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The Structure of Shihunine, a New Phthalide-Pyrrolidine Alkaloid[1]

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An alkaloid constituent of "Chukanso" (中環草), a kind of the Chinese drug "Shih-Hu" (石斛) was examined and a new alkaloid which was designated as shihunine was isolated.

From degradative and spectroscopic data, it was established that the structure of shihunine should be represented by the Formula (F).

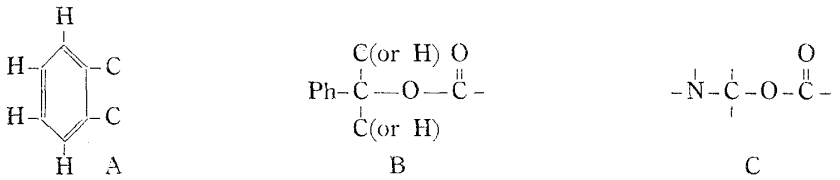
"Chukanso" (中環草) is a trade name being assigned to a kind of the Chinese drug, Shi-Hu (石斛), and this drug is available on Hong Kong market. The original plant of this drug has been studied by Takahashi, *et al.*[3] to be *Dendrobium lohohense* Tang et Wang.

In connection with studies on the alkaloids of "Chin-Shih-Hu" (金釵石斛),[4] the authors examined the alkaloid constituents of this drug and isolated a new alkaloid which was designated as shihunine.

The molecular formula $C_{12}H_{13}O_2N$ of shihunine, mp 79°, optically inactive, was established by elementary analyses of the free base and its picrate, mp 163—164° and its styphnate, mp 202—205° together with molecular weight determination by Rast method.

The NMR spectrum shihunine suggested the presence of aromatic protons (approximately 4 protons, complex peaks, at 2.0—2.7 τ) and N-methyl or Ar-methyl group (7.90 τ). Since shihunine showed a carbonyl absorption band (1743 cm^{-1}) in its IR spectrum, alkaline hydrolysis was first examined. Thus, shihunine was hydrolyzed to an amino acid, as indicated by its IR bands (see experimental), which regenerated the original base on standing or on treatment with picric acid (as its picrate). Therefore, shihunine should possess a lactone group in its molecule.

Oxidation of shihunine with potassium permanganate afforded phthalic acid establishing that this alkaloid contains the partial structure A.



A B C

Chart 1

Hydrogenation of shihunine over Adams catalyst gave a dihydro compound which seemed to be an amino acid, suggesting hydrogenolysis of shihunine. Dihydroshihunine, IR 2400 (br.), 1613, 1595, 1471 cm^{-1}, pK_a' 3.3 and 10.5, was soluble in both acidic and alkaline medium. This finding showed that shihunine has a group B or C. The fact that lithium aluminum hydride reduction of both shihunine and dihydroshihunine gave the same mono-ol, suggests

1) A preliminary communication of this work appeared in *Chem. Pharm. Bull.* (Tokyo), **12**, 749 (1964).
2) Location: *a) Yoshida-shimoadachi-cho, Sakyo-ku, Kyoto. b) Toneyama, Toyonaka, Osaka-fu.*
3) S. Takahashi, T. Namba, and Y. Hayashi, *Syôyakugaku Zasshi*, **19**, 13 (1965).
4) Y. Inubushi, H. Ishii, B. Yasui, T. Konita, and T. Harayama, *Chem. Pharm. Bull.* (Tokyo), **12**, 1175 (1964).

the latter posibility. Reduction of shihunine therefore must be accompanied by hydrogenolysis. The partial structure C was also supported by comparison of pK_a' values (Table I). The marked increase of pK_a' values in reduction products compared with that of the original base indicated that the neighboring atom of the nitrogen had been substituted by an O–acyl group which was removed by lithium aluminum hydride reduction or by hydrogenation.

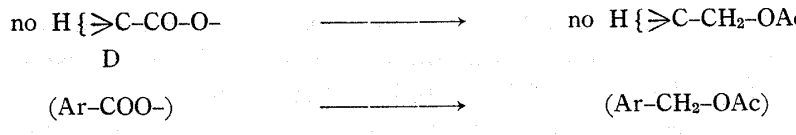


Chart 2

Table I

	pK_a'	Δ pK_a' (increase)
Shihunine	3.6	
Mono–ol	8.3	4.7
Mono–ol Acetate	7.8	4.2
Amine (E)	8.2	4.6

The NMR spectrum of mono–ol acetate showed a singlet peak due to $-CH_2-OAc$ at 4.81 τ, showing the partial structure D of shihunine. From the singlet feature and its low chemical shift, it can be inferred that this primary alcohol group attaches directly to a benzene ring ($Ar-CH_2-OAc$). Hydrogenation of this mono–ol acetate over palladium–charcoal caused hydrogenolysis, as expected, to give a product containing no oxygen atom which showed a signal due to $Ar-CH_3$ in its NMR spectrum. Thus, the partial structure ArCOO– in shihunine was firmly established.

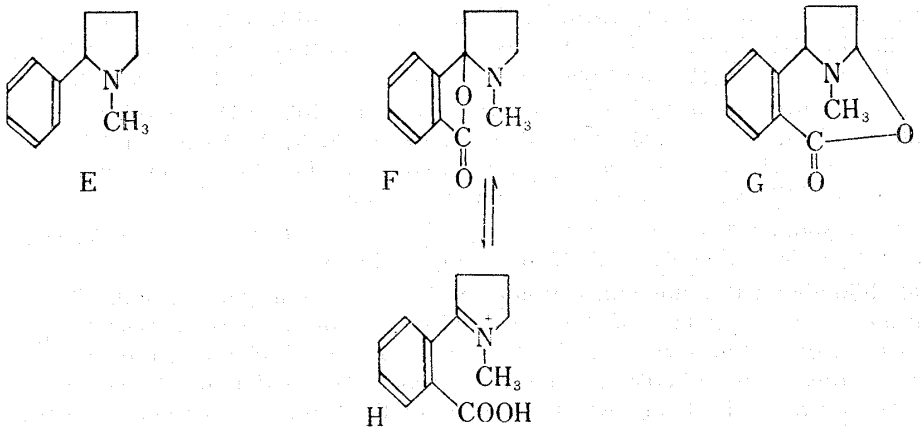


Chart 3

Decarboxylation of dihydroshihunine under acidic or neutral conditions (with solvent) did not work well but dry distillation with BaO smoothly proceeded to give an oil, a decarboxylated product, $C_{11}H_{15}N$. By direct comparison its crystalline picrate was proved to be identical with an authentic sample of 1–methyl–2–phenylpyrrolidine picrate (E).[5] Thus, the presence of N–methyl group as well as the gross structure of shihunine was settled. The structure of shihunine is now limited to F or G.

5) J.H. Burckhalter and J.H. Short, *J. Org. Chem.*, **23**, 1281 (1958). The authors acknowledge to Prof. J.H. Burckhalter for providing the authentic sample of 1–methyl–2–phenylpyrrolidine picrate.

The structure G is not rigorously excluded at the present stage of investigation since the carbonyl absorption of shihunine appeared between 1738—1761 cm^{-1} depending on the measuring state employed ($CHCl_3$; 1738, Nujol; 1743, CCl_4; 1761). The possibility of G, however, was definitely excluded by inspection of its NMR spectrum. Shihunine did not show any signal due to $>N-\overset{|}{C}H-O-CO-$ and all signals appeared were reasonable explained by assuming the formula F.

After a preliminary communication[1] of this work had been presented, the synthetic proof of the structure F for shihunine was offered by Onaka[6] in his two-step synthesis of this base from dimethylphthalate and 1-methyl-2-pyrrolidone.

The structure F possesses an asymmetric carbon atom, which however seems to be racemized by equilibrium of (F)⇄(H). Actually, its picrate showed several absorption bands between 1725—1550 cm^{-1}, the strong bands at 1690 and 1622 cm^{-1} being attributed to -COOH and $>N^+=C<$. In the plant or during the extraction procedure the optically active base, if it exists, will undergo racemization to vanish its optical activity. In fact, shihunine isolated was completely inactive within the range 200—700 mμ.

Experimental[7]

Isolation of Shihunine——A finely-cut drug (1.8 kg) was extracted four times with boiling MeOH (each 4 liter). The combined extracts were condensed under reduced pressure to give a brown residue which was dissolved in 5% HCl (2 liter) filtered and the filtrate was shaken with ether. The acidic aqueous layer was basified with conc. NH_4OH under cooling with ice and extracted several times with $CHCl_3$ (total *ca.* 10 liter) until the $CHCl_3$ layer showed a negative test to Meyer's reagent. The $CHCl_3$ solution was washed with a small amount of water, dried over anhyd. $MgSO_4$ and evaporated under reduced pressure to give 11.7 g of the basic residue which was dissolved in benzene and passed through a short column (1.5×6 cm) of alumina (15 g). Elution of the column with benzene (500 ml) and evaporation of the solvent from the eluant left a pale yellow gum. Rechromatography of this gum in benzene over alumina (10 g, 1.5×4 cm) gave shihunine (10.14 g) as a crystalline mass.

Shihunine——Shihunine formed hygroscopic colorless needles from *n*-hexane-ether, mp 78.5—79°, $[\alpha]_{200-700m\mu}=0$ (MeOH), pK_a' 3.6 (in 50% EtOH-H_2O). *Anal.* Calcd. for $C_{12}H_{13}O_2N$: C, 70.91; H, 6.45; N, 6.89. Found: C, 70.53; H, 6.54; N, 6.59. M.W. Calcd. for $C_{12}H_{13}O_2N$: 203.23. Found: 203, 209 (Rast's method). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1761, 1616; ν_{max}^{Nujol} cm^{-1}: 1743, 1616; $\nu_{max}^{CHCl_3}$ cm^{-1}: 1738, 1614. $UV_{inflection}^{95\%EtOH}$ mμ (log ε): 270 (2.13), 277 (2.05). NMR (τ): 2.0—2.7 (4H, multiplet, aromatic protons), 6.6—7.0 (2H, multiplet, $>N-CH_2-$), 7.5—8.0 (4H, multiplet, $-CH_2-CH_2-$), 7.90 (3H, singlet, $>N-CH_3$).

The picrate crystallized from MeOH as yellow prisms, mp 163—164°. *Anal.* Calcd. for $C_{12}H_{13}O_2N\cdot C_6H_3O_7N_3$: C, 50.00; H, 3.73; N, 12.96. Found: C, 49.98; H, 3.83; N, 13.00. IR_{max}^{Nujol} cm^{-1}: 1724, 1690, 1622, 1605, 1563, 1546. The picrate (100 mg) regenerated shihunine (30 mg), mp and mixed mp 78—79° on passing its $CHCl_3$-Me_2CO solution through an alumina column.

The styphnate crystallized as yellow prisms, mp 202—205°. *Anal.* Calcd. for $C_{12}H_{13}O_2N\cdot C_6H_3O_8N_3$: C, 48.22; H, 3.60; N, 12.50. Found: C, 47.67; H, 3.45; N, 12.16.

Oxidation of Shihunine with Potassium Permanganate——To a solution of $KMnO_4$ (1 g) in 20 ml of 10% aq. NaOH solution was added 200 mg of shihunine and stirred for 16 hours at room temperature. Excess $KMnO_4$ was destroyed by adding saturated Na_2SO_3 solution in 20% H_2SO_4 and the mixture was extracted several times with ether. The ethereal extract was washed with water, dried over anhyd. $MgSO_4$ and evaporated to leave white solid (118 mg) which was recrystallized from water to give prisms, mp 140—180°. The IR spectrum of this substance was superimposable with that of phthalic acid. Sublimation of the solid at 250°/2 mmHg gave lustrous needles, mp 132—134°. IR ν_{max}^{Nujol} cm^{-1}: 1845, 1790, 1765. This substance was identified with phthalic anhydride by mixture melting point determination and by IR comparison.

Alkaline Hydrolysis of Shihunine——Shihunine (54 mg) in 10% NaOH aq. (1 ml) solution was warmed on a water bath for 30 minutes and kept overnight at room temperature. The brown mixture was extracted several times with $CHCl_3$. The $CHCl_3$ extract gave a viscous gum. IR ν_{max}^{film} cm^{-1}: 1675, 1650, 1608, 1585 and 1560.

The alkaline solution was acidified with HCl and washed with $CHCl_3$ (no extract). Then, the solution was again basified with K_2CO_3 and extracted with $CHCl_3$, and evaporation of the solvent left a small amount

6) T. Onaka, *Yakugaku Zasshi*, **85**, 839 (1965).

7) All melting points are uncorrected and NMR spectra were obtained in $CDCl_3$ with tetramethylsilane as an internal standard on a Varian Associated A-60 recording spectrometer.

of viscous gum whose IR spectrum was almost identical with that of the compound obtained above. A portion of the combined gum was converted to a picrate. Recrystallization of the picrate from MeOH gave yellow prisms, mp 162—163° which was identical with shihunine picrate (IR comparison and mixed melting point).

On standing in a vacuum desiccator, the viscous gum crystallized gradually. Recrystallization from *n*-hexane–ether gave needles, mp 78—79° which was identical with shihunine (IR, TLC comparison and mixed mp).

Hydrogenation of Shihunine——Shihunine (100 mg) in abs. EtOH (10 ml) was hydrogenated over Adams catalyst (50 mg) for 2 hours. Removal of the catalyst and evaporation of the solvent left a solid (97 mg) which was recrystallized from Me_2CO–ether to give dihydroshihunine as hygroscopic colorless prisms, mp 190—201°. *Anal.* Calcd. for $C_{12}H_{15}O_2N$: C, 70.22; H, 7.37; N, 6.82. Found: C, 69.99; H, 7.45; N, 7.00. IR ν_{max}^{Nujol} cm^{-1}: 2400 (broad, $\geqslant N^+$–H), 1613 (COO^-), 1595, 1405. pK_a' 3.3 and 10.5 (50% EtOH–H_2O). Dihydroshihunine was soluble in both acidic and basic medium but insoluble in ether or $CHCl_3$.

The picrate was recrystallized from AcOEt–ether as yellow prisms, mp 155—156°. *Anal.* Calcd. for $C_{12}H_{15}O_2N \cdot C_6H_3O_7N_3$: C, 49.77; H, 4.18; N, 12.90. Found: C, 49.92; H, 4.09; N, 13.09. IR ν_{max}^{Nujol} cm^{-1}: 1684 (COOH), 1633, 1616 and 1563.

Decarboxylation of Dihydroshihunine——Dihydroshihunine (295 mg) and powdered BaO (2.45 g) were mixed and the mixture was put into a glass tube (30×1.5 cm) which was heated gently over a flame in the beginning and then drastically for about 20 minutes. A volatile oil with unpleasant odour generated smoothly and was collected and converted into a picrate (453 mg). Recrystallization of this picrate from EtOH afforded yellow prisms, mp 150—153.° *Anal.* Calcd. for $C_{11}H_{15}N \cdot C_6H_3O_7N_3$: C, 52.30; H, 4.65; N, 14.35. Found: C, 52.60; H, 4.61; N, 14.44. IR ν_{max}^{Nujol} cm^{-1}: 1632, 1617 (shoulder), 1563. This was identified with the authentic sample of 1-methyl-2-phenylpyrrolidine picrate,[5] (IR spectra and mixed melting point) and also with our synthetic specimen (see below). The free amine was obtained as an oil by passing CH_2Cl_2 solution of its picrate through an alumina column. pK_a' 8.2 (50% EtOH–H_2O), NMR: 2.72 (5 H, singlet); 6.7—7.2 (multiplet); 7.85 (3H, singlet); 7.6—8.4 (multiplet).

The methiodide formed colorless needles from Me_2CO–ether, mp 157—159°. *Anal.* Calcd. for $C_{12}H_{18}NI$: C, 47.52; H, 5.94; N, 4.62. Found: C, 47.80; H, 5.88; N, 4.74. IR ν_{max}^{Nujol} cm^{-1}: 1582. NMR 2.2—2.7 (5H, multiplet, aromatic protons), 4.53 (1H, diffused triplet, Ph–$\underset{|}{CH}$–N$<$), 5.84 (2H, diffused triplet, $>$N–CH_2–), 6.62 and 7.08 (each 3H, two singlets, $>N^+(CH_3)_2$, 7.2—7.8 (4H, multiplets, –CH_2–CH_2–).

Reduction of Shihunine with Lithium Aluminum Hydride——Shihunine (310 mg) and $LiAlH_4$ (325 mg) in anhyd. ether (10 ml) were heated under reflux for 3 hours. Water was added and the precipitates were removed by filtration. The ethereal solution was dried over anhyd. K_2CO_3 and evaporated to give a mono-ol as an oil. IR ν_{max}^{film} cm^{-1}: 3400, 1610. pK_a' 8.3 (50% EtOH–H_2O). The picrate crystallized from MeOH as yellow prisms, mp 128—130°. *Anal.* Calcd. for $C_{12}H_{17}ON \cdot C_6H_3O_7N_3$: C, 51.42; H, 4.80; N, 13.33. Found: C, 51.15; H, 4.86; N, 13.23. IR ν_{max}^{Nujol} cm^{-1}: 3497, 1631 and 1613 (shoulder).

Mono-ol from Dihydroshihunine——Dihydroshihunine (120 mg) and $LiAlH_4$ (100 mg) in anhyd. tetrahydrofuran (10 ml) were heated under reflux for 4 hours. Working up as usual, an oily residue was converted into its picrate, yellow prisms, mp and mixed mp 127—129°. IR spectrum of this picrate was identical with that of the compound obtained above.

Mono-ol Acetate——Mono-ol (373 mg) in pyridine (20 ml) and Ac_2O (5 ml) was allowed to stand overnight at room temperature. Pyridine and Ac_2O were evaporated under reduced pressure and the residue was poured into ice-water, then the mixture was made alkaline with K_2CO_3 and a separated oil was extracted with ether. The ethereal solution was shaken with 5% HCl, the acid layer was basified with K_2CO_3 and re-extracted with ether. The ether extract was washed, dried over anhyd. $MgSO_4$ and evaporated to give an oily acetate. IR ν_{max}^{film} cm^{-1}: 1740, 1610. pK_a' 7.8 (50% EtOH). The picrate crystallized from MeOH as yellow prisms, mp 174—176°. *Anal.* Calcd. for $C_{14}H_{19}O_2N \cdot C_6H_3O_7N_3$: C, 51.95; H, 4.80; N, 12.12. Found: C, 52.18; H, 4.80; N, 11.94. IR ν_{max}^{Nujol}: cm^{-1}: 1736, 1627, 1613 and 1561. The free mono-ol acetate regenerated from its picrate showed the following NMR signals. 2.3—2.9 (4H, multiplet, aromatic protons), 4.81 (2H, singlet, Ph–CH_2–OAc), 6.3—6.9 (3H, multiplets, –$\underset{|}{CH}$—N–$\underset{|}{CH_2}$–), 7.85 (3H, singlet, $>$N–CH_3 or –CO–CH_3), 7.94 (3H, singlet, –CO–CH_3 or $>$N–CH_3), 7.6—8.3 (4H, multiplets, –CH_2–CH_2–).

Hydrogenolysis of the Mono-ol Acetate——Mono-ol acetate (380 mg), in 5% HCl (5 ml) was hydrogenated over 20% Pd-C (500 mg) for 5 hours. The mixture was filtered and the filtrate was basified with Na_2CO_3, then extracted with ether. The ether extract was washed, dried over anhyd. K_2CO_3 and evaporated to leave an oily amine which showed no carbonyl absorption band in the IR spectrum. This oily amine was characterized as its picrate (121 mg). Recrystallization from EtOH gave yellow needles, mp 158—162°. *Anal.* Calcd. for $C_{12}H_{17}N \cdot C_6H_3O_7N_3$: C, 53.46; H, 4.99; N, 13.86. Found: C, 53.25; H, 4.95; N, 13.78. NMR (measured in the form of picrate): 7.12 (3H, singlet, $>$N–CH_3), 7.59 (3H, singlet, Ph–CH_3).

2-Phenylpyrrolidine——This substance was prepared by the method according to Burckhalter and Short[5] and obtained as a pale yellow oil. The picrate formed yellow prisms, mp 151—153°.

1-Methyl-2-phenylpyrrolidine——2-Phenylpyrrolidine (230 mg), 37% HCHO (2.5 ml) and 20% Pd-C (500 mg) in EtOH (10 ml) and H_2O (20 ml) were stirred under H_2 for 5 hours. The mixture was filtered and the filtrate was basified with K_2CO_3, extracted with ether. The ethereal extract was washed, dried over anhyd. K_2CO_3 and evaporated to leave 1-methyl-2-phenylpyrrolidine as a pale yellow oil. The picrate formed yellow prisms and recrystallization from EtOH gave crystals which melted at 148—151°. *Anal.* Calcd. for $C_{11}H_{15}N \cdot C_6H_3O_7N_3$: C, 52.30; H, 4.65; N, 14.35. Found: C, 52.45; H, 4.71; N, 14.15.