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PENTACYCLIC TRITERPENOIDS FROM *PRUNELLA VULGARIS**

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Key Word Index—*Prunella vulgaris*; Labiatae; triterpenoids; 2α,3α-dihydroxyursa-12,20(30)-dien-28-oic acid; 2α,3α,24-trihydroxyursa-12,20(30)-dien-28-oic acid; 2α,3α,24-trihydroxyoleana-11,13(18)-dien-28-oic acid.

Abstract—Three new pentacyclic triterpenes together with several known triterpenes have been isolated as their methyl esters from the roots of *Prunella vulgaris*.

INTRODUCTION

Our previous paper [1] dealt with the isolation and structural determination of eight triterpenes, one of which was a new compound, 2α,3α,24-trihydroxyolean-12-en-28-oic acid, from the leaves and stems of *Prunella vulgaris* var. *lilacina* Nakai. We now report the presence of three new and several known triterpenes in the roots of the same plant, their structure elucidation by spectroscopic and chemical means, and the assignment of ^{1}H NMR and ^{13}C NMR spectral signals of the novel compounds. Their ^{1}H NMR and ^{13}C NMR assignments were made using the DEPT pulse sequence, two-dimensional proton–proton and proton–carbon shift correlation data.

RESULTS AND DISCUSSION

The methanol extract of the roots of *P. vulgaris* L. var *lilacina* Nakai was separated into three fractions, two of which were then methylated with diazomethane and separated into fractions A–F.

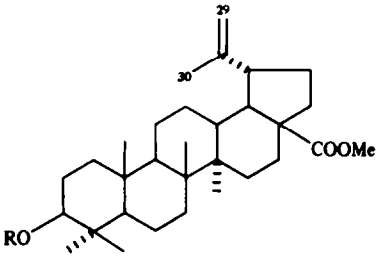
Fraction A was found to comprise methyl betulinate (**1**), methyl ursolate (**2**) and a trace of methyl oleanolate (**3**); fraction B, methyl 2α,3α-dihydroxyurs-12-en-28-oate (**4**) and small amounts of 3-epimaslinate (**5**) and a new triterpene (**6**); and fraction D, methyl 2α-hydroxyursolate (**7**) and methyl maslinate (**8**). GC of the acetylated fraction E gave three main peaks similar to those given by the corresponding fraction from leaves and stems. The first peak co-chromatographed with the peak of authentic methyl 2α,3α,24-triacetoxyolean-12-en-28-oate (Ac). Further purification of fractions B and E by reversed-phase HPLC afforded compounds **4**, **5** and **6**, and **9**, **10**, **11** and **12** with some minor compounds.

Compound **1** belonged to the lupane group of the triterpenes and its data (TLC, GC and ^{1}H NMR) were identical with those of a sample of methyl betulinate, isolated from *Zizyphus jujuba* [2].

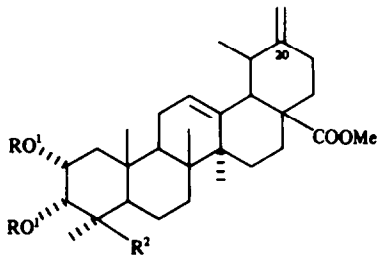
The molecular formulae of compounds **9** and **10** were identical. The ^{1}H NMR and ^{13}C NMR spectra of **9** were identical with those of authentic 2α,3α,24-trihydroxyolean-12-en-28-oate. The ^{1}H NMR (three doublets of 0.85, J = 6 Hz, 29-H$_3$; 0.94, J = 6 Hz, 30-H$_3$; and 2.22, J = 12 Hz, 18-H) and ^{13}C NMR (125.1, C-12; 138.1, C-13; 52.7, C-18; 38.9, C-19; 38.7, C-20, etc.) data of **10** indicated that it belonged to the ursene group of triterpenoids. As the former spectral pattern due to two hydroxymethines and one hydroxymethylene group in ring A was very similar to that of **9**, compound **10** was determined to be the ursene equivalent of **9**, methyl 2α,3α,24-trihydroxyurs-12-en-28-oate, which has been isolated before from *Rhododendron japonicum* [3] and *Hedyotis lawsoniae* [4].

The molecular formulae of compounds **11** and **12** were identical and had two hydrogens less than those of **9** and **10**. A signal at m/z 239 [M − retro-Diels–Alder fragment − H]$^+$ found in the mass spectra of **9** [1] and **10** was also observed in the mass spectra of **11** and **12**, from which it was concluded that their A/B rings had the same structure. On comparison of the ^{13}C NMR chemical shifts of **11** and **10**, the data for C-1 to C-16 were, as expected, similar to each other, but for C-18, C-19, C-21 and C-22 moderate differences were observed (1.6–2.1 ppm) and a very large difference for C-20 and C-30. From the ^{1}H NMR data, the number of methyls of the former was one less than that of the latter, and two broad singlets were present at δ4.64 and 4.70. As expected, the pattern of four carbinylic protons was very similar in both spectra. Therefore, the above data and IR spectral data (3078, 1642 and 886 cm^{-1}) confirmed the presence of an exomethylene group in the E-ring, and the other rings were presumed to have a structure identical with that of **10**. Further, on comparison of the two-dimensional proton–proton and proton–carbon shift correlation data of **11** and **10**, the signals of H-19 and H$_2$-21 in the former were greatly shifted (*ca* 1 ppm) downfield, and both methine protons at C-18 and C-19 were so equivalent that the doublet splitting pattern of the former, generally expected in the ursene skeleton, could not be observed and were replaced by a broad singlet. So, a C-20 position of the exomethylene group was suspected. As the signals of C-12, C-13, C-16 and C-28 in **11** and **10** were very similar, their D/E-ring junctions were presumed to be the same, i.e. *cis* [5]. This assumption was supported by hydrogenation of

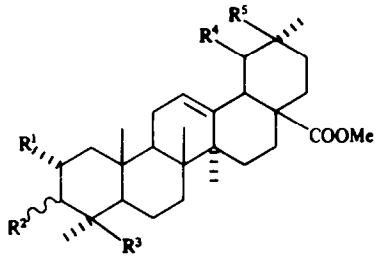
* Part 2 in the series "Constituents of the Labiatae Plants". For Part 1 see ref. [1].



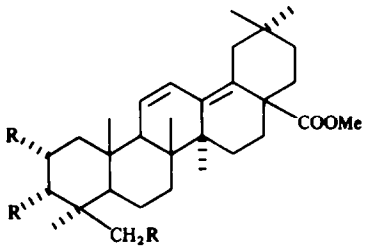
1 R = H
1a R = Ac



	R^1	R^2
6	H	Me
6a	Ac	Me
11	H	CH_2OH
11a	Ac	CH_2OAc



	R^1	R^2	R^3	R^4	R^5
2	H	β-OH	Me	Me	H
3	H	β-OH	Me	H	Me
4	OH	α-OH	Me	Me	H
4a	OAc	α-OAc	Me	Me	H
5	OH	α-OH	Me	H	Me
7	OH	β-OH	Me	Me	H
8	OH	β-OH	Me	H	Me
9	OH	α-OH	CH_2OH	H	Me
9a	OAc	α-OAc	CH_2OAc	H	Me
10	OH	α-OH	CH_2OH	Me	H
10a	OAc	α-OAc	CH_2OAc	Me	H



12 R = OH
12a R = OAc

11a in ethyl acetate over platinum oxide, because one of the two main products was identified as **10a** by ^{1}H NMR and ^{13}C NMR spectroscopy. The other main product was the isomer (**13a**) at C-20(30) of **10a**. The ^{1}H NMR and ^{13}C NMR spectral data of **13a** are shown in Table 1 and Fig. 1, respectively. Therefore, compound **11** was established to be methyl 2α,3α,24-trihydroxyursa-12,20(30)-dien-28-oate.

The ^{1}H NMR spectrum of **12** showed the presence of two vinylic protons (δ 5.64, *dd*, *J* = 11, 1 Hz and 6.44, *dd*, *J* = 11, 3 Hz), the eq (β)-H at C-22, eq (α)-H at C-19, eq (β)-H at C-1 and eq (α)-H at C-15 as a result of them being shifted downfield from the ordinary methylene region, and much the same pattern of four carbinylic protons as that of **9**. Further, the differences of the ^{13}C NMR shifts between compounds **9a** and **11a** were moderate for C-8, C-12, C-16, C-20, C-21, C-22, C-25 and C-28 (1.2–4.1 ppm); considerable for C-9, C-13, C-15, C-19 and C-27 (5.1–7.8 ppm); very large for C-11 and C-18; and the others were almost identical to one another (Table 2). Together with the typical UV absorption spectral data (243, 251 and 260 nm) [6], these results indicated that compound **12** had a heteroannular diene group which was located in rings C/D. Therefore, it was presumed to be methyl 2α,3α,24-trihydroxyoleana-11,13(18)-dien-28-oate, which was identified as **12a** by comparison with the main product obtained by oxidation of **9a** with selenium dioxide in acetic acid.

Compound **6**, previously reported as unknown [1], had one less oxygen atom than **11**. Its ^{1}H NMR and ^{13}C NMR spectra were very similar to those of **11**, except that a seventh tertiary C-methyl singlet at C-24 was observed in **6**, instead of the two two-proton doublets shown in **11**. Further, the ^{13}C NMR spectral relationship between **6a** and **4a** was very similar to that between **11a** and **10a**, and also that between **11** and **10**. Therefore, compound **6** can be easily assigned as methyl 2α,3α-dihydroxyursa-12,20(30)-dien-28-oate.

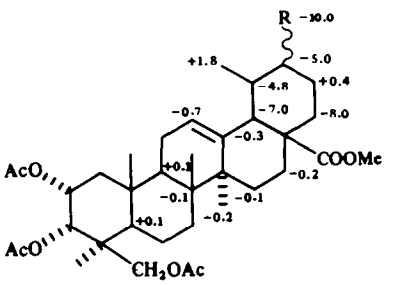
Ursolic acid was the major triterpene of the leaves and stems, from which betulinic acid was not isolated, while betulinic acid was the major and ursolic acid the minor triterpene in the roots. It is probable that ursolic and oleanolic acid may be transformed into more oxygenated compounds after biosynthesis. Moreover, 2α,3α,23-trihydroxyolean-12-en-28-oic acid present in the leaves and stems has not been detected in the roots. These results

Table 1. ^{1}H NMR spectral data of compounds **6**, **6a**, **9–12a** and **13a** ($CDCl_3$)

Assignments	6*	6a*	11	11a	9	9a	10	10a*	13a*	12*	12a*
H-1β										1.95 *dd* (12, 5)	
H-2β	4.00 *m* (23)	5.24 *ddd* (12, 5, 3)	4.00 *m* (23)	5.18 *ddd* (12, 5, 3)	4.02 *m* (23)	5.18 *ddd* (12, 5, 3)	3.99 *m* (23)	5.17 *ddd* (12, 5, 3)	5.17 *ddd* (12. 5, 3)	4.06 *m* (22)	5.25 *ddd* (12, 5, 3)
H-3β	3.43 *s* (*br*)	4.97 *d* (3)	3.87 *d* (3)	5.31 *d* (3)	3.89 *d* (3)	5.33 *d* (3)	3.86 *d* (3)	5.31 *d* (3)	5.31 *d* (3)	3.90 *d* (3)	5.35 *d* (3)
H-9										2.09 *s* (*br*)	
H-11										6.44 *dd* (11, 3)	6.46 *dd* (11, 3)
H-12	5.29 *t* (3.5)	5.28 *t* (3.5)	5.30 *t* (3.5)	5.29 *t* (3.5)	5.32 *t* (3.5)	5.30 *t* (3.5)	5.25 *t* (3.5)	5.24 *t* (3.5)	5.23 *t* (3.5)	5.64 *dd* (11, 1)	5.58 *d* (11)
H-15α										1.89 *m* (18)	
H-18	2.34 *s* (*br*)	2.34 *s* (*br*)	2.34 *s* (*br*)	2.34 *s* (*br*)	2.88 *dd* (14, 5)	2.88 *dd* (14, 5)	2.22 *d* (12)	2.24 *d* (12)	2.50 *d* (12)	—	—
H-19α										2.50 *d* (14)	2.50 *d* (14)
H-22β										2.26 *m* (18)	2.24 *m* (18)
3H-23	1.02 *s*	0.88 *s*	1.15 *s*	0.97 *s*	1.16 *s*	0.96 *s*	1.15 *s*	0.96 *s*	0.96 *s*	1.15 *s*	0.97 *s*
H_A and H_B (or 3H)-24	0.86 *s*	0.99 *s*	3.49 *d* (12) 3.59 *d* (12)	4.03 *d* (12) 4.17 *d* (12)	3.52 *d* (12) 3.72 *d* (12)	4.04 *d* (12) 4.18 *d* (12)	3.49 *d* (12) 3.70 *d* (12)	4.03 *d* (12) 4.17 *d* (12)	4.03 *d* (12) 4.17 *d* (12)	3.51 *d* (12) 3.69 *d* (12)	4.06 *d* (12) 4.17 *d* (12)
3H-25	0.97 *s*	1.02 *s*	0.94 *s*	1.04 *s*	0.94 *s*	1.03 *s*	0.93 *s*	1.04 *s*	1.04 *s*	0.94 *s*	1.04 *s*
3H-26	0.74 *s*	0.74 *s*	0.72 *s*	0.73 *s*	0.70 *s*	0.70 *s*	0.70 *s*	0.72 *s*	0.72 *s*	0.75 *s*	0.77 *s*
3H-27	1.15 *s*	1.18 *s*	1.15 *s*	1.17 *s*	1.14 *s*	1.17 *s*	1.08 *s*	1.14 *s*	1.14 *s*	0.96 *s*	1.00 *s*
3H-29	1.00 *d* (6)	1.00 *d* (6)	1.00 *d* (6)	0.99 *d* (6)	0.90 *s*	0.90 *s*	0.85 *d* (6)	0.85 *d* (6)	0.79 *d* (7)	0.79 *s*	0.80 *s*
H_A and H_B (or 3H)-30	4.64 *s* (*br*) 4.69 *s* (*br*)	4.64 *s* (*br*) 4.69 *s* (*br*)	4.64 *s* (*br*) 4.70 *s* (*br*)	4.63 *s* (*br*) 4.69 *s* (*br*)	0.93 *s*	0.93 *s*	0.94 *d* (6)	0.94 *d* (6)	0.86 *d* (7)	0.92 *s*	0.93 *s*
OMe	3.60 *s*	3.60 *s*	3.59 *s*	3.60 *s*	3.64 *s*	3.63 *s*	3.60 *s*	3.60 *s*	3.61 *s*	3.65 *s*	3.67 *s*
OAc	—	1.95 *s* 2.12 *s*	—	1.95 *s* 2.08 *s* 2.13 *s*	—	1.97 *s* 2.09 *s* 2.13 *s*	—	1.95 *s* 2.08 *s* 2.13 *s*	1.95 *s* 2.08 *s* 2.13 *s*	—	1.99 *s* 2.09 *s* 2.15 *s*

*Measured at 400 MHz; the rest at 300 MHz.

—Indicates no signal. The figures in parentheses are coupling constants in Hz except for $W_{1/2}$ values in *m*.



10a R = α-Me
13a R = β-Me

Fig. 1. Differences in the chemical shifts between **10a** (see Table 2) and **13a**.

show that different kinds of triterpenes are present in different organs of the same plant. Other rare ursene triterpenes with an exocyclic methylene at C-20 have been reported such as micromeric acid from *Micromeria benthami* [7] and dehydrouvaol from *Salvia coccinea* [8]. All the plants containing these triterpenes with this exocyclic double bond belong to the Labiatae. Other triterpenes with the same conjugated diene group as that of the new compound **12** are oleana-11,13(18)-diene-3,23,28-triol from *Scrophularia smithii* (Scrophulariaceae) [9], papyriogenins A, D, E and G from *Tetrapanax papyriferum* (Araliaceae) [10, 11], rotundioside E from *Bupleurum rotundifolia* (Umbelliferae) [12] as well as three triterpenes from *Glycyrrhiza* spp. (Leguminosae) [13–15].

EXPERIMENTAL

Mps: uncorr; TLC, IR, MS; ^{1}H NMR, ^{13}C NMR and plant: see ref. [1], unless otherwise indicated; UV: MeOH; HPLC: Shenshu Pak column (Whatman Partisil 5 ODS-3, 10 mm × 25 cm), flow rate 3.0 ml/min at room temp.

Extraction and isolation. The dried cut roots (0.7 kg) were extracted with MeOH to give a material (69.5 g), which was further extracted with *n*-hexane and Me_2CO. The Me_2CO-soluble portion (28.4 g) was chromatographed on silica gel and

Table 2. ^{13}C NMR spectral data of compounds **4a**, **6**, **6a**, **9**–**12a** ($CDCl_3$)

Carbon	4a*	6*	6a*	11	11a	9	9a	10	10a*	12*	12a*
1	39.0	42.0	38.9	41.6	38.7	41.4	38.5	41.8	38.8	41.4	38.3
2	68.3	66.4	68.1	66.1	67.7	66.2	67.7	66.2	67.9	66.2	67.5
3	77.1	78.8	77.0	73.2	72.3	73.3	72.4	73.3	72.5	73.5	72.4
4	38.4	38.2	38.3	43.8	42.0	41.4	41.6	43.8	42.0	44.0	42.0
5	49.6	48.0	49.5	48.5	50.1	48.6	50.1	48.5	50.1	48.4	49.7
6	17.8	17.9	17.7	18.1	17.9	18.2	18.0	18.2	18.0	18.3	18.0
7	32.6	32.6	32.5	32.9	32.8	32.7	32.5	33.0	33.0	32.6	32.4
8	39.7	39.5	39.5	39.5	39.5	39.3	39.3	39.5	39.6	40.9	40.8
9	47.4	47.2	47.3	47.3	47.4	47.4	47.5	47.3	47.5	54.2	54.1
10	38.1	38.1	38.0	37.9	38.0	38.0	38.1	37.9	38.1	37.7	37.7
11	23.3	23.2	23.1	23.3	23.4	23.4	23.5	23.3	23.4	125.7	125.4
12	125.2	125.7	125.5	125.5	125.3	121.9	121.8	125.1	125.0	126.2	125.9
13	138.2	137.8	137.8	137.8	137.8	143.7	143.8	138.1	138.2	136.3	136.0
14	42.1	41.8	42.0	41.9	42.0	41.6	42.0	41.9	42.1	42.1	42.0
15	28.0	27.8	27.8	27.7	27.8	27.5	27.5	27.8	28.0	32.7	32.6
16	24.2	24.2	24.2	24.1	24.1	22.9	22.9	24.1	24.1	24.9	24.9
17	48.0	48.1	48.1	48.1	48.0	46.6	46.5	47.9	48.0	48.2	48.3
18	52.8	54.7	54.6	54.6	54.6	41.1	41.1	52.7	52.8	132.0	132.4
19	39.0	37.2	37.2	37.1	37.1	45.7	45.8	38.9	39.1	40.6	40.4
20	38.9	152.8	152.7	152.7	152.6	30.6	30.6	38.7	38.8	32.5	32.0
21	30.6	32.1	32.2	32.1	32.1	33.7	33.7	30.5	30.5	36.9	36.7
22	36.6	38.6	38.6	38.6	38.5	32.2	32.2	36.5	36.6	35.5	35.4
23	27.7	28.4	27.7	22.0	22.1	22.0	22.1	22.0	22.2	22.1	22.1
24	21.6	21.7	21.5	65.7	66.1	65.5	66.1	65.5	66.2	65.2	65.8
25	16.3	16.3	16.2	16.7	16.7	16.6	16.6	16.7	16.8	19.2	19.0
26	16.9	16.8	16.8	16.6	16.6	16.6	16.7	16.7	16.8	16.2	16.1
27	23.7	23.6	23.5	23.5	23.5	25.9	25.9	23.6	23.7	19.9	19.8
28	178.0	177.2	177.2	177.2	177.1	178.2	178.1	178.0	177.9	177.1	176.9
29	17.0	16.0	16.0	16.0	16.0	33.0	33.0	16.9	17.0	24.1	24.0
30	21.2	105.0	105.0	105.0	105.1	23.5	23.5	21.1	21.2	32.2	32.3
$CO_2\underline{Me}$	51.5	51.5	51.5	51.4	51.5	51.4	51.5	51.4	51.5	51.8	51.7
Ac$\underline{C}$O	170.7		170.6		171.0		171.1		171.2		171.0
	170.4		170.3		170.3		170.3		170.4		170.4
					170.0		170.1		170.2		170.2
$\underline{Ac}$CO	21.1		21.0		20.9		21.1		21.0		21.0
	21.0		20.9		20.9		20.9		21.0		20.9
					20.8		20.8		20.9		20.8

*Measured at 100 MHz; the rest at 75.2 MHz.

divided into three fractions by elution with EtOAc. The first two fractions were each esterified with an excess of ethereal CH_2N_2 to yield two solid residues (I and II). Residue I (10.5 g) was fractionated on a silica gel column to afford **1** (0.3 g) from the *n*-hexane–Et_2O (17:3) eluate, and a mixture of triterpenes (1.5 g) from the *n*-hexane–Et_2O (1:1) and C_6H_6–Et_2O (1:1) eluates. The mixture of triterpenes was added to residue II (12.4 g) and chromatographed on silica gel to give six fractions: A (4.2 g), B (0.9 g), C (0.4 g), D (0.7 g), E (1.2 g) and F (0.6 g) by elution with C_6H_6–Et_2O mixtures (A–E, 4:1; F, 7:3).

Methyl betulinate (**1**). Recrystallization (MeOH) afforded 216.6 mg, needles, mp 224–225°, $[\alpha]_D^{26}$ +4.0° ($CHCl_3$; *c* 0.5). HRMS *m/z*: 470.375 $[M]^+$, calc. for $C_{31}H_{50}O_3$; 470.376. EIMS *m/z* (rel. int.): 470 $[M]^+$ (46), 455 $[M-Me]^+$ (6), 452 $[M-H_2O]^+$ (8), 411 $[M-CO_2Me]^+$ (17), 410 $[M-HCO_2Me]^+$ (19), 262 (100), 248 (19), 220 (25), 207 (50), 203 (66), 189 (65), 133 (21); IR ν_{max} cm^{-1}: 3550 (OH), 3075, 1640, 880 (C=CH_2), 1710 (CO_2Me); ^1H NMR: δ0.73, 0.80, 0.89, 0.94 (5 × Me, each *s*), 1.66 (*s*, H_3-30), 2.88 (*m*, H-19), 3.15 (*m*, $W_{1/2}$ = 18 Hz, H-3), 3.61 (*s*, CO_2Me), 4.56, 4.68 (2*d*, *J* = 2 Hz, H_A-29 and H_B-29); ^{13}C NMR: δ39.0 (C-1), 27.6 (C-2), 79.3 (C-3), 39.0 (C-4), 55.7 (C-5), 18.4 (C-6), 34.6 (C-7), 41.0 (C-8), 50.9 (C-9), 37.4 (C-10), 21.1 (C-11), 25.8 (C-12), 38.5 (C-13), 42.6 (C-14), 30.9 (C-15), 32.4 (C-16), 56.8 (C-17), 47.2 (C-18), 49.8 (C-19), 150.9 (C-20), 29.9 (C-21), 37.4 (C-22), 28.1 (C-23), 15.4 (C-24), 16.1 (C-25), 16.1 (C-26), 14.8 (C-27), 177.0 (C-28), 109.5 (C-29), 19.5 (C-30), 51.3 ($CO_2\underline{Me}$). This assignment by the NMR signals was readily achieved by comparison with the published spectral data of betulinic acid [16]. *Monoacetate* (**1a**), plates, mp 210–212°, $[\alpha]_D^{27}$ +16.0° ($CHCl_3$; *c* 0.5). HRMS *m/z*: 512.386 $[M]^+$, calc. for $C_{33}H_{52}O_4$: 512.386. EIMS *m/z* (rel. int.): 512 $[M]^+$ (30), 452 $[M-HOAc]^+$ (47), 262 (100), 249 (54), 248 (27), 203 (55), 189 (94), 133 (33); ^1H NMR: δ0.82, 0.91, 0.95 (*s*, Me × 5), 1.67 (*s*, Me), 2.01 (*s*, OAc), 2.89 (*m*, H-19), 3.63 (*s*, CO_2Me), 4.56, 4.69 (2*d*, *J* = 2 Hz, H_A and H_B-29), 4.43 (*m*, H-3).

Methyl ursolate (**2**), *methyl oleanolate* (**3**), *methyl 2α,3α-dihydroxyurs-12-en-28-oate* (**4**), *methyl 3-epimaslinate* (**5**), *methyl 2α-hydroxyursolate* (**7**) *and methyl maslinate* (**8**). Co-TLC and co-GC [2% OV-17 (0.5 m × 3 mm), column temp. 255°, N_2 59 ml/min] of fractions A, B and D with the appropriate mixtures of authentic samples showed that fraction A contained **1** (R_t 2.8 min and rel. amount 97.7%), **2** (3.2; 2.2) and **3** (2.5; under 0.1); fraction B: **4** (5.4; 91.0), **5** (4.6; 6.1) and **6** (6.5; 2.9), and fraction D: **7** (5.4; 89.5) and **8** (4.7; 10.5).

Methyl 2α,3α-dihydroxyursa-12,20(30)-dien-28-oate (**6**).

Further separation of fraction B on HPLC (MeOH–H_2O, 9:1) gave **6** (R_t 20.7 min), a minor compound (21.6), **5** (22.6) and **4** (23.4). Compound **6** was obtained as needles (10 mg) from MeOH, mp 128–129°, $[\alpha]_D^{23}$ + 128.0° ($CHCl_3$; *c* 0.3); HRMS *m/z*: 484.355 $[M]^+$, $C_{31}H_{48}O_4$ (required: 484.355). EIMS *m/z* (rel. int.): 484 $[M]^+$ (34), 427 $[M-CO_2Me]^+$ (11), 260 (60), 247 (75), 231 (22), 223 (20), 201 (100), 200 (57), 187 (32), 171 (19), 131 (15); IR ν_{max} cm^{-1}: 3450 (OH), 1721 (CO_2Me), 3080, 1660, 890 (C=CH_2), 822 (C=CH). *Diacetate* (**6a**), amorphous. HRMS *m/z*: 568.377, $C_{35}H_{52}O_6$ (required: 568.376). EIMS *m/z*: 568 $[M]^+$ (46), 508 (8), 434 (10), 260 (45), 247 (100), 231 (15), 215 (10), 187 (35), 133 (15), 131 (12), 119 (12); ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2.

Analysis of acetylated fraction E by GC and HPLC. Fraction E was acetylated (C_5H_5N–Ac_2O) overnight at room temp. and the reaction mixture treated in the usual way. The mixture of acetates which on GC (column temp. 275°) gave three peaks (R_t 9.9 min (**9**), 11.0 and 13.0) was subjected to repeated HPLC [MeCN–H_2O, 87:13; six peaks: R_t 27.1 min (**11a**), 28.7 (**12a**), 30.5 (minor compound), 31.4 (**9a**), 32.9 (**10a**) and 35.5 (minor compound)]. It was, however, impossible to effect a good separation between the minor compound with R_t 30.5 min and **9a** and between **10a** and the minor compound with R_t 35.5 min. Each pair of compounds was collected as a single fraction which, after removal of the solvent, was repurified (5% KOH–MeOH) for 1 hr and the reaction mixture poured into H_2O. The ppts. obtained were again purified by HPLC to give a minor compound (R_t 25.0 min) and **9** (27.5) (MeOH–H_2O, 4:1) and **10** (23.5) and a minor compound (R_t 21.4) (MeOH–H_2O, 17:3) respectively. The ^{1}H NMR and ^{13}C NMR spectra of compound **9** (154 mg) were found to be identical with those of methyl 2α,3α,24-trihydroxyolean-12-en-28-oate [1] (see Tables 1 and 2).

Methyl 2α,3α-24-trihydroxyurs-12-en-28-oate (**10**). Crystallization of **10** from MeOH afforded 140 mg of plates, mp 205–206°, $[\alpha]_D^{19}$ + 57.2° ($CHCl_3$; *c* 0.5). HRMS *m/z*: 502.366 $[M]^+$, $C_{31}H_{50}O_5$ (calc. for 502.366). EIMS *m/z* (rel. int.): 502 $[M]^+$ (2), 484 $[M-H_2O]^+$ (1), 442 $[M-HCO_2Me]^+$ (3), 425 $[M-CO_2Me-H_2O]^+$ (1), 262 (100), 249 (13), 239 (12), 203 (85), 189 (19), 133 (42); IR ν_{max} cm^{-1}: 3541 (OH), 1706 (CO_2Me), 1660 (C=C), 827 (C=CH). *Triacetate of* **10** (**10a**), amorphous. HRMS *m/z*: 628.395 $[M]^+$, $C_{37}H_{56}O_8$ (calc. for 628.397). EIMS *m/z*: 628 $[M]^+$ (2), 568 $[M-HOAc]^+$ (6), 508 (3), 367 (5), 307 (5), 262 (100), 249 (12), 203 (77), 189 (11), 173 (10), 133 (28); IR ν_{max} cm^{-1}: 1746 (OAc), 1660, 827.

Methyl 2α,3α,24-trihydroxyursa-12,20(30)-dien-28-oate (**11**). Triacetate of **11** (**11a**) was amorphous. HRMS *m/z*: 626.382 $[M]^+$, $C_{37}H_{54}O_8$ (required: 626.382). EIMS *m/z* (rel. int.): 626 $[M]^+$ (37), 566 $[M-HOAc]^+$ (13), 433 (13), 260 (42), 247 (100), 230 (18), 201 (76), 187 (29), 131 (13); IR ν_{max} cm^{-1}: 1751 (OAc), 1715 (CO_2Me), 3082, 1646, 889 (C=CH_2), 827 (C=CH). Compound **11a** was hydrolysed as mentioned above. Recrystallization from MeOH afforded **11** (122 mg) as needles, mp 212–213°, $[\alpha]_D^{19}$ + 121.2° ($CHCl_3$; *c* 0.5). HRMS *m/z*: 500.351 $[M]^+$, $C_{31}H_{48}O_5$ (required: 500.350). EIMS *m/z* (rel. int.): 500 $[M]^+$ (69), 482 $[M-H_2O]^+$ (7), 441 $[M-CO_2Me]^+$ (9), 260 (94), 247 (88), 239 (18), 231 (19), 201 (100), 200 (62), 187 (21), 133 (6); IR ν_{max} cm^{-1}: 3552 (OH), 1718 (CO_2Me), 3078, 1642, 886 (C=CH_2), 825 (C=CH).

Conversion of **11a** *into* **10a**. Compound **11a** (27 mg) in EtOAc (2 ml) was shaken at room temp. over PtO_2 (32 mg) under H_2 for 5 hr. After removal of the catalyst, the filtrate was evaporated and the residue purified by HPLC [mobile phase MeCN–H_2O (87:13); R_t 28.7 min] to yield plates (17 mg), which were shown to be a mixture of **10a** and **13a** (1:3) by ^{1}H NMR (see Table 1) and ^{13}C NMR (see Fig. 1).

Methyl 2α,3α,24-trihydroxyoleana-11,13(18)-dien-28-oate (**12**). Triacetate of **12** (**12a**) was an amorphous powder. HRMS *m/z*: 626.383 $[M]^+$, $C_{37}H_{54}O_8$ (required: 626.382). EIMS *m/z*: 626 $[M]^+$ (100), 567 (77), 507 (36), 367 (54), 247 (52), 239 (8), 201 (23), 187 (48), 133 (25). Hydrolysis of **12a** by the method mentioned above gave **12** (4 mg) as needles (from MeOH), mp 267–269°, $[\alpha]_D^{24}$ – 156.0° ($CHCl_3$; *c* 0.3). HRMS *m/z* 500.350 $[M]^+$, $C_{31}H_{48}O_5$ (required: 500.350). EIMS *m/z* (rel. int.): 500 $[M]^+$ (100), 482 $[M-H_2O]^+$ (27), 441 $[M-CO_2Me]^+$ (46), 423 $[M-CO_2Me-H_2O]^+$ (22), 367 (36), 225 (34), 247 (35), 201 (20), 189 (20), 187 (30), 133 (23); IR ν_{max} cm^{-1}: 3420 (OH), 1720 (CO_2Me), 1690 (C=C); UV λ_{max} nm: 243, 251, 260 (log ε 4.42, 4.49, 4.27). The ^{1}H NMR and ^{13}C NMR spectral assignments (see Tables 1 and 2) were made by comparison with published spectral data [12, 17, 18].

Formation of **12a** *from* **9a**. This oxidation was effected by a similar procedure to that described by King *et al.* [6]. Compound **9a** (30 mg) in HOAc (2 ml) was heated with SeO_2 (at 120° resublimed, 45 mg) under reflux for 2.5 hr. After cooling, the reaction mixture was poured into H_2O and extracted with Et_2O. Removal of the solvent gave a residue (28 mg), which was purified by HPLC [mobile phase MeOH–H_2O (9:1)] to afford **12a** (24 mg; yield 80%, R_t 20.0 min) and the 2α,3α-diacetate of **12** (2.2 mg; 15.8 min).

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