Two *p*-Quinonoid Aporphine Alkaloids from *Fissistigma balansae*

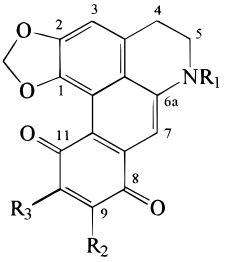
Yi-Chen Chia,[†,‡] Fang-Rong Chang,[‡] and Yang-Chang Wu*[,‡]

Graduate Institute of Natural Products, Kaohsiung Medical College Kaohsiung 807, Taiwan, and Tajen Junior College of Pharmacy, Ping Tung Hsien 900, Taiwan

Received May 15, 1998

Investigation of the ethanolic extract from the twigs of *Fissistigma balansae* resulted in the isolation of two new *p*-quinonoid aporphine alkaloids, named fissilandione (**1**) and norfissilandione (**2**). The structures of **1** and **2** were elucidated on the basis of spectral data and confirmed by chemical conversions.

A series of studies on *Fissistigma* species has been reported by our laboratories.[1−5] In these previous papers, such species were found to contain alkaloids, and a number of flavonoids were reported by Shang et al.[6,7] *Fissistigma balansae* (A. DC.) Merr. (Annonaceae) is a climbing shrub and is found in the southern part of the People's Republic of China and in Vietnam.[8] In this paper, we report the isolation and characterization of two new *p*-quinonoid aporphine alkaloids, named fissilandione (**1**) and norfissilandione (**2**), from the twigs of *F. balansae*.



1	R_1=CH_3	R_2=OCH_3	R_3=H
2	R_1=H	R_2=OCH_3	R_3=H
3	R_1=CH_3	R_2=H	R_3=OCH_3

Compound **1** was obtained as a violet amorphous solid. Its molecular formula was established as $C_{19}H_{15}NO_5$ by HREIMS (found *m*/*z* 337.0948, calcd 337.0950). The UV spectrum exhibited absorptions at 220, 284, 306, 333, and 590 nm, which corresponded to a *p*-quinonoid type of aporphine nucleus.[9−11] The IR spectrum showed a carbonyl absorption at 1670 cm^{-1}. The ^1H NMR spectrum of **1** revealed three singlets for aryl protons at δ 7.00, 6.94, and 6.05, one methylenedioxy singlet at δ 6.14, one methoxyl signal at δ 3.85, one *N*-methyl signal at δ 3.23, and two triplets for two vicinally coupled methylenes at δ 3.52 and 3.18 (J = 6.4 Hz). These data were very similar to those reported for the *p*-quinonoid, bulbodione (**3**), isolated from *Corydalis bulbosa* and prepared from bulbocapnine by oxidation with Fremy's salt.[9] To delineate the differences between **1** and **3**, bulbocapnine (isolated from *Illigera luzonensis*)[12] was treated with Fremy's salt to afford **3**.

The ^{13}C NMR and DEPT experiments on **1** and **3** showed 19 resonance lines attributable to two methyl groups, three methylenes, three methines, and 11 quaternary carbons (including two carbonyl signals; δ 181.9 and 183.5 for **1** and δ 177.2 and 186.3 for **3**). The NOESY spectra of **1** and **3** showed the same correlations between H-3/H-4, H-4/H-5, H-5/*N*-methyl, and *N*-methyl/H-7, as well as between CH_3O-9 and H-10 (Figure 1).

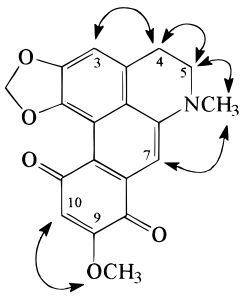


Figure 1. Key NOESY interactions observed for **1**.

Table 1. ^1H and ^{13}C NMR Spectral Data (δ/ppm) for Compounds **1**−**3** in $CDCl_3$

	^1H (mult) (Hz)			^{13}C (mult)		
position	**1**	**2**	**3**	**1**	**2**	**3**
1				141.6 s	141.4 s	141.5 s
2				147.2 s	147.6 s	147.6 s
3	6.94 s	6.96 s	6.91 s	109.2 d	109.7 d	108.6 d
3a				127.9 s	128.3 s	127.9 s
3b				119.7 s	118.8 s	119.0 s
4	3.18 t (6.4)	3.15 t (6.4)	3.17 t (6.4)	29.7 t	29.7 t	29.6 t
5	3.52 t (6.4)	3.55 t (6.4)	3.55 t (6.4)	50.2 t	40.6 t	50.3 t
Me-6	3.23 s		3.26 s	40.4 q		40.3 q
6a				150.3 s	149.6 s	151.2 s
7	7.00 s	7.00 s	7.02 s	98.0 d	100.6 d	98.6 d
7a				133.9 s	133.9 s	136.2 s
8				181.9 s	181.6 s	186.2 s
9			5.95 s	157.2 s	157.1 s	105.5 d
10	6.05 s	6.05 s		111.4 d	111.6 d	163.3 s
11				183.5 s	183.5 s	177.2 s
11a				116.2 s	116.9 s	114.7 s
11b				118.2 s	117.4 s	117.1 s
OMe-9	3.85 s	3.85 s		55.9 q	56.0 q	
OMe-10			3.89 s			56.3 q
OCH_2O	6.14 s	6.14 s	6.13 s	101.0 t	101.0 t	101.2 t

To completely assign and confirm the structure of **1**, in comparison to **3**, the HETCOR and HMBC techniques were used. The H-7 signal of **3** correlated to C-8 (δ 186.3), C-7a (δ 136.2), C-3b (δ 119.0), and C-11a (δ 114.7), and the H-9 signal at δ 5.95 correlated to C-8 (δ 186.3), C-11 (δ 177.2), C-10 (δ 163.3), and C-7a (δ 136.2), respectively. In turn, the H-7 aromatic proton of **1** at δ 7.00 correlated to C-8 (δ 181.9), C-7a (δ 133.9), C-3b (δ 119.7), and C-11a (δ 116.2). The H-10 resonance at δ 6.05 correlated to C-11 (δ 183.5), C-8 (δ 181.9), C-9 (δ 157.2), and C-11a (δ 116.2), indicating

* To whom correspondence should be addressed. Tel.: 886-7-3121101 ext 2197. Fax: 886-7-3114773. E-mail: yachwu@cc.kmc.edu.tw.
† Tajen Junior College.
‡ Kaohsiung Medical College.

10.1021/np980197x CCC: $15.00
Published on Web 10/15/1998

Table 2. HMBC Data for Compounds 1 and 3 in $CDCl_3$

	HMBC of **1** (J = 9 Hz)		HMBC of **3** (J = 9 Hz)	
position	δ_H	δ_C	δ_H	δ_C
3	6.94	147.2 (C-2), 141.6 (C-1), 119.7 (C-3b), 29.7 (C-4)	6.91	147.6 (C-2), 141.5 (C-1), 119.0 (C-3b), 29.6 (C-4)
4	3.18	127.9 (C-3a), 119.7 (C-3b), 109.2 (C-3), 50.2 (C-5)	3.17	127.9 (C-3a), 119.0 (C-3b), 108.6 (C-3), 50.3 (C-5)
5	3.52	150.3 (C-6a), 127.9 (C-3a), 29.7 (C-4)	3.55	151.2 (C-6a), 127.9 (C-3a), 29.6 (C-4)
Me-6	3.23	150.3 (C-6a), 50.2 (C-5)	3.26	151.2 (C-6a), 50.3 (C-5)
7	7.00	181.9 (C-8), 119.7 (C-3b), 116.2 (C-11a)	7.02	186.3 (C-8), 136.2 (C-7a), 119.0 (C-3b), 114.7 (C-11a)
9			5.95	186.3 (C-8), 177.2 (C-11), 163.3 (C-10), 136.2 (C-7a)
10	6.05	183.5 (C-11), 181.9 (C-8), 157.2 (C-9), 116.2 (C-11a)		
OMe-9	3.85	157.2 (C-9)		
OMe-10			3.89	163.3 (C-10)
OCH_2O	6.14	147.2 (C-2), 141.6 (C-1)	6.13	147.6 (C-2), 141.5 (C-1)

that these two carbonyl carbons in the D ring were located at the para position and that the methoxyl was situated at the C-9 position, respectively.

Although the spectral data supported the differences proposed between **1** and **3**, chemical evidence for the structure of **1** was made by simple chemical conversion of the alkaloid by means of Fremy's radical oxidation. When (+)-*N*-methylfissoldine[1] was oxidized with Fremy's radical (an excellent synthetic method for *p*-benzoquinones),[13] a product was obtained spectrally and chromatographically identical with **1**. On the basis of the NOESY, DEPT, HETCOR, and HMBC data, the ^1H and ^{13}C NMR assignments for **1** and **3** are listed (Tables 1 and 2). From the above discussion, the new compound fissilandione (**1**) was elucidated as 1,2-(methylenedioxy)-9-methoxy-8,11-dioxo-*N*-methyl-6a,7-dehydroaporphine.

Compound **2** was also isolated as a violet amorphous solid. Its HREIMS gave an $[M]^+$ ion at *m/z* 323.0793, which corresponded to the molecular formula $C_{18}H_{13}NO_5$ (calcd 323.0794). The UV spectrum exhibited absorptions at 218, 283, 300, 335, and 591 nm, typical of a compound with a *p*-quinonoid aporphine nucleus.[9−11] The ^1H NMR spectrum of **2** showed three singlets for aryl protons at δ 7.00, 6.96, and 6.05, one methylenedioxy at δ 6.14, one methoxyl at δ 3.85, and two triplets for two vicinally coupled methylenes at δ 3.55 and 3.15 (J = 6.4 Hz). Comparison of the ^1H NMR spectra of **2** and **1** (Table 1) showed the same patterns except for the absence of the *N*-methyl group. Fremy's radical oxidation of fissoldine[1] gave norfissilandione (**2**), which was assigned as 1,2-(methylenedioxy)-9-methoxy-8,11-dioxo-6a,7-dehydroaporphine.

Experimental Section

General Experimental Procedures. Melting points were determined using a Mel-Temp II apparatus and are uncorrected. The UV spectra were obtained on a Hitachi 200-20 spectrophotometer, and IR spectra were measured on a Hitachi 260-30 spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded with a Varian NMR spectrometer at 400 MHz (Unity Plus), in $CDCl_3$ using TMS as internal standard, with 2D NMR spectra recorded using the Varian standard pulse program. In the HMBC experiment, Δ = 1 s and J = 145, 9 Hz, respectively. EIMS were obtained with a JEOL JMS-SX/SX 102A mass spectrometer and a Quattro GC/MS spectrometer at 70 eV. Silica gel 60 (Macherey-Nagel and Merck), active charcoal (Wako), and Sephadex LH-20 (Pharmacia) were used for chromatographic columns, and precoated silica gel plates (Macherey-Nagel, SIL G-25 UV_{254}, 0.25 mm; aluminum) were used for analytical TLC. Similarly, precoated silica gel plates (Macherey-Nagel, SIL G/UV_{254}, 0.25 mm; glass) were used for preparative TLC.

Plant Material. The twigs of *F. balansae* were collected from Xishuangbanna, Kunming, the southern part of Yunnan Province, People's Republic of China, in May 1995. A voucher specimen is deposited in the Laboratory of Phytochemistry, Kunming Institute of Botany, Academia Sinica, Kunming.

Extraction and Isolation. The twigs of *F. balansae* (2.14 kg) were extracted repeatedly with EtOH at room temperature and evaporated under reduced pressure. Then, the EtOH residue (144.5 g) was first partitioned between $CHCl_3$ and H_2O and then the H_2O partitioned with *n*-BuOH, to yield $CHCl_3$, n-BuOH and H_2O extracts, respectively. The $CHCl_3$ solution was extracted with 3% HCl to give a neutral $CHCl_3$ layer and an acidic aqueous solution. The latter was basified with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$-soluble fraction gave a positive alkaloidal test with Dragendorff's reagent. The crude alkaloidal fraction was chromatographed over silica gel 60 (Merck, 230−400 mesh) and eluted with $CHCl_3$/EtOAc/MeOH mixtures of increasing polarity to give 19 fractions. Fraction 7, eluted with $CHCl_3$/MeOH (10:1), was further separated and purified by silica gel column chromatography and preparative TLC (silica gel, $CHCl_3$/MeOH, 10:0.5) to give fissilandione (**1**) (4.5 mg) and norfissilandione (**2**) (6 mg), respectively.

Fissilandione (1) was obtained as a violet amorphous solid ($CHCl_3$): mp 250−252 °C; UV (EtOH) λ_{max} (log ϵ) 220 (4.40), 284 (sh) (4.07), 306 (4.15), 333 (3.96), 590 (3.30) nm; IR (neat) ν_{max} 1670, 1620, 1590, 1520, 1450, 1305, 1225, 1120, 1050, 920 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; EIMS *m/z* $[M]^+$ 337 (100), 294 (19), 266 (58), 238 (15), 208 (9), 155 (20); HREIMS *m/z* 337.0948 (calcd for $C_{19}H_{15}NO_5$ 337.0950).

Norfissilandione (2) was isolated as a violet amorphous solid ($CHCl_3$): mp 258−261 °C; UV (EtOH) λ_{max} (log ϵ) 218 (4.57), 283 (4.06), 300 (4.20), 335 (3.98), 591 (3.30) nm; IR (neat) ν_{max} 1670, 1622, 1600, 1525, 1450, 1310, 1225, 1120, 1045, 920 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; EIMS *m/z* $[M]^+$ 323 (100), 280 (19), 252 (86), 224 (31), 208 (9), 139 (28); HREIMS *m/z* 323.0793 (calcd for $C_{18}H_{13}NO_5$ 323.0794).

Preparation of *p*-Quinonoids by Fremy's Radical Oxidation.[9,13] *N*-Methylfissoldine (20 mg) was reacted with Fremy's salt (Aldrich Chemical Co., Inc.). After 10 min, the reaction was quenched, and then **1** (6.5 mg) was obtained by preparative TLC. Fissoldine (20 mg) and bulbocapnine (20 mg) were treated with Fremy's salt in the same way to yield **2** (7 mg) and **3** (6 mg), respectively.

Acknowledgment. This investigation was supported by a grant from the National Science Council of the Republic of China (NSC 87-2314-B-037-017) awarded to F.-R. Chang.

References and Notes

(1) Lu, S. T.; Wu, Y. C. *Heterocycles* **1983**, *20*, 813−815.
(2) Lu, S. T.; Wu, Y. C.; Leou, S. P. *Phytochemistry* **1985**, *24*, 1829−1834.
(3) Wu, Y. C.; Lu, S. T.; Wu, T. S.; Lee, K. H. *Heterocycles* **1987**, *26*, 9−12.
(4) Wu, Y. C.; Kao, S. C.; Huang, J. F.; Duh, C. Y.; Lu, S. T. *Phytochemistry* **1990**, *29*, 2387−2388.

(5) Chia, Y. C.; Chang, F. R.; Li, C. M.; Wu, Y. C. *Phytochemistry* **1998**, *48*, 367−369.
(6) Shang, L.; Zhao, B.; Hao, X. *Yunnan Zhiwu Yanjiu* **1994**, *16*, 191−195.
(7) Hao, X.; Lu, Y.; Shang, L.; Zheng, Q. *Yunnan Zhiwu Yanjiu* **1995**, *5*, 143−145.
(8) Wu, C. Y.; Chen, C. *Flora Yunnanica*; Institutum Botanicum Kunmingense Academiae Sinicae, Ed.; Science Press: Beijing, People's Republic of China, 1991; Vol. 5, p 53.
(9) Kiryakov, H. G.; Iskrenova, E. S. *Planta Med.* **1984**, *50*, 136−138.
(10) Chen, I. S.; Chen, J. J.; Tsai, I. L.; Chang, Y. L.; Teng, C. M. *Planta Med.* **1995**, *61*, 537−539.
(11) Lee, S. S.; Chen, C. K.; Chen, I. S.; Chen, C. H. *J. Nat. Prod.* **1996**, *59*, 55−58.
(12) Chen, K. S.; Wu, Y. C.; Teng, C. M.; Ko, F. N.; Wu, T. S. *J. Nat. Prod.* **1997**, *60*, 645−647.
(13) Zimmer, H.; Lankin, D. C.; Horgan, S. W. *Chem. Rev.* **1971**, *71*, 229−246.

NP980197X