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INTERCONVERSION OF *TRANS, TRANS* AND *CIS, TRANS* FARNESOL BY ENZYMES FROM *ANDROGRAPHIS*

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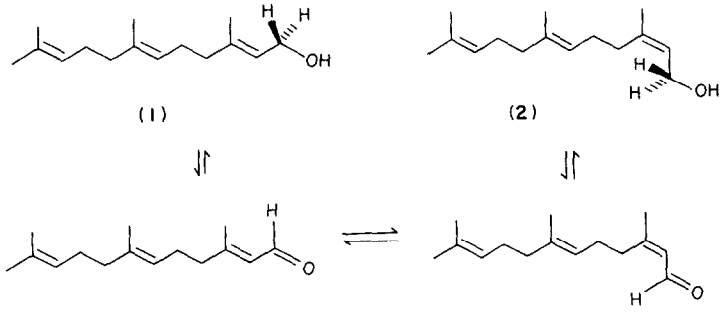
Key Word Index—*Andrographis paniculata*; Acanthaceae; *trans, trans*- and *cis, trans*-farnesol interconversion, tissue cultures

Abstract—When *trans, trans*-farnesol [4,8,12-$^{14}C_3$,1-$^{3}H_2$] is isomerized to *cis, trans*-farnesol by soluble enzymes from *Andrographis paniculata* tissue cultures, 50% of the tritium label is lost The same loss is observed when isomerization occurs in the opposite direction This is in accordance with the proposed mechanism for isomerization via aldehydes

IN RECENT experiments with cell-free preparations from tissue cultures of *Andrographis paniculata* we have found[1] that the 2-*trans*,6-*trans*-farnesol (tt-F) (**1**) formed retains all the tritium label from mevalonate 3RS-[2-^{14}C,5-$^{3}H_2$] substrate, while the 2-*cis*,6-*trans*-farnesol (ct-F) (**2**) formed retains only five-sixths of the tritium label. These results can be regarded as evidence favouring the isomerization of initially formed (**1**) to (**2**) via intermediate aldehydes, as illustrated in the Figure.



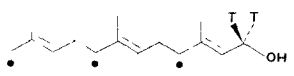
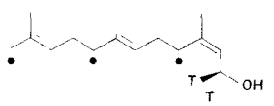
Our previous results furnished direct evidence concerning the forward reaction [(tt-F) to (ct-F) conversion], but it seemed to us desirable to obtain evidence that the forward *and* backward reactions can proceed with comparable ease and stereospecificity, a situation implied in the equilibria illustrated in the Figure. To this end samples of [4,8,12-$^{14}C_3$,1-$^{3}H_2$]tt-F and [4,8,12-$^{14}C_3$,1-$^{3}H_2$]ct-F were prepared by mixing chemically synthesized farnesol [1-$^{3}H_2$] (tt- or ct-) with the corresponding farnesol [4,8,12-$^{14}C_3$] prepared enzymically from mevalonate 3RS[2-^{14}C] by the cell-free preparation from *A. paniculata* tissue cultures (see Experimental). Each doubly labelled substrate was incubated with the 105000 *g* fraction prepared from hypocotyl suspension cultures of *A paniculata* in the presence of NADH/NADPH and ATP for 12 hr at 27° (see Experimental).

[1] OVERTON, K H and ROBERTS, F M (1973) *J C S Chem Commun* 378

The mixture of tt-F and ct-F formed from each substrate [(ct-F) or (tt-F)] was recovered from the hexane-soluble fraction by preparative-layer chromatography (PLC). GLC analysis showed that incubation of *either* tt-F or ct-F had resulted in an approx 4:1 ratio of tt-F to ct-F. The compounds were separated by PLC on $AgNO_3$-silica gel and each isomer was then converted into the acetate and TMS ether for radioactive counting. The $^3H/^{14}C$ ratios for the substrates and products formed from them are shown in Table 1. The loss of 50% of tritium label in both the tt-F to ct-F and the ct-F to tt-F conversion is clearly in accord with the equilibria set out in the figure. The shortfall of a significant proportion of tritium label (*ca* 10%) in the recovered substrates in each case, probably represents that fraction which has been converted (with tritium loss) into aldehyde and back to alcohol.

These results support the proposed mechanism, illustrated in the figure, for the interconversion of tt-F and ct-F by the 105000 *g* cell-free fraction from *A. paniculata* tissue cultures. The stereochemistry of hydrogen loss from C-1 of the isomeric farnesols during these interconversions is currently under investigation in our laboratory.

TABLE 1. LOSS OF ^3H FROM C-1 OF FARNESOLS DURING *trans* → *cis* AND *cis* → *trans* ISOMERIZATIONS BY SOLUBLE ENZYMES FROM *Andrographis paniculata* TISSUE CULTURES.

Substrate	Product	$^3H/^{14}C$ substrate	$^3H/^{14}C$ product	% ^3H retention
	*trans*-OAc	7·95	7·20	91
	trans-OTMS		7·09	89
	cis-OAc		4·08	52
	cis-OTMS		3·96	49
	*cis*-OAc	9·86	9·11	93
	cis-OTMS		8·69	88
	trans-OAc		5·25	54
	trans-OTMS		4·68	47

EXPERIMENTAL

Preparation of substrates. 2-Trans,6-trans- *and* 2-cis,6-trans- *farnesols* [1-$^3H_2$]. Commercial farnesol mixture (*trans,trans*, *cis,trans* 2:1) was oxidized to the corresponding farnesals with MnO_2 in C_6H_6.[2] An excess of this farnesol mixture (2 mg, 10 μmol) purified by PLC (silica gel; EtOAc–hexane 1:3) was reduced with NaB^3H_4 (2·5 μmol; 1·5 mCi) in *iso*-PrOH (2 ml) at 0° during 12 hr. The resulting mixture of [1-$^3H_2$]tt-F and [1-$^3H_2$]ct-F was separated from unreacted aldehydes by PLC (silica gel; $CHCl_3$–MeOH 9:4:6); the two tritiated farnesols separated one from the other by PLC[3] ($AgNO_3$-silica gel; EtOAc–HOAc 0·2%) and purified by PLC[4] (silica gel; EtOAc–hexane 1:3). Detection in each case was by radio-scanning.

2-trans,6-trans- *and* 2-cis,6-trans *farnesols* [4·8,12-$^{14}C_2$]. Mevalonate 3RS-[2-^{14}C] (50 μCi) as the K salt[4] was incubated with the cell-free preparation from *A. paniculata* hypocotyl tissues under standard conditions (*q.v.*). The ^{14}C-labelled farnesols, obtained by extraction into Et_2O, were isolated and purified in a similar manner to the ^3H-labelled farnesols. The singly labelled samples of tt-F and ct-F were mixed to afford doubly labelled samples with ^3H, ^{14}C ratios of about 10:1. Each mixed sample was re-chromatographed and portions converted into the acetate and tetramethylsilyl ether which were chromatographed and their ^3H, ^{14}C ratios determined. The ratios shown in the Table for substrate in each case represent an average value for the acetate and TMS ether.

Preparation of cell-free extracts. The culture techniques have been described.[5] Hypocotyl tissues grown in suspension-culture for 21 days were collected by filtration and homogenized in a pre-cooled homogenizer for 2 min at 0° in Na phosphate buffer (1 ml buffer 1 g fresh cells, 0·1 M, pH 7·6) containing sucrose (0·35 M), GSH (5 mM), nicotinamide (5 mM) and soluble PVP (MW 24500; 1% w/v). The homogenate was centrifuged at 105000 *g* for 1 hr at 4° and the supernatant used directly for incubation.

[2] GOLDMAN, I. M. (1969) *J. Org. Chem.* **34**, 1979.
[3] CORNFORTH, J. W., CORNFORTH, R. H., DONNINGER, C. and POPJAK, G. (1966) *Proc. Roy. Soc. (B)* **163**, 492.
[4] CORNFORTH, J. W., CORNFORTH, R. H., DONNINGER, C., POPJAK, G., RYBACK, G. and SCHROEPFER, G. J. (1966) *Proc. Roy. Soc. (B)* **163**, 436.
[5] BUTCHER, D. N. and CONNOLLY, J. D. (1971) *J. Exp. Botany* **22**, 314.

Incubations The substrate (*ca* 25000 dpm ^{14}C) was suspended in 0 5 ml Na phosphate buffer (0 1 M, pH 7 6) with 0 5 mg Tween 80 by sonicating for 10 min, and then incubated with the 105000 *g* supernatant (3 ml) containing NADH/NADPH (0 05 mM each), ATP (1 mM), $MgCl_2$ (0 1 mM), $MnCl_2$ (0 1 mM), NaF (1 mM) (total vol 4 0 ml) for 12 hr at 27° in a 10 ml Warburg flask The flask was evacuated and filled with N_2 3 × before mixing substrate and medium

Isolation and purification of farnesols Incubations were terminated by adding ice-cold EtOH (8 ml) After 30 min the protein ppt was removed by centrifugation at 4000 *g* for 5 min and the supernatant extracted with hexane (3 × 10 ml) To the combined, dried hexane extracts was added carrier farnesol mixture (10 mg) The tt-F and ct-F were isolated and separated as described under the preparation of labelled farnesols and the separation checked by GLC (3 5 m × 3 mm 1% SE30, 150°) Each alcohol was then converted into the acetate and TMS ether as before The $^3H/^{14}C$ ratios for the derivatives obtained in the two experiments are shown in the Table

Radio-assay Samples were assayed by liquid scintillation counting with 2,5-diphenyloxazole (5 g/l) and 1,4-bis-(5-phenyloxazol-2-yl) benzene (0 1 g/l) in toluene as scintillant Where necessary MeOH was added to effect soln Counting efficiencies were typically ca 90% for ^{14}C alone and 75 and 32% respectively for ^{14}C and ^3H in experiments using both nuclides Corrections for counting efficiency and quenching were automatically computed, using the channels ratio mode with ^{137}Cs as external standard Typically samples registered 500–5000 dpm from ^{14}C and at least 4×10^4 disintegrations per sample were normally accumulated, ensuring for 2σ a maximum value of ±1% Substrates were diluted with inactive material to adjust counting levels to that of the product In doubly labelled samples the maximum overlap of ^{14}C into the ^3H channel was *ca* 5%, so that for a minimum $^3H/^{14}C$ ration of 7, the ^{14}C counts recorded in the ^3H channel did not exceed 0 6% of the total

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