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BIOSYNTHETIC ORIGIN OF THE *GEM*-METHYLS OF GERANIOL*

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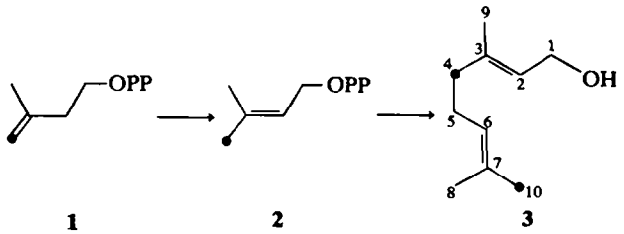
Key Word Index—*Rosa dilecta*; Rosaceae; biosynthesis; geraniol.

Abstract—Degradation of geraniol (3,7-dimethylocta-*trans*-2,6-dien-1-ol) biosynthesized in *Rosa dilecta* has proved that C-10 is exclusively derived from C-2 of mevalonate. This verifies the generally accepted but hitherto unproven view of the origin of the *gem*-methyls in this compound and of the corresponding groups in other terpenoids.

INTRODUCTION

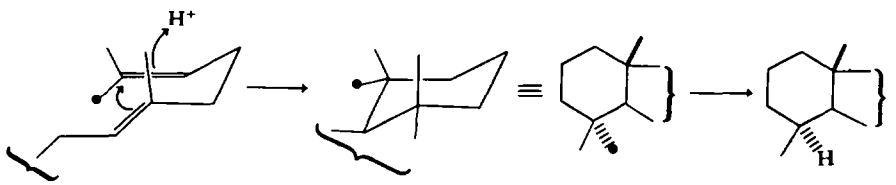
It is universally accepted that isopentenyl pyrophosphate (**1**; IPP) is converted into 3,3-dimethylallyl pyrophosphate (**2**; DMAPP) such that C-2 of the parent mevalonate (MVA) yields the methyl *trans* to the ester group in the latter (Scheme 1). It follows that



Scheme 1. ● is derived from C-2 of MVA. OPP = pyrophosphate.

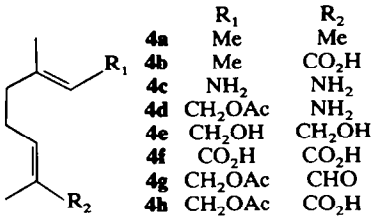
these C_5 moieties will then condense to yield geraniol (**3**; 3,7-dimethylocta-*trans*-2,6-dien-1-ol) with two carbons, as shown, derived from C-2 of MVA [1–3]. Geraniol is thought to be the parent of all classes of regular mono- and higher terpenoids, and this distinction between the *gem*-methyls is believed to be carried over into the corresponding groups of the derived compounds. Detailed interpretations of the mechanism of action of IPP–DMAPP isomerase (EC 5.3.3.2) also assume this stereochemistry for the interconversion of **1** and **2** [3, 4].

The evidence for this fundamental point in terpene biosynthesis is, however, meagre, and consists of two sets of observations. Firstly, that loss of an α-methyl from C-4 of certain sesquiterpenes and higher terpenoids formed from MVA-[2-^{14}C] resulted in loss of tracer: this is consistent with the route in Scheme 2 (see discussion in ref. [5]), but the interpretation begs questions as to the stereochemistry (inversion or retention) of the enzymic decarboxylation and also as to the possibility of an 'X-group' mechanism involving addition of HX (H–SEnz?) to the terminal double bond followed by displacement of X with overall inversion of configuration at the carbon centre by the electrons of the internal double bond. Secondly, degradation studies on the fungal metabolite mycelianamide are repeatedly quoted to be relevant for this situation [5]. This compound contains a monoterpene moiety, which was split off and converted into **4a** and thence into **4b** by means of feeding the former to a rabbit and working up the animal's urine. In the one experiment recorded, **4b** obtained from mycelianamide that had been biosynthesized from MVA-[2-^{14}C] was decarboxylated and it was shown that tracer was distributed *ca* 5:1 between the *gem*-methyls, the bulk being liberated as CO_2. An explanation for the non-stereospecificity of location of tracer, based on the preconception that the stereochemistry of interconversion of IPP and DMAPP must have been as in Scheme 1, was proposed. However, the authors were extremely careful to point out that their work did *not* provide an unequivocal proof for the

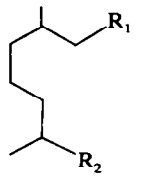


Scheme 2.

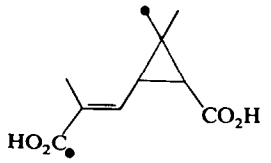
* Part 25 in the series "Terpene Biosynthesis". For Part 24 see Anand, A., Banthorpe, D. V. and Rowan, M. G. (1980) *Phytochemistry* **19**, 1433. Reprints of this paper are not available.



	R_1	R_2
4a	Me	Me
4b	Me	CO_2H
4c	NH_2	NH_2
4d	CH_2OAc	NH_2
4e	CH_2OH	CH_2OH
4f	CO_2H	CO_2H
4g	CH_2OAc	CHO
4h	CH_2OAc	CO_2H



	R_1	R_2
6a	NH_2	NH_2
6b	CH_2OH	CH_2OH
6c	CO_2H	CO_2H



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Scheme 3

stereospecificity of the step or for the stereochemical consequences for higher classes of terpenes. Later commentators have ignored this qualification completely! It is worth noting that the proof that pyrethrins biosynthesized from MVA-[2-^{14}C] contained a monoterpenoid fragment labelled as shown in **5** [6] is not relevant to the present discussion as geraniol is not a precursor of this irregular monoterpene, and indeed DMAPP may not be directly involved in its biosynthesis [7, 8].

We have investigated these questions by degrading geraniol biosynthesized from MVA-[2-^{14}C] in *Rosa dilecta* cv Lady Seton (Rosaceae).

RESULTS AND DISCUSSION

The DMAPP-derived moiety of monoterpenes is generally feebly labelled by exogenous MVA (typically less than one tenth of that of the IPP-derived portion) owing to an endogenous pool of the C_5 compound [9]. However, very high incorporations of MVA-[2-^{14}C] (up to 22% of the *R*-isomer) into geraniol and nerol and their β-glucosides occur in petals of *Rosa* species [10] and here the parts derived from IPP and DMAPP are equally labelled. In addition, it was shown that the tracer was position-specific at C-4 and C-8 plus C-10, but the latter two positions could not be distinguished by the degradation methods used [10]. Consequently, we have now developed methods for differentiating between C-8 and C-10.

Firstly, we confirmed the previous work as to specificity of labelling. We found incorporation of MVA-[2-^{14}C] into geraniol (free and as β-glucoside) was 6–10% for the *R*-isomer and that to within ±2% (actual value) the tracer was distributed 50:50 between C-4 and C-8 plus C-10. Three routes were then used to locate tracer at C-10. One involved the use of an oxidase system from *Vinca rosea* that specifically converts geraniol into 10-hydroxygeraniol [11], followed by chemical degradation (route A); and the others (routes B and C) involved direct chemical degradation by initial specific oxidation at C-10. Details are in the Experimental. Route A yielded CO_2 derived from both C-1 and C-10 of geraniol, but we know from the preliminary degradations that C-1 does not contain appreciable amounts of tracer. However, the concomitantly formed diamine **4c** could not be isolated, presumably owing to oxidation and polymerization under the conditions (Schmidt reaction) of the decarboxylation. Route B yielded CO_2 only from C-10 of geraniol, but again the counter-fragment **4d** was intractable. But route C, which involved reduction of the double bonds of the skeleton before functionalization and decarboxylation, yielded both CO_2 (from C-1 and C-10) and the diamine **6a**, and gave a satisfactory isotope balance.

The results (Table 1) show that, within the limits of the experimental error, all the tracer in the DMAPP-derived moiety of geraniol was located at C-10. Hence, the generally accepted stereochemical distinction between the *gem*-methyls of this compound, and by inference of DMAPP and of higher terpenes, is proved.

Table 1. Degradation of geraniol biosynthesised in *R. dilecta*

Route*	Geraniol†	Products†,‡	Sources§
A	4639	CO_2: 2238	C-1 plus C-10
B	309	CO_2: 152	C-10
C	428	CO_2: 209; Diamine **6a**: 201	C-1 plus C-10

* Degradation procedure: see Experimental.

† dpm/mmol (s.e. ± 3%); aliquots containing 500–700 mg of labelled compound (>10^3 dpm) were assayed.

‡ Counter fragment to CO_2 not isolated in routes A and B; see Discussion.

§ Carbon atom(s) in geraniol liberated as CO_2.

EXPERIMENTAL

Materials. MVA-[2-^{14}C] (50 μCi) was fed to flowerheads of *R. dilecta* and harvested after 1 hr as described previously [10]. β-Glucosides were hydrolysed and geraniol was separated from nerol and purified to radiochemical homogeneity [10].

Degradation schemes. All intermediates and products had the expected ^{1}H NMR (60 MHz), MS and IR spectra and elemental analysis. Labelled geraniol obtained as above (0.21–1.51 μCi) was diluted with carrier (0.39–1.2 g) and degraded. 10-Hydroxygeraniol (**4e**), bp 65–66°/2 mm Hg, 7%, for use as standard, was prepared by oxidative condensation of isoprene over a metallic catalyst [12].

Route A. Geraniol hydroxylase (20 000 g pellet; 70 mg) was prepared from seedlings of *V. rosea* [11] and was reconstituted [11] to convert geraniol-[^{4}C] into its 10-hydroxy derivative. The incubation mixture (1.5 ml) was extracted with $CHCl_3$ (5 ml) and evapd under N_2 at 20°. The product was separated by TLC on Si gel with C_6H_6–Me_2CO–EtOAc (2:1:1), R_f 0.72. Carrier was added and the product was rechromatographed and oxidized with $H_2Cr_2O_7$ [13] to yield **4f** (88%), 200–205°/1 mm Hg. Alternatively, the conversion was carried out in two steps with (a) MnO_2 [14] and (b) $AgNO_3$–NaOH [15]. Compound **4f** (60%) was then cleaved by the Schmidt procedure to yield CO_2 [16].

Route B. Geraniol was converted into its acetate (95%) bp 242°, with Py and Ac_2O (6 hr at 20°) or MeCOCl (1 hr at 0°). This was specifically oxidized at the C-10 position with SeO_2 [15, 17] to yield **4g** (40%), 115–120°/1 mm Hg, and this was further oxidized with $AgNO_3$/NaOH [15] to **4h**, which was decarboxylated by the Schmidt reaction [16] to yield CO_2 (45%).

Route C. Geraniol was converted into **4g** as above and this was reduced through 10-hydroxygeraniol to **6b** (54%), 40–45°/4 mm Hg, with $LiAlH_4$ and Raney nickel [18]. This product was oxidized [19] with CrO_3–HOAc to **6c** (70%), 123°/5 mm Hg, which on Schmidt decarboxylation [16] yielded **6a**; benzoate (60%) from EtOH, mp 162°, and CO_2 (50%).

Radiochemical methods. These have been fully described [10]. All starting materials and products were rigorously purified to constant sp. act., by recrystallization if possible.

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