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NEW METABOLITES OF *GIBBERELLA FUJIKUROI*—XII

GIBBERELLIN A_{15}[1]

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Abstract—The spectroscopic and chemical properties of gibberellin A_{15}, a metabolite of *Gibberella fujikuroi*, are consistent with the structure VII.

THE investigation of the biosyntheses of gibberellic acid (I) has involved as one of its facets, an exhaustive study[2] of the metabolites of the fungus, *Gibberella fujikuroi*. The diterpenoid metabolites, with the exception of olearyl oxide, fall into three skeletal classes, the gibberellins, the kauranoid metabolites, and the tricyclic fujenal derivatives. The participation of certain of these compounds in the biosynthesis of gibberellic acid from (−)-kaurene has been demonstrated.[3,4] The C-20 gibberellins, gibberellins A_{12} (II),[5] A_{13} (III)[6] and A_{14} (IV)[1] have been isolated from *Gibberella fujikuroi* whilst recently the isolation of two further compounds of this type, V[7] and VI,[8] has been recorded from higher plants. In a preliminary communication we proposed[9] (without detailed evidence) the structure VII for another C-20 gibberellin now named gibberellin A_{15}. In this paper we wish to present evidence for the structure.

Gibberellin A_{15} (VII) m.p. 274–276°, was isolated by careful fractionation of the residues from a commercial *Gibberella fujikuroi* ACC 917 fermentation.[10] The analytical and mass spectral data of the acid indicated that Gibberellin A_{15} had the formula, $C_{20}H_{26}O_4$. It titrated as a monobasic acid whilst with diazomethane it gave a monomethyl ester, $C_{21}H_{26}O_4$, m.p. 198–200°. The IR spectra of the acid, [ν_{max} 3239 (br) (OH of CO_2H), 1724 (δ-lactone), 1680 (CO_2H) 1652 and 889 ($>C{=}CH_2$) cm^{-1}] and the ester [ν_{max} 1738 and 1728 (δ-lactone and ester) 1660 and 885 ($>C{=}CH_2$) cm^{-1}] enabled the oxygen atoms to be accounted in δ-lactone and carboxyl groups and the unsaturation as a terminal methylene group. On hydrogenation gibberellin A_{15} monomethyl ester gave a dihydro compound, which lacked the characteristic terminal methylene absorption in the IR whilst the resonances at

[1] Previous part, B. E. Cross, *J. Chem. Soc.* 501 (1966).
[2] B. E. Cross, R. H. B. Galt and J. R. Hanson, *Tetrahedron* **18**, 451 (1962) et seq.
[3] B. E. Cross, R. H. B. Galt and J. R. Hanson, *J. Chem. Soc.* 295 (1964).
[4] B. E. Cross and K. Norton, *Chem. Comm.* 535 (1965).
[5] B. E. Cross and K. Norton, *J. Chem. Soc.* 1570 (1965).
[6] R. H. B. Galt, *J. Chem. Soc.* 3143 (1965).
[7] K. Koshimizu, H. Fukui, T. Kusaki, T. Mitsui and Y. Ogawa, *Tetrahedron Letters* 2459 (1966).
[8] S. Tamura, N. Takahashi, N. Murofushi, S. Iriuchijima, J. Kato, Y. Wada, E. Watanabe and T. Aoyama, *Tetrahedron Letters* 2465 (1966).
[9] B. E. Cross, R. H. B. Galt and J. R. Hanson, *Regulations Naturels de la Croissance Vegetale* p. 265. Centre National de la Recherche Scientifique, Paris (1964).
[10] A. Borrow, E. G. Jefferys, R. H. J. Kessel, E. C. Lloyd, P. B. Lloyd and I. S. Nixon, *Canad. J. Microbiol.* 7, 227 (1961).

τ 5·1 and 5·25 had disappeared from the NMR spectrum. Gibberellin A_{15} was thus tetracarbocyclic. Micro-ozonolysis of the methyl ester gave a nor-ketone showing additional ketonic IR absorption at 1748 cm^{-1} characteristic of a cyclopentanone indicating that the double bond was attached to a five-membered ring. Further evidence for the nature of the second oxygen function as a δ-lactone came from reduction of the methyl ester with LAH to a triol, VIII, $C_{20}H_{32}O_3$ m.p. 179–181°.

The gibberellin-like biological activity in the cucumber hypocotyl and dwarf maize tests[11] together with the source of gibberellin A_{15} suggested that it had the gibbane skeleton. The NMR spectrum of the ester provided strong support for this. This spectrum showed peaks at τ 8·85 (tertiary C—CH_3), 6·33 (O—CH_3), 5·58 and 5·97 (AB quartet J = 12 c/s; —CH_2OCO) 5·25 and 5·1 (>C· CH_2). The C-20 tetracyclic skeleton may be distinguished since the kauranoid skeleton IX contains four extra-nuclear carbon atoms whilst the gibbane skeleton X bears five. Since gibberellin A_{15} also contains a δ-lactone there are five pendant groups, and hence it seemed likely that gibberellin A_{15} had the gibbane skeleton. The presence of doublets centred at τ 7·21 and 7·79 (J = 13 c/s) is a feature highly reminisent of the quartet due to the 10:10a protons in the known gibberellins.[12]

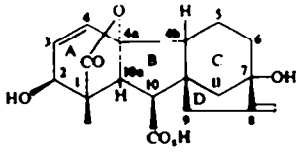
On the basis of this skeleton it is possible to arrive at structure VII for gibberellin A_{15}.

The acid shows pK°_{MCS} 7·15 characteristic of a gibbane 10-carboxyl. The single proton doublet in the NMR spectrum of the methyl ester at τ 7·21 is then assigned to the methine proton at position 10, α to the carbomethoxy group. As a doublet it is coupled to only one proton (at 10a). The position of the tertiary ring A methyl resonance as a singlet at τ 8·85 inidcates that it is deshielded by the β-carbonyl group belonging to the lactone ring. The presence of two protons at τ 5·58 and 5·97 indicate that the oxygen of the δ-lactone ring is attached to position 4a. These resonances are in the range quoted[13] for angular substituents. This evidence is strongly indicative of structure VII for gibberellin A_{15}. Furthermore it would seem likely in view of its source and structural similarity to gibberellins A_{12}–A_{14} that gibberellin A_{15} has the overall stereochemistry shown in VII.

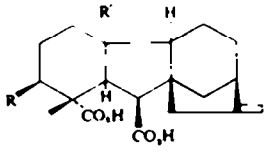
EXPERIMENTAL

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 Varian A60 and HA. 100 spectrometers in $CDCl_3$ with TMS as internal reference.

Light petroleum refers to the fraction of b.p. 60–80°. Silica gel was B.D.H. Chromatographic

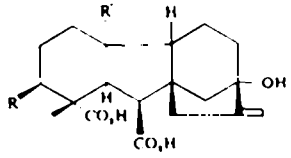


I

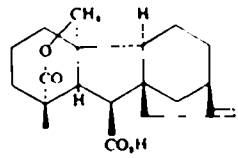


II R – H; R′ – Me
III R – OH; R′ – CO_2H
IV R – OH; R′ – Me

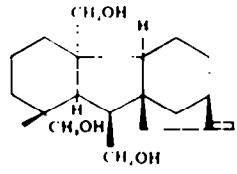
[11] P. W. Brian and H. G. Hemming, *Nature, Lond.* **189**, 74 (1961).
[12a] N. Sheppard, *J. Chem. Soc.* 3040 (1960); [b] J. R. Hanson, *Ibid.* 5036 (1965).
[13] A. Gaudemer, J. Polonsky and E. Wenkert, *Bull. Soc. Chim.* 407 (1964).



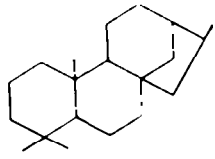
V R = OH; R′ = Me
VI R = H; R′ = CHO



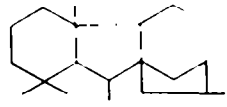
VII



VIII



IX



X

grade and the celite was Celite 545 (Johns Mandeville). Alumina for chromatography was Woelm grade II acid washed.

Isolation of gibberellin A_{16}. The crude fractions eluted in 25–30% AcOEt–Chf from a silica:celite (1:2) column chromatography[14] of the "weak acids" from a large scale *Gibberella fujikuroi* ACC 917 fermentation, were rechromatographed on silica gel. The fractions eluted in 40–50% AcOEt–light petroleum eventually crystallized from acetone–light petroleum to give *gibberellin* A_{16} (VII) as prisms m.p. 274–276° $[\alpha]_D$ +5°. (Found: C, 72·7; H, 8·2 equiv. 323. $C_{20}H_{26}O_4$ requires: C, 72·7; H, 7·9% M 330), ν_{max} (nujol) 3239, 1724, 1680, 1652, 889 cm^{-1} $pK^{\bullet}_{MCS}$ 7·15. Mass spectra *m/e* 330.

Methylation of gibberellin A_{16}. Gibberellin A_{16} methyl ester, prepared by adding ethereal diazomethane to a soln of gibberellin A_{16} in MeOH, crystallized from acetone–light petroleum as needles, m.p. 198–200°. (Found: C, 73·5; H, 8·1. $C_{21}H_{28}O_4$ requires: C, 73·25; H, 8·1%), ν_{max} (nujol) 1738, 1728, 1660, 885 cm^{-1} (in chf) 1727 (br), 1654 cm^{-1}, τ 8·85 (3), 7·79 (1), and 7·21 (1) (J = 13 c/s), 6·33 (3), 5·97 (1) and 5·58 (1) (J − 12 c/s), 5·25 (1), 5·1 (1).

Ozonoylsis on a micro-scale (5 mg) in glacial AcOH gave a nor-ketone (3 mg) which crystallized from acetone–light petroleum as needles, m.p. 238–240°, ν_{max} (nujol) 1748, 1727 cm^{-1}.

Hydrogenation of gibberellin A_{16} *methyl ester*. The ester (104 mg) in AcOEt (10 ml) was shaken under H with 30% Pd-C (100 mg) until the uptake of H ceased (1·04 moles). The catalyst was filtered off, the organic solvents evaporated and the gummy residue chromatographed on alumina. Elution with 10% AcOEt–chf gave *dihydrogibberellin* A_{16} *methyl ester* (35 mg) which crystallized from acetone–light petroleum as prisms, m.p. 126–128°. (Found: C, 72·7; H, 8·6. $C_{21}H_{30}O_4$ requires: C, 72·8; H, 8·7%), ν_{max} (nujol) 1729 (br), τ 9·08 (3) (J = 6 c/s), 8·88 (3), 7·90 (1) and 7·23 (1) (J = 13 c/s), 6·34 (3), 6·01 (1) and 5·62 (1) (J = 12 c/s).

Reduction of gibberellin A_{16} *methyl ester*. The ester (114 mg) in dioxan (15 ml) was refluxed with LAH (250 mg) for 4 hr. Water was cautiously added, the dioxan evaporated under reduced press and the organic material recovered in ether. The product was chromatographed on alumina. Elution with 80% AcOEt-light petroleum gave the *triol* VIII (51 mg) which crystallized from acetone–light petroleum as fine needles, m.p. 179–181°. (Found: C, 75·0; H, 10·2. $C_{20}H_{32}O_3$ requires: C, 75·0; H, 10·1%), ν_{max} (nujol) 3250 (br) 3070, 1650, 872 cm^{-1}.

Acknowledgement—I thank Drs. B. E. Cross and R. H. B. Galt for helpful discussions.

[14] J. R. Hanson, *Tetrahedron* **22**, 701 (1966).