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TRITERPENE DIENOLS AND OTHER CONSTITUENTS FROM THE BARK OF *PHYLLANTHUS FLEXUOSUS*

REIKO TANAKA and SHUNYO MATSUNAGA*

Osaka University of Pharmaceutical Sciences, 2-10-65 Kawai, Matsubara, Osaka 580, Japan

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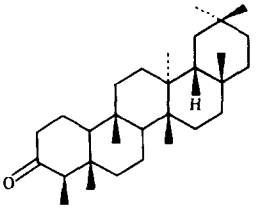
Key Word Index—*Phyllanthus flexuosus*; Euphorbiaceae; stem bark; *n*-alkane derivatives; *ent*-3β-hydroxykaur-16-ene; triterpenes; oleanadienols; phytosterols; bergenin.

Abstract—Besides four known triterpenes and bergenin, *ent*-3β-hydroxykaur-16-ene and two oleanadienols were isolated from the stem bark of *Phyllanthus flexuosus*. The bark also contained *n*-alkanes, *n*-alkanols and phytosterols.

INTRODUCTION

The *Phyllanthus* genus contains species which have useful medicinal applications [1]. A considerable number of these species have been examined and some effective constituents have been reported. In particular, the isolation of the antineoplastic bisabolene glycosides phyllanthoside [2] and phyllanthostatins 1, 2 and 3 [1, 3] from *P. accuminatus* attracted our attention.

Phyllanthus flexuosus (Sieb. et Zucc.) Muell.-Arg. is a common shrub of the Central Japan–Himalayan geographic region [4]. Up to now, nothing has been reported about the chemistry or biological activity of this plant. We have shown that the ether extract of the stem bark of *P. flexuosus* contains, in order of increasing polarity, a mixture of *n*-alkanes, friedelin (**1**), lupa-1,20(29)-dien-3-one (glochidone) (**2**), β-amyrin (**3a**), two unidentified triterpene dienols **4a** and **5a**, lupeol (**6**), a mixture of *n*-alkanols, an unknown diterpene alcohol (**7a**) and a mixture of phytosterols (**8**). Bergenin (**9**) was isolated from the methanol extract of the stem bark in good yield. This paper deals with the characterization of compounds **4a**, **5a** and **7a** and also reports on the composition of the mixtures of alkanes, alkanols and phytosterols.



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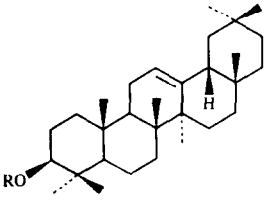
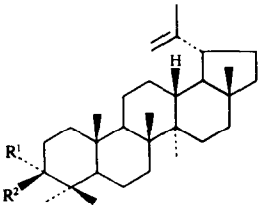


3a R = H
3b R = Ac



2 $R^1 = R^2$ = O, 1-ene
6a R^1 = H, R^2 = OH
6b R^1 = H, R^2 = OAc

RESULTS AND DISCUSSION

The alkane mixture was a minor component of the ether extract, whilst the alkanol mixture was a major one next to phytosterols. The mass and ^{1}H NMR spectra of both mixtures showed signals characteristic for *n*-alkanes. Detailed GC analyses [5] of the mixtures gave the results listed in Table 1. The alkane mixture consisted of a homologous series of *n*-alkanes with chain lengths ranging from C_{23} to C_{36}, with nonacosane, triacontane, hentriacontane and tritriacontane as the main components. The alkanol mixture consisted of a homologous series of *n*-alkanols with chain length ranging from C_{20} to C_{30} with 1-tetracosanol, 1-hexacosanol and 1-octacosanol as main components.

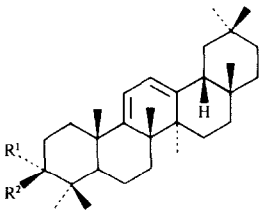
The composition of the mixture of phytosterols was determined by GC and GC/MS techniques [6]. The major component was sitosterol (72.1%) which was accompanied by campesterol (12.2 %) and stigmasterol (15.7%) [see Experimental].

The known compounds, **1**, **3a**, **6** and **9** were identified by direct comparison with authentic samples and **2** was identified on the basis of the close agreement of its physical and spectral data with those already published.

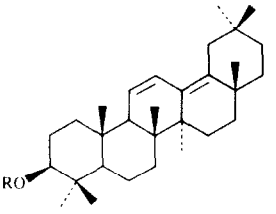
Compounds **4a** and **5a** showed UV absorptions of a homoannular diene and a heteroannular diene, respectively, despite having the same molecular formula of $C_{30}H_{48}O$.

Compound **4a** was purified as its acetate (**4b**), since it was hardly separable from **3a**. Alkaline hydrolysis of **4b** furnished **4a**, which was oxidized with chromic oxide–pyridine to give the ketone **4c**. The EIMS of **4a**–**4c** exhibited two prominent peaks at *m/z* 255 $[C_{19}H_{27}]^+$ and *m/z* 271 $[C_{19}H_{27}O]^+$ (4a) or 313 (**4b**) or 269 (**4c**), typical of $\Delta^{9(11):12}$-triterpenes [7]. The above results,

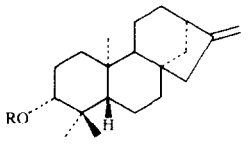
*Author to whom correspondence should be addressed.



4a R^1 = H, R^2 = OH
4b R^1 = H, R^2 = OAc
4c R^1 = R^2 = O



5a R = H
5b R = Ac



7a R = H
7b R = Ac

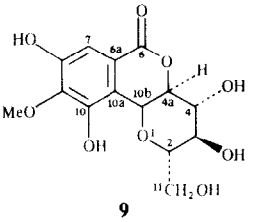


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Table 1. Compositions of *n*-alkane and *n*-alkanol mixtures of *P. flexuosus*

	Composition (%)	
C_x	*n*-Alkane	*n*-Alkanol
20	—	0.16
21	—	+
22	—	4.81
23	0.17	0.64
24	0.18	38.65
25	1.67	2.15
26	1.44	23.08
27	3.98	1.67
28	4.21	28.84
29	27.78	+
30	8.44	+
31	32.20	—
32	5.15	—
33	8.69	—
34	2.05	—
35	5.14	—
36	0.37	—

+: Minor component less than 0.1%.

along with the ^{1}H NMR and ^{13}C NMR data (Tables 2 and 3), indicated that **4a** was oleana-9(11):12-dien-3β-ol, which was identified by direct comparison of **4b** with an authentic sample of **4b** prepared from β-amyrin acetate **(3b)** [8–10]. This is the first report of the isolation of **4a** from natural sources, though **4b** and **4c** have recently been obtained from *Ferula linkii* (Umberiferae) [11] and *Vellozia compacta* (Velloziaceae) [12], respectively. The published physical and spectral data of **4a** and **4b** were very similar to those of our samples except for some inconsistency in the ^{1}H NMR data.

Compound **5a** was isolated as a minor component. Its ^{1}H NMR spectrum (Table 2) displayed signals for a C-3 (ax) carbinolic methine proton and two vinylic protons on the transoid diene as ABX types at δ5.52 and 6.38. These facts, together with the signal pattern of the ^{13}C NMR spectrum (Table 3), indicated **5a** to be oleana-11:13(18)-dien-3β-ol, which was proved by direct comparison of its acetate **(5b)** with a sample of **5b** prepared from β-amyrin acetate **(3a)** [13]. This is the first report of the isolation of **5a** from natural sources, though **5b** has been obtained from the rhizomes of *Polypodium niponicum* and *P. formosana* (Polypodiaceae) [14].

Compound **7a** was a tetracyclic diterpene alcohol with an exocyclic double bond isolated as a minor component next to **5a**. Acetylation gave the acetate **7b**. The ^{1}H and ^{13}C NMR data (Tables 2 and 3) suggested that **7a** must be *ent*-3β-hydroxykaur-16-ene, which had not been isolated from natural sources, though it had previously been prepared from the following natural products, *ent*-3β,16,17-trihydroxykaurane [15], abbeocutone [16] and linearol [17]. Finally, **7a** was identified by comparison of the ^{1}H NMR spectrum of **7b** with that of an authentic **7b** [17].

EXPERIMENTAL

General procedures. Mps: uncorr; optical rotations: $CHCl_3$; UV: EtOH; IR: KBr discs. ^{1}H NMR (90 or 300 MHz) and ^{13}C NMR (75.4 or 22.6 MHz): $CDCl_3$ with TMS as int. std. unless otherwise noted MS: 70 eV. CC: silica gel 60 and alumina 90 (70–230 mesh, Merck). TLC: silica gel HF_{254} and PF_{254} (Merck). HPLC was performed with a TOSOH CCPM-UV8000 instrument equipped with a stainless column (15 cm × 4.6 mm i.d.) packed with TSK-gel ODS-120T. GC experiments were performed with a glass column (2 m × 2 mm i.d.) packed with 1.5% SE-30 and 1.5% OV-17 using N_2 gas and the following temp conditions: (A) isothermal 250°, injector and FID temps 280°; (B) isothermal 240°, injector and FID temps 270°. GC/MS (EI, 70 eV) analysis was carried out on a glass column (1 m × 2 mm i.d.) packed with 1.5% SE-30 employing the following conditions: He 20 ml/min; isothermal 240°; injector and separator temps 270°.

Plant materials. P. flexuosus was collected on 31 July 1983, in a valley of the Sekigahara district (Gifu Pref., Japan) and was identified by Mr G. Murata of the Department of Botany, Faculty of Science, Kyoto University. A voucher specimen has been deposited at the Herbarium of the laboratory of Medicinal Chemistry, Osaka University of Pharmaceutical Sciences.

Extraction and isolation of constituents. The dried and finely chopped stem bark (3.75 kg) was extracted with Et_2O (10 l) in an automatic glass percolator at 35° for 1 week. The Et_2O extract was concd (*ca* 31) and the soln was washed with 5% Na_2CO_3, 5%NaOH and 5% HCl to remove the acidic and basic components. Neutralization followed by removal of the solvent from the Et_2O layer gave a residue (28.4 g), which was divided into seven portions by CC on silica gel (1.5 kg) using *n*-hexane, *n*-hexane–C_6H_6 mixtures (5:1, 3:1, 2:1, 1:1), C_6H_6 and C_6H_6–$CHCl_3$ (10:1) as eluants. Evaporation of each solvent afforded residues I (*n*-alkane mixture, 51 mg) II (48 mg), III (178 mg), IV (699 mg), V (3.731 g), VI (5 mg) and VII (1.203 g). Recrystallization of residues III (EtOAc) and VII (MeOH–$CHCl_3$) yielded **1** (155 mg) and **8** (1.080 g), respectively. Residue IV on repeated CC ($AgNO_3$–silica gel 1:4, 50 g; *n*-hexane–C_6H_6 5:1) afforded **2** (663 mg). Residue V was recrystallized from C_6H_6 to give the *n*-alkanol mixture (1.446 g). Removal of C_6H_6 from the filtrate furnished a solid (2.282 g), which was separated by CC ($AgNO_3$–silica gel 1:4, 230 g) into four

Table 2. 300 MHz ^{1}H NMR spectral data of compounds **4a**, **4b**, **4c**, **5a**, **5b**, **7a** and **7b**

	4a	**4b**	**4c**	**5a**	**5b**	**7a**	**7b**
Me	0.81	0.875	0.875	0.71	0.71	0.78	0.852
	0.888	0.885(×2)	0.896	0.75	0.75	0.98	0.856
	0.899	0.897	0.902	0.78	0.86	1.02	1.05
	0.99	0.906	1.009	0.89	0.87		
	1.04	0.98	1.08	0.96(×2)	0.92		
	1.14	1.13	1.12	0.99	0.95		
	1.19	1.21	1.17	1.06	0.96		
	1.25		1.26		1.06		
H-3	3.24 *dd*	4.51 *dd*		3.25 *dd*	4.52 *dd*	3.12 *dd*	4.47 *dd*
	J = 11.5, 6	J = 11.5, 6		J = 11.5, 6	J = 11.5, 6	J = 10.8, 5.5	J = 10.8, 5.5
H-11	5.58 *d*	5.56 *d*	5.63 *d*	6.38 *dd*	6.38 *dd*		
	J = 6	J = 6	J = 6	J = 11.8, 3.3	J = 11.8, 3.3		
H-12	5.53 *d*	5.48 *d*	5.51 *d*	5.52 *dd*	5.51 *dd*		
	J = 6	J = 6	J = 6	J = 11.8, 1.6	J = 11.8, 1.6		
						4.73 *s*	4.74 *s*
H-17						4.80 dif *t*	4.80 dif *t*
						J = 1.5	J = 1.5
$MeCO_2$		2.06 *s*			2.05 *s*		2.05 *s*

Table 3. 75.4 MHz ^{13}C NMR spectral data for compounds **4a**, **4b**, **5a**, **5b**, **7a** and **7b**

C	**4a**	**4b**	**5a**	**5b**	**7a**	**7b**
1	38.78	37.06	37.99	37.68	39.74	39.75
2	27.87	24.25	27.08	23.46	24.41	23.65
3	78.59	80.59	78.90	80.93	79.07	80.98
4	38.92	37.88	38.92	37.85	38.83	37.78
5	51.17	51.23	54.20	54.13	55.18	55.27
6	18.37	18.27	18.40	18.29	20.02	19.86
7	32.18	32.09	32.37	32.30	41.19	41.05
8	37.04	38.64	40.21	40.24	44.00	44.02
9	154.32	153.96	54.84	54.96	55.89	55.74
10	40.66	40.66	36.67	36.58	39.03	38.94
11	115.77	115.91	125.27	125.12	18.27	18.29
12	120.75	120.69	125.75	125.83	33.24	33.21
13	147.13	147.24	138.14	138.25	43.95	43.86
14	42.80	42.74	42.35	42.35	38.69	38.36
15	25.69	25.63	35.27	35.27	49.01	48.98
16	27.26	27.20	24.42	24.42	155.84	155.70
17	32.18	32.15	34.65	34.65	103.01	103.10
18	45.61	45.58	133.36	133.31	28.38	28.36
19	46.93	46.81	38.92	38.92	15.48	16.64
20	31.14	31.11	33.08	33.07	17.59	17.65
21	34.65	34.62	36.13	36.14		
22	37.18	36.84	37.99	37.99		
23	28.27	28.72	27.84	27.82		
24	15.06	16.72	15.06	16.18		
25	20.09	20.01	16.58	16.57		
26	20.99	21.02	17.93	17.98		
27	25.29	25.29	20.24	20.17		
28	28.75	28.16	25.34	25.38		
29	23.72	23.72	24.11	24.08		
30	33.21	33.19	32.45	32.46		
$MeCO_2$		21.33		21.33		21.30
$MeCO_2$		171.02		170.98		171.00

portions (A: 267 mg, B: 391 mg, C: 378 mg, D: 26 mg) by eluting with *n*-hexane–C_6H_6 [5:1 (A–B), 3:1 (C), 2:1 (D)]. Acetylation of residue A (Ac_2O–C_5H_5N 1:1, 16 mg) at room temp for 24 hr, followed by CC ($AgNO_3$–Al_2O_3, 1:4, 15 g; *n*-hexane–C_6H_6, 20:1 and 10:1) of the product furnished both **3b** (95 mg) and **4b** (191 mg). Residue B on repeated CC ($AgNO_3$–Al_2O_3 1:4, 25 g; *n*-hexane–C_6H_6 5:1 and 3:1) afforded **5a** (15 mg) and **6** (376 mg). Residue C on recrystallization (MeOH–$CHCl_3$) yielded **6** (351 mg). Prep. TLC of residue D [2 mm layer, 20 × 20 cm; *n*-hexane–$CHCl_3$ (3:1); UV light; eluant: $CHCl_3$] yielded **7a** (21 mg). The stem bark was further extracted with boiling MeOH (10 l) for 5 days. Concn (*ca* 1 l) and refrigeration of the MeOH extract gave a crystalline mass, which was collected and recrystallized (EtOH) to give **9** (82.5 g).

n-*Alkanes*. Powder, mp 53–59° (EtOAc); IR ν_{max} cm^{-1}: 2950, 2910, 2840, 1470, 1450, 1375, 723, 712; EIMS *m/z* (rel. int.): 492 [M^+, $C_{35}H_{72}$] (0.2), 464 [M^+, $C_{33}H_{68}$] (0.4), 450 [M^+, $C_{32}H_{66}$] (0.2), 436 [M^+, $C_{31}H_{64}$] (0.9), 422 [M^+, $C_{30}H_{62}$] (0.4), 408 [M^+, $C_{29}H_{60}$] (0.8), 394 [M^+, $C_{28}H_{58}$] (0.2), 380 [M^+, $C_{27}H_{56}$] (0.2), 209 (5), 195 (5), 181 (5), 167 (8), 153 (10), 139 (12), 125 (20), 111 (31), 97 (59), 85 (36), 83 (63), 71 (81), 69 (60), 57 (100), 55 (76), 43 (82), 41 (42). For GC analysis, C_{28}, C_{32} and C_{36} paraffins were employed as authentic standards.

n-*Alkanols*. Powder, mp 76–80°; IR: ν_{max} cm^{-1}: 3400, 2970, 2925, 2855, 1480, 1470, 735, 722; EIMS *m/z* (rel. int.): 392 [$M^+ - H_2O$, $C_{28}H_{56}$] (1.5), 378 [$M^+ - H_2O$, $C_{27}H_{54}$] (0.1), 364 [$M^+ - H_2O$, $C_{26}H_{52}$] (1.3), 350 [$M^+ - H_2O$, $C_{25}H_{50}$] (0.2), 336 [$M^+ - H_2O$, $C_{24}H_{48}$] (2), 308 [$M^+ - H_2O$, $C_{22}H_{44}$] (0.3), 195 (3), 181 (4), 167 (5), 153 (7), 139 (10), 125 (18), 111 (30), 97 (57), 85 (34), 83 (62), 71 (56), 69 (56), 57 (100), 55 (76), 43 (81), 41 (41). For GC analysis of the mixture, 1-docosanol, 1-hexacosanol and 1-triacontanol were used as standard samples.

Phytosterols **(8)**. Colourless prisms, mp 137–139° (MeOH–$CHCl_3$); IR ν_{max} cm^{-1}: 3400, 2950, 2920, 2850, 1625, 1460, 1440, 1375, 1360, 1055. On GC analysis the sterol mixture showed three peaks corresponding to campesterol (RR_t: 1.10, content: 12. 2%), stigmasterol (RR_t: 1.16, 15.7%) and sitosterol (RR_t: 1.23, 72.1%), which were identified by co-injection or comparison of RR_ts (relative to cholesterol) and GC/MS with those of standard compounds. The amounts were estimated from the area of the GC peaks. (i) EIMS of campesterol: *m/z* (rel. int.): 400 $[M]^+$ (53), 385 (20), 382 (26), 367 (23), 315 (31), 289 (36). (ii) EIMS of stigmasterol: *m/z* (rel. int.) 412 $[M]^+$ (40), 397 (1), 394 (6), 379 (27), 369 (6), 327 (6), 314 (32), 300 (40), 273 (39), 271 (58), 255 (95), 231 (53), 229 (35), 213 (100). (iii) EIMS of sitosterol (rel. int.): *m/z* 414 $[M]^+$ (77), 399 (31), 396 (35), 381 (25), 329 (46), 303 (54), 273 (36), 255 (47), 231 (52), 229 (19), 213 (100).

Friedelin **(1)**. Colourless needles, mp 265–266° (EtOAc), $[\alpha]_D^{23}$ −25.6° (*c* 0.84) (lit. [18] mp 261 264°, $[\alpha]_D$ −25°); IR ν_{max} cm^{-1}: 1700; EIMS *m/z* (rel. int.) 426 $[M]^+$ (82), 411 (28), 341 (15), 302 (53), 273 (84), 205 (75). Compound **1** was identified by direct comparison with an authentic sample of friedelin isolated from Cork [18].

Lupa-1,20(29)-*dien*-3-*one* **(2)** (Glochidone). Colourless needles, mp 168–169.5° (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ + 70.5° (*c* 0.67) {lit. [19] mp 164–165°, $[\alpha]_D$ + 73.41°($CHCl_3$)}; UV λ_{max} nm (ε): 228 (10000), 333 (98); IR ν_{max} cm^{-1}: 3075, 1663, 1642, 878, 822; ^{1}H NMR (300 MHz): δ 0.81 (3H, *s*), 0.96 (3H, *s*), 1.07 (3H, *s*), 1.08 (3H, *s*), 1.11 (3H, *s*), 1.13 (3H, *s*), 1.69 (3H, *s*, H-30), 4.59 and 4.71 (each 1H, *d*, *J* = 2.7 Hz), 5.79 (1H, *d*, *J* = 9.8 Hz, H-1), 7.10 (1H, *d*, *J* = 9.8 Hz, H-2); EIMS *m/z* (rel. int.): 422 $[M]^+$ (25), 407 (13), 285 (19), 229 (100), 205 (52), 203 (51), 191 (48), 189 (38). Physical and spectral data were in good agreement with those reported in the literature.

β-Amyrin acetate **(3b)**. Colourless needles, mp 241–243° MeOH–$CHCl_3$), $[\alpha]_D^{23}$ + 78.7° (*c*1.07) (lit. [20] mp 241–242°, $[\alpha]_D$ + 81°); IR ν_{max} cm^{-1}: 1722, 1635, 1240, 812; EIMS *m/z* (rel. int.): 468 $[M]^+$ (4), 453 (2), 408 (4), 218 (100), 203 (47). Saponification of **3b** (110 mg) with 0.05 M KOH–EtOH (30 ml) on a steam bath for 1 hr afforded *β*-amyrin **(3a)**, mp 197–198° (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ + 86.9° (*c* 1.12) (lit. [20] mp 197–197.5°, $[\alpha]_D$ + 88.4°); EIMS *m/z*: 426 $[M]^+$. Compounds **3a** and **3b** were identified by direct comparison with an authentic samples.

Lupeol **(6)**. Colourless needles, mp 219.5–221° (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ + 26.5° (*c* 0.98) (lit. [20] mp 215–216°, $[\alpha]_D$ + 26.4°); IR ν_{max} cm^{-1}: 3400, 3060, 1638, 878. Acetylation of **6** (80 mg) with Ac_2O and pyridine (each 5 ml) gave lupenyl acetate **(6a)**, mp 217–218°, $[\alpha]_D^{23}$ + 37.5° (*c* 0.86) (lit. [20] mp 217–218°, $[\alpha]_D$ + 35.5° (*c* 1.05); IR ν_{max} cm^{-1}: 1727, 1637, 1239, 865; EIMS *m/z* 468 $[M]^+$. Compounds **6** and **6a** were identified by direct comparison (mmp, IR, ^{1}HNMR, MS) with authentic samples.

Oleana-9(11):12-*dien*-3*β*-*yl acetate* **(4b)**. Colourless needles, mp 217–218° (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ + 331. 5° (lit. [8, 9] mp 217°, $[\alpha]_D$ + 342°); UV λ_{max} nm (ε): 282 (homoannular diene); IR ν_{max} cm^{-1}: 3030, 2960, 2940, 2850, 1723, 1480, 1445, 1375, 1360, 1240, 1020, 1000, 985, 975, 930, 900, 820; EIMS *m/z* (rel. int.): 466 $[M]^+$ (33), 406 (15), 313 (18), 255 (38). **4b** was identified by direct comparison (mmp, TLC, IR, ^{1}H NMR, MS) with the synthetic sample described later.

Oleana-9(11):12-*dien*-3*β*-*ol* **(4a)**. Compound **4b** (55 mg) was refluxed with 0.1 M KOH–EtOH (30 ml) on a steam bath for 1 hr. Work-up as usual afforded a residue, which was purified on CC to give **4a** (46 mg), FDMS: M^+ at *m/z* 424 ($C_{30}H_{48}O$), mp 219–221° (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ + 318° (*c* 0.56) (lit. [8] mp 213.5–214.5°, $[\alpha]_D$ + 320°); UV λ_{max} nm (ε): 282 (8000); IR ν_{max} cm^{-1}: 3300, 3030, 2970, 2940, 2910, 2860, 1632, 1480, 1460, 1438, 1375, 1360, 1033, 985, 920, 830, 816, 730, 680; EIMS *m/z* (rel. int.): 424 $[M]^+$ (100), 409 (3), 391 (1), 271 (6), 255 (17).

CrO_3 *Oxidation of* **4a**. A cold soln of **4a** (20 mg) in pyridine (1.5 ml) was added to a slurry of CrO_3 (20 mg) in pyridine (1 ml) at 0° and the mixture was stirred at 20° for 5 hr. Dilution with H_2O (15 ml) gave a ppt. Work-up as usual afforded a residue, which was purified over CC to give **4c** (14 mg), mp 204–205° (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ + 353° (*c* 0.42) (lit. [8] mp 206–208°, $[\alpha]_D$ + 373°); UV λ_{max} (ε): 283 (8,600); IR ν_{max} cm^{-1}: 3060, 2970, 2940, 2865, 1700, 1630, 1470, 1460, 1450, 1430, 1375, 1360, 1110, 1025, 1003, 990, 830, 815; EIMS *m/z* (rel. int.): 422 $[M]^+$ (100), 407 (22), 269 (34), 255 (45).

Synthesis of **4b**. To a soln of *β*-amyrin acetate **(3b)** (150 mg) in dried CCl_4 (16 ml) *N*-bromosuccinimide (89 %, 64 mg) was added and the mixture was refluxed on a steam bath for 1.5 hr [10]. Filtration and removal of CCl_4 *in vacuo* gave a pale yellow residue, which was dissolved in Et_2O. Neutralization and evapn of the Et_2O soln gave a solid (180 mg), which on CC furnished oleana-9(11):12-dien-3*β*-yl acetate {(97 mg), mp 217–219.5°, $[\alpha]_D^{23}$ + 335° (*c*1.56)}, identical in all respects with **4b**.

Oleana-11:13(18)-*dien*-3*β*-*ol* **(5a)**. Colourless needles, FDMS: M^+ at *m/z* 424 ($C_{30}H_{48}O$); mp 232–234.5° (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ − 61.3° (*c* 0.58) (lit. [13] mp 228–229°, $[\alpha]_D$ − 72°); UV λ_{max} nm (ε): 243 (22, 700), 251 (25,500), 260 (16,400) (heteroannular diene); IR ν_{max} cm^{-1}: 3470, 3022, 2950, 2930, 2099, 2830, 1637, 1600, 1470, 1458, 1450, 1440, 1370, 1348, 1075, 1020, 975, 960, 935, 758; EIMS *m/z* (rel. int.): 424 $[M]^+$ (100), 409 (22), 406 (3), 391 (4), 323 (5), 271 (13), 270 (13), 255 (30), 229 (40), 215 (47), 203 (28), 189 (31).

Acetylation of **5a**. Compound **5a** (4 mg) was acetylated (Ac_2O–pyridine 1:1, 1 ml) at room temp. for 24 hr. Usual work-up afforded a residue, which was recrystallized (MeOH–$CHCl_3$) to give oleana-11:13(18)-dien-3*β*-yl acetate **(5b)** (3 mg) as colourless needles, mp 230–233°, $[\alpha]_D^{23}$ − 61.5° (*c* 0.31) (lit. [9] mp 233–234°, $[\alpha]_D$ − 59°); IR ν_{max} cm^{-1}: 3020, 2950, 2925, 2875,

2850, 1725, 1620, 1585, 1480, 1460, 1440, 1375, 1360, 1240, 1140, 1020, 980, 900; EIMS *m/z* (rel. int.): 466 $[M]^+$ (100), 451 (30), 406 (19), 391 (16), 323 (9), 271 (11), 270 (11), 255 (26), 229 (38), 215 (45), 203 (56), 189 (44). Compound **5b** was identified by direct comparison (mmp, co-TLC, IR, ^{1}H NMR, MS) with the synthetic sample described below.

*Synthesis of oleana-*11:13(18)*-dien-*3*β-yl acetate* **(5b)**. A soln of freshly sublimed SeO_2 (40 mg) in 80% HOAc (5 ml) was added to a soln of *β*-amyrin acetate **(3b)** (80 mg) in HOAc (10 ml) and the mixture was refluxed for 1 hr [13]. After cooling, the reaction mixture was diluted with H_2O (40 ml), and the resulting ppt. was extracted with Et_2O and neutralized. Removal of the solvent gave a residue (76 mg), which on CC over $AgNO_3$–Al_2O_3 (1:4, 16 g) furnished oleana-11:13(18)-dien-3*β*-yl acetate (58 mg), mp 232–233.5° MeOH–$CHCl_3$), $[\alpha]_D^{23}$ − 63.5° (*c* 1.03), identical in all respects with **5b**.

Ent-3*β-hydroxykaur-*16*-ene* **(7a)**. Colourless needles, mp 177–178° (MeOH), $[\alpha]_D^{23}$ − 69.2° (*c* 0.32) (lit. [15] mp 176–177°); HRMS: M^+ at *m/z* 288.2450 ($C_{20}H_{32}O$ requires 288.2453); IR ν_{max} cm^{-1}: 3310, 3055, 2975, 2930, 2860, 1655, 1365, 1037, 870; EIMS *m/z* (rel. int.): 288 $[M]^+$ (100), 273 (32), 255 (83), 245 (48), 227 (62). The above data were fairly compatible with those published for synthetic *ent-*3*β*-hydroxykaur-16-ene.

Acetylation of **7a**. Compound **7a** (10 mg) was acetylated as usual to give **7b** (MeOH–$CHCl_3$) (8 mg) as colourless needles, mp 162–164°, $[\alpha]_D^{23}$ − 68.2° (*c* 0.23) (lit. [17] mp 162–163°, $[\alpha]_D$ − 62°); HRMS: M^+ at *m/z* 330.2568 ($C_{22}H_{34}O_2$ requires 330.2559); IR ν_{max} cm^{-1}: 3060, 2975, 2925, 2850, 1720, 1655, 1380, 1365, 1250, 1020, 862; EIMS *m/z* (rel. int.): 330 $[M]^+$ (64), 288 (15), 255 (100), 227(82). The ^{1}H NMR spectrum of **7b** was in good agreement with that of an authentic *ent-*3*β*-acetoxykaur-16-ene [17].

Bergenin **(9)**. Hydrate: colourless prisms, mp 139–145° (50% EtOH), anhydride: mp 247–250°, $[\alpha]_D^{23}$ − 53.5° (*c* 1.27, EtOH) (lit. [21] hydrate: mp 130°, anhydride: mp 234°, $[\alpha]_D$ − 37.3° (*c* 1.96, EtOH); HRMS: M^+ at *m/z* 328.0797 ($C_{14}H_{16}O_9$ requires 328.0794); HPLC: R_t 10.3 (MeOH:H_2O, 7:3); UV λ_{max} nm (*ε*): 219 (22 300), 276 (7000) 314 (2900); IR λ_{max} cm^{-1}: 3400 (OH), 1695 (>C=O), 1600, 1520, 1455, 1360, 1340, 1325, 1225, 1130, 1120, 1080, 1060, 1038, 980, 955, 900, 855, 760, 720; EIMS *m/z* (rel. int.): 328 $[M]^+$ (28), 237 (5), 222 (20), 208 $[C_{10}H_8O_5]^+$ (100), 195 (16), 180 (23), 165 (12), 152 (20). On acetylation (Ac_2O: Pyridine 1:1), **9** gave a pentaacetate **(9a)**, mp 209–211.5°, $[\alpha]_D^{23}$ − 34.7° (*c* 1.14, MeOH) (lit. [21] mp 208–209°, $[\alpha]_D$ − 33.85°); IR ν_{max} cm^{-1}: 1780, 1760, 1615, 1485, 805; ^{1}H NMR (90 MHz, DMSO-d_6:H_2O, 1:1): *δ* 2.06 (3H, *s*, OAc), 2.21 (6H, *s*, OAc), 2.34 (6H, *s*, OAc), 3.91 (2H, *s*, H-11), 4.35 (1H, *q*), 4.38 (1H, *d*), 5.11 (1H, *t*), 5.49 (1H, *t*), 7.67 (1H, *s*, H-7); EIMS *m/z* (rel. int.): 538 $[M]^+$ (2), 496 (6), 454 (30), 436 (3), 418 (6), 394 (5), 376 (21), 363 (25), 334 (37), 321 (38), 316 (16), 292 (69), 279 (38), 274 (100), 261 (30), 250 (29), 237 (15), 225 (25), 208 (46), 195 (36), 180 (22), 152 (20), 139 (32). Compound **9** was identified by direct comparison (mmp, HPLC, IR, MS, ^{1}H NMR, ^{13}C NMR) with an authentic sample [22].

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