UDC 581.134 : 547.566.1 : 582.893

104. **Ko Kaneko** : Biogenetic Studies of Natural Products. IV.1) Biosynthesis of Anethole by *Foeniculum vulgare*. (1).

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Anethole is contained in several Umbelliferae species and fennel oil contains 50∼60% of anethole. For the biogenesis of anethole, Robinson has suggested the C_6—C_3 type of condensation.2)

Nord, *et al.* described the biochemical pathway of lignin from phenylpyruvic acid by sugar-cane plant.3) Neish, *et al.* studied flavone biosynthesis from phenylpropane compounds, such as phenylalanine, cinnamic acid, coffeic acid, ferulic acid, and sinapic acid, by buckwheat and salvia plants. They concluded that the phenylpropane skeleton was incorporated into the B-ring and C-2, C-3, and C-4 of the flavone, without rearrangement of the side chain.4∼6)

On the other hand, Shibata, *et al.*7,8) established the pathway from phenylalanine to ephedrine via 2-phenylethylamine and ω-aminoacetophenone by Ephedra plant.

The biosynthetic study of anethole in comparison with flavone, lignin, and ephedrine is of interest. One of the possible ways of anethole biosynthesis is that acetyl-coenzyme-A (C_2 fragment) is transformed into C_7 fragment such as benzaldehyde, etc., but the result of feeding experiments showed that acetic acid[*carboxyl*-^{14}C] was not incorporated into anethole.9) The results indicated that C_7—C_2 type of condensation seems improbable.

In the present work, the origin of three carbons of the side chain and methoxyl-carbon of anethole was examined by the use of *dl*-phenylalanine[2-^{14}C] and *dl*-methionine[*methyl*-^{14}C].

Experimental

Cultivation of Plants—*Foeniculum vulgare* Miller was cultivated in the field for 3 months and it reached about 180 cm. in height, contained 0.15∼0.25% of fennel oil per fresh weight, and had a few younger flowers on the top. Two kg. of the plant was harvested by severing the stem just above the ground level. The lower leaves and stems were removed, the middle and higher leaves and side stems were cut carefully, and 1 kg. of these leaves and side stems was placed in a 1-L. beaker which contained 500 cc. of half strength of Hoagland solution. This was covered with a large glass bell jar and CO_2-free air was supplied, as shown in Fig. 1.

The hydroponic cultivation lasted for 5 days, during which the jar was cooled by tap water at 15∼20° and the plant was illuminated by Hitachi-FL 30-W lamp during the day time. The weight of plant increased to 1150 g. during the experiment.

Labeled Compounds—*dl*-Phenylalanine[2-^{14}C] and *dl*-methionine[*methyl*-^{14}C] were procured from Daiichi Chem. Co., Tokyo.

Identification of Anethole by Chromatostrip of Silica Gel—The purity of anethole was detected by color change on the chromatostrip of silica gel with H_2SO_4. The silica gel chromatostrip was prepared in the following way: 30 g. of silica gel was mixed with 12 g. of 0.5% alginic acid solu-

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1) Part Ⅲ : Yakugaku Zasshi, **80**, 268(1960).

2) R. Robinson : "The Structural Relations of Natural Products," 1st Ed., 33(1955), Oxford University Press.

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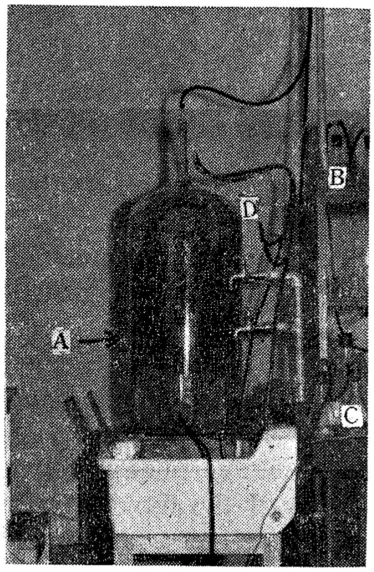

Fig. 1. Experimental Arrangement

A is the jar used for cultivation and it is supplied with CO_2-free air from B trap, C trap is collection trap for CO_2 released from the plant. Jar A is cooled by tap water from D line.

Table I. Components of Hydroponic Culture Solution

	Concentration ($\times 10^{-3}$ moles)	
	Expt. 1	Expt. 2
KNO_3	2.5	2.5
$Ca(NO_3)_2 \cdot 4H_2O$	2.5	2.5
$MgSO_4 \cdot 7H_2O$	1.0	1.0
KH_2PO_4	0.5	0.5
Supplement	trace	trace
dl-Phenylalanine	6.0 (50 μc)	6.0 (Not labeled)
dl-Methionine	6.0 (Not labeled)	6.0 (50 μc)

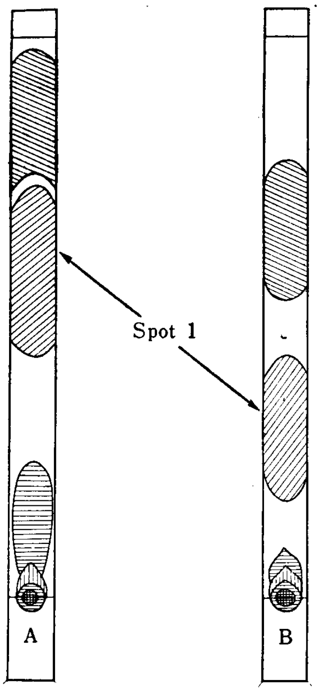

Fig. 2. Chromatostrip of Fennel Oil

A : Developed by hexane containing 15% AcOEt.

B : Developed by hexane.

Spot 1 is anethole fraction.

tion and 80 cc. of H_2O, the mixture was extended on 1.5×3.0 cm. of glass plate, dried in an air bath at 80∼90° for 1 hr., and kept in a desiccator ($CaCl_2$) overnight.

A small volume of crude anethole was spotted on this chromatostrip which was developed with hexane containing 15% AcOEt. When the developer was evaporated the chromatostrip was sprayed with conc. H_2SO_4, $KMnO_4$, and $KMnO_4$ plus dil. HCl. The change of these colors is shown in Fig. 2.

The spot corresponding to anethole showed following color changes: H_2SO_4, reddish green → orange → yellow; $KMnO_4$, brown; $KMnO_4$ plus dil. HCl, white → yellow. Rf value of anethole was 0.74~0.76 (hexane containing 15% AcOEt).

Isolation and Purification of Anethole—Plant materials administered with labeled compounds were carefully washed with tap water, minced into small pieces, and extracted by steam distillation. The distillate was extracted with Et_2O and fennel oil obtained was 2.00 g. in Expt. 1 and 2.50 g. in Expt. 2. The yield of pure anethole was 950 mg. in Expt. 1 and 1,130 mg. in Expt. 2. The purity was examined by above-mentioned glass-strip method. Pure anethole crystallized in the refrigerator. UV: λ_{max}^{EtOH} 268 mμ.

Degradation of Anethole and Determination of its Radioactivity—The scheme of degradation is shown in Chart 1. One g. of anethole was dissolved in 20 cc. of $CHCl_3$ and 5% O_3 was passed through

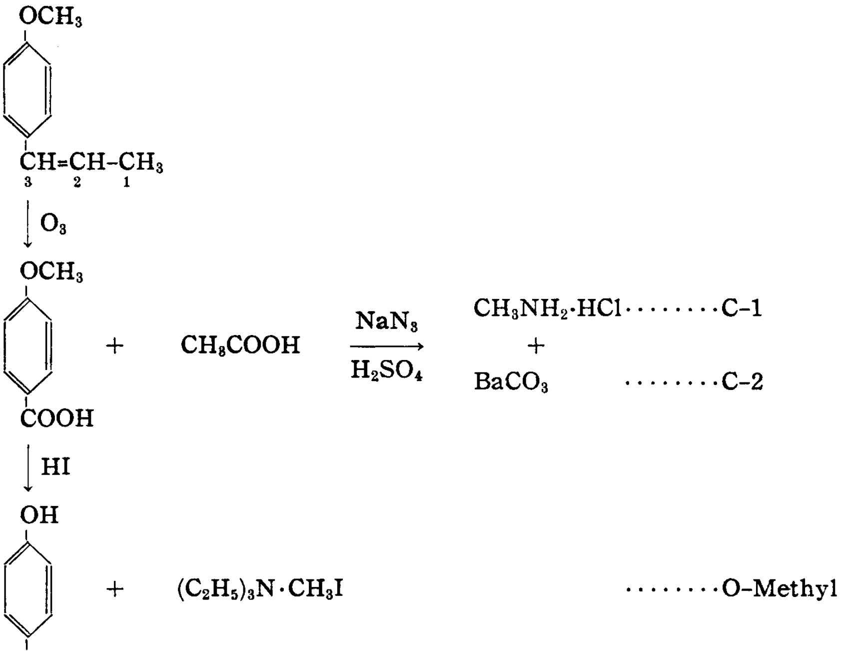

Chart 1.

this solution for 3~4 hr. under cooling. After $CHCl_3$ was evaporated, residual yellow crystals were dissolved in 20 cc. of 5% NaOH solution on a boiling water bath, then oxidized by the dropwise addition of 10 cc. of H_2O_2, and heated for 2 hr. at 80°. The solution was acidified by the addition of 10 cc. of H_3PO_4 (sp. gr., 1.7). The crude crystals of anisic acid were collected by filtration and recrystallized from water to 830 mg. of crystals, m.p. 182°. UV λ_{max}^{EtOH} mμ: 256, 210.

The filtrate was distilled and the distillate was titrated with 0.1*N* NaOH to determine the amount of generated AcOH. The crude AcONa was recrystallized from EtOH; yield, 957 mg. (88%).

A mixture of 60 mg. of AcONa with 70 mg. of NaN_3 and 1.5 cc. of conc. H_2SO_4 was heated for 60 min. at 70°, CO_2 evolved was washed with 5% $KMnO_4$, and was absorbed in 0.2*N* NaOH solution (CO_2-free). $BaCl_2$ was added to the alkali solution with exclusion of air and the resulting $BaCO_3$ was collected. The radioactivity of this $BaCO_3$ is due to that of C-2 of anethole.

5 cc. of 50% NaOH solution was added to the decarboxylated solution and the generated $MeNH_2$ was trapped with 0.1*N* HCl solution. $MeNH_2 \cdot HCl$ solution was evaporated and the crystals were recrystallized from EtOH; yield, 42 mg. (70%). The activity of $MeNH_2$-HCl crystals corresponds to that of C-1 of anethole.

A mixture of 100 mg. of anisic acid and HI (sp. gr., 1.7) was heated at 170~180° in a modified Pregl apparatus and MeI produced was absorbed in 5% EtOH solution of Et_3N. On standing overnight, about 144 mg. of white crystals of $Et_3N \cdot MeI$ were obtained upon evaporation of the solution.[10]

Upon cooling this demethylated solution, 74 mg. of *p*-hydroxybenzoic acid was obtained, which after recrystallization from water, melted at 212°. Amide, m.p. 162°.

Measurement of Radioactivity—Radioactive material was burned by the wet-combustion method and the resulting $BaCO_3$ was prepared as a thin film on a copper plate. Radioactivity was measured by the flowcounter, Low-Background Proportional Counter PC-2B (Nuclear Measurement Co.).

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[10] S. A. Brown, R. U. Byerrum: J. Am. Chem. Soc., **74**, 1523(1952).

Results and Discussion

It is evident that Foeniculum plants metabolize phenylalanine and methionine to anethole as shown in Table II, but, in Expt. 1 and 2, the ratio of radioactivity of anethole to fennel oil is 93/100 and 12/100, respectively. On silica gel chromatography of fennel oil (Expt. 2) strong radioactivity remained near top of the column.

When phenylalanine was metabolized to anethole by the Foeniculum plant, α-carbon of phenylalanine was completely incorporated into the C-2 of anethole as shown in Expt. 1 in Table III.

TABLE II.

	Radioactivity (cpm × 10^6)	
	Expt. 1	Expt. 2
Culture medium		
Initial	322.400	335.910
Final	146.000	152.020
Net	176.400	182.900
Fennel oil	0.116	0.357
Anethole	0.108	0.043
Respiratory carbon dioxide	0.531	0.548

TABLE III.

	Radioactivity (cpm/mM)	
	Expt. 1 (× 10^4)	Expt. 2 (× 10^3)
Anethole	1.33	4.17
Anisic acid	0	4.04
p-Hydroxybenzoic acid	—	0
Triethylmethylammonium iodide	—	3.88
Acetic acid	1.23	0
Methylamine hydrochloride	0	—
Carbon dioxide	1.30	—

In this plant, methyl group of methionine was transmethylated to the methoxyl group of anethole but not to the side chain of anethole. This result is summarized in Expt. 2 of Table III. If the transmethylation of methionine to the side chain of anethole occurs, the radioactivity would be distributed to the methyl-carbon of acetic acid produced from the ozone oxidation of anethole, but the activity of acetic acid was completely negative.

The radioactivity of respiratory carbon dioxide was five times higher than that of anethole during the metabolism of phenylalanine[2-^{14}C] in Foeniculum plant. It may be suggested that the phenylpropane skeleton is metabolized by the β-oxidation-like breakdown, while the products of C_7 compounds are not resynthesized to anethole because acetic acid[*carboxyl*-^{14}C] is not incorporated into anethole as mentioned before.

In view of the experiments described above it can be suggested that the phenylpropane skeleton is incorporated into anethole without rearrangement of its side chain, though not rigidly demonstrated. Additional experiments are being conducted concerning this point.

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Summary

It was found that *dl*-phenylalanine[2-^{14}C] and *dl*-methionine[*methyl*-^{14}C] are respectively incorporated into the α-carbon of the side chain and methyl-carbon of methoxyl group in anethole molecule produced by *Foeniculum vulgare* MILLER, *in vivo*.

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