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New Alkaloids from *Isatis indigotica*

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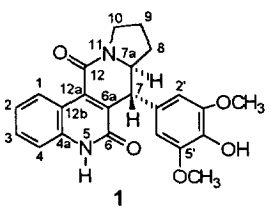
Abstract: From the roots of *Isatis indigotica*, two new alkaloids named isaindigotidione **1** and isaindigotone **2** were isolated. Their structures were elucidated by spectral and X-ray crystallographic analyses. **1** is the first discovered indolizino[7,6-c]quinoline derivative. © 1997 Elsevier Science Ltd.

INTRODUCTION

Isatis indigotica Fort. (Cruciferae) is a biennial herbaceous plant distributed widely in Changjiang River valley. Its roots as a commonly-used traditional Chinese medicine are named "Ban-Lan-Gen" in Chinese. The term "Ban-Lan-Gen" as an official crude drug has been recorded in Chinese Pharmacopeia for long time (see 1985, 1990 and 1995 eds.). The purified extracts of "Ban-Lan-Gen" have been subjected to make various preparations which are popularly used in clinical practice for treatment of influenza, epidemic hepatitis, epidemic encephalitis B, carbuncle, erysipelas etc[1,2]. Chemical investigation of this plant has led to the isolation of indigotin, indirubin, epigotrin, 2-hydroxy-3-butenyl thiocyanate etc.[3,4], among which indirubin has been proven to be an anticancer agent used in the clinic for chronic granulocytic leukemia[5]. Recently, several "Ban-Lan-Gen" injections manufactured by different factories were showed the antiendotoxic effects in experimental studies[6]. In the course of our study, the chloroform and butanol fraction of the root ethanol extracts of this plant were found to be effective for antiendotoxic tests using limulus amebocyte lysate (LAL) method. This result encouraged us to study more detail in its chemical constituents. Previously, we have reported the isolation and antiendotoxic activities of several compounds from the chloroform fraction of the root of same plant[7,8]. This paper describes the isolation and structural elucidation of two new alkaloids named isaindigotidione **1** and isaindigotone **2**, along with seven known compounds, all of which were isolated from this species for the first time.

RESULTS AND DISCUSSION

Isaindigotidione **1** showed positive reaction to Dragendorff reagent and $[M]^+$ peak at m/z 406.1531 in HRMS, corresponding to the molecular formula $C_{23}H_{22}N_2O_5$ (calcd.: 406.1529). The IR spectrum exhibited intense absorptions at 3540 and 3450 (OH and NH), 1655 and 1650 (C=O), 1600 and 1520 cm^{-1} (aromatic ring). The ^{13}C NMR spectrum contained peaks for 23 carbons (Table 1) including two carbonyl, fourteen olefinic (six methine and eight quaternary), two methoxyl, three aliphatic mehtylene and two aliphatic methine carbons. Further studies on its ^1H NMR, ^1H-^1H COSY, ^{13}C NMR and HMBC spectra (Table 1 and Fig. 1) indicated the presence of an *ortho*-substituted phenyl (δ8.76 *d*, 7.19 *t*, 7.47 *t*, 7.31 *d*, each 1H, *J*=7.7Hz), a symmetric 3,5-dimethoxy-4-hydroxy phenyl (δ6.44 *s*, H-2',6'; δ3.67 *s*, OCH_3×2) and a partial structure: -CH_2-CH_2-CH_2-CH-CH-. The calculation of the molecular unsaturation degree revealed that **1** must be a pentacyclic compound. The UV spectrum of **1** was colse to 2-quinolinone derivatives[9]. All the above consideration led us to propose **1** as 7-(3',5'-dimethoxy-4'-hydroxyphenyl)-7a,8,9,10-tetrahydroindolizino[7,6-c]quinoline-6,12(5*H*,7*H*)-dione.



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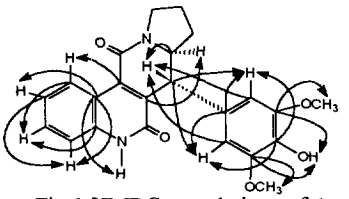

Fig.1 HMBC correlations of **1**.

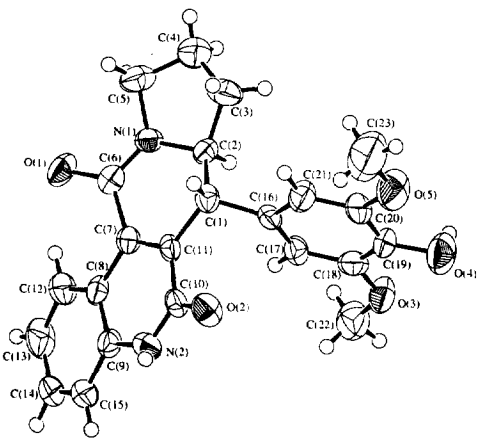

Fig. 2 Atom numbering scheme and solid-state conformation of **1**.

Table 1. NMR Data of **1** (DMSO-d_6)

Position	δ_C (ppm)	δ_H (ppm)		*J* (Hz)
1	127.6	8.76	*d*	7.7
2	121.4	7.19	*t*	7.7
3	129.5	7.47	*t*	7.7
4	114.9	7.31	*d*	7.7
4a	138.5			
NH		11.7	*s*	
6	160.3*			
6a	135.6			
7	48.5	3.89	*d*	12.2
7a	61.6	3.83	*m*	
8	32.0	1.86, 1.79	*m*	
9	22.1	1.94, 1.72	*m*	
10	45.7	3.68, 3.56	*m*	
12	159.3*			
12a	135.3			
12b	116.6			
1'	132.1			
2'	105.2	6.44	*s*	
3'	147.6			
4'	134.0			
5'	147.6			
6'	105.2	6.44	*s*	
OCH_3 × 2	56.0	3.67	*s*	
OH		8.10	*s*	

*may be interchangeable

In order to confirm the structure and determine its configuration, a single-crystal X-ray crystallographic analysis of the $HClO_4$ salt of **1** was carried out. A view of the solid-state conformation is provided in Fig. 2. Bond lengths are in accord with expectations. From the above evidence, **1** was determined to be a new alkaloid and named as isaindigotidione. The assignments of the ^1H and ^{13}C NMR signals (Table 1) were based on ^1H-^1HCOSY, ^1H-^{13}CCOSY and HMBC spectra. Literature investigation revealed that, in all known compounds, its quinoline and indolizine were fused along [1,2-*b*], [1,2-*c*], [2,3-*b*] and [2,3-*g*] forming indolizinoquinoline structures. Thus **1** was considered to be a novel derivative of indolizino[7,6-*c*]quinoline found in natural and synthetic products for the first time.

Isaindigotone **2** showed positive reaction to Dragendorff reagent. Its molecular formula was assigned as $C_{20}H_{18}N_2O_4$ by HRMS. Its IR spectrum revealed the presence of hydroxyl group (3400 cm^{-1}), conjugated carbonyl (1650cm^{-1}) and aromatic ring (1580, 1510cm^{-1}). The ^{13}C NMR spectrum contained peaks for 20 carbons (Table 2) including one carbonyl, fifteen olefinic (seven methine and eight quaternary), two methoxyl and two methylene carbons. According to the results from its ^1H NMR (Table 2), ^1H-^1H COSY, ^{13}C NMR and ^1H-^{13}C COSY spectra, following structural parts may be deduced: one *ortho*-substituted phenyl (δ7.70 *d*, 7.81 *t*, 7.47 *t*, 8.13 *d*, each 1H, *J*=7.8Hz), one symmetric 3,5-dimethoxy-4-hydroxyphenyl (δ6.95 *s*, H-2',6'; 3.85 *s*, OCH_3×2) and -CH_2-CH_2-. The two nitrogens in **2** were both considered as tertiary by ^1H and ^{13}C NMR data. The calculation of the molecular unsaturated degree revealed that **2** must be a tetracyclic compound. All the above consideration led us to propose **2** as a 3-arylidene-2,3-dihydro-pyrolo[2,1-*b*]quinazolin-9-(1*H*)-one derivative. On irradiation of the proton at δ7.74 (1H, *br s*), only enhancement of the equivalent protons at δ6.95 (2.10%) was observed, thus supporting the (*E*)-configuration of the double bond between C_3 and C_7.

To confirm the structure and its configuration, a single-crystal X-ray crystallographic analysis of **2** was carried out. A view of the solid-state conformation is provided in Fig. 3. Bond lengths are in accord with expectations. From the above evidence, **2** was determined to be (*E*)-3-[(3',5'-dimethoxy-4'-hydroxyphenyl)-methylene]-2,3-dihydro-pyrrolo[2,1-*b*]quinazolin-9-(1*H*)-one, a new alkaloid named as isaindigotone. The assignments of the ^1H and ^{13}C NMR signals of **2** were based on ^1H-^1H COSY, ^1H-^{13}C COSY and HMBC experiment.

Besides two new alkaloids **1** and **2**, other isolated compounds from the plant were identified as emodin[10], deoxyvasicinone[11], pyrophaeophorbide a[12], guanine[13], emodin-8-O-β-D-glucoside[14], homovitexin[15] and linarin[16] on the basis of spectral evidences and comparison of physical data with literature values. All of them were isolated from this species for the first time.

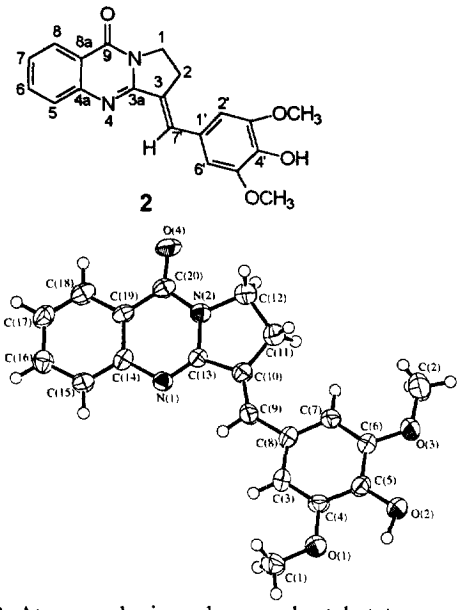

Fig. 3 Atom numbering scheme and solid-state conformation of **2**.

Table 2. NMR Data of **2** (DMSO-d_6)

Position	δ_C (ppm)	δ_H (ppm)	J (Hz)
1	44.3	4.20 *t*	7.1
2	24.9	3.30 *m*	
3	129.0		
3a	156.3		
4a	149.0		
5	126.5	7.70 *d*	7.8
6	134.4	7.81 *t*	7.8
7	125.8	7.47 *t*	7.8
8	125.8	8.13 *d*	7.8
8a	120.3		
9	160.2		
1'	125.8		
2'	107.8	6.95 *s*	
3'	148.0		
4'	137.3		
5'	148.0		
6'	107.8	6.95 *s*	
7'	136.6	7.74 *bs*	
$OCH_3 \times 2$	56.1	3.85 *s*	

MATERIALS AND METHODS

General procedures. Melting points were determined on a Kofler apparatus and uncorrected. Optical rotations were measured on a JASCO DIP-181 polarimeter and UV spectra on a Shimadzu UV-240 instrument. IR spectra were obtained on a Perkin-Elmer 599B spectrometer using KBr discs. ^1H and ^{13}C NMR, ^1H-^1HCOSY and HMBC spectra were recorded on a Bruker AM-400 spectrometer. Chemical shifts (δ) are expressed in ppm with reference to TMS. Mass spectra were recorded with a MAT-711 mass spectrometer using an electron impact ion source (70 eV). X-ray crystallographic measurements were made on a Rigaku AFC7R diffractometer with graphite monochromatized Mo-Kα radiation (λ=0.71073Å). 200-300 mesh silica gel was used for CC and silica gel GF_{254} for TLC (Qingdao Marine Chemical Factory).

Plant material. The roots of *I. indigotica* Fort. were collected in Feb. 1993 from Liaoning Province, China and identified by Prof. Zhiwei Wang of Shanghai Medical University. The voucher specimen (No. 930201) was deposited in the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and isolation. Powdered air-dried roots of *I. indigotica* (5 kg) were extracted three times with 95% EtOH (3×20 L) at room temperature by percolation. The solutions were combined and concentrated at reduced pressure. A suspension of the ethanol extracts in water was extracted with petroleum ether, chloroform, ethyl acetate and n-butanol three times successively. After evaporation, the chloroform extracts (100 g) were chromatographed on silica gel (8×140 cm, 2 kg) eluted with $CHCl_3$ and increasing amounts of MeOH (210 fractions of 1000 ml). Fractions with similar R_f (TLC) were evaporated and combined to give 25 fractions. Each fraction was subjected to repeated CC on silica gel eluted with petroleum / EtOAc or MeOH / $CHCl_3$ to give compounds **2** (18 mg), **3** (80 mg), **4** (71 mg) and **5** (53 mg). After evaporation, the n-butanol extracts (120 g) were chromatographed on Diaion HP-20 with an increasing amount of EtOH in H_2O. The 70%EtOH and 95% EtOH eluate fractions were combined and subjected to CC on silica gel with MeOH-H_2O to provide **1** (450 mg), **6** (700 mg), **7** (210 mg), **8** (9 mg) and **9** (16 mg).

Isaindigotidione (**1**). mp: 240~242°C. $[\alpha]_D^{20}$: +90.9° (DMSO; *c* 0.047), UV λ_{max} (EtOH) nm (logε): 209 (4.92), 235 (4.53) (sh), 265 (4.02), 278 (3.95) (sh), 355 (3.89), IR ν_{max} (KBr) cm^{-1}: 3540, 3450 (br), 3200~2920 (br), 1655, 1650, 1600, 1520, 1430, 1210, 1120, 840, 760, 640. EIMS 70ev m/z: 406 (M^+, 66), 337 (100). 322 (61), 305 (32), 294 (9), 277(16), 252 (15), Anal. Calcd. for $C_{23}H_{22}N_2O_5$: C,67.95, H,5.46, N,6.90, Found: C,67.78, H,5.63, N,6.77. HRMS: 406.1531 (M^+); $C_{23}H_{22}N_2O_5$ required: 406.1529. ^1H NMR and ^{13}C NMR data see Table 1.

X-Ray Analysis of 1. Crystal data: $[(C_{23}H_{22}N_2O_5)^+ClO_4^- 2H_2O]$, MW = 541.92. , triclinic, space group *P*1 (No. 2), a = 7.951(2), b = 12.231(2), c = 13.568(3) Å, V = 1235.8(5) $Å^3$, Z = 2, D_c = 1.456 g/cm^3, μ (Mo-Kα radiation, λ = 0.71073 Å) = 2.19 cm^{-1}. Crystal dimensions 0.20 × 0.10 × 0.35 mm^3. The intensity was recorded with a Rigaku AFC7R diffractometer. From a total of 17193 measurements, those 2193 reflections with $I > 3\sigma(I)$ were retained for the analysis. The crystal structure was solved by direct methods (*SIR92*)[17] and expanded by Fourier method and refined by full-matrix least-squares using the software package *TeXsan*[18] on a Silicon Graphics Indy computer. The non-hydrogen atoms were refined anisotropically. Full-matrix least-squares refinement was stopped at R = 0.139 and R_w = 0.199 (GOF = 6.34). The high R-factor was due to the low crystallinity of the crystals despite repeated recrystallization attempts. Atom coordinates for this structure have been stored at the Cambridge Crystallographic Data Centre.

Isaindigotone (**2**). yellow prisms, m.p. 247~248°C. $[\alpha]_D^{20}$: -5.0° (EtOH; *c* 0.017), UV λ_{max} (EtOH) nm (logε): 210 (3.30), 228 (3.00) (sh), 365 (3.22). IR ν_{max} (KBr) cm^{-1}: 3400 (br), 1650, 1580, 1510, 1480, 1110, 780. EIMS 70ev m/z: 350 (M^+, 100), 349 (39), 335 (12), 319 (6). HRMS: 350.1266 (M^+); $C_{20}H_{18}N_2O_4$ required: 350.1267. ^1H NMR and ^{13}C NMR data see Table 2.

X-Ray Analysis of 2. Crystal data: $C_{20}H_{18}N_2O_4$, MW = 350.37, orthorhombic, space group A*ba*2 (No. 41), a = 19.731(7), b = 9.806(5), c = 17.311(6) Å, V = 3349(4) Å^3, Z = 8, D_c = 1.390 g/cm^3, μ (Mo-Kα radiation, λ = 0.71073 Å) = 0.98 cm^{-1}. Crystal dimensions 0.20 × 0.15 × 0.25 mm^3. The intensity was recorded with a Rigaku AFC7R diffractometer. From a total of 1422 measurements, those 887 reflections with $I > 3\ \sigma\ (I)$ were retained for the analysis. The crystal structure was solved by direct methods (*SAPI*91)[19] and refined by full-matrix least squares using the software package *TeXsan*[18] on a Silicon Graphics Indy computer. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were not refined. The final cycle of full-matrix least-squares refinement converged at R = 0.034 and R_w = 0.038 (GOF = 1.40). Atom coordinates for this structure have been stored at the Cambridge Crystallographic Data Centre.

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