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Isolation of 1-(*p*-Hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (demethylcoclaurine), an Active Alkaloid from *Nelumbo nucifera*

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One of the alkaloidal components of *Nelumbo nucifera* embryo was isolated in crystalline form and identified with 1-(*p*-hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline. This is the first tetrahydroisoquinoline alkaloid with a secondary base isolated from *Nelumbo nucifera*.

In the course of a pharmacological screening program of natural products, an ethanolic extract of the embryo of *Nelumbo nucifera* was found to possess a significant activity to relax the smooth muscle and uterine strips. The active principle was isolated by ion-exchange chromatography and crystallized as the hydrochloride. Its structure was determined to be 1-(*p*-hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, which was confirmed by synthesis. Alkaloidal components contained in *Nelumbo nucifera* have been investigated by several workers[2-4)] and a number of isoquinoline alkaloids have been reported, all of which are tertiary or quaternary bases. This paper reports the isolation and identification of a benzyltetrahydroisoquinoline alkaloid with a secondary amine function from the embryo of the seeds of *Nelumbo nucifera* collected in Taiwan. The pharmacological data obtained with this compound will be reported in detail elsewhere.

Pharmacognosy of *Nelumbo nucifera*

Various parts of *Nelumbo nucifera* (lotus), *Fam. Nymphaeaceae*, have been used in oriental countries for many medicinal purposes since its first description in the oldest Chinese herbal book "Seng-Ron-Pen-Chau" (BC 500). The dried embryo of *Nelumbo nucifera* is called *Lian-zi-sin* in Chinese medicine and is generally used for tonics. It is located in the middle of the seeds of lotus, and its shape is like a small green bar of 1.2—1.6 cm length. It has two cotyledons of deep green color in branches, and the plumule 2 mm in length stands erect between the cotyledons. The radicle is a yellowish green cylinder of 2—4 mm in length with very bitter taste.

Isolation of the Active Component

The seed embryo of *Nelumbo nucifera* was successively extracted with *n*-hexane, chloroform, ethanol and water, and the pharmacological activity (smooth muscle and uterine relaxa-

1) Location: *a) 2-9-3, Shimo-meguro, Meguro-ku, Tokyo; b) No. 4-3, Lane 16, Shau An Street, Taipei, Taiwan.*

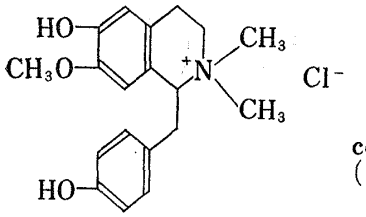
2) Y.C. Chao, Y.L. Chou, P.C. Yang and C.K. Chao, *Scientia Sinica*, **11**, 215 (1962); P.C. Pan, Y.L. Chou, T.C. Sun and Y.S. Kao, *ibid.*, **11**, 321 (1962).

3) M. Tomita, Y. Watanabe, M. Tomita and H. Furukawa, *Yakugaku Zasshi*, **81**, 469 (1961); M. Tomita, Y. Watanabe and H. Furukawa, *ibid.*, **81**, 942 (1961); M. Tomita, Y. Watanabe and H. Furukawa, *ibid.*, **81**, 1202 (1961); M. Tomita, Y. Watanabe and H. Furukawa, *ibid.*, **81**, 1644 (1961); M. Tomita and H. Furukawa, *ibid.*, **82**, 1458 (1962); J. Kunitomo, C. Yamamoto and T. Ōtsuki, *ibid.*, **84**, 1141 (1964); M. Tomita, H. Furukawa, T.H. Yang and T.J. Lin, *Chem. Pharm. Bull.* (Tokyo), **13**, 39 (1965).

4) H. Furukawa, *Yakugaku Zasshi*, **85**, 335 (1965); H. Furukawa, *ibid.*, **85**, 353 (1965); H. Furukawa, T.H. Yang and T.J. Lin, *ibid.*, **85**, 472 (1965); H. Furukawa, *ibid.*, **86**, 75 (1966).

tion) was found in the ethanol extract. The activity in the crude extract was unstable in alkaline medium, the approximate half-life period at room temperature being 45 minutes at pH 8.0 and shorter than 10 minutes at pH 9.0. The thin-layer chromatography (TLC)[5] of the crude extract showed several Dragendorff positive spots at *Rf* 0.64, 0.48, 0.30, 0.25 and 0.18. These alkaloidal components were designated as A_1, A_2, B, C and D, respectively, in the order of decreasing *Rf* value.

The usual solvent extraction procedures for the fractionation of alkaloidal mixture were not suitable to this material because of its instability in alkaline medium. However, separation and isolation of the active component was successfully accomplished by ion-exchange chromatography using a column of Amberlite CG-50 resin (H-type). The column was developed by diluted hydrochloric acid and the major activity appeared in fractions of pH 3.0 to 2.0. Th active eluates were combined, neutralized by Amberlite IR-4B resin (OH-type) and concentrated *in vacuo* to dryness. The resultant solid, in which the presence of component A_1 and a small amount of A_2 was shown by TLC, was dissolved in methanol and addition of acetone induced crystallization of the A_1 component. Repeated recrystallizations from ethanol gave colorless prisms, mp 242—244°, $C_{16}H_{17}O_3N \cdot HCl$.



component B
(lotusine)

Some activity was found in more acidic fractions of pH 2.0 to 1.7 when the above chromatographic column was further eluted with the same solvent. From these fractions component B was isolated in crystalline form but its pharmacological activity was estimated to be about 1/20 of that of component A_1. Component B melted at 210° with decomposition and was analyzed for $C_{19}H_{24}O_3NCl$. From the ultraviolet (UV), infrared (IR) and nuclear magnetic resonance (NMR) spectral data, component B was identified with 1-(*p*-hydroxybenzyl)-2,2-dimethyl-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinolinium chloride, which had been reported and named as lotusine by Furukawa, *et al.*[4]

Structure of the Active Component A_1

Component A_1 gives a positive $FeCl_3$ reaction and exhibits a quite similar ultraviolet spectrum (maxima at 224 mμ and 284 mμ) to that of lotusine, suggesting the benzyltetrahydroisoquinoline structure of component A_1. The infrared spectrum of component A_1 (Fig. 1) revealed absorptions for amino and/or hydroxy group at 3300 and 3500 cm^{-1}, and a series of small absorptions in the 2400—2800 cm^{-1} region, which are characteristic to the ammonium or immonium function.

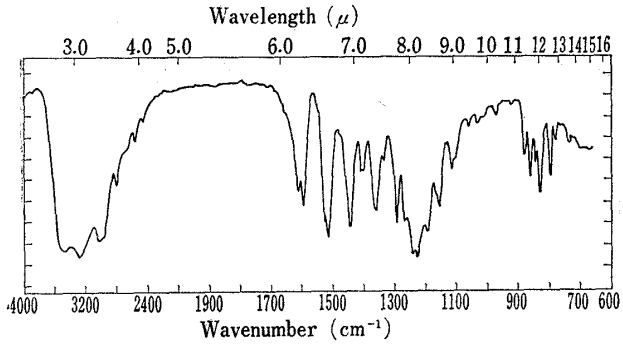


Fig. 1. IR Spectrum of Component A_1 (in KBr pellet)

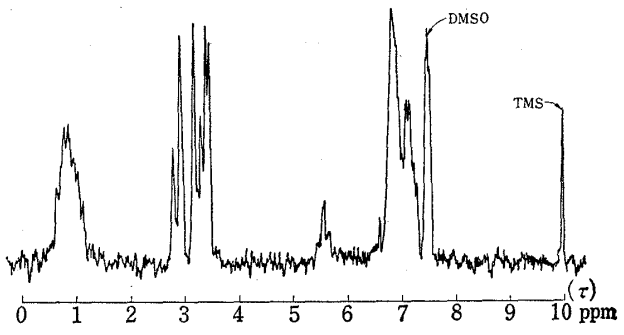
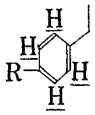
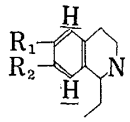
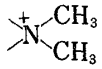


Fig. 2. NMR Spectrum of Component A_1 (60 Mc, in DMSO-d_6)

5) Silica gel plate, butanol-acetic acid-water (63:10:27).

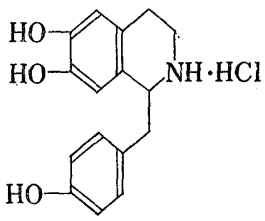
The NMR spectrum of component A_1 (Fig. 2) was compared with that of component B (lotusine)[4] and the data are summarized in Table I. The lowest signals were observed at 0.60—1.12 τ as the multiplet corresponding to five protons. In the aromatic proton region, the A_2B_2 quartet (4H) at 2.87 τ and 3.25 τ (J=8 cps) attributable to a *para*-substituted benzyl group was similar to that of lotusine. However, the chemical shifts of two singlets due to the other two aromatic protons of component A_1 (3.40 τ and 3.48 τ) are very close to each other comparing with the relative chemical shifts of the corresponding protons of lotusine (3.30 τ and 4.35 τ). The fact suggests that both of the 6 and 7 positions of component A_1 might be substituted with hydroxy group.

Table I. NMR Data of Component A_1 and Lotusine

Assignment	Component B (lotusine) τ	Component B (lotusine) Relative intensity	Component A_1 τ	Component A_1 Relative intensity
	0.42 (s)	1 proton	0.6—1.2 (m)	3 protons (OH)
OH	0.67 (s)	1		2
	3.05 (d) 3.25 (d)	2 2	2.87 (d) 3.25 (d)	2 2
	3.30 (s) 4.35 (s)	1 1	3.40 (s) 3.48 (s)	1 1
	5.25 (m)	1	5.55 (m)	1
	6.60 (s) 6.88 (s)	3 3	— —	
O—CH_3	6.72 (s)	3	—	
Others	6.22—7.30 (m)	6	6.60—7.30 (m)	6

Combination of the above analytical and spectral informations leads to the following structure for component A_1.

The mass spectrum of component A_1 free base (Fig. 3) was also consistent with the proposed structure. It gives a molecular ion peak at m/e 271 as well as a series of fragment ions



component A_1

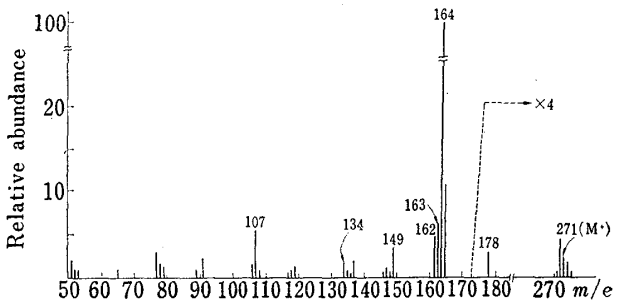


Fig. 3. Mass Spectrum of Component A_1

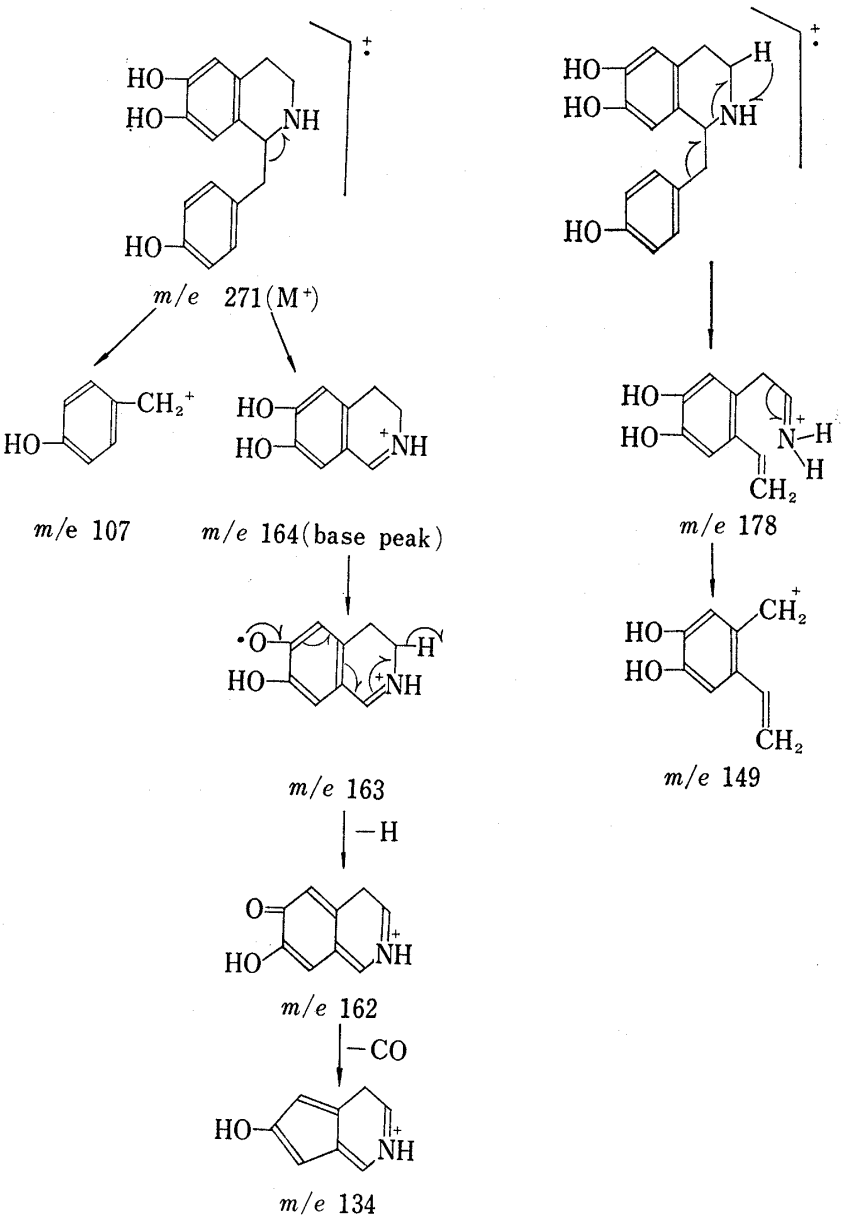


Chart 1. Fragmentation of Component A_1

characteristic to those of the benzylisoquinoline alkaloids[6,7] as shown in the following fragmentation chart.

In order to prepare a larger quantity of component A_1 for detailed pharmacological evaluation, the hydrobromide of demethylcoclaurine was synthesized according to a reported method[8] and the hydrobromide was then converted to the hydrochloride. The identity of component A_1 with the hydrochloride of 1-(*p*-hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (demethylcoclaurine) was established by the UV and IR spectra comparison.

6) M. Ōhashi, M. Wilson, H. Budzikiewicz, M. Shamma, W.A. Slusarchyk and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 2807 (1963).
7) M. Tomita, H. Furukawa, T. Kikuchi, A. Kato and T. Ibuka, *Chem. Pharm. Bull.* (Tokyo), **14**, 232 (1966).
8) H. Yamaguchi, *Yakugaku Zasshi*, **78**, 692 (1958).

Experimental

Isolation of Components A_1 and B (Lotusine)——Air-dried seed embryo of *Nelumbo nucifera*, 2 kg, was treated successively with *n*-hexane and hot-chloroform, and then extracted with three 5 liter portions of ethanol under refluxing temperature. The combined extracts were concentrated under reduced pressure to give 774 g of greenish brown solid. The solid was dissolved in 400 ml of water and filtered. The aqueous solution was extracted with three 400 ml portions of *n*-butanol, and the combined butanol layers were evaporated *in vacuo* to leave 55 g of yellowish brown solid. The solid was dissolved in 500 ml of water and applied on a column (6 cm in diameter) which contained 3000 ml of cation exchange resin (Amberlite CG-50, H-type). The column was washed with water and then developed with 1/50N hydrochloric acid. Fractions showing pH 3.0—2.0 were combined, neutralized with Amberlite IR-4B (OH-type) and then concentrated *in vacuo* to dryness. The resultant solid, 2.58 g, was crystallized from methanol–acetone (1:1) to yield 380 mg of pale yellow crystalline powder which was recrystallized from ethanol to give colorless prisms (component A_1), mp 242—244° (decomp.), $[\alpha]_D^{26.5} = +16°$ ($c=1$, MeOH). *Anal.* Calcd. for $C_{16}H_{17}O_3N \cdot HCl$: C, 62.43; H, 5.89; N, 4.55; Cl, 11.52. Found: C, 62.22; H, 5.87; N, 4.39; Cl, 11.89.

Further fractions in the above chromatography (pH 2.0—1.7) were combined and treated in the same manner as above to give 120 mg of colorless prisms (component B) which was identified with lotusine. Another lotusine was isolated in rather larger amount from the aqueous layer of the above butanol extraction process.

Synthesis of *dl*-Demethylcoclaurine Hydrobromide——*dl*-Coclaurine hydrochloride (21.6 g, 0.067 mole), which was prepared according to the method of Tomita, *et al.*[8,9] was refluxed with 300 ml of 47% aqueous hydrobromic acid for 20 minutes. The reaction mixture was diluted with 100 ml of water and then evaporated under reduced pressure. The residue was dissolved in methanol, and treated with active carbon. Evaporation of the solvent under reduced pressure gave the hydrobromide of demethylcoclaurine which was repeatedly washed with ethyl acetate and then air-dried. Yield 17 g (72%), mp 269—270° (lit[8]. 270—272°). UV λ_{max}^{EtOH} mμ (ε): 228.5 (14800), 288 (6200).

***dl*-Demethylcoclaurine Hydrochloride**——A solution of *dl*-demethylcoclaurine hydrobromide (1.0 g, 0.003 mole) in 20 ml of water was layered with 20 ml of ethyl acetate and adjusted to pH 9.0 by addition of aqueous ammonium hydroxide under stirring. The aqueous layer was further extracted with two 100 ml portions of ethyl acetate, and the combined organic layers were washed with water and then dried over anhydrous sodium sulfate. Dry hydrogen chloride was bubbled into the ethyl acetate solution which was then concentrated *in vacuo* to dryness. The solid was repeatedly washed with ethyl acetate and dried. Yield 0.5 g (60%). mp 256—263°. UV λ_{max}^{EtOH} mμ (ε) 228.5 (14400), 288 (5100). *Anal.* Calcd. for $C_{16}H_{18}O_3NCl$: Cl, 11.52. Found: Cl, 11.19.

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9) M. Tomita, K. Nakaguchi and S. Takagi, *Yakugaku Zasshi*, **71**, 1046 (1951).