Constituents of Compositae Plants. 2. Triterpene Diols, Triols, and Their 3-*O*-Fatty Acid Esters from Edible Chrysanthemum Flower Extract and Their Anti-inflammatory Effects

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The *n*-hexane soluble and the nonsaponifiable lipid fractions of the edible flower extract of chrysanthemum (*Chrysanthemum morifolium*) were investigated for triterpene diol and triol constituents. These triterpenes occur as the 3-*O*-fatty acid esters in the *n*-hexane soluble fraction from which 26 new and 6 known fatty acid esters were isolated and characterized. From the nonsaponifiable lipid fraction, 24 triterpene diols and triols were isolated, of which 3 were new compounds: (24*S*)-25-methoxycycloartane-3β,24-diol (**11**), (24*S*)-25-methoxycycloartane-3β,24,28-triol (**22**), and 22α-methoxyfaradiol (**23**). Faradiol (**9**) and heliantriol C (**19**), present in the nonsaponifiable lipid fraction and as the 3-*O*-palmitoyl esters in the *n*-hexane soluble fraction, were the most predominant triterpene diol and triol constituents. Fourteen triterpene diols and triols and 9 fatty acid esters were evaluated with respect to their anti-inflammatory activity against 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation in mice. All of the triterpenes examined showed marked inhibitory activity, with a 50% inhibitory dose (ID_{50}) of 0.03−1.0 mg/ear, which was more inhibitive than quercetin (ID_{50} = 1.6 mg/ear), a known inhibitor of TPA-induced inflammation in mice.

Keywords: *Edible chrysanthemum flowers; Compositae; triterpene diols and triols; fatty acid esters; antiedema; TPA-induced ear edema*

INTRODUCTION

Chrysanthemum morifolium Ramat. var. *sinense* Makino forma *esculentum* Makino (Japanese name: Ryourigiku; Compositae) has been widely cultivated in the northeastern part of the Honshu Island of Japan as a traditional edible flower. Our recent study has demonstrated that the methanol extract of the edible chrysanthemum ligulate flowers, among the other Compositae plant flower extracts, possesses inhibitory activity on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced ear edema in mice (*1*). In addition, we have shown that several triterpene alcohols (3-monohydroxytriterpenes) (*2*) and triterpene diols and triols such as faradiol (**9**) and heliantriol C (**19**), isolated from the nonsaponifiable lipid fraction of the methanol extracts of several Compositae plant flowers, exhibited remarkable anti-inflammatory activity against TPA-induced inflammation in mice (*3*−*5*). Furthermore, these triterpenes have been shown to inhibit the tumor-promoting activity of TPA in two-stage carcinogenesis in mouse skin (*4*, *6*). Because the triterpene constituents, especially triterpene diols and triols, were expected to be principles responsible for the anti-inflammatory activity of the methanol extract of edible chrysanthemum flowers, we were interested in the constituents of the extract. We now report an investigation on the triterpene diols and triols in the *n*-hexane soluble and the nonsaponifiable lipid fractions of the methanol extract of edible chrysanthemum flowers, which enabled the isolation and characterization of 24 triterpene diols and triols and 32 3-*O*-fatty acid esters. Fourteen triterpene diols and triols and 9 of their fatty acid esters were evaluated with respect to their anti-inflammatory activity against TPA-induced inflammation in mice and were found to possess marked inhibitory activity. The flowers of some *Chrysanthemum* species have been used as a Chinese natural medicine, Chrysanthemi Flos, which is prescribed for anti-inflammatory, analgesic, and antipyretic purposes (*7*).

MATERIALS AND METHODS

Crystallizations were performed from acetone/methanol (1:1, v/v). Melting points measured are uncorrected. Thin-layer chromatography (TLC) on silica gel (Kieselgel 60G, Merck; 0.5 mm thick; 20 × 20 cm) was developed using *n*-hexane/ethyl acetate (EtOAc) (6:1, v/v). Reversed-phase preparative high-performance liquid chromatography (RP-HPLC) was carried out on octadecyl silica columns (25 cm × 10 mm i.d.), on a Superiorex ODS S-5 μm column (Shiseido Co., Ltd., Tokyo, Japan) (HPLC I) and on a TSK ODS-120A 5 μm column (Toso Co., Tokyo, Japan) (HPLC II), at 25 °C with MeOH (4 mL/min) as the mobile phase. Normal-phase HPLC was carried

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10.1021/jf010164e CCC: $20.00
Published on Web 06/28/2001

out on a silica column (Pegasil silica 60-5 column, 25 cm × 4.6 mm i.d.; Senshu Scientific Co., Ltd., Tokyo, Japan) (HPLC III) at 25 °C with *n*-hexane/EtOAc (92:8, v/v; 1.0 mL/min) as the mobile phase. Gas−liquid chromatography (GLC) was performed on a Shimadzu GC-14B instrument (Shimadzu Co., Kyoto, Japan) using a DB-17 fused-silica capillary column (30 m × 0.3 mm i.d.; column temperature, 275 °C) and nitrogen as a carrier gas at 60 mL/min (split ratio 60:1). GLC for fatty acid methyl esters was run on a Shimadzu GC-17A instrument using a Quadrex 23 fused-silica capillary column (25 m × 0.25 mm i.d.; column temperature, 180 °C) and nitrogen as a carrier gas (60 mL/min; split ratio 60:1). For both HPLC I and II and GLC, cholesterol (retention times: GLC, 6.7 min; HPLC I, 27.2 min; HPLC II, 31.5 min) was the standard for the determination of the relative retention times (RR_t) of free triterpene diols and triols; cholesterol acetate (retention times: GLC, 10.0 min; HPLC I, 49.0 min; HPLC II, 48.0 min) was used for their acetyl derivatives. Infrared (IR) spectra were recorded on a Jasco IR-300 IR spectrometer as liquid films. Optical rotations were measured on a Jasco DIP-370 polarimeter in $CHCl_3$ at 25 °C. Electron-impact mass spectra (MS) and high-resolution MS (HR-MS) were recorded on a Hitachi M-80B double-focusing gas chromatograph−mass spectrometry (GC-MS) instrument (70 eV) using a direct inlet system. Nuclear magnetic resonance (NMR) spectra were recorded with a JEOL JNM LA-500 spectrometer at 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR) in $CDCl_3$ with tetramethylsilane (TMS; ^{1}H NMR) and $CDCl_3$ at δ 77.0 (^{13}C NMR) as internal standard. Acetylation (acetic anhydride/pyridine) and hydrolysis of acetates (5% KOH in methanol) were performed at room temperature overnight. Hydrolysis of the fatty acid esters of triterpenes was performed with 5% (w/v) KOH in methanol under reflux for 2 h. Methyl ester derivatives of fatty acids were prepared by refluxing fatty acids with 1% (w/v) H_2SO_4 in methanol for 1 h.

Chemicals and Materials. The edible ligulate flowers of *Chrysanthemum morifolium* Ramat. var. *sinense* Makino forma *esculentum* Makino were collected from plants cultivated at Mogami (Yamagata, Japan) in 1996. (−)-2-Methoxy-2-phenyl-2-(trifluoromethyl)acetic acid (MTPA), (+)-MTPA chlorides, and *N*,*N*-dimethyl-1,3-propanediamine were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). TPA was purchased from ChemSyn Laboratories (Lenexa, KS). Quercetin (3,3′,4′,5,7-pentahydroxyflavone), indomethacin, hydrocortisone, and reference fatty acid methyl esters were obtained from Sigma Chemical Co. (St. Louis, MO). Ten triterpene diols and triols [arnidiol (**1**), brein (**2**), calenduladiol (**3**), **9**, maniladiol (**10**), heliantriol B_2 (**18**), **19**, and longispinogenin (**21**) (*3*), erythrodiol (**8**) (*8*), and (24*R*)-cycloart-25-ene-3β,24-diol (**5**) (*9*)] were used as the reference specimens. The systematic names of the triterpene diols and triols are shown in Table 1, and the structures of the triterpene diols and triols described in this paper are shown in Figures 1−3.

Extraction and Isolation. *n-Hexane Soluble Fraction*. Fresh flowers of *C. morifolium* were air-dried (383 g) and extracted by soaking at room temperature three times for 3 days each with methanol to give an extract (183 g). The extract was partitioned in *n*-hexane/methanol/H_2O (19:19:2, v/v/v), giving *n*-hexane (27 g) and methanol/H_2O fractions. The latter fraction, after evaporation of the solvent, was partitioned in EtOAc/H_2O (1:1, v/v), yielding EtOAc (15 g) and H_2O fractions. The H_2O fraction was extracted with *n*-butanol, which yielded *n*-butanol (28 g) and residual H_2O fractions (112 g). The *n*-hexane soluble fraction was chromatographed on a silica gel (silica gel 60, 230−400 mesh; Merck; 700 g) column with a stepwise gradient of *n*-hexane/EtOAc [1:0 (9.9 L), 9:1 (5.1 L), 4:1 (7.8 L), 1:1 (3.3 L), 0:1 (1.5 L); v/v] as eluant, which yielded fractions containing fatty acid esters of triterpenols and sterols (R_f 0.85 on TLC; 4.6 g; fraction A), fatty acid esters of triterpene diols (R_f 0.48; 3.2 g; fraction B), free triterpenols (R_f 0.45; 2.5 g; fraction C), free sterols (R_f 0.27; 3.2 g; fraction D), and fatty acid esters of triterpene triols (R_f 0.18; 1.8 g; fraction E). Preparative HPLC of fractions B and E gave 24 and 8 compounds, respectively. No detectable free triterpene diols, triols, or their fatty acid esters were observed in the EtOAc and *n*-butanol fractions, as revealed by means of TLC.

Nonsaponifiable Lipid Fraction. Fresh flowers of *C. morifolium* were air-dried (3.0 kg) and extracted by soaking at room temperature three times for 3 days each with methanol. The nonsaponifiable lipid fraction (65.2 g) obtained from the methanol extract (1421 g) by alkaline hydrolysis (5% KOH in methanol, reflux, 3 h), followed by diisopropyl ether extraction, was chromatographed on a silica gel (700 g) column with stepwise gradient of *n*-hexane/EtOAc [1:0 (1.8 L), 95:5 (5.1 L), 9:1 (3.0 L), 4:1 (2.4 L), 1:1 (2.1 L), 0:1 (2.1 L); v/v] as eluant. *n*-Hexane/EtOAc (9:1) eluted a fraction (16.7 g; fraction A′) consisting of triterpenols; *n*-hexane/EtOAc (4:1) eluted a fraction (6.9 g; fraction B′) consisting of sterols; *n*-hexane/EtOAc (1:1) eluted a fraction (6.0 g; fraction C′) consisting mainly of triterpene diols; and EtOAc eluted a fraction (8.8 g; fraction D′) containing triterpene triols. Fractions C′ and D′ were acetylated, which gave the corresponding acetate fractions. Further chromatography on silica gel (eluant = *n*-hexane/EtOAc gradient of 9:1 → 1:1) of the acetate fractions C′ and D′ yielded purified acetate fractions C′ (3.9 g) and D′ (3.2 g), which contained predominantly the acetates of triterpene diols and triols, respectively. The acetate fraction C′, on preparative HPLC I, and when necessary on HPLC II, eventually yielded 13 triterpene acetates: **1a** (211 mg), **2a** (410 mg), **3a** (44 mg), coflodiol diacetate (**4a**; 3 mg), (24*R*)-cycloart-25-ene-3β,24-diol diacetate (**5a**; 6 mg), dammarenediol II 3-monoacetate (**6a**; 20 mg), 3-epicabraleadiol 3-monoacetate (**7a**; 3 mg), **8a** (8 mg), **9a** (1550 mg), **10a** (615 mg), (24*S*)-25-methoxycycloartane-3β,24-diol diacetate (**11a**; 66 mg), and a mixture (6 mg) of (24*R*)- (**12a**) and (24*S*)-saringosterol 3-monoacetates (**13a**). Normal-phase HPLC (HPLC III) of the mixture of **12a** and **13a** enabled the isolation of **12a** (2 mg; retention time = 10.5 min) and **13a** (1.5 mg; retention time = 10.0 min). The acetate fraction D′, on the same HPLC systems as above, eventually afforded 11 triterpene acetates: (24*R*)-cycloartane-3β,24,25-triol 3,24-diacetate (**14a**; 69 mg), (24*S*)-cycloartane-3β,24,25-triol 3,24-diacetate (**15a**; 7 mg), faradiol α-epoxide diacetate (**16a**; 2 mg), heliantriol A_1 triacetate (**17a**; 25 mg), heliantriol B_2 triacetate (**18a**; 210 mg), **19a** (1158 mg), (24*S*)-lanost-9(11)-ene-3β,24,25-triol 3,24-diacetate (**20a**; 2 mg), **21a** (35 mg), (24*S*)-25-methoxycycloartane-3β,24,28-triol triacetate (**22a**; 4 mg), 22α-methoxyfaradiol diacetate (**23a**; 7 mg), and (24*S*)-29-norcycloartane-3β,24,25-triol 3,24-diacetate (**24a**; 4 mg). Alkaline hydrolysis of the acetylated triterpene diols and triols gave free alcohols.

Preparation of (R)- and (S)-MTPA Esters of Triterpene Alcohols. A solution of a triterpene alcohol (6 mg, 12 μmol) in dried pyridine (50 μL) was treated with (+)-MTPA chloride (13 μL, 67 μmol), and the mixture was kept overnight at room temperature. *N*,*N*-Dimethyl-1,3-propanediamine (13 μL, 101 μmol) was added, the solution was kept for 10 min, and the residue obtained after evaporation of the solvent under the stream of N_2 was subjected to HPLC I to give pure (*R*)-MTPA ester (6 mg). Treatment of a triterpene alcohol with (−)-MTPA chloride in the same manner as above gave the (*S*)-MTPA ester.

Identification and Characterization. *Fatty Acid Esters of Triterpene Diols from the n-Hexane Soluble Fraction*. Four compounds among the 24 triterpene diol esters isolated from fraction B were known compounds, and their identification was done by spectral comparison with the literature: calenduladiol 3-*O*-myristate (**3m**) and calenduladiol 3-*O*-palmitate (**3p**) (by MS and ^{1}H NMR comparison) (*10*); faradiol 3-*O*-myristate (**9m**) (MS) (*11*); and faradiol 3-*O*-palmitate (**9p**) (MS and ^{1}H NMR) (*11*−*13*). Identification was confirmed by comparison of the hydrolysis products with the reference triterpene diols (by HPLC, MS, and ^{1}H NMR comparison) and fatty acids (by GLC comparison as the methyl ester derivatives). Characterization of the following 20 new compounds was performed by MS and ^{1}H NMR spectroscopy and by spectral and chromatographic comparison of the hydrolysis products with the reference triterpene diols and fatty acids (as the methyl esters): arnidiol 3-*O*-laurate (**1l**), **1m**, **1p**, **2l**, **2m**, **2p**, **6l**, **6m**, **6p**, **7m**, **7p**, **9l**, faradiol 3-*O*-stearate (**9s**), **10l**, **10m**, **10p**, **11p**, **16m**, **16p**, and

Table 1. Chromatographic Data[a] of Triterpene Diols and Triols and Their Acetyl Derivatives, and Their Compositions in Fractions C′ and D′ from the Nonsaponifiable Lipids of the Extract of Edible Chrysanthemum Flowers

compound (common and systematic names)	acetate, RR_t			free alcohol, RR_t		composition[b] (%)
	GLC	HPLC I	HPLC II	HPLC I	HPLC II	
			Fraction C′			
arnidiol (**1**) [taraxast-20(30)-ene-3β,16β-diol]	5.41	0.29	0.19	0.14	0.08	7.2
brein (**2**) (urs-12-ene-3β,16β-diol)	3.56	0.27	0.18	0.14	0.09	14.0
calenduladiol (**3**) [lup-20(29)-ene-3β,16β-diol]	4.38	0.24	0.15	0.10	0.07	1.5
coflodiol (**4**) [olean-13(18)-ene-3β,16β-diol]	3.50	0.30	0.21	0.16	0.10	0.1
(24*R*)-cycloart-25-en-3β,24-diol (**5**)	4.03	0.33	0.35	0.18	0.19	0.2
dammarenediol II (**6**) [(20*S*)-dammar-24-ene-3β,20-diol]	3.18	0.14	0.12	0.08	0.13	0.6
3-epicabraleadiol (**7**) [(20*S*,24*S*)-20,24-epoxydammarane-3β,25-diol]	3.05	0.16	0.15	0.20	0.15	0.1
erythrodiol (**8**) (olean-12-ene-3β,28-diol)	4.16	0.26	0.18	0.12	0.08	0.3
faradiol (**9**) (taraxast-20-ene-3β,16β-diol)	5.24	0.31	0.21	0.14	0.09	52.7
maniladiol (**10**) (olean-12-ene-3β,16β-diol)	3.10	0.27	0.16	0.13	0.08	20.9
(24*S*)-25-methoxycycloartane-3β,24-diol (**11**)	5.82	0.25	0.29	0.18	0.16	2.2
(24*R*)-saringosterol (**12**) [(24*R*)-stigmasta-5,24^1(24^2)-diene-3β,24-diol]	2.91	0.20	0.22	0.18	0.18	0.1
(24*S*)-saringosterol (**13**) [(24*S*)-stigmasta-5,24^1(24^2)-diene-3β,24-diol]	2.91	0.20	0.22	0.18	0.18	0.1
			Fraction D′			
(24*R*)-cycloartane-3β,24,25-triol (**14**)	np[c]	0.19	0.21	0.07	0.07	4.5
(24*S*)-cycloartane-3β,24,25-triol (**15**)	np	0.18	0.18	0.09	0.08	0.5
faradiol α-epoxide (**16**) [(20*R*,21*S*)−20,21-epoxytaraxastane-3β,16β-diol]	8.37	0.12	0.09	0.06	0.05	0.1
heliantriol A_1 (**17**) [olean-13(18)-ene-3β,16β,28-triol]	7.43	0.12	0.10	0.08	0.08	1.6
heliantriol B_2 (**18**) [lup-20(29)-ene-3β,16β,28-triol]	8.88	0.11	0.07	0.06	0.04	13.8
heliantriol C (**19**) (taraxast-20-ene-3β,16β,22α-triol)	8.06	0.16	0.12	0.04	0.04	76.0
(24*S*)-lanost-9(11)-ene-3β,24,25-triol (**20**)	np	0.16	0.16	0.22	0.16	0.1
longispinogenin (**21**) (olean-12-ene-3β,16β,28-triol)	6.48	0.07	0.07	0.06	0.04	2.3
(24*S*)-25-methoxycycloartane-3β,24,28-triol (**22**)	12.4	0.13	0.15	0.09	0.09	0.3
22α-methoxyfaradiol (**23**) (22α-methoxytaraxast-20-ene-3β,16β-diol)	5.97	0.20	0.15	0.12	0.09	0.5
(24*S*)-29-norcycloartane-3β,24,25-triol (**24**)	np	0.16	0.20	0.18	0.14	0.3

[a] Relative retention times (RR_t) relative to cholesterol acetate (RR_t = 1.00) for the acetates of the triterpenes, whereas relative to cholesterol (RR_t = 1.00) for the free alcohols. HPLC I, Superiorex ODS column; HPLC II, TSK ODS column. [b] Composition in each fraction determined on the basis of the amount of compounds isolated as the acetyl derivatives. [c] GLC peak did not appear.

25p. Identification of **25p**, of which a reference compound was unavailable, was performed by ^{1}H NMR and mass spectral comparison with the literature data (*14*). The melting points and the mass spectral data of the 20 new compounds are described below. The ^{1}H NMR spectral data of 9 representatives of the 20 new triterpene diol esters are listed in Table 3. The ^{1}H NMR data for the other 11 triterpene diol esters were essentially the same as those of their homologues shown in Table 3. Percentage composition of fraction B (Table 2) was determined on the basis of HPLC data.

*Arnidiol 3-O-laurate (**1l**):* amorphous solid; HR-MS, *m/z* 624.5448 [calcd for $C_{42}H_{72}O_3$ (M^+) 624.5481].

*Arnidiol 3-O-myristate (**1m**):* amorphous solid; HR-MS, *m/z* 652.5821 [calcd for $C_{44}H_{76}O_3$ (M^+) 652.5794].

*Arnidiol 3-O-palmitate (**1p**):* mp 78−80 °C; HR-MS, *m/z* 680.6133 [calcd for $C_{46}H_{80}O_3$ (M^+) 680.6107].

*Brein 3-O-laurate (**2l**):* amorphous solid; HR-MS, *m/z* 624.5449 [calcd for $C_{42}H_{72}O_3$ (M^+) 624.5481].

*Brein 3-O-myristate (**2m**):* mp 90−91 °C; HR-MS, *m/z* 652.5771 [calcd for $C_{44}H_{76}O_3$ (M^+) 652.5794].

*Brein 3-O-palmitate (**2p**):* amorphous solid; HR-MS, *m/z* 680.6126 [calcd for $C_{46}H_{80}O_3$ (M^+) 680.6107].

*Dammarenediol II 3-O-laurate (**6l**):* amorphous solid; HR-MS, *m/z* 608.5508 [calcd for $C_{42}H_{72}O_2$ ($M^+ - H_2O$) 608.5532].

*Dammarenediol II 3-O-myristate (**6m**):* amorphous solid; HR-MS, *m/z* 636.5820 [calcd for $C_{44}H_{76}O_2$ ($M^+ - H_2O$) 636.5845].

*Dammarenediol II 3-O-palmitate (**6p**):* amorphous solid; HR-MS, *m/z* 664.6122 [calcd for $C_{46}H_{80}O_2$ ($M^+ - H_2O$) 664.6158].

*3-Epicabraleadiol 3-O-myristate (**7m**):* amorphous solid; HR-MS, *m/z* 655.5695 [calcd for $C_{43}H_{75}O_4$ ($M^+ -$ Me) 655.5665].

*3-Epicabraleadiol 3-O-palmitate (**7p**):* amorphous solid; HR-MS, *m/z* 683.5953 [calcd for $C_{45}H_{79}O_4$ ($M^+ -$ Me) 683.5978].

*Faradiol 3-O-laurate (**9l**):* amorphous solid; HR-MS, *m/z* 624.5471 [calcd for $C_{42}H_{72}O_3$ (M^+) 624.5481].

*Faradiol 3-O-stearate (**9s**):* mp 94−97 °C; HR-MS, *m/z* 708.6380 [calcd for $C_{48}H_{84}O_3$ (M^+) 708.6420].

*Maniladiol 3-O-laurate (**10l**):* mp 93−96 °C; HR-MS, *m/z* 624.5456 [calcd for $C_{42}H_{72}O_3$ (M^+) 624.5481].

*Maniladiol 3-O-myristate (**10m**):* mp 93−94 °C; HR-MS, *m/z* 652.5792 [calcd for $C_{44}H_{76}O_3$ (M^+) 652.5794].

*Maniladiol 3-O-palmitate (**10p**):* mp 92−93 °C; HR-MS, *m/z* 680.6093 [calcd for $C_{46}H_{80}O_3$ (M^+) 680.6107].

*(24S)-25-Methoxycycloartane-3β,24-diol 3-O-palmitate (**11p**):* amorphous solid; HR-MS, *m/z* 680.6108 [calcd for $C_{46}H_{80}O_3$ ($M^+ -$ MeOH) 680.6107].

Table 2. Relative Retention Times (RR_t)[a] in the HPLC I of the Fatty Acid Esters of Triterpene Diols and Triols and the Compositions (Percent)[b] of Fractions B and E from the Extract of Edible Chrysanthemum Flowers

	laurate		myristate		palmitate		stearate	
compound	RR_t	%	RR_t	%	RR_t	%	RR_t	%
			Fraction B[c]					
1	1.63	0.2	2.34	2.7	3.36	9.5		
2	1.72	0.1	2.45	3.1	3.43	11.7		
3			2.08	0.4[e]	3.02	2.3[e]		
6	2.08	0.1	2.78	0.1	3.98	0.6		
7			2.99	0.1	4.42	0.6		
9	1.74	0.8	2.49	8.5[e]	3.56	32.0[e]	5.35	1.0
10	1.68	0.2	2.30	4.3	3.38	15.8		
11					4.63	0.6		
16			1.38	0.2	1.88	1.0		
25					2.93	0.2		
			Fraciton E[d]					
14			1.67	3.3	2.44	12.6		
18			1.36	2.7	1.98	4.8[e]		
19	0.99	1.1	1.43	14.8	2.05	53.9[e]		
23					2.90	1.3		

[a] Cholesterol $RR_t = 1.00$. [b] Composition in each fraction determined on the basis of HPLC data. [c] Other unidentified components, 3.9%. [d] Other unidentified components, 5.5%. [e] Known compounds. Others are new naturally occurring compounds.

*Faradiol α-epoxide 3-O-myristate (**16m**):* amorphous solid; HR-MS, *m/z* 668.5720 [calcd for $C_{44}H_{76}O_4$ (M^+) 668.5744].

*Faradiol α-epoxide 3-O-palmitate (**16p**):* amorphous solid; HR-MS, *m/z* 696.6043 [calcd for $C_{46}H_{80}O_4$ (M^+) 696.6057].

*Ocotillol II [(20S,24R)-20,24-epoxydammarane-3β,25-diol] 3-O-palmitate (**25p**):* amorphous solid; HR-MS, *m/z* 683.5956 [calcd for $C_{45}H_{79}O_4$ ($M^+ - Me$) 683.5978].

Fatty Acid Esters of Triterpene Triols from the *n*-Hexane Soluble Fraction. Two among the eight triterpene triol esters isolated from fraction E were known compounds, and their identification was made by spectral comparison with the literature: heliantriol B_2 3-*O*-palmitate (**18p**) (by MS and ^{1}H NMR comparison) and heliantriol C 3-*O*-palmitate (**19p**) (MS and ^{1}H NMR) (*12*). Identification was confirmed by spectral and chromatographic comparison of the hydrolysis products with the reference triterpenes and fatty acids (by GLC comparison as the methyl ester derivatives). Characterization of the following six new compounds was performed by MS and ^{1}H NMR spectroscopy and by spectral and chromatographic comparison of the hydrolysis products with the reference triterpenes and fatty acids (as the methyl esters): **14m**, **14p**, **18m**, **19l**, **19m**, and **23p**. The melting points and the mass spectral data of the six new compounds are described below. The ^{1}H NMR spectral data of the four representatives, **14p**, **18m**, **19m**, and **23p**, of the six new triterpene triol esters are listed in Table 3. The ^{1}H NMR data for the other two, **14m** and **19l**, were essentially the same as those of their homologues shown in Table 3. Percentage composition of fraction E (Table 2) was determined on the basis of HPLC data.

*(24R)-Cycloartane-3β,24,25-triol 3-O-myristate (**14m**):* amorphous solid; HR-MS, *m/z* 670.5897 [calcd for $C_{44}H_{78}O_4$ (M^+) 670.5900].

*(24R)-Cycloartane-3β,24,25-triol 3-O-palmitate (**14p**):* mp 81−82 °C; HR-MS, *m/z* 698.6213 [calcd for $C_{46}H_{82}O_4$ (M^+) 698.6213].

*Heliantriol B_2 3-O-myristate (**18m**):* amorphous solid; HR-MS, *m/z* 668.5750 [calcd for $C_{44}H_{76}O_4$ (M^+) 668.5744].

*Heliantriol C 3-O-laurate (**19l**):* amorphous solid; HR-MS, *m/z* 640.5432 [calcd for $C_{42}H_{72}O_4$ (M^+) 640.5431].

*Heliantriol C 3-O-myristate (**19m**):* mp 151−153 °C; HR-MS, *m/z* 668.5743 [calcd for $C_{44}H_{76}O_4$ (M^+) 668.5744].

*22α-Methoxyfaradiol 3-O-palmitate (**23p**):* amorphous solid; HR-MS, *m/z* 710.6213 [calcd for $C_{47}H_{82}O_4$ (M^+) 710.6213].

Triterpene Diols and Triols from the Nonsaponifiable Lipid Fraction. Identification of 10 triterpenes as the acetyl derivatives, **1a**, **2a**, **3a**, **5a**, **8a**, **9a**, **10a**, **18a**, **19a**, and **21a**, was performed by chromatographic (HPLC and GLC) and spectroscopic (MS and ^{1}H NMR) comparison with reference compounds. The following 11 compounds were identified by spectral comparison with the literature: **4a** (by MS and ^{1}H NMR spectral comparison) (*15*); **6a** (as a free alcohol; ^{13}C NMR) (*17*); **7a** (MS, ^{1}H NMR, and ^{13}C NMR) (*18*, *19*); **12a** and **13a** (^{1}H NMR) (*24*, *25*); **14a** and **15a** (MS, ^{1}H NMR, and ^{13}C NMR) (*16*); **16a** (MS and ^{1}H NMR) (*20*); **17a** (MS and ^{1}H NMR) (*21*); (24ξ)-**20a** (^{1}H NMR) (*22*); and (24ξ)-**24a** (MS, ^{1}H NMR, and ^{13}C NMR) (*23*). Characterization as the acetyl derivatives of three new compounds, **11a**, **22a**, and **23a**, was performed on the basis of IR, ^{1}H NMR, ^{13}C NMR, and mass spectral data. Stereochemistry at C-24 of three 24-hydroxytriterpenes, **11**, **20**, and **24**, was determined by measuring the ^{1}H NMR spectra of their MTPA esters (Mosher's method) (*26*). The spectral data and some physical characteristics of three new compounds, **11**, **22**, and **23**, and their acetyl derivatives, the ^{1}H NMR spectral data of the (*R*)-bis-MTPA esters (**11*R***, **20*R***, and **24*R***) and (*S*)-bis-MTPA esters (**11*S***, **20*S***, and **24*S***) of three triterpenes are shown below. The NMR spectral data of **7** (and its acetate **7a**) are also described below. The ^{13}C and ^{1}H NMR spectral data along with the HMBC data for the acetyl derivatives of three new compounds, **11**, **22**, and **23**, are shown in Table 4.

*(24S)-25-Methoxycycloartane-3β,24-diol (**11**) and its diacetate (**11a**):* (**11**) mp 140−145 °C; $[\alpha]_D$ +29.0° (*c* 0.4, $CHCl_3$); IR ν_{max} cm^{-1} 3468 (OH); MS, *m/z* (%) 474 (M^+, 1), 456 (3), 442 (2), 441 (1), 427 (3), 424 (1), 383 (1), 355 (1), 334 (2), 315 (2), 297 (1), 73 (100); HR-MS, *m/z* 474.4038 [calcd for $C_{31}H_{54}O_3$ (M^+) 474.4073]; ^{1}H NMR δ 0.33 (1H, d, *J* = 4.4 Hz; *exo*) and 0.55 (1H, d, *J* = 4.4 Hz; *endo*) (H-19), 0.81 (3H, s, H-18), 0.89 (3H, s, H-30), 0.89 (3H, d, *J* = 6.6 Hz, H-21), 0.97 (6H, s, H-18 and H-28), 1.09 and 1.13 (each 3H and s, H-26 and H-27), 3.23 (3H, s, OMe-25), 3.28 (1H, dd, *J* = 4.4, 11.0 Hz, H-3*a*). (**11a**) mp 152−155 °C; $[\alpha]_D$ +34.0° (*c* 0.9, $CHCl_3$); IR ν_{max} cm^{-1} 1732 and 1245 (OAc); MS, *m/z* (%) 558 (M^+, 1), 543 (1), 526 (2), 498 (6), 376 (1), 357 (2), 297 (2), 255 (1), 241 (1), 203 (6), 185 (14), 73 (100); HR-MS, *m/z* 558.4285 [calcd for $C_{35}H_{58}O_5$ (M^+) 558.4284].

*(R)-Bis-MTPA (**11R**) and (S)-bis-MTPA esters (**11S**) of (24S)-25-methoxycycloartane-3β,24-diol (**11**):* (**11R**) HR-MS, *m/z* 906.4869 [calcd for $C_{51}H_{68}F_6O_7$ (M^+) 906.4869]; ^{1}H NMR δ 0.35 (1H, d, *J* = 4.3 Hz; *exo*) and 0.59 (1H, d, *J* = 4.0 Hz; *endo*) (H-19), 0.80 (3H, s, H-28), 0.85 (3H, s, H-29), 0.87 (3H, d, *J* = 6.4 Hz, H-21), 0.89 (3H, s, H-30), 0.94 (3H, s, H-18), 1.07 and 1.11 (each 3H and s, H-26 and H-27), 3.17 (3H, s, 25-OMe), 4.81 (1H, dd, *J* = 4.6, 11.6 Hz, H-3α), 5.10 (1H, dd, *J* = 1.8, 10.1 Hz, H-24). (**11S**) MS, *m/z* 906 (M^+); ^{1}H NMR δ 0.32 (1H, d, *J* = 4.3 Hz) and 0.56 (1H, d, *J* = 4.3 Hz) (H-19), 0.83 (3H, d, *J* = 6.4 Hz, H-21), 0.85 (3H, s, H-29), 0.89 (6H, s, H-30, H-28), 1.14 and 1.16 (each 3H and s, H-26 and H-27), 3.20 (3H, s, 25-OMe), 4.78 (1H, dd, *J* = 4.6, 11.3 Hz, H-3α), 5.15 (1H, dd, *J* = 2.1, 10.4 Hz, H-24).

*(24S)-25-Methoxycycloartane-3β,24,28-triol (**22**) and its triacetate (**22a**):* (**22**) mp 180−185 °C; $[\alpha]_D$ +29.3°(*c* 0.1, $CHCl_3$); IR ν_{max} cm^{-1} 3388 (OH); MS, *m/z* (%) 490 (M^+, 19), 472 (34), 458 (16), 440 (19), 427 (24), 409 (29), 375 (18), 355 (12), 334 (41), 313 (23), 302 (29), 109 (100); ^{1}H NMR δ 0.38 (1H, d, *J* = 4.3 Hz; *exo*) and 0.59 (1H, d, *J* = 4.3 Hz; *endo*) (H-19), 0.89 (3H, s, H-30), 0.89 (3H, d, *J* = 6.8 Hz), 0.94 (3H, s, H-29), 0.96 (3H, s, H-18), 1.09 and 1.13 (each 3H and s, H-26 and H-27), 3.23 (3H, s, OMe-25), 3.36 (1H, br d, *J* = 10.8 Hz), 3.53 (1H, d, *J* = 10.4 Hz) and 3.75 (1H, d, *J* = 9.7 Hz) (H-28), 3.75 (1H, dd, *J* = 4.6, 9.8 Hz, H-3α). (**22a**) mp 210−213 °C; $[\alpha]_D$ +46.7° (*c* 0.1, $CHCl_3$); IR ν_{max} cm^{-1} 1729 and 1249 (OAc); MS, *m/z* (%) 616 (M^+, 8), 601 (1), 584 (18), 556 (16), 541 (4), 496 (33), 415 (13), 376 (10), 355 (78), 295 (24), 255 (20), 73 (100); HR-MS, *m/z* 616.4338 [calcd for $C_{37}H_{60}O_7$ (M^+) 616.4339].

*22α-Methoxyfaradiol (**23**) and its diacetate (**23a**):* (**23**) mp 235−236 °C; $[\alpha]_D$ +24.0° (*c* 0.2, $CHCl_3$); IR ν_{max} cm^{-1} 3399 (OH), 829 (>C=CH−); MS, *m/z* (%) 472 (M^+, 47), 457 (22), 454 (11), 440 (100), 425 (23), 397 (54), 379 (10), 273 (13), 247 (7), 207 (22), 189 (35); HR-MS, *m/z* 472.3915 [calcd for $C_{31}H_{52}O_3$ (M^+) 472.3916]; ^{1}H NMR δ 0.64 (3H, s, H-28), 0.77 (3H, s, H-24), 0.85 (3H, s, H-25), 0.97 (3H, s, H-23), 1.02 (3H, d, *J* = 6.6 Hz, H-29), 1.03 (3H, s, H-26), 1.04 (3H, s, H-27), 1.72 (3H, s, H-30), 3.20 (1H, dd, *J* = 5.1, 11.2 Hz, H-3a), 3.33 (3H, s,

Table 3. ^{1}H NMR Data of 13 New Fatty Acid Esters of Triterpene Diols and Triols from the Extract of Edible Chrysanthemum Flowers[a]

proton	1p	2p	6p	7m	9s	10p	11p	14p	16p	18m	19m	23p	25p
H-3	4.48 (dd) (5.9, 10.6)	4.50 (dd) (5.0, 10.0)	4.48 (dd) (5.5, 10.4)	4.48 (dd) (6.6, 10.7)	4.48 (dd) (5.5, 10.6)	4.50 (dd) (7.0, 8.8)	4.57 (dd) (4.7, 11.3)	4.57 (dd) (4.6, 11.3)	4.48 (dd) (5.5, 11.0)	4.46 (dd) (5.4, 10.5)	4.48 (dd) (5.5, 10.7)	4.48 (dd) (5.5, 11.0)	4.48 (dd) (5.5, 10.7)
H-12		5.19 (t) (3.7)				5.24 (t) (3.7)							
H-16	3.39 (dd) (4.4, 11.7)	4.22 (dd) (5.1, 11.0)			3.44 (dd) (4.4, 11.4)	4.20 (dd) (5.1, 11.4)			3.37 (dd) (5.2, 13.2)	3.83 (dd) (1.9, 9.5)	4.11 (dd) (4.9, 11.8)	4.13 (dd) (6.8, 12.8)	
H-18				0.88 (s)			0.96 (s)	0.97 (s)					0.87 (s)
H-19				0.88 (s)			0.34 and 0.57 (each d, 4.1)	0.34 and 0.58 (each d, 4.1)					0.86 (s)
H-21			1.14 (s)	1.15 (s)	5.31 (d) (7.0)		0.89 (d) (6.3)	0.89 (d) (7.9)	3.08 (dd) (1.2, 6.8)		5.65 (d) (6.6)	5.64 (d) (6.0)	1.13 (s)
H-22									2.34 (dd) (6.9, 14.6)		3.88 (d) (6.6)	3.45 (d) (6.0)	
H-23	0.85 (s)	0.87 (s)			0.85 (s)	0.87 (s)			0.84 (s)	0.84 (s)	0.85 (s)	0.85 (s)	
H-24	0.85 (s)	0.87 (s)	5.12 (tt) (2.0, 7.1)	3.64 (dd) (5.5, 9.9)	0.85 (s)	0.87 (s)	3.37 (dt) (2.4, 10.2)	3.29 (br d) (9.8)	0.84 (s)	0.84 (s)	0.84 (s)	0.84 (s)	3.73 (t) (7.1)
H-25	0.88 (s)	0.98 (s)			0.88 (s)	0.97 (s)			0.87 (s)	0.85 (s)	0.88 (s)	0.87 (s)	
H-26	1.03 (s)	1.03 (s)	1.69 (s)	1.10 (s)[b]	1.00 (s)	0.99 (s)	1.09 (s)[b]	1.17 (s)[b]	1.03 (s)	1.06 (s)	1.04 (s)	1.03 (s)	1.12 (s)[b]
H-27	0.98 (s)	1.15 (s)	1.63 (s)	1.19 (s)[b]		1.22 (s)	1.13 (s)[b]	1.22 (s)[b]	0.93 (s)	0.99 (s)	1.06 (s)	1.04 (s)	1.21 (s)[b]
H-28	0.85 (s)	0.77 (s)	0.87 (s)[b]	0.85 (s)	0.73 (s)	0.79 (s)	0.84 (s)	0.84 (s)	0.78 (s)	3.39 and 4.16 (each d, 10.6)	0.64 (s)	0.64 (s)	0.85 (s)
H-29	1.03 (d) (6.2)	0.79 (d) (6.2)	0.88 (s)[b]	0.85 (s)	1.00 (d) (6.2)	0.89 (s)	0.89 (s)	0.89 (s)	1.13 (d) (6.3)	4.61 and 4.69 (each s)	1.05 (d) (5.5)	1.02 (d) (6.6)	0.85 (s)
H-30	4.64 (dt) (2.0, 7.3)	0.94 (br s)	0.96 (s)	0.97 (s)	1.65 (br s)	0.91 (s)	0.90 (s)	0.90 (s)	1.33 (s)	1.68 (s)	1.72 (br s)	1.72 (br s)	0.95 (s)
22-OMe												3.26 (s)	
25-OMe							3.23 (s)						
MeCH$_2$−	0.88 (t) (7.0)	0.88 (t) (7.0)	0.88 (t) (6.9)	0.88 (t) (6.9)	0.88 (t) (6.9)	0.88 (t) (7.0)	0.88 (t) (7.1)	0.88 (t) (7.3)	0.88 (t) (7.1)	0.88 (t) (6.6)	0.88 (t) (6.6)	0.88 (t) (7.1)	0.88 (t) (6.6)
−CH$_2$−	1.25 (br s)	1.25 (br s)	1.25 (br s)	1.25 (br s)	1.25 (br s)	1.26 (br s)	1.25 (br s)	1.25 (br s)	1.25 (br s)	1.25 (br s)	1.25 (br s)	1.25 (br s)	1.25 (br s)
−CH$_2$COO	2.29 (t) (7.3)	2.29 (t) (7.0)	2.29 (t) (7.4)	2.29 (t) (6.9)	2.29 (t) (7.7)	2.29 (t) (7.0)	2.30 (t) (7.4)	2.30 (t) (7.6)	2.29 (t) (7.7)	2.29 (t) (7.3)	2.29 (t) (6.9)	2.29 (t) (7.4)	2.29 (t) (6.9)

[a] Figures in parentheses denote *J* values (hertz). [b] Values bearing the same superscript in each column are interchangeable.

Table 4. ^{13}C, ^{1}H, and HMBC NMR Spectral Data for the Acetyl Derivatives of Three New Triterpenes from the Extract of Edible Chrysanthemum Flowers

	11a			22a			23a		
carbon	δ_C	δ_H[a]	HMBC (H to C)	δ_C	δ_H[a]	HMBC (H to C)	δ_C	δ_H[a]	HMBC (H to C)
1	31.6	1.61 (α), 1.25 (*β*)	2, 9, 10, 19	31.4	1.61 (α), 1.25 (*β*)	5, 19	38.4	1.69 (α), 1.03 (*β*)	
2	26.8	1.77 (α), 1.62 (*β*)	4, 10	26.2	1.87 (α), 1.64 (*β*)	1, 4, 10	23.7	1.63 (2H)	1
3	80.7	4.56 (dd, 4.8, 11.0)	28, 29, 3-OCOMe	74.2	4.89 (dd, 4.6, 11.0)	2, 4, 28, 29, 3-OCOMe	80.9	4.48 (dd, 4.9, 11.3)	23, 24, 3-OCOMe
4	39.4			42.4			37.8		
5	47.2	1.39	4, 6, 10, 19, 29	40.9	1.78	1, 3, 4, 6, 7, 10, 19, 29	55.3	0.81	23, 24
6	20.9	1.58 (α), 0.79 (*β*)	5, 8	20.7	1.45 (α), 0.82 (*β*)	5	18.1	1.51 (α), 1.38 (*β*)	6
7	25.8	1.10 (α), 1.31 (*β*)	5, 8	25.7	1.10 (α), 1.33 (*β*)	8	34.2	1.44 (2H)	
8	47.8	1.51	7, 9, 10, 14, 15, 19, 30	48.0	1.50	7, 9, 13, 14, 15, 19, 30	41.1		
9	20.1			20.0			49.8	1.33	11
10	26.0			25.3			37.0		
11	26.4	1.97 (α), 1.13 (*β*)	9, 10, 12, 13, 19	26.4	2.01 (α), 1.10 (*β*)	9, 10, 12, 13, 19	21.5	1.55 (α), 1.27 (*β*)	8, 12, 13
12	32.8	1.62 (2H)	11, 13, 14, 18	32.8	1.61 (2H)	9, 11, 13, 14	27.0	1.24 (α), 1.65 (*β*)	13, 18
13	45.3			45.3			38.2	1.72	
14	48.8			48.8			42.7		
15	35.5	1.29 (2H)	14, 16, 30	35.5	1.28 (2H)	14, 30	32.6	1.48 (α), 1.62 (*β*)	13, 14, 16
16	28.0	1.88 (α), 1.30 (*β*)	15	28.0	1.87 (α), 1.28 (*β*)	15	73.1	5.29 (dd, 4.9, 11.6)	28
17	52.1	1.61	18	52.1	1.58	16, 18	41.9		
18	18.0	0.95 (s)	12, 13, 14, 17	18.1	0.95 (s)	12, 13, 14, 17	41.0	1.56	19
19	29.8	0.33 (1H, d, 4.4, *exo*)	1, 5, 8, 9, 10	30.0	0.39 (1H, d, 4.3, *exo*)	1, 5, 11	36.3	1.70	
		0.57 (1H, d, 4.4, *endo*)	1, 5, 8, 9, 10		0.57 (1H, d, 4.0, *endo*)	1, 5, 11			
20	36.3	1.35		36.3	1.34		145.8		
21	18.4	0.88 (d, 7.3)	17, 20, 22	18.4	0.88 (d, 6.7)	17, 20, 22	119.1	5.63 (d, 6.1)	17, 19, 30
22	32.7	1.00, 1.38		32.7	1.00, 1.38		76.8	3.15 (d, 6.1)	17, 18, 20, 21, 28, 22-OMe
23	26.1	1.37, 1.77		26.1	1.36, 1.87	22	27.9	0.85 (s)	4, 24
24	78.4	4.90 (dd, 2.2, 10.6)	22, 23, 25, 26, 27, 24-OCOMe	78.4	4.90 (dd, 2.4, 10.7)	22, 23, 25, 26, 27, 24-OCOMe	16.5	0.84 (s)	4, 23
25	76.1			76.2			16.4	0.87 (s)	1, 9, 10
26	21.1[b]	1.14 (s)[b]	24, 25, 27	21.1[b]	1.14 (s)[b]	24, 25, 27	16.1	1.03 (s)	7, 9, 10, 14
27	22.2[b]	1.15 (s)[b]	24, 25, 26	22.2[b]	1.15 (s)[b]	24, 25, 26	16.0	1.08 (s)	8, 13, 14, 15
28	25.4	0.84 (s)	3, 4, 5, 29	64.8	3.76 (1H, d, 11.3)	3, 4, 5, 29, 28-OCOMe	13.8	0.71 (s)	16, 17, 18, 22
					3.90 (1H, d, 11.3)	3, 4, 5, 29, 28-OCOMe			
29	15.1	0.89 (s)	3, 4, 5, 28	11.5	0.89 (s)	3, 4, 5, 28	22.3	1.03 (d, 5.5)	18, 19, 20
30	19.3	0.89 (s)	8, 13, 14, 15	19.4	0.90 (s)	8, 13, 14, 15	22.0	1.71 (s)	19, 20, 21
3-OCOMe	21.3	2.05 (s)	3-OCOMe	21.3	2.03 (s)	3-OCOMe	21.3	2.04 (s)	3-OCOMe
3-OCOMe	170.9			170.6			171.0		
16-OCOMe							21.3	2.00 (s)	16-OCOMe
16-OCOMe							170.3		
22-OMe							56.4	3.22 (s)	22
24-OCOMe	21.1	2.08 (s)	24-OCOMe	21.2	2.09 (s)	24-OCOMe			
24-OCOMe	171.0			171.0					
25-OMe	49.7	3.23 (s)	25	49.7	3.23 (s)	25			
28-OCOMe				21.0	2.06 (s)	28-OCOMe			
28-OCOMe				171.2					

[a] Figures in parentheses denote *J* values (hertz). [b] Values bearing same superscript in each column are interchangeable.

OMe-22), 3.45 (1H, d, J = 6.1 Hz, H-22β), 4.13 (1H, dd, J = 4.9, 11.8 Hz, H-16α), 5.64 (1H, d, J = 6.1 Hz, H-21). (**23a**) mp 220−224 °C; $[\alpha]_D$ +22.7° (c 0.2, $CHCl_3$); IR ν_{max} cm^{-1} 1737 and 1245 (OAc), 831 (>C=CH−); MS, m/z (%) 556 (M^+, 2), 541 (1), 524 (1), 509 (1), 496 (35), 481 (9), 466 (5), 451 (1), 389 (1), 371 (1), 259 (1), 217 (3), 189 (11), 163 (21), 43 (100); HR-MS, m/z 556.4109 [calcd for $C_{35}H_{56}O_5$ (M^+) 556.4128].

*(R)-Bis-MTPA (**20R**) and (S)-bis-MTPA esters (**20S**) of (24S)-lanost-9(11)-ene-3β,24,25-triol (**20**):* (**20R**) ^{1}H NMR δ 0.63 (3H, s, H-18), 0.73 (3H, s, H-30), 0.82 (3H, s, H-28), 0.83 (3H, s, H-29), 0.88 (3H, d, J = 6.4 Hz, H-21), 1.08 (3H, s, H-19), 1.14 and 1.18 (each 3H and s, H-26 and H-27), 4.72 (1H, dd, J = 4.3, 11.3 Hz, H-3α), 4.95 (1H, dd, J = 2.1, 9.5 Hz, H-24), 5.23 (1H, d, J = 5.8 Hz, H-11). (**20S**) ^{1}H NMR δ 0.59 (3H, s, H-18), 0.72 (3H, s, H-30), 0.84 (3H, d, J = 6.7 Hz, H-21), 0.84 (3H, s, H-29), 0.91 (3H, s, H-28), 1.05 (3H, s, H-19), 1.17 and 1.23 (each 3H and s, H-26 and H-27), 4.69 (1H, dd, J = 4.3, 11.9 Hz, H-3α), 4.95 (1H, dd, J = 2.1, 9.8 Hz, H-24), 5.22 (1H, d, J = 6.1 Hz, H-11).

*(R)-Bis-MTPA (**24R**) and (S)-bis-MTPA esters (**24S**) of (24S)-29-norcycloartane-3β,24,25-triol (**24**):* (**24R**) ^{1}H NMR δ 0.15 (1H, d, J = 4.3 Hz; *exo*) and 0.39 (1H, d, J = 4.0 Hz; *endo*) (H-19), 0.72 (3H, d, J = 6.7 Hz, H-28), 0.87 (3H, d, J = 6.4 Hz, H-21), 0.88 (3H, s, H-30), 0.94 (3H, s, H-18), 1.15 and 1.18 (each 3H and s, H-26 and H-27), 4.75 (1H, ddd, J = 4.6, 10.7, 10.7 Hz, H-3α), 4.95 (1H, dd, J = 2.1, 9.5 Hz, H-24). (**24S**) ^{1}H NMR δ 0.11 (1H, d, J = 4.3 Hz; *exo*) and 0.36 (1H, d, J = 4.0 Hz; *endo*) (H-19), δ 0.82 (3H, d, J = 6.4 Hz, H-21), 0.89 (3H, d, J = 6.7 Hz, H-28), 0.87 (3H, s, H-30), 0.91 (3H, s, H-18), 1.17 and 1.23 (each 3H and s, H-26 and H-27), 4.73 (1H, ddd, J = 4.9, 10.7, 10.7 Hz, H-3α), 4.95 (1H, dd, J = 2.1, 9.8 Hz, H-24).

*3-Epicabraleadiol (**7**) and its monoacetate (**7a**):* (**7**) ^{1}H NMR δ 0.78 (3H, s, H-29), 0.85 (3H, s, H-19), 0.88 (3H, s, H-18), 0.97 (6H, s, H-28 and H-30), 1.11 and 1.19 (each 3H and s, H-26 and H-27), 1.14 (3H, s, H-21), 3.20 (1H, dd, J = 5.2, 10.1 Hz, H-3α), 3.64 (1H, br d, J = 7.6 Hz, H-24). (**7a**) ^{13}C and ^{1}H NMR C-1 [δ_C 38.7; δ_H 1.06, 1.69], C-2 [23.7; 1.62 (2H)], C-3 [80.9; 4.48 (dd, J = 5.9, 10.3 Hz)], C-4 [37.9], C-5 [56.0; 0.89], C-6 [18.2; 1.44, 1.52], C-7 [35.2; 1.27, 1.55], C-8 [40.4], C-9 [50.8; 1.35], C-10 [37.1], C-11 [21.8; 1.22, 1.53], C-12 [26.4; 1.20, 1.83], C-13 [42.8], C-14 [50.0], C-15 [31.4; 1.07, 1.47], C-16 [25.8; 1.32, 1.75], C-17 [49.8; 1.86], C-18 [16.4; 0.97 (s)], C-19 [16.3; 0.87 (s)], C-20 [86.5], C-21 [27.1; 1.14 (s)], C-22 [34.9; 1.68, 1.88], C-23 [27.0; 1.79, 1.86], C-24 [86.3; 3.64 (dd, J = 5.4, 10.0 Hz)], C-25 [70.2], C-26 and C-27 [24.1 and 27.8; 1.11 (s) and 1.19 (s)], C-28 [28.0; 0.85 (s)], C-29 [16.5; 0.85 (s)], C-30 [15.5; 0.97 (s)], 3-OCOMe [21.3; 2.04 (s)], 3-OCOMe [171.0].

Assay of TPA-Induced Inflammation Ear Edema. TPA (1 μg) dissolved in acetone (20 μL) was applied to the right ear only of ICR mice by means of a micropipet. A volume of 10 μL was delivered to both the inner and outer surfaces of the ear. The samples or their vehicles, $CHCl_3$/methanol (1:1, v/v; 20 μL), as control, were applied topically ~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:

edema A is induced by TPA alone ($b - a$)

edema B is induced by TPA plus sample ($b' - a$)

inhibitory ratio (%) =

$$[(\text{edema A} - \text{edema B})/\text{edema A}] \times 100$$

Each value was the mean of individual determinations from five mice. The 50% inhibitory dose (ID_{50}) values were determined according to the method of probit-graphic interpolation for four dose levels.

Statistical Analysis. Statistical analysis was carried out by using Student's t test.

RESULTS

Thirty-two 3-*O*-fatty acid esters of triterpene diols and triols, including 26 new compounds from the *n*-hexane soluble fraction, and 24 triterpene diols and triols, including 3 new compounds from the nonsaponifiable lipid fraction, were isolated and characterized in the methanol extract of edible chrysanthemum flowers in this study. Tables 1 and 2 show the chromatographic data and the compositions of these triterpenes. Two triterpenes, **9** and **19**, which constituted the most predominant components in the triterpene diol and triol fractions, respectively, of the nonsaponifiable lipid fraction (Table 1), have been shown to be present mostly as the 3-*O*-palmitoyl esters, **9p** and **19p**, in the chrysanthemum flower extract (Table 2).

Structural determination of three new triterpenes, **11**, **22**, and **23**, as the acetyl derivatives is described below. Stereochemical determination at C-24 of **11** and two known 24-hydroxytriterpenes, **20** and **24**, by means of Mosher's method is also described below.

(24*S*)-25-Methoxycycloartane-3β,24-diol (11). Compound **11a** ($C_{35}H_{58}O_5$), the diacetyl derivative of **11**, has two secondary acetoxyl groups [ν_{max} 1732 cm^{-1}; δ_H 2.05 and 2.08 (each 3H and s); δ_H 4.56 and 4.90 (each 1H and dd)], a methoxyl group [δ_H 3.23 (s)], a secondary methyl [δ_H 0.88 (d)], six tertiary methyls (δ_H 0.84, 0.88, 0.88, 0.95, 1.14, and 1.15), and cyclopropylmethylene protons (ABq at δ_H 0.33 and 0.57), characteristic of nonequivalent protons of a cyclopropylmethylene group located most probably at C-19, which was supported by an MS fragment ion at m/z 376 [$M^+ - C_{11}H_{18}O_2$ (ring A)] (*27*). These, in combination with the diagnostic MS fragment ions at m/z 357 [$M^+ - C_{11}H_{21}O_3$ (side chain)], 297 (m/z 357 − HOAc), and 255 [m/z 297 − C_3H_6 (ring D)], suggested that **11a** possesses a 3β-acetoxycycloartane-type skeleton and a C_8 side chain with an acetoxyl and a methoxyl group (*27*, *28*). A further MS fragment ion at m/z 73 ($C_4H_9O^+$, C_{25}−C_{27}; base peak) suggested that the methoxyl group is located at C-25 and the acetoxyl group most probably at C-24. This was supported by heteronuclear multiple-bond correlation (HMBC) spectroscopy, which provided cross-correlations for H-24 (with C-22, C-23, C-25, C-26, C-27,and 24-OCOMe), H-26 (with C-24, C-25, and C-27), H-27 (with C-24, C-25, and C-26), and 25-OMe (with C-25) (Table 4). Further analysis of the ^{13}C DEPT, ^{1}H−^{1}H correlation spectroscopy (COSY), ^{1}H detected multiple quantum coherence (HMQC), and HMBC spectra and comparison of the ^{13}C and ^{1}H NMR spectral data (Table 4) with those of relevant compounds (*27*, *28*) revealed the structure of **11a** to be 25-methoxycycloartane-3β,24-diol diacetate. The absolute configuration at C-24 was determined by application of the modified Mosher's method (*26*) for the (*R*)-bis-MTPA (**11R**) and (*S*)-bis-MTPA esters (**11S**). As shown in Figure 3, the $\Delta\delta$ ($\delta_S - \delta_R$) values for the H-26 and H-27 ($\Delta\delta$ = 0.05 and 0.07) and OMe-25 signals ($\Delta\delta$ = 0.03) were found to be positive, whereas those for the H-18 ($\Delta\delta$ = −0.03) and H-21 signals ($\Delta\delta$ = −0.04) were negative, which unequivocally demonstrated that **11** possesses 24*S*-stereochemistry (*26*). The 24*R*-stereoisomer would show almost the opposite $\Delta\delta$ values for the corresponding signals. The combined evidence confirmed that the compound was (24*S*)-25-methoxycycloartane-3β,24-diol.

(24*S*)-25-Methoxycycloartane-3β,24,28-triol (22). Compound **22a** ($C_{37}H_{60}O_7$), a triacetyl derivative of **22**, has an acetoxy methylene group [ν_{max} 1729 cm^{-1}; δ_H

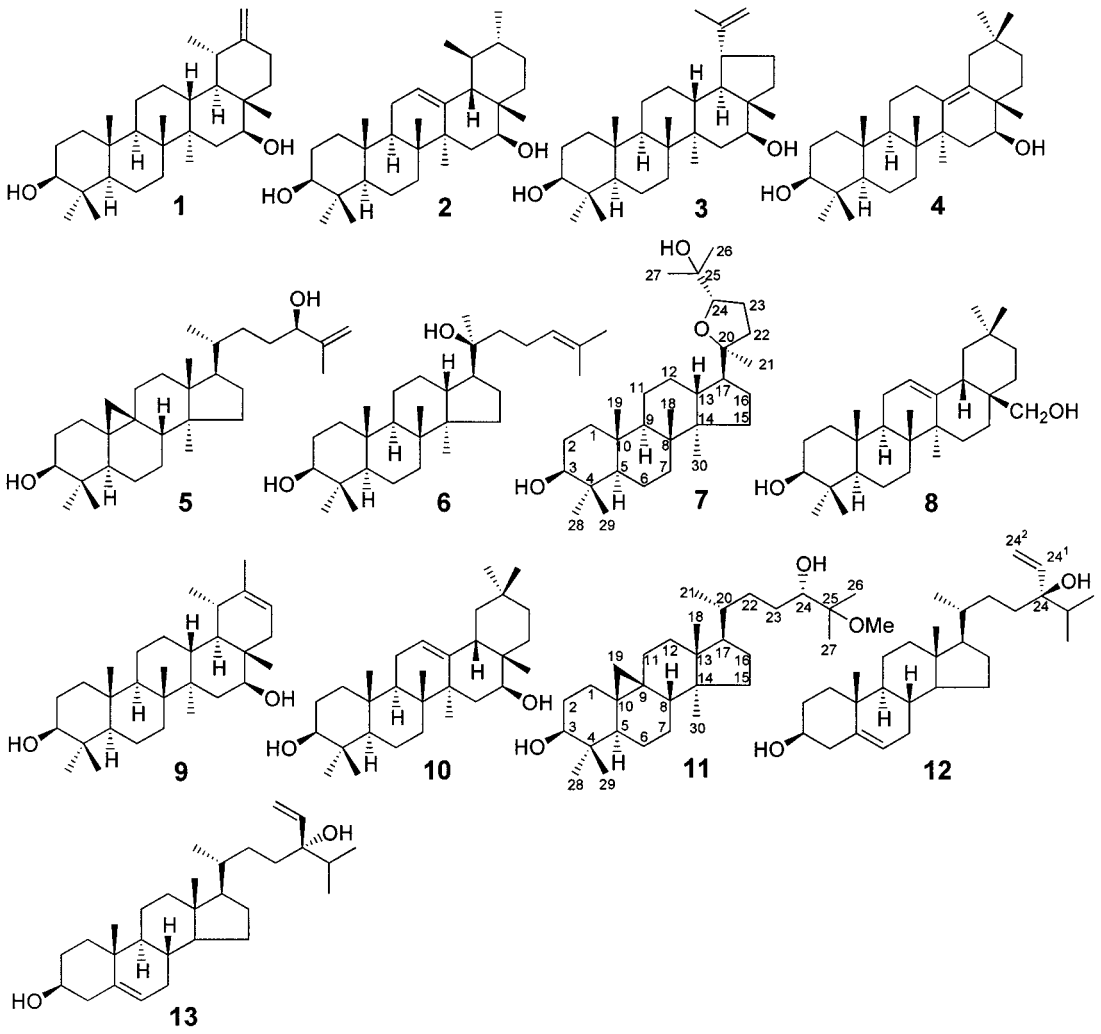

Figure 1. Structures of the triterpene diols described in this paper (see Table 1 for the systematic names).

2.06 (3H, s); δ_H 3.76 and 3.90 (each 1H and d)], two secondary acetoxyl groups [δ_H 2.03 and 2.09 (each 3H and s); δ_H 4.89 and 4.90 (each 1H and dd)], a tertiary methoxyl group [δ_H 3.23 (s)], a secondary methyl group [δ_H 0.88 (d)], and five tertiary methyl groups (δ_H 0.89, 0.90, 0.95, 1.14, and 1.15), and cyclopropylmethylene protons (ABq at δ_H 0.39 and 0.57). A close similarity of the ^{13}C and ^1H NMR spectral data (Table 4) for rings B, C, D and the side chain of **22a** with those of the corresponding signals of **11a**, which in combination with diagnostic MS fragment ions at *m/z* 415 [$M^+ - C_{11}H_{21}O_3$ (side chain)] and 376 [$M^+ - C_{13}H_{20}O_4$ (ring A)], suggested that **22a** possesses the (24*S*)-25-methoxycycloartane-3β,24-diol diacetate structure, with an acetoxy methylene group most probably at C-4. The proposed structure was supported by the HMBC experiment, which provided cross-correlations for H-28 (with C-3, C-4, C-5, C-29, and 28-OCOMe), H-29 (with C-3, C-4, C-5, C-28, and 28-OCOMe), H-24 (with C-22, C-23, C-25, C-26, C-27, and 24-OCOMe), H-26 (with C-24, C-25, and C-27), H-27 (with C-24, C-25, and C-26), and 25-OMe (with C-25) (Table 4). Compound **22a** exhibited definite nOe correlations between [H-29 (4β-Me)−H-19*endo* (9β,19-cyclopropylmethylene)−H-18 (13β-Me)−H-20] on the β-face and [H-28 (acetoxy methylene)−H-5α and H-6α] and [H-3α−H-7α−H-30 (14α-Me)−H-17α] on the α-face of the molecule in the nuclear Overhauser difference spectroscopy (NOESY), which indicated that the acetoxy methylene group (H-28) at C-4 is oriented to the α-face of the ring system. Thus, we propose that this compound is (24*S*)-25-methoxycycloartane-3β,24,28-triol triacetate. Analysis of the ^{13}C DEPT, $^1H-^1H$ COSY, HMQC, and NOESY spectra confirmed the proposed structure. Hydrolysis yielded (24*S*)-25-methoxycycloartane-3β,24,28-triol (**22**).

22α-Methoxytaraxast-20-ene-3β,16β-diol (22α-Methoxyfaradiol; 23). Compound **23a** ($C_{35}H_{56}O_5$), the diacetyl derivative of **23**, possesses two secondary acetoxyl groups [ν_{max} 1737 cm^{-1}; δ_H 2.00 and 2.04 (each 3H and s); δ_H 4.48 and 5.29 (each 1H and dd)], a secondary methoxyl group [δ_H 3.23 (3H, s); δ_H 3.15 (1H, d)], a vinyl methine [δ_H 5.63 (d)], a vinyl methyl (δ_H 1.71), a secondary methyl [δ_H 1.03 (d)], and six tertiary methyl groups (δ_H 0.71, 0.84, 0.85, 0.87, 1.03, and 1.08). Close similarity of the ^1H NMR spectral data (Table 4) of **23a** with those of the known **9a** (*20*) suggested that compound **23a** possesses a structure of faradiol with a methoxyl group most probably at C-22. The $^1H-^1H$ COSY spectrum of **23a** showed a definite cross-peak between the vinyl methine [H-21; δ_H 5.63 (d)] and the methoxyl methine signals [δ_H 3.15 (d)],which confirmed the methoxyl group located at C-22. The H-22 signal exhibited an nOe correlation with a methyl singlet at δ_H 0.71, assignable to H-28, suggesting that the methoxyl group at C-22 is oriented down from the α-face of the plane of the ring system. On the basis of the spectral

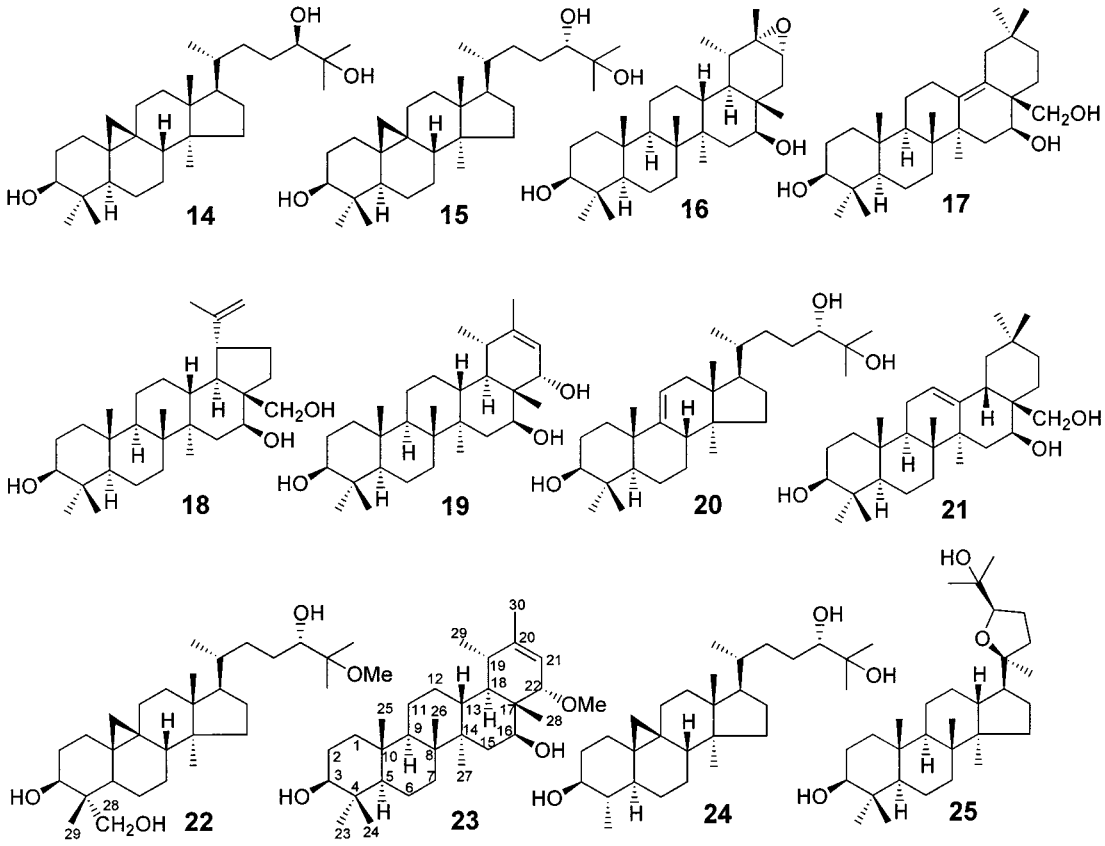

Figure 2. Structures of the triterpene triols described in this paper (see Table 1 for the systematic names).

evidence, compound **23a** was established as 22α-methoxytaraxast-20-ene-3*β*,16*β*-diol (22α-methoxyfaradiol) diacetate. Analysis of the ^{13}C DEPT, ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY spectra confirmed the proposed structure. Hydrolysis of **23a** yielded 22α-methoxyfaradiol (**23**).

Determination of the Stereochemistry at C-24 of Lanost-9(11)-ene-3*β*,24,25-triol (20) and 29-Norcycloartane-3*β*,24,25-triol (24). The stereochemistry at C-24 of the two 24-hydroxytriterpenes, **20** and **24**, was determined by application of the modified Mosher's method (*26*) for the (*R*)-bis-MTPA (**20*R*** and **24*R***) and (*S*)-bis-MTPA esters (**20*S*** and **24*S***). As shown in Figure 3, the $\Delta\delta$ ($\delta_S - \delta_R$) values for the H-26 and H-27 signals (**20**, $\Delta\delta$ = 0.03 and 0.05; **24**, $\Delta\delta$ = 0.02 and 0.05) were found to be positive, whereas those for the H-18 (**20**, $\Delta\delta$ = −0.04; **24**, $\Delta\delta$ = −0.03) and H-21 signals (**20**, $\Delta\delta$ = −0.04; **24**, $\Delta\delta$ = −0.05) were negative, which unequivocally demonstrated that both compounds, **20** and **24**, possess 24*S*-stereochemistry.

Inhibitory Effect of *Chrysanthemum* Triterpene Diols and Triols and Their 3-*O*-Fatty Acid Esters on TPA-Induced Inflammation in Mice. The methanol extract of the edible chrysanthemum flowers, the *n*-hexane, EtOAc, and *n*-butanol soluble fractions obtained from the methanol extract, nine triterpene esters isolated from the *n*-hexane soluble fraction, and six triterpenes, **6**, **8**, **11**, **14**, **16**, and **17**, isolated from the nonsaponifiable lipid fraction of the methanol extract were examined for their inhibitory effects on TPA-induced inflammation in mice. The inhibitory effects are shown in Table 5 along with those of eight other triterpenes, **1**−**3**, **9**, **10**, **18**, **19**, and **21**, which have also been isolated in this study and their anti-inflammatory activities have recently been evaluated (*3*). The inhibitory effects were compared with those of a reference compound, quercetin, a known inhibitor of TPA-induced inflammation in mice, and two commercially available anti-inflammatory drugs, indomethacin and hydrocortisone. All of the chrysanthemum triterpenes evaluated inhibited the TPA-induced inflammation with 0.03−1.0 mg per ear of the 50% inhibitory dose.

DISCUSSION

The triterpene diols and triols isolated from the nonsaponifiable lipid fraction of the methanol extract of edible chrysanthemum flowers were suggested to be present mostly as the 3-monoesterified form in the extract because the *n*-hexane soluble fraction of the methanol extract contained triterpene diols and triols only in the 3-monoesterified form. This is similar in nature to the triterpenes of marigold flowers from *Calendula officinalis* (*29*), of which preparations are widely used for topical application both in dermatology and in cosmetics owing mainly to their anti-inflammatory effect (*11*, *30*). Among 32 fatty acid esters of triterpene diols and triols isolated and characterized from the *n*-hexane soluble fraction, 6 were known compounds, and it is worthy of mention that all of the 6 known esters have so far been isolated from Compositae plant materials, that is, *Inula britannica* (**3m** and **3p**) (*10*), marigold (**9m** and **9p**) (*11*), *Dendranthema morifolium* (**18p** and **19p**) (*12*), and Chrysanthemi Flos (*Chrysanthemum morifolium*; **9p**) (*13*).

Three new methoxylated triterpenes, **11**, **22**, and **23**, isolated from the nonsaponifiable lipid fraction are possible artifacts formed from their hydroxy homologues, **15**, **19**, and (24*S*)-cycloartane-3*β*,24,25,28-tetrol, respectively, during methanol extraction of the plant

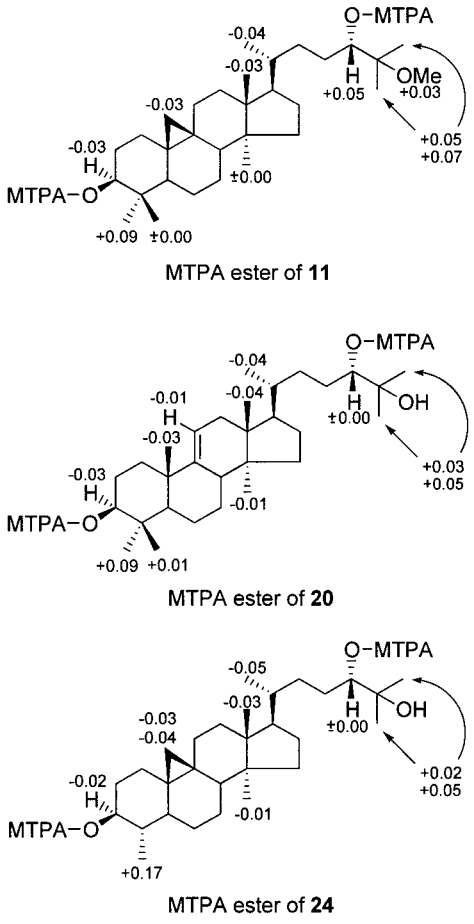

Figure 3. Chemical shift differences (Δδ) between (*S*)-bis-MTPA esters and (*R*)-bis-MTPA esters of three 24-hydroxy-triterpenes, (24*S*)-25-methoxycycloartane-3β,24-diol (**11**), (24*S*)-lanost-9(11)-ene-3β,24,25-triol (**20**), and (24*S*)-29-norcycloartane-3β,24,25-triol (**24**).

material. Triterpenes **15** and **19** are the constituents of fraction D′ (Table 1) of the nonsaponifiable lipid fraction. Whereas (24*S*)-cycloartane-3β,24,25,28-tetrol has not been detected in this study, its occurrence is highly probable in the chrysanthemum extract. Although 29-norcycloartane-3β,24,25-triol (24ξ-**24**), from pollen grains of *Ambrosia elatior* (Compositae) (*23*), and lanost-9(11)-ene-3β,24,25-triol (24ξ-**20**), from the bark of western white pine (*Pinus monticola*; Pinaceae) (*22*), have previously been isolated, their stereochemistry at C-24 remained undetermined. 3-Epicabraleadiol [**7**; (20*S*,24*R*)-20,24-epoxydammarane-3β,25-diol] has previously been isolated from the *Cistus bourgeanus* (*18*), but its stereochemistry at C-24 was erroneously assigned as 24*R*. The 24*S*-stereochemistry of **7** was assigned in this study on the basis of ^{13}C and ^{1}H NMR (see Materials and Methods section) spectral comparison with the literature data for the relevant compounds (*31*). Occurrence of saringosterols (**12** and **13**) has previously been reported only in some marine brown algae (*24*), and these have been suggested as artifacts produced during the isolation procedure by oxidation of fucosterol ([24(24^1)*E*]-stigmasta-5,24(24^1)-dien-3β-ol) (*24*).

The methanol extract [InhR (inhibitory ratio) = 87% at 1 mg/ear], the *n*-hexane (52%), EtOAc (63%), and *n*-butanol soluble fractions (49%) obtained from the extract, and the triterpene diols and triols and their fatty acid esters evaluated showed inhibitory effects on TPA-induced inflammation in mice. The higher inhibitory activity of the methanol extract compared with those of the fractionated extracts may be attributable to some synergistic effects. The inhibitory effects of the triterpenes evaluated were stronger than that of quercetin (ID_{50} = 1.6 mg/ear) and corresponded to or were stronger than that of indomethacin (ID_{50} = 0.3 mg/ear), with some exceptions. Compound **19** (0.03 mg/ear) followed by **2** (0.05 mg/ear) and **18** (0.05 mg/ear) exhibited fairly strong inhibitory effects, which were almost comparable with that of hydrocortisone (ID_{50} = 0.03 mg/ear). The inhibitory effects of the fatty acid esters were weaker than their corresponding free triterpene diols and triols. Such a reduction of the activity by esterification at C-3 has recently been observed also in croton oil-induced edema assay in mice for a triterpene diol, **9** (*11*, *30*), which could be due to kinetic reasons because highly lipophilic compounds can be trapped in the epidermis, reaching a lower concentration at the underlying action site.

Table 5. Inhibitory Effects of Triterpene Diols and Triols and Their Fatty Acid Esters from the Extract of Edible Chrysanthemum Flowers and Reference Compounds on TPA-Induced Inflammation in Mice[a]

	free alcohol		myristate		palmitate	
	ID_{50} (mg/ear)	InhR (%)	ID_{50} (mg/ear)	InhR (%)	ID_{50} (mg/ear)	InhR (%)
triterpene						
1	0.1[b]	96[b]			1.0	52
2	0.05[b]	97[b]	0.2	81	0.2	76
3	0.2[b]	67[b,c]			0.3	81
6	0.3	87				
8	0.1	97				
9	0.2[b]	94[b]	1.0	50	0.9	57
10	0.1[b]	87[b]	0.3	73	0.4	72
11	0.3	87				
14	1.0	50				
16	0.2	96				
17	0.1	96				
18	0.05[b]	97				
19	0.03[b]	100[b]			0.3	68
21	0.2[b]	95				
ref compd						
quercetin	1.6	40				
indomethacin	0.3	96				
hydrocortisone	0.03	99				

[a] ID_{50}, 50% inhibitory dose. InhR, inhibitory ratio. Unless otherwise stated, the InhR was at 1 mg per ear, and $p < 0.01$ by Student's *t* test as compared to control group. [b] Values taken from the literature (*3*). [c] InhR at 0.5 mg per ear.

The triterpenols from Compositae flowers also have been revealed to possess marked inhibitory effects (ID_{50} = 0.1−0.8 mg/ear) on TPA-induced inflammation in mice, although their inhibitory effects were somewhat lower than their corresponding diols and triols (*2*). The triterpene diols and triols as well as mono-ols, especially **9** and **19**, which occur as the principal diol and triol constituents, respectively (Tables 1 and 2), might be significant principles for the activity of the methanol extract of edible chrysanthemum flowers. The inhibitory effects against TPA-induced inflammation have been demonstrated to closely parallel those against tumor promotion (*3*, *4*, *6*, *32*), and it has recently been revealed that **9** (*4*, *6*) and **19** (*4*) markedly inhibited the tumor promotion on two-stage carcinogenesis promoted by TPA following initiation with 7,12-dimethylbenz[*a*]anthracene, a well-known initiator, in mouse back skin. Taking this into consideration, edible chrysanthemum flowers, of which the methanol extract exhibited marked anti-

inflammatory activity and contains highly anti-inflammatory triterpene diols and triols, although as the 3-*O*-fatty acid esters, along with triterpene mono-ols, might be of importance from the point of view of cancer chemoprevention.

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Received for review February 7, 2001. Revised manuscript received May 16, 2001. Accepted May 18, 2001. We gratefully acknowledge support for this research by a grant, "Research and Development of Nanoscience" from the Ministry of Education, Science, Sports and Culture to promote multidisciplinary research projects.

JF010164E