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SHORT COMMUNICATION

STUDIES IN TERPENOID BIOSYNTHESIS—III[1].

THE INCORPORATION OF GERANYL PYROPHOSPHATE INTO CINEOLE

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Abstract—1-^{14}C-Geraniol as its pyrophosphate, has been shown to be specifically incorporated (0·22%) into cineole by *Rosmarinus officinalis*.

GERANIOL or some close relative such as the pyrophosphate (I), has been recognized[2] for a long time as the likely progenitor of the monoterpenes. However, experimental evidence to support this is lacking. Although geranyl pyrophosphate has been shown[3] to form a mandatory intermediate both for steroid biosynthesis and for the biosynthesis of the terpenoid portion of the indole alkaloids,[4] its conversion into simple monoterpenes has not been reported. It is the purpose of this paper to record the conversion of geranyl pyrophosphate to cineole (II).

Mevalonic acid has been shown to act as a precursor of a number of monoterpenes.[5] In particular mevalonate was specifically incorporated[6] into cineole (II). Rosemary (*Rosmarinus officinalis*) produces an essential oil containing a large number of mono- and sesquiterpenes including cineole.[7] In the present work young plants (3–7 month old) were dried, extracted with boiling water and the monoterpene fraction steam-distilled and purified by preparative GLC. α-Pinene, cineole, borneol, *p*-cymene and camphor were separated and identified by comparison of their i.r. and NMR spectra with known samples. Although the composition of this oil changed with the season and the age of the plant, cineole formed a regular component.

The lithium salt of the pyrophosphate of 1-^{14}C-geraniol was prepared by the literature method[3,8] and shown to be chromatographically homogeneous. The salt was administered hydroponically to sixty cuttings over 7 days. The essential oils were recovered and shown by

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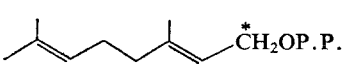
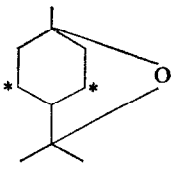
analytical GLC to contain 30 per cent cineole. Carrier cineole was added and the components separated by preparative GLC. Cineole showed an incorporation (corrected for dilution) of 0·22 per cent.



(I)



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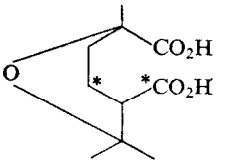

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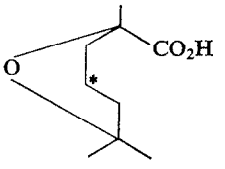
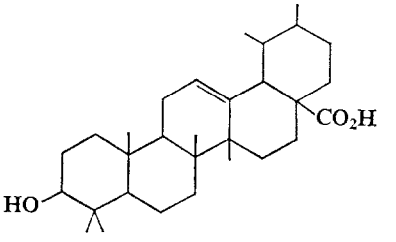


(IV)



(V)

In order to establish the specificity of this biosynthesis, the cineole was degraded by oxidation with potassium permanganate to cineolic acid (III).[9] This retained 91 per cent of the activity of the cineole. The small loss of activity was probably due to the presence of a trace labelled contaminant. The cineolic acid on refluxing with aqueous sulphuric acid gave cinnenic acid (IV)[10] and carbon dioxide. Both fragments were collected and counted—the latter as barium carbonate. The carbon dioxide contained 41 per cent and the cinnenic acid 46 per cent of the activity of the cineolic acid, i.e. the label was approximately equally distributed between the two symmetrical positions in cineole. If degradation and re-formation of the C-10 unit had occurred then the label would be more randomly distributed (in at least a ratio of 3:1).

This result does not define the double-bond isomer immediately prior to cyclization. Neryl pyrophosphate has been implicated for this role,[2] and neryl and geranyl pyrophosphates were incorporated[4] to an equivalent extent into the indole alkaloids.

In another experiment 1-^{14}C-geraniol, solubilized in Tween 80, was fed to the plant. The triterpene fraction was isolated and purified. This contained ursolic acid (V) which showed an incorporation of 0·07 per cent.

EXPERIMENTAL

General details have been described previously.[11] Analytical and preparative GLC was carried out on a Pye 105 instrument. Some samples were counted as thin films in a Nuclear Chicago D47 gas-flow detector through the courtesy of the School of Biological Sciences. All were corrected for efficiency by the use of a common standard.

The lithium salt of 1-^{14}C-geranyl pyrophosphate was prepared according to the method of Cramer and Bohm.[8] It showed a specific molar activity of $5\,61 \times 10^5$ d.p.s./m.mole.

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[10] H. RUPE and H. ALTENBERG, *Chem. Ber.* **41**, 3952 (1908).
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Feeding Experiment

The lithium salt of 1-^{14}C-geranyl pyrophosphate (50 mg; $5{\cdot}61 \times 10^5$ dps/m mole) in water (100 ml) was evenly distributed between fifteen glass tubes each containing four cuttings of *Rosmarinus officinalis*. The cuttings were fed for 7 days at 30–35°, further water being added from time to time. The cuttings were dried at 40–45°, crushed, infused with boiling water and the essential oil steam-distilled. The monoterpenes were salted out and recovered in ether.

Separation of the Monoterpenes

Analytical GLC on a Ucon oil Column showed the essential oil to contain 30 per cent cineole. The mixture (332 mg) was diluted with cineole (150 mg) and separated preparatively on a 15 ft column of 10 per cent Ucon oil on 180–100 mesh silonized firebrick operating at 65° using nitrogen (50 ml/min) as the carrier gas. Cineole (155 mg) had a retention time of 50 min and, allowing for dilution, the initial material present therefore had a specific molar activity $1{\cdot}232 \times 10^3$ dps/m mole representing a 0·22 per cent incorporation.

Oxidation of Cineole

Cineole (155 mg) was oxidized with $KMnO_4$ (500 mg) in water (50 ml) at 40° for 24 hr and then at 80–90° for a further 24 hr. Excess $KMnO_4$ was destroyed with methanol (5 ml), the solution concentrated to 10 ml, acidified and the cineolic acid collected, and recrystallized to constant activity from hot water as plates, m.p. 211° (lit.,[8] 211°) (specific activity $1{\cdot}16 \times 10^3$ d.p.s./m mole).

Decarboxylation of Cineolic Acid

Cineolic acid (28 mg) was heated at 60–70° with 40 per cent H_2SO_4 (7 ml) under a low current of N_2. The effluent gases were passed through three baryta traps. The barium carbonate (19 mg) was centrifuged, washed, dried and counted as a thin film (specific activity: $0{\cdot}51 \times 10^3$ d.p.s./m mole; 41 per cent).

The sulphuric acid solution was saturated with salt, and the cinnenic acid recovered in ether. It was recrystallized from aqueous ethanol as needles, m.p. 74° (lit.,[9] 76°) (specific activity: $0{\cdot}56 \times 10^3$ d.p.s./m mole; 46 per cent).

Ursolic Acid

1-^{14}C-Geraniol (30 mg, sp. act. $5{\cdot}2 \times 10^6$ d.p.s./m mole) was dissolved in an aqueous solution (60 ml) of Tween 80 (10 drops in 250 ml). This was fed to thirty cuttings of *R. officinalis* for 7 days. The dried plants were steam distilled to recover the essential oils and then extracted with methanol. Crude ursolic acid separated from this extract on the addition of water. The acid was purified by sublimatio and had m.p. 291° (specific activity $3{\cdot}7 \times 10^3$ d.p.s./m mole 0·07 per cent incorporation).