Triterpene Glycosides from the Flower Petals of Sunflower (*Helianthus annuus*) and Their Anti-inflammatory Activity

Motohiko Ukiya,[†] Toshihiro Akihisa,*[,†] Ken Yasukawa,[‡] Kazuo Koike,[§] Akitomo Takahashi,[†] Takashi Suzuki,[‡] and Yumiko Kimura[‡]

College of Science and Technology, Nihon University, 1-8 Kanda Surugadai, Chiyoda-ku, Tokyo 101-8308, Japan, College of Pharmacy, Nihon University, 7-7-1 Narashinodai, Funabashi-shi, Chiba 274-8555, Japan, and School of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi-shi, Chiba 274-8510, Japan

Received January 24, 2007

Two new oleanane-type triterpene glycosides, named helianthosides 4 (**4**) and 5 (**5**), along with four known triterpene glycosides, helianthosides 1 (**1**), 2 (**2**), 3 (**3**), and B (**6**), were isolated from an *n*-butanol-soluble fraction of a methanol extract of sunflower (*Helianthus annuus*) petals. The structures of the two new compounds were determined on the basis of spectroscopic and chemical methods. Upon evaluation of compounds **1**−**6** for inhibitory activity against 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation (1.7 nmol/ear) in mice, all of the compounds tested exhibited marked anti-inflammatory activity, with ID_{50} values in the range 65−262 nmol per ear.

Sunflower (*Helianthus annuus* L.; Asteraceae) is cultivated primarily for its seeds, which yield the world's second most important source of edible oil. Prior to the use of the seeds as a food, other parts of the plant, notably the petioles and young flowers, were used as savory delicacies.[1] The seed oil, shoots, and herb tincture have been employed for anti-inflammatory, antipyretic, aperitif, astringent, cathartic, diuretic, emollient, expectorant, stimulant, vermifuge, and vulnerary purposes.[2] In the course of a study on the phytochemical and pharmacological constituents of Asteraceae species,[3−9] we have found that methanol (MeOH) extracts of the ligulate and tubular flower petals of sunflower possess marked anti-inflammatory activities on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced ear edema in mice.[4] We now report the isolation and characterization of two new (**4** and **5**) and four known (**1**−**3** and **6**) oleanane-type triterpene glycosides from an *n*-butanol (*n*-BuOH)-soluble fraction of a MeOH extract of the ligulate flower petals of sunflower along with an evaluation of their anti-inflammatory activity against TPA-induced inflammation in mice.

Results and Discussion

Six triterpene glycosides, helianthoside 1 (**1**), helianthoside 2 (**2**), helianthoside 3 (**3**), helianthoside 4 (**4**), helianthoside 5 (**5**), and helianthoside B (**6**), were isolated from an *n*-BuOH-soluble fraction of a MeOH extract of the ligulate flower petals of sunflower. Among these, **4** and **5** are new compounds, and characterization and spectroscopic analysis of these compounds were performed by data comparison with literature values.[10,11] The ^{13}C and ^{1}H NMR data for these compounds are shown in Tables 1 and 2, respectively. The ^{13}C and ^{1}H NMR signal assignments were aided by HMQC, HMBC, and $^{1}H-^{1}H$ COSY experiments. All other compounds were identified as helianthosides 1 (**1**),[10] 2 (**2**),[10,11] 3 (**3**),[10] and B (**6**),[10] on the basis of ^{1}H NMR and MS comparison with the corresponding compounds in the literature.

Compound **4** gave a quasi-molecular ion at *m*/*z* 1391.6459 [M + Na]$^+$, indicating a molecular weight of 1368, in agreement with the formula $C_{64}H_{104}O_{31}$ found by elemental analysis in the HRES-IMS. The IR spectrum showed the presence of an ester carbonyl group (1726 cm^{-1}) and an olefinic group (1641 cm^{-1}). In the ^{1}H NMR spectrum of the aglycon moiety of **4**, six tertiary methyls [δ_H 0.70 (s), 0.78 (s), 0.88 (s), 0.97(s), 0.99(s), and 1.38 (s)], a primary hydroxy methylene [δ_H 3.29 (d, J = 11.0 Hz) and 3.60 (d, J = 11.0 Hz)], two secondary hydroxy methines [δ_H 3.63 (dd, J = 4.4, 11.7 Hz) and 4.48 (br s)], and an olefinic methine [δ_H 5.36 (t, J = 3.8 Hz)] were observed. The ^{13}C NMR data of the aglycone moiety of compound **4** were essentially the same as those of compound **2**[10,11] except that the former exhibited the C-23 signal as an oxygen-bearing methylene signal at δ_C 64.9 (t) instead of as a methyl signal at δ_C 28.8 (q)[10] for **2**. This suggested that **4** possesses a unit of caulophyllogenin (23-hydroxyechinocystic acid) as the aglycon moiety, with the bisdesmoside sugar moieties being the same as those of **2**. In the HMBC spectrum of **4**, long-range correlations were observed between δ_H 4.39 (H-1 of Glc I) and δ_C 83.5 (C-3 of the aglycon), δ_H 5.34 (H-1 of Rha I) and δ_C 79.2

Table 1. ^{13}C NMR Spectroscopic Data (δ values, 150 MHz, methanol-d_4) of Triterpene Glycosides **4** and **5**

carbon	**4**	**5**	carbon	**4**	**5**	carbon	**4**	**5**
aglycon			3-*O*-glycosyl			28-*O*-glycosyl		
1	39.7	40.0		Glc I	Glc I		Xyl II	Glc II
2	26.4	27.1	1	105.7	106.6	1	94.0	95.2
3	83.5	91.0	2	77.0	76.9	2	75.6	77.5
4	43.9	40.2	3	79.2	79.3	3	71.2	79.0
5	48.3	57.2	4	74.9	74.9	4	67.0	71.4
6	18.9	19.4	5	77.2	77.3	5	63.7	78.4
7	33.8	34.3	6	61.4	61.5	6		62.5
8	40.9	40.9						
9	48.2	48.2		Rha I	Rha I		Rha II	Rha II
10	37.7	38.0	1	101.8	101.8	1	101.4	101.6
11	24.5	24.5	2	72.6	72.6	2	72.1	72.0
12	123.7	123.5	3	72.0	72.0	3	72.3	72.2
13	144.8	144.7	4	74.2	74.2	4	83.5	73.9
14	42.8	42.8	5	69.3	69.3	5	69.1	70.4
15	36.4	36.5	6	18.0	18.0	6	18.2	18.3
16	74.7	74.7						
17	50.4	50.3		Xyl I	Xyl		Glc II	
18	42.2	42.5	1	104.2	104.2	1	105.9	
19	47.7	48.1	2	75.5	75.5	2	76.1	
20	31.3	31.3	3	78.2	78.2	3	78.1	
21	36.4	36.5	4	70.8	71.0	4	71.6	
22	31.9	31.5	5	66.8	66.8	5	78.2	
23	64.9	28.6				6	62.8	
24	13.5	17.1						
25	16.7	16.2						
26	18.0	17.9						
27	27.4	27.2						
28	177.1	177.3						
29	33.4	33.4						
30	25.2	25.1						

* To whom correspondence should be addressed. Tel: 81-3-3259-0806. Fax: 81-3-3293-7572. E-mail: akihisa@chem.cst.nihon-u.ac.jp.
[†] College of Science and Technology, Nihon University.
[‡] College of Pharmacy, Nihon University.
[§] Toho University.

10.1021/np078002l CCC: $37.00
Published on Web 05/05/2007

Table 2. ^{1}H NMR Spectroscopic Data (δ values, 600 MHz, methanol-d_4) of Triterpene Glycosides **4** and **5**[a]

position	**4**	**5**
aglycon		
1	0.98 (α), 1.63 (*β*)	1.00 (α), 1.64 (*β*)
2	1.94 (α), 1.74 (*β*)	1.92 (α), 1.71 (*β*)
3	3.63 (dd, 4.4, 11.7)	3.16 (dd; 4.4, 11.5)
5	1.24	0.78
6	1.49 (2H)	1.54 (α), 1.39 (*β*)
7	1.64 (a), 1.35 (b)	1.54 (α), 1.42 (*β*)
9	1.68 (t, 8.6)	1.63
11	1.91 (2H)	1.90 (2H)
12	5.36 (t, 3.8)	5.33 (t, 3.5)
15	1.44 (α; dd, 3.4, 14.8), 1.76 (*β*)	1.43 (α), 1.72 (*β*)
16	4.48 (brs)	4.46 (brs)
18	3.06 (dd, 3.7, 13.5)	2.92 (dd, 4.4, 13.0)
19	1.05 (α), 2.28 (*β*; dd, 13.5, 13.5)	1.05 (α), 2.27 (*β*; dd, 13.0, 13.0)
21	1.15 (α), 1.94 (*β*)	1.17 (α), 1.92 (*β*)
22	1.90 (α), 1.76 (*β*)	1.92 (α), 1.83 (*β*; dd, 5.5, 15.4)
23	3.29 (d, 11.0), 3.60 (d, 11.0)	1.04 (s)
24	0.70 (s)	0.84 (s)
25	0.99 (s)	0.97 (s)
26	0.78 (s)	0.78 (s)
27	1.38 (s)	1.37 (s)
29	0.88 (s)	0.88 (s)
30	0.97 (s)	0.95 (s)
3-*O*-glycosyl		
	Glc I	Glc I
1	4.39 (d, 7.9)	4.32 (d, 7.9)
2	3.33 (dd, 7.9, 9.3)	3.35 (dd, 7.9, 9.3)
3	3.67 (dd, 9.3, 9.3)	3.66 (dd, 9.3, 9.3)
4	3.69 (dd, 9.3, 9.3)	3.70 (dd, 9.3, 9.3)
5	3.37	3.35
6	3.84, 3.92	3.84 (dd, 2.0, 12.0), 3.90
	Rha I	Rha I
1	5.34 (d, 1.4)	5.35 (d, 1.7)
2	3.90 (dd, 1.4, 3.4)	3.90 (dd, 1.7, 3.4)
3	3.84	3.84 (dd, 3.4, 9.3)
4	3.37 (dd, 9.0, 9.0)	3.37 (dd, 9.3, 9.3)
5	4.37 (dq, 6.2, 9.0)	4.37 (dq, 6.2, 9.3)
6	1.21 (d, 6.2)	1.21 (d, 6.2)
	Xyl I	Xyl
1	4.38 (d, 7.9)	4.38 (d, 7.5)
2	3.19 (dd, 7.9, 9.3)	3.19 (dd, 7.5, 9.0)
3	3.28 (dd, 9.3, 9.3)	3.28 (dd, 9.0, 9.0)
4	3.57 (ddd, 5.5, 9.3, 10.3)	3.57 (ddd, 5.0, 9.0, 10.3)
5	3.12 (dd, 10.3, 10.3), 3.88	3.12 (dd, 10.3, 10.3), 3.87 (dd, 5.0, 10.3)
28-*O*-glycosyl		
	Xyl II	Glc II
1	5.63 (d, 3.7)	5.38 (d, 7.6)
2	3.79 (dd, 3.7, 5.2)	3.56 (dd, 7.6, 9.6)
3	3.88	3.54 (dd, 9.6, 9.6)
4	3.82	3.39 (dd, 9.6, 9.6)
5	3.49 (dd, 3.5, 11.0), 3.91	3.32
6		3.66, 3.79 (dd, 2.4, 12.1)
	Rha II	Rha II
1	5.04 (d, 1.3)	5.34 (d, 1.7)
2	3.84	3.92 (dd, 1.7, 3.5)
3	3.84	3.65 (dd, 3.5, 9.6)
4	3.61 (dd, 9.3, 9.3)	3.39 (dd, 9.6, 9.6)
5	3.74 (dq, 6.2, 9.3)	3.75 (dq, 6.2, 9.6)
6	1.35 (d, 6.2)	1.27 (d, 6.2)
	Glc II	
1	4.56 (d, 7.9)	
2	3.24 (dd, 7.9, 9.3)	
3	3.28	
4	3.37 (dd, 9.3, 9.3)	
5	3.31	
6	3.69 (dd, 5.1, 12.1), 3.85 (dd, 2.1, 12.1)	

[a] Figures in parentheses denote *J* values (hertz).

(C-3 of Glc I), δ_H 4.38 (H-1 of Xyl I) and δ_C 74.9 (C-4 of Glc I), δ_H 5.63 (H-1 of Xyl II) and δ_C 177.1 (C-28 of the aglycon), δ_H 5.04 (H-1 of Rha II) and δ_C 75.6 (C-2 of Xyl II), and δ_H 4.56 (H-1 of Glc II) and δ_C 83.5 (C-4 of Rha II), which suggested the substitution patterns of the aglycon by the sugar moieties assigned as shown in Chart 1. Hence, the structure of **4** was proposed as 28-*O*-*β*-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranosyl caulophyllogenin 3-*O*-α-L-rhamnopyranosyl(1→3)-[*β*-D-xylopyranosyl-(1→4)]-*β*-D-glucopyranoside and has been named helianthoside 4. Upon acid hydrolysis, compound **4** afforded L-arabinose, D-glucose, and L-rhamnose in the ratio 1:1:1, which supported the types and numbers of sugar moieties suggested.

Compound **5** exhibited a $[M + Na]^+$ peak at *m*/*z* 1243.6130 in the HRESIMS, corresponding to a molecular formula of $C_{59}H_{96}O_{26}$. The IR spectrum showed the presence of ester carbonyl (1737 cm^{-1}) and olefinic (1641 cm^{-1}) groups. Compound **5** exhibited ^{1}H NMR signals for seven tertiary methyls [δ_H 0.78 (s), 0.84 (s), 0.88 (s), 0.95 (s), 0.97 (s), 1.04 (s), and 1.37 (s)], two secondary hydroxy methines [δ_H 3.16 (dd, J = 4.4, 11.5 Hz) and 4.46 (br s)], an olefinic methine [δ_H 5.33 (t, J = 3.5 Hz)] due to the aglycon moiety, two secondary methyls [δ_H 1.21 (d, J = 6.2 Hz) and 1.27(d, J = 6.2 Hz)], and five anomeric methines [δ_H 4.32 (d, J = 7.9 Hz), 4.38 (d, J = 7.5 Hz), 5.34 (d, J = 1.7 Hz), 5.35 (d, J = 1.7 Hz), and 5.38 (d, J = 7.6 Hz)] arising from the sugar moiety. The ^{13}C NMR data of **5** were in good agreement with those of compound **3**[10] except for those arising from the sugar moieties at C-3 and C-28. Careful comparison of the ^{13}C NMR data of **5** with those of **3**[10] suggested that the former lacks the terminal glucose unit at C-28 of **3**. In the HMBC spectrum of **5**, long-range correlations were observed between δ_H 4.32 (H-1 of Glc I) and δ_C 91.0 (C-3 of the aglycon), δ_H 5.35 (H-1 of Rha I) and δ_C 79.3 (C-3 of Glc I), δ_H 4.38 (H-1 of Xyl) and δ_C 74.9 (C-4 of Glc I), δ_H 5.38 (H-1 of Glc II) and δ_C 177.3 (C-28 of the aglycon), and δ_H 5.34 (H-1 of Rha II) and δ_C 77.5 (C-2 of Glc II). On acid hydrolysis, **5** gave D-xylose, L-rhamnose, and D-glucose in the ratio 2:2:1 as the sugar units and echinocystic acid as the aglycon.[12] Hence, the structure of **5** was established as 28-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl echinocystic acid 3-*O*-α-L-rhamnopyranosyl(1→3)-[*β*-D-xylopyranosyl-(1→4)]-*β*-D-glucopyranoside and was named helianthoside 5.

The inhibitory effects were evaluated on TPA-induced inflammation in mice of the six triterpene glycosides, **1**−**6**. The results are shown in Table 3 together with those of two commercially available anti-inflammatory drugs, indomethacin and hydrocortisone. All of the triterpene glycosides tested showed potent inhibitory effects, with ID_{50} (50% inhibitory dose) values of 65−262 nmol/ear, and were more potently inhibitory than indomethacin (ID_{50} = 838 nmol/ear). Among them, compound **6** exhibited a strong inhibitory effect that was almost the same order of potency as that of hydrocortisone (ID_{50} = 83 nmol/ear). These triterpene glycosides therefore contribute to the anti-inflammatory activity of the MeOH extract of the ligulate flower petals of sunflower.[4] The inhibitory effect against TPA-induced inflammation has been demonstrated to closely parallel that of the inhibition of tumor promotion in two-stage carcinogenesis initiated by 7,12-dimethylbenz[*a*]anthracene (DMBA) and TPA, a well-known promoter, in a mouse skin model.[13] Thus, compounds **1**−**6** may have anti-tumor-promoting activity in this animal model.

Experimental Section

General Experimental Procedures. Crystallizations were performed in MeOH. Melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1030 polarimeter in MeOH at 25 °C. NMR spectra were recorded with a JEOL ECA-600 (^{1}H, 600 MHz; ^{13}C, 150 MHz) spectrometer in methanol-d_4 with tetramethylsilane as internal standard. HRESIMS were recorded on an Agilent 1100 LC/MSD TOF (time-of-flight) system (ionization mode: positive; nebulizing N_2 gas pressure: 35 psig; drying N_2 gas: flow, 12 L/min, temp, 325 °C; capillary voltage: 3000 V; fragmentor voltage: 225 V). Silica gel (silica gel 60, 230−400 mesh, Merck), C_{18} silica (Chromatorex-ODS, 100−200 mesh, Fuji Silysia Chemical Co., Ltd., Aichi, Japan), Diaion HP-20 (Mitsubishi Chemical Co., Tokyo, Japan), and Amberlite MB-3 (Rohm and Hass Co., Philadelphia, PA) were used for open column chromatography. Reversed-phase preparative HPLC was carried out on a C_{18} silica column (Pegasil ODS II column, 25 cm × 10 cm i.d.; Senshu Scientific Co., Ltd., Tokyo, Japan) at 25 °C with MeOH−H_2O

Chart 1

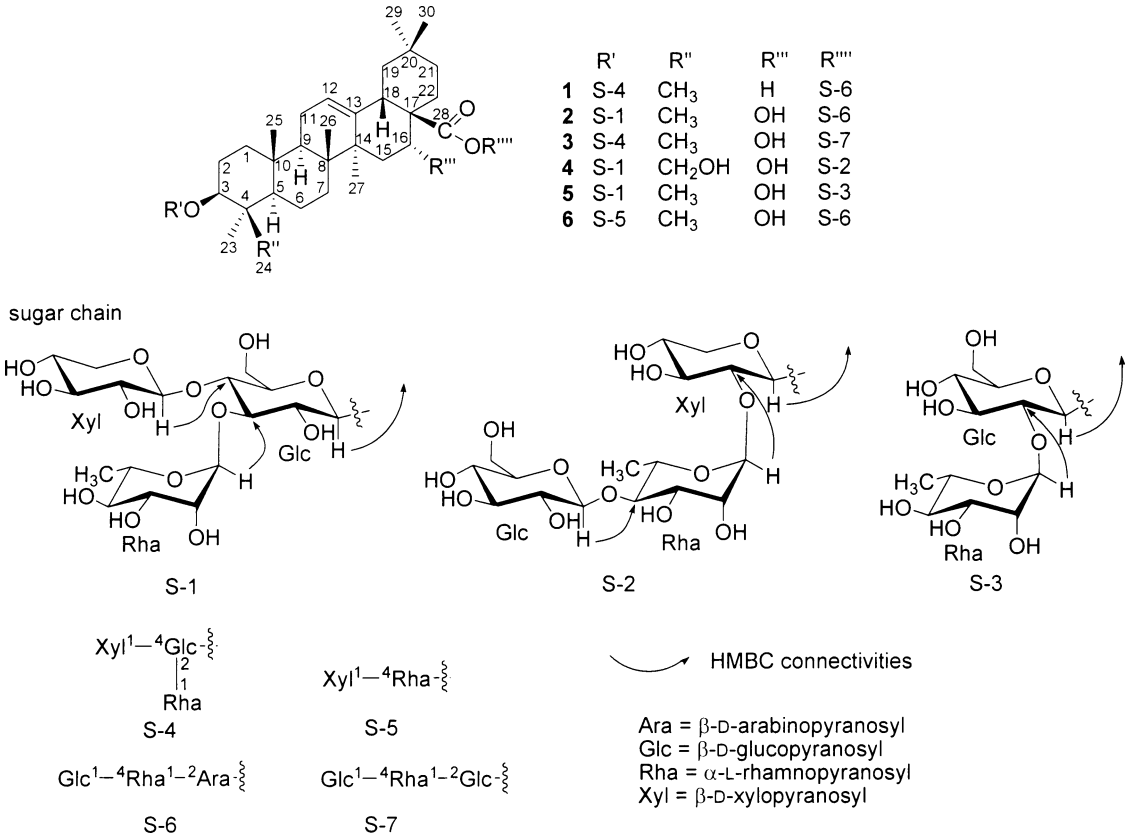

Table 3. Inhibitory Effects of Compounds **1**−**6** and Reference Compounds on TPA-Induced Inflammation in Mice

	compound	inhibition of inflammation	
		ID_{50}[a] (nmol/ear)	InhR (%)[b]
1	helianthoside 1	144	71
2	helianthoside 2	135	77
3	helianthoside 3	156	73
4	helianthoside 4	262	75
5	helianthoside 5	155	63
6	helianthoside B	65	80
	reference compound		
	indomethacin	838	96
	hydrocortisone	83	99

[a] ID_{50}: 50% inhibitory dose. [b]InhR (inhibitory ratio) was 1.0 mg/ear, and $p < 0.01$ by Student's *t*-test as compared to control group.

(3:2; 2 mL/min; HPLC system I) or MeOH−H_2O (7:3; 2 mL/min; HPLC system II) as mobile phase. GLC was performed using a 25 m × 0.25 mm i.d. 007-5 MS fused-silica capillary column (Quadrex, Woodbridge, CT) (detector: FID; column temperature: 200 °C; detector temperature: 270 °C; injector temp: 270 °C; carrier gas: N_2).

Chemicals were purchased as follows: TPA from ChemSyn Laboratories (Lenexa, KS), indomethacin and hydrocortisone from Sigma Chemical Co. (St. Louis, MO), and L-cysteine methyl ester hydrochloride, trimethylsilylimidazole, D-xylose, L-rhamnose, and D-glucose from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan).

Plant Material. *Helianthus annuus* L. (cultivar: Russian sunflower) was cultivated at an herbal garden of Toho University (Chiba, Japan), and the flower heads were harvested in August 2003. A voucher specimen (TU-HA-0308) has been deposited in the School of Pharmaceutical Sciences, Toho University. The ligulate flower petals pinched off from the flower head samples were air-dried at 60 °C.

Animals. Experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of the College of Pharmacy, Nihon University, Chiba, Japan. Specific pathogen-free female ICR mice were obtained from Japan SLC (Shizuoka, Japan). The animals were housed, five per polycarbonate cage, in an air-conditioned specific pathogen-free room at 24 ± 2 °C. Food and water were available ad libitum.

Extraction and Isolation. The air-dried ligulate flower petals of *H. annuus* (303 g) were extracted with MeOH by soaking for 3 days each at room temperature three times. Evaporation of the combined solvent under reduced pressure provided an extract (101 g). The extract was suspended in water and partitioned successively with *n*-hexane, ethyl acetate (EtOAc), and *n*-BuOH to yield separate *n*-hexane- (40.0 g), EtOAc- (13.9 g), *n*-BuOH- (14.8 g), and H_2O- (30.3 g) soluble fractions. The *n*-BuOH-soluble fraction was subjected to chromatography on a Diaion HP-20 (210 g) column. Step gradient elution was conducted with H_2O−MeOH [1:0 (2 L), 7:3 (2 L), 1:1 (3 L), 3:7 (3 L), 0:1 (3 L)]. A fraction (6.0 g) that eluted with H_2O−MeOH (3:7) was subjected to chromatography over ODS (60 g) to give two fractions, fractions A (3.2 g; eluted with 50% MeOH) and B (1.0 g; eluted with 80% MeOH). Fraction A was further separated by HPLC system I to give **2** (1301 mg, ca. 8.79% in the *n*-BuOH-soluble fraction; t_R 26.2 min), **3** (52 mg, 0.35%; t_R 19.8 min), **4** (62 mg, 0.42%; t_R 21.9 min), **5** (19 mg, 0.13%; t_R 24.9 min), and **6** (64 mg, 0.43%; t_R 35.1 min). Fraction B, upon separation using HPLC system II, afforded **1** (127 mg, 0.85%; t_R 21.2 min), **2** (229 mg, 1.54%; t_R 9.9 min), and **6** (42 mg, 0.28%; t_R 11.7 min).

Helianthoside 4 {**28-*O*-β-d-glucopyranosyl-(1→4)-α-l-rhamnopyranosyl-(1→2)-β-d-xylopyranosylcaulophyllogenin 3-*O*-α-l-rhamnopyranosyl(1→3)-[β-d-xylopyranosyl-(1→4)]-β-d-glucopyranoside**} **(4):** fine needles, mp 239−243 °C; $[\alpha]^{25}_D$ −48.2 (*c* 0.29, MeOH); IR (KBr) ν_{max} 3425, 1726, 1641 cm^{-1}; ^{13}C and ^1H NMR, see Tables 1 and 2, respectively; HRESIMS *m/z* 1391.6459 (calcd for $C_{64}H_{104}O_{31}Na$ $[M + Na]^+$, 1391.6459).

Helianthoside 5 {**28-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosylechinocystic acid 3-*O*-α-L-rhamnopyranosyl(1→3)-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranoside**} **(5):** fine needles, mp 222−227 °C; $[\alpha]^{25}_D$ −46.7 (*c* 0.15, MeOH); IR (KBr) ν_{max} 3425, 1737, 1641 cm^{-1}; ^{13}C and ^1H NMR, see Tables 1 and 2, respectively; HRESIMS *m/z* 1243.6130 (calcd for $C_{59}H_{96}O_{26}Na$ $[M + Na]^+$, 1243.6087).

Analysis of Sugar Moieties of Compounds 4 and 5. Compounds **4** and **5** were hydrolyzed separately with 1 M HCl for 2 h at 90 °C. The reaction mixture was diluted with H_2O and extracted twice with EtOAc. The H_2O layer was passed through an Amberlite MB-3 column,

and the eluate was concentrated. The residue was dried and dissolved in pyridine (0.1 mL). After addition of L-cysteine methyl ester hydrochloride (1.0 mg), the mixture was warmed at 60 °C for 1 h. The trimethylsilylimidazole (150 μL) was added, and the warming at 60 °C was continued for another 30 min. The precipitate was centrifuged off, and the supernatant (1 μL) was analyzed by GLC.[14] The standard monosaccharides were subjected to the same reaction and GLC analysis, and peaks were observed with t_R (min) of 11.9 (D-xylose), 14.5 (L-rhamnose), and 22.3 (D-glucose). D-Xylose, L-rhamnose, and D-glucose were obtained in the ratio 1:1:1 and 1:2:2 from **4** and **5**, respectively.

Assay of TPA-Induced Inflammation Ear Edema in Mice. TPA (1.7 nmol, 1 μg) dissolved in acetone (20 μL) was applied to the right ear only of ICR mice by means of a micropipette. A volume of 10 μL was delivered to both the inner and outer surfaces of the ear. The samples or their vehicles, $CHCl_3$−MeOH (1:1, 20 μL), as a control, were applied topically about 30 min before TPA treatment. For ear thickness determinations, a pocket thickness gauge with a range of 0−9 mm, graduated at 0.01 mm intervals and modified so that the contact surface area was increased to reduce the tension, was applied to the tip of the ear. The ear thickness was measured before treatment (a) and 6 h after TPA treatment (b = TPA alone; b' = TPA plus sample). The following values were then calculated:

$$\text{Edema A as induced by TPA alone } (b - a)$$

$$\text{Edema B as induced by TPA plus sample } (b' - a)$$

$$\text{Inhibitory ratio (InhR) (\%)} = [(\text{Edema A} - \text{Edema B})/\text{Edema A}] \times 100$$

Each value was the mean of individual determinations from five mice. The 50% inhibitory dose (ID_{50}) values were determined by the method of probit-graphic interpolation for four dose levels. Statistical analysis was carried out by Student's t-test.

Acknowledgment. This work was supported, in part, by a grant "Academic Frontier" Project for Private Universities. Matching fund subsidies were received from MEXT (Ministry of Education, Culture, Sports, Science and Technology) 2002−2006 and the High-Tech Research Center of Nihon University.

References and Notes

(1) Carter, J. F., Ed. In *Sunflower Science and Technology*; American Society of Agronomy: Madison, WI, 1978; p 4.
(2) Duke, J. A. In *Handbook of Medicinal Herbs*; CRC Press: Boca Raton, FL, 2002; p 707.
(3) Akihisa, T.; Yasukawa, K.; Oinuma, H.; Kasahara, Y.; Yamanouchi, S.; Takido, M.; Kumaki, K.; Tamura, T. *Phytochemistry* **1996**, *43*, 1255−1260.
(4) Akihisa, T.; Yasukawa, K.; Kasahara, Y. In *Current Topics in Phytochemistry, Vol. 1, Research Trends*; Trivandrum: India, 1997; pp 137−144.
(5) Yasukawa, K.; Akihisa, T.; Inoue, Y.; Tamura, T.; Yamanouchi, S.; Takido, M. *Phytother. Res.* **1998**, *12*, 484−487.
(6) Ukiya, M.; Akihisa, T.; Yasukawa, K.; Kasahara, Y.; Kimura, Y.; Koike, K.; Nikaido, T.; Takido, M. *J. Agric. Food Chem.* **2001**, *49*, 3187−3197.
(7) Ukiya, M.; Akihisa, T.; Tokuda, H.; Suzuki, H.; Mukainaka, T.; Ichiishi, E.; Yasukawa, K.; Kasahara, Y.; Nishino, H. *Cancer Lett.* **2002**, *177*, 7−12.
(8) Ukiya, M.; Akihisa, T.; Tokuda, H.; Koike, K.; Takayasu, J.; Okuda, H.; Kimura, Y.; Nikaido, T.; Nishino, H. *J. Agric. Food Chem.* **2003**, *51*, 2949−2957.
(9) Ukiya, M.; Akihisa, T.; Tokuda, H.; Koike, K.; Kimura, Y.; Asano, T.; Motohashi, S.; Nikaido, T.; Nishino, H. *J. Nat. Prod.* **2003**, *66*, 1476−1479.
(10) Bader, G.; Zieschang, M.; Wagner, K.; Gründemann, E.; Hiller, K. *Planta Med.* **1991**, *57*, 471−474.
(11) Hiegemann, M.; Duddeck, H.; Bader, G.; Hiller, K. *Magn. Reson. Chem.* **1995**, *33*, 337−341.
(12) Lee, K. T.; Choi, J.; Jung, W. T.; Nam, J. H.; Jung, H. J.; Park, H. J. *J. Agric. Food Chem.* **2002**, *50*, 4190−4193.
(13) Akihisa, T.; Yasukawa, K. In *Studies in Natural Products Chemistry, Vol. 25, Bioactive Natural Products (Part F)*; Atta-ur-Rahman, Ed.; Elsevier Science B.V.: Amsterdam, 2001; pp 43−87.
(14) Hara, S.; Okabe, H.; Mihashi, K. *Chem. Pharm. Bull.* **1987**, *35*, 501−506.

NP078002L