136°(sint. at 128°). A mixed m.p. with *d*-O,O,N-trimethylcoclaurine methiodide, m.p. 136°(sint. at 128°), showed a distinct depression (m.p. 120~125°, sint. at 115°).

Identity of *l*-1-(4'-Methoxybenzyl)-6-methoxy-7-hydroxy-N-methyl-1,2,3,4-tetrahydroisoquinoline (III) Picrate, m.p. 136°, with Picrate, m.p. 136°, of the Bisected Base from Epistephanine —Fujita[5] carried out the cleavage of epistephanine by the sodium-liquid ammonia process, and obtained a bisected phenolic base as a picrate crystallizing in orange yellow needles, m.p. 136°. This picrtae was confirmed by admixture to be identica with *l*-1-(4'-methoxybenzyl)-6-methoxy-7-hydroxy-N-methyl-1,2,3,4-tetrahydroisoquinoline (III) picrate, m.p. 136°. Furthermore, as a result of the paper chromatography, both gave the same Rf value as shown below :

	Rf of the base	Rf of picric acid
Picrate (from epistephanine)	0.85	0.66
Picrate (from cepharanthine)	0.85	0.66

Toyo Roshi No. 50 was used and development was effected by the ascending technique with the upper layer of a mixture of 67 cc. of BuOH, 27 cc. of water, and 10 cc. of glacial AcOH. For the detection of alkaloidal spots, fluorescence by the ultraviolet rays and the Dragendorff reagent were used.

Summary

In the previous paper of this series, it was shown that the fission of cepharanthine by the sodium-liquid ammonia process gave a base possessing two phenolic hydroxyls and no methoxyl group, corresponding to (II), as crystals, and on this basis formula (I) was proposed for cepharanthine. In the present series of experiments, an attempt to isolate (III) as the picrate from the mother liquor of (II) has been successful. From these experimental results, formula (I) previously proposed by the authors for cepharanthine was confirmed.

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86. Masao Tomita* and Eiichi Fujita** : Studies on the Alkaloids of Menispermaceous Plants. CXXI[1]. Cleavage of Epistephanine by Metallic Sodium in Liquid Ammonia.

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Epistephanine is one of the biscoclaurine alkaloids contained in *Stephania japonica* Miers (Japanese name " Hasunoha-Kazura "), and its chemical constitution was first studied by Kondo and Tanaka[2] who proposed its structure to be one of the formulae (I)~(IV). Subsequently, Tomita and Uyeo[3], by comparative study of ultraviolet absorption spectra of miscellaneous dihydroisoquinoline derivatives, pointed out that either formula (III) or (IV) would be the most appropriate for the representation of epistephanine. Furthermore, by our recent work on the fission of isotetrandrine[4], tetrandrine[5], O-methyloxyacanthine[6], and O-methylrepandine[7] by the sodium-liquid ammonia process, the differentiation in the structure between the oxyacanthine and berbamine series, which has hitherto remained

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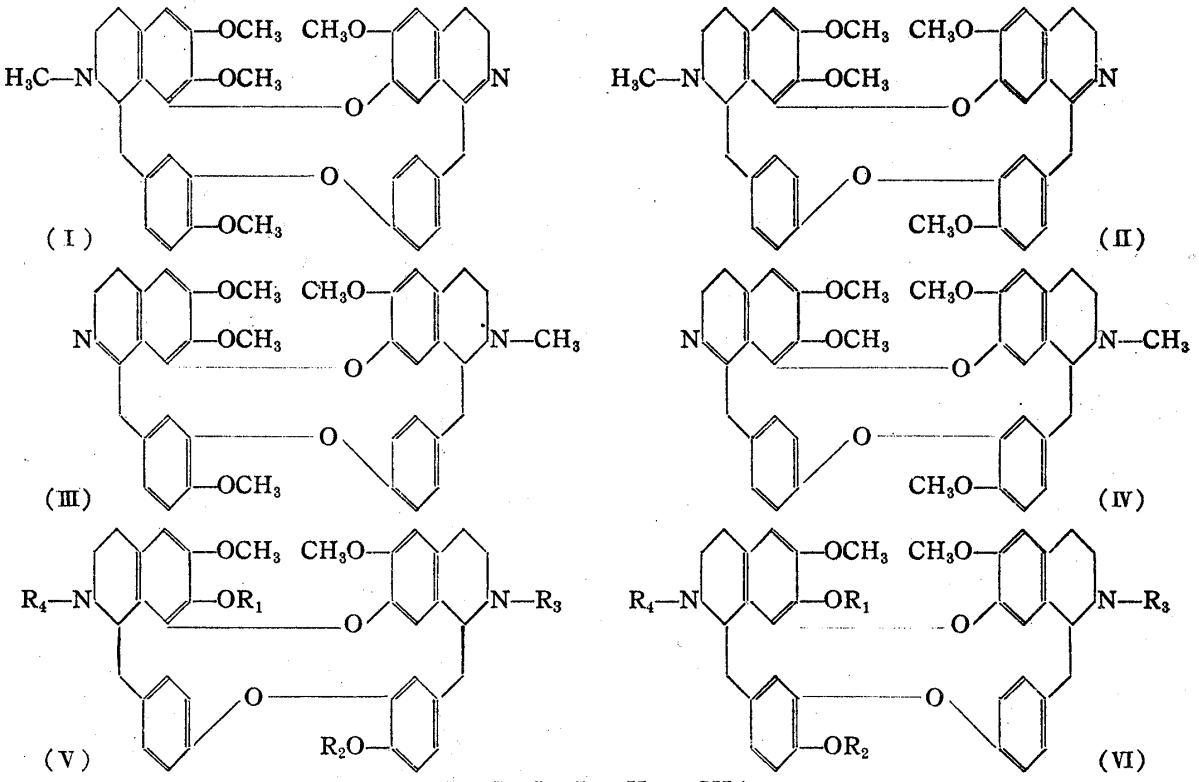
1) Part CXX. M. Tomita, Y. Sasaki : This Bulletin, **2**, 375(1954).
2) H. Kondo, K. Tanaka : J. Pharm. Soc. Japan, **63**, 267, 273(1943); *ibid.*, **64**, 28(1944).
3) M. Tomita, S. Uyeo, K. Doi, T. Miwa : *Ibid.*, **69**, 22(1949) (C.A., **44**, 4476(1950)).
4) M. Tomita, E. Fujita, F. Murai : *Ibid.*, **71**, 226, 1035(1951); M. Tomita, Y. Inubushi, H. Niwa: *Ibid.*, **72**, 211(1952).
5) E. Fujita, F. Murai : *Ibid.*, **71**, 1039(1951).
6) E. Fujita : *Ibid.*, **72**, 213, 217(1952).
7) E. Fujita, T. Saijoh : *Ibid.*, **72**, 1232(1952).

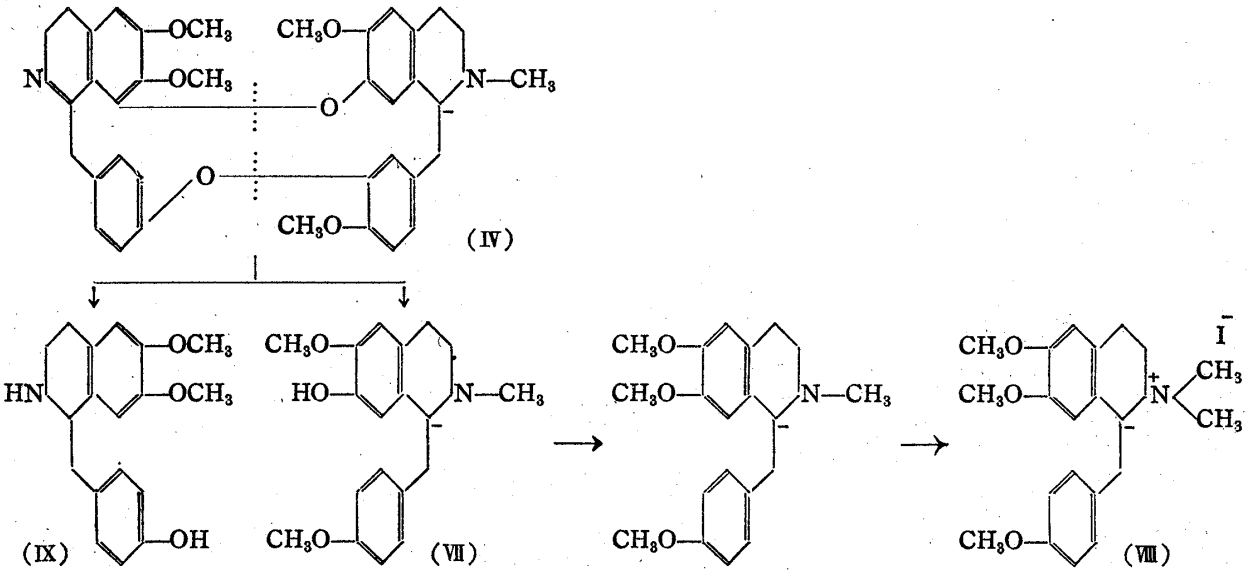
unknown, has been made possible, and hence it has been established that oxyacanthine is (V), and berbamine, (VI).



Consequently, since it is known so far that N-methylhydroepistephanine-A derivable from epistephanine is identical with oxyacanthine methyl ether (O-methyloxyacanthine), it appears most reasonable that epistephanine has formula (IV). In the present series of experiments, an attempt to carry out the cleavage of epistephanine with sodium in liquid ammonia has been made, and as a result, it has been experimentally confirmed that epistephanine should be represented by formula (IV).

As described in the experimental section, the procedure employed was essentially the same as before. By treatment of the fission products, the phenolic bases were obtained in an almost quantitative yield, only a small amount of unreacted material being recovered as the non-phenolic base. Addition of a solution of oxalic acid in ethanol to a solution of the phenolic bases in ethanol caused no immediate precipitate, but when the resulting mixture was concentrated somewhat, the oxalate deposited out. Recrystallization of this from ethanol showed m.p. 222~227°. The free base from this oxalate formed crystals of m.p. 124~128°, gave a positive Liebermann's nitroso reaction and is optically inactive. Because of the difficulty of purification of the free base itself, as well as the oxalate, and bacause of the small amount available, this was set aside. Meanwhile, the mother liquor from the above oxalate was freed from ethanol, dissolved in warm water, and the solution, after being made alkaline with aqueous ammonia, was extracted with ether. Evaporation of the ether extract left the residue of a reddish amorphous solid giving a negative Liebermann's nitroso reaction. This was then chromatographed in benzene on alumina and the purified product was converted into the picrate, which crystallized in orange yellow pillars, m.p. 134~136°. By analysis it was found to possess an empirical formula $C_{25}H_{26}O_{10}N_4$, the identity of which was confirmed by admixture with *l*-1-(4'-methoxybenzyl)-6-methoxy-7-hydroxy-N-methyl-1,2,3,4-tetrahydroisoquinoline picrate,[1] m.p. 136°, obtained from cepharanthine by the same mode of cleavage. The paper

chromatography revealed that the two picrates also gave the same Rf values. The base liberated from this picrate was difficult to crystallize. This, after methylation with diazomethane and subsequent purification by chromatography, could be readily converted into the methiodide, m.p. 134~136° (sint. at 123°), undepressed on admixture with *l*-O-methylarmepavine (*l*-O,O,N-trimethylcoclaurine) methiodide (Ⅷ), m.p. 136° (sint. at 128°). From these results, it seems certain that the original phenolic base is identical with *l*-1-(4'-methoxybenzyl)-6-methoxy-7-hydroxy-N-methyl-1,2,3,4-tetrahydroisoquinoline (Ⅶ), and if this should correspond to *l*-armepavine, the oxalate would crystallize most readily, and afford a crystalline free base, m.p. 145~146°. By the difficulty of crystallization of this base as well as the oxalate, this can be readily distinguished from *l*-armepavine. Since (Ⅶ) was obtained as one of the bisected bases in this fission it is evident that the structure of epistephanine should be represented by formula (Ⅳ), as so far considered, and degradation proceeds according to the following scheme.



It has also become apparent that, as suggested by us[8] before, the stereochemical configuration of one of the two asymmetric centers in epistephanine is of the *l*-type. Finally, for the characterization of the base which may be regarded as corresponding to (Ⅸ) and giving a positive Liebermann's nitroso reaction, an attempt to convert it into armepavine by methylation with formaldehyde solution and formic acid was attempted but without success. Also an attempt to obtain *m*-hemipinic acid by direct oxidation failed. In this case, it was keenly felt that a larger amount to carry out complete purification, which would enable us to confirm the other phenolic fragment in formula (Ⅳ), is needed.

At any rate, on the basis of the foregoing results, there is no doubt that formula (Ⅳ), so far considered to be most likely for epistephanine, is the correct one.

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Experimental[9]

Purification of Epistephanine—The crude epistephanine of m.p. 185~188° was dissolved in acetone and after filtration, the solution was concentrated to a small bulk. MeOH was then added, and the solution was allowed to stand, whereby crystals were obtained. Purification by repetition of this

8) M. Tomita, E. Fujita : This Bulletin, **1**, 101(1953).
9) All melting points are uncorrected. The microanalyses were carried out in the Microanalytical Laboratory of this Institute by Mr. K. Hozumi and his staff.

process gave a product melting around 195°. This was chromatographed in benzene on alumina and after subsequent recrystallization, showed the m.p. of 197°(sint. at 193°), which was not raised any further. Another crude sample of m.p. 188~198° was in like manner purified and showed m.p. 201° (sint. at 196°), but similar attempt to obtain a product of m.p. 200~204° recorded in the literature[2] failed.

Cleavage Reaction of Epistephanine—1.3 g. of epistephanine was dissolved in toluene and liq. NH_3 was added. Na was then added gradually with vigorous stirring, and after completion of the reaction, the mixture allowed to stand to permit evaporation of NH_3. Water was added to the residue and the layers were separated. The aq. layer, after treatment by the usual method, yielded 0.8 g. of the phenolic fraction. The non-phenolic base was recovered from the toluene layer; yield, 0.1 g.

Treatment of Bisected Phenolic Base—The crude phenolic base was dissolved in EtOH, and an ethanolic oxalic acid solution added. On concentration of the resulting solution, the oxalate deposited as crystals, which were separated from the mother liquor. The mother liquor was freed from EtOH and the residue was dissolved in warm water, made alkaline with NH_4OH, and extracted with ether. The ether extract was dried over anhyd. K_2CO_3, and the ether removed, leaving a reddish amorphous solid. Liebermann's nitroso reaction was negative. This, after being purified chromatographically in benzene on alumina, was dissloved in acetone, and a solution of picric acid in ether added, after which the mixture was kept for some time, depositing clustered yellow needles. This picrate is readily soluble in acetone and relatively sparingly soluble in MeOH. After recrystallization from MeOH, it became orange yellow crystals of m.p. 134~136°, undepressed on admixture with *l*-1-(4'-methoxybenzyl)-6-methoxy-7-hydroxy-N-methyl-1,2,3,4-tetrahydroisoquinoline picrate, m.p. 136°[1], obtained from cepharanthine by a similar mode of fission. *Anal.* Calcd. for $C_{25}H_{26}O_{10}N_4$: C, 55.35; H, 4.77. Found: C, 55.20, 55.40; H, 5.09, 5.11. As a result of paper chromatography[1], it was found that the two picrates also gave the same Rf values. When a solution of the above picrate in acetone was passed through a column of alumina, the free base was obtained with the elimination of picric acid. This could not be induced to crystallize after various treatments.

Subsequently, this base was treated in MeOH with excess ethereal CH_2N_2, and the resulting methyl ether was submitted to chromatography using alumina. The purified product was refluxed in MeOH with excess MeI. The methiodide thus formed was recrystallized from MeOH and melted at 134~136°(sint. at 123°), either alone or on admixture with *l*-O-methylarmepavine methiodide, m.p. 136° (sint. at 128°).

On the other hand, the crystalline oxalate initially obtained was recrystallized several times from EtOH, and showed m.p. 222~227°. Because of the difficulty of purification, however, attempts to obtain a sample having a definite m.p. were unsuccessful. The free base was obtained by dissolving this oxalate in warm water, making alkaline with NH_4OH, and extracting with ether. It gave a positive Liebermann's nitroso reaction, and formed crystals, m.p. 124~128°, but because of the small amount available, further recrystallization was impossible. The specific rotation measured was ±0°. An attempt to obtain armepavine by methylation of this sample with HCHO solution and formic acid failed. Oxidation with $KMnO_4$ to obtain *m*-hemipinic acid was also of no avail.

Summary

As a result of the fission of epistephanine by the sodium-liquid ammonia process, it has directly been elucidated that formula (IV), which has hitherto been accepted as the most reasonable for the representation of epistephanine, is correct.

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