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CORRELATION BETWEEN LOSS OF *PRO*-CHIRAL HYDROGEN AND *E*, *Z* GEOMETRY IN ISOPRENOID BIOSYNTHESIS

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Key Word Index—Biosynthesis; monoterpenes; sesquiterpenes; alkene formation; stereochemical correlations.

Abstract—Biosynthesis of several mono- and sesqui-terpenes that possess *E* or *Z* double bonds, or which are generally considered to be derived from precursors possessing such geometries, involved loss of the *pro*-4S hydrogen of mevalonate in the construction of the double bond. These results confirm and extend previous observations. A recent claim to have newly discovered such a stereochemical correlation is rejected.

Table 1 records the normalized isotope ratios in several mono- and sesquiterpenes biosynthesized from [^{14}C, ^{3}H]-MVA in various plant species. All products were purified to constant specific radioactivity, and although incorporations of tracer were low (except for the *Rosa* species), the values obtained were typical for this type of experiment.

The *pro*-4S hydrogen of MVA was lost in the construction of double bonds derived from IPP-derived moieties (or their biogenetic equivalents) irrespective of whether an *E* or *Z* bond was formed in products or whether (according to the usually-accepted scheme for isoprenoid biosynthesis) GPP or NPP (*E*, *Z* isomers respectively) were the precursors of the products. Formation of DMAPP (the starter unit in isoprenoid biosynthesis) from IPP is known to involve loss of the *pro*-4S hydrogen of MVA [1] and our results are consistent with this stereochemical consequence of IPP–DMAPP isomerase.

These results confirm and extend previous studies. Loss of the *pro*-4S hydrogen of MVA (or the equivalent *pro*-2R hydrogen of IPP) had been demonstrated in the formation of geraniol (*E*-isomer) and nerol (*Z*-isomer) in a *Rosa* species and in *Pelargonium* and *Tanacetum* species [1, 2]; and also in geraniol, nerol, 2(*E*),6(*E*)-farnesol, 2(*Z*),6(*E*)-farnesol and their pyrophosphates biosynthesized in cell-free preparations from *Pinus* and *Citrus* species [3].

In addition, the same prochiral hydrogen was lost in the formation of the bicyclic monoterpenes α-pinene [4], car-3-ene [5] and *trans*-thuj-3-one [2] that are generally considered to be derived from NPP (*Z*-isomer); and also in 2(*E*),6(*E*)-farnesol and higher terpenoids containing *E*-double bonds derived from it by chain extension [6–9]. The only examples of the loss of the *pro*-4R hydrogen of MVA are in the formation of *Z* double bonds in rubber [8] and in certain polyprenols [10–12]. Thus there is no obligatory correlation, as was once thought [8], between the prochirality of the hydrogen lost in the formation of double bonds in isoprenoids and the *E* or *Z* geometry about the bond. The spatial relationships between prenyltransferase and the *pro*-2R (eliminated) hydrogen of IPP are well understood for the avian liver enzyme [13], and a rationale for the formation of the *Z* double bond with loss of this prochiral hydrogen in plant enzymes has been proposed [14].

Recently, Suga *et al.* have reported that the *pro*-4S hydrogen of MVA is lost in the formation of *Z* double bonds in malloprenol and certain other isoprenoids [15, 16]. They claim that these are the first examples that break the rule that loss of the *pro*-4S hydrogen of MVA is correlated with the formation of an *E*-double bond whereas loss of the epimeric 4R hydrogen leads to the formation of a *Z* bond [cf. 8]. In view of the work mentioned above [1–4] which has been extensively reviewed and discussed [cf. 13, 14, 17, 18], this is an astonishing claim that can be categorically rejected!

EXPERIMENTAL

Feeding experiments. [^{14}C, ^{3}H]-MVA (^{3}H/^{14}C, 6–8; 0.1 g; total 10–30 μCi) in C_6H_6 were mixed, assayed and stem fed to the plant specimens [young shoots or opening flowerheads (for *Rosa* species); 15 cm; 50 g] under forced transpiration in June to September. After uptake of tracer (0.5 hr) the plants were kept in distilled H_2O for 48 hr under natural illumination and temperature, harvested, carrier added and the terpene isolated by GC (Carbowax 20 M; 6 m × 0.3 cm; 120–150°; 3–6 1 hr^{-1} N_2). Menthone was purified as the 2,4-dinitrophenylhydrazone and citronellol as its phenylurethane. Sabinyl acetate was converted into thuj-3-one which was purified as the 4-phenylsemicarbazone; 1,8-cineole was purified as its adduct with *o*-chlorophenol. Geraniol and nerol were oxidized with MnO_2 to their aldehydes and thence purified as semicarbazones. All derivatives (yields > 70%) were recrystallized to constant specific radioactivity (ex MeOH–H_2O; EtOH–H_2O; C_6H_6 variously).

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Abbreviations: MVA, mevalonate; IPP, isopentenyl pyrophosphate; DMAPP, 3,3-dimethylallyl pyrophosphate; GPP, geranyl pyrophosphate; NPP, neryl pyrophosphate; FPP, farnesyl pyrophosphate.

Table 1. Incorporation of [2-^{14}C, 4-$^{3}H_1$]MVA into isoprenoids

Product	Δ-type*	Species†	Precursor	$^{3}H/^{14}C$‡ Product(4R)	Product(4S)
Geraniol	*E*	*Rosa damascena* L.§	1.00	0.96	0.05
Nerol	*Z*	*Rosa damascena* L.	1.00	0.98	0.02
Citronellol	*E* or *Z*	*Rosa damascena* L.	1.00	0.96	0.01
Geraniol	*E*	*Rosa gallica* L.‖	1.00	1.01	0.02
Nerol	*Z*	*Rosa gallica* L.	1.00	0.98	0.01
1,8-Cineole	*Z*	*Eucalyptus globulus* L.	1.00	1.00	0.03
Menthone	*Z*	*Mentha arvensis* L.	1.00	0.95	0.03
Sabinyl acetate	*Z*	*Juniperus sabina* L.	1.00	0.98	0.01
Limonene	*Z*	*Mentha piperita* L.	1.00	0.97	0.01
Longifolene	*E*, *Z*	*Pinus longifolia* L.	1.00	0.98	0.02

*Geometry of the double bond derived from the IPP unit (for geraniol and nerol) *or* for others the geometry of the presumed parent (NPP or FPP).

†Flowerheads (*Rosa* species) or foliage (others).

‡Normalized isotope ratios for precursor ([2-^{14}C, 4R-$^{3}H_1$]-MVA and its 4S epimer) and for products derived from the 4R and 4S isomers respectively. $^{3}H/^{14}C$ in MVA was 6–8. Estimated s.e. was ±0.01 for MVA and ±0.04 for products. Values are the mean of two independent experiments carried out under each set of conditions. Typically the ^{14}C in products was 10^3 to 10^4 dpm. Incorporation of (3R)-[2-^{14}C]-MVA was 10–16% (*Rosa* species) and 0.005–0.08% (others).

§Var. *versicolora*.

‖Var. *officinalis*.

Limonene and longifolene were purified by TLC on silica gel H and $AgNO_3$–silicic acid (C_6H_6–EtOAc; Et_2O variously) until bands (4–6) cut across overloaded and smeared traces were of equal specific radioactivity.

Radiochemical techniques. The channels-ratio method was used with counting efficiencies (butyl-PBD in toluene) of *ca* 95 and 40% respectively for ^{14}C and ^{3}H. The maximum overlap of ^{14}C into the ^{3}H channel was *ca* 5%: hence for $^{3}H/^{14}C$ *ca* 6, the contribution of ^{14}C to the ^{3}H channel was < 1%, and could be allowed for. For each assay *ca* 4×10^4 disintegrations were accumulated so that 2σ was ±1%.

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