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26. **Masao Tomita and Takehiko Kugo** : Studies on the Alkaloids of Berberidaceous Plants. VIII. Alkaloids of *Berberis amurensis* Rupr. var. *japonica* (Regel) Rehd. forma *Bretschneideri* (Rehd.) Ohwi. (2).[1]

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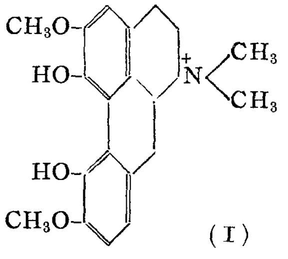
In the preceding paper[1] of this series, we reported that the bark and trunk of *Berberis amurensis* Rupr. var. *japonica* (Regel) Rehd. forma *Bretschneideri* (Rehd.) Ohwi (Japanese name " Akajiku-hebinoborazu ") contains the tertiary bases berbamine, oxyberberine, and a new alkaloid, m.p. 190∼191°, and the quaternary bases berberine, palmatine, and jatrorrhizine. We also mentioned that it is of interest from a taxonomical point of view that it furnishes only berbamine as the biscoclaurine bases not accompanied by oxyacanthine, as compared with *Berberis Thunbergii* DC. (Japanese name " Megi ")[2] belonging to the same genus Berberis.

This work has been stimulated by the discovery of substances producing a precipitate by alkaloidal reagents, which after isolating the quaternary bases (berberine, palmatine, and jatrorrhizine) as the iodides, still remained in the mother liquor.

The plant material used in this experiment was collected in the spring of 1955, at the seashore of Futami Village, Sado Island, Niigata Prefecture. The methanol extract of 30 kg. of the undried stem was treated in the usual manner, and after isolation of tertiary bases, the quaternary bases (berberine, palmatine, and jatrorrhizine) were removed by precipitating them as the iodides. The mother liquor from these iodides was then treated as described in the experimental section and yielded a quaternary base as the styphnate, m.p. 231°(decomp.). The yield was 4.5 g. The quaternary alkaloid gave a well crystallizing iodide, m.p. 254∼255°(decomp.). The analytical data and infrared spectra of the styphnate and the iodide agreed closely with those of the corresponding derivatives of magnoflorine[3] (I).



(I)

Besides magnoflorine styphnate, there was obtained a small amount of the styphnate, m.p. 211∼212°(decomp.), which differed from magnoflorine styphnate in the analytical data. The picrate showed m.p. 212∼213°(decomp.), which gave different analytical data and infrared spectrum from those of magnoflorine picrate. However, the iodide derived from the above picrate showed m.p. 254∼255° (decomp.) and its infrared spectrum was in accord with that of magnoflorine iodide. Because of the small amount of the styphnate, m.p. 211∼212°(decomp.), available, it was impossible to investigate it further, but it might be suggested that the styphnate of m.p. 211∼222°(decomp.) was a less pure state of magnoflorine styphnate.

Meanwhile, the quaternary bases (berberine, palmatine, and jatrorrhizine) precipitated as the iodides were reduced with zinc powder and dilute sulfuric acid to the tetrahydro derivatives, the mother liquor from which, after purification by the reineckate precipitation, an additional amount of 0.1 g. of magnoflorine styphnate,

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[1] Part VII. (1). M. Tomita, T. Kugo : J. Pharm. Soc. Japan, **75**, 753(1955)(C. A. **49**, 13597(1955)).

[2] H. Kondo, M. Tomita : Arch. Pharm., **261**, 1(1930); J. Pharm. Soc. Japan, **50**, 309(1930).

[3] T. Nakano : This Bulletin, **2**, 326, 329(1954).

m.p. 231°(decomp.), was obtained.

Similar treatment of the root of the plant material gave the results as shown in Table I. In this case, no such styphnate of m.p. 211~212°(decomp.), as obtained from the stem, was detected. In general, it seems that the root furnishes a higher amount of alkaloids than the stem.[1] The yields are all based upon 4.5 kg. of the undried plant material, and those of (D) and (F) are indicated as the tetrahydro derivatives.

TABLE I.

Bases from the root of *Berberis. amurensis*				m.p.(°C)	Yield (g.)
Tert. base	Phenolic base	(A)	Berbamine (benzene adduct)	124~126	15.9
		(B)	New base	190~191	0.2
	Non-phenolic base	(C)	Oxyberberine	197~199	0.2
Quat. base	Phenolic base	(D)	Jatrorrhizine		1.8
		(E)	Magnoflorine (styphnate)		3.6
	Non-phenolic base	(F)	Shobakunine (Mol. compd. of tetrahydroberberine and tetrahydro palmatine)		1.2

Recently, Nakano[4] in our laboratory has revealed that *Magnolia grandiflora* L. (Magnoliaceae) and *Cocculus trilobus* DC. (Menispermaceae) contain a quaternary alkaloid magnoflorine (I) of the aporphine type in large quantities. The occurrence of the same quaternary base in *Berberis amurensis* of the taxonomically related family Berberidaceae is of biogenetic significance.

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Experimental[5]

Isolation of Magnoflorine from the Bark and Trunk—The coarsely ground bark and trunk (30 kg. based on undried material) was extracted with warm MeOH, the solvent removed under reduced pressure, and the sticky residual mass was treated in the conventional manner.[1] The ammonia-alkaline mother liquor freed from tertiary bases, after acidification with AcOH, was saturated with KI, whereby the quaternary bases (berberine, palmatine, and jatrorrhizine) precipitated as the iodides, which were filtered off with suction. The filtrate was treated with aq. ammonium reineckate solution, and the resulting precipitate was decomposed with aq. Ag_2SO_4 and then with aq. $BaCl_2$, yielding the chloride of the quaternary base. This did not crystallize in spite of two more repetitions of similar procedure.

Consequently, this was dissolved in the minimum amount of water and treated with conc. aq. sodium styphnate (1:1 mixture of styphnic acid and NaOH) solution. The resulting precipitate was dissolved in acetone, and on concentrating the solution somewhat, yellow needles were obtained. Recrystallization was effected from acetone, m.p. 211~212°(decomp.); yield, 0.35 g.

The mother liquor from the above styphnate, when concentrated to a small volume, deposited yellow prisms (magnoflorine styphnate), which were recrystallized from acetone to show m.p. 231° (decomp.). Yield, 4.5 g. *Anal.* Calcd. for $C_{20}H_{24}O_4N \cdot C_6H_2O_8N_3$: C, 53.24; H, 4.47; N, 9.55. Found: C, 53.29; H, 4.63; N, 9.55.

0.2 g. of magnoflorine styphnate, on decomposition with 1% HCl and on subsequent treatment with KI, was converted into its corresponding iodide which crystallized from a mixture of MeOH and a small amount of acetone in colorless pillars, m.p. 254~255°(decomp.), yield, 0.1 g. *Anal.* Calcd. for $C_{20}H_{24}O_4NI$: C, 51.18; H, 5.15; N, 3.19. Found : C, 51.29; H, 5.25; N, 3.45. The infrared spectra of the styphnate, m.p. 231°(decomp.), and the iodide, m.p. 254~255°(decomp.), were identical with those of the corresponding derivatives of magnoflorine.

On the other hand, the styphnate, m.p. 211~212°(decomp.)(*Anal.* Found : C, 48.09; H, 3.45; N, 12.10), was converted by the usual method into its corresponding picrate, m.p. 212~213°

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4) T. Nakano : This Bulletin, 4, 67, 69(1956).

5) All melting points are uncorrected. We are indebted Mr. K. Hozumi and the members of the Central Analysis Room of this Institute for the microanalyses.

(decomp.)(*Anal.* Found : C, 50.06; H, 4.15; N, 12.79). 0.2 g. of the same styphnate, m.p. 211~212° (decomp.), was converted into its corresponding iodide, m.p. 254~255°(decomp.); yield, 70 mg. This iodide gave the same analytical data as those for magnoflorine iodide. *Anal.* Calcd. for $C_{20}H_{24}O_4NI$: C, 51.18; H, 5.15; N, 3.19. Found : C, 51.44; H, 5.27; N, 3.40. Its infrared spectrum is quite identical with that of magnoflorine iodide.

Subsequently, the quaternary bases which were precipitated as iodides from the mother liquor of the tertiary bases were reduced with Zn powder and 10% H_2SO_4, and the tetrahydro derivatives of berberine, palmatine, and jatrorrhizine were removed by means of ether. The mother liquor thus remaining was acidified with HCl and treated with saturated ammonium reineckate solution, yielding the reineckate as a precipitate. The reineckate was treated as described above, from which magnoflorine styphnate, m.p. 231°(decomp.), was obtained. Yield, 0.5 g.(from total material).

Isolation of the Alkaloids from the Root—The root (4.5 kg. based on undried material) was coarsely ground and treated in a similar manner. MeOH extract was dissolved in 3% AcOH with warming and filtered off after cooling. The filtrate was shaken thoroughly with ether to remove acidic and neutral substances, made alkaline with NH_4OH, and the deposited tertiary bases were taken up in ether. From the ether extract berbamine, oxyberberine, and a new base, m.p. 190~191°, were obtained (Table I).

The NH_3-alkaline mother liquor, after acidification with AcOH, was saturated with KI to precipitate the quaternary bases as the iodides and filtered. They were reduced with Zn powder and 10% H_2SO_4 to give the tetrahydro derivatives (Table I).

The mother liquor filtered from the iodides of the quaternary bases was treated by the same procedure as described for the bark and trunk, yielding magnoflorine styphnate, m.p. 231°(decomp.). Yield, 3.6 g.

Summary

A quaternary alkaloid magnoflorine (I) has been identified from the stem and root of *Berberis amurensis* Rupr. var. *japonica*(Regel) Rehd. forma *Bretschneideri* (Rehd.) Ohwi (Berberidaceae). The wide distribution of magnoflorine (I) throughout the families Magnoliaceae (*M. grandiflora* L.), Menispermaceae (*Cocculus trilobus* DC.), and Berberidaceae (*B. amurensis*) is of significance in biogenesis of alkaloids.

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