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PROTOBERBERINE ALKALOIDS FROM *FISSISTIGMA BALANSAE*

YI-CHEN CHIA, FANG-RONG CHANG, CHAO-MING LI† and YANG-CHANG WU*

Graduate Institute of Natural Products, Kaohsiung Medical College, Kaohsiung 807, Taiwan, Republic of China; † Laboratory of Phytochemistry, Kuming Institute of Botany, Academia Sinica, Kuming 650204, People's Republic of China

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Key Word Index—*Fissistigma balansae*; Annonaceae; twigs; protoberberine; fissisaine.

Abstract—Chromatography of the ethanolic extracts from the twigs of *Fissistigma balansae* led to the isolation of a new protoberberine alkaloid, fissisaine, along with four known protoberberine alkaloids thaipetaline, kikemanine, columbamine and dehydrodiscretamine. The structures of these isolates were established by means of mass and related spectral experiments. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

A series of studies on Formosan *Fissistigma* species has been reported by our laboratories [1–4] and Wu *et al.* [5]. In these previous papers, such species were found to contain many alkaloids, including two tetrahydroprotoberberines, discretamine and tetrahydropalmatine, seven aporphines, asimilobine, norannuradhapurine, crebanine, calycinine, anolobine, xylopine and anonaine, three oxoaporphines, kuafumine, oxocrebanine and liriodenine, five phenanthrenes, fissicesine, fissicesine *N*-oxide, atherosperminine, *N*-noratherosperminine and *N*-methylatherosperminium and four morphinandienones, *O*-methyl-moschatoline, *N*-methyl-2,3,6-trimethoxymorphinandien-7-one, *N*-nor-2,3,6-trimethoxy-morphinandien-7-one and *O*-methyl-flavinantine. Some flavonoids were also found by Shang *et al.* [6, 7].

Fissistigma balansae is a climbing shrub found in the southern part of Yunnan in China and also in Vietnam [8]. To our knowledge, there is no information about the constituents of this species. In this paper, we report the isolation and characterization of a new protoberberine alkaloid, fissisaine (**1**), along with four known protoberberine alkaloids, thaipetaline (**2**), kikemanine (**3**), columbamine (**4**) and dehydrodiscretamine (**5**), from the twigs of *F. balansae*.

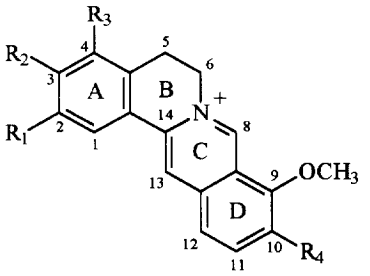
RESULTS AND DISCUSSION

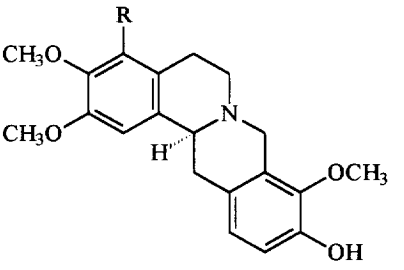
Fissisaine (**1**) was isolated as yellow needles. The HREI mass spectrum gave *m/z* 354.1323 (calcd 354.1341) for the $[M]^+$, corresponding to the molecular formula, $C_{20}H_{20}O_5N^+$. The UV spectrum showed maximal absorptions at 206, 230, 282 and 337 nm, and bathochromic shifts on adding alkali indicating the presence of a typical phenolic berberine-type alkaloid [9]. The IR spectrum showed absorptions at 3400 cm^{-1} for hydroxyl. The ^1H NMR spectrum of **1** revealed two typical signals of the berberine skeleton at δ 9.63 (1H, *s*) and 8.77 (1H, *s*), which were assigned to H-8 and H-13, two coupling methylene groups at δ 3.23 (2H, *t*, $J = 6.8$ Hz) and the down-field shifted signals caused by the quaternary amine at δ 4.88 (2H, *t*, $J = 6.8$ Hz) were assigned to H-5 and H-6, respectively. The other signals including three methoxyl groups at δ 3.89, 4.01 and 4.15 (each 3H, *s*), two *ortho*-coupling aromatic protons at δ 7.77 and 7.90 (each 1H, *d*, $J = 8.8$ Hz) and an isolated singlet at δ 7.29 (1H, *s*) should be located to the A and D rings of berberine skeleton. The complete assignments of the relative configuration of aliphatic and aromatic protons of **1** was established by ^1H-^1H COSY and NOESY (Scheme 1) experiments. Significant correlation between H-5 and H-6, H-8 and C-9 methoxyl, as well as C-3 methoxyl, C-2 methoxyl, H-1, H-13, H-12 and H-11 was observed in the NOESY spectrum.

Although the spectral data supported structure **1** for fissisaine, confirmation was obtained by the preparation of a tetrahydroberberine derivative. Treatment of **1** with sodium borohydride gave a compound that had UV, IR, TLC and ^1H NMR data identical to those of **2**. From the above discussion, the new compound fissisaine (**1**) was elucidated as 4,10-dihydroxyl-2,3,9-trimethoxyl berberine.

The HREI mass spectrum of **2** showed a $[M]^+$ at *m/z* 357.1576 (calcd 357.1576), corresponding to the molecular formula, $C_{20}H_{23}O_5N$. The spectrum exhibited characteristic retro Diels–Alder fragments associated with a tetrahydroprotoberberine skeleton

* Author to whom correspondence should be addressed.





1 $R^1=R^2=OCH^3$ $R^3=R^4=OH$
4 $R^1=OH$ $R^2=R^4=OCH^3$ $R^3=H$
5 $R^1=OCH^3$ $R^2=R^4=OH$ $R_3=H$

2 R=OH
3 R=H

possessing one methoxyl group and one hydroxyl group in the D ring (fragment at *m/z* 150), two methoxyl groups and one hydroxyl group in the A ring (fragment at *m/z* 206) [10–12]. Examination of the ^1H NMR spectrum of **2** revealed that it was closely related to those given for (−)-thaipetaline [12]. To completely assign the three methoxyl groups in the A and D rings, ^1H 2D nOe spectroscopy (NOESY) was taken. This showed a significant correlation between C-3 methoxyl, C-2 methoxyl, H-1, H-14, H-13, H-12 and H-11, as well as H-5, H-6, H-8 and C-9 methoxyl. On the basis of the above results, the three methoxyl groups should be located at C-2 (3.88), C-3 (3.90) and C-9 (3.82) and the other two phenolic hydroxyl groups were positioned at C-4 and C-10, respectively (Scheme 1). The negative rotation of (−)-thaipetaline is consistent with the 14*S*-configuration [9].

To our knowledge, only three 2,3,4,9,10-pentasubstituted tetrahydroprotoberberines have been reported previously: (−)-thaicanine and (−)-*O*-methylthaicanine, both isolated from *Parabaena sagittata* (Menispermaceae) [11] and (−)-thaipetaline, isolated from *Polyalthia stenopetala* (Annonaceae) [12]. It is interesting to note the existence of both (−)-thaipetaline and fissisaine in *F. balansae*, because the 2, 3, 4, 9, 10-pentasubstituted protoberberines are found for the second time in the Annonaceae.

The known compounds, **3–5** were identified as kikemanine, columbamine and dehydrodiscretamine by comparison of their spectral data (UV, IR, mass and NMR) with the literature [13–16], respectively.

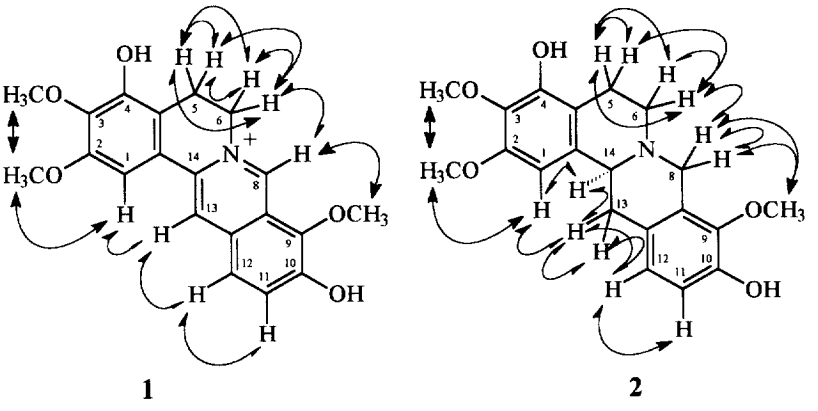
EXPERIMENTAL

Instrumental

UV spectra were obtained in EtOH. ^1H NMR (400 MHz), HETCOR, NOESY and DEPT spectra were obtained on a Varian NMR spectrometer. Low-resolution FABMS and EIMS were recorded using a direct inlet system, Silica gel 60 (Merck, 230–400 mesh) was used for CC and precoated silica gel (Merck, Kieselgel 60 F-254) for TLC. Spots were detected by spraying with Dragendorff's reagent or with 50% H_2SO_4 followed by heating.

Plant material

Twigs of *F. balansae* were collected from southern part of Yunnan, China, in May 1995. A voucher speci-



1 **2**

Scheme 1. Noe interactions observed for compounds **1** and **2**.

men is deposited in the Laboratory of Phytochemistry, Kuming Institute of Botany, Academia Sinica, Kuming 650204, P.R. China.

Extraction and isolation

Twigs (2.14 kg) were extracted repeatedly with EtOH at room temp. and the solvent evapd under red. pres. The EtOH extracts (144.5 g) were then partitioned to yield $CHCl_3$, *n*-BuOH and aq. solns, respectively. The $CHCl_3$ soln was extracted with 3% HCl to give a neutral $CHCl_3$ layer and an acidic aq. soln. The latter was basified with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$-sol. fr. gave a positive alkaloidal test with Dragendorff's reagent. The crude alkaloidal fr. was chromatographed over silica gel 60 and eluted with $CHCl_3$–EtOAc–MeOH mixts of increasing polarity to give 19 frs. Fr. 8 eluted with $CHCl_3$–MeOH (10:1) was further sepd and purified by silica gel CC and prep. TLC (silica gel, $CHCl_3$–MeOH, 10:1) to give thaipetaline (**2**) (5 mg) and kikemanine (**3**) (15 mg), respectively. Fr. 9 eluted with $CHCl_3$–MeOH (9:1) was further sepd and purified by silica gel CC and prep. TLC (silica gel, $CHCl_3$–MeOH–NH_4OH, 100:5:1) to afford columbamine (**4**) (4.5 mg), dehydrodiscretamine (**5**) (4.5 mg) and fissisaine (**1**) (6 mg), respectively.

Fissisaine (**1**). Yellow needles, mp > 300°. IR (Nujol) ν_{max} cm^{-1}: 3400, 1600, 1560, 1500, 1220. UV (EtOH) λ_{max} 206, 230, 282, 337 nm. HREIMS found $[M]^+$ *m/z*: 354.1323 ($C_{20}H_{20}O_5N^-$, calcd 354.1341). FAB-MS *m/z* (rel. int.): 354 $[M]^+$ (13), 176 (23), 154 (100), 137 (78) and 136 (84). ^1H NMR (400 MHz, CD_3OD) δ: 9.63 (1H, *s*, H-8), 8.77 (1H, *s*, H-13), 7.90 (1H, *d*, J = 8.8 Hz, H-12), 7.77 (1H, *d*, J = 8.8 Hz, H-11), 7.29 (1H, *s*, H-1), 4.88 (2H, *t*, J = 6.8 Hz, H-6), 4.15 (3H, *s*, 9-OCH_3), 4.01 (3H, *s*, 2-OCH_3), 3.89 (3H, *s*, 3-OCH_3), 3.23 (2H, *t*, H-5).

Thaipetaline (**2**). Yellow amorphous, mp 229–232°. $[\alpha]_D^{24}$ −145.1° (MeOH; *c* 0.61). UV (EtOH) λ_{max} 208, 282 nm. IR (Nujol) ν_{max} cm^{-1}: 3300, 1610, 1580, 1490, 1240. HREIMS found $[M]^+$ *m/z*: 357.1576 ($C_{20}H_{23}O_5N$, calcd 357.1576). EIMS *m/z* (rel. int.): 357 $[M]^+$ (62), 356 (57), 326 (14), 208 (100), 206 (26), 192 (26), 150 (31), 149 (52). Identified by comparison (UV, IR, EIMS and NMR) with lit. [12].

Kikemanine (**3**). Yellow powder. $[\alpha]_D^{24}$ −196.92° ($CHCl_3$, *c* 0.03), IR (Nujol) ν_{max} cm^{-1}: 3550, 2900, 1600. UV (EtOH) λ_{max} 211, 253, 283 nm. EIMS *m/z* (rel. int.): 341 $[M]^+$ (47), 340 (49), 310 (11), 192 (100), 206 (26), 192 (100), 190 (34), 150 (17), 149 (31), 135 (35). Identified by direct comparison with authentic sample (TLC, UV, IR, EIMS and NMR) and with lit. [10].

Columbamine (**4**). Yellow powder. IR (Nujol) ν_{max} cm^{-1}: 3400, 1605, 1505, 1290, 1240, 1150, 1090. UV (MeOH) λ_{max} 206, 225, 265, 345 nm. EIMS *m/z* (rel. int.): 338 $[M]^+$ (37), 323 (100), 307 (41), 294 (18), 278 (23), 250 (12), 161 (16). Identified by direct comparison with authentic sample (TLC, UV, IR EIMS and NMR) and with lit. [13].

Dehydrodiscretamine (**5**). Red amorphous. IR (Nujol) ν_{max} cm^{-1}: 1605, 1535, 1515, 1325, 1290, 1240. UV (MeOH) λ_{max} 206, 230, 286, 356 nm. EIMS *m/z* (rel. int.): 324 $[M]^+$ (8), 323 (20), 309 (19), 294 (9), 278 (5), 191 (3), 178 (3), 154 (3). Identified by direct comparison with authentic sample (TLC, UV, IR, EIMS and NMR) and with lit. [14–16].

Reduction of fissisaine (**1**). Fissisaine (4.5 mg) in dry MeOH (1 ml) was reacted with $NaBH_4$, with stirring at room temp. for 2 hr. The reaction mixture was partitioned to afford a yellow amorphous compound (3 mg), that was identified by comparison with **2** (co-TLC, UV, IR and NMR).

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