Studies on Nepalese Crude Drugs. XXIX.[1] Chemical Constituents of Dronapuspi, the Whole Herb of *Leucas cephalotes* SPRENG.

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From the whole herb of *Leucas cephalotes* SPRENG., new labdane-, norlabdane- and abietane-type diterpenes named leucasdins A (1), B (2) and C (3), respectively, and two protostane-type triterpenes named leucastrins A (4) and B (5) were isolated, together with a known triterpene, oleanolic acid, five sterols, 7-oxositosterol, 7-oxostigmasterol, 7α-hydroxysitosterol, 7α-hydroxystigmasterol and stigmasterol, and eight flavones, 5-hydroxy-7,4′-dimethoxyflavone, pillion, gonzalitosin I, tricin, cosmosin, apigenin 7-*O*-β-D-(6-*O*-*p*-coumaroyl)glucopyranoside, anisofolin A and luteolin 4′-*O*-β-D-glucuronopyranoside. The structures of 1—5 were determined as (3*S*,6*R*,8*R*,9*R*,13*S*,16*S*)-9,13,15,16-bisepoxy-3,16-diacetoxy-6-formyloxylabdane, (3*S*,6*R*)-3-acetoxy-6-formyloxy-*iso*-ambreinolide, (4*R*,9*S*,12*R*,13*R*)-12,13-dihydroxyabiet-7-en-18-oic acid, (3*S*,17*S*,20*S*,24*S*)-3,20-dihydroxy-24-methylprotost-25-en, and (3*S*,17*S*,20*S*,24*S*)-3,20,24-trihydroxyprotost-25-en respectively, based on spectral and chemical data.

Key words *Leucas cephalotes*; Labiatae; diterpene; triterpene; flavonoid

Leucas cephalotes SPRENG. (syn.: *L. capitata* SPRENG.) is a herb of the family Labiatae, which is distributed in Nepal, India, Pakistan, and Afghanistan.[2] In Nepal, the plant is called Gumpati or Gumma[3] and its whole herb is a crude drug which is known as Dronapuspi and has been used as diaphretic, anti-inflammatory, edema, and obstinate urinary troubles including diabetes for diseases due to the aggravator of *pitta* (the force governing heat, temperature, and all chemical reactions) in the Ayurvedic system of medicine.[4,5] In Pakistan, it is registered useful in the treatment of cough, cold, and gastric complaints for warm and dry in second order in the Unic system of medicine.[6]

Regarding the chemical constituents of this drug, only two sterols, β-sitosterol and its glucoside have been reported.[7] In the course of our studies on Nepalese crude drugs,[1] we have investigated the chemical constituents of Dronapuspi.

A hot MeOH extract of the material was defated with *n*-hexane, and partitioned between 1-butanol and water. Repeated chromatographic separation of the 1-butanol-soluble fraction led to the isolation of nineteen compounds (**1**—**19**), including five new ones, labdane-, norlabdane-, and abietane-type diterpenes named leucasdins A (**1**), B (**2**), and C (**3**), respectively, and two protostane-type triterpenes named leucastrins A (**4**) and B (**5**), together with a known triterpene (**6**), five sterols (**7**—**11**), and eight flavones (**12**—**19**), as described in the experimental section. This paper deals with identification of their structures.

The structures of the known compounds (**6**—**19**) were identified as oleanolic acid (**6**),[8] 7-oxositosterol (**7**),[9,10] 7-oxostigmasterol (**8**),[11] 7α-hydroxysitosterol (**9**),[9] 7α-hydroxystigmasterol (**10**),[12] stigmasterol (**11**),[13] 5-hydroxy-7,4′-dimethoxyflavone (**12**),[14] pillion (**13**),[15] gonzalitosin I (**14**),[16] tricin (**15**),[17] cosmosin (**16**),[18] apigenin 7-*O*-β-D-(6-*O*-*p*-coumaroyl)glucopyranoside (**17**),[19] anisofolin A (**18**),[20] and luteolin 4′-*O*-β-D-glucuronopyranoside (**19**),[21—23] respectively, by direct comparison with authentic samples or of the respective spectral and chemical data with those described in the literatures.

Leucasdin A (**1**) was obtained as colorless needles, mp 167—168 °C (dec.), $[\alpha]_D^{25}$ −33.4°, and showed IR absorption bands assignable to esters (1740, 1724, 1248, 1240 cm^{-1}). The molecular formula was deduced to be $C_{25}H_{38}O_8$ from the high resolution (HR)-MS and ^{13}C-NMR spectral data. Twenty-five carbon signals were observed in the ^{13}C-NMR spectrum, and their multiplicities were determined based on the distortionless enhancement by polarization transfer (DEPT) spectrum. The presence of a formyl and two acetyl groups were deduced from the ^{1}H- and ^{13}C-NMR spectra which showed characteristic signals at δ 8.11, 2.24, and 2.03, and δ 160.8, 171.2, and 170.5 (Tables 1, 2), respectively. The ^{13}C-NMR spectrum showed the remaining signals due to four methyls (δ 27.5, 19.7, 17.5, 16.9), seven methylenes (δ 65.7, 36.9, 36.5, 34.7, 30.3, 29.2, 23.6), five sp^3-hybridized methines (δ 96.7, 80.2, 70.4, 48.1, 31.3), and four quaternary sp^3-carbons (δ 92.9, 89.3, 42.3, 38.4) as shown in Table 2.

From these data and the index of hydrogen deficiency given by the molecular formula of **1**, **1** was suggested to be a tetracyclic diterpenoid.

The ^{1}H–^{1}H-shift correlation spectroscopy (COSY) analysis for the functional groups thus established led to seventeen partial structures which were supported by the ^{1}H–^{13}C long-range COSY spectrum. The connection of these partial structures was determined by the ^{1}H–^{13}C long-range COSY correlations. Based on this evidence, the planar structure of **1** was elucidated.

The relative stereochemistry of the decaline moiety of **1** was determined as follows. H-3 and H-6 were deduced as being axial and equatorial from the valu of $J_{\text{H-3,H}_2\text{-2}}$ (11, 5 Hz) and $J_{\text{H-5,H-6,H}_2\text{-7}}$ (3, 3, 3 Hz), respectively. In the difference nuclear Overhauser effect (DIFNOE) spectrum, irradiation of H_3-18 enhanced the intensities of the H-3, H-5, H-6 and H_3-19 signals, whereas irradiation of H_3-20 enhanced those of the H-8, H_3-19, H-2β (δ 1.65, brqd, J=12, 4 Hz), H-1β (δ 1.35, ddd, J=12, 4, 4 Hz), and the formylmethin proton signal as shown in Fig. 1. The above data suggested that both of the A- and B-ring existed in chair conformation with C-10 methyl and C-6 formyloxy groups in an axial arrangement and a C-3 acetoxy group in equatorial one, and the decaline

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Table 1. ^{1}H-Chemical Shifts of Compounds **1**, **1b**—**1e**, **2**, **2a** and **2b** δ (ppm) in $CDCl_3$ (J/Hz in Parentheses)

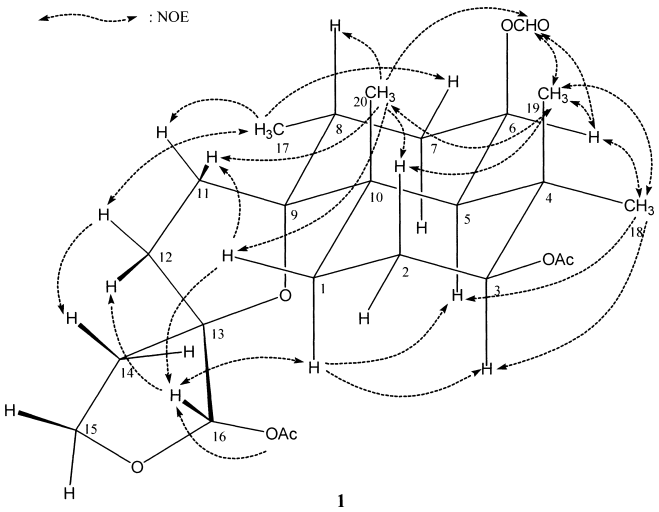
H	**1**	**1b**	**1c**	**1d**	**1e**	**2**	**2a**	**2b**
1α	1.48 ddd (12, 12, 4)	*ca.* 1.85	*ca.* 1.56	2.10 ddd (12, 12, 4)	*ca.* 1.78	1.60 ddd (12, 12, 4)	1.49 ddd (12, 12, 4)	1.74 ddd (12, 12, 4)
1β	1.35 ddd (12, 4, 4)	1.29 ddd (12, 4, 4)	1.33 m	1.46 ddd (12, 4, 4)	1.51 ddd (12, 3, 3)	1.40 ddd (12, 4, 4)	1.33 ddd (12, 4, 4)	1.54 ddd (12, 4, 4)
2α	1.62 m	1.67 m	1.67 m	1.89 m	1.93 m	1.77 m	1.70 m	1.94 m
2β	1.65 brqd (12, 4)	1.64 brqd (12, 4)	1.64 brqd (12, 4)	*ca.* 1.83	*ca.* 1.85	1.70 brqd (12, 4)	1.67 brqd (12, 4)	1.84 m
3	4.30 dd (11, 5)	3.24 ddd (11, 6, 5)	3.17 ddd (11, 6, 5)	4.77 dd (11, 4)	4.69 dd (11, 4)	4.42 dd (11, 4)	3.18 brdd (11, 4)	4.71 dd (11, 4)
5	1.63 brs	1.38 d (3)	1.31 d (3)	1.89 d (3)	1.79 d (3)	1.64 brs	1.38 d (3)	1.87 d (2)
6	5.58 ddd (3, 3, 3)	4.36 dddd (3, 3, 3, 3)	4.35 dddd (3, 3, 3, 3)	5.72 ddd (3, 3, 3)	5.71 ddd (3, 3, 3)	5.62 ddd (3, 3, 3)	4.42 brq (3)	5.77 brq (2)
7α	1.77 ddd (14, 14, 3)	1.88 ddd (14, 14, 3)	1.78 ddd (14, 14, 3)	1.93 m	1.85 m	1.77 m	1.77 ddd (14, 14, 3)	1.85 m
7β	1.55 ddd (14, 3, 3)	1.38 ddd (14, 3, 3)	1.37 ddd (14, 3, 3)	1.65 ddd (14, 3, 3)	1.63 ddd (14, 3, 3)	1.70 m	1.55 m	1.84 m
8	2.09 m	2.20 m	2.23 m	2.15 m	2.18 m	2.17 m	2.31 m	2.23 m
11α	1.79 brt (12)	1.72 m	1.72 ddd (13, 9, 3)	1.78 brt (11)	*ca.* 1.78	1.95 ddd (14, 12, 4)	1.93 ddd (14, 12, 4)	1.97 ddd (14, 12, 4)
11β	2.12 brq (11)	2.08 brq (12)	2.01 ddd (13, 9, 9)	2.17 brq (11)	2.09 ddd (13, 9, 9)	2.28 ddd (14, 12, 9)	2.25 ddd (14, 12, 9)	2.33 ddd (14, 12, 9)
12α	1.95 brq (10)	*ca.* 1.88	1.88 ddd (13, 9, 9)	*ca.* 1.92	1.91 ddd (13, 9, 9)	2.60 ddd (18, 12, 9)	2.59 ddd (18, 12, 9)	2.64 ddd (18, 12, 9)
12β	2.03 brt (12)	1.94 brt (12)	2.31 ddd (13, 9, 3)	2.01 brt (11)	2.38 ddd (13, 9, 3)	2.51 ddd (18, 12, 4)	2.48 ddd (18, 12, 4)	2.52 ddd (18, 12, 4)
14α	2.50 ddd (12, 10, 9)	2.42 brq (10)	2.14 ddd (12, 8, 4)	2.48 brq (11)	2.20 ddd (12, 8, 4)	—	—	—
14β	1.88 ddd (12, 7, 2)	1.84 m	1.99 ddd (12, 8, 8)	*ca.* 1.88	2.02 ddd (12, 8, 8)	—	—	—
15α	4.07 ddd (10, 9, 2)	3.96 ddd (10, 9, 3)	3.87 ddd (12, 8, 4)	4.01 ddd (11, 9, 3)	3.90 ddd (12, 8, 4)	—	—	—
15β	3.82 ddd (10, 9, 7)	3.77 brq (9)	3.95 brq (8)	3.80 brq (9)	3.99 brq (8)	—	—	—
16	5.63 s	4.18 s	4.76 s	4.24 s	4.83 s	—	—	—
17(14)[d]	0.88 d (6)	0.89 d (6)	0.91 d (6)	0.89 d (6)	0.91 d (6)	0.90 d (6)	0.91 d (6)	0.91 d (6)
18(15)[d]	0.93 s	1.10 s	1.08 s	1.06 s	1.03 s	0.97 s	1.09 s	1.06 s
19(16)[d]	1.02 s	1.19 s	1.18 s	1.16 s	1.15 s	1.07 s	1.20 s	1.18 s
20(17)[d]	1.23 s	1.24 s	1.24 s	1.44 s	1.44 s	1.30 s	1.30 s	1.50 s
3-OAc	2.03 s	—	—	—	—	2.05 s	—	—
16-OAc	2.24 s	—	—	—	—	—	—	—
CHO	8.11 s	—	—	—	—	8.12 s	—	—
OMe	—	3.37 s	3.35 s	3.51 s	3.39 s	—	—	—
3-OH	—	1.34 d (6)	1.33 d (6)	—	—	—	—	—
6-OH	—	1.12 d (3)	1.13 d (3)	—	—	—	—	—
p-Br-Bz				[a]	[b]			[c]

a) 7.91 d (9) ×2, 7.85 d (9) ×2, 7.61 d (9) ×2, 7.55 d (9) ×2. *b*) 7.91 d (9) ×2, 7.86 d (9) ×2, 7.61 d (9) ×2, 7.55 d (9) ×2. *c*) 7.90 d (9) ×2, 7.85 d (9) ×2, 7.63 d (9) ×2, 7.58 d (9) ×2. *d*) C-No. of **2**, **2a** and **2c**.

Table 2. ^{13}C-Chemical Shifts of Compounds **1**, **1b**—**1e**, **2**, **2a**, **2b**, **21**, **3** and **3a**, δ (ppm) in $CDCl_3$

C	1	1b	1c	1d	1e	2	2a	2b	21[e]	3[f]	3a
1	30.3	30.9[g]	30.7[g]	29.8[g]	30.8[g]	30.4	30.7	30.5	31.1	39.4	38.9
2	23.6	27.4	27.5	24.1	24.0	23.2	26.9	23.4	18.2	18.5	17.9
3	80.2	78.7	78.7	81.2	81.3	79.5	78.1	80.4	41.2	37.9	37.1[g]
4	38.4	39.8	39.8	38.9	39.0	38.5	39.6	38.8	33.2	46.5	46.5
5	48.1	48.8	48.9	48.5	48.6	47.8	48.5	48.4	46.5	45.4	45.0
6	70.4	68.2	68.2	71.9	71.8	69.5	67.4	70.7	21.2	25.9	25.4
7	36.5	40.8	41.0	36.3	36.6	36.0	40.4	36.0	30.9	121.6	123.9
8	31.3	30.1	30.4	31.7	31.7	31.8	31.3	32.1	36.7	135.5	133.0
9	92.9	93.4	93.4	92.6	92.7	92.7	93.9	92.6	93.9	51.3	50.2
10	42.3	42.4	42.7	42.6	42.8	41.9	41.9	42.1	42.1	35.2	35.0
11	29.2	29.8[g]	29.7[g]	29.7[g]	29.8[g]	24.6	24.7	24.7	24.6	29.9	29.6
12	36.9	37.5	37.7	37.5	37.8	29.4	29.6	29.4	29.4	71.5	71.3
13	89.3	90.1	92.1	90.3	92.4	176.9	177.1	176.9	177.8	75.6	75.5
14	34.7	35.1	32.0	35.1	31.3	—	—	—	—	37.5	37.0[g]
15	65.7	64.3	65.8	64.3	65.6	—	—	—	—	33.9	32.5
16	96.7	104.8	107.6	104.9	107.4	—	—	—	—	17.0	16.4
17(14)[d]	16.9	17.3[h]	17.3[h]	16.8	17.5	15.0	15.3	15.1	15.6	18.4	17.7
18(15)[d]	27.5	27.8	27.7	27.4	27.8	27.5	27.8	27.8	33.0	181.2	179.1
19(16)[d]	17.5	17.1[h]	17.8[h]	18.4	18.4	17.7	17.2	18.3	21.8	18.0	17.4
20(17)[d]	19.7	20.1	20.5	20.3	20.6	18.2	18.6	18.8	15.5	15.6	15.4
3-*O*-Ac	170.5					170.5					
	21.2					21.2					
16-*O*-Ac	171.2										
	21.2										
HCO	160.8					160.5					
16-OMe		54.4	54.8	54.7	55.0						
p-Br-Bz				[a]	[b]			[c]			
COOMe											52.0

a) 165.4, 165.3, 131.9×2, 131.7×2, 131.1×2, 131.0×2, 129.8×2, 128.1×2. *b*) 165.4, 165.3, 131.9×2, 131.7×2, 131.1×2, 131.0×2, 129.8, 129.7, 128.1, 127.9. *c*) 165.2×2, 132.0×2, 131.7×2, 131.0×4, 129.5×2, 128.0×2. *d*) C-No. of **2**, **2a** and **2c**. *e*) As reported in reference. *f*) In C_5D_5N. *g*, *h*) May be reversed in each column.



1

Fig. 1

moiety of **1** was A/B *trans*. In addition, nuclear Overhauser effects (NOEs) were observed as follows: between H-11β (δ 2.12, brq, J=11 Hz)/H_3-20 and H-1β (δ 1.35, ddd, J=12, 4, 4 Hz); H-11α (δ 1.79, brt, J=12 Hz)/H_3-17; H-12α (δ 1.95, brq, J=10 Hz)/H_3-17; and H-16/H-12β (δ 2.03, brt, J=12 Hz), H_2-1, and the acetylmethyl proton at δ 2.24. The above data indicated that the relative stereochemistry of the spiro-tetrahydrofuran rings of **1** was 9*R**, 13*S**, 16*S**.

From these findings, the relative stereochemistry of **1** is as shown in Chart 1.

The absolute stereochemistry of **1** was confirmed by the fact as follows. On treatment with 3% NaOMe/MeOH followed by neutralization with dil. HCl, **1** gave an equilibrium mixture of deacyl product of 1 (**1a**), which was methylated with AcCl/MeOH (20 : 1) to give a C-16 epimeric mixture of 16-methylether of **1a** (**1b**, **1c**) as shown in Chart 2. The product was separated by a preparative TLC to give **1b** and **1c**, and then subjected to *p*-bromobenzoylation to afford the 3,6-di-*O*-*p*-bromobenzoates (**1d**, **1e**), of which both CD spectra showed a negative first Cotton effect at 253 nm and a positive second one at 236 nm, respectively, as described in the experimental section. By applying the exiton chirality rule[24] to this result, the absolute configuration at the C-3 and C-6 position of **1d** and **1e** were estimated as *S* and *R*, respectively, as shown in Fig. 2.

Based on these data, the structure of leucasdin A (**1**) was concluded to be (3*S*,6*R*,8*R*,9*R*,13*S*,16*S*)-9,13,15,16-bisepoxy-3,16-diacetoxy-6-formyloxylabdane.

Leucasdin B (**2**) was obtained as colorless needles, mp 230—231 °C (dec.), $[\alpha]_D^{25}$ −13.6°, and its IR spectrum revealed the presence of γ-lactone (1772, 1170 cm^{-1}) and ester (1720, 1248 cm^{-1}) groups. The molecular formula was deduced to be $C_{20}H_{30}O_6$ from the HR-MS and ^{13}C-NMR spectral data. The index of hydrogen deficiency given by the molecular formula, and ^{13}C-NMR and DEPT spectral data suggested **2** to be a tricyclic compound. The presence of a formyl and an acetyl groups were deduced from the ^{1}H- and ^{13}C-NMR spectra which showed characteristic signals at δ 8.12 and 2.05, and δ 160.5 and 170.5 (Tables 1, 2), respectively. The ^{1}H- and ^{13}C-NMR spectra were similar to those of **1**, apart from the lack of the signals due to one acethyl-

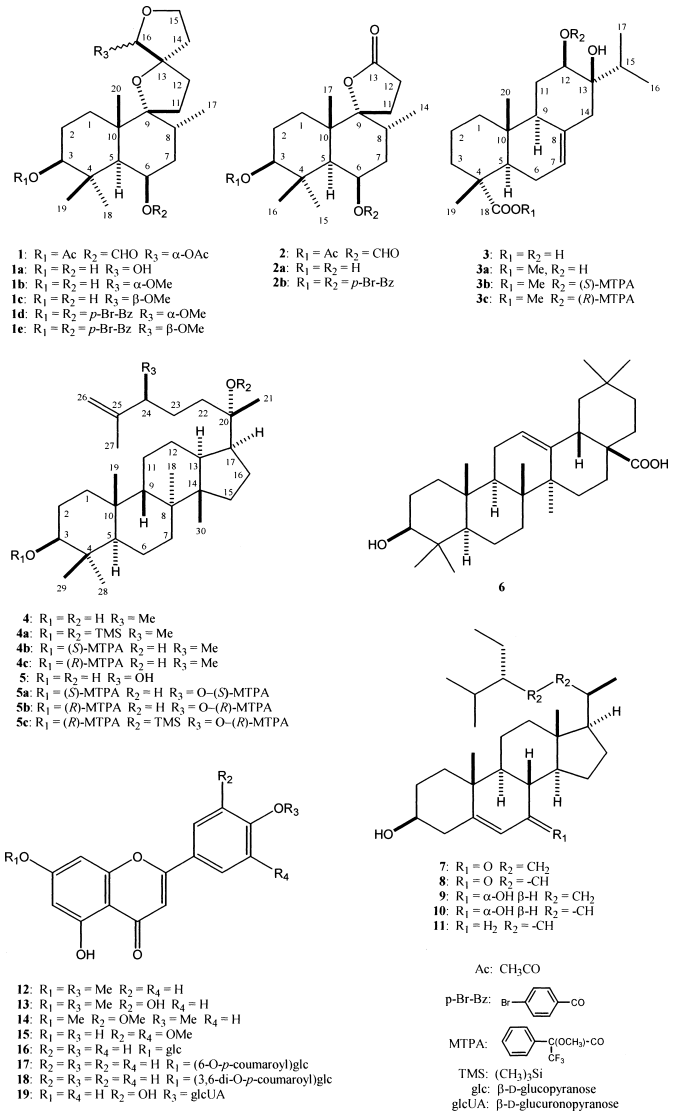
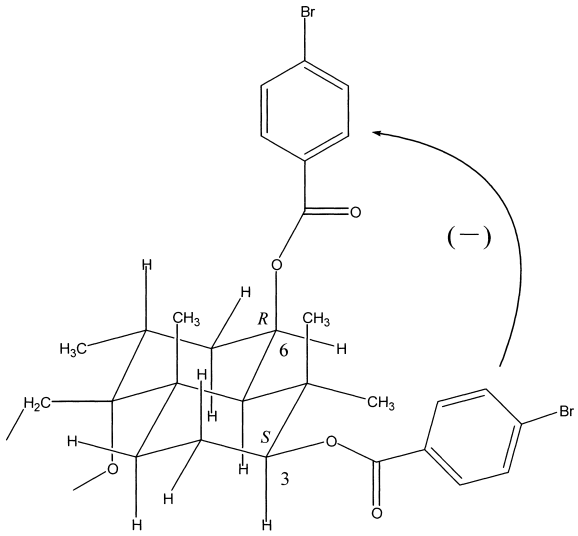


Chart 1

methyl, methylene, oxymethylene, hemiacetal ester, and one quaternary sp^3-carbon observed in the spectra of **2**.

These findings indicated that **2** is a norlabdane-type diterpene as *iso*-ambreinolide[25] (**21**) having a γ-lactone ring instead of the two spiro-tetrahydrofuran rings in **1**. This was further confirmed by the ^{13}C-NMR spectrum of **2**, in which the signal pattern of the γ-lactone moiety was almost identical with that of **21**. The position of two acyl groups were confirmed to be same as **1** by ^{1}H–^{13}C COSY data. The relative stereochemistry was confirmed by NOE experiments which gave the same results as for **1**.

On deacylation followed by *p*-bromobenzoylation in the same manner as **1**, **2** afforded the 3,6-di-*O*-*p*-bromobenzoate (**2b**) whose CD spectrum showed a negative first Cotton effect at 253 nm and a positive second one at 236 nm, denoting



1d, **1e** and **2b**

Fig. 2

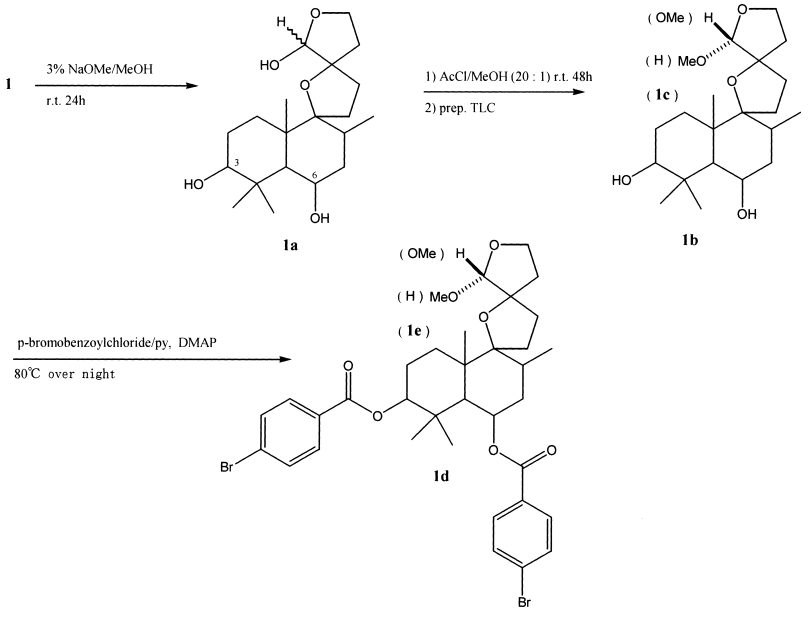


Chart 2

that the absolute configurations at the C-3 and C-6 position of **2b** were estimated as *S* and *R*, respectively, as shown in Fig. 2.

These findings led us to conclude that the structure of leucasdin B (**2**) should be (3*S*,6*R*)-3-acetoxy-6-formyloxy-isoambreinolide.

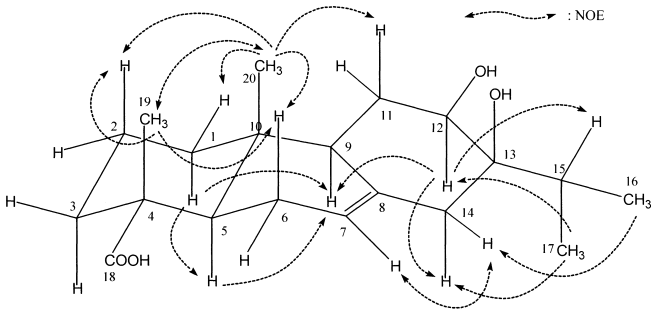
Leucasdin C (**3**) was obtained as colorless needles, mp 284—285 °C (dec.), $[\alpha]_D^{25}$ +26.8°, and showed absorption bands of hydroxy (3352 cm^{-1}) and carboxy (1690 cm^{-1}) groups in its IR spectrum. The molecular formula was deduced to be $C_{20}H_{32}O_4$ from the HR-FAB-MS, and ^{13}C-NMR and DEPT spectral data, suggesting **3** to be a tricyclic compound. The ^1H- and ^{13}C-NMR spectra indicated the presence of an isopropyl and two *tert*-methyl groups, and three quaternary sp^3-carbons in addition to a tri-substituted double bond (Tables 2, 3). These findings suggested **2** to be a tricyclic diterpenoid.

The presence of five partial structures was deduced from the ^1H–^1H COSY spectral data and the assignment of each carbon, except for a quaternary one, was based on the ^1H–^{13}C COSY spectral data. The connectivity of each partial structure was obtained from the ^1H–^{13}C long-range COSY spectral data. From these data, **3** was deduced to have a 7-abietene skeleton possessing 4-carboxy and 12,13-dihydroxy groups.

The relative stereochemistry was established by a combination of observed coupling constants and NOE experiments. In the DIFNOE spectrum of **3** in which the H_3-20 was irradiated, enhancement of the signal intensity of H_3-19, H-1β (δ 1.86, brd, J=13 Hz), H-2β (δ 1.54, brqt, J=13, 3 Hz), H-6β (δ 2.15, m), and H-11β (δ 2.15, brq, J=11 Hz) was observed, whereas irradiation of H-1α (δ 1.18, ddd, J=13, 13, 4 Hz) enhanced those of the H-5 and H-9 as shown in Fig. 3. The above data and the coupling constants from H-5 to H-6β (12 Hz) suggested that the A-ring existed in chair conformation with C-4 and C-10 methyl groups in an axial arrangement, and the decaline moiety of **3** was A/B *trans*. In addition, NOEs were observed between H-12/H-9, H-15, H_3-17, and H-14α (δ 2.09, d, J=14 Hz) indicating that the B- and C-rings exist in a half-chair and chair conformation, respectively, and the configuration of C-4, C-9, C-12, and C-13 are *R**, *S**, *R**, and *R**, respectively.

The absolute stereochemistry of **3** was determined by the advanced Mosher method.[26] After conversion from **3** to its methylester (**3a**) by treatment with CH_2N_2, 12-*O*-(*S*)- and (*R*)-methoxy (trifluoromethyl) phenylacetyl (MTPA) ester (**3b**, **3c**, respectively) were prepared and submitted to ^1H-NMR spectroscopy. The $\Delta\delta$ value ($\delta_{(S)\text{-MTPA (3b)}} - \delta_{(R)\text{-MTPA (3c)}}$) of each proton is shown in Fig. 4, indicating that the absolute configuration at C-12 should be *R*.

On the basis of these results, the structure of leucasdin C (**3**) was concluded to be (4*R*,9*S*,12*R*,13*R*)-12,13-dihydroxy-



3

Fig. 3

Table 3. ^1H-Chemical Shifts of Compounds **3** and **3a**—**3c** δ (ppm) in $CDCl_3$ (J/Hz in Parentheses)

H	**3**[a]	**3a**	**3b**	**3c**
1α	1.18 ddd (13, 13, 4)	1.12 ddd (13, 13, 4)	1.16 ddd (12, 12, 5)	1.15 ddd (12, 12, 5)
1β	1.86 brd (13)	1.86 m	1.82 m	1.80 m
2α	1.47 m	1.55 m	1.55 m	1.55 m
2β	1.54 brqt (13, 3)	1.55 m	1.55 m	1.55 m
3α	2.13 m	1.75 ddd (13, 13, 6)	1.76 ddd (13, 13, 6)	1.76 ddd (13, 13, 6.5)
3β	1.81 brd (13)	1.61 m	1.62 m	1.62 m
5	2.42 dd (12, 4)	1.91 dd (12, 3)	1.94 brd (14)	1.93 brd (14)
6α	2.03 brd (13)	*ca.* 1.58	*ca.* 1.54	*ca.* 1.54
6β	2.15 m	1.99 m	2.00 m	2.00 m
7	5.47 m	5.48 m	5.48 brd (5)	5.47 brd (5)
9	1.92 brd (12)	1.86 brd (12)	2.01 brd (12)	1.98 brd (12)
11α	1.94 ddd (12, 4, 4)	1.76 ddd (12, 4, 4)	1.87 ddd (12, 4, 4)	1.82 ddd (12, 4, 4)
11β	2.15 brq (11)	1.45 brq (12)	1.78 brq (12)	1.64 brq (12)
12	4.01 dd (11, 4)	3.73 brd (12)	5.18 dd (12, 4)	5.17 dd (12, 4)
14α	2.09 d (14)	2.01 d (14)	2.01 d (16)	2.02 d (16)
14β	2.38 d (14)	2.15 d (14)	2.18 d (16)	2.21 d (16)
15	2.61 sep (7)	2.12 sep (7)	1.50 sep (7)	1.75 sep (7)
16	1.16 d (7)	0.96 d (7)	0.83 d (7)	0.93 d (7)
17	1.10 d (7)	0.92 d (7)	0.74 d (7)	0.84 d (7)
19	1.46 s	1.26 s	1.26 s	1.25 s
20	1.00 s	0.89 s	0.90 s	0.86 s
12-OH	—	*ca.* 1.57	—	—
13-OH	—	1.43 s	1.21 brs	1.22 brs
COOMe	—	3.64 s	3.64 s	3.64 s
MTPA	—		[b]	[c]

a) In C_5D_5N. *b*) 7.56, 2H, m; 7.40, 3H, m; 3.58, 3H, s. *c*) 7.52, 2H, m; 7.42, 3H, m; 3.05, 3H, s.

Table 4. ^{1}H-Chemical Shifts of Compounds **4**, **4a**—**4c**, **5** and **5a**—**5c** δ (ppm) in $CDCl_3$-d_6 (J/Hz in Parentheses)

H	**4**	**4a**	**4b**	**4c**	**5**	**5a**	**5b**	**5c**
1α	1.39 m	1.35 m	1.40 m	1.49 m	1.40 m	1.41 m	1.48 m	1.48 m
1β	1.39 m	1.35 m	1.44 m	1.50 m	1.40 m	1.43 m	1.50 m	1.50 m
2α	1.71 m	1.55 m	1.83 m	1.90 m	1.69 m	1.83 m	1.90 m	1.90 m
2β	1.59 m	1.64 brqd (12, 5)	1.66 m	1.77 brqd (12, 5)	1.60 m	1.66 m	1.77 m	1.77 m
3	3.24 dd (12, 5)	3.20 dd (12, 5)	4.73 dd (12, 5)	4.75 dd (12, 5)	3.23 dd (12, 5)	4.73 dd (12, 5)	4.75 dd (12, 5)	4.75 dd (12, 5)
5	1.45 dd (11, 3)	1.45 brd (11)	1.49 dd (11, 3)	1.48 dd (11, 3)	1.46 dd (11, 3)	1.49 dd (11, 3)	1.49 dd (11, 3)	1.49 dd (11, 3)
6α	1.52 m	1.48 brd (12)	1.54 m	1.54 m	1.54 m	1.54 m	1.54 m	1.54 m
6β	1.19 m	1.22 brqd (12, 6)	1.22 m	1.22 m	1.19 m	1.19 m	1.19 m	1.18 m
7α	1.19 m	1.14 m	1.22 m	1.22 m	1.19 m	1.19 m	1.19 m	1.18 m
7β	1.97 m	1.94 ddd (12, 6, 6)	1.97 m	1.97 m	1.96 m	1.95 m	1.95 m	1.95 m
9	1.49 dd (13, 3)	1.49 dd (13, 3)	1.50 dd (13, 3)	1.49 dd (13, 3)	1.49 dd (13, 3)	1.48 dd (13, 3)	1.48 dd (13, 3)	1.49 dd (13, 3)
11α	1.20 m	1.13 m	1.22 m	1.22 m	1.20 m	1.20 m	1.20 m	1.14 m
11β	1.51 m	1.47 m	1.50 m	1.50 m	1.49 m	1.49 m	1.49 m	1.47 m
12α	1.96 brddd (12, 4, 4)	1.88 brd (12)	1.97 m	1.97 m	1.96 m	1.88 m	1.95 m	1.89 m
12β	1.43 m	1.41 m	1.43 m	1.43 m	1.43 m	1.35 m	1.41 m	1.42 m
13	2.06 ddd (12, 12, 3)	1.97 m	2.06 ddd (12, 12, 3)	2.06 ddd (12, 12, 3)	2.07 ddd (12, 12, 3)	2.02 ddd (12, 12, 3)	2.06 ddd (12, 12, 3)	1.97 m
15α	1.36 dd (12, 7)	1.33 m	1.36 dd (12, 7)	1.36 dd (12, 7)	1.38 m	1.34 m	1.36 m	1.34 m
15β	1.12 dd (12, 7)	1.05 dd (10, 6)	1.12 dd (11.5, 7)	1.12 dd (12, 7)	1.14 dd (12, 7)	1.12 dd (12, 7)	1.12 dd (12, 7)	1.07 dd (12, 7)
16α	1.63 m	1.57 m	1.63 m	1.63 m	1.67 m	1.57 m	1.61 m	1.56 m
16β	1.54 m	1.51 m	1.54 m	1.54 m	1.56 m	1.49 m	1.54 m	1.52 m
17	2.12 ddd (11, 11, 9)	2.00 m	2.12 ddd (11, 11, 9)	2.12 ddd (11, 11, 9)	2.15 ddd (11, 11, 9)	2.03 ddd (11, 11, 9)	2.09 ddd (11, 11, 9)	2.00 m
18	1.10 s	1.09 s	1.11 s	1.11 s	1.10 s	1.10 s	1.10 s	1.09 s
19	0.92 s	0.92 s	0.92 s	0.95 s	0.91 s	0.92 s	0.95 s	0.96 m
21	1.20 s	1.25 s	1.20 s	1.20 s	1.22 s	1.14s	1.19 s	1.25 s
22	1.38 m	1.33 m	1.38 m	1.38 m	1.47 m	1.34 m	1.40 m	1.42 m
	1.28 m	1.12 m	1.28 m	1.28 m	1.39 m	1.34 m	1.40 m	1.42 m
23	1.33 m	1.28 m	1.33 m	1.33 m	1.68 m	1.73 m	1.81 m	1.67 m
	1.31 m	1.20 m	1.31 m	1.31 m	1.58 m	1.66 m	1.71 m	1.67 m
24	2.09 m	2.05 m	2.09 m	2.09 m	4.07 dd (6, 6)	5.40 dd (6, 6)	5.36 dd (6, 6)	5.33 dd (6, 6)
26	4.674 brs	4.67 brs	4.67 brs	4.67 brs	4.96 brs	5.04 brs	4.96 brs	4.95 brs
	4.674 brs	4.67 brs	4.67 brs	4.67 brs	4.85 brs	4.97 brs	4.93 brs	4.93 brs
27	1.646 s	1.64 s	1.65 s	1.65 s	1.73 s	1.73 s	1.62 s	1.62 s
28	0.98 s	0.87 s	0.91 s	0.83 s	0.98 s	0.91 s	0.83 s	0.83 s
29	0.77 s	0.75 s	0.82 s	0.79 s	0.79 s	0.82 s	0.80 s	0.80 s
30	0.92 s	0.91 s	0.92 s	0.92 s	0.91 s	0.86 s	0.89 s	0.88 s
24-Me	1.014 d (5)	1.00 d (5)	1.01 d (5)	1.01 d (5)	—	—	—	—
TMS	—	0.10 s, 0.09 s	—	—	—	—	—	0.07 s
MTPA	—	—	*a)*	*b)*	—	*c)*	*d)*	*e)*

a) 7.53, 2H, m; 7.40, 3H, m; 3.55, 3H, s. *b*) 7.56, 2H, m; 7.39, 3H, m; 3.57, 3H, s. *c*) 7.53, 2H, m; 7.51, 2H, m; 7.40, 6H, m; 3.53, 3H, s; 3.52, 3H, s. *d*) 7.55, 2H, m; 7.51, 2H, m; 7.39, 6H, m; 3.57, 3H, s; 3.55, 3H, s. *e*) 7.55, 2H, m; 7.51, 2H, m; 7.39, 6H, m; 3.57, 3H, s; 3.56, 3H, s.

abiet-7-en-18-oic acid.

Leucastrin A (**4**) was obtained as colorless needles, mp 176—177 °C (dec.), $[\alpha]_D^{25}$ +36.3°, and showed absorption bands of a hydroxy group (3676 cm^{-1}) in its IR spectrum. The molecular formula was deduced to be $C_{31}H_{54}O_2$ from the HR-MS, and ^{13}C-NMR and DEPT spectral data, suggesting **4** to be a tetracyclic compound.

A close inspection of the ^{1}H- and ^{13}C-NMR spectra of **4** by DEPT and ^{1}H–^{13}C COSY experiments revealed the presence of eight methyl groups, including one secondary (δ 19.9) and seven tertiary (δ 29.1, 27.4, 22.5, 22.1, 18.9, 17.4, 16.1) groups; ten methylenes (δ 40.2, 35.1, 32.9, 32.5, 29.5, 29.2, 26.3, 25.9, 23.9, 18.5); six sp^3-hybridized methines (δ 79.4, 48.3, 47.7, 45.5, 43.5, 41.8); five quaternary sp^3-carbons (δ 75.2, 50.0, 40.0, 39.2, 36.8); one *exo*-methylene (δ 109.6), and one quaternary sp^2-carbon (δ 149.9) as shown in Table 5.

From these data, **1** was suggested to be a tetracyclic triterpenoid.

The nineteen partial structures were suggested from the ^{1}H–^{1}H-COSY and ^{1}H–^{13}C COSY data, and then the sequence was clarified based on the ^{1}H–^{13}C long-range COSY spectrum.

The relative stereochemistry was confirmed by NOE experiments (Fig. 5) in addition to the coupling constant of each proton. In the DIFNOE spectrum of **4**, the experiments of the A-ring gave almost of same results as for **1** suggesting that the A-ring exists in chair conformation with C-10 methyl and C-3 hydroxy groups in an axial and equatorial arrangement, respectively. In addition, NOEs were observed between H-9/H_3-19 and H_3-30; H_3-18/H-5, H-11α (δ 1.20, m), H-13, and H-15α (δ 1.36, dd, J=12, 7 Hz); and H_3-30/H-7β (δ 1.97, m), H-9, H-12β (δ 1.43, m), H-15β (δ 1.12, dd, J=12, 7 Hz), and H-16β (δ 1.54, m) indicating that the B- and C-rings exist in a boat and chair conformation, respectively, with C-8 and C-14 methyl groups in an axial arrangement, and the ring junctions of the A-, B-, C-, and D-ring in **4** are all *trans*. However, an assessment of the DIFNOE spectral data to determine the relative stereochemistry of the side chain moiety was unsuccessful. Thus, **4** was converted into the 3,20-di-trimethylsilyl (TMS) ether derivative (**4a**), and detailed NOE experiments were carried out. As a result, NOEs were observed, as depicted with dashed arrow in Fig. 6, demonstrating the relative stereochemistry of 17*S** and 20*S**.

3-*O*-(*S*)- and (*R*)-MTPA ester (**4b**, **4c**, respectively) were prepared as same manner as **3**, and submitted to ^{1}H-NMR spectroscopy. The absolute configuration at C-3 of **4** should be *S* from the $\Delta\delta$ value ($\delta_{(S)\text{-MTPA (4b)}} - \delta_{(R)\text{-MTPA (4c)}}$) of each proton to be shown in Fig. 7. The absolute stereochemistry of

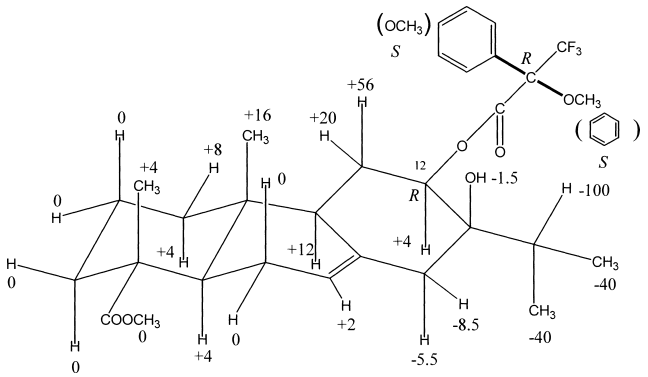


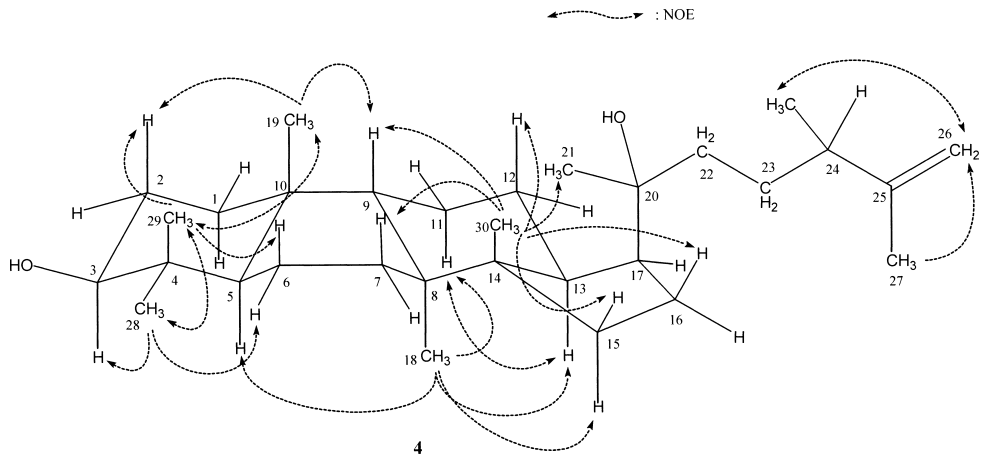
Fig. 4. Chemical Shift Differences between **3b** and **3c**

$\Delta\delta$ values ($\Delta\delta = \delta_{(S)\text{-MTPA(3b)}} - \delta_{(R)\text{-MTPA(3c)}}$) are shown in Hz.

Table 5. ^{13}C-Chemical Shifts of Compounds **4**, **4a** and **5**, δ (ppm) in $CDCl_3$

C	4	4a	5	C	4	4a	5
1	32.9	33.0	32.9	18	22.1	22.1	22.1
2	29.2	30.7	29.2	19	22.5	22.6	22.5
3	79.4	80.1	79.4	20	75.2	78.5	75.1
4	39.2	39.6	39.2	21	27.4	28.3	27.1
5	47.7	47.5	47.7	22	40.2	41.8[a)]	37.2
6	18.5	18.7	18.5	23	29.5	29.7	29.4
7	35.1	35.0	35.1	24	41.8	41.9	76.0
8	40.0	39.9	40.0	25	149.9	150.0	147.6
9	45.5	45.6	45.5	26	109.6	109.4	110.9
10	36.8	36.8	36.8	27	18.9	19.0	17.9
11	23.9	24.1	23.9	28	29.1	29.5	29.1
12	26.3	26.1	26.2	29	16.1	16.6	16.1
13	43.5	43.8	43.4	30	17.4	17.6	17.5
14	50.0	50.1	50.0	24-Me	19.9	19.7	
15	32.5	32.6	32.4	TMS		0.6	
16	25.9	25.9	26.0			2.9	
17	48.3	47.8[a)]	48.8				

a) brd.



4

Fig. 5

the 24-position was determined to be *S* by comparing data in the literature,[27] in which the ^{1}H-NMR study for differentiate (24*S*)- and (24*R*)-alkyl sterols has been reported, including two compounds, (24*S*)-methylcholesta-5,25-dien-3β-ol (codisterol,[27] **22**) and (24*R*)-methylcholesta-5,25-dien-3β-ol (epicodisterol, [27] **23**). As shown in Table 6, the $\Delta\delta$ values of H-26−H-27, H-26−C-24-metyl protons, and H-27−C-24-metyl protons in **4** were approximated to those of **22**.

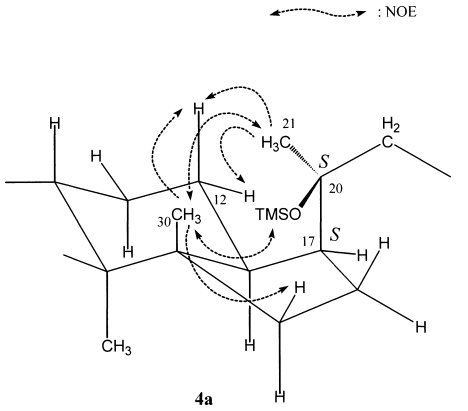


Fig. 6

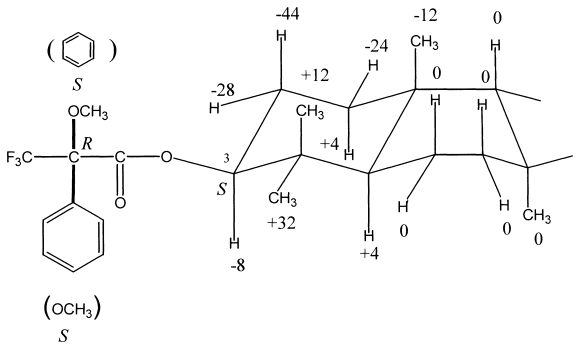


Fig. 7. Chemical Shift Differences between **4b** and **4c**

$\Delta\delta$ values ($\Delta\delta=\delta_{(S)\text{-MTPA(4b)}}-\delta_{(R)\text{-MTPA(4c)}}$) are shown in Hz.

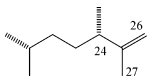
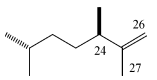
On the basis of all the above findings, the structure of leucastrin A (**4**) was concluded to be (3*S*,17*S*,20*S*,24*S*)-3,20-dihydroxy-24-methylprotost-25-en.

Leucastrin B (**5**) was obtained as colorless needles, mp 196—197 °C (dec.), $[\alpha]_D^{25}$ +71.8°, and showed absorption bands of a hydroxy group (3676 cm^{-1}) in its IR spectrum. The molecular formula was deduced to be $C_{30}H_{52}O_3$ from the HR-FAB-MS and ^{13}C-NMR spectral data. ^{1}H- and ^{13}C-NMR spectra of **5** closely resembled those of **4** except for the chemical shifts of the side chain at C-22−C-27 (Tables 4, 5), and revealed that the 24-methyl group in **4** was replaced by a hydroxyl group in **5** from observations of an oxymetine signal (δ-H 4.07, dd, J=6, 6 Hz; δ-C 76.0) and ^{1}H–^{13}C long-range COSY correlations.

The relative stereochemistry was confirmed by NOE experiments which gave the same results as for **4**.

3,24-di-*O*-(*S*)- and (*R*)-MTPA ester (**5a**, **5b**, respectively) were prepared as same manner as **3**, and submitted to ^{1}H-NMR spectroscopy. The absolute configuration at C-3 and 24 of **5** should be both *S* from the $\Delta\delta$ value ($\delta_{(S)\text{-MTPA (5a)}}-\delta_{(R)\text{-MTPA (5b)}}$) of each proton to be shown in Fig. 8.

Table 6. Comparison of ^{1}H-Chemical Shifts of Compounds **4**, **22** and **23**, δ (ppm) in $CDCl_3$

H	**4**	 **22**[a]	 **23**[b]
26	4.674	4.658	4.654
27	1.646	1.635	1.649
24-Me	1.014	0.990	0.984
$\Delta\delta$			
26—27	3.028	3.023	3.005
26—24-Me	3.660	3.668	3.670
27—24-Me	0.632	0.645	0.665

a) Side chain of codisterol in reference. *b*) Side chain of epicodisterol in reference.

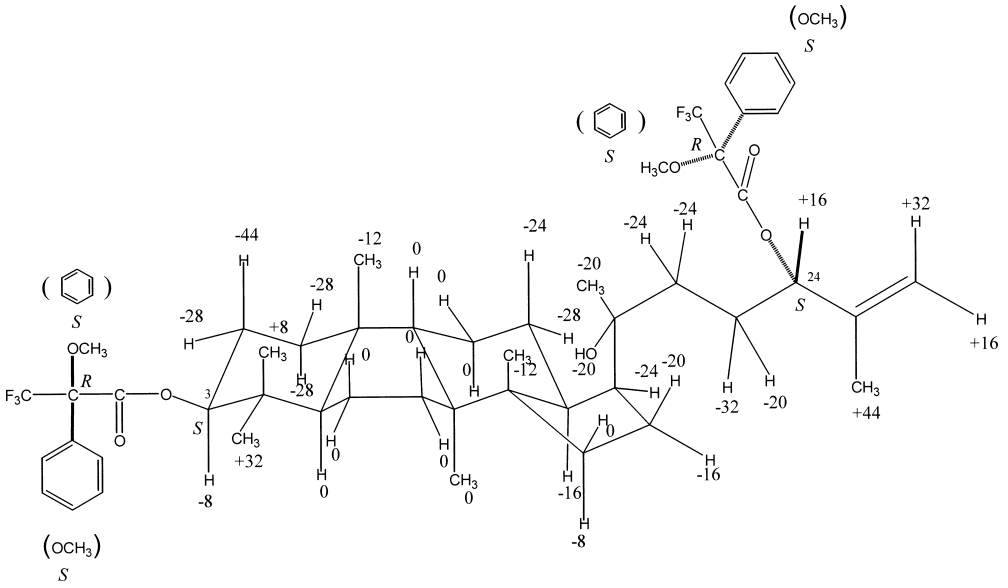
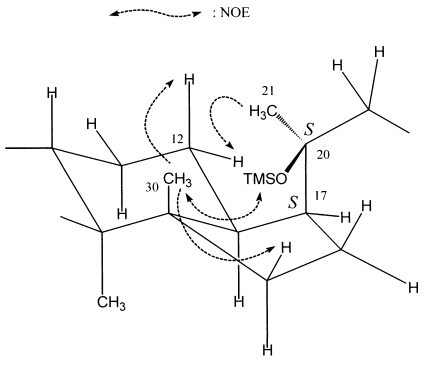


Fig. 8. Chemical Shift Differences between **5b** and **5c**

$\Delta\delta$ values ($\Delta\delta=\delta_{(S)\text{-MTPA(5b)}}-\delta_{(R)\text{-MTPA(5c)}}$) are shown in Hz.



5c

Fig. 9

To determine the absolute configuration at C-17 and 20, **5b** was converted into the 20-TMS ester derivative (**5c**), and detailed NOE experiments were carried out. As a result, NOEs were observed as shown in Fig. 9, demonstrating the absolute stereochemistry of 17*S* and 20*S*, respectively. Based on these data, the structure of leucastrin B (**5**) was concluded to be (3*S*,17*S*,20*S*,24*S*)-3,20,24-trihydroxyprotost-25-en.

Experimental

General Procedures All melting points were determined on a Yanagimoto micromelting point apparatus and were uncorrected. NMR spectra were taken in $CDCl_3$ on a JEOL GSX-400 spectrometer (^{1}H-NMR at 400 MHz and ^{13}C-NMR at 100 MHz), using TMS as an internal standard, and chemical shifts are given in δ (ppm). EI-MS and FAB-MS (positive ion mode; matrix, magic bullet) spectra were recorded on a JEOL JMS-SX-102A mass spectrometer and major peaks are indicated as *m/z*. IR spectra were recorded in KBr disks on a Hitachi 270-30 infrared spectrophotometer and data are given in cm^{-1}. Optical rotation was measured by a JASCO DIP-370 digital polarimeter. UV spectra were taken on a Shimadzu dual-wavelength/double beam recording spectrophotometer. CD spectra were recorded in MeOH on a JASCO J-720 CD dispersion spectrometer. For column chromatography, silica gel (Wako-gel C-300) and ODS (Cosmosil 140 C_{18}-OPN) were used. HPLC was performed on a Tosoh CCPS pump system with a Tosoh UV-8020 UV detector. Preparative HPLC was performed on a TSKgel ODS-80T_M column (30 i.d.×215 mm). TLC was carried out on Kieselgel 60F_{254} (Merk) with the following solvent systems: benzene–EtOAc (2 : 1, TLC-1), $CHCl_3$–MeOH–H_2O (25 : 4 : 1, TLC-2), $CHCl_3$–MeOH–H_2O–HCOOH (100 : 10 : 1 : 1, TLC-3), and *n*-hexane–acetone (2 : 1, TLC-4). Spots were detected by spraying dil. H_2SO_4 followed by heating.

Plant Material The plant material was purchased at the market, Kilagal Vaidya Pasal, in Kathmandu, Nepal, in 1995. The botanical identification was made by Dr. N. P. Manandhar, National Herbarium and Plant Laboratories, Godawari, Nepal. A voucher specimen is deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Hokuriku University, Kanazawa, Japan.

Extraction and Separation The dried whole herbs (3 kg) of *Leucas cephalotes* were extracted three times with MeOH under reflux. The MeOH extract was concentrated to dryness under reduced pressure, and the resulting residue (120 g) was defated with *n*-hexane. The insoluble part (114 g) was partitioned between 1-butanol and water. The 1-butanol layer was concentrated to dryness under reduced pressure, and the residue (104 g) was extracted with an ether. The ether soluble fraction was concentrated, and the residue (54 g) was chromatographed on silica gel (3 kg) and eluted with a gradient of $CHCl_3$–MeOH–H_2O (100 : 0 : 1→90 : 10 : 1) to give four fractions (frs. 1—4), in order of elution. Fr. 1 was subjected to a silica gel column chromatography using a gradient of *n*-hexane–acetone (6 : 1→1 : 2) to give **4** (48 mg), **1** (190 mg), **6** (6 mg), **12** (6 mg), **13** (6 mg), **15** (8 mg), and a mixture of three components, which were rechromatographed on silica gel with a gradient of benzene–EtOAc (10 : 1→3 : 1) to give **2** (24 mg), **5** (20 mg), and **14** (12 mg), respectively. Frs. 2 and 3 were found to be mixtures of steroid by NMR data, both of which were subjected to preparative HPLC (solv., CH_3CN) to give **7** (18 mg) and **8** (16 mg), and **9** (24 mg) and **10** (10 mg), respectively. Fr. 4 was chromatographed on a silica gel with a gradient of *n*-hexane–EtOAc (4 : 1→2 : 1) to give a mixure of steroid, which was separated by preparative HPLC (solv., CH_3CN), affording β-sitosterol[7] (20 mg) and **11** (16 mg).

The insoluble fraction into ether was passed through a silica gel column, using a gradient of $CHCl_3$–MeOH–H_2O (100 : 10 : 1→100 : 50 : 3) to give **17** (180 mg), **18** (10 mg), **19** (10 mg), respectively, and mixture of **16** and **3**, which was purified by an ODS column chromatography using a gradient of H_2O–MeOH (7 : 1→1 : 2) to give **16** (6 mg) and **3** (16 mg).

Identification of 6—19 Compounds (**6**—**19**) were identified as oleanolic acid (**6**),[8] 7-oxositosterol (**7**),[9,10] 7-oxostigmasterol (**8**),[11] 7α-hydroxysitosterol (**9**),[9] 7α-hydroxystigmasterol (**10**),[12] stigmasterol (**11**),[13] 5-hydroxy-7,4′-dimethoxyflavone (**12**),[14] pillion (**13**),[15] gonzalitosin I (**14**),[16] tricin (**15**),[17] cosmosin (**16**),[18] apigenin 7-*O*-β-D-(6-*O*-*p*-coumaroyl)glucopyranoside (**17**),[19] anisofolin A (**18**),[20] and luteolin 4′-*O*-β-D-glucuronopyranoside (**19**),[21—23] respectively, by direct comparison with authentic samples (**6**, **11**, **12**, **15**) or of the respective spectral and chemical data with those described in the literatures (**7**—**10**, **13**, **14**, **16**—**19**).

Leucasdin A (1) [(3*S*,6*R*,8*R*,9*R*,13*S*,16*S*)-9,13,15,16-Bisepoxy-3,16-diacetoxy-6-formyloxylabdane] Colorless needles, mp 167—168 °C (dec.), $C_{25}H_{38}O_8$. $[\alpha]_D^{25}$ −33.4° (*c*=0.33, MeOH). *Rf*: 0.56 (TLC-1), 0.78 (TLC-3). HR-MS *m/z*: 466.2563, Calcd for $C_{25}H_{38}O_8$ $[M]^+$ 466.2567. HR-FAB-MS *m/z*: 489.2466, Calcd for $C_{25}H_{38}O_{81}Na$ $[M+Na]^+$ 489.2464. EI-MS *m/z* (%): 466 $[M]^+$ (11), 420 (100). FAB-MS *m/z* (%): 489 $[M+Na]^+$ (44), 505 $[M+K]^+$ (2). IR ν_{max} (KBr) cm^{-1}: 1740, 1724, 1248, 1240 (ester). ^{1}H-NMR: Table 1. ^{13}C-NMR: Table 2.

Alkaline-Methanolysis of 1 A solution of **1** (30 mg) in 3% NaOMe/MeOH (2 ml) was allowed to stand for 24 h at 60 °C. After cooling, the reaction mixture was neutralized with 0.1 M HCl and partitioned between EtOAc and H_2O. The EtOAc soluble fraction was evaporated and the residue was partitioned between *n*-hexane–benzene/MeOH–H_2O (1 : 1.5/1 : 0.5) to give a deacylated compound (**1a**, 20 mg) from the MeOH–H_2O layer, which was dissolved in AcCl/MeOH (20 : 1, 1 ml) and left to stand for 48 h at room temperature. A reaction mixture was poured into ice-water and then extracted with EtOAc. The EtOAc phase was washed with water, and then evaporated. The residue (18 mg) was a mixture of two compounds, which were separated by preparative TLC (TLC-4) followed by a silica gel column chromatography eluting with EtOAc to give **1b** (7.9 mg) and **1c** (8.5 mg).

(16*R*)-Methyl Ether (1b) of 1a White amorphous powder, $[\alpha]_D^{25}$ +12.1° (*c*=0.04, MeOH). EI-MS *m/z* (%): 368 $[M]^+$ (6), 198 (100). IR ν_{max} (KBr) cm^{-1}: 3436 (OH). ^{1}H-NMR: Table 1. ^{13}C-NMR: Table 2.

(16*S*)-Methyl Ether (1c) of 1a White amorphous powder, $[\alpha]_D^{25}$ +57.8° (*c*=0.03, MeOH). EI-MS *m/z* (%): 368 $[M]^+$ (4), 198 (100). IR ν_{max} (KBr) cm^{-1}: 3436 (OH). ^{1}H-NMR: Table 1. ^{13}C-NMR: Table 2.

3,6-Di-*O*-*p*-bromobenzoate (1d) of 1b To a solution of **1b** (7.1 mg) in pyridine (1.5 ml) was added successively DMAP (3.5 mg) and *p*-bromobenzoyl chloride (80 mg) and the mixture was allowed to stand at 80 °C for 24 h. A reaction mixture was poured into ice-water and then extracted with EtOAc. The EtOAc phase was washed successively with 0.1 M HCl, saturated $NaHCO_3$ and water, and then evaporated. The residue was chromatographed over silica gel and eluted with $CHCl_3$ to give the 3,6-di-*O*-*p*-bromobenzoate (**1d**, 7.3 mg) as a white amorphous powder. $[\alpha]_D^{25}$ −77.6° (*c*=0.35, $CHCl_3$). EI-MS *m/z* (%): 734 $[M]^+$ (1), 534 (100). FAB-MS *m/z* (%): 757 $[M+Na]^+$ (1). IR ν_{max} (KBr) cm^{-1}: 1720, 1276 (ester), 1592 (arom. C=C), 1171, 1100. UV λ_{max} (MeOH) nm (log ε): 244 (4.56). CD (*c*=1.81×10^{-4}, MeOH) $[\theta]^{25}$ (nm): +59860 (236) (positive maximum), −130500 (253) (negative maximum). ^{1}H-NMR: Table 1. ^{13}C-NMR: Table 2.

3,6-Di-*O*-*p*-bromobenzoate (1e) of 1c To a solution of **1c** (7.8 mg) in pyridine (1.5 ml) was worked up in the same way as **1b** to give the 3,6-di-*O*-*p*-bromobenzoate (**1e**, 8.1 mg) as a white amorphous powder. $[\alpha]_D^{25}$ −29.9° (*c*=0.32, $CHCl_3$). EI-MS *m/z* (%): 734 $[M]^+$ (1), 534 (100). FAB-MS *m/z* (%): 757 $[M+Na]^+$ (1). IR ν_{max} (KBr) cm^{-1}: 1720, 1276 (ester), 1592 (arom. C=C), 1114, 1102. UV λ_{max} (MeOH) nm (log ε): 244 (4.55). CD (*c*=1.79×10^{-4}, MeOH) $[\theta]^{25}$ (nm): +40970 (236) (positive maximum), −80390 (253) (negative maximum). ^{1}H-NMR: Table 1. ^{13}C-NMR: Table 2.

Leucasdin B (2) [(3*S*,6*R*)-3-Acetoxy-6-formyloxy-isoambreinolide] Colorless needles, mp 230—231 °C (dec.), $C_{20}H_{30}O_6$. $[\alpha]_D^{25}$ −13.6° (*c*=0.28, MeOH). *Rf*: 0.46 (TLC-1), 0.61 (TLC-3). HR-MS *m/z*: 366.2037, Calcd for $C_{20}H_{30}O_6$ $[M]^+$ 366.2043. EI-MS *m/z* (%): 366 $[M]^+$ (31), 260 (85), 146 (100). FAB-MS *m/z* (%): 389 $[M+Na]^+$ (54). IR ν_{max} (KBr) cm^{-1}: 1772, 1170 (γ-lactone); 1720, 1248 (ester). ^{1}H-NMR: Table 1. ^{13}C-NMR: Table 2.

Deacyl Derivative (2a) of 2 A solution of **2** (4 mg) in 3% NaOMe/MeOH (0.5 ml) was worked up in the same way as **1** to give a deacylated compound (**2a**, 3.4 mg) as a white amorphous powder. $[\alpha]_D^{25}$ −19.7° (*c*=0.05, MeOH). EI-MS *m/z* (%): 296 $[M]^+$ (49), 153 (86), 123 (100). IR

ν_{max} (KBr) cm^{-1}: 3428 (OH), 1752, 1222 (γ-lactone). ^{1}H-NMR: Table 1. ^{13}C-NMR: Table 2.

3,6-Di-*O*-*p*-bromobenzoate (2b) of 2a To a solution of **2a** (3.1 mg) in pyridine (0.8 ml) was worked up in the same way as **1b** to give the 3,6-di-*O*-*p*-bromobenzoate (**2b**, 3.3 mg) as a white amorphous powder. $[\alpha]_D^{25}$ −23.9° (c=0.03, $CHCl_3$). EI-MS m/z (%): 662 $[M]^+$ (1), 260 (100). FAB-MS m/z (%): 685 $[M+Na]^+$ (1). IR ν_{max} (KBr) cm^{-1}: 1774, 1218 (γ-lactone); 1720, 1272 (ester), 1590 (arom. C=C), 1112, 1102. UV λ_{max} (MeOH) nm (log ε): 244 (4.54). CD (c=1.13×10^{-4}, MeOH) $[\theta]^{25}$ (nm): +32710 (236) (positive maximum), −66170 (253) (negative maximum). ^{1}H-NMR: Table 1. ^{13}C-NMR: Table 2.

Leucasdin C (3) [(4*R*,9*S*,12*R*,13*R*)-12,13-Dihydroxyabiet-7-en-18-oic Acid] Colorless needles, mp 284—285 °C (dec.), $C_{20}H_{32}O_4$, $[\alpha]_D^{25}$ +26.8° (c=0.09, MeOH). *Rf*: 0.30 (TLC-3), 0.37 (TLC-4). HR-FAB-MS m/z: 359.2198, Calcd for $C_{20}H_{32}O_4Na$ $[M+Na]^+$ 359.2198. FAB-MS m/z (%): 359 $[M+Na]^+$ (19). IR ν_{max} (KBr) cm^{-1}: 3352 (OH), 1690 (CO). ^{1}H-NMR: Table 3. ^{13}C-NMR: Table 2.

Methyl Ester Derivative (3a) of 3 **3** (8 mg) was dissolved in MeOH (10 ml), mixed with an ethereal solution (20 ml) of diazomethane and left to stand for 2 h at room temperature. The reaction mixture was evaporated, and the residue was chromatographed over silica gel and eluted with $CHCl_3$ to give a methyl ester (**3a**, 4.5 mg) as a white amorphous powder. $[\alpha]_D^{25}$ +18.9° (c=0.12, MeOH). EI-MS m/z (%): 350 $[M]^+$ (17), 289 (100). IR ν_{max} (KBr) cm^{-1}: 3428 (OH), 1746, 1250 (ester). ^{1}H-NMR: Table 3. ^{13}C-NMR: Table 2.

12-*O*-(*S*)- and (*R*)-MTPA Ester Derivatives (3b, 3c, Respectively) of 3a To a solution of **3a** (2 mg) in CH_2Cl_2 (1 ml) was added successively DMAP (1 mg), Et_3N (0.2 ml) and (−)-MTPA chloride (7 mg) and the mixture was allowed to stand at room temperature for 24 h. A reaction mixture was poured into ice-water and then extracted with EtOAc. The EtOAc phase was washed successively with 0.1 M HCl, saturated $NaHCO_3$ and water, and then evaporated. The residue was chromatographed over silica gel and eluted with $CHCl_3$ to give **3b** (1.7 mg). In the same manner as for **3b**, **3c** (1.8 mg) was obtained from **3a** (2 mg). Compound **3b**, white amorphous powder, $[\alpha]_D^{25}$ +59.7° (c=0.07, MeOH). FAB-MS m/z (%): 567 $[M+1]^+$ (14), 589 $[M+Na]^+$ (15). IR ν_{max} (KBr) cm^{-1}: 1746, 1250 (ester). ^{1}H-NMR: Table 3. Compound **3c**, white amorphous powder, $[\alpha]_D^{25}$ +59.5° (c=0.07, MeOH). FAB-MS m/z (%): 567 $[M+1]^+$ (4), 589 $[M+Na]^+$ (4). IR ν_{max} (KBr) cm^{-1}: 1746, 1250 (ester). ^{1}H-NMR: Table 3.

Leucastrin A (4) [(3*S*,17*S*,20*S*,24*S*)-3,20-Dihydroxy-24-methylprotost-25-en] Colorless needles, mp 176—177 °C (dec.), $C_{31}H_{54}O_2$, $[\alpha]_D^{25}$ +36.3° (c=0.36, MeOH). *Rf*: 0.52 (TLC-1), 0.69 (TLC-3). HR-MS m/z: 458.4123, Calcd for $C_{31}H_{54}O_2$ $[M]^+$ 458.4124. EI-MS m/z (%): 458 $[M]^+$ (27), 318 (100), 206 (75). FAB-MS m/z (%): 481 $[M+Na]^+$ (1). IR ν_{max} (KBr) cm^{-1}: 3676 (OH). ^{1}H-NMR: Table 4. ^{13}C-NMR: Table 5.

3,20-Ditrimethylsilyl Ether Derivative (4a) of 4 To a solution of **4** (6 mg) in pyridine (1 ml) was added hexamethyldisilazane (1.2 ml). The reaction mixture was left to stand at room temperature for 3 h and evaporated. A residue was passed through a silica gel column (solv.: *n*-hexane–EtOAc, 4 : 1) to give a TMS derivative (**4a**, 5.0 mg) as a white amorphous powder. $[\alpha]_D^{25}$ +29.1° (c=0.27, $CHCl_3$). HR-FAB-MS m/z: 577.4274, Calcd for $C_{37}H_{61}OSi_2$ 577.4287. FAB-MS m/z (%): 577 $C_{37}H_{61}OSi_2$ (1). IR ν_{max} (KBr) cm^{-1}: 1250 ($SiMe_3$), 1108, 1084, 1062 (SiO). ^{1}H-NMR: Table 4. ^{13}C-NMR: Table 5.

3-*O*-(*S*)- and (*R*)-MTPA Ester Derivatives (4b, 4c, Respectively) of 4 **4b** (4.5 mg) and **4c** (4.6 mg) were obtained from **4** (both 5 mg) in the same manner as for **3b**. Compound **4b**, white amorphous powder, $[\alpha]_D^{25}$ +14.7° (c=0.15, $CHCl_3$). FAB-MS m/z (%): 697 $[M+Na]^+$ (1), 713 $[M+Na]^+$ (1). IR ν_{max} (KBr) cm^{-1}: 1742, 1274 (ester). ^{1}H-NMR: Table 4. Compound **4c**, white amorphous powder, $[\alpha]_D^{25}$ +43.8° (c=0.14, $CHCl_3$). FAB-MS m/z (%): 697 $[M+Na]^+$ (1), 713 $[M+Na]^+$ (1). IR ν_{max} (KBr) cm^{-1}: 1742, 1274 (ester). ^{1}H-NMR: Table 4.

Leucastrin B (5) [(3*S*,17*S*,20*S*,24*S*)-3,20,24-Trihydroxyprotost-25-en] Colorless needles, mp 196—197 °C (dec.), $C_{30}H_{52}O_3$, $[\alpha]_D^{25}$ +71.8° (c=0.04, MeOH). *Rf*: 0.22 (TLC-1), 0.40 (TLC-3). HR-FAB-MS m/z: 483.3830, Calcd for $C_{30}H_{52}O_3Na$ $[M+Na]^+$ 483.3814. FAB-MS m/z (%): 483 $[M+Na]^+$ (1). IR ν_{max} (KBr) cm^{-1}: 3676 (OH). ^{1}H-NMR: Table 4. ^{13}C-NMR: Table 5.

3,24-Di-*O*-(*S*)- and (*R*)-MTPA Ester Derivatives (5a, 5b, Respectively) of 5 **5a** (3.6 mg) and **5b** (3.5 mg) were obtained from **5** (both 4 mg) in the same manner as for **3b**. Compound **5a**, white amorphous powder, $[\alpha]_D^{25}$ +17.1° (c=0.05, MeOH). FAB-MS m/z (%): 915 $[M+Na]^+$ (1). IR ν_{max} (KBr) cm^{-1}: 1750, 1272, 1258 (ester). ^{1}H-NMR: Table 4. Compound **5b**, white amorphous powder, $[\alpha]_D^{25}$ +53.8° (c=0.08, MeOH). FAB-MS m/z (%): 915 $[M+Na]^+$ (1). IR ν_{max} (KBr) cm^{-1}: 1750, 1272, 1258 (ester). ^{1}H-NMR: Table 4.

20-Trimethylsilyl Ether Derivative (5c) of 5b **5c** (2.1 mg) was obtained from **5b** (3 mg) in the same manner as for **4** as white amorphous powder. $[\alpha]_D^{25}$ +11.5° (c=0.34, $CHCl_3$). FAB-MS m/z (%): 965 $[M+1]^+$ (1), 987 $[M+Na]^+$ (1). IR ν_{max} (KBr) cm^{-1}: 1750, 1272, 1258 (ester), 1250 ($SiMe_3$). ^{1}H-NMR: Table 4.

Acknowledgments We are grateful to Dr. N. P. Manandhar, National Herbarium and Plant Laboratories, Ministry of Forests, His Majesty's Government of Nepal, for his botanical identification of the crude drug. Thanks are also due the staff of the analytical center of Hokuriku University for MS measurements.

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