f) Stability of colored solution : After developing the colored solution, it was found that the absorbance was not changed within 8 hr.

The authors are deeply grateful to Dr. K. Takeda, Director of this Laboratory, for his helpful advice and encouragement. They are also grateful to the members of the Elementary Analysis Department for the elementary analysis data.

Summary

2′-Mercaptosulfonanilide derivatives were prepared by the reduction of 2,2′-dithiobissulfonanilide with sodium sulfide and tested as the analytical reagents. These compounds were found to be useful reagents for the identification of copper (II), cobalt (II), and nickel (II), and the spectrophotometric determination of cobalt (II).

(Received September 21, 1964)

[Chem. Pharm. Bull. 13(1) 39 ~ 43 (1965)] UDC 547.94 : 582.671

7. Masao Tomita, Hiroshi Furukawa,*1 Tsang-Hsiung Yang, and Tsung-Jen Lin*2 : On the Alkaloids of *Nelumbo nucifera* GAERTN. VIII.*3 Studies on the Alkaloids of Loti Embryo. (1).*4 Structure of Isoliensinine, a New Biscoclaurine Type Alkaloid.

(*Faculty of Pharmaceutical Sciences, Kyoto University*1 *and School of Pharmacy, Taipei Medical College*2)

In 1962, Pan Pei-chuan, *et al.*[1] reported the isolation of liensinine from Chinese drug "Lien Tze Hsin (蓮子芯)," embryo loti, (embryo of the seed of *Nelumbo nucifera* GAERTN., Fam. Nymphaeaceae) and its structure was shown to be I, based on the result of its Hofmann degradation and permanganate oxidation.*5

Recently we isolated a new phenolic tertiary base and a new water-soluble quaternary base from Formosan "Lien Tze Hsin." This paper deals with the structure elucidation of the tertiary base for which we propose the name "isoliensinine."

The free base of isoliensinine resisted all attempts at crystallization. For the preliminary characterization, several crystalline derivatives were prepared. The hydrochloride crystallized from ethanol to yield colorless needles, m.p. 185~186° and afforded analytical results in agreement with the empirical formula $C_{37}H_{42}O_6N_2 \cdot 2HCl \cdot 4H_2O$. The perchlorate gave prisms from ethanol, m.p. 200~203°, $[\alpha]_D$ −70.0° (acetone). Analysis afforded results which supported a $C_{37}H_{42}O_6N_2 \cdot 2HClO_4 \cdot H_2O$ formula.

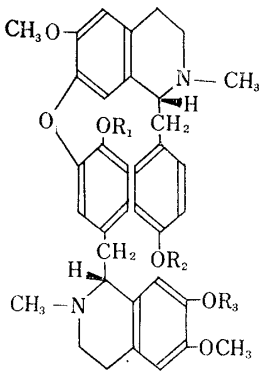
---

*1 Yoshida, Sakyo-ku, Kyoto (富田真雄, 古川 宏).
*2 Taipei, Formosa (楊 蔵雄, 林 宗仁).
*3 Part Ⅶ. Z. Kunitomo : Yakugaku Zasshi, **84**, 1100 (1964).
*4 Preliminary communication of this work appeared in Tetrahedron Letters, No. 37, 2637 (1964).
*5 The absolute configuration of two asymmetric centers of liensinine (I) had not been determined (cf. *8).

1) Chao Tse-yuan, Chou Yun-lee, Young Pao-tsin, Chou Tsan-quo : Scientia Sinica, **11**, 216 (1962); Pan Pei-chuan, Chou Yun-lee, Sun Tsun-tsi, Kao Yee-sheng : *Ibid.*, **11**, 321 (1962).

The ultraviolet spectrum of isoliensinine had a maximum absorption at 286 mμ and a minimum at 257 mμ. The nuclear magnetic resonance spectrum*[6] showed nine O-methyl protons, six N-methyl protons, two hydroxyl protons, and eleven aromatic protons. Also the IR band at 3500 cm^{-1} indicated the presence of hydroxyl group.

These spectroscopic and analytical data suggest that isoliensinine has the same rational formula as liensinine (I), $C_{32}H_{25}O(OCH_3)_3\cdot(OH)_2\cdot(NCH_3)_2$.*[7]

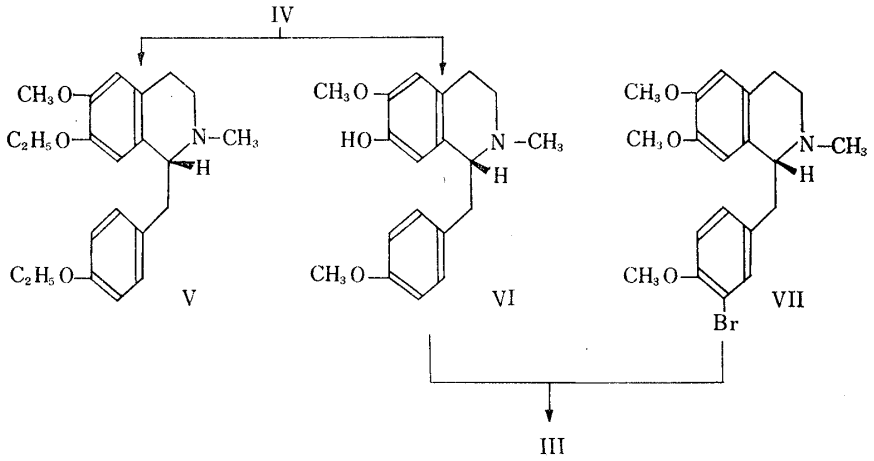


I : $R_1, R_2 = H, R_3 = CH_3$ (liensinine)

II : $R_1, R_3 = H, R_2 = CH_3$ (isoliensinine)

III : $R_1, R_2, R_3 = CH_3$

IV : $R_1, R_3 = C_2H_5, R_2 = CH_3$



Methylation of isoliensinine with diazomethane yielded O,O-dimethylisoliensinine (Ⅲ), which was characterized as the crystalline styphnate, m.p. 133~135°, $C_{39}H_{46}O_6N_2\cdot 2C_6H_3O_8N_3\cdot C_2H_5OH$ (ethanol adduct). The nuclear magnetic resonance spectrum of this O,O-dimethylisoliensinine (Ⅲ) showed fifteen O-methyl protons and six N-methyl protons.

Treatment of isoliensinine with diazoethane led to O,O-diethyl isoliensinine (Ⅳ), whose nuclear magnetic resonance spectrum revealed the presence of two ethoxyl groups. When treated with metallic sodium in liquid ammonia in the usual manner, O,O-diethylisoliensinine was cleaved to give two coclaurine type bases. The one was found to be non-phenolic base, which gave the crystalline oxalate, m.p. 177~179°, $[\alpha]_D$ −113.3° (50% MeOH, H_2O). This oxalate was shown by analysis to give the formula $C_{22}H_{29}O_3N\cdot C_2H_2O_4$ and its free base showed nuclear magnetic resonance signals at 6.20 τ (3H, OCH_3), 8.61, 8.67 τ (triplets, J=7.0 c.p.s., 6H, two $OCH_2\underline{CH_3}$), 7.48 τ (3H, NCH_3). Infrared

*[6] The NMR spectra were taken on a Varian Associated recording spectrometer (A-60) at 60 Mc. in deuterated chloroform. Chemical shifts are reported in τ values, using tetramethylsilane as the internal reference. We wish to thank Dr. T. Shingu of this Faculty for these determinations.

*[7] IR (in chloroform) and NMR spectra of isoliensinine showed distinct differences from these of liensinine (I). Also the mixed melting point of these perchlorate depressed apparently.

spectra of this bisected base and its oxalate were found to be superimposable with those of the authentic sample of D(−)-N-methyl-O,O-diethylcoclaurine (V)[2] (in chloroform solution) and the corresponding oxalate (in KBr disk).

The other, a phenolic base, was obtained as a colorless oil, $[\alpha]_D$ −87.7° (in methanol solution). This substance was identified as D(−)-1-(*p*-methoxybenzyl)-2-methyl-6-methoxy-1,2,3,4-tetrahydro-7-isoquinolinol (Ⅵ)[3] by infrared comparison in chloroform solution.

These observations led us to suppose that isoliensinine might have the analogous structure with liensinine (I). In order to confirm this assumption, we attempted to synthesize O,O-dimethylliensinine (Ⅲ).

Ullmann condensation between D(−)-3′-bromo-O-methylarmepavine (Ⅶ)[4] and D(−)-1-(*p*-methoxybenzyl)-2-methyl-6-methoxy-1,2,3,4-tetrahydro-7-isoquinolinol (Ⅵ) was carried out in pyridine solution in the presence of potassium carbonate and copper powder. Alumina chromatography of the product afforded a colorless oily base which was characterized as the crystalline styphnate, m.p. 133∼135°. As shown in Table I, properties of this synthesized compound were shown to be quite identical with those of O,O-dimethylisoliensinine.*8

Table I.

		O,O-Dimethylisoliensinine	Synthetic sample (Ⅲ)
Free base	IR ($CHCl_3$)	identical	
	NMR		
	TLC*9a,b)		
Styphnate	formula	$C_{39}H_{46}O_6N_2 \cdot 2C_6H_3O_8N_3 \cdot C_2H_5OH$	$C_{39}H_{46}O_6N_2 \cdot 2C_6H_3O_8N_3 \cdot C_2H_5OH$
	appearance	yellow needles	yellow needles
	m.p. (°C)	133∼135	133∼135
	$[\alpha]_D$ (Me_2CO)	−81.5°	−76.9°
	IR (KBr)	identical	

On the bases of these experimental evidences, the structure of isoliensinine is unambiguously assigned to the formula (Ⅱ).

Experimental

Isolation of Isoliensinine (II)——Air-dried "Lien Tze Hsin"*10 (3 kg.) of Taipei market, was extracted with hot EtOH and the extract was evaporated under red. pressure. The residue (500 g.) was dissolved in 3% AcOH, filtered, and washed with Et_2O to remove the non-basic material. The acid solution was made alkaline with conc. NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ solution, after the usual acid-alkali treatment, was washed with H_2O, dried over anhyd. K_2CO_3 and evaporated to leave a crude alkaloid mixture (34 g.). This was dissolved in a small amount of EtOH and excess of 4% HCl aq. solution was added to yield a crystalline hydrochloride (Isoliensinine (Ⅱ) hydrochloride) (9.7 g.). Examination of the mother liquor of this hydrochloride and the water-soluble quaternary base fraction is now in progress.

*8 The identity of the O,O-dimethylisoliensinine with O,O-dimethylliensinine which was afforded by the treatment of liensinine (I) with diazomethane, was confirmed by direct comparison (IR and NMR). Their styphnates were also identical in every respect. (O,O-dimethylliensinine styphnate: yellow needles, m.p. 137∼138°, $[\alpha]_D^{24}$ −78.6° (c=0.67, acetone).

Hence, the absolute configuration of liensinine is the same as that of isoliensinine (Ⅱ), (D. D).

*9 Thin-layer Chromatography: *a*) Aluminiumoxyd G nach Stahl; solvent, $CHCl_3$. *b*) Kieselgel G nach Stahl; solvent, MeOH-Me_2CO (1:1).

*10 It was said that these were collected in the southern part of Formosa in autumn, 1963.

2) M. Tomita, T. Kikuchi: Yakugaku Zasshi, **77**, 238 (1957).
3) M. Tomita, Y. Sasaki: This Bulletin, **2**, 375 (1954).
4) M. Tomita, K. Ito, H. Yamaguchi: *Ibid.*, **3**, 449 (1955).

Isoliensinine (II)——Free base : Colorless oil, $[\alpha]_D^{22}$ +49.3° (c=0.75, Me_2CO), $[\alpha]_D^{29}$ −43.3° (c=0.95, $CHCl_3$). NMR signals at 7.51, 7.62 τ (6H, two NCH_3), 6.24 τ (6H, two OCH_3), 6.30 τ (3H, OCH_3), 4.12 τ (2H, broad, two OH), and 3.02～3.69 τ (11H, aromatic-H). UV $\lambda_{max}^{95\%\ EtOH}$ 286 mμ (log ε 4.05), $\lambda_{max}^{95\%EtOH}$ 257 mμ (log ε 3.41). Perchlorate : Colorless prisms from EtOH, m.p. 200～203° (depressed to 180～185° on admixture with the authentic sample of liensinine (I) perchlorate, m.p. 212°). $[\alpha]_D^{22}$ −70.0° (c=1.32, Me_2CO). *Anal.* Calcd. for $C_{37}H_{42}O_6N_2 \cdot 2HClO_4 \cdot H_2O$. C, 53.56; H, 5.59. Found : C, 53.59; H, 5.66. Hydrochloride : Colorless needles from EtOH, m.p. 185～186°. *Anal.* Calcd. for $C_{37}H_{42}O_6N_2 \cdot 2HCl \cdot 4H_2O$. C, 58.80; H, 6.94; N, 3.69. Found : C, 59.27; H, 7.16; N, 3.71.

O,O-Dimethylisoliensinine (III)——To a solution of isoliensinine (II) (80 mg.) in MeOH (4 ml.) was added an ethereal solution (20 ml.) of diazomethane prepared from nitrosomethylurea (2 g.) and allowed to stand for 2 days at room temperature. The solvent was evaporated and the residue was dissolved in 10% AcOH. The acid solution was washed with Et_2O, made alkaline with NH_4OH and extracted with Et_2O. The combined Et_2O extract was washed successively with 5% NaOH aq. solution and with H_2O, dried with anhyd. K_2CO_3 and evaporated. The oily product (65 mg.) was chromatographed on alumina (6×40 mm.) with benzene to give a colorless oily base. TLC*[9a,b] 1 spot. NMR signals at 6.19, 6.22, 6.23, 6.29, 6.40 τ (15H, five OCH_3) and 7.56, 7.58 τ (6H, two NCH_3). The IR spectrum in $CHCl_3$, NMR spectrum, and TLC*[9a,b] were identical with those of the synthetic sample of O,O-dimethylliensinine (III) described below. Styphnate was recrystallized from EtOH to give yellow needles, m.p. 133～135°, $[\alpha]_D^{27}$ −81.5° (c=0.65, Me_2CO). *Anal.* Calcd. for $C_{39}H_{46}O_6N_2 \cdot 2C_6H_3O_8N_3 \cdot C_2H_5OH$ (ethanol adduct). C, 55.02; H, 4.93. Found : C, 55.04; H, 5.19. The IR spectrum in KBr disk was also identical with that of the synthetic sample (III) styphnate (Table I).

O,O-Diethylisoliensinine (IV)——Isoliensinine (II) (200 mg.) was dissolved in MeOH (5 ml.) and was treated with diazoethane at room temperature for a day. Usual treatment of the product yielded a colorless oil (190 mg.) from the non-phenolic fraction. TLC*[9a] 1 spot. NMR signals at 8.68, 8.72 τ (6H, two triplets, J=7.0 c.p.s., two $OCH_2\underline{CH_3}$), 7.56, 7.54 τ (6H, two NCH_3), 6.31 τ (3H, OCH_3), 6.22 τ (6H, two OCH_3).

Cleavage of O,O-Diethylisoliensinine (IV) with sodium in Liq. Ammonia——Sodium metal (300 mg.) was dissolved in liq. NH_3 (150 ml.) at −48～−50°. To this blue colored solution was added a solution of O,O-diethylisoliensinine (IV) (190 mg.) in anhyd. Et_2O (20 ml.) with stirring. After 2 hr., the excess of Na was destroyed with NH_4Cl which was added until the blue color had disappeared. The reaction mixture was allowed to stand overnight to evaporate the solvent, and the residue was separated into a non-phenolic and a phenolic base fraction in usual manner. The non-phenolic base fraction (90mg.) was chromatographed on alumina and eluted with benzene to yield a colorless oily base. TLC*[9a,b] 1 spot. NMR signals at 6.20 τ (3H, OCH_3), 8.67, 8.61 τ (6H, two triplets, J=7.0 c.p.s., two $OCH_2\underline{CH_3}$), 7.48 τ (3H, NCH_3). IR spectrum of this non-phenolic base in $CHCl_3$ solution was identical with the authentic sample of D(−)-N-methyl-O,O-diethylcoclaurine (V). It was then converted into the oxalate and recrystallized from EtOH to yield colorless needles, m.p. 177～179°, $[\alpha]_D^{26}$ −113.3° (c=0.75, 50% MeOH, H_2O). *Anal.* Calcd. for $C_{22}H_{29}O_3N \cdot C_2H_2O_4$. C, 64.70; H, 7.01. Found : C, 64.90; H, 7.14. IR spectrum of this oxalate in KBr disk was superimposable with that of D(−)-N-methyl-O,O-diethylcoclaurine (V) oxalate (m.p. 177～178°, $[\alpha]_D^{11}$ −118.6° (50% MeOH, H_2O)).[2]

The phenolic base fraction, after the usual acid-alkali treatment, afforded a colorless oil (65 mg.), $[\alpha]_D^{22}$ −87.7° (c=1.45, MeOH). This was identified as D(−)-1-(*p*-methoxybenzyl)-2-methyl-6-methoxy-1,2,3,4-tetrahydro-7-isoquinolinol (VI) ($[\alpha]_D$ −80.3° (MeOH))[3] by IR ($CHCl_3$) and TLC*[9b] comparisons.

Ullmann Condensation between D(−)-3′-Bromo-O-methylarmepavine (VII) and D(−)-1-(*p*-Methoxybenzyl)-2-methyl-6-methoxy-1,2,3,4-tetrahydro-7-isoquinolinol (VI). (Synthesis of O,O-Dimethylliensinine (III) (O,O-Dimethylisoliensinine))——D(−)-3′-Bromo-O-methylarmepavine (VII)[4] (350 mg.) and D(−)-1-(*p*-methoxybenzyl)-2-methyl-6-methoxy-1,2,3,4-tetrahydro-7-isoquinolinol (VI)[3] (218 mg.) were dissolved in dry pyridine (3 ml.), and Cu powder (330 mg.) and finely powdered anhyd. K_2CO_3 (280 mg.) were added. The mixture was heated with stirring in an oil bath at 150° under N_2 stream and additional portions of pyridine (1 ml.), Cu powder (50 mg.) and K_2CO_3 (50 mg.) were added every 5 hr. Moreover after 16 hr. from the begining of the reaction, pyridine (1 ml.), K_2CO_3 (50 mg.), and Cu powder (50 mg.) were added and temparature of the oil bath was then raised to 180～185°. After 2 hr., the reaction mixture was dissolved in 50 ml. of CH_2Cl_2, filtered and evaporated to dryness under red. pressure. The residue was dissolved in 10% AcOH and washed with Et_2O. The acidic solution was made alkaline with NH_4OH and extracted with Et_2O. The Et_2O extract was washed with 5% NaOH and then with H_2O, dried over anhyd. K_2CO_3, and evaporated to yield a oily product (400 mg.), which was chromatographed on alumina (7×150 cm.). The first benzene eluate gave a small amount of the recovered VII. Continued eluation with benzene-Et_2O (4:1) afforded a colorless oily base (60 mg.). TLC*[9a,b] 1 spot. The styphnate was prepared as usual and recrystallized from EtOH. Yellow needles, m.p. 133～135°, $[\alpha]_D^{24}$ −76.9° (c=0.78, Me_2CO). *Anal.* Calcd. for $C_{39}H_{46}O_6N_2 \cdot 2C_6H_3O_8N_3 \cdot C_2H_5OH$ (ethanol adduct). C, 55.02; H, 4.93. Found : C, 54.74, 55.05; H, 5.14, 5.08·

The authors wish to thank Professor Tzeng Kuan-fong, Professor Kao Yee-sheng, and Dr, Chou Yun-lee of Institute of Materia Medica Academic Sinica, Shanghai for supplying the authentic samples of liensinine and its perchlorate.

Thanks are also due to Dr. K. Konobu and his coworkers of this Faculty for the microanalyses.

Summary

Isoliensinine, a new phenolic biscoclaurine type alkaloid, was isolated from Formosan "Lien Tze Hsin," loti embryo (embryo of the seed of *Nelumbo nucifera* GAERTN., Fam. Nymphaeaceae) and its structure was assigned to the formula II on the basis of the cleavage reaction by sodium in liq. ammonia of its O,O-diethyl ether (IV) and of the synthesis of its O,O-dimethyl ether (III).

(Received September 30, 1964)

[Chem. Pharm. Bull. 13(1) 43 ~ 49 (1965)]

UDC 547.918.02 : 615.711.5

8. **Takayuki Wada** : Structure of Digiprolactone.*1

(*Shionogi Research Laboratory, Shionogi & Co., Ltd.**2)

Digiprolactone is a component of *Digitalis purpurea* L., (Scrophulariaceae).[1~3] As is well known, this plant contains the cardiac glycosides together with the glycosides of some pregnane modifications. Digiprolactone (I), colorless needles, m.p. 149~151°, $[\alpha]_D$ −100.5° (from acetone-petroleum ether) was obtained from the mother liquor of diginin[4] and digifolein,[5] which belong to the latter group of glycosides. The molecular formula of I, $C_{11}H_{16}O_3$, was determined on the basis of elemental analysis and vapour pressure osmometry. The presence of a secondary hydroxyl group in I was deduced from the following data : Infrared bands of I at 3580 cm^{-1} and 3440 cm^{-1}; formation of monoacetate (II) of I, m.p. 86.5°, $[\alpha]_D$ −68.5°, with pyridine acetic anhydride; reversion of II by hydrolysis with sodium carbonate to I; formation of a six membered ring ketone (III), m.p. 101.5°, $[\alpha]_D$ −162.4°, IR : ν_{max} 1715 cm^{-1}, by oxidation of I with Jones reagent (oxime of III, (IV), m.p. 156~159°); and reduction of III with sodium borohydride to I.

The nuclear magnetic resonance spectra of I, II and III show three singlet signals due to three methyl groups. Infrared peaks[6] of I at 1741 cm^{-1} and 1632 cm^{-1} and ultraviolet absorption maximum of I at 214 mμ (log ε 4.15) infer the presence of an α,β-butenolide ring. The presence of a lactone group in digiprolactone was confirmed by hydrolysis and relactonisation of its dihydroderivative (V), m.p. 85.5~86°, $[\alpha]_D$ +10.6°,

---

*1 This paper is the Part XXII of "Studies on Digitalis Glycosides" by Daisuke Satoh (Part XXI, T. Wada, D. Satoh : This Bulletin, in press.

*2 Fukushima-ku, Osaka (和田敬之).

1) D. Satoh, H. Ishii, Y. Oyama, T. Wada, T. Okumura : This Bulletin, **4**, 284 (1956).
2) T. Wada, D. Satoh : This Bulletin, **12**, 752 (1964).
3) Y. Wada : *Ibid.*, **12**, 1117 (1964).
4) C. W. Shoppee, R. E. Lack, A. V. Robertson : J. Chem. Soc., **1962**, 3610.
5) C. W. Shoppee, R. E. Lack, S. Sternhell : *Ibid* **1963**, 3281.
6) The infrared spectra of I, II, III and IV show weak doublet at 1880 cm^{-1}, instead of singlet at 1780 cm^{-1}, expected for this type of α,β-butenolide rings.