

STUDIES ON THE ALKALOIDS OF LOTUS RECEPTACLE

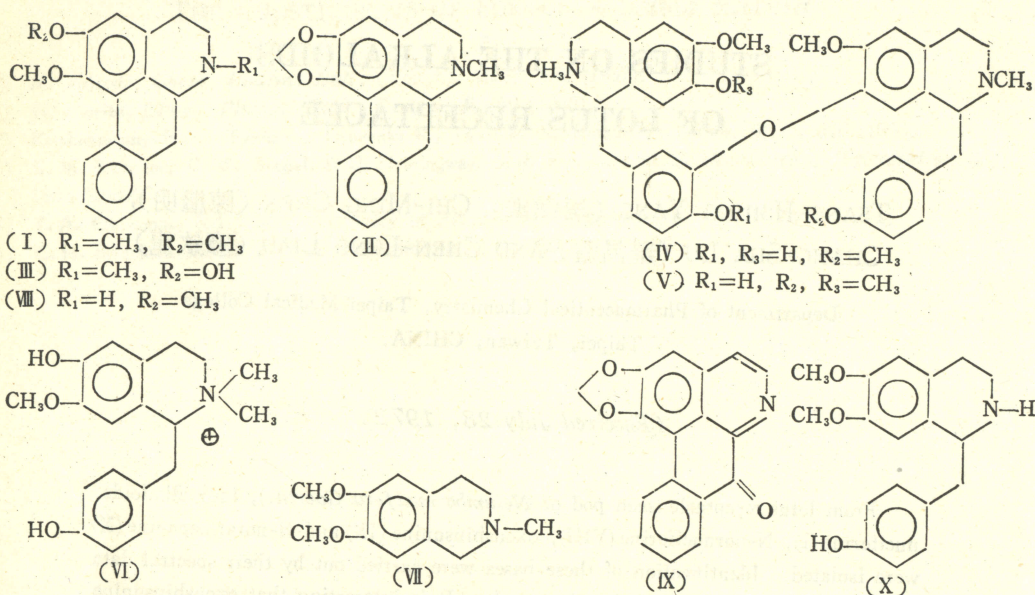
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From lotus receptacle (seed pod of *Nelumbo nucifera Gaertn.*), four alkaloids, nuciferine (I), N-nornuciferine (VIII), oxoushinsunine (IX) and N-norarmepavine (X) were isolated. Identification of these bases were carried out by their spectral data and direct comparison with authentic samples. It is interesting that oxoushinsunine (IX), a cytotoxic alkaloid shows tumor inhibitory activity against nasopharynx carcinoma reported recently by D. Warthen et al¹³. The biscoclaurine (IV and V) and quaternary water soluble base (VI) only occurs in the embryo. Comparison their alkaloids distribution in each part of Formosan lotus was listed in the Table 1.

The genus *Nelumbo* (Nymphaeaceae) produces a lot of alkaloids including aporphine, oxoaporphine, proaporphine, tetrahydroisoquinoline, benzyltetrahydroisoquinoline and biscoclaurine¹⁻⁸). In the course of continuing search for alkaloids from Formosan *Nelumbo nucifera Gaertn.*, the ethanol extracts of leaf, petiole and seed-embryo were investigated respectively to isolate seven alkaloids, nuciferine (I), roemerine (II), O-nornuciferine (III), isoleinsinine (IV), neferine (V), lotusine (VI) and methylcorypalline (VII), reported in the earlier papers⁶⁻⁸). Up to now, no studies on the chemical principles of lotus receptacle has been reported. In this paper, we wish to describe the isolation and characterization of alkaloids from receptacle in order to clarify the occurrence and difference of chemical constituents in each part of the whole plant. The receptacle or seed pod of lotus is called "Lien-fang" (蓮房) in Chinese. After the seeds have been removed, it looks something like the nozzle of a garden sprinkler. Its medicinal action is regarded as antihemorrhagic and it is also employed to promote the expulsion of the afterbirth and in watery decoction to counteract the poison of deleterious fungi in Chinese drugs⁹). From seed pod, there are four bases (A, B, C and D) obtained from the alcoholic extract. A detailed description of extraction and isolation procedure was given in the experimental part.



Base-A was crystallized from alkali-insoluble fraction as a major alkaloid. It is colorless prisms from ethanol, mp. 163-164°, $[\alpha]_D^{27} -145^\circ$ ($c=0.67$, EtOH). The ultraviolet maximum absorption in ethanol at 310 $m\mu$ ($\log \epsilon$ 3.83), 272 (4.32) and 230 (4.36) indicates Base-A is very similar to 1,2-disubstituted aporphine alkaloids¹⁰. It shows a bright green fluorescence in solution under radiation with UV light. The direct comparison by infrared spectrum, mixed melting point and thin-layer chromatogram was found identical with an authentic sample of nuciferine (I), isolated from lotus leaf.

The filtrate of nuciferine (I) was performed on alumina column chromatography to afford two non-phenolic bases, Base-B and Base-C. The free base of Base-B is an oily liquid and its crystalline hydrochloride melts at 156-157° (EtOH). The ultra-violet maximum absorption of this hydrochloride at 311 $m\mu$ ($\log \epsilon$ 3.67) 271 (4.34) 230 (4.37) and 218 (4.42) shows an aporphine skeleton having substituents at C-1 and C-2. The NMR signal in deuteriochloroform appeared two methoxyl at 6.30 and 6.10 τ , one low field aromatic proton as singlet at 1.60 τ attributed to C-11, one high field chemical shift of aromatic proton at 3.32 τ assigned to C-3 and the other three aromatic protons as doublet at 2.70 τ . Methylation with formalin and formic acid gave a crystalline product from ethanol, mp. 163-164°, $[\alpha]_D^{27} -145^\circ$ ($c=0.5$, EtOH), identical to nuciferine (I). Naturally, Base-B was proved to be N-nornuciferine (VIII), originally isolated from *N. lutea* by S.M. Kupchan et al¹¹.

Base-C was crystallized from chloroform as yellowish needles, mp. 278-280° (decomp.) and is optical inactive. It forms a cherry red with diluted mineral acids. The infrared spectrum at 1650 cm^{-1} indicated the presence of conjugated carbonyl group. Base-C shows ultra-violet absorption maximums at 249 $m\mu$ ($\log \epsilon$ 4.37) 271 (4.34)

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310 (4.08) and it also has absorption in the visible region. From above data, Base-C was assumed to be one of oxoaporphine alkaloids and superimposable with an authentic sample of oxoushinsunine (IX)¹¹ in comparison of their IR(KBr), tlc and mixed melting point.

From alkali-soluble fraction, a minor Base-D was obtained as crystalline oxalate, mp. 209-210° (MeOH), $[\alpha]_D^{27} +20^\circ$ (c=0.2, MeOH). It reveals negative to Labat's test. The ultraviolet spectrum had a maximum absorption at 283 $m\mu$ and minimum at 254 $m\mu$ indicating the pattern of benzyltetrahydroisoquinoline series. The infrared spectrum of this free base in chloroform and its tlc shows identical with those of an authentic sample of N-norarmepavine (X)¹².

Oxoushinsunine (liriodenine) (IX), a cytotoxic principle shows tumor inhibitory activity against nasopharynx carcinoma, reported recently by D. Warthen et al¹³. The leaf extract of *N. nucifera* exhibited antimitotic activity and it induce disturbances in chromosome structure and also arrested mitosis in root meristem cell of *Allium cepa*¹⁴. The isolation of nuciferine (I), N-nornuciferine (VIII), oxoushinsunine (IX) and N-norarmepavine (X) from lotus receptacle will afford a valuable information in the *N. nucifera* biosynthetic transformation from embryo via petiole, leaf to receptacle. It is interesting that the biscoclaurine (IV, V) and 'quaternary water soluble base (VI) only occurs in the embryo and these can not be found in the other part (leaf, petiole, and receptacle). Comparison their alkaloidal distribution in each part of Formosan lotus was shown in Table 1.

Table 1

	leaf	petiole	embryo	receptacle
nuciferine (I)	+	+		+
roemerine (II)	+			
O-nornuciferine (III)	+			
isoliensinine (IV)			+	
neferine (V)			+	
lotusine (VI)			+	
methylcorypalline (VII)			+	
N-nornuciferine (VIII)				+
oxoushinsunine (IX)				+
N-norarmepavine (X)				+

EXPERIMENTAL

Isolation of alkaloids

Lotus receptacle (9.5 kg.), purchased from Taipei market was refluxed with hot ethanol and the extract was evaporated under vacuum distillation. The residue (350 g.)

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was triturated with 5% AcOH and the filtrate was washed with ether to remove the non-basic substances. The acidic layer was made basic with $c\text{-NH}_4\text{OH}$ and extracted with ether. Separation into non-phenolic base (1.0 g.) and phenolic base (0.7 g.) was carried out by shaking between the ether extract and 3% aq. NaOH. The phenolic base was recovered from alkali solution by saturating it with excess NH_4Cl and extracting with ether.

The alkali-insoluble fraction was treated with ethanol and recrystallized with same solvent to afford a prism crystal of Base-A (0.15 g.), mp. 163-164° (EtOH). The mother liquid of Base-A was chromatographed on alumina column, eluted with benzene to give a small amount of Base-A and a pale yellow oily Base-B (hydrochloride, mp. 156-157°) (0.1 g.). The chloroform eluted fraction yielded a minor component of Base-C (3 mg.), mp. 278-280° (decomp.) (CHCl_3).

The acetone solution of phenolic base was added with saturated oxalic acid in acetone to give Base-D as crystalline oxalate (5 mg.). After recrystallization with methanol, it melts at 209-210°. The free base failed to crystallize due to a few samples obtained.

Base-A, nuciferine (I)

Colorless prisms, mp. 163-164° (EtOH), $[\alpha]_D^{27} -145^\circ$ ($c=0.67$, EtOH). Labat's test (-), fluorescence under uv radiation. UV $\lambda_{\text{max}}^{\text{EtOH}}$ $m\mu(\log \epsilon)$: 310 (3.83) 271 (4.32) 232 (4.36). Its IR (nujol), tlc and mixed melting point were identified with those of nuciferine (I).

Base-B, N-nornuciferine (VIII)

Free base: oily liquid, hydrochloride: colorless crystals, mp. 156-157°, $[\alpha]_D^{27} -144^\circ$ ($c=0.5$, EtOH), Labate's test (-). UV $\lambda_{\text{max}}^{\text{EtOH}}$ $m\mu(\log \epsilon)$: 311 (3.67) 271 (4.34) 230 (4.37) 218 (4.42). IR (CHCl_3) cm^{-1} : 3250 (=NH) 1590 (phenyl). NMR (CDCl_3) τ : 6.30, 6.10 (6H, two OCH_3) 3.32, 1.60 (2H, aromatic-H) 2.70 (3H, aromatic-H, doublet). N-methyl derivative (I), mp. 163-164° (EtOH), $[\alpha]_D^{27} -145^\circ$ ($c=0.5$, EtOH), identical to Base-A of nuciferine (I).

Base-C, oxoushinsunine (IX)

Yellowish microneedles, mp. 278-280° (decomp.) (CHCl_3) and optical inactive. It formed a cherry red with diluted mineral acid and exhibited a bright yellow fluorescence under uv radiation. UV $\lambda_{\text{max}}^{\text{EtOH}}$ $m\mu(\log \epsilon)$: 249 (4.37) 271 (4.34) 310 (4.08) UV $\lambda_{\text{min}}^{\text{EtOH}}$ $m\mu(\log \epsilon)$: 264 (4.29) 293 (4.04), 341 (3.82). IR (KBr) cm^{-1} : 1650 (conjugated C=O) 960, 1050 ($\text{—O—CH}_2\text{—O—}$). It shows identical to oxoushinsunine (IX) by direct comparison with an authentic sample.

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Base-D, N-norarmepavine (X)

Oxalate: colorless needles, mp. 209-210° (MeOH), $[\alpha]_D^{27} +20^\circ$ (c=0.2, MeOH).

UV $\lambda_{\min}^{\text{MeOH}}$ $m\mu(\log \epsilon)$: 254 (3.42). UV $\lambda_{\max}^{\text{MeOH}}$ $m\mu(\log \epsilon)$: 283 (3.83) 227 (4.20). IR(CHCl₃) cm^{-1} : 3250 (=NH) 1590 (phenyl). The IR(CHCl₃) spectrum of this free base shows superimposable with that of N-norarmepavine (X).

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