Biosynthesis of Bisabolene by Callus Cultures of *Andrographis paniculata*

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Summary Experiments with intact callus cultures of *Andrographis paniculata* and a derived cell-free system indicated that (a) the biosynthesised γ-bisabolene has the *Z*-configuration (**3**); (b) the biosynthetic intermediate is 2-*cis*,6-*trans*-(**1**)– and not 2-*cis*,6-*cis*-(**2**)–farnesol pyrophosphate; (c) in paniculide B (**5**) the ring carbon derived from C-2 of mevalonate is *anti* to the side chain.

ON a speculative level γ-bisabolene or cations derived from it are important early intermediates in the biosynthesis of a variety of natural sesquiterpenoids.[1,2] Suggestions for the biosynthesis of γ-bisabolene itself were made by Ruzicka in 1962[3] but, so far as we are aware, have not been subjected to experimental scrutiny. Indeed there is, to our knowledge, no convincing recorded evidence that identifies natural γ-bisabolene as either the *Z*- or *E*-isomer.

The suggested pathways to γ-bisabolene [(**3**) or (**4**)] pose two questions: (a) is the ring carbon atom derived from C-2 of mevalonate *anti* (**3**) or *syn* (**4**) to the side chain and (b) is 2-*cis*,6-*trans*-(**1**)– or 2-*cis*,6-*cis*-(**2**)–farnesol pyrophosphate the intermediate? In addition, since an enzyme-mediated double bond isomerisation of either (**3**) or (**4**) cannot be excluded *a priori*, independent evidence is desirable to identify natural γ-bisabolene as either the *Z*- or *E*-isomer.

We have attempted to distinguish between these alternatives by using callus cultures of *A. paniculata* and cell-free systems derived from them.[4,5] The callus cultures grown in suspension in presence of oxygen and light accumulate the sesquiterpene lactones paniculides A, B, and C, previously described.[6] The derived cell-free system under anaerobic conditions accumulates γ-bisabolene, as well as *trans*,*trans*- and *cis*,*trans*-farnesols.[5]

TABLE

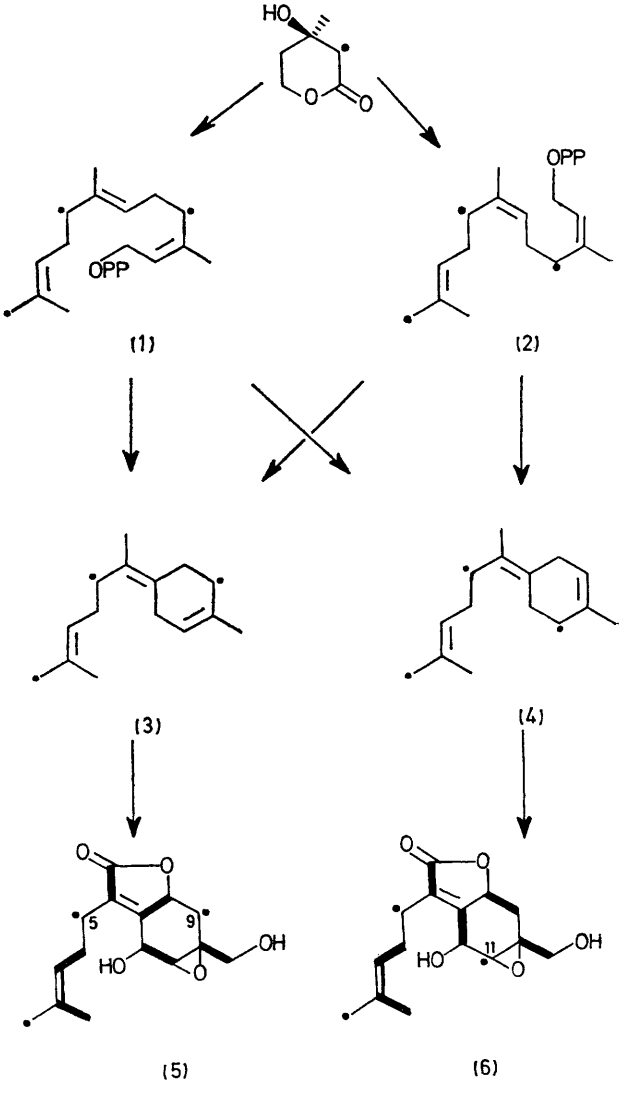
^{13}C Chemical shifts of paniculide B and coupling constants [$^1J(^{13}C–^{13}C)$/Hz] of [1,2-$^{13}C_2$]acetate-enriched paniculide B.

Carbon	1	2	3	4	5	6	7	8
δ/p.p.m.[a]	25·3	131·3	123·5	27·1	22·8	126·4	161·0	75·2
$^1J(^{13}C–^{13}C)$	—	42	43	44	—	62	35	35

Carbon	9	10	11	12	13	14	15
δ/p.p.m.	32·7	60·1	61·8	67·2	17·4	173·3	63·6
$^1J(^{13}C–^{13}C)$	—	49	46	46	42	63	49

[a] Relative to internal Me_4Si.

An answer to question (a) above came from examination of the ^{13}C n.m.r. spectrum of paniculide B [(**5**) or (**6**)], biosynthesised from [1,2-$^{13}C_2$]acetate by callus tissues. Carbon 2 of mevalonic acid will appear in paniculide B either at C-9 (**5**) or at C-11 (**6**) (and also at C-1 and C-5). Unlike the corresponding carbon atoms in the γ-bisabolene precursor, C-9 and C-11 of paniculide B are readily distinguishable in its ^{13}C n.m.r. spectrum and indeed the complete spectrum was unambiguously assignable (see Table) using samples enriched in turn by [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]-acetates. When [1,2-$^{13}C_2$]acetate served as precursor [151 mg, 91·7 atom %, administered to callus tissue (dry weight 2·55 g) grown in suspension for 20 days following transfer from solid medium[4,5]] and paniculide B (117 mg, t.l.c.-pure) was harvested after 10 days, C-9, (δ 32·7 p.p.m. from Me_4Si; Varian XL-100 at 25·2 MHz) appeared essentially as a singlet and therefore derives from C-2 of mevalonate,[7] while C-11 (δ 61·8 p.p.m.) appeared as a triplet [singlet + doublet ($J_{11,12}$ 45·9 Hz)] (see Table). It follows that paniculide B is represented by (**5**) and not (**6**) and its γ-bisabolene precursor probably by (**3**) and not (**4**). This



conclusion is supported by incorporation of radioactivity from labelled mevalonate into *Z*-γ-bisabolene (**3**), but not into the *E*-isomer (**4**). Thus co-injection (Pye 104 gas chromatograph with Panax Nucleonics Radiogas Detection System; 1% SE30 at 110 °C) of γ-bisabolene biosynthesised

by the cell-free system[5] from (3*R*)-[2-^{14}C]mevalonate, and a mixture of synthetic *Z*- and *E*-γ-bisabolenes, located radioactivity in only the *Z*-isomer.[8]

That *cis,trans*- and not *cis,cis*-farnesol pyrophosphate is the biosynthetic intermediate to γ-bisabolene was established as follows. (3*R*)-[2-^{14}C,5-$^{3}H_2$]mevalonate was incorporated into γ-bisabolene (1·2% incorporation, estimated as crystalline trihydrochloride of constant radio-activity) with loss of one-sixth of the tritium label (%^{3}H retention 85·4, 80·2; one-sixth ^{3}H loss requires 83·3). This supports the intermediacy of *cis, trans*-farnesol pyrophosphate (loss of one-sixth ^{3}H in *trans,trans*- to *cis,trans*-interconversion[5]), but not of *cis,cis*-farnesol pyrophosphate which should lose an additional one-sixth ^{3}H label at the C_{10} stage during geraniol to nerol interconversion.[5,9] More directly, [4,8,12-$^{14}C_3$]-*cis,trans*-farnesol[5] was incorporated (1·2%) into γ-bisabolene, but [2-^{14}C]-*cis,cis*-farnesol[10] was not (0·02%).

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