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# 19. Ko Kaneko : Biogenetic Studies of Natural Products. VI.[1] Biosynthesis of Anethole by *Foeniculum vulgare*. (3).

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It was shown previously[1,2] that the administration of *dl*-phenylalanine[2-$^{14}$C] to Foeniculum plants yielded radioactive anethole and degradation indicated that the tracer element was all located in the anethole. Administration of phenylpyruvic acid, cinnamic acid, and *p*-hydroxycinnamic acid to the cell-free enzyme systems prepared from the same plant indicated these compounds are precursors of anethole in biosynthesis.

These findings have considerable degree of correspondance with the formation of lignin and flavonoids.[3,4] In a preliminary experiment, *p*-hydroxycinnamic acid was found to be a convenient intermediate in anethole biosynthesis.[1] *p*-Hydroxycinnamic acid has been found to serve as a common precursor for lignin, flavonoids, and phenyl ethers. The difference of the possible function of *p*-hydroxycinnamic acid in each process is not clear.

In the present work, examinations were made on the fate of *p*-hydroxycinnamic acid, labeled with carbon-14 in carboxyl group, in the anethole formation.

## Experimental

**Cultivation of the Plant and Administration of a Tracer**—*Foeniculum vulgare* M. was cultivated in a greenhouse during the winter season, reached about 100~150 cm. in height and had a few young flowers on the top in early March. The hydroponic cultivation was as described in a previous paper,[2] except that it contained 300 mg. of *p*-hydroxycinnamic acid[*carbonyl*-$^{14}$C] instead of *dl*-phenylalanine[2-$^{14}$C]. The cultivated plants increased their weight to 1150 g. during the experiment, which started from 1000 g., and 2 g. of fennel oil was obtained by steam distillation. The purification of anethole and degradation of anethole was conducted in the same manner as described in the previous report.[2]

**Synthesis of *p*-Hydroxycinnamic Acid[*carbonyl*-$^{14}$C]**—a) Labeled Acetic Anhydride : 1.2 g. of AcCl(freshly distilled) was added to 2.0 g. of fused AcONa, which contained 16.4 mg. of sodium acetate-[*carbonyl*-$^{14}$C] (500 μc) in small portions, and at the end of the reaction, 0.8 g. of AcONa was added supplementarily. The mixture was extracted with $Et_2O$ and the extract was evaporated to leave an oily mass, which distilled at b.p. 125~135°. Yield, 1.4 g. (60%).

b) Acetylation of *p*-Hydroxybenzaldehyde : A mixture of 6 g. of *p*-hydroxybenzaldehyde, 30 g. of fused AcONa, and 30 cc. of $Ac_2O$ was heated on a water bath for 2 hr. with occasional stirring and the warm solution was poured into ice water with vigorous stirring. Resulting emulsion was extracted with $Et_2O$, the $Et_2O$ extract was washed with $H_2O$ and saturated $NaHCO_3$ solution, and evaporated. The oily residue was distilled in a diminished pressure and the fraction of $b.p_{18}$ 145~150° was collected. Yield, 5.4 g., antioxime, m.p. 113~115°.

c) Synthesis of *p*-Hydroxycinnamic Acid[*carbonyl*-$^{14}$C] : A mixture of 1.4 g. of radioactive $Ac_2O$, 1.0 g. of acetoxybenzaldehyde, 0.9 g. of fused AcONa, and 3 drops of dehyd. pyridine was heated for 10 hr. at 180~200°. The reaction mixture was allowed to stand at room temperature, saponified with 20 cc. of 5% NaOH solution, and the solution was acidified with $H_3PO_4$ (sp. gr. 1.70). After repeated recrystallizations from water, 0.62 g. of *p*-hydroxycinnamic acid was obtained as colorless crystals of m.p. 208~209°. After three recrystallizations, the radioactivity reached the plateau, $5.24 \times 10^6$ c.p.m./ mmole.

**Detection of Carbon-14 in *p*-Hydroxycinnamic Acid**—From a solution of 3 mg. of radioactive *p*-hydroxycinnamic acid and 197 mg. of non-radioactive *p*-hydroxycinnamic acid dissolved in 20 cc. of EtOH, 2 cc. was used for the determination of the radioactivity of *p*-hydroxycinnamic acid and 18 cc. was hydrogenated over Pd-C. The reduction mixture was treated in the usual manner and gave

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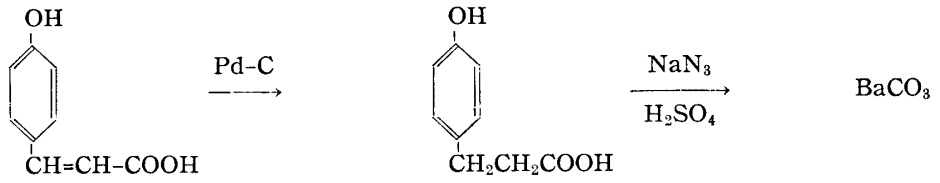
1) Part V : This Bulletin, 8, 875 (1960).
2) Part IV : *Ibid.*, 8, 611 (1960).
3) S. N. Acerbo, W. Walter, F. F. Nord : J. Am. Chem. Soc., **80**, 1990 (1958).
4) D. R. McCalla, A. C. Neish : Can. J. Biochem. Physiol., **37**, 537 (1959).

165 mg. of phloretic acid, m.p. 130°. Phloretic acid was decarboxylated by the Schmidt reaction, as described previously.[1]




$1.435 \times 10^5$ c.p.m./m$M$ $1.360 \times 10^5$ c.p.m./m$M$ $1.400 \times 10^5$ c.p.m./m$M$

This result indicates that the distribution of carbon-14 in *p*-hydroxycinnamic acid is limited to the carbonyl carbon.

**Measurement of Radioactivity**—Radioactive material was burned by the wet-combustion method, as previously described, and the radioactivity was measured by the flow-counter, CE-14 Low-background Beta Counter (Tracer Lab. Inc., U. S. A.).

## Result and Discussion

The distribution ratio of the radioactivity of *p*-hydroxycinnamic acid[*carbonyl*-$^{14}$C] absorbed by Foeniculum plant was 0.1% in anethole and 1.2% in respiratory carbon dioxide. As indicated in Table I, the radioactive element was all located in 1-position of the side chain in anethole. Therefore, it seems reasonable to assume that, in the synthesis of anethole by Foeniculum plant, phenylpropane derivatives are incorporated into anethole without rearrangement of the side chain.

Table I. Radioactivity of Anethole and its Degradation Products

| | Radioactivity c.p.m. $\times 10^4$/mmole |
|---|---|
| Anethole | 1.026 |
| Acetic Acid | 1.017 |
| Methylamine hydrochloride | 1.005 |
| $BaCO_3$ | 0 |
| Anisic Acid | 0 |

The series of successive reduction of *p*-hydroxycinnamic acid to anethole, as compared to the processes of lignin and flavonoids biosynthesis, is still unknown and further experiments will have to be made to elucidate this mechanism.

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## Summary

The radioactivity of *p*-hydroxycinnamic acid[*carbonyl*-$^{14}$C] fed to the plant was found in the methyl-carbon of the side chain of anethole produced by *Foeniculum vulgare* M.

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