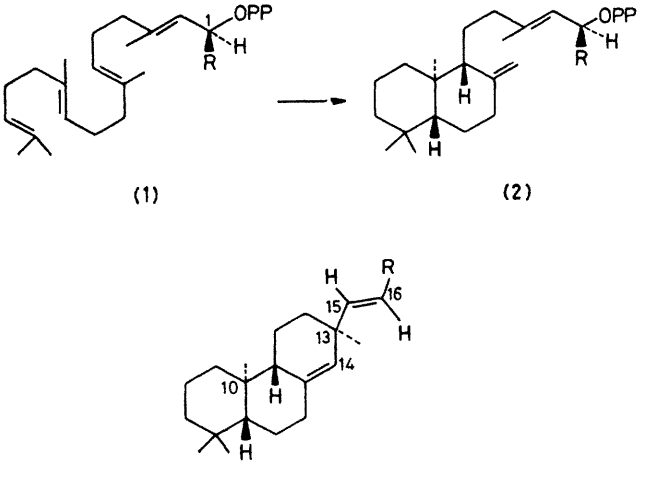
Stereochemistry of the S_N' Cyclization in the Biosynthesis of *ent*-Sandarocopimaradiene with Enzyme Extracts from Seedlings of *Ricinus communis* L.

By KEITH A. DRENGLER and ROBERT M. COATES*
(*Department of Chemistry, University of Illinois, Urbana, Illinois* 61801)

Summary Incubation of (*S*)-[1-$^2H_1$]geranylgeranyl pyrophosphate (**1b**) with an enzyme extract from castor bean (*Ricinus communis* L.) seedlings produced (*E*)-[16-$^2H_1$]-*ent*-sandarocopimaradiene (**3b**); thus, the S_N' cyclization of the intermediate copalyl pyrophosphate (**2b**) occurs with *anti*-stereochemistry.

THE S_N' cyclization of copalyl pyrophosphate (**2a**) (or its enantiomer) is a common step in the biosynthesis or biogenesis of many types of tricyclic (*e.g.*, *ent*-sandarocopimaradiene, **3a**) and tetracyclic diterpenes.[1] The stereochemistry of this enzyme-catalysed S_N' reaction is of interest as a probe for the conformation of the allyl pyrophosphate at the time of cyclization and for comparison with the stereochemistry of chemically induced S_N' processes in solution.[2] Cane and Murthy have recently reported that the biosynthesis of rosenonolactone from (5*R*)- and (5*S*)-[5-$^2H_1$]mevalonic acid in cultures of *Trichothecium roseum* proceeds by *anti*-S_N' cyclization of the enantiomer of (**2a**).[3] We relate experiments which establish the stereochemistry of the S_N' cyclization of copalyl pyrophosphate (**2a**) in the biosynthesis of *ent*-sandarocopimaradiene (**3a**) from geranylgeranyl pyrophosphate (**1a**) in enzyme extracts from castor bean (*Ricinus communis* L.) seedlings (Scheme).[4]



SCHEME. **a**; R = H
b; R = D

(*E,E,E*)-Geranylgeraniol was prepared from (*E,E*)-farnesylacetone by condensation with trimethyl phosphonoacetate (NaH, 1,2-dimethoxyethane, 60 °C, 1 h, then room temperature, 18 h),[5] followed by medium pressure liquid chromatography to remove the (2*Z*)-ester, and reduction with aluminium hydride (ether, 0 °C, 1 h). Deuterium or tritium label was introduced at C-1 by oxidation with activated manganese dioxide (hexane, 0 °C, 3 h) and subsequent reduction with sodium borodeuteride or tritium-labelled sodium borohydride (ethanol, room temperature, 2 h).[6] The alcohol was converted into the pyrophosphate by the Cramer procedure and purified by ion exchange chromatography.[6] A reference sample of (−)-sandarocopimaradiene was prepared from sandarocopimaric acid[7] to assist the isolation and identification of the biosynthetic product.

The biosynthesis of (+)-sandarocopimaradiene (**3a**) from geranylgeranyl pyrophosphate (**1a**) was carried out with soluble enzyme extracts from 2·5—3-day old seedlings of castor bean as described by Robinson and West.[4] The seedlings (30 g) were homogenized in the presence of Polyclar AT (3 g), Amberlyte XAD-4 resin (3 g),[8] β-mercaptoethanol (72 μl), and buffer solution (80 ml, pH 7·4) consisting of 50 mM Tris base, 50 mM potassium hydrogen carbonate, 10 mM magnesium chloride, 0·5 mM manganese chloride, 10 mM magnesium chloride, and 10 mM β-mercaptoethanol. The homogenate was centrifuged at 30,000 g for 20 min and 150,000 g for 60 min.

Large-scale incubations of [1-$^3H_1$]geranylgeranyl pyrophosphate (*e.g.*, 3 μmol; specific activity 13·6 mCi/mmol) with 520 ml of the final supernatant fraction (S-150) for 20—24 h at 30 °C afforded, after denaturation with 1:1 methanol–acetone, extraction with light petroleum, and filtration of the light petroleum extract over silica gel, a mixture (*ca.* 190—280 μg, 23—34% of substrate radioactivity)† of (−)-trachylobane, (−)-kaurene, (+)-sandarocopimaradiene, (+)-beyerene, and casbene. The diterpene hydrocarbons were then separated by elution from a column of silver nitrate-impregnated silica gel with a gradient of benzene in hexane. The third component to be eluted was identified as sandarocopimaradiene (28—57 μg, 3—7% of substrate radioactivity) by comparison of its t.l.c. mobility on silver nitrate-impregnated silica gel, g.c. retention time, mass spectrum, and ^1H n.m.r. spectrum with those of authentic (−)-sandarocopimaradiene. The 220 MHz ^1H n.m.r. spectrum of sandarocopimaradiene in [$^2H_6$]benzene exhibits a singlet at δ 5·35 for the vinyl hydrogen at C-14 and a typical ABX pattern for the three hydrogens on the vinyl group: δ_A 4·98 (H at C-16, *cis* to H_X), δ_B 5·05 (H at C-16, *trans* to H_X), and δ_X 5·87 (H at C-15); J_{AB} = 1·4 Hz, J_{AX} = 10·7 Hz, J_{BX} = 17·5 Hz.

The stereochemistry was revealed by stereospecific deuterium labelling. A mixture of (*S*)-[1-$^2H_1$]- and (*S*)-[1-$^3H_1$]geranylgeraniol (94% $^2H_1$ by m.s.; specific activity 1·0 μCi/μmol) was prepared by enzymic reduction of a mixture of [1-$^2H_1$]- and [1-$^3H_1$]-geranylgeraniol with liver alcohol dehydrogenase and NAD^+ (0·1 M aqueous phosphate buffer containing 0·57 M ethanol and Tween 80, 30 °C, 19 h).[9] The (*S*) stereochemistry of the [1-$^2H_1$]geranylgeraniol prepared by this procedure has been independently verified by the

† The yield calculations are corrected for the loss of 50% of the tritium in the biosynthesis of beyerene and casbene.

n.m.r. method of Gerlach and Zagalak.[9] Incubation of the corresponding pyrophosphate (**1b**) with the S-150 enzyme extract from castor bean seedlings provided [16-$^2H_1$]sandarocopimaradiene following the isolation and chromatographic purifications described above. The sandarocopimaradiene was further purified by preparative high pressure liquid chromatography on a 3·9 mm × 30 cm μ-Bondapak-C_{18} reversed-phase column with 9:1 methanol–water as eluant. The 220-MHz n.m.r. spectrum of the [16-$^2H_1$]sandarocopimaradiene (76 μg; 90% $^2H_1$ by g.c./m.s. analysis; 5—7% of substrate radioactivity) in [$^2H_6$]benzene obtained by Fourier transform accumulation of 4000 pulse sequences showed a clean pair of doublets for the deuterium-substituted vinyl group: δ 5·04 (d, $W_{\frac{1}{2}}$ 5·0 Hz, J 17·3 Hz, 16-H) and 5·87 (d, $W_{\frac{1}{2}}$ 5·0 Hz, J 17·2 Hz, 15-H). Consequently the hydrogens at C-15 and C-16 are *trans* as shown in (**3b**) and the stereochemistry of the S_N' cyclization of (**2b**) has occurred with the *anti*-stereochemistry.

It is interesting that the cyclases produced by *R. communis*, a higher plant, and *T. roseum*,[3] a fungus, which utilize enantiomeric substrates for the biosynthesis of diterpenes differing in the relative configurations at C-10 and C-13 both effect the cyclization of their allyl pyrophosphate substrates *via* the *anti*-S_N' pathway. Similar *anti*-S_N' cyclizations have also been found in the biosynthesis of pleuromutilin by cultures of *P. mutilus*,[2,10] and, with the assumption of a 'least motion' mechanism, in the conversion of (**2**) into *ent*-kaurene in enzyme preparations from *M. macrocarpus*.[9,11] Examples of both *syn*[12] and *anti*[13] S_N' cyclizations of allylic compounds have been noted in the recent literature.

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