69. Shoji Shibata** and Izumi Imaseki***: Phytochemical Investigation on Cultivation of Medicinal Plants. VII*. On the Alkaloid Biogenesis in Ephedra. (1).

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Numerous works concerning the biosynthetic pathway of alkaloid formation in the plant have been reported. For the study on the site of alkaloid formation, two botanical techniques are generally available^{1,2}). One of these is grafting between a pair of alkaloid bearing plant and non-alkaloid forming one, or in a pair of genetically related plants forming different alkaloids³). Secondly, culture of the excised plant organ is investigated.

Recently a technique employing labeled isotopic element has become very useful in the studies of plant metabolism⁴), but it is very rare to find any work using N¹⁵ as a tool for the experiment on the alkaloid biogenesis.

In the previous paper⁵), we reported that a small increase of alkaloid could be observed in the scion of *Datura* grafted upon a tomato stock, and it may be concluded, as some workers pointed out²), that the aerial portion of *Datura tatula* L. would respond but little to the biogenesis of alkaloid, which takes place chiefly in the roots.

In connection with the previous work, an experiment on the biosynthesis of Ephedra alkaloids using N¹⁵ as a tracer element was carried out, about which the present paper describes.

The detached aerial portion of *Ephedra distachya* L. was cultivated in a medium containing (N¹⁵H₄)₂SO₄ as the sole source of nitrogen. After one week's cultivation, it was estimated whether N¹⁵ was introduced or not into ephedrine isolated from the plant material.

Experimental

Experimental material—The plant material used was *Ephedra distachya* L. grown in the Experimental Field for Medicinal Plants attached to Tokyo University Forestry Experimental Station at Tojo, Chiba Pref. The plant was harvested on September 29, 1952, when the alkaloidal content of the plant appeared to reach maximum in the growing season⁵). The aerial portion of the plant was detached at the wood stems and weighed (210 g.). The content of total alkaloid was estimated: Grassy stems: 0.374±0.012%; roots: 0.018±0.002%.

Medium—Original (N¹⁵H₄)₂SO₄ solution prepared by the Scientific Research Institute, Tokyo, contained 7.89% total N, and 11.52 atom% excess N¹⁵. The medium for cultivation was prepared by dilution of the original ammonium sulfate solution to give a concentration of 250 mg. N in 500 cc. pH of the medium was adjusted to 6.0.

Cultivation—The excised aerial portion of the plant was dipped into the medium by its wood stem, held by glass wool. The experiment was carried out for 1 week (from Sept. 30 to Oct. 7, 1952), in a room, at 16~20°, avoiding direct sunlight.

At the end of the experiment, the plant material was weighed: Grassy portion: 152 g.; wood portion: 44 g.; total: 196 g. Wood stem was cautiously cut off to avoid the mechanical contamination of (N¹⁵H₄)₂SO₄.

* Part VII: S. Shibata, I. Imaseki: J. Pharm. Soc. Japan 73, in press.

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1) R. F. Dawson: "Advances in Enzymology", Vol. VIII, p. 209, New York (1948).

2) W. O. James: Alkaloids in the Plant, in "The Alkaloids" (Edited by R. H. F. Manske & H. L. Holmes) Vol. I, p. 15, Academic Press, N. Y. (1950).

3) W. C. Evans, N. W. Patridge: Nature, 169, 333 (1952).

4) cf. H. B. Vickery, et al.: J. Biol. Chem., 135, 531 (1940); C. C. Delwiche: Ibid., 189, 167 (1951); R. MacVicari, R. H. Burris: Ibid., 176, 511 (1948).

5) S. Shibata, I. Imaseki: J. Pharm. Soc. Japan, 73, in press.

6) S. Shibata, I. Imaseki: Unpublished data.

Fractionation procedure—The detached grassy portion was dried and pulverized. 54 g. of the pulverized material was extracted as follows to isolate *l*-ephedrine.

N-content of ephedrine isolated was determined by the Kjeldahl method using 50 mg. of the sample. The solution of ammonium sulfate resulting from the above determination as acidified by 1~2 drops of 0.1N H_2SO_4 , and shaken with chloroform to remove the indicator, methyl red. The aqueous solution was concentrated to 1~2 cc., which was used for N^{15} -determination by the mass-spectrometer.

Results and Discussion—The results obtained by the present experiment are shown in Tables I and II.

TABLE I
Content and Amount of Non-alkaloidal Nitrogen Absorbed

	%	Total amount*	Ratio	N^{15} atom% excess	Amount of N absorbed	Ratio
Total N	3.33 ± 0.04	1798.2 mg.	100	0.42 ± 0.01	66.1 mg.	100
Protein-N	2.34 ± 0.05	1263.6	70	0.27 ± 0.01	29.7	45
Nonprotein-N		517.4	29		36.3	55

* The amount (mg.) in 54 g. of the plant material.

TABLE II
Analysis of Ephedra Alkaloid

Total ephedra alkaloid accumulated	Percentage	$0.374 \pm 0.012\%$
	Total amount in the plant material	202.0 mg.*
	Amount of N	17.2 mg.
	$\frac{\text{Ephedra alkaloid N}}{\text{Total N}} \times 100 = 0.96\%$	

N ¹⁵ -containing ephedrine formed	N ¹⁵ atom% excess	0.10±0.01%
	N absorbed during experi- mental period	0.15 mg.*
	Ephedrine formed during experimental period	1.77 mg.*
$\frac{\text{Ephedrine formed}}{\text{Total ephedra alkaloid}} \times 100 = 0.84\%$		$\frac{\text{Ephedrine-N formed}}{\text{Total N absorbed}} \times 100 = 0.23\%$

*Amount (mg.) in 54 g. of the dried grassy stems.

This experimental results have shown that N¹⁵ was apparently fixed in the ephedrine isolated, proving that the formation of ephedrine takes place in the aerial portion of Ephedra plant. Since the proportion of N¹⁵ in the total ephedrine-N was not so small, it can scarcely be believed that the fixation of N¹⁵ in ephedrine might be effected by the exchange of N¹⁵ with normal N¹⁴ in the preformed ephedrine.

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Summary

The detached aerial portion of *Ephedra distachya* L. was cultivated for a week in a medium containing (N¹⁵H₄)₂SO₄. N¹⁵ was proved to be introduced into *l*-ephedrine isolated from the plant material. By this result it could be concluded that ephedrine is biosynthesized in the aerial part of the Ephedra plant.

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70. Morizo Ishidate, Shunsaku Noguchi, and Akira Tamano: Studies on Chemotherapeutics for Acid-fast Bacilli. VII¹⁾. Further Studies on *p*-Aminophenyl- and *p*-Aminomethylphenyl Alkyl Sulfones and Related Compounds.

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In the first paper of this series¹⁾ some *p*-aminophenyl alkyl sulfones and *p*-aminomethylphenyl alkyl sulfones, which possessed tuberculostatic activity *in vitro*, were described. Of these, the compounds having alkyl chain of 5 to 6 carbon atoms seemed to exhibit activities of the same degree as that of *p,p'*-diaminodiphenyl sulfone. This time, the authors prepared several compounds of the same type in which alkyl moiety has been replaced by alkoxyethyl groups (II and IV), and tested their bacteriostatic activities *in vitro*, comparing with those of the corresponding alkyl sulfones (I and III). The bacteriostatic data are summarized in Table I.

When the compounds of type II are compared with the corresponding compounds of type I having almost the same chain length, the bacteriostatic activities are decreased in the former, and the compounds having ether linkage possess a higher solubility in water and alcohol. On the other hand, between the two benzylamine type compounds, (III) and

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1) Part VI. M. Ishidate, M. Tomoeda, H. Oya: J. Pharm. Soc. Japan, 72, 88(1952); cf. M. Ishidate, K. Maruyama: *Ibid.*, 72, 52(1952).