73. Tatsuhiko Nakano: Studies on the Alkaloids of Magnoliaceous Plants. XII[1]. Alkaloids of *Magnolia grandiflora* L. (1)*.

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The present paper deals with the isolation and identification of two kinds of quaternary alkaloids from the root of *Magnolia grandifloara* L. (Japanese name "Daisanboku"), belonging to Magnoliaceae, growing in Japan; the detection of candicine (V) will form the main subject of this communication.

The plant material used in this investigation was collected by Mr. H. Yamada of Kobe University in April, 1952, and a portion of the methanolic extract was kindly prepared by him. The isolation of alkaloids from this extract was carried out by a procedure essentially similar to that so far employed. The amount of tertiary bases present was too small to permit any further examinations. For the isolation of crystalline quaternary alkaloids, the following two methods were used; one employing phosphotungstic acid and mercuric chloride as the base precipitant and the other utilizing ammonium reineckate for this purpose. Of these two, the repetition of the latter process seemed more satisfactory than the former.

The reineckates of the quaternary bases were decomposed with silver sulfate and then converted into the chlorides, but they did not readily crystallize and gave a syrupy oil (M). Consequently, a major portion of (M) was led to the picrates, which by taking advantage of the relative degree of solubility in ethanol, could be separated into picrate (A) and picrate (B). Picrate (A) crystallized from ethanol in the form of slender yellow pillars, m.p. 164~165.5°. This substance gave a negative test for a methylenedioxy group with the Gaebel reagent and showed no methoxyl group in the Vieböck and Brecher estimation. The analytical values represent the composition of $C_{11}H_{18}ON \cdot C_6H_2O_7N_3 \cdot {}^1/_2H_2O$. On the other hand, picrate (B) formed from ethanol orange yellow pillars, m.p. 181~182°, the identity of which was confirmed by admixture with an authentic sample of salicifoline picrate, m.p. 181~182°.

Another portion of (M), on treatment with methanolic potash and methyl iodide, afforded a product which crystallized from a mixture of methanol and a little water in the form of colorless pillars, m.p. 225~226.5°. This substance is optically inactive, and contains no methoxyl and one $N(CH_3)_3$ group (Vieböck-Brecher). By analysis it was found to possess the empircal formula $C_{11}H_{18}ONI$. The infrared spectrum (Nujol mull) showed the presence of a hydroxyl function.

From these experimental results, it seemed most probable that the iodide, m.p. 225~226.5°, would be identical with either (II) or (III).

King and Holmes[3] already synthesized β-hydroxyphenethyltrimethylammonium iodide[2] (II) and recorded for it m.p. 226°, which is quite in accord with that of the present iodide (m.p. 225~226.5°). In order to allow a direct comparison to be made, (II) was

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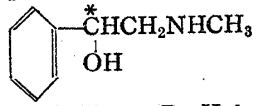
1) Part XI. M. Tomita, T. Kugo: This Bulletin, **2**, 115(1954).

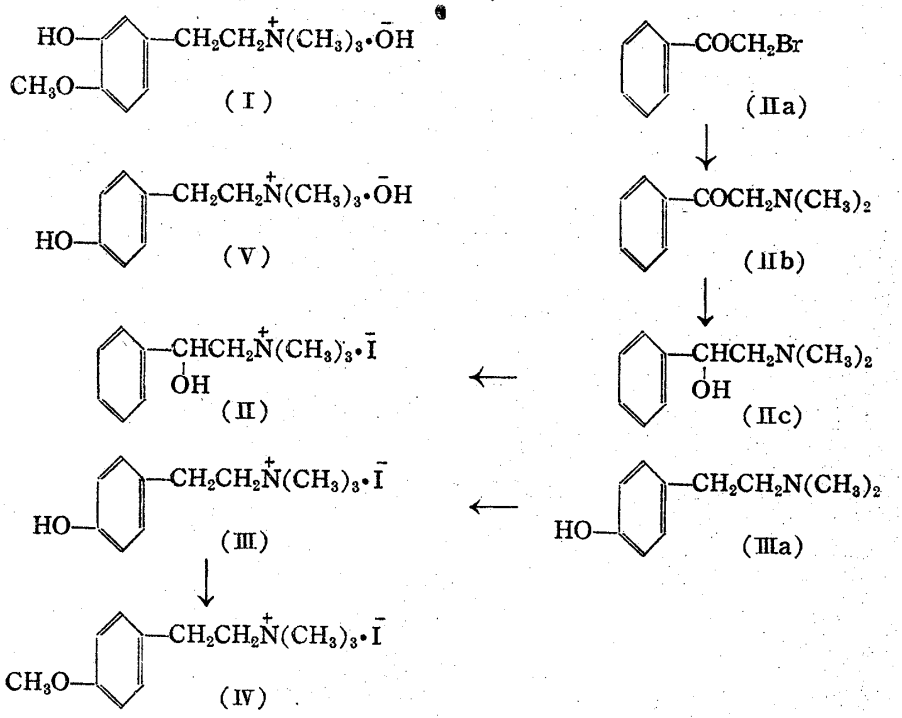
2) Although this substance itself has not yet been found in nature, its parent alkaloid, *l*-β-hydroxyphenethylmethylamine (halostachine) is known to occur in *Halostachis caspica* (Hennopodiaceae). cf.



G. P. Menshikov, M. M. Rubinstein: J. Gen. Chem. (U. S. S. R.), **13**, 801 (1943)(C. A., **39**, 1172(1945)); G. P. Menshikov, G. M. Borodina: J. Gen. Chem. (U. S. S. R.), **17**, 1569(1947)(C. A., **42**, 2245(1948)).

3) F. E. King, D. Holmes: J. Chem. Soc., **1947**, 168.

synthesized by a route essentially similar to that employed by them; in the preparation of phenyldimethylaminomethylcarbinol (IIc) from dimethylaminoacetophenone (IIb), the reducton with lithium aluminum hydride gave a better yield (89%) than the catalytic hydrogenation using platinum oxide (78%).[3] (II) thus obtained crystallized from methanol as colorless pillars, m.p. 225~226°. This substance gave a distinct depression in m.p. when mixed with the iodide, m.p. 225~226.5°, isolated from *Magnolia grandiflora* L. (II) formed a picrate crystallizing from ethanol–acetone in the form of microscopic yellow pillars of m.p. 196~197.5° after drying, which was found to be markedly different from that of picrate (A), m.p. 165°.



β-(*p*-Hydroxyphenyl)-ethyltrimethylammonium iodide[4] (III) is also referred to as hordenine methiodide, viz., candicine iodide[5]. The literature records for this substance m.p. 228~229°[4] or m.p. 230~231°,[5] and for its corresponding picrate, m.p. 162~163°.[5] In view of the close similarity in melting points between these, attempts were made to prepare hordenine methiodide (III) from hordenine (IIIa) sulfate (Merck) for direct comparison. (III) crystallized from ethanol as colorless pillars, m.p. 230~231°. A sample dried at 80° *in vacuo* over phosphorus pentoxide gave m.p. 229~230.5°. On admixture with the iodide, m.p. 225~226.5°, obtained from *Magnolia grandiflora*, no depression of the m.p. was observed, and the two iodides also gave identical infrared spectra (Nujol mull), thus confirming them to be identical. (III) formed a picrate which crystallized from ethanol–acetone in the from of yellow pillars, m.p. 165°, and one more recrystallization from methanol and drying gave m.p. 167~168°, either alone or on admixture with picrate (A), m.p. 165°.

Subsequently, a portion of hordenine (IIIa) sulfate(Merck), after being refluxed with methanolic potash and methyl iodide, yielded O-methylhordenine methiodide (IV) which crytallized from methanol–acetone in the form of colorless prisms, m.p. 209~210.5°. After drying at 80° *in vacuo* over phosphorus pentoxide, it showed m.p. 210~211°(reported m.p. 204°[4] or 206°[4]; m.p. 96~97°[4,6] with $1^1/_2$ moles of water of crystallization).

4) K. W. Rosenmund: Ber., **43**, 310, 311 (1910).
5) L. Reti: "Fortschr. Chem. org. Naturstoffe," **6**, 255(1950).
6) E. Leger: Compt. rend., **144**, 210(1907).

Since the above result was inconsistent with the fact, as mentioned before, that a portion of (M), after treatment with methanolic potash and methyl iodide, furnished a product (the iodide, m.p. 225~226.5°) whose chlorine atom was only replaced with iodine and whose hydroxyl group did not undergo methylation, it has become necessary to reinvestigate this situation. Because of the very small amount of the iodide, m.p. 225~226.5°, available, the corresponding picrate (A), m.p. 165°, was considered for this purpose. Picrate (A), after being freed from picric acid, was methylated with methanolic potash and methyl iodide in the usual manner and yielded colorless prisms, m.p. 210~211° (after drying at 85° *in vacuo* over phosphorus pentoxide), not identical with the original iodide, m.p. 225~226.5°. When mixed with O-methylhordenine methiodide (IV), m.p. 210~211°, no melting point depression was observed, thus confirming them to be identical.

As is apparent from Fig. 1, the ultraviolet absorption spectrum of the iodide, m.p. 225~226.5°, agrees completely with that of hordenine methiodide (candicine iodide) (III),

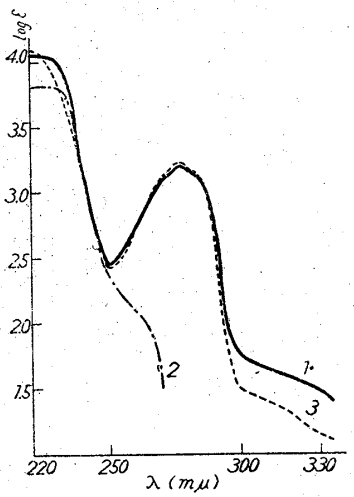


Fig. 1

1. Iodide, m.p. 225~226.5° (Methanol)
2. β-Hydroxyphenethyltrimethylammonium Iodide (II)(Methanol)
3. Hordenine Methiodide (III)(Methanol)

whereas that of β-hydroxyphenethyltrimethylammmonium iodide (II) is quite different form either of these.

From the foregoing results, it is concluded that of the two quaternary alkaloids found in the root of *Magnolia grandiflora*, one is salicifoline (I) and the other, candicine (V).

The fact that candicine (V) is present together with salicifoline (I) in the root of *Magnolia grandiflora* L. stimulates much interest on the biogenesis of alkaloids in plants. In addition, it is of importance from the taxonomical as well as physiological point of view to note that salicifoline is structurally closely related to coryneine[7] (3,4-dihydroxy-β-phenethyltrimethylammonium hydroxide, hydroxycandicine), an alkaloid of the Argentine *Stetsonia coryne* (Cactaceae), and that candicine is also known to occur in certain species of the same family.

The author acknowledges his indebtedness to Prof. Dr. M. Tomita for his valuable suggestions in this work, and expresses his thanks to Mr. Y. Matsui for carrying out the infrared determinations. The author's thanks are also due to the Ministry of Education for the Grant in Aid for Scientific Research.

Experimental[8]

Isolation of Alkaloids from the Root of *Magnolia grandiflora* **L.**—This experiment was carried

7) L. Reti: "Fortschr. Chem. org. Naturstoffe," **6**, 257(1950); R. H. F. Manske, H. L. Holmes: "The Alkaloids: Chemistry and Physiology," Vol. III, 321(1953).

8) All melting points are uncorrected. The author is indebted to Miss H. Iwata and Messrs. K. Hozumi and K. Imaeda in the Microanalytical Laboratory of this Institute for carrying out the microanalyses reported herein.

out with a portion of the methanolic extract prepared from the root of *Magnolia grandiflora* L., which was collected in April, 1952, by Mr. H. Yamada of Kobe University, and kindly supplied by him. The extract which still retained a considerable amount of water, was digested with approximately 5 L. of 3% HCl solution, in which nearly all of it dissolved, leaving only a very small amount of insoluble resinous material. The acid extract was concentrated *in vacuo* at 45° to about 500 cc. and freed by means of ether from extraneous acidic and neutral substances. After making alkaline with Na_2CO_3 it was extracted with solvents such as ether, $CHCl_3$, benzene, and AcOEt. Each extract was dried and the solvent removed, yielding only a trace of the residue, too small to be worked up. The isolation and purification of the quaternary bases from the aqueous layer remaining after extraction with the above solvents was effected by precipitating as the reineckate. The repetition of this process was found more satisfactory than phosphotungstic acid–mercuric chloride process.

The reineckates of the quaternary bases were decomposed with Ag_2SO_4 and then converted into the chlorides, but they did not readily crystallize and gave a syrupy oil (M). Consequently, a major portion of (M) was led to the crystalline picrates, which by the relative degree of solubility in EtOH, could be separated into picrate (A) and picrate (B). Picrate (A) crystallized from EtOH in the form of slender yellow pillars, m.p. 165°; after drying at 85° *in vacuo* over P_2O_5, m.p. 164~165.5°. It gave a negative test for a methylenedioxy group with Gaebel's reagent and showed no methoxyl group (Vieböck–Brecher). *Anal.* Calcd. for $C_{11}H_{18}ON \cdot C_6H_2O_7N_3 \cdot {}^1/_2 H_2O$: C, 48.62; H, 5.07; N, 13.43; NCH_3, 10.81. Found: C, 48.44, 48.71; H, 4.93, 5.10; N, 13.35, 13.04; NCH_3, 9.63, 9.58. Picrate (B) formed orange yellow pillars from EtOH and showed at first m.p. of 174~176.5°, rising after further recrystallization to 181~182°, the identity of which was confirmed by admixture with an authentic sample of salicifoline (I) picrate, m.p. 181~182°. *Anal.* Calcd. for $C_{12}H_{20}O_2N \cdot C_6H_2O_7N_3$: C, 49.31; H, 5.06; N, 12.78. Found: C, 48.91; H, 5.21; N, 13.01.

Another portion of (M), on treatment with methanolic KOH and MeI, furnished 0.1 g. of a product, which crystallized from a mixture of MeOH and a little acetone in the form of colorless pillars, m.p. 220~223°. After recrystallization from a mixture of MeOH and a small portion of water and drying at 85° *in vacuo* over P_2O_5, it gave m.p. 225~226.5°, is optically inactive, and contains no methoxyl group (Vieböck–Brecher). The infrared spectrum (Nujol mull) showed the presence of a hydroxyl function; ultraviolet absorption spectrum (MeOH solution)(Fig. 1), maximum at 277 mμ ($\log \varepsilon$ 3.21), minimum at 250 mμ ($\log \varepsilon$ 2.45). *Anal.* Calcd. for $C_{11}H_{18}ONI$: C, 43.01; H, 5.91; NCH_3, 14.67. Found: C, 43.02, 43.16; H, 6.08, 6.24; NCH_3, 14.01. By adding ether to the mother liquor from the above product, there was obtained an additional crop (50 mg.) of crystals, m.p. 215~221°, which on recrystallization from acetone (sparingly soluble) and drying at 85° *in vacuo* over P_2O_5, showed m.p. 224~225°, undepressed on admixture with the initial product, m.p. 225~226.5°.

Subsequently, a portion of picrate (A) was decomposed by dissolving it in a small amount of acetone, adding an equal volume of 1% HCl, and exhaustively extracting with ether until all of the picric acid was removed. The aq. solution was evaporated *in vacuo* at 45° to yield a syrupy residue, which was then methylated in the usual way with methanolic KOH and MeI. The O-methyl product thus obtained was recrystallized from MeOH–acetone to form colorless prisms, which after drying at 85° *in vacuo* over P_2O_5, gave m.p. 210~211°. Admixture with the iodide, m.p. 225~226.5°, originally obtained, gave a distinct depression in the m.p. (180~205°).

Preparation of β-Hydroxyphenethyltrimethylammonium Iodide[3] (II)

(1) Dimethylaminoacetophenone (II b)[9,10]—To a solution of 8.8 g. of ω-bromoacetophenone (IIa) in 60 g. of benzene was added dropwise with cooling 15 g. of 40% ethanolic dimethylamine (2.5 moles). The reaction occurred simultaneously with the evolution of heat and white crystalline precipitate resulted. The mixture was allowed to stand for 24 hrs. at room temperature, after which the resulting precipitate was taken up in 3% aq. HCl. The acid extract was then made alkaline with Na_2CO_3 and extracted with ether. The ether extract was dried over anhyd. K_2CO_3, and the solvent removed, yielding 5.7 g. of a yellowish brown syrupy residue. This was readily induced to crystallize by conversion into its hydrobromide. The hydrobromide, after recrystallization from EtOH–acetone, occurred in colorless pillars, m.p. 182~183° (reported m.p. 184~186°[10]); yield, 6.4 g. (60%). For characterization, a portion of the hydrobromide was converted into the picrate, which after recrystallization from EtOH, formed yellow needles, m.p. 142~143°; after drying at 80° *in vacuo* over P_2O_5, m.p. 145~146° (reported m.p. 150°[9]). *Anal.* Calcd. for $C_{10}H_{13}ON \cdot C_6H_3O_7N_3$: C, 48.98; H, 4.11. Found: C, 48.88; H, 4.19.

(2) Phenyldimethylaminomethylcarbinol (II c)—A solution of the base (regenerated from 4.3 g. of the above hydrobromide) in 20 cc. of abs. ether was added dropwise during 15 mins. to a stirred suspension of 0.3 g. of $LiAlH_4$ in 100 cc. of abs. ether. After the addition was complete, the mixture was refluxed for 1 hr. Then, while cooling, 4 cc. of AcOEt and subsequently 6 cc. of water were added slowly with stirring to decompose the excess of the reagent, and the complex was treated with 5 cc.

9) J. v. Braun, K. Weissbach: Ber., **62**, 2425(1929).
10) E. Schmidt, H. Rumpel: Arch. Pharm., **237**, 235(1899).

of 20% aq. NaOH, whereby white granular solid precipitated. The ether layer was decanted and the product was extracted with ether. The ether extracts were combined and dried over anhyd. K_2CO_3. Removal of the ether left 2.8 g. of a pale straw-colored oil (89% based on the methiodide obtained below).

(3) **β-Hydroxyphenethyltrimethylammonium Iodide (II)**—2.8 g. of the above carbinol was refluxed in MeOH with excess MeI for 30 mins. Crystals separated during concentration of the solution and then ether was added to ensure the completeness of crystallization. Colorless pillars, m.p. 224~226°; yield, 4.8 g. A sample was recrystallized once from MeOH and dried at 80° *in vacuo* over P_2O_5 for analysis; m.p. 225~226° (reported m.p. 226°[3]). *Anal.* Calcd. for $C_{11}H_{16}ONI$: C, 43.01; H, 5.91. Found: C, 42.81; H, 5.81. A mixed m.p. with the iodide, m.p. 225~226.5°, isolated from *Magnolia grandiflora*, showed a marked depression (192~215°).

This substance formed a picrate crystallizing from EtOH–acetone in the form of microscopic yellow pillars, m.p. 196~198° (sparingly soluble in EtOH); after drying at 80° *in vacuo* over P_2O_5, it had m.p. 196~197.5°, which was quite different from that of picrate (A)(m.p. 165°) obtained from *Magnolia grandiflora* L. *Anal.* Calcd. for $C_{11}H_{18}ON \cdot C_6H_2O_7N_3$: C, 50.00; H, 4.94. Found: C, 50.28; H, 5.06.

β-(*p*-Hydroxyphenyl)-ethyltrimethylammonium Iodide[4] (Hordenine Methiodide, Candicine Iodide[5]) (III)—Hordenine (IIIa) obtained from hordenine sulfate (Merck) was dissolved in a small portion of MeOH and treated with MeI for 30 mins. After concentration of the solution to small bulk, ether was added, whereupon crystallization began. Recrystallization from EtOH yielded colorless pillars, m.p. 230~231°, less soluble in EtOH than in MeOH. The analytical sample was dried at 80° *in vacuo* over P_2O_5 before analysis; m.p. 229.5~230.5° (reported m.p. 228~229°[4] or m.p. 230~231°[5]), ultaviolet absorption spectrum (MeOH solution)–(Fig. 1) maximum at 277 mμ ($\log \varepsilon$ 3.23), minimum at 250 mμ, ($\log \varepsilon$ 2.43). *Anal.* Calcd. for $C_{11}H_{18}ONI$: C, 43.01; H, 5.91. Found: C, 43.14; H, 6.06. No depresion of the m.p. was observed on admixture with the iodide, m.p. 225~226.5°, obtained from *Magnolia grandiflora*, and the two iodides also gave identical infrared spectra (Nujol mull).

Hordenine methiodide so obtained was in like manner converted into the corresponding picrate, which after recrystallization from EtOH–acetone, formed yellow needles, m.p. 165° (relatively sparingly soluble in EtOH); one more recrystallization from MeOH and drying at 80° *in vacuo* over P_2O_5 gave m.p. 167~168° (reported m.p. 162~163°[5]), undepressed on admixture with picrate (A), m.p. 165°. *Anal.* Calcd. for $C_{11}H_{18}ON \cdot C_6H_2O_7N_3$: C, 50.00; H, 4.94. Found: C, 49.72; H. 5.19.

O-Methylhordenine Methiodide[4] (O-Methylcandicine Iodide)(IV)—A portion of hordenine sulfate (Merck) was dissolved in a small volume of MeOH and excess MeI was added, followed by methanolic potash. The mixture was refluxed for 2 hrs., after which the solution was evaporated *in vacuo* to dryness, and the residue was extracted with successive portions of $CHCl_3$. The $CHCl_3$ extracts were dried over anhyd. K_2CO_3 and on removal of the solvent, the residue solidified to a crystalline mass. It is readily soluble in MeOH and sparingly soluble in acetone. By recrystallization from MeOH–acetone, it crystallized in the form of colorless prisms, m.p. 209~210.5°. The analytical sample, after drying at 80° *in vacuo* over P_2O_5, melted at 210~211° (reported m.p. 204°[4] or m.p. 206°[4]); m.p. 96~97°[4,6] with $1^1/_2$ moles of water of crystallization). *Anal.* Calcd. for $C_{12}H_{20}ONI$: C, 44.87; H, 6.28. Found: C, 44.70; H, 6.45. This substance gave no depression in m.p. when mixed with the O-methyl iodide, m.p. 210~211°, derived from picate (A).

Summary

Two kinds of quaternary alkaloids were isolated from the root of *Magnolia grandiflora* L. (Magnoliaceae), growing in Japan. As a result of detailed investigations, one was identified as salicifoline (I) and the other, as candicine (V).

(Received June 28, 1954)