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CHEMISTRY

STUDIES ON THE ALKALOIDS OF EMBRYO LOTI, *NELUMBO NUCIFERA* GAERTN.

I. ISOLATION AND CHARACTERISATION OF LIENSININE*

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The Chinese drug "Lien Tze Hsin", identified as embryo loti, *Nelumbo nucifera* Gaertn. Fam. Nymphaeaceae, is the embryo of the seed of lotus. It has been used as a febrifuga in folk medicine for ages in China, and also, was recorded in "Pen Tsao Kang Mu", for antipyretic treatment, etc.[1]

There have been only a few studies on the constituents of embryo loti published so far in literature. For instance, the presence of an alkaloid, nelumbine, in the seeds and cotyledon of lotus had been reported about seventy years ago by Greshoff *et al.*[2], with no detailed records given, however. Recently, the isolation from the leaves and petiole of lotus of the three alkaloids, structurally belonging to the aporphine type, namely, nuciferine, nornuciferine, and roemerine, was reported by Arthur *et al.*[3] and Tomita *et al.*[4]

In this paper, studies on the constituents of embryo loti will be described. The total alkaloid amounts to 1—1.25% of the dry material and is found to be present almost all in the aqueous extract of the material. The pharmacologic as well as preliminary clinical tests all show that this fraction possesses hypotensive effects. In consequence, one of the active principles, a new alkaloid named liensinine, has been isolated from the aqueous extract.

Liensinine is an amorphous phenolic alkaloid, and forms a well crystalline perchlorate, m.p. 212—4°C; $[\alpha]_D^{30.6}$: − 45.13° (C = 0.842 in acetone), although many other salts of both inorganic and organic acids fail to give any crystalline substance. The free alkaloid is liberated from the pure perchlorate by dissolving it in an excess of sodium hydroxide solution and then precipitating out with ammonium chloride in the form of a white powder, m.p. 95—99°C, $[\alpha]_D^{30.6}$: +15.85° (C=0.883 in acetone).

Spectroscopic studies on crystalline liensinine perchlorate show that the salt has the following absorptions in the ultraviolet region, λ max 207 mμ,

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log ε 4.56; 285 mμ, log ε 3.72, and the following absorptions in the infra-red region, 3480, 1060, 1124 cm^{-1}, the latter figures indicating the presence of hydroxyl, methoxyl, and N-methyl groups respectively. Analytical data show that the alkaloid has the composition of $C_{37}H_{42}O_6N_2$, which is well checked by the analytical results of its crystalline perchlorate, having the composition of $C_{37}H_{42}O_6N_2 \cdot 2HClO_4 \cdot H_2O$.

Analysis of the functional groups shows the presence of three methoxyl and two methylimino groups, the latter being also evidenced by the qualitative test by oxidation of liensinine with acidic permanganate to give formaldehyde. Besides, it contains two active hydrogens as shown by Zerewitinoff's method. Nitroso reaction and methylene dioxide and carbonyl tests, however, are all negative.

The alkaloid forms a diacetyl derivative by acetylation of pure or crude alkaloid, in the form of colourless rods, m.p. 124°C, having the composition $C_{37}H_{40}O_6N_2(COCH_3)_2 \cdot H_2O$ which has been proved by elementary analysis as well as the acetyl group analysis. The molecular weight determination of the vacuum-dried sample gives a rather low value of 450; probably some decomposition takes place above its melting point during the employment of Rast's camphor method. No molecular weight determination has been made for the perchlorate since the salt is insoluble in camphor. The diacetate of liensinine, after hydrolysis with alcoholic alkali, is, however, reconverted into liensinine.

From the above results it may be concluded that liensinine contains two methylimino, three methoxyl, and two hydroxyl groups. There remains only the function of one oxygen atom to be solved. Therefore, the partial formula of liensinine can be expressed as

$$C_{32}H_{25}O(OH)_2(OCH_3)_3(NCH_3)_2.$$

Experimental

1. *Extraction of the Total Alkaloid*

The dry powder of embryo loti (10 kg) was ground with 5% aqueous sodium carbonate solution (6 l), macerated with benzene (36 l) below 60°C under occasional stirring for ½ day, and then allowed to stand at room temperature for a further 1½ days. The benzene extract was filtered and the residue washed with benzene and pressed. The filtrate and washings were combined and repeatedly extracted by shaking with 3% hydrochloric acid. The solution thus obtained was made strongly alkaline with potassium carbonate until turbidity appeared, and then extracted with chloroform. The chloroform extract, when dried and distilled, gave the total alkaloid as a faint brown colour solid. The yield was 110 g or 1.1% based on the dry powder used.

2. *Isolation of Liensinine by Means of Its Perchlorate*

The total alkaloid of embryo loti was refluxed with ether in a Soxhlet-apparatus. The ether insoluble fraction was dissolved in chloroform and shaken with 2% aqueous sodium hydroxide under ice cooling in order to extract the phenolic bases. To the alkaline aqueous extract, a quantity of ammonium chloride was added to precipitate the phenolic bases, which were then extracted with chloroform. The chloroform solution, when dried and distilled, furnished a brown powder of phenolic base. The yield was 60—70% of the total alkaloid.

To the absolute alcoholic solution of phenolic base, an aqueous solution of 70% perchloric acid was added dropwise under cooling with stirring until acid to Congo red. The solution was allowed to stand overnight, whereupon white crystals deposited. The yield was 60—70% of the phenolic base used. After several recrystallizations from methyl or ethyl alcohol, the liensinine perchlorate in the form of white prismatic crystals was obtained, m.p. 212—4°C.

Analysis: Calcd. for $C_{37}H_{42}O_6N_2 \cdot 2HClO_4 \cdot H_2O$:
C, 53.56; H, 5.59; N, 3.38; Cl, 8.55; H_2O, 2.17;
found: C, 53.73; 53.74; H, 5.46, 5.54; N, 3.52, 3.23; Cl, 8.88, 8.95;
H_2O, 2.14, 2.46.

Liensinine perchlorate is insoluble in water and ordinary mineral acids, sparingly soluble in diluted aqueous acetic acid and ascorbic acid, soluble in aqueous sodium hydroxide, and very soluble in acetone, hot methanol or ethanol.

Free liensinine base is obtained in quantity by addition of ammonium chloride to the solution of perchlorate salt in 5% aqueous sodium hydroxide.

Liensinine (11 g) was again purified by dissolving in chloroform (50 ml), chromatographed on alumina (100 g), and then eluted with chloroform. From the elution, the pure liensinine (10.8 g), m.p. 95—99°C, white powder, was obtained.

Analysis: Calcd. for $C_{37}H_{42}O_6N_2$:
C, 72.76; H, 6.93; N, 4.59; 2 active H, 0.34;
found: C, 72.75; H, 6.93; N, 4.71; 2 active H, 0.38.

Liensinine is soluble in chloroform, ethanol, or methanol. Its alcoholic solution gives a pink brownish precipitate with ferric chloride, and also gives precipitates with many alkaloidal reagents.

3. *Preparation of Diacetyl Liensinine*

Liensinine (0.5 g), acetic anhydride (1 g), and pyridine (1 drop) were mixed together, and heated on a water bath at 60°C for 15 minutes. The

whole was set aside overnight, and then decomposed with ice water. After making it alkaline with 10% aqueous sodium hydroxide the white precipitate was filtered and washed with water. It was dried in vacuum, and then recrystallized from acetone in the form of colourless rhombics, m.p. 124°C. The yield was 480 mg.

Analysis: Calcd. for $C_{37}H_{40}O_8N_2(COCH_3)_2 \cdot H_2O$:
C, 69.08; H, 6.79; N, 3.93; $2COCH_3$, 12.03;
found: C, 69.35; H, 6.57; N, 4.06; $2COCH_3$, 12.01.

Diacetyl liensinine is soluble in acids, chloroform, acetone, hot ethanol, or hot methanol, and insoluble in aqueous sodium hydroxide.

4. *Acetylation of Total Alkaloid and Isolation of Diacetyl Liensinine*

Total alkaloid of embryo loti (1 g) and acetic anhydride (1.5 g) were mixed together and warmed on a water bath until the solution became clear. One drop of pyridine was added and the mixture warmed again for about 10 minutes. The whole was allowed to stand for 24 hr at room temperature, decomposed with water, and then made alkaline carefully with 20% aqueous sodium hydroxide under cooling. The solid separated out was filtered off, and washed with water. It was dried and recrystallized from ethanol to yield 0.6 g of acetyl compound. After further recrystallization, it formed colourless rhombics, m.p. 124°C, giving no depression when admixed with diacetyl liensinine prepared in the foregoing experiment.

5. *Qualitative Test of N-methyl Group*

Liensinine (1 g) was dissolved in aqueous sulphuric acid (10 ml), to which 5% aqueous solution of potassium permanganate was added dropwise under ice cooling and stirring. The addition took about one hour, and stopped until the disappearance of the pink colour subsided. The whole was filtered, the filtrate distilled, and the distillate collected in a receiver cooled with ice. One per cent aqueous dimedone (50 ml) was added to the distillate, and the mixture was heated on a boiling water bath for a few minutes, whereupon a white emulsion appeared. The whole was allowed to stand for the night. The condensation product thus obtained, m.p. 190°C, is identical with the condensation product of formaldehyde with dimedone, giving no depression when admixed with authentic sample.

Analysis: Calcd. for $C_{32}H_{27}O_3(NCH_3)_2(OCH_3)_3$:
OCH_3, 15.24; (N)—CH_3, 4.92;
found: OCH_3, 15.60; (N)—CH_3, 4.81.
Calcd: for $C_{32}H_{27}O_3(NCH_3)_2(OCH_3)_3 \cdot 2HClO_4 \cdot H_2O$:
OCH_3, 11.12; (N)—CH_3, 3.62;
found: OCH_3, 11.32; (N)—CH_3, 3.88.

Summary

From embryo loti, the embryo of the seeds of *Nelumbo nucifera* Gaertn., a Nymphaeaceous plant, a new phenolic alkaloid, liensinine, has been isolated, m.p. 95—99°C, molecular formula $C_{37}H_{42}O_6N_2$, $[\alpha]_D^{30.6}$: +15.85° (C=0.883 in acetone). Its perchlorate salt has a m.p. 212—4°C, a molecular formula $C_{37}H_{12}O_6N_2 \cdot 2HClO_4 \cdot H_2O$, and $[\alpha]_D^{30.6}$: −45.13° (C=0.842 in acetone).

By acetylation of liensinine, a diacetyl liensinine, m.p. 124°C, is obtained, having the molecular formula $C_{37}H_{10}O_6N_2(COCH_3)_2 \cdot H_2O$. Hydrolysis of the latter gives liensinine in return. Liensinine contains two methylimino, three methoxyl, and two hydroxyl groups. Thus, only the function of one oxygen atom remains to be solved. Therefore, the partial formula of liensinine can be expressed as $C_{32}H_{25}O(OH)_2(OCH_3)_3(NCH_3)_2$.

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