215. Masao Tomita and Tadashi Abe: Studies on the Alkaloids of Berberidaceous Plants. III^{1,2)} Alkaloids of *Mahonia japonica* DC.

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Among Berberidaceous plants, *Mahonia* has in late years been treated as quite distinct a genus from *Berberis* by Fedde³⁾, who recommended from systematic, anatomical and morphological points of view, that *Mahonia* should be treated separately from *Berberis*. In Japan, only a few species of *Mahonia*, including *Mahonia japonica* DC. (Japanese name "Hiiragi-nanten"), *Mahonia Fortunei* Fedde (Japanese name "Hosoba-hiiragi-nanten"), etc., grow wild.

A large number of plants of the *Berberis* genus contain biscoclaurine alkaloids, such as oxyacanthine, berbamine, etc., and H. Kondo and M. Tomita¹⁾ once proved the presence of these bases in *Berberis Thunbergii* DC. (Japanese name "Megi") growing in Japan. In the genus *Nandina*, however, belonging to the same Berberidaceae family, biscoclaurine bases have not yet been discovered. This has recently been confirmed by M. Tomita²⁾ and his collaborators in the examination of *Nandina domestica* Thunb. (Japanese name "Nanten").

Recently Chatterjee and Guha⁴⁾ examined a large number of the Indian species of of the *Mahonia* genus, including *M. acanthifolia* Don, *M. Griffithii* Takeda, *M. borealis* Takeda, *M. Simonsii* Takeda, *M. leschenaultii* Takeda, *M. manipurensis* Takeda, *M. sikkimensis* Takeda, etc., out of which they found that all these contained oxyacanthine or berbamine of the biscoclaurine type as the tertiary base, and berberine, palmatine, jatrorrhizine, etc. as the quaternary bases.

The authors attempted to make a survey of the alkaloids contained in *Mahonia japonica* DC. for the purpose of ascertaining the presence of biscoclaurine bases in the *Mahonia* genus growing in Japan. In Japan many of this plant are cultivated as garden-trees, and the authors used as materials the trunk bark of that growing in Kyoto City. By treating with the method described in the experimental section, the authors first isolated the tertiary bases, secondly the quaternary bases, of which the iodides were converted into tetrahydro derivatives on reduction, and finally separated into their alkaloidal constituents. As a result, two kinds of alkaloids, isotetrandrine (I, R=CH₃) and berbamine (I, R=H) were identified from those isolated as the tertiary bases.

As special attention was paid for the detection of oxyacanthine (II), according to

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1) Report I. H. Kondo, M. Tomita: This Journal, **50**, 309 (1930); Arch. Pharm., **268**, 1 (1930).

2) Report II. M. Tomita, Y. Inubushi, S. Ishii, M. Yamagata: This Journal, **71**, 381 (1951); C. A. **45**, 8208 (1951).

3) F. Fedde: Engler's Botanische Jahrbucher, **30**, 133 (1901).

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

the isolation method by Späth and Kolbe⁵⁾, but it could not be discovered. Previously, isotetrandrine was known to occur in large quantities solely in *Stephania cepharantha* Hayata⁶⁾, belonging to the Menispermaceae, along with cepharanthine. This is the first time that this base appeared in the Mahonia genus belonging to the different Berberidaceae family. Further, it has become clear that the Indian Mahonia contain both oxyacanthine and berbamine in many cases, whereas the Japanese Mahonia yield isotetrandrine and berbamine, both of the berbamine type, instead of oxyacanthine. In this connection, it is of special interest to observe these facts from the light of the relationship between the state of the distribution of plants of the same genus and its allied on the earth, and the variety of the alkaloids present in these plants. It seems apparent from the alkaloids contained that Mahonia which lies between Berberis and Nandina is most akin to the former.

Moreover, the authors confirmed the presence of the three quaternary bases as their tetrahydro derivatives, *viz.*, tetrahydroberberine, tetrahydropalmatine and tetrahydrojatrorrhizine, the last being the most predominant in amount. Tetrahydropalmatine was proved as tetrahydroshobakunine¹⁾, a molecular compound⁷⁾ of tetrahydropalmatine and tetrahydroberberine. Thus it has become clear that this plant contains berberine (III), palmatine (IV) and jatrorrhizine (V).

A summary of the amounts of the alkaloids isolated from *Mahonia japonica* is given in Table I (the berberine type bases being shown as their tetrahydro derivatives).

Table I.

	Yield	Percentage in 440 g. of viscous residue
Isotetrandrine	3.9	0.890 %
Berbamine	0.03	0.007
Jatrorrhizine	5.3	1.210
Berberine } Palmatine }	3.2	0.730
N-free Substance.	0.025	0.006

Comparison of the alkaloids contained in the Japanese Mahonia with those of the Indian Mahonia shows that both genera contain almost the same quaternary bases, but although both furnish the alkaloids of the biscoclaurine type as the tertiary bases, the Japanese Mahonia yield isotetrandrine in large quantities which has never been discovered in the Indian Mahonia. These results are summarized in Table II.

Chatterjee and Guha⁴⁾ discovered neprotine⁸⁾ (VI) from many of the Indian species of Mahonia. Further, the same investigators⁹⁾ discovered umbellatine¹⁰⁾ (VII) along

5) F. Späth, A. Kolbe : Ber., 58, 2280 (1925).

6) H. Kondo, I. Keimatsu : This Journal, 55, 234, 894 (1935); Ber., 68, 1503 (1935); M. Tomita, E. Fujita, F. Murai : This Journal, 71, 226, 1035 (1951).

7) M. Tomita, T. Tani : This Journal, 61, 251 (1941).

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

9) R. Chatterjee : J. Am. Pharm. Assoc., 33, 210 (1944).

10) R. Chatterjee : J. Am. Pharm. Assoc., 38, 149 (1949).

Table II.

	Indian Mahonia	Japanese Mahonia
Tert. Base	Oxyacanthine Berbamine	Isotetrandrine Berbamine
Quat. Base	Berberine Palmatine Jatrorrhizine	Berberine Palmatine Jatrorrhizine

with neprotine from *Mahonia neparensis* DC., but the authors were unable to identify these bases from the Japanese Mahonia. The authors isolated from *Mahonia japonica* a crystalline substance, m.p. 211~212.5° in small quantities. This nitrogen-free neutral substance is a new one which has not hitherto been described in literature.

On the alkaloids contained in the roots, a further report is scheduled to be made later.

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Experimental

Extraction and Isolation of the Alkaloids—The finely-cut trunk bark was extracted with hot 50% hydrated methanol (1% acidulated with hydrochloric acid), and then again extracted five times with boiling methanol for 7 hours per time. The extracts were combined and methanol evaporated under reduced pressure. 4 kg. of plant material yielded 440 g. of viscous residue (11% of the total trunk bark). Five or six volumes of water weakly acidulated with hydrochloric acid was added to the residue, warmed under stirring, and after standing for some time, filtered. The filtrate was then treated with 0.5 *N* lead subacetate solution (1 L.) and the resultant precipitate filtered off. The filtrate was freed from lead salts by precipitation with sulfuric acid. After filtering off the lead sulfate, the clear filtrate was made alkaline with ammonia, and the total tertiary bases deposited were extracted several times with ether. The ether extracts were combined and concentrated to a small volume. The ammonia-alkaline solution separated from the total tertiary bases was acidified with acetic acid, and on addition of potassium iodide the total quaternary bases precipitated as the iodide. The precipitate was collected, mixed with zinc dust and diluted sulfuric acid, and reduction carried out at a bath temperature of 70°, under stirring, for six hours. A colorless solution containing the tetrahydro derivatives of the quaternary bases was obtained.

Tertiary Bases—The ether solution of the total tertiary bases yielded, after standing overnight, a large colorless octahedral crystals. The crystals were dissolved in 2% hydrochloric acid, alkalinized with ammonia, and the alkaline solution extracted with chloroform. The chloroform extracts, after being dried with anhydrous potassium carbonate, was evaporated to dryness, leaving an oily residue. Recrystallized from a large amount of acetone, colorless prisms (A crystals), m.p. 180.5 to 182°, were obtained. The ether solution separated from A crystals was extracted with 2% sulfuric acid solution, to which potassium bromide was added, and the hydrobromides that precipitated was dissolved in warm water, made alkaline with ammonia and extracted with ether. The ether solution was again extracted with 2% sulfuric acid solution, which was saturated with sodium sulfate, and on standing, the bases deposited as the sulfate. The sulfate was dissolved in warm water, made alkaline with ammonia, and extracted with ether. The ethereal solution, after being dried with anhydrous potassium carbonate, was concentrated by which colorless octahedral crystals

deposited. The crystals were dissolved in chloroform and recrystallized from hot acetone to colorless prisms* (A' crystals), m.p. 180~181°. The mother-liquor left after the separation of the sulfate of A' crystals was alkalinized with ammonia, and extracted with ether, which was dried with anhydrous potassium carbonate, and evaporated to dryness, leaving a brownish substance. This was dissolved in benzene, concentrated to a small volume and after standing, a crystalline precipitate was obtained. Recrystallization from benzene yielded colorless needles (B crystals), m.p. 124~126° (decomp. with effervescence). The ether layer left after the separation of the total tertiary bases was concentrated to a small volume. The deposited crystals were dissolved in acetic acid, alkalinized with ammonia, and extracted with ether. The ethereal solution was dried with anhydrous potassium carbonate, and on removal of the solvent the crystals were deposited, which were dissolved in acetone, insoluble materials in acetone removed. The crystals deposited on concentration of the acetone solution were recrystallized from methanol to colorless prisms (C crystals), m.p. 211~212.5°.

(1) A crystals, m.p. 180~182° (Isotetrandrine): Recrystallization from a large amount of hot acetone yielded colorless prisms, m.p. 180.5~182°; yield, 2.6 g. They were readily soluble in chloroform, but almost insoluble or sparingly soluble in other organic solvents when cold but dissolve slightly on warming. They were readily soluble in diluted acid, insoluble in alkali, and gave no color with ferric chloride solution. Gaebel's reaction was negative. $[\alpha]_D^{10} = +147^\circ$ (chloroform). No depression occurred by the mixed melting point determination with isotetrandrine, m.p. 181~182°. *Anal.* Calcd. for $C_{38}H_{42}O_5N_2$: C, 73.27; H, 6.74; N, 4.51. Found: C, 73.20; H, 6.40; N, 4.88.

(2) A' crystals, m.p. 180~181° (Isotetrandrine): Recrystallized from a large amount of hot acetone to colorless prisms, m.p. 180~181°; yield, 1.3 g. They gave the same properties as A crystals, and did not depress the melting point of isotetrandrine, m.p. 181~182°.

(3) B crystals, m.p. 124~126° (Berbamine-benzene adduct): The benzene solution was kept for a long time, by which crystalline residue deposited, which recrystallized from benzene to colorless needles, m.p. 124~126° (decomp. with effervescence); yield, 30 mg. They were soluble in acids, insoluble in weak alkalis, and soluble in caustic alkali. Gaebel's reaction, negative. By admixture of this substance with berbamine-benzene adduct, m.p. 124~126°, it showed no melting point depression. Dried at 110° in vacuo over P_2O_5 , they gave m.p. 176°.

Methylation of B crystals by diazomethane. Approx. 10 mg. of B crystals were dissolved in a small portion of ether, mixed with the ether solution of diazomethane, and kept for three days, depositing colorless prisms. On removal of ether the residue was recrystallized from acetone, and yielded colorless prisms, m.p. 165~171°, in minute quantities, which did not permit further recrystallization. They did not depress the melting point of isotetrandrine, m.p. 180~182°.

(4) C crystals, m.p. 211~212.5° (N-free substance): Recrystallization from methanol yielded colorless needles, m.p. 211~212.5°, yield 25 mg. Nitrogen test, negative. They were sparingly soluble or insoluble in alkalis, soluble in acetone and methanol, and comparatively slightly soluble in ether. *Anal.* Found: C, 64.05; 63.97; H, 5.61, 5.87.

Tetrahydro Derivatives of the Quaternary Bases—The colorless solution of the tetrahydro derivatives, obtained by the reduction of quaternary base iodides with zinc dust and diluted sulfuric acid, gave precipitates on cooling, which were separated from the mother liquor. The mother liquor was made alkaline with ammonia, and extracted with ether. The ether solution was treated with 2% sodium hydroxide solution to separate into the phenolic and non-phenolic bases. The sodium hydroxide solution of the phenolic bases deposited the bases on addition of ammonium carbonate, which were extracted with ether, followed by chloroform. The extracts were combined, dried with anhydrous potassium carbonate, and ether and chloroform distilled off, depositing crystals. Recrystallization from methanol yielded colorless needles, m.p. 212~214° (E crystals). The ether solution of non-phenolic bases was dried with anhydrous potassium carbonate, and ether distilled off. Recrystallization from methanol, the residue gave light yellow needles or long plates, m.p. 169~171° (F crystals). The mother liquor separated from F crystals was again concentrated, and kept, depositing stellate crystals, m.p. 139~141° (G crystals).

On the other hand, the precipitate which after reduction deposited on cooling and was separated from the mother liquor, was dissolved in warm water, made alkaline with ammonia, and by treating by the same method as described for the mother liquor, it was separated into phenolic and non-phenolic bases, giving colorless needles (E' crystals), m.p. 211~213°, and light yellow needles (F' crystals), m.p. 169~171°, respectively.

(1) E crystals, m.p. 212~214° (Tetrahydrojatrorrhizine): Recrystallization from methanol yielded colorless needles, m.p. 212~214°; yield, 2.9 g. Gaebel's reaction negative. They did not depress the melting point of tetrahydrojatrorrhizine, m.p. 211~213°. *Anal.* Calcd. for $C_{20}H_{23}O_4N$: C, 70.38; H, 6.74; N, 4.11. Found: C, 70.52; H, 6.59; N, 4.24.

(2) E' crystals, m.p. 211~213° (Tetrahydrojatrorrhizine): Recrystallization from methanol also yielded colorless needles, m.p. 211~213°; yield 2.4 g. They gave quite the same properties as E

crystals, and did not depress the melting point of tetrahydrojatrorrhizine, m.p. 211~213°.

(3) F crystals, m.p. 169~171° (Tetrahydroberberine). Repeatedly recrystallized from methanol, they were obtained in light yellow needles or long plates, m.p. 169~171°; yield, approx. 3 g. They gave positive Gaebel's reaction, and did not depress by the mixed melting point determination with tetrahydroberberine, m.p. 169~171°. *Anal.* Calcd. for $C_{20}H_{21}O_4N$: C, 70.81; H, 6.25; N, 4.14. Found: C, 71.00; H, 6.25; N, 4.40.

(4) F' crystals, m.p. 169~171° (Tetrahydroberberine). Recrystallization from methanol yielded slightly yellow needles in minute quantities, m.p. 169~171°. Their properties quite agreed with those of F crystals.

(5) G crystals, m.p. 139~141° (Tetrahydroshobakunine). Repeated recrystallization from methanol gave clusters, m.p. 139~141°. They gave quite the same properties as tetrahydroshobakunine (a molecular compound of tetrahydroberberine and tetrahydropalmatine), and by the admixture of this substance with tetrahydroshobakunine, m.p. 139~141°, it shows no melting point depression.

Summary

The authors identified from domestic *Mahonia japonica* DC. isotetrandrine (I, R=CH₃) and berbamine (I, R=H) as the tertiary bases; berberine, palmatine and jatrorrhizine as the quaternary bases, and in addition a nitrogen-free neutral substance, m.p. 211~212.5°, in minute quantities. Although the Indian *Mahonia* usually yielded oxyacanthine, the authors showed that oxyacanthine was not to be found in the Japanese *Mahonia*, isotetrandrine being contained in large quantities instead. This is the first evidence of the occurrence of isotetrandrine in families other than the Menispermaceae.

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216. Yoshihisa Mizuno and Kikuo Adachi: Benzothiazoles. III. Synthesis of 2-Methylbenzothiazoles with Electron-attracting Groups.

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In one¹⁾ of the preceding papers of this series, the effect of para-nitro group in the benzene ring upon benzothiazole ring-formation of thioacyl-anilides by the action of potassium ferricyanide was examined. Based on the results obtained, we suggested that, in the case of *p*-nitro-thioacetanilide, enolization of thio-carbonyl group, which was essential to the ring formation, was so difficult owing to the considerable contribution of the structure (II) to resonance that the ring-formation of the thioacetanilide via the enol form was suppressed and the formation of *p*-nitro-acetanilide became favorable.

To check the general validity of this suggestion and also to study the scope and limitation of the improved procedure devised by one²⁾ of the present authors, other thioacetanilides with electron-attracting groups such as nitro, methyl, sulfonyl and cyano groups or with both nitro and methoxyl groups on the benzene ring were subjected to the

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1) Annual Report of the Faculty of Pharmacy, Kanazawa University, **1**, 8 (1951).

2) This Journal, **70**, 540 (1950).