Triterpenoid Saponins from *Dianthus versicolor*

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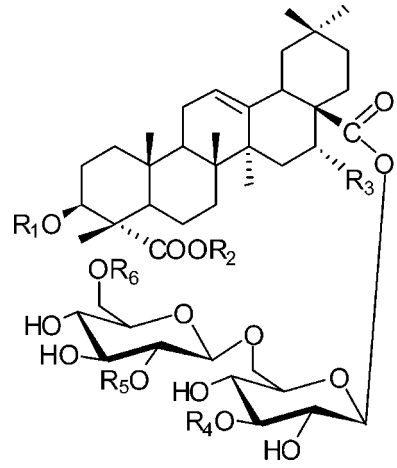
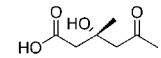
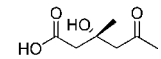
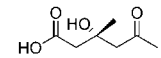
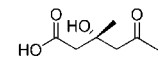
Seven new pentacyclic triterpenoid saponins, named dianversicosides A−G (**1**−**7**), together with nine known compounds, were isolated from the aerial parts of *Dianthus versicolor*. The structures of **1**−**7** were elucidated on the basis of spectroscopic data and chemical evidence. The absolute configuration of the 3-hydroxyl-3-methylglutaryl (HMG) group in **1**−**4** was ascertained by chemical analysis combined with a chiral HPLC method. Cytotoxic activities of the isolated compounds were evaluated against a small panel of cancer and other cell lines.

Dianthus versicolor Fisch. ex Link. (Caryophyllaceae) is a small herb distributed in Shandong Province and elsewhere in the northeast of mainland China. This plant, an important Chinese herbal folk medicine known as "Jumai", is used as a diuretic, as an anti-inflammatory agent, and in the treatment of urinary infections, carbuncles, and carcinomas.[1] Many bioactive saponins have been isolated from the aerial parts of *D. chinensis*[2−4] and *D. superbus* L. var. *longicalycinus* Williams.[5−8] To the best of our knowledge, there have been no reports on the chemistry or biological activity of *D. versicolor*. As part of an ongoing research project on the chemistry of bioactive triterpenoid saponins, we have isolated 16 saponins from the aerial parts of *D. versicolor*, including seven new substances. The known compounds were identified as dianchinenoside A,[3] dianchinenoside B,[3] dianchinenoside C,[2] dianchinenoside D,[2] dianoside G,[7] dianoside H,[7] vaccaroid A (**8**),[9] vaccaroid C (**9**),[10] and hainanenside,[11] respectively, on the basis of their physical and spectroscopic data. The inhibitory effects of **1**−**9** were evaluated against human fetal lung fibroblasts (HFL-I) and human umbilical vascular endothelial 304 (EVC-304), human gastric cancer 803 (BGC-803), human breast cancer (MCF-7), and human hepatocellular carcinoma (Hep G2) cells.



	R_1	R_2	R_3	R_4	R_5	R_6
1	H	Glc	OH	H	Glc	
2	H	H	OH	Glc	Glc	
3	H	Glc	H	Glc	Glc	
4	H	H	H	Glc	Glc	
5	H	Glc	OH	Glc	Glc	H
6	H	Glc	OH	H	Glc	H
7	Ara	H	H	Glc	Glc	H

Results and Discussion

The dried aerial parts of *D. versicolor* were pulverized and extracted exhaustively with 80% ethanol. The ethanol solution was concentrated to dryness under a vacuum. The residue was suspended in H_2O and partitioned successively with petroleum ether, EtOAc, and *n*-BuOH. The *n*-BuOH-soluble extract was subjected to further column chromatography and then purified by preparative HPLC, to afford seven new triterpenoid saponins, dianversicosides A−G (**1**−**7**), together with nine known saponins. Determination of the absolute configuration[12,13] of the 3-hydroxyl-3-methylglutaryl (HMG) group present in each of the saponins **1**−**4**, a biosynthetic precursor of various isoprenoids, was resolved by converting these HMG groups to a six-membered lactone followed by chiral HPLC analysis.

Dianversicoside A (**1**) was obtained as a white, amorphous powder. The HRESIMS exhibited a pseudomolecular ion peak at *m/z* 1317.5732 $[M + Na]^+$ (calcd 1317.5722), which implied that the compound has the molecular formula $C_{60}H_{94}O_{30}$. The IR spectrum showed the presence of an ester carbonyl group (1729 cm^{-1}), an olefin (1640 cm^{-1}), and hydroxyl groups (3441, 1071 cm^{-1}). The NMR data (Tables 1−4) revealed that compound **1** possesses six tertiary methyl protons at δ 0.90 (s, H_3-29), 0.96 (s, H_3-25), 1.01 (s, H_3-30), 1.06 (s, H_3-26), 1.59 (s, H_3-24), and 1.65 (s, H_3-27), and an olefinic proton at δ 5.54 (brs, H-12), connected (according to the HMQC spectrum) to carbons at δ 34.0 (C-29), 17.2 (C-25), 25.5 (C-30), 18.3 (C-26), 13.0 (C-24), 28.0 (C-27), and 123.2 (C-12), respectively, and indicative of an olean-12-ene skeleton.[4] The aglycon was suggested by the NMR spectra to be 3β,16α-dihydroxyolean-12-en-23,28-dioic acid.[2] Thus, one hydroxyl group was positioned at C-3 [δ 4.65 (brs, H-3α), 76.0] and a second at C-16 [δ 5.19 (brs, H-16β), 75.3], and COOR groups, C-28 (δ 176.9) and C-23 (δ 178.7), were also observed. A tetrasaccharide moiety was inferred by the presence of four β-anomeric protons, at δ 6.41 (d, J = 8.4 Hz, H'), 6.20 (d, J = 7.8 Hz, H″), 4.92 (d, J = 7.8 Hz, H‴), and 5.28 (d, J = 7.8 Hz, H⁗), which correlated with carbons at δ 97.4 (C-1′), 96.5 (C-1″), 103.5 (C-1‴), and 107.0 (C-1⁗) in the HMQC spectrum. The β-anomeric

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Table 1. ^{13}C NMR Data for Aglycon Moieties of **1**−**7**

position	**1**[a]	**2**[b]	**3**[a]	**4**[b]	**5**[a]	**6**[a]	**7**[a]
1	39.8 t	40.6 t	38.7 t	38.4 t	38.8 t	39.0 t	38.8 t
2	29.2 t	28.5 t	27.3 t	26.3 t	27.0 t	27.5 t	26.2 t
3	76.0 d	77.1 d	74.9 d	75.0 d	75.0 d	75.1 d	84.9 d
4	56.0 s	55.4 s	55.0 s	54.3 s	55.0 s	55.1 s	53.3 s
5	53.3 d	53.4 d	52.1 d	51.3 d	52.2 d	52.2 d	52.1 d
6	22.1 t	19.4 t	23.0 t	20.7 t	21.1 t	21.3 t	23.2 t
7	34.0 t	34.0 t	33.7 t	32.1 t	32.6 t	33.0 t	33.9 t
8	41.2 s	41.8 s	40.0 s	39.6 s	40.1 s	40.4 s	40.2 s
9	50.6 d	48.5 d	48.1 d	48.1 d	47.2 d	47.5 d	48.3 d
10	38.1 s	38.2 s	36.7 s	36.1 s	36.7 s	37.0 s	36.7 s
11	24.7 d	22.9 d	23.6 d	23.1 d	23.5 d	23.8 d	23.8 d
12	123.2 d	124.1 d	122.4 d	122.3 d	123.0 d	123.3 d	122.7 d
13	145.5 s	145.4 s	144.0 s	143.4 s	144.2 s	144.6 s	144.0 s
14	42.8 s	43.3 s	41.9 s	41.5 s	41.8 s	42.0 s	42.1 s
15	37.1 t	37.1 t	28.1 t	27.5 t	35.8 t	36.0 t	28.2 t
16	75.3 d	75.6 d	23.0 t	22.7 t	76.3 d	74.0 d	21.3 t
17	49.9 s	49.1 s	46.8 s	47.1 s	48.8 s	49.1 s	47.0 s
18	42.0 d	42.7 d	41.5 d	41.1 d	41.0 d	41.2 d	41.7 d
19	48.3 t	47.2 t	46.0 t	46.6 t	46.9 t	47.2 t	46.1 t
20	31.7 s	32.0 s	30.6 s	30.1 s	30.6 s	30.7 s	30.7 s
21	36.9 t	36.9 t	32.6 t	33.4 t	35.6 t	35.8 t	32.8 t
22	33.0 t	32.7 t	32.0 t	31.8 t	31.9 t	31.9 t	32.3 t
23	178.7 s	182.2 s	177.7 s	180.1 s	177.6 s	177.6 s	180.4 s
24	13.0 q	12.3 q	11.8 q	10.2 q	11.9 q	12.0 q	12.5 q
25	17.2 q	17.2 q	16.0 q	15.0 q	16.1 q	16.3 q	16.0 q
26	18.3 q	18.5 q	17.1 q	16.3 q	17.2 q	17.4 q	17.3 q
27	28.0 q	28.0 q	25.8 q	25.0 q	26.9 q	27.1 q	26.0 q
28	176.9 s	177.7 s	176.1 s	176.4 s	175.6 s	176.0 s	176.3 s
29	34.0 q	34.5 q	32.9 q	32.1 q	33.0 q	33.0 q	33.0 q
30	25.5 q	25.8 q	23.5 q	22.5 q	24.9 q	24.7 q	23.7 q

[a] Measured in pyridine-d_5. [b] Measured in methanol-d_4.

configurations of the glucopyranose units were determined from their $^3J_{H1,H2}$ coupling constants (7.8−8.4 Hz). The sugar unit was confirmed to be glucose by co-TLC with standard sugars after hydrolysis, and the D-configuration was proved by GC-MS results of a derivatizated sample. The β-anomeric configurations for the glucopyranose units were determined from their $^3J_{H1,H2}$ coupling constants (7.8−8.4 Hz).[3] The HMBC correlations of C-23 (δ 178.7)/ H-1′, C-28 (δ 176.9)/ H-1″, C-6″ (δ 70.3)/H-1‴, and C-2‴ (δ 83.9)/ H-1⁗ were used to determine the sugar moieties as 23-*O*-β-D-glucopyranosyl and 28-*O*-{[β-D-glucopyranosyl(1→2)][β-D-glucopyranosyl(1→6)]}-β-D-glucopyranosyl ester units, respectively. The signals of a *tert*-methyl group at δ_H 1.69 (3H, s, H-6⁗) and δ_C 28.4 (C-6⁗), two overlapped methylenes δ 3.59 (2H × 2, s, H-2⁗ and H-4⁗) and δ 41.2 (C-2⁗ and C-4⁗), two overlapped carbonyl groups δ 172.6 (C-1⁗ and C-5⁗), and one quaternary carbon at δ 71.0 (C-3⁗) helped construct the 3-hydroxyl-3-methylglutaryl (HMG) moiety, which was confirmed from the HMBC spectrum. The esterification position of the HMG unit at C-6⁗ was also suggested by the HMBC correlation of C-1⁗ (δ 172.6) and H-6⁗ (δ 4.65).

To establish the absolute configuration of the HMG unit,[14] its ester functional group was selectively reduced by $LiEt_3BH$ in dry THF in an ice bath with an inflow of high-purity Ar. The reduced product was then hydrolyzed, and the acid hydrolysate was stirred at room temperature under high-purity Ar to allow the formation of a lactone. The reaction mixture was partitioned with an equal amount of EtOAc. The EtOAc solution was concentrated to dryness and afforded a mevalonolactone, identified by ^{1}H NMR and ESIMS. The retention time of the sample was 22.9 min by chiral HPLC, while those of authentic (3*S*)- and (3*R*)-mevalonolactones were 18.9 and 22.8 min, respectively. Therefore, the HMG group was identified as (3*S*)-3-hydroxyl-3-methylglutarate. Hence, the entire structure of **1** was elucidated as 23-*O*-β-D-glucopyranosyl-3β,16α-dihydroxyolean-12-en-23α,28β-dioic acid 28-*O*-{[β-D-glucopyranosyl(1→2)][β-D-6-*O*-((3*S*)-3-hydroxyl-3-methylglutaryl)glucopyranosyl(1→6)]}-β-D-glucopyranoside.

Dianversicoside B (**2**) was purified as a white, amorphous powder. Its HRESIMS positive ion at *m*/*z* 1317.5709 $[M + Na]^+$ (calcd 1317.5722) indicated that the compound has a molecular formula of $C_{60}H_{94}O_{30}$. The proton and carbon signals in the ^{1}H NMR and ^{13}C NMR spectra of **2** were similar to those of **1** except for the downfield shifted carbon signal of C-23 at δ 182.1, suggesting de-esterification of the C-23-COOH group. By comparing the NMR data of the sugar chains attached to C-28 of **2** with those of **1**, it was observed that there is one more glucose residue and an additional glycosylation shift at Glc′-C-3 (δ 88.0) in **2**. These results suggested that the sugar moiety in **2** is [β-D-glucopyranosyl(1→3)]{[β-D-glucopyranosyl(1→2)][β-D-6-*O*-((3*S*)-3-hydroxyl-3-methylglutaryl)glucopyranosyl(1→6)]}-β-D-glucopyranoside. This was confirmed by the HMBC correlations between H-1′ (δ 5.40) and C-28 (δ 177.7), H-1″ (δ 4.60) and C-3′ (δ 88.0), H-1‴ (δ 4.50) and C-6′ (δ 70.8), and H-1⁗ (δ 4.68) and C-2⁗ 81.8). Moreover, the location of the HMG group at the C-6‴ position of the glucosyl residue in **2** was also determined by the HMBC cross-peak from the glucosyl H-6‴ δ 4.21 to the HMG carbonyl carbon signal at δ 173.1. The absolute configuration of HMG was established as 3*S* by the same method as for **1**. The structure of **2** was shown to be 3β,16α-dihydroxyolean-12-en-23α,28β-dioic acid 28-*O*-[β-D-glucopyranosyl(1→3)]{[β-D-glucopyranosyl(1→2)] [β-D-6-*O*-((3*S*)-3-hydroxyl-3-methylglutaryl)glucopyranosyl(1→6)]}-β-D-glucopyranoside.

Dianversicoside C (**3**) was obtained as a white, amorphous powder. The HRESIMS showed a molecular ion at *m*/*z* 1439.6384 $[M - H]^-$ (calcd 1439.6336) and indicated a molecular formula of $C_{66}H_{104}O_{34}$. On comparing its 1D and 2D NMR data with those of **1**, the aglycon moiety of **3** was revealed to be identical to that of **1** except for the absence of a 16-hydroxy group signal. This was supported by the upfield shifts of C-15 (δ 28.1) and C-16 (δ 23.0) in the ^{13}C NMR spectrum of **3** with respect to the corresponding value of δ 37.1 and 75.3 in **1**. The aglycon moiety of **3** was thus identified as gypsogenic acid.[5] Comparison of the 1D and 2D NMR data of **3** with those of **1** and **2** suggested that compound **3** has the same sugar units at C-23 as **1** and the same glycosylation pattern at C-28 as **2**. The absolute configuration of HMG was also established to be 3*S*. Thus, the structure of **3** was determined to be 23-*O*-β-D-glucopyranosyl-3β-hydroxyolean-12-en-23α,28β-dioic acid 28-*O*-[β-D-glucopyranosyl(1→3)]{[β-D-glucopyranosyl(1→2)][β-D-6-*O*-((3*S*)-3-hydroxy-3-methylglutaryl)glucopyranosyl(1→6)]}-β-D-glucopyranoside.

Dianversicoside D (**4**) was isolated as a white, amorphous powder, and its molecular formula determined as $C_{60}H_{94}O_{29}$ from the HRESIMS at *m*/*z* 1301.5786 $[M + Na]^+$ (calcd 1301.5773). Comparing the ^{1}H NMR and ^{13}C NMR data of **4** with those of **3** showed that **4** had the same aglycon as **3**, but differed in its saccharide units. On comparison of the ^{1}H NMR and ^{13}C NMR data with those of **2**, the sugar linkage at position C-28 of **4** was found to be the same as in **2**. The absolute configuration of HMG was established as 3*S*. The structure of **4** was determined as 3β-hydroxyolean-12-en-23α,28β-dioic acid 28-*O*-[β-D-glucopyranosyl(1→3)]{[β-D-glucopyranosyl(1→2)][β-D-6-*O*-((3*S*)-3-hydroxy-3-methylglutaryl)glucopyranosyl(1→6)]}-β-D-glucopyranoside.

Dianversicoside E (**5**), a white, amorphous powder, exhibited a $[M + Na]^+$ peak at *m*/*z* 1335.5883 in the HRESIMS (calcd 1335.5828), corresponding to an elemental formula of $C_{60}H_{96}O_{31}$. The NMR spectrum showed that there was no substitution at C-6⁗ of Glc⁗, while the C-23 and C-28 sugar chains of **5** were similar to those of **3** except for the absence of a HMG group. This indicated that **5** possesses the same aglycon moiety as **1**. From these findings, the structure of **5** was determined to be 23-*O*-β-D-glucopyranosyl-3β,16α-dihydroxyolean-12-en-23α,28β-dioic acid 28-*O*-[β-D-glucopyranosyl(1→3)]{[β-D-glucopyranosyl(1→2)][β-D-glucopyranosyl(1→6)]}-β-D-glucopyranoside.

Table 2. ^{1}H NMR Data for Aglycon Moieties of **1**−**7** (*J* in Hz)

position	**1**[a]	**2**[b]	**3**[a]	**4**[b]	**5**[a]	**6**[a]	**7**[a]
1	1.12, 1.59 m	1.08, 1.65 m	1.02, 1.50 m	1.10, 1.70 m	1.17, 1.60 m	1.12, 1.61 m	1.52, 1.58 m
2	1.69 m	1.62 m	1.87 m	1.64 m	1.90 m	1.87, 1.91 m	1.92, 2.20 m
3	4.65 br s	3.98 br s	4.62, br s	3.99 br s	4.64 br s	4.64 br s	4.61 br s
5	1.91 m	1.50 m	1.82 m	1.53 m	1.86 m	1.91 m	1.89 m
6	1.72 m	1.98 m	1.87, 1.92 m	1.10 m	1.48, 1.69 m	1.54, 1.61 m	1.83 m
7	2.04, 2.28 m	1.26, 1.52 m	1.07 m	1.26, 1.50 m	1.25, 1.64 m	1.35, 1.61 m	1.02, 1.22 m
9	2.70 m	1.68 m	1.68 m	1.66 m	1.86 m	1.87 m	1.74 m
11	1.88, 1.99 m	1.90 m	1.87, 1.92 m	1.92 m	1.87 m	2.0 m	1.89, 1.94 m
12	5.54 br s	5.32 br s	5.37 br s	5.26 br s	5.57 br s	5.55 br s	5.38 br s
15	1.62, 2.42 m	1.17 m	0.97, 1.74 m	ND[c]	2.32 m	1.61, 2.41 m	1.73 m
16	5.19 br s	4.53 br s	1.43, 1.67 m	1.71 m	4.06 br s	5.18 br s	1.43 m
18	3.43 m	3.0 dd,(2.0, 13.0)	3.10 m	2.86 dd, (3.4, 12.4)	3.42 m	3.43 m	3.20 m
19	3.08 m	1.16, 2.25 t, (14.0)	1.63 m	1.70 m	1.28, 2.70 m	1.29, 2.69 m	1.65 m
21	1.22, 2.32 m	1.35 m	1.53, 1.75 m	1.39 m	1.17, 2.31 m	1.18, 2.23	1.23, 1.58 m
22	1.32, 1.71 m	1.97 m	1.75, 1.84 m	1.69 m	2.01, 2.23 m	2.04, 2.28 m	1.58, 1.78 m
24	1.59 s	1.16 s	1.58 s	1.12 s	1.58 s	1.58 m	1.57 m
25	0.96 s	0.93 s	0.92 s	0.96 s	0.97 s	0.99 s	0.93 s
26	1.06 s	0.89 s	1.00 s	0.80 s	1.04 s	1.09 s	1.04 s
27	1.65 s	1.36 s	1.06 s	1.17 s	1.65 s	1.66 s	1.15 s
29	0.90 s	0.78 s	0.80 s	0.92 s	0.89 s	0.90 s	0.82 s
30	1.01 s	0.92 s	0.85 s	0.94 s	0.91 s	0.98 s	0.83 s

[a] Measured in pyridine-d_5. [b] Measured in methanol-d_4. [c] ND, not detected.

Table 3. ^{13}C NMR Data for Sugar Moieties of **1**−**7**

position	**1**[a]	**2**[b]	**3**[a]	**4**[b]	**5**[a]	**6**[a]	**7**[a]
Glc′	23-*O*-		23-*O*-		23-*O*-	23-*O*-	3-*O*-ara
1′	97.4		96.4		96.4	96.5	106.4
2′	75.0		74.2,		73.0	74.3	74.2
3′	79.0		78.5		78.5	78.5	72.7
4′	71.5		71.3		71.1	71.2	69.2
5′	80.3		79.4		79.4	79.3	66.3
6′	62.9		61.9		61.9	62.2	
Glc″	28-*O*-	28-*O*-	28-*O*-	28-*O*-	28-*O*-	28-*O*-	28-*O*-
1″	96.5	95.8	94.4	93.6	94.8	95.7	94.8
2″	75.0	74.2	72.9	72.1	74.2	74.1	73.1
3″	78.9	88.0	88.5	85.9	87.5	78.6	88.1
4″	71.2	69.4	70.8	67.3	69.1	71.0	69.0
5″	78.6	78.0	76.7	75.9	78.2	77.1	76.8
6″	70.3	70.8	70.0	68.7	68.7	69.4	68.9
Glc‴							
1‴	103.5	105.6	105.8	103.5	105.7	102.7	105.8
2‴	83.9	76.0	75.9	74.0	73.9	84.3	76.2
3‴	79.5	78.5	77.7	76.4	77.9	78.1	78.1
4‴	71.9	71.4	70.9	69.3	70.9	70.5	71.2
5‴	79.4	78.7	76.2	76.6	78.4	78.2	78.3
6‴	65.4	63.3	62.0	61.4	62.1	61.1	62.4
Glc⁗							
1⁗	107.0	104.2	102.4	102.1	102.4	106.0	102.6
2⁗	75.9	81.8	82.7	79.7	83.5	76.2	83.6
3⁗	77.1	75.9	77.9	73.8	77.8	78.1	78.0
4⁗	71.0	72.1	70.6	70.0	70.9	70.7	71.0
5⁗	79.6	78.8	78.4	76.7	78.3	78.7	78.2
6⁗	63.0	65.3	63.9	63.2	62.3	61.1	62.2
Glc⁗′							
1⁗′		105.0	105.4	102.9	105.6		105.8
2⁗′		76.1	75.2	74.1	76.7		75.7
3⁗′		78.6	77.9	76.5	78.0		78.0
4⁗′		72.2	71.1	70.1	70.6		71.3
5⁗′		79.1	78.2	77.0	78.4		78.5
6⁗′		63.2	62.3	61.2	62.0		62.3
HMG							
1⁗″	172.6	173.1	171.7	171.0			
2⁗″	41.2	48.1	47.5	45.0			
3⁗″	71.0	72.2	69.6	70.1			
4⁗″	41.2	46.7	47.5	44.6			
5⁗″	172.6	175.7	171.7	173.5			
3⁗″CH_3	28.4	28.5	28.4	26.4			

Dianversicoside F (**6**), a white, amorphous powder, showed a $[M + Na]^+$peak at *m/z* 1173.5263 in the HRESIMS (calcd 1173.5300), ascribable to a molecular formula of $C_{59}H_{94}O_{29}$. The two aglycon moieties in **6** and **1** were judged to be the same by comparing the NMR data of these compounds. The NMR data revealed that the signals of the sugar moieties at C-23 and C-28 were the same as those in **5**. Therefore, the structure of **6** was determined as 23-*O*-*β*-D-glucopyranosyl-3*β*,16α-dihydroxyolean-12-en-23α,28*β*-dioic acid 28-*O*-{[*β*-D-glucopyranosyl(1→2)] [*β*-D-glucopyranosyl(1→6)]}-*β*-D-glucopyranoside.

Dianversicoside G (**7**) was obtained as a white, amorphous powder. The HRESIMS results showed a quasimolecular ion peak at *m/z* 1289.5730 (calcd 1289.5773), indicating that **7** has a molecular formula of $C_{59}H_{94}O_{29}$. NMR and MS data analysis indicated the aglycon of **7** to be gypsogenic acid. Five sugar anomeric proton signals (*δ* 4.95, 4.98, 5.30, 5.34, 6.18) were observed. Acid hydrolysis of **7** gave arabinose and glucose, when compared with standard sugars by co-TLC (*n*-BuOH−HOAc−H_2O, 4:1:1). All protons were assigned sequentially within each saccharide system with the aid of the TOCSY spectrum, leading to the assignment of the sugar moiety. HMBC correlations were observed between H-1′ (*δ* 4.95) and C-3 (*δ* 84.9), H-1″ (*δ* 6.18) and C-28 (*δ* 176.3), H-1‴ (*δ* 5.34) and C-3′ (*δ* 88.1), H-1⁗ (*δ* 4.98) and C-6‴ (*δ* 68.9), and H-6⁗′ (*δ* 5.30) and C-2⁗′ (*δ* 83.6). The configurations of the anomeric positions of the glucose and arabinose moieties were assigned as *β* and α, respectively, from the coupling constants of the anomeric proton signals at *δ* 6.18 (*J* = 7.8 Hz), 5.34 (*J* = 7.8 Hz), 4.98 (*J* = 7.8 Hz), 5.30 (*J* = 7.8 Hz), and 4.95 (*J* = 6.6 Hz).[3] Thus, the structure of **7** was determined as 3-*O*-α-L-arabinopyranosylolean-12-en-23,28-dioic acid 28-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranoside.

Compounds **1**−**9** were evaluated for their cytotoxic activities against human fetal lung fibroblasts (HFL-I), human umbilical vascular endothelial cells 304 (EVC-304), human gastric cancer cells 803 (BGC-803), human breast cancer (MCF-7), and a human hepatocellular carcinoma cell line (Hep G2), with cisplatin as a positive control. In general, these compounds exhibited more potent activities against HFL-I, EVC-304, and BGC-803 cells than against MCF-7 and Hep G2 cells (Table 5).

Experimental Section

General Experimental Procedures. Optical rotations (ORD) were measured on a JASCO P-1020 spectropolarimeter. IR spectra were run on a Bruker Tensor 27 spectrophotometer. 1D and 2D NMR spectra were recorded in C_5D_5N and CD_3OD at 25 °C on a Bruker AV-500 NMR (^{1}H: 500 MHz, ^{13}C: 125 MHz) and a AV-600 NMR (^{1}H: 600 MHz, ^{13}C: 150 MHz) spectrometer with tetramethylsilane (TMS) as an internal standard, in which coupling constants are given in Hz. Mass spectra were obtained on a MS Agilent 1100 Series LC/MSD ion trap mass spectrometer (ESIMS) and a MS Agilent 1100 Series LC/MSD TOF (time-of-flight) system. Analytical HPLC was performed on an Agilent 1100 series system with a DAD UV detector and a Shimadzu

Table 4. ^{1}H NMR Data for Sugar Moieties of Compounds **1**−**4** (*J* in Hz)

position	**1**[a]	**2**[b]	**3**[a]	**4**[b]	**5**[a]	**6**[a]	**7**[a]
Glc′	23-*O*-		23-*O*-		23-*O*-	23-*O*-	3-*O*-ara
1′	6.41 d (8.4)		6.43 d (7.8)		6.41 d (7.8)	6.38 d (8.0)	4.95 d (6.6)
2′	4.17 m		4.19 m		4.20 m	4.16 m	4.07 m
3′	4.26 m		3.91 m		3.90 m	4.22 m	4.38 m
4′	4.02 m		4.20 m		4.15 m	4.25 m	4.25 m
5′	3.98 m		3.98 m		3.98 m	3.97 m	3.74, 4.28 m
6′	4.32, 4.51, m		4.19, 4.35 m		4.39, 4.52 m	4.32, 4.50 m	
Glc″	28-*O*-	28-*O*-	28-*O*-	28-*O*-	28-*O*-	28-*O*-	28-*O*-
1	6.20 d (7.8)	5.40 d (8.3)	6.18 d (7.8)	5.44 d (8.2)	6.17 d (8.4)	6.18 d (7.5)	6.18 d (7.8)
2″″	4.17 m	3.45 m	4.26 m	3.59 m	4.16 m	4.22 m	4.27 m
3″	4.17 m	3.65 m	4.27 m	3.71 m	4.26 m	4.18 m	4.28 m
4″	4.32 m	3.94 m	3.98 m	3.98 m	4.26 m	4.19 m	4.35 m
5″	4.05 m	3.52 m	4.0 m	3.58 m	3.70 m	4.01 m	4.09 m
6″	4.31, 4.61 m	3.85, 4.10 m	4.15, 4.35 m	3.88, 4.12 m	4.47 m	4.30, 4.52 m	4.27, 4.50 m
Glc‴							
1‴	4.92 d (7.8)	4.60 d (7.6)	5.31 d (7.8)	4.62 d (7.6)	5.33 d (7.8)	4.95 d (7.5)	5.34 d (7.8)
2‴	4.01 m	3.45 d	4.05 m	3.38 m	4.15 m	3.98 m	4.08 m
3‴	3.90 m	3.40 m	4.15 m	3.45 m	3.26 m	4.12 m	4.15 m
4‴	4.17 m	3.30 m	4.15 m	3.38 m	4.15 m	4.22 m	4.13 m
5‴	3.72 m	3.42 m	4.01 m	3.38 m	3.90 m	3.75 m	3.91 m
6‴	4.64 m 4.92 m	3.73 dd (6.0, 12.0), 3.89 m	4.32, 4.51 m	3.57, 3.75 m	4.39, 4.52 m	4.31 m	4.26, 4.56 m
Glc⁗							
1⁗	5.28 d (7.8)	4.50 d (7.4)	5.10 d (7.8)	4.52 d (8.0)	4.97 d (7.8)	5.25 d (7.5)	4.98 d (7.8)
2⁗	4.65 m	3.57 m	4.19 m	3.61 m	4.02 m	4.02 m	4.07 m
3⁗	4.05 m	3.35 m	4.15 m	3.48 m	4.26 m	4.12 m	4.26 m
4⁗	4.51 m	3.35 m	4.15 m	3.38 m	4.15 m	4.19 m	4.19 m
5⁗	3.90 m	3.58 m	3.91 m	3.62 m	3.70 m	3.88 m	3.78 m
6⁗	4.32, 4.51 m	4.21 dd (6.0, 12.0), 4.42 m	5.20 m	4.23 dd (6.0, 12.0), 4.44 dd, (1.7, 12.0)	4.31, 4.42 m	4.31 m	4.32, 4.38 m
Glc′′′′′							
1′′′′′	4.68 d (7.6)	5.31 d (7.8)	4.68 d (7.5)	5.28 d (7.8)		5.30 d (7.8)	
2′′′′′		3.45 m	4.05 m	3.48 m	4.06 m		4.07 m
3′′′′′		3.35 m	4.19 m	3.42 m	4.15 m		4.15 m
4′′′′′		3.35 m	4.15 m	3.38 m	4.15 m		4.17 m
5′′′′′		3.30 m	4.27 m	3.33 m	3.90 m		3.91 m
6′′′′′		3.67 dd (6.0, 11.5), 3.86 m	4.19, 4.35 m	3.72, 3.92 m	4.39, 4.52 m		4.33, 4.46 m
HMG							
2′′′′′′	3.59 s	2.73 d (14.5)	3.60 s	2.72 d (13.0)			
4′′′′′′	3.59 s	2.70 d (14.5)	3.60 s	2.66 d (13.0)			
3′′′′′′CH_3	1.69 s	1.38 s	1.64 s	1.38 s			

Table 5. Cytotoxic Activity of Compounds **1**−**9** (IC_{50}, μM)

compound	HFL-I	EVC-304	BGC-803	MCF-7	Hep G2
1	2.8	8.4	6.2	>10	>10
2	3.1	7.7	5.7	>10	>10
3	3.2	3.6	3.1	>10	>10
4	3.4	3.9	2.9	>10	>10
5	3.5	9.1	7.4	>10	>10
6	4.4	9.6	9.7	>10	>10
7	3.3	6.2	6.8	>10	>10
8	7.8	4.1	5.3	>10	>10
9	3.8	8.7	9.4	>10	>10
cisplatin	0.11	0.49	6.8	3.9	7.7

LC 20 system with a UV detector. Preparative HPLC was carried out on an Agilent 1100 series system equipped with a Shimpack ODS column, 5 μm; 20.0 = 250 mm. Gas chromatography was done on a Varian CP-3800 gas chromatograph equipped with a Saturn 2200 mass detector and a CP-sil 5 CB capillary column (30 m, 0.25 mm i.d., 0.25 μm). L-Cysteine methyl ester hydrochloride, trimethylchlorosilane, and the authentic standard compounds L- and D-arabinose, L- and D-glucose, and (3*R*)- and (3*S*)-mevalonolactone were purchased from Sigma-Aldrich (Shanghai). TLC was performed on precoated silica gel 60 F_{254} (Qingdao Haiyang Chemical Co., Ltd.) plates, and detection of saponins was achieved using 20% H_2SO_4−EtOH. Silica gel H (Qingdao Haiyang Chemical Co., Ltd.), Sephadex LH-20 (20−100 mesh, Pharmacia), ion-exchange resin Amberlite MB-3, and ODS-C_{18} (40−63 μm, Fuji) were used for column chromatography. Tetrahydrofuran (THF) was distilled from sodium-benzophenone ketyl. $LiEt_3BH$ and Ar were purchased from Beijing Sanshengtengda Co., Ltd., and Nanjing Special Gases Factory Co., Ltd., respectively. A Sunrise (Tecan) microplate spectrophotometer was used.

Plant Material. The plant material was collected in Changqing, Shangdong Province, People's Republic of China, in June 2004 and identified as *Dianthus versicolor* Fisch. ex. Link. by Prof. Ming-jian Qin, Department of Medicinal Botany, China Pharmaceutical University. A voucher specimen was deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and Isolation. The dried whole plants (20.0 kg) of *D. versicolor* were ground and exhaustively extracted with 80% ethanol (200 L × 3). The combined extracts were concentrated to dryness. The residue was suspended in H_2O (3.0 L) and partitioned with petroleum ether (5 L × 6), EtOAc (4 L × 6), and *n*-BuOH (3 L × 6), successively. The *n*-BuOH-soluble residue (105.0 g) was chromatographed on a silica gel column ($CHCl_3$−MeOH, 20:1; 10:1; 5:1; 2:1; 1:1; 100% MeOH) to afford fractions 1−6. Fractions 2−6 (82.8 g) were combined and subjected to MCI gel column chromatography and eluted with water and 30%, 50%, 70%, 90%, and 100% MeOH. The 70% and 90% MeOH elutions were combined and gave a total saponin fraction (1.01 g). This was separated over ODS C_{18}, eluted with 60%, 70%, 80%, and 90% MeOH, to afford fractions A−D. Fraction A (80 mg) was purified by preparative C_{18} HPLC (28% MeCN containing 0.03% AcOH) and gave **1** (15 mg, t_R 30.3 min), **6** (18 mg, t_R 35.0 min), and **9** (9 mg, t_R 38.3 min). Eluting with 30% MeCN containing 0.03% AcOH, **5** (17 mg, t_R 28.1 min) and **7** (16 mg, t_R 34.6 min) were obtained from fraction B (120 mg). Fraction C (200 mg) was subjected to Sephadex LH-20 column chromatography with methanol as the mobile phase to obtain dianchinenoside A (5 mg), dianchinenoside C (2 mg), and dianoside G (1 mg). Other eluents from Sephadex LH-20 separation were combined and then separated, using preparative C_{18} HPLC with 75% MeOH containing 0.03% AcOH to give compounds **3** (11 mg, t_R 40.0 min), **2** (20 mg, t_R 48.0 min), **4** (26 mg, t_R 52.0 min), and **8** (30 mg, t_R 80 min). Fraction D (30 mg) was subjected repeatedly to Sephadex LH-20 column chromatography with methanol as mobile phase to afford dianoside H (1 mg) and hainanenside (1 mg). Other eluents were combined and further purified by preparative C_{18} HPLC (80% MeOH containing 0.03% AcOH) to yield dianchinenoside B (1 mg, t_R 27.3 min) and dianchinenoside D (1 mg, t_R 29.5 min).

Dianversicoside A (1): white, amorphous powder; $[\alpha]_D^{25}$ +19.6 (*c* 0.06, MeOH); IR (KBr) ν_{max} 3441, 2933, 1729, 1640, 1071 cm^{-1}; ^{1}H

NMR and ^{13}C NMR, see Tables 1−4; ESIMS *m/z* 1293.7 $[M - H]^-$; HRESIMS *m/z* 1317.5732 $[M + Na]^+$ (calcd for $C_{60}H_{94}O_{30}$ Na, 1317.5722).

Dianversicoside B (2): white, amorphous powder; $[\alpha]_D^{25}$ −12.3 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3441, 2942, 1722, 1642, 1074 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1−4; ESIMS *m/z* 1293.7 $[M - H]^-$; HRESIMS *m/z* 1317.5709 $[M + Na]^+$ (calcd for $C_{60}H_{94}O_{30}Na$, 1317.5722).

Dianversicoside C (3): white, amorphous powder; $[\alpha]_D^{25}$ +22.8 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3432, 2924, 1722, 1640, 1075 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1−4; ESIMS *m/z* 1439.7 $[M - H]^-$; HRESIMS *m/z* 1439.6384 $[M - H]^-$ (calcd for $C_{66}H_{104}O_{34}$,1439.6336).

Dianversicoside D (4): white, amorphous powder; $[\alpha]_D^{25}$ +8.4 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3424, 2940, 1723, 1640, 1385, 1075 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1−4; ESIMS *m/z* 1277.9 $[M - H]^-$; HRESIMS *m/z* 1301.5786 $[M + Na]^+$ (calcd for $C_{60}H_{94}O_{29}$ Na, 1301.5773).

Dianversicoside E (5): white, amorphous powder; $[\alpha]_D^{25}$ −16.8 (*c* 0.08, MeOH); IR (KBr) ν_{max} 3442, 2933, 1641, 1072 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1−4; ESIMS *m/z* 1311.5 $[M - H]^-$; HRESIMS *m/z* 1335.5883 $[M + Na]^+$ (calcd for $C_{60}H_{96}O_{31}Na$, 1335.5883).

Dianversicoside F (6): white, amorphous powder; $[\alpha]_D^{25}$ +8.5 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3424, 2927, 1640, 1074 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1−4; ESIMS *m/z* 1149.5 $[M - H]^-$; HRESIMS *m/z* 1173.5263 $[M + Na]^+$ (calcd for $C_{54}H_{86}O_{26}Na$, 1173.5300).

Dianversicoside G (7): white, amorphous powder; $[\alpha]_D^{25}$ −6 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3432, 2924, 1772, 1640, 1075 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1−4; ESIMS *m/z* 1265.4 $[M - H]^-$; HRESIMS *m/z* 1289.5730 $[M + Na]^+$ (calcd for $C_{59}H_{94}O_{29}Na$, 1289.5773).

Acid Hydrolysis of 1−7 and Determination of the Absolute Configuration of Monosaccharides. Compounds **1**−**7** (3.0 mg each) were dissolved in 1 mL of 1 N HCl (dioxane−H_2O, 1:1), heated at 80 °C for 2 h in a water bath. After the removal of the dioxane under vacuum, the solutions were extracted with EtOAc (1 mL × 3). The EtOAc solutions were washed with H_2O and concentrated to give amorphous powders. 3β,16α-Dihydroxyolean-12-en-23,28-dioic acid was identified in the samples of compounds **1**, **2**, **5**, and **6**, while gypsogenic acid was confirmed in **3**, **4**, and **7** by co-TLC with authentic samples. The water-soluble parts containing monosaccharides were neutralized by passing through ion-exchange resin (Amberlite MB-3) columns. The neutralized solutions were concentrated to dryness, and these residues dissolved in pyridine (each 2 mL). L-Cysteine methyl ester hydrochloride (2 mg each) was added to pyridine solutions subsequently. The resulting mixtures were heated at 60 °C for 1 h, then trimethylchlorosilane (0.5 mL each) was added, and the mixture was heated at 60 °C for 30 min. The reaction solutions were then concentrated to dryness, and the residues suspended in water (1 mL each) and extracted with *n*-hexane (1 mL × 3).[15] The supernatant was subjected to GC-MS analysis under the following conditions: Varian CP-3800 gas chromatograph equipped with a Saturn 2200 mass detector (detection temperature 220 °C). Column, CP-Sil 5 CB capillary column (30 m, 0.25 mm i.d., 0.25 μm); column temperature, 150−260 at 8 °C/min; carrier gas was He (0.8 mL/min), split ratio 1/10; injection temperature, 250 °C; injection volume, 0.5 μL. The absolute configurations of the glucose units in compounds **1**−**6** were confirmed as D-glucose by comparison of the retention times with standard glucose samples (14.01 min). Similarly, the absolute configurations of the sugar units in compound **7** were determined as L-arabinose and D-glucose by comparison of the retention times with standard samples: L-arabinose (12.88 min) and D-glucose (14.01 min), respectively.

Determination of the Absolute Configuration of HMG in Compounds 1−4. Reductive hydrolysis reactions of **1**−**4** were worked up as reported,[14] with some modifications. A solution of $LiEt_3BH$ in dry THF (100 μL) was added to solutions of **1** (10.0 mg), **2** (15.0 mg), **3** (5.0 mg), and **4** (15.0 mg), respectively, in dry THF (400 μL), under an ice bath with an inflow of Ar. The reaction mixtures were stirred under ice in Ar gas for 30 min. After adding an excessive amount of H_2O to each reaction mixture, 0.1 M HCl was added to adjust the pH to 3. The reaction mixtures were then stirred at room temperature under Ar gas for 48 h to allow the lactones to form. Each reaction mixture was partitioned with EtOAc (1.0 mL × 3), and the EtOAc layer containing a mevalonolactone was analyzed by chiral HPLC with a column of CHIRALPAK AS-H (46 × 150 mm; Daicel) using a mobile phase of hexane−2-propanol, 9:1; wavelength, 220 nm; flow rate, 1.0 mL/min, column temperature, 35 °C. Authentic (3*S*)- and (3*R*)-mevalonolactones had retention times of 18.9 and 22.8 min, respectively. The retention times of these samples were all at 22.9 min, so the HMG group was elucidated as (3*S*)-3-hydroxy-3-methylglutaryate.

Cytotoxicity Assay. The compounds to be tested were dissolved in DMSO and diluted with RPMI-1640 medium. The final concentration of the DMSO solution was less than 1%. EVC-304 and BGC-803 were routinely cultivated at 37 °C in an atmosphere of 5% CO_2 in RPMI-1640 medium (Gibco) supplemented with 10% fetal calf serum (Sijiqing Biomaterial Co., Hangzhou, People's Republic of China) and subcultured twice per week to maintain continuous logarithmic growth. HFL-I, MCF-7, and Hep G2 cells were grown in DMEM supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in an atmosphere of 5% CO_2. The cells (10^4 cells/well/100 μL) were cultured in 96-well microtiter plates and allowed to adhere for 24 h before the compounds were introduced. Serial drug dilutions were added to each culture. At the time of drug addition (parallel triplicate wells were set) and following 24 h of culture, MTT (10 μL; 5 mg/mL) was added to each well. After a further 4 h of incubation, DMSO (150 μL) was added to each well, and the formazan crystals were dissolved in DMSO. Absorbance was read out on a system plate reader (Sunrise Tecan) at 570 nm as a measure of cell viability.

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Supporting Information Available: Figures S1 and S2 showing ^1H NMR and ^{13}C NMR of **1**. Figures S3, S4, S5, S6, and S7 showing ^1H NMR, ^{13}C NMR, TOCSY, HSQC, and HMBC of **2**. Figures S8 and S9 showing ^1H NMR and ^{13}C NMR of **3**. Figures S10 and S11 showing ^1H NMR and ^{13}C NMR of **4**. Figures S12 and S13 showing ^1H NMR and ^{13}C NMR of **5**. Figures S14, S15, S16, S17, and S18 showing ^1H NMR, ^{13}C NMR, TOCSY, HSQC, and HMBC of **6**. Figures S19 and S20 showing ^1H NMR and ^{13}C NMR of **7**. Figure S21 showing the reaction proceeding from HMG group to mevalonelacton. Figure S22 showing the HPLC chiral chromatograms of authentic (*S*)- and (*R*)-mevalonolactone and S23 showing the HPLC chiral chromatogram of mevalonolactone obtained by chemical reaction from HMG of **1**. These materials are available free of charge via the Internet at http://pubs.acs.org.

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