# THE CHEMISTRY OF GLAUCONIC ACID

A thesis presented by

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## SUMMARY

In the introduction the biogenesis of non-nitrogenous mould metabolites is briefly reviewed and a possible biogenetic route for the formation of glauconic, glaucanic and byssochlamic acids is discussed.

The early work on glauconic acid, $C_{18}H_{20}O_7$, and its pyrolysis product, glauconin, $C_{11}H_8O_6$, is reviewed and the structure of the latter is discussed. Various synthetic approaches to glauconin are described culminating in a successful synthesis from acetonedicarboxylic ester.

An account of the work carried out on glauconic acid is given and a structure derived, showing it to be a ninemembered ring compound containing two unsaturated five ring anhydrides and an hydroxyl group. In particular, the zinc dust and acetic acid reduction of glauconic acid ketone is discussed and the conversion of one of the products to an indanone is described.

The synthesis of the indanone is described.

# INTRODUCTION

## BIOGENESIS OF MOULD METABOLITES

Interest in the determination of the structures of natural products has, in recent years, been supplemented by an increasing curiosity concerning the way in which these products are synthesised in the living organism. Many speculations about possible biosynthetic routes are being tested experimentally using radioactive isotopes to trace the various pathways taken by simple starting materials, such as acetate. Moulds have been specially useful in these studies because they can be grown under rigorously defined conditions and because of the short period required for the incorporation of the precursors studied and the ease of isolation of the metabolites produced.

The comparative study of the structure of natural products as well as the study of the biochemical processes involved in their formation has encouraged the development of hypothetical biosynthetic routes which have helped the chemist in the elucidation of the structure of many natural products, and the biochemist to search for the hypothetical precursors and study the enzymatic reactions involved. Two main hypotheses, the "isoprene rule"[1], and the "acetate theory"[3] have proved of great use in the

elucidation of the structure of natural products and the correlation of their structural patterns.

## ACETATE HYPOTHESIS

Collie[2] appears to have been the first to draw attention to the role of acetic acid in the biosynthesis of natural products and to suggest that the "head-to-tail" linkage of acetic acid might be involved in the biosynthesis of many phenolic compounds. His hypothesis was based on the study of condensation reactions of β-polyketones which led to substances such as orcinol, a well-known natural product. The ring closure of a β-polyketone of type I, through aldol condensation (a), or C-acylation (b), would produce phenols of the orcinol (II) or acyl-phloroglucinol (III) type respectively. This hypothesis seems to have been forgotten until 1953 when Birch and Donovan published a paper[4] in which the "head-to-tail" condensation of acetate was considered to lead to the synthesis of many naturally occurring phenolic compounds. Birch[3] based his hypothesis on the known importance of the acetic acid unit in the biosynthesis of fatty acids and terpenoids. The basic concepts of the acetic acid hypothesis can be summarised as follows[5]:

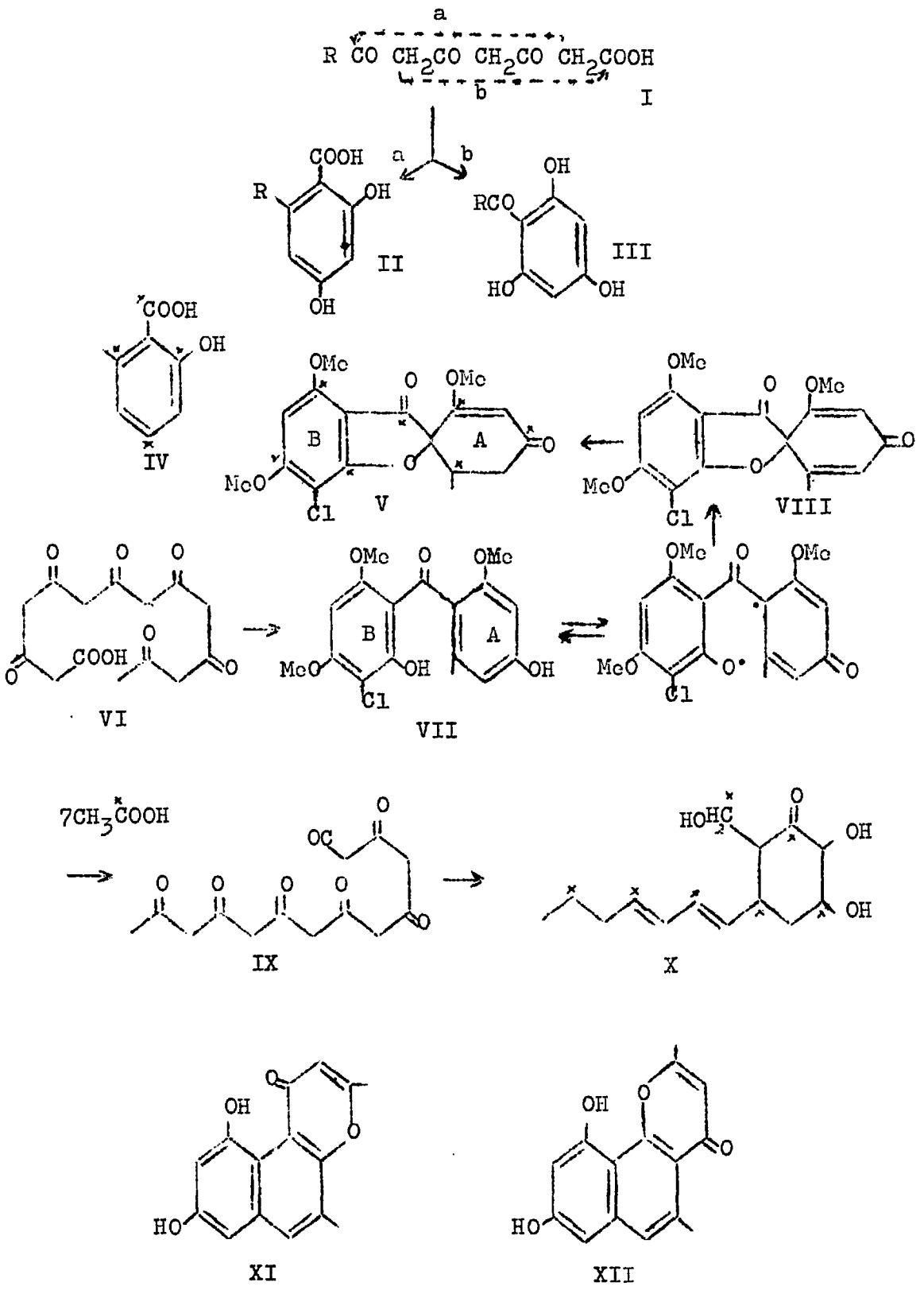
a) acetic acid units are joined by formal elimination of water in "head-to-tail" linkage with each other or with a naturally occurring carboxylic acid to form β-polyketone chains;

b) secondary changes in the β-polyketone take place such as cyclisation through C-acylation or aldol condensation to form aromatic rings;

c) modification of the original skeleton by introduction of methyl, isopentyl or polyisoprenoid groups;

d) secondary processes of reduction, dehydrogenation and oxidation.

The obvious way to prove the validity of the acetate hypothesis was through the use of isotopically labelled acetic acid in feeding experiments. The first experiment which supported this view[6] was the incorporation of (1-$^{14}$C) acetate in the 6-methyl salicylic acid produced by Penicillium griseofulvum Dierckx. The "head-to-tail" condensation of the acetic acid units was shown[6] by degradation studies which proved the distribution of $^{14}$C to be as in (IV). Griseofulvin (V), isolated from the same cultures, was shown[14] to have a distribution pattern of isotopically labelled acetate as indicated (V). It was assumed[15] that the β-polyketoacid precursor (VI) undergoes an aldol condensation to form the orcinol type ring A and a Claisen condensation to form the phloroglucinol type ring B. The intermediate ketone, griseophenone, (VII), would undergo an oxidative coupling reaction as postulated by Barton and Cohen[16], to yield dehydrogriseofulvin (VIII) and then is enzymatically reduced to griseofulvin. This hypothetical


a
R CO CH2CO CH2CO CH2COOH
b
I
COOH
OH
R
OH
II
a
b
OH
RCO
HO
OH
III
COOH
OH
IV
OMe
O
OMe
B
A
MeO
Cl
V
OMe
O
OMe
MeO
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OH
OH
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VII
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O
OMe
MeO
Cl
7CH3COOH
OC
O
O
O
O
O
IX
HOH2C
O
OH
OH
X
OH
O
HO
XI
OH
O
O
HO
XII

route has been supported by a chemical synthesis[17]. Griseophenone was synthesised and then oxidised by potassium ferricyanide to dehydrogriseofulvin which was reduced catalytically to griseofulvin.

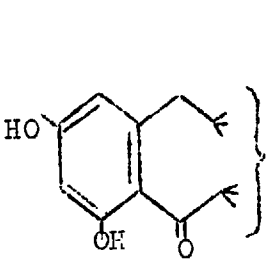
The acetate hypothesis has been corroborated in many other cases and its validity established. For example, palitantin, produced by P.palitans or P.cyclopium, can be derived from an hypothetical β-polyketone chain (IX) formed from seven acetate units. This is supported by the pattern of incorporation of (1-$^{14}$C) acetate which has been shown[7,8] to be as in (X).

The acetic acid hypothesis has been a useful tool for the elucidation of the structure of natural products. Eleutherinol[3] was formulated on chemical grounds by Schmid as (XI). Birch and Donovan proposed structure (XII) assuming a precursor formed by the usual linkage of acetic acid units. This was proved[3] to be correct on reinvestigation of the chemistry of eleutherinol. A partial structure (XIII) was assigned[9] to curvularin, a metabolite of Curvularia species. Birch et al.[10], continued the degradation studies and proposed structure (XV) which could arise from "head-totail" condensation of acetic acid units as shown in (XIV). The distribution pattern of $^{14}$C in the side chain of curvularin isolated from a culture fed with (1-$^{14}$C) acetate, was shown to be as indicated in (XV) in accordance with the hypothetical precursor.

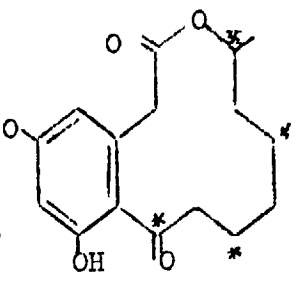
The true nature of the process involved in the formation of the natural products derived from β-polyketone precursors has not been elucidated. The study of the intermediate stages in the biosynthesis of fatty acids suggests that a reduction to a saturated chain takes place prior to the condensation with a further acetate unit.[37] The frequency with which meta oxygen substituents is found in natural phenolic compounds[3,32] and the distribution pattern of carbonyl groups in the macrolides[33] suggests on the other hand, that the β-polyketone character exists throughout the biosynthetic process. Gatenbeck and Mosbach[11] studied the incorporation of (1-$^{14}$C, $^{18}$O) acetate in orsellinic acid produced by Chaetomium cochliodes Pall. The distribution pattern of $^{18}$O and $^{14}$C was found as indicated in (XVII), as would have been expected if a direct cyclisation of a β-polyketoacid precursor such as (XVI) had taken place. A study of the $^{18}$O content in islandicin (XVIII)[12] and emodin (XIX)[13] obtained from Penicillium islandicum grown in (1-$^{14}$C, $^{18}$O) acetate medium, suggests that the $^{18}$O distribution is as expected from retention of the oxygen function after building up of the molecule, but attempts to establish the actual position of the $^{18}$O failed. A study of the $^{14}$C distribution pattern in both metabolites[12,13] suggests a "head-to-tail" linkage of acetic acid units, as shown (XVIII and XIX). These

data support the idea that the secondary changes, such as reduction, take place after the condensation of the β-polyketone chain to form aromatic products, has been effected.

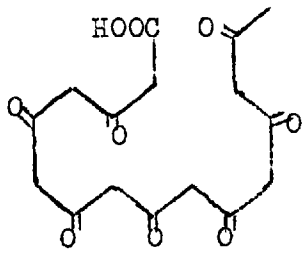
Another important route for the formation of phenolic products in nature was found by Davis et al.[18,19] in their study of the precursors of aromatic amino acids such as tyrosine, tryptophane, phenylalanine and p-aminobenzoic acid in mutants of Escherichia coli, Aerobacter aerogenes and Neurospora crassa. The use of enzymatic blockage techniques and replacement experiments allowed them to isolate the intermediates and to establish the sequence in which they were formed, (scheme I). The incorporation of isotopically labelled shikimic acid (XXI) or prephenic acid (XXII) in aromatic mould metabolites has not been studied as yet. Shikimic acid has been proved[23] to be incorporated exclusively into the ring B of quercetin (XXIII) isolated from buckwheat. The authors[23] found that shikimic acid and phenylalanine were the most efficient precursors of quercetin among a series of hypothetical precursors tested by them. The transformation of dihydroshikimic acid (XX) into gallic acid (XXIV) is suggested by growth experiments of Phycomyces blakeslecanus in media containing the precursor.[20]



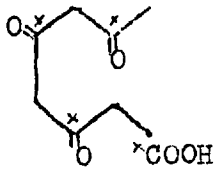

XIII



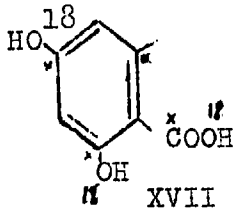

XV



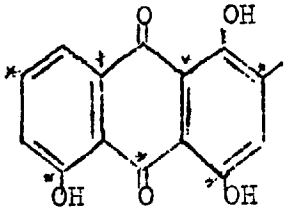

XIV



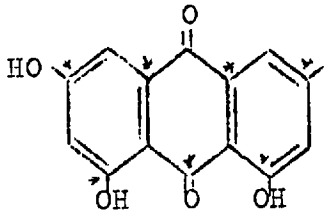

XVII




XVII

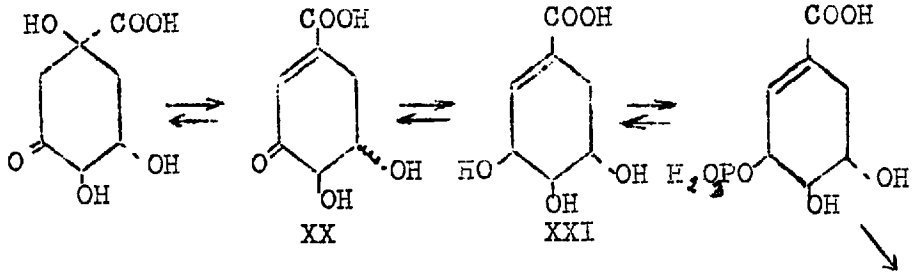



XVIII



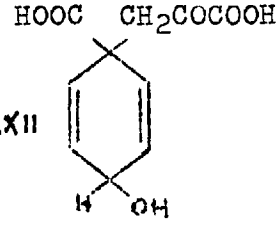

XIX

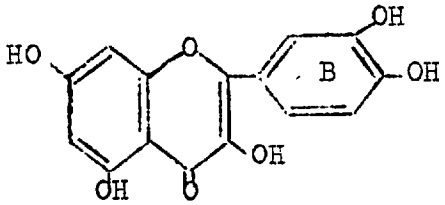
Scheme I



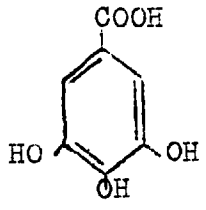

XX

XXI




XXIII

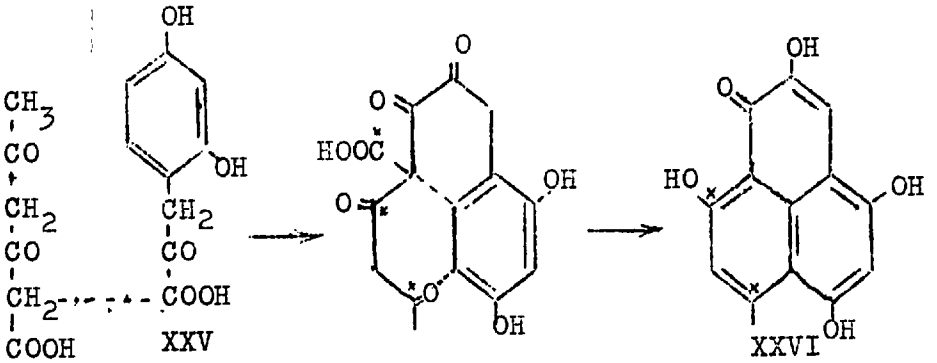



XXIV

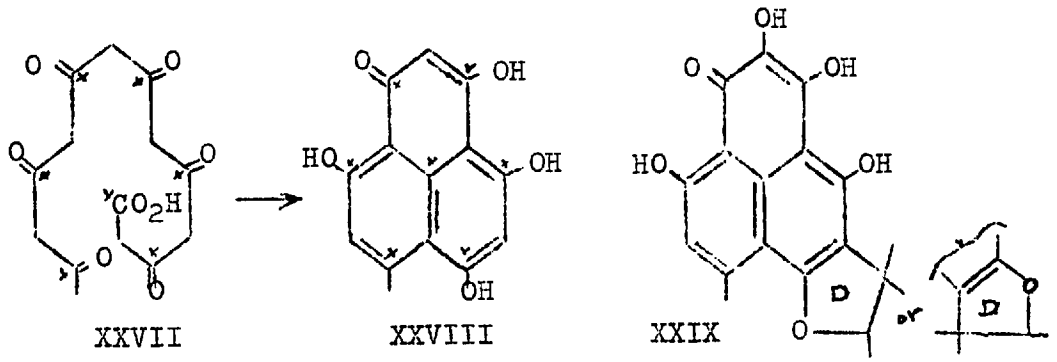
The information, available up to now, on the biogenesis of phenolic mould metabolites other than the aminoacids mentioned, seems to indicate a direct formation from a β-polyketone chain rather than through the shikimic acid route. Thomas[21] studied the incorporation of (1-$^{14}$C) acetate into atrovenetin (XXIX) produced by P. herquei. (Norherqueinone ($C_{19}H_{18}O_7$), whose structure is not fully established but is related to atrovenetin, was the actual metabolite on which the degradation studies were carried out). The isotopically labelled acetate was introduced after the mould had been incubated for 13 days. It was assumed that if the perinaphthenones are formed from the hypothetical precursor 2,4-dihydroxyphenylpyruvic acid (XXV) derived from prephenic acid, the carbon atoms marked in formula (XXVI) would show a higher content of $^{14}$C. The results obtained[21] support a β-polyketoacid precursor (XXVII) as was postulated by Barton et al.[22]

The structure of some mould metabolites such as patulin (XXX) and penicillic acid (XXXI) do not suggest a β-polyketone as direct precursor.

Patulin is produced by Penicillium patulum together with gentisyl alcohol, gentisic acid, and 6-methylsalicylic acid. Birkinshaw[24] proposed the sequence of transformations outlined in scheme (II) for its biosynthesis. This has been proved to be so by the study of the distribution of the $^{14}$C in patulin produced

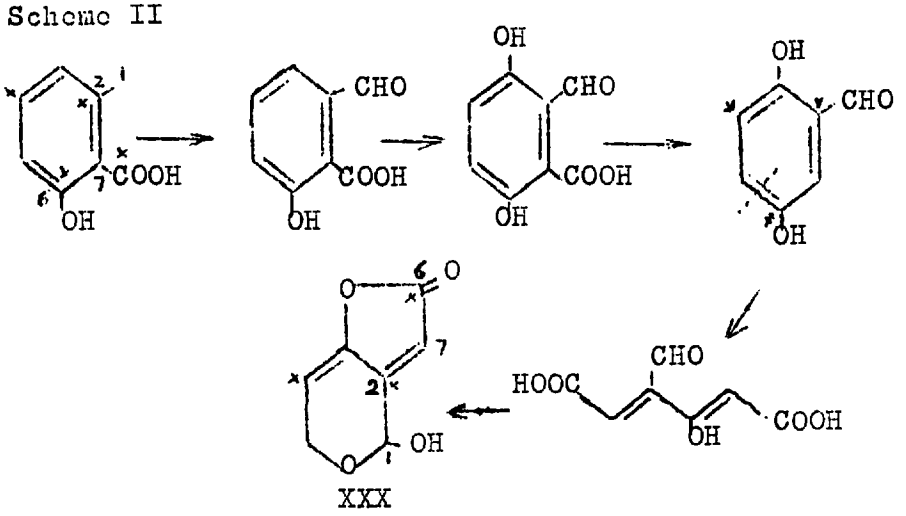


XXV XXVI

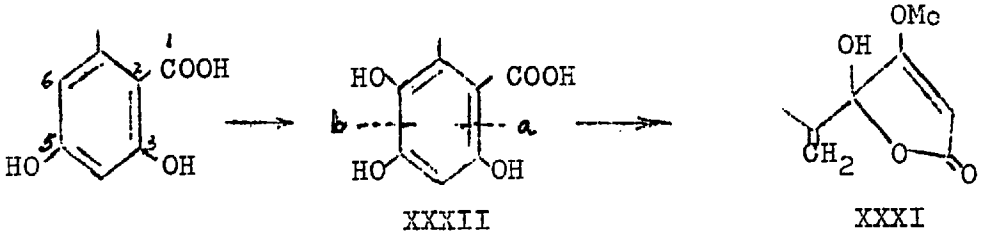


XXVII XXVIII XXIX

Scheme II



XXX



XXXII XXXI

by P. patulum fed with 6-methylsalicylic acid previously formed from (1-$^{14}$C)[26] acetate, which was shown to be as in (XXX).

The penicillic acid isolated from P.cyclopium grown in media containing (1-$^{14}$C) acetate, was found[28] to have the $^{14}$C pattern distribution as shown in (XXXI). Birch et al.,[28] proposed orsellinic acid as a precursor, the acid being formed by oxidation and breakage of the 2,3 bond (XXXIIa). Mosbach[29] fed P.barnensis with 1,3 isotopically labelled orsellinic acid, and demonstrated that orsellinic acid is indeed the precursor of penicillic acid but the bond which undergoes fission is the 5,6-bond as shown in (XXXIIb).

The pattern of distribution of isotopic carbon of polyacetylenes such as nemotinic acid (XXXIV), isolated from Basidomycetes B841 grown in a medium containing (1-$^{14}$C) acetate, is suggestive of a β-polyketone chain precursor,[25] in which the methyl from the starting acetate unit has been lost. The higher homologue odyssic acid (XXXV), is also isolated from the same culture.

The methyl groups extraneous to the acetate derived β-polyketone chain have been found to derive in some cases from $C_1$ donors such as formic acid or methionine. When P. brevi compactum was incubated in a medium containing (methyl$^{14}$C) methionine the incorporation of the methyl group into the ether function of mycophenolic acid (XXXVI)

was found to be higher than into the methyl group in the nucleus.[30] $C_1$ incorporation was also found[31] in citrinin (XXXVII) and sclerotionin (XXXVIII) (the structure of sclerotionin has been demonstrated[27] to be as shown in XXXVIIIa) grown on a medium containing ($^{14}C$) formic acid. The pattern distribution of (1-$^{14}C$) acetate incorporation was found[31] to agree with a "head-to-tail" linkage of acetate units (XXXVII and XXXVIII). An examination of the distribution pattern of the extraneous methyl groups in citrinin and sclerotionin suggests that propionic acid might be incorporated.

Robinson[32] drew attention to the possibility of incorporation of acetic and propionic acid units, or their equivalents, in the formation of the branched fatty acids such as mycolipenic (XXXIX) and tuberculostearic acids (XL).

An examination of the distribution pattern of oxygenated atoms and of methyl groups in macrolides such as magnamycin (XLI), methymycin (XLII) and erythromycin (XLIII) led Woodward[33] to suggest the participation of propionic acid in the formation of the β-polyketo chain precursor.

The incorporation of propionic acid in the aglycone of erythromycin (erythronolide, XLIII, R=H) has been demonstrated[34] by the study of incorporation of (1-,2-,3-$^{14}C$) propionic acid. Kuhn-Roth oxidation of

the erythronolide showed that propionic acid is incorporated directly into the chain without suffering randomisation.

Methymycin (XLII) has been postulated[35] to derive from an hypothetical β-polyketone chain formed from five propionic acid units, with one acetate interposed in it. Studies on incorporation of (1-$^{14}$C) propionate and (1-$^{14}$C) acetate revealed a distribution pattern of $^{14}$C as shown in (XLII).

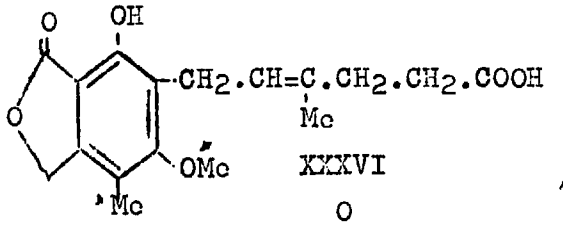
$C_1$ in ε-pyrromycin (XLIV) has been also found to derive from the $C_1$ of propionic acid. A study of (1-$^{14}$C) acetate incorporation showed that the rest of the molecule is formed by a "head-to-tail" linkage of acetate units.[36]

As in the biogenesis of fatty acids, the β-polyketone chain precursors of mould metabolites are supposed to be formed by condensation of acetic acid through its active form, the acetyl coenzyme A, but recently the demonstration of the importance of malonyl Coenzyme A in the biosynthesis of fatty acids[37] suggested its intervention in the formation of mould metabolites.

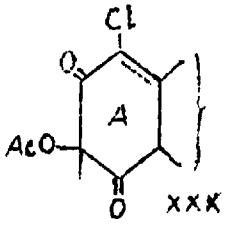
The role of malonate in the biosynthesis of aromatic compounds was first investigated by Bentley and Keil[38] who studied the incorporation of (1-, and 2-$^{14}$C) acetate and methylene-and carboxyl-labelled malonate, among other hypothetical precursors, in the biosynthesis of

$HC\equiv C.C\equiv C.CH=C=CH.CH(OH).CH_2.CH_2.COOH$ XXXIV

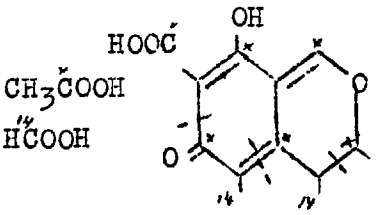
$CH_3.C\equiv C.C\equiv C.CH=C=CH.CH(OH).CH_2.CH_2.COOH$ XXXV



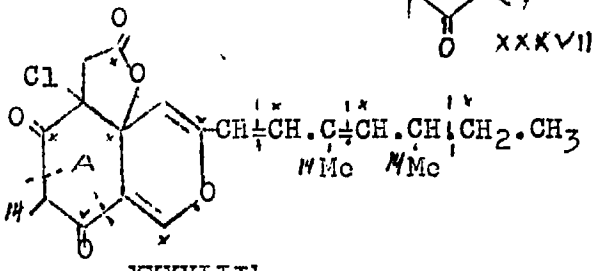
XXXVI



XXXVIIIa

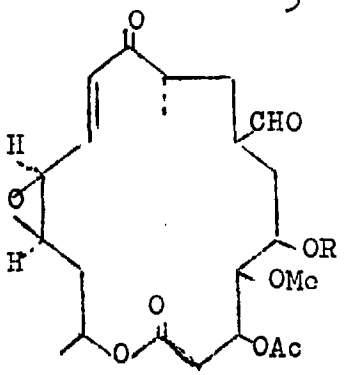


XXXVII

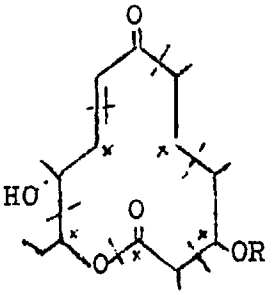


XXXVIII

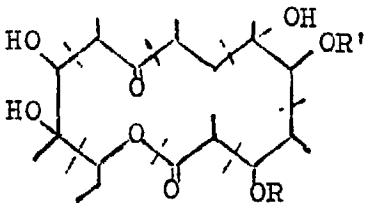
$CH_3(CH_2)_{17}CH(Me)-CH_2-CH(Me)-CH=C(Me)-COOH$ XXXIX



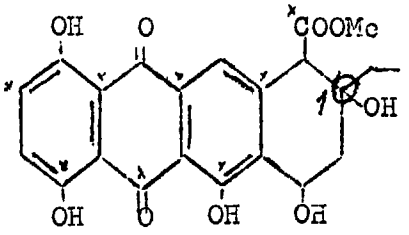
XLI



XLII



XLIII



XLIV

$CH_3(CH_2)_7CH(Me)-CH_2-(CH_2)_7-COOH$ XL

penicillic acid by P. cyclopium. They found that the labelling pattern is different in penicillic acid derived from isotopically labelled acetate and malonate. A study of the labelling pattern of the penicillic acid derived from the different precursors prompted them to postulate (XLV) as an intermediate in the biosynthesis of orsellinic acid from which penicillic acid is derived, as was shown by Mosbach,[29] (scheme III). They concluded[38] that $C_7$ and $C_5$ of penicillic acid were derived respectively from $C_2$ and $C_1$ of acetic acid and that orsellinic acid is formed from one molecule of acetyl-CoA and three molecules of malonyl-CoA and further postulated that other "head-to-tail" condensations, apparently of acetic acid, may also involve malonate as an intermediate.

The incorporation of malonate into 6-methylsalicylic acid has been demonstrated by degradation studies of the product obtained from cultures of P. urticae grown in media containing diethyl (2-$^{14}$C) malonate.[39] In experiments in which the mould was incubated in media containing (1-$^{14}$C) acetate and unlabelled malonate[40] a higher isotope content was found in $C_6$. This was attributed to a preferential utilization of malonate in the formation of the β-polyketone chain.

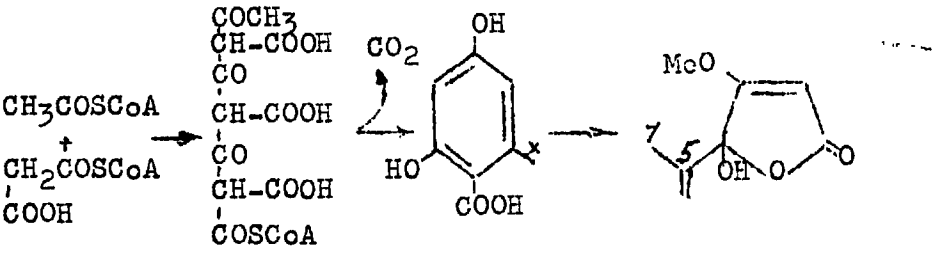
Lynen and Tada[41] studied the incorporation of (1-$^{14}$C) acetyl-CoA (enzymatically) and the utilization of malonyl-CoA in the formation of 6-methylsalicylic acid by P. patulum. They found that, as in the biosynthesis

of fatty acids, triphosphopyridine nucleotide was necessary and that the enzymatic complex contained a free sulphydril group necessary for the biosynthesis of the metabolite (suggested by inhibition by iodoacetamide). The utilisation of malonyl-CoA was demonstrated by its effect on the incorporation of the isotopically labelled acetyl-CoA.

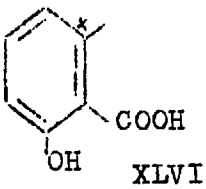
Acetyl-CoA is supposed to be the initial unit or the "starter" in the formation of the β-polyketone chain. The carboxylation of acetyl-CoA to malonyl-CoA has been shown[41,37] to be catalysed by a biotin containing enzyme and, further, it has been postulated[41,37] that biotin intervenes in the process activating the carbon dioxide as shown (scheme IV). The condensation of acetyl-CoA with malonyl-CoA is facilitated by the activating effect of the carboxylic groups on the methylene of malonic acid.[41] The formation of the β-polyketone chain could proceed through condensation of the acetyl-CoA with n-molecules of malonyl-CoA with simultaneous decarboxylation as shown in the scheme (V).

The incorporation of propionic acid into mould metabolites could proceed also through carboxylation of propionyl-CoA to form methylmalonyl-CoA.[41,58]

Scheme III

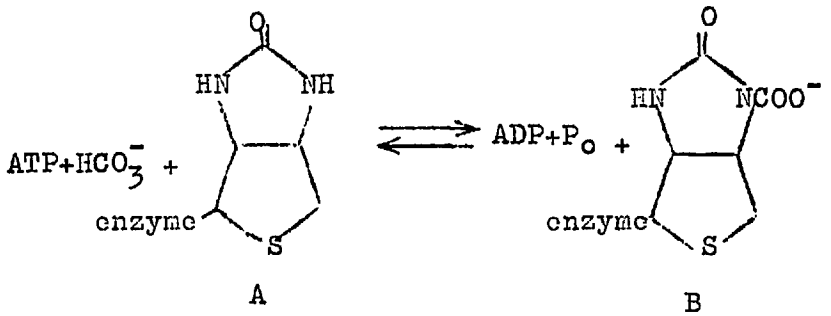


XLV

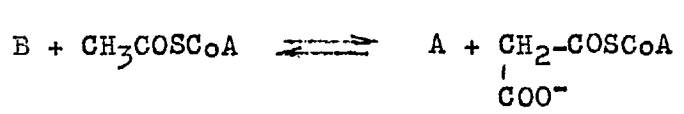


XLVI

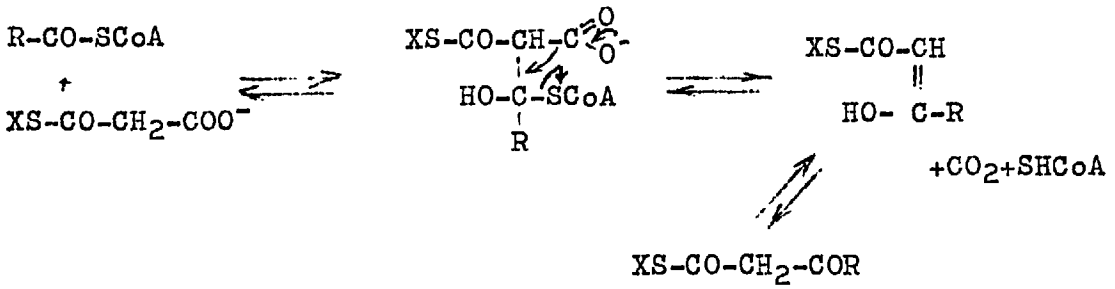
Scheme IV



A B



Scheme V



## THE ISOPRENE RULE.

The study of the structure of terpenes induced Ruzicka[1] to formulate the, now well known, "isoprene rule" which stated that "the carbon skeleton of the terpenes is composed of isoprene units linked in a regular (head-to-tail) or irregular arrangement". The study on the biogenesis of steroids and terpenoids suggested a revised version,[42] called "biogenetic isoprene rule" which stated that "terpenes are compounds formed by combination of isoprene units or can be derived from so formed aliphatic precursors such as geraniol, farnesol, squalene by accepted reaction mechanisms". Both rules have proved to be useful working hypotheses in the elucidation of the structure and biogenesis of terpenoid compounds.

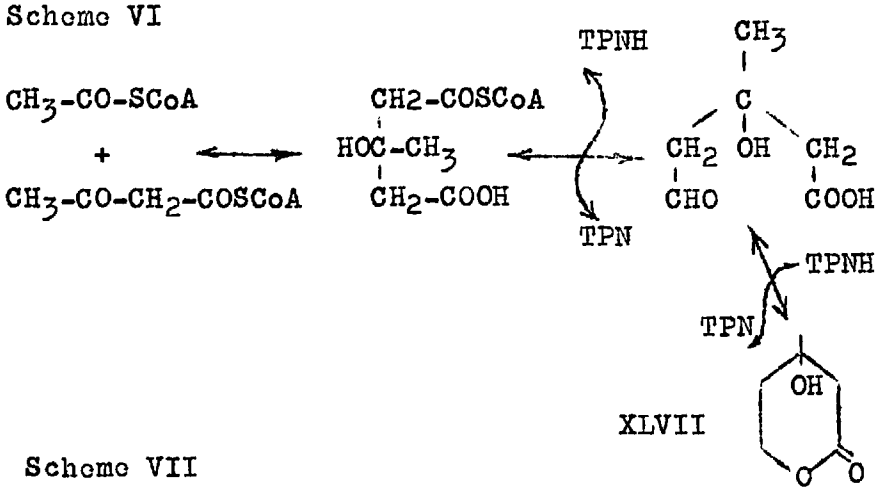
When Bloch (cited by Ruzicka[1]) found that acetic acid was incorporated into cholesterol the study of the intermediates in the biogenesis of terpenoids and steroids received new impetus. The discovery of mevalonic acid lactone[43](XLVII) as a precursor in the biogenesis of cholesterol suggested it as the precursor of the isoprenoid unit in nature. The intermediate stages in the biosynthesis of the mevalonic acid lactone from acetic acid have been established as shown (scheme VI)[51].

The problems involved in the transformation of mevalonic acid lactone to cholesterol have been very

intensively studied in recent years. It was a well known fact that ATP (adenosinetriphosphate) was necessary in the early stages of this transformation, and Bloch[52] found that the mevalonic acid lactone was phosphorylated, first to a monophosphate and then to a diphosphate. He postulated that the mevalonic acid diphosphate would undergo a concerted decarboxylation dephosphorylation to give the monophosphate ester of an isopentane derivative. Lynen et al.[53], on incubation of (2-$^{14}$C) mevalonic acid phosphate with $Mg^{+2}$, ATP and purified yeast enzyme, were able to isolate isopentenyl pyrophosphate and farnesyl pyrophosphate. The same authors[55] isolated an enzymatic system which catalysed the isomerization of the double bond of the isopentenyl pyrophosphate to yield γ,γ-dimethylallyl-pyrophosphate. The enzymatic system was shown to be labile to iodoacetamide suggesting the participation of free thiol groups in the process. They also isolated[54] an enzymatic system which catalysed the condensation of the two isopentenyl pyrophosphates described and from which they were able to isolate geranyl and farnesyl pyrophosphates. Lynen et al[54] postulated that this series of transformations take place as shown in scheme (VII).

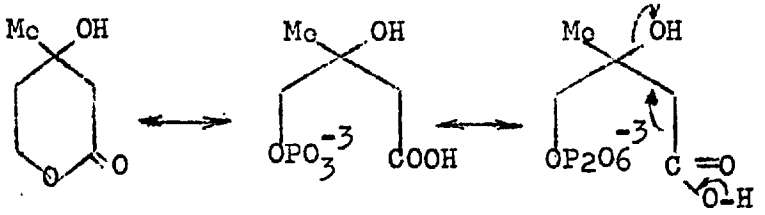
Squalene is a symmetrical molecule and it appears to be formed by a tail-to-tail condensation of two $C_{15}$ units, such as farnesol, or its equivalent. That the

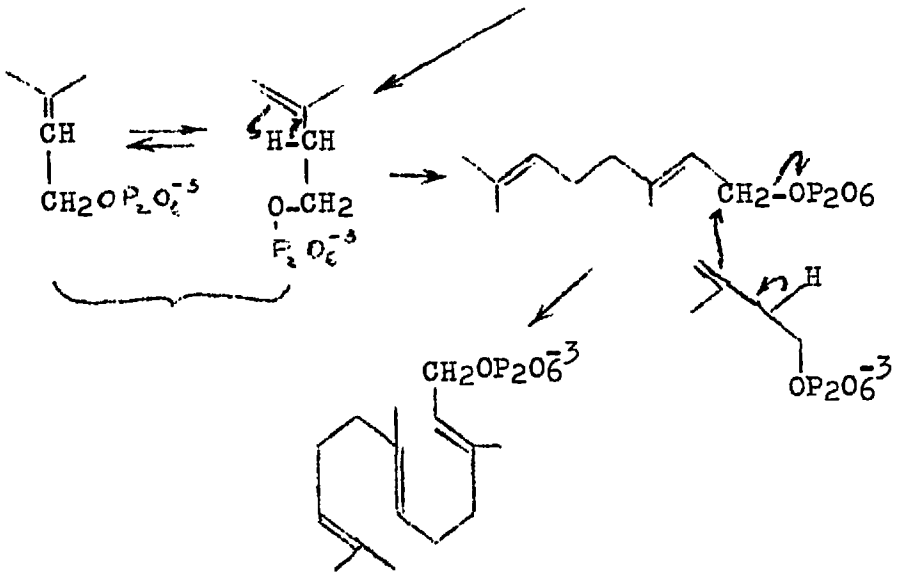
Scheme VI




XLVII

Scheme VII

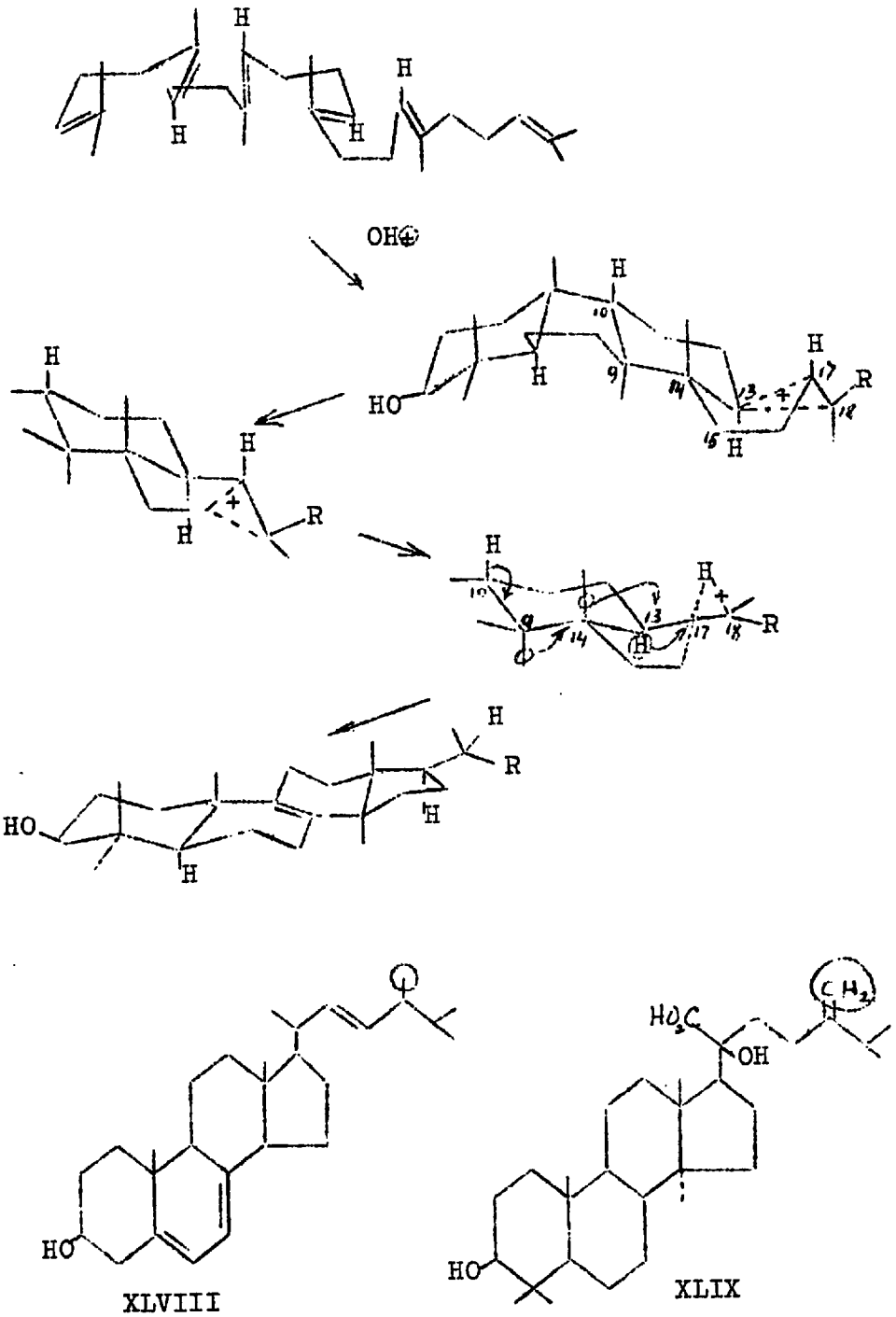


formation of squalene from farnesyl pyrophosphate is a reductive process has been shown by Popjak et al.[56] When (1-$T_2$,2-$^{14}C$) farnesyl pyrophosphate was incubated with a "microsomal squalene synthetase" system and reduced triphosphopyridine nucleotide, the $T/^{14}C$ proportion found in the isolated squalene (0.77) suggested the loss of one out of four tritium atoms. The exchanged hydrogen was found to be supplied by TPNH[3].

The cyclisation of all-trans-squalene in a chair-boat-chair-boat conformational arrangement to lanosterol has been suggested[1] to proceed as shown in scheme (VIII). Tchen and Bloch (cited by Ruzicka[1]) found that the cyclisation of squalene to lanosterol requires aerobic conditions; the fact that no deuterium was found in the molecule when the reaction was carried out in $D_2O$ showed that it goes through a concerted mechanism as was suggested by Ruzicka. Cornforth et al.[59], as well as Maudgal, Tchen and Bloch (cited by Ruzicka[1]) showed that the displacement of the two central methyl groups takes place by two single 1,2-shifts and not by one 1,3-shift. The intermediates in the transformation of lanosterol to cholesterol have been identified by Bloch and col.[52], as 14-norlanosterol, $\Delta^{8,24}$-4,4-dimethylcholestadiene-3-one and $\Delta^{8}$-4α-methylcholestadiene-3-one. The tetra- and penta-cyclic triterpenes as well as the steroids found in nature can all be derived theoretically[1] by cyclisation

Scheme VIII




XLVIII

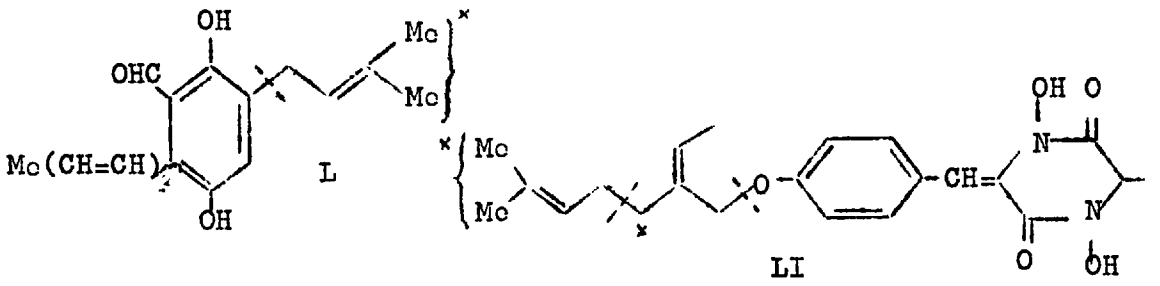
XLIX

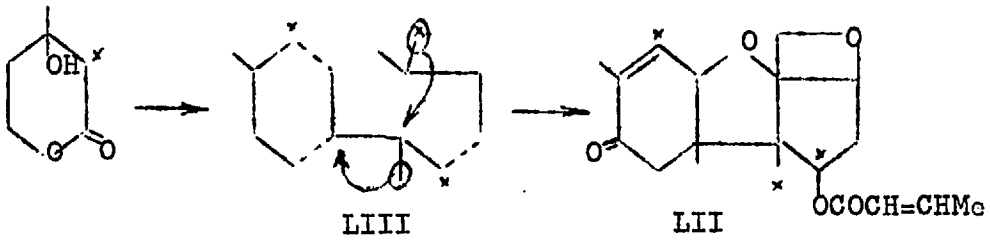
of all-*trans*-squalene in well-defined conformational arrangement, and subsequent rearrangements and degradation processes in accordance with accepted reaction mechanisms.

The "extra" methyl groups found in some steroid mould metabolites such as ergosterol (XLVIII) and eburicoic acid (XLIX) have been found[57,60] by tracer experiments, to be provided by methionine.

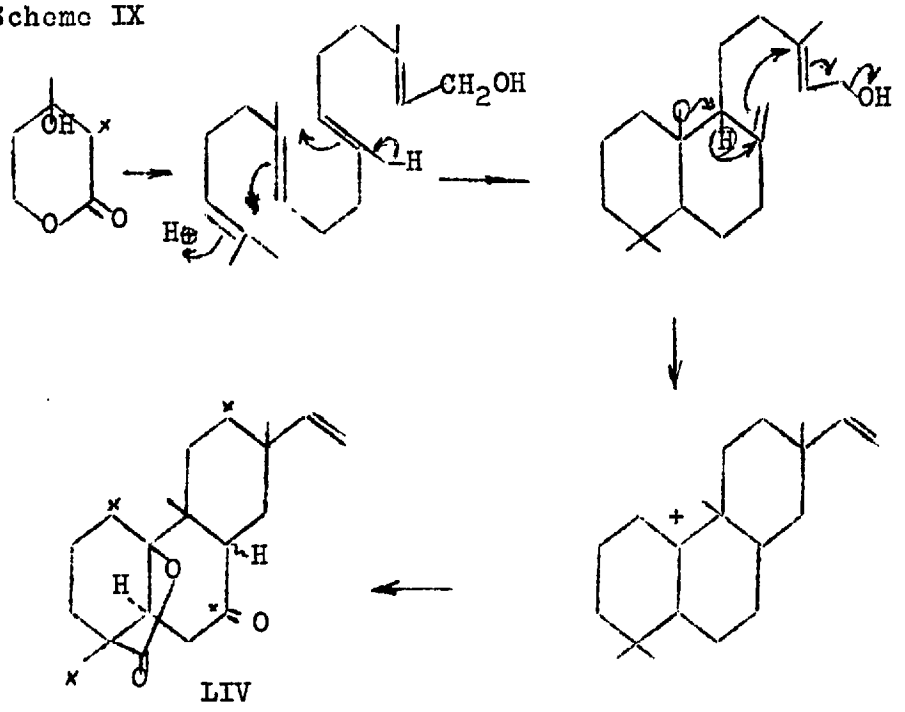
The isopentyl and isoprenoid groups found in some mould metabolites have been shown to derive from mevalonic acid lactone using (2-$^{14}$C) isotopically labelled precursor, as in auroglaucin (L)[44] and mycelianamide (LI).[45] In the last case the incorporation was found exclusively on the side chain and not in the nucleus which suggests mevalonic acid lactone as a specific intermediate in the terpene biosynthesis.

The formation of several mould metabolite terpenes from mevalonic acid lactone has been demonstrated. Trichothecin (LII) produced by *Tricothecium roseum* L. was found to derive from mevalonic acid lactone by Jones *et al.*[49]; its formation involves a double 1,2-methyl migration in an hypothetical sesquiterpenoid precursor (LIII). No incorporation was found in the isocrotonic acid when isotopically labelled mevalonic acid lactone was used; however it was found to derive from acetic acid units. Britt and Arigoni[46] and Birch *et al.*[47,48] found that (2-$^{14}$C) mevalonic acid lactone was incorporated


OH
OHC
Me
Me
Me(CH=CH)
OH
L
Me
Me
O
CH=
OH O
N
N
O
OH
LI


OH
O
O
LIII
O
O
O
OCOCH=CHMe
LII

Scheme IX

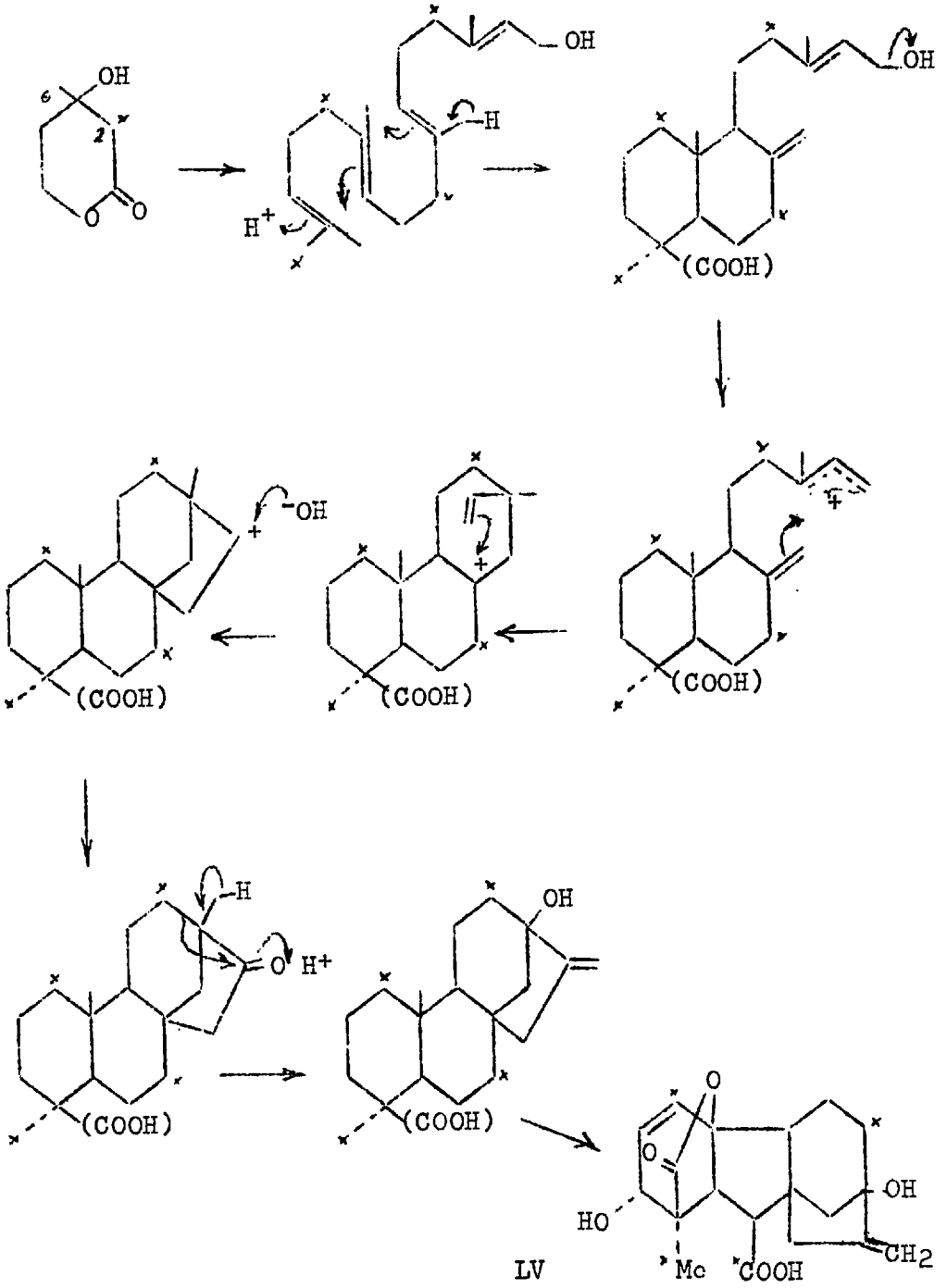

OH
O
O
CH2OH
H
H⊕
OH
H
+
H
H
O
O
O
LIV

into rosenonolactone (LIV) also isolated from T. roseum. Its formation also involves a 1,2-methyl shift as shown in the diagram (scheme IX). Only the methyl group and not the carbonyl attached to $C_4$, was found to be derived from $C_2$ of mevalonic acid lactone. The biogenetic route proposed for its formation is shown in scheme (IX).

The biogenesis of gibberellic acid (LV) from mevalonic acid lactone has been studied by Birch and col.[48,50] As for rosenonolactone (LIV), they proposed a diterpene precursor which would undergo several rearrangements as shown in scheme (X). The bridge ring system could be formed by the mechanism proposed by Wenkert (cited by Birch et al.[48]) as shown in scheme (X). They found that the methyl group is derived specifically from $C_2$ of the mevalonic acid lactone and the carboxyl group from the $C_9$ of the hypothetical diterpenoid precursor.

The fact that the methyl groups in rosenonolactone and gibberellic acid derive specifically from the $C_2$ of mevalonic acid lactone shows that the assymetric character of the $C_2$ and $C_6$ of the lactone persists in the precursor of the terpene chain.[46,48]

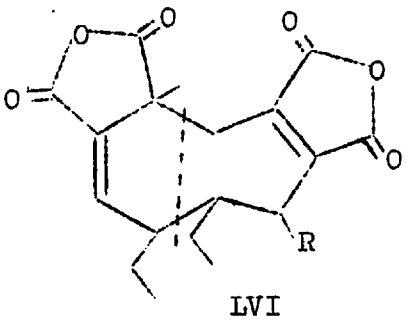
Scheme X


OH
6
2
O
O
H+
H
OH
(COOH)
OH
+
(COOH)
+
(COOH)
+
(COOH)
H
O
H+
(COOH)
OH
(COOH)
O
O
HO
OH
Me
COOH
CH2

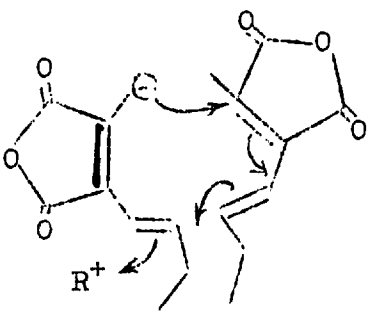
LV

Biogenesis of Glauconic, Glaucanic and Byssochlamic acids. The symmetrical structure of glauconic (LVI, R=OH) and glaucanic (LVI, R=H) acids (discussion in the next section) both produced by Penicillium glaucum or P. purpurogenum, suggested[62] a possible biogenetic route for its formation. The condensation of two units of an hypothetical precursor (LVII) through a concerted reaction would give rise to glauconic and glaucanic acids as shown. If $OH^+$ (or equivalent) plays a role in the formation of glauconic acid, anaerobic conditions should increase the yield of formation of glaucanic acid which is usually isolated in much poorer yield than glauconic acid. Byssochlamic acid (LIX),[62] isolated from Byssochlamis fulva cultures, could be formed by condensation of one molecule of the hypothetical precursor (LVII) and an isomer (LVIII) as shown.

The formation of the precursor (LVII) might involve condensation of oxaloacetic acid with butyryl-malonyl-CoA as shown (scheme XI). Several mould metabolites have been isolated in which a condensation of an acid from the Krebs cycle and a β-polyketone chain or fatty acid, can be envisaged. Agaricic acid (LX) (Polyporus officinalis), caperatic acid (LXI (Parmelia caperata), and minioleuteric acid (LXII) (Penicillium minioluteum), are some examples.[60]

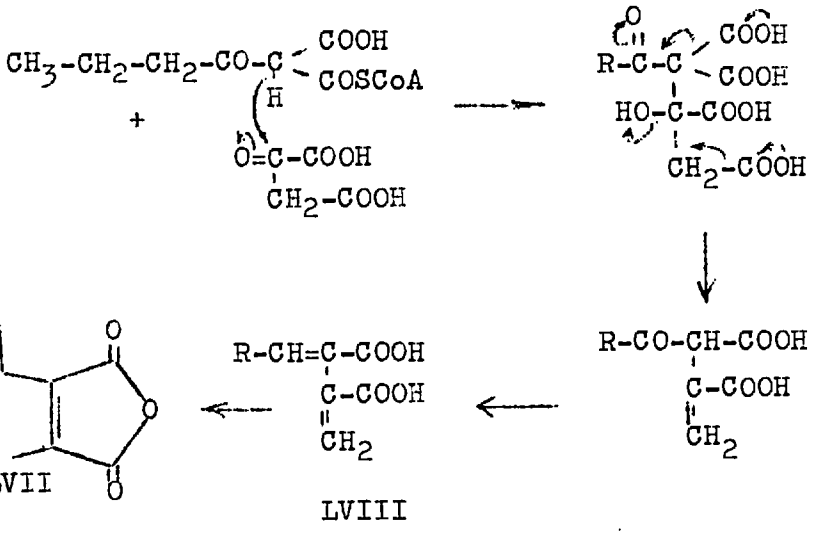


LVI



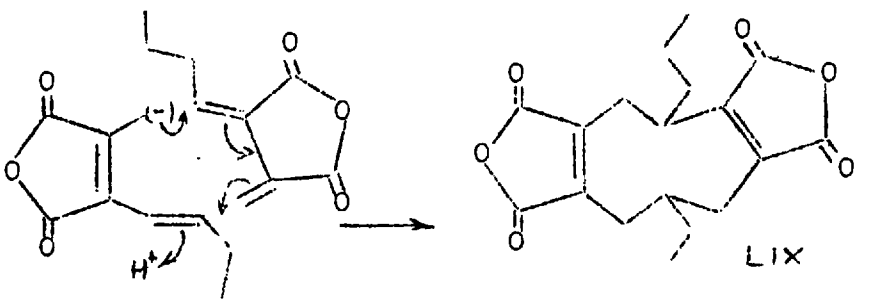
($R^+$=$OH^+$ or $H^+$)

Scheme XI



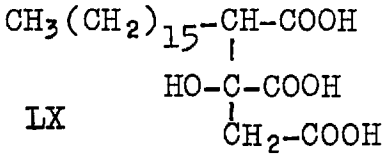
LVII

LVIII

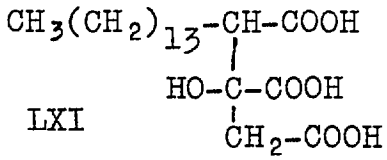


LIX

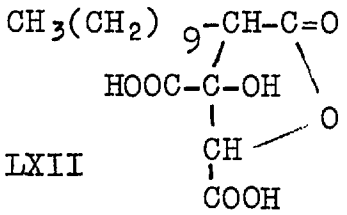
Lubing and Reio (cited by Ehrensvärd)[61] have determined the distribution pattern in carlosic acid (LXIII) and carolic acid (LXIV), isolated from Penicillium carlesii cultures incubated in media containing glucose and carboxyl-labelled acetate. The results obtained gave a strong indication that only part (B) is formed from three acetate units, while (A) is probably derived from another source related to the carbohydrate metabolism. An analysis of the portion (A) in carlosic and carolic acids suggest oxaloacetic or pyruvic acids respectively, as the possible condensing precursors.



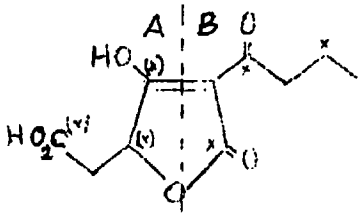
LX



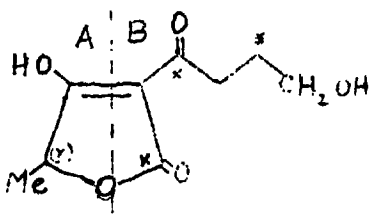
LXI



LXII



LXIII



LXIV

## THE CHEMISTRY OF GLAUCONIC ACID

Glauconic acid ($C_{18}H_{20}O_7$; m.p. 202°), together with glaucanic acid ($C_{18}H_{20}O_6$; m.p. 186°), was first isolated in 1931 by N. Wijkman[63] from Penicillium glaucum, reclassified as Penicillium purpurogenum[100].

The chemistry of glauconic and glaucanic acids was studied by Wijkman, Sutter, Kraft and their collaborators[63-68], who showed that glauconic acid has an active hydrogen atom (Zerwitinoff test), identified[63] as an acylable hydroxylic group; the acetyl, benzoyl, and nitrate derivatives were prepared by Wijkman. The active hydrogen is absent in glaucanic acid (negative Zerwitinoff test).[65] On zinc dust-acetic acid reduction, glauconic acid gave a dihydro derivative ($C_{18}H_{22}O_7$; m.p. 235°) which did not contain a free alcoholic hydroxyl group and formed monomethyl[66] and trimethyl[68] esters. The reduction of a disubstituted maleic anhydride group was assumed[65] to have taken place with subsequent lactonization leaving a free carboxyl group, as shown in the partial structures (a) to (b). On zinc dust-acetic acid reduction of glaucanic acid three products were isolated.[65] The main product of the reaction was a diacid ($C_{18}H_{24}O_7$; m.p. 209°) which gave a dimethyl ester and titrated as tetracarboxylic acid. The authors[65] suggested that the reduction of a disubstituted maleic anhydride had taken

place as shown in the partial structures (c) to (d). A neutral product ($C_{18}H_{22}O_6$; m.p. 192°) and a second acid ($C_{20}H_{24}O_7$ or $C_{20}H_{26}O_7$) were also isolated from this reaction.

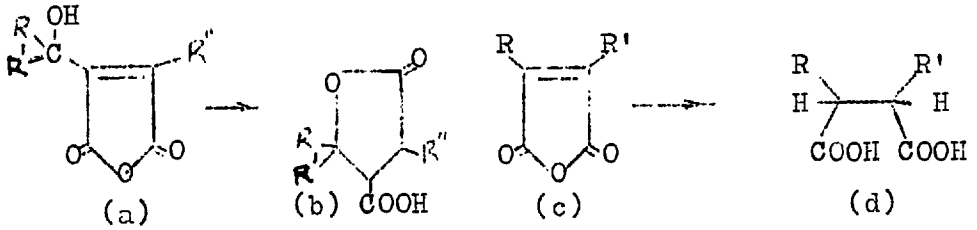
On pyrolysis (230-250°) glauconic acid breaks into two fragments, $C_7H_{12}O$ and $C_{11}H_8O_6$. Most of the work of the German group[63-68] was directed to the elucidation of the structures of these products.

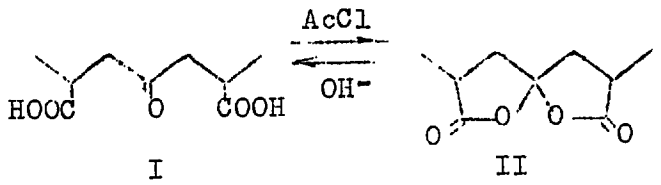
The $C_7H_{12}O$ compound (b.p. 42°/11 mm.) was identified as diethylacrolein by degradation and synthesis in the following fashion. On ozonolysis and catalytic hydrogenation of the ozonide,[64] the dinitrophenyl-hydrazones of propionaldehyde and ethyl glyoxal were obtained and identified by mixed melting point with authentic samples. On catalytic reduction over palladised charcoal[64] it gave a saturated aldehyde which was oxidised to 2-ethylpentanoic acid, identified by mixed melting point of its amide with an authentic sample.

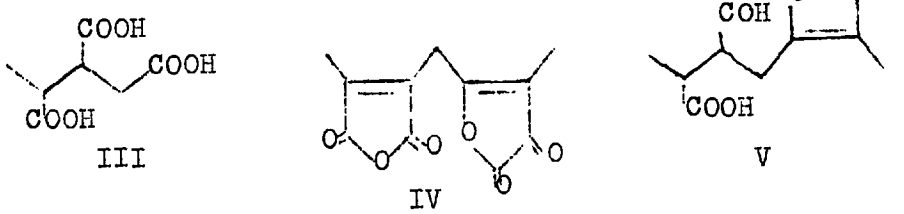
To prove the position of the double bond 2,3-diethylacrylic acid was synthesised.[65] The mixed melting point of its amide with that of the acid obtained by oxidation of the aldehyde with silver oxide showed no depression. The ultraviolet absorption of its dinitrophenyl hydrazone (m.p. 173°; $\lambda_{max}^{CHCl_3}$ 380 mμ ($\varepsilon$ 31500)) supports the conjugated character of the aldehyde.

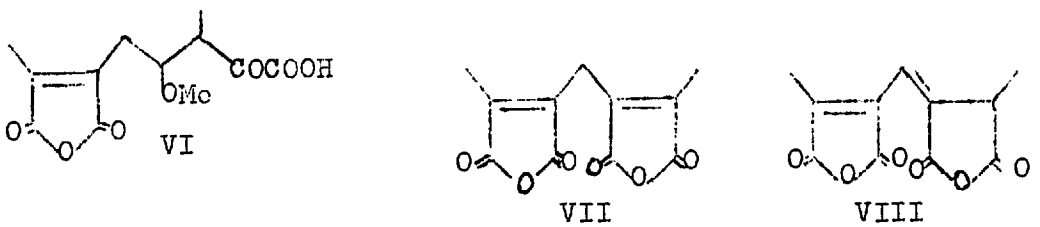
Glauconin, $C_{11}H_8O_6$ (m.p. 171°) is optically inactive,[66] contains no active hydrogen (Zerewitinoff) and retains the tetra acid character of glauconic acid (titration in warm solution).[63] Its dipotassium salt gave a dimethyl ester, $C_{13}H_{14}O_7$, m.p. 77°, on treatment with dimethyl sulphate.[67] It gives no colour with tetranitromethane and does not react with bromine in acetic acid or with perbenzoic acid.[67] On ozonolysis and catalytic hydrogenation of the ozonide[66] it gave more than one mole of pyruvic acid, identified by mixed melting point of its dinitrophenylhydrazone with an authentic sample. Oxalic, formic and oxaloacetic acids were also obtained in small amounts. The last could be the source of some of the pyruvic acid found, as was shown by treatment of oxaloacetic acid with dinitrophenylhydrazine reagent.[66] An unidentified osazone was also obtained in these experiments. Pyruvic acid was also obtained on ozonolysis of glauconin dimethyl ester.[67] All the data indicate the presence of at least one $CH_3-C=$ / $-C=O$ group. Kuhn chromic acid oxidation of glauconin[67] gave 1.5 moles of acetic acid, indicating the presence of two C-Me groups.

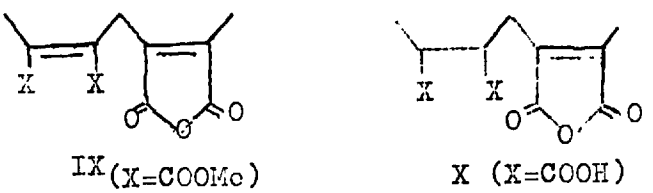
On reaction with 8N hydrochloric acid at 200°, glauconin[66] lost 2 moles of carbon dioxide and gave a mixture of two acids, $C_9H_{14}O_5$, and a neutral fraction, $C_9H_{12}O_4$. The relationship between them was found to


OH
R
R'
R''
(a)
(b)
COOH
(c)
(d)
H
COOH


AcCl
OH⁻
HOOC
COOH
I
II


COOH
III
IV
COH
V


COCOOH
OMe
VI
VII
VIII


X
IX (X=COOMe)
X (X=COOH)

be $C_9H_{14}O_4 \underset{AcCl}{\overset{OH^-}{\rightleftharpoons}} C_7H_{12}O(COOH)_2$. On treatment with nitric acid the acids gave pyrotartaric acid. A study of the ultraviolet spectrum together with the data described, suggested the possible structures I and II for the acids and the neutral fraction respectively. This was proved by synthesis[66] of the meso and racemic forms of the diacid ketone and of the ketodilactones derived from them (3 forms).

Glauconin is partially reduced on heating with hydriodic acid and red phosphorus.[68] The product obtained, dihydroglauconinic acid ($C_{11}H_{12}O_7$, m.p. 200°) showed the addition of 1 mol of hydrogen and 1 mol of water. It had a higher titre than glauconin in the cold and titrated as a tetrabasic acid in hot solution. Reduction and opening of a disubstituted maleic anhydride ring was assumed to have taken place[68] by analogy with known examples.[69]

On ozonolysis of the dimethyl ester of dihydroglauconinic acid followed by catalytic reduction of the ozonide and esterification of the product an ester was obtained, $C_{10}H_{16}O_6$, which on hydrolysis gave an acid, $C_7H_{10}O_6$, m.p. 123°. The latter titrated as a tribasic acid and was identified as methyl carballylic acid (III) by synthesis and mixed melting point.[68]

Glauconin could not be reduced catalytically under normal conditions but at 100° and 150 atm. over platinum oxide, an acid was obtained whose methyl ester analysed correctly for 2OMe groups.

On catalytic reduction of glauconin dimethyl ester over platinum oxide in anhydrous methanol under normal conditions, 1 mol of hydrogen was consumed[68] and on hydrolysis of the product some glauconin was recovered unchanged (mixed melting point). An acid was also obtained,[68] m.p. 195°, which showed a clear depression on mixed melting point (m.p. 187°) with dihydroglauconinic acid (m.p. 200°) obtained by hydriodic acid treatment of glauconin. The experimental data of this section are confused and the analytical results ambiguous. On the basis of these data the authors[68] concluded that glauconin has structure (IV). On treatment with hydriodic acid-red phosphorous the disubstituted maleic anhydride group would be reduced and remain open in the diacid form. This treatment would not affect the enol lactone group. K. Kraft[68] proposed for this compound (m.p. 200°) the structure (V). Catalytic hydrogenation, on the other hand, would not affect the disubstituted maleic anhydride group but would reduce the enol lactone group. On this basis the authors[68] proposed the structure (VI) for the product (m.p. 195°) obtained on catalytical reduction of glauconin dimethyl ester. (The analytical data do not

agree very well with this formulation).

In support of the presence of a keto group in the enol lactone ring the authors[67] studied the behaviour of glauconin and glauconin dimethyl ester towards titration with hydroxylamine and compared them with that of compounds of known structures. A lactone (valerolactone) and an enol lactone (coumarin) did not consume any hydroxylamine; an anhydride (phthalic anhydride) consumed 1 mol of hydroxylamine; glauconin consumed 2 mols and glauconin dimethyl ester 1 mol. This result was taken as evidence[67] of the presence of a keto group in glauconin and glauconin dimethyl ester, but it could also arise from two and one anhydride groups respectively.

K. Kraft[68] claims that the ultraviolet absorption of glauconin does not agree with the symmetrical structure VII proposed by H. Sutter.

In the present work glauconin was obtained as described by the German authors (op.cit.). The infra-red absorption in chloroform showed the presence of unsaturated five membered ring anhydride ($\nu_{max}^{CHCl_3}$ 1773, 1825 cm.$^{-1}$) and a conjugated double band ($\nu_{max}^{CHCl_3}$ 1675 cm.$^{-1}$).

The ultraviolet spectrum of glauconin was determined in various solvents and its behaviour studied. The results are shown in the table:

| Solvent | $\lambda_{max}$ fresh solution | $\lambda_{max}$ after 24 hrs. at room temperature. |
|---|---|---|
| | $\varepsilon$ | $\varepsilon$ |
| Ethanol | 250 (8100) | end absorption. |
| Dioxane | 255 (10900) | 250 mμ (ε 12400) |
| Cyclohexane | 250 (10200) | 250 mμ (lower ε due to insolubility). |
| Trifluoro acetic acid | 255 (10500) | end absorption after 4 hrs. |

As is shown, the stability of the chromophore depends on the prototropic character of the solvent and the disappearance of the maxima at 250 mμ could be explained by opening of the anhydride ring, in which case the absorption would be that of a disubstituted maleic type acid. The maximum absorption for this type of compound has been shown[89] to lie between 210 and 225 mμ.

The ultraviolet absorption of several model compounds was determined in ethanol. The results are shown in the table:

| | $\lambda_{max}^{EtOH}$ $\varepsilon$ |
|---|---|
| Maleic anhydride | 206 mμ (6900) |
| Itaconic anhydride | 215 mμ (6100) |
| Citraconic anhydride | 208 mμ (9750), inflexion at 230 mμ (3900) |
| Dimethyl maleic anhydride | 208 mμ (7500), $\lambda_{max}$ 248 mμ (4800) |
| Glauconin | 208 mμ (14900), $\lambda_{max}$ 250 mμ (7450) |

It clearly shows that alkyl substitution of the maleic anhydride produces a gradual emergency of a maximum near 250 mμ. The ultraviolet absorption of glauconin could be due to the presence of two disubstituted maleic anhydride groups. The intensities at 208 mμ and 250 mμ are almost double the value of dimethyl maleic anhydride. The fact that they are not exactly double and that there is found some variation in the intensities from one determination to another could be due to some interaction between the two chromophores.

The N.M.R. spectrum of glauconin confirms the symmetrical structure (VII) proposed by H. Sutter. When dioxane was used as solvent a singlet was found at $\tau$ 7.91 p.p.m. which was attributed to the methyl protons. The rest of the spectrum could not be studied due to interference by the protons of the solvent. The determination in trifluoroacetic acid showed a singlet at $\tau$ 7.7 p.p.m. attributed to $CH_3-\overset{1}{C}=$ protons and a singlet at 6.26 p.p.m. due to $CH_2$ protons. The relative intensities were found to be 3:1. These results rule out the possibility of a conjugated distribution of the double bonds as shown in (VIII).

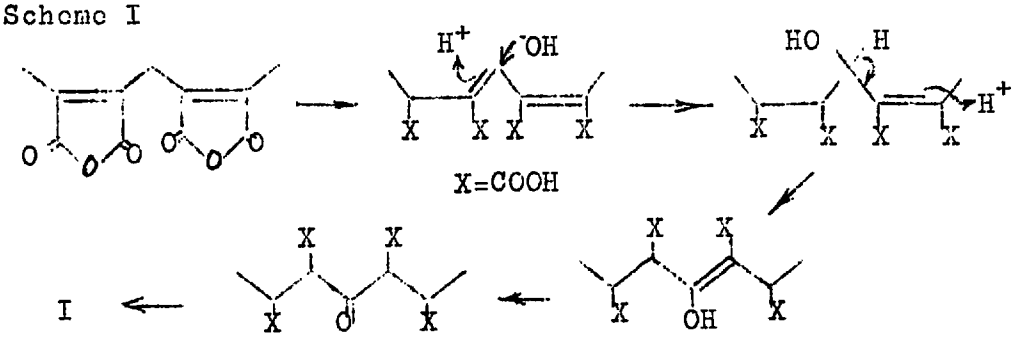
Glauconin dimethyl ester was prepared as described by K. Kraft and Porsch,[57] (m.p. 74-77°). Its infrared absorption ($\nu_{max}^{CCl_4}$ 1645, 1730, 1773, 1825 cm.$^{-1}$) and ultraviolet absorption ($\lambda_{max}^{CHCl_3}$ 248 mμ (ε 6400); $\lambda_{max}$ 208 mμ (ε 15400), inflexion at 240 mμ (ε 7400))

suggest the opening of one disubstituted maleic anhydride and retention of the other one as in glauconin, i.e. an unsymmetrical structure as shown in IX. The N.M.R. spectrum confirmed this postulate. It showed (in carbon tetrachloride) two peaks at $\tau$ 8.051 and 8.016 p.p.m. attributed to two $CH_3$-C= in different environment, a singlet at $\tau$ 6.563 p.p.m. due to the $CH_2$ protons and two peaks $\tau$ 6.368, 6.279 p.p.m. attributed to the two methyls of the methoxyl groups.

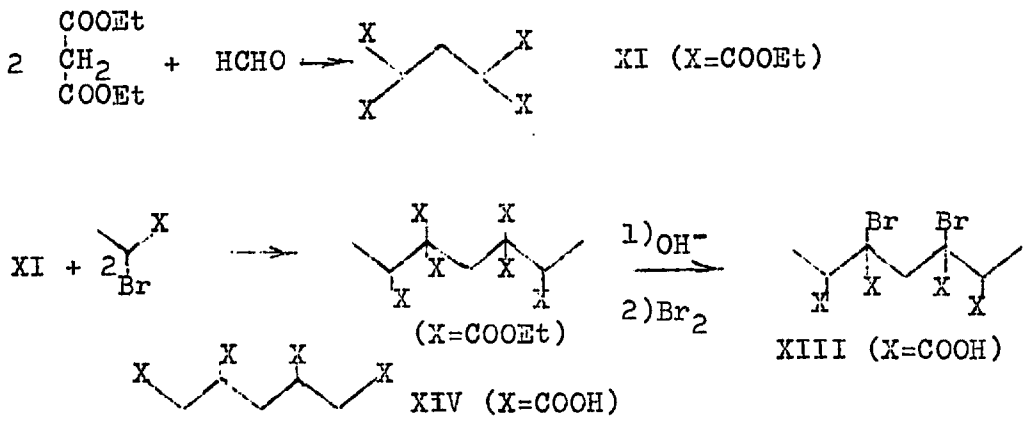
The structures (VII) and IX) proposed for glauconin and glauconin dimethyl ester, explain most of the results outlined at the beginning of this section.[63-68] Scheme I shows the possible mechanism of the formation of the ketodiacid I on treatment of glauconin with hydrochloric acid.

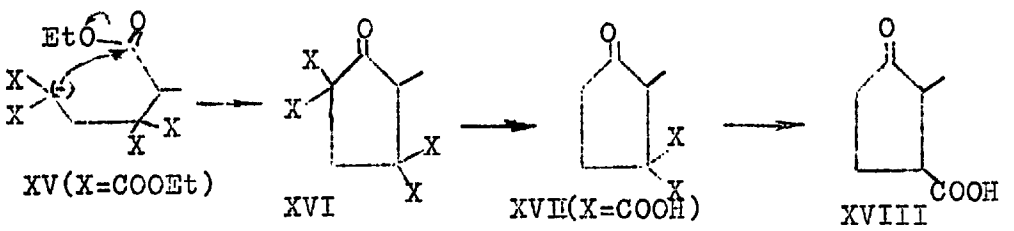
Catalytic reduction of glauconin dimethyl ester gave, in very poor yield, an acid, m.p. 155-70° ([68]m.p. 195°). The relatively easier catalytic hydrogenation of dimethyl glauconin with respect to glauconin could be explained by the opening of one of the disubstituted maleic anhydrides to the unsaturated diester thus facilitating the attack. It is known[69] that the catalytic reduction of a maleic anhydride group is a very slow process.

Scheme I

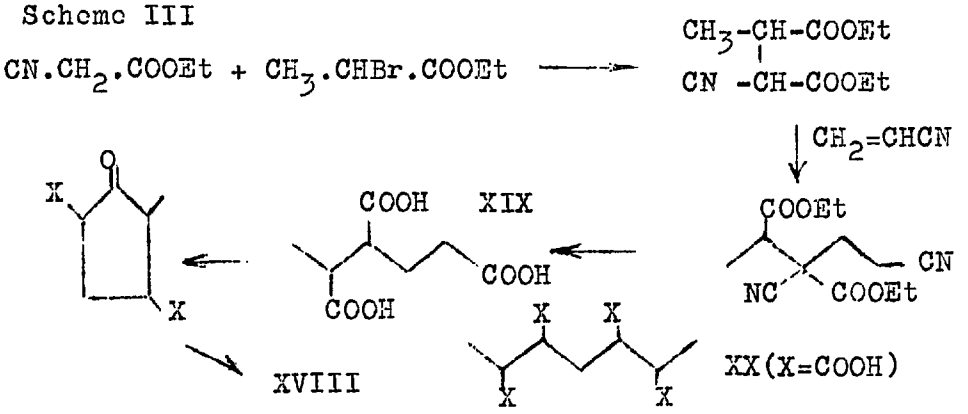



Scheme II




Scheme III



On the treatment with hydriodic acid and red phosphorus,[68] glauconin gave an acid which on repeated recrystallisation from water showed m.p. 160-76° ([68]m.p. 200°) (mixed m.p. with product m.p. 155-70°, 155-72°). The product was subjected to different treatments but on no occasion did the melting point rise to 200°. Its infrared spectrum, in nujol, showed the characteristic absorption of a maleic type anhydride ($\nu_{max}$ 1773, 1838 cm.$^{-1}$) conjugated double band at 1634 cm.$^{-1}$ and the typical carboxyl absorption in the 3000-2500 cm.$^{-1}$ region. These data, together with its ultraviolet absorption ($\lambda_{max}$ 208 mμ (ε 8800), 250 mμ (ε 3650) suggest the presence of a disubstituted maleic anhydride group, and, as Kraft concluded,[68] only one maleic anhydride group is reduced and opened. The wide range in the melting point of both products could be due to a mixture of isomers. Dihydroglauconinic acid can be represented by (X), which explains the production of methyl carballylic acid (III) on ozonolysis of its dimethyl ester.

In order to confirm the structures proposed for glauconin and dihydroglauconinic acid their syntheses were attempted through several routes which will be described in chronological order.

Alkylation of 2,4-dicarbethoxyglutarate with ethyl bromoacetate, using sodium ethoxide as condensing agent, has been described by H. Guthzeit and Engelman.[70] On acid hydrolysis of the product they obtained methylendisuccinic acid (XIV). Having this in mind we prepared ethyl 2,4-dicarbethoxyglutarate (XI) by condensation of diethyl malonate with formalin in presence of diethyl amine.[71] Sodium hydride has been used for the type of alkylation we intended, and we chose it as condensation reagent in our work. To avoid the possible Dieckmann condensation taking place an excess of bromoester was used. The ethyl 2,4-dicarbethoxyglutarate was condensed with ethyl α-bromopropionate in presence of sodium hydride. The product obtained on removal of the unchanged starting material and saponification gave on chromatography two main products, acids A and B, and other minor products which were not identified.

The infrared spectrum of acid A showed $\nu_{max}$ 1742 $cm.^{-1}$ which suggests the presence of a cyclopentanone group, it also showed $\nu_{max}$ 1704 $cm.^{-1}$ and the typical carboxyl absorption in the 3000-2500 $cm.^{-1}$ region. Such a product could arise through a Dieckmann condensation of the monoalkylated 2,4-dicarbethoxyglutarate to give 2-methylcyclopentanone-3,3,5,5-tetracarboxylic ester as shown in the sequence (XV-XVI), which on alkaline hydrolysis would decarboxylate to yield

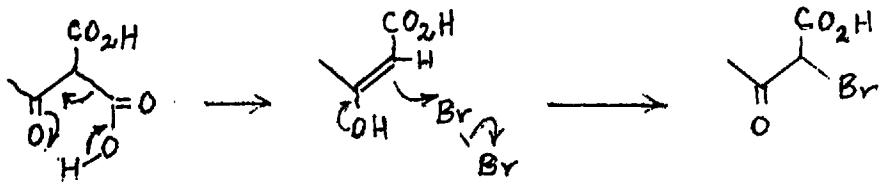
2-methylcyclopentanone-3,3-carboxylic acid (XVII). The analytical data (experimental section) support this idea.

Acid A, loses very easily carbon dioxide when it is heated above its melting point, yielding an acid C, m.p. 92-4°, whose infrared spectrum showed the presence of the cyclopentanone group ($\nu_{max}$ 1742 cm.$^{-1}$) and carboxylic group, ($\nu_{max}$ 1710 cm.$^{-1}$ and carboxyl absorption at 3000-2500 cm.$^{-1}$). Acid C was identified as 2-methylcyclopentanone-3-carboxylic acid (XVIII) by synthesis as described by Haworth and Perkin, and Newman and McPherson[72] through the sequence of reactions shown in scheme (III).

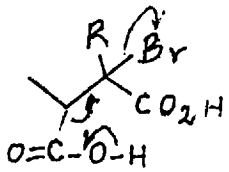
The mixed melting point of the acids and the semicarbazones of the methyl esters of the synthetic and the pyrolysis product showed no depression. The infrared spectra of the methyl esters were identical. The isolation of the cyclopentanone dicarboxylic acid as the main product of the reaction suggests that, under the conditions of reaction, the Dieckmann cyclisation is preferred to the introduction of the second alkyl group.

The second major product obtained, acid B, showed $\nu_{max}$ 1700 cm.$^{-1}$. The analytical data suggested either structure (XIX) or (XX) for it. The mixed melting point of acid B with (XIX) showed no depression but the infrared spectra (in nujol) showed some differences in the finger print region. It was not studied further.

The simultaneous bromination and decarboxylation depicted in scheme II was conceived by analogy with the behaviour of β-ketoacids which decarboxylate to give the enol form which reacts instantaneously with the bromine present resulting in overall bromination and decarboxylation as shown.

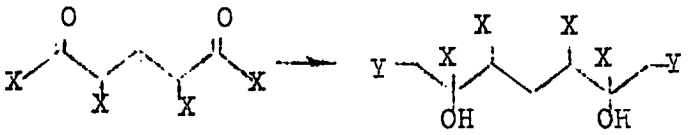


It was hoped that on treatment of the dibromide-tetracarboxylic acid (XIII) with base, the elimination of hydrobromic acid would be favoured and not the debromination with simultaneous decarboxylation which is also possible.



In view of the failure to obtain the hexacarboxylic acid and the difficulties which the following steps involved we turn our attention to another possible route.

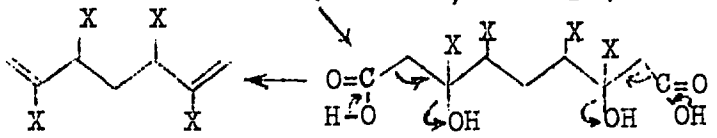
The alkylation of the diketo ester (XXI) with benzyl bromoacetic ester should give, under Reformatsky reaction conditions, the dibenzyl ethyl ester (XXII) which on hydrogenolysis followed by thermal decomposition would furnish the dimethylene tetraester (XXIV). This product would yield glauconin on hydrolysis.



XXI (X=COOEt)

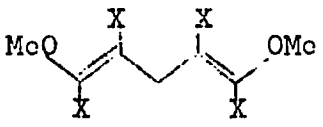


XXII (X=COOEt; Y=COOBz)

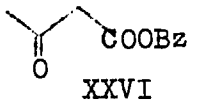


XXIV

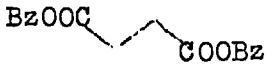
XXIII(X=COOEt)



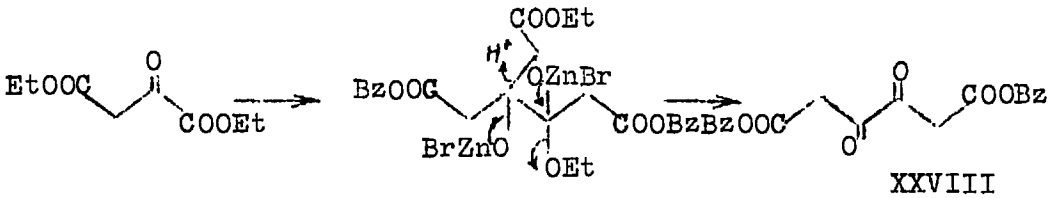
XXV (X=COOEt)



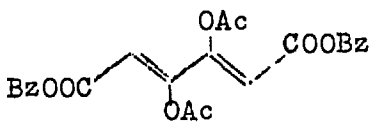
XXVI



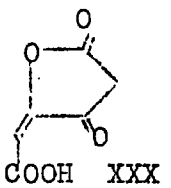
XXVII



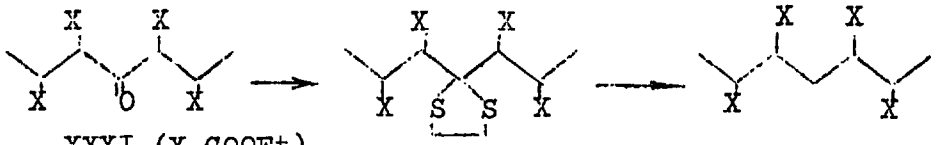
XXVIII



XXIX



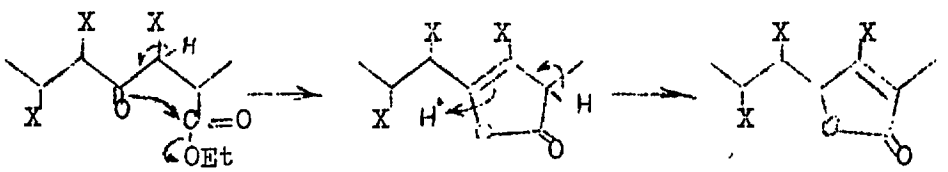
XXX



XXXI (X=COOEt)

XXXII

XXXIII



XXXIV (X=COOEt)

XXXV (X=COOEt)

XXXVI

The diketo ester XXI was prepared by Dr. Sutherland as described by Blaise and Gault.[74] When it was treated with methyl iodide it gave the dienol ether (XXV). Active hydrogen determination (Zerewitinoff) showed that the compound existed mainly in the enol form. This observation excluded the possibility of a Grignard reaction with methyl magnesium iodide. The Reformatsky reaction could present the same difficulties. In a preliminary study the reaction was tried on oxaloacetic acid as a model compound.

Ethyl citrate was prepared by Reformatsky reaction of oxaloacetic ester and ethyl bromoacetate, by Lawrence.[75a] He was able to obtain citric acid in 6 per cent. yield.

An attempt to produce, first, the zinc complex and react it with the ketoester yielded only self-condensation products of the haloester[75b] as might be expected by the possibility of attack of the zinc complex on the benzyl ester (XXVI) and elimination of the halogen to give the symmetrical condensation product (XXVII). A model experiment carried out on acetophenone showed that the simultaneous addition of the bromoester and the ketoester to the zinc gave much better results than if the haloester was allowed to react first with the zinc.

When the reaction was carried out by simultaneous addition of both reactants to the zinc a solid product was isolated (m.p. 115-8°) which gave a positive reaction

with ferric chloride solution (wine red colour) showing the presence of an enolic hydroxyl. Its infrared spectrum in chloroform ($\nu_{max}$ 1730, 1706, 1656, 1637, 1582 cm.$^{-1}$) and nujol ($\nu_{max}$ 1656, 1637, 1582 cm.$^{-1}$ only) and the ultraviolet spectrum ($\lambda_{max}^{CHCl_3}$ 298 mμ (ε 16200) $\lambda_{max}^{OH^-}$ 322 mμ (ε 14200), shoulder at 295 mμ (ε 10900)) confirms the presence of an enolised system. The difference in the infrared absorption in chloroform and nujol suggested that in the solid state the compound exists mainly in the enolic form which, in solution, is in equilibrium with the keto one. The infrared spectrum also suggests the presence of a substituted benzene. Analysis of the compound showed the absence of ethoxyl groups and bromine (experimental section). With this information in mind the possible structure (XXVIII) was assigned to it, which could arise in the way depicted in the diagram.

The enol acetate of the product, m.p.115-8°, was prepared. The analytical data are closer to the calculated value for a diacetate than for monoacetate. The infrared spectrum ($\nu_{max}^{CHCl_3}$ 1780, 1715 cm.$^{-1}$) and ultraviolet absorption ($\lambda_{max}$ 269 mμ (ε 26300)) also favour the structure (XXIX).

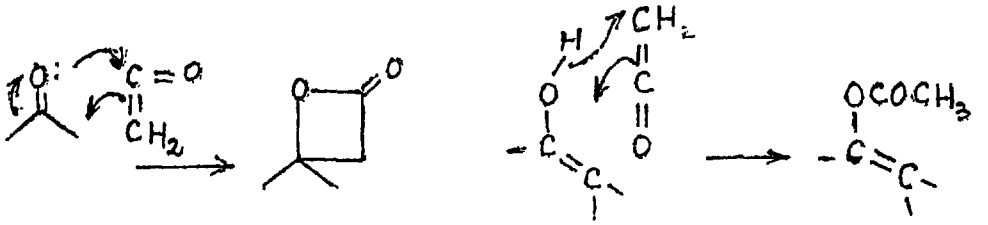
On catalytic hydrogenation over palladised charcoal the product, m.p. 115-8°, consumed two moles of hydrogen and gave an acid which could not be obtained crystalline.

On pyrolysis of the acid in toluene the only product which could be isolated was an oily acid ($\nu_{max}^{CHCl_3}$ 1773, 1718 and carboxyl absorption at 3000-2500 cm.$^{-1}$) which failed to crystallise. Its formation could be explained by a partial lactonisation to (XXX). To confirm the structure (XXVIII) proposed for the product, m.p. 115-8°, the synthesis of the dibenzyl ketipinic ester was attempted by Reformatsky reaction of benzyl bromoacetic ester and diethyl oxalate. Daimler and Keller[76] prepared the diethyl ketipinic ester by the reaction of ethyl chloroacetate and diethyl oxalate with zinc. In our case the problem is complicated by the favoured possibility of self-condensation of the halo ester and the difficulty of purification of the product of the reaction due to the high boiling point of the products formed. Attempts at distillation led to decomposition. Purification by chromatography was also unsuccessful.

We turned our attention to the preparation of diethyl ketipinic ester and subsequent transesterification. Diethyl ketipinic ester was prepared as described by Wislenum.[77] On transesterification with benzyl alcohol an oil was obtained whose infrared spectrum ($\nu_{max}^{CHCl_3}$ 1736, 1629, 1527 cm.$^{-1}$) and ultraviolet absorption ($\lambda\lambda_{max}$ 212 mμ (ε 5800), 315 mμ (ε 16500); $\lambda_{max}^{OH^-}$ flat abs. at 270-8 mμ (ε 5900); $\lambda_{max}$ 334 mμ (ε 10800)) suggested the possibility of formation of a monobenzyl ether

together with the desired transesterification. The product, m.p. 115-8°, was subjected to the same conditions but it was recovered unchanged due to its insolubility in the benzyl alcohol.

Ketene can react with carbonyl compounds to form enolacetates or β-lactones as shown:[78]



Which product is formed depends on the conditions of reaction and the catalyst used.[78] Enolacetates are usually formed when an enolisable ketone reacts with ketene in presence of acidic esterification catalysts such as sulphuric, p-toluensulphonic or, preferentially, acetylsulphoacetic acids.[78] In the formation of β-lactones Friedel Craft types of catalysts are used, particularly boron trifluoride etherate.[78,79] β-lactones under pyrolytic conditions (100-120°) lose carbon dioxide and give the corresponding olefin.

We thought it possible to react the ketoester (XXI) with ketene to give the corresponding <u>bis</u>-β-lactone which, on pyrolysis, would yield the tetraester (XXIV). The reaction was studied first on two model compounds. Ethyl acetoacetate reacted with ketene in presence of boron fluoride etherate to give on distillation the

expected ethyl isopropenylacetate in 9 and 23 per cent. yields when the condensation was carried out in ether and in absence of solvent, respectively. The reaction of ketene with oxaloacetic ester under the same conditions gave either starting material unchanged ($-10^{o}$) or the enolacetate of oxaloacetic ester (at $0^{o}$ and room temperature), characterised by analysis and comparison of its infrared spectrum with that of an authentic sample. The formation of enolacetate took place even after 5 min. of reaction as was shown when the reaction was followed by infrared spectroscopy. The formation of enolacetate could be explained by the high enolizability of the keto group in oxaloacetic ester. The keto ester (XXI) would present the same difficulties, and this approach was abandoned.

2,3,5,6-tetracarbethoxyheptanone-4 (XXXI) was prepared by Sutter and Wijkman[66] by alkylation of ethyl acetonedicarboxylate with ethyl α-bromopropionate. They used sodium ethoxide as condensing reagent. In our preparation potassium ethoxide was used instead as it has been shown[80] that it is a better catalyst when secondary haloesters are the alkylating agents. The product was purified by distillation under high vacuum ($10^{-5}$mm.) to avoid decomposition.

Several attempts to reduce the keto group of the ketotetraester to methylene, through ethanedithioketal formation (XXXII) and treatment with Raney Ni, to (XXXIII) proved unsuccessful. The ketotetraester failed to give the ethanedithioketal when it was treated with ethanedithiol and, boron fluoride etherate[81] or p-toluensulphonic acid in acetic acid.[82] Starting material was recovered unchanged as proved by the isolation of the keto acid (I) when the product of the reaction was treated with Raney Ni[83] and saponified. When diethyl acetonedicarboxylate was subjected to the same series of reactions, glutaric acid was obtained.

The failure of the keto ester (XXXI) to form the ethanedithioketal could be attributed to steric reasons. It also failed to give a crystalline dinitrophenylhydrazone when treated with Braude's reagent.[84]

To force the reaction a mixture of the ketoester (XXXII), ethanedithiol and p-toluensulphonic acid in benzene was boiled under reflux with azeotropic separation of water. The product isolated showed, $\nu_{max}$ 1805, 1773, 1730, 1656 cm.$^{-1}$ and $\lambda_{max}$ 228 mμ (ε 11000). These data suggest the formation of unsaturated γ-lactone. The same product was obtained when the reaction was carried out in absence of ethanedithiol.

The γ-lactone was prepared on a large scale using methanesulphonic acid as it is easier to eliminate the ethyl methane/sulphonate which is formed in the reaction than the corresponding ester of p-toluensulphonic acid. An analysis of the spectroscopic data of the product ($\nu_{max}$ 1805, 1773, 1730, 1656 cm.$^{-1}$; $\lambda_{max}$ 228 mμ (ε 11000); $\lambda_{max}^{OH^-}$ 285 mμ (ε 16400-18500)) suggests that it is a mixture of enol-γ-lactone and α,β-unsaturated-γ-lactone, although it is difficult to predict what should be the spectral characteristics of these products as there is the complication due to conjugation with the ester groups. It has been reported in the literature[85] that the β,γ-unsaturated-γ-lactones show $\nu_{max}$ at 1800 cm.$^{-1}$ and no maxima in the ultraviolet down to 205 mμ (ε 1000).[86] On the other hand, α,β-unsaturated-γ-lactones exhibit $\nu_{max}$ 1750-1775 cm.$^{-1}$, depending on the environment[85] and a distinct maximum at 215-225 mμ (log ε=4)[86,87,88] and maleic esters show a maximum at 205-225 mμ (log ε=4).[89] The position of the maximum depends on the degree of substitution and the geometrical configuration.[89]

The infrared spectrum of our product suggests the presence of β,γ- and α,β-unsaturated-γ-lactones-esters. The ultraviolet absorption in the neutral solution ($\lambda_{max}$ 228 mμ (ε 11000)) could be attributed better to α,β-unsaturated-γ-lactone, but the ultraviolet absorption

in alkaline solution ($\lambda_{max}^{OH^-}$ 285 mμ (ε 18500)) shows the presence of enol lactone, unless the isomerisation of the double bond, from α,β to β,γ position, under these conditions of high dilution is too quick to measure exactly the enollactone/α,β-unsaturated lactone proportion. The formation of the γ-lactones could be explained by an attack of the enol on the ester group with elimination of ethoxy anion as shown (XXXIV-XXXV), and migration of the double bond to the α,β position (XXXVI). The period of reaction (48 hrs. - 8 days) did not seem to influence the characteristics of the mixture formed. Attempts at purification by chromatography or crystallisation were unsuccessful.

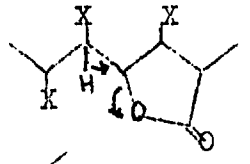
It has been found[90] that treatment of the β,γ-unsaturated γ-lactones with a base such as triethylamine produce isomerisation of the double bond to the more stable α,β-unsaturated position. When the mixture of unsaturated γ-lactones was heated with triethyl amine on the steam bath, the product isolated did not show much change in the spectral characteristics ($\nu_{max}$ 1802, 1773, 1730, 1653, 1639 $cm.^{-1}$; $\lambda_{max}$ 228 mμ (ε 9000), $\lambda_{max}^{OH^-}$ 285 mμ (ε 17000), so little more isomerisation had taken place.

In an attempt to prepare dihydroglauconinic acid the mixture of unsaturated lactones was catalytically hydrogenated over palladised charcoal. The reduction was not complete even after several changes of catalyst

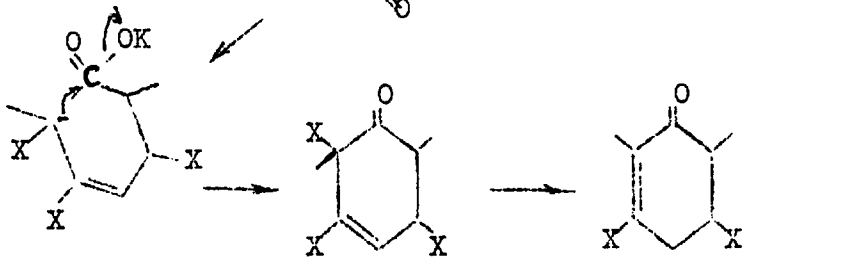
and the percentage of unsaturated material present varied from 10 to 20 per cent. as measured by the ultraviolet absorption in alkaline solution.

The saturated γ-lactone ester ($\nu_{max}$ 1780, 1730 cm.$^{-1}$) was treated with potassium tertiary butoxide in tertiary butyl alcohol. The product of the reaction was saponified and chromatographed over silica gel. Chloroform elution yielded a product whose infrared spectrum ($\nu_{max}$ 1700 cm.$^{-1}$ and carboxyl absorption at 3000-2500 cm.$^{-1}$) and ultraviolet absorption ($\lambda_{max}$ 247 mμ (ε12500)) suggested the presence of a substituted α,β-unsaturated ketone.[91] This was confirmed by the preparation of the dinitrophenylhydrazone of the methyl ester whose ultraviolet absorption ($\lambda_{max}^{CHCl_3}$ 385 mμ (ε 18700)) agrees very well with the expected value. These data and the analytical results (experimental section) suggested the compound had the structure (XXXIX). This could arise from a Dieckmann cyclisation of the opened lactone to the keto triester (XXXVIII). If decarboxylation of the β-ketoester occurred during saponification with migration of the double bond to the more stable conjugated position then structure (XXXIX) would be formed.

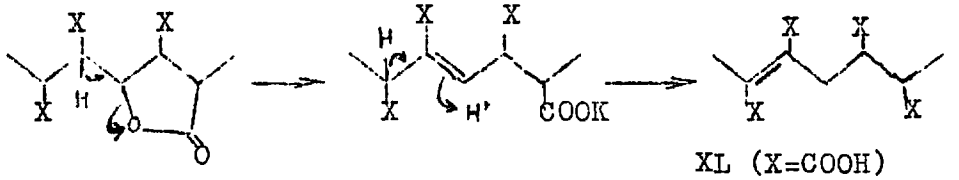
On elution with acetone a second crystalline product was obtained whose analysis and ultraviolet and infrared spectra ($\lambda_{max}$ 210 mμ (ε 12300); $\nu_{max}$ 1700 cm.$^{-1}$) suggested the possible structure (XL). It could be formed by



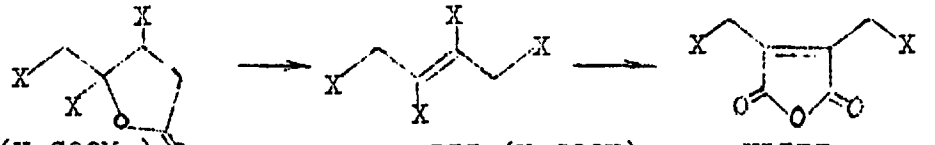
XXXVII (X=COOEt)



XXXVIII(X=COOEt) XXXIX (X=COOH)

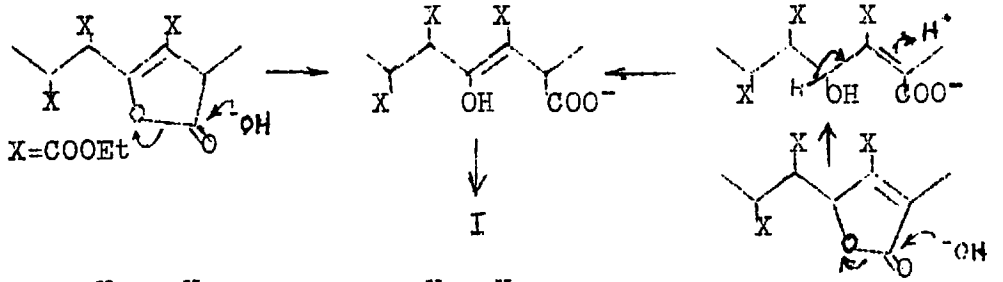


XL (X=COOH)



XLI(X=COOMe) XLII (X=COOH) XLIII

Sequence IV



X=COOEt X=COOH

opening of the lactone ring and rearrangement of the double bond as shown. The fact that it does not form the anhydride on acidification suggests that the carboxylic groups are in trans configuration. The low yield in which this product was obtained prompted us to devise other routes to glauconin or dihydroglauconinic acid.

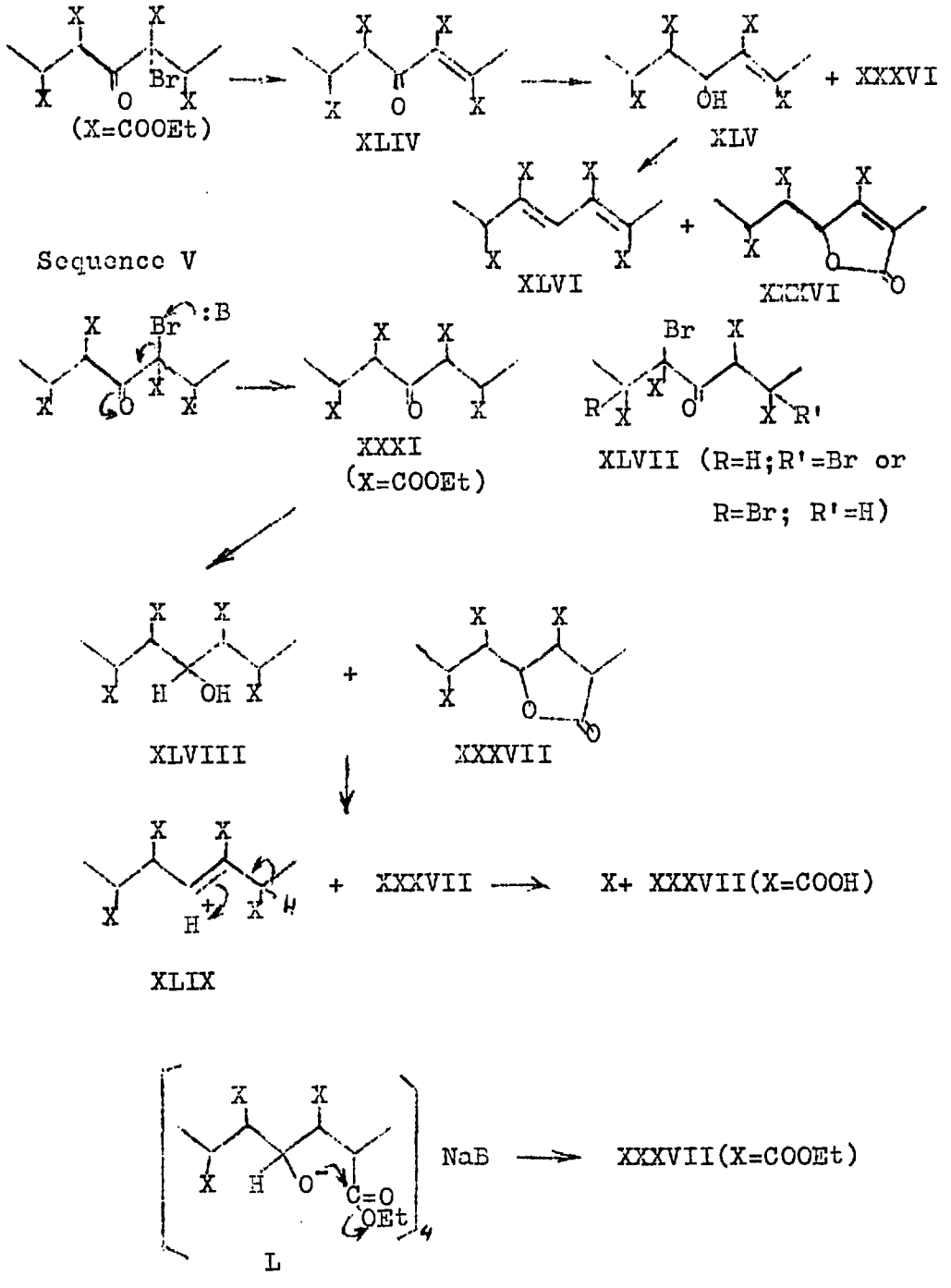
Mayer, Wick and Schmidt[92] obtained the unsaturated tetraacid (XLII) (the position of the double bond is uncertain) and an anhydride (m.p.152-4°) (to which they did not ascribe any structure but it could be (XLIII)) as byproducts on saponification of the lactone ester (XLI); so the unsaturated γ-lactone mixture was saponified and fractional crystallisation of the crude acid obtained gave mainly the keto acid (I) as expected. The mother liquors showed the presence of α,β-unsaturated lactone ($\nu_{max}$ 1776 cm.$^{-1}$). Prolonged heating and sublimation gave an oily crystalline material which on maceration with ether afforded glauconin (m.p. 166-71°). The mixed melting point with the natural product showed no depression and the spectra were identical. The formation of glauconin could be explained as shown in the sequence of reactions (IV).

The reaction was repeated and the product of saponification treated with acetyl chloride to facilitate the separation of the main product of the reaction as its

spirolactone (II). On acidification and continuous ether extraction of the bicarbonate soluble material, an oily product was obtained from which glauconin (m.p. 168-71°) was isolated on maceration with ether. More glauconin was obtained by sublimation of the ether soluble fraction.

The low yield in which glauconin was obtained (0.15 - 0.4 per cent.), (calculated from the γ-lactone used) could be explained by the possibility of isomerisation of the double bond α,β to β,γ in the lactone on treatment with base. The opening of the β,γ-unsaturated γ-lactone gives the β-keto acid which decarboxylates in an irreversible manner. This would act as a driving force thus favoring the isomerisation of the double bond to the β position, and only the molecules in which the double bonds are in positions 2,3 and 4,5 will survive the basic treatment (sequence IV).

In an attempt to increase the yield of glauconin an unambiguous synthesis of the α,β-unsaturated-γ-lactone was devised. Bromination of the ketoester followed by dehydrobromination should give the α,β-unsaturated ketoester (XLIV) which on reduction with sodium borohydride would produce a mixture of α,β-unsaturated-γ-lactone and the alcohol (XLV). Dehydration of the alcohol and hydrolysis of the product (XLVI and XXXVI) should give glauconin.


(X=COOEt)
XLIV
XLV
+ XXXVI
XLVI
+
XXXVI
Sequence V
:B
XXXI
(X=COOEt)
XLVII (R=H;R'=Br or
R=Br; R'=H)
XLVIII
+
XXXVII
+ XXXVII ⟶ X+ XXXVII(X=COOH)
XLIX
NaB ⟶ XXXVII(X=COOEt)
L

On bromination of the ketoester with 1 mol of N-bromosuccinimide a monobromo compound was obtained which, on treatment with lithium chloride in dimethylformamide,[93] gave the ketoester back. This could be explained by a direct attack of the base on the bromine leading to debromination as shown (sequence V). On treatment of the ketoester with 2 moles of bromine in acetic acid a dibromo compound was obtained whose ultraviolet spectrum in alkaline solution ($\lambda_{max}^{OH^-}$ 285 mμ (ε 13400)) suggested that the second bromine atom is not in α' position to the ketone but in either of the positions α to the carbethoxy groups as shown in (XLVII). Treatment of the dibromo ketoester under the usual dehydrobrominating conditions (heating with dimethyl formamide, lithium chloride in dimethyl formamide, or γ-collidine)[93] gave the same results as in the case of the monobromo compound.

Sodium boron hydride reduction of the ketoester gave a mixture of saturated γ-lactone ester (XXXVII) and ester alcohol (XLVIII) ($\nu_{max}$ 1730, 1780, 3450 cm.$^{-1}$). The formation of the saturated γ-lactone under these conditions could be explained by an attack of the alkoxide ion on the ester group with simultaneous elimination of the ethoxy group as shown (L). Dehydration of the mixture by treatment with phosphorus oxychloride in pyridine was followed by saponification. The

product obtained on acidification was chromatographed over silica gel. Elution with benzene-ether (6:4) gave an oil ($\lambda_{max}$ 215 mμ (ε 2300)) from which a solid could be separated on crystallisation from chloroform-ether. This did not show any U.V. absorption and was not studied further. The oily residue was sublimed, washed with chloroform and crystallised from water. The product obtained (m.p. 165-70°; $\lambda_{max}$ 208 mμ (ε 7700), 250 mμ (ε 3560); $\nu_{max}$ 1690, 1778, 1865 cm.$^{-1}$ and the characteristic carboxyl absorption in the 3000-2500 cm.$^{-1}$ region) showed no depression on mixed melting point with the product obtained by hydriodic acid reduction of glauconin. Dihydroglauconinic acid was also obtained when the product of debromination was subjected to the same series of reactions.

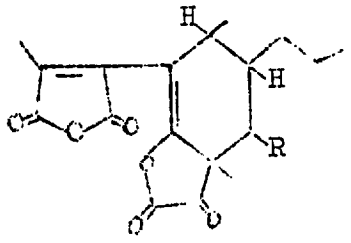
## Glauconic acid.

The facility with which diethyl acrolein and glauconin are formed on pyrolysis of glauconic acid together with the results obtained on zinc dust-acetic acid reduction led Kraft[68] to suggest the structure (LI, R=OH) for glauconic acid and (LI, R=H) for glaucanic acid. Structure (LI) could be modified to (LII) taking into account the proven structure of glauconin; neither of them explains satisfactorily the chemical reactions of

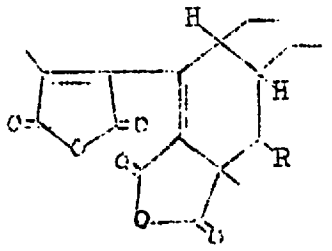
glauconic acid.

In the present work, glauconic acid ($[\alpha]_D$ + 32° (c=1.5)) was isolated as described by Wijkman[63] and purified by chromatography and recrystallisation from benzene or chloroform (experimental section). Its infrared spectrum confirmed the presence of an hydroxyl group ($\nu_{max}$ 3560 cm.$^{-1}$) and showed the presence of one or more cyclic five membered anhydride functions, probably conjugated to a double bond ($\nu_{max}$ 1825, 1770, 1667 cm.$^{-1}$). The fact that some of the derivatives of glauconic acid in which one anhydride ring has been opened, still show in the IR the presence of an anhydride ring suggest that there are two such functions present in glauconic acid. Using the $\Delta'$ and $\Delta^2$-tetrahydrophthalic anhydrides (LIII and LIV) as models, the UV spectrum of glauconic acid ($\lambda_{max}$ 223 mμ (ε 10500), inflexion near 260 mμ) could be accounted[62] for by the presence of one $\Delta'$-type ($\lambda_{max}$ 250 mμ (ε 3540)) and one $\Delta^2$-type chromophore ($\lambda_{max}$ 223 mμ (ε 7600)).

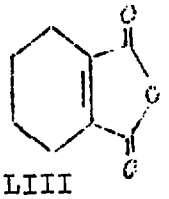
Reduction of glauconic acid with zinc dust in acetic acid[62] gives dihydroglauconic acid ($[\alpha]_D$ + 57°(c=1.01); $\lambda_{max}$ 230 mμ (ε 8000); $\nu_{max}$ 1835, 1767, 1720 cm.$^{-1}$) whose UV and IR spectra indicate the retention of the itaconic type of chromophore as expected. It gives a monomethyl ester ($[\alpha]_D$ + 34°(c=1.21); $\lambda_{max}$ 232 mμ (ε 8550); $\nu_{max}$ 1840, 1780, 1740 cm.$^{-1}$). Dihydroglauconic acid



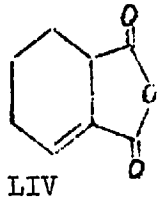
LI



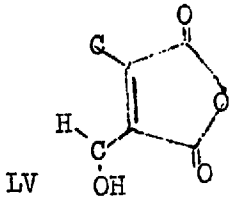
LII



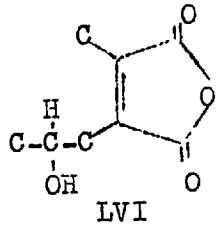
LIII



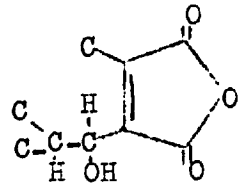
LIV



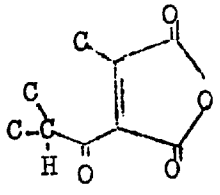
LV



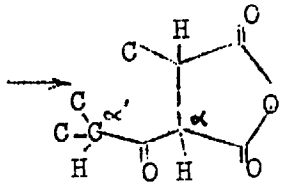
LVI



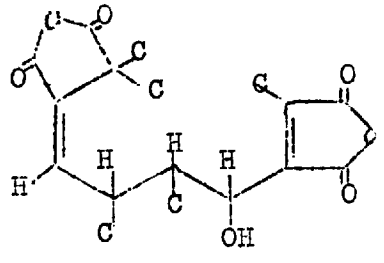
LVII



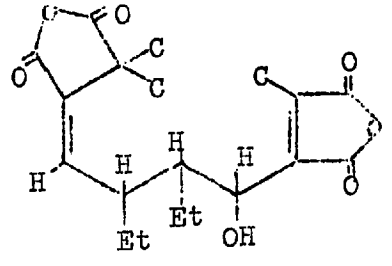
LVIII



LIX



LX



LXI

methyl ester does not show hydroxyl absorption in the IR, thus confirming the observation made by Sutter and Wijkman[66] that it cannot be bezoylated. On treatment with dimethyl sulphate and alkali dihydroglauconic acid gives[62] a trimethyl ester whose IR absorption ($\nu_{max}$ 1775, 1745 cm.$^{-1}$), confirms the presence of a γ-lactone ring. This could be explained if the reduction of the maleic anhydride is assumed to have taken place followed by a γ-lactonization. The partial structures (LV) and (LVI) could account for this transformation. The partial structure (LV) is preferred for the following reasons. Glauconic acid acetate ($[\alpha]_D$ + 35°(c=1.20); $\lambda_{max}$ 221 mμ (ε 10600) inflexion near 250 mμ; $\nu_{max}$ 1835, 1770, 1740 cm.$^{-1}$) on zinc dust-acetic acid reduction[62] gives glaucanic acid ($[\alpha]_D$ + 185°(c=1.55); $\lambda_{max}$ 220 mμ (ε 10700), inflexion near 250 mμ; $\nu_{max}$ 1840, 1775 cm.$^{-1}$) which thus must be desoxyglauconic acid. The removal of the acetoxy group under such mild conditions can be explained[62] if it is in an allylic position to a double bond. The N.M.R. spectra of glauconic acid and its acetate show that the hydrogen atom attached to the carbon atom carrying the hydroxyl or acetoxyl groups is split into a doublet (τ = 4.15 p.p.m.; J=5c/s) indicating the presence of one hydrogen atom on the neighbouring carbon atom.[102] In dihydroglauconic acid methyl ester this proton appears as a triplet (τ = 5.06 p.p.m.; J=4c/s) indicating that

it is split by two hydrogen atoms. Only in the partial structure (LV) is this condition fulfilled by reduction of the maleic anhydride; the change of position for the signal of this proton from $\tau$ = 4.15 p.p.m. in glauconic acid acetate to $\tau$ = 5.06 p.p.m. in the dihydroderivative is a further support for this hypothesis. The partial structure (LV) can be expanded to (LVII) on these grounds.

On treatment with chromic acid in acetone,[109] glauconic acid is oxidised to a ketone ($C_{18}H_{18}O_7$; $[\alpha]_D$ + 45°(c=0.56); $\lambda_{max}$ 220 mμ (ε 10000); $\nu_{max}$ 1830, 1770, 1695 cm.$^{-1}$). When the glauconic acid ketone was reduced with zinc dust in boiling acetic acid a ketotricarboxylic acid was obtained ($C_{17}H_{24}O_7$; $[\alpha]_D^{acetone}$ - 90°(c=1)) which was readily converted into an anhydride acid on sublimation and gave a trimethyl ester on treatment with diazomethane. The IR spectrum of the tricarboxylic acid ($\nu_{max}$ 1742 cm.$^{-1}$) showed that the carbonyl group was present in a five membered ring. The absence of UV absorption ($\varepsilon_{220}$ 485) suggested that a cyclisation had taken place and that the ketotricarboxylic acid is a bicyclic compound. The disappearance of the itaconic anhydride group, which is not usually attacked under these conditions, suggested an internal Michael addition[110] as responsible for the cyclisation. On reduction of the maleic anhydride group (LVIII) there are two enolisable hydrogens in α and α' position to the carbonyl group (LIX) which could act as

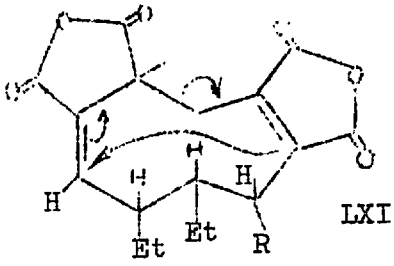
anion source for the Michael reaction. The one at position α would be preferred because it is stabilised by the keto group and the carboxyl group in position α to the ketone. Along with the bicyclic compound a monocyclic acid is isolated from the reaction ($C_{17}H_{22}O_6$; $[\alpha]_D$ + 8°(c=1)) whose UV spectrum ($\lambda_{max}$ 228 mμ (ε 8600)) and IR absorption ($\nu_{max}$ 1830, 1770, 1716, 1700, 1670 $cm.^{-1}$) show that the itaconic anhydride is intact. The monocyclic acid does not cyclise when it is isolated and subjected to the reaction with zinc dust in boiling acetic acid. This product is also obtained on zinc dust-acetic acid reduction of the glauconic acid ketone at room temperature. The fact that the monocyclic compound does not cyclise under the conditions of the reaction suggests that the carboxylic group α to the carbonyl group is essential for the cyclisation. The cyclisation could be also explained if a concerted mechanism is assumed involving the maleic and itaconic type of double bonds in which case the monocyclic compound, in which the maleic anhydride has been saturated, would not undergo cyclisation under the conditions of the reaction. The itaconic anhydride unit can now be joined to the maleic one in such a manner that the keto group will appear in a five membered ring on cyclisation and the partial structure (LVII) can be expanded to (LX). The N.M.R. spectrum of glauconic acid acetate shows the presence of

one olefinic proton split as a doublet ($\tau$ = 3.11 p.p.m.; J=13 c/s) which is retained in the same position and split as a doublet in glauconic acid ketone and in the monocyclic anhydride acid, this excludes the possibility of the hydroxyl group being in the allylic position to the itaconic unit since in the ketones the hydrogen atom which is splitting the olefinic hydrogen would disappear, i.e. there must be an hydrogen in the allylic position to the itaconic double bond. The possibility of coupling across the double bond is excluded by the magnitude of the coupling constant.[102]

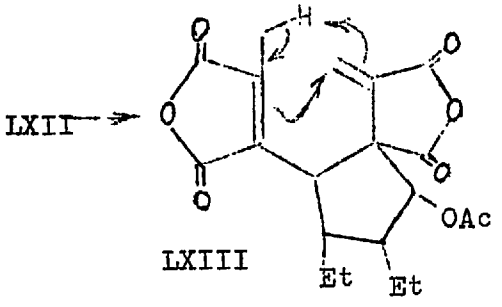
The nature of the two alkyl substituents in the bridge between the two chromophores is established by the formation of diethyl acrolein on pyrolysis and the isolation of meso-diethylsuccinic acid on chromic acid/acetic acid oxidation of glauconic acid and glauconic acid ketone.[101] These facts allow the extension of the partial structure (LX) to (LXI), leaving only two carbon atoms to be located. The N.M.R. spectra of glauconic acid and its derivatives show the presence of a quaternary C-methyl group. The remaining carbon atom must be a methylene group between two fully substituted carbon atoms, and it appears as an AB quartet in the N.M.R. spectrum of glauconic acid ketone ($\tau$ = 6.677, 6.96 p.p.m., J=15 c/s) this establishes (LXII, R=OH) as the structure for glauconic acid and (LXII', R=H) for glaucanic acid.

The relative stereochemistry of glauconic and glaucanic acids has been deduced[62] mainly on grounds of N.M.R. spectra data. The position and relative large coupling constant for the splitting of the vinylic proton in glauconic and glaucanic acids ($\tau$ = 2.97, 3.18 and 2.90, 3.09 p.p.m. respectively, J=13 c/s; $\Delta^2$-tetrahydrophtalic acid anhydride $\tau$ = 2.95 p.p.m.) suggest that the dihedral angles between the two C-H bonds involved must be about 180°.[102] The molecular models suggest[62] that this condition is fulfilled only if the angular methyl group and the ethyl group at $C_{12}$ (LXII) are trans to each other. The two ethyl groups must be cis to each other on basis of the formation of the meso-diethylsuccinic acid unless there is an inversion during the process of its formation.

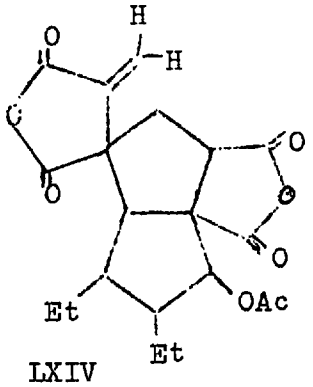
The relative conformation of the hydroxyl group is clarified by a study of the N.M.R. of the isoglauconic acid acetate[62] (m.p. 257-8°; $[\alpha]_D$ + 5° (c=1.36); $\lambda_{max}$ 220 mμ ($\varepsilon$ 10500); $\nu_{max}$ 1835, 1776, 1742 cm.$^{-1}$) which is obtained[104] by pyrolysis of glauconic acid acetate. It shows in the N.M.R. spectrum two vinyl protons ($\tau$ = 3.26, 3.95 p.p.m.) each split as a doublet (J=1c/s). On ozonolysis formaldehyde is isolated as the dimedone derivative[104] which together with the N.M.R. data confirms the presence of itaconic anhydride with a terminal methylene group. Its formation could be rationalised[62]



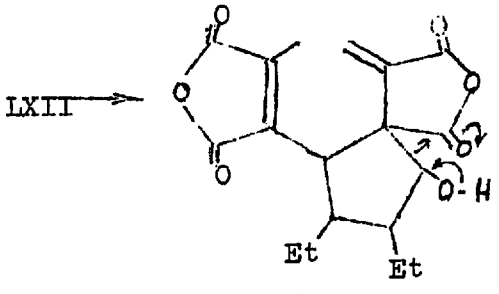

LXII



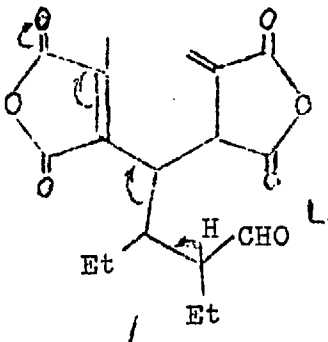

LXIII



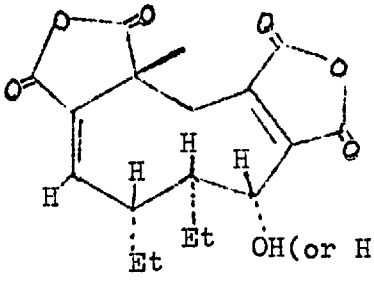

LXIV



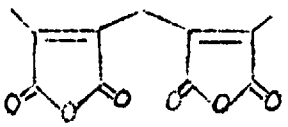

LXVI




LXVII




LXV



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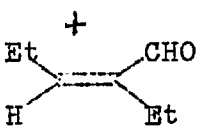


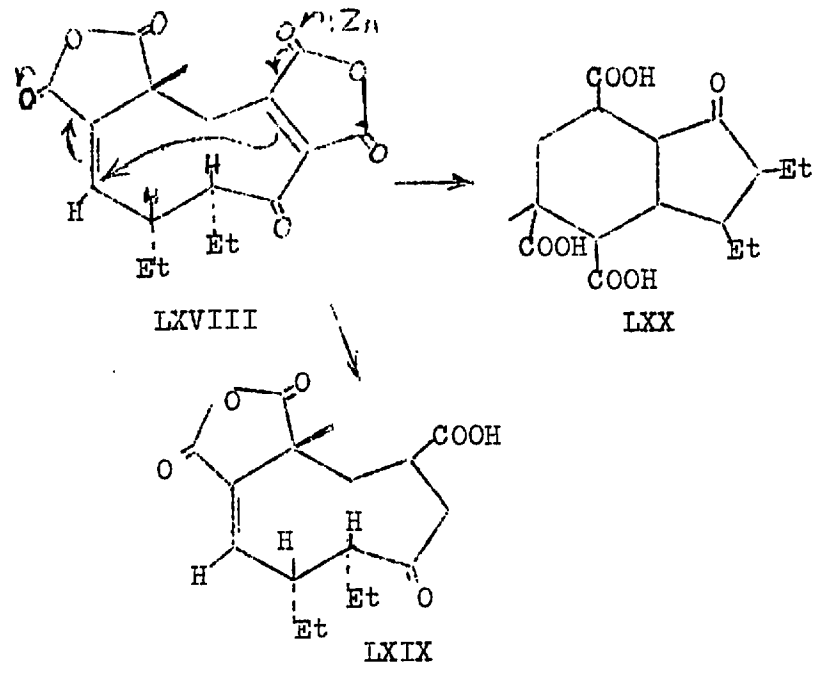
if a Cope rearrangement[111] is assumed to take place in the pyrolysis of glauconic acid acetate, followed by a cyclisation as shown in (LXIII) to give the isoglauconic acid acetate (LXIV). The hydrogen atom attached to the carbon carrying the acetoxyl group is split as a doublet ($\tau$ = 5.09, J=8 c/s). If the H–C–OAc is present in a five membered ring the dihedral angle can only be 0 or 120°. The coupling constant found is better explained[105] if the angle is near 0°, so that the acetoxyl residue and the adjacent ethyl group must be <u>cis</u> to each other. The relative stereochemistry of glauconic and glaucanic acids are thus established as shown in (LXV).

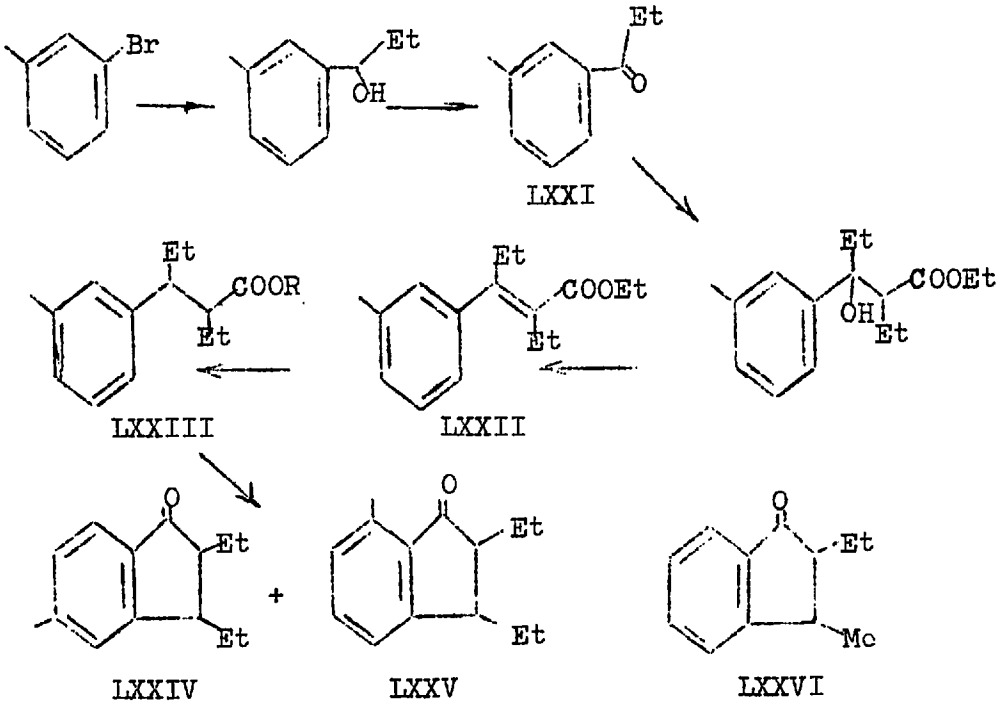
The formation of diethylacrolein and glauconin on pyrolysis of glauconic acid can now be explained.[62] An initial Cope rearrangement[111] (arrows in LXII) would yield (LXVI) in a reversible manner. A reverse aldol reaction to (LXVII) followed by a reverse Michael reaction[110] should give glauconin and diethylacrolein in an irreversible manner as shown. The formation of trans-diethyl acrolein could be explained by an inversion taking place during this process.

We can go back to the products of the zinc dust-acetic acid reduction of glauconic acid ketone. The structure (LXIX) could be ascribed to the monocyclic anhydride acid ketone (acid A, experimental section).

the physical properties found for this compound agree very well with this formulation. Its formation would involve reduction of the double bond of the maleic anhydride group followed by decarboxylation of the resulting β-ketoacid.

As it was pointed out previously (see above), the formation of the bicyclic acid (acid B, experimental section) could be envisaged as an internal Michael addition or a concerted mechanism as shown in (LXVIII, arrows). To prove the structure assigned to the bicyclic ketotricarboxylic acid (LXX) the dehydrogenation of the crude methyl ester anhydride was attempted by heating at 300° with 20 per cent. palladised charcoal in an open tube. The starting material was recovered unchanged due, perhaps, to the fact that the ester did not keep in contact with the catalyst throughout the reacting period. Attempts to cause the dehydrogenation of the ketotricarboxylic acid in an open tube were also unsuccessful for the same reason. When naphthalene was used as a carrier for the dehydrogenation, starting material was recovered unchanged even after 18 hrs. of heating under reflux. Dehydrogenation of the ketotricarboxylic acid in a vacuum sealed tube with an equal amount (w/w) of 20 per cent. palladised charcoal, heating for 12 hrs. at 330°, gave an oil ($\lambda\lambda_{max}$ 208 mμ (ε 18000), 250 mμ (ε 8100), 274 mμ (ε 2500), 297 mμ (ε 1800); $\nu_{max}$ 1700, 1610 cm.$^{-1}$).


M:Zn
Et
H
O
COOH
LXVIII
LXX
LXIX


Br
Et
OH
LXXI
COOR
COOEt
LXXIII
LXXII
LXXIV
LXXV
LXXVI
Me

If the structure (LXX) ascribed to the ketotricarboxylic acid is correct, the expected product of dehydrogenation, unless a migration of the methyl group is involved, would be p-methyl-2,3-diethylindanone (LXXIV). The synthesis of the indanone (LXXIV) was undertaken following the pattern of the synthesis of 2,3-diethylindanone as described by Barltrop et al.[98], and using ethyl-m-tolylketone[96](LXXI) as starting material (scheme VI). The ethyl-m-tolylketone was obtained by Grignard reaction of the m-bromotoluene on propionaldehyde followed by chromic acid oxidation of the alcohol. Reformatsky reaction of (LXXI) with ethyl α-bromobutirate followed by dehydration gave the ethyl α-ethyl-β(m-tolyl)-pent-2-enoate (LXXII), which on catalytic hydrogenation and saponification gave the α-ethyl-β(m-tolyl)-valeric acid (LXXIII, R=H). Some features in the synthesis deserve comment. The UV absorption spectrum found for the unsaturated ester (LXXII) (strong end absorption and flat maximum at 227 mμ (ε 6500)) is not mormal, (α,β-dimethylcinnamic acid shows $\lambda_{max}^{106}$ 271 mμ (logε 4.4)), it could be explained by lack of conjugation caused by steric hindrance.[107] On catalytic hydrogenation of (LXXII) over platinum oxide in ethanol, starting material was recovered unchanged. Barltrop et al.[98], do not report any difficulties in the catalytic hydrogenation of ethyl-α-ethyl-β-phenyl-valerate.

The catalytic hydrogenation of (LXXII) did not present any difficulty when it was carried out in glacial acetic acid over 10 per cent. palladised charcoal. No ethoxy group could be detected in the saturated ester (LXXIII, R=Et) when the determination was done under the normal conditions with prolonged period of heating. The saponification of the saturated ester (ethyl α-ethyl-β(m-tolyl)-valerate) (LXXIII, R=Et) presented some difficulties; a proportion of the ester was recovered unchanged even after prolonged periods of heating under reflux with 10 per cent. sodium or potassium hydroxide in ethanol water (1:1).

Cyclisation of the α-ethyl-β(m-tolyl)-valeric acid (LXXIII, R=H) by heating with polyphosphoric acid, yielded a mixture of p and o-methyl-2,3-diethylindanones (LXXIV and LXXV) as expected ($\nu_{max}$ 1700, 1610, 1600 cm.$^{-1}$; $\lambda\lambda_{max}$ 205 mμ (ε 27500), 252 mμ (ε 14500), 294 mμ (ε 3250)). The N.M.R. spectrum of the mixture showed the expected signals for the aromatic methyl groups in p-methyl-2,3-diethylindanone (τ = 7.58 p.p.m.) and the one corresponding to the o-methyl-2,3-diethylindanone (τ = 7.41 p.p.m.) (o- and p-methylindanones were used as reference).[99] The N.M.R. spectrum of an impure sample of the product of dehydrogenation of the bicyclic keto acid (LXX) showed two signals assigned to aromatic methyl groups (τ = 7.72 and 7.58 p.p.m.) the pattern of the

aromatic protons is arranged as in the p-methylindanone[99] but there is present an additional peak whose intensity (as well as the peak at 7.72 p.p.m.) is reduced by half on a more purified sample.

To be able to compare the product obtained on dehydrogenation of the ketotricarboxylic acid with the synthetic product, both were converted into the respective dinitrophenylhydrazones. On treatment of the synthetic mixture of indanones with the dinitrophenyl hydrazine reagent a solid product crystallised directly (m.p. 152-3°; $\lambda_{max}^{CHCl_3}$ 395 mμ (ε 30200)). On working up the mother liquors (experimental section) a second product was obtained (m.p. 174-80°; $\lambda_{max}^{CHCl_3}$ 392 mμ (ε 27200). On cyclisation of the acid precursor the para-compound is expected to be formed in higher yield than the ortho for steric reasons. A comparison of the UV spectra of the products obtained with authentic samples of p- and o-methyl indanones[99] (experimental section) together with the assumption outlined above suggested that the product m.p. 152-3° corresponds to the dinitrophenyl hydrazone of the p-methyl-2,3-diethyl-indanone (LXXIV). The IR spectrum of this product (in nujol) and that of the dinitrophenyl hydrazone of the product obtained from the natural source (m.p. 166-8°; $\lambda_{max}^{CHCl_3}$ 395 mμ (ε 30000); $[\alpha]_D$ ± 0°(c=1)) are essentially

identical but show some minor differences. The mixed melting point (153-63°) showed no marked depression. The difference in the melting points of the product obtained from the natural source (m.p. 166-8°) and the synthetic material (m.p. 152-3°) could be due to difference in the stereochemistry of the ethyl groups. Granger and collaborators[108] suggested that the methyl and ethyl groups in synthetic 2-ethyl-3-methylindanone (LXXVI) are trans to each other whatever the method of synthesis used. Their suggestion was based on a study of models which revealed that the steric hindrance is lower in the trans than in the cis configuration. On this basis the two ethyl groups in the synthetic material can be considered to be trans to each other. If we assume that there has not occurred a racemisation during the cyclisation and dehydrogenation involved in the formation of the product obtained from the natural source, the ethyl groups would be expected to be cis to each other. Alkaline treatment of the natural product (experimental section) did not produce any measurable change in the indanone (identical IR), the dinitrophenyl hydrazone showed the same m.p. (166-8°) as the one obtained from the product not subjected to this treatment. Assuming that the product obtained on dehydrogenation of the keto tricarboxylic acid (LXX) is in the most

stable configuration under the conditions of the reaction different from the one obtained on polyphosphoric acid cyclisation of the acid precursor (synthetic product), the synthetic mixture of indanones was heated with palladised charcoal under the same conditions used on the dehydrogenation. The dinitrophenyl hydrazone of the product obtained showed a constant m.p. 145-53° and could not be purified further.

The data and experiments described do not prove that the product of dehydrogenation of the keto tricarboxylic acid is the p-methyl-2,3-diethylindanone, (LXXIV), but they do not disprove it either. The problem remains open and a new approach is needed to solve it.

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Recently the preparation of the dinitrophenylhydrazones of the synthetic mixture of methyl-2,3-diethylindanones was repeated. Chromatography over alumina followed by slow crystallisation from chloroform-ethanol yielded a product which showed the same melting point as the product obtained from the natural source, mixed melting point showed no depression and the IR spectra (KBr disc)

were identical.[62] It remains to be conclusively proved whether this product is the ortho- or the para-methyl-2,3-diethylindanone, though the N.M.R. spectra of the two crude products (natural and synthetic) each show two aromatic methyl singlet peaks, only one of these peaks is common to both samples and this is the peak which is ascribed to the para-methyl group in the mixture of synthetic indanones. This strongly suggests that the dehydrogenation product is the para compound.

## EXPERIMENTAL

M.p.'s were determined on a Koefler block and are uncorrected. Except when otherwise stated, rotations were measured in chloroform. The ultraviolet absorption spectra were determined in absolute ethanol. The Unicam SP700 was used in the determinations. The infrared absorption spectra were measured with the Infracord 137.

The solvents used for elution in chromatography were "AnalaR" quality. The alumina was acid-washed, neutralised and standardised according to Brockman's method. The silica gel was supplied by B.D.H.

Anhydrous sodium sulphate was used as drying agent. Nitrogen, when used, was dry and oxygen free.

The analytical samples were dried in vacuum over phosphorous pentoxide at room temperature for 48 hours.

The microanalyses were carried out by Miss J. Cuckney (Imperial College) and her associates.

The N.M.R. spectra were carried out at 56.45 Mc/s using tetramethylsilane as an internal reference. The determination and interpretation were done by Professor Jackman, Dr. Lown and Mr. Forster, to whom we express our gratitude.

Synthesis of diethyl 2,4-dicarbethoxyglutarate.[71]

Diethylamine (10 ml.) was added dropwise to a stirred mixture of diethylmalonate (320 g.) and formalin (40 per cent, 80 g.) at 0°. The reaction mixture was left stirring overnight at room temperature and then heated on the steam bath for 4 hrs. The aqueous layer formed was separated and the organic phase washed with water and dried. Fractional distillation gave a liquid (119 g., 22 per cent yield) b.p. 160-4°/1 mm.; $n_D^{20}$ = 1.4370.

Reaction of diethyl 2,4-dicarbethoxyglutarate and α-bromopropionate in presence of sodium hydride.

Diethyl 2,4-dicarbethoxyglutarate (54 g. = 0.2 moles) was added dropwise to a stirred suspension of sodium hydride (10 g. = 0.4 moles) in dry benzene (50 ml.). Ethyl-α-bromopropionate (90 g. = 0.5 moles) in dry benzene (50 ml.) was added dropwise over a period of two hours. The reaction mixture was stirred at room temperature for 12 hr. It showed a strong alkaline reaction. After heating on the steam bath for 48 hr. it still had an alkaline reaction. Water was added. The organic phase was washed with water and dried. After removal of the solvent the unreacted starting material was removed by distillation under vacuum. On fractional distillation of a sample two high boiling fractions were obtained

A. b.p. 180-200°/1.5 mm., $n_D^{20}$ = 1.4582 (found C,57.65; H,7.65; OEt,47.9 per cent.)

B. b.p. 200-230°/1.5 mm., $n_D^{20}$= 1.4560 (found C,55.13; H,6.60 per cent.)

$C_{18}H_{26}O_9$ requires C,55.95; H,6.78; [$C_{25}H_{40}O_{12}$ requires C,56.30; H,7.58 per cent.])

The residue (24 g.) was hydrolysed by heating on the steam bath with 4N sodium hydroxide (100 ml.) for 9 hr. After acidification and continuous extraction with ether an oily product was obtained (12.3 g.) which was chromatographed over silica gel (400 g.). On elution with benzene-ether (3:2) an acid was isolated, A, (XVII), (3.35 g.) which after repeated crystallisations from ether-methanol showed melting point 170-5°.

Found: C,52.22, 51.86; H,5.63, 5.38; C-Me, 8.92, 5.73 per cent.

$C_8H_{10}O_5$ requires C,51.61; H,5.41; C-Me, 8.1 per cent.

Equivalent weight (titration) found, 85.2; calc. for $C_8H_{10}O_5$, 93. It did not show U.V. absorption; $\nu_{max.}^{nujol}$ 1742,1704 cm.$^{-1}$ and characteristic COOH absorption in the 3000-2500 cm.$^{-1}$ region. On treatment of the acid with an ethereal solution of diazomethane the methyl ester was obtained. It was purified by sublimation at 100°/1x10$^{-5}$mm. (m.p. 28-32°). It showed in the I.R. a broad absorption at 1754-1724 cm.$^{-1}$.

Found C,55.99, 55.60; H,6.65, 6.40; OMe,29.39, 29.83 per cent.

$C_{10}H_{14}O_5$ requires C,56.07, H,6.59, OMe, 29 per cent.

On further elution with benzene-ether (1:4) a second product, B, (XIX or XX), (600 mg.) was obtained which on repeated recrystallisations from ether-methanol showed melting point 165-71° (mixed melting point with acid A 150-160°). It showed only one carbonyl band in the I.R. at 1700 cm.$^{-1}$ and typical COOH absorption in the 3000-2500 cm.$^{-1}$ region.

Found C,47.57; H,6.12 per cent.

Calc. for $C_8H_{12}O_6$ (XIX) C,47.06; H,5.92 per cent.

$C_{11}H_{16}O_8$ (XX) requires C,47.86; H,5.84 per cent.

The acid A (XVII) (43.2 mg.) was pyrolysed heating under nitrogen at 210° for $\frac{1}{2}$ hr. After sublimation and repeated recrystallisations from chloroform-n-hexane the acid C (XVIII) showed m.p. 92-4°.

Found C,58.28; H,7.25 per cent.

Calc. for $C_7H_{10}O_3$ C,59.14; H,7.09 per cent.

The semicarbazone was prepared. It was recrystallised from aqueous methanol to constant m.p. 200-202°.

Found C,48.75; H,6.49; N,23.61 per cent.

Calc. for $C_8H_{13}O_3N_3$ C,48.23; H,6.58; N,21.1 per cent.

Ethyl β-methylcyanosuccinate.[72] To a solution of sodium (5.75 g.) in absolute ethanol (90 ml.) a molar equivalent of ethyl cyanoacetate (28.5 g.) was added dropwise. To the paste so formed the ethyl α-bromopropionate (45 g., 10 per cent. excess) was added with caution. The reaction

flask was kept in an ice bath during the addition of the haloester. The floculent precipitate of ethyl cyanoacetate sodium salt was dissolved in the process and sodium bromide separated. After the addition was complete (1 hr.) the reaction mixture was heated under reflux for $1\frac{1}{2}$ hr. It was cooled to room temperature, water was added and the aqueous solution extracted with ether. The ethereal solution was washed with water and dried. After removal of the solvent the residue (46.3 g.) was distilled under vacuum (b.p. 114-6/2 mm.). The product (30.04 g.)($n_D^{20^\circ}$ = 1.4332) was obtained in 55 per cent. yield.

Pentane-β,γ,ε-tricarboxylic acid.[72] The ethyl β-methylcyanosuccinate (4.26 g.) was added to ethanol (10 ml.) in which a small piece of sodium was dissolved. The acrylonitrile (1.22 g.) was added dropwise to it at $0^\circ$. The reaction mixture was left at room temperature for $1\frac{1}{2}$ hr. It was neutralised with acetic acid and the ethanol removed under vacuum at room temperature. Water was added to the residue which was extracted with ether. After removal of the solvent, the residue (5.08 g.) was distilled under vacuum. The product so obtained (b.p. 178-82°/2 mm. 3.87 g.) was hydrolysed with concentrated hydrochloric acid (15 ml.) by heating on the steam bath for 24 hrs. The aqueous solution was extracted

with ether. On removal of the solvent a solid was obtained (2.74 g.) which after several recrystallisations from ether-methanol and 24 hrs. drying under vacuum over phosphorous pentoxide showed m.p. 160-5° (reported[72] m.p. 177°). (Mixed melting point with product B (m.p. 165-71°), 160-5°). The I.R. spectra of both samples in nujol showed differences in the finger print region.

2-Methylcyclopentanonecarboxylic acid.[72] Ethyl β-methylcyanosuccinate (15.2 g.) was added to a stirred solution of sodium ethoxide in ethanol (a small piece of sodium dissolved in 60 ml. of ethanol). The acrylonitrile (5.5 ml., 15 per cent. excess) was added dropwise during $\frac{1}{2}$ hr. maintaining the temperature at 0°. The stirring was continued for $1\frac{1}{2}$ hr. at room temperature. The sodium ethoxide was neutralised with acetic acid and the ethanol removed under vacuum at room temperature. Concentrated hydrochloric acid (60 ml.) was added portionwise (ice bath). After standing for two hours at room temperature the reaction mixture was heated at 70-80° for 48 hrs. The water was removed under vacuum. Benzene was added and the distillation continued up to b.p. 80°. Ethanol (40 ml.) was added, the reaction mixture was saturated with hydrochloric acid gas and heated, with azeotropic separation of water, for 24 hrs. The inorganic material was removed by filtration. After

removal of the solvents the residue (18 g.) was distilled under vacuum. The main fraction distilled at 135-42°/1 mm., $n_D^{20°}$ = 1.4390 (10.576 g.; 52 per cent. yield). The ester (10.57 g.) was added dropwise to a stirred suspension of sodium hydride (0.88 g., equimolar amount) in ether (50 ml. containing 0.05 ml. of ethanol). The temperature was maintained at 0° for 4 hrs. after the addition was complete. Stirring was continued at room temperature until all the sodium hydride had reacted (48 hrs.). The reaction mixture was acidified with 6N hydrochloric acid (10 ml.) and the aqueous phase extracted with ether. The ethereal solution was washed and dried. After removal of the ether, the residue (7.213 g.) was distilled under vacuum. The product (b.p. 124-34°, $n_D^{20°}$ = 1.4512; 6.03 g.) was obtained in 68 per cent. yield. The diethyl 2-methylcyclopentanone-3,5-dicarboxylate so obtained was hydrolysed with 10 per cent. hydrochloric acid (30 ml.) by heating on the steam bath for 20 hrs. The ether extraction left as a residue a solid (2.89 g.) which on repeated recrystallisation from chloroform-n-hexane showed m.p. 88-94° (reported[72] m.p. 95°) (mixed m.p. with the product obtained on pyrolysis of acid A (m.p. 92-94°) 90-94°). Both products showed $\nu_{max.}^{CHCl_3}$ 1742, 1712 cm.$^{-1}$ and the characteristic COOH absorption in the 3000-2500 cm.$^{-1}$ region. The finger print region of

the spectra taken in nujol showed some differences. The methyl ester of both products were prepared and purified by sublimation. The I.R. spectra were identical ($\nu_{max}^{film}$ 1742 cm.$^{-1}$). The semicarbazones of the methyl esters (m.p. 195-6°) showed no depression on mixed melting point determination.

Reformatsky reaction.

The benzyl bromoacetic ester used in the reactions described in this section was prepared following Rottingen and Wenzel.[95] (B.p. 104-6°/1 mm.). The ethyl oxaloacetate (b.p. 92°/2.5 mm.) was obtained from the sodium salt. Zinc wool was activated by washing successively with dilute sodium hydroxide, glacial acetic acid, water, acetone, ethanol, ether and dried at 100° under vacuum.

Reformatsky reaction of benzyl bromoacetic ester and ethyl oxaloacetate.

To zinc (8 g.) covered with sodium dried benzene (25 ml.) a portion of the benzyl bromoacetic ester in benzene (20 g. in 30 ml.) was added. The reaction mixture was heated under reflux and stirred occasionally. After 1½ hr. the reaction started and the rest of the bromoester was added dropwise (½ hr.). Heating was continued for half-an-hour more. The oxaloacetic ester (5 g. in 20 ml. of benzene) was added dropwise. The reflux was continued for 6 hrs. (The reaction was

followed, without success, by working up a sample at regular intervals and measuring the U.V. absorption of an alkaline solution in ethanol; the oxaloacetic ester shows $\lambda_{max}^{OH^-}$ 302 mμ (ε 13390) under these conditions). A large portion of zinc was left unreacted. Dilute sulphuric acid (100 ml.) was added to the ice cooled reaction mixture. The aqueous phase was extracted with benzene. The benzene solution was washed with aqueous sodium carbonate solution and dilute sulphuric acid until no precipitate was formed on addition of alkali, and, finally, washed with water and dried. After removal of the solvent and unreacted starting material, the residue was distilled under vacuum. A portion (b.p. 122-8°/2 mm.; 1.1 g.) showed a positive ferric chloride reaction ($\nu_{max}^{film}$ 1740, 1724 cm.$^{-1}$, no OH, and characteristic absorption for benzene ring at 800-700 cm.$^{-1}$).

Found, C,69.35; H,6.30 per cent.

$C_{11}H_{12}O_3$ (XXVI) requires C,68.73; H,6.29 per cent.

On sublimation of the residue at 100°/5.95 x $10^{-5}$ mm. a thick oil was obtained.

Found C,72.64; H,6.25 per cent.

(XXVII) $C_{18}H_{18}O_4$ requires C,72.46; H,6.08 per cent.

Reformatsky reaction of benzyl bromoacetic ester on acetophenone. When the reaction was carried out as described in the preceding section the product (benzyl

β-methylmandelate, b.p. 174°/2 mm.) was obtained in 12 per cent. yield. If a mixture of acetophenone and benzyl bromoacetic ester was added to the zinc the product was obtained in 78 per cent. yield.

Reformatsky reaction of benzyl bromoacetic ester on ethyl oxaloacetate. To zinc (3.5 g.) covered with benzene, a portion of a mixture of benzyl bromoacetic ester (8.5 g. = 0.0365 moles) and ethyl oxaloacetate (5 g. = 0.0265 moles) in benzene (50 ml.) was added. The reaction mixture was heated under reflux and stirred occasionally. A smooth reaction took place after 15 min. heating and the rest of the solution was added dropwise maintaining the reflux (45 min.). The reflux was continued for 3 hrs. The reaction mixture was worked up as described previously. After removal of the unreacted starting material, the residue decomposed on an attempt to distill it. The reaction was repeated using a large excess of benzyl bromoacetic ester (3 moles:1 mol of oxaloacetic ester). The reaction mixture was heated under reflux for 1 hr. after the addition was complete. After removal of the unchanged starting material, the solid residue (7.55 g. from 5 g. of oxaloacetic ester) was recrystallised from ethyl acetate-petroleum ether to constant melting point 115-8°. It showed a positive ferric chloride reaction; $\nu_{max}^{CHCl_3}$ 1730, 1706, 1656, 1637,

1582; $\nu_{max}^{nujol}$ 1656, 1637, 1582 cm.$^{-1}$ only; $\lambda_{max}^{CHCl_3}$ 298 mμ (ε 16200); $\lambda_{max}$ (not very soluble) 290 mμ (ε 7660); $\lambda_{max}^{OH^-}$ shoulder 295 mμ (ε 10900), $\lambda_{max}$ 322 mμ (ε 14200). (no Br, no OEt) Found C,67.48; 67.78; 67.35; H,5.22; 5.38; 5.07 per cent.

M.W. (cryoscopic) 354.6

$C_{20}H_{18}O_6$ (XXVIII) requires C,67.79; H,5.18 per cent.; M.W. 354.34.

The enolacetate was prepared in the usual way. To the solid (m.p. 115-8°) (40 mg.) dissolved in dry pyridine (5 ml.) acetic anhydride was added (2 ml.) at 0°. After working up a solid was obtained (55 mg.) which was recrystallised from chloroform-petroleum ether to constant m.p. 152-4°.

$\nu_{max}^{CHCl_3}$ 1780, 1715, 1626 cm.$^{-1}$; $\lambda_{max}$ 208 mμ (ε 16600), 269 mμ (ε 26300)

Found C,64.71; H,5.07 per cent.

monoacetate $C_{22}H_{20}O_7$ requires C,66.66; H,5.09 per cent.

diacetate $C_{24}H_{22}O_8$ (XXIX) requires C,65.74; H,5.06 per cent.

Hydrogenolysis of the product m.p. 115-8°. The solid (73 mg.) was dissolved in ethylacetate (25 ml.), 10 per cent palladised charcoal (23 mg.) was used as a catalyst. 9.5 ml. of hydrogen were consumed (two moles equivalent = 9.25 ml.). On removal of the solvent an oil was obtained (30 mg.) which showed in the I.R. $\nu_{max}^{film}$ 1724 cm$^{-1}$ and

characteristic absorption for COOH group. It was heated in toluene under reflux for 24 hrs. On treatment of the volatile fraction with diaminophenylene no quinoxaline could be isolated. The oily residue showed $\nu_{max}^{film}$ 1773, 1718 cm.$^{-1}$ and COOH absorption in the 3000 cm.$^{-1}$ region.

Attempted synthesis of dibenzyl ketipinic ester.
Reformatsky reaction.[76] To the zinc (14.08 g. = 0.22 moles) covered with dry benzene (50 ml.) a mixture of diethyl oxalate (14.6 g. = 0.1 moles) and benzyl bromoacetic ester (45.8 g. = 0.2 moles) was added dropwise. The reaction mixture was heated under reflux and stirred until a vigorous reaction took place. Addition was continued maintaining the reflux ($2\frac{1}{2}$ hrs.). The reaction mixture was heated under reflux for 1 hr. more and worked up as described in previous section. The oily residue obtained (18 g.) after removal of the unchanged starting material, showed $\nu_{max}^{film}$ 3350(w), 1730(s), 1667(s), 1639 cm.$^{-1}$. All attempts of purification failed. In an attempt to form the enol acetate, starting material was recovered unchanged (I.R.).
Claisen condensation.[77] Dry sodium ethoxide was prepared as follows: the sodium (0.2 moles = 4.6 g.) was dissolved in an excess of ethanol. Dry benzene was added and the azeotropic mixture was distilled until the temperature rose to 80° and then all the solvent was

removed. All operations were carried out under nitrogen. Diethyl oxalate (15 ml.) in ether (150 ml.) was added. The suspension was heated on the steam bath under reflux and nitrogen. Purified and dry ethyl acetate (25 ml.) was added dropwise ($\frac{1}{2}$ hr.). The reaction mixture was heated under reflux for 1 hr. The original slurry was completely dissolved. After cooling in an ice bath some water was added and the reaction mixture acidified with glacial acetic acid. The aqueous phase was extracted with ether, the ethereal solution washed once with dilute sodium bicarbonate solution and water, and dried. On removal of the solvent an oil was obtained (2.073 g.) which was crystallised from ether-petroleum ether, m.p. 74-7°, (0.39 g.)(reported[77] m.p. 76-7° or 82-3°). It showed in the I.R. bands at 1730, 1672 $cm.^{-1}$. In the U.V. it had $\lambda_{max}$ 245 mμ (ε 5000), shoulder 275 mμ (ε 1700), $\lambda_{max}^{OH^-}$ 275 mμ (ε 8200), shoulder 308 mμ (ε 2700).

Transesterification. The crude solid diethyl ketipinic ester (176 mg.) was suspended in benzyl alcohol (5 ml.). Dry hydrochloric acid gas was passed through for $\frac{1}{2}$ hr. at 0°. All solid material was dissolved in the process. The reaction mixture was left at 0° for 40 hrs. It was poured into cold aqueous sodium bicarbonate solution and extracted with ethyl acetate. The organic solution was washed with water and dried. On removal of the solvents an oil was obtained (196 mg.) which showed positive

reaction with a ferric chloride solution. It was sublimed at 100°/1x10$^{-5}$mm. to give an oil $\nu_{max}^{film}$ 1736, 1629, 1527 cm.$^{-1}$; $\lambda\lambda_{max}$ 212 mμ (ε 5800), 315 mμ (ε 16500) shoulders 304 mμ (ε 15300) and 328 mμ (ε 11800); $\lambda_{max}^{OH^-}$ flat at 270-278 mμ (ε 5900), $\lambda_{max}$ 334 mμ (ε 10800). On treatment of the product m.p. 115-8° under the same conditions it was recovered unchanged due, perhaps, to its insolubility in benzyl alcohol.

Ketene reaction on acetoacetic ester.[78,79] Ketene, generated by pyrolysis of diketene, was passed during one hour through a solution of ethyl acetoacetate (5 g.) in ether (50 ml.) containing 40 per cent. boron trifluoride etherate (1 ml.). The reaction mixture was maintained at -10° during the process. Solid sodium acetate was added, the ether was removed and the residue distilled at atmospheric pressure (b.p. 132-56°; 1.65 g.). The product had a positive reaction to ferric chloride solution. It was dissolved in ether, the ethereal solution was washed with dilute sodium hydroxide solution and water and dried. After removal of the ether, the residue (0.69 g.) was distilled under vacuum (b.p.67-8°/ 50 mm.). The product obtained ($n_D^{20°}$ = 1.4157; 0.4534 g.: 9 per cent. yield) showed a weak end absorption in the U.V. ($\varepsilon_{220}$ 228; $\nu_{max}^{film}$ 1740, 1650, 900 cm.$^{-1}$).

Found C,65.73; H,9.68 per cent.

Calc. for $C_7H_{12}O_2$ C,65.59; H,9.44 per cent.

When the reaction was carried out without solvent the product was obtained in 23 per cent. yield (b.p. 50-52°/25 mm.; reported[78] b.p. 54.5°/20 mm.).

Ketene reaction on oxaloacetic ester. A stream of ketene was passed during $1\frac{1}{2}$ hr. through ethyl oxaloacetate (5 ml.) containing 40 per cent. boron trifluoride etherate (1 ml.). The reaction was carried out at room temperature. Samples were taken after 5 min. and 15 min. of reaction and worked up. The infrared spectra showed a progressive formation of a band at 1780 $cm.^{-1}$. The reaction mixture was worked up as described in the previous section. The product obtained (b.p. 86-90°/0.6 mm.; 0.5 g.) showed $\nu_{max}^{film}$ 1780, 1725, 1670 $cm.^{-1}$; $\lambda_{max}$ 220 mμ (ε 11500).

Found, C,52.41; H,6.30; OEt,38.75; Ac,18.45 per cent.

Calc. for $C_{10}H_{14}O_6$, C,52.17; H,6.13; OEt,39.1; Ac,18.7 per cent.

An authentic sample prepared in the usual way showed the same I.R. Oxaloacetic ester enolacetate was also obtained when the reaction was carried out at 0°. At -10° and -40°, starting material was recovered unchanged.

Synthesis of 2,3,5,6-tetracarbethoxyheptanone-4. (XXXI) Diethyl acetonedicarboxylate (11 g. = 0.054 moles) and ethyl α-bromopropionate (8.8 g. = 0.054 moles) were mixed in a three neck flask provided with a stirrer, an efficient condenser and a dropping funnel. An alcoholic

solution of potassium ethoxide(2.1 g. of potassium = 0.054 moles, dissolved in 20 ml. ethanol) was added dropwise over 15 min. The reaction mixture was heated under reflux for 1 hr., after the addition was complete. The remaining ethyl α-bromopropionate (8.8 g., 0.054 moles) was added quickly and an equivalent amount of potassium ethoxide (2.1 g. in 30 ml.) was added dropwise. The reaction mixture was heated under reflux for 4 hrs. and then left stirring overnight at room temperature. The heavy precipitate of potassium bromide was filtered off. The ethanol was removed under vacuum and the remaining oil dissolved in ether. The ethereal solution was washed and dried. The viscous oil, obtained on removal of the solvent was distilled twice at $110^{\circ}/1 \times 10^{-6}$cm. The product obtained (10.64 g.; 50 per cent yield; $n_D^{20^{\circ}} = 1.4510$) showed positive ferric chloride reaction. $\nu_{max}^{film}$ 1742 cm.$^{-1}$; $\lambda_{max}$ 232 mμ (ε 3100), 308 mμ (ε 380) $\lambda_{max}^{OH^-}$ 285 mμ (ε 19800).

Found C,56.79; H,7.4; OEt,44.45 per cent.

$C_{19}H_{30}O_9$ (XXXI), requires C,56.7; H,7.51; OEt,44.7 per cent.

When the reaction was carried out under nitrogen the product was obtained in 73 per cent. yield.

The keto ester failed to give a crystalline dinitrophenylhydrazone on treatment with Brædde's reagent.[84]

Ethanedithiol ketal of diethyl acetonedicarboxylate. To a mixture of diethyl acetonedicarboxylate (o.24 g.) and ethanedithiol (0.2 ml.), 40 per cent. boron trifluoride etherate (0.2 ml.) was added at 0°. The mixture was shaken at room temperature for 1 hr., poured into ice and extracted with petroleum ether. The petroleum ether solution was washed with dilute aqueous sodium hydroxide solution and water and dried. The oil obtained after removal of the solvent (0.2465 g.) was dissolved in ethanol and heated with (modified)[83] Raney Ni W1 for 24 hrs. under reflux. The solid material was filtered off and the solvent removed. The residue was saponified by heating with 2N sodium hydroxide solution on the steam bath. On acidification and extraction with ether an oil was obtained which on sublimation gave a solid (m.p. 80-90°). After repeated recrystallisation from benzene the product showed m.p. 97-8° (glutaric acid m.p. 97-8°; mixed m.p. 97-8°).

Ethanedithiol ketal of the ketotetraester (xxxi) A mixture of ketoester (5.17 g.) ethanedithiol (5.3 ml.) and 40 per cent boron trifluoride etherate (5.3 ml.) was shaken at room temperature for 4 hrs. After working up the reaction mixture as described in the preceding section, the residue (3.7 g.) was dissolved in ethanol and heated under reflux with Raney Ni (20 g.) for 24 hrs. The residue obtained after removal of the solid material and solvent (1.7 g.)

was saponified by heating on the steam bath with dilute sodium hydroxide solution (25 ml.). On acidification and ether extraction a product was obtained (0.988 g.) which was repeatedly recrystallised from ether to constant m.p. 115-28°. On titration the equivalent weight was found to be 116, 103.

Found: C,53.43; H,6.9 per cent.

Calc. for $C_9H_{14}O_5$ (I): C,53.46; H,6.98 per cent.

On sublimation it gave a product (II) which was recrystallised from ether-chloroform to constant melting point 117-30° ($\nu_{max}^{film}$ 1780 cm.$^{-1}$). The conditions of the thiolation reaction were varied but starting material was always recovered unchanged.

When the reaction was carried out using p-toluensulphonic acid as catalyst in boiling benzene with azeotropic separation of water, the product isolated showed $\nu_{max}^{film}$ 1805, 1773, 1730, 1656 cm.$^{-1}$ and $\lambda_{max}$ 228 mμ (ε 11000). The same product was obtained in absence of ethanedithiol.

Preparation of unsaturated γ-lactone mixture. (XXXV + XXXVI). A mixture of keto ester (24 g.) and freshly distilled methanesulphonic acid (20 ml.) in benzene (250 ml.) was heated under reflux, with azeotropic separation of water, for 48 hrs. The reaction mixture was poured into ice. The aqueous phase was extracted with ether, the combined organic solutions were washed with saturated sodium bicarbonate solution and water.

The oil obtained after removal of the solvents was distilled at 110-120°/1x10$^{-6}$mm. The product obtained (17.95 g., $n_D^{20°}$ = 1.4692) showed $\nu_{max}^{film}$ 1656, 1730, 1773, 1805 cm.$^{-1}$; $\lambda_{max}$ 228 mμ (ε 11000), $\lambda_{max}^{OH^-}$ 285 mμ (ε 16400-18500).

Found, C,56.9, 56.61, 57.98; H,6.24, 6.59, 6.75;
OEt,29.28 per cent.

Calc. for $C_{17}H_{24}O_8$: C,57.29; H,6.79; OEt,37.8 per cent.

The period of reflux did not affect the infrared and ultraviolet absorptions but it had some effect on the refractive index, (after 8 days of reflux the product had $n_D^{20°}$ = 1.4707 (distilled at 110-120°) and $n_D^{20°}$ = 1.4770 (120-130°).

Treatment of the unsaturated γ-lactone mixture with triethylamine. The γ-lactone mixture (8.29 g.) was heated on the steam bath with freshly distilled triethylamine (10 ml.) for 24 hrs. On addition of the triethylamine a violet colour appeared which changed to magenta on heating. The reaction mixture was poured into cold dilute sulphuric acid, (50 ml.), the aqueous phase was extracted with ether. The ethereal solution was washed with saturated sodium bicarbonate solution and water,and dried. After removal of the solvent, the residue (5.529 g.) was distilled under high vacuum at 100°/1x10$^{-5}$ cm. The product obtained (2 g., $n_D^{20°}$ = 1.4645) showed $\nu_{max}^{film}$ 1802, 1773, 1730, 1653, 1639 cm.$^{-1}$;

$\lambda_{max}$ 228 mμ (ε 9000); $\lambda^{OH^-}_{max}$ 285 mμ (ε 17000).

Catalytic hydrogenation of the unsaturated γ-lactone ester. The unsaturated γ-lactone ester (2.47 g.) in ethanol (25 ml.) was catalytically reduced over 10 per cent. palladised charcoal (0.500 g.). The reaction was followed by U.V. absorption in alkaline solution and the catalyst had to be dhanged three times. The oil obtained after removal of the solvent was distilled at $105^{o}/1x10^{-6}$mm. It showed $\nu^{film}_{max}$ 1780, 1730 cm.$^{-1}$; $\lambda^{OH^-}_{max}$ 285 mμ (ε 1560).

Treatment of saturated γ-lactone with potassium-t-butoxide. Freshly cut potassium (3 g.) was dissolved in t-butanol (100 ml.) (distilled from sodium after heating under reflux overnight). The saturated γ-lactone (1.515 g., $\lambda^{OH^-}_{max}$ 285 mμ (ε 4450) in t-butanol (50 ml.) was added dropwise. All operations were carried out under nitrogen. The reaction mixture was stirred overnight at room temperature. It was cooled and dilute sulphuric acid added (25 ml.). The t-butanol was removed under vacuum at $50^{o}$ and the aqueous solution was extracted continuously with ether. The oil obtained on removal of the solvent (1.26 g.) was saponified with dilute sodium hydroxide solution (50 ml.) by heating on the steam bath for 4 hrs. under nitrogen. It was acidified with dilute sulphuric acid (52 ml.) and extracted continuously with ether. The oily product obtained (0.814 g.) was chromatographed over acid washed silica gel (17 g.), benzene, chloroform

and acetone were used as eluents. On elution with chloroform a solid product was obtained (188 mg.) which was recrystallised from chloroform to constant m.p. 140-158°. It showed $\lambda_{max}$ 247 mμ (ε 12500), $\nu_{max}^{CHCl_3}$ 1700 cm.$^{-1}$ and characteristic COOH absorption in the 3000-2500 cm.$^{-1}$ region.

Found: C,56.58; H,5.61 per cent.

$C_{10}H_{12}O_5$ (XXXIX) requires C,56.6; H,5.30 per cent.

The methyl ester was obtained as an oil, it showed $\nu_{max}^{film}$ 1730-1701 cm.$^{-1}$, $\lambda_{max}$ 244 mμ (ε 8400). The dinitrophenylhydrazone of the dimethyl ester was prepared and purified by recrystallisation from ethyl acetate to constant m.p. 213-5°. It showed $\lambda_{max}^{CHCl_3}$ 385 mμ (ε 18700). On elution with acetone/chloroform (1:9) a solid product was obtained (315 mg.) which was recrystallised from acetone-chloroform mixture to a constant m.p.150-160°; $\nu_{max}^{nujol}$ 1700 cm.$^{-1}$; $\lambda_{max}$ 210 mμ (ε 12300).

Found C,48.08; H,5.09 per cent.

$C_{11}H_{14}O_8$ (XL) requires C,48.18; H,5.15 per cent.

On treatment of the unsaturated γ-lactone ester with potassium t-butoxide under the same conditions only the diacidketone (I) and the spirolactone (II) could be isolated.

Hydrolysis of the unsaturated γ-lactone mixture. Synthesis of glauconin. The mixture of unsaturated γ-lactones (5 g.) was saponified with 2N sodium hydroxide

solution (70 ml.) by heating on the steam bath for 3 hrs. under nitrogen. The solution was acidified in cold with dilute sulphuric acid solution and extracted continuously with ether for two days. The oily product obtained on removal of the solvent (3.5454 g.) was fractionally crystallised from ether and a mixture of ethyl acetate-benzene. The solid product obtained (total amount 1.03 g., m.p. 90-130°) did not show any U.V. absorption and was identified as the ketodiacid (I) (IR identical). The I.R. spectrum of the oily residue suggested the presence of unsaturated γ-lactone acid in the mixture. It was chromatographed over acid washed silica gel. On elution with benzene-ether (4:1) an oily product was obtained whose I.R. spectrum showed it to be a mixture of the spirolactone (II) ($\nu_{max}^{CHCl_3}$ 1780 cm.$^{-1}$) and α,β-unsaturated γ-lactone acid ($\nu_{max}^{CHCl_3}$ 1760, 1710 cm.$^{-1}$). The fractions obtained on elution with higher proportion of ether showed an increase in acid content in relation to the α,β-unsaturated γ-lactone ($\nu_{max}^{CHCl_3}$ 1760 cm.$^{-1}$ band). After elimination of the neutral fraction (spirolactone) and some ketodiacid (I) by direct crystallisation from ether-petroleum ether, the residue was sublimed at 120-130°/1x10$^{-6}$ cm. The oily product obtained was washed with ether. The ether soluble fraction showed $\lambda_{max}$ 228 mμ (ε 10600, M.W.242) $\nu_{max}^{CHCl_3}$ 1720, 1760, 1820 cm.$^{-1}$, it was not identified. The product not soluble in ether was separated as white needles m.p.166-71°.

(4 mg., 0, 15 per cent yield). It was identified as glauconin (m.p. 170-1°) mixed melting point (168-71°). It showed $\lambda_{max}$ 208 mμ (ε 16000), 250 mμ (ε 8900) (glauconin shows $\lambda_{max}$ 208 mμ (ε 15000), 250 mμ (ε 8100)), $\nu_{max}^{CHCl_3}$ 1675, 1773, 1825 $cm.^{-1}$. The I.R. spectra in potassium chloride disc of the synthetic material and the product obtained by pyrolysis of glauconic acid, were identical.

The reaction was repeated. The mixture of unsaturated γ-lactones (10.g) was saponified with 2N sodium hydroxide solution (100 ml.) by heating on the steam bath for 4 hrs. under nitrogen. On acidification and continuous extraction with ether ($2\frac{1}{2}$ days) an oil was obtained (5.92 g.) which was heated under reflux with acetyl chloride (50 ml.) for $\frac{1}{2}$ hr. The acetyl chloride was removed under vacuum, water added to the residue and the mixture extracted with chloroform. The chloroform solution was extracted three times with a saturated solution of sodium bicarbonate and washed with water. The sodium bicarbonate solution was acidified and extracted continuously with ether. After removal of the solvent, the oily product obtained was sublimed at $120°/1x10^{-6}$ cm. The product so obtained, mainly spirolactone (II) ($\nu_{max}^{CHCl_3}$ 1780 $cm.^{-1}$) was washed repeatedly with ether. Glauconin (m.p.168-71°, 2 mg.) was so obtained.

The chloroform soluble fraction was extracted several times with saturated sodium bicarbonate solution. The

neutral fraction was mainly spirolactone (II) ($\nu_{max}$ 1780 cm.$^{-1}$). The sodium bicarbonate solution was acidified and extracted continuously with chloroform. After removal of the solvent the oily product (85.6 mg.) was washed repeatedly with ether. Glauconin was isolated (13 mg., m.p. 168-71°). The ether soluble fraction gave more glauconin (10 mg., m.p. 170-1°) on sublimation and ether trituration. Glauconin was obtained (25 mg.) in 0.4 per cent yield.

Synthesis of dihydroglauconinic acid. Sodium borohydride (2 g.) in a mixture of methanol-ethanol (1:1) was added slowly to a solution of the keto ester (10.287 g.) in methanol (20 ml.). The addition (1 hr.) was carried out at 0°. The reaction mixture was left during 1 hr. at room temperature, glacial acetic acid was added (pH 4-5) and the alcohol removed under vacuum at 50°. Water was added and the aqueous solution extracted with ether. The ethereal solution was washed with water and dried. On removal of the ether the product obtained (7,751 g.) showed $\nu_{max}^{film}$ 1730, 1780, 3450 cm.$^{-1}$. It was treated with phosphorous oxychloride (25 ml.) and pyridine (200 ml.) at room temperature for 24 hrs. The pyridine was removed under vacuum, the residue was poured into cold dilute hydrochloric acid and extracted with chloroform. The chloroform solution was washed with dilute hydrochloric acid, bicarbonate solution and water, and dried. After

removal of the solvent the residue was distilled at $100^{\circ}/1x10^{-6}$ cm. The product obtained (3.90 g.), ($\nu_{max}^{film}$ 1724, 1780 $cm.^{-1}$; $\lambda_{max}$ 215 mμ (ε 4100), no change in alkaline solution) was saponified with 2N sodium hydroxide solution (100 ml.) by heating on the steam bath for 8 hrs. under nitrogen. On acidification and continuous extraction with ether an oil was obtained (2.1 g.) which was chromatographed over silica gel (100 g.). On elution with benzene-ether (4:1) an oil was obtained which showed $\lambda\lambda_{max}$ 215 mμ (ε 3000), 250 mμ (ε 2000), it was sublimed at $120^{\circ}/1x10^{-4}$ mm. yielding an oil.

Elution with benzene-ether (6:4) gave an oil which showed only $\lambda_{max}$ 215 mμ (ε 2300) in the U.V. On crystallisation from chloroform-ether a solid was obtained (m.p. 200°) which did not have any U.V. absorption and was not studied further. The residue was sublimed at $100\text{-}120^{\circ}/1x10^{-6}$ cm. to give an oil. Both sublimation products were dissolved in water, the aqueous solution was extracted with chloroform, and then repeatedly with ether. The product obtained after removal of the ether (200 mg.) was crystallised from water to constant melting point 165-70° (11.6 mg., 0.16 per cent yield). Mixed melting point with dihydroglauconinic acid obtained on hydroiodic acid reduction of glauconin (m.p. 160-72°) showed no depression ($\lambda\lambda_{max}$ 208 mμ (ε 7700), 250 mμ (ε 3560); $\nu_{max}^{nujol}$ 1690, 1778, 1865 $cm.^{-1}$ and the typical COOH absorption in the 3000-2500 $cm.^{-1}$ region).

Found: C,51.58; H,4.87 per cent.

$C_{11}H_{12}O_7$ requires C,51.56; H,4.72 per cent.

Bromination of the ketoester with N-bromosuccinimide. The ketoester (3.47 g.), N-bromosuccinimide (1.58 g., 1 molar equivalent) and few crystals of benzoyl peroxide in carbon tetrachloride, were heated under reflux for 4 hrs. The succinimide was filtered off and the solvent removed under vacuum. The residue was washed with hot water and extracted with ether. On removal of the solvent an oil was obtained (4 g., $n_D^{20°}$ = 1.4650). It analysed for monobromo derivative (found Br, 14.05, calc. Br, 16.3 per cent) ($\nu_{max}^{film}$ 1730, shoulder at 1748 cm.$^{-1}$; $\lambda_{max}$ 208 mμ (ε 2680), $\lambda_{max}^{OH^-}$ 285 mμ (ε 14800).

Bromination of the ketoester with bromine in acetic acid. A solution of bromine (4.8 g., 2 molar equivalents + 10 per cent. excess) in glacial acetic acid (50 ml.) was added dropwise during 75 min. to a solution of the ketoester (5.27 g.) in glacial acetic acid (10 ml. containing 0.1 ml. of a 50 per cent. solution of hydrobromic acid in acetic acid). The bromine was rapidly consumed. The reaction mixture was left overnight at room temperature, then poured onto ice, solid sodium metabisulphite added and the mixture extracted with chloroform. The organic solution was washed with sodium bicarbonate solution and water. On removal of the solvent an oil was obtained

(6.3 g., $n_D^{20°}$ = 1.4690) which analyses for a dibromo compound (found Br,25.05 per cent., calc.Br,28.6 per cent.) $\nu_{max}^{film}$ 1742 cm.$^{-1}$; $\lambda\lambda_{max}$ 208 mμ (ε 2340), 266 mμ (ε 320), $\lambda_{max}^{OH^-}$ 285 mμ (ε 13400).

Experiments on dehydrobromination of the bromo compounds.

a) The dibromoketoester (1.166 g.) in purified and dry dimethylformamide (10 ml.) containing lithium chloride (0.51 g.) was heated at 100° for 3 hr. under nitrogen. Cold water was added and the mixture extracted with chloroform. On removal of the solvents the oily residue (0.848 g.) was distilled at 100°/1x10$^{-6}$ cm. to give an oil (0.6634 g.) which showed $\nu_{max}^{film}$ 1730 cm.$^{-1}$; $\lambda_{flat}$ 208-227mμ (ε 1840), $\lambda_{max}^{OH^-}$ 285 (ε 14100).

b) The dibromoketoester (0.5 g.) in dry dimethylformamide (5 ml.) was heated at 100° for 24 hrs. under nitrogen. It was worked up as described in the preceding section giving an oil which after distillation showed ($n_D^{20°}$ = 1.4556) $\nu_{max}^{film}$ 1730 cm.$^{-1}$; $\lambda_{flat}$ 210-228 mμ (ε 3440), shoulder 264 mμ (ε 1100) $\lambda_{max}^{OH^-}$ 285 mμ (ε 18200).

c) The dibromoketoester (0.3 g.), in freshly distilled γ-collidine (10 ml.), was heated at 100° for 3 hr. under nitrogen. It was allowed to reach room temperature, ether was added and the ethereal solution washed several times with dilute hydrochloric acid and water and dried. The residue obtained after removal of the ether was distilled

at $100^{\circ}/1x10^{-4}$ mm. yielding an oil (150 mg., $n_D^{20^{\circ}} = 1.4550$) which showed $\lambda_{max}$ 210 mμ (ε 2880), shoulder at 264 mμ (ε 1510), $\lambda_{max}^{OH^-}$ 285 mμ (ε 18400).

The same results were obtained on treatment of the monobromoketoester under the same conditions.

Dihydroglauconinic acid from the product of debromination. To the product (1.1 g.) obtained on the dehydrobromination experiments described above, in methanol at $0^{\circ}$, solid sodium borohydride (250 mg.) was added slowly. The reaction mixture was worked up as described previously. An oil was so obtained (0.877 g.) ($\nu_{max}^{film}$ 3450, 1780, 1718 $cm.^{-1}$, λ 210-228 mμ (ε 1500) $\lambda_{max}^{OH^-}$ 285 mμ (ε 3000)), which was left overnight at room temperature with phosphorous oxychloride (5 ml.) and pyridine (25 ml.). On working up the reaction mixture an oil was obtained (0.607 g.) ($\nu_{max}^{film}$ no OH, 1783, 1730 $cm.^{-1}$, $\lambda_{max}$ 220 mμ (ε 2140), $\lambda_{max}^{OH^-}$ 285 mμ (ε 1280). It was saponified with 2N sodium hydroxide solution (10 ml.) by heating at $100^{\circ}$ for 4 hr. under nitrogen. The solution was acidified and extracted continuously with ether. The oily product obtained on removal of the solvent (0.445 g.) was heated on the steam bath with acetyl chloride (5 ml.) for $\frac{1}{2}$ hr. The acetyl chloride was removed under vacuum. The residue (276 mg.) was dissolved in chloroform and the solution extracted with sodium bicarbonate. The alkaline solution was acidified and extracted continuously with ether. The

oily residue obtained on removal of the solvent, was sublimed at 110-120°/1x10$^{-6}$ cm. and washed with chloroform. A solid product was so obtained (m.p. 158-71°, 17 mg., 1.5 per cent. yield) which was recrystallised from water to constant m.p. 160-72°. ( $\lambda\lambda_{max}$ 210 mμ (ε 6750), 250 mμ (ε 4150); $\nu_{max}^{nujol}$ 1684, 1773, 1838 cm.$^{-1}$, and characteristic COOH absorption in the 3000-2500 cm.$^{-1}$ region). The mixed melting point with a sample of dihydroglauconinic acid obtained from glauconin and subjected to the same treatment (sublimation and recrystallisation from water), m.p. 160-72°, did not show depression.

Purification of glauconic acid. Glauconic acid was purified by chromatography over silica gel, benzene-ether mixture (9:1) was used as eluent, and recrystallised from benzene-ethyl acetate (m.p. 200-202°) or chloroform (m.p. 196-8°). $[\alpha]_D$ + 32°(c=1.5); $\nu_{max}^{CHCl_3}$ 1667, 1770, 1825, 3300, 3560 cm.$^{-1}$, $\lambda_{max}$ 223 mμ (ε 10500), inflexion ca 260 mμ.

Pyrolysis of glauconic acid.[63] Glauconic acid (3.77 g.) was heated under nitrogen in a quinoline bath (b.p.230-240°) for 30 min. The outlet was connected to a cold trap (-55°) and then to a trap containing dinitrophenylhydrazine hydrochloride in methanol. The diethyl acrolein collected

in the cold trap was purified by distillation. The N.M.R. spectrum revealed a trans configuration.
The remaining diethyl acrolein was transformed into its dinitrophenylhydrazone. It was purified by chromatography over bentonite-celite (1:1) and recrystallisation from methanol (75 mg., m.p. 171-3°; $\lambda_{max}^{CHCl_3}$ 280 mμ (ε 31500). The solid residue was recrystallised from a mixture of ether-chloroform. Glauconin was so obtained (782 mg., m.p. 162-71°, yield 30 per cent.). It was recrystallised to constant m.p. 170-71°. ($\nu_{max}^{CHCl_3}$ 915, 945, 1675, 1773, 1825, shoulder at 1873 $cm.^{-1}$; $\lambda\lambda_{max}$ 208 mμ (ε 14900), 250 mμ (ε 7450); N.M.R. (dioxane) showed a singlet at τ 7.91 p.p.m.; N.M.R. (trifluoroacetic acid) a singlet at τ 7.71 p.p.m. and singlet at τ 6.26 p.p.m. The relative intensities were 3:1.

Reduction of glauconin with hydroiodic acid, and red phosphorous.[68] Glauconin (150 mg.) in hydroiodic acid (3.75 ml., sp.gr. 1.94) and a little red phosphorous, was heated under reflux in an oil bath (140-150°) for 5 hr. The red phosphorous was filtered off and the solution left at 0° for two days. A solid separated under these conditions (10 mg.). On evaporation of the mother liquor more solid product was obtained (29.6 mg.). Both products were recrystallised from water to constant melting point 160-76° (reported [68] m.p. 198-200°) ($\lambda\lambda_{max}$

208 mμ (ε 8800), 250 mμ (ε 3650); $\nu_{max}^{nujol}$ 1684, 1773, 1838 cm.$^{-1}$ and characteristic COOH absorption in the 3000-2500 cm.$^{-1}$ region).

Glauconin dimethyl ester.[57] Glauconin (292 mg.) was heated with 2N potassium hydroxide solution (2.63 ml.) for $\frac{1}{2}$ hr. at 100°. The solution was cooled to room temperature, dimethyl sulphate (0.6 g.) was added and the mixture shaken for 45 hrs. at room temperature. The solid formed was filtered off and extracted continuously with ether (Soxhlet) for 48 hrs. The unchanged glauconin crystallised out (120 mg.). The ethereal solution was reduced to 2 ml. and carbon tetrachloride added. Dimethyl glauconin was so obtained (70.6 mg., m.p.70-77°). It was repeatedly recrystallised from carbon tetrachloride to constant m.p. 74-77°. ($\nu_{max}^{CCl_4}$ 915, 930, 1645, 1730, 1773, 1825 cm.$^{-1}$; $\lambda_{max}^{CHCl_3}$ 248 mμ (ε 6400), $\lambda_{max}$ 208 mμ (ε 15400), inflection at 240 mμ (ε 7400). The N.M.R. spectrum in carbon tetrachloride showed τ 8.051, 8.016; 6.563; 6.368; 6.279 p.p.m.

Catalytic hydrogenation of dimethyl glauconin.[68] Dimethyl glauconin (22 mg.) in absolute methanol (3 ml.) was reduced catalytically over platinum oxide (10.8 mg.) The hydrogenation was stopped after 1 mol. of hydrogen was consumed. The solvent was removed under vacuum, ether was added and the solid filtered off. On removal

of the ether an oil was obtained (20 mg.), which was saponified by heating with 2N potassium hydroxide solution for 2 hr. at 100°. On acidification a solid separated (5 mg.) which was identified as glauconin (mixed m.p. showed no depression). The aqueous solution was extracted with ether. The product obtained on removal of the ether (12 mg.) was crystallised from ether (m.p. 155-70°). (reported[68] m.p. 195°). Mixed melting point with the product obtained from hydroiodic acid treatment (m.p. 160-72°) showed no depression, (155-72°).

Glauconic acid ketone. Glauconic acid (1.5 g.) in acetone (25 ml., AnalaR) was oxidised by titration with an 8N chromic acid solution (17.5 g. of chromic oxide in 50 ml. water and 14.8 ml. concentrated sulphuric acid) at 0°. It consumed 1.05 ml. of the chromic acid solution (1 mol. equivalent). The solution was poured into ice water, solid sodium methabisulphite was added and the solution extracted with chloroform (250 ml.). The chloroform solution was washed twice with water and dried. On removal of the solvent an oil was obtained which solidified on addition of ether. It was recrystallised from acetone-ether mixture. (944 mg., m.p. 163-73°)($\nu_{max}^{CHCl_3}$ 1661, 1712, 1776, 1825 cm.$^{-1}$)(A pure sample,[62] m.p.174-6° (dec), $[\alpha]_D$ + 45 (c=0.56) $\lambda_{max}$ 220 mμ (ε 10000); $\nu_{max}^{nujol}$ 1695, 1770, 1830 cm.$^{-1}$, $\nu_{max}^{CHCl_3}$ 1715, 1770, 1827 cm.$^{-1}$.)

Found: C,62.76; H,5.36 per cent.

$C_{18}H_{18}O_7$ requires C,62.42; H,5.36 per cent.

Zinc dust reduction of glauconic acid ketone. Glauconic acid (5.01 g.) was oxidised to the glauconic acid ketone as described (3.7 ml. of 8N chromic acid solution were consumed, the titration was carried out over a period of $1\frac{1}{2}$ hr. The crude glauconic acid ketone (4 g.) was heated with zinc dust (20 g. AnalaR) in glacial acetic acid (100 ml., AnalaR) under reflux for 24 hrs. The solid material was filtered off and the acetic acid removed under vacuum. Water and a few drops of hydrochloric acid were added to the oily residue and the whole extracted with ethyl acetate. On removal of the solvent an oil was obtained (3.7 g.) which was chromatographed over silica gel (400 g.). On elution with benzene-ether (9:1) a solid product (540 mg.) was obtained (acid A). It was recrystallised from chloroform-n-hexane to constant m.p. 170-80° (76 mg.) ( $[\alpha]_D$ + 8°(c=1); $\lambda_{max}$ 225-8 mμ (ε 8600); $\nu_{max}^{CHCl_3}$ 1670, 1700, 1716, 1770, 1830 cm.$^{-1}$, and typical COOH absorption at 3000-2500 cm.$^{-1}$).

Found: C,62.94; H,6.81 per cent.

$C_{17}H_{22}O_6$ requires C,63.34; H,6.88 per cent.

Its methyl ester was prepared by treatment with an ethereal solution of diazomethane. It was purified by recrystallisation from ether-n-hexane to constant melting point 104-105.5° or 115-7°. ( $[\alpha]_D$ ± 0(c=1); $\lambda_{max}$ 225 mμ

($\varepsilon$ 8300); $\nu_{max}^{CHCl_3}$ 1670, 1700, 1725, 1770, 1830 cm.$^{-1}$).

Found: C,64.52; H,7.24 per cent.

$C_{18}H_{24}O_6$ requires C,64.27; H,7.19 per cent.

The acid A (94 mg.) was heated with zinc (2 g.) in glacial acetic acid (10 ml.) under reflux for 12 hr. On working up as described, starting material was quantitatively recovered unchanged.

Elution with ether yielded a second solid product (1.3 g.), (acid B), which was recrystallised to constant m.p. 167-70° from acetonitrile or ethyl acetate-n-hexane. ($[\alpha]_D^{acetone}$ -90°(c=1); in the U.V. it only showed end absorption ($\varepsilon_{220}$ 485); $\nu_{max}^{nujol}$ 1742, 1709 cm.$^{-1}$ and characteristic COOH absorption in the 3000-2500 cm.$^{-1}$ region.)

Found: C,60.47; H,7.22 per cent.

$C_{17}H_{24}O_7$ requires C,59.99; H,7.11 per cent.

Equivalent (titration) 113, calc. 113.3.

The sodium salt showed $\nu_{max}^{KBr}$ 1725 cm.$^{-1}$.

The trimethyl ester was prepared by reaction with an ethereal solution of diazomethane. It was recrystallised from ether-n-hexane to constant m.p. 104-7° ($[\alpha]_D$ -101° (c=1), $\nu_{max}^{CHCl_3}$ 1730 cm.$^{-1}$)

Found: C,63.31, 63.34, 63.04; H,7.94, 7.97, 8.07 per cent.

$C_{20}H_{30}O_7$ requires C,62.81; H,7.91 per cent.

The triacid, on sublimation at $160^{\circ}/1 \times 10^{-5}$ mm., gave a solid which was recrystallised to constant m.p. 160-8°, from chloroform-n-hexane. ($[\alpha]_D$ -59°(c=1.1), U.V. only showed end absorption ($\varepsilon_{220}$ 410); $\nu_{max}^{CHCl_3}$ 1730, 1780, 1848 cm.$^{-1}$, $\nu_{max}^{nujol}$ 1701, 1730, 1770, 1838 cm.$^{-1}$ and the typical COOH absorption at 3000-2500 cm.$^{-1}$)

Found: C,64.62, 64.04; H,7.17, 7.30 per cent.

$C_{17}H_{22}O_6$ requires C,63.34; H,6.88 per cent.

Attempts to prepare the monomethyl ester anhydride by reaction with diazomethane in ether, gave mainly the trimethyl ester and an oil which showed in the I.R. ($\nu_{max}^{CHCl_3}$ 1730, 1780, 1848 cm.$^{-1}$) which could not be obtained crystalline.

Zinc dust reduction of glauconic acid ketone at room temperature. Crude glauconic acid ketone (270 mg.) was shaken overnight at room temperature with zinc dust (2 g.) in glacial acetic acid (25 ml.). The reaction mixture was worked up as described, avoiding heating during the process. The oily product obtained (150 mg.) was purified by chromatography over silica gel (10 g.). On elution with benzene-chloroform (1:4) a solid product was obtained which was recrystallised from chloroform-n-hexane to constant m.p. 170-80° (mixed m.p. with acid A showed no depression) (7 mg.), ($\lambda_{max}$ 228 mμ ($\varepsilon$ 7400); $\nu_{max}^{CHCl_3}$ 1672, 1709, 1724, 1776, 1838 cm.$^{-1}$ and COOH absorption at 3000-2500 cm.$^{-1}$).

Dehydrogenation of the tricarboxylic acid anhydride methyl ester. Crude methyl ester anhydride (40 mg.) was heated under nitrogen at 300° with 20 per cent. palladised charcoal[94] (10 mg.). The ester did not keep in contact with the catalyst due to distillation of the oil. On working up the starting material was recovered unchanged (I.R.).

Dehydrogenation of the tricarboxylic acid in an open tube. The acid (18.6 mg.) was heated under nitrogen with 20 per cent. palladised charcoal (10 mg.) at 300° for 2 hr. The material sublimed leaving the catalyst dry. It was cooled to room temperature, ethyl acetate was added and the palladised charcoal filtered off. On removal of the solvent an oil was obtained (6.2 mg.) which gave an oily solid on sublimation at 110°/1x10$^{-5}$mm. ($\lambda_{max}$ 235 mμ (ε 24.75 x M.W.); $\nu_{max}^{CHCl_3}$ 1848, 1783, 1695, 1639 cm.$^{-1}$ and COOH absorption at 3000-2500 cm.$^{-1}$).

Dehydrogenation of the Tricarboxylic Acid in naphthalene. The tricarboxylic acid (50 mg.) in naphthalene (2.03 g.) was heated under reflux (nitrogen) with 20 per cent. palladised charcoal (15 mg.) for 18 hrs. Ether was added, the catalyst removed and the ethereal solution extracted with a saturated sodium bicarbonate solution. On acidification and continuous extraction with ether an oil was obtained (31 mg.) which was sublimed at 160°/1x10$^{-5}$ cm. The infrared spectrum was identical to the

tricarboxylic acid anhydride.

Dehydrogenation of the acid in a vacuum sealed tube. The tricarboxylic acid (50 mg.) was heated with 20 per cent. palladised charcoal[94] (50 mg.) in a vacuum sealed tube for 12 hrs. at 330°. Ethyl acetate was added and the catalyst removed. On evaporation of the solvent an oil was obtained (21 mg.) which showed $\nu_{max}^{film}$ 1700, 1610 cm.$^{-1}$; $\lambda\lambda_{max}$ 208 mμ (ε 18000), 250 mμ (ε 8100), 274 mμ (ε 2500), 297 mμ (ε 1800). The dinitrophenylhydrazone was prepared and purified by chromatography over bentonite-celite (1:1) (eluent benzene) and recrystallisation from ethanol-chloroform to constant m.p. 160-65°. ($\lambda_{max}^{CHCl_3}$ 395 mμ (ε 33700) Found C,62.99; H,6.07; N,14.73 per cent.

$C_{20}H_{22}O_4N_4$ requires C,62.81, H,5.80; N,14.65 per cent.

b) The tricarboxylic acid (260 mg.) was heated with 20 per cent. palladised charcoal[94] (70 mg.) in a vacuum sealed tube for 24 hrs. at 290°. The product obtained (153 mg.) showed in the I.R. the presence of acidic material ($\nu_{max}^{film}$ 1610, 1700, 1730, 1780 cm.$^{-1}$ and COOH absorption at 3000-2500 cm.$^{-1}$). It was dissolved in chloroform and extracted with saturated sodium bicarbonate solution. The neutral fraction (90 mg.) was distilled at 100°/0.05 mm. The oil obtained (50 mg.; $\nu_{max}^{film}$ 1610, 1701, weak absorption at 1730, 1780 cm.$^{-1}$) showed in the

N.M.R. - two aromatic methyl groups at 7.72 p.p.m., and 7.58 p.p.m. The pattern of the aromatic protons is arranged as in p-methyl indanone but there was present an additional peak. The product was passed through alumina (eluent benzene) and the oil obtained (26 mg.) ($\nu_{max}^{film}$ 1610, 1701 cm.$^{-1}$; $[\alpha]_D \pm 0^o$(c=1); $\lambda_{max}$ 208 mμ (ε17800), 255 mμ (ε 8500), 277 mμ (ε 2120)), showed the same N.M.R. spectrum as before but the extraneous peaks were decreased to almost half of their original intensity. The dinitrophenyl hydrazone (m.p. 166-8°; $\lambda_{max}^{CHCl_3}$ 295 mμ (ε 30000)) did not show optical activity, $[\alpha]_D \pm 0^o$(c=1). A sample (29 mg.) of the dehydrogenation product was heated for 3 hrs. at 100° with 10 drops of triethylamine in 10 ml. of ethanol. The oil obtained on removal of the solvents (17 mg.; $\lambda\lambda_{max}$ 210 mμ (ε 13600), 255 mμ (ε 10300) and same infrared spectrum as the starting material) was converted to the dinitrophenyl hydrazone, (m.p. 166-8°).

Ethyl m-tolyl-carbinol.[96] To magnesium turnings (5 g., plus a crystal of iodine) covered with ether (10 ml.) a small portion of m-bromotoluene (34.7 g.) in dry ether (50 ml.) was added.[97] The reaction mixture was heated under reflux with stirring until a vigorous reaction took place. Heating was stopped and the remaining m-bromotoluene solution was added dropwise (1 hr.). The stirring was continued for 15 min. at room temperature

and 15 min. under reflux. The flask was cooled in an ice bath and freshly distilled propionaldehyde (11.8 g.) in ether (100 ml.) was added dropwise at room temperature avoiding a too vigorous reaction (30 min.). The stirring was continued for 30 min. at room temperature. The reaction mixture was cooled in an ice bath and a 25 per cent ammonium chloride solution was added. The ethereal solution was decanted and the solid mass washed with ether. The ethereal solutions were dried and the ether removed under vacuum. The residue was distilled. b.p. 72-80°/1 mm. (17.22 g., yield 54 per cent.). The infrared spectrum shows a strong OH absorption and no carbonyl band.

Ethyl m-tolyl ketone.[96] The ethyl m-tolylcarbinol (10 g.) in acetone (60 ml., AnalaR) was oxidised with an 8N chromic acid solution by titration at 0°. (It consumed 17 ml., 1 molar equivalent, in $1\frac{1}{2}$ hr.). Water was added and the excess of chromic acid destroyed with solid sodium methabisulphite. The aqueous solution was extracted with ether, the ethereal solution was washed and dried. The residue obtained on removal of the solvents was distilled under vacuum (b.p. 74-7°/1 mm.; 9.19 g. yield 85 per cent.) ($\nu_{max}^{film}$ 1685 cm.$^{-1}$; $\lambda\lambda_{max}$ 240 mμ (ε 11300), 278 mμ (ε 1000)).

Ethyl-α-ethyl-β-hydroxy-β(m-tolyl)-valerate. To zinc (7 g., activated as described) in dry benzene (20 ml.) a small portion of a mixture of ethyl m-tolylketone (12.6 g.) and ethyl α-bromobutyrate (18 g.) in benzene (to 100 ml.) was added. The reaction mixture was heated under reflux with stirring until a reaction started. The remaining reaction mixture was added dropwise (1 hr.) maintaining a gentle reflux. The reaction mixture was heated under reflux and stirred for one additional hour, cooled, and a dilute sulphuric acid solution added. The organic phase was separated, the aqueous phase was extracted with ether. The combined organic solutions were washed with dilute sulphuric acid solution, sodium bicarbonate solution, and water, and dried. The solvents were removed under vacuum. The residue (25 g.) was distilled twice under vacuum. The product obtained (14.90 g., 66 per cent yield; $n_D^{20^\circ}$ = 1.4945, b.p.112-4°/0.8 mm.) showed $\nu_{max}^{film}$ 3550, 1710 cm.$^{-1}$

Found: C,73.3; H,9.13; OEt, 16.67 per cent.

$C_{16}H_{24}O_3$ requires C,72.69; H,9.15; OEt, 17 per cent.

Ethyl α-ethyl-β(m-tolyl)-pent-2-enoate. The crude Reformatsky reaction product (16.6 g.) was heated under reflux with phosphorous pentoxide (11.5 g.) in benzene for 3 hrs. The benzene solution was decanted, the phosphorous pentoxide washed with benzene, and the

combined benzene solutions were left for $\frac{1}{2}$ hr. over anhydrous potassium carbonate, and the solvent removed under vacuum. The product obtained on fractional distillation (b.p. 122-8°/2 mm.; 9.2 g.; 60 per cent. yield, $n_D^{20°}$ = 1.5050) showed $\nu_{max}^{film}$ 1724 cm.$^{-1}$; U.V. strong end absorption, and flat absorption at 227 mμ (ε 6500).

Found: C,79.03; H,9.37; OEt, 14.44 per cent.

$C_{16}H_{22}O_2$ requires C,78.01; H,9.00; OEt, 18.3 per cent.

α-Ethyl-β-(m-tolyl)-valeric acid. The unsaturated ester (5.2 g.) in glacial acetic acid was catalytically hydrogenated over 10 per cent. palladised charcoal (500 mg.). It consumed 1 mole of hydrogen over a period of 1 hr. The catalyst was filtered off and the solvent removed under vacuum. The residue was distilled (b.p. 110-112°/1.5 mm.; 4.24 g., yield 80 per cent.; $n_D^{20°}$ = 1.4880; $\nu_{max}^{film}$ 1725 cm.$^{-1}$; $\lambda\lambda_{max}^{cyclohexane}$ strong end absorption; 265, 273 mμ (ε 530).

Found: C,77.98; H,9.9 (no OEt could be detected).

$C_{16}H_{24}O_2$ requires C,77.37; H, 9.74 per cent.

The ester (4.24 g.) was saponified with 10 per cent. potassium hydroxyde in ethanol-water (1:1) (100 ml.) by heating under reflux on the steam bath for 4 hrs. On working up most of the ester was recovered unchanged (3 g.) and some acid (1.5 g.) was isolated from the bicarbonate soluble fraction. The recovered ester was

saponified again by heating overnight with 10 per cent. sodium hydroxyde solution (50 per cent. ethanol). On working up some more acid was obtained (1.6 g.) which was distilled together with the previous product. (b.p. 146°/1.5 mm.; 2.278 g.; $n_D^{20°}$ = 1.5042; $\nu_{max}^{film}$ 1710 cm.$^{-1}$ and COOH absorption at 3000-2500 cm.$^{-1}$; $\lambda\lambda_{max}^{cyclohexane}$ strong end absorption, 260 mμ (ε 410), 273 mμ (ε 390).

p and o-methyl-2,3-diethylindanones. The acid (550 mg.) in ether (1 ml.) was added to polyphosphoric acid(from 80 per cent orthophosphoric acid (8 ml.) and phosphorous oxide (12 g.) heated on the steam bath for 2 hrs. to give an almost clear solution). The mixture was heated at 100° for 3 hrs. with occasional shaking. It was cooled to room temperature and poured into ice-water. The aqueous phase was extracted with chloroform. The chloroform solution was washed with saturated sodium bicarbonate solution and water. On removal of the solvent, the neutral residue (500 mg.) was distilled under vacuum, (b.p. ± 100°/1 mm.). The mixture of indanones obtained (360 mg.; $n_D^{20°}$ = 1.5360) showed $\nu_{max}^{film}$ 1700, 1610, 1600 cm.$^{-1}$; $\lambda\lambda_{max}$ 205 mμ (ε 27500), 252 mμ (ε 14500), 294 mμ (ε 3250). The N.M.R. spectrum (carbon tetrachloride solution) showed a distorted triplet at 46.5, 53.1, 60.5 c/s corresponding to the methyl groups

of the ethyl; a distorted quartet at 82.0, 88.6, 95.6, 102 c/s due to the methylene groups of the ethyl and two peaks, at 7.58 p.p.m. due to the aromatic methyl of the para-methyl-indanone, and 7.41 p.p.m. corresponding to the aromatic methyl of the ortho-methyl-indanone.

(Reference:[99] p-methyl indanone, aromatic methyl group 7.60 p.p.m.

o-methyl indanone, aromatic methyl group 7.46 p.p.m.)

The mixture of indanones (97 mg.) was heated for $\frac{1}{2}$ hr. on the steam bath with dinitrophenylhydrazine (200 mg.) in methanol (2 ml.) and the necessary amount of concentrated sulphuric acid to have a clear solution. A solid product separated on standing at room temperature (187 mg.) which was chromatographed over bentonite-celite (1:1) (eluent benzene) and recrystallised from ethanol-chloroform to constant m.p. 152-3° ($\lambda_{max}^{CHCl_3}$ 395 mμ (ε 30200).

Found: C,62.53; H,5.88; N, 14.03 per cent.

$C_{20}H_{22}O_4N_4$ requires C,62.81, H,5.8; N, 14.65 per cent.

The aqueous solution was extracted with chloroform. The chloroform solution was washed with water. The residue obtained on removal of the solvent was chromatographed over bentonite-celite (1:1) (eluent benzene) and recrystallised from ethanol/chloroform to constant melting point 174-80°; ($\lambda_{max}^{CHCl_3}$ 392 mμ (ε 27200)).

(The p-methylindanone dinitrophenylhydrazone (m.p.275-6°) showed[99] $\lambda_{max}^{CHCl_3}$ 395 mμ (ε 29400). The dinitrophenylhydrazone of o-methylindanone (m.p.281-2°) showed[99] $\lambda_{max}^{CHCl_3}$ 392 mμ (ε 30600)).

The mixture of indanones was heated with 20 per cent. palladised charcoal in a vacuum sealed tube for 24 hrs. at 280°. The dinitrophenyl hydrazone of the product obtained showed m.p. 145-53°.

REFERENCES.


1. Ruzicka, Experientia, 1953, 9, 357; Proc.Chem.Soc. 1959, 341.
2. Collie, J.Chem.Soc., (London), 1907, 91, 1806.
3. Birch in "Fortschritte der Chemie Organisher Naturstoffe", Zechmeister, vol.14, p.186 (1957).
4. Birch, Donovan, Austral. J. Chem., 1953, 6, 360.
5. Birch, Smith, Chem. Soc. Special Publ., 1958, 12, 1.
6. Birch, Massy-Westropp, Moye, Austral.J.Chem., 1955, 8, 539.
7. Chaplen, Thomas, Biochem.J., 1960, 77, 91.
8. Birch, Kocor, J.Chem.Soc., 1960, 866.
9. Musgrave, J.Chem.Soc., 1957, 1104.
10. Birch, Musgrave, Rickards, Smith, J.Chem.Soc., 1959, 3146.
11. Gatenbeck, Mosbach, Acta Chem.Scand., 1959, 13, 1561.
12. Gatenbeck, ibid., 1958, 12, 1211.
13. ibid., 1960, 14, 296
14. Birch, Massy-Westropp, Rickards, Smith, Proc.Chem. Soc., 1957, 98.
15. ibid., J.Chem.Soc., 1958, 360.
16. Barton, Cohen, "Festschrift A. Stoll", Basle, 1957, p.117.
17. Day, Nabeny, Scott, J.Chem.Soc., 1961, 4067.
18. Weiss, Davis, Miglioli, J.Amer.Chem.Soc., 1953, 75, 5572.

19. Davis in "Advances in Enzymology", 1955, vol.16, p.287.

20. Halsane, Haworth, Knowles, J.Chem.Soc., 1961, 1854.

21. Thomas, Biochem. J., 1961, 78, 807.

22. Barton, de Mayo, Morrison, Raistrick, Tetrahedron, 1959, 6, 48.

23. Underhill, Watkin, Neish, Canad.J.Biochem.Physiol., 1957, 35, 219.

24. Birkinshaw, Ann.Rev.Biochem., 1953, 22, 379.

25. Bu'Lock, Gregory, Biochem.J., 1959, 72, 322.

26. Bu'Lock, Ryan, Proc.Chem.Soc., 1958, 222.

27. Dean, Staunton, Whalley, J.Chem.Soc., 1959, 3004.

28. Birch, Blance, Smith, ibid., 1958, 4582.

29. Mosbach, Acta Chem.Scand., 1960, 14, 457.

30. Birch, English, Massy-Westropp, Slaytor, Smith, Proc.Chem.Soc., 1957, 204.

31. Birch, Fitton, Pride, Ryan, Smith, Whalley, J.Chem.Soc., 1958, 4676.

32. Robinson, "The Structural Relations of Natural Products", Clarendon Press, London, 1955, p.7.

33. Woodward, Angew.Chem., 1956, 68, 13; ibid., 1957, 69, 50; "Festschrift A. Stoll", Basle, 1957, p.524.

34. Corcoran, Kaneda, Butte, J.Biol.Chem., 1960, 235, PC29.

Grisebach, Achenback, Grisebach, Naturwiss., 1960, 47, 206.

35. Birch, Pride, Rickards, Thomson, Dutcher, Perlman, Djerassi, Chem. and Ind., 1960, 1245.

36. Ollis, Sutherland, Codner, Gordon, Miller, Proc.Chem.Soc., 1960, 347.

37. Stumpf, Ann.Rev.Biochem., 1960, 29, 261; Langdon, Phillips, ibid., 1961, 30, 189.

38. Bentley, Keil, Proc.Chem.Soc., 1961, 111.

39. Bu'Lock, Smalley, ibid., 1961, 209.

40. Birch, Cassera, Rickards, Chem. and Ind., 1961, 792.

41. Lynen, Tada, Angew.Chem., 1961, 73, 513.

42. Ruzicka, Eschenmoser, Heuser, Experientia, 1953, 9, 362.

43. Tavormina, Gibbs, Huff, J.Amer.Chem.Soc., 1956, 78, 4498;

Wolf, Hoffman, Wright, Folkers, Aldrich, Skeggs, ibid., 1956, 78, 4499;

Tavormina, Gibbs, ibid., 1956, 78, 6210.

44. Birch, Schofield, Smith, Chem. and Ind., 1958, 1321.

45. Birch, English, Massy-Westropp, Smith, J.Chem.Soc., 1958, 369.

46. Britt, Arigoni, Proc.Chem.Soc., 1958, 224.

47. Birch, Rickards, Smith, Harris, Whalley, ibid., 1958, 223.

48. Birch, Rickards, Smith, Harris, Whalley, Tetrahedron, 1959, 7, 241.

49. Fishman, Jones, Lowe, McWhiting, Proc.Chem.Soc., 1959, 127; ibid., J.Chem.Soc., 1960, 3959.

50. Birch, Rickards, Smith, Proc.Chem.Soc., 1958, 192.

51. Rudney in "Ciba Foundation Symposium on the Biosynthesis of Terpenes and Sterols", Churchill, London, 1959, p.75.

52. Bloch, ibid., p.4.

53. Lynen, Eggerer, Henning, Kessel, Angew. Chem., 1958, 70, 738.

54. Lynen, Agranoff, Eggerer, Henning, Moslein, ibid., 1959, 71, 657.

55. Eggerer, Henning, Agranoff, Lynen, J.Amer.Chem.Soc., 1959, 81, 1254.

56. Popjak, Goodman, Cornforth, Ryhazo, Cornforth, Biochem.J., 1961, 80, 15p.

57. Dauben, Richards, J.Amer.Chem.Soc., 1957, 79, 968.

58. Flavin, Castro-Mendoza, Ochoa, J.Biol.Chem., 1957, 229, 965, 981.

59. Cornforth, Cornforth, Horning, Pelter, Popjak, in "Ciba Foundation Symposium on the Biosynthesis of Terpenes and Sterols", 1959, p.119.

60. "The Pfizer Handbook of Microbial Metabolites", Max W. Miller; McGraw Hill, New York, 1961.

61. Ehrensvard, Chem.Soc.Special publ., 1958, 12, 13.

62. Professor Barton, Dr. Sutherland, personal communication.

63. Wijkman, Annalen, 1931, 485, 61.

64. Sutter, Wijkman, ibid., 1933, 505, 248.

65. Sutter, Rootmayr, Porsch, ibid., 1936, 521, 189.

66. Sutter, Wijkman, ibid., 1935, 519, 97.

67. Kraft, Porsch, ibid., 1937, 527, 168.

68. Kraft, ibid., 1937, 530, 20.

69. Ott, Schroter, Behr, Ber., 1928, 61, 2124.

70. Guthzeit, Engelman, J.Pr.Chem., (2), 66, 104, (1902).

71. Organic Synthesis, Coll. Vol.1, p.290.

72. Haworth, Perkin, J.Chem.Soc., 1908, 58;
Newman, McPherson, J.Org.Chem., 1954, 19, 1717.

73. Bartlett in "Organic Chemistry", Gillman, Chapman and Hall, New York, 1953, vol.3, p.104.

74. Blaise, Gault, Compt.rend., 1904, 139, 137.

75. (a) Lawrence, J.Chem.Soc., 1897, 71, 457;
(b) Shriner in "Organic Reaction", Roger Adams, John Wiley and Sons, New York, 1942, Vol.1, p.5.

76. Daimler, Keller, Annalen, 1888, 249, 182.

77. Wislenum, ibid., 1888, 246, 328.

78. Hagemeyer, Ind.Eng.Chem., 1949, 41, 765.

79. Caldwell, Chem.Abs., 1956, 50, P1559i; ibid., 1954, 48, P12169c; ibid., 1952, 46, P8672h.

80. Renfrow, Renfrow, J.Amer.Chem.Soc., 1946, 68, 1801.

81. Fieser, J.Amer.Chem.Soc., 1954, 76, 1945; Kovacs, Herout, Horak, Sorm, Coll.Czech, Chem.Comm., 1956, 21, 225.

82. Ralls, Riegel, J.Amer.Chem.Soc., 1954, 76, 4479.

83. Bernstein, Dorfman, ibid., 1946, 68, 1152.

84. Braude, Timmons, J.Chem.Soc., 1953, 3136.

85. Grove, Willis, ibid., 1951, 877.

86. Dauben, Hance, J.Amer.Chem.Soc., 1953, 75, 3352.

87. Ruzicka, Reichstein, Furst, Helv.Chim.Acta, 1941, 24, 76, 716; ibid., 1942, 25, 79.

88. Haynes, Jones, J.Chem.Soc., 1946, 954.

89. Rekke, Brombacher, Hamann, Nanta, Rec.trav.Chim., 1954, 73, 410.

90. Thiele, Annalen, 1901, 319, 144.

91. Woodward, J.Amer.Chem.Soc., 1941, 63, 1123.

92. Mayer, Wick, Schmidt, Chem.Ber., 1953, 86, 488.

93. Djerassi, Burnstein, Tetrahedron, 1959, 7, 37; Holysz, J.Amer.Chem.Soc., 1953, 75, 4432.

94. Newman, Zahn, J.Amer.Chem.Soc., 1943, 65, 1097.

95. Rottingen, Wenzel, Monatsh., 1913, 34, 1885.

96. Wallach, Ruishler, Annalen, 1908, 360, 61.

97. Gilman, McCracken, J.Amer.Chem.Soc., 1923, 45, 2462.

98. Barltrop, Acheson, Philpott, MacPhee, Hunt, J.Chem.Soc., 1956, 2928.

99. Mr. Forster, personal communication.

100. Mr. G. Smith of the London School of Hygiene and Tropical Medicine; personal communication to Professor Barton and Dr. Sutherland.

101. Professor Barton, Dr. Sutherland, Mr. Baldwin, personal communication.

102. Jackman, Wiley, J.Chem.Soc., 1960, 2881.

103. Jackman,"Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", Pergamon Press, London, 1959.

104. Professor Barton, Dr. Sutherland, Mr. Bloomer; personal communication.

105. Conroy, in "Advances in Organic Chemistry, Methods and Results", Interscience, New York, 1960, Vol.2, p.265.

106. Ramart-Lucas, Hoch, Bull.Soc.Chim.France, 1949, (5) 16, 447.

107. Braude, Experientia, 1955, 11, 457.

108. Granger, Corbier, Vinas,Nau, Bull.Soc.Chim.France, 1957, 810, 815.

109. Bowden, Heilbron, Jones, Weedon, J.Chem.Soc., 1946, 39.

110. Bergman, Ginsberg, Pappo, in "Organic Reactions", Roger Adams, 1959, Vol.10, p.179.

111. Cope, et al., J.Amer.Chem.Soc., 1940, 62, 441; ibid., 1941, 63, 1843, 1852.