UDC 581.134 : 547.566.1 : 582.893

176. Ko Kaneko : Biogenetic Studies of Natural Products. VIII.[1] Biosynthesis of Anethole by *Foeniculum vulgare*. (4).

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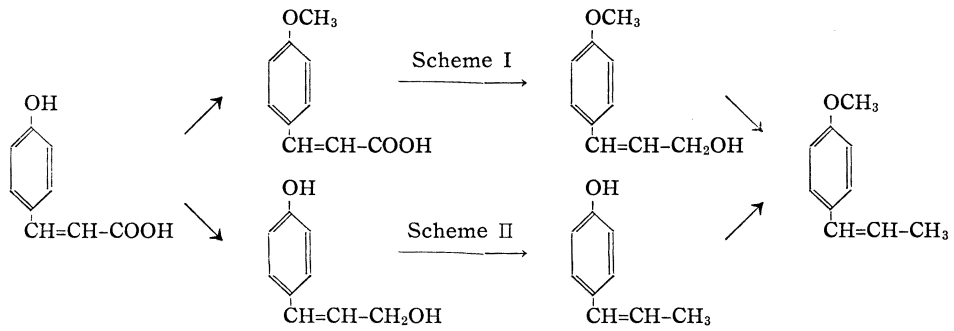
In the previous papers[2~4] of this series on the biosynthesis of anethole by *Foeniculum vulgare*, it was shown that anethole was synthesized from a phenylalanine path through phenylpyruvic acid, cinnamic acid and 4-hydroxycinnamic acid. These results, when estimated by isotopic and enzymatic methods, were in good agreement with those obtained from the biosynthesis of other phenylpropanoide, such as lignin and flavones.[5~7]

It was from this point of view that the author was interested in the relationship between the reduction of the carboxyl group and the transmethylation of 4-hydroxycinnamic acid.

As regards the reduction and the transmethylation of 4-hydroxycinnamic acid, two possibilities are to be conceivable.



In this paper, an attempt was made to ascertain the actual route by the use of the inhibition method, as described previously.[3]

Experimental

Cultivation of Plant, Extraction and Isolation of Enzyme System——They were conducted in the same manner as described in the previous paper.[3]

Assay of Enzymatic Synthesis of Anethole and its Assumed Intermediates——The condition of incubation was described in a previous paper.[3]

The change of the enzymatic reactions was measured at the end of every hour. The aliquots of the reaction mixture were acidified with 10% HCl, treated with 20 mg. of pure anethole, 4-methoxycinnamic acid, 4-methoxycinnamyl alcohol and cinnamyl alcohol, and extracted with Et_2O for 5 hr. They were then treated in the same manner as described in the previous paper.[3] 4-Hydroxycinnamic acid and 4-methoxycinnamic acid obtained from the reaction mixture were purified by paper chromatography,[3] the Rf values of which were 0.35 and 0.27, respectively.

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1) Part Ⅶ : This Bulletin, **10**, 1119 (1962).
2) K. Kaneko : *Ibid.*, **8**, 611 (1960).
3) *Idem* : *Ibid.*, **8**, 875 (1960).
4) *Idem* : *Ibid.*, **9**, 108 (1961).
5) S. N. Acerbo, W. J. Schubert, F. F. Nord : J. Am. Chem. Soc., **80**, 1990 (1958).
6) S. A. Brown, A. C. Neish : Can. J. Biochem. Physiol., **34**, 769 (1956).
7) D. R. McCalla, A. C. Neish : *Ibid.*, **37**, 537 (1959).

Also, 4-methoxycinnamyl alcohol and anethole were purified by silica gel chromatostrip,[3] and the Rf values were 0.17 and 0.74, respectively.

Preparation of 4-Methoxycinnamyl Alcohol——Ethyl 4-methoxycinnamate was reduced by $LiBH_4$, as described by Kollonitsch.[8]

A solution of 1.55 g. of the ester, 2.6 g. of LiI and 0.7 g. of $NaBH_4$ dissolved in dehyd. tetrahydrofuran was heated at 60° for 2 hr. with vigorous stirring. Then the reaction mixture was evaporated to dryness dissolved in H_2O and extracted with Et_2O. After the Et_2O extract was evaporated, the resulting oily residue was hydrolysed with 5% NaOH for 2 hr. and extracted with Et_2O. After evaporation of Et_2O, the resulting oily residue was destilled in a reduced pressure and the fraction of $b.p_2$ 132~138° was collected; yield, 0.6 g. This fraction was separated by silica gel chromatography into the original ester and 4-methoxycinnamyl alcohol, which were further submitted to silica gel chromatostrip.[3] The Rf values obtained were 0.45 and 0.17, respectively. The lower spot was extracted with Et_2O. Yield, 0.2 g. (18%); its phenylurethane melted at 46°. *Anal.* Calcd. for $C_{17}H_{17}O_3N$: C, 72.06; H, 6.05. Found: C, 71.97; H, 6.21.

Results and Discussion

The previous paper[3] of this series demonstrated that the cell free enzyme system from *Foeniculum vulgare* synthesized anethole from phenylalanine-[2-^{14}C] in the presence of ATP, DPN, phosphate, and ascorbate. In this study, the enzyme system was also shown to synthesize anethole from 4-hydroxycinnamic acid-[1-^{14}C], and these results coincided with those of the previous one[4] using phenylalanine-[2-^{14}C]. The change of radioactivity of 4-hydroxycinnamic acid-[1-^{14}C] in this enzyme reaction is shown in Fig. 1, which shows clearly that after 3 hours the radioactivity of 4-hydroxycinnamic acid-[1-^{14}C] distributed to 4-methoxycinnamic acid in a large quantity, and to anethole and 4-methoxycinnamyl alcohol in a minute amount. This was followed by the rapid decrease in the radioactivity of 4-methoxycinnamyl alcohol and anethole showed a gradual increase and reached its maximum value after 8 hours. From the data given in Fig. 1, it seems to indicate that 4-hydroxycinnamic acid is methylated with methionine and ATP at the first stage in which the rate of reaction is relatively high. And then, the amount of 4-methoxycinnamic acid reduced gradually resulting in the formation of 4-methoxycinnamyl alcohol and anethole.

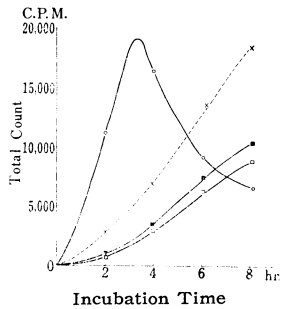

Fig. 1.
—○— 4-Methoxycinnamic acid
—□— 4-Methoxycinnamyl alcohol
—■— Anethole
---×--- Anethole+4-Methoxycinnamyl alcohol

Experiments were made to observe the effects of 4-methoxycinnamic acid and 4-methoxycinnamyl alcohol on the synthesis of anethole from 4-hydroxycinnamic acid-[1-^{14}C] by the inhibition method, as described previously.[3]

From Table I, it is apparent that 4-methoxycinnamic acid and 4-methoxycinnamyl alcohol have strong dilution effect of the radioactivity which incorporated into anethole from 4-methoxycinnamic acid-[1-^{14}C] hence these compounds have an inhibitory action on this reaction.

8) J. Kollonitsch, O. Fushs, V. Gabor: Nature, **173**, 125 (1954).

TABLE. I.

Exp. No.	Addition	Concentration (M)	Radioactivity c.p.m./mmole	Radioactivity Ratio
1.	None		8,870	100.0
	4-Methoxycinnamic acid	0.015	3,017	34.1
	4-Methoxycinnamyl alcohol	0.015	2,359	26.6
	Cinnamyl alcohol	0.015	7,896	89.0
2.	None		14,805	100.0
	4-Methoxycinnamic acid	0.015	4,250	28.6
	4-Methoxycinnamyl alcohol	0.015	3,892	26.6

The incubation mixture contained 10 μc of 4-hydroxycinnamic acid-[1-^{14}C]. The assay conditions were described in the previous paper.[3] In Exp. 1, each incubation tube contained 135 mg. of protein and in Exp. 2, each tube contained 182 mg. of protein. Both tubes were incubated at 30° for 6 hr.

Scheme II was not examined under an identical condition but it may be concluded that Scheme I will furnish an important route of anethole biosynthesis in *Foeniculum* plant.

Sincere gratitude is expressed to Prof. H. Mitsuhashi of Hokkaido University for his kind encouragement. A part of the expenses of the present work was defrayed by the Grant-in-Aid for Institutional Research of Education and Rockefeller Foundation Grant which are gratefully acknowledged.

Summary

The anethole biosynthesis, using the cell-free enzyme system of *Foeniculum* plant was established as follows: 4-Hydroxycinnamic acid→4-methoxycinnamic acid→4-methoxycinnamyl alcohol→anethole.

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177. Yasuo Yura: Studies on Acetylenic Compounds. XXV.*2 Ring Closure. (5). New Synthetic Method of Heterocyclic Compounds from α-Amino- and α-N-substitued Aminoacetylenic Compounds.

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In the previous paper[1,2] the author reported that 2-aminothiazole, 2-thiazolethiol, and 2-aminoimidazole derivatives were easily synthesized by refluxing alcoholic solution of α-haloacetylenic compounds with thiourea, ammonium dithiocarbamate, and guanidine, respectively. As a general application of this reaction to another new ring closure, α-haloacetylenic compounds were allowed to with urea, amide, thioamide, or S-benzylisothiourea to obtain derivatives of imidazole, oxazole, or thiazole, respectively. In these

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*2 Part XXIV. I. Iwai, T. Konotsune: Yakugaku Zasshi, **82**, 601 (1962).
1) Part XXI. Y. Yura: This Bulletin, **10**, 372 (1962).
2) Part XXII. *Idem*: *Ibid.*, **10**, 376 (1962).