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NEW METABOLITES OF *GIBBERELLA FUJIKUROI*—X

GIBBERELLIN A_{10}[1]

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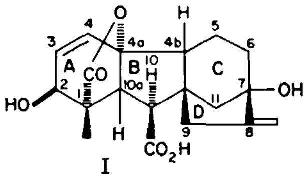
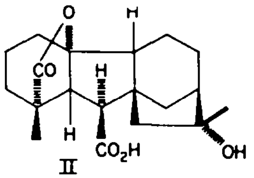
Abstract—Gibberellin A_{10} has been isolated from the culture filtrate of *Gibberella fujikuroi* and shown to have the structure II.

Gibberellic acid (I) is the best known[2] of the diterpenoid metabolites of the fungus *Gibberella fujikuroi*. Interest in its biosynthesis[3] has involved a careful examination of the culture filtrate and this has led to the isolation of gibberellins A_7,[4] A_9,[4] A_{12}[5] and A_{13}[1] In this paper we wish to present evidence, briefly reported elsewhere,[6] for the structure and stereochemistry of gibberellin A_{10}.

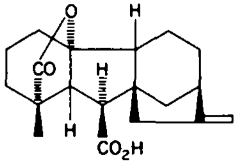
Gibberellin A_{10} (II), m.p. 245–246°, $[\alpha]_D$ +3°, was isolated as a minor metabolite by repeated chromatography of the weakly acidic fraction from a large scale *Gibberella fujikuroi* ACC 917 fermentation. It titrated as a monobasic acid whilst with diazomethane it gave a methyl ester, m.p. 157–168°. Analytical data of the acid and its ester indicated that Gibberellin A_{10} had the formula $C_{19}H_{28}O_5$. The IR spectra of the acid, [ν_{max} 3360 (OH), 2605 (OH of CO_2H), 1766 (γ-lactone), 1680 (CO_2H) cm^{-1}] and the ester [ν_{max} 3520 (OH), 1757 (γ-lactone), 1729 (ester) cm^{-1}] enabled the oxygen



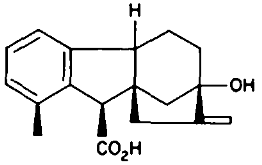
I



II



III



IV

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[1] Previous part. R. H. B. Galt, *J. Chem. Soc.* 3143 (1965).

[2] For review see J. F. Grove, *Quart. Rev.* **15**, 56 (1961).

[3] B. E. Cross, R. H. B. Galt and J. R. Hanson, *J. Chem. Soc.* 295 (1964).

[4] B. E. Cross, R. H. B. Galt and J. R. Hanson, *Tetrahedron* **18**, 451 (1962).

[5] B. E. Cross and K. Norton, *J. Chem. Soc.* 1570 (1965).

[6] B. E. Cross, R. H. B. Galt and J. R. Hanson, *Regulateurs Naturels de la Croissance Vegetale* p. 265. Centre National de la Recherche Scientifique, Paris (1964).

atoms to be accounted for in hydroxyl, γ-lactone and carboxyl groups. Furthermore the UV and IR spectra showed no olefinic absorption whilst the ester was inert to microhydrogenation. Gibberellin A_{10} was thus tetracarbocyclic whilst its source and gibberellin-like biological activity in the cucumber hypocotyl test[7] indicated that it had the gibbane skeleton. The structure II followed from the NMR spectrum of the methyl ester which showed, *inter alia*, two tertiary $-\overset{|}{\underset{|}{C}}-CH_3$ at τ 8·9 and 8·62, the characteristic[8] gibbane 10 : 10a quartet at τ 7·53 and 7·23; (J = 15 c/s), and a methoxyl at τ 6·22. Compared to gibberellin A_9 (3) this revealed loss of the olefinic resonances at τ 5·15 and 5·05 and the appearance of a new $-\overset{|}{\underset{|}{C}}-CH_3$ at 8·62 deshielded by an adjacent oxygen substituent.

This relationship was confirmed experimentally by treatment of gibberellin A_9[4] with dilute hydrochloric acid at room temperature for 68 hr which gave gibberellin A_{10}. It has been shown[9] with (−)-kaurene that hydration of the C-16 terminal methylene which possesses the same stereochemical environment as C-8 of the gibberellins, leads stereospecifically to (−)-kauran-16α-ol (also a metabolite[10] of *Gibberella fujikuroi*). We, therefore, propose the structure and stereochemistry (II) for gibberellin A_{10} in which gibberellin A_{10} is related to gibberellin A_9 in the same way as gibberellin A_2 is related to gibberellin A_4. It has been shown[6] that [^{14}C] gibberellin A_9 is converted *inter alia* into gibberellin A_{10} by *Gibberella fujikuroi*.

During the course of this work 4b-epiallogibberic acid (IV)[10] was isolated from the fermentation. Dehydroallogibberic acid has previously been isolated[11] from the culture filtrate. This possibly microbiological degradation, is in contrast to the acid-catalysed degradation of gibberellic acid which leads to mainly allogibberic acid.[2]

EXPERIMENTAL

M.ps were determined on a Kofler hot-stage apparatus and are corrected; IR spectra were measured on a Perkin–Elmer 221; NMR spectra were measured on a Varian A.60 instrument in $CDCl_3$ with tetramethylsilane as internal reference.

Light petroleum refers to the fraction of b.p. 60–80°. Silica gel was B.D.H. Chromatographic grade and the celite was Celite 545 (Johns Mandeville).

Isolation of gibberellin A_{10}. The crude "neutral" fraction from a large-scale *Gibberella fujikuroi* ACC 917 fermentation was carefully extracted with $NaHCO_3$aq. This extract was acidified and the organic material recovered in ethyl acetate. These "weak acids" were then chromatographed on silica : celite (1 : 2). Elution with 30% ethyl acetate—$CHCl_3$ gave a gummy fraction (7·9 g), a portion (2·1 g) of which was rechromatographed on silica gel (21 × 1·2 cm). Elution with 30% ethyl acetate–light petroleum gave 4b-epiallogibberic acid (IV; 195 mg) m.p. 242–245° (lit.[10] m.p. 244°) identified by comparison with an authentic specimen. Elution with 50% ethyl acetate–light petroleum gave *gibberellin* A_{10} (II; 156 mg) which crystallized from acetone–light petroleum as felted needles, m.p. 245–246°, $[\alpha]_D$ +3°. (Found: C, 68·2; H, 7·9. $C_{19}H_{26}O_5$ requires: C, 68·2; H, 7·8%), ν_{max} (nujol) 3360, 2605, 1766, 1680 cm^{-1}.

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[7] P. W. Brian and H. G. Hemming, *Nature, Lond.* **189**, 74 (1961).

[8] [a] N. Sheppard, *J. Chem. Soc.* 3040 (1960);
[b] J. R. Hanson, *Ibid.* in press.

[9] [a] J. R. Hanson, *J. Chem. Soc.* 5061 (1963);
[b] J. R. Hanson, J. MacMillan and M. Barnes, unpublished work.

[10] J. F. Grove and T. P. V. Mulholland, *J. Chem. Soc.* 3007 (1960).

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

Methylation of Gibberellin A_{10}. *Gibberellin* A_{10} *methyl ester*, prepared with diazomethane, crystallized from acetone–light petroleum in needles, m.p. 167–168°. (Found: C, 69·2; H, 8·15. $C_{20}H_{28}O_5$ requires: C, 68·9; H, 8·1%), ν_{max} 3545, 1757, 1729 cm^{-1}, τ 8·9, 8·62, 7·53, 7·23 (J = 15 c/s) 6·22.

Action of hydrochroric acid on gibberellin A_9. Gibberellin A_9 (III; 50 mg) in MeOH (0·5 ml (was treated with dil. HCl (1·0 ml) at room temp for 68 hr. The solution waas diluted with water and extracted with ethyl acetate, the extract washed with water dried over Na_2SO_4 and evaporated to give II (46 mg) which crystallized from acetone–light petroleum as needles, m.p. 244–246° identical to the material isolated from *Gibberella fujikuroi.*

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