[Chem. Pharm. Bull. 32(10)3906—3911(1984)]

Studies on the Constituents of Medicinal and Related Plants in Sri Lanka. I. New Triterpenes from *Hedyotis lawsoniae*

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(Received February 18, 1984)

Three new triterpene acids were isolated from the twigs and leaves of *Hedyotis lawsoniae*, together with sixteen known ones. The new compounds were determined to be 3β,23-dihydroxyurs-12-en-28-oic acid, 3β,24-dihydroxyurs-12-en-28-oic acid, and 2α,3β,24-trihydroxyurs-12-en-28-oic acid on the basis of spectroscopic evidence.

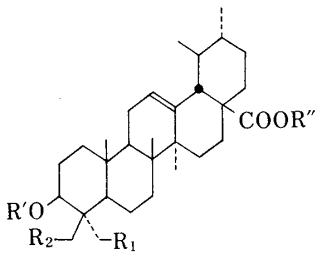
Keywords——*Hedyotis lawsoniae*; Rubiaceae; triterpene; 3β,23-dihydroxyurs-12-en-28-oic acid; 3β,24-dihydroxyurs-12-en-28-oic acid; 2α,3β,24-trihydroxyurs-12-en-28-oic acid

Some of the *Hedyotis* plants (Rubiaceae) are used as folk medicines in India and Sri Lanka. For instance, *H. auricularia* L. was used for the treatment of diarrhea and dysentery in India, while it is taken as a vegetable effective to reduce high blood pressure in Sri Lanka.[1] In the course of our studies on the biologically active constituents of medicinal plants and related plants in Sri Lanka, we obtained three new triterpenes from *Hedyotis lawsoniae* (DC.) WIGHT *et* ARN., along with sixteen known ones. This paper describes the structure assignments of these triterpenes.

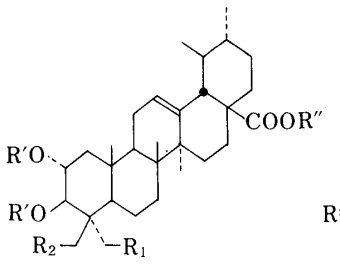
The methanolic extract of dried twigs and leaves of *Hedyotis lawsoniae* was separated into the ethyl acetate-soluble part and the water-soluble one. The former was roughly separated by silica gel column chromatography, and the fractions were further separated by preparative layer chromatography (PLC), or high-performance liquid chromatography (HPLC), giving new triterpenes (**1a**—**3a**) along with known ones (**4a**—**19a**) (see Experimental). Among these, ursolic acid (**4a**), 3-epiursolic acid (**5a**),[2] ursonic acid (**6a**), asiatic acid (**8a**),[3] euscaphic acid (**12a**),[4] oleanolic acid (**13a**), oleanonic acid (**14a**), sumaresinolic acid (**15a**),[5] hederagenin (**16a**), arjunolic acid (**17a**),[3] betulinic acid (**18a**), and betulin (**19a**) were identified by direct comparisons of the proton nuclear magnetic resonance (^{1}H-NMR) spectra, gas chromatographic (GC) retention times, and gas chromatography-mass spectrometry (GC-MS) data with those of corresponding authentic samples. On the other hand, identification of 2α-hydroxyursolic acid (**7a**),[6] 2α-hydroxy-3-epiursolic acid (**9a**),[7] 2α,3α,23-trihydroxyurs-12-en-28-oic acid (**10a**),[8] and benthamic acid (**11a**)[9] was performed by comparisons of the MS and ^{1}H-NMR spectra with those described in the literature.

Compounds **1a** and **2a** were isolated as the methyl ester diacetates: **1b**, mp 180—181.5 °C, $C_{35}H_{54}O_6$, $[\alpha]_D$ +64.1° ($CHCl_3$), and **2b**, mp 180—182 °C, $C_{35}H_{54}O_6$, $[\alpha]_D$ +50.8° ($CHCl_3$), after methylation and acetylation. The purification of **1b** was difficult, but it was achieved by preparative HPLC.

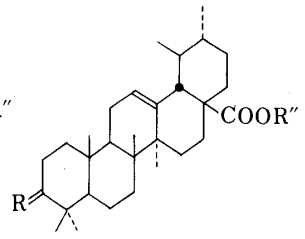
In the MS, both compounds showed the base peak at *m/z* 262, ascribable to the



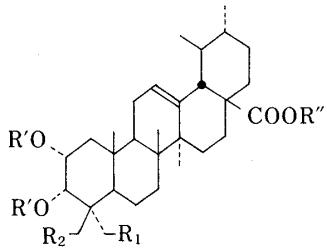
1 : R_1=OR′, R_2=H
2 : R_1=H, R_2=OR′
4 : R_1=R_2=H



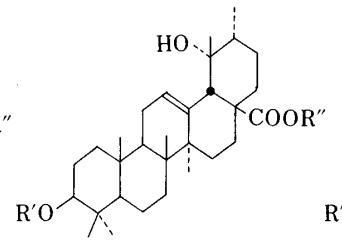
3 : R_1=H, R_2=OR′
7 : R_1=R_2=H
8 : R_1=OR′, R_2=H



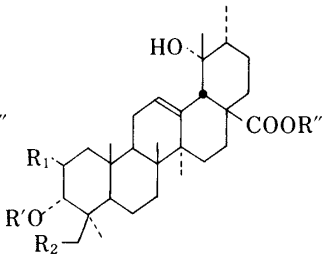
5 : R=α-OR′, β-H
6 : R=O



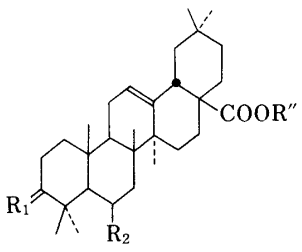
9 : R_1=R_2=H
10 : R_1=OR′, R_2=H
20 : R_1=H, R_2=OR′



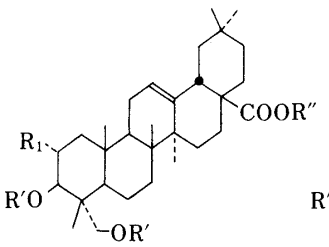
11



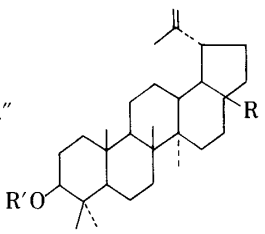
12 : R_1=OR′, R_2=H
21 : R_1=H, R_2=OR′



13 : R_1=α-H, β-OR′, R_2=H
14 : R_1=O, R_2=H
15 : R_1=α-H, β-OR′, R_2=OH



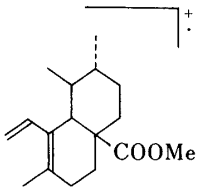
16 : R=H
17 : R=OR′



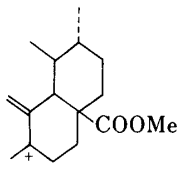
18 : R=COOR″
19 : R=CH_2OR′

a : R′=R″=H, **b** : R′=Ac, R″=Me

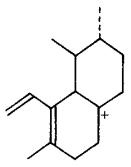
Chart 1



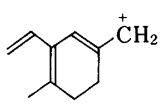
a : *m/z* 262



b : *m/z* 249



c : *m/z* 203



d : *m/z* 133

Chart 2

retro-Diels–Alder fragment ion (a), along with peaks at *m/z* 249 (b), 203 (c), 189 (c-14), and 133 (d), which are characteristic of Δ^{12}-oleanene or Δ^{12}-ursene type triterpenes (Chart 2).[10] The ^{1}H-NMR spectra of **1b** and **2b** showed significant signals assignable to the 18-proton at δ 2.25 (d, J=11 Hz) and the 12-olefinic proton at δ 5.25 (br t, J=3.5 Hz), together with two

sec-methyl and four *tert*-methyl signals, whose signal pattern is suggestive of the structural features of urs-12-en-28-oic acid.[11] In view of these data, **1b** and **2b** were presumed to be methyl urs-12-en-28-oate derivatives having two acetoxyl groups. These two acetoxyls may be present at ring A rather than ring B, since the acetylation of **1a** and **2a** leading to **1b** and **2b**, respectively, proceeded smoothly under very mild conditions. The positions and configuration of these acetoxyls in each compound were determined by ^{1}H-NMR analyses as described below.

The ^{1}H-NMR spectrum of **1b** showed signals due to a methine proton at δ 4.81 (dd, J= 10, 5.5 Hz, CHOAc) and methylene protons at δ 3.71 and 3.88 (each 1H, d, J=12 Hz, CH_2OAc), along with two acetyl methyl signals (δ 2.02 and 2.07). This spectral pattern closely resembles that of authentic **16b**. Thus, the compound was determined to be methyl 3β,23-diacetoxyurs-12-en-28-oate (**1b**).

On the other hand, compound **2b** showed a ^{1}H-NMR spectrum very similar to that of **1b**, except for signals arising from a CH_2OAc group and a *tert*-methyl group. The signals due to the acetoxyl-bearing methylene (δ 4.13 and 4.36, each 1H, d, J=12 Hz) were shifted downfield relative to the 23-methylene signals of **1b**, and these chemical shifts are virtually the same as those of the 24-methylene protons of methyl 2α,3α,24-triacetoxyurs-12-en-28-oate (**20b**)[12] and methyl barbinervate diacetate (**21b**).[13] In addition, the methyl group at the C-4 position resonated downfield (*ca.* 0.08 ppm) relative to the 23-methyl signal of **4b**. This behavior was also parallel with those of **20b** and **21b**, whose 23-methyl signals appeared downfield relative to those of **9b** and **5b**, as shown in Table I. On the other hand, in the case of 23-acetoxylated triterpenes, the signal of the 24-methyl group was reported to appear at about the same region as the corresponding signal of non-substituted compounds.[11a] Therefore, these data indicated that the primary acetoxyl group of **2b** is located at the 24-position. Since the signal due to the 3-proton was observed at δ 4.60 (dd, J=9, 7 Hz), this compound was established to be methyl 3β,24-diacetoxyurs-12-en-28-oate (**2b**).

Compound **3a** was also obtained as the methyl ester triacetate (**3b**), amorphous powder, $C_{37}H_{56}O_8$, $[\alpha]_D$ +51° ($CHCl_3$). The gross structure of this compound was suggested by the MS base peak at m/z 262 (a) and the ^{1}H-NMR pattern, which is similar to that of **2b**. Furthermore, the ^{1}H-NMR spectrum showed signals due to the methine protons geminal to acetoxyl groups at δ 4.86 (d, J=10.5 Hz) and 5.18 (td, J=10.5 and 4.5 Hz), indicating the presence of a 2α,3β-glycol diacetate system. The signals arising from the methylene protons at δ 4.21 (s) and a methyl group at δ 1.02 (Table I) suggested that the third acetoxyl group should be located at the 24-position. In addition, the 3α-proton of **2b** and **3b** resonated downfield by 0.09 and 0.08 ppm, respectively, relative to the corresponding proton of **4b** and **7b**,

TABLE I. Chemical Shifts of Methyl Signals in Several 24-Acetoxylated Triterpenes (δ in $CDCl_3$)

Compound (Position of AcO group)	Methyl signals			
	23	24	25	26
4b (3β)	0.87	0.88	0.94	0.74
2b (3β, 24)	0.95[a]	—	1.01[a]	0.74
5b (3α)	0.86	0.90	0.95	0.76
21b (3α, 24)	0.95[a]	—	0.93[a]	0.68
7b (2α, 3β)	0.90	0.90	1.05	0.74
3b (2α, 3β, 24)	1.02	—	1.06	0.73
9b (2α, 3α)	0.87	0.98	1.04	0.75
20b (2α, 3α, 24)	0.98	—	1.05	0.75

a) Assignments may be interchanged as indicated in each compound.

TABLE II. Chemical Shifts of Methine and Methylene Protons of Carbons Carrying Acetoxyl Groups (δ in $CDCl_3$)

Compound (Position of AcO group)	Chemical shifts		
	2β-H	3α-H	23-H_2 or 24-H_2
4b (3β)	—	4.51 (dd, J=9, 7 Hz)	—
1b (3β, 23)	—	4.81 (dd, J=10, 5.5 Hz)	3.71, 3.88 (each d, J=12 Hz)
2b (3β, 24)	—	4.60 (dd, J=9, 7 Hz)	4.13, 4.36 (each d, J=12 Hz)
7b (2α, 3β)	5.14 (td, J=10, 4.5 Hz)	4.78 (d, J=10 Hz)	—
8b (2α, 3β, 23)	5.2 (td, J=10, 4.5 Hz)	5.12 (d, J=10 Hz)	3.62, 3.89 (each d, J=12 Hz)
3b (2α, 3β, 24)	5.18 (td, J=10.5, 4.5 Hz)	4.86 (d, J=10.5 Hz)	4.20 (s)

whereas that of **1b** and **8b** appeared further downfield by 0.30 and 0.34 ppm, respectively, in comparison with that of **4b** and **7b**, as shown in Table II. These results supported the *trans* relationship between the acetoxymethyl group and the 3α-proton in **3b**. From the above findings, compound **3b** was determined to be 2α,3β,24-triacetoxyurs-12-en-28-oate (**3b**).

Experimental

Melting points were determined with a Kofler-type apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 automatic polarimeter. ^{1}H-NMR spectra were recorded on a Varian Associates XL-200 spectrometer and tetramethylsilane (TMS) in $CDCl_3$ was used as an internal standard. MS and high resolution MS were obtained with a JEOL JMS-D 300 spectrometer. Column chromatography was carried out using Mallinckrodt silica gel and the columns were eluted with MeOH–$CHCl_3$ mixtures of increasing MeOH contents, unless otherwise stated. The eluted solutions were concentrated *in vacuo*. For thin layer chromatography (TLC), Kieselgel 60 F_{254} (Merck) was used and spots were detected by spraying a $Ce(SO_4)_2$–aq. H_2SO_4 reagent. For PLC, Kieselgel PF_{254} or F_{254} (Merck) was employed and the plates were examined after exposure to I_2 vapor or under ultraviolet (UV) light. Extraction of substances from the silica gel was done with MeOH–CH_2Cl_2 mixture. The organic solutions were dried over anhydrous $MgSO_4$. Gas chromatography (GC) was done on a GC-6AM instrument (Shimadzu) with a 3% SE-300 or a 2% OV-17 column (2 m × 3 mm i.d. glass tube; injection temp. 300 °C, column temp. 280 °C, carrier gas N_2).

Isolation of Triterpenes from *H. lawsoniae*——Dried twigs and leaves (1.69 kg) of *H. lawsoniae*, collected at Horton Plains in Sri Lanka, were extracted with boiling MeOH (5 l × 3) and the extracts were combined and concentrated *in vacuo*. The residue was diluted with water (1 l) and extracted with AcOEt (500 ml × 3). The combined AcOEt extract was washed with water, dried, and concentrated *in vacuo* to give a syrupy residue (20 g). This was suspended in MeOH–CH_2Cl_2 (1 : 9) (200 ml) and the insoluble material (F-I) (4.58 g) was separated by filtration. The filtrate was concentrated *in vacuo* and the residue (15 g) was subjected to silica gel (450 g) column chromatography, giving four fractions containing triterpenes: *viz.* the MeOH–$CHCl_3$ (2 : 98) eluate (F-II, 2.57 g), the earlier part of the MeOH–$CHCl_3$ (5 : 95) eluate (F-III, 1.81 g), the later part of the MeOH–$CHCl_3$ (5 : 95) eluate (F-IV, 1.45 g), and the MeOH–$CHCl_3$ (1 : 9) eluate (F-V, 1.12 g).

F-I was rechromatographed on a silica gel (200 g) column. The eluate with MeOH–$CHCl_3$ (1 : 99) was concentrated to give ursolic acid (**4a**) (1.45 g) as a colorless powder. The subsequent eluate with MeOH–$CHCl_3$ (5 : 95) (1.27 g) was methylated with CH_2N_2 and subjected to PLC with acetone–$CHCl_3$ (5 : 95) as the eluent. The less polar band gave methyl ursolate (0.97 g), which was identified by GC, MS, and ^{1}H-NMR comparisons with an authentic sample. The more polar band afforded the methyl ester of benthamic acid (**11a**) (60 mg), amorphous powder, $[\alpha]_D^{22}$ +29.8° (c=0.55, $CHCl_3$). MS m/z: 486 (M^+), 468, 278, 260, 201, 179. High resolution MS: Found 486.3699, Calcd for $C_{31}H_{50}O_4$ (M^+) 486.3708. This ester was acetylated with Ac_2O–pyridine and recrystallized from MeOH to give the monoacetate (**11b**) (31 mg), colorless needles, mp 197—199 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.69 (3H, s,

26-H_3), 0.87 (3H, s, 23-H_3), 0.88 (3H, s, 24-H_3), 0.94 (3H, s, 25-H_3), 0.96 (3H, d, J=6 Hz, 30-H_3), 1.23 (3H, s, 27-H_3), 1.25 (3H, s, 29-H_3), 2.07 (3H, s, OAc), 2.62 (1H, s, 18-H), 3.63 (3H, s, $COOCH_3$), 4.54 (1H, dd, J=9, 7 Hz, 3-H), and 5.39 (1H, t, J=3.5 Hz, 12-H). The above data are practically identical with those of methyl benthamate monoacetate (**11b**) reported in the literature.[9]

F-II and F-III were each suspended in CH_2Cl_2 and the insoluble substances (450 mg and 710 mg, respectively) were collected by filtration and methylated with CH_2N_2 to give an additional crop of methyl ursolate. The filtrate from F-II was concentrated and the residue (2.1 g) was diluted with dil. NaOH (100 ml), then extracted with CH_2Cl_2 (50 ml × 3). The CH_2Cl_2 layer was washed with water, dried, and concentrated to give a residue (1.5 g). This residue was roughly separated by silica gel (80 g) column chromatography and the fractions were further purified by repeated PLC using acetone–$CHCl_3$ (5 : 95) as the eluent to give oleanolic acid (**13a**) (130 mg), 3-epiursolic acid (**5a**) (4.2 mg), a mixture of ursonic acid (**6a**) and oleanonic acid (**14a**) (3.9 mg), betulinic acid (**18a**) (13 mg), and betulin (**19a**) (2 mg). These compounds except for **5a** were methylated with CH_2N_2 and identified by GC, MS, and ^{1}H-NMR comparisons with corresponding authentic samples. Compound **5a** (1.6 mg) was methylated with CH_2N_2 and oxidized with CrO_3–AcOH at room temperature for 2 h. The product was purified by PLC (developed with acetone–$CHCl_3$, 5 : 95) to give **6b** (1 mg), which was identified by GC and ^{1}H-NMR comparison with an authentic sample.

F-IV was acetylated with Ac_2O–pyridine at room temperature for 4 h and the product was subjected to silica gel (70 g) column chromatography with AcOEt–hexane as the eluent. The AcOEt–hexane (2 : 8) eluate (178 mg) was methylated with CH_2N_2 and the product was subjected to PLC with $CHCl_3$ to afford the following compounds.

1) Methyl 2α-hydroxyursolate diacetate (**7b**) (18 mg), amorphous powder, $[\alpha]_D^{22}$ +17° (c=1.7, $CHCl_3$). MS m/z: 570 (M^+), 510, 450, 262, 249, 203, 189, 133. High resolution MS: Found 570.3912, Calcd for $C_{35}H_{54}O_6$ (M^+) 570.3919. ^{1}H-NMR ($CDCl_3$) δ: 0.74 (3H, s, 26-H_3), 0.83—0.90 (6H, 29- and 30-H_3), 0.90 (6H, s, 23- and 24-H_3), 1.05 (3H, s, 25-H_3), 1.07 (3H, s, 27-H_3), 1.98 and 2.07 (each 3H, s, OAc), 2.25 (1H, d, J=11 Hz, 18-H), 3.62 (3H, s, $COOCH_3$), 4.78 (1H, d, J=10 Hz, 3-H), 5.14 (1H, td, J=10, 4.5 Hz, 2-H), and 5.23 (1H, m, 12-H). These spectral data are compatible with those of **7b** reported in the literature.[6]

2) A mixture of methyl 3β,23-diacetoxyurs-12-en-28-oate (**1b**) and methyl hederagenin diacetate (**16b**) (12 mg, approximately 7 : 3 on GC analysis). Purification of this mixture by HPLC is described below.

3) Methyl 3β,24-diacetoxyurs-12-en-28-oate (**2b**) (2 mg), colorless needles (from MeOH), mp 180 −182 °C, $[\alpha]_D^{22}$ +50.8° (c=0.12, $CHCl_3$). MS m/z: 570 (M^+), 510, 450, 262, 249, 203, 189, 133. High resolution MS: Found 570.3882, Calcd for $C_{35}H_{54}O_6$ (M^+) 570.3919; Found 262.1922, Calcd for $C_{17}H_{26}O_2$ (a) 262.1932; Found 249.1815, Calcd for $C_{16}H_{25}O_2$ (b) 249.1854; Found 203.1790, Calcd for $C_{15}H_{23}$ (c) 203.1800; Found 189.1627, Calcd for $C_{14}H_{21}$ (c-14) 189.1642; Found 133.0991, Calcd for $C_{10}H_{13}$ (d) 133.1016. IR ν_{max}^{KBr} cm^{-1}: 1730, 1230. ^{1}H-NMR ($CDCl_3$) δ: 0.74 (3H, s, 26-H_3), 0.85 and 0.94 (each 3H, d, J=6 Hz, 29- and 30-H_3), 0.95 (3H, s, 23-H_3), 1.01 (3H, s, 25-H_3), 1.07 (3H, s, 27-H_3), 2.05 and 2.07 (each 3H, s, OAc), 2.25 (1H, d, J=11 Hz, 18-H), 3.61 (3H, s, $COOCH_3$), 4.13 and 4.36 (each 1H, d, J=12 Hz, 24-H_2), 4.60 (1H, dd, J=9, 7 Hz, 3-H), and 5.26 (1H, br t, J=3.5 Hz, 12-H).

4) Methyl 2α-hydroxy-3-epiursolate diacetate (**9b**) (3 mg), amorphous powder, $[\alpha]_D^{22}$ +15° (c=0.25, $CHCl_3$). MS m/z: 570 (M^+), 510, 450, 262, 249, 203, 189, 133. High resolution MS: Found 570.3876, Calcd for $C_{35}H_{54}O_6$ (M^+) 570.3919. ^{1}H-NMR ($CDCl_3$) δ: 0.75 (3H, s, 26-H_3), 0.85 and 0.93 (each 3H, d, J=6 Hz, 29- and 30-H_3), 0.87 (3H, s, 23-H_3), 0.98 (3H, s, 24-H_3), 1.04 (3H, s, 25-H_3), 1.11 (3H, s, 27-H_3), 1.97 and 2.12 (each 3H, s, OAc), 2.25 (1H, d, J=11 Hz, 18-H), 3.62 (3H, s, $COOCH_3$), 4.99 (1H, d, J=2.5 Hz, 3-H), and 5.20—5.32 (2H, m, 2- and 12-H). These spectral data are compatible with the reported values.[7]

5) Methyl euscaphate diacetate (**12b**) (4 mg), colorless needles, mp 115—118 °C, $[\alpha]_D^{22}$ +17° (c=0.35, $CHCl_3$). This compound was identified by direct comparison of the TLC behavior, ^{1}H-NMR, and GC with those of an authentic sample.

6) Methyl sumaresinolate monoacetate (**15b**) (4 mg), colorless needles (from EtOH), mp 220—222 °C. This was identified by GC and ^{1}H-NMR comparisons with an authentic sample.

F-V (1.0 g) was also acetylated in the same manner as described above and the product was chromatographed on a silica gel (50 g) column. The eluate with MeOH–$CHCl_3$ (2 : 98) (120 mg) was treated with CH_2N_2 and the resulting methyl ester was separated by PLC with AcOEt–benzene (2 : 8) as the eluent. The least polar band afforded methyl 2α,3α,23-triacetoxyurs-12-en-28-oate (**10b**) (10 mg), amorphous powder, $[\alpha]_D^{22}$ +37° (c=0.35, $CHCl_3$). MS m/z: 628 (M^+), 568, 508, 448, 262, 249, 203, 189, 133. High resolution MS: Found 628.4022, Calcd for $C_{37}H_{56}O_8$ 628.3975. ^{1}H-NMR ($CDCl_3$) δ: 0.75 (3H, s, 26-H_3), 0.85 and 0.93 (each 3H, d, J=6 Hz, 29- and 30-H_3), 1.07 (3H, s, 24-H_3), 1.11 (6H, s, 25- and 27-H_3), 1.95, 2.00, and 2.06 (each 3H, s, OAc), 2.25 (1H, d, J=11 Hz, 18-H), 3.61 (3H, s, $COOCH_3$), 3.73 and 4.11 (each 1H, d, J=12 Hz, 23-H_2), and 5.18—5.37 (3H, m, 2-, 3-, and 12-H).[8] The middle band gave a mixture of methyl asiatate triacetate (**8b**) and methyl arjunolate triacetate (**17b**) (40 mg) (approximate ratio of 62 : 38 by GC analysis). Both compounds were identified by GC and GC-MS comparisons with corresponding authentic samples. The most polar band gave impure methyl 2α,3β,24-triacetoxyurs-12-en-28-oate (**3b**) (6 mg), purification of which is described below.

Separation of the Mixture of Methyl 3β,23-Triacetoxyurs-12-en-28-oate (1b) and Methyl Hederagenin Diacetate (16b) by HPLC——Preparative HPLC was performed on a Waters Associates ALC/GPC 201D compact type liquid chromatograph using a TSK-GEL ODS-120A column (column size, 4.6 mm i.d. × 25 cm; detector setting, UV

215 nm) with acetonitrile as the eluent (flow rate 0.5 ml/min). The mixture (**1b** and **16b**) (12 mg) gave **16b** (1.0 mg) and **1b** (3.7 mg). The former was identified by GC, MS, and ^{1}H-NMR comparisons with an authentic sample. Methyl 3β,23-diacetoxyurs-12-en-28-oate (**1b**) was obtained as colorless needles (from EtOH), mp 180—181.5 °C, $[\alpha]_D^{21}$ +64.1° (c=0.22, $CHCl_3$). MS m/z: 570 (M^+), 510, 450, 262, 249, 203, 189, 133. High resolution MS: Found 570.3913, Calcd for $C_{35}H_{54}O_6$ (M^+) 570.3919; Found 262.1937, Calcd for $C_{17}H_{26}O_2$ (a) 262.1933; Found 249.1842, Calcd for $C_{16}H_{25}O_2$ (b) 249.1854; Found 203.1789, Calcd for $C_{15}H_{23}$ (c) 203.1799. IR ν_{max}^{KBr} cm^{-1}: 1730, 1230. ^{1}H-NMR ($CDCl_3$) δ: 0.75 (3H, s, 26-H_3), 0.83 (3H, s, 24-H_3), 0.87 and 0.94 (each 3H, d, J=6 Hz, 29- and 30-H_3), 0.98 (3H, s, 25-H_3), 1.07 (3H, s, 27-H_3), 2.02 and 2.07 (each 3H, s, OAc), 2.25 (1H, d, J=11 Hz, 18-H), 3.62 (3H, s, $COOCH_3$), 3.71 and 3.88 (each 1H, d, J=12 Hz, 23-H_2), 4.81 (1H, dd, J=10, 5.5 Hz, 3-H), and 5.23 (1H, br t, J=3.5 Hz, 12-H).

Purification of Methyl 2α,3β,24-Triacetoxyurs-12-en-28-oate (3b) by HPLC——Preparative HPLC of the above-mentioned impure **3b** under the same conditions as used for **1b**, gave a pure sample (**3b**) (1.4 mg), amorphous powder, $[\alpha]_D^{21}$ +51° (c=0.1, $CHCl_3$). MS m/z: 628 (M^+), 568, 508, 262, 249, 203, 189, 133. High resolution MS: Found 628.3954, Calcd for $C_{37}H_{56}O_8$ (M^+) 628.3974; Found 262.1918, Calcd for $C_{17}H_{26}O_2$ (a) 262.1932; Found 249.1820, Calcd for $C_{16}H_{25}O_2$ (b) 249.1854; Found 203.1785, Calcd for $C_{15}H_{23}$ (c) 203.1799; Found 189.1629, Calcd for $C_{14}H_{21}$ (c-14) 189.1642; Found 133.1000, Calcd for $C_{10}H_{13}$ (d) 133.1016. IR ν_{max}^{KBr} cm^{-1}: 1740, 1235. ^{1}H-NMR ($CDCl_3$) δ: 0.73 (3H, s, 26-H_3), 0.83 and 0.94 (each 3H, d, J=6 Hz, 29- and 30-H_3), 1.02 (3H, s, 23-H_3), 1.06 (3H, s, 25-H_3), 1.07 (3H, s, 27-H_3), 1.97, 2.05, and 2.06 (each 3H, s, OAc), 2.25 (1H, d, J=11 Hz, 18-H), 3.60 (3H, s, $COOCH_3$), 4.20 (2H, s, 24-H_2), 4.86 (1H, d, J=10.5 Hz, 3-H), 5.18 (1H, td, J=10.5, 4.5 Hz, 2-H), and 5.25 (1H, br t, J=3.5 Hz, 12-H).

Acknowledgement We are grateful to Dr. T. Kawasaki of Kyushu University for the gift of the sample of arjunolic acid, to Dr. I. Kitagawa of Osaka University for the samples of ursolic acid and betulin, to Dr. H. Hikino of Tohoku University for the sample of sumaresinolic acid, to Dr. J. Sakakibara of Nagoya City University for the samples of 2α,3α,24-trihydroxyurs-12-en-28-oic acid and asiatic acid, and to Dr. M. Takani of Kanazawa University for the samples of barbinervic acid and euscaphic acid.

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