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seco-Adianane-type triterpenoids from *Dorstenia brasiliensis* (Moraceae)

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Abstract

Two *seco*-adianane-type triterpenoids, dorstenic acid A and B, were isolated, along with a known isopimarane-type diterpenoid and six coumarins, from the roots of *Dorstenia brasiliensis*. Their structures were elucidated on the basis of their spectral data. The two triterpenoids showed moderate cytotoxicity against leukemia cells (L-1210 and HL-60). © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Dorstenia brasiliensis; Moraceae; Dorstenic acid A; Dorstenic acid B; Cytotoxicity; *seco*-Adianane-type triterpenoid; Psoralen; Coumarin

1. Introduction

In a search for biologically active substances from South American medicinal plants (Satoh et al., 2000; Ohkoshi et al., 2000), the roots of *Dorstenia brasiliensis*, called Carapia in Brazil, which have been used as a folk medicine for the treatment of digestive system disease and typhoid fever (Hashimoto, 1996), were studied. There is one report on the isolation of furocoumarin derivatives from this plant (Kuster et al., 1994), and a review on the chemistry of the genus *Dorstenia* has been published (Abegaz et al., 2000). In this paper, we report the isolation and structure elucidation of two 3,4-*seco*-adianane-type triterpenoids, named dorstenic acid A (**1**) and dorstenic acid B (**2**), along with seven known compounds, 14α-hydroxy-7,15-isopimaradien-18-oic acid (**3**) (Bruno et al., 1986), psoralen (**4**) (Elgamal et al., 1979), (2′*S*,3′*R*)-3′-hydroxymarmesine 4′-*O*-β-D-glucopyranoside (**5**) (Lemmich et al., 1983), (2′*S*)-marmesin 4′-*O*-α-L-rhamnopyranosyl(1→6)-*O*-β-D-glucopyranoside (**6**) (Srivastava and Srivastava, 1993), (2′*S*, 3′*R*)-3′-hydroxymarmesin (**7**) (Vilegas and Pozetti, 1993), 2′-(1″-hydroxy-1″-methylethyl)-psoralen (**8**) (Quader et al., 1992; Zubia et al., 1992; Stanjek et al., 1997), and 7-hydroxycoumarin (**9**) (Cussans and Huckerby, 1975), as well as the cytotoxic activities of **1** and **2** against leukemia cells.

2. Results and discussion

Dorstenic acid A (**1**) was obtained as a white amorphous powder, $[\alpha]_D$ −18.1° (*c* 0.67, $CHCl_3$). Its high resolution-EIMS spectrum showed a molecular ion peak at *m*/*z* 458.3760 $[M]^+$ corresponding to the molecular formula $C_{30}H_{50}O_3$ and indicating six degrees of unsaturation. The ^1H NMR spectrum of **1** showed the presence of four tertiary methyls (δ 0.79, 0.93, 0.95, 1.04), a vinyl methyl (δ 1.78, *br s*), two secondary methyls [δ 0.83, 0.89 (each 3H, *d*, *J* = 6.5 Hz)], and an *exo*-methylene [δ 4.89, 5.12 (each 1H, *d*, *J* = 1.0 Hz)] group. The ^{13}C NMR (Table 1) spectra, including DEPT, exhibited thirty signals due to a double bond (δ 111.1 and 150.7), seven methyls, ten methylenes, six methines, four quaternary carbons, including an oxygen-bearing carbon (δ 78.0), and a carboxyl carbon (δ 178.5). The ^1H–^1H COSY and HMQC spectra revealed the presence of isopropyl and isopropenyl groups and a propionic acid moiety (C-1–C-3). The above spectral data suggested that **1** should be a tetracyclic *seco*-adianane-type triterpenoid (Tanaka et al., 1989) and the connectivities of these groups were investigated by HMBC experiments. As shown in Fig. 1, the HMBC spectrum of **1** displayed cross peaks due to the long-

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range couplings between the following proton and carbon signals: δ 2.28 (H-2) and 178.5 (C-3), 54.2 (C-10); 1.26 (H-10) and 78.0 (C-5), 19.6 (C-25); 4.89 and 5.12 (H-23) and 78.0 (C-5); 1.04 (H-25) and 54.2 (C-10), 49.0 (C-8), 38.8 (C-9); 0.94 (H-26) and 49.0 (C-8), 38.9 (C-13), 40.1 (C-14), 29.2 (C-15); 0.96 (H-27) and 28.4 (C-12), 38.9 (C-13), 40.1 (C-14), 51.6 (C-18); 0.79 (H-28) and 42.6 (C-17), 51.6 (C-18), 60.0 (C-21); 0.83 and 0.89 (H-29 and H-30) and 60.0 (C-21), 30.7 (C-22). Finally, the relative stereochemistry of **1** was determined by analysis of the NOESY spectrum. In the NOESY spectrum, correlations were observed between the following proton signals, as shown in Fig. 2: δ 1.25 (H-1) and 1.04 (H-25); 1.26 (H-10) and 1.79 (H-24); 1.04 (H-25) and 1.25 (H-1 and OH), 0.94 (H-26); 0.94 (H-26) and 1.04 (H-25), 1.57 (H-18); 0.79 (H-28) and 0.96 (H-27), 0.83 and 0.89 (H-29 and H-30). Thus, the structure of dorstenic acid A was confirmed as the 3,4-*seco*-adianane-type triterpenoid **1** with a carboxyl group at C-2.

Dorstenic acid B (**2**) was obtained as a white amorphous powder, $[\alpha]_D$ +39.5° (*c* 0.86, $CHCl_3$). Its high resolution-EIMS spectrum exhibited a molecular ion peak at *m/z* 416.3301 $[M]^+$ corresponding to the molecular formula $C_{27}H_{44}O_3$ and indicating six degrees of unsaturation. The ^1H and ^{13}C NMR spectra (Table 1) of **2** were very similar to those of **1** except for the presence of a carbonyl group (δ_C 212.0) and the disappearance of an isopropenyl group. The HMBC spectrum of **2** showed cross peaks due to the long-range correlations between H-10 (δ 2.26) and C-2 (δ 32.8), C-5 (δ 212.0), and C-6 (δ 42.1). Therefore, the carbonyl group was assigned at C-5 and the structure of dorstenic acid B (**2**) was elucidated to be a deisopropenyl derivative of **1**.

Dorstenic acid A (**1**) and B (**2**) showed moderate cytotoxicity against leukemia cells (L-1210: IC_{50} = 5 and 40 μM, and HL-60: IC_{50} = 10 and 40 μM), respectively.

Table 1
^{13}C NMR spectral data for compounds **1** and **2** in $CDCl_3$ (100 MHz, δ in ppm)

	1	2
1	17.4 *t*[a]	17.1 *t*
2	35.8 *t*	32.8 *t*
3	178.5 *s*	178.5 *s*
4	150.7 *s*	
5	78.0 *s*	212.0 *s*
6	39.2 *t*	42.1 *t*
7	20.1 *t*	23.2 *t*
8	49.0 *d*	47.8 *d*
9	38.8 *s*	41.8 *s*
10	54.2 *d*	64.0 *d*
11	34.3 *t*	33.2 *t*
12	28.4 *t*	28.7 *t*
13	38.9 *s*	38.8 *s*
14	40.1 *s*	40.4 *s*
15	29.2 *t*	29.4 *t*
16	35.8 *t*	35.5 *t*
17	42.6 *s*	42.7 *s*
18	51.6 *d*	51.5 *d*
19	19.8 *t*	20.0 *t*
20	28.1 *t*	28.3 *t*
21	60.0 *d*	60.0 *d*
22	30.7 *d*	30.7 *d*
23	111.1 *t*	
24	20.5 *q*	
25	19.6 *q*	18.2 *q*
26	16.2 *q*	15.8 *q*
27	15.5 *q*	15.3 *q*
28	16.4 *q*	16.2 *q*
29	21.9 *q*	21.9 *q*
30	22.8 *q*	22.8 *q*

[a] Multiplicities were obtained from DEPT spectra.

3. Experimental

3.1. General

The ^1H- (400 or 500 MHz) and ^{13}C NMR (100 or 125 MHz) spectra were obtained using a Jeol JNM lambda-400 and 500 spectrometer in $CDCl_3$ containing TMS as an internal standard. The MS were recorded on a Jeol JMS-GCMATE. Optical rotations were measured using a JASCO DIP-360 digital polarimeter. CC: Diaion HP-20 (Nippon Rensui). SiO_2 gel CC: Wako gel C-200 (Wako). TLC: SiO_2 gel 60 F_{254} plates (Merck). HPLC: normal phase (Shodex SIL-5E, 250×10 mm (I), Showa Denko), reversed phase (CAPCELL PAK C_{18} UG120Å 250×10 mm (II), Shiseido). Leukemia cells were either obtained from the National Cancer Center Research

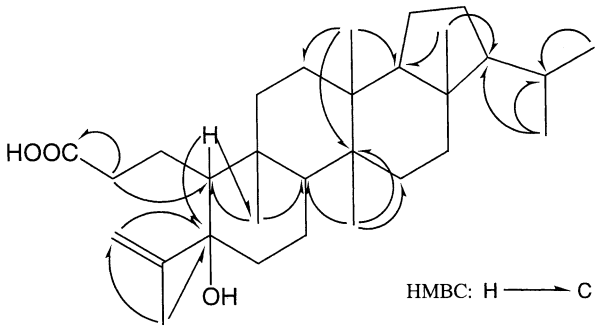


Fig. 1. Selected HMBC correlations for dorstenic acid A (**1**).

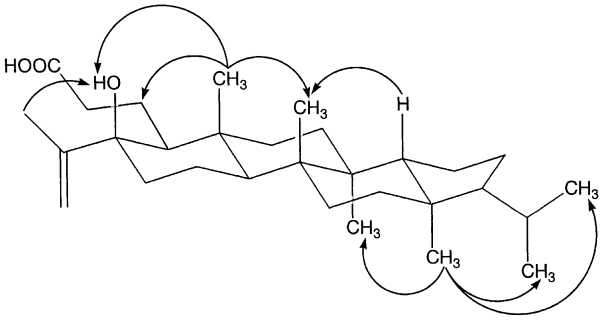
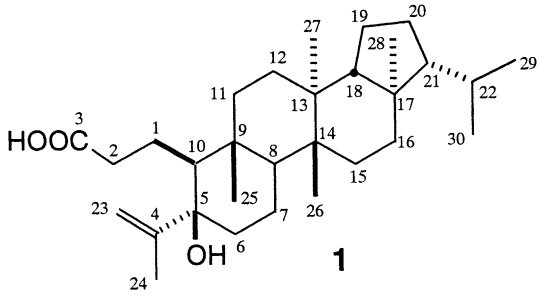
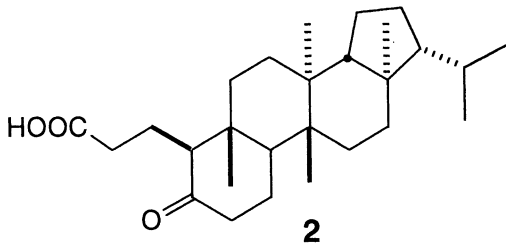


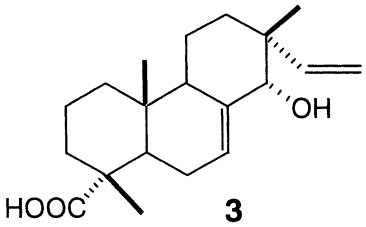
Fig. 2. NOESY correlations for dorstenic acid A (**1**).



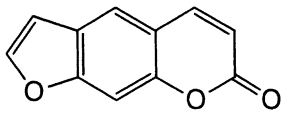
1



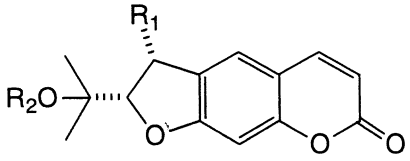
2



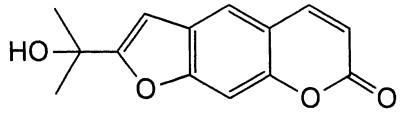
3



4
(psoralen)



5: R_1 = OH R_2 = β-D-Glc
6: R_1 = H R_2 = α-L-Rha(1→6)β-D-Glc-
7: R_1 = OH R_2 = H



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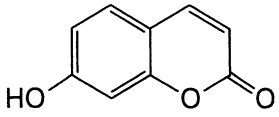

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Institute (L-1210) or purchased from Dainippon Pharmaceutical Co., Ltd. (HL-60).

3.2. Plant material

The plant material was purchased from Laboratorio Farmaervas Ltda. in São Pãulo, Brazil and identified by Pharmacist Girberto Rubens Biancalana. A voucher specimen (YF0083) was deposited in the herbarium of College of Pharmacy, Nihon University.

3.3. Extraction and isolation

The dried roots of *Dorstenia brasiliensis* (3 kg) were crushed and extracted with EtOH (36 l) under ultrasonication. The EtOH extract was conc. in vacuo to give a crude extract (139 g) which was applied to Diaion HP-20 CC (1.5 l) eluted successively with MeOH–H_2O (4:6, 6 l), MeOH–H_2O (7:3, 6 l), MeOH (6 l), and acetone (6 l). The growth inhibition of each fraction against leukemia cells (L-1210) was tested, and the MeOH fraction was found to be the most active (79% inhibition at 50 μg/ml). The MeOH fraction was conc. in vacuo to give a MeOH eluate (41 g) which was subjected to SiO_2 gel CC eluted with a stepwise gradient of $CHCl_3$, $CHCl_3$:MeOH = 50:1, 30:1, 10:1, 1:1 and MeOH to give six fractions (frs. 1–6). Fr. 1 (21.8 g) was recrystallized from MeOH to afford **4** (psoralen, 18.0 g). Fr. 3 was separated by normal phase (np)-HPLC (I, *n*-hexane:acetone = 2:1) to give seven fractions (frs. 3.1–3.7). Fr. 3.2 was purified by np-HPLC (I, *n*-hexane: acetone = 10:1) to give **1** (5.5 mg) and **2** (6.5 mg). Fr. 3.3 was purified by np-HPLC (I, *n*-hexane:EtOAc = 3:1) to give **3** (2.6 mg). The MeOH–H_2O (7:3) fraction was conc. in vacuo to give an oily material (6 g) which was subjected to SiO_2 gel CC eluted successively with solvent of increasing polarity ($CHCl_3$:MeOH = 20:1, 10:1, $CHCl_3$:MeOH:H_2O = 8:1:0.1, 6:4:1, MeOH) to give six fractions (frs. 7–12). Fr. 9 (993 mg) and fr. 10 (3.55 g) were combined and recrystallized from MeOH to afford

5 (721 mg) and **6** (1.14 g), respectively. Fr. 7 (211 mg) was separated by reversed phase (rp)-HPLC [II, MeOH–H_2O (1:1)] to give four fractions (frs. 7.1–7.4) and the fr. 7.4 (152 mg) was purified by rp-HPLC [II, H_2O–CH_3CN (3:1)] to give **7** (31.0 mg), **8** (4.3 mg), and **9** (23.7 mg). All known compounds (**3**–**9**) were identified by comparison of their spectral data with those described in the literature.

3.4. Dorstenic acid A (*1*)

White amorphous powder: $[\alpha]_D$ −18.1° ($CHCl_3$, *c* 0.67); ^1H NMR ($CDCl_3$, 400 MHz): δ 5.12 (1H, *d*, $J=1.0$ Hz, H-23a), 4.89 (1H, *d*, $J=1.0$ Hz, H-23b), 2.28 (2H, *m*, H-2), 1.79 (3H, *s*, H-24), 1.26–1.25 (*m*, H-1 and H-10, overlapped with –OH), 1.04 (3H, *s*, H-25), 0.96 (3H, *s*, H-27), 0.94 (3H, *s*, H-26), 0.89 and 0.83 (each 3H, *s*, H-29 and 30), 0.79 (3H, *s*, H-28); ^{13}C NMR spectral data, see Table 1; HR–EIMS *m*/*z*: 458.3760 (calc. for $C_{30}H_{50}O_3$, 458.3760; LR–EIMS *m*/*z* (rel. int.): 458 $[M]^+$ (36), 440 $[M–H_2O]^+$ (10), 361 $[M–C_6H_9O]^+$ (100).

3.5. Dorstenic acid B (*2*)

White amorphous powder: $[\alpha]_D$ +39.5° ($CHCl_3$, *c* 0.86); ^1H NMR ($CDCl_3$, 400 MHz): δ 2.50 (1H, *ddd*, $J=16.5$, 8.5, 5.0 Hz, H-2a), 2.42 (1H, *ddd*, $J=14.0$, 5.0, 1.5 Hz, H-6a), 2.30 (1H, *dd*, $J=14.0$, 7.5 Hz, H-6b), 2.26 (1H, *br d*, $J=10.0$ Hz, H-10), 2.15 (1H, *dt*, $J=16.5$, 7.5 Hz, H-2b), 1.04 (3H, *s*, H-27), 0.94 (3H, *s*, H-26), 0.89 and 0.83 (each 3H, *s*, H-29 and 30), 0.82 (3H, *s*, H-28); ^{13}C NMR spectral data, see Table 1; HR–EIMS *m*/*z*: 416.3301 (calc. for $C_{27}H_{44}O_3$, 416.3290); LR–EIMS *m*/*z* (rel. int.): 416 $[M]^+$ (71), 401 $[M–CH_3]^+$ (100), 331 $[M–C_4H_5O_2]^+$ (65).

3.6. Cytotoxic activity

L-1210 and HL-60 leukemia cells were maintained in RPMI 1640 medium containing 10% fetal bovine serum supplemented, respectively, with L-glutamine, sodium bicarbonate, 100 units/ml penicillin, and 100 μg/ml streptomycin. The cells (1×10^5 cells/ml) were incubated for 72 h at 37 °C with or without test compound ranging from 50 to 0.1 μg/ml, and cell growth was estimated by colorimetric measurement of stained living cells with Alamar Blue® assay (Räz et al., 1997). Optical density was determined at 595 and 570 nm on a microtiter plate reader (BioRad). A dose-response curve was plotted for each compound, and the concentration giving 50% inhibition (IC_{50}) was calculated.

Acknowledgements

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