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THE MICROBIOLOGICAL PREPARATION OF TWO 'ATISAGIBBERELLINS'

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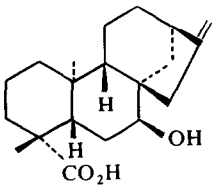
Tetracyclic diterpenoids may be divided in terms of their ring C/D structure into the kaurene, 13β-kaurene (phyllocladene), atisene and beyerene (stachene) classes [1]. Although the naturally occurring gibberellins, which have hitherto been isolated, all belong to the *ent*-kaurene series, there is nothing known about gibberellin biosynthesis which would preclude the occurrence of gibberellin-like substances with other tetracyclic diterpenoid skeletons. The biosynthesis of the gibberellins in the fungus *Gibberella fujikuroi* utilizes *ent*-7α-hydroxykaur-16-en-19-oic acid (**1**) as a key intermediate (32% incorporation into gibberellic acid (**2**)) immediately prior to the contraction of ring B [2]. A number of kaurenoids differing from the normal metabolites have been transformed along part or the whole of this pathway depending upon their oxygenation pattern [3–6]. In this paper we record the preparation of the first 'atisagibberellins' using *ent*-7α-hydroxyatis-16-en-19-oic acid (**3**) [7] as an artificial substrate.



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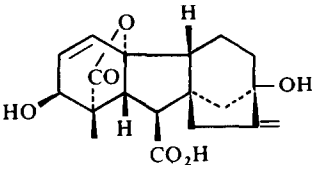

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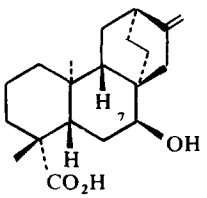
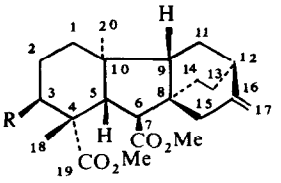


3

When *Gibberella fujikuroi* is cultured in the presence of AMO 1618, the biosynthesis of *ent*-kaur-16-ene is inhibited and thus the metabolites derived from it are not formed [8]. However, the post-kaurene metabolism is not perturbed and hence it is possible to utilize the biosynthetic enzymes for the transformation of artificial substrates. *Ent*-7α-hydroxyatis-16-en-19-oic acid (**3**), obtained by the hydrolysis of gummiferolic acid, was incubated with *Gibberella fujikuroi* in the presence of AMO 1618. After six days the metabolites were isolated and compared (TLC) with a control fermentation. Two new metabolites were detected in the acid fraction and isolated by chromatography of their methyl esters. The first of these, a minor component, $C_{22}H_{32}O_4$, showed ester (ν_{max} 1730 cm^{-1}) and olefinic (880 cm^{-1}) absorption in its IR spectrum. The ^1H NMR spectrum contained two

—C(Me)—, two OMe and two olefinic ($C=CH_2$) signals.

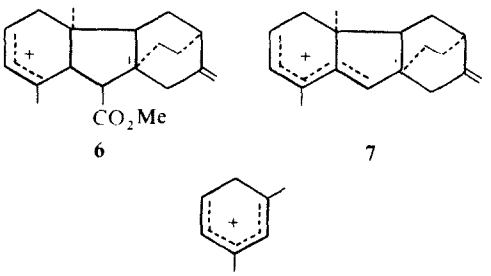
In addition, decoupling experiments confirmed that it contained the typical gibberellin AB system (δ 1.87 and 3.06, J = 12 Hz) assigned to the *trans* 5-H and 6-H protons, respectively. The compound was thus the dimethyl ester of atisagibberellin A_{12} (**4**). The MS shows similarities to that of gibberellin A_{12} dimethyl ester [9] by possessing ions at M = 32, M − 60, M − 91 and strong ions at M-119/120 characteristic of the loss of two methoxycarbonyl groups from a C_{20} gibberellin. Fragments at *m/e* 225 [M − (120 + 15)], 197 [M − (120 + 15 + 28)], and 185 [M − (120 + 15 + 40)] represent the further loss of a methyl group and ethylene or the ring D bridge. The presence of a significant ion at *m/e* 109 is associated with an unsubstituted ring A in gibberellins.



4 R = H
5 R = OH

The second major metabolite, isolated as its dimethyl ester $C_{22}H_{32}O_5$, showed hydroxyl (3500 cm^{-1}), ester (1720 cm^{-1}) and olefinic (1650, 880 cm^{-1}, $C=CH_2$) absorption in its IR spectrum. The ^1H NMR spectrum contained resonances assigned to two —C(Me)— groups two O—Me groups, two ($C=CH_2$) olefinic protons and a secondary alcohol. Spin decoupling experiments confirmed the presence of the typical gibberellin AB system (δ 2.34 and 3.05, J = 12 Hz) assigned to the C-5 and C-6 protons. The location of the hydroxyl group at C-3 followed from the pyridine induced solvent shifts ($\delta_{CDCl_3} - \delta_{Py}$) [10] for the C-4 methyl (18-H) (Δδ 0.35) and 5-H (Δδ 0.55) resonances in the ^1H NMR spectrum. The 3-H signal was a triplet (J = 3 Hz) in accord with the axial configuration of the alcohol. Hence the compound was assigned the structure **5**, atisagibberellin A_{14} dimethyl ester. The ^{13}C NMR resonances were assigned by comparison with other gibberellins [11] and *ent*-atisene derivatives [12]. Whilst most resonance occurred within the anticipated ranges, there was no high-field SFORD triplet around 16 ppm which is characteristic of C-11 in the kaurenes and gibberellins, possibly reflecting the changes in conformation of rings C and D in the bicyclo-

(2,2,2)-octane. The MS showed the anticipated similarities to that of gibberellin A_{14} dimethyl ester [9] with significant ions at M − 32, M − 60 and M − 120 and also strong ions at *m/e* 298, 239 and 107 (base peak) associated with the fragments **6**, **7** and **8**.



6 **7**

8

An interesting feature of this bio-transformation is that, in contrast to some kaurenoids, the substrate was only metabolized for a few steps along the pathway. In particular, the atisagibberellin metabolites were not oxygenated at C-20, a major site of oxidation in the natural gibberellins. Molecular models suggest that this may be a reflection of the increased steric crowding at C-20 by the two-carbon bridge (C-13 and C-14) as compared to the methylene (C-14) of gibberellin A_{12}/A_{14}.

EXPERIMENTAL

General experimental details have been described previously [2].

Hydrolysis of gummiferolic acid. Gummiferolic acid (1.0 g) in 10 N methanolic KOH (25 ml) was heated under reflux for 24 hr. The soln was acidified and the product recovered in $CHCl_3$ and purified by dry column chromatography on Si gel. Elution with EtOAc–petrol (3:7) gave *ent*-7α-hydroxyatis-16-en-19-oic acid (750 mg) which crystallized from MeOH as needles, mp 216–218 (lit. [7] 215–221°).

Incubation with Gibberella fujikuroi. *Gibberella fujikuroi* (ACC 917) inhibited with 10^{-4} M AMO 1618, was grown in shake culture at 25° for 1 day in 50 flasks (250 ml) each containing sterile medium (100 ml) [2]. *Ent*-7α-hydroxyatis-16-en-19-oic acid (300 mg) in EtOH (48 ml) was distributed equally between 48 flasks and the remaining were retained as a control. The incubation was allowed to continue for a further 5 days. The broth was filtered and acidified with dil HCl and extracted with EtOAC. The extract was separated into 'acidic' and 'neutral' fractions with aq. $NaHCO_3$. Chromatography of the neutral fraction gave the starting material (10 mg) identified by its ^{1}H NMR spectrum. TLC of the acidic fraction showed that it contained two new compounds compared to the control. The fraction was dissolved in MeOH and methylated with ethereal CH_2N_2. The solvent was evapd and the residue was chromatographed on Si gel (Merck). Elution with EtOAc–petrol (1:1) gave atisagibberellin A_{12} dimethyl ester (15 mg) as an oil (M^+ 360.230. $C_{22}H_{32}O_4$ requires: 360.230). IR ν_{max} cm^{-1}: 1730 (br), 880; ^{1}H NMR: 0.75 (3H, *s*, 20-H), 1.04 (3H, *s*, 18-H), 1.87 (1H, *d*, *J* = 12 Hz, 5-H), 3.06 (1H, *d*, *J* = 12 Hz, 6-H), 3.61 and 3.65 (each 3H, *s*, OMe), 4.62 and 4.75 (each 1H, *s(br)*, 17-H); MS *m/e* (rel. int.): 360(8), 328(47), 300(100), 285(26), 269(9), 241(43), 240(31), 225(17), 197(14), 185(17), 173(11), 171(12), 159(11), 157(12), 145(12), 143(14), 131(12), 129(13), 119(14), 117(11), 109(16), 107(13), 105(24). Further elution gave atisagibberellin A_{14} dimethyl ester (58 mg) as an oil (M^+ 376.225. $C_{22}H_{32}O_5$ requires 376.225). IR ν_{max} cm^{-1}: 3500, 3060, 1720 (br), 1650, 880; ^{1}H NMR ($CDCl_3$): 0.77 (3H, *s*, 20-H), 1.14 (3H, *s*, 18-H), 2.34 (1H, *d*, *J* = 12 Hz, 5-H), 3.05 (1H, *d*, *J* = 12 Hz, 6-H), 3.64 and 3.67 (each 3H, *s*, OMe), 4.11 (1H, *t*, *J* = 3 Hz, 3-H), 4.65 and 4.78 (each 1H, *s(br)*, 17-H); ^{1}H NMR (Py): δ 0.89 (3H, *s*), 1.49 (3H, *s*), 2.89 (1H, *d*), 3.35 (1H, *d*, each *J* = 12 Hz), 3.63 and 3.67 (each 3H, *s*) 4.47 (1H, *m*) 4.69 and 4.85 (each 1H, *s(br)*); ^{13}C NMR (Py): δ 13.6 (*q*, C-20), 24.1 (*q*, C-18), 27.1 (*t*, C-14)[a], 27.1 (*t*, C-13)[a], 27.9 (*t*, C-11)[a], 32.2 (*t*, C-2)[b], 33.8 (*t*, C-1)[b], 37.0 (*d*, C-12), 42.0 (*t*, C-15), 42.7 (*s*, C-10), 42.7 (*s*, C-8), 49.0 (*s*, C-4), 51.1 (*d*, C-6)[c], 51.3 (*q*, OMe), 51.3 (*q*, OMe), 56.1 (*d*, C-9), 70.2 *d*, C-3), 107.6 (*t*, C-17), 151.8 (*s*, C-16), 175.3 (*s*, C-19), 177.8 (*s*, C-7) ([a, b, c] these assignments may be interchanged); MS *m/e* (rel. int.): 376(0.8), 344(8), 316(3), 298(6), 183(3), 270(1), 256(1), 239(3), 231(1), 208(2), 166(30), 149(5), 134(12), 123(10), 121(30), 119(34), 107(100).

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