^{2}H N.M.R. Determination of the Stereochemistry of an Allylic Displacement in the Biosynthesis of Virescenol B

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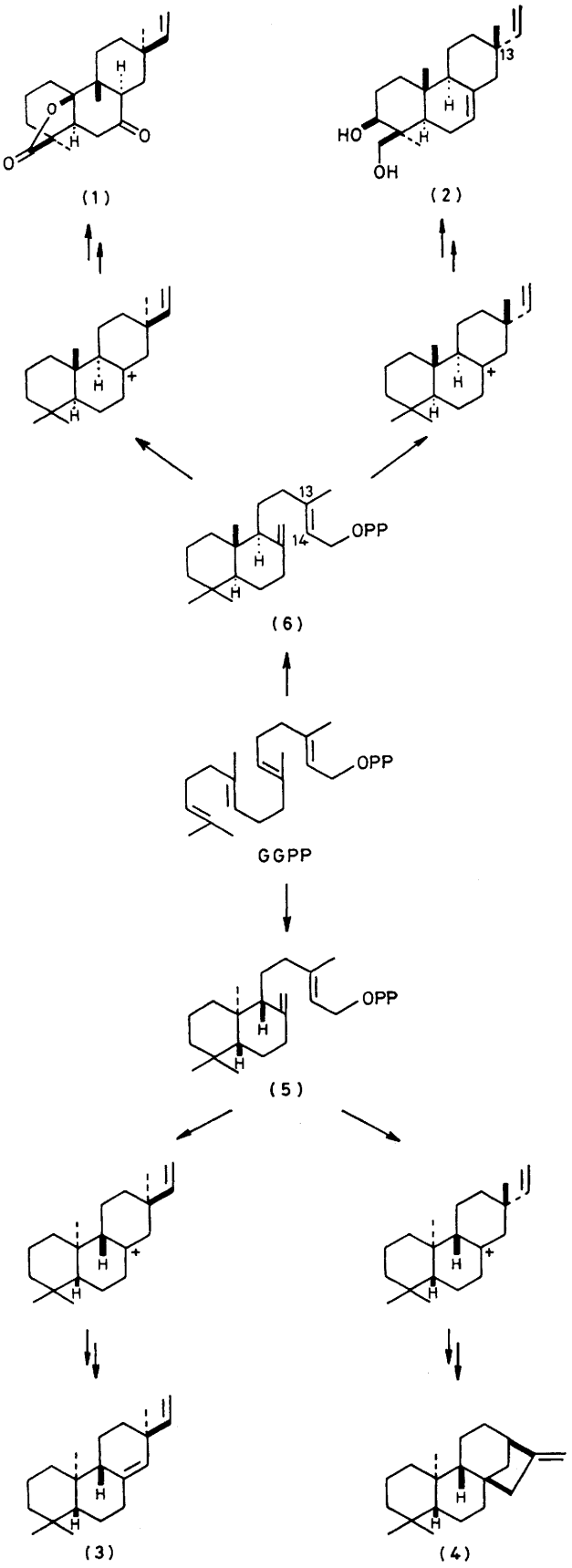
Summary Feeding of (5*R*)-[5-^{2}H]mevalonate to *Oospora virescens* and ^{2}H n.m.r. analysis of a derivative of the resulting virescenol B establish that the allylic displacement of pyrophosphate which generates ring c takes place with overall *anti* stereochemistry.

RECENT investigations at Brown University have established that in the biosynthesis of the diterpenoid fungal metabolite rosenonolactone (**1**), the allylic displacement of pyrophosphate in labdadienyl pyrophosphate (**6**) which generates ring c takes place with net *anti* stereochemistry.[1] An analogous *anti*-displacement occurs in the formation of the related diterpenes (+)-sandarocopimaradiene (**3**)[2] and (−)-*ent*-kaurene (**4**).[3,4] Each of these three diterpenes can be considered as the product of a single type of biochemical reaction, arising from a common bicyclic precursor, copalyl pyrophosphate (**5**) or its enantiomer (**6**); their distinction lies in the diastereomeric relationship of the tricyclic cationic intermediates, which differ in the configuration of the A/B ring system, and of the resulting angular vinyl and methyl groups (Scheme 1). The remaining member of this series is virescenol B (**2**),[5] the aglycone of the natural altrosyl compound virescenoside B. Biosynthetic studies utilizing [1-^{13}C]-, [2-^{13}C]- and [1,2-$^{13}C_2$]-acetate are consistent with a biosynthetic pathway to the virescenosides proceeding from geranylgeranyl pyrophosphate *via* (**6**) to the 13-*epi*-pimarene skeleton.[6] From the known 13*S* configuration of the virescenols[5] it is evident that during the allylic displacement by which ring c is formed cyclization occurs on the *re* face of the 13,14 double bond of (**6**). We report the results of a ^{2}H n.m.r. study[7] which establishes that this allylic displacement takes place with overall *anti* stereochemistry.

Feeding of 4·85 mmol of sodium [5-$^2H_2$]mevalonate (**7a**),[8] containing $3·67 \times 10^6$ d.p.m. of [2-^{14}C]mevalonate, to 0·5 l of an 8-day-old culture of *Oospora virescens* (synonymous with *Acremonium luzulae*), followed by incubation for a further 22 h gave, after isolation and mild acidic hydrolysis, 0·093 g of purified virescenol B (**2a**) ($8·87 \times 10^4$ d.p.m./mmol, 2·9% enrichment per labelled site). This product was acetylated and treated with 1·1 equiv. of *m*-chloroperbenzoic acid (Et_2O, 4 h, 25 °C), yielding a mixture of 7,8-epoxy-diacetates (**8**) and (**9**).† The two epoxides were separated by p.l.c. on silica with CH_2Cl_2 (3 developments): (**8a**) (45%), R_f 0·15, $[\alpha]_D^{25}$ −2·10° (*c* 1·00, $CHCl_3$), m.p. 88—89 °C; (**9a**) (17%), R_f 0·25 $[\alpha]_D^{25}$ −25·8° (*c* 1·00, $CHCl_3$), m.p. 169 °C; unchanged virescenol B diacetate (16%), R_f 0·45, m.p. 134 °C. Examination of several derivatives of (**2**) established that the ^{1}H n.m.r. spectrum of the α-epoxy-diacetate in C_6D_6 showed the best separation of the terminal vinyl proton resonances: δ 5·11 (16-H_A), 5·00 (16-H_B), and 6·36 (15-H); J(15-H,16-H_A) 17·4, J(15-H, 16-H_B) 10·6, and J(16-H_A,16-H_B) 1·1 Hz. The 41·44 MHz ^{2}H n.m.r. spectrum of (**8a**) obtained on 26 mg in 0·5 ml of benzene displayed a poorly resolved pair of overlapping peaks of unequal line width at δ_D 5·05 (narrow) and 4·97 (br)‡ (Figure). In addition to these olefinic resonances a group of signals centred at δ_D 1·88 (1D), 1·46 (3D), 1·06 (1D), and 0·88 (1D) due to deuterons at C-2, C-6, and C-11 was also observed.

† The major product (**8**) has recently been assigned the α-configuration (M. Curini, P. Ceccherelli, R. Pellicciari, and E. Sisani, *Gazz. Chim. Ital.*, in the press). The interpretation of the ^{2}H n.m.r. spectra, however, is independent of the epoxide assignment.

‡ A similar disparity in line widths has previously been observed for the analogous terminal vinyl deuterons of (**1**) [$\nu_{\frac{1}{2}}$ (H_A) 3·5 Hz, $\nu_{\frac{1}{2}}$ (H_B) 7·5 Hz] (ref. 1) and may be explained by the somewhat longer T_1 of 16-H_A resulting from local rotation about the C-13, 15 bond (ref. 7b).



SCHEME 1

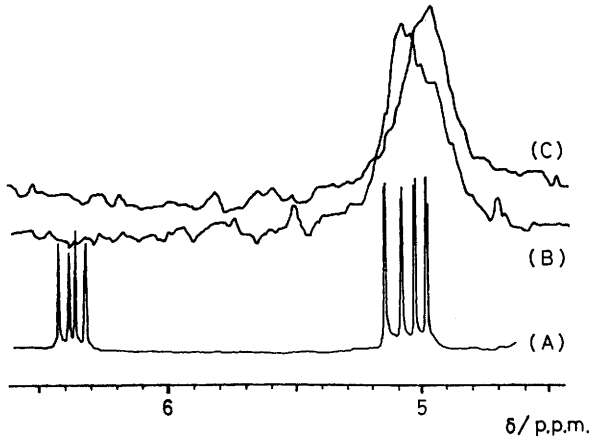
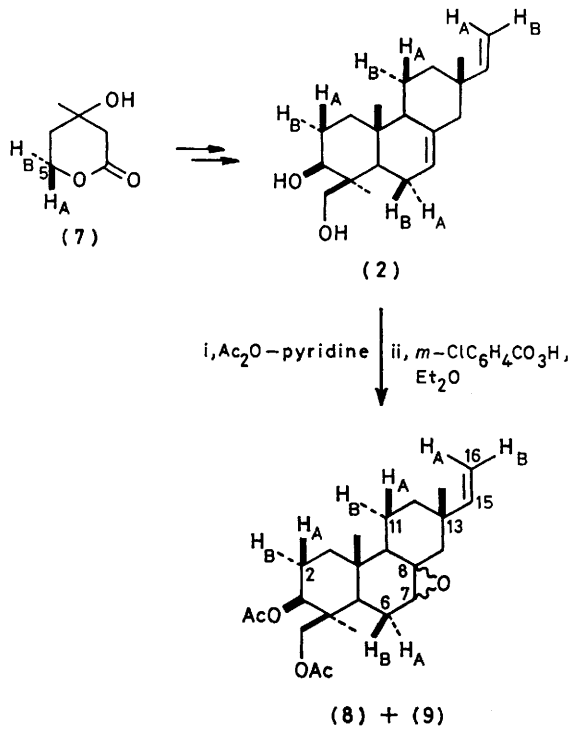


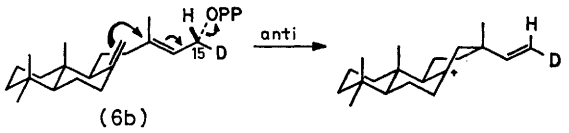
FIGURE. (A) 270 MHz ^{1}H n.m.r. spectrum of vinyl protons of (**8**); (B) Bruker HX 270, 41·44 MHz ^{2}H n.m.r. of (**8a**), 4*K* data points, quadrature detection, 90° pulse, spectral width 1000 Hz, 9067 transients, LB (line broadening) = 0·5; (C) 41·44 MHz ^{2}H n.m.r. of (**8b**), 7961 transients, LB = 0·5.

Feeding of 2·13 mmol of (3*RS*,5*R*)-[5-^{2}H]mevalonate (**7b**), (85%-D) prepared as previously described[1] and containing $3·67 \times 10^6$ d.p.m. of [2-^{14}C]mevalonate gave, after hydrolysis, labelled (**2**) which was once again analysed as the corresponding α-epoxy-diacetate (**8b**) ($2·02 \times 10^5$ d.p.m./mmol, 2·5% enrichment per labelled site). The ^{2}H n.m.r. spectrum showed δ_D 4·97 (s, $\nu_{1/2}$ 8·6 Hz, 16-D_B). Since the intermediate deuteriated (**6b**) has the 15*R* configuration, unchanged from that of the (5*R*)-[5-^{2}H]-mevalonate precursor, the observed result is consistent with



a; $H_A = H_B = D$
b; $H_A = H$, $H_B = D$

an overall *anti* displacement of pyrophosphate in the second cyclization step§ (equation 1).



(6b) (1)

The demonstration that the biosynthesis of (**2**) involves an *anti* allylic displacement, as do the formation of the rosane, *ent*-sandaracopimarane, and *ent*-kaurane classes of diterpenes, establishes that the stereochemical course of these biochemical transformations is independent of both the relative and the absolute configuration of substrates and products, and therefore must reflect some fundamental property of the catalytic mechanism itself.¶ These results take on added significance with the recognition that the vast majority of tri- and tetra-cyclic diterpenes can be derived from one of the four diastereomeric pimarenyl cations.[9]

This research was supported by a grant from the N.S.F. (D.E.C.). The Bruker HX270 Spectrometer at Yale University is supported as part of the Northeast Regional NSF-NMR Facility. 270 MHz ^{1}H n.m.r. spectra of (**8**) and (**9**) were recorded by D.E.C. on a Bruker WH 270 at the University of Chicago.

(*Received, 28th October* 1980; *Com.* 1164.)

§ As little as 10% *syn* displacement would have been detected. Although we cannot rigorously exclude a smaller *syn* component we have made the reasonable assumption that the enzyme-catalysed process is completely stereospecific.

¶ It has been shown that a structurally unrelated allylic displacement in the biosynthesis of the diterpene pleuromutilin also occurs with exclusive *anti* stereochemistry (H. Hasler, *Diss. ETH Zurich*, 1979, No. 6359; D. E. Cane, *Tetrahedron*, 1980, **36**, 1109).

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