Biosynthesis of the Sesquiterpenoids, Botrydial and Dihydrobotrydial

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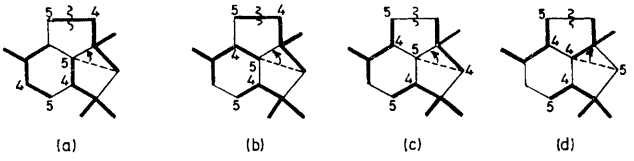
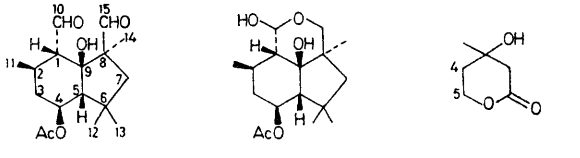
Summary The origin of the carbon skeleton of botrydial and dihydrobotrydial has been defined using [4,5-$^{13}C_2$]-mevalonic acid.

THE fungal metabolites, botrydial (**1**) and dihydrobotrydial (**2**), are produced by the plant pathogen *Botrytis cinerea*.[1] Although these novel structures do not obey the simple isoprene rule, their carbon skeletons may be derived from three isoprene units in several ways [(a)—(d)]. Each of these biogenetic routes involves a rearrangement and a



(a) (b) (c) (d)



(1) (2) (3)

bond fission. We have distinguished between them by a combination of ^{13}C n.m.r. spectroscopy and ^{3}H labelling.

[4,5-$^{13}C_2$]Mevalonic acid was prepared[2] from 90% $^{13}CH_3$-$^{13}CO_2H$. It was fed to *B.cinerea* for the optimum period (determined by [2-^{14}C]mevalonate experiments) of 8 days. The anticipated labelling pattern is shown for each case and the coupling patterns serve to distinguish (a) and (b) from (c) and (d). The ^{13}C n.m.r. assignments together with the coupled centres in the enriched sample of botrydial and dihydrobotrydial are shown in the Table. There were only

TABLE. The ^{13}C n.m.r. spectra of botrydial and dihydrobotrydial ($CDCl_3$, 25·15 MHz, p.p.m. from Me_4Si)

Carbon atom	Botrydial	Dihydrobotrydial
1	67·2	55·0[a]
2	35·6	35·9
3	38·7	39·9
4	72·3	72·7[b]
5	63·8	59·8[b]
6	39·4	38·8
7	51·5	50·4[c]
8	59·0	45·4
9	89·6	83·4[c]
10	204·3	92·2[a]
11	19·8	20·0
12	20·4	25·4
13	27·8	27·2
14	28·0	28·6
15	206·7	67·4

Acetate 21·4 and 170·3 p.p.m.

[a] Coupling constant 41 Hz; [b] coupling constant 38 Hz; [c] Enriched centres. Incorporation based on added ^{14}C MVA (18 μC) was 0·5 and 1%.

two pairs of ^{13}C–^{13}C couplings; between C(4) and C(5) (J 38 Hz) and C(1) and C(10) (J 41 Hz). The resonances associated with C(7) and C(9) were enriched. Thus two mevalonate C(4)–C(5) bonds have remained intact and one has been cleaved. The ^{13}C–^{13}C coupling patterns could be accommodated by (c) or (d).

These two probabilities were then distinguished by the number of [5-$^3H_2$]mevalonoid hydrogen atoms which were incorporated by the metabolites. Arrangement (c) would require two [5-$^3H_2$]mevalonoid labels whereas (d) would require four [5-$^3H_2$]mevalonoid labels in the metabolites.

[5-$^3H_2$,2-^{14}C]Mevalonic acid (^3H:^{14}C, 16·22:1, atom ratio 6:3, 80 μC ^{14}C) was fed to *B. cinerea*. Botrydial (**1**) (^3H:^{14}C, 10·2:1, atom ratio, 3·8:3, 0·06% incorporation) was isolated. The number of [5-$^3H_2$]-mevalonoid labels which were incorporated was in accord with (d).

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