576. *New Metabolites of* Gibberella fujikuroi. *Part IX.*[1] *Gibberellin* A_{13}

By R. H. B. Galt

Gibberellin A_{13}, a biogenetically interesting new metabolite of *Gibberella fujikuroi*, is shown to be 2β-hydroxy-1β-methyl-8-methylenegibbane-1α,4aα,10β-tricarboxylic acid (I).

The fungus *Gibberella fujikuroi* ACC.917 [2] is a rich source of the plant-growth hormones, the gibberellins,[3] and of related natural products.[4] During a study of the biosynthesis of gibberellic acid, mutants of the fungus were examined and attempts were made to induce metabolic blocks with enzyme inhibitors. By such techniques, intermediates which normally have only a transient existence can often be accumulated. One mutant (B.47), and a fermentation to which the cholesterol biosynthesis inhibitor triparanol [5] had been added, both produced a new metabolite, gibberellin A_{13}; * it was later isolated in lower

* "Fujic acid" isolated by M. Sternberg, *Arch. Biochem. Biophys.*, 1962, **98**, 299, but assigned the molecular formula $C_{14}H_{18}O_6$, is probably gibberellin A_{13}.

[1] Part VIII, B. E. Cross, and K. Norton, *J.*, 1965, 1570.

[2] A. Borrow, P. W. Brian, V. E. Chester, P. J. Curtis, H. G. Hemming, C. Henehan, E. G. Jefferys, P. B. Lloyd, I. S. Nixon, G. L. F. Norris, and M. Radley, *J. Sci. Food Agric.*, 1955, **6**, 340.

[3] J. F. Grove, *Quart. Rev.*, 1961, **15**, 56.

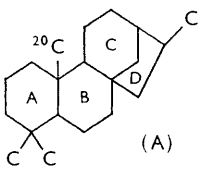
[4] B. E. Cross, R. H. B. Galt, J. R. Hanson, and (in part) P. J. Curtis, J. F. Grove, and A. Morrison, *J.*, 1963, 2937.

[5] R. D. Mackenzie and T. R. Blohm, *Fed. Proc.*, 1959, **18**, 417.

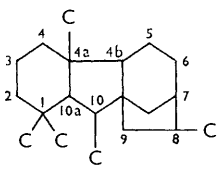
yield from a normal ACC.917 fermentation. Like gibberellin A_{12},[6] it belongs to the new class of C_{20} gibberellins.

Elemental analyses of gibberellin A_{13} were never satisfactory owing to solvent of crystallisation but analyses of derivatives and degradation products established the molecular formula as $C_{20}H_{26}O_7$. It showed infrared absorption at 3500br., 1723—1700br, and 1660 cm.$^{-1}$, had no ultraviolet absorption between 220 and 350 mμ, and titrated as an acid but gave no sharp end-point. On microhydrogenation, it absorbed one mol. of hydrogen and split off formaldehyde on ozonolysis. Methylation of gibberellin A_{13} produced a trimethyl ester * (III) which readily formed a monoacetate which had nuclear magnetic resonance (n.m.r.) signals at τ 8·90 (tertiary methyl), 7·92 (acetate), 6·39, 6·32, and 6·28 (methyl esters), 5·15 (H–$\underset{|}{\overset{|}{C}}$–O·CO·$CH_3$; multiplet) and 5·00 (C=CH_2); in addition, two doublets centred at 7·48 and 6·15 (J = 15 c./sec.) were very reminiscent of the 10,10a-quartet of the known gibberellins and their derivatives.[7] Gibberellin A_{13} was therefore a monohydroxy-tricarboxylic acid with a terminal methylene group and was tetracarbocyclic.

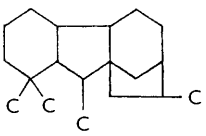
Metabolic products based on only two tetracarbocyclic skeletons, the C_{20} kaurane (A) and the C_{19} gibberellin (C), have been isolated from *Gibberella fujikuroi*, and each has four



(A)



(B)



(C)

pendant carbon groups. Gibberellin A_{13} has five such groups, and the intermediary C_{20} gibberellin nucleus (B), in which ring-B contraction had occurred but the C-20 carbon atom had been retained, seemed a likely possibility. Gibberellin A_{13} was oxidised to a gummy ketone which was decarboxylated easily. The n.m.r. spectrum of the methyl ester of the resultant ketone (II) showed signals at τ 9·08 (>CH·CH_3 doublet; J = 7 c./sec.), 6·30 and 6·22 (methyl esters), and 5·10 (>C=CH_2). The C-10 hydrogen was a doublet at 6·50 (J = 13 c./sec.) and a quartet centred at 7·19 (J = 7 and 13 c./sec.) probably represented the C-10a proton. The hydroxyl group was therefore secondary and adjacent to a carbon atom bearing both a tertiary methyl and a carboxyl group (*i.e.*, at C-2). The presence of a gibbane AB-quartet in gibberellin A_{13} derivatives requires a further carboxyl group at C-10 and thus the terminal methylene group at C-8. The third carboxyl group was placed at 4a and hence early in the chemical investigation the structure (I) was envisaged for gibberellin A_{13}. It is acceptable on biogenetic grounds, representing the penultimate stage of oxidative loss of the diterpenoid angular methyl group at C-20 in the conversion into the C_{19} gibberellins [cf. (A) ⟶ (B) ⟶ (C)]. Further studies confirmed this structure.

Under normal acetylation conditions, gibberellin A_{13} was converted into an acetate anhydride (VII), $\nu_{max.}$ 1804 and 1766 (unstrained anhydride), 1752 (acetate), 1729 and 1681 (acid), and 1660 and 897 (>C=CH_2) cm.$^{-1}$. Since the di-acid (II) was recovered when similarly treated, the C-1 carboxyl was assumed to be involved in anhydride formation.

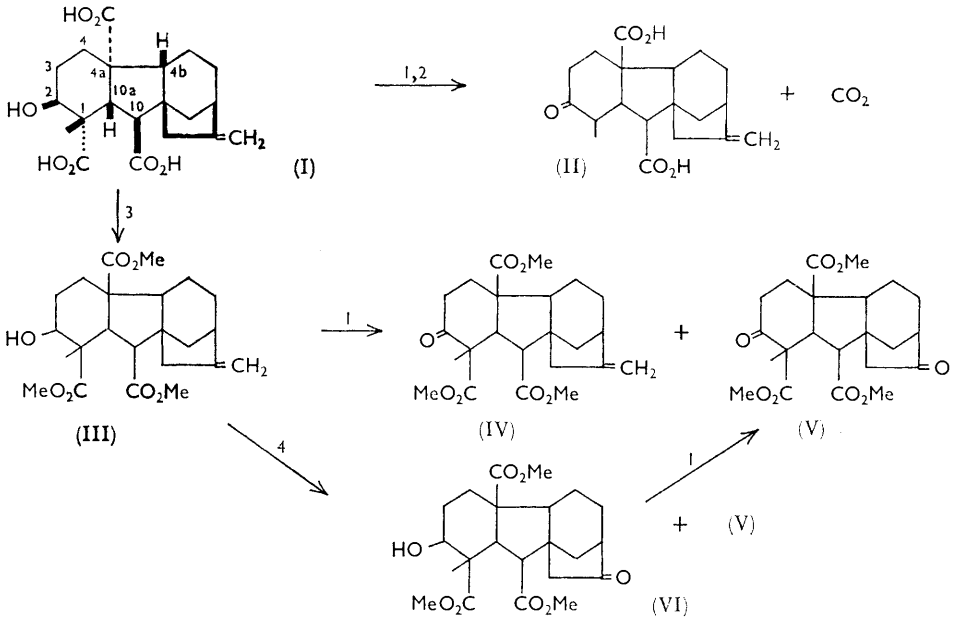
The decomposition of gibberellin A_{13} at its melting point was found to be a dehydration.

* In one methylation the endocyclic double-bond isomer was also isolated after chromatography.

[6] B. E. Cross, and K. Norton, *J.*, 1965, 1570.

[7] (*a*) N. Sheppard, *J.*, 1960, 3040; (*b*) D. C. Aldridge, J. F. Grove, R. N. Speake, B. K. Tidd, and W. Klyne, *J.*, 1963, 143.

At 220° it was converted into the hydroxy-acid-anhydride (VIII) which formed a methyl ester, $\nu_{max.}$ 3542 (OH), 1802 and 1764 (anhydride), 1729 (ester), and 812 (–CH=C<) cm.$^{-1}$; the double bond had isomerised to the endocyclic position. In another pyrolysis at 280° two hydroxyl-free products, shown to be the anhydride (IX) and the di-acid (X; R = H), were obtained; the latter was converted into the former at 280° but, significantly, not by acetic anhydride and pyridine at room temperature or by refluxing acetic anhydride.



Reagents: 1, CrO_3; 2. heat; 3, CH_2N_2; 4, O_3

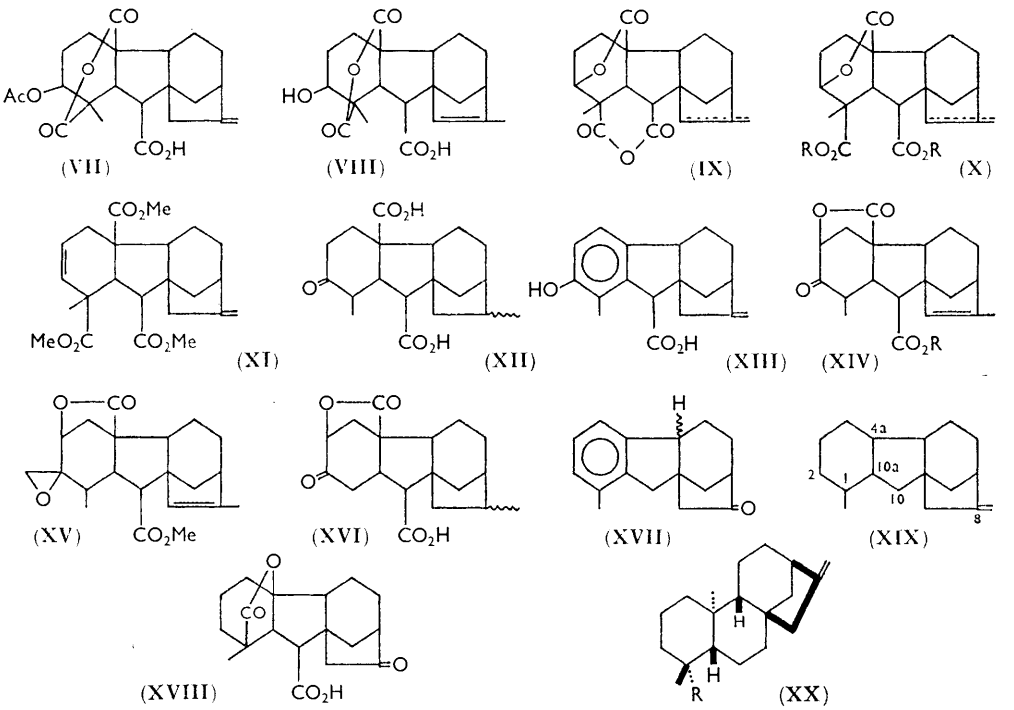
The n.m.r. spectrum of the ester (X; R = Me), $\nu_{max.}$ 1730—1740 (δ-lactone and esters) cm.$^{-1}$, revealed it to be a mixture of exocyclic and endocyclic double-bond isomers; the exocyclic isomer was later obtained pure (see below). For the formation of (IX) and (X) (R = H), ring A must adopt the boat conformation, a recurrent feature of the chemistry.

Gibberellin A_{13} trimethyl ester (III) absorbed one mol. of hydrogen to give a dihydro-derivative. The gummy toluene-*p*-sulphonate of (III) was converted by boiling collidine into the Δ^2-olefin (XI), τ 8·72 (C–CH_3), 7·58 and 6·10 (10,10a-quartet; J = 13 c./sec.), 6·44, 6·34, 6·25 (methyl esters), 5·13 (>C=CH_2) and 4·2 ⟶ 4·6 (–C=CH–). A minor product of this reaction was the δ-lactone (X; R = Me).

Attempts to aromatise ring A of gibberellin A_{13} to relate it to a known or readily accessible gibberellin derivative, *e.g.*, (XIII), failed. When the ketone (II) was brominated with phenyltrimethylammonium perbromide, dehydrobrominated with lithium chloride, and methylated, two products (XIV; R = Me) and (XV), resulted. The former showed infrared absorption at 1773 (γ-lactone) and 1730s (ester and ketone) cm.$^{-1}$, and n.m.r. signals at τ 8·97 (>CH·CH_3; doublet) 8·34 (C=C·CH_3; doublet; J = 2 c./sec.), 6·32 (methyl ester), 5·45 (C-3 hydrogen; doublet; 4·5 c./sec.), and 4·55 (C-9 hydrogen; doublet; 2 c./sec.). In the spectrum of the epoxide, the secondary methyl group moved upfield to τ 9·3 and the C-3 proton to 6·03. The AB-quartet of the epoxide ring was centred at 7·21 (J = 4 c./sec.). The ketone (XIV; R = Me) was converted in high yield into the epoxide by treatment with diazomethane. The ketone (XII), prepared to remove the

complication of double-bond isomerisation, behaved similarly to (II) in bromination–dehydrobromination experiments; on treatment of (XII) with selenium dioxide in acetic acid mainly intractable products resulted. The lactone (XVI) was partially recovered after reaction with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

Confirmation of the carbon skeleton came from selenium dehydrogenation of gibberellin A_{13} nor-ketone (cf. dehydrogenations in ref. 8). In the hydrocarbon products there was



ultraviolet absorption evidence for the presence of a fluorene. The major dehydrogenation product was difficult to purify by chromatography or crystallisation (best m. p. 126—132°) although it gave only one spot on thin-layer chromatography in two solvent systems. The infrared [$\nu_{max.}$ 1741 (5-ring ketone) and 1600 (aromatic) cm.$^{-1}$] and ultraviolet [$\lambda_{max.}$ 262sh, 266 and 274 mμ (log ε 2·68, 2·72, and 2·64)] spectra suggested the structure (XVII) the 4bα-epimer of which has been synthesised.[9] Support for this assignment came from selenium dehydrogenation of gibberellin A_9 nor-ketone (XVIII) which gave the same compound, $C_{16}H_{18}O$, m. p. 136—139°, in higher yield. Presumably slightly different mixtures of epimers at 4b were produced in the two experiments; the infrared spectra were virtually identical and the two compounds behaved identically on thin-layer chromatography. The structure of gibberellin A_{13} can now be constructed with (XIX) as the foundation with the hydroxyl and carboxyl groups placed as in (I).

The nor-ketone (VI) showed a large positive Cotton effect indicative in tetracyclic diterpene chemistry [10,11] of a β-orientated ring D. The dimethyl ester of (II) had a negative Cotton effect of similar magnitude but of opposite sign to a 4α-methyl-3-oxo-5α-steroid, revealing a *trans*-antipodal A/B ring junction. The triester (IV) showed a smaller negative Cotton effect, owing to the positive contribution from the axial substituent at C-1. The

[8] B. E. Cross, J. F. Grove, J. MacMillan, and T. P. C. Mulholland, *J.*, 1958, 2520.
[9] K. Mori, M. Matsui, and Y. Sumiki, *Argric. Biol. Chem.*, 1961, **25**, 907.
[10] L. H. Briggs, B. F. Cain, R. C. Cambie, and B. R. Davis, *Tetrahedron Letters*, 1960, No. 24, 18.
[11] R. A. Finnegan and C. Djerassi, *J. Amer. Chem. Soc.*, 1960, **82**, 4342.

high coupling constants between the 10 and 10a hydrogen atoms are better explained by a β-carboxyl group at C-10. The case of anhydride formation is only explained satisfactorily by an axial (α) carboxyl group at C-1. An equatorial (β) carboxyl group could only form an anhydride with the C-10β carboxyl, the spatial proximity (Dreiding models) to which is not altered significantly by a ring A boat conformation, yet the anhydride (IX) requires extremely vigorous conditions for its formation. An axial carboxyl at C-1 can link readily with the 4a carboxyl in anhydride formation and more difficultly with the C-10 carboxyl when ring A becomes a boat. The possibility of epimerisation of the C-10 carboxyl group in (IX) at 280° is ruled out by the high coupling constant of the 10,10a-quartet (J = 12 c./sec.) which favours retention of the gibberellin stereochemistry at this centre.

Furthermore, when free to rotate, the carbonyl groups of the C-1 and C-4a substituents appear to deshield the C-10 hydrogen atom and to do so they must be axial.[7b] The proton signal moves upfield in compounds lacking the C-1 carboxyl group and again in the anhydride (VII) in which the deshielding cones of both carbonyl groups are held rigidly away. For the C-10 proton, τ values are: acetate of (III), 6·15; (XI), 6·10; dimethyl ester of (II), 6·50; (VII), >7·00.

Reduction of the ketone (IV) with sodium borohydride would be expected to take place from the less-hindered β-face of the molecule, yielding a 2α-alcohol. The latter was not isolated but cyclised immediately to the exocyclic isomer of the δ-lactone (X; R = Me),[12] which had n.m.r. signal at τ 8·64 () and 6·27 and 6·32 (methyl esters); the 10 and 10a doublets were centred at 6·97 and 7·65 (J = 13 c./sec.) and a broad three-proton signal (5·05 ⟶ 5·22) embraced the terminal methylene protons and the C-2 hydrogen atom. The hydroxyl group in gibberellin A_{13} must therefore be β and axial as in the other gibberellins.

The determination of the stereochemistry at 4b or 9 (terpene numbering) has always been difficult in diterpene chemistry. Since *syn*-backbones are now considered unlikely,[13] the 4b-hydrogen is almost certainly β. I am indebted to a colleague, J. R. Hanson, for suggesting a means of confirmation. Attempts were in progress at the time to label likely biogenetic precursors of gibberellic acid for feeding experiments. Owing to shortage of material, a synthesis of [17-^{14}C]kaur-16-en-19-oic acid (XX; R = CO_2H) ended at the alcohol (XX; R = CH_2·OH) stage.[14] The latter was added to a small fermentation of *G. fujikuroi*.

Radioautography of thin-layer plates run in two solvent systems showed that both gibberellic acid and gibberellin A_{13} were radioactive. The incorporation of (−)-kaur-16-en-19-ol of known stereochemistry into gibberellin A_{13} strongly suggested a 4bβ stereochemistry; there is also the implication that (XX; R = CH_2·OH) can act as a precursor of gibberellic acid. The results of this experiment are purely qualitative and are based on the assumption that the alcohol (XX; R = CH_2·OH) is specifically incorporated. This does not seem unreasonable since (−)-kaurene is specifically incorporated into gibberellic acid.[15]

Experimental

The following chromatographic materials were used: silica gel M.F.C. (Hopkin and Williams), activated charcoal (B.D.H.), Celite 545 (Johns-Manville), and alumina (Woelm neutral alumina, grade II).

[12] W. L. Meyer and A. S. Levinson, *J. Org. Chem.*, 1963, **28**, 2184.
[13] A. I. Scott, F. McCapra, F. Comer, S. A. Sutherland, P. W. Young, G. A. Sim, and G. Ferguson, *Tetrahedron*, 1964, **20**, 1339.
[14] R. H. B. Galt and J. R. Hanson, *Chem. and Ind.*, 1964, 837; cf. P. R. Jefferies and C. A. Henrick, *ibid.*, 1963, 1801.
[15] B. E. Cross, R. H. B. Galt, and J. R. Hanson, *J.*, 1964, 295.

M. p.s were determined on a Kofler hot-stage apparatus and are corrected; infrared and ultraviolet spectra were determined for Nujol mulls and ethanol solutions, respectively, and optical rotations for ethanol solutions.

Microhydrogenations were carried out in acetic acid with a palladium black catalyst. " Light petroleum " refers to the fraction of b. p. 60—80°. Ethyl acetate extracts were dried over anhydrous sodium sulphate.

Unless otherwise stated, n.m.r. spectra were measured on a Varian A 60 instrument in deuterochloroform with tetramethylsilane as internal reference.

Isolation of Gibberellin A_{13}.—(a) *Fermentation in presence of triparanol* (by Dr. B. E. Cross). Triparanol (17·5 g.) in ethanol (500 ml.) was added, after exhaustion of inorganic nitrogen (164 hr.), to a fermentation (ACC.917) of *Gibberella fujikuroi* grown on a glucose–ammonium nitrate medium. The mycelium was harvested after 402 hr. The culture filtrate (41·5 l.) gave crude gibberellic acid (23 g.), a crude acidic gum (29 g.), and a neutral gum (6 g.). The acidic gum was chromatographed on Celite–charcoal (2 : 1; 1500 g.; 78 × 7·5 cm.) by gradient elution with aqueous acetone.[4] Fractions 1—7 were of 2 l.; later fractions were of 1 l. Fraction 3 contained 5-hydroxymethyl-2-furoic acid and fraction 5 contained gibberellic acid. Fraction 6 was rechromatographed on silica gel and eluted with increasing concentrations of ethyl acetate in light petroleum, but very small fraction weights were obtained. Elution with ethyl acetate containing 2·5% methanol gave *gibberellin* A_{13} which crystallised from ethyl acetate–light petroleum in plates, m. p. 194—196° (decomp.), $[\alpha]_D^{17}$ −48° (*c* 0·25) (Found: C, 61·4, 61·8; H, 7·1, 7·0; O, 31·6. $C_{20}H_{26}O_7,C_4H_8O_2$ requires C, 61·8; H, 7·35; O, 30·9%), $\nu_{max.}$ 3500br, 1723—1700br, and 1660 cm.$^{-1}$.

(b) *Mutant B47 of* Gibberella fujikuroi. This mutant was grown on a maize meal–ammonium tartrate medium. Metabolites were extracted in the usual way [4] and separated into acids and neutrals with sodium hydrogen carbonate. Most of the gibberellic acid was removed from the acids by crystallisation and a portion (30—40 g.) of the mother-liquors was chromatographed on Celite–charcoal (2 : 1; 1500 g.), eluting with increasing concentrations of acetone in water. The fraction (2 l.) eluted with 42·5% acetone was crude gibberellic acid (5·5 g.). The next fraction, eluted with 48% acetone, contained gibberellin A_{13} (5 g.) which sometimes required further chromatography on Celite–silica gel (2 : 1) before crystallisation.

(c) *Gibberellic acid mother-liquors from a normal* 917 *fermentation* (by Dr. B. E. Cross). Gibberellic acid mother-liquors (32 g.) were chromatographed on Celite–charcoal as described under (a) except that all fractions were of 1 l. Fraction 7 contained gibberellic acid. Fractions 8 and 9 crystallised and gave gibberellin A_{13}. Fractions 10—13 (2·4 g.) were gums and were combined and rechromatographed on Celite–silica gel (2 : 1; 45 × 3·2 cm.). Elution with increasing concentrations of ethyl acetate in chloroform gave small amounts of gum until the ethyl acetate concentration reached 75%. At this point 1·0 g. of gum was eluted in two 200 ml. fractions.

Rechromatography of this gum on silica gel (100 g.; 26 × 2·6 cm.) and elution with increasing concentrations of ethyl acetate in light petroleum gave, with 85% ethyl acetate, a gum which slowly crystallised. Recrystallisation gave gibberellin A_{13} (260 mg.).

Methylation of Gibberellin A_{13}.—(*a*) *Gibberellin A_{13} trimethyl ester* (III), prepared with diazomethane, crystallised from acetone–light petroleum in needles, m. p. 117—119° (Found: C, 65·9; H, 7·8; OMe, 20·0. $C_{23}H_{32}O_7$ requires C, 65·7; H, 7·7; 3OMe, 22·1%), $\nu_{max.}$ 3400, 1743, 1724, 1716, 1656, and 877 cm.$^{-1}$.

(*b*) Another sample of gibberellin A_{13} (0·04 g.) on methylation gave a gum which was chromatographed on alumina. Elution with 20% ethyl acetate–light petroleum gave crystals (0·03 g.) which crystallised from acetone–light petroleum in rosettes, m. p. 121—122° (Found: C, 65·4; H, 7·6%), $\nu_{max.}$ 3521, 1739, 1715, 1662, and 824 cm.$^{-1}$. This ester is the endocyclic-double-bond isomer of (III) and was prepared from it by treatment in acetone solution with a few drops of concentrated sulphuric acid.

The *acetate* of (III), prepared in pyridine solution with excess of acetic anhydride, crystallised in prisms from aqueous methanol, m. p. 151—154° (Found: C, 64·9; H, 7·5; OMe, 18·4. $C_{25}H_{34}O_8$ requires C, 64·9; H, 7·4; 3OMe 20·1%), $\nu_{max.}$ 1740—1720br, 1737sh, 1730, 1720sh, 1662, and 880 cm.$^{-1}$.

The *dihydro-derivative* of (III), prepared in ethyl acetate solution using a 10% palladium–charcoal catalyst, crystallised from acetone–light petroleum, m. p. 166—168° (Found: C, 65·6, H, 8·1. $C_{23}H_{34}O_7$ requires C, 65·4; H, 8·1%). $\nu_{max.}$ 3435, 1713, and 1697 cm.$^{-1}$.

Treatment of Gibberellin A_{13} with Acetic Anhydride in Pyridine.—Gibberellin A_{13} (0·08 g.) in pyridine (2 ml.) was treated with acetic anhydride (0·5 ml.) at room temperature for 2 days. The mixture was poured into dilute hydrochloric acid and the product was recovered in ethyl acetate and chromatographed on silica gel (ethyl acetate–light petroleum). The fractions eluted with 10 and 15% ethyl acetate were solid and were recrystallised from acetone–light petroleum, giving 2β-*acetoxy*-1β-*methyl*-8-*methylenegibbane*-1α,4aα,10β-*tricarboxylic acid* 1 ⟶ 4*a*-*anhydride* (VII) (0·048 g.), m. p. 264—267° (Found: C, 65·8; H, 6·7. $C_{22}H_{26}O_7$ requires C, 65·7; H, 6·5%), $\nu_{max.}$ 3230 (OH of carboxyl), 1804, 1766, 1752, 1729, 1681, 1660, and 897 cm.$^{-1}$.

Pyrolyses of Gibberellin A_{13}.—(*a*) Gibberellin A_{13} (0·028 g.) was heated between 205° and 220° for 0·5 hr. under nitrogen. Recovery gave a solid which was chromatographed on silica gel (ethyl acetate–light petroleum). Elution with 30 and 40% of ethyl acetate gave 2β-*hydroxy*-1β,8-*dimethylgibb*-8(9)-*ene*-1α,4aα,10β-*tricarboxylic acid* 1 ⟶ 4*a*-*anhydride* (VIII) which crystallised in needles (0·017 g.) from acetone–light petroleum, m. p. 218—225° (Found: C, 66·5; H, 6·6. $C_{20}H_{24}O_6$ requires C, 66·65; H, 6·7%). The methyl ester, prepared with diazomethane, was chromatographed on alumina (ethyl acetate–light petroleum). Elution with 25% ethyl acetate gave the methyl ester, which crystallised from acetone–light petroleum in prisms, m. p. 133—135°, $\nu_{max.}$ 3542, 1802, 1764, 1729, and 812 cm.$^{-1}$.

(*b*) Gibberellin A_{13} (1·94 g.) was heated at 260—280° for 2 hr. under nitrogen and the product was chromatographed on silica gel (ethyl acetate–light petroleum). Elution with 40% ethyl acetate gave first the *anhydride* (IX) (0·55 g.) which crystallised from acetone–light petroleum, m. p. 262—265° (Found: C, 70·0; H, 6·5. $C_{20}H_{22}O_5$ requires C, 70·2; H, 6·5%), $\nu_{max.}$ 1800, 1757, and 1739 cm.$^{-1}$. Further elution with 40% ethyl acetate gave the *di-acid* (X; R = H) (0·575 g.), which crystallised from acetone–light petroleum, m. p. 280—283° (Found: C, 66·2; H, 6·7. $C_{20}H_{24}O_6$ requires C, 66·65; H, 6·7%), $\nu_{max.}$ 1741 and 1703 cm.$^{-1}$. The *dimethyl ester* (X; R = Me) crystallised in needles from light petroleum, m. p. 164—168° (Found: C, 68·4; H, 7·2. $C_{22}H_{28}O_6$ requires C, 68·0; H, 7·3%), $\nu_{max.}$ 1740—1727br cm.$^{-1}$.

Preparation of the Ketone (II).—Gibberellin A_{13} (0·15 g.) in acetone (15 ml.) was treated with the Jones (chromium trioxide) reagent (0·4 ml.) at room temperature for 0·5 hr. The excess reagent was destroyed with methanol, water was added, and the solution was extracted with ethyl acetate and separated into acid and neutral fractions. The acid fraction (0·14 g.) was heated with a little water on the steam-bath for 1 hr. under nitrogen. Carbon dioxide was evolved (baryta trap) and the product was recovered in ethyl acetate and chromatographed on silica gel (ethyl acetate–light petroleum). Elution with 40% ethyl acetate gave 1-*methyl*-8-*methylene*-2-*oxogibbane*-4aα,10β-*dicarboxylic acid* (II) which crystallised from acetone–light petroleum in prisms (0·068 g.), m. p. 252—257° (decomp.) (Found: C, 68·7; H, 7·3. $C_{19}H_{24}O_5$ requires C, 68·65; H, 7·3%), $\nu_{max.}$ 1710, 1700—1680br, and 877 cm.$^{-1}$.

The *dimethyl ester* crystallised from light petroleum (b. p. 40—60°) in feathery needles, m. p. 114—115° (Found: C, 69·5; H, 7·8. $C_{21}H_{28}O_5$ requires C, 70·0; H, 7·8%), $\nu_{max.}$ 1726, 1713sh, 1710, 1660, and 887 cm.$^{-1}$.

The *dihydro-derivative* of (II), prepared similarly from dihydro-gibberellin A_{13}, crystallised from ethyl acetate–light petroleum in rosettes of needles, m. p. 244—247° (Found: C, 67·9; H, 7·9. $C_{19}H_{26}O_5$ requires C, 68·2; H, 7·8%).

Ozonolysis of Gibberellin A_{13}.—A stream of ozonised oxygen (15 mg. O_3 per min.) was passed into a solution of gibberellin A_{13} (0·2 g.) in ethyl acetate (100 ml.) at room temperature for 4 min. The solution was shaken vigorously with an equal volume of water and the aqueous layer run into a saturated aqueous solution of dimedone. After 24 hr. formaldehyde dimethone (0·067 g., 43%) was collected. No crystalline product was isolated from the organic fraction.

Ozonolysis of Gibberellin A_{13} Trimethyl Ester.—A solution of (III) (0·105 g.) in ethyl acetate (20 ml.) was treated similarly for 2 min. The product was separated into acids (trace) and neutrals (0·1 g.) with sodium hydrogen carbonate solution, and the neutral fraction was chromatographed on alumina (ethyl acetate–light petroleum). Elution with 30% of ethyl acetate gave *methyl* 1β-*methyl*-2,8-*dioxogibbane*-1α,4aα,10β-*tricarboxylate* (V) which crystallised in plates (0·003 g.) from acetone–light petroleum, m. p. 181—184° (Found: C, 63·2; H, 6·8. $C_{22}H_{28}O_8$ requires C, 62·8; H, 6·7%), $\nu_{max.}$ 1737 and 1719 cm.$^{-1}$. The fraction eluted with 40% of ethyl acetate contained *methyl* 2β-*hydroxy*-1β-*methyl*-8-*oxogibbane*-1α,4aα,10β-*tricarboxylate* (VI) which crystallised from acetone–light petroleum in rhombs (0·047 g.), m. p. 186—189° (Found: C, 62·9; H, 7·2. $C_{22}H_{30}O_8$ requires C, 62·5; H, 7·2%), $\nu_{max.}$ 3518, 1735, 1720, and

1709 cm.$^{-1}$. The nor-ketone (VI) was readily oxidised to the diketone (V) with the Jones reagent.

Oxidation of Gibberellin A_{13} Trimethyl Ester.—The ester (III) (0·2 g.) in acetone (20 ml.) was treated with the Jones reagent (0·5 ml.) for 1 hr. at room temperature. The excess of reagent was destroyed with methanol, water was added, and the solution extracted with ethyl acetate. The neutral fraction (0·19 g.) was chromatographed on alumina (ethyl acetate–light petroleum). Elution with 20% of ethyl acetate gave *methyl* 1β-*methyl*-8-*methylene*-2-*oxogibbane*-1α,4aα,10β-*tricarboxylate* (IV) which crystallised in rhombs (0·129 g.) from acetone–light petroleum, m. p. 138—140° (Found: C, 66·5; H, 7·4. $C_{23}H_{30}O_7$ requires C, 66·0; H, 7·2%), $\nu_{max.}$ 1728—1720br, 1715sh, 1708sh, and 879 cm.$^{-1}$. The fraction eluted with 40% of ethyl acetate yielded the diketone (V) (0·018 g.).

Preparation of the Δ^2-Olefin (XI).—Toluene-*p*-sulphonyl chloride (1 g.) was added to gibberellin A_{13} trimethyl ester (0·8 g.) in pyridine (6 ml.), and the solution left at room temperature for 92 hr. The mixture was poured into dilute hydrochloric acid and the product was recovered in ethyl acetate and chromatographed on silica gel (ethyl acetate–light petroleum). Elution with 20% of ethyl acetate gave the gummy toluene-*p*-sulphonate (0·664 g.). Starting material (0·132 g.) was recovered from the 30% ethyl acetate fraction. The toluene-*p*-sulphonate was dissolved in collidine (10 ml.) and the solution refluxed for 6 hr. The work-up was as in the isolation of the toluene-*p*-sulphonate and the Δ^2-*olefin* (XI), eluted with 15% of ethyl acetate, crystallised from light-petroleum (b. p. 40—60°) in prisms (0·268 g.), m. p. 133—134·5° (Found: C, 68·8; H, 7·5. $C_{23}H_{20}O_6$ requires C, 68·6; H, 7·5%). Elution with 25% ethyl acetate gave the exocyclic-double-bond isomer of (X; R = Me) (0·103 g.), m. p. 167—169°.

Attempts to Aromatise Ring A *of Gibberellin A_{13}.*—(*a*) The diketone (II) (0·81 g.) in tetrahydrofuran (10 ml.) was treated with phenyltrimethylammonium perbromide (1·18 g.) at room temperature for 1·5 hr. The mixture was poured into water and the gummy product was recovered in ethyl acetate. It was dissolved in dimethylformamide (10 ml.) and treated with lithium chloride (0·7 g.) at room temperature for 40 hr. The mixture was poured into dilute hydrochloric acid and the product, which was recovered in ethyl acetate, was chromatographed on silica gel (ethyl acetate–light petroleum). Elution with 40% of ethyl acetate gave the γ-lactone (XIV; R = H) which crystallised in needles (0·679 g.) from acetone–light petroleum, m. p. 112—115°. The n.m.r. spectrum showed it to consist mainly of the endocyclic-double-bond isomer with a trace of terminal methylene absorption. Without further purification, a portion (0·4 g.) was methylated with diazomethane and the product was chromatographed on alumina (ethyl acetate–light petroleum). Elution with 20% of ethyl acetate gave the *epoxide* (XV) which crystallised in plates (0·117 g.) from acetone–light petroleum, m. p. 180—182° (Found: C, 70·3; H, 7·4. $C_{21}H_{26}O_5$ requires C, 70·4; H, 7·3%), $\nu_{max.}$ 1772 and 1725 cm.$^{-1}$. The fractions eluted with 40% of ethyl acetate contained *methyl* 4aα-*carboxy*-3α-*hydroxy*-1,8-*dimethyl*-2-*oxogibb*-8(9)-*ene*-10β-*carboxylate* 4a ⟶ 3-*lactone* (XIV; R = Me) which crystallised from acetone–light petroleum in needles (0·199 g.), m. p. 167—171° (Found: C, 69·9; H, 7·1. $C_{20}H_{24}O_5$ requires C, 69·75; H, 7·0%), $\nu_{max.}$ 1773 and 1730s cm.$^{-1}$.

(*b*) Compound (XII) behaved similarly and *methyl* 4aα-*carboxy*-3α-*hydroxy*-1,8-*dimethyl*-1-*oxogibbane*-10β-*carboxylate* 4a ⟶ 3-*lactone* crystallised from acetone–light petroleum in needles, m. p. 224—226° (Found: C, 68·65; H, 7·4. $C_{19}H_{24}O_5$ requires C, 68·65; H, 7·3%), $\nu_{max.}$ 1772, 1739, and 1695 cm.$^{-1}$. Treatment with diazomethane followed by chromatography on alumina once again produced two compounds. The dihydro-derivative of (XV) crystallised from acetone–light petroleum in needles, m. p. 196—202° (Found: C, 70·4; H, 7·9. $C_{21}H_{28}O_5$ requires C, 70·0; H, 7·8%), $\nu_{max.}$ 1772 and 1728 cm.$^{-1}$. The dihydro derivative of (XIV; R = Me) crystallised from the same solvents, m. p. 145—150° (Found: C, 69·6; H, 7·8. $C_{20}H_{26}O_5$ requires C, 69·3; H, 7·6%), $\nu_{max.}$ 1772 and 1732s cm.$^{-1}$; the latter was converted into the former by treatment with diazomethane overnight.

Selenium Dehydrogenation of Gibberellin A_9 Nor-ketone (XVIII).—Gibberellin A_9 (0·5 g.) in glacial acetic acid (30 ml.) was treated with excess ozone. Water was added and the mixture shaken for 1 hr. Recovery in ethyl acetate produced a gum (0·471 g.) which, without purification, was heated with selenium (0·5 g.) under nitrogen (metal block temperature ~330—340°). After 1 hr., the mixture was cooled and extracted with ether. The products were chromatographed on alumina. Elution with light petroleum gave a fluorescent gum. The fractions eluted with light petroleum–ether (5 : 1) contained the *ketone* (XVII) (0·15 g.) which was crystallised from aqueous methanol, light petroleum (b. p. 40—60°), and sublimed, m. p. 136—139° (Found: C, 84·9; H, 8·0. $C_{16}H_{18}O$ requires C, 84·9; H, 8·0%), $\lambda_{max.}$ 262sh, 267, and 274

mμ (log ε 2·68, 2·72, and 2·64), $\nu_{max.}$ 1741 and 1600 cm.$^{-1}$, τ 3·0 (Ar–H, 3), 6·8—7·3 (Ar–CH, 3), 7·4—7·7 (CO–C*H*, 3), 7·75 (Ar–CH_3, 3), and 7·9—8·4 (–CH_2–, 6).

Selenium Dehydrogenation of Gibberellin A_{13} Nor-ketone.—Gibberellin A_{13} (0·5 g.) was ozonised as in the previous experiment. Extraction with ethyl acetate gave a gum (0·073 g.). The aqueous fraction was evaporated to dryness, yielding a glass (0·42 g.) which was combined with the gum and treated with selenium (0·5 g.) as above. The product was chromatographed on alumina. Elution with light petroleum gave a fluorescent gum (0·01 g.) which, after distillation on to a cold finger, had $\lambda_{max.}$ 260, 265, 290, and 300 mμ (log ε 3·97, 3·98, 3·15, and 3·13). It was probably impure 1-methylfluorene. Elution with light petroleum–ether (5 : 1) gave crystals (0·021 g.) which were crystallised from aqueous methanol, sublimed, and further recrystallised, m. p. 126—132°. The spectrum was virtually identical with that of the ketone (XVII) and both compounds behaved identically on thin-layer chromatography (R_F 0·67 on silica with 5% glacial acetic acid in di-isopropyl ether).

Sodium Borohydride Reduction of (IV).—The ketone (IV) (0·09 g.) in methanol (8 ml.) was treated with excess of sodium borohydride overnight at room temperature. Water was added and the product was recovered in ethyl acetate and chromatographed on alumina (ethyl acetate–light petroleum). Elution with 25% ethyl acetate gave gummy crystals (0·033 g.) which crystallised from light petroleum in needles of the exocyclic-double-bond isomer of the δ-lactone (X; R = Me).

(–)-[17-^{14}C]*Kaur*-16-*en*-19-*ol* (XX; R = CH_2·OH).—(–)-Kaur-16-en-19-oic acid [14] was methylated with diazomethane, and the ester (0·148 g.) treated with excess of ozone as in the previous ozonolysis. The product was chromatographed on alumina (ethyl acetate–light petroleum). The fractions eluted with 10% ethyl acetate contained *methyl* 16-*oxo*-17-*nor*-*kauran*-19-*oate* which was best purified by sublimation, m. p. 142—144° (Found: C, 75·7; H, 9·6. $C_{20}H_{30}O_3$ requires C, 75·4; H, 9·5%).

[^{14}C]Triphenylmethylphosphonium iodide (0·87 g., 2 mol.), prepared from [^{14}C]methyl iodide and triphenylphosphine, was suspended in dry ether under nitrogen, and butyl-lithium in pentane (15% w/v) was added to the stirred suspension until the solution was almost homogeneous and the characteristic yellow-orange colour appeared. Methyl 16-oxo-17-norkauran-19-oate (0·34 g.) in ether (5 ml.) was added rapidly and the mixture was stirred for 5 min. then refluxed for 3 hr. Water was added and the product, recovered in ether, was chromatographed on alumina (ethyl acetate–light petroleum). Elution with light petroleum gave crude methyl [17-^{14}C]kaur-16-en-19-oate (0·083 g.) which, without purification, was dissolved in ether (10 ml.) and refluxed with excess of lithium aluminium hydride for 3 hr. The excess of reagent was destroyed with ethyl acetate and water. The organic fraction yielded gummy crystals which were chromatographed on alumina (ethyl acetate–light petroleum). Elution with 20% of ethyl acetate gave (–)-[^{14}C]kaur-16-en-19-ol which crystallised in needles (0·02 g.; 2·31 μc) (Found: r.m.a. $\times 10^{-4}$, 472) from acetone–light petroleum, m. p. 141—143° (Found: C, 83·1; H, 11·3. $C_{20}H_{32}O$ requires C, 83·8; H, 11·2%). The alcohol, dissolved in ethanol (10 ml.), was added to a stirred aerated fermentation (350 ml.) of *Gibberella fujikuroi* as soon as the inorganic nitrogen was exhausted. The fermentation was run for a further 72 hr. but did not grow as copiously as usual. The metabolites were isolated in the usual way [4] and the acid fraction (0·023 g.) was chromatographed on silica gel (ethyl acetate–light petroleum). Elution with 60% ethyl acetate gave a fraction (0·004 g.) which on methylation and thin-layer chromatography in two systems (silica, 5 and 15% glacial acetic acid–di-isopropyl ether) was shown to contain mainly gibberellic acid and gibberellin A_{13}. Exposure of the thin-layer plates to *X*-ray film (cf. ref. 14) revealed that the spots corresponding to these two compounds were radioactive.

Optical Rotary Dispersion Curves.—Values are for [*M*], in methanol, for the ketones. (VI); positive Cotton effect (400 mμ) +120°; (323, peak) +3000°; (306) 0°; (282, trough) –5160°; (231) –4500°. Dimethyl ester of (II); negative Cotton effect (400 mμ) –290°; (310, trough) –2490°; (290) 0°; (264·5, peak) +3060°; (250) +2580°. (IV); negative Cotton effect (400 mμ) –615°; (310, trough) –2800°; (266, peak) –265°; (241) –2640°.

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