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Limonoids and alkaloids of the root bark of *Dictamnus angustifolius*

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Abstract

Six limonoids, limonin, limonin diosphenol, limonexic acid, obacunone, fraxinellone and fraxinellonone; nine alkaloids, dictangustine-A, iso-γ-fagarine, dictamine, γ-fagarine, skimmianine, isodictamine, isomaculosidine, *N*-methylflindersine and preskimmianine; five coumarins, angustifolin, umbelliferone, herniarin, scopoletin and scoparone; three steroids, sitosterol, stigmast-4-ene-3,6-dione and stigmast-4-ene-3-one, together with dictafolin-A and dictafolin-B were isolated from the root bark of *Dictamnus angustifolius*. Their structures were elucidated by spectral analysis. Among them, dictangustrine-A, iso-γ-fagarine, dictafolin-A and dictafolin-B are reported for the first time from a natural source.

Keywords: Dictamnus angustifolius; Rutaceae; Limonoids; Alkaloids; Coumarins; Chroman; Furan

1. Introduction

Dictamnus angustifolius G. Don growing only in the Xin Jiang province of China, have been used as an alternative for folk medicine, *Dictamnus dasycarpus* in the treatment of rheumatism, bleeding, itching, jaundice, chronic hepatitis, skin diseases and as an anti-inflammatory agent, febrifugal and detoxicant drug in Wu (1991). (Hu, Han, Zhao, Song, Li, Yin, & 1989) have isolated several tetranortriterpenoids from this plant. In the course of our search for antiplatelet aggregation active components from Chinese herbs, we have isolated several antiplatelet aggregation and vascular relaxing active compounds from the root bark of *Dictamnus dasycarpus* (Wu, Wang, Shyur, Leu, Chan, Teng & Kuo, 1994). We found that the methanolic extract of the root bark of *Dictamnus angustifolius* showed significant vascular relaxing activity which led us to investigate the antiplatelet aggregation and vascular relaxing active components from *Dictamnus angustifolius*. The present paper describes the structure determination of the compounds isolated from the root bark of *Dictamnus angustifolius*.

2. Results and discussion

Dictangustine-A (**1**), was isolated as a yellowish powder and its HREI mass spectrum exhibited $[M]^+$ at m/z 215.0582 corresponding to a molecular formula $C_{12}H_9NO_3$. The UV absorptions at 242, 255, 263, 348 and 364 nm, coupled with a carbonyl band at 1600 cm^{-1} in the IR spectrum, suggested compound **1** being characteristic of isofuroquinoline derivative (Ayafor, Sondengam & Ngadjui, 1982). The ^1H NMR spectrum of **1** showed a *N*-methyl signal at δ 3.96 (3H, *s*). In the aromatic region, two characteristic α- and β-furan protons appeared at δ 7.53 (*d*, $J = 2.4$ Hz) and 6.95 (*d*, $J = 2.4$ Hz). An ABX pattern signals at δ 7.31 (1H, *dd*, $J = 8.8$, 3.2 Hz), 7.65 (1H, *d*, $J = 8.8$ Hz) and 7.88 (1H, *d*, $J = 3.2$ Hz) were assigned to H-7, H-8 and H-5, respectively. The deshielding of H-5 is reasonable because it lies in the *peri*-position with respect to the 4-carbonyl moiety. A hydroxy group signal was observed at δ 8.76 (exchangeable with D_2O) as a singlet. To confirm the location of the hydroxyl group, a NOESY experiment was conducted (Fig. 1). The result showed H-8 (δ 7.65) to be within NOE distance from *N*-methyl (δ 3.96) and H-7 (δ 7.31). Based on the above data, structure **1** was assigned to dictangustine-A.

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Fig. 1. The NOESY correlations of **1** and **2**.

Iso-γ-fagarine (**2**) was obtained as colorless needles and its high resolution mass spectrum established a molecular formula as $C_{13}H_{11}NO_3$. The UV and IR spectra of **2** showed a close resemblance to that of **1**, thus suggesting a isofuroquinoline nucleus for the compound. The ^{1}H NMR spectrum revealed the presence of two three proton singlets at δ 4.01 and 4.20 due to a methoxyl and *N*-methyl groups. Two α- and β-furan protons appeared at δ 7.56 and 6.94 each 1H (*d*, $J = 2.2$ Hz). In the aromatic region, an ABC type proton signals appeared at δ 7.28 (1H, *t*, $J = 7.9$ Hz), 7.35 (1H, *dd*, $J = 7.9$, 1.9 Hz) and 8.02 (1H, *dd*, $J = 7.9$, 1.9 Hz). The lower signal at δ 8.02 is characteristic of H-5 in isofuroquinoline. In the NOESY experiment (Fig. 1), which showed a methoxyl signal (δ 4.01) to be within NOE distance from the *N*-methyl (δ 4.20) and H-7 (δ 7.35). From the above results, we suggest the structure **2** for *iso*-γ-fagarine.

Dictafolin-A (**3**) was isolated as optically active colorless oil. It exhibited $[M]^+$ at *m*/*z* 192.0786 corresponding to a molecular formula $C_{11}H_{12}O_3$. The UV and IR spectra showed characteristic of a chroman derivative (Saengchantara & Wallace, 1990). In the aromatic region of the ^{1}H NMR spectrum, an ABC type system at δ 6.88 (1H, *d*, $J = 7.2$ Hz), 7.04 (1H, *d*, $J = 8.8$ Hz) and 7.49 (1H, *dd*, $J = 8.8$, 7.2 Hz) were attributed to H-6, H-8 and H-7, respectively. A methoxy signal appeared at δ 3.86 which was suggested to attach at the C-5 position because of the up field shift of the two proton signals at δ 6.88 and 7.04. In addition, an ABX pattern at δ 2.27 (1H, *dd*, $J = 16.4$, 12.8 Hz), 2.95 (1H, dd, $J = 16.4$, 3.2 Hz) and 4.50 (1H,

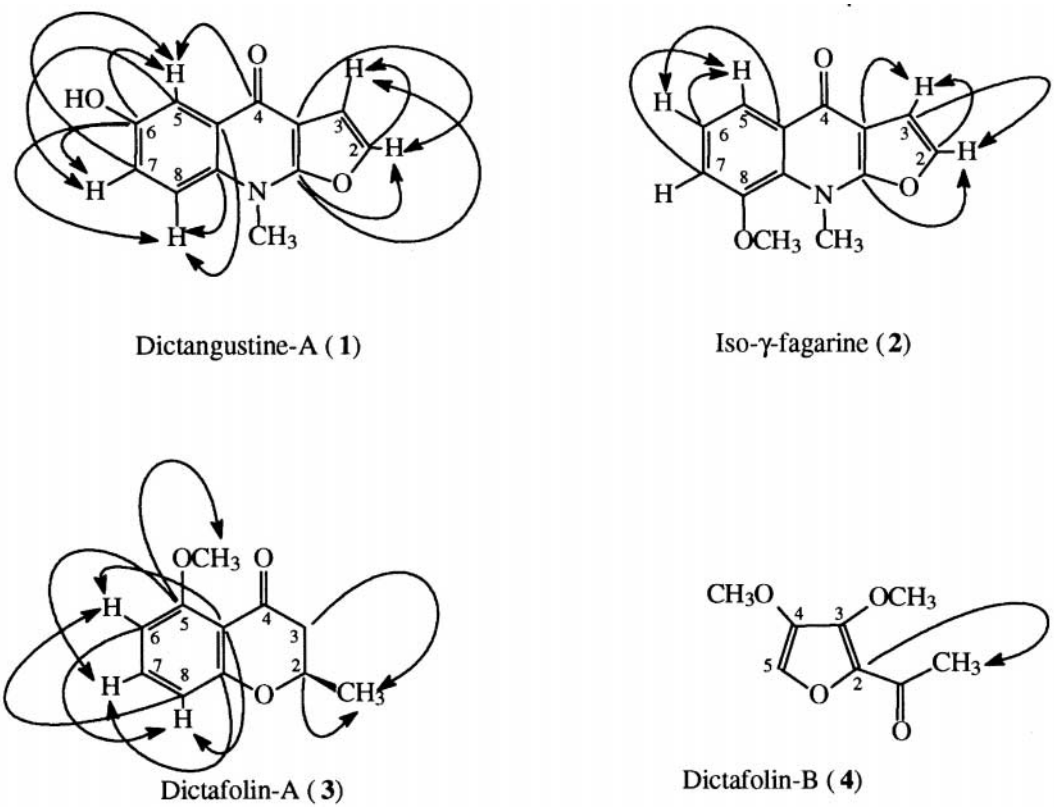

Fig. 2. The HMBC correlations of **1**, **2**, **3** and **4**.

dqd, $J = 12.8$, 6.4, 3.2 Hz) were assigned to H-3_{ax}, H-3_{eq} and H-2_{ax}, respectively. The H-2 also coupled with a methyl signal at δ 1.39. All ^{13}C NMR assignments of **3** were resolved by a combination of 1D and 2D-NMR techniques comprising HMBC (Fig. 2) and HMQC. (Wallace, *et al.* 1990) have synthesized (*S*)-2-methylchroman-4-one and shown a negative optical rotation. However, compound **3** displayed a positive optical rotation. Therefore the absolute configuration of **3** at C-2 was presumed to be *R*. On the basis of the above results, the structure of dictafolin-A was assigned as **3**.

Dictafolin-B (**4**) was separated as a yellowish oil. Its molecular formula was determined as $C_8H_{10}O_4$ by high resolution mass spectrometry. The UV spectrum of **4** at 219 and 275 nm was considered the characteristic of furan absorption (Drueckhammer, Barbas III, Nozaki & Wong, 1994). The presence of an acetyl group in the molecule was inferred by a methyl signal at δ 2.32 in the ^1H NMR spectrum and a carbonyl and a methyl carbon at δ 187.4 and 14.9 in ^{13}C NMR spectrum together with IR band at 1722 cm^{-1}. A furanoid α-proton appeared at δ 7.49. Two singlet methoxy signals were observed at δ 3.76 and 3.86. To confirm the location of methoxy and acetyl groups, an HMBC experiment was conducted (Fig. 2). The methyl proton (δ 2.32) showed ^3J correlation with a signal for C-2 (δ 158.4). Therefore two methoxy groups must be located at C-3 and C-4. The above data were in accordance with structure **4** for dictafolin-B which was synthesized by Wong *et al.* 1988.

The known compounds, limonin (**5**) (Wu *et al.* 1994), limonin diophenol (**6**) (Hu *et al.* 1989), limonexic acid (**7**) (Kondo, Suzuki & Nozoe, 1985), obacunone (**8**) (Wu *et al.* 1994), fraxinellone (**9**) (Wu *et al.* 1994), fraxinellonone (**10**) (Boustie, Moulis, Gleye, fouraste, Servin & Bon, 1990), dictamine (**11**) [(Wu *et al.* 1994), (Chen, Wu, Leu, Tsai & Wu, 1996)], γ-fagarine (**12**) [(Wu *et al.* 1994), (Chen *et al.* 1996)], skimmianine (**13**) (Chen *et al.* 1996), isodictamine (**14**) (Collins, Gray, Grundon, Harrison & Spyropoulos, 1973), isomaculosidine (**15**) (Ayafor *et al.* 1982), *N*-methylflindersine (**16**) (Moulis, Wirasutisna, Gleye, Loiseau, Stanislas & Moretti, 1983), preskimmianine (**17**), [(Ayafor *et al.* 1982), (Wu, Kuoh & Furukawa, 1983)] angustifolin (**18**) (Galan, Massanet, Pando, Luis & Salva, 1989), umbelliferone (**19**) (Lin & Wu, 1994), herniarin (**20**) (Nathan, Dominguez & Ortega, 1984), scopoletin (**21**) (Lin *et al.* 1994), scoparone (**22**) (Nathan *et al.* 1984) sitosterol (**23**) (Wu *et al.* 1994) stigmast-4-ene-3,6-dione (**24**) (Itokawa, Akasu & Fujita, 1973) and stigmast-4-ene-3-one (**25**) (Wu *et al.* 1994) were also isolated and characterized by comparison of their spectroscopic data (UV, IR and mass spectroscopy) with literature values. Dictangustrine-A (**1**), iso-γ-fagarine (**2**), dictafolin-A (**3**) and dictafolin-B (**4**) are reported from a natural source for the first time.

3. Experimental

M.p.'s: uncorr. ^1H, ^{13}C NMR: Me_2CO-d_6 and $CDCl_3$, TMS as int. standard except where noted. UV: MeOH. IR: KBr, unless otherwise stated. MS: 70 eV. Column chromatography: silica gel 60 (70–230 and 230–400 mesh) (E. Merck). TLC: silica gel 60 GF254 (E. Merck).

3.1. Plant material

The root bark of *Dictamnus angustifolius* was collected from the Xin Jiang province and verified by Professor Q. G. Liu. A voucher specimen (NCKU-WU-960701) is deposited in the Herbarium of the Cheng Kung University, Tainan, Taiwan.

3.2. Extraction and separation

The root bark of *Dictamnus angustifolius* (1 kg) was extracted with EtOH ($\times$5) at room temp., and concd to give a deep brown syrup (180 g). The crude extract was partitioned between H_2O and $CHCl_3$. The $CHCl_3$ soluble layer was acidified with 5% aq. HCl to give the acidic layer and the $CHCl_3$ layer. The acidic layer was neutralized by aq. NH_4OH and extracted with $CHCl_3$ to give an alkaloid layer. The $CHCl_3$ layer was directly chromatographed on silica gel eluting with a gradient of $CHCl_3$–Me_2CO to afford 12 fractions. Fr. 2 was rechromatographed on silica gel using *n*-C_6H_{14}–EtOAc (39:1) as eluent to give **9** (15 mg). Fr. 3 was CC on silica gel and eluted with *n*-C_6H_{14}–$CHCl_3$ (14:1) to afford **9** (1.3 mg). Fr. 4 underwent CC over silica gel using *n*-C_6H_{14} and Me_2CO (9:1) as eluent to yield **10** (24 mg), **20** (1.2 mg), **23** (32 mg) and **25** (32 mg), successively. Fr. 5 was rechromatographed on silica gel eluted with *n*-C_6H_{14}–$CHCl_3$ (1:1) to give **3** (1.7 mg), **11** (50 mg), **12** (20 mg), **22** (4.5 mg) and **24** (15 mg). Fr. 6 was treated in a similar manner as fr. 5 to afford **4** (2.5 mg), **8** (15 mg), **13** (3.2 mg), **16** (5.2 mg), **17** (3.8 mg) **18** (1.2 mg) and **21** (4.2 mg). Fr. 7 underwent CC over silica gel eluted with $CHCl_3$–Me_2CO (24:1) to give **6** (2.1 mg) and **19** (16 mg). Similarly, fr. 8 eluting with $CHCl_3$–Me_2CO (9:1) gave **5** (3.2 g) and **6** (0.4 g). Fr. 9 was rechromatographed on silica gel and eluted with a gradient of $CHCl_3$–MeOH to yield **5** (4.9 g), and **7** (8.0 mg). The alkaloid layer was chromatographed on silica gel and eluted with a gradient of C_6H_6–Me_2CO to afford **1** (4.7 mg), **2** (1.2 mg), **4** (1.7 mg), **11** (600 mg), **12** (2.5 mg) **14** (3.3 mg) and **15** (5.1 mg), successively.

3.3. Dictangustine-A (*1*)

Yellowish powders (Me_2CO), m.p. > 280°. HRMS: calcd. for $C_{12}H_9NO_3$, *m/z* 215.0583 $[M]^+$, found 215.0582. UV λ_{max}^{MeOH} nm: 242, 255, 263, 348, 364. IR ν_{max}^{KBr} cm^{-1}: 3452, 1600, 1556, 1541, 1454, 1240. EIMS *m/z* (rel. int): 215 ($[M]^+$, 100), 200 (15), 172 (3), 144 (2), 63 (16). ^1H NMR (Me_2CO-d_6, 400 MHz): δ 8.76 (1H, *s*, OH), 7.88 (1H, *d*, *J* = 3.2 Hz, H-5), 7.65 (1H, *d*, *J* = 8.8 Hz, H-8), 7.53 (1H, *d*, *J* = 2.4 Hz, H-2), 7.31 (1H, *dd*, *J* = 8.8, 3.2 Hz, H-7), 6.95 (1H, *d*, *J* = 2.4 Hz, H-3), 3.96 (3H, *s*, NMe). ^{13}C NMR (Me_2CO-d_6, 100 MHz): δ 172.9 (C-4), 157.1 (C-1b), 154.3 (C-8a), 139.4 (C-2), 133.6 (C-6), 127.8 (C-4a), 122.4 (C-7), 117.6 (C-8), 111.2 (C-5), 108.4 (C-3), 106.2 (C-3a), 32.1 (NMe).

3.4. Iso-γ-fagarine (*2*)

Colorless needles (Me_2CO), m.p.: 160–161°. HRMS: calcd. for $C_{13}H_{12}NO_3$, *m/z* 229.0739 $[M]^+$, found 229.0739. UV λ_{max}^{MeOH} nm: 246, 255, 301, 322, 336, 350. IR ν_{max}^{KBr} cm^{-1}: 2925, 2858, 1604, 1537, 1269, 1080. EIMS *m/z* (rel. int): 229 ($[M]^+$, 11), 214 (8), 144 (14), 129 (15), 115 (22), 109 (15), 97 (25), 83 (41), 69 (68), 57 (100). ^1H NMR (Me_2CO-d_6, 400 MHz): δ 8.02 (1H, dd, *J* = 7.9, 1.9 Hz, H-5), 7.56 (1H, *d*, *J* = 2.2 Hz, H-2), 7.35 (1H, *dd*, *J* = 7.9, 1.9 Hz, H-7), 7.28 (1H, *t*, *J* = 7.9 Hz, H-6), 6.94 (1H, *d*, *J* = 2.2 Hz, H-3), 4.20 (3H, *s*, NMe), 4.01 (3H, *s*, OMe). ^{13}C NMR (Me_2CO-d_6, 100 MHz): δ 183.7 (C-4), 157.2 (C-1b), 152.0 (C-8a), 139.7 (C-2), 131.8 (C-8), 131.4 (C-4a), 123.9 (C-5), 119.9 (C-6), 115.7 (C-7), 111.2 (C-3a), 108.2 (C-3), 57.3 (OMe), 37.7 (NMe).

3.5. Dictafolin-A (*3*)

Colorless oil. $[\alpha]_D$ +62.3° (*c* = 0.015, Me_2CO); +30.0° (*c* = 0.02, $CHCl_3$). HRMS: calcd. for $C_{11}H_{12}O_3$ *m/z* 192.0786 $[M]^+$, found 192.0786. UV λ_{max}^{MeOH} nm: 209, 242, 306. IR ν_{max}^{KBr} cm^{-1}: 2932, 1732, 1598, 1475, 1244, 1085. EIMS *m/z* (rel. int.): 192 ($[M]^+$, 60), 149 (100), 131 (24), 105 (42), 90 (61), 77 (34). ^1H NMR ($CDCl_3$, 400 MHz): δ 7.49 (1H, *dd*, *J* = 8.8, 7.2 Hz, H-7), 7.04 (1H, *d*, *J* = 8.8 Hz, H-8), 6.88 (1H, *d*, *J* = 7.2 Hz, H-6), 4.50 (1H, *dqd*, *J* = 12.8, 6.4, 3.2 Hz, H-2_{ax}), 3.86 (3H, s, OMe), 2.95 (1H, *dd*, *J* = 16.4, 3.2 Hz, H-3_{eq}), 2.27 (1H, *dd*, *J* = 16.4, 12.8 Hz, H-3_{ax}), 1.39 (3H, *d*, *J* = 6.4 Hz, 2-Me). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 186.8 (C-4), 161.2 (C-5), 141.9 (C-8a), 134.3 (C-7), 119.1 (C-8), 113.8 (C-4a), 110.9 (C-6), 74.0 (C-2), 56.2 (OMe), 36.1 (C-3), 20.7 (2-Me).

3.6. Dictafolin-B (*4*)

Yellowish oil. HRMS: calcd. for $C_8H_{10}O_4$, *m/z* 170.0579 $[M]^+$, found 170.0578. UV λ_{max}^{MeOH} nm: 219, 275. IR ν_{max}^{KBr} cm^{-1}: 2922, 2850, 1722, 1593, 1456, 1298, 1128. EIMS *m/z* (rel. int.): 170 ($[M]^+$, 100), 155 (13), 140 (15), 97 (21), 69 (17). ^1H NMR ($CDCl_3$, 400 MHz): δ 7.49 (1H, *s*, H-5), 3.86 (3H, *s*, 3-OMe), 3.76 (3H, *s*, 4-OMe), 2.32 (3H, *s*, Me). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 187.4 (C=O), 158.4 (C-2), 149.0 (C-3), 144.3 (C-4), 137.1 (C-5), 60.1 (3-OMe), 56.9 (4-OMe), 14.9 (Me).

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