PERGAMON

Phytochemistry 53 (2000) 837–840

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Iridoids from *Dunnia sinensis*[☆]

Xiaoyi Wei*, Haihui Xie, Xuejun Ge, Fengxian Zhang

South China Institute of Botany, Chinese Academy of Sciences, Guangzhou, 510650, People's Republic of China

Received 12 July 1999; received in revised form 14 September 1999

Abstract

A plumieride type iridoid glucoside, dunnisinoside, and a non-glucosidic iridoid, dunnisinin, were isolated from the leaves of *Dunnia sinensis*. Their structures were established by 1D and 2D NMR and FABMS experiments.

Keywords: *Dunnia sinensis*; Rubiaceae; Iridoids; Dunnisinoside; Dunnisinin

1. Introduction

Dunnia sinensis Tutch. (Rubiaceae), a rare plant endemic to the southern Guangdong Province of China, is used in folk medicine as an anti-inflammatory drug. *Dunnia*, a monotypic genus, is comprised of the single species *D. sinensis*, which has not previously been chemically studied. In this paper, we describe the isolation and the structure elucidation of two new iridoids obtained from the leaves of this plant.

2. Results and discussion

The EtOH percolate of the powdered dry leaves was fractionated with petroleum, $CHCl_3$ and N-BuOH. The N-BuOH-soluble fraction was subjected to chromatography on a silica gel column, followed by recrystallization. A major iridoid, trivially named dunnisinoside (**1**), was isolated. The $CHCl_3$-soluble fraction was separated by Al_2O_3 and silica gel column chromatography to yield a minor iridoid named dunnisinin (**2**).

The negative FAB mass spectrum of **1** gave a base ion peak at m/z 549 ($[M-H]^-$), indicating a molecular weight of 550. By the combined analysis of FABMS, ^{13}C-NMR and DEPT data, its molecular formula was suggested as $C_{26}H_{30}O_{13}$. The IR spectrum of **1** indicated the presence of hydroxyl groups (3406 cm^{-1}), a saturated γ-lactone (1774 cm^{-1}), an iridoidic enol ether system conjugated with an ester carbonyl group (1712, 1637 cm^{-1}) and a *p*-substituted phenyl group (1614, 1516 and 837 cm^{-1}).

The ^{1}H-NMR spectrum of **1** showed a singlet for the carbomethoxy group at δ 3.65, a doublet (J = 6.5 Hz) for the C-1 proton at δ 5.13, a doublet (J = 1.2 Hz) for the C-3 proton characteristic of iridoids at δ 7.43, two double doublets (J = 5.6, 2.4 Hz) for disubstituted olefinic protons at δ 6.07 and 6.23, a doublet (J = 8.0 Hz) for C-1′ proton at δ 4.58, suggesting that the cyclopentanopyran ring system and the sugar moiety of **1** were identical with those of plumieride (Schmid, Bickel & Meijer, 1952; Halpern & Schmid, 1958; Yamauchi, Abe & Taki, 1981). In addition, a pair of two-proton doublets (J = 8.4 Hz) at δ 6.65 and 7.01, along with a broad singlet at δ 9.25 (D_2O exchangeable), indicated the presence of a *p*-hydroxyphenyl group, and this was supported by the bathochromic shift of the UV absorption at 279 nm under

☆ The investigation here presented is part of the program numbered KZ952S1-113 of the Bureau of Resources and Ecological Environments, the Chinese Academy of Sciences.

* Corresponding author. Fax: +86-20-8770-1031.
E-mail address: weixiaoy@scib.ac.cn (X. Wei).

0031-9422/00/$ - see front matter
PII: S0031-9422(99)00581-6

alkaline condition. The corresponding carbon signals in the ^{13}C-NMR spectrum (see Experimental) of **1** assigned by HMQC and COLOC spectra confirmed the partial structures above.

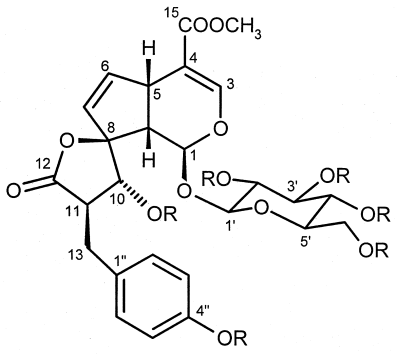
The remaining carbon resonances of **1** showed two quaternary carbons at δ 173.9 (C-12) and δ 96.7 (C-8), indicating characteristic iridoids with a five-membered spiro-lactone ring at C-8, an oxygenated methine carbon at δ 69.5, a methine carbon at δ 47.6 and a methylene carbon at δ 30.6. Based on the HMQC spectrum, the corresponding proton signals were found as a doublet (J = 8.0 Hz) overlapping with C-1′ proton at δ 4.58, a multiplet at δ 3.12, and two double doublets at δ 2.83 (J = 12.0, 4.0 Hz) and 2.75 (J = 12.0, 6.0 Hz), respectively. The multiplet at δ 3.12, which could only be assigned to H-11, correlated in ^{1}H–^{1}H COSY with the doublet at δ 4.58 and the signals at δ 2.83 and 2.75. In the COLOC spectrum of **1**, cross peaks were observed between the methylene proton signals (δ 2.83 and 2.75) and the carbon signals of C-2″/6″ at δ 131.0, C-1″ at δ 127.5, and of C-12 at δ 173.9. Thus, the oxygenated methine carbon was assigned as C-10 and the methylene carbon as C-13, to which the *p*-hydroxyphenyl group was attached. Acetylation of **1** afforded a hexaacetate (**1a**); the signal of H-10 in **1a** was shifted downfield to δ 5.58 (*d*, J = 8.4 Hz), indicating the substitution of a hydroxyl group on C-10 in **1**. Compound **1** was thus determined as shown, disregarding stereochemistry.

In order to determine the stereochemistry of **1**, NOESY measurements were carried out on **1** and **1a**. In the NOESY spectra of both **1** and **1a**, the presence of strong cross peaks between H-1 and H-10 and between H-11 and H-7 indicated that the linkage between C-8 and C-10 was α orientation and that H-1 was α and in an equatorial conformation as in plumieride (Abe, Chen & Yamauchi, 1988). Further, it was shown that H-10 was in the β position, and H-11 was α. The large coupling constant (J = 8.0 Hz in **1**, 8.8 Hz in **1a**) between H-10 and H-11 showed that the five-membered lactone ring assumes an E_{11}-conformation with a *trans*-diaxial proximity between these protons. In conclusion, dunnisinoside (**1**) has the structure depicted. It is noted that a similar compound, oruwacin, was obtained from *Morinda lucida* (Adesogan, 1979).

The UV spectrum of compound **2** showed no absorption for the conjugated carbonyl group. The positive FAB mass spectrum of **2** showed $[M + H]^+$ at m/z 227, which was consistent with a molecular formula of $C_{11}H_{14}O_5$.

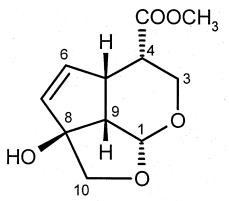
The ^{1}H-NMR spectrum of **2** resembled that of gardiol (Jensen, 1983) or gardenogenin (Ishiguro, Yamaki & Takagi, 1983), except that the signal for the acetalic C-3 proton was absent in **2**. Instead, signals for two protons corresponding to an extra methylene group appeared at δ 3.77 (1H, *ddd*, J = 11.6, 4.0, 1.2 Hz) and δ 3.69 (1H, *t*, J = 11.6 Hz), which could be assigned to the C-3 protons by ^{1}H–^{1}H COSY. The assignment was supported by the ^{13}C-NMR spectrum (see Experimental), in which the corresponding carbon signal for C-3 was found at δ 56.3 by HMQC and DEPT experiments.

The configuration at C-4 in **2** could be deduced from the ^{1}H-NMR and the NOESY experiment of **2**. The coupling constants, $J_{4,3\beta}$ = 4.0 Hz, $J_{4,3\alpha}$ = 11.6 Hz and $J_{4,5}$ = 6.0 Hz, along with $J_{5,3\beta}$ = 1.2 Hz (probably due to W long-range coupling), indicated that H-4 was in a β orientation and in the axial conformation. Careful examination of Dreiding models showed that if the tetrahydropyran ring assumed a half-chair conformation $^{4}H_3$ with 4-methoxycarbonyl group in α-configuration, the expected coupling constants among the ring protons were in accord with those measured for **2**. This was confirmed by the NOESY experiment, in which the cross peaks were observed between H-4 and H-3β, respectively, H-5 and H-9. Furthermore, in the NOESY, the presence of cross peaks between H-1 and H-10β and between H-7 and H-10α showed that the tetrahydrofuran ring assumes an E_o-conformation with 1,3-diaxial proximity between H-1 and H-10β (Jensen, 1983). Compound **2** was thus elucidated as shown.



1 : R = H

1a : R = Ac



2

3. Experimental

3.1. Plant material

The leaves of *Dunnia sinensis* were collected from southern Guangdong Province, China, in April, 1997. A voucher specimen (GXJ97001) has been deposited at the herbarium of the South China Institute of Botany, the Chinese Academy of Sciences, Guangzhou, People's Republic of China.

3.2. General

Mps: uncorr. NMR: 400 MHz (^{1}H) or 100 MHz (^{13}C), chemical shifts as δ values (ppm) relative to TMS, DMSO-d_6 as solvent in compound **1** and $CDCl_3$ in others. FABMS: *m*-nitrobenzyl alcohol (*m*NBA) as a matrix for negative ion mode and glycerol (Gly) as that for positive ion mode. TLC: silica gel 60 F_{254}, $CHCl_3$–MeOH (9:1) and hexane–acetone (2:1), spray reagent H_2SO_4 (10%) in EtOH followed by heating. CC: silica gel 60 (100–200 mesh) and neutral Al_2O_3 (100–200 mesh).

3.3. Extraction and isolation

The powdered air-dried leaves (2.7 kg) were extracted by percolation with 90% EtOH three times at room temp. The EtOH extracts were concd. to a syrup in vacuo. This syrup was suspended in H_2O and the aq. suspension was extracted three times subsequently with petroleum, $CHCl_3$ and *n*-BuOH. The combined *n*-BuOH extract, after concentration in vacuo, yielded 250 g of brown syrup. This syrup was further fractionated by Diaion HP-20 CC eluted successively with H_2O, 50% MeOH in H_2O and MeOH. The 50% MeOH eluate gave, on concentration in vacuo, a yellowish powder (55 g). Part of the powder (20 g) was then subjected to a silica gel CC, eluted with $CHCl_3$–MeOH mixts. of increasing polarity [(19:1) to (9:1)], yielding five fractions (I–V). Fraction IV, on recrystallization from MeOH, afforded compound **1** (1.5 g).

The combined $CHCl_3$ extract, upon evaporation, yielded a dark syrup (9.4 g). This syrup was subjected to a neutral Al_2O_3 CC, eluted with a gradient of hexane–EtOAc (9:1, 5:1, 4:1 and EtOAc). The hexane–EtOAc (5:1) eluate, on concentration, afforded a brown powder (250 mg). This was subjected to further silica gel column chromatography eluted with hexane–acetone (9:1), followed by recrystallization from the mix of acetone and hexane, to afford compound **2** (50 mg).

3.4. Compound *1*, dunnisinoside

Colourless prisms, mp 221–223°C; $[\alpha]_D^{25}$ + 28.4° (MeOH, c 0.25); UV (MeOH) λ_{max} nm (*log* ε): 206 (4.02), 228 (4.19), 279 (3.17); IR (KBr) ν_{max} cm^{-1}: 3406, 1774, 1712, 1637, 1614, 1516, 1433, 1286, 1209, 1114, 1076, 1027, 977, 927, 837; FABMS, negative ion mode, *m*/*z* (rel. int.): 703 $[M + mNBA]^-$ (58), 549 $[M-H]^-$ (100); ^{1}H-NMR spectral data: δ 5.13 (1H, *d*, *J* = 6.5 Hz, H-1), 7.43 (1H, *d*, *J* = 1.2 Hz, H-3), 3.66 (1H, *m*, H-5), 6.23 (1H, *dd*, *J* = 5.6, 2.4 Hz, H-6), 6.07 (1H, *dd*, *J* = 5.6, 2.4 Hz, H-7), 2.47 (1H, *t*, *J* = 6.5 Hz, H-9), 4.58 (2H, *d*, *J* = 8.0 Hz, H-10 and H-1′), 3.12 (1H, *m*, H-11), 2.83 (1H, *dd*, *J* = 12.0, 4.0 Hz, H-13a), 2.75 (1H, *dd*, *J* = 12.0, 6.0 Hz, H-13b), 3.00 (1H, *t*, *J* = 8.0 Hz, H-2′), 3.16–3.25 (3H, *m*, H-3′, H-4′ and H-5′), 3.72 (1H, *dd*, *J* = 11.6, 2.0 Hz, H-6′a), 3.47 (1H, *dd*, *J* = 11.6, 6.4 Hz, H-6′b), 7.01 (2H, *d*, *J* = 8.4 Hz, H-2″ and H-6″), 6.65 (2H, *d*, *J* = 8.4 Hz, H-3″ and H-5″), 3.65 (3H, *s*, OCH_3), 9.25 (1H, *br s*, OH-4″); ^{13}C-NMR spectral data: δ 93.3 (*d*, C-1), 151.7 (*d*, C-3), 107.9 (*s*, C-4), 39.0 (*d*, C-5), 139.6 (*d*, C-6), 129.2 (*d*, C-7), 96.7 (*s*, C-8), 47.0 (*d*, C-9), 69.5 (*d*, C-10), 47.6 (*d*, C-11), 173.9 (*s*, C-12), 30.6 (*t*, C-13), 166.4 (*s*, C-15), 99.1 (*d*, C-1′), 72.9 (*d*, C-2′), 76.3 (*d*, C-3′), 70.0 (*d*, C-4′), 76.9 (*d*, C-5′), 60.8 (*t*, C-6′), 127.5 (*s*, C-1″), 131.0 (*d*, C-2″ and C-6″), 115.0 (*d*, C-3″ and C-5″), 155.9 (*s*, C-4″), 51.3 (*q*, OCH_3).

3.5. Acetylation of dunnisinoside (*1*)

Dunnisinoside (**1**) (50.0 mg) was acetylated with Ac_2O–pyridine (each **1** ml) by the usual method and the product was subjected to prep. TLC with hexane–acetone (2:1) as eluant. The major band gave dunnisinoside hexaacetate (**1a**) (44.0 mg) as white amorphous powder, $[\alpha]_D^{25}$ − 73.6° (MeOH, *c* 0.25); FABMS, positive ion mode, *m*/*z* (rel. int.): 893 $[M + Gly - H]^+$ (34), 802 $[M]^+$ (100), 759 $[M - CH_3CO]^+$ (72), 717 $[M - CH_3CO–CH_3CO + H]^+$ (34), 657 (15), 615 (10); ^{1}H-NMR spectral data: δ 4.90 (1H, *d*, *J* = 2 Hz, H-1), 7.09 (1H, *d*, *J* = 1.6 Hz, H-3), 3.52 (1H, *m*, H-5), 6.36 (1H, *dd*, *J* = 6.0, 2.8 Hz, H-6), 5.69 (1H, *dd*, *J* = 6.0, 1.6 Hz, H-7), 2.92 (1H, *dd*, *J* = 8.4, 2.0 Hz, H-9), 5.58 (1H, *d*, *J* = 8.8 Hz, H-10), 3.03 (1H, *m*, H-11), 3.10–3.12 (2H, *m*, H_2-13), 4.70 (1H, *d*, *J* = 8.0 Hz, H-1′), 4.88 (1H, *dd*, *J* = 9.6, 8.4 Hz, H-2′), 5.15 (1H, *t*, *J* = 9.6 Hz, H-3′), 5.03 (1H, *t*, *J* = 9.6 Hz, H-4′), 3.70 (1H, *m*, H-5′), 4.05 (1H, *dd*, *J* = 12.8, 2.4 Hz, H-6′a), 4.28 (1H, *dd*, *J* = 12.8, 4.0 Hz, H-6′b), 7.33 (2H, *d*, *J* = 8.4 Hz, H-2″ and H-6″), 6.98 (2H, *d*, *J* = 8.4 Hz, H-3″ and H-5″), 3.66 (3H, *s*, OCH_3), 2.25, 2.08, 1.98, 1.95, 1.84 and 1.78 (each 3H, *s*, 6 × CH_3CO); ^{13}C-NMR spectral data: δ 92.1 (*d*, C-1), 149.0 (*d*, C-3), 110.9 (*s*, C-4), 36.6 (*d*, C-5), 138.3 (*d*, C-6), 129.0 (*d*, C-7), 94.7 (*s*, C-8), 47.4 (*d*, C-9), 71.7 (*d*, C-10), 48.3 (*d*, C-11), 173.0 (*s*, C-12), 32.4 (*t*, C-13), 166.1 (*s*, C-15), 95.9 (*d*, C-1′), 70.4 (*d*, C-2′), 72.3 (*d*, C-3′ or C-5′), 67.8 (*d*, C-4′), 72.0 (*d*, C-5′ or C-3′), 61.4 (*t*, C-6′), 134.5 (*s*, C-1″), 130.8 (*d*, C-2″ and C-6″), 121.6 (*d*, C-3″ and C-5″), 149.5 (*s*, C-4″), 51.4 (*q*, OCH_3), 21.0, 20.7, 20.5 × 2, 20.3, 20.1 (each *q*, 6 × $\underline{C}H_3CO$), 170.6, 170.1, 169.2 × 3, 168.8 (each *s*, 6 × $CH_3\underline{C}O$).

3.6. Compound *2*, dunnisinin

Colourless needles, mp 178–179°C; $[\alpha]_D^{25}$ + 213.5° (MeOH, *c* 0.20); IR (KBr) ν_{max} cm^{-1}: 3446, 1734,

1607, 1416, 1284, 1200, 1105; FABMS, positive ion mode, *m/z* (rel. int.): 411 $[M + Gly + Gly + H]^+$ (21), 319 $[M + Gly + H]^+$ (19), 227 $[M + H]^+$ (100), 209 $[M - H_2O + H]^+$ (6), 191 (25), 177 (10), 163 (22); ^{1}H-NMR spectral data: δ 5.38 (1H, *d*, *J* = 7.2 Hz, H-1), 3.77 (1H, *ddd*, *J* = 11.6, 4.0, 1.2 Hz, H-3β), 3.69 (1H, *t*, *J* = 11.6 Hz, H-3α), 2.90 (1H, *ddd*, *J* = 11.6, 6.0, 4.0 Hz, H-4), 3.65 (1H, *m*, H-5), 5.80 (1H, *dd*, *J* = 5.6, 2.4 Hz, H-6), 5.70 (1H, *dd*, *J* = 5.6, 1.6 Hz, H-7), 2.44 (1H, *dd*, *J* = 8.0, 7.2 Hz, H-9), 3.55 (1H, *d*, *J* = 9.2 Hz, H-10β), 3.88 (1H, *d*, *J* = 9.2 Hz, H-10α), 3.70 (3H, *s*, OCH_3); ^{13}C-NMR spectral data: δ 99.8 (*d*, C-1), 56.3 (*t*, C-3), 40.8 (*d*, C-4), 42.0 (*d*, C-5), 136.4 (*d*, C-6), 135.7 (*d*, C-7), 92.5 (*s*, C-8), 45.7 (*d*, C-9), 70.6 (*t*, C-10), 172.2 (*s*, C-11), 51.8 (*q*, OCH_3).

Acknowledgements

We thank Dr. Keyang Ding and Mr. Ruiqiang Chen, Guangzhou Institute of Chemistry, the Chinese Academy of Sciences, for 1D and 2D NMR spectroscopic measurements, and Prof. Huiling Liang, Kunming Institute of Botany, the Chinese Academy of Sciences, for FABMS experiments.

References

Abe, F., Chen, R.-F., & Yamauchi, T. (1988). *Chemical and Pharmaceutical Bulletin*, *36*(8), 2784–2789.

Adesogan, E. K. (1979). *Phytochemistry*, *18*(1), 175–176.

Halpern, O., & Schmid, H. (1958). *Helvetica Chimica Acta*, *41*, 1109–1154.

Ishiguro, K., Yamaki, M., & Takagi, S. (1983). *Journal of Natural Products*, *46*(4), 532–536.

Jensen, S. R. (1983). *Phytochemistry*, *22*(8), 1761–1765.

Schmid, H., Bichel, H., & Meijer, T. M. (1952). *Helvetica Chimica Acta*, *35*, 415–427.

Yamauchi, T., Abe, F., & Taki, M. (1981). *Chemical and Pharmaceutical Bulletin*, *29*(10), 3051–3055.