Biosynthesis of Bisabolene by Callus Cultures of *Andrographis paniculata*

By KARL H. OVERTON* and DOUGLAS J. PICKEN
(*Plant Tissue Culture Unit, Department of Chemistry, The University of Glasgow, Glasgow* G12 8QQ)

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

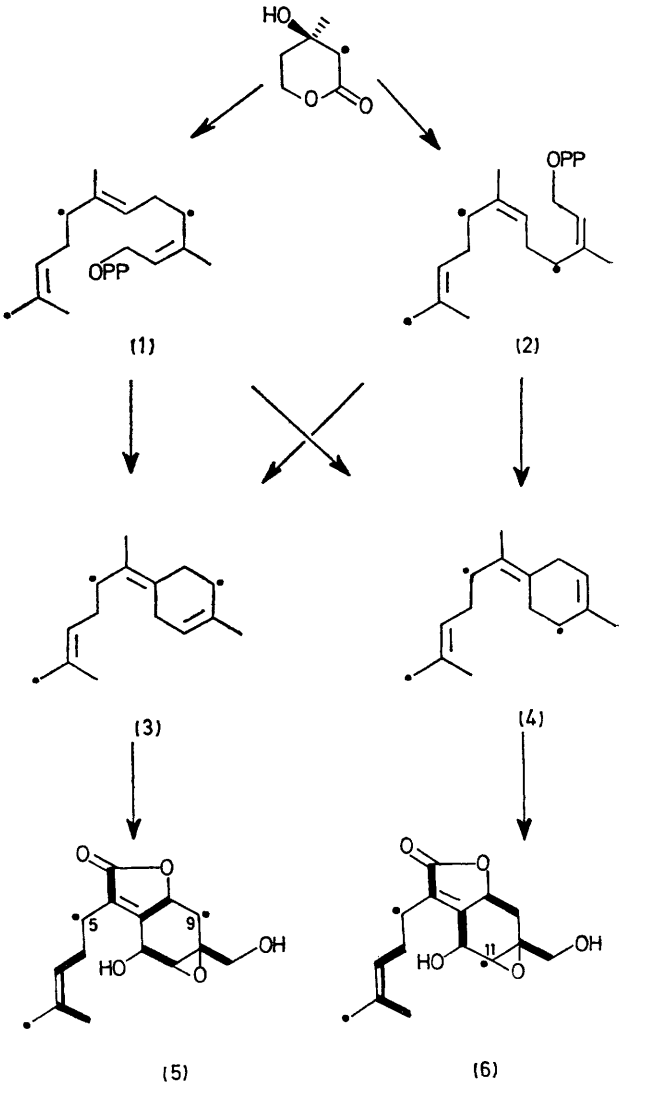
^{13}C Chemical shifts of paniculide B and coupling constants [$^1J(^{13}C-^{13}C)$/Hz] of [1,2-$^{13}C_2$]acetate-enriched paniculide B.

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%).

We thank Miss I. Freer for the maintenance and culturing of Andrographis tissue, the S.R.C. for a studentship (to D.J.P.), Dr. I. H. Sadler, Edinburgh University, for ^{13}C n.m.r. spectra, and Dr. D. J. Faulkner, La Jolla, California, for synthetic *E*- and *Z*-bisabolene.

(*Received*, 19*th November*, 1975; *Com.* 1295.)

[1] W. Parker, J. S. Roberts, and R. Ramage, *Quart. Rev.*, 1967, **21**, 331.
[2] G. Rücker, *Angew. Chem. Internat. Edn.*, 1973, **12**, 793.
[3] L. Ruzicka, *Pure Appl. Chem.*, 1963, **6**, 493.
[4] D. N. Butcher and J. D. Connolly, *J. Exper. Botany*, 1971, **22**, 314.
[5] F. M. Roberts and K. H. Overton, *Biochem. J.*, 1974, **144**, 585.
[6] A. J. Allison, D. N. Butcher, J. D. Connolly, and K. H. Overton, *Chem. Comm.*, 1968, 1493.
[7] M. Tanabe and K. T. Suzuki, *J.C.S. Chem. Comm.*, 1974, 445.
[8] The *E*- and *Z*-isomers have recently been identified by *X*-ray structure analysis of a synthetic intermediate: L. E. Wolinsky, D. J. Faulkner, J. Finer, and J. Clardy, *J. Org. Chem.*, in the press. We are indebted to Dr. Faulkner for pre-publication information.
[9] E. Jedlicki, G. Jacob, F. Faini, and O. Cori, *Arch. Biochem. Biophys.*, 1972, **152**, 590.
[10] Prepared from methyl bromo[2-^{14}C]acetate, triethyl phosphite, and neryl acetone; see 'Methods in Enzymology,' ed. R. B. Clayton, Vol. XV, Academic Press, New York, 1969; pp. 378, 379.