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THE METABOLISM OF LINALOOL IN CITRUS PLANTS*

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Abstract—Citrus fruit and leaf explants were fed linalool-3-^{14}C. Volatile oils were recovered by steam distillation and fractionated by gas–liquid and thin-layer chromatography. α-Terpineol and other terpenoid alcohols were the predominant metabolites in leaves. Fruit also produced α-terpineol, but significant label could also be found in (+)-limonene and other hydrocarbons.

INTRODUCTION

IT WAS observed in *Citrus* species, that the concentration of linalool in the essential oils decreased during the course of the normal growing season.[1] Consequently, a study of the metabolism of linalool was initiated. In an earlier report,[2] the formation of α-terpineol was indicated. Further metabolites in the volatile oils originating from linalool are the subject of this paper.

RESULTS

Steam distilled volatile oils of young rough lemon (*Citrus jambhiri* Lush.) leaves and Dancy tangerine (C. *reticulata* Blanco) fruit, after the application of linalool-3-^{14}C, indicated the accumulation of ^{14}C label in α-terpineol from analysis by TLC. Recovery of the α-terpineol region showed a clean separation from linalool, but indicated the presence of more than one labelled alcohol in addition to α-terpineol. In rough lemon leaves, maximum incorporation of ^{14}C into the α-terpineol region was observed about 2 days after application of linalool-3-^{14}C, the activity approaching 20 per cent of the total volatile radioactivity. The amount in α-terpineol declined after this time.[2]

Gas chromatographic fractionation of rough lemon leaf oil confirmed the presence of labelled α-terpineol, and indicated the presence of activity in other components (Table 1). Fractionation of tangerine peel oil indicated, in addition to the alcohols, the presence of labelled hydrocarbons (Table 1).

DISCUSSION

Examination of the components of steam-distilled rough lemon leaf oil and Dancy tangerine peel oil indicated the formation of labelled metabolites derived from linalool-3-^{14}C. Most activity was found in tertiary cyclic alcohols, both in the leaf and in the peel oils. The major components in the leaf oil were unchanged linalool, α-terpineol and terpinen-4-ol, with significant activity in an unidentified fraction following linalool, perhaps linalyl acetate. Tangerine peel oil also showed trans-2,8-*p*-menthadien-1-ol, and hydrocarbons.

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[1] J. A. ATTAWAY, A. P. PIERINGER and L. J. BARABAS, *Phytochem.* **6**, 25 (1967).
[2] J. A. ATTAWAY and B. S. BUSLIG, *Biochim. Biophys. Acta.* **164**, 609 (1968).

TABLE 1. DISTRIBUTION OF RADIOACTIVITY AMONG VOLATILE COMPONENTS OF LEAF AND PEEL OILS

Gas chromatographic peak number	Compound	Radioactivity in CPM: Rough lemon leaf oil	Radioactivity in CPM: Tangerine peel oil
1	α-pinene	0	0
2	β-pinene	0	45
3	sabinene		—
4	myrcene	14	45
5	α-terpinene	0	—
6	(+)-limonene	23	286
7	cis-β-ocimene	0	—
8	γ-terpinene		222
9	octanal	—	85
10	terpinolene	0	
11	methylheptenone	300	—
12	unidentified	0	—
13	unidentified	—	34
14	linalool	61200	8610
15	unidentified	615	—
16	terpinen-4-ol	700	430
17	trans-2,8-p-menthadien-1-ol	—	570
18	β-caryophyllene (?)	490	—
19	α-terpineol	1030	1350
20	citral	237	88
21	geraniol	0	102

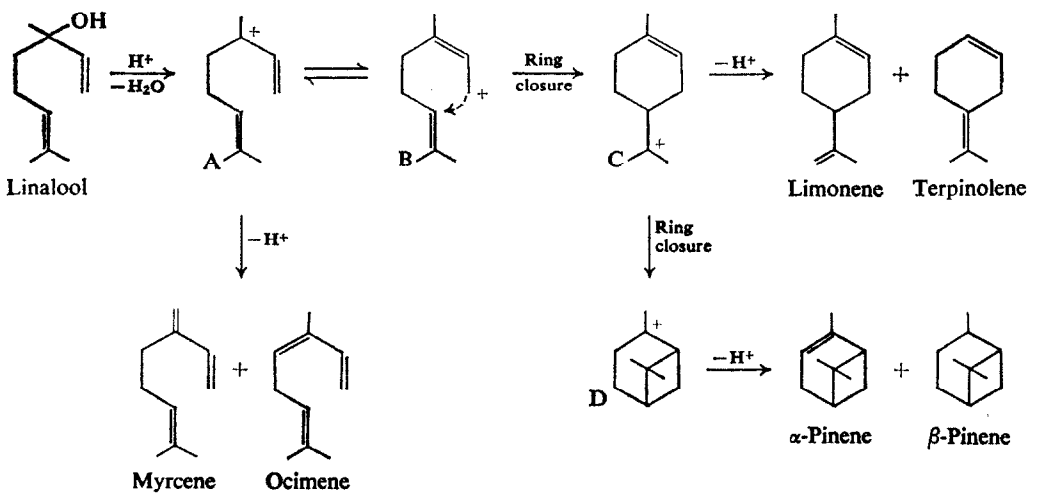

FIG. 1. PROPOSED MECHANISM OF CITRUS TERPENE FORMATION THROUGH LINALOOL INTERMEDIATE (Attaway *et al.*[4])

The mechanism of conversion to α-terpineol is not clear, although indications from nonenzymic hydrolysis of the phosphate esters of monoterpene alcohols suggest an anchimerically assisted nucleophilic attack of the 2,3-double bond on C-8 of linalool.[3] A similar

[3] W. RITTERSDORF and F. CRAMER, *Tetrahedron* **24**, 43 (1968).

mechanism is probably involved in the formation of trans-2,8-*p*-menthadien-1-ol. The reactions occurring probably involve a phosphorylated intermediate of linalool, which would account for the formation of its isomer, geraniol, in the oil of the fruit, and the appearance of citral.

A mechanism for the formation of hydrocarbons was indicated earlier,[4] and is shown in Fig. 1. The appearance of labelled β-carophyllene cannot be accounted for by simple rearrangements. Its formation probably involves the condensation of an intermediate monoterpene alcohol pyrophosphate, derived from linalool, with isopentenyl pyrophosphate and subsequent hydrolysis of the pyrophosphate followed by dehydration and cyclization.

EXPERIMENTAL

Application of Linalool-3-^{14}C

Isotopically tagged linalool was fed to leaves in one of two ways. At first a solution was made of linalool-3-^{14}C (0·285 μc) in dimethylsulfoxide (DMSO) and diluted with water to 1% DMSO concentration. Small volumes of this solution were fed to the stems of cuttings which had been allowed to wilt slightly to facilitate uptake, after which water was added as necessary to keep the cuttings alive. Later, 5 μl of the alcohol (0·285 μc) was dissolved in 1 ml of isopentane and the solution spread on the surface of young leaves. Linalool-3-^{14}C was fed to fruit by direct application to the abscission zone, followed by the administration of water to prevent drying. All materials were allowed to metabolize for 4–6 days with continuous illumination by a 100-W incandescent lamp.

Preparation of Samples

Steam distillation was performed, as described earlier.[5] The small amounts of oil were trapped in isopentane which was subsequently removed using a rotary evaporator.

Gas–Liquid Chromatographic Fractionation

The chromatographic separation and collection was performed as described by Attaway *et al.*,[4] using a 14 ft × ¼ in. column of 4% Carbowax 20 M on GasChrom Z in an F & M Model 500 gas chromatograph modified for dual column operation. Identification of components were by enrichment with known compounds and mass spectroscopy.

Thin-Layer Chromatography

Analyses were performed according to the procedure of Attaway *et al.*,[6] using Alumina G with benzene as solvent and the vanillin/H_2SO_4 spray for identification.

Radioactivity Analysis

Components separated by TLC were scraped from the appropriate areas of the plates and counted in suspension with 4% Cab-O-Sil in 15 ml of 0·475% PPO and 0·025% dimethyl-POPOP in toluene, using a Packard Series 4000 liquid scintillation system. Gas chromatographically separated fractions were collected by condensing in capillary tubes which were subsequently broken into 15 ml of toluene counting solution without Cab-O-Sil.

[4] J. A. ATTAWAY, A. P. PIERINGER and L. J. BARABAS, *Phytochem.* **5**, 141 (1966).
[5] A. P. PIERINGER, G. J. EDWARDS and R. W. WOLFORD, *Proc. Am. Soc. Hort. Sci.* **84**, 204 (1964).
[6] J. A. ATTAWAY, R. W. WOLFORD and G. J. EDWARDS, *Anal. Chem.* **37**, 74 (1965).