STUDIES WITH SOME TERPENOIDS

by

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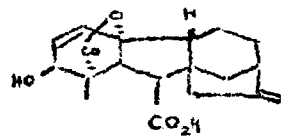
Abstract

The thesis describes some aspects of the chemistry of the terpenoid metabolites of the mould Gibberella fujikuroi. The chemistry of the gibberellins is briefly reviewed and illustrated by the chemistry of the 7-deoxygibberellins. Gibberellin A_7 is shown to have the structure and stereochemistry (1) by degradation and interrelationship with gibberellic acid. Its alkaline rearrangement is described. Gibberellins A_{10} and A_{11} are assigned the structures (2) and (3) respectively. The value of solvent shifts in the study of nuclear magnetic resonance spectra is illustrated by a study of the spectra of gibberellin derivatives in both pyridine and deuterochloroform solution.

The evidence which led to the assignment of the structures (4), (5) and (6) to fujenal, 7-hydroxykaurenolide and 7,18-dihydroxykaurenolide is reviewed. The interrelationship of (-)-kaurene and (+)-phyllocladene through the enantiomers of (7) is described. Nuclear magnetic resonance evidence for a 'twisted boat' conformation of ring B of the kaurenolides is presented and the consequences are explored in the photochemical activation of the C-10 methyl group from a 7α-hydroxyl group. The conversion of fujenal and 7-hydroxykaurenolide to compounds



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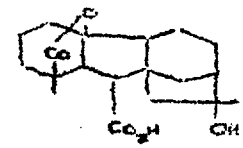

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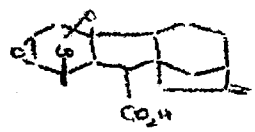

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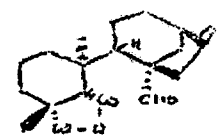

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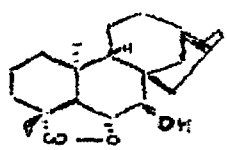

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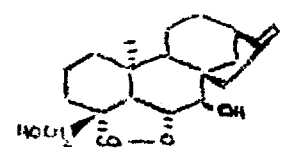

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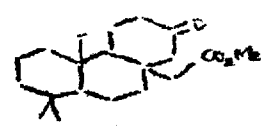

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of the gibbane skeleton is described. Structures are assigned to some oxidation by-products of 7-hydroxy-kaurenolide. Methods of preparing 6-ketones are described and the properties of this keto-group explored.

The partial synthesis of (-)-kaur-16-en-19-oic acid, recently isolated from Ricinocarpus stylosus, from 7-hydroxykaurenolide is described. The partial synthesis of 19-hydroxystachene, isolated from Erythoxylon monogynum, from isosteviol is described providing confirmatory evidence for the stereochemistry of the former.

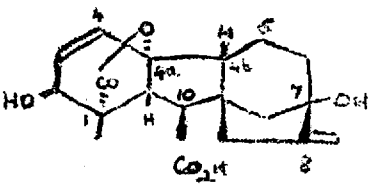
CHAPTER 1

Some Aspects of the Chemistry of the Gibbane Skeleton

1.1. The Gibberellins

The culture filtrate of some strains of the fungus Gibberella fujikuroi - the causative organism of the 'bakanae' disease of rice seedlings - contains a group of metabolites known as the gibberellins. The earlier chemical work,[1] which was limited almost exclusively to Japan, was hampered by the fact that the so-called 'gibberellin A' was a mixture of closely related compounds. Gibberellic acid,[2] which is the best known of the gibberellins, was isolated from strain ACC 917 at the Akers Research Laboratories of I.C.I. in the early 1950's. This metabolite affected many aspects of normal plant growth and development such as increasing stem length and enhancing apical dominance in bushy plants particularly genetic dwarfs. Subsequently small amounts of related gibberellins have been isolated from higher plants and are regarded as plant-growth hormones. The chemical and biological properties of these compounds have been the subject of a number of recent reviews.[3]

Gibberellic acid, $C_{19}H_{22}O_6$ (1) was characterized[4] as a tetracarbocyclic dihydroxy-γ-lactonic acid containing two ethylenic bonds. Insight into its structure was obtained by studying the products of acidic degradation.



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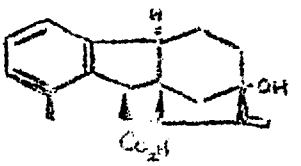

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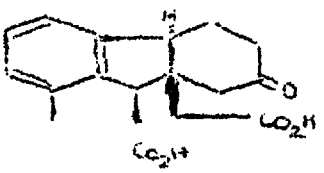

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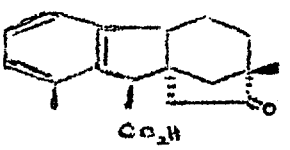

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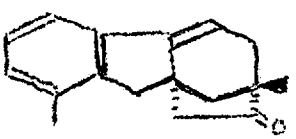

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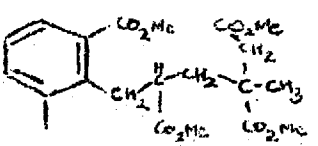

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Treatment with mineral acid at room temperature afforded carbon dioxide and two isomeric aromatic hydroxy-acids, allogibberic acid, $C_{18}H_{22}O_3$(2),[4,5,6] and 4b-epiallogibberic acid whilst more vigorous conditions gave an isomer, gibberic acid and its 4b- epimer. On dehydrogenation with selenium allogibberic acid gave gibberene[6] (1,7-dimethylfluorene)[7] thus revealing the unique carbon skeleton of the gibberellins. The hydroxyl group of allogibberic acid was tertiary and allylic to the double bond since on ozonolysis it gave formaldehyde and a five-ring ketol which could be oxidized to a keto-acid (3). The latter was dehydrogenated to 8-methylfluoren-2-ol thus locating the position of one end of the C_2 bridge forming ring D. The dicarboxylic acid (3) formed a <u>cis</u> six-ring anhydride which was hydrolysed by base to the parent acid, in turn relating the five-membered ring and the carboxyl group of allogibberic acid.[8]

On treatment with boiling mineral acid, both gibberellic acid and allogibberic acid gave a keto-acid, gibberic acid (4)[9] whilst epiallogibberic acid gave epigibberic acid. Under similar conditions the methyl esters of gibberellic acid and allogibberic acid gave methyl gibberate. The ketone of gibberic acid, which from its infra-red spectrum was situated in a five-membered ring, was oxidised to a non-enolisable α-diketone,

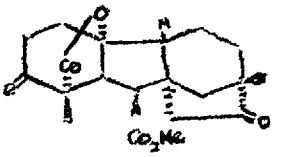
gibberdionic acid, the methyl ester of which was dehydrogenated to 9-methoxycarbonyl-1, 7-dimethylfluorene This established the position of the carboxyl group on the fluorene nucleus and showed that the five-membered ring of gibberic acid formed a meta-bridge. Gibberone (5), a dehydrogenation product of gibberic acid which has recently been synthesized, was subjected to a stepwise degradation to the tetramethyl esters (6) which were then synthesized.[10] This firmly established the structure of gibberic acid and hence that of allogibberic acid as (4) and (2) respectively. Thus the conversion of allogibberic acid to gibberic acid required [9,11] a Wagner-Meerwein re-arrangement.

The relationship of the 4b-epi series was established[12] by inversion of the acetic acid side chain of (3) through an internal Claisen reaction. This, with inversion of the 9-carboxyl group, gave a compound enantiomorphic to a ring D seco-acid from epiallogibberic acid thus establishing its isomeric relationship with allogibberic acid at position 4b and the consequent relationship of gibberic and epigibberic acid.[13]

The Cotton effects in the optical rotatory dispersion curves of the nor-ketone of allogibberic acid, its ring D seco keto-ester together with their 4b and 9a epimers indicated that allogibberic acid had the absolute stereochemistry (2).

Ring A of gibberellic acid therefore contains[14] the second double bond, a saturated γ-lactone and a secondary hydroxyl group. The latter was shown to be part of an allylic system by oxidation with manganese dioxide to an αβ -unsaturated ketone ($\lambda_{max.}$ 228 mμ log ε 3·99). The position of the ring A hydroxyl group and hence the double bond was then established by dehydrogenation of the diketone (7) obtained by Wagner-Meerwein isomerization and oxidation of gibberellin A_1, to 1,7-dimethylfluoren-2-ol.[15] Since hydrogenation of the ring A double bond was accompanied by considerable hydrogenolysis of the lactone ring whilst the heteroannullar dienic acid, gibberellenic acid (8)[16] was obtained from the aqueous decomposition of gibberellic acid, the lactone ring was assigned the 1→4a position.[17]

Methyl gibberellate nor-ketone showed[18] a positive Cotton effect closely similar to that shown by the analogues in the allogibberic acid and phyllocladene series and hence it was assigned the same stereochemistry. Since a cis anhydride was formed[19] between the 8a acetic acid side chain and the 10-carboxyl the latter was assigned the β configuration. The coupling constant between the 10 and 10a protons in the nuclear magnetic resonance spectrum was taken[20] as evidence for a trans relationship between these protons and hence to a β 10a proton. Further evidence for the β-configuration of the proton at 10a came from



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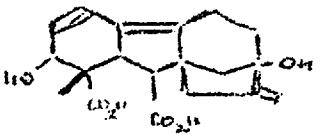

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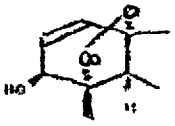

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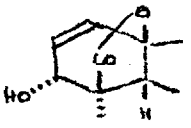

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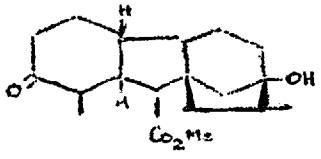

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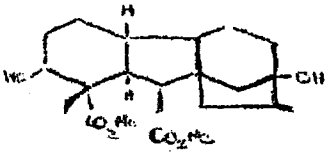

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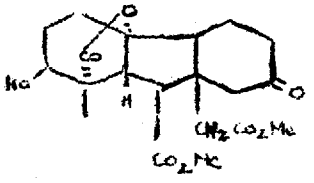

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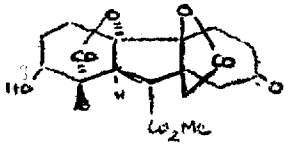

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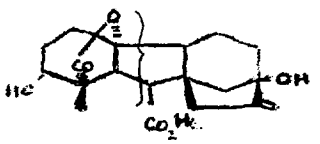
the optical rotatory dispersion curves of the hydrogenolysis products.

The stereochemistry of ring A and in particular the orientation of the lactone ring, was a source of some controversy.[18,21] The epimerization of the 2-hydroxyl in gibberellin A_1 derivatives implied[22] that it possessed an axial conformation and hence the distinction[23] had to be made between the two absolute configurations (9) and (10) for ring A. Hydrogenolysis of the lactone ring takes place with inversion at C-4a. The ring A ketone (11) obtained by decarboxylation and oxidation of the hydrogenolysis acid (12) showed a negative Cotton effect which was taken to imply a cis A/B ring fusion. Furthermore methyl 2-ketotetrahydrogibberellate showed a strong positive Cotton effect pointing to an α-oriented lactone bridge. However arguments based on the molecular rotation differences on opening the lactone ring, suggested that the lactone ring was in fact β. An α-oriented lactone ring requires the more stable trans A/B ring junction in contrast to a β-oriented lactone ring which requires the cis fusion. The former was more consistent with the ready relactonization and rearrangement of a 1 → 4a lactone to a 1 → 3 lactone together with the elimination reactions of the 2(ax.) hydroxyl group. Treatment of gibberellin A_1 methyl ester with 2N-hydrochloric acid gives rise through a 4a



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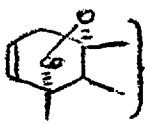

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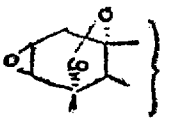

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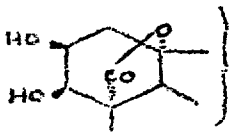

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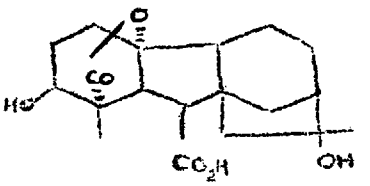

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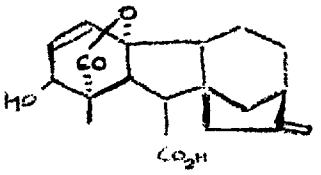

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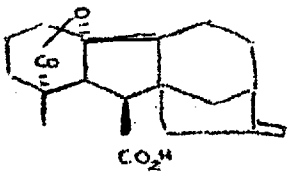

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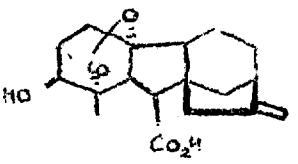

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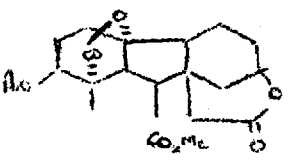

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carbonium ion to an equilibrium mixture of 4b epimers together with the 4a:4b unsaturated ester. Under these conditions there was no inversion at the adjacent 10a suggesting that this was the more stable A/B fusion. Inversion at C-4a is reflected[24] in the difference of reactivity between the hydrogenolysis products and the corresponding gibberellin A_1 derivatives.

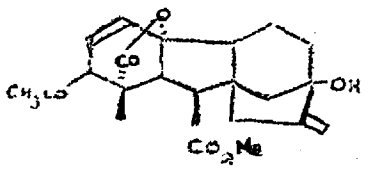
The ring D seco keto-ester (13) showed a positive Cotton effect similar to that of the related compound derived from allogibberic acid and hence gibberellic acid was initially assigned[18] the same B/C/D ring fusion. However further optical rotatory dispersion studies[19] by the same group of workers comparing 4b epimers and a cis-fused lactone of the type (14) demonstrated[25] the weakness of this analogy. Meanwhile independent circular dichroism studies implied the opposite conclusion. X-ray analysis[26] of methyl bromogibberellate led to a final clarification of the problem and revealed a β-hydrogen atom at 4b (1).

Gibberellins A_1,[27] A_5, A_6 and A_8[28] have been related to gibberellic acid by methods which establish them to be (15) to (18). Gibberellins A_2 (19),[29] A_7 (20) and[30] A_9 (21)[31] were related to gibberellin A_4 (22) which in turn was related[32] to gibberellic acid through

the common degradation product ($\frac{2}{3}$3) in which all the centres of asymmetry were retained.

The chemistry of the different gibberellins may be distinguished by two features; the presence or absence of the $\Delta^{3,4}$ double bond on the one hand and the 2 and 7 hydroxyl on the other. Thus in the absence of 7-hydroxyl the 8-methylene is hydrated by treatment with mineral acid. However the presence of a 7-hydroxyl permits a Wagner-Meerwein rearrangement to the 7α-gibbane system - a rearrangement which may also be brought about by positive halogen. The double bonds of gibberellic acid differ in their reactivity and thus the $\Delta^{3,4}$ double bond may be selectively hydrogenated over a partially poisoned catalyst to form gibberellin A_1. In an interesting acylation reaction[33] methyl gibberellate gives a 2-acetyl derivative (24) with zinc and boiling acetic anhydride.

Ring A of the gibberellin system shows both alkali and acid instability.[22] In the absence of a $\Delta^{3,4}$ double bond the 2 (ax.) hydroxyl of for example gibberellin A_1, is epimerized in dilute alkali to an equilibrium mixture with the 2 (eq.) hydroxyl, giving pseudogibberellin A_1. A retroaldol mechanism has been proposed for this. The presence of a $\Delta^{3,4}$ double bond alters this completely.



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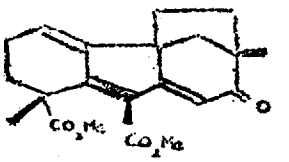

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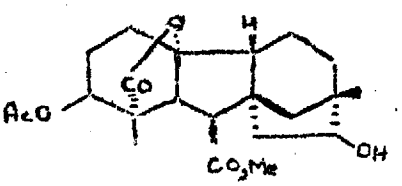

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In alkali allylic rearrangement of the lactone ring takes place giving 1 → 3 lactones without inversion of the 2(ax.) substituent whilst in acid solution a facile aromatization of ring A takes place giving predominantly allogibberic acid (with inversion at 4b) together with some 4b-epiallogibberic acid. Gibberellic acid in cold concentrated sulphuric acid gives an intense wine-red colour with a strong blue fluorescence, this forming the basis of its fluorometric estimation. A compound which can be reversibly converted into the fluorogen in sulphuric acid has been isolated[34] and shown to have the structure (25) arising by a Wagner-Meerwein rearrangement.

Treatment of the 7α-gibbane alcohol (26) with phosphorus pentachloride led[35] to a reversal of the Wagner-Meerwein rearrangement and hence to a partial synthesis of the 7-deoxy gibberellin system.

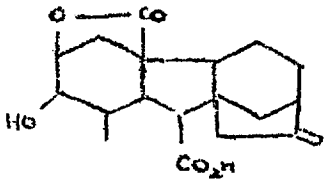
1.2. The Chemistry of the 7-Deoxygibberellins

The gibberellins fall into two classes distinguished by the presence or absence of a hydroxyl group at position 7. At the outset of this work two 7-deoxygibberellins had been described in the literature.[29] The structure (27), later modified to (28), had been proposed for gibberellin A_4 on the basis of its spectral characteristics and its relationship to gibberellin A_2 (29). The latter had in turn been related to gibberellin A_1 through the 'anti-Bredt' intermediate (30) and the deacetoxylation of (31).[29] Neither relationship could be repeated. Concurrent work at the Frythe on these gibberellins led to the suggestion that they possessed the structure (28) and (29).

When the fungus Gibberella fujikuroi (Saw.) Wr. strain ACC 917 is grown in stirred culture at the natural pH on a Raulin Thom or a glucose-ammonium nitrate medium it produces gibberellic acid (1). However when the fungus is grown at the normal pH until the inorganic nitrogen is exhausted from the medium and the pH is then adjusted to 7 by the addition of alkali, gibberellin A_7[30] and gibberellin A_9[31] are produced in yields of about 25 and 3-4 mg/l. of the culture filtrate. The gibberellins were extracted from the culture filtrate by the addition of

charcoal (17 g/l.) and subsequent elution of the charcoal with acetone. These extracts were separated into acidic and neutral fractions with sodium hydrogen carbonate solution and the acidic fraction recovered in ethyl acetate. Concentration of the ethyl acetate gave crude crystalline gibberellic acid whilst the mother liquors afforded a gum containing the new gibberellins. These were chromatographed firstly on a charcoal-celite column which was eluted with water containing an increasing concentration of acetone and the gibberellin containing fractions were then further purified by chromatography on celite-silica gel in chloroform-ethyl acetate.

Gibberellin A_7 (32) $[\alpha]_D^{24}$ + 20°, crystallised in two polymorphic forms, melting points 169-172° and 202° (decomp.) respectively. It titrated as a monobasic acid whilst with diazomethane it formed a mono-methyl ester, m.p. 152-153° or 168-170°. On acetylation it gave a mono-acetate, m.p. 190-192°. On hydrogenation the methyl ester took up 2·3 mol. of hydrogen. Analytical data were consistent with the molecular formula $C_{19}H_{22}O_5$ for gibberellin A_7 and this together with its biological activity, suggested that it was related to gibberellic acid. In chloroform solution the infrared spectrum of



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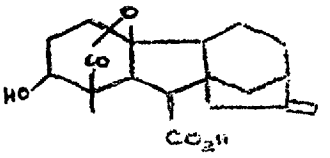

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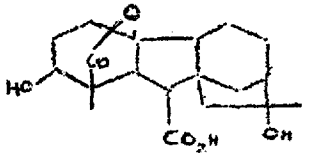

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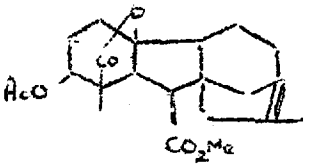

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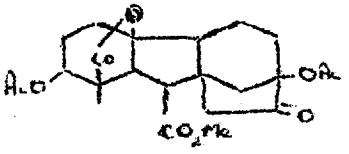

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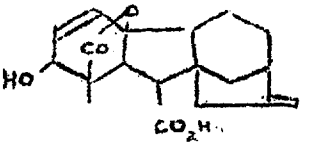

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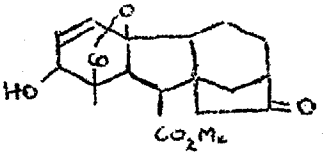

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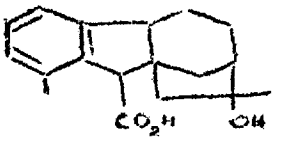


(34)

gibberellin A_7 methyl ester showed absorption due to hydroxyl (3578 cm.$^{-1}$), γ-lactone (1767 cm.$^{-1}$), ester (1732 cm.$^{-1}$), olefin (1657 cm.$^{-1}$) and terminal methylene (887 cm.$^{-1}$). These assignments accounted for all the oxygen functions. The terminal methylene group was shown to be exocyclic to a five-membered ring by ozonolysis of the methyl ester which gave formaldehyde (0·43 mol.) and a nor-ketone $C_{19}H_{22}O_6$ (33) which showed an additional carbonyl absorption at 1755 cm.$^{-1}$ ascribed to a cyclopentanone. Although this nor-ketone gave a mono-acetate, it was stable to periodate and hence gibberellin A_7 was a 7-deoxygibberellin.

The structure of ring A was identical with that of gibberellic acid.[14] Thus treatment of gibberellin A_7 with dilute mineral acid at 20° led to the formation of an aromatic hydroxy-acid, $C_{18}H_{22}O_3$. Under similar conditions gibberellic acid gives the aromatic acid, allogibberic acid[8] whilst the 7-deoxygibberellin, gibberellin A_4 is converted[29] to gibberellin A_2 by hydration of the terminal methylene group. Hence the acid was assigned the structure (34) and this was supported by the aromatisation of gibberellin A_7 methyl ester nor-ketone with boiling dilute mineral acid to form a ketonic aromatic ester $C_{18}H_{20}O_3$. The presence of an allylic hydroxyl group in ring A was confirmed by the oxidation with chromium

trioxide in sulphuric acid which led to an αβ-unsaturated ketone, $C_{20}H_{22}O_5$ (35) ($\lambda_{max.}$ 228 mμ ε 6,900) which was also prepared[32] by Dr. R. H. B. Galt by oxidation with active manganese dioxide.

Selective catalytic reduction of the ring A double bond over a 2% palladium on barium carbonate catalyst poisoned with pyridine led to gibberellin A_4 and a gummy mixture of hydrogenolysis acids. Treatment of the latter with boiling dilute mineral acid afforded gibberellin A_2 and dihydrogibberellin A_4 presumably through relactonisation of unsaturated acids of the type (36). Repetition of this work on a larger scale by Dr. T.P.C. Mulholland showed[36] the presence of a $\Delta^{8,9}$ isomer of gibberellin A_4 and a C-8 epimer of gibberellin A_2 in this mixture. The high yield of hydrogenolysis acids provides further support for the Δ^{3} position of the ring A double-bond.

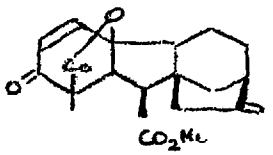
Occasionally an acid, isomeric with gibberellin A_7, was also isolated from these fermentations. This acid, m.p. 186-190° $[\alpha]_D^{20}$ + 59°, gave a mono-methyl ester, m.p. 226-228°. Both these, unlike gibberellin A_7 and its methyl ester, showed trisubstituted double-bond absorption in the infrared at 826 $cm.^{-1}$. The methyl ester could be prepared by the action of 0·03 N-sodium hydroxide on gibberellin A_7

methyl ester thus establishing the relationship of the parent acid to gibberellin A_7 and indicating that the isomerization paralleled[22] the alkaline isomerization of methyl gibberellate. A similar change in the ultraviolet end absorption and in the nuclear magnetic resonance spectrum was consistent with this 1 → 3 lactone structure (37). In particular the nuclear magnetic resonance spectrum showed the characteristic[20] gibbane 10:10a AB quartet at τ = 7·45 and 6·72 (J = 7 c/sec), the latter also coupled to the C-4 proton (τ = 4·18, J = 3 c/sec). Furthermore the coupling constant of 6 c/sec between the C-2, C-3 and C-4 protons corresponded to an angle of about 30° between these protons and hence to an axial 2-hydroxyl group.

In view of the isolation of this alkaline isomerization product of gibberellin A_7 from the fermentation, the alkaline isomerization products of gibberellin A_4 and its methyl ester were prepared. Thus gibberellin A_4 was oxidised with chromium trioxide in sulphuric acid to form the corresponding 2-ketone which in turn was reduced with sodium borohydride in methanol to give predominantly the 2 (eq.) epimer, m.p. 215-220°. Similarly oxidation of gibberellin A_4 methyl ester and reduction of the ketone with sodium borohydride gave the corresponding 2 (eq.) hydroxy-ester.

Gibberellin A_9 (38), m.p. 208-211° $[\alpha]_D^{22}$ -12°, and its mono-methyl ester, m.p. 136° gave analyses which were consistent [31,32] with the formula $C_{19}H_{24}O_4$ for the acid. Microhydrogenation revealed the presence of one double bond whilst the infrared spectrum of the methyl ester showed absorption due to γ-lactone (1777 cm.$^{-1}$), ester (1738 cm.$^{-1}$) and terminal methylene groups (1659 and 873 cm.$^{-1}$). Ozonolysis of gibberellin A_9 gave a nor-ketone $C_{18}H_{22}O_5$.[37] This accounted for the functional groups of gibberellin A_9 and led to the structure (13) which was confirmed [32] by inter-relationship with gibberellin A_4 performed by Dr. R. H. B. Galt.

The structure (32) and (38) which were proposed for gibberellins A_7 and A_9 were both dependent upon that of gibberellin A_4 and hence an unambiguous relationship between gibberellin A_4 and a gibberellin of established structure was essential. Catalytic reduction of gibberellin A_7 methyl ester nor-ketone over palladised charcoal led to gibberellin A_4 methyl ester nor-ketone. Baeyer-Villiger oxidation of the acetyl derivative with perbenzoic acid led to the δ-lactone (39). Ozonolysis of gibberellic acid followed by methylation led to the keto-ester (40). Reduction and acetylation of this gave a compound which on hydrogenation over Adams' catalyst in acetic acid containing a trace of perchloric acid led to the above δ-lactone (39). Thus



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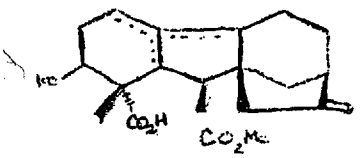

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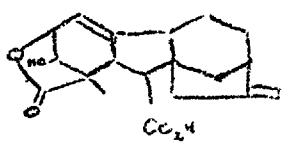

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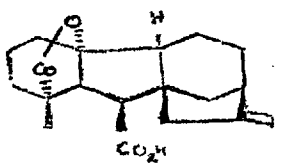

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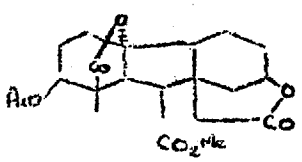

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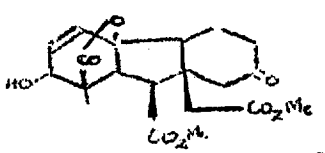

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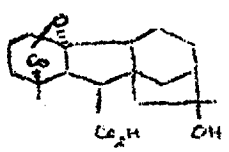

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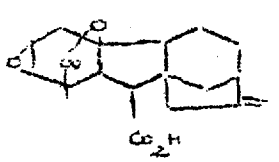


(42)

gibberellin A_4 and hence the other 7-deoxygibberellins were related to gibberellin A_1 at all centres of assymetry.

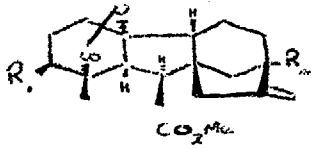
Gibberellin A_{10}, $C_{19}H_{26}O_5$ (41), was isolated[38] from Gibberella fujikuroi ACC 917 in low yield. On methylation it gave a mono-methyl ester, $C_{20}H_{28}O_5$. The infrared spectrum of the latter revealed the presence of a hydroxyl group ($\nu_{max.}$ 3545 cm.$^{-1}$) and a γ-lactone ($\nu_{max.}$ 1757 cm.$^{-1}$) thus accounting for the oxygen functions. The nuclear magnetic resonance spectrum of the ester contained the characteristic gibbane AB quartet (τ = 7·53 and 7·22 J 15 c/sec.) and showed the presence of two tertiary methyl groups (τ, 8·9 and 8·62) and an absence of olefinic protons. The low end-absorption (λ_{205} ε 400) indicated the absence of a fully substituted ethylenic bond and hence the structure (41) was proposed for gibberellin A_{10}. This relationship to gibberellin A_9 and hence the other gibberellins was confirmed by hydration of the terminal methylene group of gibberellin A_9 with mineral acid giving gibberellin A_{10}. Gibberellin A_{11} gave analytical data for $C_{19}H_{22}O_5$. Its infrared spectrum accounted for four oxygen atoms in the carboxyl and γ-lactone. The remaining oxygen was placed as a ring A epoxide from the N.M.R. spectrum (doublet at τ 6·7) and by analogy with gibberellin A_6. This is the subject of confirmatory studies.

1.3 The Nuclear Magnetic Resonance Spectra of some Gibberellin Derivatives.

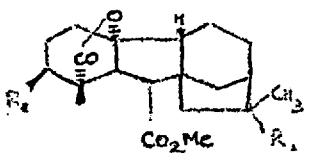
The diamagnetic anisotropy of pyridine and its consequent effect on the nuclear magnetic resonance spectra of solute molecules renders it unsuitable[39] as a normal solvent for this form of spectroscopy. However examples have appeared of its use in separating steroidal methyl resonances[40] and in a study of β-lumicolchicine. Nuclear magnetic resonance spectroscopy has played a very important part in the study of gibberellin chemistry.[20,23,33] Because of their low solubility in deuterochloroform a number of derivatives were also examined in pyridine solution. As a result it was possible to associate shifts between the two solvents with certain structural features and thus to appreciate the value of comparing spectra determined in the two solvent systems.

A methyl group at C-1 is a characteristic of all the known gibberellins. Although it is an equatorial substituent, its position is sensitive to the presence of other ring A substituents. Thus the deshielding by a 2β-hydroxyl group has been noted previously. (cf. spectra 1-5). Similar deshielding by an 2α-hydroxyl (13), by a 2-ketone (11, 12), by a Δ^2-olefin (14) and from a Δ^3-olefin (cf. 2 and 15, 9 and 17) is also apparent. On the other hand an acetyl group apparently removes this deshielding. When the spectra

Table 1 - Structures



(I)



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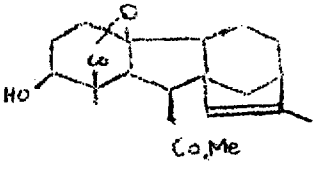

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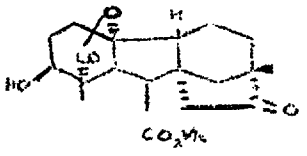

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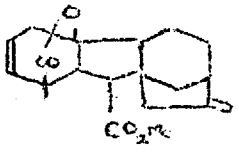

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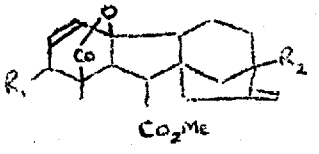

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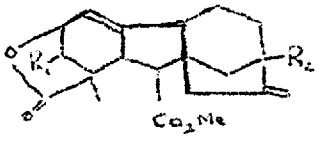

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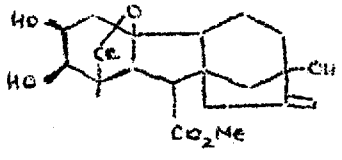


(VIII)

TABLE 1

Chemical shifts (τ values) of protons in some gibberellin derivatives.

(i) in deuterochloroform; (ii) in pyridine solution

Compound	C-1	C-8 substituent		C-10	C-10a	C-2	C-3	C-4
1. I $R_1=R_2=H$.	8·92	5·15	5·05	7·28	7·51			
	8·88	5·13	5·03	7·14	7·39			
2. I $R_1=OH$; $R_2=H$.	8·85	5·15	5·05	7·29	6·78	6·15		
	8·56	5·14	5·04	7·07	6·32	5·94		
3. I $R_1=R_2=OH$.	8·85	4·96	5·05	7·33	6·78	6·15		
	8·56	4·41	4·95	7·03	6·27	5·92		
4. II $R_1=OH$; $R_2=H$.	8·87					6·17		
	8·56	9·10	6c/sec	7·05	6·32	5·95		
5. III	8·85					6·18		
	8·55	8·38	J 1·5 c/sec	7·15	6·34	5·95		
6. IV	8·78	8·96		7·36	6·78	6·14		
	8·53	8·98		6·96	6·28	5·95		
7. II $R_1=R_2=OH$.	8·97							
	8·55	8·55		6·98	6·21	5·92		
8. 8 epimer of 7								
	8·96	8·73		7·51	6·85	6·40		
	8·55	8·55		7·08	6·32	5·93		
9. I $R_1=OAc$; $R=H$.	8·94	5·15	5·05	7·36	6·83	5·15		
	8·79	5·12	5·02	7·15	6·61	4·86		
10. VIII (ii)	8·55	5·09	4·45	7·05	6·20	5·85	5·21(?)	

Compound	C-1	C-8 substituent		C-10	C-10a	C-2	C-3	C-4
11. I								
R_1=O; R_2=H.	8·83	5·15	5·05	7·22	6·92			
	8·66	5·12	5·02	7·02	6·63			
12. II								
R_1=O; R_2=H.	8·85	9·05		7·23	6·96			
	8·62	9·08		7·03	6·61			
13. II								
R_1=α·OH.								
R_2=H. (7-OH)	8·85	9·08		7·28	7·51	6·40		
	8·53	8·82		6·96	7·28	6·15		
14. V	8·78	5·15	5·05	7·21	7·42	4·28	4·28	
	8·72	5·12	5·02	7·05	7·21	4·31	4·31	
15. VI								
R_1=OH; R_2=H.	8·77	5·15	5·05	7·29	6·78	5·87	4·14	3·70
	8·48	5·15	5·03	7·03	6·38	5·58	3·96	3·65
16. VI								
R_2=R_2=OH.								
(ii)	8·46	4·55	4·98	6·95	6·31	5·52	3·89	3·59
17. VI								
R_1=OAc; R_2=H.	8·86	5·15	5·05	7·27	6·70	4·70	4·18	3·62
	8·70	5·13	$5·0_3$	7·06	6·52	4·42	4·14	3·52
18. VI								
R_2=R_2=OAc.	8·86	4·84	5·03	7·25	6·67	4·68	4·16	3·68
	8·69	4·41	4·95	6·99	6·46	4·42	4·13	3·52
19. VII								
R_1=OH; R_2=H.	8·80	5·09		7·44	6·72	5·77	5·28	4·15
	8·62	5·10		7·22	6·22	5·49	5·12	4·15
20. VII								
R_1=R_2=OH.	8·82	4·91	5·06	7·46	6·74	5·79	5·30	4·22
	8·64	4·56	4·96	7·12	6·20	5·49	5·12	4·12
21. VII								
R_1=OAc;	8·80	4·96		7·44	6·70	4·96	4·96	4·25
R_2=OH.	8·64	4·96	4·55	7·17	6·37	4·89	4·67	4·15
22. VII								
R_1=R_2=OAc;								
(i)	8·81	4·98		7·43	6·70	4·98	4·98	4·25
(ii)	8·65	4·95	4·65	7·15	6·38	4·65	4·82	4·20

in deuterochloroform and pyridine solution are compared it is possible to distinguish between effects on this methyl group due to olefinic unsaturation and those due to oxygen substituents. Whereas pyridine magnifies the deshielding due to oxygen substituents (from $\Delta\tau$ = 0·07 to 0·29), it leaves unchanged the deshielding due to olefinic unsaturation. Thus even the acetyl derivatives (9) shows a difference between the two solvent systems. Hence the use of the two solvent systems serves to distinguish in this case between effects due to oxygen and those due to olefinic unsaturation. Furthermore it is apparently diagnostic of an oxygen function at C-2. Thus gibberellin A_8 methyl ester (VIII) with an additional hydroxyl group at C-3 has the 1-methyl resonance at 8·56 in pyridine solution identical to that of gibberellin A_1 methyl ester.

The 10:10a AB quartet is a characteristic and important feature of the nuclear magnetic resonance spectra of the gibbane skeleton. The 10a proton is a β-axial substituent on ring A and hence 1:3 diaxial transannular effects might well be expected from the 2-position. On the other hand the 10-proton is an α-substituent on ring B. Thus the position of the 10-proton resonance remains fairly constant within the range τ7·22-7·36 in deuterochloroform and at lower field

(τ6·96 to 7·15) in pyridine. This is in agreement[23] with some previous assignments made by Grove et al by comparison with methyl cyclopentane carboxylate. However the 10a proton is remarkably susceptible to ring A substitution. Thus in gibberellin A_9 the 10a proton resonance appears at τ7·31 not far removed from the predicted value (τ7·6) for the chemical shift of a hydrogen at a ring junction in a rigid system and β to a methoxycarbonyl group. The shifts from this position in spectra 2 and 4 etc, are consistent with transannular 1-3-diaxial interactions. Furthermore the 2-epimer (13) lacking this interaction, shows the 10a resonance τ7·51. Deshielding may also be noted from a 2β-acetoxyl and a 2-carbonyl group. Thus the earlier explanation of the position of this resonancce is wrong. The deshielding due to a 1:3-diaxial interaction with a hydroxyl group is again amplified by pyridine (τ from 0·73 to 1·07), a smaller effect being noted from the 2-epimer. Thus the effect shows some stereochemical specificity. This point may be illustrated more clearly in an examination of the 8-methylene resonances. The C-8 methylene proton resonances are readily distinguished at τ5·15 and 5·05 by comparison with the corresponding dihydro derivatives (cf. 11 and 12). An adjacent 7-hydroxyl brings about a significant shift in the position of one of these resonances which is further changed by pyridine (cf. 1 and 3). Models

in fact show that one of these protons will lie closer to the hydroxyl than the other. A similar difference in position between the two solvent systems is apparent in the resonances of the C-2 and C-3 protons. Indeed a further application of this effect can be seen in those regions where olefinic and C<u>H</u>-(O) resonances co-occur and it is necessary to distinguish between them. Thus in gibberellin A_4 acetate methyl ester the 2-proton resonance appears at $\tau 5{\cdot}05$ together with the C-8 methylene proton resonances. However on determining the spectrum in pyridine solution the former is shifted to $\tau 4{\cdot}86$ whilst the latter remain constant.

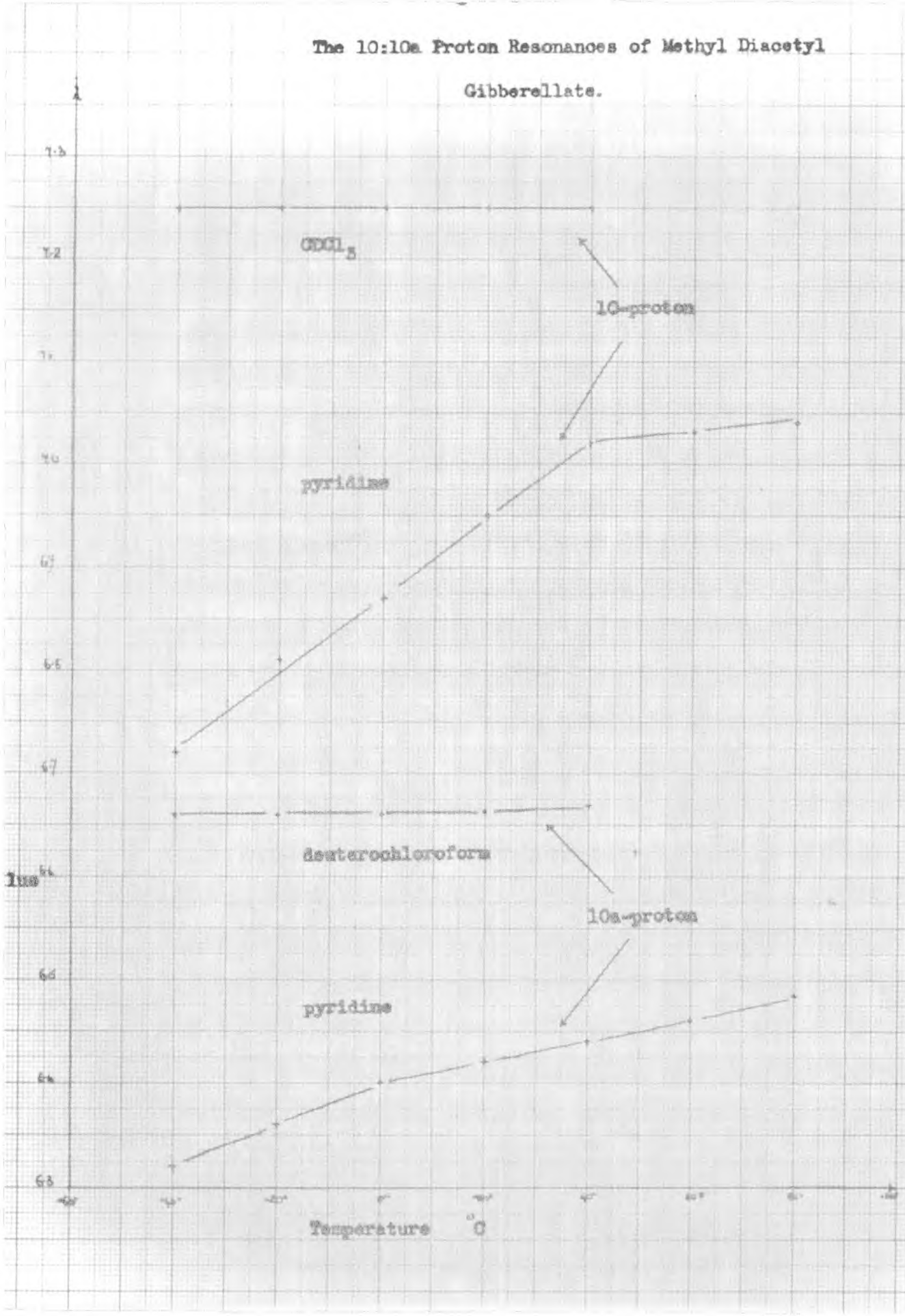
The interpretation of spectra may be confused in this region by overlapping multiplets and again choice of a suitable solvent may serve to separate out these resonances and thus to assist in the determination of coupling patterns. An example of this is furnished by the ester (VII R_1=OAc; R_2=OH.) The spectrum was originally recorded in chloroform solution at 40 mc. The 60 mc. deuterochloroform spectrum agrees in general with this. In particular the resonances from the $C{=}CH_2$ and those due to the C-2 and C-3 protons overlap. However when the spectrum was redetermined in pyridine solution this region was resolved revealing a triplet at $\tau 4{\cdot}67$ J = 5 c/sec. due to the C-3 proton, a doublet at τ 4·89 J = 5 c/sec. due to the C-2 proton and the broader olefinic resonances at τ 4·55 and 4·96.

Since the effect of pyridine apparently involves co-ordination at specific sites in the molecule it should show a temperature dependence. The spectrum of methyl diacetyl gibberellate, which is sufficiently soluble in the two solvent systems, was observed over the range -40° to + 80° in pyridine and compared to that in deuterochloroform over the range -40° to + 40°. The variation in position of the 10 and 10a resonances are shown graphically. Confirming the part played by solvation, the effect shown by pyridine is least at the higher temperatures.

Acetone-d_6 was used as a solvent for the poorly soluble 8-epigibberellin A_2 derivative (8). Comparison of this spectrum with that of gibberellin A_4 methyl ester (2) and in particular the position of the C-2 resonance suggests that this solvent unlike pyridine, shields certain protons. The position of the 10-proton resonance appears anomolous.

Thus in those cases where material is at a premium necessitating the application of as wide a range of physical methods as possible, the comparison of nuclear magnetic resonance spectra in two solvent systems such as pyridine and deuterochloroform may provide useful information. However, as the effect is due to solvation and may thus vary from series to series, caution must be exercised in the choice of model compounds.

The 10:10a Proton Resonances of Methyl Diacetyl Gibberellate.



Experimental Section

1.4 M.ps were determined on a Kofler block. Infrared spectra were determined for Nujol mulls on a Perkin-Elmer 221 or Unicam S.P. 500 spectrometer. Nuclear magnetic resonance spectra were determined in deuterochloroform (unless other wise stated) on Varian Associates A 60 spectrometers at Imperial College and at the Akers Research Laboratories of I.C.I. Microanalyses were performed by the micro-analytical laboratories of these departments. [Tetramethylsilane was used as an internal reference] Alumina for chromatography was acid-washed grade II-III. Silica gel was supplied by B.D.H. Light petroleum refers to the fraction b.p. 60-80°. Extracts were dried over sodium sulphate.

Gibberellin A_7 (32) - Gibberellin A_7 crystallised from acetone-light petroleum as needles m.p. 169-172°, or as prisms m.p. 202° (decomp.), or as a mixture of these two with intermediate melting point. They gave two distinct infrared spectra (i) $\nu_{max.}$ 3450 (OH), 1742 (γ-lactone), 1722 (carboxyl) and 1654 (double bond) and (ii) 3340, 1778, 1711 and 1674 $cm.^{-1}$ Both forms gave identical infrared spectra in chloroform solution, $\nu_{max.}$ 1765 and 1708 $cm.^{-1}$

(Found: C, 69·05; H, 7·0%; equiv. 321. $C_{19}H_{22}O_5$ requires C, 69·1; H, 6·7% M. 330), $[\alpha]_D^{20}$ + 20° (c 0·5). With cold conc. sulphuric acid, it gave a brownish-yellow colour with a green fluorescence.

The methyl ester, prepared with diazomethane, crystallised from acetone-light petroleum as needles, m.p. 152-153° or 168-170°, $[\alpha]_D^{23}$ + 33° (c 0·7), (Found: C, 69·9; H, 7·2. $C_{20}H_{24}O_5$ requires C, 69·75; H, 7·0%) $\nu_{max.}$ 3460 and 3320 (OH). 1774 (γ-lactone), 1733 and 1718 (ester), 1659 and 887 (terminal methylene) $cm.^{-1}$ $\nu_{max.}$ (in $CHCl_3$) 3578, 1767, 1732, 1657 and 887 $cm.^{-1}$

The acetate, prepared with acetic anhydride in pyridine crystallised from acetone-light petroleum as prisms, m.p. 190-192°, $[\alpha]_D^{24}$ + 87° (c 0·9), (Found: C, 67·3; H, 6·7% $C_{21}H_{24}O_6$ requires C, 67·7; H, 6·5%).

Determination of Exocyclic Methylene. (43) - Gibberellin A_7 (1·5 mg.) was dissolved in water (5 ml.) in a volumetric flask (25 ml.) with the addition of 0·1N-potassium carbonate solution to bring the pH to 7-7·5. 0·02M-Sodium metaperiodate (10 ml.) and 0·005M-potassium permanganate (1 ml.) were added, the solution made up to 25 ml. with distilled water and left for 15 min. 1 Ml. was transferred to a test tube, the chromotropic acid reagent (chromotropic acid 1 g.

dissolved in water 100 ml., filtered, and made upto 500 ml. with 2:1 v/v sulphuric acid-water) (10 ml.) added, and the solution heated on a water bath for 30 min. The percentage transmission at 270 mμ in 1 cm. cells was determined and compared to mesoerythritol and gibberellic acid standards. In 15 min. the latter gave 30% available formaldehyde against mesoerythritol whilst gibberellin A_7 gave 30·5%.

Ozonolysis of Gibberellin A_7 Methyl Ester. - Ozonised oxygen (1·1 mol.) was passed through a solution of gibberellin A_7 methyl ester (41 mg.) in acetic acid (15 ml.). After being kept for 0·5 hr., the solution was diluted with water to 100 ml. and rapidly steam distilled. The distillate (100 ml.) was treated with aqueous dimedone and after 5 days formaldehyde-dimethone (13 mg.) m.p. 189-190° was collected. The acetic acid solution was neutralized with aqueous sodium hydrogen carbonate solution and extracted with ethyl acetate. The extract was washed with water, dried and the solvent evaporated to give a gum which was chromatographed on silica gel. Elution with 1:4 ethyl acetate-light petroleum gave gibberellin A_7 methyl ester nor-ketone (33) (23 mg.) which crystallised from ethyl acetate-light petroleum as prisms, m.p. 185° $[\alpha]_D^{22}$ + 88°

(c 0·8) (Found: C, 65·75; H, 6·5. $C_{19}H_{22}O_6$ requires C, 65·9; H, 6·4%), $\nu_{max.}$ 3440, 1784, 1755 and 1738 $cm.^{-1}$

Acetylation with acetic anhydride in pyridine gave the acetate which crystallised from acetone-light petroleum as needles, m.p. 187-189° (Found: C, 65·2; H, 6·2. $C_{21}H_{24}O_7$ requires C, 64·9; H, 6·2%) $\nu_{max.}$ 1781 and 1738 $cm.^{-1}$

Stability of the Nor-ketone to Sodium Periodate. - The nor-ketone (15 mg.) in methanol (5 ml.) was treated with 0·1N-sodium periodate solution (2 ml.) at room temperature for 24 hr. Dilution with water and recovery with ethyl acetate afforded the starting material (13 mg.) as needles, m.p. 186°, identified by its infrared spectrum.

Aromatisation of Gibberellin A_7. - A suspension of gibberellin A_7 (95 mg.) in dilute hydrochloric acid (25 ml.) was allowed to stand at room temperature for 5 days. The solution was extracted with ethyl acetate and the gummy product chromtographed on silica gel. Elution with 1:1 ethyl acetate-light petroleum gave the aromatic acid (34) (33 mg.) which crystallised from acetone-light petroleum in needles, m.p. 220-225°, (Found: C, 75·5; H, 8·0. $C_{18}H_{22}O_3$ requires C, 75·5; H, 7·7%) $\nu_{max.}$ 3410, and 1684 $cm.^{-1}$ $\lambda_{max.}$ 269, 272 mu (ε 418, 377).

Aromatisation of Gibberellin A_7 Methyl Ester Nor-ketone (33). The nor-ketone (49 mg.) in acetone (2 ml.) was added to 3N-hydrochloric acid (20 ml.) and heated under reflux for 1 hr. Recovery with ethyl acetate and chromatography of the residue on silica gel gave, in the fractions eluted with 1:4 ethyl acetate-light petroleum, the aromatic keto-ester (22 mg.) which crystallised from ether-light petroleum in needles, m.p. 119-120° (Found: C, 76·2; H, 7·3. $C_{18}H_{20}O_3$ requires C, 76·0; H, 7·1%), $\nu_{max.}$ 1744, 1714, and 1590 $cm.^{-1}$

Oxidation of Gibberellin A_7 Methyl Ester. - Gibberellin A_7 methyl ester (50 mg.) in acetone (5 ml.) was treated with the chromic oxide reagent[44] (0·1 ml.) for 0·5 hr. at room temperature. Methanol was added and the solution then concentrated, and diluted with water to 100 ml. The solution was extracted with ethyl acetate, and the extract washed with sodium hydrogen carbonate solution, water, and dried. The solvent was evaporated and the residue crystallised from acetone-light petroleum to give the αβ-unsaturated ketone (35) (35 mg.) as needles, m.p. 138-140°, $\nu_{max.}$ 1775, 1732, 1688, 1654, 880 $cm.^{-1}$ This compound was identical with the product of manganese dioxide oxidation of gibberellin A_7 methyl ester first prepared by Dr. R. H. B. Galt.

Hydrogenation of Gibberellin A_7. - (a) in methanol. Gibberellin A_7 (300 mg.) in dry methanol (15 ml.) and pyridine (1 ml.) was hydrogenated over 2% palladium on barium carbonate (300 mg.) until uptake ceased (19 ml. at N.T.P.; 0.98 mol.). The catalyst was removed by filtration and the solvent evaporated to give a gum which was chromatographed on celite-silica gel (2:1; 24 x 1.5 cm.). Careful elution with 1:7 ethyl acetate-chloroform gave gibberellin A_4 (25 mg.) which crystallised from acetone-light petroleum in prisms, m.p. 212-215° (decomp.), identical with an authentic specimen.

(b) in ethyl acetate. Gibberellin A_7 (500 mg.), dissolved in ethyl acetate (30 ml.) and pyridine (2 ml.) was hydrogenated over 2% palladium on barium carbonate (500 mg.) until the uptake ceased (30 min. 43 ml. at N.T.P.; 12 mol.). The catalyst was removed by filtration, the solution diluted with ethyl acetate, extracted with dilute hydrochloric acid, washed with water and dried. The solvent was evaporated and the residue chromatographed on silica gel. Elution with 3:17 ethyl acetate-chloroform gave gibberellin A_4 as prisms (200 mg.) m.p. 211-214° (decomp.) $[\alpha]_D^{22}$ - 16° (c 0.9). Further elution with 25-30% ethyl acetate-chloroform gave a gummy mixture of hydrogenolysis acids (250 mg.).

Relactonisation of the Hydrogenolysis Acids. - The acids (250 mg.) from the preceding experiment, in acetone (10 ml.) were refluxed with dilute hydrochloric acid (15 ml.) for 1 hr. The solution was diluted with water and extracted with ethyl acetate. This extract was washed with water, dried and evaporated to give a gum which was chromatographed on silica:celite. Elution with 1:9 ethyl acetate-chloroform gave dihydrogibberellin A_4 (80 mg.) which crystallised from acetone-light petroleum as needles, m.p. 252-254° (decomp.) (Found: C, 68·3; H, 7·5. $C_{19}H_{26}O_5$ requires C, 68·2; H, 7·8%) $\nu_{max.}$ 3530, 1733 and 1712 $cm.^{-1}$ Further elution with 1:3 ethyl acetate-chloroform gave gibberellin A_2 (41 mg.) which crystallised from acetone-light petroleum as prisms m.p. 238-242° (with a polymorphic change to needles at 230°) identified by its infrared spectrum.

Hydrogenation of Gibberellin A_7 Methyl Ester. - The ester (18 mg.) in ethyl acetate (4 ml.) was hydrogenated over 25% palladised charcoal (10 mg.)(uptake 2·3 mol.). The catalyst was filtered, the solution diluted with water, extracted with ethyl acetate and separated into acidic and neutral fractions with sodium hydrogen carbonate solution. The acidic fraction (2 mg.) was intractable but the neutral fraction (7 mg.) gave dihydrogibberellin A_4 methyl ester which crystallised from acetone-light petroleum as needles,

m.p. 148-151° identified by its infrared spectrum.

Hydrogenation of Gibberellin A_7 Methyl Ester Nor-ketone. - The nor-ketone (75 mg.) in ethyl acetate (10 ml.) was hydrogenated over 25% palladised charcoal (60 mg.)(uptake 4.7 ml. at N.T.P.; 1.1 mol.). After filtration, the solution was diluted with ethyl acetate and separated into acidic and neutral fractions with sodium hydrogen carbonate solution. The acidic fraction (30 mg.) was an intractable gum. The neutral fraction gave gibberellin A_4 methyl ester nor-ketone (45 mg.) as prisms, m.p. 206-208°, identified by its infrared spectrum.

Acetylation with acetic anhydride in pyridine gave the acetyl derivative which crystallised from ethyl acetate-light petroleum as needles, m.p. 189° (Found: C, 64.5; H, 7.0. $C_{21}H_{26}O_7$ requires C, 64.6; H, 6.7%) $\nu_{max.}$ 1767 and 1732 $cm.^{-1}$

Baeyer-Villiger Oxidation of Acetylgibberellin A_4 Methyl Ester Nor-ketone. - The nor-ketone (60 mg.) in chloroform (2 ml.) was treated with 0.43N-perbenzoic acid (2 ml.) and toluene-p-sulphonic acid (10 mg.) at 0° for 16 hr. The solution was diluted with ethyl acetate, extracted with sodium hydrogen carbonate solution, washed with water and dried. The solvent was evaporated and the residual gum

chromatographed on silica gel. This gave the δ-lactone (39) (36 mg.) which crystallised as needles, m.p. 190-192° from ethyl acetate-light petroleum identical with the sample obtained below.

Preparation of the δ-Lactone (39) from Gibberellic Acid - The seco-ester (40) was prepared from methyl gibberellate by ozonolysis, reduction and methylation. Acetylation with acetic anhydride in pyridine gave the 2-acetyl - derivative which crystallised from ethyl acetate-light petroleum as needles, m.p. 193-195°. (Found: C, 60·8; H, 6·55. $C_{22}H_{28}O_9$ requires C, 60·5; H, 6·5%).

Reduction of the Acetyl Derivative. - The acetyl derivative (52 mg.) in acetic acid (10 ml.) was hydrogenated over Adam's catalyst (60 mg.) in the presence of perchloric acid (5 drops) until the uptake of hydrogen ceased (25 min.). After filtration, the solution was diluted with ethyl acetate and extracted with sodium hydrogen carbonate solution, water and dried. The solvent was evaporated to give a gum which was chromatographed on silica gel. Elution with 1:1 ethyl acetate-light petroleum gave the δ-lactone (39) (13 mg.) which crystallised from acetone-light petroleum as needles, m.p. 193-194° (Found: C, 62·3; H, 6·7. $C_{21}H_{26}O_8$ requires C, 62·1; H, 6·45%) $\nu_{max.}$ 1764, 1737, and 1722 $cm.^{-1}$ This was identical to the product of Baeyer-Villiger oxidation described above.

Alkaline Isomerization of Gibberellin A_7 Methyl Ester. - Gibberellin A_7 methyl ester (50 mg.) in methanol (5 ml.) was shaken with 0·05N-sodium hydroxide solution (10 ml.) for 3 hr. The solution was diluted with water and acidified. The solution was then extracted with ethyl acetate and the extract washed with sodium hydrogen carbonate solution, water and dried. The solvent was evaporated and the residual gum crystallised from acetone-light petroleum to give the hydroxy-ester (37) (35 mg.) as prisms, m.p. 226-228° (Found: C, 69·7; H, 7·1. $C_{20}H_{24}O_5$ requires C, 69·75; H, 7·0%) $\nu_{max.}$ 3490, 1771, 1710, 928, 879, 822 $cm.^{-1}$

The infrared spectrum of this ester was identical to that of the ester of an acid, m.p. 186° (decomp.) (Found: C, 69·0; H, 6·7. $C_{19}H_{22}O_5$ requires C, 69·05; H, 6·7%) $\nu_{max.}$ 3460, 1774, 1722 $cm.^{-1}$ which had been isolated from Gibberella fujikuroi.

Oxidation of Gibberellin A_4 Methyl Ester. - Gibberellin A_4 methyl ester (50 mg.) in acetone (5 ml.) was treated with the chromic oxide reagent (0·1 ml.) for 1 hr. Methanol was added and the solution concentrated and then diluted with water. It was extracted with ethyl acetate and the extract washed with sodium hydrogen carbonate solution, water and dried. The solvent was evaporated and

the residue crystallised from acetone-light petroleum as prisms (45 mg.) of the 2-keto-ester, m.p. 112-113° (Found: C, 70·3; H, 7·2. $C_{20}H_{24}O_5$ requires C, 69·75; H, 7·0%) $\nu_{max.}$ 1790, 1770, 1725, 1654 and 880 $cm.^{-1}$

Reduction of the Keto-ester with Sodium Borohydride.- The above keto-ester (25 mg.) in methanol (2 ml.) was treated with sodium borohydride (50 mg.) at room temperature for 2 hr. The solution was acidified with dilute hydrochloric acid and diluted with water. This was extracted with ethyl acetate and the extract washed with sodium hydrogen carbonate solution, water and dried. The solvent was evaporated and the residue crystallised from acetone-light petroleum as prisms (15 mg.) of 2-epigibberellin A_4 methyl ester, m.p. 166-167° (Found: C, 69·35; H, 7·6. $C_{20}H_{26}O_5$ requires C, 69·3; H, 7·6%) $\nu_{max.}$ 3508, 1770, 1720, 1660 and 884 $cm.^{-1}$

Gibberellin A_9 (39) - Gibberellin A_9 crystallised from ethyl acetate-light petroleum as needles, m.p. 208-210° (Found: C, 72·1; H, 7·5. $C_{19}H_{24}O_4$ requires C, 72·1; H, 7·65%) $\nu_{max.}$ 1740, 1723, 1659 and 893 $cm.^{-1}$

The methyl ester prepared with diazomethane, crystallised from acetone-light petroleum as prisms, m.p. 131-132° (Found: C, 73·1; H, 8·08. $C_{20}H_{26}O_4$ requires C, 72·7; H, 7·93%) $\nu_{max.}$ 1774, 1733, 1658 and 880 $cm.^{-1}$

Ozonolysis of Gibberellin A_9 - Gibberellin A_9 (50 mg.) in ethyl acetate (5 ml.) was treated with a stream of ozonised oxygen at -40° for 5 min. The solution was allowed to attain room temperature and shaken with water. Evaporation of the solvent and crystallisation from acetone-light petroleum gave gibberellin A_9 nor-ketone as prisms, m.p. 125-128° (Found: C, 64·2; H, 7·1. $C_{18}H_{22}O_5$, H_2O requires C, 64·3; H, 7·2%) $\nu_{max.}$ 3469, 1753, 1725, 1700 $cm.^{-1}$

Gibberellin A_{10} - Gibberellin A_{10}(41) crystallised from acetone-light petroleum as needles, m.p. 245-246° (Found: C, 68·2; H, 7·9. $C_{19}H_{26}O_5$ requires C, 68·2; H, 7·8%) $\nu_{max.}$ 3360, 1766 and 1680 $cm.^{-1}$

The methyl ester, prepared with diazomethane, crystallised from acetone-light petroleum as needles, m.p. 167-168° (Found: C, 69·2; H, 8·18. $C_{20}H_{28}O_5$ requires C, 68·9; H, 8·1%) $\nu_{max.}$ 3545, 1757, and 1735 $cm.^{-1}$

Hydration of Gibberellin A_9. - Gibberellin A_9 (50 mg.) in methanol (0·5 ml.) was treated with dilute hydrochloric acid (1·0 ml.) at room temperature for 68 hr. The solution was diluted with water and extracted with ethyl acetate, the extract washed with water, dried and evaporated to give gibberellin A_{10} (41) (46 mg.) which crystallised from acetone-light petroleum as needles, m.p. 244-246° identical to the material isolated from Gibberella fajikuroi.

Gibberellin A_{11}. - Gibberellin A_{11} crystallised from acetone-light petroleum as needles, m.p. 242-245° (Found: C, 69·3; H, 6·8. $C_{19}H_{22}O_5$ requires C, 69·1; H, 6·7%) $\nu_{max.}$ 3150, 1777, 1730, 1658 and 896 cm.$^{-1}$ $[\alpha]_D^{20}$ + 11° (c 0·5).

The methyl ester crystallised from acetone-light petroleum as needles, m.p. 179-181° (Found: C, 70·4; H, 7·1. $C_{20}H_{24}O_5$ requires C, 69·8; H, 7·0%) $\nu_{max.}$ 1765, 1729, 1659 and 886 cm.$^{-1}$

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CHAPTER II

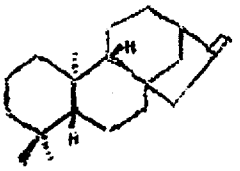
Some Aspects of the Chemistry of the Kaurene Ring System

2.1. The Kaurenolide Metabolites of Gibberella fujikuroi

The gibberellins are well-known metabolites of the fungus Gibberella fujikuroi ACC 917. Their chemistry and biological significance have been extensively explored during the past few years. In addition the mould elaborates[1] a further group of diterpenoid metabolites which, whilst lacking the intrinsic biological interest of the gibberellins, nevertheless take their place amongst the expanding group of tetracyclic diterpenes. The structure of these metabolites are summarized in fig. I. It is the purpose of this part of the thesis to examine the chemistry of these metabolites and the light that it sheds on the stereochemical consequences of the kaurene ring-system.

The major kaurenolides, (3) and (4), were characterized[2] through their derivatives and spectral properties as mono- and di-hydroxy-γ-lactones containing one double bond present as an exocyclic methylene group on a five-membered ring. They were thus tetracarbocyclic. The isolation[1] of the known natural products, (-)-kaurene and (-)-kauranol, from the culture filtrate implied a relationship with these metabolites which was subsequently established experimentally. The spectral and analytical data of fujenal (6) together with the related acid, fujenoic

fig. 1.



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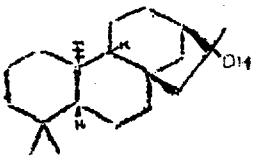

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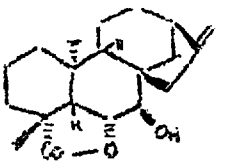

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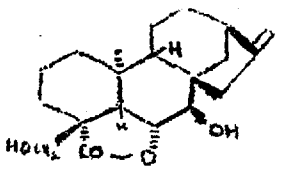

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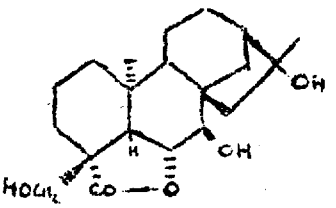

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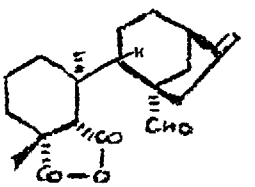

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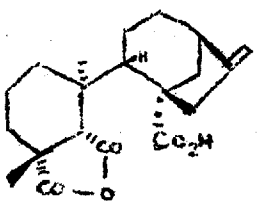


(7)

acid (7), showed them to be tricarbocyclic containing a five-ring anhydride, an aldehyde (and its corresponding acid) together with an exocyclic methylene grouping. The structural studies were therefore resolved into a number of phases which involved firstly the inter-relationship of the metabolites, secondly the determination of the position and relative stereochemistry of the oxygen substituents, thirdly the assignment of the absolute stereochemistry to the ring system and finally the biosynthesis.

The interrelationship of the metabolites in which 7-hydroxykaurenolide formed the key intermediate, was achieved as follows. The primary hydroxy group of 7,18-dihydroxykaurenolide[3] was acylated with toluene-p-sulphonyl chloride in pyridine to form the 18-monotoluene-p-sulphonate. This on reduction with lithium aluminium hydride, gave a triol which was also obtained by reduction of 7-hydroxykaurenolide. 7,16,18-Trihydroxy-kaurenolide, which can be obtained from 7,18-dihydroxy-kaurenolide by the action of mineral acid, was degraded to an 18-nor-16-hydroxy-7-ketone which was also obtained from 7β,18-dihydroxykaurenolide.

7-Hydroxykaurenolide[4] was reduced to (-)-kaurane through a reaction sequence which precluded epimerization at any of the ring junctions. The epimeric 16-dihydro

compounds were separated and the 6-oxygen function eliminated by hydrogenolysis of the 7-keto-lactone with zinc dust and acetic anhydride. The 7-ketone was subsequently removed through a Wolff-Kishner reduction and the 19-carboxyl converted to a methyl group through the alcohol and its corresponding aldehyde. This degradation at the same time formed a link with steviol and the garryfoline alkaloids.

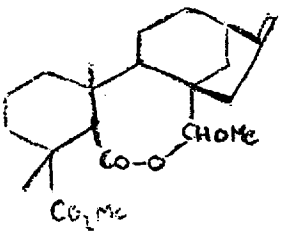
Fujenal[5] was related to fujenoic acid by oxidation. On methanolysis fujenal formed a pseudo-ester shown by its nuclear magnetic resonance spectrum to be (8). Ozonolysis of this gave the 16-oxo-17-nor derivative. Cautious hydrolysis with mineral acid then distinguished between the ketal methoxyl and the ester methoxyl to give a lactonol which on subsequent oxidation formed the dicarboxylic acid (9). The latter was obtained, inter alia, through oxidation of methyl 6,7-dihydroxy-16-oxo-17-norkauran-19-oate derived by ozonolysis and hydrolysis of 7-hydroxykaurenolide.

Implicit in the degradation of 7-hydroxykaurenolide to (-)-kaurane was the presence of an acylated α-glycol. 7-Hydroxykaurenolide was oxidized to a cyclohexanone, the presence of which was substantiated by the conversion of the 6,7-secodicarboxylic acid (9) through an unstrained adipic anhydride to a cyclopentanone. Furthermore

oxidation of the α-glycol led to the formation of a diketone enolizing to a dicsphenol the ultraviolet spectrum of which (λ_{max} 281 mμ) required a fully substituted chromophore possible only on rings B and C. Elimination of the 7-tosyloxy group from the 7-toluene-p-sulphonyl derivative of 7-hydroxykaurenolide with the formation of an enol-lactone [was only sterically possible with the lactone carbonyl at the geminal position. Furthermore the 6,7-secodicarboxylic acid (9) did not behave as a β-keto-acid and indeed on refluxing with water it reformed the five-ring anhydride. Thus the diol system of 7-hydroxykaurenolide was placed at the 6 and 7-positions.

The nuclear magnetic resonance spectrum 7,18-dihydroxy-kaurenolide showed that it contained only one C-CH_3 group and that a primary alcohol replaced the second methyl group of 7-hydroxykaurenolide. Two facets of its chemistry, namely the thermal loss of formaldehyde by a retroaldol reaction and the oxidative decarboxylation of the primary alcohol indicated that the geminal methyl groups of (-)-kaurene both bore oxygen substituents in 7,18-dihydroxy-kaurenolide.

The stereochemistry of the kaurenolides and hence that of (-)-kaurene (which was open to doubt at the time of this work) involved a number of pieces of evidence. At C(4) it was possible to distinguish between the axial carbonyl of the lactone ring and the equatorial hydroxymethyl



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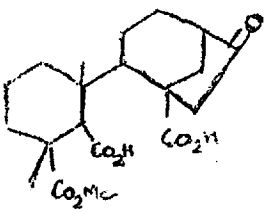

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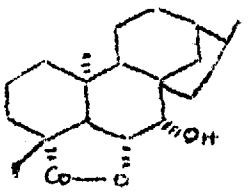

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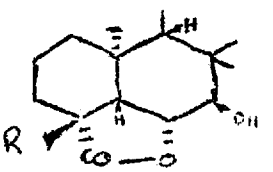


(11)

substituent by hydrogenolysis of the 18-toluene-p-sulphonate as opposed to hydrolysis of the 19-toluene-p-sulphonate with lithium aluminium hydride and by pK_a measurements of the derived acids.

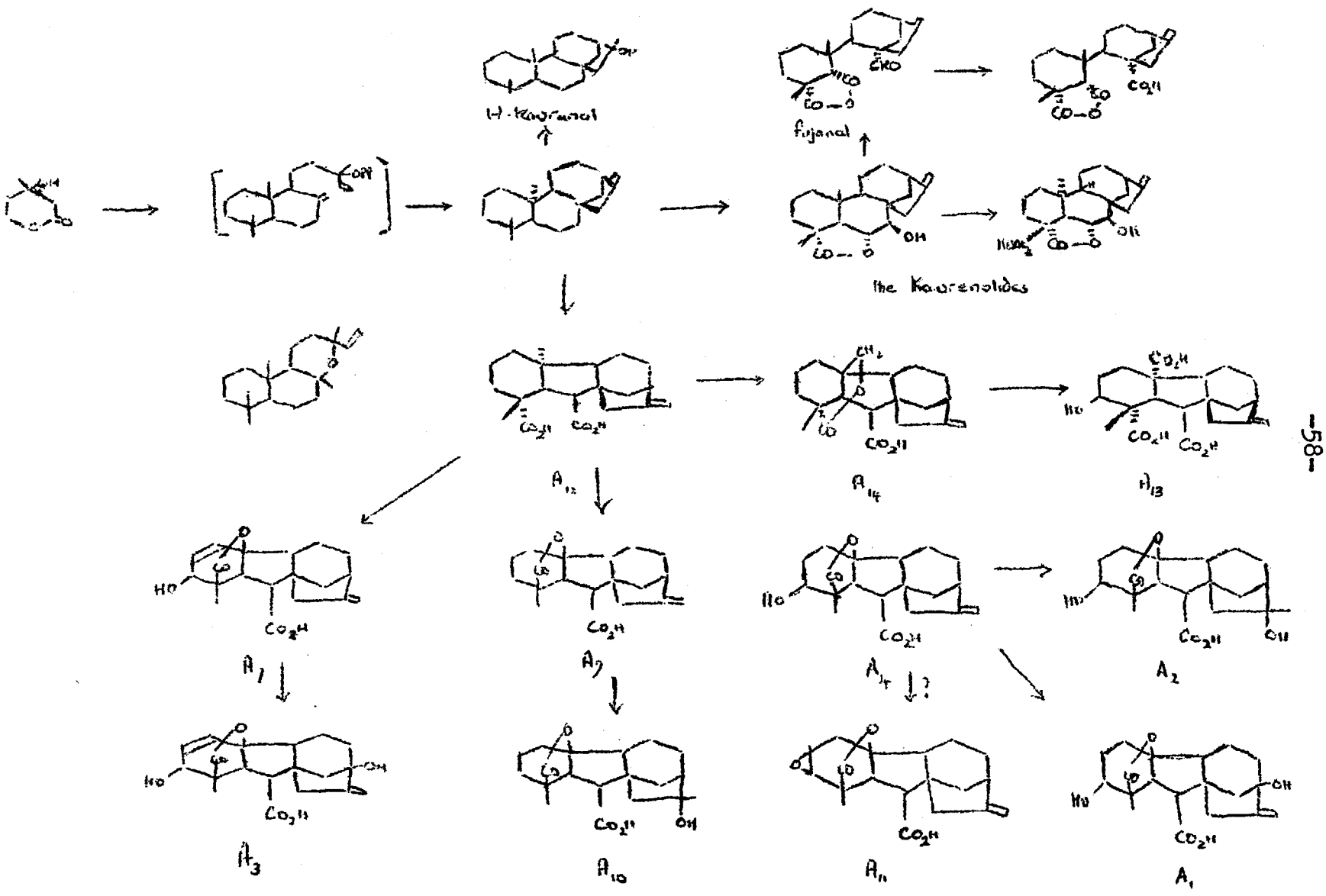
The trans diaxial relationship of the oxygen atoms on ring B was demonstrated in a number of ways. Reduction of the 7-ketones with sodium borohydride led to a 7-alcohol epimeric at this centre with the natural alcohols. Comparison of the relative rates of elimination of the 7-toluene-p-sulphonates showed little difference between the epimers and hence there was no trans diaxial relationship between a α-hydrogen atom and the leaving group. Furthermore whereas hydrolysis of the natural kaurenolides gave the corresponding 6,7-diols in high yield, hydrolysis of their 7-epimers gave a 25-30% yield of the 6-deoxy-7-ketones. The ready trans diaxial elimination of water required an axial 7-hydrogen atom. Furthermore the natural diols were inert to oxidation with sodium periodate whilst their 7-epimers reacted rapidly. Hence in the kaurenolides their is a trans relationship between the 6 and 7 substituents.

The presence of a 19-6 diaxial lactone ring precluded the existance of a cis A/B ring junction. Furthermore

oxidation of the 7-epihydroxy-19-6-lactone (10) with lead tetraacetate led to the formation of a 7-20 ether thus demonstrating a cis relationship between the hydroxyl group and the angular methyl group. Therefore the relative stereochemistry of the oxygen functions and the angular methyl group required the presence of the fragment (11; R = CH_3 and CH_2OH respectively) in 7-hydroxy- and 7,18-dihydroxy-kaurenolide. Since methyl 7-oxo-(-)-kauran-19-oate showed a large positive Cotton effect in its optical rotatory dispersion curve whilst the corresponding lactone showed a negative effect superimposed on a positive background, the lactone ring on formation must close axially into a negative quadrant and thus (11) represents the absolute stereochemistry of rings A and B. 16-Oxo-17-nor(-)-kaurane showed[6] a positive Cotton effect like a number of other 16-ketones and hence ring D possessed a β-absolute configuration. Thus the overall stereochemistry of (-)-kaurene and the kaurenolides must be represented by (4) and (5) respectively, in agreement with deduction drawn from studies with the diterpene alkaloids.

2C^{14}-Mevalonic acid and 17C^{14}-(-)-kaurene were incorporated[7] by Gibberella fujikuroi into gibberellic acid[8], 7-hydroxykaurenolide and 7,18-dihydroxykaurenolide. However 7-hydroxykaurenolide and its corresponding diol were only

transformed into 7,18-dihydroxykaurenolide and hence the biosynthetic pathways leading to the kaurenolides and to the gibberellins diverged prior to the formation of 7-hydroxykaurenolide. Furthermore 7,18-dihydroxykaurenolide derived from $2C^{14}$-mevalonate contained a quarter of the radioactivity at C(18) which therefore corresponded to the 1-methyl group of gibberellic acid. Since the absolute stereochemistry of the kaurenolides was known with confidence at this centre it was concluded that the γ-lactone ring of gibberellic acid was α-oriented. At that time this feature was in dispute although further chemical and X-ray measurements substantiated the stereochemistry of ring A described by the I.C.I. workers. The proposed biogenetic relationship between the metabolites is outlined in the accompanying diagram.


ent-Kaurenal
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Some Studies with (-)-Kaurene

2.2

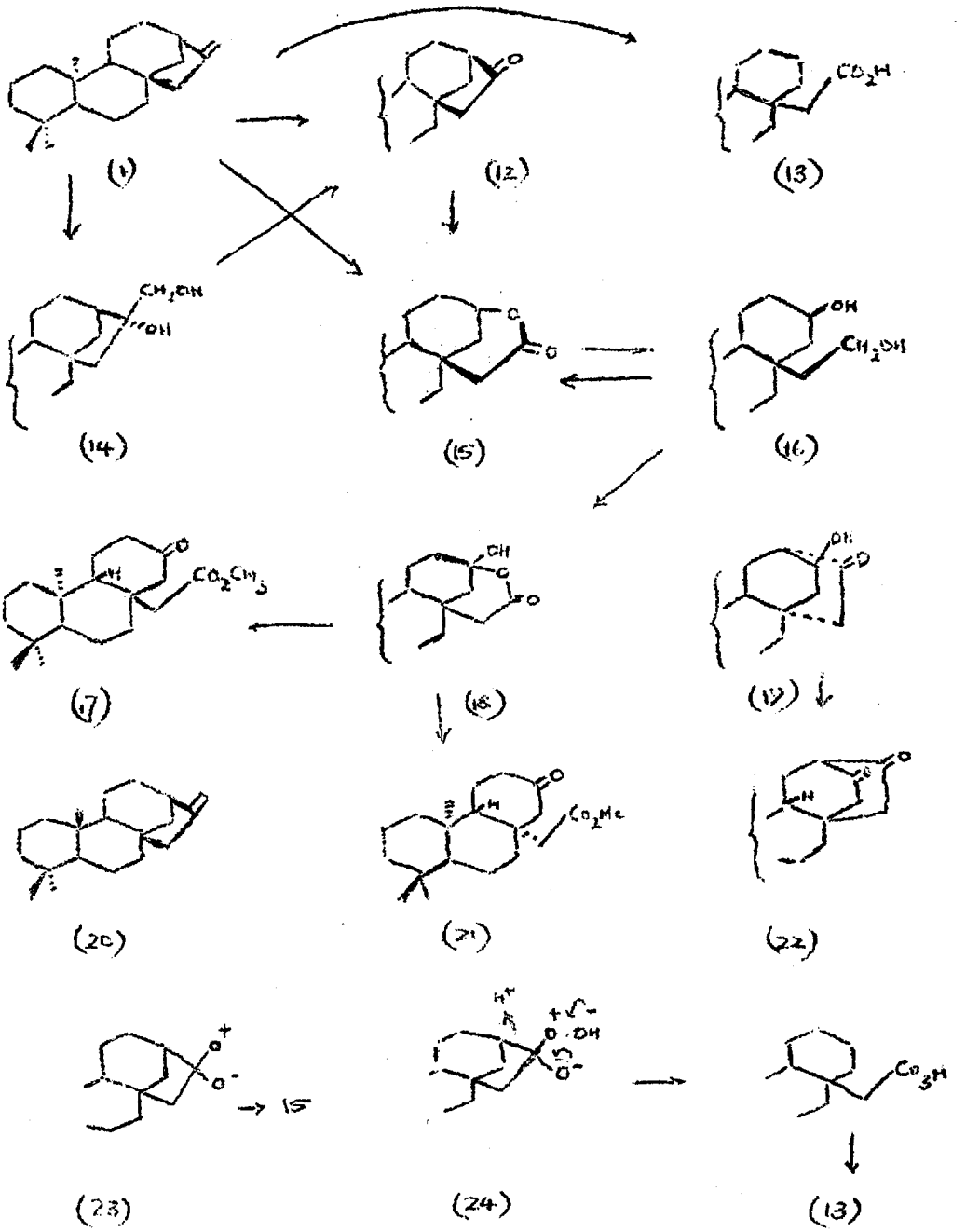
Both trans-anti-cis and trans-anti-trans skeleta exist amongst the tetracyclic diterpenes. However the predominant stereochemistry now appears to be that of the cis B/C fusion of (-)-kaurene to which the diterpene alkaloids of the atisine[9] and garryfoline group,[10] (-)-kauranol,[11] the kaurenolides,[2] and steviol[12] have been related. Cafestol,[13] the gibberellins[14] and the grayanotoxins[15] with an identical B/C/D fusion have an obvious biogenetic relationship. As part of the problem of determining the structure and stereochemistry of the kaurenolides,[2] we turned our attention to the then (1960) unproved stereochemistry of (-)-kaurene. These studies[16] form the subject of this chapter.

The isolation of (-)-kaurene (1) from Gibberella fujikuroi has been described.[1] Ozonolysis of (-)-kaurene in glacial acetic acid gave[17] as the major product 17-nor-(-)-kauran-16-one (12) ($\nu_{max.}$ 1745 cm.$^{-1}$) which showed a positive Cotton effect in the optical rotatory dispersion cruve. Amongst the other products of ozonolysis was a δ-lactone (15) ($\nu_{max.}$ 1724, 1216 cm.$^{-1}$), identical with the product of Baeyer-Villiger oxidation of the nor-ketone. A monobasic acid, $C_{19}H_{32}O_2$ ($\nu_{max.}$ 2667, 1704 cm.$^{-1}$), which was saturated and hence tricarbocyclic, was also

isolated from the ozonolysis and has been assigned the structure (13). Although we could not exclude the possibility of Baeyer-Villiger oxidations during this reaction, these by-products could arise[18] from rearrangement of the zwitterions (23) and (24) and in the case of the latter subsequent decomposition of the per-acid.

Treatment of kaurene in ether-pyridine with osmium tetroxide gave (-)-kaurane-16α,17-diol in which hydroxylation was assumed to have taken place from the less hindered α-face of the molecule to form the 16α-hydroxy derivative. Cleavage of the diol with sodium periodate in aqueous methanol gave 17-nor-(-)-kauran-16-one.

The δ-lactone (15) was prepared in quantity by the Baeyer-Villiger oxidation of the 17-nor-16-ketone using perbenzoic acid catalysed by toluene-p-sulphonic acid. Migration of the more highly substituted bond under these conditions has been observed[17] in the oxidation of camphor and in the degradation[20] of gibberellin A_7. However the free acid could not be obtained pure by alkaline hydrolysis of the lactone. Thus refluxing with sodium hydroxide and cautious acidification at 0° followed by rapid work-up returned the starting material. Similar difficulties have been recently observed in the related degradation[21] of


(1)
(12)
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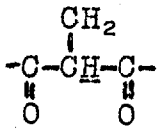
(13)

veatchine. This was avoided by reduction of the δ-lactone with lithium aluminium hydride to the diol (16) which on oxidation with chromium trioxide in sulphuric acid afforded a lactonol (18) [$\nu_{max.}$ 3350 and 1703 cm.$^{-1}$] as the major product. The lactone and a monohydroxy-ketone, $C_{19}H_{30}O_2$, were isolated as minor products from this oxidation.

The hydroxy-ketone [$\nu_{max.}$ 3450 and 1710 cm.$^{-1}$], showed no significant end-absorption in the ultraviolet whilst resonances which might be attributed to olefinic protons are absent from its nuclear magnetic resonance spectrum and hence the compound was tetracarbocyclic. Further analysis of this spectrum suggested the presence of a secondary alcohol (one-proton multiplet, τ 5·9) together with a ketone flanked by an α-methylene group (τ 7·7) and an α-methine proton (τ 7·15). On oxidation the hydroxy-ketone formed a diketone ($\nu_{max.}$ 1740 and 1721 cm.$^{-1}$) the nuclear magnetic resonance spectrum of which, whilst excluding the presence of an aldehyde, showed a one-proton triplet at τ 6·8 J = 3c/sec. due to the system



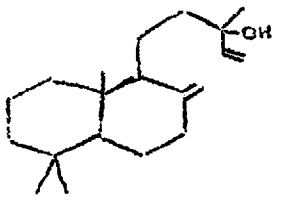
It followed that the hydroxy-ketone had the atisine skeleton (19) arising from an internal aldol condensation

of a keto-aldehyde formed during the oxidation. It has a structure related to that of the intermediate in the inversion of the acetic acid side chain (vide infra).

The lactonol (18) could not be methylated by ethereal diazomethane. However the methyl ester (17) was obtained by refluxing with methyl iodide in dry acetone in the presence of two moles of potassium hydroxide. This keto-ester had m.p. 128-129° and showed absorption in the infrared at 1733 and 1706 $cm.^{-1}$ However, on methylation with methyl iodide in methanol in the presence of excess sodium methoxide an isomeric methyl ester (21) was isolated, m.p. 178-179·5° ($\nu_{max.}$ 1738 and 1711 $cm.^{-1}$). Furthermore the optical rotatory dispersion curve of this ester displayed a negative Cotton effect. The ester was shown[16] to be the optical antipode of a degradation product of (+)-phyllocladene (20) kindly supplied by Drs. R. C. Cambie and P. S. Rutledge of the University of Auckland. The infrared spectra were identical in solution and in nujol mull whilst the optical rotatory dispersion curves were mirror images. This example of the inversion of the acetic acid side chain is analogous to the rearrangement[22] of some degradation products of allogibberic acid for which a 1:3-diketone intermediate has been proposed. The inter-

relationship with phyllocladene clearly demonstrated the antipodal nature of the A/B ring fusion of (-)-kaurene together with the position of the angular methyl group and serves to link the (-)-kaurene group of diterpenes not only with (+)-phyllocladene but also with the bicyclic diterpenes of the manöol series. Edwards et al[23] have reported a similar link between podocarpic acid and a degradation product of the alkaloid atisine.

Before the relationship with phyllocladene was complete, an attempt was made to remove the ring D C-15, C-16 bridge of (-)-kaurene in the hope of achieving a similar link with a degradation product of (+)-manöol (25). Isomerisation of (-)-kaurene with methanolic sulphuric acid gave (-)-isokaurene and the methyl ether (26) as a minor product.[24] Hydroxylation of isokaurene with osmium tetroxide gave the 15,16-diol (27) which on oxidation with lead tetraacetate in benzene followed by crystallization of the gummy residue from methanol gave the tri-ether $C_{22}H_{38}O_3$. The structure of the ether followed from its nuclear magnetic resonance spectrum which showed resonances at τ 6·81 and 6·59 due to two methoxyl groups and deshielded $-C-CH_3$ resonance at τ 8·77 in addition to the three methyl resonances at



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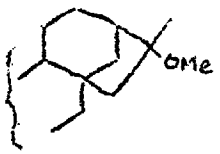

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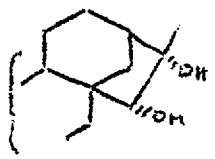

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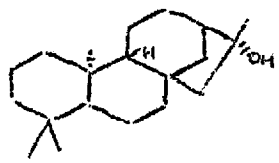

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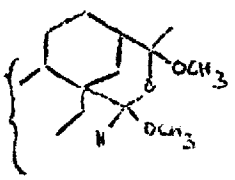
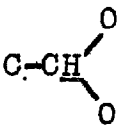


(28)

τ 9·16, 9·14 and 8·96 and a sharp singlet at τ 6·09 due to  leading to the internal ketal structure (28) for the ether.

The hydrated terminal methylene group of phyllocladanol[25], gibberellin A_2[26] and gibberellin A_{10}[27] is a characteristic feature of the tetracyclic diterpenes. (-)-Kauranol,[11] which has also been isolated from Gibberella fujikuroi, was of undefined stereochemistry at C(16). Epoxydation of (-)-kaurene gave a monoepoxide which following the pattern of hydrogenation, was assumed to be the 16α-epoxide, i.e. attack of perbenzoic acid from the less hindered α-face of the molecule. Reduction of this epoxide with lithium aluminium hydride gave (-)-kauranol which must therefore have the 16α hydroxyl group (2).[17] Treatment with mineral acid gave the diol (14).

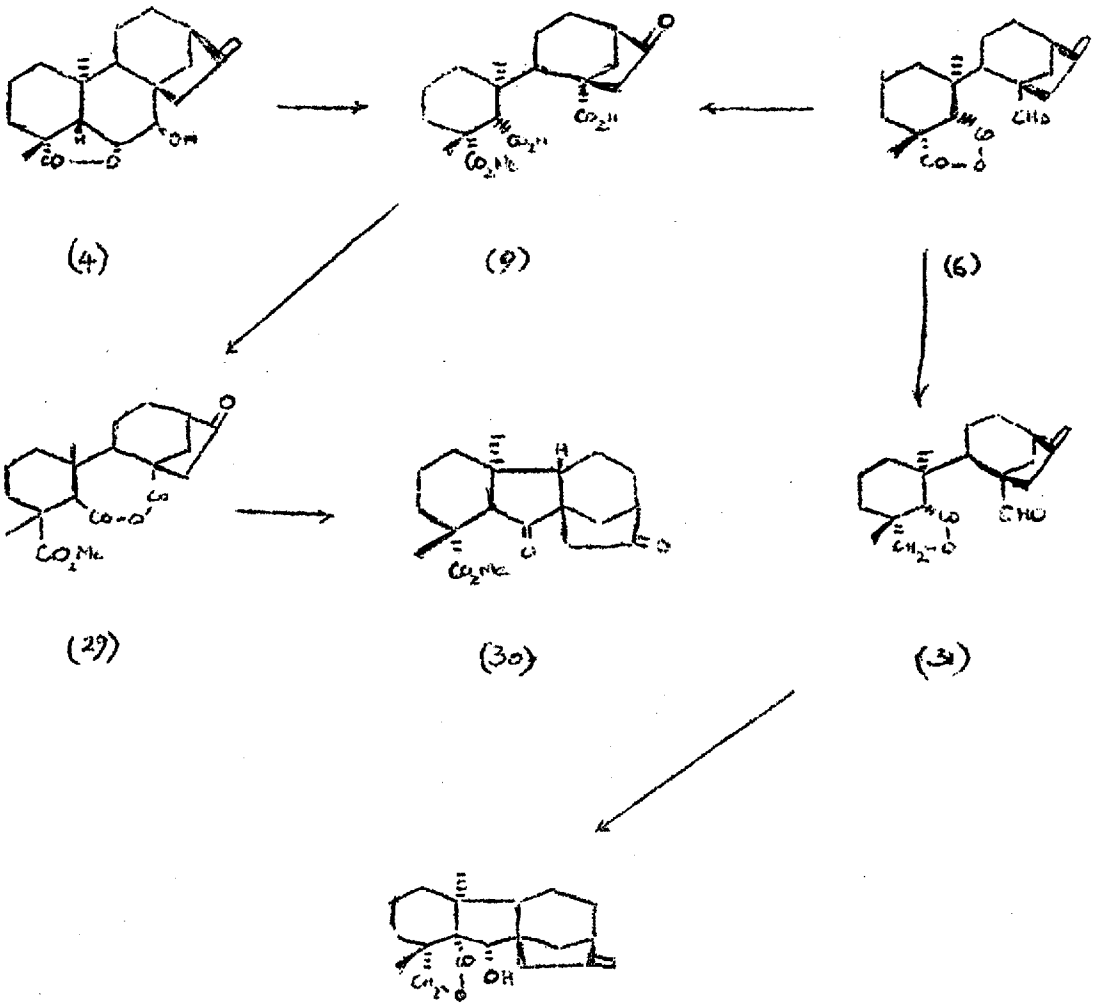
2.3 The Preparation of some Ring B Nor-derivatives

Considerable attention has been directed at the biological activity of compounds possessing the gibbane skeleton.[28] 7-Hydroxykaurenolide (4) occurs along with gibberellic acid as a diterpenoid metabolite of the fungus Gibberella fujikuroi. Furthermore it belongs to the same enantiomorphic series whilst the parent hydrocarbon, (-)-kaurene, has been shown to be converted[7] in vivo to gibberellic acid. In this section the conversion of fujenal[5] (6) and 7-hydroxykaurenolide[4] to compounds of the gibbane skeleton will be described.[29] Two successful methods of ring contraction have been explored involving in the first the cyclization of ring B seco derivatives and in the second the rearrangement of ring B.

The dicarboxylic acid (9) can be obtained in three stages from 7-hydroxykaurenolide[4] and in four stages from fujenal.[5] On refluxing with acetic anhydride it formed the internal 6,7-anhydride (29) (no acetoxyl), $\nu_{max.}$ 1794, 1742, and 1717 $cm.^{-1}$ The anhydride was hydrolysed to the parent acid with aqueous methanolic sodium hydroxide whilst on pyrolysis at 280° it evolved carbon dioxide to give a diketone shown to have the gibbane structure (30) by its infrared spectrum ($\nu_{max.}$ 1753, 1741 (cyclopentanones), and

1729 (ester) cm.$^{-1}$). However attempts to obtain confirmation of this by dehydrogenation to a fluorene were unsuccessful. Pyrolysis of the barium salt of the dicarboxylic acid gave intractable material showing infrared absorption characteristic of a five-membered anhydride whilst pyrolysis of the free dicarboxylic acid at 210° led to the formation of 17-nor-16-oxofujenoic acid.

An alternative route to the gibbane skeleton involved reduction of fujenal with lithium aluminium hydride to form the hydroxy-lactone (31). On one occasion the corresponding dihydroxyacid was isolated from this reduction. The hydroxy-lactone, on oxidation with chromium trioxide, gave the aldehyde which on refluxing with 0·5N-methanolic sodium hydroxide, underwent an internal aldol condensation to form a hydroxy-γ-lactone, $C_{20}H_{28}O_3$. Since microhydrogenation of this showed the presence of only one double bond, present from the infrared spectrum ($\nu_{max.}$ 3065, 1650 and 875 cm.$^{-1}$) as a terminal methylene group, the lactone was tetracarbocyclic. Oxidation of the hydroxy-lactone with chromium trioxide gave a keto-lactone which decarboxylated on pyrolysis. The gibbane structure (32) for the condensation product is consistent with its nuclear magnetic resonance spectrum which showed a one proton singlet at τ 5·98 and a two-proton



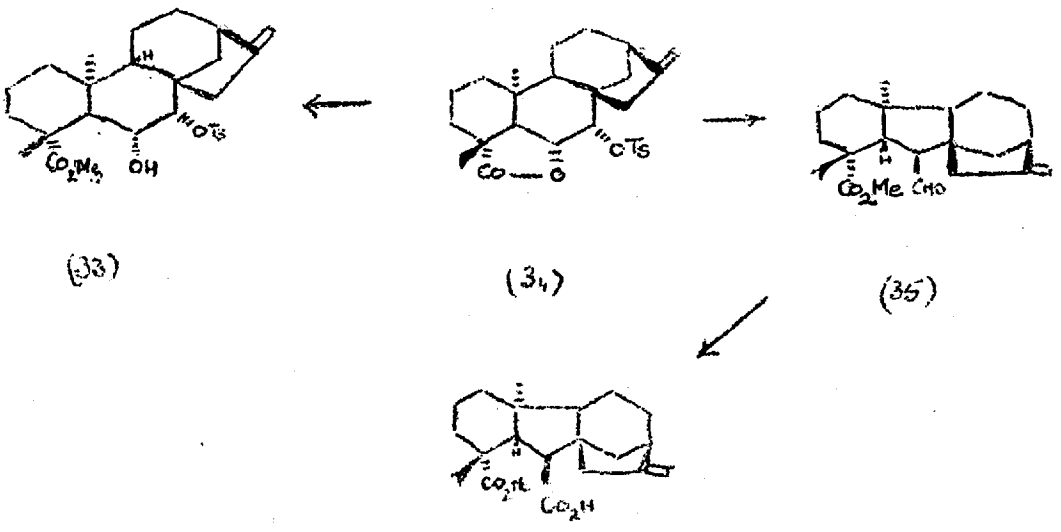
(4) (9) (6)

(29) (30) (31)

(32)

double doublet at τ 5·8 and 6·3 J = 9 c/sec. assigned to the CH.OH and C-CH_2O protons respectively. Reduction of the keto-lactone with sodium borohydride regenerated the parent hydroxy-lactone. Attack of the reagent from the less hindered β-face of the molecule leads to the α-configuration for the hydroxyl group as in (32).

The solvolysis of 11α-acetoxy-12β-mesyloxyrockogenin has been shown[30] to lead to the extrusion of a carbon atom with the formation of a ring C nor-aldehyde. However the kaurenolides possess a diaxial diol system whereas rearrangement requires the elimination of an equatorial group. Indeed it is possible that the kaurenolides retain the perhydrophenanthrene skeleton since they are 'wrongly' substituted on ring B. The equatorial 7-alcohols were readily available through reduction of the 7-oxo-kaurenolides with sodium borohydride and these on alkaline hydrolysis and methylation, gave inter alia the corresponding 6 (ax.), 7(eq.)-diols. On treatment with toluene-p-sulphonyl chloride in pyridine, methyl 6α,7α-dihydroxy-kaur-16-en-19-oate gave only a mono-toluene-p-sulphonate (33) in which the free hydroxyl is shown to be at the 6-position. This compound was recovered unchanged after treatment with refluxing 10% methanolic potassium hydroxide, sodium methoxide and potassium t-butoxide. However treatment of the toluene-p-sulphonate of



(33) (34) (35)

(36)

7α-hydroxykaurenolide refluxing 10% methanolic potassium hydroxide gave, after methylation of the crude product, a 10-15% yield of ring B nor-aldehyde (35). The major product from this reaction was the toluene-p-sulphonate (33) thus showing that the free hydroxyl group was at the 6-position. Presumably hydrolysis of the lactone ring provided some anchimeric assistance for the elimination of the 7-toluene-p-sulphonate with concomitant migration of the 5-6 bond. Under the same conditions hydrolysis of the 7 (ax.) toluene-p-sulphonate gave mainly intractable material. The aldehyde (35) showed absorption in the infrared at 2738 $cm.^{-1}$ (aldehyde C-H) and 1730 and 1712 $cm.^{-1}$ (ester and aldehyde). It was oxidised to a gibbane mono-carboxylic acid (36). The latter possessed the characteristic[3] 10:10a quartet in the nuclear magnetic resonance spectrum with a coupling constant of 11 c/sec. Thus the carboxyl group existed in the more stable β-configuration typical of the gibberellins.

2.4 The Shape of Ring B of the Kaurenolides

The reactions of ring B of the kaurene-atisine group of diterpenes reveal the participation of both boat and chair forms. Thus on the one hand the elimination and cleavage reactions of 7-hydroxykaurenolide[4] are those diagnostic of chair forms whilst on the other hand the ether formation and internal hydrogen transfer reactions of the alkaloids imply the participation of boat forms.[9] Therefore in order to define the ground state conformation of ring B in the kaurenolides we measured the coupling constants between the C(5), C(6) and C(7) protons for a series of 7-epimers. These results are tabulated.

The parameters of the Karplus equation[32] appear to be dependent on the chemical nature of the coupled fragment.[33] A set of parameters closely related to those of other workers was found to be satisfactory.

$$J_{AB} = 12{\cdot}4 \cos^2 \Phi \quad 0 < \Phi < 90^\circ$$

$$J_{AB} = 14{\cdot}3 \cos^2 \Phi \quad 90 < \Phi < 180^\circ$$

Since the coupling constants lie in the steeply changing portion of the curve variation of $\pm$ 0·5 in the parameters of the Karplus equation introduce variations of only $\pm 3^\circ$ in the calculated dihedral angles. Furthermore since epimeric pairs of compounds were used in these calculations a cross

check can be obtained. The average value of $J_{6\beta:7\alpha}$ of 6·75 corresponds to a dihedral angle of 146° ± 3° between these protons whilst $J_{6\beta:7\beta}$ of 7·75 corresponds to an angle of 22° ± 3°. Hence we conclude that in the kaurenolides in which the lactone ring is present, ring B exists in a twisted boat form[34] with C(6) at one of the points.

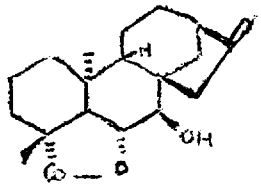
The compounds used in this study were prepared by methods described previously.[3,4] Thus reduction of the 7-ketones with sodium borohydride gave the corresponding 7α-alcohols. Contrary to an earlier report[3] 7,18-dihydroxy-kaurenolide forms both a mono and di-toluene-p-sulphonyl derivative together with a dibenzoate and a dimethane-sulphonate. Oxidation of the monotoluene-p-sulphonate with chromium trioxide gave the 7-ketone which was reduced with sodium borohydride to the 7α-alcohol. Treatment of the monotoluene-p-sulphonate with sodium hydrogen carbonate in refluxing dimethyl sulphoxide gave the 18-nor derivative in better yield than the pyrolytic procedure described previously.[3]

The spectra of a number of 6:7 diols and 7-acylated diols were also examined. In most cases the 6- and 7-protons were incompletely resolved and it was impossible to obtain a complete set of coupling constants. However

$J_{6\beta:7\beta}$ is approximately 2-3 c/sec. as in the acetyl derivative (37). This corresponds to an angle of about 60° indicating that in these compounds ring B is nearer to an undistorted chair form thus accommodating the earlier experimental results.

Williamson and Johnson derived[35] an expression of the Karplus equation for some steroidal α-acetoxy ketones. This equation was used for the analogous 7-keto-lactones which showed an average $J_{5:6}$ of 6 c/sec. corresponding to an angle of 39° ± 3° between these protons and hence to a partially twisted form. In addition to showing a doublet at $\tau = 5.32$ $J_{5:6} = 6$ c/sec., the spectrum of the keto-lactone (38) shows a triplet at $\tau = 7.35$ arising from the 5-proton in which $J_{4:5} = J_{5:6} = 6$ c/sec. Hence the lactone ring remains diaxial despite the loss of an equatorial 18-substituent. These results suggest that in the kaurenolides distortion of ring A of the type displayed[36] by the 4,4-dimethyl steroids may be relayed to ring B by the lactone ring.

One consequence of this conformation of ring B is the juxta-position of the angular C-10 methyl and C-7α-hydroxyl groups. A six-membered transition state has been proposed[37] for photochemical substitution reactions. However a 7-20 ether has been reported from a thermal reaction with lead



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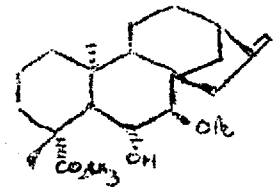

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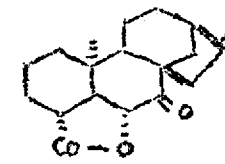

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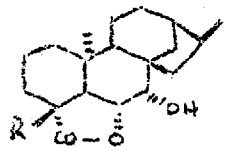

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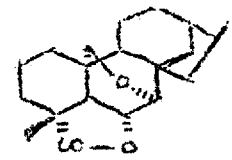

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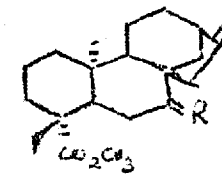

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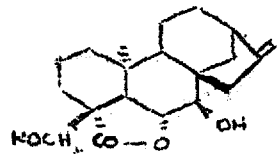

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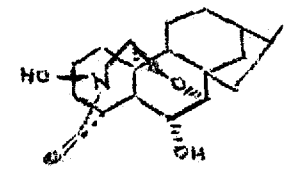

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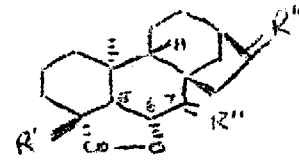

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Table 2

The Chemical Shifts of the 6 and 7-protons in the Kaurenolides

Compound 43	R'	R''	R'''	τ_6	$J_{5:6}$	τ_7	$J_{6:7}$
1.	CH_3	OH H	$=CH_2$	5·20	6·5	6·65	6·5
2	CH_3	H OH	$=CH_2$			5·92	7·5
3	CH_2OH	OH H	$=CH_2$	5·21	7 0	5·68	7·0
4	CH_2OH	H OH	$=CH_2$			5·95	7·5
5	CH_3	OAc H	$=CH_2$	5·35		4·10	6·5
6	CH_3	H OAc	$=CH_2$			4·70	8·0
7	CH_3	OAc H	$=O$	5·35	6·5	4·20	6·5
8	CH_3	H OAc	$=O$			4·7o	8·o
9	CH_2OTs	OH H	$=CH_2$	5·25	6·5	5·55	6·5
10.	CH_2OTs	H OH	$=CH_2$	5·1		5·88	8·0
11.	CH_2OTs	OTs H	$=CH_2$	5·40	6·5	4·60	6·5
12.	CH_3	OTs H	$=CH_2$	5·38	6·5	4·57	6·5
13.	CH_2OAc	OAc H	$=CH_2$	5·30	7·0	4·23	7·0
14.	CH_2OAc	OAc H	$=O$	5·31	7·0	4·23	7·0

Table 2 continued

	R^{I}	R^{II}	R^{III}	τ_6	$J_{5:6}$
15.	CH_3	=O	$=CH_2$	5.11	5.5
16.	CH_2OH	=O	$=CH_2$	4.95	6.0
17.	CO_2CH_3	=O	=O	5.21	6.5
18.	H	=O	$=CH_2$	5.32	6.5

tetraacetate in this series and it was therefore of interest to apply photochemical transformations to this series to seek exceptions to the rule. Irradiation of the 7α-alcohol (39; R=CH_3) in the presence of iodine and lead tetraacetate for 3 hr. gave a good yield of an ether, $C_{20}H_{28}O_3$ together with only a trace of the corresponding 7-ketone. Comparison of the nuclear magnetic resonance spectrum with that of the starting material revealed the loss of the C-10 methyl resonance and the appearance of a methyleneoxy group at τ = 6.42. The C-7 proton remained coupled to the C-6 proton which appeared as a double doublet at τ 5.28. Further evidence for the structure (40) was obtained as follows.

Although the ether was recovered unchanged after attempted hydrolysis with N-sodium hydroxide, reduction with lithium hydride afforded a diol-ether. This on oxidation with chromium trioxide, gave a lactonol in which the C-7 proton resonance had collapsed to a singlet. Furthermore reduction of the lactonol with lithium aluminium hydride furnished the parent ether (40) thus removing the possibility of C-C bond fission during the reaction sequence and establishing the stereochemistry at C-6 which could have been inverted during photolysis. Hence we may conclude that the photochemical ether formation reaction may under these favourable circumstances utilize a seven-membered transition state.

Reduction of the 7-keto-ester (41, R=O) with sodium borohydride gave the 7α-alcohol (41, R=OH) which on irradiation with lead tetraacetate and iodine in benzene gave only intractable material.

Owing to the scarcity of 7-hydroxykaurenolide, the Barton reaction[38] was examined in the 18-nor series. 7,18-Dihydroxykaurenolide (5) on vigorous oxidation gave, inter alia, the keto-lactone (38) which was reduced with sodium borohydride to the 7α alcohol (39, R=H). Catalytic hydrogenation followed by careful crystallization led to the isolation of the α-dihydro compound. Alternatively reduction of 7,18-dihydroxykaurenolide over 10% palladised charcoal followed by fractional crystallization gave pure α-dihydro-7,18-dihydroxykaurenolide. This was related to the corresponding α-dihydro-7-hydroxykaurenolide by reduction of its mono-toluene-p-sulphonyl derivative with lithium aluminium hydride to a triol. The same triol, 6α, 7β, 19-trihydroxykaurane, was obtained by reduction of α-dihydro-7-hydroxykaurenolide. Oxidation of the kauranolide with the chromium trioxide reagent followed by decarboxylation gave the corresponding 18-nor-7-ketolactone which on reduction with sodium borohydride formed the 7-epialcohol. Reduction of this alcohol with lithium aluminium hydride gave a triol. The nuclear magnetic resonance spectrum of

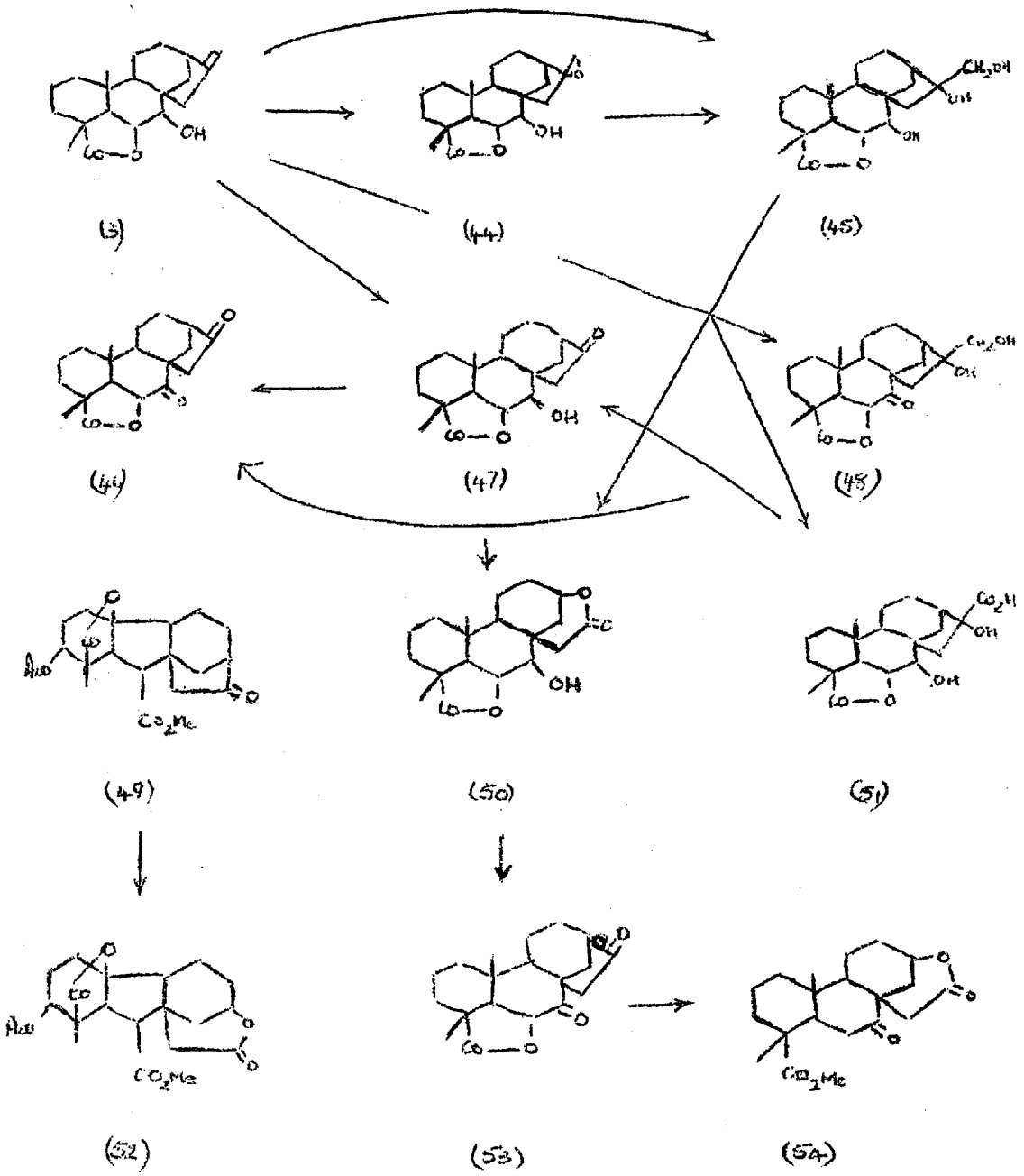
this and the corresponding triol from 7-epihydroxy-kaurenolide both possess a two-proton resonance at τ = 6.10 which may be assigned to the axial hydroxymethyl group. Thus in accord with the evidence discussed earlier, epimerization at C(4) has not taken place during the preparation of the 18-nor lactones.

Irradiation of the 18-nor-7α-hydroxykauranolide in the presence of lead tetraacetate and iodine gave as expected, a 7-20 ether. Photolysis of the nitrite followed by heating in isopropanol gave a lactam, $C_{19}H_{27}O_4N$ $\nu_{max.}$ 1640 $cm.^{-1}$ Comparison of the nuclear magnetic resonance spectrum with that of the starting material revealed the loss of the angular methyl group and the appearance of a singlet at 4.95. The structure (42) is proposed for this product which is formed from the initial oxime undergoing firstly 7-20 ether formation, which finds a ready analogy in the reactions of ajaconine, and subsequently the lactone undergoes internal hydrolysis and lactamization to form the system reminiscent of the diterpene alkaloids.[39] Thus this reaction forms an exception to the currently accepted six-membered transition state theory for the Barton reaction.

2.5 Some Oxidation Products of 7-Hydroxykaurenolide

7-Hydroxykaurenolide was shown to have the structure and stereochemistry (3).[4] Its oxidative degradation led to the formation of a number of compounds which, whilst they do not contribute to the proof of the structure of the kaurenolides, nevertheless shed some light on the reactivity of the ring system. Their properties form the subject of this section.

On treatment with perbenzoic acid, 7-hydroxykaurenolide formed a single epoxide (44) which in turn gave a triol (45) on treatment with mineral acid. This triol was also obtained, together with the corresponding 7-ketone by hydroxylation of 7-hydroxykaurenolide with osmium tetraoxide in pyridine. Both these 16,17-glycols were cleaved with sodium periodate in aqueous methanol to form their corresponding 16-ketones (47 and 46 respectively). In the case of the latter this served to define the position of the carbonyl group at C-7. Although these two steps could be combined to form the 16-ketones by making use of the Rudloff-Lemieux procedure,[40] oxidation of 7-hydroxykaurenolide with excess neutral potassium permanganate led not only to the formation of the 16-ketone (47) but also to the triol (45) and the corresponding α-hydroxy-acid. This acid in support of its formulation as (51) was oxidised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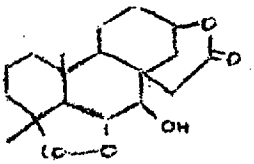
with sodium bismuthate to the 16-ketone. It is interesting to note that the 7(ax.) hydroxyl was unattacked in this oxidation in contrast[4] to the action of chromium trioxide in sulphuric acid. The configuration with an 16α-hydroxyl group is preferred for this series of compounds in view of the propensity of the reagents used for attacking the less hindered face of the molecule.

Although the 16-ketones were more conveniently prepared by ozonolysis of 7-hydroxykaurenolide in glacial acetic acid, this also led to the isolation of a δ-lactone (50). This lactone could be prepared by Baeyer-Villiger oxidation of the 16-ketone (47) with perbenzoic acid. In the analogous oxidation[20] of gibberellin A_4 8-ketone (49), the orientation of the corresponding δ-lactone as (52) has been unambiguously established by inter-relationship with gibberellic acid. Oxidation of the δ-lactone with chromium trioxide gave the 7-ketone (53) which on hydrogenolysis with zinc dust in acetic anhydride followed by methylation, formed the corresponding 6-deoxy-7-ketone (54). A broad single-proton resonance in the nuclear magnetic resonance spectra of (50) and (53) at 5·15 and 5·25 respectively which is absent from the spectrum of 7-hydroxykaurenolide may be associated with the proton at C-13, thus providing some further evidence for the orientation of the lactone ring.

Hydrolysis of the δ-lactone with N-sodium hydroxide for 2 hr. followed by methylation of the product gave a γ-lactonic methyl ester $C_{20}H_{30}O_6$. On oxidation with chromium trioxide in sulphuric acid this formed a diketone, $C_{20}H_{26}O_6$, with the loss of four hydrogen atoms and hence the parent ester was a diol. The diketone showed no ultra-violet absorption characteristic of a conjugated system whilst products typical[4] of the fission of an unprotected 6,7-glycol were absent from this oxidation. Hence we must conclude that either the 6 or the 7-hydroxyl participates in the lactone ring. The nuclear magnetic resonance spectrum of the diketone apart from containing resonances from the two tertiary methyl groups (τ 8·52 and 8·85) and methoxyl (τ 6·3) showed a one proton singlet at τ 4·9 due to the 7-proton. These features are accommodated by structure (56) for the diketone and hence by (55) for the parent γ-lactone in which isomerization of a 16→13 δ-lactone to a 16→7 γ- lactone has occurred. On the other hand Baeyer-Villiger oxidation of the 7α-acetate (57) led to the corresponding δ-lactone which on alkaline hydrolysis formed a δ-lactonic methyl ester ($\nu_{max.}$ 1706 $cm.^{-1}$) showing the characteristic hydrogen bonded hydroxyl absorption of the 6α,7α-diol.



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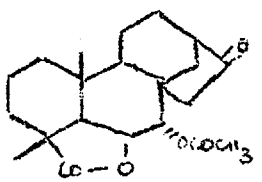

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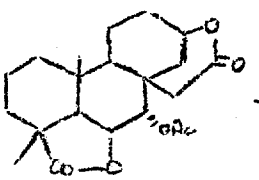

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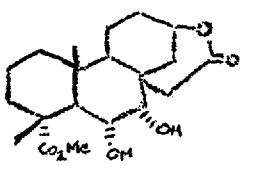

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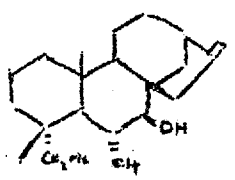

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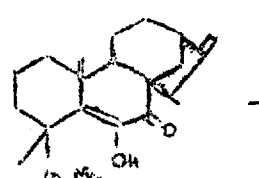

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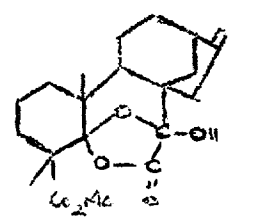

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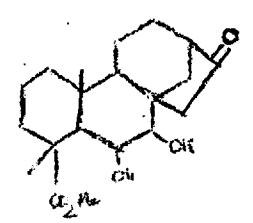

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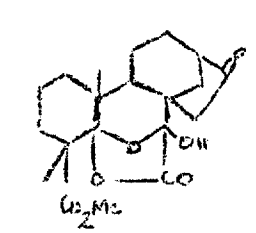

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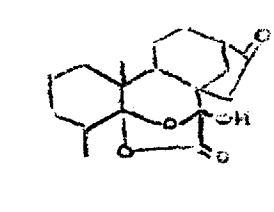


(65)

Hydrolysis of 7-hydroxykaurenolide led to a 6α,7β-diol (60) which on oxidation with chromium trioxide in sulphuric acid formed a crystalline diosphenol (61) ($\lambda_{max.}$ 281 mμ) and a lactonol $C_{21}H_{28}O_6$. Similarly oxidation of the diol (63) gave in addition to the diosphenol and dicarboxylic acid isolated earlier by Dr. R. H. B. Galt, a lactonol $C_{20}H_{26}O_7$. The latter could also be obtained by further oxidation of the diosphenol and by ozonolysis of the lactonol $C_{21}H_{28}O_6$. The lactonol, $C_{20}H_{26}O_7$, consumed one mol. of alkali on microtitration whilst on refluxing with sodium hydroxide solution and acidification of the solution it gave one mol. of carbon dioxide and a second lactonol, $C_{18}H_{24}O_5$. The latter whilst retaining the high frequency infrared absorption of the lactone ring (1795 $cm.^{-1}$) did not contain methoxyl and hence the parent lactonol must have contained a potential β-keto-ester. The lactonols lacked absorption in the ultraviolet characteristic of an α-diketone. Furthermore their infrared spectra contained bands at 1795-1801 $cm.^{-1}$ which could be assigned to a γ-lactone. We therefore propose structure (62) and (64) for the lactonols.

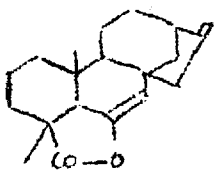
2.6. The Preparation and Properties of some 6-Keto-Kaurene Derivatives

The elimination reactions of 7-hydroxykaurenolide and the nuclear magnetic resonance spectra of its derivatives were examined[4] in order to define the conformation of ring B. Treatment of both the 7-axial and equatorial toluene-p-sulphonates with refluxing collidine led to the formation of Δ^6-enol-lactones at comparable rates and as a result of this study it was concluded that neither group was trans and axial to a 6-proton. The enol-lactone (67) was prepared by elimination of toluene-p-sulphonic acid from the toluene-p-sulphonate of 7-hydroxykaurenolide with refluxing collidine. Hydrolysis of the enol-lactone with refluxing mineral acid gave the mono-hydroxyketo-acid (68) which on methylation with diazomethane, gave a crystalline monomethyl ester. An alternative method for the preparation of 6-ketones made used of the concomitant elimination of the toluene-p-sulphonate of 7-hydroxykaurenolide and hydrolysis of the Δ^6-enol-lactone with dry lithium iodide in collidine giving directly the keto-acid (69). A small amount of the γ-lactone described below was also isolated from this reaction.

The optical rotatory dispersion curves of 6-ketones throw some light on the nature of the A/B ring fusion although it is difficult to predict the sign of the Cotton effect from the Octant[41] rule owing to the possibility of near octant effects. However the 6-ketones from marrubiin and 6-oxodihydrocativic acid, both with the normal A/B ring fusion, have a positive Cotton effect.[42] The 6-ketones from the (-)-kaurene system described above showed negative Cotton effects of an amplitude consistent with an antipodal A/B ring fusion.

Since the tetrasubstituted centre at C(8) forms part of a bridgehead, it precluded the formation of a trigonal carbonium ion and thus the use of skeletal rearrangements to characterize the conformation of the 7-hydroxyl group. The action of phosphorus pentachloride on both the 7-(ax.)- and 7-(eq.)-hydroxykaurenolides led to the isolation of the Δ^6-enol-lactone, 6-oxo-kaur-16-en-19-oic acid (69) and a γ-lactone. Thionyl chloride on the other hand led to the formation of a dimeric sulphite.

Attention was therefore turned to the structure of the γ-lactone $C_{20}H_{26}O_2$. The infrared spectrum showed absorption at 1760 $cm.^{-1}$ characteristic of a saturated γ-lactone whilst apart from terminal methylene absorption ($\nu_{max.}$ 1655 and 882 $cm.^{-1}$) it showed no further evidence of



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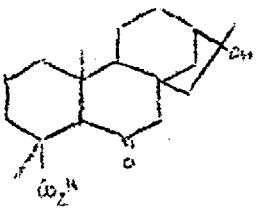
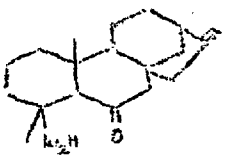


(68)



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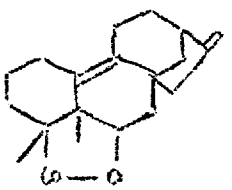

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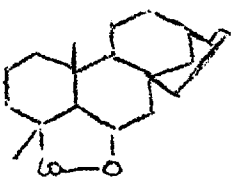

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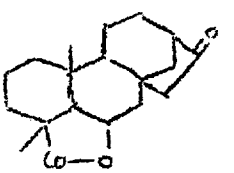

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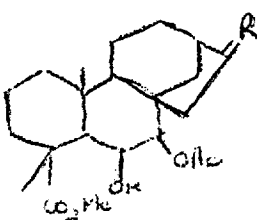

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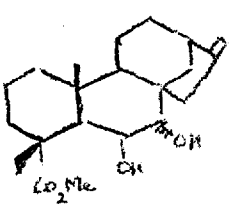

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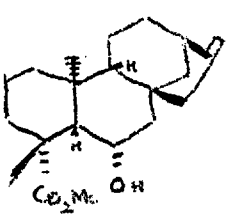

(75)

olefinic protons. The nuclear magnetic resonance spectrum showed no proton resonances below $\tau = 5{\cdot}0$. However the ultraviolet spectrum showed high end-absorption ($\varepsilon_{205} = 10{,}000$) and thus the molecule contained a further, presumably tetrasubstituted, double bond in addition to the terminal methylene group. The nuclear magnetic resonance spectrum also revealed a multiplet (partially obscured by the terminal methylene protons) at $\tau = 5{\cdot}1$ which is ascribed to the group $\underset{\underset{O.CO}{|}}{C\text{-}CH\text{-}C}$, together with two tertiary methyl groups at τ 8·8. The structure (70) is therefore proposed for this by-product, the rearrangement having a strong analogy in the formation of Westphalen's diol.[xxx] During the rearrangement all the migrating groups possess a trans diaxial relationship to one another.

Both the 6-keto acids (68) and (69) together with the methyl ester (68) showed an unusual carbonyl absorption in which the cyclohexanone frequencies lay in the range 1730-1740 $cm.^{-1}$ whilst the carboxylic acid and ester frequencies were lowered to 1660 and 1706 $cm.^{-1}$ respectively. This suggested marked interaction between the two groups. A similar effect may be noted in the weakening of a 19-carboxylic acid by a 6-ketone (pK'_{DMS} 8·63 to 9·25). This implies a limitation in the use of the relationship between the pK' and the number of 1:3 interactions to assign the position of a carbo[x]yl group.[44]

Preface

This thesis is concerned with the chemistry of tetracyclic diterpenes. This work stems from studies on the biosynthesis of gibberellic acid begun in 1959 by myself in association with Drs. B. E. Cross and R. H. B. Galt at the Akers Research Laboratories. On the closure of these laboratories some aspects of this work have been continued at Imperial College under the generous guidance and hospitality of Professor D. H. R. Barton F.R.S. to whom I should like to express my sincere thanks. The study of the biosynthesis of the gibberellins is being continued by Dr. B. E. Cross at the University of Leeds whilst the investigation of the C-20 gibberellin, gibberellin A_{13}, has been completed by Dr. R. H. B. Galt. In this part of the work I have therefore tried to explore features of the chemistry of the tetracyclic diterpenes bearing in mind their relevance to the biosynthetic pattern of the group as a whole.

J. MacLaurin

March 1965

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Reduction of the 6-keto acid with sodium borohydride followed by methylation gave a 6-hydroxy-ester. The stereochemistry of this hydroxyl group was demonstrated by further reduction of the ester with lithium aluminium hydride to give the known 6α,19-dihydroxykaurene. The latter had been obtained by reduction of the toluene-p-sulphonate of 7-hydroxykaurenolide with lithium aluminium hydride. Reduction of the ditoluene-p-sulphonate of 7,18-dihydroxykaurenolide led to the same diol. Thus, typically, reduction of the hindered 6-carbonyl group led to the axial alcohol. The 6(ax.) hydroxyl group was inert to acetylation with acetic anhydride in pyridine. Indeed acetylation of the 6α,7β-diol (60) gave the 7-monoacetate whilst the use of more vigorous conditions such as sodium acetate and refluxing acetic anhydride, conditions which serve to acetylate the inert 6(ax.) hydroxyl of sumaresinolic acid,[45] led to relactonization and the formation of 7-acetylkaurenolide. Pyrolysis of the parent hydroxy-acid at 220° led to relactonization and the formation of kaurenolide (71). Ozonolysis of the hydroxy-ester formed the 16-oxo derivative which underwent a similar relactonization to give the γ-lactone (72).

2.7 Experimental Section

For general details see the experimental section of Chapter 1

(-)-Kaurene, m.p. 50-50·5°, $[\alpha]_D$ - 80°, $\nu_{max.}$ 3065, 1657, and 870 $cm.^{-1}$ used for this work was isolated from Gibberella fujikuroi.

Ozonolysis of (-)-Kaurene (1) - Ozonised oxygen (15 mg. of O_3 per min.) was passed through (-)-kaurene (600 mg.) in acetic acid (15 ml.) for 6 min. The acetic acid was evaporated at the water-pump and the residue diluted with water, neutralised with sodium hydrogen carbonate, and extracted with ether. The extract was washed with water, dried, and evaporated to give a semi-crystalline residue. This was 17-nor-(-)-kauran-16-one (12) (405 mg.) which crystallised from methanol as plates, m.p. 114-116° (lit. 114-115°)(Found: C, 83·0; H, 11·0. Calc. for $C_{19}H_{30}O$: C, 83·15; H, 11·0%), $\nu_{max.}$ 1745 $cm.^{-1}$ Chromatography on alumina and elution with 1:3 ethyl acetate-light petroleum gave 7β-hydroxy-(-)-podocarpan-14β-ylacetic acid lactone (15) (35 mg.) which crystallised from light petroleum as needles, m.p. 146-148° (Found: C, 78·2; H, 10·5. $C_{19}H_{30}O_2$ requires C, 78·6; H, 10·4%), $\nu_{max.}$ 1724, 1216 $cm.^{-1}$

The aqueous phase was acidified with dilute hydrochloric acid and the organic material recovered in ether and chromatographed on silica gel. Elution with 3:17 ethyl acetate-light petroleum gave (-)-podocarpan-14β-ylacetic acid (13) (46 mg.) which crystallised from acetone-light petroleum as needles, m.p. 144-145° (Found: C, 77·75; H, 11·0. $C_{19}H_{32}O_2$ requires C, 78·0; H, 11·0%), ν_{max} 2667 and 1704 cm.)$^{-1}$

Baeyer-Villiger Oxidation of 17-Nor-(-)-kauran-16-one (12) The nor-ketone (400 mg.) in a 0·7 N-solution of perbenzoic acid in chloroform (10 ml.) containing toluene-p-sulphonic acid (15 mg.) was kept at 0° for 18 hr. The solution was diluted with chloroform and extracted with aqueous ferrous sulphate, dilute hydrochloric acid, aqueous sodium hydrogen carbonate, and water. Recovery gave a crystalline residue which was recrystallised from light petroleum to give the above δ-lactone (15) (350 mg.) as needles, m.p. 147-148°, identified by its infrared spectrum.

The lactone (55 mg.) in methanol (2 ml.) was heated under reflux with 0·5N-sodium hydroxide for 2 hr. The solution was diluted with a large excess of water and extracted with ether. The aqueous phase was cooled to 0°, cautiously acidified, and again extracted, this time with

ethyl acetate. After drying and evaporation at 30° the gum was treated with ethereal diazomethane and chromatographed on alumina to give the starting material (35 mg).

Hydroxylation of (-)-Kaurene (1). Osmium tetroxide (500 mg.) was added to a solution of (-)-kaurene (350 mg.) in ether (10 ml.) and pyridine (5 ml.). The brown suspension was kept at 0° for 18 hr., diluted with ether to 100 ml. and treated with a solution of mannitol (5 g.) and potassium hydroxide (5 g.) in water (50 ml.). The mixture was refluxed for 2 hr. The organic phase was separated and the aqueous phase extracted with ether. The combined extracts were washed with diluted hydrochloric acid and water and dried. The solvent was evaporated and the residue crystallised from acetone, to give (-)-kaurane-16α,17-diol (14) (310 mg.) as needles, m.p. 189-190° (Found: C, 75·8; H, 11·4. $C_{20}H_{34}O_2$, 0·5 H_2O requires C, 76·1; H, 11·2%), $\nu_{max.}$ 3370 (br.) cm.$^{-1}$

Oxidation of Kaurane-16,17-diol with Sodium Periodate. The diol (250 mg.) in methanol (15 ml.) was treated with sodium periodate (500 mg.) in water (5 ml.) at room temperature for 18 hr. The solution was concentrated and then diluted with water and extracted with ether. The extract was washed with water, dried and evaporated. The residue

crystallised from methanol as plates of 17-nor-(-)-kauran-16-one (190 mg.) m.p. 115-116°, identical with the sample described above.

Reduction of the δ-Lactone (15). - The lactone (15) (244 mg.) in dry ether (15 ml.) was refluxed with lithium aluminium hydride (245 mg.) for 2·5 hr., then cooled and the excess reagent was destroyed with ethyl acetate and water. The aqueous phase was extracted with ether and the combined ether extracts were washed with dilute hydrochloric acid and water and dried. The solvent was evaporated and the residue chromatographed on alumina. Elution with ether gave 13,16-seco-17-nor-(-)-kaurane-13β, 16-diol (16) (169 mg.) which crystallised from light petroleum as needles, m.p. 155-156° (Found: C, 77·4; H, 11·8. $C_{19}H_{34}O_2$ requires C, 77·5; H, 11·6%), $\nu_{max.}$ 3255 (OH) $cm.^{-1}$

Oxidation of the Diol (16). - The diol (210 mg.) in acetone (5 ml.) was treated at room temperature for 1 hr. with a solution (0·5 ml.) prepared from chromic oxide (26·67 g.) in concentrated sulphuric acid (23 ml.) and water (40 ml.) made up to 100 ml. Methanol was added and the solution concentrated, diluted with water and extracted with ethyl acetate. The extract was washed with water, dried, and evaporated, to give a semi-crystalline residue of 13-oxo-13,16-seco-17-nor-(-)-kauran-16-oic acid (16→13-

lactone form)(18) (91 mg.) which crystallised from light petroleum as needles, m.p. 131-135° (Found: C, 75·1; H, 10·1. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), ν_{max} 3350 (OH of hydroxy-lactone), 1703 (C = O of lactone) $cm.^{-1}$ The product could not be methylated with ethereal diazomethane. The residue was chromatographed on silica gel. Elution with 1:9 ether-light petroleum gave the above δ-lactone (15) (10 mg.); further elution gave the hydroxy-ketone (19) (34 mg.) which crystallised from acetone light petroleum as needles, m.p. 196-197° (Found: C, 78·3; H, 10·45. $C_{19}H_{30}O_2$ requires C, 78·6; H, 10·4%) $\nu_{max.}$ 3450 (OH), 1710 (cyclohexanone) $cm.^{-1}$ nuclear magnetic resonance peaks at 9·3, 9·2, 8·8 ($3\text{-}C\text{-}CH_3$), 8·6-8·1 (ring protons), 7·7 ($CH_2.CO$), 6·4 (OH), 5·9 (multiplet)(CH.OH).

Oxidation of the Hydroxy-ketone (19). - The hydroxy-ketone (94 mg.) in acetone (3 ml.) was treated with the above chromic oxide reagent (0·1 ml.) at room temperature for 1 hr. The solution was treated with methanol, concentrated, then diluted with water and extracted with ether. The extract was washed with aqueous sodium hydrogen carbonate and water and dried. The solvent was evaporated, giving a gum which crystallised from acetone as needles (58 mg.) of the diketone (22), m.p. 210-211° (Found: C, 79·5; H, 9·7. $C_{19}H_{28}O_2$ requires C, 79·1; H, 9·8%) $\nu_{max.}$ 1740 and 1721 $cm.^{-1}$ nuclear magnetic resonance

peaks at 9·18, 9·15, 9·12, 7·8, 6·8 (triplet, J 3 c/sec.).

Alkaline Hydrolysis of the Hydroxy-lactone (18).- (a) The compound (50 mg.) in pure acetone (5 ml.) was refluxed with methyl iodide (0·4 ml.) and potassium hydroxide (25 mg.) for 3 hr. The solution was concentrated, diluted with water, acidified with dilute hydrochloric acid, and extracted with ether. The extract was washed with sodium hydrogen carbonate solution and water and dried. The solvent was evaporated and the residue crystallised from light petroleum to give methyl 7-oxo-(-)-podocarpan-14β-yl acetate (17) (25 mg.) as needles, m.p. 128-129° (Found: C, 74·3; H, 10·2. $C_{20}H_{32}O_3$ requires C, 74·9; H, 10·1%), $\nu_{max.}$ 1733, 1706 $cm.^{-1}$

(b) The hydroxy-lactone (208 mg.) and methyl iodide (0·5 ml.) were added to a solution of sodium (53 mg.) in methanol (10 ml.) and refluxed for 4·5 hr. and the solution was then concentrated *in vacuo*. The residue was diluted water and extracted with ether. The extract was washed with dilute hydrochloric acid and water and dried. Evaporation of the solvent gave a residue which was chromatographed successively on silica gel and alumina. Elution with 1:9 ethyl acetate-light petroleum gave methyl 7-oxo-(-)-podocarpan-14α-yl acetate (21) (74 mg.) which crystallised from acetone

as needles, m.p. 178-179·5° $[\alpha]_D^{20}$ -24° (c 0·2)(Found: C, 75·2; H, 10·0. $C_{20}H_{32}O_3$ requires C, 74·9; H, 10·1%) $\nu_{max.}$ 1738 and 1711 $cm.^{-1}$ The infrared spectra for a chloroform solution and nujol mull were identical with those of the corresponding enantiomer from phyllocladene. However the mixed m.p. was depressed to 150-156°.

Isomerisation of (-)-Kaurene. - (-)-Kaurene (450 mg.) in methanol (25 ml.) was treated with concentrated sulphuric acid (2·5 ml.) at 0° for 18 hr. The solution was concentrated in vacuo, diluted with water and extracted with ether. The extract was dried and evaporated and the residue crystallised from ethanol, to give (-)-isokaurene (210 mg.), m.p. 63°. Chromatography of the residue on alumina and elution with light petroleum gave a mixture of kaurene and isokaurene, m.p. 51-55°; further elution gave 16-methoxy-(-)-kaurene (26) (39 mg.) which crystallised from acetone as needles, m.p. 103-104° (Found: C, 83·4; H, 11·9. $C_{21}H_{36}O$ requires C, 82·8; H, 11·9%).

Hydroxylation of Isokaurene. - Isokaurene (0·75 g.) in dry ether (45 ml.) containing pyridine (5 ml.) was treated with osmium tetroxide (1 g.) at 0° for 18 hr. The solution was then refluxed with mannitol (15 g.), potassium hydroxide (15 g.), ethanol (100 ml.), and water (100 ml.) for 1 hr. The organic solvents were removed

in vacuo and the solution extracted with ether. The extract was washed with sodium hydroxide solution, dilute hydrochloric acid, and water and dried. The solvent was evaporated to give a semi-crystalline residue (0·5 g.) which was chromatographed on alumina. Elution with 3:17 ethyl acetate-light petroleum gave (-)-kaurane-15α,16α-diol (27) (240 mg.) which crystallised from acetone-light petroleum as needles, m.p. 174-175° (Found: C, 78·0; H, 11·3. $C_{20}H_{34}O_2$ requires C, 78·4; H, 11·2%).

Oxidation of the Diol (27) by Lead Tetra-acetate. - The diol (200 mg.) in acetic acid (10 ml.) was refluxed with lead tetra-acetate (500 mg.) for 3 hr. The solution was poured into water and extracted with ether. The extract was washed with aqueous sodium hydrogen sulphite, sodium hydrogen carbonate solution, and water and dried. Evaporation furnished a gum which was crystallised from methanol to give the diether (28) (100 g.) as needles, m.p. 124-125° (Found: C, 75·7; H, 10·95. $C_{22}H_{38}O_3$ requires C, 75·4; H, 10·9%), nuclear magnetic resonance (40 Mc/sec. instrument) peaks at 9·16, 9·14, 8·96, and 8·77 (4 $>C{=}CH_3$), 6·81 and 6·59 (MeO), and 6·09 [$CH(O)_2$].

Epoxidation of (-)-Kaurene. - (-)-Kaurene (250 mg.) was dissolved in a 0·7N-solution of perbenzoic acid in chloroform (5 ml.) and kept at 0° for 24 hr. The solution

was diluted with chloroform and extracted with aqueous ferrous sulphate, water, sodium hydrogen carbonate solution, and again water and dried. The solvent was evaporated and the residue crystallised from methanol to give 16α,17-epoxy-(-)-kaurane (156 mg.), as needles m.p. 113-115°, (Found: C, 83·3; H, 11·2. $C_{20}H_{32}O$ requires C, 83·3; H, 11·2%).

Reduction of Kaurene Epoxide. - The epoxide (100 mg.) in dry ether (15 ml.) was heated under reflux with lithium aluminium hydride (200 mg.) for 4 hr. The excess of reagent was destroyed with ethyl acetate followed by dilute hydrochloric acid. The solution was extracted with ether, and the extract washed with water and dried. Evaporation of the solvent and crystallisation of the residue from acetone-light petroleum gave (-)-kauranol (2) (52 mg.) as needles, m.p. 212-214°, $[\alpha]_D^{20}$ -40° (c 0·3).

Treatment of (-)-Kaurene Epoxide with Mineral Acid. - The epoxide (10 g.) in methanol (1 ml.) was refluxed with dilute hydrochloric acid (10 ml.) for 5 hr. The solution was diluted with water, and the organic material recovered with ether, to give a gum which crystallised from acetone-light petroleum as needles of (-)-kaurene-16α,17-diol (14) (4 mg.) m.p. 189-190°, identified by the infrared spectrum.

Anhydride Formation from the Acid (9). - The dicarboxylic acid (9) (80 mg.) in acetic anhydride (5 ml.) was heated under reflux for 5 hr. The excess reagent was then removed in vacuo to leave a gum which crystallised from acetone-light petroleum as plates of 16-oxo-6,7-seco-17-nor-(-)-kauran-6,7,19-trioic acid 6-7 anhydride 19-methyl ester (40 mg.), m.p. 195-198° (Found: C, 65·9; H, 7·4; OMe, 8·9% no acetyl. $C_{20}H_{26}O_6$ requires C, 66·3; H, 7·2; OMe, 8·6%), $\nu_{max.}$ (in $CHBr_3$) 1794 and 1742 (adipic anhydride and cyclopentanone), 1717 (ester) $cm.^{-1}$, $\nu_{max.}$ 1775, 1748 and 1728 $cm.^{-1}$

Hydrolysis of the Anhydride, - The anhydride (30 mg.) in methanol (5 ml.) was treated with 0·5N-sodium hydroxide solution (4 ml.) for 16 hr. at 0°. The solution was poured into dilute hydrochloric acid (30 ml.) and extracted with ethyl acetate. The extract was washed with water and dried. The solvent was evaporated to give a gum which slowly deposited crystals (10 mg.) from acetone-light petroleum of the dicarboxylic acid (9), m.p. 172° reset and remelt 204-206°, identified by its infrared spectrum.

Pyrolysis of the 6-7-Anhydride (29). - The anhydride (45 mg.) was heated in a pyrex tube under nitrogen for 16 hr. at 350° during which time carbon dioxide (baryta trap) was

evolved. The distillate was chromatographed on silica gel (10 x 1 cm.). Elution with 10% of ethyl acetate in light petroleum gave methyl 1β,4aα-dimethyl-8,10-dioxogibbane-1α-carboxylate (15 mg.) which crystallised from acetone light petroleum as plates, m.p. 150-152° (Found: C, 71.6; H, 8.3. $C_{19}H_{26}O_4$ requires C, 71.7; H, 8.2%), $\nu_{max.}$ 1753 and 1741 (cyclopentanones) 1729 (ester) $cm.^{-1}$, $\nu_{max.}$ (in CCl_4) 1753m 1742 and 1725 $cm.^{-1}$ Attempts to dehydrogenate this (50 mg.) with selenium were unsuccessful.

Pyrolysis of the Dicarboxylic Acid (9) - The dicarboxylic acid (50 mg.) was heated in a pyrex tube under nitrogen at 205° for 2 hr. The organic material (gum; 47 mg.) was recovered with ethyl acetate. It crystallised from acetone-light petroleum (charcoal) as prisms, m.p. 248-250° (decomp.), identical with fujenoic acid norketone.

On one occasion fujenoic acid norketone (50 mg.) was heated in a pyrex tube at 285° under nitrogen for 3hr. and the effluent gases passed through a solution of barium hydroxide. When the temperature reached 270°, carbon dioxide (0.8 mol.) was evolved. The residue was recovered with ethyl acetate and chromatographed on silica gel. Elution with 10% of ethyl acetate in light petroleum gave a compound (20 mg.) m.p. 133°, which crystallised from acetone-light petroleum as needles, $\nu_{max.}$ (in $CHBr_3$) 1851, 1780 (5-ring anhydride) and 1759 (cyclopentanone) $cm.^{-1}$

The lactone (31) was prepared by the reduction of fujenal with lithium aluminium hydride in refluxing ether followed by oxidation in acetone solution with the 8N-chromium trioxide reagent.[3]

Action of Alkali on the Lactone (31) - The lactone (390 mg.) in methanol (5 ml.) was heated under reflux with aqueous 1·5N-sodium hydroxide solution (40 ml.) for 5½ hr. The solution was cooled and the precipitate which separated (119 mg.) filtered off. Recrystallisation from acetone-light petroleum gave 10α-hydroxy-1α-hydroxymethyl-1β,4aα-dimethyl-8-methylenegibbane-10a-carboxylic acid 10a→1'-lactone (32) as needles, m.p. 164-165° (Found: C, 75·9; H, 8·8. $C_{20}H_{28}O_3$ requires C, 75·9; H, 8·9%), $\nu_{max.}$ 3453, 3065, 1754 (γ-lactone), 1650 and 875 $cm.^{-1}$ (C = CH_2), τ = 8·95, $-\overset{|}{C}-CH_3$; 8·78 $-\overset{|}{C}-CH_3$; 8·6-8·52 (ring protons), 7·85 and 7·7 (allylic protons) 6·3 and 5·8, (J = 9 c/sec.) (lactone protons) 5·98 (10-proton), 5·15 (terminal methylene protons).

The alkaline filtrate was acidified and the organic material recovered with ethyl acetate. The solvent was washed, dried and evaporated to give a gum which was methylated with diazomethane and chromatographed on alumina. Elution with 25% of ethyl acetate in light petroleum gave the starting material (110 mg.).

The hydroxy-lactone was recovered unchanged after treatment with freshly purified pyridine and acetic anhydride for 18 hr.

Oxidation of the hydroxy-lactone with chromium trioxide gave gummy crystals, m.p.156-160° (decomp.) $\nu_{max.}$ 1745, 1720, 1650 and 875 $cm.^{-1}$, which evolved carbon dioxide on heating at 200° under nitrogen.

The Toluene-p-sulphonate of 7α-Hydroxykaurenolide. - This was prepared by treatment of the corresponding alcohol with toluene-p-sulphonyl chloride in dry pyridine at room temperature for 72 hr. The toluene-p-sulphonate of 6α,7α-dihydroxy-(-)-kaur-16-en-19-oic acid 19→6-lactone crystallised from acetone-light petroleum as needles, m.p. 160-161° (Found: C, 69·2; H, 7·5. $C_{27}H_{34}O_5S$ requires C, 68·9; H, 7·3%), $\nu_{max.}$ 1771, (γ-lactone), 1667, (double bond), 1598 (ar.), 893 ($C=CH_2$) $cm.^{-1}$

The toluene-p-sulphonate of methyl 6α,7α-dihydroxy-(-)-kaur-16-en-19-oate crystallised from acetone-light petroleum as needles, m.p. 190-192° (Found: C, 67·2; H, 7·4. $C_{28}H_{36}O_6S$ requires C, 67·2; H, 7·25%), $\nu_{max.}$ 3410, 1695, 654, 1590 and 890 $cm.^{-1}$

The toluene-p-sulphonate of 6α,7β-dihydroxy-16-oxo-17-norkauran-19-oic acid 19→6α-lactone crystallised from acetone-light petroleum as needles, m.p. 178-180° (Found: C, 66·4;

H, 6·9. $C_{26}H_{32}O_6S$ requires C, 66·1; H, 6·8%), $\nu_{max.}$ 1788, 1743 and 1599 cm.$^{-1}$

The toluene-p-sulphonate of 6α,7α-dihydroxy-16-oxo-17-(-)-kauran-19-oic acid 19→6-lactone crystallised from acetone-light petroleum as needles, m.p. 210-212° (Found:,C, 65·5; H, 6·8. $C_{26}H_{32}O_6S$ requires C, 66·1; H, 6·8%), ν_{max} 1759, 1740 and 1595 cm.$^{-1}$

Ring Contraction Reactions - (a) The toluene-p-sulphonate (34) was recovered after heating under reflux with 10% methanolic potassium hydroxide solution (4½ hr.), methanolic sodium methoxide (3 hr.) and potassium-t-butoxide in t-butanol-benzene (4 hr.) followed by methylation and chromatography.

(b) The toluene-p-sulphonate of 6α,7α-dihydroxykaur-16-en-19-oic acid 19-6-lactone (210 mg.), potassium hydroxide (3 g.) and methanol (30 ml.) were heated under reflux for 4½ hr. The solution was concentrated under reduced pressure, diluted with water, acidified and extracted with ether. The extract was washed with aqueous sodium hydrogen carbonate water, dried and evaporated to give a neutral gum (150 mg) which was chromatographed on alumina. Elution with light petroleum gave methyl 10β-formyl-1β,4aα-dimethyl-8-methylene-gibbane-1α-carboxylate (35) (22 mg.) which crystallised from

light petroleum as needles, m.p. 68-70° (Found: C, 76·1; H, 9·45. $C_{21}H_{30}O_3$ requires C, 76·3; H, 9·15%), $\nu_{max.}$ 3073 ($C{=}CH_2$), 2738 (aldehydic C→H), 1730, 1712 (ester and aldehyde) 1655 and 890 ($C{=}CH_2$) $cm.^{-1}$

Further elution with 10-20% of ethyl acetate in light petroleum gave the mono-toluene-p-sulphonate of methyl 6α,7α-dihydroxykaur-16-en-19-oate (61 mg.) as needles, m.p. 189-190°, identified by its infrared spectrum. In subsequent experiments the total (acidic and neutral) fractions were methylated with diazomethane prior to chromatography thus giving slightly higher yields.

Oxidation of the Aldehyde (35) The aldehyde (71 mg.) in acetone (5 ml.) was treated with the 8N-chromium trioxide reagent[9] (0·25 ml.) at room temperature for 2 hr.. Methanol was added, the solution concentrated, diluted with water and extracted with ethyl acetate. The extract was washed with water, dried and evaporated to give a gum which was chromatographed on silica gel (12 x 1 cm.). Elution with 15-20% of ethyl acetate in light petroleum gave 1α-methoxycarbonyl-1β,4aα-dimethyl-8-methylenegibbane-10β-carboxylic acid (22 mg.) which crystallised from acetone-light petroleum as needles, m.p. 196-198° (Found: C, 72·8; H, 8·7. $C_{21}H_{30}O_4$ requires C, 73·2; H, 8·8%), $\nu_{max.}$ 2623 (carboxyl OH), 1724 (ester), 1697 (carboxyl), 1655, 1622 and 891 $cm.^{-1}$ ($C{=}CH_2$).

7-Hydroxy-18-Norkaurenolide - The 18-monotoluene-p-sulphonate of 7,18-dihydroxykaurenolide (210 mg.) and sodium hydrogen carbonate (500 mg.) in dimethylsulphoxide (5 ml.) were heated under reflux for 1 hr. The solution was cooled, diluted with water and extracted with ether, The extract was washed with aqueous sodium hydrogen carbonate, water, dried and evaporated to give 6α,7β-dihydroxy-18-nor-(-)-kaur-16-en-19-oic acid 19→6α-lactone (72 mg.) as needles from acetone-light petroleum, m.p. 196-197°, identified by its infrared spectrum.

Oxidation of 6α,7α-Dihydroxy-(-)-kauran-19-oic Acid 19→6α-Lactone (39; R=CH_3) with Iodine and Lead Tetra-acetate - The hydroxy-lactone (400 mg.), iodine (730 mg.) with lead tetra-acetate (750 mg.) in benzene (30 ml.) were irradiated under reflux under nitrogen for 3 hr. The solution was poured into water and extracted with ether. The extract was washed with sodium thiosulphate solution, aqueous sodium hydrogen carbonate, water and dried. Recovery gave 7α,20-epoxy-6α-hydroxy-(-)-kauran-19-oic acid 19-6α-lactone (275 mg.) which crystallised from acetone-light petroleum as needles, m.p. 195-196° (Found: C, 75·4; H, 8·7. $C_{20}H_{28}O_3$ requires C, 75·9; H, 8·9%), $\nu_{max.}$ 1765 $cm.^{-1}$ The infrared spectrum of the crude product showed the presence of traces of the corresponding 7-ketone.

The lactone was recovered unchanged after 5 hr. from attempted hydrolysis with refluxing aqueous methanolic N-sodium hydroxide.

Reduction of the Ether (40). - The ether (180 mg.) and lithium aluminium hydride (200 mg.) in ether (10 ml.) and tetrahydrafuran (5 ml.) were left at room temperature overnight. Dilute hydrochloric acid was added and the solution extracted with ether. The extract was washed with aqueous sodium hydrogen carbonate, water, dried and evaporated to give 7α,20-epoxy-6,19-dihydroxy-(-)-kaurane (120 mg.) which crystallised from acetone-light petroleum as needles, m.p. 158-160° (Found: C, 74·5; H, 9·9. $C_{20}H_{32}O_3$ requires C, 75·0; H, 10·1%) $\nu_{max.}$ 3180 (sh.), 3100 cm.$^{-1}$

Oxidation of the Diol - The diol (75 mg.) in acetone (10 ml.) was treated with the chromic oxide reagent at room temperature overnight. Methanol was added, the solution concentrated, diluted with water and extracted with ether. The extract was washed with water, dried and evaporated. 7α,20-epoxy-6-oxo-(-)-kauran-19-oic acid (lactonol form) (35 mg.) crystallised as needles from acetone-light petroleum, m.p. 226-228° (Found: C, 71·9; H, 8·4. $C_{20}H_{28}O_4$ requires C, 72·3; H, 8·5%), $\nu_{max.}$ 3300, 1770 cm.$^{-1}$

Reduction of the Lactonol - The above lactonol (25 mg.) in ether (10 ml.) and tetrahydrofuran (5 ml.) was treated with lithium aluminium hydride (100 mg.) at room temperature overnight. The solution was treated dropwise with water and then poured into dilute hydrochloric acid and extracted with ether. Recovery gave 7α,20-epoxy-6α-hydroxy-(-)-kauran-19-oic acid 19→6α-lactone form (8 mg.) m.p. 194-195°, identified by its infrared spectrum.

Reduction of Methyl 7-Oxo-(-)-kaur-16-en-19-oate - The keto-ester (360 mg.) in methanol (10 ml.) was treated with sodium borohydride (200 mg.) at room temperature for 2 hr. The solution was acidified, concentrated, poured into water and extracted with ether. Recovery gave a crystalline residue which was chromatographed on alumina. Elution with 2:3 ether-light petroleum gave methyl 7α-hydroxy-(-)-kaur-16-en-19-oate (240 mg.) as needles, m.p. 145-146° (Found: C, 75·7; H, 9·8. $C_{21}H_{32}O_3$ requires C, 75·9; H, 9·7%), $\nu_{max.}$ 3588, 1728, 1650, 875 and 868 $cm.^{-1}$

Oxidation of the alcohol with lead tetra-acetate and iodine in benzene under irradiation as described earlier a gave mainly intractable material from which a low yield of methyl 7-oxo-(-)-kaur-16-en-19-oate was recovered.

Hydrogenation of 6α,7α-Dihydroxy-(-)-18-norkaur-16-en-19-oic Acid 19→6α-Lactone - 10% Palladised charcoal (100 mg.) suspended in ethyl acetate (50 ml.) was saturated with hydrogen and then the lactone (650 mg.) in ethyl acetate (150 ml.) was added and the mixture shaken. One mol. of hydrogen was absorbed rapidly. Recovery gave a solid which after several recrystallisations from acetone-light petroleum gave 6α,7α-dihydroxy-(-)-18-norkauran-19-oic acid 19→6α-lactone as needles, (300 mg.), m.p. 189-190° (Found: C, 74·9; H, 9·1. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), $\nu_{max.}$ 3510, 1760 $cm.^{-1}$

Hydrogenation of 7,18-Dihydroxykaurenolide - 10% Palladised charcoal (500 mg.) suspended in ethyl acetate (100 ml.) was saturated with hydrogen and then the kaurenolide (3 g.) in ethyl acetate (200 ml.) was added and the mixture shaken until the rapid uptake of hydrogen ceased. Recovery gave a solid which was recrystallised from acetone-light petroleum in needles of 7,18-dihydroxykauranolide, m.p. 223-224°.

Preparation of the Toluene-p-sulphonates of 7,18-Dihydroxykauranolide. 7,18-Dihydroxykauranolide (250 mg.) was treated with toluene-p-sulphonyl chloride (650 mg.) in freshly purified pyridine (2 ml.) at room temperature for 2 days. The mixture was poured into dilute hydrochloric acid and the product recovered in ethyl acetate and

chromatographed on alumina. Elution with 9:1 light petroleum-ether gave the ditoluene-p-sulphonate of 7,18-dihydroxykauranolide (71 mg.) which crystallised from light petroleum as needles, m.p. 243-244°.

Further elution with 3:2 ether-light petroleum gave the 18-mono-toluene-p-sulphonate of 7,18-dihydroxykauranolide (130 mg.) which crystallised from acetone-light petroleum as needles, m.p. 205-206° (Found: C, 66·3; H, 7·2. $C_{27}H_{36}O_6S$ requires C, 66·4; H, 7·4%), $\nu_{max.}$ 3550, 1770 and 1600 $cm.^{-1}$

Reductions with Lithium Aluminium Hydride - (a) The 18-monotoluene-p-sulphonate of 7,18-dihydroxykauranolide (102 mg.) in ether (10 ml.) and dioxan (5 ml.) was treated with lithium aluminium hydride (104 mg.) overnight. Ethyl acetate followed by dilute hydrochloric acid was cautiously added and the solution extracted with ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate, water and dried. Recovery gave a gum which was chromatographed on alumina. Elution with 9:1 ether:methanol gave 6α,7β,19-trihydroxy-(-)-kaurane (35 mg.) which crystallised as needles from aqueous methanol, m.p. 246-247° (Found: C, 73·9; H, 10·6. $C_{20}H_{34}O_3$ requires C, 74·5; H, 10·6%), $\nu_{max.}$ 3300 (br.) $cm.^{-1}$

(b) 7-Hydroxykauranolide (100mg.) was reduced with lithium aluminium hydride (120 mg.) in ether:dioxan (30 ml.) as above, to give the identical (by infrared and mixed m.p.) triol (63 mg.).

(c) Methyl 6α,7α-dihydroxy-(-)-kaur-16-en-19-oate (75 mg.) in dry ether (25 ml.) and lithium aluminium hydride (100 mg.) were heated under reflux for 1·5 hr. The solution was cooled and the excess reagent destroyed with ethyl acetate and the organic material recovered in ether. 6α,7α,19-Trihydroxy-(-)-kaur-16-ene (55 mg.) crystallised from acetone-light petroleum as needles, m.p. 211-213° (Found: C, 73·4; H, 10·2. $C_{20}H_{32}O_3.0{\cdot}5H_2O$ requires C, 72·9; H, 10·1%), $\nu_{max.}$ 3480, 3220, 1655 and 870 $cm.^{-1}$

Oxidation of the Kauranolide - 7,18-Dihydroxykauranolide (1·3 g.) in acetone (50 ml.) was treated with the chromic oxide reagent (3 ml.) at room temperature for 3 hr. Methanol was added, the solution concentrated and heated with water on a steam-bath for 1 hr. The product was recovered in ethyl acetate and crystallised from acetone-light petroleum to give 6α-hydroxy-7-oxo-(-)-18-norkauran-19-oic acid 19-6α-lactone (1·01 g.) as prisms, m.p. 162-163° (Found: C, 74·9; H, 8·9. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%) $\nu_{max.}$ 1775 and 1705 $cm.^{-1}$

Reduction of the keto-lactone. - The keto-lactone (900 mg.) in tetrahydrofuran (25 ml.) and methanol (15 ml.) was treated with sodium borohydride (250 mg.) for 3·5 hr. The solution was acidified and concentrated *in vacuo*. It was diluted with aqueous ammonium chloride and extracted with ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate, dried and evaporated to give 6α,7α-dihydroxy-(-)-18-norkauran-19-oic acid 19-6α-lactone (0·53 g.) which crystallised from acetone-light petroleum as needles, m.p. 189-191° identical with the material prepared above.

Oxidation of 6α,7α-Dihydroxy-(-)-18-norkauran-19-oic Acid 19-6α-Lactone with Iodine and Lead Tetra-acetate. - The hydroxy-lactone (180 mg.), iodine (210 mg.) and lead tetra-acetate (300 mg.) in benzene (25 ml.) were irradiated under reflux under nitrogen for 3 hr. The solution was poured into water and extracted with ether. The extract was washed with sodium thiosulphate solution, aqueous sodium hydrogen carbonate, water and dried. Recovery gave 7α,20-epoxy-6-hydroxy-(-)-18-norkauran-19-oic acid 19-16α-lactone which crystallised from acetone-light petroleum as needles, m.p. 207-208° (Found: C, 74·9; H, 8·5. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%), $\nu_{max.}$ 1770 cm.$^{-1}$

Photolysis of the Nitrite of 6α,7α-Dihydroxy-(-)-18-norkauran-19-oic Acid 19-6α-lactone - The hydroxy-lactone (350 mg.) was treated with excess nitrosyl chloride in pyridine (3 ml.) at -10° for 15 min. The solution was poured into ice-water and rapidly worked up in ether. The extract was washed several times with ice-cold water, dried and evaporated at room temperature. The residue was dissolved in benzene and irradiated under nitrogen with water cooling for 0·5 hr. The benzene was evaporated and the residual gum in isopropanol (10 ml.) heated under reflux for 1 hr. The solvent was evaporated and the residue chromatographed on silica gel. Elution with 3:2 light petroleum-ether gave 6-hydroxy-7-oxo-(-)-18-norkauran-19-oic acid 19-6-lactone (20 mg.) identified by its infrared spectrum. Subsequent elution with ether:methanol gave the lactam (42) (190 mg.), m.p. 243-244° (Found: C, 69·0; H, 8·4. $C_{19}H_{27}O_4N$ requires C, 68·4; H, 8·16%), $\nu_{max.}$ 3490, 3140 (br.), 1640 cm.$^{-1}$

Epoxydation of 7-Hydroxykaurenolide. - 7-Hydroxykaurenolide (400 mg.) was dissolved in a 1·3N-solution of perbenzoic acid in chloroform (5 ml.) and left at 0° for 18 hr. The solution was diluted with ethyl acetate (100 ml.) and washed successively with aqueous ferrous sulphate, water, sodium hydrogen carbonate solution, and dried. Evaporation of the solvent and crystallisation of the residue from acetone-light petroleum gave 16,17-epoxy-6α,7β-dihydroxy-(-)-kauran-19-oic acid 19-6α-lactone as needles (228 mg.), m.p. 250-252° (Found: C, 72·0; H, 8·6. $C_{20}H_{28}O_4$ requires C, 72·3; H, 8·5%), $\nu_{max.}$ 3452, 3393, and 1759 cm.$^{-1}$

Hydrolysis of the Epoxide with Mineral Acid. - The epoxide (44)(101 mg.) in methanol (5 ml.) was heated under reflux with dilute hydrochloric acid (50 ml.) for 3 hr. The solution was left overnight during which time it deposited needles (64 mg.) of 6α,7β,16α,17-tetrahydroxy-(-)-kauran-19-oic acid 19-6α-lactone (45) which crystallised from aqueous methanol, m.p. 239-242° (Found: C, 69·0; H, 8·2. $C_{20}H_{30}O_5$ requires C, 68·5; H, 8·6%), $\nu_{max.}$ 3540, 1761, 1739 cm.$^{-1}$

Hydroxylation of 7-Hydroxykaurenolide. - 7-Hydroxykaurenolide (254 mg.) in ether (10 ml.) was treated with osmium tetroxide (500 mg.) in pyridine (5ml.) at room temperature

overnight. The suspension was heated for 1 hr. with potassium hydroxide (2·5 g.), mannitol (5 g.) and water (100 ml.) and then acidified with dilute hydrochloric acid. Recovery with ethyl acetate gave a crystalline residue which was chromatographed on silica gel. Elution with ethyl acetate gave 6α,16α,17-trihydroxy-7-oxo-(-)-kauran-19-oic acid 19-6-lactone (48)(75 mg.) which crystallised from acetone-light petroleum as needles, m.p. 248-250° (Found: C, 69·05; H, 8·2. $C_{20}H_{28}O_5$ requires C, 68·9; H, 8·1%), $\nu_{max.}$ 3535, 3475, 3414, 1786 and 1699 $cm.^{-1}$

Further elution with ethyl acetate and 1:20 ethyl acetate : methanol gave the triol described above (109 mg.) which crystallised from aqueous methanol, m.p. 239-244°.

Oxidation of the α-Glycol (45). - The glycol (84 mg.) in methanol (10 ml.) was treated at room temperature with a solution of sodium periodate (390 mg.) in water (2 ml.) for 18 hr. The solution was concentrated, diluted with water and the organic material recovered with ethyl acetate. Crystallisation of the residue from acetone-light petroleum gave 6α,7β-dihydroxy-16-oxo-17-nor-(-)-kauran-19-oic acid 19-6 lactone (47)(26 mg.), m.p. 303-306°, which was identified by its infrared spectrum.

Oxidation of the α-Glycol (48). - The glycol (6 mg.) in methanol (0·5 ml.) was treated with a solution of sodium periodate (24 mg.) in water (0·5 ml.) at room temperature overnight. The solution was diluted with water and the organic material recovered with ethyl acetate. On crystallisation from acetone-light petroleum it gave 6α-hydroxy-7,16-dioxo-17-nor-(-)-kauran-19-oic acid 19→6-lactone (46), (3 mg.) as needles, m.p. 289-292°, identified by its infrared spectrum.

Rudloff-Lemieux Oxidation of 7-Hydroxykaurenolide. - 7-Hydroxykaurenolide (306 mg.) in fresh dioxan (3 ml.) was added to a solution of potassium permanganate (79 mg.) potassium carbonate (0·5 g.) and sodium periodate (1·05 g.) in water (25 ml.), and left for 18 hr. The suspension was diluted with water, sodium hydrogen sulphite was added and the organic material recovered with ethyl acetate. On crystallisation from acetone-light petroleum it gave 6α,7β-dihydroxy-16-oxo-17-nor-(-)-kauran-19-oic acid 19→6α-lactone (47)(210 mg.), m.p. 305-307°, identified by its infrared spectrum.

Oxidation of 7-Hydroxykaurenolide with Potassium Permanganate. - Potassium permanganate (1 g.) was added to a solution of 7-hydroxykaurenolide (750 mg.) in purified

acetone (20 ml.). After 6 hr., the precipitated manganese dioxide was filtered off and thoroughly washed with acetone The solutions were combined and decolourised with sulphurous acid, concentrated and diluted with water. The solution was extracted with ethyl acetate and the extract separated into acidic and neutral fractions with sodium hydrogen carbonate solution. The neutral fraction was chromatographed on alumina. Elution with 1:1 ethyl acetate-light petroleum gave 6α,7β-dihydroxy-16-oxo-17-nor-(-)-kauran-19-oic acid 19→6-lactone (47)(210 mg.) m.p. 304-306°, identified by its infrared spectrum. Elution with ethyl acetate gave 6α,7β,16α,17-tetrahydroxy-(-)-kauran-19-oic acid 19→6β-lactone (45)(130 mg.) m.p. 239-241°, which was identified by its infrared spectrum. The acid fraction gave 6α,7β,16α-trihydroxy-(-)-kauran-17,19-oic acid 19→6α-lactone (51)(57 mg.) which crystallised from acetone-light petroleum as prisms, m.p. 148-150° (decomp) (Found: C, 62·9; H, 7·8. $C_{20}H_{28}O_6.H_2O$ requires C, 62·8; H, 7·9%), $\nu_{max.}$ 3406, 2480, 1980, 1750, 1690 $cm.^{-1}$

Oxidation of the Hydroxy Acid (51) with Sodium Bismuthate. The hydroxy-acid (24 mg.) in acetic acid (80%) (2 ml.) was treated with sodium bismuthate (26 mg.) for 4 hr. The solution was made alkaline with 3N-sodium hydroxide solution, diluted with water, and extracted with ethyl acetate. The extract was washed with sodium hydrogen carbonate solution,

water and dried. The solvent was evaporated and the residue crystallised from acetone-light petroleum to give 6α,7β-dihydroxy-16-oxo-17-nor-(-)-kauran-19-oic acid 19→6α-lactone (47)(10 mg.), m.p. 304-306°, identified by its infrared spectrum.

Isolation of the δ-lactone (50). - 7-Hydroxykaurenolide (1.5 g.) in glacial acetic acid (75 ml.) was treated with a stream of ozonised oxygen (17 mg/min.) for 15 min. The acetic acid was neutralised with sodium carbonate, diluted with water and the product recovered in ethyl acetate. It crystallised from acetone-light petroleum to give 6α,7β-dihydroxy-16-oxo-17-nor-(-)-kauran-19-oic acid 19→6α-lactone (47) (905 mg.) as needles, m.p. 304-307°. Chromatography of the mother liquors on alumina gave, in the fractions eluted with 1:3 light petroleum-ethyl acetate, the δ-lactone (50)(148 mg.), which crystallised from acetone-light petroleum as prisms, m.p. 284-285° (Found: C, 68·0; H, 8·0. $C_{19}H_{26}O_5$ requires C, 68·2; H, 7·8%), $\nu_{max.}$ 3450, 1761 and 1695 $cm.^{-1}$.

Baeyer-Villiger Oxidation of the Ketone (47). - The ketone (150 mg.) and toluene-p-sulphonic acid (26 mg.) were dissolved in a 1·3N-solution of perbenzoic acid in chloroform (10 ml.) and stood at 0° for 17 hr. The solution was diluted

with chloroform, washed with sodium hydrogen carbonate solution, aqueous ferrous sulphate, water and dried. The solvent was evaporated and the residual gum chromatographed on alumina. Elution with 1:1 ethyl acetate-light petroleum gave the starting material (24 mg.) whilst elution with 3:1 ethyl acetate-light petroleum gave the δ-lactone (94 mg.) which crystallised from acetone-light petroleum as prisms, m.p. 284-285°, identical with the sample prepared above.

Hydrolysis of the δ-Lactone (50). - The lactone (53 mg.) in methanol (5 ml.) was heated under reflux with N-sodium hydroxide (10 ml.) for 2 hr. The solution was cooled, diluted with water, cautiously acidified and extracted with ethyl acetate. The extract was washed with water, dried and evaporated to give a gum which was methylated with diazomethane. The residue was filtered through alumina to give the methyl ester (55)(41 mg.) as needles, m.p. 216-218° (Found: C, 65·6; H, 8·45. $C_{20}H_{30}O_6$ requires C, 65·6; H, 8·25%) $\nu_{max.}$ 3500, 3339, 1748 and 1704 $cm.^{-1}$

Oxidation of the Lactone (55). - The lactone (19 mg.) in acetone (2 ml.) was treated with the 8N-chromic oxide reagent (0·13 ml.) for 1 hr. Methanol was added and the solution poured into water and extracted with ethyl acetate.

The extract was separated into acidic and neutral fractions with sodium hydrogen carbonate solution. The neutral fraction (15 mg.) gave the diketone (56) which crystallised from acetone-light petroleum as needles (13 mg.), m.p. 214-215° (Found: C, 66·3; H, 7·4. $C_{20}H_{26}O_6$ requires C, 66·3; H, 7·2%) $\nu_{max.}$ 1785, 1730, 1710 $cm.^{-1}$

Oxidation of the δ-Lactone (50).- The lactone (84 mg.) in acetone (5 ml.) was treated with the chromic oxide reagent (0·13 ml.) at room temperature for 1 hr. Methanol was added, and the solution concentrated and diluted with water and extracted with ethyl acetate. The extract was washed with dilute hydrochloric acid, water and dried. The solvent was evaporated and the residue crystallised from acetone-light petroleum to give the keto-lactone (53)(58 mg.) as prisms, m.p. 275-279° (Found: C, 68·7; H, 7·4. $C_{19}H_{24}O_5$ requires C, 68·65; H, 7·3%), $\nu_{max.}$ 1777, 1738 and 1730 $cm.^{-1}$

Hydrogenolysis of the Keto-lactone (53). - The keto-lactone (43 mg.) in acetic anhydride (5 ml.) was heated under reflux with zinc dust (1 g.) overnight. More zinc dust (1 g.) was then added and the solution refluxed for a further 2 hr. The acetic anhydride was evaporated and the zinc dust extracted with ethyl acetate. The extract was washed with water, dilute hydrochloric acid, dried and evaporated to give a residue which was methylated with

diazomethane. On crystallisation from acetone-light petroleum it gave the keto-ester (54)(31 mg.) as prisms, m.p. 184-186° (Found: C, 69·2; H, 7·3. $C_{20}H_{28}O_5$ requires C, 68·9; H, 8·1%), $\nu_{max.}$ 1724, 1716 and 1704 cm.$^{-1}$

Oxidation of the Diol (63). - The diol (2 g.) in acetone (50 ml.) was treated, with cooling, with the chromic oxide reagent (6 ml.) and allowed to stand at room temperature for 1.6 hr. It was treated with methanol (1 ml.) and the solution then concentrated at 30°. The concentrate was diluted with water and extracted with ethyl acetate. The extract was separated into acidic and neutral fractions with sodium hydrogen carbonate. The acidic fraction crystallised from acetone-light petroleum to give the dicarboxylic acid (9)(450 mg.) as needles, m.p. 169-171° and 202-204°. The neutral fraction was chromatographed on silica gel. Elution with 3:17 ethyl acetate-light petroleum gave the diosphenol (61, R=O) (500 mg.) as a gum ($\lambda_{max.}$ 281 mμ log. ε 3·9) identified by its infrared spectrum. Elution with 1:3 ethyl acetate-light petroleum gave the lactonol (64) (400 mg.) which crystallised from acetone-light petroleum as prisms, m.p. 242-243° (decomp.)(Found: C, 63·7; 63·8; H, 7·0, 7·05. OMe, 8·25% equiv. 334. $C_{20}H_{26}O_7$ requires C, 63·5; H, 6·9; OMe 8·2%; M, 350), $\nu_{max.}$ 3415, 1801 and 1727 cm.$^{-1}$

Alkaline Hydrolysis of the Lactonol (64). - The lactonol (200 mg.) in ethanol (5 ml.) was refluxed with 0·5N-sodium hydroxide (5 ml.) under nitrogen for 1 hr. The solution was then acidified with dilute hydrochloric acid. There was a copious evolution of carbon dioxide (baryta trap). The solution was diluted with water and extracted with ethyl acetate and the extract separated into acidic and neutral fractions with sodium hydrogen carbonate. The neutral fraction was chromatographed on silica gel in 1:4 ethyl acetate-light petroleum to give the lactonol (65) (40 mg.) which crystallised from acetone-light petroleum as needles, m.p. 246-251° (Found: C, 67·6, 67·8; H, 7·8, 7·8. $C_{18}H_{24}O_5$ requires C, 67·5; H, 7·55%), $\nu_{max.}$ 3393, 1788 and 1744 $cm.^{-1}$

6-Hydroxy-(-)-kaur-6,16-dien-19-oic Acid 19→6-Lactone. - The toluene-p-sulphonate of 7-hydroxykaurenolide (250 mg.) was refluxed in pure collidine (15 ml.) for 4 hr. The solution was poured into dilute hydrochloric acid and extracted with ethyl acetate. The extract was washed with dilute hydrochloric acid and water and dried. Evaporation of the solvent gave a gum which was chromatographed on alumina. Elution with 1:19 ethyl acetate:light

petroleum as needles, m.p. 205° (Found: C, 79·9; H, 8·8. $C_{20}H_{26}O_2$ requires C, 80·5; H, 8·8%), $\nu_{max.}$ 1793, 1693, 1655, 871 and 826 cm.$^{-1}$

Hydrolysis of the Enol-lactone (67) with Mineral Acid. - The enol-lactone (75 mg.) in acetone (10 ml.) was refluxed with dilute hydrochloric acid (10 ml.) for 2 hr., the acetone was evaporated, the solution diluted to 250 ml. with water, and the product recovered in ethyl acetate. Crystallisation from acetone-light-petroleum gave 16-hydroxy-6-oxo-(-)-kauran-19-oic acid (68) as needles (35 mg.), m.p. 274-275° (Found: C, 71·6; H, 9·0. $C_{20}H_{30}O_4$ requires C, 71·8; H, 9·0%), $\nu_{max.}$ 3500, 2745, 1745, and 1661 cm.$^{-1}$

The methyl ester, prepared with diazomethane, crystallised from light petroleum as needles, m.p. 112-113° (Found: C, 72·5; H, 9·3. $C_{21}H_{32}O_4$ requires C, 72·4; H, 9·3%), $\nu_{max.}$ 3430, 1741 and 1706 cm.$^{-1}$ (in $CHCl_3$) 3480, 1728 and 1717 cm.$^{-1}$

6-Oxo-(-)-kaur-16-en-19-oic Acid (69). - The toluene-p-sulphonate of 7-hydroxykaurenolide (400 mg.) and dry lithium iodide (500 mg.) in dry collidine (15 ml.) were refluxed for 5 hr., poured into dilute hydrochloric acid (100 ml.) and extracted with ether. The extract was washed with sodium

thiosulphate solution and water, and dried. Evaporation of the solvent gave a crystalline residue which was crystallised from acetone-light petroleum to give 6-oxo-(-)-kaur-16-en-19-oic acid as needles (320 mg.), m.p. 205-206° (Found: C, 75·8; H, 8·9. $C_{20}H_{28}O_3$ requires C, 75·9; H, 8·9%), $\nu_{max.}$ 2720, 1740, 1659 (s) and 893 $cm.^{-1}$

The methyl ester, prepared with diazomethane, was a gum.

The Action of Phosphorus Pentachloride on 7-Hydroxykaurenolide. - 7-Hydroxykaurenolide (620 mg.) suspended in ether (5 ml.) was treated with phosphorus pentachloride (510 mg.) at room temperature for 2 hr. The solution was poured into dilute hydrochloric acid and the organic material recovered in ether. Chromatography on silica gel in light petroleum gave the enol-lactone (67) (115 mg.), m.p. 202-204° followed by the γ-lactone (70)(160 mg.) which crystallised from light petroleum as needles, m.p. 233-234° (Found: C, 80·1; H, 8·7. $C_{20}H_{26}O_2$ requires C, 80·5; H, 8·7%), $\nu_{max.}$ 1760, 1655 and 882 $cm.^{-1}$ Nuclear magnetic resonance peaks at τ = 8·8 (2 $-\overset{|}{\underset{|}{C}}-CH_3$), 7·8 (br. multiplet of 7-protons) 5·15 (multiplet 3 protons).

Further elution with 1:9 ethyl acetate-light petroleum gave 6-oxo-(-)-kaur-16-en-19-oic acid (295 mg.), m.p. 263-265°.

The Action of Phosphorus Pentachloride on 7-Epihydroxykaurenolide. - 7-Epihydroxykaurenolide (250 mg.) suspended in ether (5 ml.) was treated with phosphorus pentachloride (210 mg.) at room temperature for 2 hr. The solution was diluted with ether, poured into dilute hydrochloric acid and the organic material recovered in ether. Chromatography on silica gel gave successively the Δ^6-enol-lactone (67) (106 mg.), the γ-lactone (70) (29 mg.), and the 6-keto-acid (93 mg.) identified by their infrared spectra.

Isolation of the γ-Lactone (70) from the Preparation 6-Oxo-(-)-Kaur-16-en-19-oic Acid. - The combined crude residues (0·26 g.) from three preparations of the keto-acid described previously (from 1·5 g. of the monotoluene-p-sulphonate of 7-hydroxykaurenolide) were chromatographed on alumina. Elution with 1:19 ethyl acetate-light petroleum gave the γ-lactone (70) (39 mg.). Subsequent fractions (126 mg.) contained the Δ^6-enol-lactone admixed with the γ-lactone as indicated by the infrared spectrum.

Acetylation of the Diols (60) and (63). - (a) The diol (60) (325 mg.) in pyridine (2 ml.) was treated with acetic anhydride (0·5 ml.) for 3 days. Isolation with ethyl acetate gave methyl 7β-acetoxy-6α-hydroxy-(-)-kaur-16-en-19-oate which crystallised from acetone-light petroleum as needles, m.p. 190-192° (Found: C, 70·2; H, 8·9. $C_{23}H_{34}O_5$ requires C, 70·7; H, 8·8%), $\nu_{max.}$ 3410, 1732, 1694, 1662 and 884 $cm.^{-1}$

(b) Under similar conditions the diol (63) gave methyl 7β-acetoxy-6α-hydroxy-16-oxo-17-nor-(-)-kauran-19-oate which crystallised from acetone-light petroleum as needles, m.p. 196-198° (Found: C, 67.8; H, 8.4. $C_{22}H_{32}O_6$ requires C, 67.3; H, 8.2%), $\nu_{max.}$ 3382, 1752, 1734, 1696 cm.$^{-1}$

Preparation of the Toluene-p-sulphonates of the Diols (60) and 74). - The diol (60) (750 mg.) in dry pyridine (10 ml.) was treated with toluene-p-sulphonyl chloride (1.1 g.) for 2 days at room temperature. The solution was poured into dilute hydrochloric acid and the organic material removed in ether. Chromatography on alumina gave the 7β-toluene-p-sulphonate of methyl 6α,7β-dihydroxy-(-)-kaur-16-en-19-oate which crystallised from light petroleum as needles (0.72 g.), m.p. 144-145° (Found: C, 67.0; H, 7.3. $C_{28}H_{38}O_6S$ requires C, 67.2; H, 7.25%), $\nu_{max.}$ 3400, 1690, 1655, 1600 cm.$^{-1}$

(b) The 7α-toluene-p-sulphonate of methyl 6α,7α-dihydroxy-(-)-kaur-16-en-19-oate, prepared similary, had m.p. 190-192°.

Oxidation of the Acetates (73; R=CH_2 and R=O). - (a) The 7-acetyl derivative of the diol (73, R=CH) (200 mg.) in acetone (5 ml.) was treated with the chromic oxide reagent (0.25 ml.) for 4 hr. Methanol was added, the solution concentrated and diluted with water and the neutral organic material recovered with ethyl acetate to give methyl 7β-

acetoxy-6-oxo-(-)-kaur-16-en-19-oate (85 mg.) which crystallised from acetone-light petroleum as needles, m.p. 120-121° (Found: C, 70·7; H, 8·5. $C_{23}H_{32}O_5$ requires C, 71·1; H, 8·3%), $\nu_{max.}$ 1741, 1728, 1658, 895 and 885 cm.$^{-1}$

Chromatography of the mother-liquors on alumina gave, in the fraction eluted with 2:3 ethyl acetate-light petroleum, the nor-ketone (21 mg.) described below.

(b) Under similar conditions the acetyl derivative (73; R=O) gave methyl 7β-acetoxy-6,16-dioxo-17-nor-(-)-kauran-19-oate which crystallised from acetone-light petroleum as needles, m.p. 174-175° (Found: C, 67·6; H, 7·7. $C_{22}H_{30}O_6$ requires C, 67·7; H, 7·7%), $\nu_{max.}$ 1753, 1745, 1729 and 1721 cm.$^{-1}$

Oxidation of the 6α-Hydroxy-7α-monotoluene-p-sulphonate (33) - The toluene-p-sulphonate (280 mg.) in acetone (5 ml.) was treated with the chromic oxide reagent (0·25 ml.) at room temperature overnight. Methanol was added, the solution concentrated, diluted with water, extracted with ethyl acetate and the extract washed with sodium hydrogen carbonate solution, water and dried. The solvent was evaporated and the residue chromatographed on alumina. Elution with 25% ethyl acetate in light petroleum gave the toluene-p-sulphonate of methyl 7α-hydroxy-6-oxo-(-)-kaur-16-en-oate (45 mg.) which crystallised from acetone light petroleum as thick needles, m.p. 165-166° (Found: C, 67·7; H, 7·4.

$C_{28}H_{34}O_6S$ requires C, 67·45; H, 6·9%), $\nu_{max.}$ 1738, 1719, 1662, 1600 and 894 $cm.^{-1}$

Elution with 7·5% of ethyl acetate in light petroleum gave the <u>toluene-p-sulphonate</u> of methyl 7α-hydroxy-6,16-dioxo-17-nor-(-)-kauran-19-oate (109 mg.) which crystallised from acetone-light petroleum as needles, m.p. 194-195° (Found: C, 64·3; H, 6·9. $C_{27}H_{32}O_7S$ requires C, 64·8; H, 6·4%), $\nu_{max.}$ 1747, 1722, and 1601 $cm.^{-1}$

<u>Reduction of the 7-Acetate of Diol (60) with Lithium Aluminium Hydride.</u> - The acetate (54 mg.) and lithium aluminium hydride (73 mg.) in ether (10 ml.) were heated under reflux for 5·5 hr. Moist ether and dilute hydrochloric acid were cautiously added and the organic material recovered in ether to give 6α,7β,19-trihydroxy-(-)-kaur-16-ene (21 mg.) which crystallised slowly from acetone-light petroleum as needles, m.p. 207-209°, identified by its infrared spectrum.

<u>Reduction of the 6-Keto-acid (69) with Sodium Borohydride.</u> The keto-acid (500 mg.) in methanol-tetrahydrofuran (1:1; 50 ml.) was treated with sodium borohydride (500 mg.) for 1 hr. at room temperature. The solution was concentrated, poured into water, acidified with dilute hydrochloric acid and extracted with ether. The extract was dried and evaporated

to give a semi-crystalline residue which was taken up in methanol and methylated with diazomethane. The solvent was evaporated and the residue filtered through alumina in 1:9 ethyl acetate-light petroleum to give methyl 6α-hydroxy-(-)-kaur-16-en-19-oate (75) (490 mg.) which crystallised from light petroleum as needles, m.p. 158-159° (Found: C, 75·6; H, 9·7. $C_{21}H_{32}O_3$ requires C, 75·9; H, 9·7%), $\nu_{max.}$ 3437, 1705, 1654, 870 cm.$^{-1}$

Reduction of the Hydroxy-ester (75). - The above hydroxy-ester (45 mg.) in dry ether (5 ml.) was treated with lithium aluminium hydride (49 mg.) at room temperature for 2 hr. The excess reagent was destroyed with ethyl acetate, the solution diluted with ether, washed with dilute hydrochloric acid, water, dried and evaporated to give 6α,19-dihydroxy-(-)-kaur-16-ene, m.p. 173-175° identified by its infrared spectrum.

Pyrolysis of the Ester (75). - The ester (30 mg.) was heated on a Kofler block at 220° for 0·5 hr. The residue was extracted with acetone. On crystallisation from acetone-light petroleum it gave 6α-hydroxy-(-)-kaur-16-en-19-oic acid 19→6α-lactone (10 mg.), m.p. 204-205° (Found: C, 79·5; H, 9·3. $C_{20}H_{28}O_2$ requires C, 79·95; H, 9·4%), $\nu_{max.}$ 1754, 1655 and 880 cm.$^{-1}$

Ozonolysis of Methyl 6α-Hydroxy-(-)-kaur-16-en-19-oate. - A stream of ozonised oxygen (13-5 mg./min.) was passed through a solution of the hydroxy-ester (210 mg.) in glacial acetic acid (10 ml.) for 5 min. After being left for 1 hr., the acetic acid was neutralised with aqueous sodium hydrogen carbonate and the organic material recovered in ether. The residue crystallised from acetone-light petroleum as needles, (195 mg.) of methyl 6α-hydroxy-16-oxo-(-)-17-norkauran-19-oate m.p. 172-174° (Found: C, 71·6; H, 9·0. $C_{20}H_{30}O_4$ requires C, 71·8; H, 9·0%) $\nu_{max.}$ 3406, 1735 and 1693 cm.$^{-1}$

Pyrolysis of the above Ester. - The nor-ketone (35 mg.) from the above experiment was heated in a pyrex tube at 250° for 0·75 hr. The residue was extracted with acetone and crystallised from acetone-light petroleum to give needles (12 mg.) of 6α-hydroxy-16-oxo-17-nor-(-)-kauran-19-oic acid 19→16-lactone, m.p. 264-265° (Found: C, 74·9; H, 8·8. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·6%), $\nu_{max.}$ 1755 and 1737 cm.$^{-1}$

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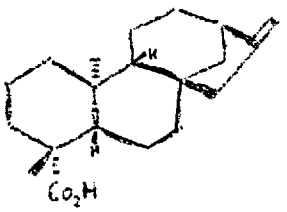
CHAPTER III

Some Partial Syntheses

3.1 The Partial Synthesis of (-)-Kaur-16-en-19-oic Acid

(-)-Kaur-16-en-19-oic acid (1) was required in connection with the study of the biosynthesis of gibberellic acid.[1,2] Furthermore it was recently isolated[3] from Ricinocarpus stylosus. Hence its partial synthesis from 7-hydroxykaurenolide[4] was attempted.[5]

Oxidation of 7-hydroxykaurenolide with chromium trioxide gave the corresponding 7-ketone which was converted to the keto-acid (3, R=H) by hydrogenolysis with calcium in liquid ammonia. Wolff-Kishner reduction of the latter led to (-)-kaur-16-en-19-oic acid, m.p. 165-167° $[\alpha]_D^{17}$ -112° (c. 0·25) (1). Alternatively the 7-keto-lactone was reduced with sodium borohydride to give the 7-epi-alcohol. This, on hydrolysis and methylation, gave a separable mixture of the keto-ester (74; R=Me) and a dihydroxy ester. The former, on Wolff-Kishner reduction with concomitant hydrolysis, was converted to the required acid. The corresponding ester was resistant to hydrolysis but readily furnished the 19-alcohol on reduction with lithium aluminium hydride.



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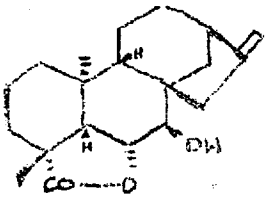

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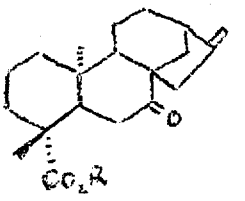

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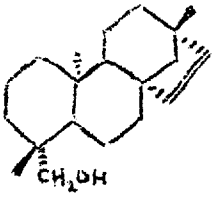

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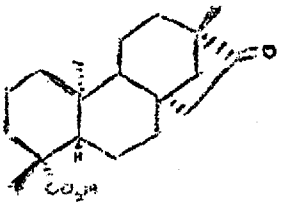

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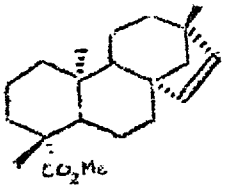

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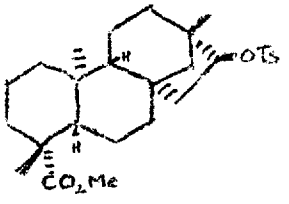

(7)

3·2 The Partial Synthesis of Monogynol from IsoSteviol

Monogynol, isolated[6] from Erythoxylon monogynum, has been assigned the structure of 19-hydroxystach-15-ene (4)[7] or its optical antipode.[8] These in turn do not accord with the structure for stachenone recently considered by Scott et al.[9] In view of these discrepancies monogynol was related to isosteviol (5). Steviol and hence isosteviol has been related to (-)-kaurene of known absolute stereochemistry.[4,10] Furthermore beyerol, isostevane and stachenol have also been interrelated[11] and thus this work provides additional confirmation of the stereochemistry of the latter.

Steviol is the aglycone of stevioside which forms the sweet principle of Caa-ehe (Stevia rebaudiana) a Paraguyan plant. On treatment with mineral acid it undergoes a Wagner-Meerwein rearrangement to form isosteviol. The latter may also be extracted direct from the plant by acidifying the aqueous extract and boiling for 2-3 hr. Reduction of isosteviol methyl ester with sodium borohydride gave the known[12] 16α-alcohol which was converted to its toluene-p-sulphonyl derivative. The latter, on refluxing with collidine formed the Δ^{15} unsaturated ester (6) which was subsequently reduced with lithium aluminium hydride to give the required 19-alcohol (4) m.p. 119° $[\alpha]_D$ + 27°. This was identical by mixed m.p. and infrared with a sample from Erythroxylon

monogynum generously provided by Dr. R. D. H. Murray and thus the absolute stereochemistry assigned by the Indian workers to mongynol must be inverted and the β-15-16 bridge predicted by Scott for stachenone replaced by an α-bridge.

Comparison of the position of the C-20 proton resonances of the alcohol and ester with their saturated derivatives shows the shielding effect of an α-15-16 double bond noted[13] by Jefferies et al. for the beyerol series. A smaller effect may also be seen in comparing isosteviol with its 16-deoxy derivative. The latter was prepared by Wolff-Kishner reduction of isosteviol.[2] The corresponding saturated 19-alcohol was prepared by subsequent reduction of the ester with lithium aluminium hydride.

3.3 Experimental Section

Hydrogenolysis of 7-Oxokaurenolide. - Calcium metal (1 g.) was shredded and dissolved in liquid ammonia (300 ml.). The ketone (0.65 g.) in dioxan (50 ml.) was added with stirring. The solution was left until the blue colour returned and then ammonium chloride (5 g.) was added and the ammonia evaporated. Water was added and the solution was extracted with ether. The extract was washed with dilute hydrochloric acid, water, dried and evaporated to give a gum which was methylated with diazomethane. This ester was taken up in acetone (5 ml.) and oxidised with the chromic oxide reagent (0.3 ml.). Methanol was added, the solution concentrated and diluted with water and extracted with ether. The extract was washed with dilute hydrochloric acid, water, dried and evaporated to give a gum which was chromatographed on alumina. Elution with light petroleum gave methyl 7-oxo-(-)-kaur-16-en-19-oate (0.4 g.) which crystallised from light petroleum as needles, m.p. 110-112° (Found: C, 76.3; H, 9.3. $C_{21}H_{30}O_3$ requires C, 76.3; H, 9.15%), $\nu_{max.}$ 1706, 1656, and 871 $cm.^{-1}$

Hydrolysis of the Keto-ester (3; R=Me). - The keto-ester (123 mg.) in methanol (1 ml.) and 3N-sodium hydroxide (15 ml.) was heated under reflux overnight. The solution was washed with ether and acidified and the organic material

recovered in ether. Chromatography on silica gel in 3:17 ethyl acetate-light petroleum gave 7-oxo-(-)-kaur-16-en-19-oic acid (85 mg.) which crystallised from acetone-light petroleum as needles, m.p. 208-210° (Found: C, 75·2; H, 9·0. $C_{20}H_{28}O_3$ requires C, 75·9; H, 8·9%), $\nu_{max.}$ 3205, 1724, 1689, 1655 and 870 $cm.^{-1}$

Wolff-Kishner Reduction of the above Keto-Acid. - The keto-acid (72 mg.) in diglyme (5 ml.) and hydrazine hydrate (0·5 ml.) was heated at 150° for 2 hr. Potassium hydroxide pellets (0·6 g.) were added and the temperature raised to 210° for 1 hr. The solution was poured into water, acidified with dilute hydrochloric acid and extracted with ether. Recovery gave (-)-kaur-16-en-19-oic acid (1) (41 mg.) which crystallised from acetone-light petroleum as needles, m.p. 165-167° (Found: C, 79·0; H, 10·1. $C_{20}H_{30}O_2$ requires C, 79·4; H, 10·0%), $\nu_{max.}$ 2704, 2639, 1691, 1658 and 872 $cm.^{-1}$

The methyl ester, prepared with diazomethane, crystallised from methanol as needles, m.p. 71-73° (Found: C, 80·2; H, 10·3. $C_{21}H_{32}O_2$ requires C, 79·7; H, 10·2%), $\nu_{max.}$ 1724, 1655 and 874 $cm.^{-1}$

The Toluene-p-sulphonate (7). - Isosteviol methyl ester was reduced as described previously.[12] The toluene-p-sulphonate of methyl 16-hydroxystachan-19-oate, prepared with toluene-p-sulphonyl chloride in pyridine, crystallised

from light petroleum as needles, m.p. 112-113° (Found: C, 69·0; H, 8·7. $C_{28}H_{40}O_5S$ requires C, 68·8; H, 8·3%), $\nu_{max.}$ 1710 and 1600 cm.$^{-1}$

Preparation of the Unsaturated Ester (6). - The above toluene-p-sulphonate (240 mg.) and collidine (5 ml.) were heated under reflux for 4 hr. The solution was poured into dilute hydrochloric acid and extracted with ether. The extract was washed with water, dried and evaporated. Methyl stach-15-en-19-oate (110 mg.) crystallised from aqueous methanol as plates, m.p. 107-109° (Found: C, 79·0; H, 10·0. $C_{21}H_{32}O_2$ requires C, 79·7; H, 10·2%), $\nu_{max.}$ 1710 cm.$^{-1}$

Reduction of the Unsaturated Ester (6). - The ester (75 mg.) from the above experiment, in ether (10 ml.) was treated with lithium aluminium hydride (100 mg.) at room temperature for 3 hr. Water followed by dilute hydrochloric acid was cautiously added and the solution extracted with ether. The extract was washed with water, dried and evaporated to give 19-hydroxystach-15-ene (44 mg.) which crystallised from aqueous methanol as needles, m.p. 120-121° (Found: C, 82·7; H, 11·5. $C_{20}H_{32}O$ requires C, 83·3; H, 11·2%), $[\alpha]_D^{20}$ + 27°. $\nu_{max.}$ 3300 cm.$^{-1}$ The sample was identical with a sample of the natural product generously provided by Dr. R. D. H. Murray.

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