Helianol [3,4-*seco*-19(10→9)*abeo*-8α,9β,10α-eupha-4,24-dien-3-ol], a Novel Triterpene Alcohol from the Tabular Flowers of *Helianthus annuus* L.

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A novel 3,4-*seco*-triterpene alcohol with a migrated euphane-type skeleton, called helianol, was isolated together with seven known triterpene alcohols from the nonsaponifiable lipid of the tabular flower extract of *Helianthus annus* L. The structure was determined by extensive spectroscopic analyses.

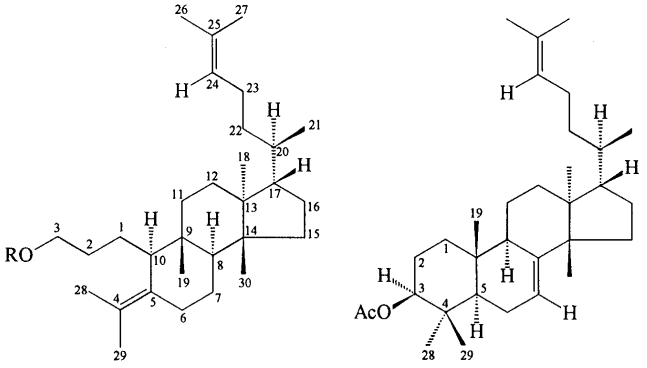
Key words *Helianthus annuus*; tabular flower; *seco*-triterpene alcohol; helianol; Compositae

In the course of our research on the phytochemical and pharmacological aspects of Compositae plants, we have demonstrated that some sterols[1] and alkane-6,8-diols[2,3] isolated from the flowers of *Carthamus tinctorius* L., and some triterpenes isolated from the flowers of *C. tinctorius*, *Chrysanthemum morifolium* Ramat., and *Helianthus annuus* L.[4] exhibited activity against 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammatory ear edema in mice[1,3,4] and tumor promotion in mouse skin.[1,3] This paper describes our continued study which led to the isolation of a novel *seco*-triterpene alcohol from the tabular flowers of *H. annuus* (sunflower) and its characterization as 3,4-*seco*-19(10→9)*abeo*-8α,9β,10α-eupha-4,24-dien-3-ol (**1**) which we called "helianol". There is no previous report on the triterepene alcohol constituents of the tabular flowers of *H. annuus*, whereas the ligulate flowers were shown to contain α-amyrin (urs-12-en-3β-ol), β-amyrin (olean-12-en-3β-ol), lupeol [lup-20(29)-en-3β-ol], taraxasterol [taraxast-20(30)-en-3β-ol], and ψ-taraxasterol (taraxast-20-en-3β-ol).[5]

Column chromatography on silica gel of the nonsaponifiable lipid obtained by alkaline hydrolysis from the MeOH extract of the dried tabular flowers of *H. annuus* yielded a triterpene alcohol fraction. Acetylation of the fraction followed by preparative reverse phase HPLC of the acetate fraction led to the isolation of a novel *seco*-triterpene alcohol, helianol (**1**), as the acetyl derivative (**2**) (78.3% of the triterpene acetate mixture as determined based on the HPLC and GLC data), together with seven known compounds as the acetyl derivatives[6,7]: α-amyrin (3.9%), β-amyrin (11.1%), lupeol (0.5%), ψ-taraxasterol (0.7%), cycloartenol (cycloart-24-en-3β-ol; 0.6%), 24-methylenecycloartanol (24-methylenecycloartan-3β-ol; 1.0%), and dammaradienol (dammara-20,24-dien-3β-ol; 0.5%).[8]

The molecular formula of **2** was determined to be $C_{32}H_{54}O_2$ on the basis of the high-resolution mass spectrum (HR-MS) [*m/z* 470.4116 (M^+)], while the ^{1}H-NMR spectrum (Table 1) showed the presence of four olefinic methyls, corresponding to two isopropylidene groups and indicating of it to be a tricyclic *seco*-triterpene. The presence of the signal of an acetoxymethylene group (δ 4.03, t) suggested it to be a 3,4-*seco*-triterpene-3-yl acetate structure.[9] Further distinctive signals observed in the ^{1}H-NMR of **2** were an olefinic methine proton (δ 5.10), three methyl singlets and one methyl doublet. The mass fragmentations observed at *m/z* 359 [loss of one (C_8H_{15}) of the two side chains], 287 [loss of one side chain ($CH_2=CHCH_2OAc$; $C_5H_8O_2$) and part of the other side chain (C_6H_{11}; C-22—C-27)], 259 (loss of both side chains $C_5H_8O_2$ and C_8H_{15}), and 69 [$CH_2CH=C(Me)_2]^+$ (C-23—C-27; base peak) supported the proposed structure and further suggested the presence of a C-24 mono-unsaturated C_8 side chain.[9] The other double bond might be located at C-4(5). Further diagnostic fragmentation ions at *m/z* 274 ($C_{20}H_{34}$), corresponding to the loss of *seco*-ring A and ring B by cleavages of the C-7–C-8 and C-9–C-10 bonds,[10,11] and 163 ($C_{12}H_{19}$; 274 − C_8H_{15}) indicated the absence of a methyl group at C-10 and suggesting **2** a 3,4-*seco*-cucurbitane or a 3,4-*seco*-19(10→9)*abeo*-euphane (or tirucallane).

The analysis of ^{13}C distortionless enhancement by polarization transfer (DEPT), ^{1}H–^{1}H correlated spectroscopy (COSY), ^{13}C–^{1}H COSY, and heteronuclear



(1) R = H (2) R = Ac (3)

Chart 1

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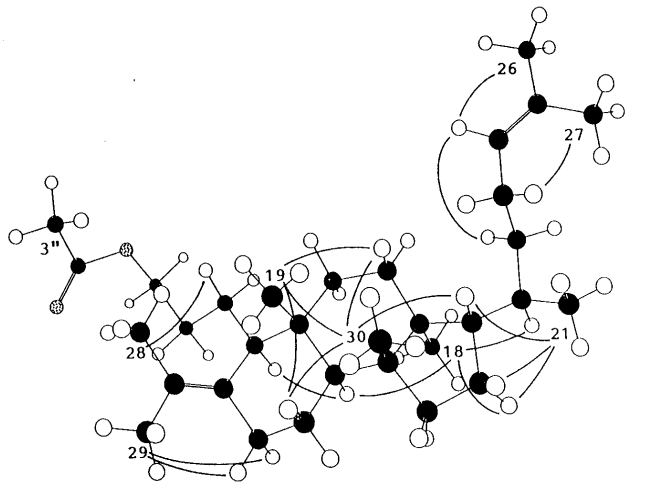
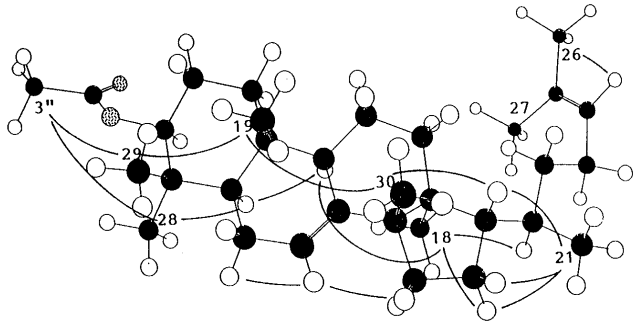


(2)



(3)

Fig. 1. CAChe Drawings and Some Representative NOE Correlations (−) for Helianyl Acetate (**2**) and Butyrospermyl Acetate (**3**)

NOE correlations between *gem*-protons were omitted from the figure.

multiple-bond correlation (HMBC) spectra supported the above assumption. That **2** possesses the stereochemistry of an 19(10→9)*abeo*-8α,9β,10α-euphane-type skeleton was established by comparison of the difference nuclear Overhauser effect (NOE) of **2** with that of the known butyrospermyl (eupha-7,24-dien-3β-yl) acetate (**3**). The difference NOE spectrum of **2** exhibited cross peaks between H-30 and H-17, H-17 and H-21; and H-21 and H-16αβ, H-16α and H-18, H-18 and H-20 (Table 1, Fig. 1) which were consistent with those observed for **3** (Fig. 1) suggesting that **2** possesses the stereochemistry of the C and D ring juncture (13α, 14β), C-17 (17β-H), and C-20 (20*R*) as does **3**. Furthermore, **2** showed other marked cross peaks between H-30 and H-19, H-18 and H-8, and H-8 and H-10, revealing 19(10→9)*abeo*-8α,9β,10α-euphane stereochemistry.

The most stable conformation of **2** and **3** with minimum steric energy was simulated using CAChe, and drawings[12] are shown in Fig. 1 accompanied by appreciable NOE's (−). The conformer of simulated **2** orients C-22 of the side chain at C-17 into a "left-handed" conformation (C-22 *cis*-oriented to C-13) similar to that of **3** and to the crystal structure of another euphane, eupha-8,24-dien-3β-yl (euphyl) acetate.[13] This was fairly consistent with the NOE experiment done in solution, thus confirming the proposed structure of **2**. The acetate **2**, upon hydrolysis,

Table 1. ^{13}C- and ^{1}H-NMR Spectral Data (δ/ppm) for Helianol (**1**) and Helianyl Acetate (**2**)[a]

C No.	Helianol (**1**)		Helianyl acetate (**2**)	
	^{13}C	^{1}H	^{13}C	^{1}H
1	32.1	1.55 (2H)	26.4	1.36, 1.52
2	26.3	1.33, 1.56	27.8	1.62 (2H)
3	63.9	3.62 (2H, t, 6.3)	65.3	4.03 (2H, t, 6.6)
4	122.2	—	122.3	—
5	134.2	—	134.0	—
6	25.1	2.28 (α), 2.07 (β)	25.4	2.24 (α), 2.09 (β)
7	23.6	1.34 (α), 1.59 (β)	23.7	1.33 (α), 1.51 (β)
8	44.0	1.61	44.2	1.59
9	38.6	—	38.6	—
10	54.9	2.29	54.9	2.26
11	39.1	1.54 (2H)	39.1	1.50 (2H)
12	30.3	1.53 (α), 1.72 (β)	30.3	1.54 (α), 1.70 (β)
13	46.1	—	46.1	—
14	47.8	—	47.8	—
15	34.1	1.13 (2H)	34.1	1.07 (α), 1.15 (β)
16	28.1	1.25 (α), 1.87 (β)	28.1	1.25 (α), 1.88 (β)
17	50.4	1.48	50.4	1.45
18 (13α)	15.4	0.80 (s)	15.4	0.80 (s)
19 (9β)	18.1	0.90 (s)	18.1	0.90 (s)
20	35.8	1.45	35.8	1.43
21	18.6	0.91 (d, 6.1)	18.6	0.91 (d, 6.1)
22	36.5	1.07, 1.43	36.5	1.04, 1.42
23	24.9	1.84, 2.03	24.9	1.84, 2.02
24	125.2	5.10 (tt, 1.3, 7.1)	125.3	5.10 (tt, 1.5, 7.1)
25	130.9	—	130.9	—
26	25.7	1.68 (s)	25.7	1.68 (s)
27	17.6	1.60 (s)	17.6	1.60 (s)
28	20.9	1.66 (s)	21.0	1.66 (s)
29	20.9	1.66 (s)	20.8	1.65 (s)
30 (14β)	19.0	0.83 (s)	19.0	0.83 (s)
COMe (3′)	—	—	171.3	—
COMe (3″)	—	—	21.1	2.04 (s)

a) Determined at 100.62 MHz (^{13}C-NMR) and 400 MHz (^{1}H-NMR) in $CDCl_3$ with $CDCl_3$ at δ 77.0 (^{13}C-NMR) or TMS (^{1}H-NMR) as internal standard. Figures in parentheses on ^{1}H-NMR denote *J* values (Hz). If not otherwise specified in parentheses, multiplicity of ^{1}H-NMR signals was not determined.

yielded a free alcohol, 3,4-*seco*-19(10→9)*abeo*-8α,9β,10α-eupha-4,24-dien-3-ol (**1**) (*m/z* 428.3987, M^+, $C_{30}H_{50}O$).[14] The fully assigned ^{13}C- and ^{1}H-NMR spectral data of **1** and **2** are shown in Table 1.

A 19(10→9)*abeo*-8α,9β,10α-euphane (called "reissantane") triterpene, reissantenol oxide [(24*S*)-24,25-epoxy-reissant-5-en-3β-ol],[15] and three other reissantane-type triterpenes[11] have been isolated from *Reisssantia indica* (Celastraceae), but this study constitutes the first isolation of the triterpene with a 3,4-*seco*-reissantane skeleton[16] from a natural source. Although a number of 3,4-*seco*-triterpenes are known to occur in nature,[17,18] only a few compounds possessing a 3-hydroxyl group have so far been reported as natural products, *i.e.*, 3,4-*seco*-dammara-4,24-dien-3-ol as the acetyl derivative, isolated from the roots of *Abrotanella forsterioides* (Compositae),[9] and 3,4-*seco*-D : B-friedo-B′ : A′-neogammacer-4(23)-en-3,5α-diol (espinendiol A) and its 5β-epimer (espinendiol B) isolated from the whole herb of *Euphorbia supina* (Euphorbiaceae).[19]

Experimental

Crystallizations were performed from acetone–MeOH. Preparative HPLC was carried out on an octadecyl silica column (Superiorex ODS

S-5 μm column, 25 cm × 10 mm i.d.; Shiseido Co., Ltd., Tokyo) with MeOH (4 ml/min) using an SSC Flow System 3100K (Senshu Scientific Co., Tokyo) and an ERC-7520 refractive index detector (Erma Optical Works, Ltd., Tokyo). Gas-liquid chromatography (GLC) was run on a Shimadzu GC-14A apparatus using a DB-17 fused silica capillary column (30 m × 0.3 mm i.d., column temp. 275 °C). In both HPLC and GLC, cholesterol (cholest-5-en-3β-ol) was the standard for the determination of Rt_R(I) of triterpene alcohol, whereas cholesteryl acetate was the standard for the determination of Rt_R(II) for the triterpene acetate. Electron-impact MS and HR-MS were taken on a Hitachi M-80B double focusing gas chromatograph-mass spectrometer (70 eV) using a direct inlet system. NMR spectra were recorded with a JEOL GSX-400 spectrometer at 400 MHz (^{1}H-NMR) and 100.62 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 standards, and chemical shifts were recorded in δ values. Acetylation was performed in Ac_2O–pyridine at room temperature overnight. Hydrolysis of the acetates was done in 5% KOH in MeOH also at room temperature overnight. Tabular flowers of sunflower were obtained from the plants cultivated in the Medicinal-Plant Garden of the College of Pharmacy of Nihon University. Butyrospermyl acetate (**3**) was used as the reference compound.[20] The assigned ^{13}C- and ^{1}H-NMR data of **3** are shown below.

Isolation Procedure Fresh tabular flowers (916 g) of sunflower were air dried (218 g) and extracted 3 times on 3 succesive days with MeOH at room temperature to give an extract (40 g). Nonsaponifiable lipids (2.71 g) obtained from the extract by alkaline hydrolysis (5% KOH in MeOH, reflux, 3 h) were subjected to column chromatography on silica gel (200 g) using the gradient solvent system (*n*-hexane : EtOAc = 1 : 0—1 : 4, v/v) to yield a triterpene alcohol fraction (473 mg) which, upon acetylation, gave the acetate fraction (366 mg). Preparative HPLC of the acetate fraction yielded **2** and the acetates[6,7] of the known α-amyrin, β-amyrin, lupeol, ψ-taraxasterol, cycloartenol, 24-methylenecycloartanol, and dammaradienol. Identification of known compounds was made based on the chromatographic (HPLC, GLC) and spectroscopic (MS, ^{1}H-NMR) data.

Helianyl [3,4-*seco*-19(10→9)*abeo*-8α,9β,10α-Eupha-4,24-dien-3-yl] Acetate (2) Amorphous gum. Rt_R(II): 0.63 (HPLC), 1.32 (GLC). MS *m/z* (%): 470 (M^+, 4), 455 (3), 427 (1), 410 (1), 401 (1), 386 (2), 359 (2), 357 (2), 299 (2), 287 (13), 274 (7), 259 (2), 221 (6), 205 (5), 203 (4), 191 (5), 189 (6), 175 (7), 163 (17), 149 (14), 69 (100). HR-MS *m/z*: 470.4116 [Calcd for $C_{32}H_{54}O_2$ (M^+): 470.4120]; 359.2958 [Calcd for $C_{24}H_{39}O_2$: 359.2948]; 287.2755 [Calcd for $C_{21}H_{35}$: 287.2737]; 274.2655 [Calcd for $C_{20}H_{34}$: 274.2659]; 259.2445 [Calcd for $C_{19}H_{31}$: 259.2424]; 163.1459 [Calcd for $C_{12}H_{19}$: 163.1486]; 69.0695 [Calcd for C_5H_9: 69.0704].

Helianol [3,4-*seco*-19(10→9)*abeo*-8α,9β,10α-Eupha-4,24-dien-3-ol] (1) Alkaline hydrolysis of the acetate (**2**) yielded helianol (**1**): Amorphous gum. Rt_R(I): 0.63 (HPLC), 1.44 (GLC). MS *m/z* (%): 428 (M^+, 8), 413 (1), 317 (1), 287 (14), 274 (8), 273 (5), 259 (3), 217 (3), 205 (5), 191 (4), 163 (18), 149 (10), 69 (100). HR-MS *m/z*: 428.3987 [Calcd for $C_{30}H_{52}O$ (M^+): 428.4015].

Butyrospermyl [Eupha-7,24-dien-3β-yl] Acetate (3) ^{13}C- and ^{1}H-NMR δ: C-1 [^{13}C: 36.8; ^{1}H : 1.22(α), 1.66(β)], C-2 [24.2; 1.67 (2H)], C-3 [81.1; 4.52, dd, J = 4.0, 11.0 Hz], C-4 [37.8], C-5 [50.8; 1.41, dd, J = 5.9, 12.1 Hz], C-6 [23.8; 2.13(α), 1.96(β)], C-7 [117.6; 5.25, dd, J = 2.7, 6.6 Hz], C-8 [146.0], C-9 [48.8; 2.22], C-10 [34.8], C-11 [18.1; 1.52 (2H)], C-12 [33.7; 1.66(α), 1.80(β)], C-13 [43.5], C-14 [51.3], C-15 [33.9; 1.45 (2H)], C-16 [28.5; 1.27(α),1.92(β)], C-17 [53.2; 1.49], C-18 [22.1; 0.80, s], C-19 [13.1; 0.77, s], C-20 [35.8; 1.40], C-21 [18.6; 0.85, d, J = 6.2 Hz], C-22 [35.2; 0.99, 1.59], C-23 [25.4; 1.88, 2.04], C-24 [125.1; 5.10, br t, J = 7.0 Hz], C-25 [131.0], C-26 [25.7; 1.69, s], C-27 [17.7; 1.61, s], C-28 [27.6; 0.85, s], C-29 [15.9; 0.93, s], C-30 [27.3; 0.97, s], C-3′ (COMe) [171.0], C-3″ (COMe) [21.3; 2.05, s].

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