8. **Tatsuhiko Nakano:** Studies on the Alkaloids of Magnoliaceous Plants. IX.[1] Alkaloids of *Magnolia liliflora* Desrouss.*

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A survey has been made of the alkaloids contained in the leaves, bark, trunk, and root of *Magnolia liliflora* Desrouss (Japanese name "Mokuren"), belonging to the Magnoliaceae, which grows in Japan. The results obtained are reported in the following. The plant material used was collected late in May in a garden in the environs of Kyoto City. The present study deals with the isolation and identification of the alkaloids of each part of the plant.

First, a survey was made of the base contained in the leaves. The methanol extract of the leaves was treated by the procedure described in the experimental part and gave a crystalline hydrochloride as a tertiary base, which recrystallized from alcohol in the form of colorless prisms, m.p. 260~262°(decomp.). This substance produced a precipitate by the Mayer reagent and the Millon test was positive. No further investigation, however, was made because of its very minute amount. Following the procedure[2] so far employed, the quaternary base was examined, but its content was so small that no satisfactory results were obtained.

Second, the investigation of the bases of the bark was undertaken. The methanol extract of the stem bark was treated by the same procedure as in the case of the leaves, and furnished, as a tertiary base, a hydrochloride crystallizing in the form of colorless prisms, m.p. 254°(decomp.), in small quantities. This substance precipitated by the Mayer reagent and the Millon test was positive. Also the quaternary bases of the bark were treated by the same method so far employed. By purification through phosphotungstic acid and the mercuric chloride salts, colorless prisms, $C_{12}H_{20}O_2NCl$, m.p. 260~261°(decomp.), was isolated as the chloride, which was identified as salicifoline chloride by the mixed melting point determination. This substance formed a picrate which crystallized in the form of orange yellow prisms, m.p. 182~182.5°, undepressed by an authentic sample of salicifoline picrate, m.p. 181~182°. Subsequently, the residual bases which remained in the mother liquor left after separation of the above chloride were precipitated as the picrates, and by taking advantage of the relative degree of solubility in acetone, separated into orange yellow prisms, m.p. 181~182°, and yellow needles, m.p. 181~182°. When the above two kinds of the picrates were fused with authentic samples of salicifoline picrate, m.p. 181~182°, and magnocurarine picrate, m.p. 181~182°, respectively, no melting point depressions occurred.

Also, in the isolation of the base of the trunk, the methanol extract prepared was treated in the usual manner, and the tertiary base was extracted with ether followed by benzene, but its amount was not sufficient to make any further examination. On the other hand, the quaternary base was precipitated utilizing ammonium Reineckate as the base precipitant instead of phosphotungstic acid so far used, and then purified as the mercuric chloride salt, as a results of which colorless prismatic crystals, m.p. 260~261°(decomp.), were obtained as the chloride, undepressed by an authentic sample of salicifoline chloride, m.p. 260~261°(decomp.). Its picrate forms orange yellow prisms, m.p. 181~182°, either alone or on admixture with a sample of salicifoline picrate, m.p. 181~182.5°. These results

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2) M. Tomita, T. Nakano: J. Pharm. Soc. Japan, 72, 200, 727, 766, 1260 (1952).

indicate that this base is identical with salicifoline chloride. The residual alkaloid remained in the mother liquor after separation of this chloride was isolated as a picrate by the usual method, and yielded a picrate crystallizing in the form of orange yellow prisms, m.p. 181~183°, the identity of which was confirmed by mixing with an authentic sample of salicifoline picrate, m.p. 181~182.5°.

Finally, as a result of the investigation of the alkaloid contained in the root, it was clarified that it furnishes no appreciable amount of a tertiary base, and salicifoline chloride in small quantities was obtained as a quaternary base.

For convenience of comparison the distribution of the alkaloids obtained as crystals from each part of *Magnolia liliflora* Desrouss is summarized in Table I.

TABLE I

		Magnolia liliflora Desrouss			
		leaves	bark	trunk	root
Tert. base	m.p. 260~262°(decomp.)	+			
	m.p. 254°(decomp.)		+		
Quat. base	salicifoline		+	+	+
	magnocurarine		+		

It has been revealed that the bark furnishes the highest percentage of the alkaloid content, and salicifoline is widely distributed throughout every organ except the leaves of the plant.

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Experimental[3]

Extraction and Isolation of the Alkaloids of *Magnolia liliflora* Desr. — (1) Leaves — The fresh leaves (660 g.) were roughly cut and extracted three times with warm methanol. The solvent was removed under reduced pressure and the sirupy residue was treated with 1% aqueous tartaric acid. The acid extract was freed from acidic and neutral materials by means of ether, and after alkalization with sodium carbonate, extracted exhaustively with ether. The ether extracts were combined, dried over anhydrous potassium carbonate, and the ether distilled off, yielding a small amount of brownish oily residue. This was again dissolved in ether, and hydrochloric acid gas was passed through the latter, depositing the hydrochloride of the base. This was collected and when recrystallized from alcohol, colorless prisms, m.p. 260~262°(decomp.), were obtained. This substance gave precipitates by the Mayer reagent, and the Millon test was positive. Because of its very small amount further examination was not made. The alkaline solution separated from the tertiary base by extraction with ether was acidified with sulfuric acid, and the quaternary base was treated as the phosphotungstic acid salt and then as the mercuric chloride salt, but its amount was not sufficient to give any satisfactory result.

(2) Bark—Raw, roughly cut bark (1.8 kg.) was extracted three times with warm methanol, and the methanol was evaporated to a viscous residue. This was dissolved in 1% aqueous tartaric acid, and extracted with ether to remove acidic and neutral materials. Then the aqueous layer was made alkaline with sodium carbonate, and shaken first with ether and then with chloroform. The ether extract was dried over anhydrous potassium carbonate, and the ether removed, leaving a yellowish brown, oily residue. This was again dissolved in ether, and hydrochloric acid gas passed through the latter, when the hydrochloride of the base deposited. This was collected, dissolved in alcohol, and after decolorization by charcoal, concentrated, yielding colorless prisms, m.p. 253°(decomp.); yield, 10 mg. Recrystallization was affected from alcohol; m.p. 254°(decomp.). This substance yielded precipitates by the Mayer reagent and gave a positive Millon reaction. Meanwhile, the chloroform extract was dried over anhydrous potassium carbonate, and the solvent

3) All melting points are uncorrected. The author wishes to express his appreciation to Mr. K. Hozumi and Mr. K. Imaeda in the Microanalytical Laboratory of the Pharmaceutical Institute, University of Kyoto, for performing the microanalyses reported herein.

distilled off, leaving a brownish oily residue. When this was further purified through the acid-alkali process, very minute amount of crystalline substance appeared, but its amount did not permit any further investigation. As soon as possible following the extraction by the above solvents, the alkaline solution was acidified with sulfuric acid, and submitted to the phosphotungstic acid-mercuric chloride process giving colorless prisms as the quaternary base chloride. By recrystallization from a mixture of methanol and a few drops of water they melted at 260~261° with decomposition, undepressed by a sample of salicifoline chloride. The yield was 1.5 g. Dried at 85° in vacuo over phosphorus pentoxide. *Anal.* Calcd. for $C_{12}H_{20}O_2NCl$: C, 58.65; H, 8.20. Found: C, 58.92; H, 8.39.

This substance, when reacted with picric acid, yielded the picrate crystallizing in the form of orange yellow prisms, m.p. 182~182.5°, undepressed by a sample of salicifoline picrate. The mother liquor remaining after separation of the above chloride was diluted with a small portion of water, and treated with saturated aqueous sodium picrate solution. The resulting black brownish, muddy precipitate was filtered, dissolved in a small amount of acetone, and extracted with water by gently warming on a water bath. After cooling, the resinous material was decanted and discarded. This manipulation was repeated until no trace of the base could be recognized in the aqueous extract. The aqueous extracts were combined and evaporated in vacuo to dryness, yielding an orange yellow crystalline residue. This was dissolved in acetone and, by taking advantage of the relative degree of solubility in acetone, separated into orange yellow prisms, m.p. 179~180.5°, and yellow needles, m.p. 176~178°. The former, when further recrystallized from acetone, melted at 181~182° either alone or on admixture with a sample of salicifoline picrate. Yield, 0.9 g. The latter, when recrystallized once more from acetone and dried at 85° in vacuo over phosphorus pentoxide, showed the melting point of 181~182°, undepressed by a sample of magnocurarine picrate, m.p. 181~182°. Yield, 30 mg. *Anal.* Calcd. for $C_{19}H_{24}O_3N \cdot C_6H_2O_7N_3$: C, 55,35; H, 4.83. Found: C, 55.42; H, 5.10.

The filtrate left after separation of the black brownish muddy precipitate resulting by the addition of aqueous sodium picrate solution was evaporated in vacuo to dryness, and the residue treated, but no appreciable amounts of crystals were obtained.

(3) Trunk—Dried, powdered trunk (4.9 kg.) was extracted three times with boiling methanol, and the methanol removed. The residue was extracted with 1% aqueous tartaric acid solution, and then treated with a concentrated solution of lead subacetate. The filtrate was freed from lead salts by precipitation with hydrogen sulfide, concentrated to approx. 400 cc., and shaken with ether to remove ether-soluble impurities. The aqueous layer, after making alkaline with sodium carbonate, was extracted with ether followed by benzene. The ether and benzene extracts were dried over anhydrous potassium carbonate, and the solvents removed, yielding a slightly yellowish brown and a light yellow oily residue, respectively. Because of the very small amounts they were not further examined. Immediately after the ether and benzene extractions the alkaline solution was acidified with hydrochloric acid and a saturated aqueous solution of ammonium Reineckate added. The light pink chalky precipitate which resulted was washed thoroughly with water. The Reineckate was dissolved in acetone and a 0.6% solution of silver sulfate was added to the acetone solution with mixing until no further precipitation of silver Reineckate occurred. Before filtering off the precipitated silver Reineckate, an amount of barium chloride solution containing a molecular equivalent corresponding to the silver sulfate used for precipitation was added to convert the quaternary base sulfate to the chloride and to precipitate any excess silver sulfate which had been added. The combined silver Reineckate and barium sulfate precipitates were filtered off and washed throughly with water. The filtrate and washings were combined and concentrated in vacuo at 40° to approx. 50 cc. Then the solution was saturated with powdered mercuric chloride to precipitate the base as the mercuric chloride salt, which was decomposed by hydrogen sulfide and gave the chloride as colorless prisms, m.p. 260~261°(decomp.). Yield, 1.2 g. This substance formed a picrate which crystallized in the form of orange yellow prisms, m.p. 181~182°, either alone or on admixture with a sample of salicifoline picrate. The residual alkaloid remaining in the mother liquor was purified as the picrate, which crystallized in the form of orange yellow prisms, m.p. 181~182°, undepressed by a sample of salicifoline picrate. Yield, 5.1 g. The mother liquor from the above salicifoline picrate was further treated, but no other alkaloid could be identified.

(4) Root—Dried, roughly chopped root (2 kg.) was extracted three times with boiling methanol, and the solvent removed. The sirupy residue was treated by the same procedure as employed in the case of the trunk, but no appreciable amounts of the tertiary base could be identified. The quaternary base was precipitated as the Reineckate and, by treating as described in the case of the trunk, colorless prisms, m.p. 260~261°(decomp.), were obtained as the chloride. Yield, 0.1 g. When mixed with salicifoline chloride, no melting point depression occurred. The remaining alkaloid in the mother liquor was isolated as the picrate and a further small amount of salicifoline was obtained, but no other new base could be detected.

Summary

A survey was made of the alkaloids contained in the leaves, bark, trunk, and root of *Magnolia liliflora* Desrouss, belonging to the Magnoliaceae, which grows in Japan. The bases identified are: as a tertiary base, colorless prisms, m.p. 260~262° (decomp.) (hydrochloride) from the leaves; as a tertiary base, colorless prisms, m.p. 254° (decomp.) (hydrochloride), and as quaternary bases salicifoline chloride and magnocurarine from the stem bark; and as a quaternary base, salicifoline chloride from the trunk and root.

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9. Takeo Ueda, Tomohiko Kawai, and Tadakazu Tsuji: Studies on Anthelmintics. I. Studies on Hydroxytetralin as an Anthelmintic.

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In the course of investigations on the anthelmintic action of santonin, it was not found to possess such a direct ascaricidal activity as shown by hexylresorcinol *in vitro*[1]. Trendelenburg[2] has suggested that its activity was due to the convulsive action produced in the ascaris by its lactone, while Shirane[3] has claimed that its activity was due to the paralysing action produced in the ascaris by the quinol substance produced from santonin itself. The latter theory has been accepted as an almost established theory in Japan.

Very recently, Kobayashi and Bando[4] explained that the activity of santonin was due to the abnormal kinetic state and the fern-like curling-motion made by the ascaris by santonin.

On the other hand, desmotroposantonin, the isomeric compound of santonin, has been found to possess a slight direct ascaricidal activity *in vitro*.

As to the main skeleton of tetralin ring, santonin possesses one hydroxyl group in the 1-position of tetralin, while desmotroposantonin possesses two hydroxyl groups in the 1,7-positions.

On the whole, the reason why desmotroposantonin has stronger direct ascaricidal properties than santonin might be due to its hydroxyl group in the 7-position.

Judging by the studies of Kobayashi, Bando, or Trendelenburg, and also by Lamson[5], who has studied the activity of the hydroxyl group of alkylbenzene, it seems necessary to investigate not only the behavior of the hydroxyl group in the 7-position of tetralin, but also of that in the 1-position.

In order to confirm this assumption, compounds related to the structure of santonin and desmotroposantonin such as the hydroxytetralin were examined for their ascaricidal activities against *Ascaris lumbricoides* by Lamson-Nakamura method[6] and their curling-motions against *Ascaris lumbricoides* by Kobayashi-Bando method[4].

This paper describes the interesting relationship between the structure and activity of several *ar*- and *al*-hydroxytetralins.

Syntheses of Hydroxytetralins Three series of hydroxytetralins were synthesized, *viz.*

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