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THE BIOSYNTHESIS OF GERANIOL IN GERANIUM

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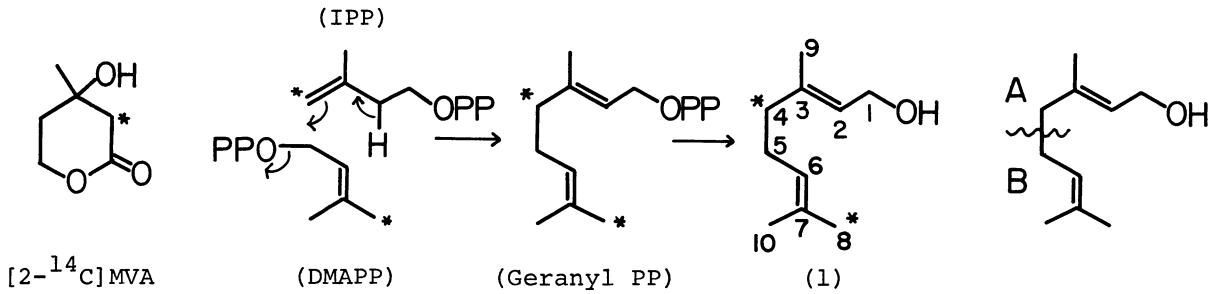
The labelling pattern in geraniol biosynthesized from [2-^{14}C]-mevalonic acid in leaves of *Pelargonium roseum* Bourbon was consistent with the pathway shown in the scheme. The radioactivities at C-4 and C-8 (and/or C-10) were unbalanced, in opposition to the results observed for geraniol biosynthesized in flowers of the rose.

The biosynthesis of monoterpenes is said to involve the conversion of mevalonic acid (MVA) into isopentenyl pyrophosphate (IPP) and 3,3-dimethylallyl pyrophosphate (DMAPP), followed by the condensation of IPP with DMAPP directed towards the formation of geranyl pyrophosphate, the presumed precursor of monoterpenes. Incorporated tracers in geraniol[1] and mycelianamide[2] biosynthesized from [2-^{14}C]MVA in petals of the rose and in the blue mold respectively are distributed symmetrically in each moiety derived from IPP and DMAPP respectively. On the other hand, the biosynthesis of monoterpenes in leaves and stems of several higher plants has been reported to localize the tracer mainly in the part derived from IPP,[3-5] although one exception has been observed for linalool in which the radioactivities were almost balanced.[6] Both linalool and geraniol are considered to be biosynthetically close to geranyl pyrophosphate. We now have tested the labelling pattern in geraniol (1) biosynthesized



[2-^{14}C]MVA (DMAPP) (Geranyl PP) (1)

from [2-^{14}C]MVA in leaves of a geranium, *Pelargonium roseum* Bourbon.

Feeding experiments were carried out on small terminal branches (ca. 7 cm long) of young plants in Summer. A phosphate buffered solution (4 ml, pH 7.3) of [2-^{14}C]MVA (0.1 mCi, 17 μmol) and ATP (5 mmol) was fed through a cut-stem into the plant (60 g) for 4 hr. The leaves and stems were then subjected to steam-distillation. The essential oil thus obtained (260 mg) was chromatographed on silica gel impregnated with 3% silver nitrate (w/w) to give geraniol (1) (24 mg) in a higher purity than 99.5%. The purity of the alcohol (1) was checked by radio- and gas chromatographys. The incorporation of the tracer into geraniol (1) was ca. 0.038%.

Geraniol (1) was degraded to acetone and levulinic acid by permanganate-periodate oxidation. By hypoiodite oxidation, then, acetone was degraded to iodoform and acetic acid, and levulinic acid to iodoform and succinic acid. This acid was further cleaved into carbon dioxide and ethylenediamine by the Schmidt reaction. All degradation products, after the conversion into the solid derivative when the product is a liquid, were purified by the recrystallization or the sublimation. The origin of tracer carbons in the degradation products are described in the parentheses in the rear of the products shown in Table 1. The purified degradation products and geraniol (1) were converted to barium carbonate by Van Slyke-Folch oxidation in order to determine their radioactivities, shown in the table.

Table 1. Specific activities of geraniol (1) and its degradation products

Compounds (Carbons originated from 1)	Specific activity dpm×10^{-3}/mmol	(%)
Geraniol (C-1∿C-10)	271	(100)
Levulinic acid (C-3∿C-6 and C-9)	220	(81.3)
Iodoform (C-9)	4.2	(1.9)
Succinic acid (C-3∿C-6)	210	(77.5)
Ethylenediamine (C-4 and C-5)	197	(72.7)
Carbon dioxide (C-3 and C-6)	14.5	(5.4)
Acetone (C-7, C-8, and C-10)	—	—
Iodoform (C-8 and/or C-10)	25.7	(9.5)
Acetic acid (C-7 and C-8, and/or C-7 and C-10)	23.6	(8.7)

More than 90 per cent of the total radioactivity was detected in ethylenediamine and iodoform derived respectively from C-4 and C-5 and from C-8 (and/or C-10) of geraniol (1). Since C-1, C-2, C-3, and C-9 were almost unlabelled, a main site of labelling on geraniol (1) is considered to be C-4. Thus, locations of the tracer accord with those, shown in the scheme, assumed for the biogenesis. Iodoform derived from levulinic acid contained little tracer. This means that the methyl group and the terminal methylene of IPP are non-equivalent in the process of the condensation with DMAPP, in the same manner as they are in biosyntheses of other mono- and higher-terpenes.[7-9] On the other hand, in opposition to the results observed for geraniol (1) biosynthesized in flowers of the rose, the radioactivities of C-4 derived from IPP and of C-8 derived from DMAPP were unbalanced; the former contained more than 70 per cent of the total activity and the latter did only 20 per cent. Such unbalanced labelling that the tracer predominantly resided in the moiety A of geraniol may be rationalized in terms of the operation of several factors; a) a pool of DMAPP in the plant may exist which can react with IPP generated from exogenous radioactive MVA before the labelled IPP can be isomerized to DMAPP, b) DMAPP may not be the direct mevalonoid origin, c) compartmentation effects may intervene, and d) the excess of MVA unavoidably used may inhibit IPP-isomerase.[10] The unbalanced labelling shown in geraniol (1) biosynthesized in leaves is interesting, since the phenomenon has not been observed yet in triterpenes and steroids biosynthesized from the same precursor as in geraniol. The incorporation of MVA into geraniol,[1] nerol,[1] and pyrethrins[11] in petals resulted in the nearly equal labelling in moieties derived from IPP and DMAPP. In the biosynthesis in leaves of several higher plants, on the contrary, the unbalanced labelling has been observed in thujane derivatives,[3] camphor,[4] pulegone,[5] and artemisia ketone,[12] as well as in geraniol as described above. Those findings related to the labelling pattern suggest that the nature of the synthetic site in leaves of higher plants may be different from that in petals. The presence of an oil gland in leaves seems to be responsible for the unbalanced labelling.

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