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Triterpene Alcohols from Camellia and Sasanqua Oils and Their Anti-inflammatory Effects

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The nonsaponifiable lipids of camellia and sasanqua oils from the seeds of *Camellia japonica* L. and *C. sasanqua* THUNB., respectively, were investigated for their triterpene alcohol constituents. This led to the isolation of twenty-seven triterpene alcohols of which seven were novel naturally occurring compounds, tirucalla-5,7,24-trien-3β-ol (1), lemmaphylla-7,21-dien-3β-ol (2), isoeuphol (3), isotirucallol (4), (24*R*)-24,25-epoxybutyrospermol (5) and its 24*S*-epimer (6), and isoaglaiol (7). The structures were determined by spectroscopic and chemical methods. The inhibitory effects of 3, 4, a mixture of 5 and 6, a mixture of 7 and its 24*S*-epimer (aglaiol), and eight known triterpene alcohols isolated in this study were evaluated in ear inflammation in mice induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). The 50% inhibitory dose of these triterpenes for TPA-induced inflammation (1 μg per ear) was 0.2—0.9 mg/ear.

Key words *Camellia japonica*; *Camellia sasanqua*; seed oil; triterpene alcohol; antioedema; 12-*O*-tetradecanoylphorbol-13-acetate-induced ear oedema

The triterpene alcohol fractions of Theaceae seed oils contain a significant amount of euphane/tirucallane-type compounds, *viz.*, butyrospermol (**9**) and tirucalla-7,24-dien-3β-ol (**11**).[1,2] We undertook detailed re-investigation of the constituents of the triterpene alcohol fractions separated from the nonsaponifiable lipids (NSL) of camellia (*Camellia japonica* L.) and sasanqua (*Camellia sasanqua* THUNB.) seed oils and isolated seven novel compounds, **1**—**7**, along with twenty known compounds. This paper deals with the structure elucidation of the seven novel compounds and the anti-inflammatory activity of fourteen triterpene alcohols, isolated in this study, on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation in mice.

Results and Discussion

Column chromatography on silica-gel of the NSL obtained by alkaline hydrolysis of camellia and sasanqua oils yielded triterpene alcohol fractions. These were acetylated and the resulting acetate fractions were subjected to argentation TLC and HPLC, which enabled the isolation of twenty-seven compounds including seven novel ones, **1**—**7**, as their acetyl derivatives (**1a**—**7a**, respectively). Table 1 shows the compositions of triterpene alcohol fractions from the NSL of camellia and sasanqua oils along with their chromatographic data. Among the twenty known compounds **8**—**27**, aglaiol (**8**),[3,4] δ-amyrin (**21**),[5] and 17-epilupeol (**26**)[6] were identified as their acetyl derivatives by spectral comparison with literature data, whereas the others were identified by direct comparison of chromatographic and spectral data with reference compounds.

All the novel compounds, **1a**—**7a**, have a secondary acetoxyl group [ν_{max} 1729—1733, 1241—1250 cm^{-1}; δ_H 2.05—2.07 (3H, s)] associated with an adjacent methine [δ_H *ca.* 4.5—4.6 (1H, dd), J = *ca.* 4—6, 10—11 Hz]. The shift and coupling constants of the methine ^1H signal suggested that the acetoxyl group of the seven triterpene acetates is oriented equatorially (β) at C-3.[7]

The molecular formula of **1a** was determined as $C_{32}H_{50}O_2$ on the basis of the high-resolution mass spectrum (HR-MS) (M^+, m/z 466.3789). The compound had a $\Delta^{5,7}$-conjugated diene [λ_{max} 273 (log ε 4.04), 281 (4.00) nm; ν_{max} 1646, 837, 820 cm^{-1}; δ_H 5.55 (1H, d, J = 2.7, 5.5 Hz) and 5.89 (1H, d, J = 5.5 Hz)],[8] a terminal isopropylidene group [δ_H 1.61 (s), 1.68 (s)], and one secondary and five tertiary methyl groups. These data, in combination with fragment ions having m/z 451 (M^+ − Me), 391 (M^+ − Me − HOAc), 295 [loss of side-chain (s.c.; C_8H_{15}) and HOAc], 253 (295 − 42), 239 (295 − 42 − CH_2)[9] and 69 [$CH_2CH{=}C(Me)_2^+$], suggested that **1a** had a 3β-acetoxy tetracyclic triterpene skeleton possessing a $\Delta^{5,7}$-diene system and a C_8-side-chain containing an isopropylidene group. The diagnostic fragment ion at m/z 171 ($C_{13}H_{15}^+$) formed by the loss of ring D plus side-chain due to cleavage of the C-8–C-14 and C-9–C-11 bonds with concomitant HOAc loss supported the $\Delta^{5,7}$-unsaturation.[7,10] Furthermore, analysis of the ^1H–^1H and ^{13}C–^1H correlation spectroscopies (COSYs) and heteronuclear multiple-bond correlation (HMBC) spectra enabled the structure of **1a** to be formulated as 4,4,14-trimethyl-$\Delta^{5,7,24}$-statrien-3β-yl acetate with an as-yet-to-be-determined stereochemistry. In order to establish the stereostructure of **1a**, a difference nuclear Overhauser effect (NOE) experiment was undertaken. Thus, **1a** showed significant NOE correlations between [H-29(4β-Me)–H-19(10β-Me)–H-30(14β-Me)–H-17β–H-21] on the β-face, [H-3α–H-28(4α-Me)] and [H-9α–H-18(13α-Me)–H-20] on the α-face and [H-12α–H-21] of the molecule. These NOE correlations were consistent with those of two tirucallane-type triterpenes, tirucalla-5,24-dien-3β-yl acetate and **11a** (the acetate of **11**).[11] Compound **9a** (the acetate of **9**), a euphane-type triterpene, on the other hand, did not exhibit the NOE correlation between [H-12α–H-21].[11] Further-

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Table 1. Composition (%) and Chromatographic Data of Acetates of Triterpene Alcohols from the Saponified Neutral Fraction of Camellia and Sasanqua Oils

Code	Compound[a]	Acetate, Rt_R[b]			Composition (%)[c]	
		GLC	HPLC I	HPLC II	Camellia	Sasanqua
1	Tirucalla-5,7,24-trienol[d]	1.42	0.67	0.41	0.2	0.2
2	Lemmaphylla-7,21-dienol[d]	1.86	0.86	0.52	1.6	1.2
3	Isoeuphol [(20*R*)-dammara-13(17),24-dienol][d]	1.21	0.61	0.44	0.1	0.1
4	Isotirucallol [(20*S*)-dammara-13(17),24-dienol][d]	1.28	0.69	0.38	1.7	2.1
5	(24*R*)-24,25-Epoxybutyrospermol [(24*R*)-24,25-epoxyeuph-7-enol][d]	2.53	0.18	0.28	0.1	0.1
6	(24*S*)-24,25-Epoxybutyrospermol [(24*S*)-24,25-epoxyeuph-7-enol][d]	2.53	0.18	0.28	0.1	0.1
7	Isoaglaiol [(24*R*)-24,25-epoxydammar-20-enol][d]	2.53	0.22	0.16	0.1	0.1
8	Aglaiol [(24*S*)-24,25-epoxydammar-20-enol]	2.53	0.22	0.16	0.1	0.1
9	Butyrospermol (eupha-7,24-dienol)	1.66	0.80	0.47	16.3	16.9
10	Euphol (eupha-8,24-dienol)	1.30	0.76	0.44	0.5	0.4
11	Tirucalla-7,24-dienol	1.89	0.83	0.58	25.6	22.4
12	Tirucallol (tirucalla-8,24-dienol)	1.47	0.80	0.53	0.4	0.1
13	Dammaradienol (dammara-20,24-dienol)	1.66	0.61	0.39	6.0	6.9
14	24-Methylenedammarenol [24-methyldammara-20,24(24[1])-dienol]	1.79	0.68	0.41	0.1	0.1
15	Cycloartenol (cycloart-24-enol)	1.83	0.98	0.82	0.6	0.9
16	24-Methylenecycloartanol [24-methylcycloart-24(24[1])-enol]	2.00	1.07	0.90	0.4	2.0
17	24-Methyllanosta-8,24(24[1])-dienol	1.71	0.98	0.87	0.3	0.4
18	24-Methyllanosta-9(11),24(24[1])-dienol	1.95	0.93	0.67	0.1	0.1
19	Bacchara-12,21-dienol	2.10	0.93	0.76	2.8	3.3
20	β-Amyrin (olean-12-enol)	1.70	0.92	0.60	24.4	24.6
21	δ-Amyrin [olean-13(18)-enol]	1.71	0.95	0.62	3.0	3.3
22	Germanicol (olean-18-enol)	1.70	0.88	0.88	1.4	0.9
23	α-Amyrin (urs-12-enol)	1.91	1.01	0.66	1.0	1.8
24	Taraxerol (tarax-14-enol)	1.63	0.88	0.56	3.6	4.9
25	Lupeol [lup-20(29)-enol]	2.00	0.74	0.44	4.4	2.9
26	17-Epilupeol (17-epilup-20(29)-enol)	2.07	0.86	0.50	0.3	0.4
27	ψ-Taraxasterol (taraxast-20-enol)	2.49	1.06	0.72	1.5	0.7
	Others, unidentified				3.3	3.0

a) All compounds have a hydroxyl group at C-3β. All compounds, with the exception of C-5 unsaturated ones, are 5α-compounds. *b*) Standard: cholesteryl acetate (Rt_R: 1.00). *c*) Percentage composition of the triterpene alcohol fraction determined based on HPLC and GLC data. *d*) New compounds reported in this paper.

more, although the side-chain ^{13}C-NMR signals (C-20–C-27) of **1a** (Table 2) were fully consistent with those of two tirucallanes, tirucalla-5,24-dien-3β-yl acetate and **11a**,[11] small but significant differences for some side-chain carbons (C-21–C-23) were observed between **1a** and euphane **9a**,[11] *viz.*, C-21 ($\delta_{1a}-\delta_{9a}=-0.3$), C-22 (1.0), and C-23 (-0.4). We concluded that **1a** is tirucalla-5,7,24-trien-3β-yl acetate. The most stable conformation of **1a** with minimum steric energy,[12] simulated by using the CAChe and MM2 program,[13] orients C-22 in a "right-handed" conformation (C-22 trans-oriented with respect to C-13), as shown in Fig. 1, similar to that of **11a**[11] and the crystal structure of another tirucallane, tirucallyl acetate (**12a**; the acetate of **12**).[14] This conformation of **1a** was fairly consistent with results from the NOE experiment carried out in solution. Alkaline hydrolysis of **1a** yielded tirucalla-5,7,24-trien-3β-ol (**1**; $C_{30}H_{48}O$; M^+, *m/z* 424.3712).

Compound **2a**, which showed M^+ at *m/z* 468.3984 ($C_{32}H_{52}O_2$) in the HR-MS, possesses two trisubstituted double bonds [ν_{max} 817, 800 cm^{-1}; δ_H 5.07 (1H, tt, $J=1.4$, 7.4 Hz) and 5.37 (1H, dd, $J=3.1$, 3.6 Hz)], a terminal isopropylidene group [δ_H 1.59 (s) and 1.68 (s)], and six tertiary methyl groups. These data, in combination with mass fragmentations at *m/z* 453 (M^+-Me), 393 ($M^+-Me-HOAc$), 325 [loss of s.c.(C_6H_{11}) and HOAc], and 69 [$CH_2CH=C(Me)_2^+$], suggested that **2a** was a 3β-acetoxy triterpene with a six-membered tetracyclic skeleton possessing one double bond and a C_6-side-chain containing an isopropylidene functionality.[15] Further fragment ions at *m/z* 255 ($C_{19}H_{27}^+$; loss of ring D plus s.c., due to cleavage of C-13–C-18 and C-14–C-15, with concomitant loss of HOAc), and 203 ($C_{15}H_{23}^+$) and 189 ($C_{14}H_{21}^+$) formed by the loss of ring D plus side-chain due to cleavage of the C-8–C-14 and C-12–C-13 bonds, and C-8–C-14 and C-11–C-12 bonds, respectively, with concomitant HOAc loss suggested **2a** had the skeleton of a migrated baccharane having a Δ^7- or a $\Delta^{9(11)}$-double bond.[15] The ^{1}H-NMR signals of **2a** were, with the exception of those arising from the ring A substituents, in agreement with the corresponding ^{1}H signals for lemmaphylla-7,24-diene but not for its $\Delta^{9(11)}$-isomer[15] and, thus, **2a** was proposed to have the structure lemmaphylla-7,24-dien-3β-yl acetate (D: C-friedo-18,19-seco-lupa-7,19-dien-3β-yl acetate). The proposed structure and stereochemistry of **2a** were confirmed by analysis of its two-dimensional (2D) NMR (^{1}H–^{1}H and ^{13}C–^{1}H COSYs, and HMBC) spectra and difference NOE spectra.[16] Compound **2a** showed a significant NOE correlation between [H-24(4β-Me)–H-25(10β-Me)–H-26(14β-Me)–H-16β, H-18β–H-28(17β-Me)] on the β-face and [H-3α–H-23(4α-Me)–H-5α–H-9α–H-27(13α-Me)] on the α-face of the molecule. The most stable conformation of **2a** with minimum steric energy,[17] simulated by using the CAChe and MM2 program[13] is shown in Fig. 1. This conformation of **2a** was fairly consistent with results from

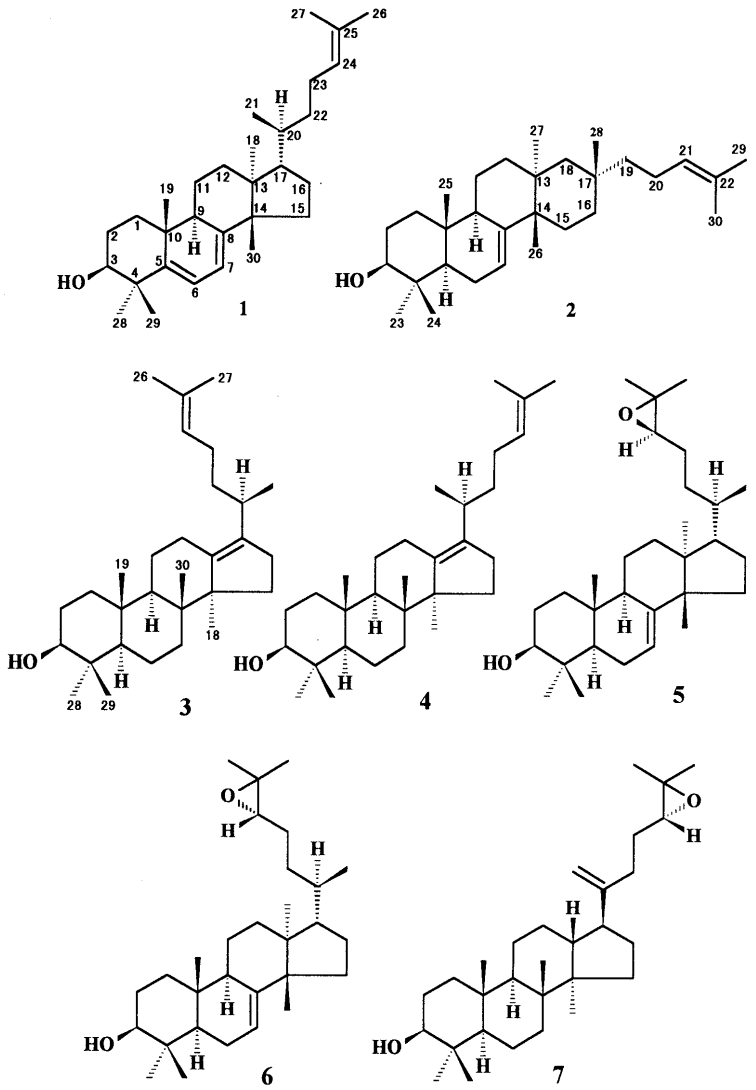

Chart 1. Structures of Seven Novel Triterpene Alcohols from Camellia and Sasanqua Oils

the NOE experiment carried out in solution. Alkaline hydrolysis of **2a** yielded lemmaphylla-7,24-dien-3β-ol (**2**; $C_{30}H_{50}O$; M^+, m/z 426.3872).

Compound **3a**, which showed M^+ at m/z 468.3944 ($C_{32}H_{52}O_2$) in the HR-MS, possesses a trisubstituted [ν_{max} 828 cm^{-1}; δ_H 5.13 (1H, tt, J=1.2, 7.1 Hz)] and a tetrasubstituted [δ_C 134.7 (s) and 138.9 (s)] double bonds, a terminal isopropylidene group [δ_H 1.59 (s) and 1.68 (s)], and one secondary and five tertiary methyl groups. This, in combination with a mass fragment at m/z 399 [loss of part of s.c. by cleavage of C-22–C-23], 357 (loss of s.c.), 355 (357−2H), and 297 (357−HOAc), suggested that **3a** was a 3β-acetoxy tetracyclic triterpene possessing a monounsaturated ring system with a tetrasubstituted double bond and a Δ^{24}-unsaturated C_8-side-chain. Diagnostic fragment ions at m/z 263 (A, B rings formed by cleavages of C-8–C-14 and C-11–C-12), and 249 (A, B rings formed by cleavages of C-8–C-14 and C-9–C-11) further suggested that **3a** had a dammara-$\Delta^{13(17),24}$-dien-3β-yl acetate structure[18] with an undetermined stereochemistry. The structure containing the stereostructure of **3a** was determined by direct comparison with an authentic synthetic compound as follows. Thus, **9a**, upon treatment with BF_3-etherate in ether,[18] yielded (20R)-dammara-13(17),24-dien-3β-yl acetate (**3a**; isoeuphyl acetate) in almost quantitative yield. The synthetic **3a** and its alkaline-hydrolysis product, isoeuphol (**3**; $C_{30}H_{50}O$; M^+, m/z 426.3830), were identical following chromatographic and spectral comparison with natural **3a** and its hydrolysis product, **3**, respectively, and hence, natural **3** was determined as isoeuphol.

The HR-MS of compound **4a** showed M^+ at m/z 468.3974 ($C_{32}H_{52}O_2$), and its fragmentation pattern (see Experimental section) was essentially identical with that of **3a**. Furthermore, the ^{1}H- and ^{13}C-NMR (Tables 2, 3), and IR spectral properties of **4a** were very similar to those of **3a** suggesting that **4a** had the same structure as that of **3a** except for stereochemistry. Furthermore, **4a** and its alkaline hydrolysis product, **4** ($C_{30}H_{50}O$; M^+, m/z 426.3840), were identical following chromatographic and spectral comparison with an authentic specimen of (20S)-dammara-13(17),24-dien-3β-yl acetate (**4a**; isotirucallyl acetate), prepared from tirucalla-7,24-dien-3β-yl acetate (**11a**) by treatment with BF_3-etherate in ether, and its hydrolysis product **4**, respectively. Hence, natural **4** was characterized as isotirucallol.

Compounds **5a** and **6a** were a homogeneous mixture in reverse-phase HPLC and GLC. The mixture **5a**/**6a**,

Table 2. ^{1}H-NMR Data (δ/ppm, 400 MHz, $CDCl_3$) of the Acetates of Seven Novel Triterpene Alcohols from Camellia and Sasanqua Oils[a)]

Proton	1a	2a	3a	4a	5a	6a	7a
1	1.40 (α), 1.71 (β)	1.20 (α), 1.64 (β)	1.08 (α), 1.74 (β)	1.10 (α), 1.75 (β)	1.25 (α), 1.69 (β)	1.22 (α), 1.68 (β)	1.06 (α), 1.72 (β)
2	1.75 (α), 1.69 (β)	1.66 (2H)	1.65 (2H)	1.65 (2H)	1.68 (2H)	1.66 (2H)	1.64 (2H)
3	4.61 (dd, 5.0, 10.7)	4.51 (dd, 4.4, 10.7)	4.50 (dd, 6.2, 10.3)	4.50 (dd, 5.9, 11.0)	4.52 (dd, 4.0, 10.6)	4.52 (dd, 4.0, 11.0)	4.48 (dd, 5.8, 10.4)
OAc-3	2.07 (s)	2.05 (s)	2.05 (s)	2.05 (s)	2.06 (s)	2.05 (s)	2.05 (s)
5		1.41 (dd, 6.2, 12.1)	0.85	0.85	1.42	1.42	0.85
6	5.55 (dd, 2.7, 5.5)	2.15 (α), 1.98 (β)	1.53 (α), 1.38 (β)	1.54 (α), 1.37 (β)	2.13 (α), 1.95 (β)	2.13 (α), 1.98 (β)	1.53 (α), 1.42 (β)
7	5.89 (d, 5.5)	5.37 (dd, 3.1, 3.6)	1.48 (α), 1.36 (β)	1.50 (α), 1.38 (β)	5.26 (dd, 3.0, 3.6)	5.26 (dd, 3.0, 3.6)	1.57 (α), 1.30 (β)
9	2.36	2.36	1.42	1.42	2.23	2.23	1.34
11	1.56 (2H)	1.45 (α), 1.52 (β)	1.51 (α), 1.20 (β)	1.52 (α), 1.20 (β)	1.50 (2H)	1.53 (2H)	1.52 (α), 1.24 (β)
12	1.70 (α), 1.86 (β)	1.33 (α), 1.40 (β)	1.82 (α), 2.37 (β)	1.83 (α), 2.37 (β)	1.65 (α), 1.84 (β)	1.70 (α), 1.82 (β)	1.10 (α), 1.58 (β)
13							1.68
15	1.60 (α), 1.42 (β)	1.58 (α), 1.46 (β)	1.23 (α), 1.88 (β)	1.25 (α), 1.89 (β)	1.49 (2H)	1.46 (2H)	1.12 (α), 1.62 (β)
16	1.30 (α), 1.97 (β)	1.54 (α), 1.43 (β)	2.24 (α), 2.06 (β)	2.14 (2H)	1.30 (α), 1.94 (β)	1.30 (α), 1.93 (β)	1.92 (α), 1.42 (β)
17	1.53				1.52	1.50	2.20
18	0.70 (s)	1.30 (α), 1.15 (β)	1.06 (s)	1.08 (s)	0.82 (s)	0.83 (s)	0.87 (s)
19	0.87 (s)	1.14, 1.62	0.87 (s)	0.87 (s)	0.77 (s)	0.77 (s)	0.87 (s)
20	1.38	1.88, 1.94	2.46	2.47	1.47	1.47	
21	0.89 (d, 6.6)	5.07 (tt, 1.4, 7.4)	0.92 (d, 6.6)	0.96 (d, 7.0)	0.86 (d, 6.6)	0.86 (d, 7.0)	4.72 (d, 1.5), 4.78 (br s)
22	1.04, 1.39		1.32 (2H)	1.32 (2H)	1.20, 1.67	1.06, 1.78	2.06, 2.18
23	1.84, 2.06	0.84 (s)	1.85, 1.96	1.74, 1.80	1.38, 1.65	1.40, 1.58	1.68 (2H)
24	5.10 (tt, 1.4, 7.1)	0.93 (s)	5.13 (br t, 7.0)	5.07 (tt, 1.5, 7.3)	2.70 (t, 6.0)	2.70 (t, 6.2)	2.75 (t, 6.2)
25		0.77 (s)					
26	1.68 (s)	0.95 (s)	1.68 (s)	1.67 (s)	1.32 (s)	1.31 (s)	1.32 (s)
27	1.61 (s)	1.06 (s)	1.59 (s)	1.56 (s)	1.27 (s)	1.27 (s)	1.28 (s)
28	1.10 (s)	0.86 (s)	0.87 (s)	0.86 (s)	0.85 (s)	0.85 (s)	0.86 (s)
29	1.13 (s)	1.68 (s)	0.85 (s)	0.84 (s)	0.94 (s)	0.93 (s)	0.85 (s)
30	1.06 (s)	1.59 (s)	0.85 (s)	0.81 (s)	0.98 (s)	0.98 (s)	0.98 (s)

a) Figures in parentheses denote J values (Hz). J values not included in the Table were not determined.

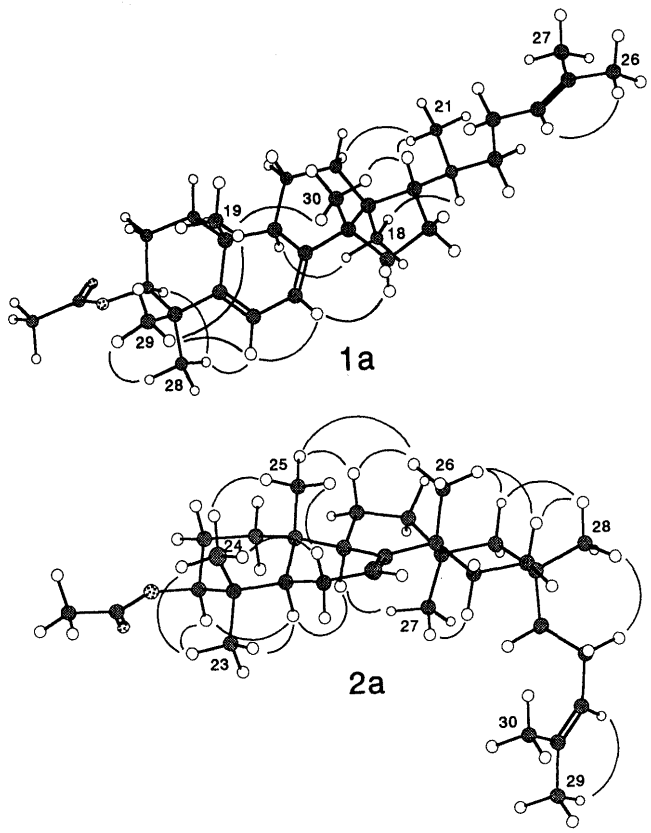

Fig. 1. CAChe Drawings and Some Representative NOE Correlations (—) for Tirucalla-5,7,24-trien-3β-yl Acetate (**1a**) and Lemmaphylla-7,21-dien-3β-yl Acetate (**2a**)

which possesses a trisubstituted double bond (ν_{max} 824, 799 cm^{-1}), showed M^+ at m/z 484.3900 ($C_{32}H_{52}O_3$) accompanied with fragment ions at m/z 409 ($M^+ - Me - HOAc$), 355 [$M^+ - s.c.(C_8H_{15}O) - 2H$], 315 ($M^+ - s.c. - 42$), 255 (315 − HOAc), and 241 (255 − CH_2).[9] This indicated that **5a**/**6a** was a mixture of 3β-acetoxy tetracyclic triterpenes containing a monounsaturated ring system and a mono-oxygenated C_8-side-chain. The ring system ^{1}H signals (see Experimental section) were consistent with the corresponding signals of **9a** and **11a**,[11] whereas the side-chain ^{1}H signals were very close to those of 24,25-epoxylanosteryl acetate (24,25-epoxylanosta-8,24-dien-3β-yl acetate).[19] Thus, **5a**/**6a** were supposed to be either 24,25-epoxylated euph-7-en-3β-yl (20*R*) or tirucall-7-en-3β-yl (20*S*) acetate. Chromatographic and spectral coincidence of **5a**/**6a** with an authentic specimen of (24*R*/*S*)-24,25-epoxybutyrospermyl acetate possessing a 20*R*-chirality, prepared from **9a** by treatment with *m*-chloroperbenzoic acid (*m*-CPBA) in dichloromethane,[20] revealed that **5a**/**6a** has a euphane (20*R*) group. Separation of **5a** and **6a** from the mixture was achieved by normal-phase HPLC in which the less-polar component was designated as **5a** and the more-polar one **6a**. The stereochemical assignment at C-24 of **5a** and **6a** was undertaken based on the ^{13}C-NMR chemical shift differences in the side-chain ^{13}C signals. Thus, 24*R*- and 24*S*-epimers of 24,25-epoxylanosteryl acetate, which possess the same chirality at C-20 (20*R*) as that of **5a** and **6a**, exhibited ^{13}C-NMR chemical shift differences ($\delta_R - \delta_S$) in the side-chain ^{13}C signals as C-20 ($\delta_R - \delta_S = -0.1$), C-21 (0.1), C-22 (−0.2), C-23 (−0.3), C-24 (−0.2), C-25 (0.3), C-26

Table 3. ^{13}C-NMR Data (δ/ppm, 100.6 MHz, $CDCl_3$) of the Acetates of Seven Novel Triterpene Alcohols from Camellia and Sasanqua Oils

^{13}C	1a	2a	3a	4a	5a	6a	7a	^{13}C	1a	2a	3a	4a	5a	6a	7a
1	36.9	36.5	38.7	38.7	36.8	36.8	38.8	17	52.6	32.7	134.7	134.6	53.0	53.3	47.6
2	23.9	24.2	23.7	23.7	24.2	24.2	23.7	18	21.0	46.6	22.8	23.1	22.1	22.1	15.9
3	79.2	81.1	80.9	81.0	81.1	81.1	80.9	19	15.6	39.8	16.5	16.5	13.1	13.1	16.3
4	39.8	37.8	37.9	37.9	37.8	37.8	37.9	20	35.9	23.0	31.9	31.6	35.8	35.9	151.9
5	150.5	50.8	56.0	56.0	50.8	50.7	56.0	21	18.3	125.3	19.9	20.1	18.7	18.6	107.9
6	119.2	24.0	18.1	18.2	23.8	23.7	18.2	22	36.2	130.7	35.6	35.7	31.5	31.8	31.0
7	115.3	116.1	35.4	35.5	117.7	117.7	35.3	23	25.0	27.5	26.7	26.4	26.0	26.4	27.8
8	147.6	144.7	41.0	41.3	145.9	145.8	40.5	24	125.2	15.7	124.9	125.0	64.7	64.8	64.2
9	46.0	48.0	51.5	51.6	48.8	48.8	50.8	25	131.0	12.9	131.1	131.0	58.4	58.1	58.4
10	37.1	35.2	37.3	37.3	34.8	34.8	37.1	26	25.7	23.8	25.7	25.7	24.9	24.9	25.0
11	16.6	16.4	21.8	22.0	18.1	18.1	21.4	27	17.7	26.8	17.6	17.6	18.8	18.7	18.8
12	33.2	34.8	22.8	23.0	33.9	33.9	24.9	28	26.5	32.1	28.0	28.1	27.6	27.6	28.0
13	43.6	40.2	138.9	139.1	43.6	43.5	45.4	29	25.7	25.7	16.6	16.6	15.9	15.9	16.5
14	50.9	34.4	56.5	56.4	51.3	51.3	49.5	30	24.3	17.6	17.2	16.7	27.3	27.3	15.7
15	32.9	29.5	30.6	30.8	33.8	33.9	31.3	OCOMe	21.4	21.3	21.3	21.3	21.3	21.3	21.3
16	28.1	35.2	29.0	29.1	28.4	28.4	29.0	OCOMe	170.9	171.0	171.0	171.0	171.0	171.0	171.0

(0.0), and C-27 (0.1).[19] Almost the same differences as those of the lanostanes were observed in the side-chain ^{13}C signals between **5a** and **6a**, *viz.*, C-20 ($\delta_{5a}-\delta_{6a}=-0.1$), C-21 (0.1), C-22 (−0.3), C-23 (−0.4), C-24 (−0.1), C-25 (0.3), C-26 (0.0), and C-27 (0.1), as calculated from the ^{13}C-NMR data in Table 3, and hence, **5a** and **6a** were attributed to the 24*R*- and 24*S*-epimers of 24,25-epoxybutyrospermyl acetate, respectively. On alkaline hydrolysis, **5a** and **6a** yielded (24*R*)- (**5**) and (24*S*)-24,25-epoxybutyrospermol (**6**), respectively.

Compounds **7a** and **8a**, which were a homogeneous mixture in reverse-phase HPLC and GLC, showed the presence of a terminal methylene group (ν_{max} 3072, 1642, 876 cm^{-1}) in the IR spectrum and M^+ at *m/z* 484.3887 ($C_{32}H_{52}O_3$) in the HR-MS accompanied with fragment ions at *m/z* 409 ($M^+-Me-HOAc$), 357 [M^+−s.c. ($C_8H_{13}O$)−2H], and 297 (357−HOAc). This suggested that the mixture **7a**/**8a** was a 3β-acetoxy saturated tetracyclic triterpene containing a mono-oxygenated C_8-side-chain with a terminal methylene group. The ^{1}H-NMR signals (see Experimental section) of **7a**/**8a** were consistent with the corresponding signals of aglaiyl acetate [(24*S*)-24,25-epoxydammar-20-en-3β-yl acetate; (24*S*)-24,25-epoxydammaradienyl acetate][3,4] and an authentic specimen of (24*R*/*S*)-24,25-epoxydammaradienyl acetate prepared from the acetate of dammaradienol (**13**) by epoxidation.[20] Thus, **7a**/**8a** were supposed to be a mixture of (24*R*/*S*)-24,25-epoxydammar-20-en-3β-yl acetate. Normal-phase HPLC enabled the mixture to be separated into less-polar **7a** and more-polar **8a**. The more-polar **8a** had a melting point (mp 167—169 °C) close to the known 24*S*-epimer, aglaiyl acetate (lit.: mp 161—162 °C,[3] mp 159—164 °C[4]), while the less-polar **7a** had a distinctly higher melting point (mp 185—187 °C) than **8a**. We, therefore, concluded that **7a** was a 24*R*-epimer, which we named isoaglaiyl acetate. Compounds **7a** and **8a**, upon alkaline hydrolysis, yielded isoaglaiol (**7**) and aglaiol (**8**), respectively.

Tables 2 and 3 show the assigned ^{1}H- and ^{13}C-NMR data, respectively, of seven compounds, **1a**—**7a**. Signal assignments were aided by ^{13}C distortionless enhancement by polarization transfer (DEPT), ^{1}H–^{1}H and ^{13}C–^{1}H COSYs, HMBC, and difference NOE experiments.

Several naturally occurring sterols with a $\Delta^{5,7}$-conjugated diene system, represented by ergosterol [(22*E*)-ergosta-5,7,22-trien-3β-ol], have been reported.[2] However, the tirucallane-type triterpene **1** is the first example of a triterpene alcohol with a $\Delta^{5,7}$-diene system as a natural product. The natural occurrence of lemmaphyllane (D: C-friedo-18,19-seco-lupane)-type triterpene is extremely rare and the only previously known compound of this type is lemmaphylla-7,21-diene from a fern *Lemmaphyllum micropyllum* var. *obovatum*.[15] As for the (20*R*)-dammar-13(17)-ene (isoeuphane)-type triterpene, the only compound of this type previously reported as a natural product is (20*R*)-dammara-13(17),24-diene isolated from *Polypodium ferns*.[18] Aglaiol (**8**), isolated in this study along with its 24*R*-epimer **7**, has so far been isolated only from the leaves of *Aglaia odorata* (Meliaceae).[3,4] Among the known compounds described in this paper, baccharа-12,21-dien-3β-ol (**19**) has previously been isolated only from the seeds of *Glycine max* (Leguminosae) and some other Leguminosae seeds,[21] and 17-epilupeol (**26**), as the acetyl derivative, only from the whole herb of *Ixeris chinensis* (Compositae).[6]

Triterpene alcohols and plant sterols have recently been demonstrated to possess inhibitory effects on TPA-induced inflammation in mice.[22-24] Thus, the fourteen triterpene alcohols, **3**, **4**, **5**/**6** (examined as a C-24 epimeric mixture), **7**/**8** (C-24 epimeric mixture), **9**, **10**, **12**, **14**, **18**, **19**, **21**, and **22**, isolated from the Theaceae oils in this study were examined for their inhibitory effects.[25] These are shown in Table 4 along with those of nine other triterpene alcohols, which have already been evaluated for their anti-inflammatory activity.[23] These consist of Theaceae triterpene alcohols and the reference compounds,[23] quercetin (3,3′,4′,5,7-pentahydroxyflavone), a known inhibitor of TPA-induced inflammation in mice, and two commercially available anti-inflammatory drugs, indomethacin and hydrocortisone. All the triterpene alcohols examined here inhibited the TPA-induced inflammation with 0.2—0.9 mg/ear of the 50% inhibitory dose. Although the inhibitory effects of these triterpenes were weaker than that of hydrocortisone, some of them inhibit-

Table 4. Inhibitory Effect of Triterpene Alcohols Isolated from Camellia and Sasanqua Oils, and Reference Compounds on TPA-Induced Inflammation in Mice[a]

Code	Compound	ID_{50}[b] (mg/ear)
3	Isoeuphol	0.3
4	Isotirucallol	0.3
5/6	(24*R*/*S*)-24,25-Epoxybutyrospermol[c]	0.5
7/8	(24*R*/*S*)-24,25-Epoxydammaradienol[c]	0.5
9	Butyrospermol	0.6
10	Euphol	0.2
12	Tirucallol	0.4
14	24-Methylenedammarenol	0.5
18	24-Methyllanosta-9(11),24(24^1)-dienol	0.4
19	Bacchara-12,21-dienol	0.8
21	δ-Amyrin	0.3
22	Germanicol	0.9
11	Tirucalla-7,24-dienol[d]	0.8
13	Dammaradienol[d]	0.8
15	Cycloartenol[d]	0.3
16	24-Methylenecycloartanol[d]	0.2
20	β-Amyrin[d]	0.4
23	α-Amyrin[d]	0.2
24	Taraxerol[d]	0.3
25	Lupeol[d]	0.6
27	ψ-Taraxasterol[d]	0.4
	Quercetin[d]	1.6
	Indomethacin[d]	0.3
	Hydrocortisone[d]	0.03

a) Compounds dissolved in $CHCl_3$–MeOH (1 : 1, v/v) were applied 30 min before TPA treatment. Ear thickness was determined 8 h after TPA treatment. *b*) 50% Inhibitory dose. *c*) C-24 Epimeric mixture in almost equal proportion of each epimer. *d*) Data taken from refs. 22 and 23*b*.

ed at a grade almost corresponding to that of indomethacin, and were far more potent than quercetin. As far as the euphane/tirucallane-type compounds were concerned, the euphanes (**9**, **10**) were more potent than the tirucallanes (**11**, **12**), while the Δ^8-(**10**, **12**) were more potent than the Δ^7-(**9**, **11**) isomers. There was an effect of double bond isomerism, moreover, with three pentacyclic oleanaes **20**—**22**, in which **21**, possessing a tetrasubstituted $\Delta^{13(18)}$-double bond, was most inhibitory. 24-Methylenation of tetracyclic triterpenes increased the activity as has been shown with the dammaranes (**13**, **14**) and cycloartanes (**15**, **16**). Inhibitors of TPA-induced inflammation have been demonstrated to have an almost parallel inhibitory effect against tumor promotion,[23a,24] and further study is necessary to investigate the correlation between the structural features of triterpenes and their anti-inflammatory and anti-tumor promoting activities.

Experimental

Crystallizations were performed from acetone–MeOH. Melting points measured were uncorrected. Reverse-phase 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) (HPLC I) and on a TSK ODS-120A 5 μm column (Toso Co., Tokyo) (HPLC II), with MeOH (4 ml/min) as mobile phase. Normal-phase HPLC was done on a Senshu Pak Silica-4251-N column (25 cm × 10 mm i.d.; Senshu Scientific Co., Tokyo) with *n*-hexane–EtOAc (97 : 3, v/v; 4 ml/min) as mobile phase. GLC was performed using a DB-17 fused-silica capillary column (30 m × 0.3 mm i.d., column temp. 275 °C). In both HPLC and GLC, cholesteryl (cholest-5-en-3β-yl) acetate was the standard for the determination of Rt_R of triterpene acetate. Electron-impact MS and HR-MS were recorded at 70 eV. NMR spectra were recorded at 400 MHz (^{1}H-NMR) and 100.6 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, and chemical shifts were recorded in δ values. IR and UV spectra were recorded in KBr and EtOH, respectively. Instrumental details and general procedures were the same as described previously.[26] Crude camellia and sasanqua oils were donated by Takada Oil Manufacturing Co. (Ohshima, Tokyo) and Nikko Fine Products Co. (Tokyo), respectively. Sources of seventeen reference triterpene alcohols (**9**—**20**, **22**—**25**, **27**) were described in our previous article.[1,23b,26,27]

Isolation Procedures Alkaline hydrolysis (5% KOH in MeOH, reflux, 3 h) of crude Theaceae oils (camellia oil, 3.0 kg; sasanqua oil, 5.0 kg) followed by diisopropyl ether extraction yielded neutral NSL (10.4 g; 19.2 g). Column chromatography of the NSL over silica-gel afforded triterpene alcohol fractions (3.3 g; 6.7 g) which, upon acetylation, gave acetylated fractions (2.9 g; 5.2 g). Isolation of individual components from the acetylated fractions was performed by argentic TLC followed by HPLC.

Tirucalla-5,7,24-trien-3β-yl Acetate (1a) and Tirucalla-5,7,24-trien-3β-ol (1) **1a**: mp 123—124 °C. IR ν_{max} cm^{-1}: 1733, 1646, 1241, 837, 820. UV λ_{max} nm: 273 (log ε 4.04), 281 (4.00). MS *m/z* (%): 466 (M^+, 31), 451 (4), 406 (5), 391 (53), 337 (6), 295 (1), 253 (3), 239 (4), 201 (20), 187 (17), 186 (11), 185 (16), 171 (21), 157 (18), 145 (16), 69 (100). HR-MS: *m/z* 466.3789 [Calcd for $C_{32}H_{50}O_2$ (M^+): 466.3807]; 451.3569 [Calcd for $C_{31}H_{47}O_2$: 451.3573]; 391.3309 [Calcd for $C_{29}H_{43}$: 391.3362]; 295.2431 [Calcd for $C_{22}H_{31}$: 295.2424]; 253.1915 [Calcd for $C_{19}H_{25}$: 23.1954]; 239.1820 [Calcd for $C_{18}H_{23}$: 239.1798]; 171.1181 [Calcd for $C_{13}H_{15}$: 171.1173]; 69.0704 [Calcd for C_6H_9: 69.0704]. Alkaline hydrolysis of **1a** yielded a free alcohol **1**. **1**: Amorphous gum. IR ν_{max} cm^{-1}: 3430, 1650, 830, 820. MS *m/z* (%): 424 (M^+, 33), 409 (6), 391 (28), 337 (7), 311 (6), 271 (5), 253 (5), 239 (3), 201 (6), 187 (10), 186 (6), 185 (10), 171 (13), 157 (13), 149 (16), 69 (100). HR-MS: *m/z* 424.3712 [Calcd for $C_{30}H_{48}O$ (M^+): 424.3703]. ^{13}C- and ^{1}H-NMR: C-1 [δ_C 37.4; δ_H 1.43(α), 1.71(β)], C-2 [27.5; 1.71(α), 1.67(β)], C-3 [77.2; 3.39, dd, *J*=6.2, 9.2 Hz], C-4 [41.1], C-5 [151.5], C-6 [119.0; 5.91, d, *J*=5.5 Hz], C-7 [115.4; 5.55, dd, *J*=2.9, 5.5 Hz], C-8 [147.0], C-9 [46.1; 2.35; ddd, *J*=3.3, 8.1, 11.8 Hz], C-10 [37.3], C-11 [16.7; 1.58 (2H)], C-12 [33.3; 1.66(α), 1.85(β)], C-13 [43.6], C-14 [50.8], C-15 [32.9; 1.58(α), 1.41(β)], C-16 [28.1; 1.30(α), 1.99(β)], C-17 [52.6; 1.53], C-18 [21.1; 0.71, s], C-19 [15.5; 0.85, s], C-20 [35.9; 1.38], C-21 [18.3; 0.89, d, *J*=6.6 Hz], C-22 [36.2; 1.05, 1.42], C-23 [25.0; 1.86, 2.04], C-24 [125.2; 5.10, brt, *J*=7.0 Hz], C-25 [130.9], C-26 [25.7; 1.69, s], C-27 [17.7; 1.61, s], C-28 [26.5; 1.22, s], C-29 [24.3; 1.07, s], C-30 [24.4; 1.06, s].

Lemmaphylla-7,21-dien-3β-yl Acetate (2a) and Lemmaphylla-7,21-dien-3β-ol (2) **2a**: Amorphous gum. IR ν_{max} cm^{-1}: 1731, 1249, 817, 800. MS *m/z* (%): 468 (M^+, 16), 453 (29), 408 (1), 393 (18), 371 (1), 325 (1), 311 (5), 289 (2), 271 (3), 257 (3), 255 (3), 241 (4), 229 (11), 215 (6), 203 (9), 201 (7), 189 (10), 187 (7), 69 (76), 43 (100). HR-MS: *m/z* 468.3984 [Calcd for $C_{32}H_{52}O_2$ (M^+): 468.3965]; 453.3721 [Calcd for $C_{31}H_{49}O_2$: 453.3729]; 393.3512 [Calcd for $C_{29}H_{45}$: 393.3518]; 325.2933 [Calcd for $C_{24}H_{37}$: 325.2894]; 255.2115 [Calcd for $C_{19}H_{27}$: 255.2112]; 203.1790 [Calcd for $C_{15}H_{23}$: 203.1798]; 189.1648 [Calcd for $C_{14}H_{21}$: 189.1642]; 69.0698 [Calcd for C_5H_9: 69.0704]. Alkaline hydrolysis of **2a** yielded a free alcohol **2**. **2**: mp 142—143 °C. IR ν_{max} cm^{-1}: 3380, 840, 817, 800. MS *m/z* (%): 426 (M^+, 33), 411 (72), 393 (26), 343 (1), 325 (8), 311 (6), 271 (4), 255 (4), 247 (7), 241 (5), 229 (20), 215 (10), 203 (11), 201 (11), 189 (17), 187 (10), 69 (100). HR-MS: *m/z* 426.3872 [Calcd for $C_{30}H_{50}O$ (M^+): 426.3859]. ^{13}C- and ^{1}H-NMR: C-1 [δ_C 36.8; δ_H 1.14(α), 1.60(β)], C-2 [27.7; 1.60 (2H)], C-3 [79.3; 3.24, dd, *J*=4.0, 11.4 Hz], C-4 [38.9], C-5 [50.7; 1.32, dd, *J*=5.5, 11.7 Hz], C-6 [24.1; 2.16(α), 1.99(β)], C-7 [116.3; 5.38, dd, *J*=3.1, 3.7 Hz], C-8 [144.6], C-9 [48.1; 2.34], C-10 [35.3], C-11 [16.4; 1.42(α), 1.56(β)], C-12 [34.8; 1.37 (2H)], C-13 [40.2], C-14 [34.4], C-15 [29.5; 1.56(α), 1.48(β)], C-16 [35.2; 1.52(α), 1.42(β)], C-17 [32.7], C-18 [46.6; 1.30(α), 1.14, d, *J*=14.3 Hz(β)], C-19 [39.8; 1.16, 1.64], C-20 [23.0; 1.92 (2H)], C-21 [125.3; 5.07, brt, *J*=7.0 Hz], C-22 [130.7], C-23 [27.5; 0.96, s], C-24 [14.6; 0.85, s], C-25 [12.9; 0.75, s], C-26 [23.8; 0.95, s], C-27 [26.9; 1.06, s], C-28 [32.1; 0.86, s], C-29 [25.7; 1.67, s], C-30 [17.6; 1.59, s].

Isoeuphyl Acetate (3a) and Isoeuphol (3) **3a**: mp 92—93 °C. IR ν_{max} cm^{-1}: 1733, 1250, 828. MS *m/z* (%): 468 (M^+, 26), 453 (2), 399 (12), 384 (5), 357 (11), 355 (9), 342 (5), 339 (5), 297 (3), 276 (3), 263 (2), 249 (4), 229 (4), 218 (12), 205 (32), 203 (23), 189 (32), 149 (43), 43 (100). HR-MS: *m/z* 468.3944 [Calcd for $C_{32}H_{52}O_2$ (M^+): 468.3965]; 399.3265 [Calcd for $C_{27}H_{43}O_2$: 399.3261]; 357.2807 [Calcd for $C_{24}H_{37}O_2$: 357.2792]; 355.2597 [Calcd for $C_{24}H_{35}O_2$: 355.2635]; 297.2572 [Calcd

for $C_{22}H_{33}$: 297.2580]; 263.2050 [Calcd for $C_{17}H_{27}O_2$: 263.2009]; 249.1851 [Calcd for $C_{16}H_{25}O_2$: 249.1851]. Alkaline hydrolysis of **3a** gave a free alcohol **3**. **3**: Amorphous gum. IR ν_{max} cm^{-1}: 3387, 828. MS *m/z* (%): 426 (M^+, 42), 411 (5), 393 (1), 357 (31), 342 (7), 339 (5), 315 (20), 313 (19), 300 (10), 297 (4), 234 (4), 229 (6), 221 (7), 218 (8), 207 (45), 205 (29), 203 (25), 189 (30), 149 (77), 55 (100). HR-MS: *m/z* 426.3830 [Calcd for $C_{30}H_{50}O$ (M^+): 426.3859]. ^{13}C- and ^1H-NMR: C-1 [δ_C 39.0; δ_H 1.01(α), 1.75(β)], C-2 [27.4; 1,56(α), 1.63(β)], C-3 [79.0; 3.22, dd, J=5.1, 11.4 Hz], C-4 [38.9], C-5 [55.9; 0.74], C-6 [18.2; 1.54(α), 1.36(β)], C-7 [35.4; 1.48(α), 1.38(β)], C-8 [41.0], C-9 [51.6; 1.40], C-10 [37.4], C-11 [21.8; 1.52(α), 1.20(β)], C-12 [22.9; 1.84(α), 2.37(β)], C-13 [139.0], C-14 [56.5], C-15 [30.7; 1.22(α), 1.88(β)], C-16 [29.1; 2.24(α), 2.06(β)], C-17 [134.7], C-18 [22.8; 1.06, s], C-19 [16.4; 0.85, s], C-20 [31.9; 2.47], C-21 [19.9; 0.92, d, J=7.0 Hz], C-22 [35.6; 1.31 (2H)], C-23 [26.7; 1.84, 1.98], C-24 [125.0; 5.13, br t, J=7.0 Hz], C-25 [131.1], C-26 [25.7; 1.69, s], C-27 [17.6; 1.59, s], C-28 [28.1; 0.99, s], C-29 [15.5; 0.77, s], C-30 [17.2; 0.85, s].

Preparation of Isoeuphyl Acetate (3a) from Butyrospermyl Acetate (9a) **9a** (20 mg) in 20% BF_3-etherate in dry diethyl ether (Et_2O; 4 ml) was stirred for 24 h at room temperature. The reaction mixture, after usual work-up followed by reverse-phase HPLC, yielded **3a** (11 mg) which was identical with the natural **3a** by HPLC, GLC, ^1H-NMR, and MS.

Isotirucallyl Acetate (4a) and Isotirucallol (4) **4a**: mp 85—87 °C. IR ν_{max} cm^{-1}: 1730, 1248, 829. MS *m/z* (%): 468 (M^+, 13), 453 (1), 399 (13), 384 (2), 357 (5), 355 (4), 339 (4), 297 (3), 249 (4), 229 (2), 205 (22), 203 (16), 189 (23), 43 (100). HR-MS: *m/z* 468.3974 [Calcd for $C_{32}H_{52}O_2$ (M^+): 468.3965]. Alkaline hydrolysis of **4a** afforded a free alcohol **4**. **4**: mp 155—156 °C. IR ν_{max} cm^{-1}: 3393, 832, 820. MS *m/z* (%): 426 (M^+, 36), 411 (2), 357 (45), 342 (4), 339 (6), 315 (17), 313 (11), 300 (7), 297 (4), 234 (2), 229 (5), 221 (6), 218 (7), 207 (42), 205 (26), 203 (23), 189 (26), 175 (16), 161 (39), 149 (69), 69 (100). HR-MS: *m/z* 426.3840 [Calcd for $C_{30}H_{50}O$ (M^+): 426.3859]. ^{13}C- and ^1H-NMR: C-1 [δ_C 39.0; δ_H 1.00(α), 1.74(β)], C-2 [27.4; 1,56(α), 1.64(β)], C-3 [79.0; 3.22, dd, J=5.1, 11.4 Hz], C-4 [38.9], C-5 [55.9; 0.74], C-6 [18.2; 1.54(α), 1.40(β)], C-7 [35.3; 1.47(α), 1.38(β)], C-8 [41.3], C-9 [51.7; 1.41], C-10 [37.4], C-11 [22.0; 1.52(α), 1.20(β)], C-12 [23.0; 1.83(α), 2.37(β)], C-13 [139.1], C-14 [56.4], C-15 [30.7; 1.25(α), 1.90(β)], C-16 [29.1; 2.12(α), 2.16(β)], C-17 [134.6], C-18 [23.1; 1.08, s], C-19 [16.4; 0.85, s], C-20 [31.6; 2.47], C-21 [20.1; 0.96, d, J=7.0 Hz], C-22 [35.7; 1.30 (2H)], C-23 [26.4; 1.81 (2H)], C-24 [125.0; 5.07, tt, J=1.5, 7.0 Hz], C-25 [131.0], C-26 [25.7; 1.69, d, J=1.1 Hz], C-27 [17.6; 1.56, s], C-28 [28.1; 0.99, s], C-29 [15.5; 0.77, s], C-30 [16.7; 0.82, s].

Preparation of Isotirucallyl Acetate (4a) from Tirucalla-7,24-dien-3β-yl Acetate (11a) **11a** (26 mg) in 20% BF_3-etherate in dry Et_2O (5 ml) was stirred for 24 h at room temperature, and after usual work-up and reverse-phase HPLC, yielded **4a** (17 mg). The synthetic **4a** was identical with the natural **4a** by HPLC, GLC, ^1H-NMR, and MS.

A Mixture of (24*R*)- (5a) and (24*S*)-24,25-Epoxybutyrospermyl Acetate (6a) and a Mixture of (24*R*)- (5) and (24*S*)-24,25-Epoxybutyrospermol (6) **5a**/**6a**: Amorphous gum. IR ν_{max} cm^{-1}: 1729, 1247, 869, 824, 799. MS *m/z* (%): 484 (M^+, 6), 469 (12), 451 (4), 409 (16), 391 (2), 355 (1), 341 (1), 315 (1), 273 (2), 255 (2), 241 (2), 227 (3), 215 (3), 43 (100). HR-MS: *m/z* 484.3900 [Calcd for $C_{32}H_{52}O_3$ (M^+): 484.3913]; 409.3449 [Calcd for $C_{29}H_{45}O$: 409.3468]; 355.2633 [Calcd for $C_{24}H_{35}O_2$: 355.2635]; 315.2287 [Calcd for $C_{21}H_{31}O_2$: 315.2322]; 255.2148 [Calcd for $C_{19}H_{27}$: 255.2111]; 241.1988 [Calcd for $C_{18}H_{25}$: 241.1955]. ^1H-NMR: δ 0.77 (3H, s, H-19), 0.82, 0.83 (each 3H, s, H-18), 0.85 (3H, s, H-28), 0.86 (3H, d, J=6.0 Hz, H-21), 0.93 (3H, s, H-29), 0.98 (3H, s, H-30), 1.27 (3H, s, H-27), 1.31, 1.32 (each 3H, s, H-26), 2.05 (3H, s, OAc-3), 2.70 (1H, t, J=6.0 Hz, H-24), 4.52 (1H, dd, J=4.7, 11.5 Hz, H-3), 5.25 (1H, dd, J=3.0, 3.6 Hz, H-7). Alkaline hydrolysis of a portion of the mixture **5a**/**6a** yielded a mixture of free alcohols **5**/**6**. **5**/**6**: mp 57—60 °C. IR ν_{max} cm^{-1}: 3485, 865, 824, 799. MS *m/z* (%): 442 (M^+, 20), 427 (47), 409 (52), 391 (6), 327 (4), 313 (7), 297 (3), 295 (2), 287 (6), 273 (12), 255 (7), 241 (6), 43 (100). HR-MS: *m/z* 442.3807 [Calcd for $C_{30}H_{50}O_2$ (M^+): 442.3807]. Normal-phase HPLC of the other portion of the acetate mixture **5a**/**6a** yielded isolated less-polar **5a** (Rt_R=6.09 on HPLC) and more-polar **6a** (Rt_R=6.42 on HPLC).

(24*R*)-24,25-Epoxybutyrospermyl Acetate (5a) and (24*R*)-24,25-Epoxybutyrospermol (5) **5a**: mp 144—146 °C. MS: *m/z* 484 (M^+). Alkaline hydrolysis of **5a** gave a free alcohol **5**. **5**: Amorphous gum. MS: *m/z* 442 (M^+). ^1H-NMR: δ 0.75 (3H, s, H-19), 0.82 (3H, s, H-18), 0.86 (3H, d, J=6.0 Hz, H-21), 0.86, 0.97 (each 3H, s, H-28, H-29), 0.98 (3H, s, H-30), 1.27 (3H, s, H-27), 1.32 (3H, s, H-26), 2.70 (1H, t, J=6.3 Hz, H-24), 3.24 (1H, dd, J=4.1, 11.0 Hz, H-3), 5.26 (1H, dd, J=3.0, 3.6 Hz, H-7).

(24*S*)-24,25-Epoxybutyrospermyl Acetate (6a) and (24*S*)-24,25-Epoxybutyrospermol (6) **6a**: mp 169—171 °C. MS: *m/z* 484 (M^+). Alkaline hydrolysis of **6a** gave a free alcohol **6**. **6**: Amorphous gum. MS: *m/z* 442 (M^+). ^1H-NMR: δ 0.75 (3H, s, H-19), 0.83 (3H, s, H-18), 0.86 (3H, d, J=6.0 Hz, H-21), 0.86, 0.97 (each 3H, s, H-28, H-29), 0.98 (3H, s, H-30), 1.27 (3H, s, H-27), 1.31 (3H, s, H-26), 2.70 (1H, t, J=6.3 Hz, H-24), 3.24 (1H, dd, J=4.1, 11.0 Hz, H-3), 5.26 (1H, dd, J=3.0, 3.6 Hz, H-7).

Preparation of a Mixture of (24*R*)- (5a) and (24*S*)-24,25-Epoxybutyrospermyl Acetate (6a) from Butyrospermyl Acetate (9a) To a solution of **9a** (50 mg) in dry CH_2Cl_2 (6 ml) was added *m*-CPBA (20 mg). After stirring overnight at room temperature, the mixture was extracted with Et_2O and, after washing with $NaHCO_3$ aq. soln. and usual work-up (47 mg) followed by reverse-phase HPLC, yielded **5a**/**6a** (30 mg). The synthetic **5a**/**6a** was identical with the natural **5a**/**6a** by HPLC, GLC, ^1H-NMR, and MS.

A Mixture of (24*R*)- (7a) and (24*S*)-24,25-Epoxydammaradienyl Acetate (8a) and a Mixture of (24*R*)- (7) and (24*S*)-24,25-Epoxydammaradienol (8) **7a**/**8a**: mp 175—176 °C. IR ν_{max} cm^{-1}: 3072, 1729, 1642, 1245, 876, 865. MS *m/z* (%): 484 (M^+, 2), 469 (1), 466 (2), 424 (2), 409 (1), 357 (1), 353 (1), 299 (3), 297 (1), 289 (6), 273 (2), 249 (5), 229 (7), 203 (8), 189 (26), 43 (100). HR-MS: *m/z* 484.3887 [Calcd for $C_{32}H_{52}O_3$ (M^+): 484.3913]; 409.3453 [Calcd for $C_{29}H_{45}O$: 409.3468]; 357.2844 [Calcd for $C_{24}H_{37}O_2$: 357.2792]; 297.2567 [Calcd for $C_{22}H_{33}$: 297.2580]. ^1H-NMR: δ 0.85 (3H, s, H-29), 0.86 (3H, s, H-28), 0.87 (6H, s, H-18, H-19), 0.98 (3H, s, H-30), 1.28 (3H, s, H-27), 1.32 (3H, s, H-26), 2.05 (3H, s, OAc-3), 2.74, 2.75 (each 1H, d, J=6.0 Hz, H-24), 4.48 (1H, dd, J=5.8, 10.4 Hz, H-3), 4.72 (1H, t, J=1.4 Hz), 4.78 (1H, br s) (H-21). Alkaline hydrolysis of a portion of the mixture **7a**/**8a** yielded a mixture of free alcohols **7**/**8**. **7**/**8**: mp 113—115 °C. IR ν_{max} cm^{-1}: 3412, 3080, 1640, 885, 860. MS *m/z* (%): 442 (M^+, 8), 427 (4), 424 (6), 409 (3), 371 (1), 355 (1), 317 (3), 315 (5), 299 (5), 297 (3), 275 (3), 257 (3), 255 (2), 247 (38), 234 (7), 229 (18), 207 (90), 189 (43), 43 (100). HR-MS: *m/z* 442.3839 [Calcd for $C_{30}H_{50}O_2$ (M^+): 442.3807]. Normal-phase HPLC of the other portion of the acetate mixture **7a**/**8a** yielded less-polar **7a** (Rt_R=4.60 on HPLC) and more-polar **8a** (Rt_R=4.69 on HPLC).

(24*R*)-24,25-Epoxydammaradienyl (Isoaglaiyl) Acetate (7a) and (24*R*)-24,25-Epoxydammaradienol (Isoaglaiol) (7) **7a**: mp 185—187 °C. MS: *m/z* 484 (M^+). Alkaline hydrolysis of **7a** gave a free alcohol **7**. **7**: mp 94—96 °C. MS: *m/z* 442 (M^+). ^1H-NMR: δ 0.78, 0.98 (each 3H, s, H-28, H-29), 0.85 (3H, s, H-19), 0.87 (3H, s, H-18), 0.98 (3H, s, H-30), 1.28 (3H, s, H-27), 1.32 (3H, s, H-26), 2.75 (1H, t, J=6.3 Hz, H-24), 3.20 (1H, dd, J=5.0, 11.3 Hz, H-3), 4.72 (1H, d, J=1.4 Hz) and 4.78 (1H, br s) (H-21).

(24*S*)-24,25-Epoxydammaradienyl (Aglaiyl) Acetate (8a) and (24*S*)-24,25-Epoxydammaradienol (Aglaiol) (8) **8a**: mp 167—169 °C (lit.: mp 161—162 °C,[3] mp 159—164 °C[4]). MS: *m/z* 484 (M^+). ^1H-NMR: δ 0.85 (3H, s, H-29), 0.86 (3H, s, H-28), 0.87 (6H, s, H-18, H-19), 0.98 (3H, s, H-30), 1.28 (3H, s, H-27), 1.32 (3H, s, H-26), 2.05 (3H, s, OAc-3), 2.75 (1H, t, J=6.0 Hz, H-24), 4.48 (1H, dd, J=5.8, 10.4 Hz, H-3), 4.72, 4.78 (each 1H, br s, H-21). Alkaline hydrolysis of **8a** gave a free alcohol **8**. **8**: mp 101—105 °C (lit.: mp 113—115 °C[3]). MS: *m/z* 442 (M^+).

Preparation of a Mixture of (24*R*)- (7a) and (24*S*)-24,25-Epoxydammaradienyl Acetates (8a) from Dammaradienyl Acetate (13a) To a solution of **13a** (50 mg) in dry CH_2Cl_2 (6 ml) was added *m*-CPBA (20 mg). After stirring overnight at room temperature, the mixture was extracted with Et_2O and, after washing with $NaHCO_3$ aq. soln. and usual work-up (48 mg) followed by reverse-phase HPLC, yielded **7a**/**8a** (27 mg; mp 176—178 °C). The synthetic **7a**/**8a** was identical with the natural **7a**/**8a** by HPLC, GLC, ^1H-NMR, and MS.

δ-Amyrin Acetate (21a) and δ-Amyrin (21) **21a**: mp 197—200 °C (lit.: mp 208 °C[5a]; mp 197—198 °C[5b]) HR-MS: *m/z* 468.3959 [Calcd for $C_{32}H_{52}O_2$ (M^+): 468.3964]. The ^1H-NMR data of **21a** were consistent with those reported for **21a**.[5a] Alkaline hydrolysis of **21a** gave a free alcohol **21**. **21**: mp 204—207 °C (lit.: mp 191 °C[5a]; mp 212—213 °C[5b]). HR-MS: *m/z* 426.3875 [Calcd for $C_{30}H_{50}O$ (M^+): 426.3859]. The MS data of **21** were identical with those reported for **21**.[5c]

17-Epilupeyl Acetate (26a) mp 206—209 °C (lit.: mp 219—221 °C[6]). HR-MS: *m/z* 468.3916 [Calcd for $C_{32}H_{52}O_2$ (M^+): 468.3964]. The MS, ^1H-NMR, and ^{13}C-NMR data of **26a** were identical with those reported for **26a**.[6]

Assay of TPA-Induced Inflammation in Mice The assay procedures were the same as those described in our previous article.[23,24]

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