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DIANCHINENOSIDES A AND B, TWO NEW SAPONINS FROM *DIANTHUS CHINENSIS*

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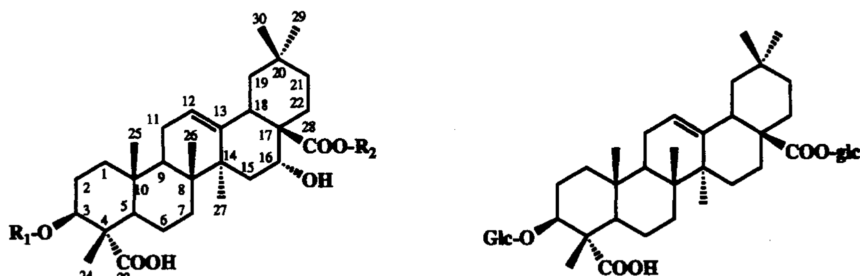
ABSTRACT.—From *Dianthus chinensis*, two new triterpene saponins, named dianchinenosides A [1] and B [2], were isolated. Their structures were determined as 3β-*O*-α-L-arabinopyranosyl-16α-hydroxyolean-12-ene-23α, 28β-dioic acid 28-*O*-β-D-glucopyranoside [1] and 3β-*O*-β-D-xylopyranosyl-16α-hydroxyolean-12-ene-23α, 28β-dioic acid 28-*O*-D-glucopyranoside [2] by fabms, various 2D nmr techniques, and chemical reactions.

The whole plant of *Dianthus chinensis* L. (Caryophyllaceae) (Chinese name, Jumai), an important drug in Chinese folk medicine, is used as a diuretic and anti-inflammatory agent (1). *D. chinensis* is endemic in China, mainly in the northeast. Many saponins from the aerial parts of *Dianthus superbus* L. var. *longicalycinus* Williams and *Dianthus deltoides* L. have been characterized (2–7), and pharmacological studies on *D. superbus* var. *longicalycinus* saponins have indicated analgesic and antihepatotoxic activities (8,9). In our research on biologically active substances of *D. chinensis* we found two new saponins, dianchinenoside A [1] and dianchinenoside B [2], and their structures are reported. In this paper, we describe the isolation and structure elucidation of those compounds.

RESULTS AND DISCUSSION

The 95% EtOH extract of the dried aerial part of *D. chinensis* was partitioned between $CHCl_3$ and H_2O. The aqueous layer was further extracted successively with EtOAc and *n*-BuOH. The *n*-BuOH-soluble fraction was passed through a highly porous polymer resin (HP-20) column. The 30% MeOH eluent, a glycoside mixture, was repeatedly chromatographed on polyamide, Toyopearl HW-40F, and Si gel, and further purified by mplc to afford a mixture of dianchinenosides A [1] and B [2]. Hplc was conducted to separate **1** and **2**.



1 R_1=ara, R_2=glc
2 R_1=xyl, R_2=glc
3 R_1=R_2=H

4

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TABLE 1. [1]H-nmr Spectral Data for Dianchinenosides A [1] and [2].

Proton	Compound	
	1	**2**
Aglycone moiety		
H-3	4.62 (1H, dd, 11.9, 4.0)	4.63 (1H, dd, 11.9, 4.0)
H-12	5.61 (1H, brt, 3.4)	5.61 (1H, brt, 3.4)
H-15	ca. 1.68	ca. 1.68
H-15	2.52 (1H, dd, 15.3, 3.6)	2.52 (1H, dd, 15.4, 4.0)
H-16	5.24 (brs)	5.24 (brs)
H-18	3.51 (1H, dd, 14.4, 4.0)	3.51 (1H, dd, 14.1, 4.0)
H-19	1.37	1.37
H-19	2.77 (1H, t, 13.4)	2.77 (1H, t, 13.4)
H-24	1.53 (3H, s)	1.54 (3H, s)
H-25	0.99 (3H, s)	0.99 (3H, s)
H-26	0.99 (3H, s)	0.99 (3H, s)
H-27	1.78 (3H, s)	1.78 (3H, s)
H-29	1.05 (3H, s)	1.05 (3H, s)
H-30	1.13 (3H, s)	1.13 (3H, s)
Sugar moiety		
C-28		
Glc-1	6.31 (1H, d, 8.2)	6.30 (1H, d, 8.2)
Glc-2	4.14 (1H, t, 8.6)	4.14 (1H, t, 8.6)
Glc-3	4.25[a]	4.25[a]
Glc-4	4.28[a]	4.28[a]
Glc-5	4.02[a]	4.02[a]
Glc-6	4.43 (1H, dd, 11.9, 2.8)	4.43 (1H, dd, 11.9, 2.4)
Glc-6	4.62 (1H, dd, 11.9, 4.0)	4.62 (1H, dd, 11.9, 4.0)
C-3		
Ara-1	4.95 (1H, d, 6.7)	
Ara-2	4.04[a]	
Ara-3	4.22[a]	
Ara-4	4.20[a]	
Ara-5	3.75 (1H, d, 11.0)	
Ara-5	4.23[a]	
Xyl-1		4.99 (1H, d, 7.3)
Xyl-2		3.93 (1H, t, 7.7)
Xyl-3		4.08[a]
Xyl-4		4.30[a]
Xyl-5		3.67 (1H, t, 10.0)
Xyl-5		4.33[a]

[a]Obscured by other signals; couplings could not be accurately determined.

Dianchinenoside A [**1**] was obtained as colorless needles from MeOH, mp 225–227° (dec), $[\alpha]^{20}D$ +14.9° (pyridine), and indicated to have molecular formula $C_{41}H_{64}O_{15}$ from a positive fabms $[M+K]^+$ ion at *m/z* 835, $[M+Na]^+$ ion at *m/z* 819, and $[M+H]^+$ ion at *m/z* 797, and the presence of 41 ^{13}C signals, which were attributed to six Me, nine CH_2, four CH, six quaternary carbons, two CH_2-O, ten CH-O, two O-CH-O, one C=CH and two CO in DEPT 90 and 135 experiments. The ir spectrum indicated the presence of hydroxyl, ester, and carboxyl groups and a double bond. The ^1H-nmr spectrum (500 MHz, pyridine-d_5) of **1** showed signals of six tertiary methyl groups (δ 0.99×2, 1.05, 1.13, 1.53, and 1.78), two carbinol protons [δ 4.62 (1H, dd, *J*=11.9 and 4.0 Hz) and 5.24 (1H, brs)], an olefinic proton [δ 5.61 (1H, brt, *J*=3.4 Hz)], and two anomeric

protons [δ 4.95 (1H, d, J=6.7 Hz) and 6.31 (^{1}H, d, J=8.2 Hz)]. The ^{13}C-nmr (125 MHz, pyridine-d_5) spectrum indicated signals of six C-C bonded quaternary carbons (δ 30.8, 36.8, 40.5, 42.1, 53.4, and 49.2), two carbinol carbons (δ 74.4 and 85.2), a pair of olefinic carbons (δ 122.6 and 144.5), and two anomeric carbons (δ 95.9 and 106.0). The above spectral data demonstrated dianchinenoside A [**1**] to be a triterpenoid glycoside similar to dianoside A [**4**] (2). Acid hydrolysis of **1** gave D-glucose and L-arabinose. The ^{1}H-^{1}H COSY of **1** showed spin systems of L-arabinose and D-glucose units. The HMBC spectrum gave cross peaks between C-1 (δ 106.0) of the arabinopyranosyl unit and H-3 (δ 4.62) of the aglycone and between H-1 (δ 6.31) of the glucopyranosyl unit and C-28 (δ 176.0) of the aglycone. The fabms of **1** gave fragment ions at *m/z* 657 $[M+Na-162]^+$ due to loss of one glucose unit and a fragment at *m/z* 503 $[M+H-294]^+$ arising from the loss of one glucose and one arabinose unit. The configurations of the anomeric position of the glucose and arabinose moieties were assigned to be β and α, respectively, from the coupling constant of the anomeric proton signals at δ 6.31 (J=8.2 Hz) and 4.95 (J=6.7 Hz). The configuration of H-16 was determined by the rotating frame NOESY (ROESY) spectrum (10) as β. NOe effects were registered between H-16β and H-15, H-18 and Me-8. These results are consistent with structure **1** in which the hydroxyl group at C-16 is α-oriented. Thus, dianchinenoside A [**1**] is 3β-*O*-α-L-arabinopyranosyl-16α-hydroxyolean-12-ene-23α, 28β-dioic acid 28-*O*-β-D-glucopyranoside.

Dianchinenoside B [**2**] was obtained as colorless needles from MeOH, mp 230–232°, $[\alpha]^{20}$D +2.6° (pyridine). The ir spectrum indicated the presence of hydroxyl, ester, and carboxyl groups and a double bond. The molecular formula was determined as $C_{41}H_{64}O_{15}$ based on fabms and ^{13}C-nmr spectroscopy. The ^{1}H-nmr spectrum showed signals due to six tertiary methyls [δ 0.99×2, 1.05, 1.13, 1.56, and 1.78], carbinol protons [δ 4.63 (dd, J=11.9 and 4.0 Hz) and 5.24 (brd)], an olefinic proton [δ 5.61 (brt, J=3.4 Hz)], and two anomeric protons [δ 4.99 (d, J=7.3 Hz) and 6.30 (d, J=8.2 Hz)]. The ^{13}C-nmr spectrum indicated signals of six C-C bonded quaternary carbons (d, 30.8, 36.8, 40.5, 42.1, 49.2, and 53.4), two carbinol carbons (d, 74.4 and 85.3), a pair of olefinic carbons (d, 122.6 and 144.5), and two anomeric carbons (d, 95.9 and 106.2). Acid hydrolysis of **2** gave aglycone **3**, D-glucose, and D-xylose. The ^{1}H- and ^{13}C-nmr spectra of **2** were similar to those of **1** except that the arabinose moiety was replaced by a xylose moiety in **2**. The HMBC experiment showed cross peaks between C-3 at δ 85.3 of the aglycone and the anomeric proton at δ 4.99 of the xylopyranosyl unit between C-28 ester carbonyl at δ 176.0 and the anomeric proton at δ 6.30 of the glucopyranosyl unit. Thus, dianchinenoside B [**2**] was identified as 3β-*O*-β-D-xylopyranosyl-16α-hydroxyolean-12-ene-23α, 28β-dioic acid 28-*O*-β-D-glucopyranoside.

EXPERIMENTAL

PLANT MATERIAL.—Aerial parts of *D. chinensis* were collected by one of the authors (H.-Y. Li) from Nansan mountain, Dongliao, People's Republic of China in September 1989 and identified by Prof. C.-K. Xie, Department of Pharmacognosy, School of Pharmacy, West China University of Medicinal Sciences, Chengdu, China. A voucher specimen is deposited there.

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Ir spectra were recorded on a JASCO 7300 FTIR spectrophotometer. Optical rotation was determined on a JASCO DIP-4 digital polarimeter. Eims and fabms were measured on JEOL D-300 and DX-303 mass spectrometers, respectively. ^{1}H-, ^{13}C- and 2D nmr spectra were recorded with JEOL A-500 (500 MHz for ^{1}H nmr and 125 MHz for ^{13}C nmr) and JEOL EX-400 (400 MHz for ^{1}H nmr and 100 MHz for ^{13}C nmr) spectrometers in pyridine-d_5, using TMS as the internal standard. Si gel (BW-820MH, Fuji Davison), Diaion HP-20 (Mitsubishi Kasei), Toyopearl HW-40F (Tosoh), and Polyamide C-200 (Wako) were used for cc. Mplc was carried out on Si gel [CQ-3, Fuji Gel, 24 mm i.d.×360 mm, detector uv 210 nm, solvent system $CHCl_3$-MeOH-H_2O (6:4:1)]. Preparative hplc was performed using an ODS column [Capcell Pak C18 SG120, Shiseido, 10 mm i.d.×250 mm, detector UV 210 nm,

TABLE 2. ^{13}C-nmr Spectral Data for Dianchinenosides A [**1**] and B [**2**] and Dianoside A [**4**].

Carbon	Compound		
	1	**2**	**4**[a]
Aglycone moiety			
C-1	39.0	39.0	38.7
C-2	26.3	26.4	26.1
C-3	85.2	85.3	85.1
C-4	53.4	53.4	56.3
C-5	52.2	52.2	52.0
C-6	21.4	21.4	21.3
C-7	33.2	33.2	32.8
C-8	40.5	40.5	40.2
C-9	47.5	47.5	48.1
C-10	36.8	36.8	36.6
C-11	23.9	23.9	23.6
C-12	122.6	122.6	123.8
C-13	144.5	144.5	144.1
C-14	42.1	42.1	42.0
C-15	36.2	36.2	28.1
C-16	74.4	74.4	23.3
C-17	49.2	49.2	46.9
C-18	41.4	41.4	41.6
C-19	47.2	47.2	46.1
C-20	30.8	30.9	30.7
C-21	36.0	36.0	33.9
C-22	32.2	32.2	32.5
C-23	180.5	180.5	180.9
C-24	12.7	12.8	12.5
C-25	16.2	16.2	15.9
C-26	17.5	17.5	17.3
C-27	27.2	27.2	26.1
C-28	176.0	176.0	176.4
C-29	33.2	33.2	33.1
C-30	24.7	24.7	23.6
Sugar moiety			
C-28			
Glc-1	95.9	95.9	95.6
Glc-2	74.2	74.2	74.0
Glc-3	78.9	78.9	78.7
Glc-4	71.2	71.2	71.0
Glc-5	79.4	79.4	79.2
Glc-6	62.3	62.2	62.1
C-3			
Ara-1	106.0		
Ara-2	74.4		
Ara-3	72.8		
Ara-4	69.2		
Ara-5	66.5		
Xyl-1		106.2	
Xyl-2		75.2	
Xyl-3		78.0	
Xyl-4		71.1	
Xyl-5		67.1	

[a]Data from Oshima *et al.* (2). Data of second glucose signals are not given.

solvent system MeOH-H_2O (2:3)]. For glc 2% SE-30 on Chromosorb W (60–80 mesh, 3 mm i.d.×1.5 m, column temperature 150°, carrier gas N_2, flow rate 40 ml/min] was used.

ISOLATION OF DIANCHINENOSIDES A [**1**] AND B [**2**].—Dried aerial parts (6 kg) of *D. chinensis* were extracted with 95% EtOH (10 liters) (four times) under reflux for 1 h. The combined EtOH extract was concentrated under reduced pressure to give a residue (312 g), and the aqueous MeOH extract (3 liters) was washed with $CHCl_3$ (500 ml, three times). The aqueous solution was concentrated in vacuo to a small volume and partitioned with EtOAc (500 ml, three times). The aqueous layer was further extracted with *n*-BuOH (500 ml, three times) to give an *n*-BuOH residue (59 g). The *n*-BuOH extract (59 g) was applied onto a column of Diaion HP-20 (4.5 kg) with H_2O/MeOH as the eluent with increasing MeOH content [H_2O-MeOH (7:3), (5:5), (3:7) and then MeOH] to afford 400 fractions. Diaion HP-20 fractions 51–66 (1.5 g) were placed on a column of Polyamide C-200 and washed with H_2O and MeOH. The saponin fraction was chromatographed over Toyopearl HW-40F. Development with MeOH-H_2O (1:1) gave a mixture of **1** and **2** (50.3 mg). Fractions 71–80 gave a residue (3.8 g) which was chromatographed on a Si gel (150 g) column. Elution was performed with 10, 22, 35, 44, and 70% MeOH in $CHCl_3$ and then MeOH. Residue from the 35% MeOH in $CHCl_3$ fraction was recrystallized from MeOH to give a mixture ov **1** and **2** (52 mg). The combined saponin mixture (102 mg) was subjected to mplc using Si gel to give a mixture of **1** and **2** (48 mg) (ratio 7:3). Carefully repeated preparative hplc of the saponin mixture led to isolation of dianchinenoside A [**1**] (19 mg) and dianchinenoside B [**2**] (10 mg), both as colorless needles.

DIANCHINENOSIDE A [**1**].—Colorless needles (MeOH): mp 230–232°; $[\alpha]^{23}D$ +14.9° (c=1.4, pyridine); fabms *m/z* $[M+K]^+$ 835, $[M+Na]^+$ 819, $[M+H]^+$ 797, $[M+Na-glc]^+$ 657, $[M+Na-glc-ara]^+$ 503; Ft-ir (KBr) 3379 (br), 1734, 1718, 1651, 1556, 1457, 1388, 1074 (br) cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2.

DIANCHINENOSIDE B [**2**].—Colorless needles (MeOH): mp 225–227°, $[\alpha]^{23}D$ +2.6° (c=0.9, pyridine); fabms *m/z* $[M+K]^+$ 835, $[M+Na]^+$ 819, $[M+H]^+$ 797, $[M+Na-glc]^+$ 657, $[M+Na-glc-xyl]^+$ 503; Ft-ir (KBr) 3414 (br), 1718, 1653, 1556, 1460, 1389, 1248, 1076 (br), 1044 (br) cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2.

ACID HYDROLYSIS OF **1** AND **2**.—Dianchinenoside A [**1**] 6 mg was heated in MeOH (2 ml) with 1 N HCl (1 ml) for 2 h at 80°. After MeOH was removed, the aqueous solution was diluted with H_2O and extracted with EtOAc. The solvent was evaporated to give aglycone **3**. The aqueous layer was neutralized with ion-exchange resin (Amberlite MB-3), and then evaporated. The residue was treated with 1-(trimethylsilyl)imidazole at 90° for 1 h, and H_2O was added to the reaction mixture to decompose the excess reagent. The reaction product was extracted with hexane (1 ml×3), and the hexane layer was washed with H_2O (1 ml×3). The hexane solution was subjected to glc for identification of the sugar moiety. The TMSi derivatives were identified as derivatives of D-glucose and L-arabinose.

By the same method, **2** (6 mg) was hydrolyzed to aglycone **3**, D-glucose, and D-xylose.

Aglycone **3**.—Amorphous solid: mp 272–274°; $[\alpha]^{26}D$ +44.0° (c=0.2, MeOH); fabms *m/z* $[M+Na]^+$ 525, eims (rel. int.) *m/z* $[M-H_2O]^+$ 484 (1), 440 (71), 425 (14), 407 (4), 378 (4); Ft-ir (KBr) 3429 (br), 2927, 1698, 1456, 1387, 1261, 1170, 1043, 1003 cm^{-1}; ^1H nmr (400 MHz) δ 1.03^a (3H, s, Me-25), 1.04^a (3H, s, Me-26), 1.07 (3H, s, Me-29), 1.18 (3H, s, Me-30), 1.65 (3H, s, Me-24), 1.80 (3H, s, Me-27), 3.63 (1H, dd, J=14.7 and 5.5 Hz, H-3), 5.20 (1H, brs, H-16), 5.66 (1H, brs, H-12); ^{13}C nmr (100 MHz) δ 12.3 (C-24), 16.2 (C-25), 17.5 (C-26), 21.8 (C-6), 23.9 (C-11), 24.8 (C-30), 26.2 (C-2), 27.3 (C-27), 31.0 (C-20), 32.7 (C-22), 33,3^b (C-7), 33.4^b (C-29), 36.2 (C-15 and C-21), 37.0 (C-10), 39.2 (C-1), 40.4 (C-8), 41.5 (C-18), 42.2 (C-14), 47.3 (C-19), 47.6 (C-9), 49.0 (C-17), 52.0 (C-5), 54.4 (C-4), 74.7 (C-3), 75.6 (C-16), 123.0 (C-12), 145.3 (C-13), 180.2 (C-28), 180.8 (C-23) (values with the same superscript may be interchangeable.

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LITERATURE CITED

1. "Zhong Yao Da Zi Dian, Jiangsu," New Medical College, Jiangsu, China, 1977, p. 2702.
2. Y. Oshima, T. Ohsawa, K. Oikawa, C. Konno, and H. Hikino, *Planta Med.*, **50**, 40 (1984).
3. Y. Oshima, T. Ohsawa, and H. Hikino, *Planta Med.* **50**, 43 (1984).
4. Y. Oshima, T. Ohsawa, and H. Hikino, *Planta Med.*, **50**, 154 (1984).
5. G. Bukharov, S.P. Shcherbak, and A.P. Beschekova, *Khim. Prir. Soedin.*, **33** (1971).
6. G. Bukharov and S.P. Shcherbak, *Khim. Prir. Soedin.*, 420 (1971).
7. M. Schimizu and T. Takemoto, *Yakugaku Zasshi*, **87**, 250 (1967).

8. R. Koster, *Fed. Proc.,* **22**, 249 (1958).
9. H. Hikino, T. Ohsawa, Y. Kiso, and Y. Oshima, *Planta Med.,* **50**, 353 (1984).
10. A. Bax and D.G. Davis, *J. Magn. Reson.,* **63**, 207 (1985).

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