Potency, MLD 0.12 γ . Yield 200 mg.

Recrystallization—The crystalline powder obtained through the chromatopile is washed several times with absolute alcohol, and the insoluble solid is dissolved in 0.5 cc. of water containing 1 drop of glacial acetic acid. This is filtered, 5 cc. of alcohol added to the filtrate, and the precipitate that separates out is removed by filtration. To this filtrate, 5 cc. of ether is added, allowed to stand overnight, and the globular crystals that separate out are collected and washed with water. These globules, when broken, reveal silky needle crystals. These crystals are dissolved in 0.5 cc. of diluted acetic acid, 5 cc. each of alcohol and ether added, the small amount of precipitate removed by filtration, and the filtrate allowed to stand overnight. The prismatic crystals that separate out are collected, washed with water, and dried in a vacuum desiccator over phosphorus pentoxide. The crystals gradually turn black from around 220°, but do not melt even at 300°. *Anal.* Found: C, 41.88; H, 5.98; N, 11.81.

Summary

Tetrodotoxin, which had previously been purified through chromatographic column of starch and active charcoal, was further purified through chromatopile and recrystallization by which crystalline pure tetrodotoxin, possessing a potency of MLD 0.012 γ /g. body wt. of mouse, was obtained as prismatic crystals.

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225. Masao Tomita and Tadashi Abe: Studies on the Alkaloids of Berberidaceous Plants. IV¹⁾. Alkaloids of *Mahonia Fortunei* Fedde.

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In the previous paper¹⁾, it was reported that as a result of the investigation of the alkaloids present in the domestic *Mahonia japonica* DC. (Japanese name "Hiiraginanten") the authors identified isotetrandrine and berbamine, which belong to the biscoclaurine type alkaloids, as tertiary bases, and jatrorrhizine, berberine and palmatine, as quaternary bases. The authors also clarified that in comparison with the alkaloids of the Indian *Mahonia*, studied by Chatterjee, et al.²⁾, the Japanese differed from the Indian in that in many cases the latter furnishes oxyacanthine as the biscoclaurine base, whereas the former contains isotetrandrine instead.

Now the authors have examined the alkaloids of the domestic *Mahonia Fortunei* Fedde (Japanese name "Hosoba-hiiraginanten"), and the results thereby obtained are reported in the following.

The plant material was obtained from the garden of Dr. K. Kimura, Professor of the Osaka University, where it grows wild, by his courtesy, and the alcoholic extract of the trunk was prepared as usual. The process for isolation of the alkaloids was quite the same as that described in the previous paper¹⁾. A comparison of the alkaloids contained in the Japanese and the Indian *Mahonias* is summarized in Table I.

As shown in Table I, the Japanese *Mahonia Fortunei* furnishes quite the same alkaloids, oxyacanthine and berbamine as the biscoclaurine bases, as the Indian *Mahonia*. In this connection, it is interesting to note that in Japan only two species, *Mahonia japonica* and *Mahonia Fortunei* grow wild, and of these two species, one furnishes isotetrandrine, and the other oxyacanthine. Besides, the fact gives a great suggestion

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1) Part V : M. Tomita, T. Abe : This Journal, **72**, 735 (1952).

2) R. Chatterjee, M. P. Guha : Science & Culture, **15**, 163 (1949) ; J. Am. Pharm. Assoc. Sci. Ed., **39**, 181, 577 (1950) ; *ibid.*, **40**, 36, 229 (1951).

to the taxonomical position of the two species from the view point of alkaloid chemistry.

Table I

	Japanese Mahonia		Indian Mahonia
	<i>M. japonica</i>	<i>M. Fortunei</i>	
Tert. Base	Isotetrandrine Berbamine	Oxyacanthine Berbamine	Oxyacanthine Berbamine
Quat. Base	Jatrorrhizine Berberine Palmatine	Jatrorrhizine Berberine Palmatine	Jatrorrhizine Berberine Palmatine

Table II gives the comparison of the yield of alkaloids isolated from *M. Fortunei* and from *M. japonica*.

Table II

	<i>Mahonia Fortunei</i>		<i>Mahonia japonica</i>	
	Yield g.	% in 55 g. of viscous residue	Yield g.	% in 440 g. of viscous residue
Oxyacanthine	0.25	0.455	—	—
Berbamine	0.21	0.382	0.03	0.007
Isotetrandrine	—	—	3.90	0.890
Jatrorrhizine	0.79	1.436	5.30	1.210
Berberine	0.31	0.563	3.20	0.730
Palmatine				

In this Table, the yield of the quaternary bases is given as the tetrahydro derivatives. It is also of interest that, as shown in Table II, each percentage of the tertiary and the quaternary bases of both species is almost the same as the total bases, but in the tertiary bases of *M. japonica* all except a small amount of berbamine is occupied by isotetrandrine, whereas in *M. Fortunei* oxyacanthine and berbamine occupy almost the same percentage. It is found that neproline³⁾ or umbellatine⁴⁾, which Chatterjee, et al., discovered from several species of the Indian Mahonia, is not contained in *Mahonia Fortunei*.

Furthermore, the authors examined the alkaloids of the root of *Mahonia japonica*, and have been able to clarify that the root furnishes quite the same alkaloids as the trunk, as was reported in the previous paper¹⁾. The process for isolation of the alkaloids of the root was quite the same as that of the trunk.

The authors wish to thank Dr. K. Kimura, Professor of the Osaka University, who provided them with the plant material used in this experiment.

Experimental

Extraction and Isolation of the Alkaloids—The finely-cut trunk of *Mahonia Fortunei* Fedde was extracted with warm 50% aqueous methanol acidulated with hydrochloric acid in the proportion of 1%. After cooling, the extract was filtered and the remaining plant material was again extracted several times with warm methanol. The extracts were combined and the solvent was removed under reduced pressure, leaving a viscous residue. Yield, 55 g. from 530 g. of the plant material (10.4% of the plant material).

To the residue, six or seven volumes of diluted hydrochloric acid solution was added, and after having been treated with basic lead acetate solution, the mixture was rendered alkaline with ammo-

3) R. Chatterjee, M. P. Guha : J. Am. Pharm. Assoc. Sci. Ed., **40**, 233 (1951).

4) R. Chatterjee : J. Am. Pharm. Assoc., **33**, 210 (1944) ; *ibid.*, **38**, 149 (1949).

nia, and the liberated total tertiary bases were extracted with ether. The ammonia alkaline mother liquor was acidified with acetic acid, and potassium iodide was added to precipitate the quaternary bases as their iodides.

Tertiary Bases—The total tertiary bases were purified as the sulfates, and when the crystalline sulfate sparingly soluble in water was treated, colorless needles, m.p. 209~211° (from acetone), were obtained. Yield, 250 mg. This substance showed the same properties as those of oxyacanthine, and, on admixture with an authentic sample, was confirmed to be oxyacanthine.

The treatment of the mother liquor separated from the above sulfate of oxyacanthine yielded colorless needles, m.p. 124~126° (decomp.) (from benzene). It weighed 210 mg. When this substance was admixed with berbamine-benzene adduct, m.p. 124~126°, no melting point depression was observed. When this substance was dried at 110° in vacuo over phosphorus pentoxide, it indicated m.p. 175~176°. It follows that this substance is berbamine.

Quaternary Bases—The iodides of the quaternary bases were converted into tetrahydro derivatives by reduction with zinc dust and diluted sulfuric acid, and were separated into the phenolic and the non-phenolic bases. They were respectively purified as usual, and separated into three kinds of bases. By admixture with corresponding samples and by comparison of their chemical properties, the following three kinds of bases were identified.

Tetrahydrojatrorrhizine, m.p. 212~214°, colorless needles (from methanol). Yield, 790 mg. Tetrahydroberberine, m.p. 169~170°, colorless elongated plates (from methanol). Yield, 250 mg. Tetrahydroshobakunine (molecular compound of tetrahydroberberine and tetrahydropalmatine), m.p. 138~140°, colorless clusters (from methanol). Yield, 60 mg.

Summary

As a result of the investigation of alkaloids in the Japanese *Mahonia Fortunei* Fedde, the authors identified oxyacanthine, berbamine, jatrorrhizine, berberine and palmatine, and confirmed that it furnishes almost the same alkaloids as the Indian Mahonia. It has also been clarified that the Japanese *Mahonia japonica* DC. contains isotetrandrine and berbamine as the tertiary bases, whereas *Mahonia Fortunei* Fedde furnishes oxyacanthine and berbamine. Furthermore, it has been found that in *Mahonia japonica* DC. the root contains quite the same alkaloids as the trunk.

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226. Masaiti Yanagita : The Constitution of Usnic Acid. II.*

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Usnic acid, one of the characteristic products of lichens, has been known over hundred years and attention has recently been called to this acid by its antibacterial activity¹⁾.

In spite of the intensive research carried out before the World War II in the laboratories of Robertson, Schöpf and Asahina, it is not yet possible to present a structural formula of usnic acid that is certain in every detail. In 1937, Robertson and Curd²⁾ proposed formula (I) for usnic acid, which has become generally regarded as correct. Schöpf and Ross³⁾ obtained the diacetate of 7-acetyl-3,5-dimethyl-4,6-dihydroxycoumaranone-(2) (III) and ethyl acetoxalate by ozonization of the diacetate of usnic acid (II), followed by treatment with alcohol, and supported structure (I).

Asahina and Yanagita⁴⁾ reported that gentle refluxing of a solution of the diacetate

* Part I : This Journal, **71**, 54 (1951).

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1) A. Stoll, et al.: *Experimentia*, **3**, 111, 115 (1947) ; S. Shibata, et al.: *J. Japan. Med.*, **1**, 152 (1948).

2) A. Robertson, F. H. Curd : *J. Chem. Soc.*, **1937**, 894. 3) C. Schöpf, F. Ross : *Ann.*, **546**, 1 (1941).

4) Y. Asahina, M. Yanagita : *Ber.*, **72**, 1140 (1937).