299. *New Metabolites of* Gibberella fujikuroi. *Part VIII.*[1] *Gibberellin* A_{12}

By B. E. Cross and K. Norton

Gibberellin A_{12} has been isolated from culture filtrates of the fungus *Gibberella fujikuroi* and shown to have structure (V; R = H).

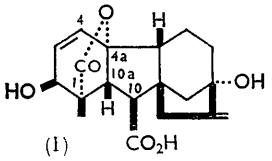
In continuation of work aimed [2-4] at discovering new metabolites of *Gibberella fujikuroi* which might be of use in elucidating the biosynthetic pathway to the gibberellins, several new C_{20} acids have been isolated.[4] Tentative structures for four of these compounds and brief speculations as to their role in the biosynthesis of gibberellic acid (I) have been advanced.[4] The gibberellins A_1 to A_9 [5,6] and gibberellins A_{10} and A_{11} [4,7] are C_{19} compounds which possess the 1 ⟶ 4a-lactone ring in common. Although the new C_{20} acids differ from the older gibberellins in carbon content and also lack the γ-lactone ring, they are known (see below and ref. 8), or are believed,[4] to possess the gibbane [9] skeleton (II) and they show gibberellin-like biological activity.[4] Hence, they should be regarded as gibberellins (cf. ref. 10). The present Paper describes our work on one of these acids which has been named gibberellin A_{12}.

The gibberellins A_7 and A_9 were isolated [5] from fermentations of *G. fujikuroi* in which the broth was adjusted to pH ~7 after the inorganic nitrogen in the medium had been utilised. Careful chromatography of the neutral (insoluble in aqueous sodium hydrogen carbonate) fractions (cf. ref. 2) from such fermentations afforded not only the known metabolites [2] 13-epi-(−)-manoyl oxide, (−)-kaurene, (−)-kauranol, fujenal, 7-hydroxykaurenolide, and 7,18-dihydroxykaurenolide, but also gibberellin A_{12} in very low yield (~0·3 mg./l. of culture filtrate).

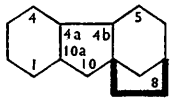
Gibberellin A_{12} is a dibasic acid, $C_{20}H_{28}O_4$. It was shown from its infrared spectrum to

[1] Part VII, Galt and Hanson, preceding Paper.
[2] Cross, Galt, Hanson, Curtis, Grove, and Morrison, *J.*, 1963, 2937.
[3] Cross, Galt, and Hanson, *J.*, 1964, 295.
[4] Cross, Galt, and Hanson, "Régulateurs Naturels de la Croissance Végétale," Centre National de la Recherche Scientifique, Paris, 1964, p. 265.
[5] Grove, *Quart. Rev.*, 1961, **16**, 56.
[6] Cross, Galt, and Hanson, *Tetrahedron*, 1962, **18**, 451.
[7] Cross and Hanson, unpublished work.
[8] Galt, unpublished work.
[9] Grove and Mulholland, *J.*, 1960, 3007.
[10] Phinney and West, *Ann. Rev. Plant. Physiol.*, 1960, **11**, 411.

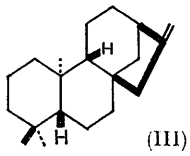
contain a terminal methylene group (bands at 3078, 1658, and 882 cm.$^{-1}$) whilst its nuclear magnetic resonance (n.m.r.) spectrum revealed 3-proton singlets at τ 9·21 and 8·87 which were assigned to two tertiary methyl groups. Thus, gibberellin A_{12} has five extranuclear carbon atoms, so that structures [2,11] based on the kaurene nucleus (III) are excluded.



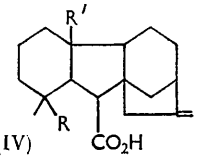
(I)



(II)



(III)



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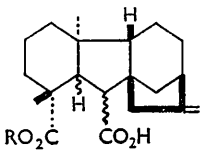
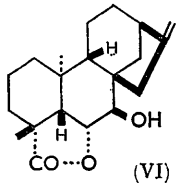


(V)



(VI)

However, in view of the co-occurrence of gibberellin A_{12} with C_{19} gibberellins, *e.g.*, gibberellic acid (I), the gibbane skeleton (II) becomes a possibility if, unlike the C_{19} compounds, it carries a carbon substituent at position 4a. The most likely structures are then (IV; R = Me, R′ = CO_2H) and (IV; R = CO_2H, R′ = Me).

The structure of gibberellin A_{12} was established by relating it to that of the half-ester (V; R = Me) prepared from 7-hydroxykaurenolide (VI) by the method of Galt and Hanson.[1] Methylation of gibberellin A_{12} and of the half-ester (V; R = Me), with diazomethane, gave in each case intractable gums. However, the infrared and n.m.r. spectra of the two gums were virtually identical. Finally, reductive hydrolysis of the half-ester (V; R = Me) with lithium in ammonia [12] gave gibberellin A_{12} in good yield. This partial synthesis of gibberellin A_{12} not only established its structure but also determined the absolute configuration at all centres except 10 and 10a. Thus, gibberellin A_{12} is 1α,10ξ-dicarboxy-1β,4aα-dimethyl-8-methylenegibbane (V; R = H).

If the carboxyl group at C-10 of gibberellin A_{12} has the β-configuration, gibberellin A_{12} may be an intermediate in the biosynthesis of gibberellic acid. This point is under investigation since a positive result would help to determine the reaction sequence by which *G. fujikuroi* transforms (−)-kaurene into gibberellic acid.[3,4]

Experimental

Chromatographic materials and general experimental methods were as described in Part II.[2] Nuclear magnetic resonance spectra were measured on a Varian Associates A.60 spectrometer (60 Mc.) and had tetramethylsilane as internal standard with τ = 10·00. The identification of mould metabolites was confirmed by comparison of infrared spectra.

Fermentation and Isolation of Neutral and Acidic Fractions.—Gibberella fujikuroi (Saw.) Wr. strain ACC. 917 [13] was grown under the conditions described [6] for the production of gibberellins A_7 and A_9, except that an 80 l. fermenter was used. The neutral and acidic fractions were isolated as previously described.[2]

Isolation of Gibberellin A_{12}.—The combined neutral fractions (42 g.) from 4 fermentations (250 l. of culture filtrate) were chromatographed on Celite–silica gel (2 : 1; 1500 g.; 90 × 6·6 cm.) (cf. ref. 2). Elution of the column with increasing concentrations of ethyl acetate in light petroleum gave the following results (% of ethyl acetate and volume of fractions in parentheses): Fractions 1 and 2 (5%; 1 l.) were rejected. Fraction 3 (10%; 1 l.) was an oil (3·2 g.) which, after rechromatography on alumina (30 × 3 cm.) in light petroleum (b. p. 40—60°), afforded (−)-kaurene. Fraction 4 (10%; 1 l.) was an oil (1·1 g.) which, after rechromatography on

[11] Cross, Galt, and Hanson, *J.*, 1963, 2944, 3783, 5052.
[12] Wenkert and Jackson, *J. Amer. Chem. Soc.*, 1958, **80**, 217.
[13] Borrow, Jefferys, Kessell, Lloyd, Lloyd, and Nixon, *Canad. J. Microbiol.*, 1961, **7**, 227.

silica gel (24 × 2 cm.) in ethyl acetate–light petroleum, gave (−)-kaurene and 13-epi-(−)-manoyl oxide. Fraction 5 (10%; 1 l.), solid (0·7 g.), gave (−)-kauranol. Fractions 6—8 (10%; 2 l.), crystals (4·6 g.), yielded fujenal. Fractions 9—11 (10%; 2 l.), crystals (3·6 g.), gave 7-hydroxykaurenolide. Fractions 12—14 (15%; 2 l.) and 15—19 (20%; 2 l.) were red gums (total 6·3 g.) which are being investigated. Trituration of fractions 20 and 21 (22·5%; 2 l.) with ether gave prisms, m. p. 188—196°, which were chromatographed on silica gel (8·0 × 1·3 cm.). Elution with ethyl acetate–light petroleum (1 : 3) and crystallisation from ethanol–light petroleum gave *gibberellin* A_{12} as rosettes of rods, m. p. 245—248°, pK^*_{MCS} 8·44 (7·63 and 9·19; not quite resolved) [Found: C, 72·5; H, 8·45%; Equiv. (potentiometric), 148. $C_{20}H_{28}O_4$ requires C, 72·3; H, 8·5%; Equiv. (dibasic), 166], $\nu_{max.}$ 3400—2460 (OH of CO_2H), 1692br (C=O or CO_2H), 3078, 1658, and 882 cm.$^{-1}$ (C=CH_2), τ (in dioxan) 9·21 and 8·87 (>C·CH_3), 7·92 (−CH_2−C=C), and 5·94 (C=CH_2). Fraction 22 (22·5%; 2 l.) was a solid (0·5 g.). Fractions 23—25 (27·5%; 2 l.), solid (4·6 g.), yielded 7,18-dihydroxykaurenolide. The remaining fractions were intractable.

The methyl ester of gibberellin A_{12}, prepared with diazomethane in ether–methanol at 0°, was an intractable gum. Its n.m.r. spectrum (in CCl_4) showed singlets at τ 9·36 and 8·97 (2>C·CH_3), doublets centred at 8·18 and 6·78 (J = 12 c./sec.) (10,10a protons), a singlet at 6·37 (2 O·CH_3), and a broad peak at 5·2 (C=CH_2).

The n.m.r. and infrared spectra ($\nu_{max.}$ 1735, 1660, and 880 cm.$^{-1}$) of the ester were almost identical with those of the gum obtained by methylating the half-ester (V; R = Me).

Methyl 10ξ-*Carboxy*-1β,4a*α*-*dimethyl*-8-*methylenegibbane*-1*α*-*carboxylate* (V; R = Me).—The half-ester (V; R = Me) was prepared by the method of Galt and Hanson.[1] Methylation with diazomethane in ether–methanol at 0° gave a gum.

Reductive Hydrolysis of the Half-ester (V; R = Me).—The ester (40 mg.) in tetrahydrofuran (20 ml.) was added to liquid ammonia (75 ml.). Lithium was then added until a persistent blue colour was obtained, and the solution was allowed to evaporate at room temperature. The residue was acidified with 10% hydrochloric acid, and the product was recovered in ethyl acetate and chromatographed on silica gel (14 × 0·9 cm.). Elution with 22·5% of ethyl acetate in light petroleum gave crystals (27 mg,) which were recrystallised from ethanol–light petroleum, giving needles (20 mg.), m. p. 244—247°, identical (infrared spectrum) with gibberellin A_{12}. On thin-layer chromatography on Silica Gel G (Merck) in di-isopropyl ether–acetic acid (98 : 2) gibberellin A_{12} had R_F 0·79.

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