DOI 10.1007/s10600-017-2032-1
Chemistry of Natural Compounds, Vol. 53, No. 3, May, 2017

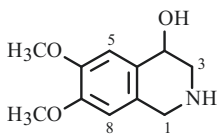
ISOQUINOLINE ALKALOIDS FROM *Michelia fuscata*

H. T. Li,[1] C. L. Kao,[2] C. R. Tsai,[3] W. J. Li,[4*] and C. Y. Chen[5*]

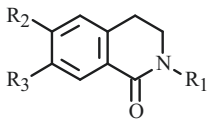
*Two new isoquinoline alkaloids, fuscatine A (**1**) and fuscatine B (**2**), along with 21 compounds including eight isoquinoline alkaloids, northalifoline (**3**), thalifoline (**4**), corydaldine (**5**),* N-*methylcorydaldine (**6**), (6,7-dimethoxyisoquinolinyl)-(4′-methoxyphenyl)-methanone (**7**),(6,7-dimethoxyisoquinolinyl)-(4′-hydroxyphenyl)-methanone (**8**), liriodenine (**9**), and corydine (**10**); two steroids,* β-*sitosterone and stigmasterone; six benzenoids,* p-*hydroxybenzaldehyde,* p-*hydroxybenzoic acid, 3,4-dimethoxybenzoic acid, methylparaben, syringic acid, and coniferyl aldehyde; one quinone 2,6-dimethoxy-*p-*benzoquinone, and one sesquiterpene, caryophyllene oxide, are isolated from the stems of* Michelia fuscata *(Magnoliaceae). These compounds were characterized and identified by physical and spectral analysis.*

Keywords: *Michelia fuscata*, Magnoliaceae, fuscatine, isoquinoline.

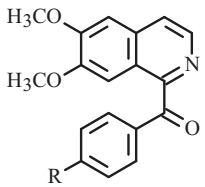
Species belonging to *Michelia* genus are arboreous plants, growing in the temperate zone of oriental India, southern China, Malaysia, and Indonesia. The species more utilized is *Michelia champaca*: its cortex and seeds are used as febrifuge and tonic-aromatic, roots are employed as emmenagogue, leaves as astringent, and gemmae in the treatment of hemorrhage; its flowers and fruits are believed to possess curative properties in enteritis [1]. Less known species, as *Michelia fuscata*, are used as ornamental plants and to obtain essences [1]. *M. fuscata* is an evergreen medium shrub, commonly called banana shrub, because of the heavy, sweet fragrance banana scent of its purple flowers. The plant is also known in Indian folk medicine as a remedy for hypertension [2]. To further understand the chemotaxonomy of the *Michelia* species [3–8], *M. fuscata* was chosen for phytochemical investigation. The compounds derived from the stem include 10 isoquinoline alkaloids, fuscatine A (**1**), fuscatine B (**2**), northalifoline (**3**) [9], thalifoline (**4**) [9], corydaldine (**5**) [10], *N*-methylcorydaldine (**6**) [11], (6,7-dimethoxyisoquinolinyl)-(4′-methoxyphenyl)-methanone (**7**) [12], (6,7-dimethoxyisoquinolinyl)-(4′-hydroxyphenyl)-methanone (**8**) [13], liriodenine (**9**) [14], and corydine (**10**) [14]; two steroids, *β*-sitosterone [14] and stigmasterone [14]; six benzenoids, *p*-hydroxybenzaldehyde [9], *p*-hydroxybenzoic acid [9], 3,4-dimethoxybenzoic acid [9], methylparaben [9], syringic acid [9], and coniferyl aldehyde [9]; one quinone, 2,6-dimethoxy-*p*-benzoquinone [9]; and one sesquiterpene, caryophyllene oxide [15]. In addition to the two new compounds **1** and **2**, all of these compounds were found for the first time from this plant.



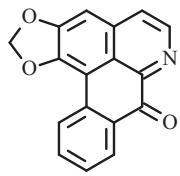
1



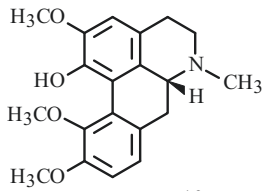
2 - 6



7, 8



9



10

2: $R_1 = R_2 = H$, $R_3 = OCH_3$; **3:** $R_1 = H$, $R_2 = OCH_3$, $R_3 = OH$
4: $R_1 = CH_3$, $R_2 = OCH_3$, $R_3 = OH$; **5:** $R_1 = H$, $R_2 = R_3 = OCH_3$
6: $R_1 = CH_3$, $R_2 = R_3 = OCH_3$; **7:** $R = OCH_3$; **8:** R = OH

1) Department of Medical Laboratory Science and Biotechnology, Fooyin University, Ta-Liao, 83102, Kaohsiung, Taiwan; 2) Tzu Hui Institute of Technology, Pingtung County, Taiwan; 3) Department of Nutrition and Health Science, Fooyin University, Ta-Liao, 83102, Kaohsiung, Taiwan; 4) Department of Nursing, Fooyin University, Ta-Liao, 83102, Kaohsiung, Taiwan, e-mail: mt082@fy.edu.tw; 5) School of Medical and Health Sciences, Fooyin University, Ta-Liao, 83102, Kaohuing, Taiwan, e-mail: xx377@fy.edu.tw. Published in *Khimiya Prirodnykh Soedinenii*, No. 3, May–June, 2017, pp. 428–430. Original article submitted November 3, 2015.

 0009-3130/17/5303-0504

Fuscatine A (**1**) was obtained as yellow needles from CH_2Cl_2. The molecular formula, $C_{11}H_{15}NO_3$, was established by HR-ESI-MS (*m/z* 232.0952 $[M + Na]^+$; calcd 232.0950). The UV spectrum of **1** showed intense absorption bands at 289 and 300 nm, which were typical of a isoquinoline skeleton [16]. The IR spectrum of **1** exhibited absorption bands at ν 3200 cm^{-1}, indicating the hydroxyl group. The ^1H NMR spectrum of **1** showed two methoxy groups at δ 3.92 (3H, s) and 3.97 (3H, s), two aromatic protons at δ 6.68 (1H, s) and 7.32 (1H, s), one methine proton at δ 4.23, and two methylene protons at δ 3.64/3.80 and 3.88 (2H, m), indicating that **1** was probably a 1,2,3,4-tetrahydro-6,7-dimethoxy-4-isoquinolinol. The ^{13}C NMR and DEPT experiments for **1** showed 11 resonance lines consisting of two methyl, two methylenes, three methines, and four quaternary carbons. The structure of **1** was also confirmed by 2D NMR experiments. A COSY correlation was observed between H-3 and H-4. The HETCOR experiment showed that the carbon signals at δ 61.3 for C-1, 70.7 for C-3, 49.4 for C-4, 103.5 for C-5, and 106.0 for C-8 were correlated to the proton signals at δ 3.64/3.80 for H-1, δ 3.88 for H-3, δ 4.23 for H-4, δ 7.32 for H-5, and δ 6.68 for H-8, respectively. Proton 5 displayed significant correlations to 6-OMe and H-4, and H-8 was correlated with 7-OMe and H-1 in the NOESY spectrum. The above results support the structure of **1** as a new alkaloid, 1,2,3,4-tetrahydro-6,7-dimethoxy-4-isoquinolinol, which we name fuscatine A.

Fuscatine B (**2**) was obtained as colorless needles from CH_2Cl_2. The molecular formula, $C_{10}H_{11}NO_2$, was established by HR-ESI-MS (*m/z* 177.0789 $[M]^+$; calcd 177.0790). The UV spectrum of **2** showed intense absorption bands at 213, 261, 274, and 300 nm, which were typical of an isoquinolone skeleton [17]. The IR spectrum of **2** exhibited absorption bands at ν 1685 cm^{-1}, indicating the carbonyl group. The ^1H NMR spectrum of **1** showed one methoxy group at δ 3.96 (3H, s), three ABX aromatic protons at δ 6.97 (1H, d, J = 8.4 Hz, H-5), 7.58 (1H, d, J = 1.6 Hz, H-8), and 7.71 (1H, dd, J = 8.4, 1.6 Hz, H-6), and two methylene protons at δ 2.36 (2H, t, J = 6.8 Hz, H-4) and 3.64 (2H, t, J = 6.8 Hz, H-3), indicating that **2** was probably a methoxy-1,2,3,4-tetrahydroisoquinolone. The structure of **2** was also confirmed by 2D NMR experiments. A COSY correlation was observed between H-3 and H-4 and between H-5 and H-6. Proton 5 displayed significant correlations to H-6 and H-4, and 7-OMe was correlated with H-8 and H-6 in the NOESY spectrum. The above results support the structure of **2** as a new alkaloid, 7-methoxy-1,2,3,4- tetrahydroisoquinolin-1-one, which we name fuscatine B.

EXPERIMENTAL

General. UV spectra were obtained on a Jasco UV-240 spectrophotometer in MeCN. IR spectra were measured on a Hitachi 260-30 spectrophotometer (Hitachi, Tokyo, JP). ^1H NMR (400/500 MHz) and ^{13}C NMR (100 MHz), HSQC, HMBC, COSY, and NOESY spectra were obtained on a Varian (Unity Plus) NMR spectrometer (Varian, CA, USA). For each sample, 128 scans were recorded with the following settings: 0.187 Hz/point; spectral width, 14400 Hz; pulse width, 4.0 μs; relaxation delay, 2s. Low-resolution ESI-MS spectra were obtained on an API 3000 (Applied Biosystems, CA, USA), and high-resolution ESI-MS spectra on a Bruker Daltonics APEX II 30e spectrometer (Bruker, Bremen, Germany). Silica gel 60 (Merck, 70–230 mesh, 230–400 mesh) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F-254), 0.20 mm and 0.50 mm, were used for analytical TLC and preparative TLC, respectively, and visualized with 10% H_2SO_4.

Plant Material. The stems of *M. fuscata* were collected from Taoyuan City, Taiwan, July 2011. Plant material was identified by Prof. Fu-Yuan Lu (Department of Forestry and Natural Resources, College of Agriculture, National Chiayi University). A voucher specimen (*Michelia* 5) was deposited in the School of Medical and Health Sciences, Fooyin University, Kaohsiung City, Taiwan.

Extraction and Isolation. The air-dried stems of *M. fuscata* (3.1 kg) were extracted with MeOH (3 L × 5) at room temperature, and a MeOH extract (68.5 g) was obtained upon concentration under reduced pressure. The MeOH extract was chromatographed over silica gel (950 g, 70–230 mesh) using *n*-hexane–EtOAc–MeOH mixtures as eluents to produce six fractions. Part of fraction 1 (2.04 g) was subjected to silica gel chromatography by eluting with *n*-hexane–EtOAc (50:1) and enriched gradually with EtOAc to furnish five fractions (1-1–1-5). Fraction 1-2 (0.53 g) was further purified on a silica gel column using *n*-hexane–EtOAc mixtures to obtain coniferyl aldehyde (15 mg). Part of fraction 2 (6.02 g) was subjected to silica gel chromatography by eluting with *n*-hexane–EtOAc (50:1) and enriched gradually with EtOAc to furnish five fractions (2-1–2-5). Fraction 2-1 (1.43 g) was further purified on a silica gel column using *n*-hexane–EtOAc mixtures to obtain a mixture of *β*-sitosterone and stigmasterone (7 mg). Fraction 2-3 (1.06 g) was further purified on a silica gel column using CH_2Cl_2–MeOH (30:1) mixtures to obtain liriodenine (17 mg). Part of fraction 3 (18.54 g) was subjected to silica gel chromatography by eluting with *n*-hexane–EtOAc (50:1) and enriched with EtOAc to furnish six further fractions (3-1–3-6).

Fraction 3-2 (2.11 g) was further purified on a silica gel column using CH_2Cl_2–MeOH mixtures to obtain corydaldine (2 mg), *N*-methylcorydaldine (3 mg), and fuscatine B (**2**) (2 mg). Fraction 3-3 (4.32 g) was further purified on a silica gel column using CH_2Cl_2–MeOH mixtures to obtain northalifoline (5 mg), thalifoline (5 mg), and fuscatine A (**1**) (3 mg). Fraction 3-4 (2.68 g) was further purified on a silica gel column using CH_2Cl_2–MeOH mixtures to obtain liriodenine (3 mg) and (6,7-dimethoxyisoquinolinyl)-(4′-methoxyphenyl)-methanone (4 mg). Fraction 3-5 (1.88 g) was further purified on a silica gel column using CH_2Cl_2–MeOH mixtures to obtain (6,7-dimethoxyisoquinolinyl)-(4′-hydroxyphenyl)-methanone (5 mg). Fraction 3-6 (3.12 g) was further purified on a silica gel column using CH_2Cl_2–MeOH mixtures to obtain corydine (6 mg). Part of fraction 4 (14.76 g) was subjected to silica gel chromatography by eluting with CH_2Cl_2–MeOH (50:1) and enriched with MeOH to furnish five fractions (4-1–4-5). Fraction 4-3 (2.89 g) eluted with CH_2Cl_2–MeOH (30:1) was further separated using silica gel column chromatography and preparative TLC (CH_2Cl_2–MeOH, 50:1) to give *p*-hydroxybenzaldehyde (5 mg), *p*-hydroxybenzoic acid (6 mg), and 3,4-dimethoxybenzoic acid (2 mg). Part of fraction 5 (13.47 g) was subjected to silica gel chromatography by eluting with CH_2Cl_2–MeOH (40:1) and enriched with MeOH to furnish three fractions (5-1–5-3). Fraction 5-2 (1.64 g) eluted with CH_2Cl_2–MeOH (40:1) was further separated using silica gel column chromatography and preparative TLC (CH_2Cl_2–MeOH, 45:1) to give methylparaben (3 mg) and syringic acid (2 mg). Part of fraction 6 (3.78 g) was subjected to silica gel chromatography by eluting with CH_2Cl_2–MeOH (35:1) and enriched with MeOH to furnish seven fractions (6-1–6-7). Fraction 6-2 (0.34 g) eluted with CH_2Cl_2–MeOH (40:1) was further separated using silica gel column chromatography and preparative TLC (CH_2Cl_2–MeOH, 50:1) to give 2,6-dimethoxy-*p*-benzoquinone (5 mg) and caryophyllene oxide (3 mg).

Fuscatine A (1). Yellow needles, mp 137–139°C. IR (neat, ν_{max}, cm^{-1}): 3200 (br, OH), 1485. UV/Vis (CH_3CN, λ_{max}, nm) (log ε): 300 (3.21), 289 (2.24). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 3.64 (1H, m, H-1a), 3.80 (1H, m, H-1b), 3.88 (2H, m, H-3), 3.92 (3H, s, 7-OMe), 3.97 (3H, s, 6-OMe), 4.23 (1H, m, H-4), 6.68 (1H, s, H-8), 7.32 (1H, s, H-5). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 49.4 (C-4, CH), 61.3 (C-1, CH_2), 56.4 (6-OMe, CH_3), 56.5 (7-OMe, CH_3), 70.7 (C-3, CH_2), 103.5 (C-5, CH), 106.0 (C-8, CH), 115.5 (C-4a, C), 119.2 (C-8a, C), 129.9 (C-6, C), 129.9 (C-7, C). ESI-MS *m/z* (%) 232 $[M + Na]^+$; HR-MS-ESI *m/z* 232.0952 $[M + Na]^+$, calcd for $C_{11}H_{15}O_3NNa$, 232.0950).

Fuscatine B (2). Colorless needles, mp 195–196°C. IR (neat, ν_{max}, cm^{-1}): 1685 (C=O), 1610. UV/Vis (CH_3CN, λ_{max}, nm) (log ε): 300 (3.36), 274 (3.34), 261 (4.43), 213 (4.52). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.36 (2H, t, J = 6.8, H-4), 3.64 (2H, t, J = 6.8, H-3), 3.96 (3H, s, 7-OMe), 6.97 (1H, d, J = 8.4, H-5), 7.58 (1H, d, J = 1.6, H-8), 7.71 (1H, dd, J = 8.4, 1.6, H-6). ESI-MS *m/z* (%): 177 $[M]^+$; HR-MS-ESI *m/z* 177.0789 $[M]^+$ (calcd for $C_{10}H_{11}O_2N$, 177.0790).

ACKNOWLEDGMENT

This work was financially supported by the Ministry of Science and Technology of the Republic of China under Grant No. MOST 104-2320-B-242-001-MY3 (C. Y. Chen).

REFERENCES

1. C. T. Huang, S. J. Chen, H. M. Wu, Y. F. Kang, H. L. Chen, W. J. Li, H. T. Li, and C. Y. Chen, *Chem. Nat. Compd.*, **50**, 1047 (2014).
2. S. Chericoni, L. Testai, E. Campeol, V. Calderone, I. Morelli, and E. Martinotti, *J. Ethnopharmacol.*, **91**, 263 (2004).
3. W. L. Lo, L. Y. Huang, H. M. Wang, and C. Y. Chen, *Chem. Nat. Compd.*, **46**, 664 (2010).
4. H. M. Wang, W. L. Yang, S. C. Yang, and C. Y. Chen, *Chem. Nat. Compd.*, **46**, 327 (2010).
5. M. J. Cheng, W. L. Lo, J. C. Huang, Y. T. Yeh, Z. L. Hong, Y. C. Lu, M. S. Chang, and C. Y. Chen, *Nat. Prod. Res.*, **24**, 682 (2010).
6. H. M. Wang, W. L. Lo, L. Y. Huang, Y. D. Wang, and C. Y. Chen, *Nat. Prod. Res.*, **24**, 398 (2010).
7. C. Y. Chen, L. Y. Huang, L. J. Chen, W. L. Lo, S. Y. Kuo, Y. D. Wang, S. H. Kuo, and T. J. Hsieh, *Chem. Nat. Compd.*, **44**, 137 (2008).
8. C. Y. Chen, T. Z. Liu, W. C. Tseng, F. J. Lu, R. P. Hung, C. H. Chen, and C. H. Chen, *Food Chem. Toxicol.*, **46**, 2694 (2008).
9. C. Y. Chen, F. R. Chang, C. M. Teng, and Y. C. Wu, *J. Chin. Chem. Soc.*, **46**, 77 (1999).

10. N. Kucukboyaci, F. Bingol, B. Sener, J. P. Kutney, and N. Stoynov, *Nat. Prod. Sci.*, **4**, 257 (1998).
11. Y. Su, S. Li, N. Li, L. Chen, J. Zhang, and J. Wang, *J. Med. Plants Res.*, **5**, 5428 (2011).
12. P. Pudjiastuti, M. R. Mukhtar, A. H. A. Hadi, N. Saidi, H. Morita, M. Litaudon, and K. Awang, *Molecules*, **15**, 2339 (2010).
13. H. W. Gibson, F. D. Popp, and A. C. Noble, *J. Heterocycl. Chem.*, **3**, 99 (1966).
14. C. Y. Chen, F. R. Chang, and Y. C. Wu, *J. Chin. Chem. Soc.*, **44**, 313 (1997).
15. C. Thebtaranonth, Y. Thebtaranonth, S. Wanauppathamkul, and Y. Yuthavong, *Phytochemistry*, **31**, 2645 (1999).
16. W. Werner and S. Dieter, *Tetrahedron Lett*., **21**, 1927 (1980).
17. C. Weimar, S. Angerer, and W. Wiegrebe, *Arch. Pharm*., **324**, 509 (1991).