junction, which it was known could readily be introduced at any convenient stage in the synthesis.

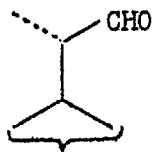
d) Introduction of the side chain moiety

The synthesis was now at a stage where all the tetracyclic features had been introduced, except for the A/B cis fused ring junction. The only outstanding problem was that of introduction of the necessary side chain. It had been planned that the reaction between a 20-formyl derivative and a suitable Grignard reagent should lead to the completion of the synthesis.

The 20-formyl group was readily introduced by ozonolysis of the side chain double bond in the diacetate (27). In order to achieve optimum conditions for this ozonolysis, the cyclopropyl ketone (3) was used as a suitable model compound. When this 22-ene compound (3) was ozonised in a solution of methylene chloride (containing 1% pyridine by volume),[21] the desired aldehyde (31) was obtained in excellent yield.

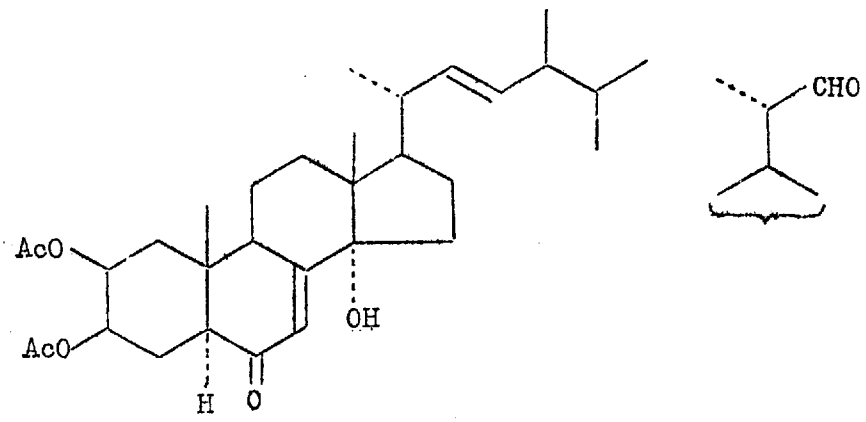


(3)



(31)

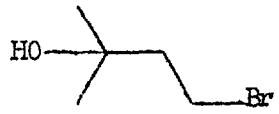
By a similar treatment of the ketone (27) the required main series aldehyde (32) was obtained in good yield.



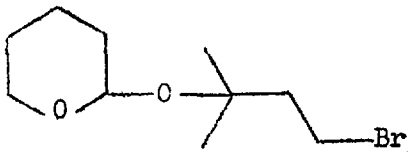
(27) (32)

It was now hoped to introduce the required side chain by reaction of the aldehyde (32), with the Grignard reagent derived from 4-bromo-2-methyl-2-butanol (33) protected as its tetrahydropyranyl ether (34).

The preparation of the bromoalcohol (33) was achieved by the Grignard reaction between ethyl-3-bromoproprionate and excess methyl magnesium iodide. The tosyl acid catalysed reaction between the bromoalcohol (33) and dihydropyran then gave the pyranyl ether (34).



(33)

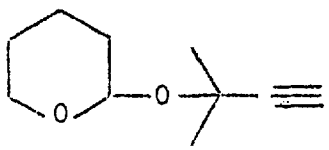


(34)

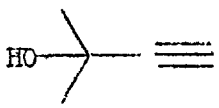
However, despite many attempts under a variety of conditions, the pyranyl ether (34) could not be induced to form a Grignard reagent, and this approach was set aside.

An alternative approach[12,22] was to utilise a Grignard reaction between the acetylenic ether (35) and the aldehyde (32), with subsequent reduction of the acetylenic bond.

The tetrahydropyranyl ether (35) was readily formed by the tosyl acid catalysed reaction between dihydropyran and the known acetylenic alcohol (36).



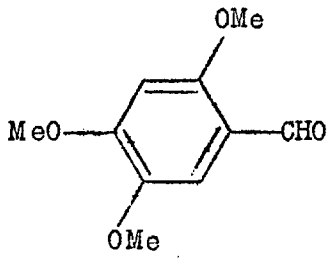
(35)



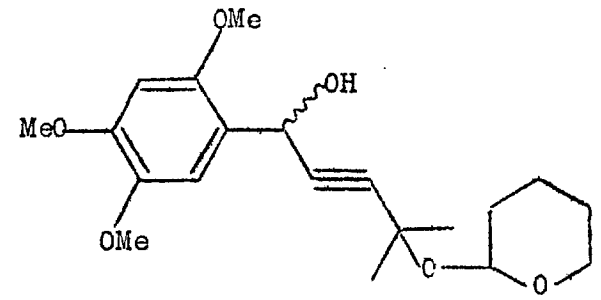
(36)

The lithium derivative of the acetylenic ether (35) was readily prepared by an exchange reaction between the ether (35) and methyl lithium.

As an initial model experiment, the reaction between the lithium derivative of the acetylenic ether (35) and 2, 4, 5-trimethoxybenzaldehyde (36a) (a hindered, crystalline aldehyde), was carried out to give the crystalline trimethoxy ether (37) in excellent yield.

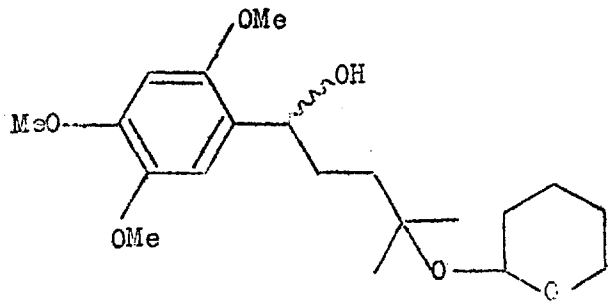


(36a)



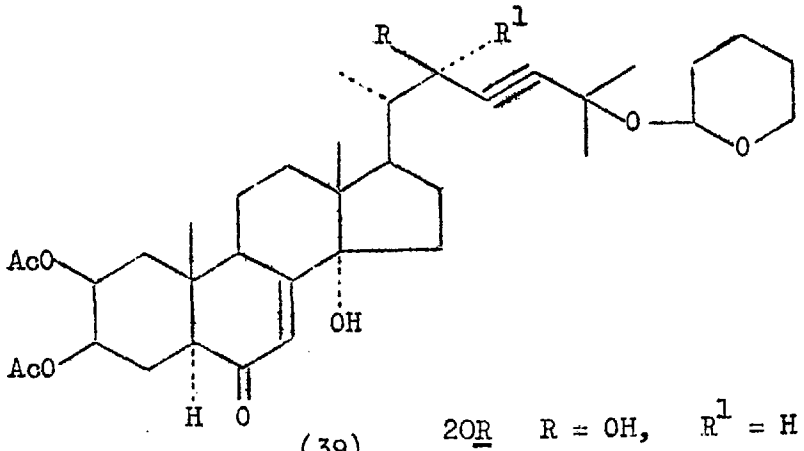
(37)

As the next stage in the synthesis was to be a reduction of the acetylenic bond, the trimethoxy ether (37) was also used as a model compound in hydrogenation experiments. Reduction of the ether (37) over 5% Palladium on carbon smoothly gave the saturated ether (38).



(38)

A return was now made to the main series. The reaction between the aldehyde (32) and the lithium derivative of the acetylenic ether (35) gave a mixture of the two expected[22] isomeric (20 S and 20 R) alcohols (39) in good yield. The required, less polar, 22S isomer (39), R=H, R^1=OH, was separated by P.L.C. Mass spectroscopic examination of this compound confirmed that the desired alkylation had taken place.



(39)

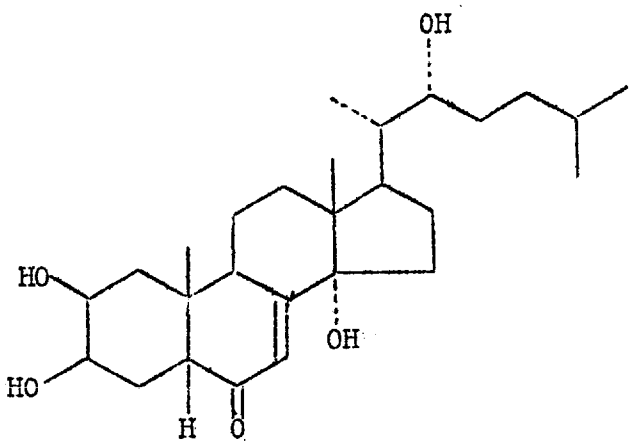
20R R = OH, R^1 = H

20S R = H, R^1 = OH

The reduction of the acetylenic bond in the diol (39), R = H, R^1=OH, proved to be more difficult than the model compound experiments had indicated.

In one experiment reduction of the triple bond was accompanied by extensive hydrogenolysis of the 25-oxygen function.

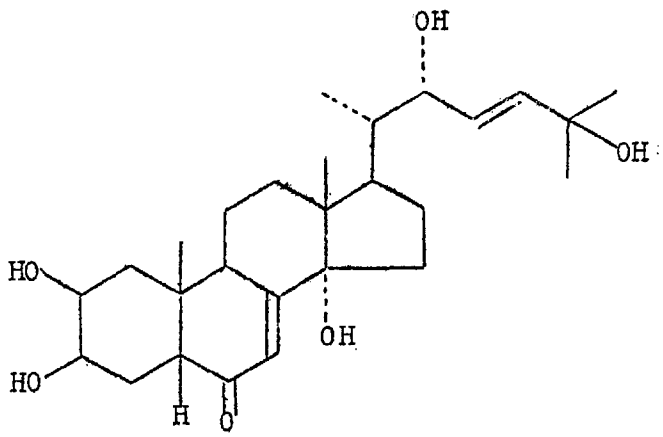
This result was indicated by mass spectroscopic examination of the tetraol (40), produced after removal of the protecting groups, which showed the molecular ion at m/e 448 and not, as expected, at m/e 464. [N.B. the 25-desoxy nature of this compound was inferred from later experiments (see p. 103)].



(40)

In a further experiment, using a different batch of catalyst, the reduction ceased at the 23-ene stage. This result, once again, was indicated by mass spectroscopic examination of the completely

hydrolysed, equilibrated hydrogenation product (41).



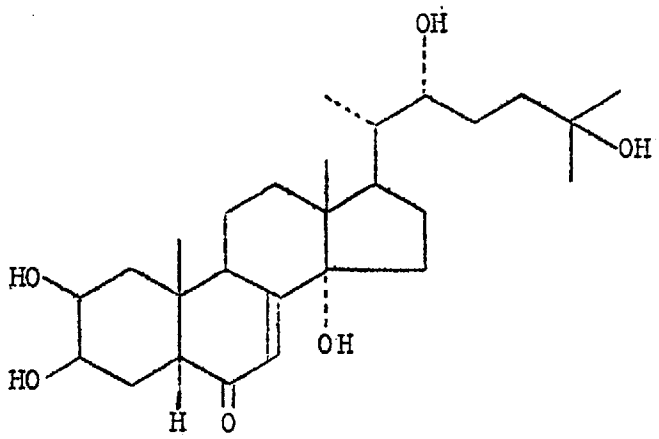
(41)

The reduction of the acetylenic ether (39), R=H, R^{1}= OH, was finally achieved, using 10% Palladium on carbon as the catalyst, to give the saturated diol, which now had the required mass spectrum.

Removal of the pyranyl ether protecting group with dilute hydrochloric acid, followed by basic hydrolysis of the 2, 3-diacetate moiety, (with simultaneous equilibration at C-5), gave, after removal of the less polar 5 α isomer, a compound which had its mass spectrum in good agreement with that published for ecdysone[4](1).

The melting point of this material was 236-9°C, only 2°C lower than that reported for ecdysone. It showed positive activity in the "Calliphora test" bioassay for insect moulting hormones.[23] However,

on direct comparison with an authentic sample[24] of ecdysone the compound was found to be somewhat more polar and therefore it must be isomeric with the naturally occurring hormone (1). Insufficient material was available for further characterisation to be carried out.

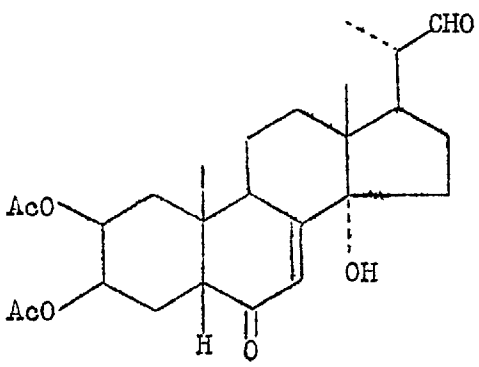


(1)

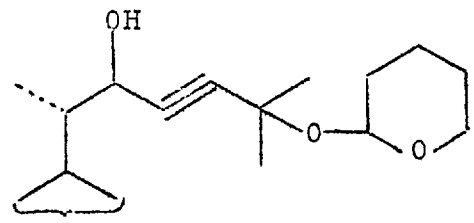
Further small scale experiments indicated that the bromomagnesium derivative of the ether (35) reacted more smoothly with the aldehyde (32) than the lithium compound. Hence the complete sequence from the aldehyde (32) was repeated, alkylating with the bromomagnesium derivative of the acetylenic ether (35).

From this reaction the 20R diol (39), R=CH, R^1=H, was obtained in excellent yield, with insufficient quantities of the other (20S)

isomer being formed for isolation. The marked difference in stereospecifity between the lithium and bromomagnesium derivatives used in the alkylation reaction, can only be due to differences between the metal derivative used. In support of these results it has also been independently shown that when the alkylation, using the bromomagnesium derivative, is carried out on the 14 α-hydroxylated aldehyde (42) only the 20R alkylated isomer (43) could be isolated.[4]

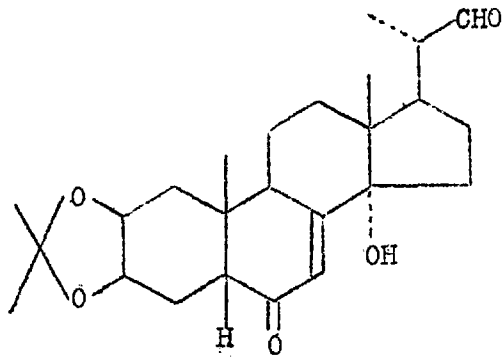


(42)



(43)

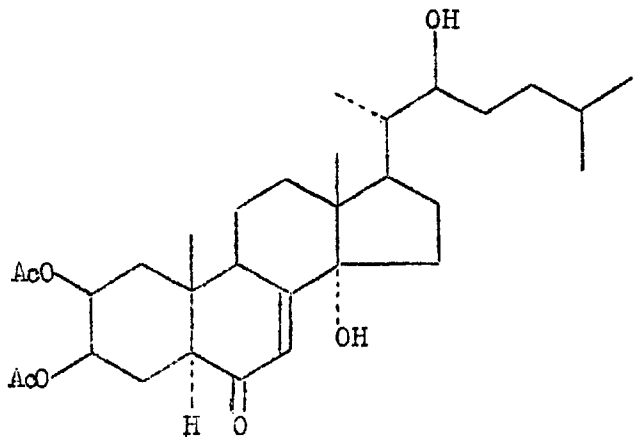
On the other hand it has been reported that use of the lithium derivative of the acetylenic ether (35), on the aldehyde (44) gave both possible isomers.[22]



(44)

Both the aldehydes (42) and (44) are A/B cis steroids, whereas in the present case the reactions were carried out on a A/B trans system with thealdehyde (32), but however, this should have little influence on the course of the reaction.

The initial reduction experiment on the acetylenic 20R diol (39), R=OH, R^1=H, (produced by use of the bromomagnesium derivative), also gave a hydrogenolysis product (45).

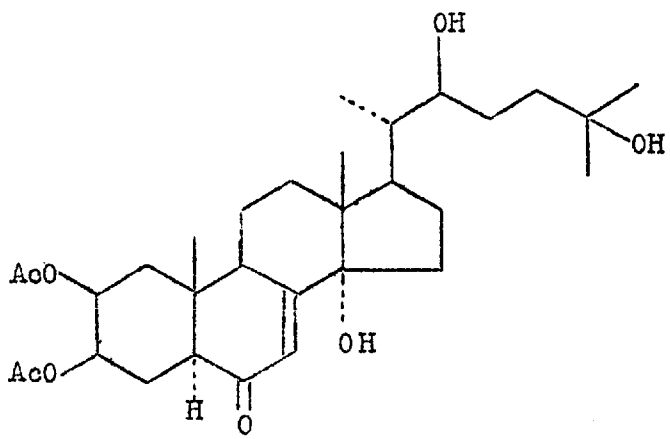


(45)

The 25-desoxy nature of this compound was shown by comparison with the product formed by alkylation of the aldehyde (32) with iso-amyl magnesium bromide [25]. Both compounds had identical melting points. A mixed melting point showed no depression.

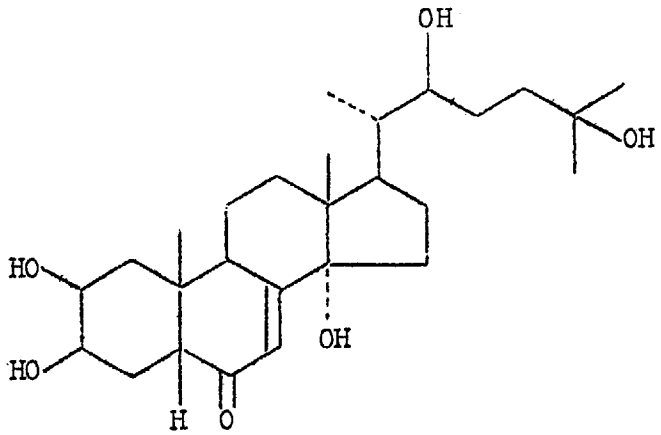
This experiment also allowed the assignment of a structure to a previous desoxy compound (40) obtained (see p. 98).

The reduction of the acetylenic diol (39), R=OH, R^1=H, was finally achieved, using a different batch of the 5% palladium on carbon catalyst, to give a non-crystalline diol. This compound after dilute acid removal of the tetrahydropyranyl ether protecting group, gave the crystalline triol (46).



(46)

Mild base hydrolysis, under equilibrating conditions, of the triol (46) gave a mixture of C-5 epimeric pentaols. Separation of the required 5β isomer gave 22-*iso*- ecdysone (47), identical to an authentic sample.



(47)

e) Summary

A new, acid catalysed, ring opening procedure of 3 α, 5 α -cyclo-6-keto steroids which gives directly a 2-ene system has been devised. In the present case, where a 7-ene double bond is featured, isomerisation of this double bond leads to mixtures of 14 α and 14β steroids.

A novel method, via a enolacetate, whereby the 14 α -hydroxyl group occurring in insect moulting substances can readily be

introduced, has been developed. An alternative method, hitherto unreported, for simultaneously introducing both a 7-ene and 14 α-hydroxy moiety into a 6-keto steroid has also been found .

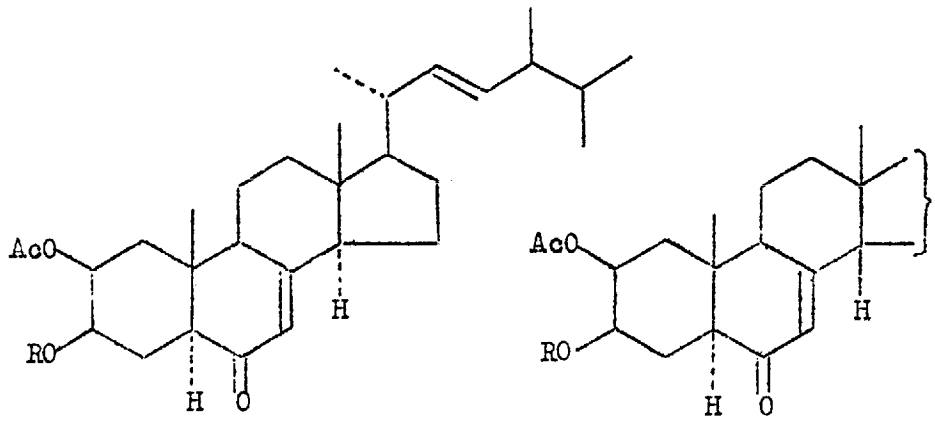
The shortest sequence yet reported (12 stages), to the penta-hydroxylated insect moulting analogue, 22-iso-ecdysone has been achieved. This sequence should be readily adaptable to a synthesis of ecydsone by a simple change in the alkylating reagent used in the preparation of 22-iso-ecydsone.

SECTION II. Synthetic studies on C-20 hydroxylated insect moulting substances.

a) Introduction

There are potentially several ways in which the 20, 22-diol system of crustecdysone, and the other C-20 hydroxylated insect moulting substances, could be introduced.

As it was preferred to use an intermediate, made available during the course of the earlier ecdysone synthetic work (see SECTION I), the choice of method was narrowed. The common intermediate chosen as being most suitable was the mixture of C-14 epimeric monoacetate diols (20) and (22), R=H, since this could readily be degraded to a 22-aldehyde by the same ozonolysis procedure used in the ecdysone work.



(20) R = H
R = Ac

(22) R = H
R = Ac

A method had to be derived then, for introduction of a hydroxyl group at C-20, whilst still retaining the 20-formyl function, (in order that the side chain could be elaborated by a Grignard reaction, with formation of the C-22 hydroxyl group).

Several methods by which this could be done were considered. For example, α-halogenation of the aldehyde function, followed by hydroxyl substitution, would give the desired type of structure. Since this approach may have been difficult to control it was rejected.

Two other general approaches were considered and have been explored. The possibility of direct autoxidation in basic media, of the aldehyde, to a suitable derivative merited investigation.

Similarly, the correct type of structure could possibly be achieved by expoxidation of the enamine, enolacetate or enol-ether, derived from the aldehyde function.

Present efforts along these lines will be discussed in detail below.

b) Autoxidation experiments

It is well known that the base catalysed autoxidation of suitable oxo steroids affords the corresponding α-hydroxy compounds. The autoxidation has been shown to proceed via the α-hydroperoxy intermediate, which in some cases is stable enough to be isolated. The hydroperoxide can be decomposed by either warming a solution of the compound with base, or by reducing, to give the corresponding α-hydroxy oxo steroid[26]. An example is outlined in Fig. 8.

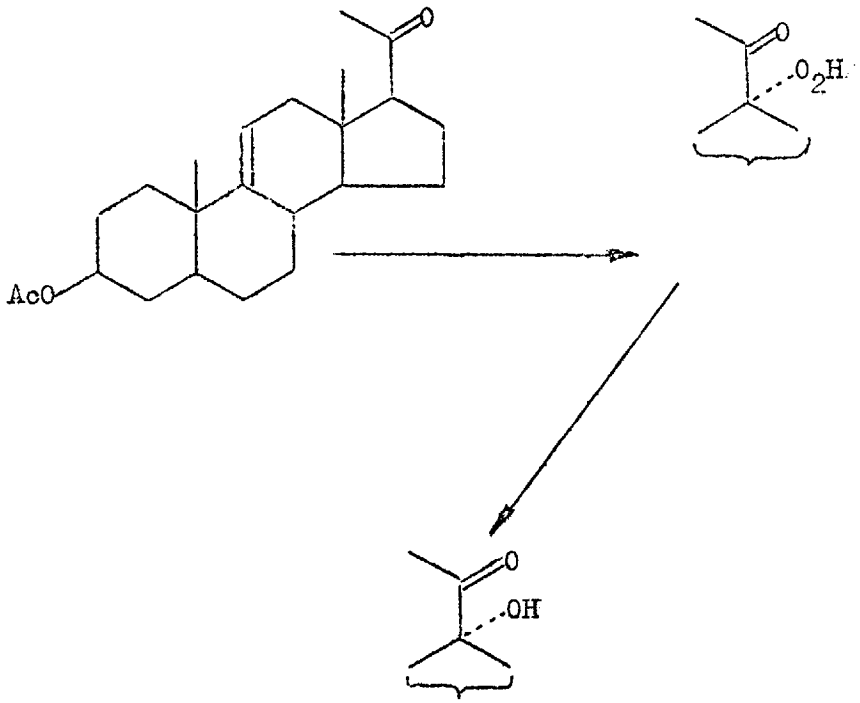
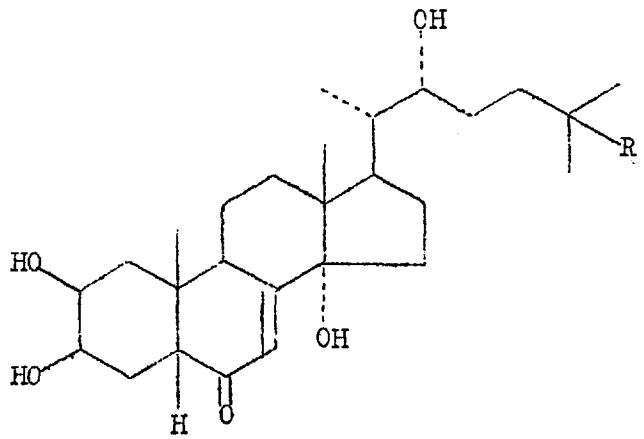


Fig. 8.

It was considered that in principle the reaction could be utilised to produce the required C-20 hydroxylated intermediates necessary for the synthesis of a C-20 hydroxylated insect moulting hormone e.g. crustecdysone (48) R=OH, or ponasterone A (48) R=H.

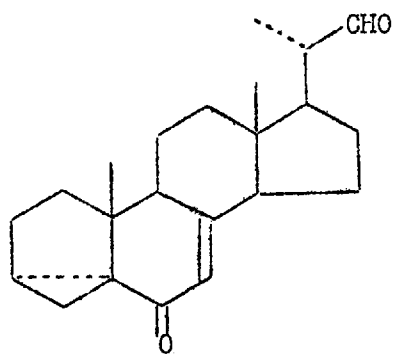


(48) R = OH
R = H

For the initial experiment the cyclopropyl aldehyde (31) was chosen as a suitable model compound. It is possible that such C-20 formyl steroids could undergo C-20, C-22 bond fission when subjected to autoxidation under the normal conditions, i.e. potassium t.-butoxide in t-butanol,[27] to give a methyl ketone. Such a bond fission must proceed via an unstable C-20

hydroperoxide. However, if this hydroperoxide could be reduced, *in situ*, as soon as it is formed, then the C-20 hydroxylated aldehyde would be produced. The reaction sequence outlined in Fig. 8 (see above), has been achieved on one step, (without isolation of the intermediate hydroperoxide), by carrying out the autoxidation in the presence of trivalent phosphorous compounds.[28]

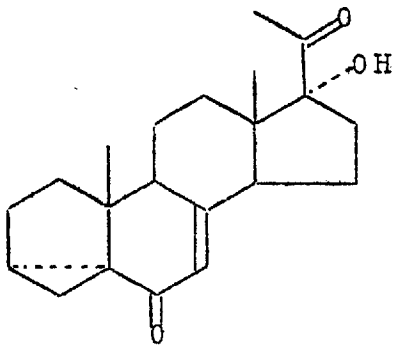
Triphenyl phosphine was chosen as a suitable reducing agent in the autoxidation experiments carried out on the cyclopropyl aldehyde (31).



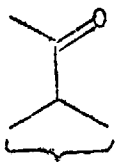
(31)

The initial experiment, conducted at room temperature, using the usual excess of base but in the presence of a large quantity of triphenylphosphine, proceeded much too fast to follow and yielded a complex mixture. However, when the reaction was repeated at 0°C and the base concentration reduced to two equivalents, the oxidation proceeded smoothly to give, in high yield, the hydroxylated

methyl ketone (49).



(49)



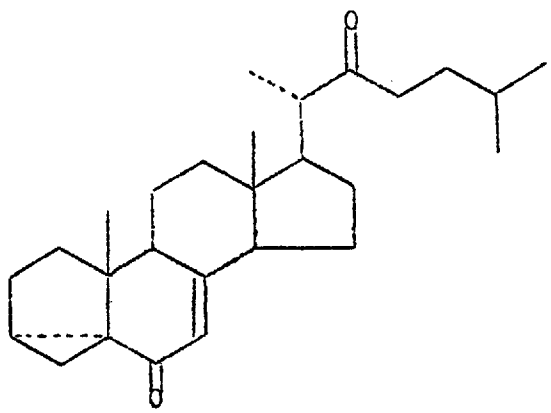
(50)

The structure of this material (49) was deduced from its n.m.r. spectrum which showed a 3 proton singlet at 2.13 p.p.m. (characteristic of a methyl ketone). The hydroxylated nature of the compound was inferred by the disappearance of the signal at 6.15 p.p.m. after exchange with deuterium oxide, and confirmed from its infrared spectrum. The position of the hydroxylgroup at C-17 and its stereochemistry was by analogy with known autoxidations.[26]

When the experiment was repeated under the same conditions, but stopped before completion, both the hydroxylated (49) and unhydroxylated ketone (50) were present in the reaction mixture. At no time was any compound observed which may have been the required C-20 hydroxylated aldehyde. If the reaction temperature

was reduced still further to -70°C, the rate of oxidation became too slow to be of practical use.

As an alternate possibility, attention was then directed at the autoxidation of a suitable 22-keto steroid, and the dione (51) was chosen as a suitable model compound.

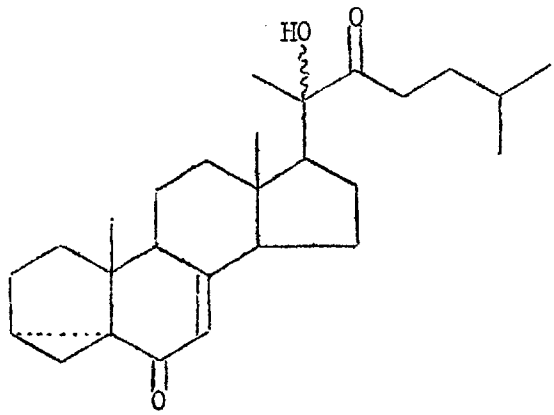


(51)

The model dione (51) was readily prepared by a Grignard reaction between the aldehyde (31) and iso-amyl magnesium bromide to give the correspnding C-22 alcohol, which was smoothly oxidised to the required ketone (51) with Jones reagent.

Autoxidation of the dione (51) at room temperature, under the usual conditions, in the presence of triphenylphosphine or triethylphosphite, proceeded rapidly with the uptake of four equivalents of oxygen, to give three major products. Separation of these products

followed by infrared spectroscopic examination, showed none of them could have been the required 20-hydroxylated compound (52).



(52)

Even when the reaction was stopped after the uptake of 1, 2 or 3 equivalents of oxygen the same three compounds were recovered, together with unreacted starting dione (51).

At this point it was decided to cease the autoxidation experiments and to concentrate on an alternate approach to the problem.

c) Enol acetylation experiments.

The introduction of a hydroxyl group α to a carbonyl group is often accomplished by epoxidation of the corresponding enolacetate followed by ring opening of the epoxide. If the resulting epoxide is opened by base catalysed hydrolysis, opening occurs with retention of configuration about the hydroxyl bearing carbon atom. In contrast, on heating epoxy acetates, rearrangement occurs[29,30] to give the

α -acetoxy ketone in which inversion of configuration has occurred about the α-carbon atom (see Fig. 9).

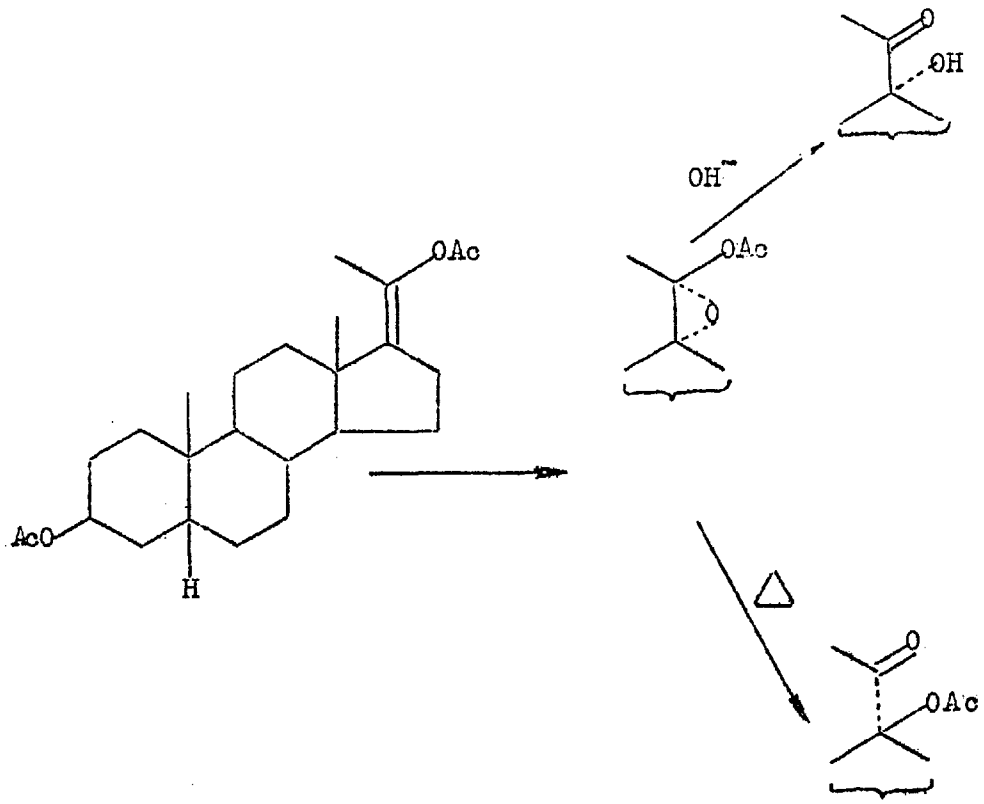
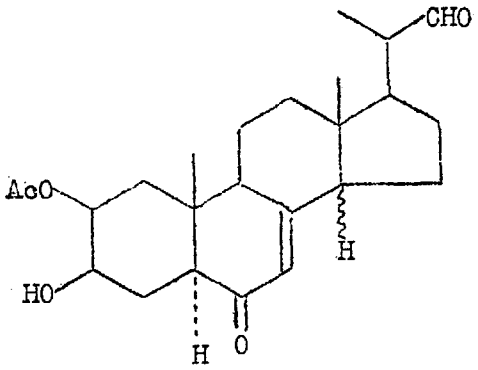
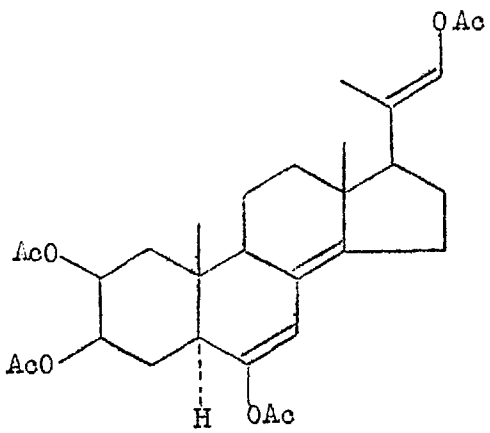


Fig. 9.[30]

It was intended to utilise the reaction by oxidation of the bis-enolacetate (52) derived from the mixture of C-24 epimeric aldehydes (53).



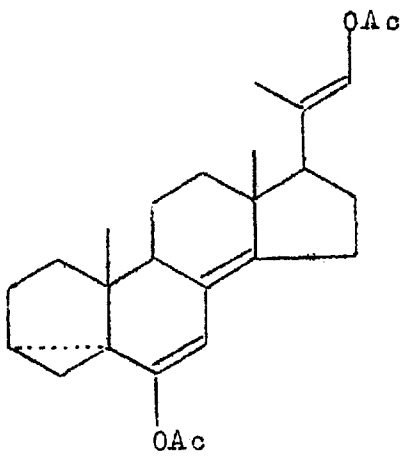
(53)



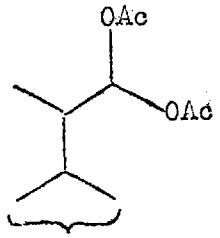
(52)

Hence, by subsequent treatment with peracid, simultaneous introduction of the 14 α-hydroxyl group and an oxygen at C-20 could be achieved. It was hoped that the epoxide formation on the side chain enolacetate would be stereospecific and therefore, either by simple base opening or by thermal rearrangement of the epoxy acetate, the necessary stereochemistry at C-20, i.e. 20R could be achieved.

For the initial experiments the cyclopropyl aldehyde (31) was chosen as a suitable model compound. Enolacetylation under the perchloric acid catalysed conditions (used earlier in the ecydsone synthetic work), failed to give the desired bis-enolacetate (54) and gave instead the non-crystalline diacetate (55).

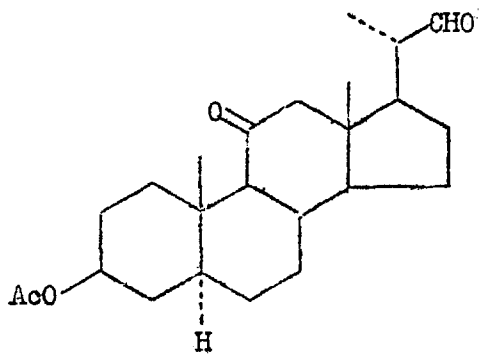


(54)



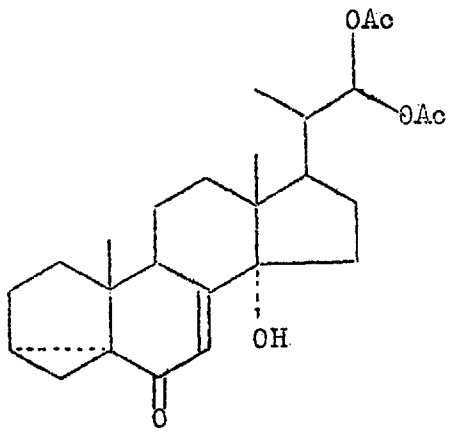
(55)

A search of the literature revealed a similar result had been observed by Cameron et. al.[31], when the aldehyde (56) was treated under identical conditions.



(56)

Treatment of the diacetate (55) with peracid yielded the 14 α -hydroxy diacetate (57) which was completely characterised by all the usual methods.

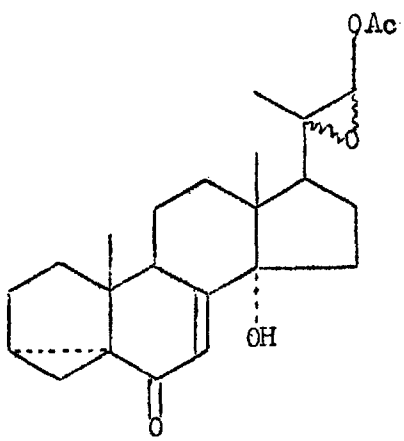


(57)

During these experiments an observation was made which was to prove of some importance (see below). In the course of the enolacetylation reaction, the α,β-unsaturated ketone moiety appeared to react faster than the aldehyde function.

In contrast, treatment of the cyclopropyl aldehyde (31), with sodium acetate and acetic anhydride gave the required bis-enolacetate (54) in excellent yield. In this case it was observed that the aldehyde moiety had reacted first. Subsequent treatment of the bis-enolacetate (54) with excess monoperphthalic acid gave, not as hoped a stereochemically pure epoxide, but an inseparable mixture of two isomeric epoxides (58) in the ratio 3:2. The amount of each

isomer was determined from the n.m.r. spectrum obtained on the mixture. The signals due to both the 18-methyl and C-22 protons for each isomer, appeared at quite different points in the spectrum and hence the ratio of each isomer in the mixture could be calculated. At this point it was not possible to assign any stereochemistry to either of these isomers.

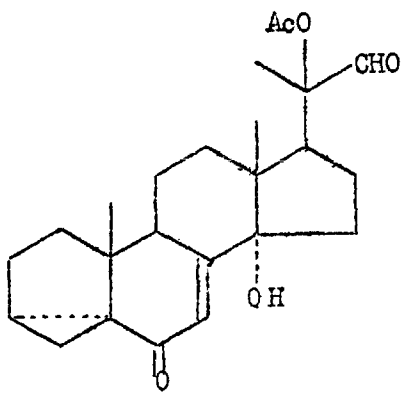


(58)

Attempts were made to achieve steric control of the reaction using a variety of conditions and expoxidising agents. The effect of temperature on the steric result of the epoxidation proved to be very minor. Some degree of control was obtained when the reaction was carried out using an optically active peracid *viz.* L. percamphoric acid, the ratio of isomers now being 2:3 *m*-chloroperbenzoic acid

altered the ratio to 1:1 and when used at low temperatures to 2:3. No better degree of control could be achieved. In view of this result, it was decided to use the mixture in further experiments and hope to achieve a separation at a later stage.

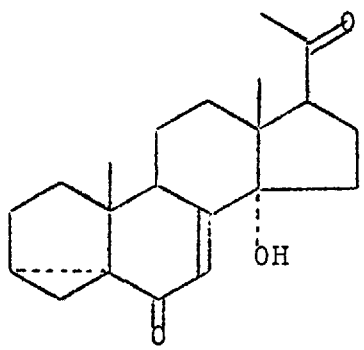
Thermal rearrangement of the epoxy acetate (58) was preferred to base catalysed ring opening since, in the main series other acetate groups would be present. However, it was not known if the 14 α-hydroxyl group would be affected by heat. In the event, upon heating the mixture of epoxides (58) to 160°C, the envisaged rearrangement reaction occurred smoothly to yield the α-acetoxy aldehyde mixture (59) in excellent yield. This mixture <u>again</u> proved to be inseparable and hydrolysis to the corresponding α-hydroxy aldehyde mixture was then attempted.



(59)

The initial experiment proved to be a rather interesting one. This hydrolysis was carried out in aqueous methanolic/ tetrahydrofuran solution, using potassium carbonate as the base, and smoothly gave two compounds which were readily separated by P.L.C.

The less polar material was not one of the required isomeric α -hydroxy aldehydes as expected, but instead was shown to be the methyl ketone (60).



(60)

Its n.m.r. spectrum showed a signal at 2.15 p.p.m., characteristic of a methyl ketone, and the absence of formyl protons. The assigned structure was supported by the infrared spectrum which showed the required peak for the 20-keto function at 1695 cm^{-1}.

The more polar compound was shown to be one of the expected α-hydroxy aldehydes by infrared and n.m.r. spectroscopic studies. It was interesting to note that the material obtained was stereochemically pure (the n.m.r. spectrum had only one signal for the C-18 and C-21 methyl groups and it was assigned the 20 S configuration (61), (the reasons for this assignment will be discussed in detail below).

The formation of the methyl ketone (60) is presumably due to the reaction of the hydroxy aldehydes with peroxides present in the tetrahydrofuran, (a possible mechanism is outlined in Fig. 10).

This oxidation reaction appears to have taken place selectively, with the 20 R epimer (62) reacting faster, essentially leaving the 20 S epimer (61) unreacted.

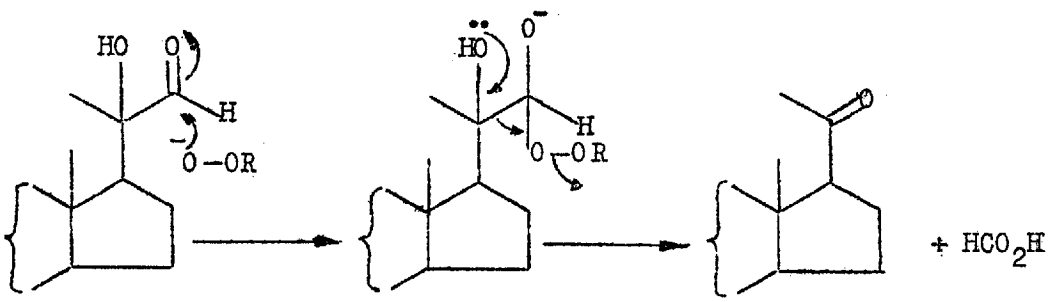


Fig. 10.

When the hydrolysis was repeated in the absence of tetrahydrofuran the expected mixture of α-hydroxy aldehydes was obtained. An important point noted during the course of this reaction was that the hydrolysis was complete in a matter of seconds, presumably due to the synartetic effect of the formyl group, (see Fig. 11).

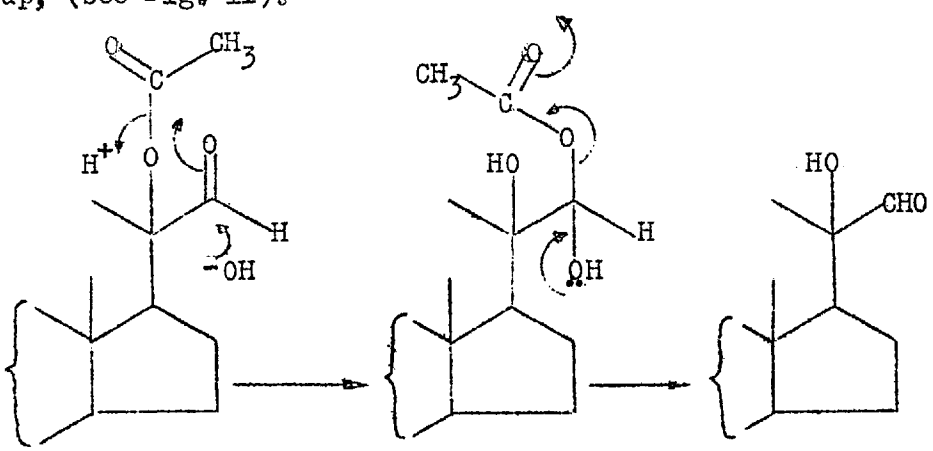
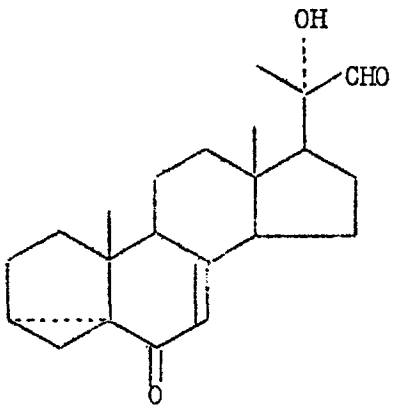


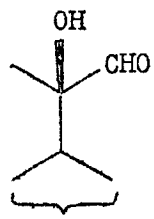
Fig. 11.

The mixture of aldehydes obtained in this way were separated, with some difficulty, into the pure components (61) and (62).

The stereochemistry of each epimer was assigned at this point by comparison of their n.m.r. spectra with the n.m.r. spectra of



(61)

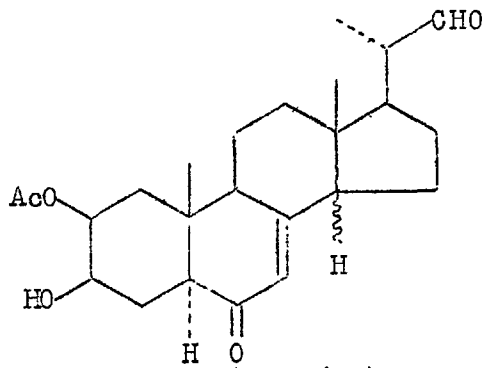


(62)

20-hydroxy pregnanes[32,33], and 20-hydroxy cholesterols[34] of known absolute stereochemistry. The Syntex group, in their synthesis of crustecdysone, also assigned the stereochemistry of their α-hydroxy aldehydes on these grounds.[35] (for a detailed comparison see Table 1.p 130)

Having successfully introduced the 20-hydroxyl group these experiments could now be repeated in the main series.

Ozonolysis of the mixture of monoacetate diols (20), R=H, and (22), R=H, in methylene chloride/pyridine, gave the required aldehyde mixture (53) in good yield.

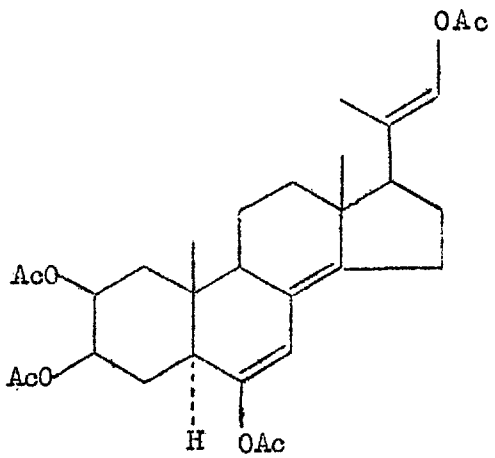


(53)

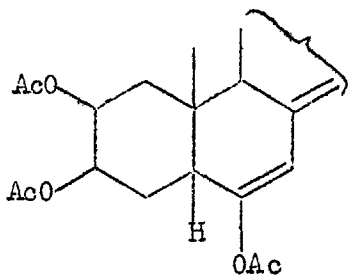
Treatment of the aldehyde mixture (53) under the base catalysed enolacetylation conditions described above, gave, not as expected a single product, but two, difficult to separate compounds. These were shown to be isomeric by mass spectral studies. N.m.r. spectroscopic examination of the separated products suggested that they were the two possible C-5 epimers (63) and (64).

This conclusion was based on the multiplicity of the C-7 proton signal in the spectrum. In the case of the 5 α - epimer the signal appeared as the expected doublet, (due to allylic coupling with the C-5 proton), and as a broad singlet in the case of the 5β epimer.

This was not an unexpected result as the reaction times required for the enolacetylation reaction (ca. 20 hours), were quite sufficient for equilibration at C-5 to have taken place.

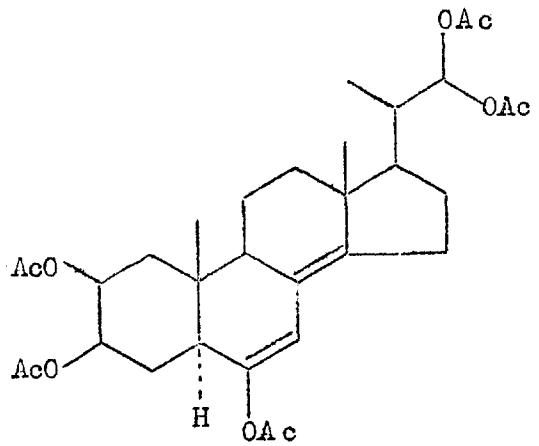


(63)



(64)

Due to the extreme difficulty of separating these isomers, (made more difficult by their unstable nature), it was decided to investigate conditions whereby the required bis-enolacetylation reaction could be achieved without equilibration at C-5. It had been previously shown (in the ecdysone work), that an acid catalysed enolacetylation of the 7-en-6-one moiety, without equilibration at C-5, could be abhieved. A number of acid catalysed procedures were attempted but all gave the geminal diacetate (65).



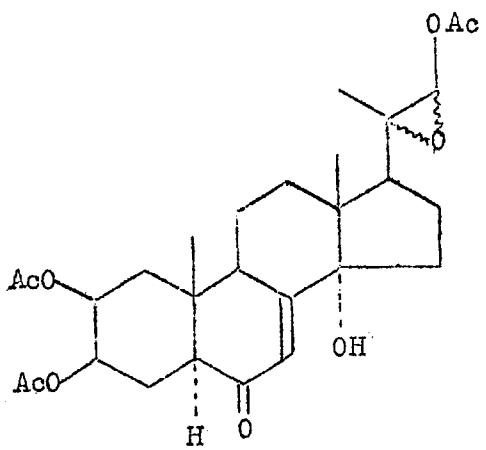
(65)

At this point it was decided that the problem could be overcome by using a kinetically controlled, two step, enolacetylation reaction, with little or no equilibration at C-5 occurring. The reasons for arriving at this conclusion were based on the earlier observations, in which it was noted that the aldehyde moiety could be rapidly enolacetylated under basic conditions. It had also been noted that the 7-en-6-one function smoothly reacted under acid catalysed conditions without equilibration at C-5. Therefore, by using a two step procedure, firstly under basic conditions, and then under acidic conditions, a short enough reaction time may be achieved

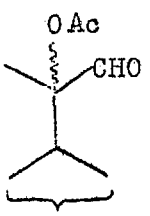
so that no equilibration would occur at C-5. In practice this proved to be the case, the bis-enolacetate formed being the 5 ∝ epimer (63). This same two step procedure also gave some of the geminal diacetate (65).

Epoxidation of the bis-enolacetate (63) with excess monoperphthalic acid gave, as expected, a mixture of the epimeric epoxides (66) which, as in the case of the cyclopropyl model compound, proved to be inseparable.

As before, rearrangement to the ∝ -acetoxy aldehyde mixture (67) was smoothly achieved by heating to 160°C. Once again this mixture proved to be inseparable.



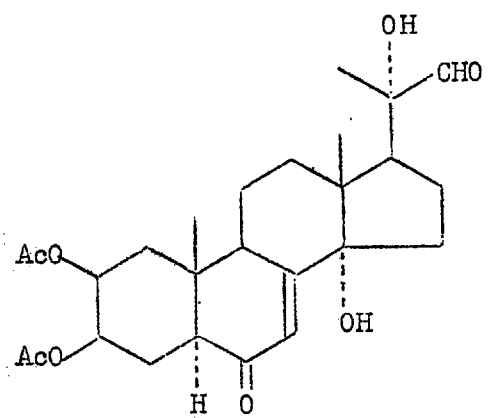
(66)



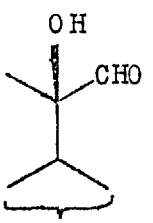
(67)

The earlier model work had also shown that if the 20-acetoxy group could be selectively removed the resulting α-hydroxy aldehydes would be separable by P.L.C.

The observation, noted in the case of the cyclopropyl model compound (59), that the 20-acetoxyl group was rapidly hydrolysed to the alcohol, was now put to practical use. Brief treatment (two minutes), of the α-acetoxy aldehyde mixture (67) with dilute methanolic potassium carbonate gave the desired products (68) and (69) in excellent yield.



(68)

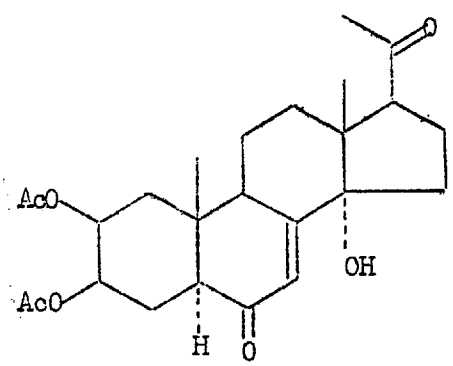


(69)

The mixture could now be separated, with great difficulty, using the successive elution technique by P.L.C. into its individual

components (68) and (69). Once again, the absolute stereochemistry of these were assigned by comparison of the n.m.r. spectrum of each compound with those of 20-hydroxy pregnanes[32,33] and 20-hydroxy cholesterols [34] of known absolute stereochemistry. (see Table 1.- the Syntex α -hydroxy aldehydes[35] are included for further comparison).

The formation of the methyl ketone (70) could also be accomplished in a similar way to that used in the cyclopropyl series (see p.120). An ethereal solution of the isomeric hydroxy aldehydes (68) and (69) was left for 48 hours in the presence of air. Once again, a methyl ketone (70) was isolated together with the stereochemically pure 20S epimer (68).



(70)

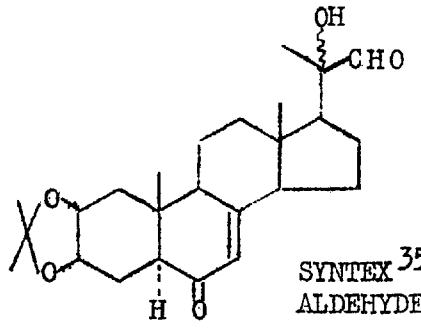
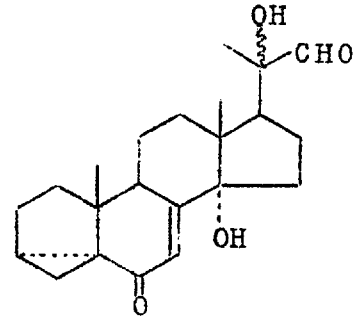
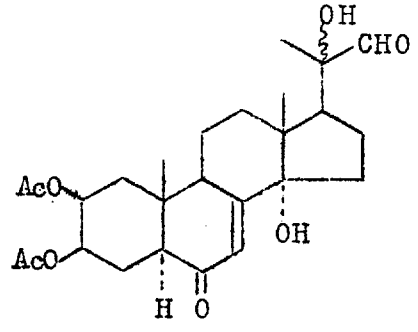
Compound	Position of 21-methyl Group (δ)		$\Delta\delta$
 SYNTEX[35] ALDEHYDES	$20\beta_F$	$20\alpha_F$	
	1.36 20R	1.27 20S	-0.09
	1.37 20R	1.31 20S	-0.06
	1.36 20R	1.30 20S	-0.06
3β-acetoxy-20-hydroxy-pregn-5-ene[32, 33]	1.24 20S	1.14 20R	-0.11
3β-acetoxy-20-hydroxy-cholest-5-ene[34]	1.17 20S	1.00 20R	-0.17

TABLE 1.

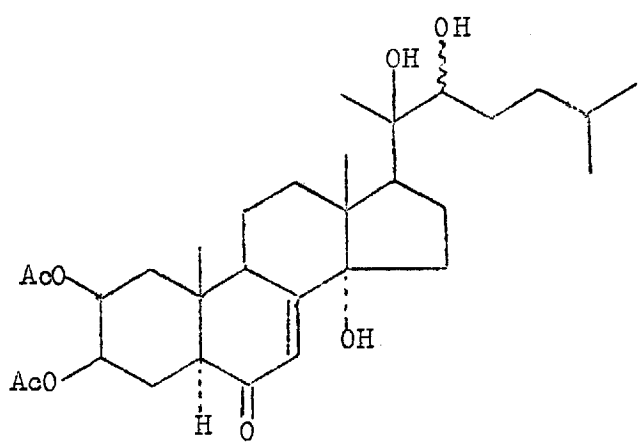
It would therefore, seem that this reaction can be achieved by the interaction of any ether peroxide with the ∝ hydroxy aldehydes.

Having arrived at the desired 20R-hydroxy aldehyde (69), albeit in small (mg) quantities, it was now possible to attempt the addition of a suitable side chain.

d) Attempted addition of a side chain

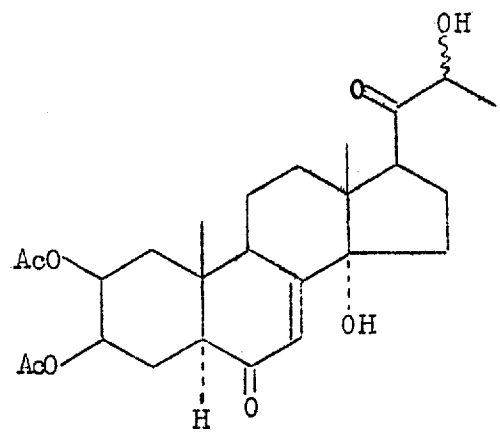
It had been decided to attempt a synthesis of ponasterone A (48), R = H. There were two reasons for this choice. Firstly a synthesis of crustecdysone had appeared in the literature[35] during the course of this work and secondly, the absence of a 25-hydroxyl group in ponasterone A meant that a Grignard reaction between the aldehyde (69) and iso-amyl magnesium bromide would lead directly to the required side chain.

An initial experiment was carried out on the pure, main series, 20R ∝-hydroxy aldehyde (69). The Grignard reaction smoothly gave a new product. However, thin layer chromatography indicated that the compound formed was unlikely to be the required alkylated product (71).



(71)

This conclusion was further confirmed by mass spectroscopic studies on the product. These studies showed that the new compound was, in fact, isomeric with the starting aldehyde (69). The small amount of material available made further characterisation difficult. The ketol structure (72) was assigned mainly on the basis of information received from the Syntex group[36] (a similar reaction had been attempted by them in the course of their studies).



(72)

A simple, base. catalysed, rearrangement mechanism can be written to explain the nature of the product. (See Fig. 12).

A further experiment was carried out on the mixture of α-acetoxy aldehydes in the cyclopropyl series (59). In this case the 20-hydroxyl group is protected and the rearrangement cannot

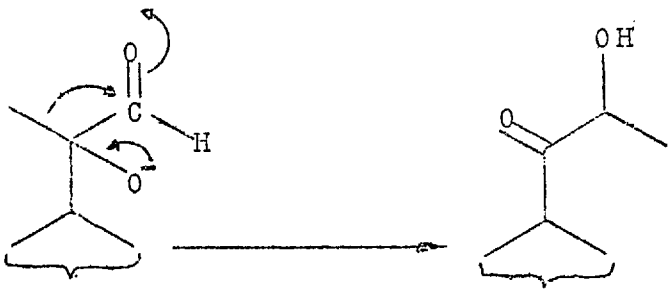
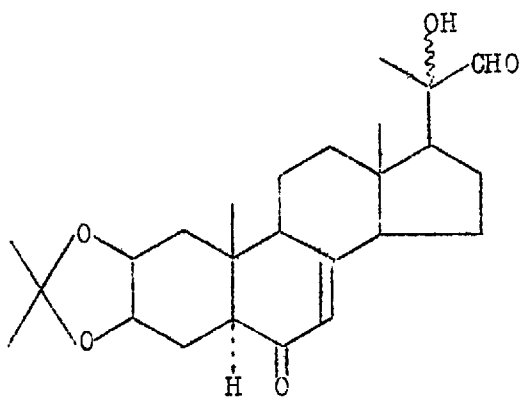


Fig. 12.

take place. An objection to this route was that the reaction would lead from a mixture of two compounds to a mixture of four possible isomeric products, i.e. the four possible C-20, C-22 diols (assuming no stereospecifity was obtained). It was hoped that the mixture would be fairly amenable to separation. But when the reaction was carried out a complex mixture was obtained. The n.m.r. spectrum of the crude reaction product indicated that the desired alkylation had taken place (by the appearance of additional methyl group signals). Unfortunately, the mixture proved too complex to allow ready separation of any pure products and further work in this direction was discontinued.

Thus, two problems remained, A suitable protecting group had to be found for the 20-hydroxyl group and, if possible, derivatives of the 20-hydroxy or 20-acetoxy aldehyde mixtures which would allow ready separation into the pure components.

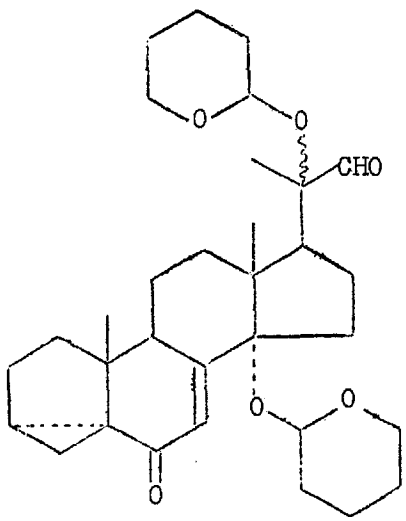
It was believed the cause of the separation difficulties of the α-hydroxy aldehydes (68) and (69) was the influence of the 14 α-hydroxyl group. This belief was based on the report that the Syntex group had separated their mixtures of α-hydroxy aldehydes (73), in which the 14 α-hydroxyl group is absent, without any apparent difficulties.[35]



(73)

Hence if a derivative could be found that protected both the 14 and 20-hydroxyl groups the separation may become easier and both objectives would have been achieved.

It was reported that the 20-hydroxyl group could be protected by formation of the tetrahydropyranyl ether[35]. Attempts to form the bis-ether (74) in the cyclopropyl series afforded such a compound. However, no separation of the isomeric mixture could be achieved.

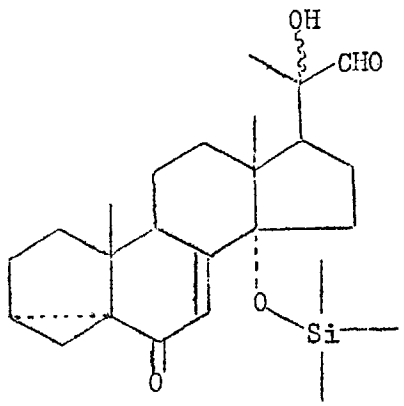


(74)

It was considered that this was due to the formation of diastereomeric tetrahydropyranyl ethers, and hence the introduction of further centres of asymmetry.

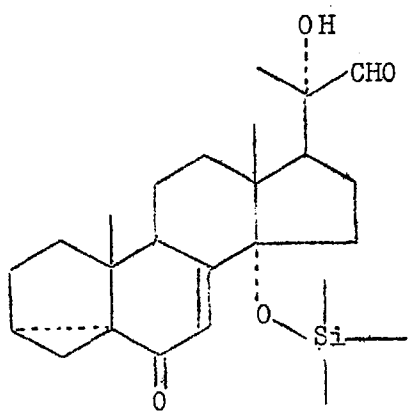
Attention was then focused on the use of silyl ethers as protecting groups. When the α-hydroxy aldehyde mixture in the cyclopropyl series was treated with bis-trimethylsilyl-acetamide in dimethylformamide,[37,38] rearrangement of the α-hydroxy aldehyde moiety occurred (c.f. p.131). This result was indicated from the n.m.r. spectrum of the reaction product, which showed no aldehyde protons.

Silylation, under the same conditions, of the α-acetoxy aldehyde mixture (59) gave a crystalline mixture of the required C-4 silyl ethers (75).

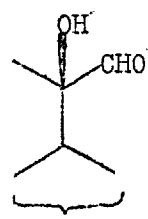


(75)

This mixture could not be separated, but after hydrolysis of the 20-acetoxyl group, separation was found to be possible and the α-hydroxy aldehydes (76) and (77) were obtained in a stereochemically pure form.

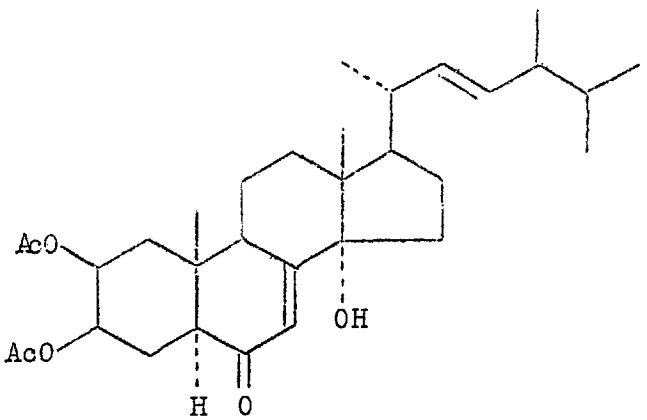


(76)



(77)

However, an attempted silylation of the 14 α-hydroxyl group of the diacetate (27) gave a mixture of two silyl ethers.



(27)

N.m.r. spectroscopic examination of each ether indicated that isomerisation at C-5 had again occurred. The change from the A/B <u>trans</u> to the A/B <u>cis</u> fused system is indicated, in such types of compound, by an increase in the difference between the positions of the two acetate signals[39]. In this case the less polar, 5 α compound, showedthe two acetate signals at 2.00 and 2.06 p.p.m., whilst in the more polar 5β compound, the signals appeared at 1.97 and 2.08 p.p.m.

As a result of this experiment further silylation work was discontinued. Time did not permit further work on this problem which remains to be solved.

e) Summary

A method for the introduction of a 20-hydroxyl group into a C-20 formylated steroid has been devised. The method yields both possible C-20 epimers, which in the general case should be readily separable. A novel reaction of the 20-hydroxyl-20-formyl steroids studied, is one in which ether peroxides have been found to react preferentially with 20R epimers to yield a 20-ketopregnane. In addition, these compounds have been observed to readily undergo a base catalysed rearrangement with a 1, 2 shift of the 20-methyl group, to give an α-ketol.

When the problem of separation of the C-20 hydroxy epimers has been solved the scheme outlined will represent a convenient synthetic approach to C-20 hydroxylated insect moulting hormones.

P A R T I I I

EXPERIMENTAL

Unless otherwise stated, the following data applies to experiments described in this section.

Melting points were determined on a Kofler block and are uncorrected. Infrared spectra were taken as nujol mulls on a Unican S.P.200 spectrometer. Ultraviolet spectra were determined in ethanol solution using a Unicam S.P. 700 spectrometer. N.m.r. spectra were measured in deuterochloroform solutions, using trimethylsilane as an internal standard, on a Varian A.60 spectrometer. Mass spectra were determined with a A.E.I. high resolution M.S.9 instrument using the direct inlet technique. Rototations were determined in a 10 cm. cell using ethanol solutions. All reactions were followed, wherever possible, by thin layer chromatography (T.L.C.) using 0.1 m.m. plates prepared from Merck silica gel type GF_{254}. Preparative layer chromatogrphy (P.L.C.) was carried out on 0.1 x 20 x 60 cm. or 0.05 x 20 x 60 cm. plates prepared from Merck silica gel type GF_{254}. T.L.C. plates were developed using the phosphomolybdic acid spray.[40] Solvents were dried, when necessary, using standard techniques. Petrol refers to light petroleum fraction (60 - 80°C).

The following refers to n.m.r. data:

s.....singlet

d.....doublet

t.....triplet

q.....quartet

bs.....broad singlet

m.....multiplet

SECTION I

3 α ,5 α -cyclo-ergosta-7,22-dien-6-one. (3)[41]

Ergosterol (2) (10 g.) was dried by dissolving in benzene and removal of the solvent by distillation. The residue was dissolved in dried pyridine (50 ml.) and p-toluene sulphonyl chloride (12 g.) added. The resulting solution was allowed to stand for 24 hours in the dark at room temperature. The solution was poured into ice-cold potassium hydrogen carbonate solution (4%, 1000ml.) with stirring. The crude tosylate which separated, was collected by filtration and washed, until free from pyridine, with further portions of ice-cold bicarbonate solution. The crude product was then added to a refluxing solution of potassium hydrogen carbonate (6.0 g.) in acetone (2 l.) and water (500 ml.). The refluxing was continued for 5 minutes and then the solvent distilled off until ca. 1250 ml. had been collected. The resulting turbid solution in the distillation flask was cooled to 0°C and the solid cyclopropyl alcohol which separated out was collected by filtration. The residue on the filter was dissolved in chloroform (100 ml.) and any aqueous layer discarded. The organic layer was washed with water, dried (Na_2SO_4), and the volume adjusted to ca. 250 ml. with chloroform. Active manganese dioxide (60 g.) was added and the mixture stirred overnight at room temperature under dried nitrogen. The manganese dioxide was filtered off, washed well with chloroform, and the filtrate evaporated to dryness, under reduced pressure, to yield the crude ketone (3) (9.2 g.). Crystallisation from ether gave the

pure ketone m.p. 165 - 66°C (lit. m.p. 166°C)[42]. Yields varied from 57 - 64% ν max. 1650 $cm.^{-1}$

The mother liquors from the crystallisation were evaporated to dryness and chromatographed on alumina (Grade V). to yield initially, 3 α, 5 α -cyclo-ergosta-6, 8, (14), 22-triene (4). which crystallised from acetone as plates m.p. 100 - 102°C (lit. m.p. 101°C)[43]. Further elution gave an additional quantity of the ketone (3).

3 α, 5 α -cyclo-cholest -6-one (8)

3 α, 5 α -cyclo-cholest -6β-ol[8] (3.0 g.) (9) was dissolved in analar acetone (100 ml.) and cooled to 0°C. Jones reagent (2.3 ml.) was then added dropwise to the stirred solution over a period of 5 minutes. The mixture was stirred a further 5 minutes, neutralised with saturated sodium bicarbonate solution, and evaporated to low bulk. Water (25 ml.) was then added and the mixture extracted with chloroform (2 x 100 ml.). The separated organic layer was dried (Na_2SO_4) and evaporated under reduced pressure to give the crude ketone (8) (2.8 g.). Chromatography over alumina (Grade III. 100 g.) and elution with benzene/petrol (1:9) gave the pure ketone (8) (2.6 g.) m.p. 98°C (lit. m.p. 96.5-7.5°)[44].

Attempted base catalysed ring opening of 3α, 5α-cyclo-cholest-6-one(8)

a) With quinoline.[7]

The ketone (8) (50 mg.) was dissolved in quinoline (5 ml.) and refluxed for 16 hours. T.L.C. indicated no reaction. The mixture was poured into ice-cold hydrochloric acid (6N), extracted with ether, the organic layer separated and dried (Na_2SO_4). Evaporation under reduced pressure gave a ketone (46 mg.) identical by n.m.r. and infrared spectroscopic examination to the starting ketone (8).

b) With sodium hydroxide

The ketone (8) (60 mg.) was dissolved in a methanolic solution of sodium hydroxide (10 ml. 20%) and refluxed for 20 hours. T.L.C. again indicated no reaction had taken place.

c) With sodium hydride

The ketone (8) (50 mg.) was dissolved in dried toluene (5 ml.) and sodium hydride (10 mg. 50% suspension in oil) added. The mixture was refluxed overnight. T.L.C. showed no reaction had taken place.

d) With Potassium t-Butoxide

Potassium (390 mg.) was dissolved in dried t-butanol (7.4 g.) and the resulting solution filtered. The ketone (8) (120 mg.) was added and the solution refluxed, under dried nitrogen, for 1 hr. T.L.C. indicated the reaction was complete and had given a complex mixture. Water was added, and the mixture acidified with dilute hydrochloric acid. Extraction with ether gave an oil, which showed no olefinic protons in its n.m.r. spectrum.

Treatment of 3 α , 5 α cyclo-ergosta-7,22 - dien-6-one (3) with NaOH

The ketone (3) (100 mg.) was dissolved in methanol (10 ml.) and sodium hydroxide (0.5 g.) added. The solution was refluxed, under dried nitrogen for 2 hours. After dilution with water and extraction with ether, the organic phase was dried (Na_2SO_4) and evaporated under reduced pressure to yield an oil (95 mg.). T.L.C. indicated the presence of the ketone (3) and a less polar new compound. Separation by P.L.C. gave 3 α,5 α -cyclo-ergosta-8, (14), 22-dien-6-one (10) (40 mg.) as an oil. ν max. 1675 $cm.^{-1}$ λ max. no absorption above 210 mμ.

Attempted base catalysed ring opening of 14 α -hydroxy,-3 α ,5 α -cyclo-cholest-7-en-6-one (11).

a) With sodium hydroxide

The hydroxy ketone (11) (20 mg.) was dissolved in methanol (5 ml.) and sodium hydroxide (100 mg.) added. The mixture was refluxed 1 hour, cooled, and diluted with water. Extraction with ether in the usual way gave an oil (17 mg.). T.L.C. indicated the starting ketone (11) was present and a slightly more polar new product. Infrared spectroscopic examination of the crude product showed carbonyl absorption at 1650 (starting ketone) and 1690 $cm.^{-1}$

b) With Lithium N, N-dimethylamide.

To a solution of the hydroxy ketone (11) (25 mg.) in dried benzene (10 ml.) was added a solution of lithium N, N-dimethylamide in ether (1.5 ml. 16% w/v), and the mixture refluxed, under dried

nitrogen, for 1½ hours. T.L.C. indicated the same reaction product as obtained by sodium hydroxide treatment (see above).

c) With lithium N-phenyl-N-methylamide

The hydroxy ketone was treated with lithium N-phenyl-N-methylamide as described above. T.L.C. indicated essentially the same reaction product had been obtained.

d) With sodamide

The hydroxy ketone (20 mg.) was refluxed in dried benzene (5 ml.), containing sodaamide (100 mg.) for 16 hours. T.L.C. indicated no reaction had occurred.

e) With collidine

The hydroxy ketone (25 mg.) was refluxed for 48 hours with dried collidine (5 ml.). T.L.C. indicated no reaction had taken place.

3B-chloro-cholest-6-one (13)

3α,5α-cyclo-cholest-6-one(8) (550 mg.) was refluxed with dried pyridine (5 ml.), into which hydrogen chloride gas had been passed for 10 seconds, for 1 hour. The mixture was cooled, poured into ice-cold dilute (6N) hydrochloric acid, extracted with ether, and the organic layer dried (Na_2SO_4). Evaporation under reduced pressure gave an oil (555 mg.) which crystallised from methanol as fine needles m.p. 126°C (lit. m.p. 128-30°C)[45] ν max. 1710 cm^{-1} n.m.r. 3.8 p.p.m. (1 Hm).

Reaction of 3 α, 5 α -cyclo-cholest-6-one (8) with pyridine tosylate

The ketone (8) (50 mg.) was refluxed with p-toluene sulphonic acid (250 mg.) in dried pyridine (5 ml.) for 24 hours. The mixture was poured into ice-cold dilute hydrochloric acid and extracted with ether in the usual way to yield an oil (46 mg.). T.L.C. comparison of the crdue reaction product with cholest-2-en-6-one[9] indicated that this compound was present. The n.m.r. spectrum had a signal at 5.6 p.p.m. (m). (olefinic protons).

Treatment of 14 α -hydroxy-3 α ,5 α -cyclo-cholest-7-en-6-one (11) with Pyridine tosylate

The hydroxy ketone (11) (20 mg.) and p-toluene sulpho ic acid (250 mg.) were refluxed overnight in dried pyridine (5 ml.) T.L.C. showed that dehydration had occurred to yield the possible dehydration products (see below).

3 α,5 α -cyclo-cholest-7, 14-dien-6-one (14)

The hydroxy ketone (11) (50 mg.) was stirred, at room temperature, with glacial acetic acid (5 ml.) for 24 hours. T.L.C. showed no reaction had occurred. p-toluene sulphonic acid (50 mg.) was then added and stirring continued for a further 2 hours. The mixture was poured into water, extracted with chloroform, washed with saturated

sodium bicarbonate solution, water and then dried (Na_2SO_4). The solution was evaporated to dryness. P.L.C. separation gave the pure dienone (14) (25 mg) which crystallised from methanol. mp. 104 - 5°C. ν max. 1657 cm^{-1}, λ max. 301 mμ (ε, 13,400). The n.m.r. spectrum had signals at 6.03, (1 Hm) and 6.23, (1 Hd). The mass spectrum showed the molecular ion at m/e 380 corresponding to $C_{27}H_{40}O$.

Treatment of 14α-hydroxy-3α, 5α-cyclo-cholest-7-en-6-one (11) with lithium iodide in pyridine.

The hydroxy ketone (11) (20 mg.) was dissolved in dried pyridine (5 ml.) and lithium iodide (100 mg.) added. The mixture was refluxed for 16 hours. T.L.C. indicated dehydration had occurred to give 3α, 5α-cyclo-cholest-7,8(14)-dien-6-one (14) and 3α, 5α-cyclo-cholest-8 (9), 14-dien-6-one (15).

Reaction of 3α,5α-cyclo-ergosta-7,22-dien-6-one (3) with Pyridine tosylate.

The ketone (3) (45 mg.) and p-toluene sulphonic acid (250 mg.) were refluxed in dried pyridine (5 ml.) for 24 hours. The mixture was poured into ice-cold dilute hydrochloric acid, extracted with ether, separated and dried (Na_2SO_4). Evaporation of the solvent gave an oil (40 mg.) which T.L.C. examination showed to contain the starting ketone (3) and the same isomerised ketone (10) obtained by sodium hydroxide treatment (see p.144). The n.m.r. spectrum of the crude product

showed no additional olefinic protons.

3β-tosyl-5α-14α and β-ergosta-7, 22-dien-6-one (16)

The cyclopropyl ketone (3) (100 mg.) was dissolved in dried benzene (10 ml.) and p-toluene sulphonic acid (50 mg.) added. The mixture was refluxed for 3½ hours. The solvent was distilled off and the residue chromatographed over alumina (20 g. grade V). Elution with petrol containing increasing amounts of benzene yielded the tosylate (16) (70 mg.) as an oil which crystallised from methanol as fine needles. m.p. 142 - 145°C, ν max. 1680, 1620, 1610, 800 and 890 cm^{-1} The n.m.r. spectrum had signals at 7.88 (1 Hs), 7.74 (1 Hs), 7.39 (1 Hs), 7.27 (1 Hs), 5.74 (1 Ht), 5.26 (2 Hm) 2.43 (3 Hs) p.p.m. The mass spectrum showed the molecular ion at m/e 566 and a strong peak at m/e 394. Found; C, 73.43; H, 8.74; $C_{35}H_{50}O_4S$ requires C, 73.17; H, 8.21%.

Thermal treatment of 3β-tosyl-5α-14α and β-ergosta-7, 22-dien-6-one (16)

The tosylate (16) (20 mg.) was heated at 220°C, under dried nitrogen, in a sealed flask for 10 minutes. T.L.C. comparison of the crude product with 14α-5α-ergosta-2,7,22-trien-6-one showed this compound was present in the complex reaction mixture. Infrared spectroscopic examination of the crude reaction product showed ν max 1710 and 1675 cm^{-1}. The n.m.r. spectrum had additional olefinic proton

signals at 5.7 p.p.m.

Thermal treatment of 3α, 5α-cyclo-ergosta-7, 22-dien-6-one (3) with tosyl acid.

The ketone (3) (50 mg.) and p-toluene sulphonic acid (0.5 mg.) where heated together, under dried nitrogen, in a sealed flask at 225°C for 10 minutes. T.L.C. and spectroscopic examination of the crude reaction product indicated essentially the same result had been achieved as in the previous experiment.

Treatment of 3α,5α-cyclo-ergosta-7, 22-dien-6-one (3) with tosyl acid in dimethyl acetamide

A mixture of the ketone (3) (50 mg.) and p-toluene sulphonic acid (5 mg.) was refluxed in dried dimethylacetamide (3 ml.) for 8 hours. T.L.C. showed a cleaner reaction product than in the heterogeneous reaction (see above), had been obtained.

Direct ring opening of 3α, 5α-cyclo-ergosta-7, 22-dien-6-one (3)

The ketone (3) (5 g) and p-toluene sulphonic acid (250 mg.) was stirred, under nitrogen, with dried freshly distilled, sulpholane

(30 ml.) at 160°C for 1½ hours. The resulting green solution was poured into water to give a cloudy suspension of the crude 2-ene products. The suspension was extracted with benzene/petrol (1:4, 3 x 50 ml.) and the combined extracts filtered through alumina (grade V, 60 g.) and further eluted with an additional 150 ml. of the benzene/petrol mixture. (N.B. if a emulsion was obtained during the extraction, sodium chloride was added to the aqueous layer). The collected filtrate was evaporated under reduced pressure to yield a crude mixture of 2-ene products as an oil (4.3 g.).

Perchloric acid catalysed ring opening of
3α, 5α-cyclo-ergosta-7, 22-dien-6-one (3)

The ketone (3) (50 mg.) was dissolved in a mixture of dried benzene (3 ml.) and dried dioxan (2 ml.). 3 drops of perchloric acid (60% aqueous) were then added and the mixture refluxed for 1 hour. T.L.C. indicated the presence of the required 2-ene products but a large base line spot was also present.

14α-ergosta-2, 7, 22-trien-6-one(5) and 14β-ergosta-2, 7, 22-trien-6-one (18).

The crude mixture of tosylates (16) (2.4 g.), obtained as described above (p. 148), was refluxed with lithium bromide (2.0 g) in dried dimethylformamide (20 ml.) for 1 hr. The bulk of the solvent was removed under high vacuum and the residue diluted with water. Extraction with ether in the usual way, followed by evaporation of the solvent under reduced pressure gave the crude product (1.91 g.) Chromatography on alumina (grade V, 220 g) and elution with petrol and then petrol/benzene (20:1) yielded the 14β isomer (18) (95 mg.) which crystallised from methanol as fine needles m.p. 103-4°C. ν max. 1665 and 1615 cm^{-1}. λ max. 249 mμ (ε,11,300).

The n.m.r. spectrum had signals at 5.80 (1 Hd), 5.65 (2 Hm) and 5.25 (2 Hm). The mass spectrum showed the molecular ion at m/e 394 and strong peaks at m/e 340, 54. Found; C, 85.23; H, 10.71; $C_{28}H_{42}O$. requires C, 85.22; H, 10.73%. $[\alpha]_D^{24}$ + 28.5° (c = 0.186). Further elution gave a mixture of isomeric 2-ene compounds (5) and (18) (740 mg.) and finally the pure 14α epimer (5) (84 mg.) which crystallised from methanol as fine needles. m.p. 142-3°C. ν max. 1675 and 1635^{-1}. λmax. 246 mμ (ε , 10,700). The n.m.r. spectrum had singals at 5.7 (3 Hm) and 5.25(2 Hm) p.p.m. The mass spectrum was very similar to that of the 14β epimer and showed the molecular ion at m/e 394 and peaks at m/e 340 and 54. Found; C, 85.29; H, 10.78; $C_{28}H_{42}O$ requires C, 85.22; H, 10.73%. $[\alpha]_D^{26}$ + 22.5° (c = 0.212).

Equilibration of 3 α, 5 α-cyclo-ergosta-7, 22-dien-6-one (3) with hydrochloric acid

A solution of the ketone (3) in ethanol was made up of sufficient concentration to give an ultraviolet absorbance of approximately 0.6. Sufficient hydrochloric acid was added to make the solution 0.1 N. The absorbance at 249 mμ was measured at intervals. Equilibrium was taken as the point when the absorbance became constant.

Results

Time (hrs)	0	0.25	1	2	3
Absorbance	0.576	0.375	0.342	0.342	0.344

∴ Relative concentration of ketone (3) at equilibrium =

$$\frac{0.342}{0.576}$$

and the relative concentration of unconjugated ketone at equilibrium =

$$\frac{0.234}{0.576}$$

∴ Ratio of conjugated : unconjugated =

$$\frac{0.342}{0.576} : \frac{0.234}{0.576}$$

$$\simeq 6 : 4$$

Equilibration of 14α-ergosta-2, 7, 22-trien-6-one with hydrochloric acid (5)

The procedure outlined above was used with the following results:

Time (hrs)	0	0.25	1	2	3
Absorbance	0.539	0.510	0.486	0.484	0.484

$\therefore$ Relative concentration of conjungated ketone (5) at equilibrium $= \frac{0.484}{0.539}$

and relative concentration of unconjungated ketone at equilibrium $= \frac{0.045}{0.539}$

$\therefore$ Ration of conjugated : unconjugated $= \frac{0.484}{0.539} : \frac{0.045}{0.539}$

$\simeq$ 10 : 1

cis-Hydroxylation of the crude 2-ene products (see p.150).

The crude 2-ene product (4.3 g.), was dissolved in glacial acetic acid (100 ml., freshly distilled from chromium trioxide) and silver acetate (4.0 g.) added. The mixture was stirred, under dried nitrogen, and finely powdered iodine (2.9 g.) added in portions over a period of 15 minutes. After a further 15 minutes water was added (0.5 ml.) and stirring continued overnight. The precipitated silver iodide was filtered off, and the residue washed well with chloroform. The collected filtrate was diluted further with chloroform, washed twice with water, then with saturated sodium bicarbonate solution, and finally with water. The dried (Na_2SO_4) organic layer was evaporated under reduced pressure and the residue chromatographed on alumina (grade V, 200 g.) Elution with petrol/benzene (4:1, 500 ml.) removed non polar impurities. Further elution was with ether, 25 ml. fractions being collected. Fractions 10 - 24 were combined and the solvent removed to yield a mixture of C-14 eperimeric monoacetate diols (20), R=H and (22) R=H, (887 mg.) The product crystallised from ether as needles, m.p. 181 - 87°C. νmax. 3500, 1718, 1665 and 1620 cm^{-1}. Found; C, 76.29; H, 9.62; $C_{30}H_{46}O_4$ requires C, 76.55; H, 9.85%.

2β, 3β-diacetoxy-14α-ergosta-7, 22-dien-6-one (20), R=Ac, and 2β, 3β-diacetoxy-14β-ergosta-7, 22, dien-6-one (22), R=Ac.

The crude monoacetate diol mixture (840 mg.), prepared as above but without chromatographic purification, was dissolved in dried pyridine (5 ml.) and acetic anhydride (10 ml.) added. The solution was left overnight and then the excess acetic anhydride was destroyed by shaking with water. The products were extracted into chloroform, the organic layer separated and washed successively with dilute hydrochloric acid (2N), saturated sodium bicarbonate solution and water. The chloroform layer was dried (Na_2SO_4) and evaporated to give the crude diacetates (20) R=Ac, and (22), R=Ac, (880 mg.) A partial purification was effected by chromatography on alumina (grade V, 100 g.) and elution with petrol containing increasing amounts of benzene yielded a purer sample of the diacetate mixture (248 mg.). Crystallisation from methanol gave a sample (55 mg.) of the C-14 epimers uncontaminated with other impurities, m.p. 135-50°C. Separation of the epimers was finally achieved by P.L.C. using the successive elution technique (12 ×) in an acetone/petrol system (1:12) to yield, first, the 14β epimer (30 mg.), which crystallised as needles from methanol m.p. 153-56°. ν max. 1740, 1655, 1615 and 1260 $cm.^{-1}$ The n.m.r. showed the C-7 proton as the required doublet at 5.83 p.p.m. (J = 3 c.p.s.). Found; C, 74.69; H, 9.13 $C_{32}H_{48}O_5$ requires C, 74.96; H, 9.44%. λ max. 252 mμ. (ε, 11,500). $[\alpha]_D^{26}$ + 32° (C = 0.047).

The 14 α -epimer which was obtained (19 mg.) crystallised as fine needles from methanol. m.p. 193-96°C (lit. m.p. 194-96°C).[4] ν max. 1738, 1665, 1623 and 1250 cm.$^{-1}$ The n.m.r. spectrum showed the C-7 proton as the required triplet at 5.75 p.p.m.

2β, 3β-diacetoxy-20S-formyl-14β-5α -pregn-7-en-6-one (24)

The residue (160 mg.), obtained by evaporation of the mother liquours from the original crystallisation of the crude product described in the previous experiment, was dissolved in methylene chloride (10 ml.) and Pyridine (0.1 ml.) added. The mixture was cooled to -70°C and treated with ozone until T.L.C. indicated none of the starting diacetate was present. Water was added, followed by a few drops of dilute hydrochloric acid. The organic layer was separated, washed with water, and finally dried (Na_2SO_4). Evaporation of the solvent gave an oil (141 mg.). Purification by P.L.C. yielded the aldehyde (24) (30 mg.) as an oil which crystallised from methanol as plates. Recrystallisation from iso-propyl ether/diethyl ether gave the analytical sample. m.p. 186-190°C. ν max. 1740, 1740(sh), 1670, and 1618 cm.$^{-1}$ The n.m.r. spectrum showed signals at 9.70 (1 Hd), 5.90 (1 Hd), 5.3 (1 Hm), 4.8 (1 Hm), 2.03 (3 Hs) and 2.08 (3 Hs) p.p.m.

14 α-hydroxy-3 α, 5 α-cyclo-cholest-7-en-6-one (11) and

9 α, 14 α-dihydroxy-3 α, 5 α-cyclo-cholest-7-en-6-one (24)

The cyclopropyl ketone (8) (500 mg.) was refluxed with selenium dioxide (720 mg.) in t-butanol (40 ml.) and pyridine (10 ml.) for $1\frac{1}{2}$ hours. The solvent was distilled off, and the residue extracted with hot chloroform. The chloroform extracts were filtered and the filtrate washed successively with ammonium sulphide solution, ammonium hydroxide (5N), saturated sodium chloride solution and finally water. The organic layer was separated, dried (Na_2SO_4), and evaporated to dryness to leave a oily residue (550 mg.). Chromatography on alumina (grade V, 50 g.) and elution with petrol containing increasing amounts of benzene, then finally benzene, gave initially the monohydroxy compound (11) (215 mg.) which crystallised from petrol as silky needles m.p. 206-110°C. ν max. 3380, 1640, 1610. $^{-1}$ λ max. 240 mμ (ε, 13, 310). The n.m.r. spectrum showed an exchangeable signal at 1.7 (1 Hs) and a further signal at 5.95 (1 Hd, J = 2.5 c.p.s.) p.p.m. The mass spectrum showed the molecular ion at m/e 398 corresponding to $C_{27}H_{42}O_2$.

Further elution with benzene gave the diol (24) (81 mg.) which crystallised from petrol as needles. m.p. 189-91°C. ν max. 3150, 1648, 1610 cm.$^{-1}$ λ max. 233 mμ (ε, 12,700). The n.m.r. spectrum showed an exchangeable signal at 3.7 (2 Hs) and a further signal at 6.05 (1 Hs) p.p.m. The mass spectrum showed the molecular ion at m/e 414 corresponding to $C_{27}H_{42}O_3$. Found; C, 78.36; H, 10.21; $C_{27}H_{42}O_3$ requires C, 78.40; H, 10.13.%.

Dehydration of 14 α -hydroxy-3 α , 5 α -cyclo-cholest-7-en-6-one (11)

The alcohol (11) (2.8 mg) was warmed with ethanolic hydrochloric acid (0.1 N)(10 ml.) at 60°C for 10 minutes. After appropriate dilution the ultraviolet spectrum was taken. The spectrum showed two bands, one at 290 mμ due to a conjugated dienone (14) and the other at 245 mμ due to a diene system (15).

Dehydration of 9 α , 14 α -dihyrdoxy-3 α , 5 α -cyclo-cholest-7-en-6-one (24).

The diol (24) (1.14 mg) was treated in an identical manner to that described above. The ultraviolet spectrum showed only one band at 308 mμ due to the conjugated dienone (25).

Reoxidation of 14 α -hydroxy-3 α , 5 α -cyclo-cholest-7-en-6-one (11)

The alcohol (11) (10 mg.) were treated with selenium dioxide in t-butanol/pyridine in exactly the same way as described above (p.157). T.L.C. examination of the reaction product showed the alcohol (11) had been smoothly converted to the diol (24), no side products being formed.

14 α -hydroxy-3 α , 5 α -cyclo-ergosta-7, 22-dien-6-one (26)

The cyclopropyl ketone (3) (600 mg.) was dissolved in t-butanol (8 ml.) and pyridine (2 ml.). Selenium dioxide (800 mg.) was added and the mixture refluxed $\frac{1}{2}$ hour. The solvent was removed by distillation under reduced pressure. The residue was dissolved in chloroform, filtered and successively washed with hydrochloric acid (2N), saturated sodium bicarbonate solution, and water. After filtration through alumina (grade V) the solvent was removed under raduced pressure. The residue was purified by P.L.C. to give the alcohol (26) (408 mg). Crystallisation from methanol gave a pure sample m.p. 190-91°C. A mixed melting point with a sample prepared via the enolacetate (see p.161) showed no depression. The spectroscopic data was also identical in every respect.

6-acetoxy-3 α ,5 α -cyclo-ergosta-6-, 8 (14), 22-triene (28)

The cyclopropyl ketone (3) (200 mg.) was dissolved in the perchloric acid catalysed enolacetylating solution (5 ml.), [prepared from acetic anhydride (5 ml.), carbon tetrachloride (95 ml.) and perchloric acid (60%, 0.005 ml.)], and allowed to stand for 2 hours at room temperature. After washing well with water, the dried (Na_2SO_4) organic layer was evaporated under reduced pressure. The residue, after P.L.C., afforded the enolacetate (28) (176 mg.) as an oil. ν max. 1760, 1625 $cm.^{-1}$ λ max. 263 mμ. The n.m.r. spectrum showed signals at 5.95 (1 Hs), 5.25 (2 Hm) p.p.m.

2β, 3β, 6-triacetoxy-5α-ergosta-6, 8 (14), 22-triene (30)

2β-acetoxy-3β-hydroxy-5α-14α and 14β-ergosta-7-en-6-one (20), R=H and (22), R=H, (700 mg.) was dissolved in a mixture of carbon tetrachloride (475 ml.), acetic anhydride (2.5 ml.) and perchloric acid (60% aqueous, 0.005 ml.) and left at room temperature for $3\frac{1}{2}$ hours. The solution was then washed with water, saturated sodium bicarbonate solution, and finally with water. The organic layer was separated, dried (Na_2SO_4), and evaporated under reduced pressure to give an oily residue. Purification by P.L.C. gave the enolacetate (30) (570 mg.) as a glass which failed to crystallise. ν max 1740 $cm.^{-1}$ λ max. 254 mμ. The n.m.r. spectrum showed a signal at 5.90 (1 Hd, J = 3 c.p.s.) p.p.m.

The same enolacetate was obtained when the pure 14β-diacetate (22), R=Ac, was treated under indentical conditions.

14α-hydroxy-3α, 5α-cyclo-ergosta-7, 22 dien-6-one (26).

The enolacetate (28) (104 mg.) in ether (3 ml.) was treated with monoperphthalic acid in ether (2 ml. - containing 29.1 mg./ml.) at room temperature overnight. The solution was washed with saturated sodium bicarbonate solution and then water. The organic

layer was dried ($MgSO_4$) and evaporated to dryness. P.L.C. afforded the pure product (58 mg.) which crystallised from methanol as needles, m.p. 192-4°C. ν max. 3450, 1650, 990 $cm.^{-1}$ λ max. 244 mμ (ε, 12,100). The n.m.r. spectrum showed signals at; 6.00 (1 Hd), 5.27 (2 Hm). p.p.m. $[\alpha]_D^{26}$ + 115° (c = 0.3). Found; C, 81.72; H, 10.20; $C_{28}H_{42}O_2$ requires C, 81.90; H, 10.31. %.

2β, 3β-diacetoxy-14 α-hydroxy-5 α-ergosta-7, 22-dien-6-one (27)[4]

The enolacetate (30) (500 mg.) was dissolved in an ethereal solution of monoperphthdic acid (6.5 ml.), (the ether solution contained 33.1 mg./ml. of the peracid), and left at room temperature for 24 hours. The solution was diluted with ether, washed with saturated sodium bicarbonate solution and then, finally, with water. The ether layer was separated and dried (Na_2SO_4). Evaporation to drynass followed by P.L.C. (system; acetone/petrol 1:5) separation of the major, less polar, product gave the hydroxy ketone (27) which crystallised from methanol as needles, m.p. 220-23°C. ν max. 3347, 1733, 1655 $cm.^{-1}$ λ max. 241 mμ (ε, 14,600). $[\alpha]_D^{26}$, + 66° (c = 0.364). The n.m.r. spectrum had signals at 5.97 (1 Hd), 5.25 (3 Hm). Found; C, 72.67; H, 8.99; $C_{32}H_{48}O_6$ requires C, 72.69; H, 9.15 %.

3α, 5α-cyclo-20S-formyl-pregn-7-en-6-one(31)

The cycloropyl ketone (3) (520 mg.) was dissolved in methylenechloride (20 ml.) containing pyridine (0.2 ml.). Ozone was passed through the solution at -70°C until T.L.C. indicated the reaction was just complete. (N.B. it is important not to over ozonise as yields suffer). The solution was allowed to warm to room temperature, then washed with dilute hydrochloric acid (c a. 0.1 N), and water. The separated organic layer was dried (Na_2SO_4), and evaporated under reduced pressure to give an oil (456 mg.). Filtration over alumina (grade V) with benzene as solvent afforded, eventually, the unstable aldehyde (31), (402 mg, 94.4%), m.p. 138-41°C. ν max. 1725, 1650, cm^{-1}, λmax. 247 mμ (ε, 9,600). $[\alpha]_D^{26}$ + 91° (c= 0.44, $CHCl_3$). The n.m.r. spectrum had signals at 9.71 (1 Hd), 5.78 (1 Ht) p.p.m. The mass spectrum showed the molecular ion at m/e 326 (base peak), corresponding to $C_{22}H_{30}O_2$. Found; C, 80.32; H, 8.75; $C_{22}H_{30}O_2$ requires C, 80.93; H, 9.25. %.

2β, 3β-diacetoxy-14α-hydroxy-20S-formyl-5α-pregn-7-en-6-one (32)

The hydroxy ketone (27) (400 mg.) was dissolved in methylene chloride (100 ml.) containing pyridine (1.0 ml.), cooled to -70°C and treated with ozone in the usual way until T.L.C. indicated the reaction to be complete. After work up as outlined in the previous experiment, the crude aldehyde was purified by P.L.C.

(system acetone/petrol, 1:3) to give the aldehyde (32) (305 mg.) which crystallised from ether as needles, m.p. 234-36°C. ν max. 3475, 1730, 1715, 1670 cm.$^{-1}$ λ max. 244 mμ, (ε, 12,400). $[\alpha]_D^{26}$ + 49° (c = 0.41). The n.m.r. spectrum had signals at 9.6 (1 Hd), 5.93 (1 Hd), 2.08 (3 Hs), 2.03 (3 Hs). The mass spectrum had the molecular ion at m/e 460 (base peak 43), corresponding to $C_{26}H_{36}O_7$. Found; C, 67.71; H, 8.18; $C_{26}H_{36}O_7$ requires C, 67.80; H, 7.88 %.

4-bromo-2-methyl-butan-2-ol. (33)

To a stirred solution of methyl magenesium iodide, [prepared by reacting methyl iodide (24.3 g.) with magnesium (4.15 g.) in dried ether (200 ml.)], was added, dropwise, a solution of ethyl-3-bromoproprionate (14 g.) in dried ether (50 ml.). The solution was then refluxed for 20 minutes and then worked up in the usual way with ammonium chloride solution. The product extracted with ether, the extracts dried (Na_2SO_4) and the ether removed under reduced pressure. The oily residue was distilled under vacuum and the fraction distilling between 64-65°C at 8 m.m. gave the bromoalcohol (33) (6.4 g.). ν max. 3420 cm.$^{-1}$ (film). The n.m.r. spectrum showed signals at 3.45 (2 Ht, J= 10 c.p.s.), 2.80 (1 Hs) (exchangeable), 2.10 (2 Ht, J = 10 c.p.s.), 1.30 (6 Hs). The analytical sample was obtained after purification by P.L.C. (system acetone/petrol 1:9). Found; C, 36.11; H, 6.43; $C_5H_{11}OBr$ requires C, 35.94; H, 6.63%.

4-bromo-2-methyl-butan-2-ol, tetrahydropyranyl ether (34)

To a mixture of the bromoalcohol (33) (420 mg.) and dihydropyran (210 mg.) was added one crystal of p-toluene sulphonic acid. The mixture was left a room temperature overnight. Purification by P.L.C. (system; acetone/petrol 1:19) gave the unstable ether which darkened rapidly in air (600 mg.). ν max. 995 cm^{-1}, the n.m.r. spectrum had signals at 4.70 (1 Hm), 3.42 (2 Ht), 2.07 (2 Ht), 1.18 (6 Hs) p.p.m. Found; C, 48.38; H, 7.24; $C_{10}H_{19}O_2Br$ requires C, 47.82; H, 7.62%.

Attempted preparation of the Grignard reagent of the ether (34)

To a stirred suspension of magnesium (25 mg.) in dried ether (0.5 ml.) was added(dropwise) a solution of the bromoether (34) (251 mg.) in dried ether (3 ml.). No reaction was observed even after addition of a crystal of iodine, or a small portion of methyl magnesium iodide or dibromoethane.

Attempts at preparing the lithium compound by exchange with methyl lithium also failed.

Tetrahydrofuran was also tried as a solvent with no success.

2-methylbut-3-yne-2-ol-tetrahydropyranyl ether (35)

The acetylenic alcohol (36) (4.2 g.) was mixed with dihydropyran (4.6 g.) and a crystal of p-toluene sulphonic acid added. The mixture was left overnight at room temperature. Excess dihydropyran was then removed by bubbling a stream of dry nitrogen through the mixture. Distallation at 3.5 m.m. and collection of the fraction b.p. 57°C gave the pure ether (35) (6.5 g.). n_D^{24} 1.4459. νmax. 3250 cm.$^{-1}$ The n.m.r. spectrum had signals at 5.10 (1 Hm), 2.35 (1 Hs), 1.43 (3 Hs), 1.48 (3 Hs) p.p.m. Found; C, 71.11; H, 9.50, $C_{10}H_{16}O_2$ requires C, 71.39; H, 9.59%.

Alkylation of 2, 4, 5-trimethoxy-benzaldhyde (36a) with 2-methyl but-3-yne-2-ol-tetrahydropyranyl ether (35).

To a solution of the acetylenic ether (35) (200 mg.) in dried ether (10 ml.) was added a ethereal solution of methyl lithium (1 ml, 1.1 M). The resulting solution was left at room temperature for 10 minutes and then added dropwise to a stirred solution of the aldehyde (36 a) (100 mg.) in dried ether (5 ml.). The stirring was continued for a further 10 minutes and then the reaction was quenched with ammonium chloride solution and worked up in the usual way to yield the crude alcohol (37) which crystallised from benzene/petrol to give 4- (2, 4, 5-trimethoxy phenyl) -penta-3-yne-2, 4-diol-2-tetrahydropyranyl ether (37) (127 mg.) m.p. 92-3°C. νmax. 3480, 1610 cm.$^{-1}$ The n.m.r. spectrum had signals at; 7.15 (1 Hs), 6.50 (1 Hs), 5.69 (1 Hd),

5.05 (1 Hm), 3.81 (9 Hd), 3.08 (1 Hd) (exchangeable), 1.50 (12 Hd). Irradiation at 3.08 caused the doublet at 5.69 to collapse to a singlet. Found; C,65.83; H, 7.67; $C_{20}H_{28}O_6$ requires C, 65.92; H, 7.74%.

4-(2, 4, 5-trimethoxy-phenyl)-pentane-2, 4-diol-2-tetra hydropyranyl ether (38)

The acetylenic alcohol (37) (37 mg.) in dried ethyl acetate (10 ml.) was hydrogenated over 5% palladium on carbon (2.5 mg.) until the calculated amount of hydrogen had been taken up (4.5 ml.). The catalyst was filtered off and the solvent removed under reduced pressure to yield the alcohol (38) (35 mg.). as a non-crystalline semi-solid. ν max. 3450, 1610 cm.$^{-1}$ The n.m.r. spectrum showed signals at; 6.85 (1 Hs), 6.30 (1 Hs), 4.70 (2 Hm), 2.60 (1 Hm) (exchangeable). p.p.m.

2β, 3β-diacetoxy-14α, 22S-dihydroxy-25-(tetrahydropyran-2-yloxy)-5α-cholest-7-en-23-yne-6-one (59) R = H, R^1 = OH

To a solution of 2-methyl-3-butyne-2-ol-tetrahydropyranyl ether (500 mg.) in dried ether (5 ml.) was added a ether solution of methyl lithium (2.5 ml. 1.1M). The mixture was left a room temperature for 30 minutes and then added dropwise to a stirred solution of 2β, 3β-diacetoxy-14α-hydroxy-20S-formyl-5α-pregn-7-en-6-one (32) (200 mg.) in dried tetrahydrofuran (10 ml.). Stirring was continued

at room temperature for a further 10 minutes. After quenching the reaction with ammonium chloride solution and extraction with ether the separated ether layer was dried (Na_2SO_4), and evaporated to give a crude mixture of the C-22 epimeric alcohols. P.L.C. (system; acetone/petrol 1:5, 2 elutions) separation afforded the required, less polar, 22S isomer (100 mg.) as a non-crystalline semi-solid. ν max. 3470, 1735, 1670 $cm.^{-1}$ The n.m.r. spectrum showed signals at; 5.88 (1 Hd), 5.24 (1 Hm), 5.03 (2 Hm), 4.47 (1 Hd), 3.83 (1 Hm), 3.46 (1 Hm), 2.06 (3 Hs), 1.99 (3 Hs), 1.52 (3 Hs), 1.47 (3 Hs), 1.06 (3 Hd), 0.98 (3 Hs), 0.67 (3 Hs). p.p.m. The mass spectrum showed the molecular ion at m/e 628 corresponding to $C_{36}H_{60}O_9$ and strong peaks at m/e 527, 526, 85.

Reduction of 2β, 3β-diacetoxy-14α, 22S, dihydroxy-25-(tetrahydropyran-2-yloxy)-5α-cholest-7-en-23-yne-6-one (39) R = H, R = OH.

a) The acetylenic alcohol (39), R=H, R = OH (47 mg.) was dissolved in a mixture of methanol (10 ml.) and acetic acid (0.1 N, 0.2 ml.). 5% Palladium on carbon catalyst (10 mg.) was added and the mixture hydrogenated in the usual way until the uptake of hydrogen ceased. The mixture was acidified to ca. 0.1N with dilute hydrochloric acid, stood at room temperature for 1 hour, then filtered and potassium carbonate (1.0 g.) added. After standing overnight at room temperature

the mixture was diluted with saturated sodium chloride solution and extracted (3x) with ethyl acetate. The separated organic layer was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. P.L.C. separation (system; methanol/chloroform 1:9) afforded the more polar 5β isomer (40) (11 mg.). Mass spectroscopic examination of the product showed the molecular ion a m/e 448 corresponding to $C_{27}H_{44}O_5$ and not to the required formula $C_{27}H_{44}O_6$.

b) The acetylenic alcohol (39), R=H, R^1=OH (50 mg.) was dissolved in dried, sodium bicarbonate washed, ethyl acetate (5 ml.) and hydrogenated in the usual way over 5% Palladium on carbon (3 mg.) until the uptake of hydrogen ceased. After removal of the protecting groups, with equilibration, as outlined above, the required more polar, 5β isomer was separated by P.L.C. (system; methanol/phloroform 1:9, 2 elutions). (7 mg.). Mass spectroscopic examination of the product showed an ion at m/e 444 (molecular ion - 18), and strong peaks at m/e 97, 79. (c.f. ecdysone, strong peaks at m/e 99, 81). The result showed that only partial hydrogenation had taken place to give the 23-ene compound (41).

c) The acetylenic alcohol (39), R=H, R^1=OH (50 mg.) was dissolved in ethyl acetate (5 ml. washed with sodium bicarbonate and dried) and then hydrogenated over 10% palladium on carbon until the uptake of hydrogen ceased. The catalyst was filtered off and the solvent

removed under reduced pressure. The residue was purified by P.L.C. (system; acetone/petrol 30:70) to give the reduced ether. (46 mg.). The mass spectrum showed no molecular ion but had peaks at m/e 531 , 530, 99, 85, 81.

Removal of protecting groups from the reduced acetylenic ether (39) R=H, R^1=OH to give an unknown isomer of Ecdysone.

The reduced ether (46 mg.) was dissolved in dilute methanolic hydrochloric acid (0.05N, 10 ml.) and stood at room temperature for 10 minutes. The solution was diluted with water, extracted with ethylacetate (3 ×), and the separated organic layer evaporated to dryness. The residue was dissolved in a mixture of water (1 ml.), methanol (2 ml.) and tetrahydrofuran (1 ml.) and potassium carbonate (14 mg.) added. The solution was refluxed, under nitrogen, for $1\frac{1}{2}$ hours and then the solvent removed under high vacuum at room temperature. The required, more polar, 5β epimer was separated by P.L.C. (system; methanol/chloroform 1:9, 2 elutions). Crystallisation from methanol/ether gave the product, m.p. 236-9°C. The mass spectrum corresponded very closely to a spectrum obtained from an authentic sample of ecydsone.[24] The material gave a positive reaction in the "Calliphora test" bioassay.[23] T.L.C. comparison showed it to be somewhat more polar than ecdysone but less polar than iso-ecdysone.[24]

2β, 3β-diacetoxy-14α, 22R-dihydroxy-25-(tetrahydropyran-2-yloxy)-5α-cholest-7-en-23-yne-6-one (39) R^1= H, R =OH.

A solution of the magnesium bromide derivative of the acetylenic ether (35) was prepared by the addition of the ether (35) (2.4 ml.) to a solution of ethyl magnesium bromide [prepared by the reaction between magnesium (300 mg.) and ethyl bromide (1.0 ml.) in dried ether (15 ml.)], and allowing the mixture to stand at room temperature for 1 hour. The Grignard reagent prepared in this way was added dropwise over 5 minutes to a stirred solution of 2β, 3β-diacetoxy-14α-hydroxy-20S-formyl-5α-pregn-7-en-6-one (32) (300mg.) in dried tetrahydrofuran (15 ml.) at -10°C. The solution was allowed to warm to 0°C and stirring was continued for a further 15 minutes. After addition of ammonium chloride solution and extraction with ether the separated organic layer was dried (Na_2SO_4) and evaporated under high vacuum to yield the crude ether (39) R=OH, R^1=H which was purified by P.L.C. (system; acetone/petrol 35:65) to give a non crystalline mixture (340 mg.) which was predominantly the 20R epimer. ν max. 3480, 1735, 1665, 1625 $cm.^{-1}$. The n.m.r. spectrum showed signals at 5.88 (1 Hd), ca. 5.24 (1 Hm), ca. 5.03 (1 Hm), 4.9 (1 Hm), 4.47 (1 Hd), 3.83 (1 Hm), 3.46 (1 Hm), 2.06 (3 Hs), 1.99 (3 Hs), 1.52 (3 Hs), 1.47 (3 Hs), 1.06 (3 Hd), 0.98 (3 Hs), 0.67 (3 Hs).

2β, 3β-diacetoxy-14α, 22S-dihydroxy-5α-cholest-7-en-6-one (45)

The acetylenic diol (39) R=OH, R^1= H (340 mg.) was dissolved in ethyl acetate (50 ml.)(washed with sodium carbonate and dried) and 10% Palladium on carbon (100 mg.) added. Hydrogenation in the usual way until the uptake of hydrogen ceased followed by filtration and subsequent evaporation of the filtrate gave a crude residue (300 mg.). Separation of the major product by P.L.C. (system; acetone/petrol 35:65) gave the hydrogenolysis product (110 mg.) which crystallised from ether as needles m.p. 243-46°C. A mixed melting point with a sample prepared by Mr. J.P. Poyser by an alternate route showed no depression.[25] The infrared spectra of each sample were superimposable. The mass spectrum showed the molecular ion at m/e 532 corresponding to $C_{31}H_{48}O_7$.

2β, 3β-diacetoxy-14α, 22R-dihydroxy-25(tetrahydropyran-2-yloxy)-5α-cholest-7-ene-6-one.

The acetylenic diol (39), R = OH, R^1= H, (200 mg.) was dissolved in ethylacetate (dried over anhydrous sodium carbonate) (25 ml.) and hydrogenated over 5% Palladium on carbon (20 mg.) in the usual way. The catalyst was removed by filtration and the solvent evaporated under reduced pressure to give the crude reduced diol (190 mg.) νmax. 3550, 1735, 1665 $cm.^{-1}$ The n.m.r. spectrum showed signals at 5.88 (1 Hd), 2.07 (3 Hs), 1.99 (3 Hs), 1.24 (3 Hs), 1.21 (3 Hs), 0.99 (3 Hs), 0.90 (3 Hd), 0.68 (3 Hs) p.p.m.

2β, 3β-diacetoxy-14α, 22S, 25-trihydroxy-5α-cholest-7-en-6-one (46).

The reduced diol (190 mg.) was dissolved in methanolic hydrochloric acid (10 ml., N/20) and left at room temperature for 10 minutes. Water was then added and the product extracted with ether. The separated ether layer was dried (Na_2SO_4) and evaporated to give the crude triol (46) (140 mg.) P.L.C. (system; acetone/petrol 30:70, 3 elutions) afforded the pure product (110 mg.) which cyrstallised from ether/petrol as long needles m.p. 241-2°C. ν max. 3500, 3420, 1720, 1645 $cm.^{-1}$ λ max. 239 mμ, (ε, 10,700). $[\alpha]_D^{24}$ + 56° (c = 0.305). The n.m.r. spectrum showed signals at 5.90 (1 Hd) 5.27 (1 Hm), 4.85 (1 Hm), 2.06 (3 Hs), 2.00 (3 Hs), 1.23 (6 Hs), 0.99 (3 Hs), 0.91 (3 Hd), 0.68 (3 Hs) p.p.m. Found; C. 67.64; H, 8.62; $C_{31}H_{48}O_8$ requires C, 67.89; H, 8.82%.

2β, 3β, 14α, 22S, 25-pentahydroxy-5β-cholest-7-en-6-one (47).

The triol (46) (100 mg.) was dissolved in aqueous methanolic (90%) potassium carbonate (0.3 %, 10 ml.) and refluxed under nitrogen for 3 hours. The mixture was diluted with saturated sodium chloride solution and extracted with ethylacetate (3×). The combined extracts were dried (Na_2SO_4) and evaporated to yield a crude mixture of C-5 epimers. P.L.C. separation afforded the more polar 5β isomer (system, methanol/chloroform 1:9) (48 mg.) which crystallised from water m.p. 246-48°C. A mixed m.p. with authentic 22-iso-ecdysone showed no depression. The O.R.D. curves of both compounds were

identical as were the infrared spectra. T.L.C. comparison showed identical R_F values.

SECTION II

17α hydroxy-3α, 5α -cyclo-pregn-7-ene-6, 20-dione (49)

Potassium (9 mg.) was added to t-butanol (5 ml.) and allowed to dissolve. Dimethylsulphide (5 ml.) and triphenyl phosphine (100 mg.) was then added and the mixture cooled to 0°C. After addition of 3α, 5α -cyclo-20S- formyl-pregn-7-en-6-one (31) (32 mg.) the mixture was autoxidised in the usual way until ca. 2 equivalents of oxygen had been consumed. The reaction was quenched with glacial acetic acid (3 drops) and the solvent removed under reduced pressure. P.L.C. purification (system; acetone/petrol 1:4) gave the ketol (49) (26 mg.) as a non-crystalline semi-solid. ν max 3300, 1703, 1655 $cm.^{-1}$ The n.m.r. spectrum showed signals at 6.15 (1 Hbs), (exchangeable), 5.80 (1 Hd), 2.13 (3 Hs), 1.09 (3 Hs), 0.64 (3 Hs) p.p.m.

Partial autoxidation of the cyclopropyl aldehyde (31).

The autoxidation experiment outlined above was repeated under identical conditions except the reaction was quenched after uptake of ca. 1 equivalent of oxygen. T.L.C. now showed two products, the more polar being identical to the ketol (49). P.L.C. separation (system; acetone/petrol, 1:4) of the least polar product gave 3 α, 5 α-cyclo-pregn-7-ene-6, 20-dione (50) (14 mg.). ν max. 1703, 1635 $cm.^{-1}$

A repeat of the experiment at -70°C was attempted but the uptake of oxygen was too slow to be of practical use, i.e. ca. 0.1 equivalents in 36 hours.

3 α, 5 α-cyclo-cholest-7-ene-6, 22-dione (51).

The cyclopropyl aldehyde (31) (600 mg.) was dissolved in dried ether (10 ml.) and added to a stirred solution of isoamyl magnesium bromide [prepared by reacting magnesium (250 mg.) with isoamyl bromide (1:8 ml.) in dried ether (5 ml.)] over a period of 5 minutes. The solution was refluxed for a further 5 minutes and then the insoluble Grignard complex decomposed with ammonium chloride solution in the usual way. The mixture was ether extracted, the extracts dried (Na_2SO_4) and evaporated to give crude C-22 alcohol. The crude mixture was dissolved in acetone (10 ml.) and oxidised with Jones reagent in the usual way. After addition of

methanol, to destroy excess Jones reagent, water was added and the mixture extracted with ether. The dried extracts (Na_2SO_4) were evaporated to give the crude diketone (400 mg.) which crystallised from methanol as needles, m.p. 158-59°C. ν max. 1702, 1644, 1619 cm.$^{-1}$ λmax 246 mμ, (ε, 13,300) $[\alpha]_D^{26}$ + 115°C, (c = 0.229). The n.m.r. spectrum showed signals at 5.77 (1 Ht), 1.15 (3 Hd), 1.08 (3 Hs), 0.90 (3 Hd), 0.70 (3 Hs), p.p.m. Found; C, 81.53; H, 9.92; $C_{27}H_{40}O_2$ requires C, 81.76; H, 10.17%.

Autoxidation of the diketone (51)

a) Potassium (26 mg.) was dissolved in t- butanol (5 ml.) and triethylphosphite (0.25 ml.) added. After addition of the diketone (51) (25 mg.) the solution was autoxidised in the usual way until the uptake of oxygen had ceased (ca. 6.4 ml.) After addition of glacial acetic acid (0.25 ml.) the solvent was removed under reduced pressure. The three major products in the reaction mixture were separated by P.L.C. (system; acetone/petrol 30:70). Infrared spectroscopic examination of each product showed only the second fraction could contain a α,β-unsaturated ketone moiety and a hydroxyl group, but no aldehyde peak was present.

b) The experiment was repeated as outlined above but quenched after the uptake of ca. 2 ml. of oxygen. T.L.C. examination showed the same three products as previously observed were present and in addition the starting diketone (51) was also present.

c) When the reaction was repeated as above but quenched after the uptake of ca. 4 ml. of oxygen essentially the same result was obtained as outlined in b).

d) The above experiments were repeated in the presence of triphenylphosphine (instead of triethylphosphite) and essentially the same result was observed.

7, 22, 22^1-triacetoxy-3α, 5α-cyclo-bisnorchol-6, 8 (14)-diene (55)

3α, 5α-cyclo-20S-formyl-pregn-7-en-6-one (31) (400 mg.) was dissolved in the perchloric acid enolacetylating solution (25 ml.) [prepared by mixing carbon tetrachloride (95 ml.), acetic anhydride (5 ml.) and perchloric acid (0.005 ml.)] and left at room temperature for 1 hour. Water was added and the mixture vigorously shaken for 10 minutes. The organic layer was separated, dried (Na_2SO_4), and evaporated to leave the crude product as an oil.

Purification by P.L.C. (system; acetone/petrol), 1:4) gave the pure non-crystalline product (55) (305 mg.). ν max (film from $CHCl_3$), 1760, 1635 cm^{-1}. The n.m.r. spectrum had signals at 6.85 (1 Hd), 5.92 (1 Hs), 2.08 (9 Hs), 1.09 (3 Hd), 0.94 (3 Hs), 0.84 (3 Hs) p.p.m. The mass spectrum showed the molecular ion at m/e 470 corresponding to $C_{28}H_{38}O_6$, with the base peak at m/e 83.

22, 22^{I}-diacetoxy-14α-hydroxy-3α, 5α-cyclo-bisnorchol-7-en-6-one (57).

The enolacetate (55) (42 mg.) was dissolved in ether (2 ml.) and an excess of a solution of monoperphthalic acid in ether (1 ml.) added. The mixture was left overnight at room temperature then washed with saturated sodium bicarbonate solution and water. The separated, dried (Na_2SO_4) ether layer was evaporated and the residue purified by P.L.C. (system; acetone/petrol 30:70) to give the ketone (57) (21 mg.) which crystallised from methanol, m.p. 194-7°C. Recrystallisation from ethylacetate/petrol afforded the analytical sample m.p. 202-4°C ν max. 3460, 1760, 1650 $cm.^{-1}$ λ max. 241 mμ, (ε,12,950). $[\alpha]_D^{26}$ + 111° (c = 0.254). The n.m.r. spectrum showed signals at; 6.25 (1 Hd), 5.95 (1 Hd), 2.13 (3 Hs), 2.07 (3 Hs), p.p.m. The mass spectrum had the molecular ion at m/e 444 corresponding to $C_{26}H_{36}O_6$. Found; C, 70,08; H, 8.22; $C_{26}H_{36}O_6$ requires C, 70.24; H, 8.16%.

7. 22-diacetoxy-3 α, 5 α -cyclo-bisnorchol-6, 8 (14), 20-triene (54)

The aldehyde (31) (200 mg.) was dissolved in a mixture of aceticanhydride (7.5 ml.) and freshly fused sodium acetate (130 mg.) and refluxed, under nitrogen, for 20 hours. The solvent was distilled off and the residue dissolved in chloroform and filtered. The chloroform was then removed under reduced pressure to give the crude enolacetate (236 mg.). A sample was purified by P.L.C. (system; acetone/petrol 1:9) to give the pure oily enolacetate (54). ν max. 1755, 1635 cm.$^{-1}$ λ max. 263 mμ. The n.m.r. spectrum had signals at; 6.95 (1 Hq), 5.95 (1 Hs), 2.15 (3 Hs), 2.13 (3 Hs), 1.75 (3 Hd), 0.84 (6 Hs). The mass spectrum showed the molecular ion at m/e 410.24551, $C_{26}H_{34}O_4$ requires m/e 410.24569, and showed the base peaks at m/e 43 and 242.

Treatment of the bisenolacetate (54) with peracid.

a) With monoperphthalic acid.

The bisenolacetate (54) (70 mg.) was dissolved in ether (5 ml.) and an excess of a ether solution of monoperphthalic acid added (1 ml.) The mixture was left at room temperature for 48 hours. After work up in the usual way the crude epoxide was obtained. P.L.C. separation (system; acetone/petrol, 1:4) afforded a non-crystalline mixture of 22-acetoxy-14 α hydroxy-20 (R and S), 22-epoxy-3 α , 5 α-cyclo-bisnorchol-7-en-6-one (58) (62 mg.) ν max. 3420, 1740, 1650 cm.$^{-1}$ The n.m.r. spectrum had signals at 5.95

(1 Hd), 5.64 (2/5 Hs), 5.31 (3/5Hs), 2.14 (3 Hs), 1.46 (3 Hbs), 1.09 (3 Hs), 0.87 (9/5 Hs), 0.83 (6/5 Hs) p.p.m.

b) With L percamphoric acid.[46]

The enolacetate (54) (50 mg.) was dissolved in a chloroform solution of Lpercamphoric acid (4 ml.) and stood overnight at room temperature. After work up in the usual way the expoxide mixture obtained was examined by n.m.r. spectroscopy. The signals at 5.64 and 5.31 p.p.m. were now in the ratio 3:2 as were the signals at 0.87 and 0.83 p.p.m.

c) With m-chloroperbenzoic acid.

The experiment was repeated essentially as outlined above. The n.m.r. spectrum now showed the ratio of signals as being ca. 1:1.

20 (R and S)-acetoxy-14α-hydroxy-3α, 5α-cyclo-20(R and S) - formyl-pregn-7-en-6-one (59).

The mixture of epoxides (58) (250 mg.), prepared by the monoperphthalic acid oxidation was outlined in the previous experiment, was heated at 160°C for 10 minutes under nitrogen. The mixture solidified on cooling but could not be crystallised from any solvent tried. P.L.C. (system; acetone/petrol, 30:70) gave a pure sample of the aldehyde mixture (59). ν max. 3450, 1725, 1650 $cm.^{-1}$ The n.m.r. spectrum had signals at; 9.70 (3/5 Hs), 9.63 (2/5 Hs), 5.95 (1 Hd), 2.12 (3 Hs), 1.55, 1.60 (3 Hs), 1.15 (3 Hs), 0.95 (6/5 Hs), 0.85 (9/5 Hs) p.p.m.

14 α-hydroxy-3 α, 5 α-cyclo-pregn-7-ene-6, 20-dione (60) and 14 α, 20S-dihydroxy-20-formyl-3 α, 5 α-cyclo-pregn-7-en-6-one (61).

The mixture of acetoxy aldehydes (59) (250 mg.) was dissolved in a mixture of water (15 ml.), methanol (20 ml.), tetrahydrofuran (5 ml.) and potassium carbonate (1.25 g.). The reaction was complete in 5 minutes, but was allowed to proceed for 30 minutes. After dilution with water the mixture was extracted with ether, the organic layer separated, dried (Na_2SO_4), and evaporated. Separation of the residue by P.L.C. (system; acetone/petrol, 15:85, 5 elutions) afforded the methyl ketone (60) (80 mg.) which crystallised from ethyl acetate as needles, m.p. 214-16°C. ν max. 3470, 1695, 1655, 1625 $cm.^{-1}$ λ max. 237 $m\mu$, (ε,10,800). $[\alpha]_D^{26}$ + 161° (c = 0.388).

The n.m.r. spectrum had signals at; 5.90 (1 Hd), 2.15 (3 Hs), 1.08 (3 Hs), 0.70 (3 Hs) p.p.m. Found; C, 76.69; H, 8.39; $C_{21}H_{28}O_3$ requires C, 76.79; H, 8.59.%

The aldehyde (61) fraction (100 mg.) crystallised from ethylacetate as needles, m.p. 205-7°C. ν max. 3470, 3380, 1725, 1640, 1615 $cm.^{-1}$ λ max. 245 $m\mu$ (ε,12,300). $[\alpha]_D^{24}$ + 116° (c = 0.500). The n.m.r. spectrum had signals at; 9.58 (1 Hs), 5.90 (1 Hd), 129 (3 Hs), 1.04 (3 Hs), 0.74 (3 Hs). p.p.m. Found; C, 73.71; H, 8.29; $C_{22}H_{30}O_4$ requires C, 73.71; H, 8.44 %.

14 α, 20S-dihydroxy-20-formyl-3 α, 5 α-cyclo-pregn-7-en-6-one (61) and 14 α, 20R-dihyrdoxy-20-formyl-3 α, 5 α-cyclo-pregn-7-en-6-one (62).

The acetoxy aldehyde mixture (59) (700 mg.) was dissolved in methanol (100 ml.). A solution of potassium carbonate (2 g.) in water (20 ml.) and methanol (80 ml.) was added and the mixture stirred at room temperature for 15 minutes. The solution was diluted with a large volume of water and extracted (2 x) with ether. The combined extracts were dried (Na_2SO_4) and evaporated to give a crude mixture of hydroxy aldehydes (61) and (62) (600 mg.). A portion (60 mg.) of the crude mixture was separated by P.L.C. (system; acetone/petrol 12:88, 14 elutions) to give the less polar 20S isomer (10 mg. which crystallised from ethylacetate, m.p. 206-8°C. A mixed m.p. with the hydroxy aldehyde obtained in the previous experiment showed no depression. The n.m.r. and infrared spectra were also identical. Found; C, 73.59; H, 8.33; $C_{22}H_{30}O_4$ requires C, 73.71; H, 8.44%.

The more polar 20R epimer (8 mg.) cyrstallised from ethylacetate/petrol as needles. m.p. 192°C. ν max. 3450, 1715, 1650 cm.$^{-1}$ The n.m.r. spectrum had signals at 9.56 (1 Hs), 5.93 (1 Hd), 1.37 (3 Hs), 1.09 (3 Hs), 0.87 (3 Hs) p.p.m. Found; C, 73.64; H. 8.44; $C_{22}H_{30}O_4$ requires C, 73.71; H. 8.44 %.

2β-acetoxy-3β-hydroxy-5 α, 14 α and β-20S-formyl-pregn -7-en-6-one (53)

The monoacetate diol mixture (20), R = H and (22), R = H (500 mg.) was dissolved in methylene chloride (150 ml.) and pyridine(1.5 ml.) added. Ozonolysis and work up in the usual way, followed by P.L.C.

purification (system; acetone/petrol, 30:70) gave the product (53) (280 mg.) as a non-crystalline solid. ν max. 3450, 1725, 1713, 1665, 1618 cm.$^{-1}$

2β, 3β, 7, 22-tetraacetoxy-5α-bisnorchol-6, 8 (14), 20-triene (63) and 2β, 3β, 7, 22-tetraacetoxy-5β-bisnorchol-6, 8 (14), 20-triene (64).

The aldehyde (53) (50 mg.) was dissolved in acetic anhydride (5 ml.) and added to a slowly distilling mixture of aceticanhydride (10 ml.) and freshly fused sodium acetate (50 mg.). After ½ hour the reaction was worked up in the usual way. Infrared examination of the product indicated only the aldehyde moiety had enolacetylated, a peak at 1665 cm^{-1} being present. The crude product was redissolved in acetic anhydride (10 ml.) and more freshly fused sodium acetate added (50 mg.). The mixture was refluxed for a further 20 hours and then worked up in the usual way. P.L.C. separation (system; acetone/petrol, 30:70) afforded a crude mixture of C-5 epimers. (30 mg.). Further separation by P.L.C. (system; acetone/petrol 5:95, 10 elutions) gave the less polar 5β epimer (3 mg.) as a semi-solid oil. ν max. 1740, 1660 cm.$^{-1}$ The n.m.r. spectrum (100 m/c) showed the signal due to the 7 proton as a singlet at 5.85 p.p.m.

The more polar 5α epimer (5 mg.) was also obtained as a non-crystalline solid. ν max. 1740, 1640 cm.$^{-1}$ The n.m.r. spectrum (100 m/c) showed the signal due to the 7 proton as a doublet at 5.82 p.p.m. (J = 3 c.p.s.).

The mass spectra of each compound were very similar and showed the molecular ion at m/e 528 corresponding to $C_{30}H_{40}O_8$.

2β, 3β, 7, 22, 22^1-pentacetoxy-5α-bisnorchol-6, 8 (14)-diene (65).

The aldehyde (53) (100 mg.) was dissolved in the perchloric acid enolacetylating mixture (5 ml.) and left at room temperature 4 hours. After work up in the usual way, P.L.C. separation (system; acetone/petrol 1:4) of the major product gave the geminal diacetate (65) (60 mg.) as an oil. ν max. 1740, 1650 $cm.^{-1}$ The n.m.r. spectrum showed signals at 5.86 (1 Hd) p.p.m. The mass spectrum had the molecular ion at m/e 588 corresponding to $C_{32}H_{44}O_{10}$.

Two step enolacetylation of the monoacetate diol aldehyde (53) mixture

The aldehyde (230 mg.) was dissolved in acetic anhydride (2 ml.) and added to a slowly distilling solution of acetic anhydride (15 ml.) and freshly fused sodium acetate (230 mg.). The distillation was continued for ½ hour, and then the remainder of the solvent reduced rapidly to low bulk (ca. 2 ml.) by distillation under reduced pressure. The residue was diluted with carbon tetrachloride (25 ml.), filtered, and perchloric acid (0.005 ml., 60% aqueous) added. The mixture

was shaken vigorously for a few minutes and then stood at room temperature of 1½ hours. After work up in the usual way, separation of the major product by P.L.C. (system; acetone/petrol, 30:70) gave the required 2β, 3β, 7, 22-tetracetoxy-5α-bisnorchol-6, 8 (14), 20-triene (63) (110 mg.) as a semi-solid foam. ν max. 1760 cm.$^{-1}$ λ max. 254 mμ. The n.m.r. spectrum showed signals at; 6.96 (1 Hm), 5.88 (1 Hd), 1.72 (3 Hd), 0.90 (3 Hs), 0.80 (3 Hs).

The geminal diacetate (62 mg.) (65) was obtained from the P.L.C. separation as a by-product.

2β, 3β, 22 triacetoxy-14α-hydroxy-20 (R and S), 22-epoxy-5α-bisnorchol-7-en-6-one (66).

The bisenolacetate (63) (140 mg.) was dissolved in ether (10 ml.) and an excess of an ether solution of monoperphthalic acid added (5 ml.). The mixture was stood overnight at room temperature, and then worked up in the usual way to yield the crude product. Purification by P.L.C. (system; acetone/petrol 30:70) gave the pure mixture (66) (110 mg.) which crystallised from ether. ν max. 3470, 1740, 1718, 1675, 1630 cm.$^{-1}$. The n.m.r. spectrum had signals at; 5.38 (1 Hd), 5.58 (ca. 2/5 Hs), 5.24 (ca. 3/5 Hs), 1.45 (3 Hs), 0.99 (3 Hs), 0.78 (3 Hbs).

2β, 3β, 20 (R and S)-triacetoxy-14α-hydroxy-20-formyl-5α-pregn-7-en-6-one (67)

The epoxy acetate mixture (66) (110 mg.) was heated at 150°C for 10 minutes under nitrogen. P.L.C. (system; acetone/petrol, 35:65) gave the pure mixture of epimers (100 mg.) which crystallised from ethylacetate/petrol, m.p. 254-56°C. ν max. 3480, 1725, 1670. The n.m.r. spectrum showed signals at; 9.61 (3/5 Hs), 9.55 (2/5 Hs), 5.90 (1 Hs), 1.55 (3 Hbs), 0.98 (3 Hs), 0.85 (9/5 Hs), 0.75 (6/5 Hs). Found; C, 64.68; H, 7.47; $C_{28}H_{38}O_9$ requires C, 64.85, H, 7.39%.

2β, 3β-diacetoxy-14α, 20S-dihydroxy-20-formyl-5α-pregn-7-en-6-one (68) and 2β, 3β-diacetoxy-14α, 20R-dihydroxy-20-formyl-5α-pregn-7-en-6-one (69).

The acetoxy aldehyde mixture (67) (100 mg.) was dissolved in methanol (10 ml.) and a solution of potassium carbonate (100 mg.) in methanol (7 ml.) and water (3 ml.) added. The reaction was quenched after two minutes at room temperature by addition of acetic acid (1 ml.). After work up in the usual way the crude product obtained (84 mg.) was separated by P.L.C. (system; acetone/petrol, 1:9, 12 elutions). The less polar 20S epimer (36 mg.) thus obtained was crystallised from chloroform/ether as needles, m.p. 239-41°C. ν max. 3540, 1722, 1670, 1630. λ max. 237 mμ, (ε, 12,100). $[\alpha]_D^{23}$ + 12° (c = 0.118). The n.m.r. spectrum showed signals at; 9.58 (1 Hs), 5.90 (1 Hd), 1.30 (3 Hs), 0.97 (3 Hs), 0.68 (3 Hs). Found; C, 65.45; H, 7.54;

$C_{26}H_{36}O_8$ requires C, 65.53; H, 7.61 %.

The more polar 20R epimer (26 mg.) crystallised from chloroform/ether as needles, mp. 237-8°C. ν max. 3520, 1730, 1720, 1670, 1625 cm.$^{-1}$ λ max. 238 mμ, (ε, 11,900) $[\alpha]_D^{23}$ + 11° (c = 0.05). The n.m.r. spectrum had signals at 9.56 (1 Hs), 5.90 (1 Hd), 1.36 (3 Hs), 0.98 (3 Hs), 0.79 (3 Hs). Found; C, 65.39; H, 7.77; $C_{26}H_{36}O_8$ requires C, 65.53; H, 7.61 %.

2β, 3β-diacetoxy-14 α-hydroxy-5 α-pregn-7-ene-6, 20-dione (70).

The hydroxy aldehyde mixture (68) and (69)(50 mg.) was dissolved in ether (20 ml.) and allowed to stand 3 days at room temperature exposed to daylight. After removal of the ether by evaporation the two products were weparated by P.L.C. (system; acetone/petrol 1:4, 3 elutions) to give the less polar methyl ketone (70) (21 mg.) which crystallised from ethyl acetate/petrol as needles, m.p. 237-39°C. ν max. 3450, 1728, 1715, 1670 cm.$^{-1}$ λ max. 237 mμ, (ε,10,400). $[\alpha]_D^{24}$ + 243° (c = 0.07). The n.m.r. spectrum showed signals at; 2.14 (3 Hs), 0.98 (3 Hs), 0.62 (3 Hs). Found; C, 67.18; H, 7.69, $C_{25}H_{34}O_7$ requires C, 67.24; H, 7.68 %.

The more polar hydroxy aldehyde (68) was recovered (14 mg.) and was identical with the pure 20S epimer (68) by n.m.r. and T.L.C. comparison.

Attempted Grignard reaction on the pure 20S and 20R hydroxy aldehydes (68) and (69).

To a stirred solution of isoamyl magnesium bromide, [prepared by the reaction of magnesium (12 mg.) with isomyl bromide (150 mg.) in dried ether (3 ml.)] at 0°C, was added a solution of the 20R hydroxy aldehyde (69) (16 mg.) in dried tetrahydrofuran (5 ml.). Stirring was continued for a further 15 minutes and then the reaction was worked up in the usual way with ammonium chloride solution. P.L.C. separation (system; acetone/petrol 35:65) gave the more polar product (6 mg.) (72). ν max. 3500, 1740, 1710, 1675 cm.$^{-1}$ The mass spectrum had the molecular ion at m/e 476 corresponding to $C_{26}H_{36}O_8$. The 20S epimer gave thesame compound when treated under indentical conditions.[26]

Grignard reaction on 14 α-hydroxy-20 (R and S)-diacetoxy-20-formyl-3α-5α-cyclo-pregn-7-en-6-one (59).

To a stirred Grignard solution of isoamyl magnesium bromide [prepared by the reaction between magnesium (20 mg.) and isoamyl bromide (250 mg.) in dried ether (2 ml.)], at room temperature, was added a solution of the aldehyde mixture (59) (25 mg.) in dried tetrahydrofuran (3 ml.). Stirring was continued for 10 minutes and then a solution of ammonium chloride in water was added. The mixture was ether extracted, the separated ether layer dried (Na_2SO_4) and evaporated under high vacuum to yield the crude product (28 mg.). T.L.C. indicated a complex

mixture had been produced. The n.m.r. spectrum of the crude product showed additional signals in the methyl region at 0.94, 1.09, 1.28 p.p.m. No aldehyde protons were present. No separation of the complex mixture was attempted.

14 α,20 (R and S)-(bistetrahydropyran-2-yloxy)-20-formyl-3 α, 5 α -cyclo-pregn-7-en-6-one (74).

The cyclopropyl hydroxy aldehyde mixture (61) and (62) (30 mg.) was dissolved in dried ether (3 ml.) and dihydropyran (0.5 ml.) A crystal of p-toluene sulphonic acid was added and the mixture left overnight at room temperature. The solvent was removed under reduced pressure and the residue separated by P.L.C. (system; acetone/petrol, 1:4). The least polar fraction (74) was obtained as an oil (8 mg.) which failed to crystallise. ν max. 1725, 1665, 1080, 1040 cm.$^{-1}$

Attempts at separation of the product by T.L.C. (system; acetone/ petrol 1:9, 10 elutions) failed. (The products just streaked along the plate).

Attempted silylation of the cyclopropyl diol mixture (61) and (62)

The diol mixture (61) and (62) (30 mg.) was refluxed with dried dimethylformamide (3 ml.) and bis-(trimethylsilyl)-acetamide (0.5 ml.) for 24 hours. The solvent was removed under high vacuum and the crude product separated into two components by P.L.C. (system; acetone/petrol 1:4). The least polar material obtained (14 mg.) failed to crystallise. Spectroscopic examination indicated that rearrangement of the side chain moiety had occurred. i.e. ν max. 3450, 1710, 1655, 855, 760. The n.m.r. spectrum had no aldehyde protons but showed signals at: 5.96 (1 Hd), 3.62 (1 Hm), 1.26 (3 Hs), 1.07 (3 Hs), 0.76, 0.71 (3 Hs), 0.15 (9 Hs). The more polar material also could not have been the required bis-silyl ether as the infrared spectrum showed only one carbonyl band at 1650 cm.$^{-1}$

20 (R and S)-acetoxy-14α-trimethylsilyloxy-20-formyl-3α, 5α-cyclo-pregn-7-en-6-one (75).

The acetoxy aldehyde mixture (59) (150 mg.) was dissolved in dried dimethylformamide (4 ml.) and bistrimethylsilylacetamide (B.S.A.) (0.2 ml.) and refluxed for 3 hours. Further portions of B.S.A. (0.1 ml.) were added after 1 and 2 hours. The solvent was removed under high vacuum and the residue purified by P.L.C. (system; acetone/petrol 1:4) to give the required ether mixture (75) (74 mg.) which crystallised as needles from ether, m.p. 153-56°C. ν max. 1725, 1655, 1260, 855,

765 cm.$^{-1}$ The n.m.r. spectrum showed signals at, 9.58, 9.48, (1 Hs), 5.88 (1 Hdd), 1.99 (3 Hs), 1.41 (3 Hs), 0.95 (3 Hs), 0.76, 0.64 (3 Hs), -0.11 (9 Hs). Found; C, 68.60; H, 8.38; $C_{27}H_{40}O_5Si$ requires C, 68.61; H, 8.53 %. No further separation of the epimers could be achieved by P.L.C.

20S-hydroxy-14α-trimethylsilyloxy-20-formyl-3α, 5α-cyclo-pregn-7-en-6-one (76) and 20R-hydroxy-14α-trimethylsilyloxy-20-formyl-3α, 5α-cyclo-pregn-7-en-6-one (77).

The acetoxy ether (75)(50 mg.) was dissolved in methanol (2 ml.) and a solution (2 ml.) of potassium carbonate (100 mg.) in methanol (8.5 ml.) and water (1.5 ml.) added. After standing for 2 minutes at room temperature the reaction was quenched by addition of glacial acetic acid (3 drops). Saturated sodium chloride solution was added and the mixture extracted with ether. The separated organic layer was dried (Na_2SO_4) and the solvent removed under reduced pressure to yield the crude mixture of C-20 epimers (48 mg.). Separation by P.L.C. (system; acetone/petrol 1:19, 3 elutions), afforded initially the less polar 20S epimer (20 mg.) which crystallised from petrol as needles, m.p. 125-27°C. ν max. 34500, 1720, 1655, 1250, 850, 760 cm.$^{-1}$

λmax. 236 mμ, (ε, 10,600). $[\alpha]_D^{23}$ + 26° (c = 0.192). The n.m.r. spectrum showed signals at; 9.57 (1 Hs), 5.87 (1 Hd), 1.21 (3 Hs), 0.97 (3 Hs), 0.62 (3 Hs), 0.01 (9 Hs). (benzene as internal standard). Found; C, 69.82; H, 8.98; $C_{25}H_{38}O_4Si$ requires C, 69.73; H, 8.90 %.

The more polar 20R epimer (14 mg.) crystallised from chloroform/petrol as needles, m.p. 152-3°C. ν max. 3470, 1715, 1650, 1245, 850, 760 cm.$^{-1}$ λ max. 237 mμ (ε,10,750). $[\alpha]_D^{23}$ + 125° (c = 0.132). The n.m.r. spectrum had signals at: 9.56 (1 Hs), 5.88 (1 Hd), 1.29 (3 Hs), 1.01 (3 Hs), 0.75 (3 Hs), 0.01 (9 Hs). (benzene as internal standard). Found; C, 69.78; H, 8.87; $C_{25}H_{38}O_4Si$ requires C, 69.73; H, 8.90 %.

2β, 3β-diacetoxy-14α-trimethylsilyloxy-5α-ergost-7-en-6-one and 2β, 3β-diacetoxy-14α-trimethylsilyloxy-5β-ergost-7-en-6-one.

2β, 3β-diacetoxy-14α-hydroxy-5α-ergost-7-en-6-one (27) (50 mg.) was dissolved in dried dimethylformamide (2 ml.) and bistrimethylsilylacetamide (0.2 ml.) and warmed to 70°C for 6 hours.

Additional portions of B.S.A. (0.2 ml.) were added every 2 hours. The solution was stood overnight at room temperature and then the solvent removed under high vacuum. P.L.C. separation afforded initially the less polar 5α epimer (14 mg.) as an oil. ν max. 1738, 1675, 1620, 1250, 860, 770 $cm.^{-1}$ The n.m.r. spectrum showed signals at 5.90 (1 Hd), 5.22 (1 Hm), 4.82 (1 Hm), 2.06 (3 Hs), 2.00 (3 Hs), 0.07 (9 Hs).

The more polar 5β epimer was also obtained as an oil (11 mg.). ν max. 1735, 1670, 1250, 860, 770 $cm.^{-1}$ The n.m.r. spectrum showed signals at; 5.88 (1 Hd), 5.26 (1 Hm), 4.89 (1 Hm), 2.08 (3 Hs), 1.97 (3 Hs), 0.11 (9 Hs).

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