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

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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 $3\beta,23$ -dihydroxyurs-12-en-28-oic acid, $3\beta,24$ -dihydroxyurs-12-en-28-oic acid, and $2\alpha,3\beta,24$ -trihydroxyurs-12-en-28-oic acid on the basis of spectroscopic evidence.

Keywords—*Hedyotis lawsoniae*; Rubiaceae; triterpene; $3\beta,23$ -dihydroxyurs-12-en-28-oic acid; $3\beta,24$ -dihydroxyurs-12-en-28-oic acid; $2\alpha,3\beta,24$ -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⁾ ursolic 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 (¹H-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⁾ $2\alpha,3\alpha,23$ -trihydroxyurs-12-en-28-oic acid (**10a**),⁸⁾ and benthamic acid (**11a**)⁹⁾ was performed by comparisons of the MS and ¹H-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, C₃₅H₅₄O₆, [α]_D + 64.1 ° (CHCl₃), and **2b**, mp 180—182 °C, C₃₅H₅₄O₆, [α]_D + 50.8 ° (CHCl₃), 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

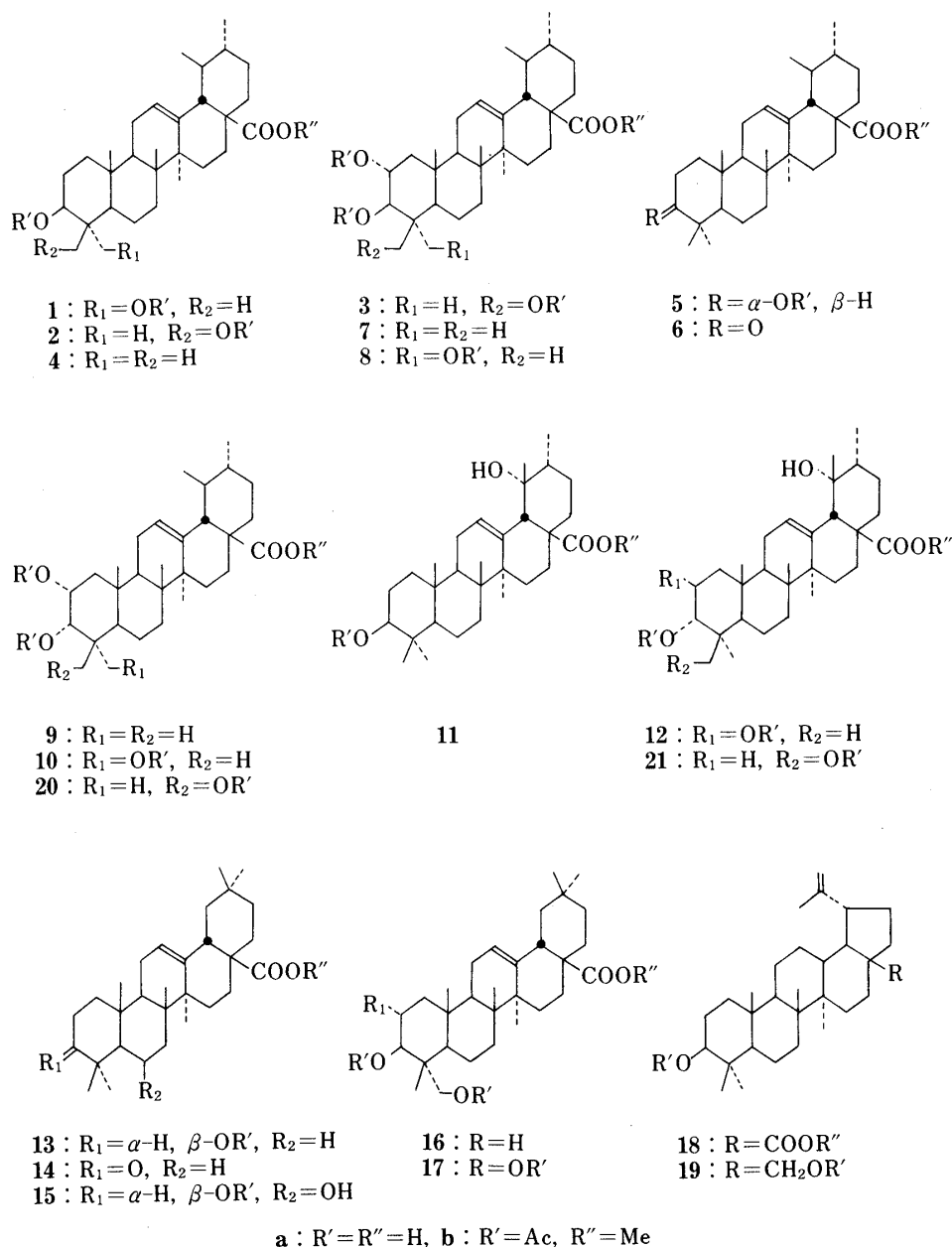


Chart 1

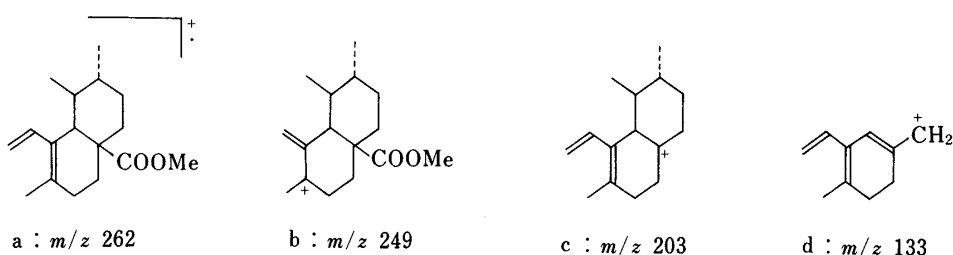


Chart 2

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 1H -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 ¹H-NMR analyses as described below.

The ¹H-NMR spectrum of **1b** showed signals due to a methine proton at δ 4.81 (dd, J = 10, 5.5 Hz, CH₂OAc) and methylene protons at δ 3.71 and 3.88 (each 1H, d, J = 12 Hz, CH₂OAc), 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 3 β ,23-diacetoxyurs-12-en-28-oate (**1b**).

On the other hand, compound **2b** showed a ¹H-NMR spectrum very similar to that of **1b**, except for signals arising from a CH₂OAc 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 2 α ,3 α ,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 3 β ,24-diacetoxyurs-12-en-28-oate (**2b**).

Compound **3a** was also obtained as the methyl ester triacetate (**3b**), amorphous powder, C₃₇H₅₆O₈, [α]_D + 51° (CHCl₃). The gross structure of this compound was suggested by the MS base peak at m/z 262 (a) and the ¹H-NMR pattern, which is similar to that of **2b**. Furthermore, the ¹H-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 2 α ,3 β -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 3 α -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-Acetoxyated Triterpenes (δ in CDCl₃)

Compound (Position of AcO group)	Methyl signals			
	23	24	25	26
4b (3 β)	0.87	0.88	0.94	0.74
2b (3 β , 24)	0.95 ^{a)}	—	1.01 ^{a)}	0.74
5b (3 α)	0.86	0.90	0.95	0.76
21b (3 α , 24)	0.95 ^{a)}	—	0.93 ^{a)}	0.68
7b (2 α , 3 β)	0.90	0.90	1.05	0.74
3b (2 α , 3 β , 24)	1.02	—	1.06	0.73
9b (2 α , 3 α)	0.87	0.98	1.04	0.75
20b (2 α , 3 α , 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		
	$2\beta\text{-H}$	$3\alpha\text{-H}$	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 $2\alpha,3\beta,24$ -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. $^1\text{H-NMR}$ 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 $\text{Ce}(\text{SO}_4)_2\text{-aq. H}_2\text{SO}_4$ 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 $\text{MeOH-CH}_2\text{Cl}_2$ 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 $\times$ 3 mm i.d. glass tube; injection temp. 300 $^\circ\text{C}$, column temp. 280 $^\circ\text{C}$, 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 $\times$ 3) and the extracts were combined and concentrated *in vacuo*. The residue was diluted with water (1 l) and extracted with AcOEt (500 ml $\times$ 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 $\text{MeOH-CH}_2\text{Cl}_2$ (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 $^1\text{H-NMR}$ comparisons with an authentic sample. The more polar band afforded the methyl ester of benthamic acid (**11a**) (60 mg), amorphous powder, $[\alpha]_D^{25} + 29.8^\circ$ ($c=0.55$, CHCl_3). MS m/z : 486 (M^+), 468, 278, 260, 201, 179. High resolution MS: Found 486.3699, Calcd for $\text{C}_{31}\text{H}_{50}\text{O}_4$ (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 $^\circ\text{C}$. $^1\text{H-NMR}$ (CDCl_3) δ : 0.69 (3H, s,

26-H₃), 0.87 (3H, s, 23-H₃), 0.88 (3H, s, 24-H₃), 0.94 (3H, s, 25-H₃), 0.96 (3H, d, $J=6$ Hz, 30-H₃), 1.23 (3H, s, 27-H₃), 1.25 (3H, s, 29-H₃), 2.07 (3H, s, OAc), 2.62 (1H, s, 18-H), 3.63 (3H, s, COOCH₃), 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 CH₂Cl₂ and the insoluble substances (450 mg and 710 mg, respectively) were collected by filtration and methylated with CH₂N₂ 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 CH₂Cl₂ (50 ml $\times$ 3). The CH₂Cl₂ 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-CHCl₃ (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 CH₂N₂ and identified by GC, MS, and ¹H-NMR comparisons with corresponding authentic samples. Compound **5a** (1.6 mg) was methylated with CH₂N₂ and oxidized with CrO₃-AcOH at room temperature for 2 h. The product was purified by PLC (developed with acetone-CHCl₃, 5:95) to give **6b** (1 mg), which was identified by GC and ¹H-NMR comparison with an authentic sample.

F-IV was acetylated with Ac₂O-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 CH₂N₂ and the product was subjected to PLC with CHCl₃ to afford the following compounds.

1) Methyl 2 α -hydroxyursolate diacetate (**7b**) (18 mg), amorphous powder, $[\alpha]_D^{22} + 17^\circ$ ($c=1.7$, CHCl₃). MS m/z : 570 (M⁺), 510, 450, 262, 249, 203, 189, 133. High resolution MS: Found 570.3912, Calcd for C₃₅H₅₄O₆ (M⁺) 570.3919. ¹H-NMR (CDCl₃) δ : 0.74 (3H, s, 26-H₃), 0.83–0.90 (6H, 29- and 30-H₃), 0.90 (6H, s, 23- and 24-H₃), 1.05 (3H, s, 25-H₃), 1.07 (3H, s, 27-H₃), 1.98 and 2.07 (each 3H, s, OAc), 2.25 (1H, d, $J=11$ Hz, 18-H), 3.62 (3H, s, COOCH₃), 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 3 β ,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 3 β ,24-diacetoxyurs-12-en-28-oate (**2b**) (2 mg), colorless needles (from MeOH), mp 180–182 °C, $[\alpha]_D^{22} + 50.8^\circ$ ($c=0.12$, CHCl₃). MS m/z : 570 (M⁺), 510, 450, 262, 249, 203, 189, 133. High resolution MS: Found 570.3882, Calcd for C₃₅H₅₄O₆ (M⁺) 570.3919; Found 262.1922, Calcd for C₁₇H₂₆O₂ (a) 262.1932; Found 249.1815, Calcd for C₁₆H₂₅O₂ (b) 249.1854; Found 203.1790, Calcd for C₁₅H₂₃ (c) 203.1800; Found 189.1627, Calcd for C₁₄H₂₁ (c-14) 189.1642; Found 133.0991, Calcd for C₁₀H₁₃ (d) 133.1016. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 1730, 1230. ¹H-NMR (CDCl₃) δ : 0.74 (3H, s, 26-H₃), 0.85 and 0.94 (each 3H, d, $J=6$ Hz, 29- and 30-H₃), 0.95 (3H, s, 23-H₃), 1.01 (3H, s, 25-H₃), 1.07 (3H, s, 27-H₃), 2.05 and 2.07 (each 3H, s, OAc), 2.25 (1H, d, $J=11$ Hz, 18-H), 3.61 (3H, s, COOCH₃), 4.13 and 4.36 (each 1H, d, $J=12$ Hz, 24-H₂), 4.60 (1H, dd, $J=9, 7$ Hz, 3-H), and 5.26 (1H, br t, $J=3.5$ Hz, 12-H).

4) Methyl 2 α -hydroxy-3-epiursolate diacetate (**9b**) (3 mg), amorphous powder, $[\alpha]_D^{22} + 15^\circ$ ($c=0.25$, CHCl₃). MS m/z : 570 (M⁺), 510, 450, 262, 249, 203, 189, 133. High resolution MS: Found 570.3876, Calcd for C₃₅H₅₄O₆ (M⁺) 570.3919. ¹H-NMR (CDCl₃) δ : 0.75 (3H, s, 26-H₃), 0.85 and 0.93 (each 3H, d, $J=6$ Hz, 29- and 30-H₃), 0.87 (3H, s, 23-H₃), 0.98 (3H, s, 24-H₃), 1.04 (3H, s, 25-H₃), 1.11 (3H, s, 27-H₃), 1.97 and 2.12 (each 3H, s, OAc), 2.25 (1H, d, $J=11$ Hz, 18-H), 3.62 (3H, s, COOCH₃), 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, $[\alpha]_D^{22} + 17^\circ$ ($c=0.35$, CHCl₃). This compound was identified by direct comparison of the TLC behavior, ¹H-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 ¹H-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-CHCl₃ (2:98) (120 mg) was treated with CH₂N₂ and the resulting methyl ester was separated by PLC with AcOEt-benzene (2:8) as the eluent. The least polar band afforded methyl 2 α ,3 α ,23-triacetoxyurs-12-en-28-oate (**10b**) (10 mg), amorphous powder, $[\alpha]_D^{22} + 37^\circ$ ($c=0.35$, CHCl₃). MS m/z : 628 (M⁺), 568, 508, 448, 262, 249, 203, 189, 133. High resolution MS: Found 628.4022, Calcd for C₃₇H₅₆O₈ 628.3975. ¹H-NMR (CDCl₃) δ : 0.75 (3H, s, 26-H₃), 0.85 and 0.93 (each 3H, d, $J=6$ Hz, 29- and 30-H₃), 1.07 (3H, s, 24-H₃), 1.11 (6H, s, 25- and 27-H₃), 1.95, 2.00, and 2.06 (each 3H, s, OAc), 2.25 (1H, d, $J=11$ Hz, 18-H), 3.61 (3H, s, COOCH₃), 3.73 and 4.11 (each 1H, d, $J=12$ Hz, 23-H₂), and 5.18–5.37 (3H, m, 2-, 3-, and 12-H).⁸⁾ The middle band gave a mixture of methyl asiaticate 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 2 α ,3 β ,24-triacetoxyurs-12-en-28-oate (**3b**) (6 mg), purification of which is described below.

Separation of the Mixture of Methyl 3 β ,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. $\times$ 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 $^1\text{H-NMR}$ comparisons with an authentic sample. Methyl $3\beta,23$ -diacetoxyurs-12-en-28-oate (**1b**) was obtained as colorless needles (from EtOH), mp $180\text{--}181.5^\circ\text{C}$, $[\alpha]_{\text{D}}^{21} + 64.1^\circ$ ($c=0.22$, CHCl_3). MS m/z : 570 (M^+), 510, 450, 262, 249, 203, 189, 133. High resolution MS: Found 570.3913, Calcd for $\text{C}_{35}\text{H}_{54}\text{O}_6$ (M^+) 570.3919; Found 262.1937, Calcd for $\text{C}_{17}\text{H}_{26}\text{O}_2$ (a) 262.1933; Found 249.1842, Calcd for $\text{C}_{16}\text{H}_{25}\text{O}_2$ (b) 249.1854; Found 203.1789, Calcd for $\text{C}_{15}\text{H}_{23}$ (c) 203.1799. IR $\nu_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$: 1730, 1230. $^1\text{H-NMR}$ (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 $2\alpha,3\beta,24$ -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, $[\alpha]_{\text{D}}^{21} + 51^\circ$ ($c=0.1$, CHCl_3). MS m/z : 628 (M^+), 568, 508, 262, 249, 203, 189, 133. High resolution MS: Found 628.3954, Calcd for $\text{C}_{37}\text{H}_{56}\text{O}_8$ (M^+) 628.3974; Found 262.1918, Calcd for $\text{C}_{17}\text{H}_{26}\text{O}_2$ (a) 262.1932; Found 249.1820, Calcd for $\text{C}_{16}\text{H}_{25}\text{O}_2$ (b) 249.1854; Found 203.1785, Calcd for $\text{C}_{15}\text{H}_{23}$ (c) 203.1799; Found 189.1629, Calcd for $\text{C}_{14}\text{H}_{21}$ (c-14) 189.1642; Found 133.1000, Calcd for $\text{C}_{10}\text{H}_{13}$ (d) 133.1016. IR $\nu_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$: 1740, 1235. $^1\text{H-NMR}$ (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).

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References and Notes

- 1) R. N. Chopra, J. C. Chopra, K. L. Handa, and L. D. Kapur, "Chopra's Indigenous Drugs of India," 2nd ed., U. N. Dhur & Sons Private Ltd., Calcutta, 1958, p. 339.
- 2) K. S. Mukherjee, M. K. Bhattacharya, and P. K. Ghosh, *Phytochemistry*, **21**, 2416 (1982).
- 3) H. T. Cheung and M. C. Feng, *J. Chem. Soc., (C)*, **1968**, 1047.
- 4) K. Takahashi, S. Kawaguchi, K. Nishimura, K. Kubota, Y. Tanabe, and M. Takani, *Chem. Pharm. Bull.*, **22**, 650 (1974).
- 5) C. Djerassi, G. H. Thomas, and O. Jeger, *Helv. Chim. Acta*, **38**, 1304 (1955).
- 6) A. T. Glen, W. Lawrie, J. McLean, and M. El-Garby Younes, *J. Chem. Soc., (C)*, **1967**, 510; W. M. Bandaranayake, S. P. Gunasekera, S. Karunanayake, S. Sotheeswaran, and M. U. S. Sultanbawa, *Phytochemistry*, **14**, 2043 (1975).
- 7) H. W. A. Biessels, A. C. van der Kerk-van Hoof, J. J. Kettenes-van der Bosch, and C. A. Saleminck, *Phytochemistry*, **13**, 203 (1974).
- 8) R. Tandon, G. K. Jain, R. Pal, and N. M. Khanna, *Indian J. Chem.*, **19B**, 819 (1980). The $^1\text{H-NMR}$ spectrum of **10b** was not consistent with that reported in this paper, but was very similar to that of methyl $2\alpha,3\alpha,19,23$ -tetrahydroxyurs-12-en-28-oate triacetate. See C. H. Brieskorn and W. Riedel, *Arch. Pharm.*, **310**, 910 (1977).
- 9) J. Bermejo, J. L. Bretón, and G. de la Fuente y A. G. González, *Tetrahedron Lett.*, **1967**, 4649.
- 10) H. Budzikiewicz, J. M. Wilson, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 3688 (1963).
- 11) a) H. T. Cheung and D. G. Williamson, *Tetrahedron*, **25**, 119 (1969); b) H. T. Cheung and T. C. Yan, *Aust. J. Chem.*, **25**, 2003 (1972).
- 12) J. Sakakibara and T. Kaiya, *Phytochemistry*, **22**, 2547 (1983).
- 13) M. Takani, K. Kubota, K. Nozawa, T. Ushiki, and K. Takahashi, *Chem. Pharm. Bull.*, **25**, 981 (1977).