U. D. C. 615.32 : 581.134 : 547.94 : 582.49

17. Shoji Shibata, Izumi Imaseki, and Mikio Yamazaki : Phytochemical Investigation on Cultivation of Medicinal Plants. XII.* On the Alkaloid Biogenesis in Ephedra. (3).**

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As mentioned in the previous paper,[1] by the isotopic tracer technique using ^{15}N-labeled compounds, we gave a conclusion that phenylalanine would take part as a precursor in the biosynthesis of *l*-ephedrine in Ephedra plant.

For the purpose of analysing the biosynthetical pathway more closely, we investigated the source of N-methyl group in ephedrine molecule, on which the present paper is chiefly concerned.

Although there have been reported numerous works concerning biological methylation in animal tissues and cells, the actual evidence of this biochemical process in the higher living plants has not fully been provided.

As far as we are aware, the following works dealing with higher plant metabolism would be of considerable value. Barrenscheen and Vályi-Nagy[2] suggested the occurrence of transmethylation in wheat germ, and Byerrum *et al.*,[3,4] using ^{14}C-tracer technique, gave an evidence for the incorporation of methyl group of methionine into N-methyl of nicotine in tabacco leaves.

Dubeck and Kirkwood[5] observed that methyl group of methionine serves as a precursor of both O- and N-methyl of ricinine in *Ricinus communis*, and Marion *et al.*,[6,7] after feeding methionine[*methyl*-^{14}C] and formate[^{14}C], established that N-methyl of hordenine in the germinating barley arises from tyrosine through transmethylation.

The present study was carried out by administration of ^{14}C-labeled methionine to the medium of hydroponic cultivation of intact Ephedra plant. The radioactivity of *l*-ephedrine isolated from the plant after one week's cultivation and the localised activity in the N-methyl grouping were determined.

Experimental

Synthesis of DL-Methionine[*methyl*-^{14}C]—DL-Methionine[*methyl*-^{14}C] was prepared by the method of Melville, Rachele, and Keller[8] by the reaction of DL-benzylhomocysteine with $^{14}CH_3I$ (1 mc., 26 mg.; The Radiochemical Centre). The apparatus used for the preparation was given by Melville *et al.*[8,9] The product recrystallized from aq. EtOH gives m.p. 275°(decomp.). On the paper chromatogram developed with BuOH·H_2O·AcOH (4:2:1), after spraying with ninhydrin reagent, the product showed two visible spots, one of which with Rf 0.55 was radioactive, corresponding to methionine (Rf 0.55), and the other inactive one represented homocysteine (Rf 0.28), along with an invisible but slightly radioactive spot at Rf 0.85. After repeated crystallization from aq. EtOH, the pure crystals

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** (2) : This Bulletin, **4**, 277(1956).
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1) S. Shibata, I. Imaseki : This Bulletin, **4**, 277(1956).
2) H. K. Barrenscheen, T. Vályi-Nagy : Z. physiol. Chem., **277**, 97(1942).
3) S. A. Brown, R. U. Byerrum : J. Am. Chem. Soc., **74**, 1523(1952).
4) L. J. Dewey, R. U. Byerrum, C. D. Ball : *Ibid.*, **76**, 3997(1954).
5) M. Dubeck, S. Kirkwood : J. Biol. Chem., **199**, 307(1952).
6) T. J. Machett, L. Marion, S. Kirkwood : Can. J. Chem., **31**, 488(1953).
7) E. Leete, L. Marion : *Ibid.*, **32**, 646(1954).
8) D. B. Melville, J. R. Rachele, E. B. Keller : J. Biol. Chem., **169**, 419(1947).
9) M. Calvin, *et al.* : "Isotopic Carbon," John Wiley & Sons, Inc., N. Y., 222(1949).

gave a single radioactive spot at Rf 0.55. The radioactivity of the synthesized methionine was 1.3×10^8 c.p.m./mM.

The radioactivity was all estimated with a "Q"-gas flow counter (Nuclear Instrument and Chemical Corp.) attached to the Scaler (Kaken Model 32) and appropriate correction was made.

Experimental Plant Meterial—*Ephedra distachya* L. grown in the Experimental Field for Medicinal Plants attached to Tokyo University Forestry Experimental Station at Tojo, Chiba Pref., was lifted from the ground on June 18, 1956. The hydroponic cultivation of the intact plant started from the following day employing the Houghland solution added with DL-methionine[*methyl*-^{14}C] (Table I.).

TABLE I. Component of Hydroponic Solution

KNO_3	5×10^{-3} *M*/L.
KH_2PO_4	1×10^{-3}
$Ca(NO_3)_2 \cdot 4H_2O$	5×10^{-3}
$MgSO_4 \cdot 7H_2O$	2×10^{-3}
DL-Methionine[*methyl*-^{14}C]	4.5×10^{-4}
Minor elements	Trace
pH	6.2

The plant was dipped in the medium by its root and the pot was placed in a glass cylinder with a tight glass lid, through which inlet and outlet tubings were fitted. Dried air was gradually allowed into the cylinder to keep the internal atmosphere in appropriate humidity. Air was occasionally bubbled into the nutrient solution for aeration (2 hrs./day) (Fig. 1).

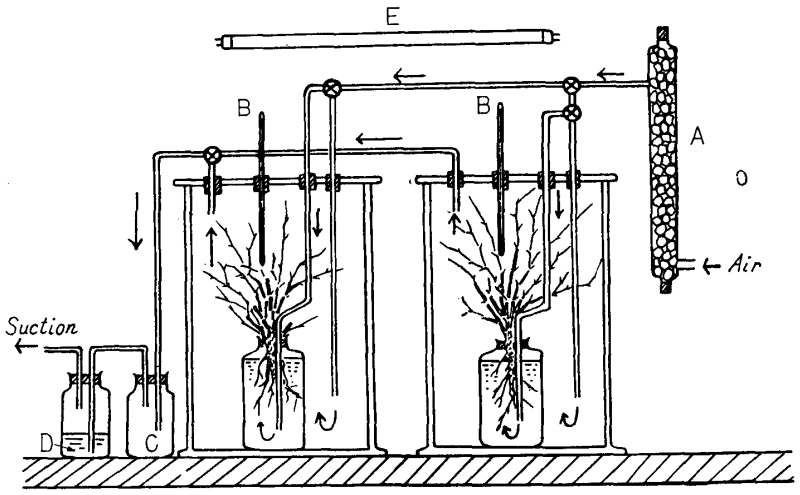

Fig. 1.

A : $CaCl_2$
B : Thermometer
C : Trap
D : $Ba(OH)_2$
E : Light

The outlet tubing was connected to a trap bottle containing $Ba(OH)_2$ solution to hold radioactive CO_2 generated from the plant, whose radioactivity became detectable from the 3rd day of cultivation. The temperature range recorded during the cultivation was 20~30°.

The plant material was removed from the solution on the 7th day of cultivation and the grassy portion was dried cautiously at 40~45°, under an infrared lump. *l*-Ephedrine was extracted by the procedure mentioned in the previous paper[10] and its radioactivity was estimated.

Degradation of *l*-Ephedrine—*l*-Ephedrine isolated from the plant material was decomposed as described by Nagai[11] to separate N-methyl grouping whose specific activity was determined. *l*-Ephedrine (100 mg.) was heated with 25% HCl (0.5 cc.) in a sealed tube at 160~170° for 8 hrs. The reaction mixture was separated into basic and neutral portions. Methylamine obtained from the basic portion was isolated as the chloroplatinate, m.p. 228°(decomp.); yield, 103 mg. (72%). Methyl benzyl ketone isolated from the neutral portion was determined as its 2,4-dinitrophenylhydrazone, m.p. 122°; yield, 45 mg. (26%).

Results and Consideration

At the end of the cultivation, the weight of fresh and dried plant material was determined and the content of *l*-ephedrine was recorded.

10) S. Shibata, I. Imaseki : This Bulletin, **1**, 285(1953).
11) N. Nagai : J. Pharm. Soc. Japan, No. 121, 206(1892).

Table II.

Whole plant	Grassy portion		Amt. of solution absorbed	*l*-Ephedrine isolated as hydrochloride
Fresh	Fresh	Dried		
560 g.	129 g.	48.5 g.	170 cc.	288 mg. (0.49%)

The radioactivity of the methionine administered and that of *l*-ephedrine produced were measured, and the localization of activity in ephedrine molecule was determined.

Table III.

	DL-Methionine-[*methyl*-^{14}C] administered	Ephedrine-HCl	CH_3NH_2 Chloroplatinate	$C_6H_5CH_2COCH_3$ 2,4-Dinitrophenylhydrazone
Specific activity	1.3×10^8 c.p.m./mM.	3.6×10^3 c.p.m./mM.	3.8×10^3 c.p.m./mM.	Nil.
Total activity	9.0×10^7 c.p.m.	—	—	—

It was evidently shown, as given in Table III, that the radioactivity of methyl grouping in methionine administered to the intact Ephedra plant is transferred to N-methyl group of *l*-ephedrine during 7 days' experimental cultivation, and any detectable localization of radioactivity in the remaining portion of ephedrine molecule could not be observed.

This experiment presents an evidence that the active methyl group of methionine is a source of N-methyl group of ephedrine and that transmethylation occurs in the course of alkaloid formation in Ephedra.

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Summary

By the ^{14}C-tracer experiment it was proved that methionine takes part as a source of N-methyl group of *l*-ephedrine in Ephedra plant.

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