Biosynthesis of a C_{16}-Terpenoid Lactone, a Plant Growth Regulator

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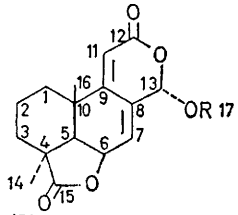
Summary Biosyntheses of a C_{16}-terpenoid lactone (I) from [2-^{13}C]acetic acid and [2-^{14}C,5-$^{3}H_2$]mevalonic acid show that the lactone (I) is derived from a diterpenoid precursor.

THE terpenoid lactones (I) and (II) isolated from the metabolite of a mould, Acrostalagmus species NRRL-3481, are antifungal substances.[1] We were interested in these compounds because of their structural resemblance to inumakilactones,[2] nagilactones,[3] and podolactones,[4] some of which have potent inhibitory activity of the expansion and mitosis of plant cells.[4,5] The lactone (I) has a strong inhibitory activity on the growth of an *Avena coleoptile* section comparable to those of the lactones mentioned.



(I) R = Me
(II) R = H

The lactones (I) and (II) have a novel C_{16}-carbon skeleton. Two possibilities may be envisaged for their biosynthesis: the carbon skeleton arises from (i) a diterpene with oxidative loss of four carbon atoms, or (ii) a sesquiterpenoid precursor of the drimenin family with the addition of a C_1-unit. We report that the lactone (I) is biosynthesized from a diterpenoid precursor.

Isotope-enriched acetic acid or mevalonic acid was added to the culture medium of the Acrostalagmus mould at the most active period of production of antibiotic. Fermentation was terminated 100 h after the addition of the precursor and the labelled lactone (I) was isolated.

The ^{13}C-natural abundance n.m.r. spectrum of (I) showed seventeen peaks, and these were assigned (Table) from an analysis of the off-resonance decoupled spectrum and comparison of the chemical shifts with those of diterpenes.[6] The enhancement of the signal intensities of ten carbon atoms in the spectrum of a sample enriched biologically with [2-^{13}C]acetic acid (Table) shows clearly that these carbon atoms originate from the methyl group of acetic acid. Furthermore, the almost equal enhancement of the signal intensities of C-12 and C-15 in the labelled lactone shows that the compound (I) is biosynthesized from

TABLE. ^{13}C-N.m.r. spectrum of the lactone (I) and enhancement of the signal intensities of the lactone biosynthesized from [2-^{13}C]acetic acid

Carbon number	Chemical[a] shift	Relative[b] enhancement
1	27·8(t)[c]	1·66
2	17·4(t)	1·00
3	29·9(t)[c]	1·72
4	42·8(s)	0·89
5	48·0(d)	1·40
6	71·3(d)	1·00
7	124·0(d)	1·54
8	132·9(s)	—
9	157·5(s)	1·90
10	35·1(s)	0·99
11	111·8(d)	1·01
12	162·7(s)	1·94
13	101·2(d)	1·56
14	24·7(q)[c]	1·85
15	180·8(s)	2·10
16	24·2(q)[c]	1·97
17	57·4(q)	1·34

[a] Chemical shifts relative to Me_4Si; multiplicity observed on off-resonance spectrum in parentheses. [b] The ratio of spectral intensity enhanced by incorporation of [2-^{13}C]acetic acid and that of natural abundance spectrum, assuming the ratio of C-2 as unity. [c] The assignments made to C-1, C-3 and to C-14, C-16 may need to be reversed.

a diterpene derived from acetic acid through mevalonic acid. This observation was supported by the experiment using a doubly labelled mevalonic acid.

Administration of [2-^{14}C,5-$^{3}H_2$]mevalonic acid (^{3}H/^{14}C = 5·4) to the mould afforded a doubly labelled lactone (I) whose ^{3}H/^{14}C ratio was shown to be 2·5 (calculated 2·7). This shows that (I) is constructed from four molecules of mevalonic acid with loss of four tritium atoms. If the lactone were derived from three molecules of mevalonic acid, the ^{3}H/^{14}C ratio of the lactone would be 3·6.

These experiments show that the C_{16}-skeleton of (I) and (II) is derived from microbiological degradation of a normal diterpene such as labdadienol by oxidative cleavage between C-12 and C-13.

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