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METABOLITES FROM THE SUCCINATE ESTER OF (-)-KAURENOL

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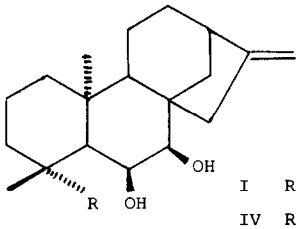
The available evidence on the formation of the gibbane skeleton in Gibberella fujikuroi cultures[1,2,3] suggests that 6β, 7β-dihydroxy-(-)-kaur-16-en-19-oic acid (I) might be a key intermediate[1,4]. However the isolation or preparation of a diol of this stereochemistry has not yet been described.

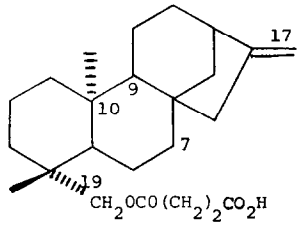
We have observed the formation of 6β, 7β-dihydroxy derivatives from the metabolism of various (-)-kaurene derivatives in G. fujikuroi and report here on metabolites of the hemi-succinate ester of (-)-kaur-16-en-19-ol (II).

The hemi-succinate ester (II) m.p. 125-8°, prepared by reaction of (-)-kaurenol with succinic anhydride, was incubated with a suspension of the mycelium of G.fujikuroi at pH7. After hydrolysis of the acidic fraction from the metabolism, a diol (III) $C_{20}H_{32}O_2$, m.p. 189-90°, $[\alpha]_D^{23}$-21°($CHCl_3$) and a triol (IV), M^+ 320.2343 ($C_{20}H_{32}O_3$ requires 320.2351), m.p. 192-3°, $[\alpha]_D^{23}$ -73°(MeOH) were isolated in 12% and 20% yields respectively.

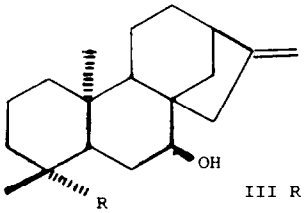


I R = CO_2H

IV R = CH_2OH

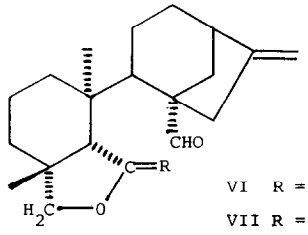


II



III R = CH_2OH

V R = CO_2H



VI R = $\langle^{H}_{OH}$

VII R = O

The diol metabolite was recognized as 7β, 19-dihydroxy-(-)kaurene (III) from the close correspondence of the m.p. with the literature value[4] and from comparison (m.s., t.l.c. in two solvent systems) with a small sample obtained by methylation and $LiAlH_4$ reduction of 7β-hydroxy-(-)kaurenoic acid (V). The i.r. and n.m.r. spectra of the metabolite are consistent with the structural assignment showing two tertiary methyls, terminal methylene and primary and secondary hydroxyl groups.

The triol metabolite has the following spectral characteristics: ν_{max}^{nujol} 3300(O-H), 3055, 1660, 868cm^{-1} ($C=CH_2$); m.s. peaks at m/e 320(M^+), 302($M^+ - H_2O$), 284($M^+ - 2H_2O$), 271($M^+ - H_2O - CH_2OH$), 253($M^+ - 2H_2O - CH_2OH$); n.m.r. (d_5-pyridine) singlets at 8.82, 8.43τ (2 tertiary Me), one proton doublet at 7.76τ (J 11Hz), partially-resolved doublet at 6.13τ (J~2-3Hz, H-C-OH), broadened doublet centred at 5.52τ(peak separation ~ 11Hz, H-C-OH) overlapping an AB quartet at 5.51, 5.68, 6.18, 6.35τ (J 11Hz, -C-CH_2OH) and a broadened signal at 5.07τ ($>C=CH_2$). This indicates that the metabolite has two 2° hydroxyls substituted on the original (-)-kaurenol nucleus. Furthermore,the splitting of the n.m.r. signals,centred at 7.76, 6.13 and 5.52τ,suggests that they are due to an isolated system of three methine protons on contiguous carbon atoms in which the central methine proton,giving a signal at 5.52τ, has a small coupling (~2-3Hz) to the other proton geminal to a hydroxyl and a large coupling (11 Hz) to the remaining proton. This is accommodated by location of the 2° hydroxyls at the 6β and 7β positions i.e. by 6β, 7β, 19-trihydroxykaurene (IV).

Periodate oxidation of the triol confirmed the 1,2-diol grouping. The gummy product (VI) had M^+318, $\nu_{max}^{CS_2}$, 1725 cm^{-1} (-CHO) and n.m.r. signals at 0.15τ (-CHO) and 4.65τ (hemiacetal proton). The base peak of the mass spectrum at m/e 168 is attributed to an ion, incorporating the A-ring, resulting from cleavage of the 9-10 bond with loss or transfer of a hydrogen. The B-ring cleaved structure of VI is thus demonstrated confirming the position of the vicinal diol grouping of the metabolite (IV).

Further support for the structural assignments came from selective oxidation of VI with silver carbonate[5] to form the γ-lactone (VII) of known structure[6], m.s. peaks at m/e 316(M^+), 167(A-ring from fission of the 9-10 bond); $\nu_{max}^{CS_2}$ 1720(-CHO), 1770cm^{-1} (γ-lactone). The n.m.r. spectrum of the product (three proton singlets at 8.87, 9.06τ, two proton AB quartet at 5.85, 6.00, 6.16, 6.30τ,two proton broad signal at 5.12τ and a one proton singlet at 0.2τ) showed close correspondence with data published for VII[6].

17-^{14}C- labelled triol (IV , 347,000dpm), prepared by the metabolic method, was fed to a growing culture of G.fujikuroi. An appreciable proportion of the radioactivity was converted into acids but purification of gibberellic acid through the methyl ester showed that < 0.2% of the radioactivity had been incorporated into this compound. In contrast 17-^{14}C-labelled diol (III, 73,000dpm) gave a gibberellic acid fraction which after dilution and methylation reached constant specific activity (13.7 dpm/mg, 1.8% incorporation) after two crystallizations.

The incorporation from the diol (III), while appreciable, is far less than has been observed with the related known intermediate, 7β-hydroxykaurenoic acid (V, 32.3%)[7]. This is consistent with the reported normal pathway of gibberellin biosynthesis in which B- ring modification of the (-)-kaurene nucleus occurs after a carboxylic acid has been formed from C-19[8]. Nevertheless, the very much smaller incorporation from the triol (IV) into gibberellic acid than from the diol (III) indicates that a 19-oxygenated-6β, 7β-dihydroxykaurene is unlikely as an intermediate in gibberellic acid biosynthesis. B-ring contraction of 7β- hydroxykaurenoic acid (I) without an enzyme-free intermediate is an attractive alternative.

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