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# NEW METABOLITES OF GIBBERELLA FUJIKUROI—XIV. GIBBERELLIN $A_{16}$ METHYL ESTER[1]

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**Abstract**—Gibberellin $A_{16}$ methyl ester, isolated after methylation of acidic metabolites of *Gibberella fujikuroi*, has the structure III (R = OH).

DURING the study of the biosynthesis of gibberellic acid (I; R = OH) mutants of the fungus *Gibberella fujikuroi* were examined and some were grown under abnormal pH conditions. The mutant (B47) grown at pH 7 was one of the best sources of gibberellin $A_7$[3] (I; R = H) and it also produced the $C_{20}$ gibberellin, $A_{13}$[4] (II), in good yield.

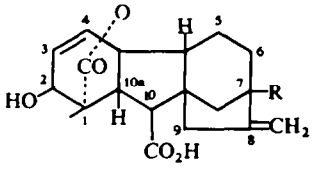
In the structural determination of gibberellin $A_{13}$ a quantity of crude acid from the fermentation was methylated with diazomethane and chromatographed on alumina. In addition to the expected gibberellin $A_{13}$ trimethyl ester, a new monomethyl ester was isolated which, from analytical and mass spectral data, had the formula $C_{20}H_{26}O_6$.

Gibberellin $A_{16}$ methyl ester shows IR absorption at 3560, 3461 (OH groups), 1780 ($\gamma$-lactone), 1716 (ester) and 1650 and 901 ($C{=}CH_2$) $cm^{-1}$. It readily forms a diacetate, $C_{24}H_{30}O_8$, $\nu_{max}$ 1779 ($\gamma$-lactone), 1750 and 1741 (acetates), 1724 (ester) and 1655 and 900 ($C{=}CH_2$) $cm^{-1}$.

The NMR spectrum of III (R = OH) shows signals at $\tau$ 8·88 (tertiary Me), 6·31 (Me ester) and a doublet at 5·05 ($C{=}CH_2$); in addition (a) double doublets centred at 7·28 and 6·80 ($J$ = 12 c/s) strongly resembles the 10,10a-quartet of the known gibberellins and their derivatives[5] and (b) a doublet at 6·05 ($J$ = 2 c/s) identical in chemical shift and splitting to the 2-equatorial hydrogen atom in 2-hydroxylated gibberellins. The spectrum is almost superimposable on that of gibberellin $A_4$ methyl ester (III; R = H); there is however an additional proton of the type $>C(\underline{H})OH$ at 5·80 appearing as a double-doublet ($J$ = 6 c/s and 10 c/s), the pattern of an axial proton adjacent to one $—CH_2—$ in a 6-membered ring. Spin-decoupling shows that gibberellin $A_{16}$ methyl ester is a 2*a*, 4*e*-glycol. Decoupling at $\tau$ 8·3 (the axial proton at C3) reduces (a) H2 to virtually a singlet and (b) H4 to a doublet ($J$ = 6 c/s) removing the larger *aa* splitting. There is no other site in the gibbane skeleton where a 1,3-disecondary glycol system can be placed to fit the NMR spectrum. The only other possibility is a 2*e*, 4*a*-glycol; a 2*e*-hydroxyl group is unlikely biogenetically and in synthetic samples the 2*a*-proton appears at higher field (e.g. in IV it is an ill-defined multiplet at $\tau$ 6·40).

The NMR spectrum of the diacetate shows signals at $\tau$ 8·95 (tertiary Me), 7·94 and 7·84 (acetates), 7·28 and 6·70 (10,10a-quartet, $J$ = 12 c/s), 5·04 (doublet, $C{=}CH_2$), 4·95 (doublet, $J$ = 2 c/s, H-2) and 4·80 (double doublet, $J$ = 6 and 10 c/s, H-4).

Biogenetically an axial OH group might have been expected at C-4 since gibberellic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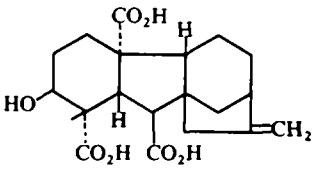
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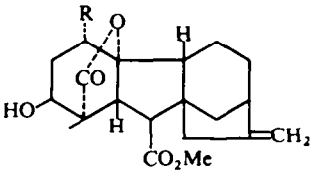
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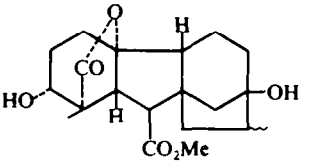
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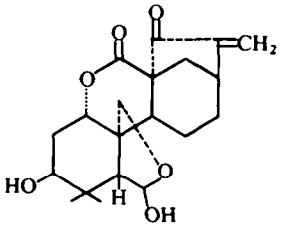
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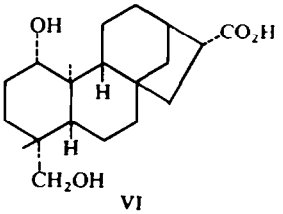
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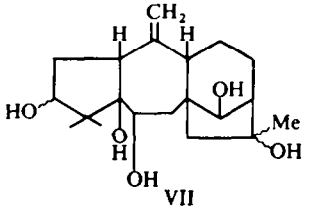
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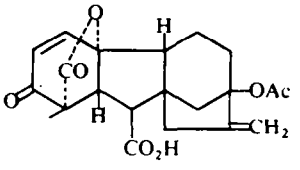
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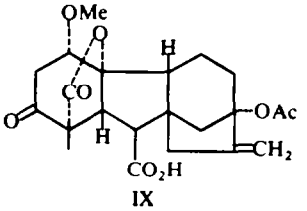
IX

acid (I; R = OH) and gibberellin $A_7$ (I; R = H) have 3,4-double bonds. An equatorial 1-hydroxyl group is, however, fairly common in diterpenes. Enmein[6] (V), for example, has the same hydroxylation pattern in ring A as gibberellin $A_{16}$ methyl ester and 1α, 19-dihydroxy-16α-(−)-kauran-17-oic acid[7] (VI) has been isolated from *Ricinocarpus stylosus*. 1α-Hydroxykaurenes are likely biogenetic precursors of the grayanotoxin skeleton[8] (e.g. VII).

The methyl ether IX and its 4-epimer have been prepared from VIII by prolonged treatment with methanol.[9]

## EXPERIMENTAL

M.ps were determined on a Kofler hot stage and are corrected; IR spectra were measured on a Perkin–Elmer 221; NMR spectra were measured on Varian A-60 and HA-100 spectrometers in $CDCl_3$ with TMS as internal standard.

*Isolation of gibberellin* $A_{16}$ *methyl ester.* Mutant B47 *Gibberella fujikuroi* was grown on a maize meal-ammonium tartrate medium keeping the pH around 7 by addition of alkali. Metabolites were extracted in the usual way[10] and separated into acids and neutrals with $NaHCO_3$. Most of the gibberellic acid was removed from the acid fraction by crystallization and a portion (30 g) of the mother liquors was chromatographed on Celite–charcoal (2:1; 1500 g) eluting with increasing concentrations of acetone in water. The fraction eluted with 48% acetone contained crude gibberellin $A_{13}$ (5 g). This was dissolved in MeOH and methylated with ethereal diazomethane and the product chromatographed on neutral alumina (Woelm grade II) eluting with increasing concentrations of AcOEt in light petroleum. The fractions eluted with 30 and 40% AcOEt contained gibberellin $A_{13}$ trimethyl ester (3·8 g) and elution with 55 and 60% AcOEt gave traces of methyl gibberellate. The fraction eluted with 90% AcOEt contained *gibberellin* $A_{16}$ *methyl ester* (0·103 g), which crystallized from acetone–light petroleum in prisms, m.p. 189–192°. (Found: C, 66·70; H, 7·43, M 362. $C_{20}H_{26}O_6$ requires: C, 66·30; H, 7·23% M 362), $\nu_{max}$ (Nujol) 3560, 3461, 1780, 1716, 1650 and 901 $cm^{-1}$, $\tau$ 8·88 (3), 7·28 (1) and 6·80 (1) ($J$ = 12 c/s), 6·31 (3), 6·05 (1) ($J$ = 2 c/s), 5·80 (1) ($J$ = 6 and 10 c/s) and 5·05 (2).

*Acetylation of gibberellin* $A_{16}$ *methyl ester.* The ester (0·03 g) in pyridine (2 ml) was treated with excess $Ac_2O$ overnight at room temp. The soln was poured into dilute hydrochloric acid and extracted with ethyl acetate. The gummy crystals were chromatographed on neutral alumina eluting with increasing concentrations of AcOEt in light petroleum. The fraction eluted with 20% AcOEt gave the *diacetate* (0·03 g) which crystallized from acetone–light petroleum, m.p. 216–218°. (Found: C, 66·43; H, 6·83. $C_{24}H_{30}O_8$ requires: C, 66·56; H, 6·77%), $\nu_{max}$ (Nujol) 1779, 1750, 1741, 1724, 1655, 900 and 893 $cm^{-1}$, $\tau$ 8·95 (3), 7·94 (3), 7·84 (3), 7·28 (1) and 6·70 (1) ($J$ = 12 c/s), 5·04 (2), 4·95 (1) ($J$ = 2 c/s) and 4·80 (1) ($J$ = 6 and 10 c/s).

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