Reprinted from
Journal of the Chinese Chemical Society
Ser. II, Vol. 17, No. 4

On the Alkaloids of *Nelumbo nucifera* Gaertn. Studies on the Alkaloids of Loti Embryo.

TSANG-HSIUNG YANG (楊藏雄) and CHI-MING CHEN (陳繼明)

Department of Pharmaceutical Chemistry, Taipei Medical College.

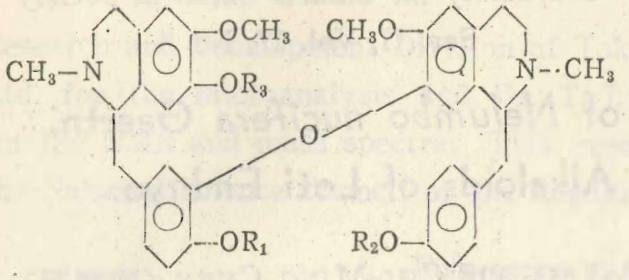
(*Received August 28, 1970*)

A new tertiary tetrahydroisoquinoline alkaloid, methylcorypalline (V) and biscoclaurine base, neferine (II) were isolated from a Formosan lotus embryo (embryo of the seed of *Nelumbo nucifera* Gaertn.) (Nymphaeaceae), besides the earlier reported isoliensinine (I) and lotusine (IV).

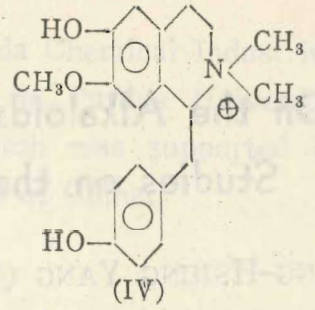
Characterization of this new base, methylcorypalline (I), mp. 58–59° (n-hexane), $C_{12}H_{17}O_2N \cdot \frac{1}{2}H_2O$, by spectral data and direct comparison with synthetic sample prepared from vanillin have proved to be 6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (V).

Methylcorypalline (V) is the first instance occurring in nature having coronary dilator action.

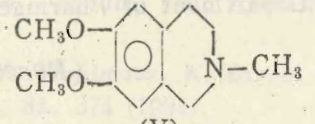
In the previous paper of this series, two new alkaloids isoliensinine[1](I) and lotusine[3](IV) had been isolated from Formosan "Lien Tze Hsin", embryo loti, (embryo of the seed of *Nelumbo nucifera* Gaertn., Family, Nymphaeaceae), and their structures were shown to be (I) and (IV). Further investigation of the mother liquid of isoliensinine hydrochloride by thin layer chromatography revealed the presence of a number of other alkaloids on the chromatogram. The present paper deals with the further study on the isolation and characterization of a new minor Base-A which was clarified as methylcorypalline[1](V) and a colorless oily Base-B which identified as neferine[4](II). These alkaloids were obtained from the tertiary non-phenolic fraction of loti embryo after chromatography on alumina, elution with benzene and benzene chloroform mixture (1-1) respectively.



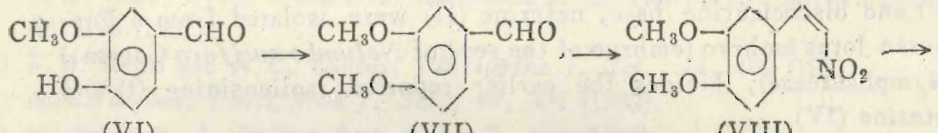
(I) R_1, R_3=H, R_2=CH_3
(II) R_1=H, R_2, R_3=CH_3
(III) R_1, R_2, R_3=CH_3



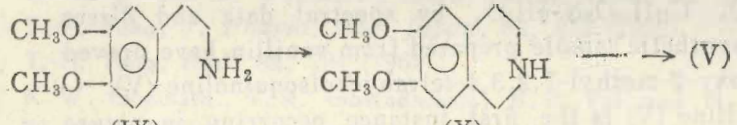
(IV)



(V)



(VI) (VII) (VIII)



(IX) (X) (V)

Base-A, $C_{12}H_{17}O_2N \cdot \frac{1}{2}H_2O$, was crystallized as feather crystals from n-hexane, *mp.* 58-59°, $[\alpha]_D^{20} \pm 0°$ ($CHCl_3$), negative Labat's and ferric chloride test. Several crystalline salts had been prepared as the hydrochloride, *mp.* 216-217° and picrate, *mp.* 152-153°. The methiodide of this base was crystallized from methanol and acetone as scaly crystals, *mp.* 242-243° and afforded an analytical results which supported a $C_{12}H_{17}O_2N \cdot CH_3I \cdot 1/3\ CH_3COCH_3$ formula.

Base-A showed a characteristic UV spectrum λ_{max}^{EtOH} at 290*mμ* (sh.) (log ε 3.39), 285 (3.47), 281 (3.47) indicating more than just simple benzenoid absorption. The IR broad band at 3250 cm^{-1} indicated the presence of hydroxyl group of semihydrate. The NMR spectrum exhibited three protons singlet of one *N*-methyl signal at 7.58τ, six protons singlet of two methoxyl signal at 6.18τ, and two one proton singlets of two aromatic protons at 3.40 and 3.48τ. The mass spectrum gave a molecular ion peak at *m/e* 207 ($C_{12}H_{17}O_2N$).

The base peak at *m/e* 164 (270-C_2H_5N) and other intense peaks were found at *m/e* 206, 121, 103, 91 and 76. These spectral and analytical data suggest that this base is a dimethoxy-N-methyl-tetrahydroisoquinoline derivative, most likely comprising two methoxyl groups at C_6 and C_7.

In order to confirm this assumption, a six step synthesis of 6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (V) was developed begining with vanillin (VI) which was converted to β-(3,4-dimethoxyphenyl) ethylamine (IX) via veratraldehyde (VII) and 3,4-dimethoxy-ω-nitrostyrene (VIII) by known methods. According to the report of J. S. Buck[5], 6,7-dimethoxy-1,2,3,4-tetrahydroisquinoline (X) was prepared by formylation of this amine IX with formalin and cyclization with *c*-HCl. *N*-methylation of X by treating with formic acid and formalin yielded compound V. The resulting product, *mp.* 58-59°, was completely identiceal to IR (nujol) spectrum and tlc with Base-A. In addition, there was no depression of the *mp.* when the two were admixed, confirming the identity of the synthesized sample and Base-A. On the basis of these experimental evidences, the structure of this base is unambiguously assigned to V, 7-O-methylcorypalline[6].

On the other hand, the second Base-B is an oily liquid or amorphous solid, *mp.* 60-61° (acetone and n-hexane), tlc 1 spot, $[\alpha]_D^{27}$ −100° (MeOH), positive ferric chloride and Millon test, negative Gibbs' reaction, and afforded analytical results in agreement with the formula, $C_{38}H_{44}O_6N_2$. The methiodide yielded upon methylation with methyliodide in methanol as white microneedles *mp.* 179-181°. The styphnate is an amorphous powder (EtOH), *mp.* 140-141°, $[\alpha]_D^{25}$ −82.11° (acetone).

The UV spectrum of this base had maximum at 284 *mμ* and minimum at 255 *mμ* indicating the benzyltetrahydroisoquinoline absorption. The IR ($CHCl_3$) band at 3550cm^{-1} indicated the presence of hydroxyl group. The NMR signals showed twelve protons of four methoxyl groups at 6.20, 6.22, 6.29, 6.48τ, six protons of two *N*-

methyl groups at 7.51, 7.54τ, one hydroxyl proton at 4.92τ, disappeared with deuterium oxide, and eleven aromatic protons at 3.01-3.98τ.

Methylation Base-B with diazomethane yielded *O*-methyl compound which was characterized as crystalline styphnate from ethanol, *mp.* 139-140°. According to the above experimental facts, this oily base-A was identified as biscoclaurine phenolic alkaloid, neferine (III) by IR ($CHCl_3$), NMR and tlc comparison with the authentic sample and its *O*-methyl derivative (III).

The synthetic compound of V had been prepared by J. S. Buck[5] from the corresponding phenylethylamine. However, this is the first instance from the natural product. Methylcorypalline (V) is now found to occur with isoliensinine (I), neferine (II) lotusine (IV) and probably one of the important biogenetic precursor in biosynthetic transformation. C. Hanna and J. H. Shutt[7] reported the substituted isoquinoline including the synthesized V are papaverine analogues having the coronary dilator action.

Experimental

All melting points are uncorrected. The optical rotations and IR spectra were measured with Rex Photoelectric Polarimeter, model NEP-2 and Hitachi Grating IR Spectrophotometer, model EPI-G2. The NMR were obtained in $CDCl_3$ and reported in τ units using a Varian A-60A Spectrometer. The mass spectrum were recorded on Hitachi, model RMU-6A using a direct inlet system at an ionizing energy of 75eV. Thin-layer chromatogaphy was performed on silical gel F254 (F. Merck) with $CHCl_3$-MeOH(6-4) as developing solvent and detection was carried out by spraying with Dragendorff's reagent.

Isolation of Alkaloids:

Air-dried "Lien Tze Hsin" (5.7 *kg.*) of Taipei market, was extracted with hot EtOH until negative Mayer's test and the extracts was evaporated under reduced pressure. The dark brown residue (1.5 *kg.*) was dissolved in 3% AcOH, filtered, and washed

with Et_2O to remove the non-basic substances. The acidic solution was made alkaline with *c*-NH_4OH and extracted with $CHCl_3$. The combined $CHCl_3$ solution was shaken with 3% NaOH solution to separate the phenolic and non-phenolic base. The lower $CHCl_3$ layer, after acid and alkali treatment, was washed with H_2O, dried over anhydrous K_2CO_3 and evaporated to leave a crude non-phenolic base (10*g.*). This crude alkaloids was chromatographed on alumina column (4×40*cm*). The C_6H_6 eluted fractions gave a small amount of crystalline residue. Repeated recrystallizations with n-hexane yielded white feathery crystals (90*mg.*), *mp.* 58-59° (Base-A). Continued elution with C_6H_6-$CHCl_3$ (1-1) afforded a colorless oily base (3.6*g.*), chromatographycally pure (Base-B).

Base-A. methylcorypalline (V):

Free base: white feathery crystals, *mp.* 58-59° (n-hexane), $[\alpha]_D^{20} \pm 0°$ (*c*=0.56, $CHCl_3$), Labat's (−), $FeCl_3$ (−). Mass spectrum: M^+ *m/e* 207 ($C_{12}H_{17}O_2N$); base peak, *m/e* 164 ($M^+ - C_2H_5N$); other intense peaks at *m/e* 206, 121, 103, 91, 76. UV λ_{max}^{EtOH} $m\mu$ (log ε): 290 (sh.) (3.39), 285 (3.47), 281 (3.47). IR (nujol): 3250 cm^{-1} (hydrate), 1580 cm^{-1} (phenyl). NMR ($CDCl_3$): 6.18τ (6H, two OCH_3); 7.58τ (3H, one NCH_3); 3.40, 3.48τ (2H, aromatic H). Anal. calcd. for $C_{12}H_{17}O_2N \cdot \frac{1}{2}H_2O$. C, 66.67; H, 8.33; N, 6.48. Found: C, 67.02; H. 8.51; N, 6.50. The IR (nujol), tlc and mixed *mp.* were identical with synthetic sample. Hydrochloride: white granulars, *mp.* 216-217° (EtOH). Picrate: yellow microneedles, *mp.* 152-153° (EtOH-Me_2CO).

Methylcorypalline (V) methiodide:

Methyliodide (3*ml.*) was added to the solution of V (30*mg.*) in MeOH (3*ml.*) and the mixture was gently refluxed on the water bath for 2.5 *hrs.* Evaporation of the excess solvent and recrystallization with MeOH and Me_2CO yielded methiodide, colorless scaly crystals, *mp.* 242-243° (24*mg.*). Anal. Calcd. for $C_{12}H_{17}O_2N \cdot CH_3I \cdot 1/3$ CH_3COCH_3 (acetone adduct). C, 45.62; H, 5.97; N, 3.83. Found: C, 45.68; H, 6.16; N, 4.06.

Synthesis of 6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (V):

The requisite β-(3,4-dimethoxyphenyl) ethylamine (IX) oxalate (1.5*g*) was prepared from vanillin (VI) (9.4*g*.) via veratraldehyde (VII) and 3,4-dimethoxy-ω-nitrostyrene (VIII) by known methods.

According to the method reported by J. S. Buck[5], this amine IX from its oxalate (1.4*g*.) was dissolved in MeOH (10*ml*.) and refluxed with formalin (37%) (0.8*ml*.) on water bath for 30 *min*. After evaporating the excess reagents, the mixture was treated with *c*-HCl (6*ml*.) on water bath for 4 *hrs*. The reaction mixture was poured into 20*ml*. of water, made alkaline with KOH and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O and dried over anhyd. K_2CO_3 and evaporated to leave an oily product, 6, 7,-dimethoxy-1,2,3,4-tetrahydroisoquinoline (X) as hydrochloride (270 *mg*.), *mp*. 254-255° (EtOH).

This oily base X from its hydrochloride (250 *mg*.) was treated with formalin (37%) (4*ml*.) and formic acid (85%) (4*ml*.) on water bath for 3*hrs*. The mixture was poured into 10*ml*. of water, made alkaline with KOH and extracted with $CHCl_3$, washed with H_2O and dried over anhyd. K_2CO_3. Evaporation the solvent afforded a crystilline residue which was chromatographed on alumina. Elution with C_6H_6 and crystallization from n-hexane yielded white feathery crystals, V (150 *mg*.), *mp*. 58-59°. Hydrochloride, white granulars (EtOH), *mp*. 214-215°. Picrate, yeliow microneedies (EtOH-Me_2CO), *mp*. 153-154°.

Base-B. neferine (II):

Colorless oil or amorphous solid (Me_2CO-n-hexane), *mp*. 60-61°, $[\alpha]_D^{27}$ −100° (*c*=0.24, MeOH), tlc 1 spot, $FeCl_3$ (+), Millon (+), Gibbs', (−). UV λ_{max}^{MeOH} mμ (log ε): 284 (3.38), λ_{min}^{MeOH}: 255 (3.47). IR ($CHCl_3$): 3550cm^{-1} (−OH). NMR ($CDCl_3$): 7.51, 7.54τ (6H, two NCH_3); 6.20, 6.22, 6.29, 6.48τ (12H, four OCH_3); 4.92τ (1H, broad, one OH) and 3.01-3.98τ

(11H, aromatic H). Anal. calcd. for $C_{38}H_{44}O_6N_2$. C, 73.05; H, 7.10. Found: C, 73.09; H, 7.36. This oily base was identified as neferine (II) by NMR, IR ($CHCl_3$) and tlc comparison. Styphnate: yellow amorphous (EtOH), *mp.* 140-141°, $[\alpha]_D^{25}$ −82.11° (c=0.55, Me_2CO).

O-methylneferine (III):

To a solution of II (100 *mg*). in MeOH (5 *ml.*) was added an etheral solution (30 *ml.*) of diazomethane prepared from p-tolylsulfonylmethylnitrosamide (5*g.*) and allowed to stand for 3 days at room temperature. The solvent was removed and the residue was dissolved in 10% AcOH. The acidic solution was made alkaline with NaOH and extracted with Et_2O. The combined Et_2O layer was dried over anhyd. K_2CO_3 and evaporated to leave a colorless base (80*mg.*). This *O*-methyl derivative was identical with *O,O*-dimethylisoliensinine (III) as styphnate in IR spectra. Styphnate: yellow needles, *mp.* 139-140° (EtOH).

Neferine (II) methiodide:

II (50*mg.*) was dissolved in MeOH (4 *ml.*) and was refluxed with CH_3I (5 *ml.*), on water bath for 4 *hrs.* After evaporating off the excess solvent, the residue was recrystallized with Me_2CO to give microneedles of (II) methiodide, *mp.* 179-181°.

Acknowledgement

We are indebted to Professor Y. Inubushi, Kyoto University for the microanalysis and Dr. Furukawa for the sample of neferine. The authors also thank Dr. T. J. Lin, Purdue University for the measurement of NMR and mass spectra. This work was supported by the National Science Council of the Republic of China.

References

(1) Preliminary Communication of this work appeared in *J. Chinese Chem. Soc.*, 17, No. 1, 54 (1970).
(2) M. Tomita, H. Furukawa, T.-H. Yang and T.-J. Lin, *Tetrahedron Letters*, 2637 (1964); *Chem. Pharm. Bull.* (Tokyo), 13, 39 (1965).
(3) H. Furukawa, T.-H. Yang and T.-J. Lin, *Yakugaku Zasshi*, 85, 472 (1965).
(4) H. Furukawa, *Yakugaku Zasshi*, 85, 335 (1965).
(5) J. S. Buck, *J. Am. Chem. Soc.*, 56, 1769 (1934).
(6) R. H. F. Manske, Canad. *J. Research*, 15B, 159 (1937).
(7) C. Hanna and J. H. Shutt, *Naunynschmiedebergs Arch. Exptl. Pathol. Pharmakol.* 220, 43-45 (1953).