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TRITERPENOIDS FROM *EUCALYPTUS PERRINIANA* CULTURED CELLS*

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Key Word Index *Eucalyptus perriniana*; Myrtaceae; plant cell culture; triterpene; arjunolic acid; asiatic acid; hederagenin; 23-hydroxyursolic acid; maslinic acid; 2α-hydroxyursolic acid; oleanolic acid; ursolic acid.

Abstract—Eight triterpenes were isolated from the methanol extracts of *Eucalyptus perriniana* cultured cells as their methyl esters. The structures were elucidated by spectral data to be oleanolic acid, ursolic acid, maslinic acid, 2α-hydroxyursolic acid, hederagenin, 23-hydroxyursolic acid, arjunolic acid and asiatic acid.

INTRODUCTION

It is well known that the leaves of *Eucalyptus globulus* contain an essential oil, called eucalyptus oil, which is used for medicinal, industrial, perfumery purposes and recently new energy sources. Many studies on clonal propagation [1] and secondary metabolites, flavonoids [2] and steroids [3] of *Eucalyptus* cell and tissue cultures have been reported. In order to produce monoterpenes, especially 1,8-cineole, by plant cell culture, seven species of *Eucalyptus perriniana*, *E. globulus*, *E. calophylla*, *E. radiata*, *E. dives*, *E. citriodora* and *E. polybractea* were examined for callus production and their chemical components. Various good growing callus strains have been obtained previously, but monoterpene production was not successful until now. In this paper we report the isolation and identification of major triterpenoids from *E. perriniana* cultured cells.

RESULTS AND DISCUSSION

Static cultured *Eucalyptus perriniana* cells were harvested after 3 weeks culture and then extracted with methanol. The methanol extracts were then partitioned between ethyl acetate and water and the ethyl acetate layer was evaporated and chromatographed on silica gel to yield four fractions (Epe-1, 2, 3 and 4).

Epe-1 was identified as a mixture of phytosterols by TLC, HPLC and GLC, and the main component (**1**) of Epe-1 was collected by HPLC. Compound **1** was isolated as colourless flakes, mp 138–138.5°, and had the composition $C_{29}H_{50}O$ on the basis of high resolution mass spectroscopy and elemental analysis. The ^{13}C NMR spectra of **1** and its acetate (**1a**) agreed with the data of sitosterol and its acetate, respectively [4, 5].

Fraction Epe-4 was methylated with diazomethane in ether and the product was separated two compounds, **2a** and **3a**, by HPLC. Compounds **2a** and **3a** were isolated as colourless needles, mp 227–237° and 229–231°, respectively, and had the same elemental composition, $C_{31}H_{50}O_5$, on the basis of high resolution mass spectroscopy. The mass spectra of these two compounds were quite similar to each other and the intense fragment ion peaks at *m/z* 262 and 203 are due to retro-Diels–Alder type fragmentation of the C ring of a methyl olean- or urs-12-en-28-oate which is not substituted on the C, D and E rings [6].

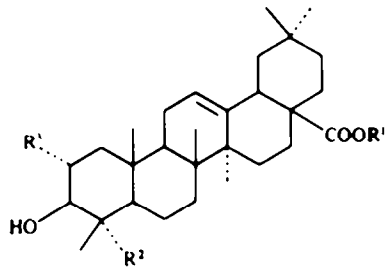
As three acetyl signals were observed in ^1H NMR spectra of the acetylation products of **2a** and **3a** (**2b** and **3b**), it is supposed that three hydroxyl groups exist on the A and B rings of **2a** and **3a**.

As shown in Table 1, in the ^1H NMR spectrum of **2a** six methyl signals were observed as singlets (δ0.68, 0.86, 0.90, 0.90, 0.99, 1.09), and the 18-H signal was observed at δ2.84 (*dd*, *J* = 4, 14 Hz). On the other hand six methyl signals (four of them singlets and two doublets) were also observed, but the 18-H signal was observed at δ2.20 (*d*, *J* = 12 Hz) in the case of compound **3a**. From these data it is thought that **2a** and **3a** should possess methyl olean-12-en-28-oate and methyl urs-12-en-28-oate skeletons, respectively.

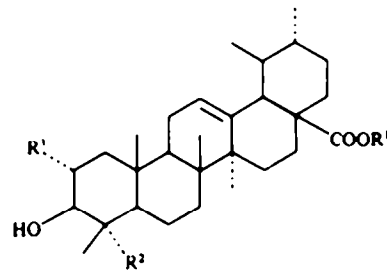
The signals of the protons attached to the carbons bearing an oxygen atom (H-2, H-3 and H-23) of **2a** were identical with those in the spectrum of **3a**. The coupling constant between H-2 and H-3 is comparatively large (*J* = 10 Hz) so that the two hydroxyl groups should have the 2α- and 3β-configurations. Since other proton signals observed at δ3.44 and 3.71 in the spectrum of **2a** were coupled to each other and other couplings were not recognized, there is an hydroxymethyl group attached to the quarternary carbon.

The assignments of the ^{13}C NMR carbon signals of **2a** and **3a** were performed by reference to their multiplicity and to the data of methyl maslinate (**6a**) and methyl 2α-hydroxyursolate (**7a**) [7], as shown in Table 2. The hydroxymethyl group signal was assigned to C-23 on the basis of the gamma effect from this hydroxyl group which was observed at C-3, C-5 and C-24, 4–7 ppm upfield from the unsubstituted compounds. Consequently the structures of compounds **2a** and **3a** were deduced to be methyl 2α,3β,23-trihydroxyolean-12-en-28-oate (methyl arjunolate) and methyl 2α,3β,23-trihydroxyurs-12-en-28-oate (methyl asiatate), respectively. Compound **3a** was identi-

*Part 48 in the series "Studies on Plant Tissue Culture". For Part 47 see Ayabe, S., Udagawa, A., Iida, K., Yoshikawa, T. and Furuya, T. (submitted for publication).



	R¹	R²	R³
2	H	CH_2OH	OH
2a	Me	CH_2OH	OH
4	H	CH_2OH	H
4a	Me	CH_2OH	H
6	H	Me	OH
6a	Me	Me	OH
8	H	Me	H
8a	Me	Me	H



	R¹	R²	R³
3	H	CH_2OH	OH
3a	Me	CH_2OH	OH
5	H	CH_2OH	H
5a	Me	CH_2OH	H
7	H	Me	OH
7a	Me	Me	OH
9	H	Me	H
9a	Me	Me	H

fied with authentic methyl asiatate by direct comparison of their IR spectra.

Epe-3 was methylated and four compounds were isolated by HPLC. Compounds **4a**, **6a** and **7a** were isolated as colourless needles, and compound **5a** was colourless prisms. These four compounds have all the same composition, $C_{31}H_{50}O_4$, on the basis of high resolution mass spectroscopy, and retro-Diels–Alder fragmentation peaks at *m/z* 262 and 203 are observed in all of their mass spectra. As in the case of compounds **2a** and **3a**, it is supposed that compounds **4a**, **5a**, **6a** and **7a** have a ring system that is based upon methyl olean- or urs-12-en-28-oates unsubstituted on the C, D and E rings, and that the residual two hydroxyl groups exist on the A and B rings [6].

In the ^{1}H NMR spectra six methyl signals are observed as singlets in **4a** but two methyl groups appear as doublets and another four as singlets in **5a**. Additionally the H-18 signals are observed as a double doublet in **4a** and as a doublet in **5a** so that it was determined that **4a** is an oleanene derivative and **5a** is an ursene derivative. On the other hand the proton signals attached to the oxygen bearing carbon in **4a** and **5a** are quite similar to each other. As the H-3 proton signal is observed at δ3.62 (**4a**) and 3.69 (**5a**) as a double doublet (J = 8, 8 Hz) and the H-23 proton signals are observed at almost the same chemical shifts in the case of compounds **2a** and **3a**, it is supposed that the two hydroxyl groups of compounds **4a** and **5a** exist at the C-3 and C-23 positions. Consequently compounds **4a** and **5a** are methyl 3β,23-dihydroxyolean-12-en-28-oate (hederagenin methyl ester) and methyl 3β,23-dihydroxyurs-12-en-28-oate (methyl 23-hydroxyursolate), respectively. Additionally compound **4a** is identified with hederagenin methyl ester by direct comparison of IR spectra of **4a** and an authentic sample.

These structures are also supported by the ^{13}C NMR chemical shifts shown in Table 2, because ^{13}C chemical shifts of the A and B rings of **2a** and **3a** are in good agreement with each other, as are those for the A and B rings of **4a** and **5a**, the D and E rings of **2a** and **4a**, and the D and E rings of **3a** and **5a**.

As seven methyl signals are observed as singlets in **6a** and two of them are observed as a doublet in **7a** in the ^{1}H NMR spectra, it is supposed that **6a** and **7a** are oleanene and ursene type compounds, respectively. The signals of protons attached to an oxygen bearing carbon in both compounds are observed at δ2.97 (*dd*, J = 3, 10 Hz H-3) and 3.67 (*ddd*, J = 4, 10, 10 Hz H-2), and the coupling constant of these two signals is 10 Hz so that the configuration of the two hydroxyl groups are 2α and 3β, respectively, as found in compounds **2a** and **3a**. From these results it is presumed that **6a** and **7a** are methyl 2α,3β-dihydroxyolean-12-en-28-oate (methyl maslinate) and 2α,3β-dihydroxyurs-12-en-28-oate (2α-hydroxyursolate), respectively.

Compounds **8a** and **9a** were isolated from Epe-2 as colourless needles, mp 200–203° and mp 111–114°, respectively, by HPLC after methylation. In the mass spectra of **8a** and **9a** characteristic retro-Diels–Alder fragment peaks at *m/z* 262 and 203 are observed. By means of direct comparison with authentic samples by TLC, HPLC, IR and mass spectrometry, it was concluded that compounds **8a** and **9a** are methyl 3β-hydroxyolean-12-en-28-oate (methyl oleanolate) and methyl 3β-hydroxyurs-12-en-28-oate (methyl ursolate), respectively.

When triterpene constituents of cultured cells were compared with the original *E. perriniana* leaves extract by TLC and HPLC after methylation, compounds **2a** and **3a** were the main components of cultured cells. By contrast compounds **2a**, **3a**, **4a** and **5a**, all 23-hydroxymethyl triterpenes, could not be detected, but the 23-methyl triterpenes **8a**, **9a**, **6a** and **7a** were detected in the original leaves. From these results it is apparent that *E. perriniana* cultured cells possess a greater 23-hydroxylation capacity than the original plants and this hydroxlation ability in *E. perriniana* cultured cells is now under investigation.

EXPERIMENTAL

Mps were determined on a Yanagimoto micro melting point apparatus and were uncorr. The ^{1}H NMR spectra were recorded at 90 MHz and 300 MHz, and the ^{13}C NMR spectra were recorded at 25 MHz and 68 MHz, with TMS as an internal standard. MS (70 eV and 20 eV) were taken with a direct inlet.

Table 1. ^{1}H NMR chemical shifts (δ) of compounds **2a**, **3a**, **4a**, **5a**, **6a** and **7a**

	2a	**3a**	**4a**	**5a**	**6a**	**7a**
CH_3	0.68	0.71	0.67	0.72	0.67	0.69
	0.86	0.82 (*d*, J = 6 Hz)	0.85	0.83 (*d*, J = 6 Hz)	0.77	0.78
	0.90	0.89	0.85	0.91 (*d*, J = 6 Hz)	0.85	0.80 (*d*, J = 6 Hz)
	0.90	0.91 (*d*, J = 6 Hz)	0.88	0.92	0.88	0.89 (*d*, J = 6 Hz)
	0.99	1.00	0.91	0.95	0.93	0.94
	1.09	1.04	1.08	1.05	0.98	0.98
					1.08	1.03
H-18	2.84 (*dd*, J = 4, 14 Hz)	2.20 (*d*, J = 12 Hz)	2.82 (*dd*, J = 4, 14 Hz)	2.21 (*d*, J = 12 Hz)	2.82 (*dd*, J = 4, 12 Hz)	2.19 (*d*, J = 12 Hz)
$COOCH_3$	3.61	3.60	3.60	3.60	3.60	3.58
H-2	3.79 (*ddd*, J = 4.5, 10, 11.5 Hz)	3.79 (*ddd*, J = 4.5, 9.5, 11.5 Hz)			3.67 (*ddd*, J = 4, 10, 10 Hz)	3.67 (*ddd*, J = 4, 10, 10 Hz)
H-3	3.48 (*d*, J = 10 Hz)	3.48 (*d*, J = 9.5 Hz)	3.62 (*dd*, J = 8, 8 Hz)	3.69 (*dd*, J = 8, 8 Hz)	2.97 (*dd*, J = 3, 10 Hz)	2.97 (*dd*, J = 3, 10 Hz)
H-23	3.44 (*d*, J = 10.5 Hz)	3.43 (*d*, J = 10.5 Hz)	3.40 (*d*, J = 10 Hz)	3.44 (*d*, J = 10 Hz)		
	3.71 (*d*, J = 10.5 Hz)	3.70 (*d*, J = 10.5 Hz)	3.70 (*d*, J = 10 Hz)	3.73 (*d*, J = 10 Hz)		
H-12	5.28 (*t*, J = 4 Hz)	5.24 (*t*, J = 4 Hz)	5.26 (*t*, J = 4 Hz)	5.25 (*t*, J = 4 Hz)	5.26 (*t*, J = 4 Hz)	5.23 (*t*, J = 4 Hz)

Table 2. ^{13}C NMR chemical shifts (δ) of compounds **2a**, **3a**, **4a** and **5a**

Carbon no.	2a	3a	4a	5a	Carbon no.	2a	3a	4a	5a
1	46.2	46.5	38.3	38.5	16	23.2	24.4	23.2	24.2
2	68.9	68.9	27.0	27.0	17	46.9	48.2	46.9	48.3
3	80.7	80.5	77.0	77.0	18	41.4	53.0	41.5	53.1
4	42.6	42.7	42.0	42.0	19	46.0	39.2*	46.1	39.2†
5	49.4	49.2	50.0	50.0	20	30.8	39.0*	30.8	39.0†
6	18.5	18.5	18.7	18.6	21	34.0	30.8	34.0	30.8
7	32.5	32.8	32.6	32.6	22	32.5	36.8	32.5	36.8
8	39.5	39.7	39.5	39.7	23	70.8	70.4	72.3	72.3
9	47.8	47.6	47.8	47.8	24	12.9	13.0	11.5	11.6
10	38.4	38.3	37.1	37.0	25	17.0‡	17.1§	15.8	16.0
11	23.6	23.5	23.5	23.4	26	17.1‡	17.2§	17.0	17.2
12	122.3	125.4	122.4	125.6	27	26.2	23.9	26.1	23.8
13	144.0	138.4	143.9	138.3	28	178.4	178.2	178.4	178.2
14	41.9	42.3	41.8	42.2	29	33.2	17.3§	33.2	17.2
15	27.8	28.2	27.9	28.2	30	23.8	21.3	23.8	21.3
					$-COOCH_3$	51.6	51.6	51.7	51.6

*,†,‡,§Assignments may be reversed in each vertical column.

Callus induction and culture conditions. Young stems of *Eucalyptus perriniana* (Japanese name: Tsukinuki-Yuhkari) were sterilized with 70% EtOH and a saturated soln of bleaching powder and then rinsed with sterile H_2O and cut into 1 cm segments. These segments were placed on DK agar medium [Murashige and Skoog's basal medium (MS medium) containing 3% sucrose, 0.9% agar, 2,4-dichlorophenoxyacetic acid (2,4-D) 1 ppm and kinetin (K) 1 ppm] and CM agar medium (DK medium containing 7% coconut milk) in May, 1980 and subcultured at 25° in the dark every 3 weeks. These growing calli were also subcultured on other agar media and a year later well grown white cell lines were obtained by subculture on BA1 agar medium [MS medium containing 3% sucrose, 0.9% agar and benzylaminopurine (BA) 1 ppm] and K1 agar medium (MS medium containing sucrose 3%, agar 0.9% and K1 ppm) and coded Epe-BA1 and Epe-K1, respectively.

Extraction and separation of Epe-1, 2, 3 and 4. Freshly harvested Epe-BA1 and Epe-K1 cells (fr. wt 6.29 kg) were homogenized with MeOH and extracted with MeOH (total 20 l.) 3 × (each several days) at room temp. The MeOH extracts were then concentrated under red. pres. and partitioned between EtOAc and H_2O, and the EtOAc layer was dried with dry Na_2SO_4 and evapd. (EtOAc fraction 18.38 g). A part of this fraction was methylated with CH_2N_2 for HPLC analysis. The EtOAc fraction was then chromatographed on a silica gel column repeatedly followed by recrystallization, giving four fractions, Epe-1 (324 mg), Epe-2 (412 mg) and Epe-3 (440 mg) (eluted with EtOAc C_6H_{14}, 1:1) and Epe-4 (1.41 g) (eluted with EtOAc and EtOAc MeOH, 95:5).

Sitosterol **1** *from Epe-1.* Epe-1 was subjected to HPLC (eluent 100% MeOH), and the main peak was collected (161 mg) and recrystallized from MeOH giving pure **1** as colourless flakes (93 mg), mp 138–138.5°; $[\alpha]_D^{22.5}$ − 32.5° (*c* 0.67, $CHCl_3$); (found: C, 84.00; H, 12.10, required for $C_{29}H_{50}O$: C, 83.99; H, 12.15%); EIMS *m/z* (rel. int.): 414 [M]$^+$ (100), 399 (30), 396 (40), 381 (25), 329 (28), 303 (41), 273 (20), 255 (22), 231 (18), 213 (27), 163 (18), 161 (24), 159 (13), 145 (32), 107 (43), 105 (34), 95 (37), 93 (32), 91 (31), 81 (37), 69 (32), 43 (90); IR ν_{max}^{KBr} cm^{-1}: 3250 3550 (OH); ^1H NMR (100 MHz, $CDCl_3$): δ0.75 (3H, *s*), 0.78 (3H, *d*), 0.86 (3H, *d*), 0.93 (3H, *d*), 1.00 (3H, *s*), 1.19 (3H, *t*), 3.5 (1H, *br*, H-3), 5.3 (1H, *m*, H-6); ^{13}C NMR (25 MHz, $CDCl_3$): δ 141.1 (*s*), 121.9 (*d*), 71.9 (*d*), 56.9 (*d*), 56.3 (*d*), 50.3 (*d*), 46.0 (*d*), 42.4 (*s*, *t*), 39.9 (*t*), 37.4 (*t*), 36.6 (*s*), 36.2 (*d*), 34.1 (*t*), 32.0 (*d*, *t*), 31.7 (*t*), 29.3 (*d*), 28.3 (*t*), 26.3 (*t*), 24.4 (*t*), 23.2 (*t*), 21.2 (*t*), 19.9 (*q*), 19.1 (*q*), 18.8 (*q*), 12.0 (*q*), 11.9 (*q*).

Acetylation of sitosterol (**1**). Compound **1** was acetylated by Ac_2O and pyridine in the usual manner and recrystallized from MeOH to yield **1a** (47 mg); colourless needles; mp 120–121°; $[\alpha]_D^{22.5}$ − 36.7° (*c* 1.04, $CHCl_3$); (found: C, 81.66; H, 11.48, required for $C_{31}H_{52}O_2$: C, 81.52; H, 11.48%); EIMS *m/z* (rel. int.): 456 [M]$^+$ (1), 399 (1), 397 (36), 396 (100), 381 (12), 288 (10), 275 (8), 255 (9), 213 (7), 163 (45), 161 (5), 159 (5), 147 (20), 145 (10), 107 (6), 105 (3), 95 (5), 93 (3), 81 (11), 43 (1); ^1H NMR (100 MHz, $CDCl_3$): δ0.67 (3H, *s*), 0.79 (3H, *d*), 0.85 (3H, *d*), 0.93 (3H, *d*), 1.00 (3H, *s*), 1.17 (3H, *t*), 2.00 (3H, *s*, $COCH_3$), 4.6 (1H, *br*, H-3), 5.3 (1H, *m*, H-6); ^{13}C NMR (25 MHz, $CDCl_3$): δ170.7 (*s*), 139.9 (*s*), 122.8 (*d*), 74.1 (*d*), 56.8 (*d*), 56.2 (*d*), 50.1 (*d*), 46.0 (*d*), 42.4 (*s*), 39.8 (*t*), 38.2 (*t*), 37.1 (*t*), 36.6 (*s*), 36.2 (*d*), 34.0 (*t*), 31.9 (*d*, *t*), 29.3 (*d*), 28.2 (*t*), 27.8 (*t*), 26.3 (*t*), 24.3 (*t*), 23.1 (*t*), 21.4 (*q*), 21.1 (*t*), 19.8 (*q*), 19.3 (*q*), 19.1 (*q*), 18.8 (*q*), 12.0 (*q*), 11.8 (*q*).

Compounds **2a** *and* **3a** *from Epe-4.* Epe-4 was subjected to HPLC (eluent 80% MeOH), and the main peak was collected (853 mg). This purified Epe-4 (734 mg) was treated with CH_2N_2 in Et_2O; the methylation products were further purified on a silica gel column (CH_2Cl_2, MeOH, 20:1) and by HPLC (80% MeOH). After recrystallization from MeOH compounds **2a** (55 mg) and **3a** (165 mg) were obtained.

Methyl arjunolate (**2a**). Colourless needles; mp 227 237°; $[\alpha]_D^{22.5}$ + 53.1° (*c* 0.58, $CHCl_3$); (found: C, 73.80; H, 10.07, required for $C_{31}H_{50}O_5$: C, 74.06; H, 10.03%); EIMS *m/z* (rel. int.): 502 [M]$^+$ (3), 484 (1), 466 (2), 442 (4), 407 (5), 262 (73), 249 (11), 203 (100), 189 (17), 133 (7); high resolution EIMS: [M]$^+$, 502.3650 required for $C_{31}H_{50}O_5$, 502.3657; IR ν_{max}^{KBr} cm^{-1}: 3225 3575 (OH), 1730 ($COOCH_3$).

Methyl asiatate (**3a**). Colourless needles; mp 229 231°; $[\alpha]_D^{22.5}$ + 53.3° (*c* 1.03, $CHCl_3$); EIMS *m/z* (rel. int.): 502 [M]$^+$ (3), 484 (1), 466 (2), 442 (3), 407 (1), 262 (100), 249 (14), 203 (71), 189 (12), 133 (22); high resolution EIMS: [M]$^+$, 502.3649, required for $C_{31}H_{50}O_5$, 502.3657; IR ν_{max}^{KBr} cm^{-1}: 3225–3550 (OH), 1735 ($COOCH_3$).

Acetylation of compounds **2a** *and* **3a**. Compounds **2a** and **3a** (each 40 mg) were acetylated by Ac_2O–pyridine and purified by

silica gel column chromatography (CH_2Cl_2), and the acetylation products **2b** (41 mg) and **3b** (40 mg) were obtained. Methyl triacetylarjunolate (**2b**), amorphous solid; ^{1}H NMR (90 MHz, $CDCl_3$): δ0.72 (3H, *s*), 0.89 (3H, *s*), 0.92 (3H, *s*), 1.08 (3H, *s*), 1.10 (3H, *s*), 1.97 (3H, *s*), 2.00 (3H, *s*), 2.07 (3H, *s*), 3.55 (1H, *d*, J = 12 Hz), 3.60 (3H, *s*), 3.84 (1H, *d*, J = 12 Hz), 5.2 (3H, *m*). Methyl triacetylasiatate (**3b**), amorphous solid; ^{1}H NMR (90 MHz, $CDCl_3$): δ0.75 (3H, *s*), 0.88 (3H, *s*), 1.07 (3H, *s*), 1.09 (3H, *s*), 1.96 (3H, *s*), 2.00 (3H, *s*), 2.07 (3H, *s*), 3.54 (1H, *d*, J = 12 Hz), 3.58 (3H, *s*), 3.84 (1H, *d*, J = 12 Hz), 5.2 (3H, *m*).

Compounds **4a**, **5a**, **6a** *and* **7a** *from Epe*-3. Epe-3 (341 mg) was treated with CH_2N_2 in Et_2O and chromatographed on a silica gel column (CH_2Cl_2–MeOH, 100:1) followed by HPLC (90% MeOH). After recrystallization from MeOH, compounds **4a** (16 mg), **5a** (84 mg), **6a** (6 mg) and **7a** (18 mg) were obtained.

Hederagenin methyl ester (**4a**). Colourless needles; mp 219 222°; EIMS *m/z* (rel. int.): 486 [M]$^+$ (4), 468 (2), 426 (2), 262 (100), 249 (7), 223 (12), 203 (97), 189 (10), 133 (7); high resolution EIMS: [M]$^+$, 486.3705, required for $C_{31}H_{50}O_4$, 486.3706; IR ν_{max}^{KBr} cm^{-1} 3250–3575 (OH), 1710 ($COOCH_3$).

Methyl 23-hydroxyursolate (**5a**). Colourless prisms; mp 254 257°; $[\alpha]_D^{22.5}$ + 65.1° (*c* 0.98, $CHCl_3$); EIMS *m/z* (rel. int.): 486 [M]$^+$ (3), 468 (1), 426 (2), 262 (100), 249 (8), 223 (15), 203 (57), 189 (10), 133 (13); high resolution EIMS: [M]$^+$, 486.3707, required for $C_{31}H_{50}O_4$, 486.3706; IR ν_{max}^{KBr} cm^{-1}: 3225 3500 (OH), 1725 ($COOCH_3$).

Methyl maslinate (**6a**). Colourless needles; mp 225–227°; EIMS *m/z* (rel. int.): 486 [M]$^+$ (13), 468 (6), 426 (6), 391 (2), 262 (100), 249 (16), 223 (10), 203 (90), 189 (16), 133 (3); high resolution EIMS: [M]$^+$, 486.3707, required for $C_{31}H_{50}O_4$, 486.3706; IR ν_{max}^{KBr} cm^{-1}: 3225 3550 (OH), 1730 ($COOCH_3$).

Methyl 2α-hydroxyursolate (**7a**). Colourless needles; mp 204 207°; EIMS *m/z* (rel. int.): 486 [M]$^+$ (4), 468 (1), 426 (4), 262 (100), 249 (13), 233 (6), 223 (11), 203 (72), 189 (12), 133 (19); high resolution EIMS: [M]$^+$, 486.3707, required for $C_{31}H_{50}O_4$, 486.3706; IR ν_{max}^{KBr} cm^{-1}: 3200–3550 (OH), 1735 ($COOCH_3$).

Compounds **8a** *and* **9a** *from Epe*-2. Epe-2 (272 mg) was methylated as mentioned above and compounds **8a** (22 mg) and **9a** (179 mg) were obtained by HPLC (97% MeOH) and recrystallization from MeOH.

Methyl oleanolate (**8a**). Colourless needles; mp 200–203°; $[\alpha]_D^{22.5}$ + 66.7° (*c* 0.87, $CHCl_3$); EIMS *m/z* (rel. int.): 470 [M]$^+$ (2), 452 (3), 410 (2), 262 (49), 249 (3), 203 (100), 189 (25), 133 (15); IR ν_{max}^{KBr} cm^{-1}: 3250–3550 (OH), 1725 ($COOCH_3$).

Methyl ursolate (**9a**). Colourless needles; mp 111–114°; $[\alpha]_D^{22.5}$ + 65.5° (*c* 1.02, $CHCl_3$); EIMS *m/z* (rel. int.): 470 [M]$^+$ (2), 452 (6), 410 (3), 262 (95), 249 (9), 207 (24), 203 (100), 189 (35), 133 (68); IR ν_{max}^{KBr} cm^{-1}: 3200–3525 (OH), 1730 ($COOCH_3$).

Extraction of triterpenes from E. perriniana *leaves*. After steam distillation of *E. perriniana* leaves (fr. wt 50 g) they were extracted with MeOH (1 l. × 2) and the solvent evapd. The MeOH extracted material was then extracted with EtOAc (250 ml × 3) and the EtOAc fraction was washed with 5% $NaHCO_3$ to remove acidic components. The EtOAc fraction was treated with dry. Na_2SO_4 and the solvent evapd. This fraction was methylated with CH_2N_2 in Et_2O for HPLC.

HPLC conditions. Analysis and purification by HPLC were performed on a Shimadzu liquid chromatograph LC-3A instrument with a Shimadzu spectrophotometric detector model SPD-2A and a Showa Denko differential refractometer model Shodex RI SE-11. A column (7.6 × 300 mm) packed with Unisil Q C18 (5 μm) was used and solvents are shown for each experiment.

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