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THE BIOSYNTHESIS OF A NON-HEAD TO TAIL MONOTERPENE, ARTEMISIA KETONE

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Artemisia ketone (1) biosynthesized from [2-^{14}C]mevalonic acid by *Artemisia annua* L. was found to contain 80% and 13% of the incorporated tracer in the internal and terminal methyls respectively. The unbalance of labelling means that the C_5-precursors of two halves of (1) are enzymatically differentiated.

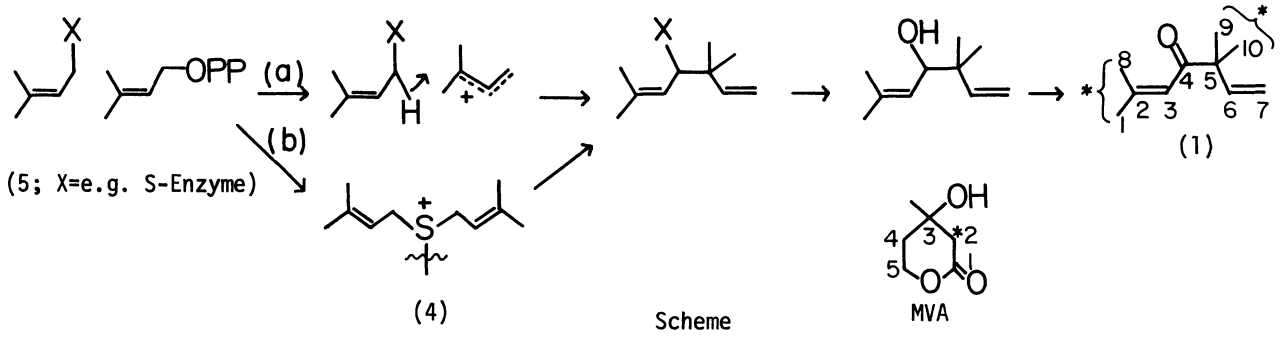
Artemisia ketone (1) is known as a novel non-head to tail monoterpene occurring in *Artemisia annua* L.[1] Some proposals for the biosynthesis of (1) have been presented; a) the ring opening of 3-carene,[2] b) the 1,3-condensation of two dimethylallyl pyrophosphates (DMAPPs),[3] c) the transformation of chrysanthemyl skeleton to artemisyl one,[4] and d) the transformation of bis-(γ,γ-dimethylallyl)-sulfonium salt precursor to artemisyl skeleton.[5] To test these proposals, we examined the labelling pattern in artemisia ketone (1) biosynthesized from [2-^{14}C]mevalonic acid (MVA) in *Artemisia annua* L.

A phosphate buffer solution (pH 7.3) of [2-^{14}C]MVA (0.1 mC, 0.017 mmol) and ATP (30 mg) was fed through a cut-stem into the plant (180 g) for one day. The twigs were then subjected to steam-distillation followed by column chromatography to isolate (1). The labelled ketone (1) was reduced with sodium borohydride to artemisia alcohol (2), which was purified through the recrystallization of its 3,5-dinitrobenzoate, followed by saponification. The incorporation of the tracer was ca. 0.001%.

The alcohol (2) was degraded to acetone and dimethylmalonic acid (3) by permanganate-periodate oxidation.[6] Acetone was further degraded to iodoform, while the acid (3) was subjected to Kuhn-Roth oxidation to give acetic acid, which was further degraded to carbon dioxide and methylamine. The degradation products and the original artemisia alcohol (2) were converted to barium carbonate by Van Slyke-Folch oxidation in order to determine their radioactivities, shown in Table 1.

Table 1. Specific activities of artemisia alcohol (2) and its degradation products

Compounds (Carbons originated from 1)	dpm/mmol
Artemisia alcohol (C-1∿C-10)	26,100
Iodoform (C-1 and/or C-8)	1,750
Dimethylmalonic acid (C-4∿C-6, C-9, and C-10)	19,120
Acetic acid (C-5 and C-9 (and/or C-10))	9,910
Methylamine (C-9 and/or C-10)	10,380
Carbon dioxide (C-5)	460



Scheme

Iodoform derived from C-1 and/or C-8 of (1) contained 13% of the total radioactivity, while methylamine derived from C-9 and/or C-10 of (1) did 80% of the activity. Thus, the terminal (C-1 and/or C-8) and internal methyls (C-9 and/or C-10) were found to be originated from C-2 of MVA. The locations of the tracer were accord with those assumed from the several hypothetical proposals,[2-5] a), b), c), and d). However, a large portion (80%) of the incorporated tracer resided in the right-half C_5-unit (C-9, C-10, C-5, C-6, and C-7) of artemisyl skeleton. This means that two halves of (1) are not derived equally from administered MVA. Thus, the unbalance of labelling could not be explained by the proposals b)[3] and c)[4], as well as by the proposal a)[2]; the b) and c) have proposed that the condensation of two DMAPPs, derived directly from NVA, forms artemisyl skeleton. On the other hand, the proposal d)[5] has given an explanation that the two dimethylallyl units of bis-(γ,γ-dimethylallyl)-sulfonium salt precursor (4), shown in Scheme, are enantiotopic and would be treated nonequivalently by an enzymatic system. The precursor (4) is the monoterpene equivalent corresponding to an intermediate in the conversion of farnesyl pyrophosphate into squalene.[7] Squalene is labelled symmetrically, although two farnesyl units of the intermediate are enantiotopic. Therefore, only that two units are enantiotopic seems not to be responsible for the unbalanced labelling in (1). It is sure that the precursor (4) has been already labelled nonequivalently during its formation.

The unbalanced labelling analogous to (1) has been observed for bicyclic monoterpenes and it was rationalized in terms of the operation of several factors,[8,9] one of which is the existence of a metabolic pool of isotopically normal DMAPP of the mevalonoids origin. The similar situation may occur in the biosynthesis of (1). It was considered by way of a probable explanation for this unbalanced labelling in (1), that a pool of dimethylallyl-precursor (5) bound with a negatively-charged group X (e.g. -S-Enzyme) may exist in the plant, and the precursor (5) is condensed with labelled DMAPP derived from administered MVA, through route (a) or (b) as shown in Scheme.

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