Triterpenoid Saponins from *Trevesia sundaica*

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Six new bisdesmosidic saponins **1**−**6**, along with four known triterpenoid saponins were isolated from the aerial parts of *Trevesia sundaica* (Araliaceae). Their structures were determined by ^{1}H−^{1}H correlation spectroscopy (COSY, TOCSY, ROESY) and ^{1}H−^{13}C heteronuclear correlation (HSQC, HMBC) NMR experiments, FABMS, and chemical data.

Chemical and pharmacological investigations have indicated that triterpenoid saponins are important bioactive components existing in plants of the Araliaceae family.[1] Saponins isolated and identified from Araliaceae are reported to have various pharmacological activities, including increasing mental efficiency, recovering physical balance, stimulation of metabolic function, and other general health promoting effects.[2,3]

Trevesia sundaica Mig. (Araliaceae) is a plant native of Java and is used as a folk medicine for rheumatism and as a general tonic. This work on *T. sundaica* is part of a series of studies on chemical constituents of the Araliaceae. In the present work we have examined polar extracts obtained from both flowers and leaves of *T. sundaica*. The present paper deals with the isolation and structure determination of six new oleanolic acid saponins (**1**−**6**) from the aerial parts of *T. sundaica*.

Results and Discussion

The dried leaves of *T. sundaica* were extracted successively with petroleum ether and MeOH. The MeOH extract was partitioned between H_2O and *n*-BuOH, and the *n*-BuOH extract was subjected to Sephadex LH-20 CC followed by DCCC to give saponins **1**, **2**, **5**, **6**. Isolation and purification of compounds of dried flowers of *T. sundaica*, obtained by the same procedure, gave saponins **3**, **4**.

Several known compounds were also isolated. The known saponins were 3*β*-*O*-*β*-D-galactopyranosyl-(1→3)-[*β*-D-glucopyranosyl-(1→4)]-*β*-D-glucopyranosylolean-12-en-28-oic acid;[4] 3*β*-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→3)-[*β*-D-xylopyranosyl-(1→2)]-α-L-arabinopyranosylolean-12-en-28-oic acid *β*-D-glucopyranosyl ester;[5] 3*β*-*O*-*β*-D-glucopyranosyl-(1→3)-[*β*-D-xylopyranosyl-(1→2)]-*β*-D-galactopyranosyloleanolic acid 28-*O*-*β*-D-glucopyranosyl ester;[5] 3*β*-*O*-*β*-D-glucopyranosyl-(1→2)-[*β*-D-galactopyranosyl-(1→3)]-*β*-D-glucopyranosyloleanolic acid[6] by spectral data and direct comparison of their physical properties with those reported previously for these compounds.[4,5] The molecular formulas for all compounds were determined by ^{13}C,^{13}C-DEPT NMR data and FABMS in negative ion mode.

^{1}H and ^{13}C NMR spectra indicated that saponins **1**, **2**, **5**, and **6** had identical aglycon portions but differed in the saccharide chains. Acid hydrolysis of **1**, **2**, **5**, and **6** afforded oleanolic acid which was identified by comparing its ^{1}H and ^{13}C NMR data with published values.[5,6]

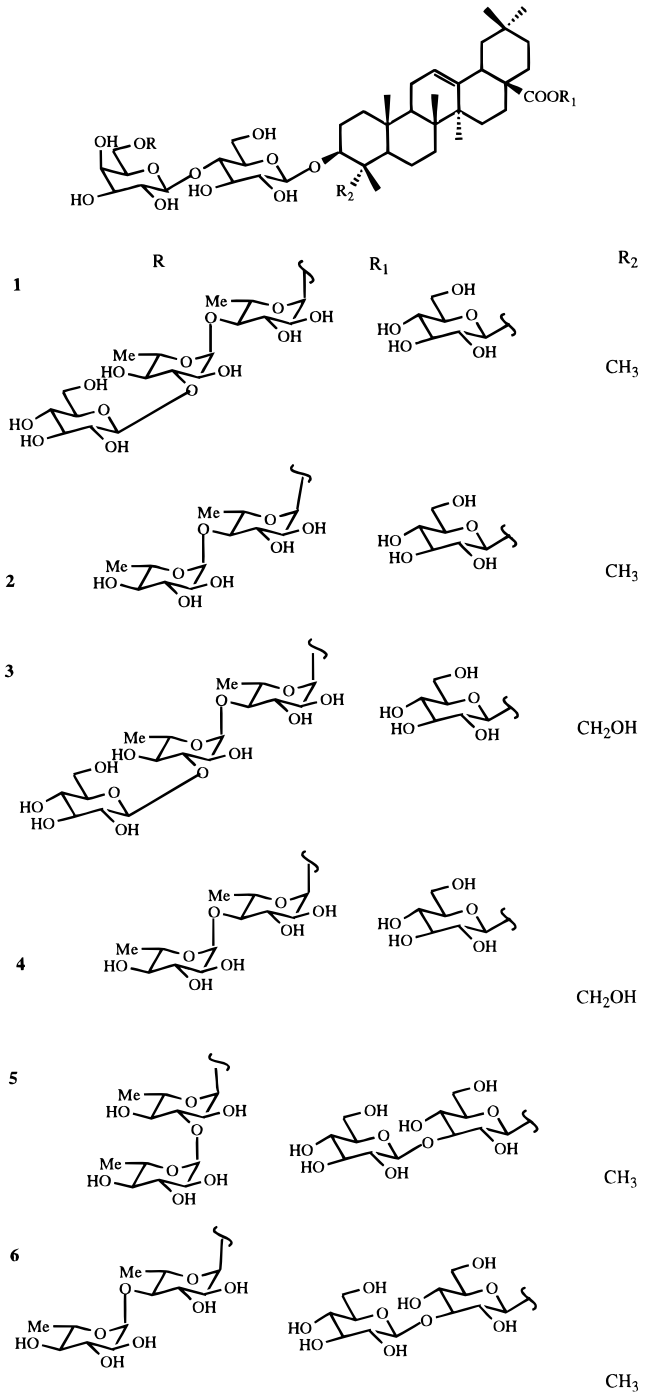

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Table 1. ^{1}H NMR Data for the Oligosaccharide Moieties of Compounds **1**, **2**, **5**, **6** in CD_3OD

	1	**2**	**5**	**6**
Glc at C-28	5.36, d, J = 7.6	5.36	5.39, d, J = 7.0	5.35, d, J = 7.8
1				
2	3.32, dd, J = 7.6, 9.0	3.44	3.60, dd, J = 7.0, 9.0	3.60, dd, J = 7.8, 9.0
3	3.64, t, J = 9.0	3.52	3.69, t, J = 9.0	3.72, t, J = 9.0
4	3.71, dd, J = 9.0, 10.0	3.64	3.62, dd, J = 9.0, 10.0	3.63, dd, J = 9.0, 10.0
5	3.40, m	3.38	3.51, m	3.50, m
6	3.68, dd, J = 12.0, 5.0	3.77	3.64, dd, J = 12.0, 5.0	3.65, dd, J = 12.0, 5.0
	3.84, dd, J = 12.0, 2.5	3.89	3.84, dd, J = 12.0, 2.5	3.82, dd, J = 12.0, 2.5
Glc III			4.57, d, J = 7.8	4.56, d, J = 7.8
			3.35, dd, J = 7.8, 9.0	3.28, dd, J = 7.8, 9.0
			3.46, t, J = 9.0	3.42, t, J = 9.0
			3.38, dd, J = 9.0, 10.0	3.35, dd, J = 9.0, 10.0
			3.42, m	3.38, m
			3.66, dd, J = 12.0, 5.0	3.72, dd, J = 12.0, 5.0
			3.83, dd, J = 12.0, 2.5	3.88, dd, J = 12.0, 2.5
Glc at C-3				
1	4.46, d, J = 7.5	4.43	4.48, d, J = 7.5	4.48, d, J = 7.5
2	3.42, dd, J = 7.5, 9.0	3.35	3.26, dd, J = 7.5, 9.0	3.36, dd, J = 7.5, 9.0
3	3.60, J = 9.0	3.55	3.48, t, J = 9.0	3.54, t, J = 9.0
4	3.28, t, J = 9.0	3.65	3.60, t, J = 9.0	3.61, t, J = 9.0
5	3.24, m	3.38	3.34, m	3.38, m
6	3.70, dd, J = 12.0, 5.0	3.74	3.70, dd, J = 12.0, 5.0	3.72, dd, J = 12.0, 5.0
	3.89, dd, J = 12.0, 2.5	3.92	3.87, dd, J = 12.0, 2.5	3.90, dd, J = 12.0, 2.5
Gal 1	4.57, d, J = 6.8	4.56	4.61, d, J = 7.0	4.58, d, J = 7.0
2	3.80, dd, J = 9.3, 6.8	3.87	3.60, dd J = 9.5, 7.0	3.85, dd, J = 9.5, 7.0
3	3.75, dd, J = 9.3, 3.5	3.75	3.51, dd, J = 9.5, 3.5	3.73, dd, J = 9.5, 3.5
4	3.58, m	3.59	3.86, m	3.59, m
5	3.62, m	3.67	3.50, m	3.67, m
6	3.90, dd J = 12.0, 2.5	3.91	3.85, dd J = 12.0, 2.5	3.89, dd, J = 12.0, 2.5
	3.65, dd, J = 12.0, 5.0	3.59	3.70, dd, J = 12.0, 4.5	3.59, dd, J = 12.0, 5.0
Rha I 1	4.85, d, J = 1.6	4.85	4.83, d, J = 1.5	4.83, d, J = 1.5
2	3.92, dd, J = 1.6, 3.0	3.94	4.18, dd, J = 1.5, 3.0	3.93, dd, J = 1.5, 2.8
3	3.71, dd, J = 9.0, 3.0	3.73	3.95, dd, J = 9.0, 3.0	3.72, dd, J = 9.0, 2.8
4	3.56, t, J = 9.0	3.59	3.66, t, J = 9.0	3.57, t, J = 9.0
5	3.66, dd, J = 9.0, 6.2	3.67	3.85, dd, J = 9.0, 6.2	3.65, dd, J = 9.0, 6.4
6	1.30, d, J = 6.2	1.30	1.30, d, J = 6.2	1.29, d, J = 6.4
Rha II 1	5.20, d, J = 1.5	5.14	5.16, d, J = 1.5	5.16, d, J = 1.5
2	4.25, dd, J = 1.5, 3.5	3.98	3.92, dd, J = 1.5, 3.5	3.96, dd, J = 1.5, 3.5
3	3.95, dd, J = 9.0, 3.5	3.74	3.73, dd, J = 9.0, 3.5	3.72, dd, J = 9.0, 3.5
4	3.66, t, J = 9.0	3.48	3.50, t, J = 9.0	3.49, t, J = 9.0
5	3.85, dd, J = 9.0, 6.2	3.92	3.94, dd, J = 9.0, 6.5	3.92, dd, J = 9.0, 6.2
6	1.26, d, J = 6.2	1.28	1.28, d, J = 6.2	1.26, d, J = 6.2
Glc II 1	4.61, d, J = 7.5			
2	3.44, dd, J = 7.5, 9.0			
3	3.55, t, J = 9.0			
4	3.30, t, J = 9.0			
5	3.26, m			
6	3.70, dd, J = 12.0, 5.0			
	3.87, dd, J = 12.0, 2.5			

In compounds **1**−**6**, C-28 appeared at δ 177.3 in the ^{13}C NMR and H-18 δ 2.92 (dd, J = 12.0 and 4.0 Hz) in the ^{1}H NMR, indicating that the carboxyl group was glycosylated.[5] Attachment of the glycosidic chain at C-3 was indicated by the significant downfield shift observed for this carbon in **1**−**6** relative to the corresponding signal in oleanolic acid and was subsequently confirmed by 2D-NMR experiments,[4−7]

Acid methanolysis of **1** gave glucose, rhamnose, and galactose in a 3:2:1 ratio. FABMS of **1** ($C_{66}H_{108}O_{31}$) showed the $[M - H]^-$ ion at m/z 1395 with prominent fragments at m/z 1233 $[(M - H) - 162]^-$ and 1217 $[(M - H) - 178]^-$ (cleavage of a hexose unit with or without the glycosidic oxygen) and at m/z 1071 $[(M - H) - (162 + 162)]^-$ due to the subsequent loss of two hexose units, and at m/z *925* $[(M - H) - (162 + 162 + 146)]^-$ due to the subsequent loss of two hexose units and one deoxyhexose unit.

Six anomeric protons were easily identified in **1**. They resonated at δ 5.36 (d, J = 7.6 Hz), 5.20 (d, J = 1.5 Hz), 4.85 (d, J = 1.6 Hz), 4.61 (d, J = 7.5 Hz), 4.57 (d, J = 6.8 Hz), and 4.46 (d, J = 7.5 Hz) and correlated in the HSQC experiments to carbons at δ 95.26, 102.30, 102.42, 104.00, 104.63, and 105.00, respectively. By a combination of 1D and 2D TOCSY[10] and 2D DQF-COSY[11] experiments, the 1D TOCSY subspectra of the six monosaccharidic units could be easily interpreted and, at the same time, the type of sugar, its configuration and conformation assigned (Table 1). The isolated ^{1}H NMR signals resonating at uncroweded regions of the spectrum, between δ 4.30 and 5.40, were the starting point for the 1D TOCSY experiments.[8] Because of the selectivity of multistep coherence transfer, the 1D TOCSY subspectra of the single monosaccharide unit could be extracted from the crowded overlapping region between δ 3.10 and 4.20. Each subspectrum could be attributed to one set of coupled protons such as H−C(1) to H−C(4) or H−C(6) of a sugar moiety. Moreover the 1D TOCSY subspectra obtained irradiating at δ 5.36, 5.20, 4.85, 4.61, and 4.46 recognized these protons as belonging to hexopyranose units. Irradiation of the anomeric signals at δ 5.20 and 4.85 allowed the identification of two deoxyhexose units. Irradiating the signal at δ 4.57 showed connectivities to three methines.

Table 2. ^{13}C NMR Data for the Oligosaccharide Moieties of Compounds **1**, **2**, **5**, **6** in CD_3OD

	δC **1**	δC **2**	δC **5**	δC **6**
Glc at C-28 1	95.26	96.00	95.40	96.0
2	75.00	75.10	73.50	74.00
3	77.76	78.00	**87.30**	**87.00**
4	71.10	70.73	70.00	70.00
5	78.10	77.80	78.30	78.10
6	62.40	63.67	62.60	62.80
GlcIII 1			104.90	105.0
2			75.00	75.00
3			78.20	78.10
4			71.00	70.90
5			77.80	77.80
6			62.40	62.15
Glc at C-3 1	104.00	104.00	103.80	104.30
2	75.13	75.13	74.93	74.60
3	77.50	77.30	77.30	77.38
4	**79.00**	**79.23**	**78.80**	**79.00**
5	76.80	76.30	76.90	76.48
6	62.10	61.80	62.50	61.90
Gal 1	104.63	104.63	104.50	104.47
2	73.70	73.70	73.40	73.16
3	74.00	73.70	73.70	73.90
4	70.20	70.20	69.75	69.5
5	76.40	76.47	76.60	76.80
6	**68.60**	**69.00**	**68.70**	**68.70**
Rha I 1	102.30	102.67	101.70	102.57
2	72.30	72.25	71.00	72.35
3	72.40	72.40	**79.00**	72.40
4	**82.80**	**83.00**	**73.60**	**82.85**
5	70.30	70.30	69.00	70.30
6	18.30	18.10	18.50	18.30
RhaII 1	102.42	101.70	101.70	101.70
2	71.10	71.79	71.90	71.90
3	**79.40**	73.60	73.70	73.70
4	74.00	74.47	74.50	74.50
5	69.40	70.30	70.45	70.45
6	18.10	17.70	17.90	17.90
GlcII 1	105.00			
2	75.0			
3	78.20			
4	70.00			
5	78.00			
6	62.30			

The coherence transfer to H-5 was not obtained because of the small coupling constant H-4−H-5[9] of the sugar galactose. A HSQC[12] experiment correlated all proton resonances with those of the corresponding carbons (Table 2). Data from the above experiments determined the position of the interglycosidic linkages by comparison of the carbon chemical shift observed with those of the corresponding methyl pyranoside and taking in account the known effects of glycosidation.[13] Unambigous determination of the interglycosidic linkages and sugar sequences was obtained from long-range CH correlation (HMBC spectrum) and 2D ROESY.[14]

The HMBC[12] spectrum of **1** was useful in the determination of the linkages of the sugar moieties to the aglycon. It identified the β-D-glucopyranosyl unit as linked to C-28, while the five-membered oligosaccharidic chain was bonded to C-3 through a β-D-glucopyranosyl unit.

Thus, compound **1** was assigned the structure 3β-*O*-β-D-glucopyranosyl-(1→3)-α-L-rhamopyranosyl-(1→4)-α-L-rhamopyranosyl-(1→6)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosylolean-12-en-28-oic acid -β-D-glucopyranosyl ester.

The FABMS spectrum of compound **2** ($C_{60}H_{98}O_{26}$) showed the $[M - H]^-$ ion at *m/z* 1233 and prominent fragments at *m/z* 1071 $[(M - H) - 162]^-$, 925 $[(M - H) - (162 + 146]^-$ (cleavage of one hexose and one deoxyhexose), and 617 $[(M - H) - (162 + 146 + 162]^-$ due to the subsequent loss of an hexose unit. The ^{13}C and DEPT ^{13}C NMR spectra showed 60 signals, of which 30 were assigned to the saccharide portion and 30 to a triterpene moiety. Analysis of NMR data of compound **2** and comparison with those of **1** showed **2** to differ from **1** only in the absence of the terminal glucopyranosyl unit (Tables 1 and 2). Therefore, the structure 3β-*O*-α-L-rhamopyranosyl-(1→4)-α-L-rhamopyranosyl-(1→6)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosylolean-12-en-28-oic acid β-D-glucopyranosyl ester was assigned to **2**.

Comparison of the NMR spectral data of compound **3** ($C_{66}H_{108}O_{32}$) with those of **1** showed these to be identical in the sugar portion but different in the aglycon portion. In particular, hydrogens and carbons due to the C, D, and E rings of **3** resonated near the same frequencies as the corresponding signals in oleanolic acid acid, while the A- and B-ring ^1H and ^{13}C signals were shifted somewhat. The NMR spectra of **3** contained one less methyl and more signals [^1H NMR δ 3.30 (1H, d, *J* = 11.8 Hz), 3.70 (1H, d, *J* = 11.8 Hz); ^{13}C NMR δ 64.7 (CH_2)] than those of **1**, suggesting that one of the Me groups was replaced by a hydroxymethyl group in **3**. In addition, one of the methyl signals was shifted upfield to δ 0.71 in **3** due to the CH_2OH group. The most significant features of the ^{13}C NMR spectrum of **3**, which suggested placement of the CH_2OH group at C-23, were the downfield shifts exhibited by C-4 and the upfield shifts experienced by C-3, C-5, and Me-24. The aglycon of **3** was elucidated to be hederagenin.[15,16] Thus **3** was determined to be 3β-*O*-β-D-glucopyranosyl-(1→3)-α-L-rhamopyranosyl-(1→4)-α-L-rhamopyranosyl-(1→6)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl]hederagenin 28-*O*-β-D-glucopyranosyl ester.

The FABMS of compound **4** ($C_{60}H_{98}O_{27}$) displayed a molecular ion peak at *m/z* 1249 $(M - H)^-$ and prominent fragments at *m/z* 1087 $[(M - H) - 162]^-$ and 941 $[(M - H) - 162 + 146]^-$ due to the sequential losses of a hexose and deoxyhexose. The ^{13}C aglycon signals were similar to those of **3**, allowing identification of the aglycon of **4** as hederagenin. Analysis of the spectral data for **4** revealed that this compound had the same glycosidic chain as **2**. Therefore, compound **4** was determined to be 3β-*O*-[α-L-rhamopyranosyl-(1→4)-α-L-rhamopyranosyl-(1→6)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosylhederagenin 28-*O*-β-D-glucopyranosyl ester.

Acid methanolysis of **5** gave glucose, rhamnose, and galactose in the ratio 3:2:1. FABMS of **5** ($C_{66}H_{108}O_{31}$) showed the $[M - H]^-$ ion at *m/z* 1395 with prominent fragments at *m/z* 1233 $[(M - H) - 162]^-$ and 1217 $[(M - H) - 178]^-$ (cleavage of a hexose unit with or without the glycosidic oxygen) and at *m/z* 1071 $[(M - H) - (162 + 162]^-$ due to the subsequent loss of two hexose units, and at *m/z* 925 $[(M - H) - (162 + 162 + 146)]^-$ due to the subsequent loss of two hexose units and one deoxyhexose unit. The ^{13}C and DEPT ^{13}C NMR spectra showed 66 signals, of which 36 were assigned to the saccharide portion and 30 to the triterpene moiety. The oligosaccharide structure was determined by 2D NMR. Even at high field (600 MHz) the 1D sugar spectral region of **5** was complex as most of the shifts were found between δ 5.00 and 3.00 and were overlapped by the aglycon signals. 1D and 2D TOCSY spectroscopy[8] experiments allowed resolution of the overlapped spec-

tra of oligosaccharides into a subset of individual monosaccharide spectra. In the 1D and 2D TOCSY spectrum of **5** the anomeric proton signal ascribable to an β-D-glucopyranose (H-1′, δ 5.39, $J = 7.0$ Hz) showed connectivities to four methines (δ 3.60, 3.70, 3.69, 3.51). This together with the 2D DQF-COSY spectrum established the proton sequence within this sugar fragment as H-1 (δ 5.39), H-2 (δ 3.40), H-3 (δ 3.69), H-4 (δ 3.62) and H-5 (δ 3.51), H-6a (δ 3.64), H-6b (δ 3.84) (Table 1). Similar observations of the TOCSY and COSY experiments for all the other sugar residues (Table 1 and 2) allowed complete sequential assignments for all proton resonances starting from the anomeric proton signals. HSQC experiments which correlated all proton resonances with those of each corresponding carbon (Tables 1 and 2) permitted assignments of the interglycosidic linkages by comparison of the ^{13}C shifts observed with those of the corresponding methyl pyranosides and taking into account the known effects of glycosidation.[16]

The absence of any ^{13}C glycosidation shift for one glucopyranosyl and one rhamnopyranosyl residues suggested that these sugars were terminal units, while glycosidations shift on C-3 (∼+9 ppm) of the glucopyranosyl unit linked at C-28 by an ester bond allowed us to establish the presence of two glucopyranosyl residues at C-28. Glycosidation shifts on C-4 (∼+6 ppm) of the glucopyranosyl unit, C-6 (∼+6 ppm) of the galactopyranosyl unit, and C-3 (∼+7) of ramnopyranosyl unit allowed us to define the structure of the saccharidic chain linked to C-3. The position of each sugar unit was deduced from a HMBC experiment. The ^1H NMR and ^{13}C NMR data indicated the β configuration at the anomeric positions for glucopyranosyl and galactopyranosyl units and the α configuration at the anomeric positions for the rhamnopyranosyl unit (Table 1 and 2). Therefore the structure 3β-*O*-α-L-rhamopyranosyl-(1→3)-α-L-rhamopyranosyl-(1→6)-β-D-galactopyranosyl (1→4)-β-D-glucopyranosylolean-12-en-28-oic acid β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl ester was assigned to **5**.

Compound **6** had molecular formula ($C_{66}H_{108}O_{31}$). FAB-MS of **6** ($C_{66}H_{108}O_{31}$) showed the $[M - H]^-$ ion at m/z 1395 with prominent fragments at m/z 1233 $[(M - H) - 162]^-$ and 1217 $[(M - H) - 178]^-$ (cleavage of a hexose unit with or without the glycosidic oxygen) and at m/z 1087 $[(M - H) - (162 + 146]^-$ due to the subsequent loss of one hexose and one deoxyhexose unit, and at m/z 925 $[(M - H) - (162 + 162 + 146)]^-$ due to the subsequent loss of two hexose units and one deoxyhexose unit. The ^{13}C and DEPT ^{13}C NMR spectra showed 66 signals, of which 36 were assigned to the saccharide portion and 30 to the triterpene moiety. Analysis of NMR data of compound **6** and comparison with those of **2** showed **6** to differ from **2** only in the presence of one additional glucopyranosyl unit (Tables 1 and 2). The additional glucopyranosyl unit was located at C-3 of glucopyranosyl esterified at C-28 on the basis of the analysis of his spectral data. Therefore the structure 3β-*O*-α-L-rhamopyranosyl-(1→4)-α-L-rhamopyranosyl-(1→6)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-olean-12-en-28-oic acid β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl ester was assigned to **6**.

Experimental Section

General Experimental Procedures. A Bruker AMX-600 spectrometer operating at 599.19 MHz for ^1H and 150.858 MHz for ^{13}C, using the UXNMR software package was used for NMR measurements in CD_3OD solutions. 2D experiments: $^1H-^1H$ DQFCOSY and ROESY[9,11] $^1H-^{13}C$ HSQC and HMBC, experiments were obtained as described previously.[14,15] The selective excitation spectra, 1D TOCSY[13] were acquired using waveform generator-based GAUSS shaped pulses, mixing time ranging from 80 to 100 ms and a MLEV-17 spin−lock field of 10 kHz preceded by a 2 ms trim pulse. The repetition rates for all kind of spectra were about 1.5 s. Optical rotations were measured on a Perkin-Elmer 141 polarimeter using a sodium lamp operating at 589 nm in 1% w/v solutions in MeOH. Fast atom bombardment mass spectra (FABMS) were recorded in a glycerol matrix in the negative ion mode on a VG ZAB instrument (XE atoms of energy of 2−6 kV). HPLC separations were performed with a Waters model 6000A pump equipped with a U6K injector and a Model 401 refractive index detector.

Plant Material. The plant *Trevesia sundaica* (Araliaceae) was collected in Palermo, Italy, in September of 1995; a sample (1223/3OBP) has been deposited in the Herbarium of the Botanical Garden of Palermo.

Extraction and Isolation. Dried flowers of *T. sundaica* (300 g) were defatted with petroleum ether then extracted with MeOH to give 13 g of residue. The methanolic extract was dissolved in H_2O. The H_2O extract was partioned between AcOEt and *n*-BuOH to afford an *n*-BuOH-soluble portion (4 g) and an AcOEt portion (1 g). The *n*-BuOH extract (2 g) was chromatographed on a Sephadex LH-20 column (100 × 5 cm) with MeOH as the eluent. Fractions (8 mL) were collected and checked by TLC. Fractions 11−15 (340 mg) from Sephadex containing a mixture of oleanolic glycosides **3**, **4** were separated by RP-HPLC on a C18 μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 mL/min) with MeOH−H_2O (4:6) to yield pure compounds [**3**] (t_R = 8 min, 12.5 mg), [**4**] (t_R = 12 min, 16 mg). Fractions 16−18 (400 mg) from Sephadex containing a mixture of **3** and three known glycosides[4−6] were separated by RP-HPLC on a C18 μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 mL/min) with MeOH−H_2O (35:65) to yield pure compounds [**3**] (t_R = 6.5 min, 9.5 mg), 3β-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosylolean 12-en-28-oic acid β-D-glucopyranosyl ester (t_R = 10 min, 12 mg), 3β-*O*-β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-β-D-galactopyranosyloleanolic acid 28-*O*-β-D-glucopyranosyl ester (t_R = 15 min, 8 mg), 3β-*O*-β-D-glucopyranosyl-(1→2)-[β-D-galactopyranosyl-(1→3)]-β-D-glucopyranosyloleanolic acid (t_R = 19 min, 13 mg).

The air-dried leaves of *T. sundaica* (300 g) were defatted with petroleum and then extracted with MeOH to give 26 g of residue. The methanolic extract was dissolved in H_2O. The H_2O extract was partioned between AcOEt and *n*-BuOH to afford an *n*-BuOH-soluble portion (8.0 g) that was chromatographed on a Sephadex LH-20 column (100 × 5 cm) with MeOH as the eluent. Fractions (8 mL) were collected and checked by TLC.

Fractions 18−25 (540 mg) from Sephadex were purified by DCCC with BuOH−EtOH−AcOH−H_2O (8:4:2:1) in which the stationary phase consisted of the higher phase (ascending mode, flow 10 mL/h). About 300 fractions (4 mL) were collected. DCCC fractions 60−

150 (250 mg) containing a mixture of **1**, **2**, **5**, **6** and two known oleanolic glycosides were separated by RP-HPLC on a C18 μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 mL/min) with MeOH−H_2O (1:1) to yield pure compounds [**1**] (t_R = 25 min, 20 mg), [**2**] (t_R = 18 min, 14 mg), [**5**] (t_R = 22 min, 18 mg), [**6**] (t_R = 24.0 min, 15.8 mg), 3β-*O*-β-D-galactopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosylolean-12-en-28-oic acid (**4**) (t_R = 14.0 min, 10.5 mg), 3β-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosylolean-12-en-28-oic acid β-D-glucopyranosyl ester (**5**) (t_R = 7.6 min, 7 mg).

Methanolysis of Compounds 1−6, Carbohydrate Constituents. A solution of each compound (2 mg) in anhydrous 2 N HCl−MeOH (0.5 mL) was heated at 80 °C in a stoppered reaction vial for 12 h. After cooling, the solution was neutralized with Ag_2CO_3 and centrifuged and then the supernatant was evapored to dryness under N_2. The residue was reacted with TRISIL-Z (Pierce) and analyzed by GLC. Retention times were identical to those of authentic methyl sugars.

Compound 1: $[\alpha]^{25}_D$ = +16.5 (*c* 1, MeOH); $C_{46}H_{72}O_{19}$, negative FABMS *m/z* 1395 $(M - H)^-$, 1233 $[(M - H) - 162]^-$, 1217 $[(M - H) - 178]^-$, 1071 $[(M - H) - (162 + 162)]^-$, 925 $[(M - H) - (162 + 162 + 146)]^-$. For NMR data of the aglycon moiety, see refs 5 and 6. For the sugar moiety, see Tables 1 and 2.

Compound 2: $[\alpha]^{25}_D$ = +5.5 (*c* 1, MeOH); $C_{47}H_{74}O_{19}$, negative FABMS *m/z* $[M - H]^-$ 1233 $[(M - H) - 162]^-$, 1217 $[(M - H) - 178]^-$, 1071 $[(M - H) - (162 + 162]^-$, 925 $[(M - H) - (162 + 162 + 146)]^-$. For NMR data of the aglycone moiety, see refs 5 and 6. For the sugar moiety, see Tables 1 and 2.

Compound 3: $[\alpha]^{25}_D$ = +10.0 (*c* 1, MeOH); $C_{41}H_{64}O_{14}$, negative FABMS *m/z* $[M - H]^-$ 1411, $[(M - H) - 162)]^-$ 1249, $[(M - H) - (162 + 162)]^-$ 1087, $[(M - H) - (162 + 162 + 146)]^-$ 941, $[(M - H) - (162 + 162 + 146 + 146)]^-$ 795; NMR data for the sugar units are superimposable on those for compound **1**. For NMR data of aglycon moiety, see refs 15 and 16.

Compound 4: $[\alpha]^{25}_D$ = +8.0 (*c* 1, MeOH); $C_{36}H_{56}O_{10}$, negative FABMS *m/z* $[M - H]^-$ 1289, $[(M - H) - 162)]^-$ 1087, $[(M - H) - (162 + 162)]^-$ 941; NMR data for the aglycone moiety are identical to those of compound **3**, NMR data for the sugar units are superimposable on those reported for compound **2**.

Compound 5: $[\alpha]^{25}_D$ = +22.0 (*c* 1, MeOH); $C_{42}H_{66}O_{15}$, negative FABMS *m/z* $[M - H]^-$ 1395, 1233 $[(M - H) - 162]^-$, 1217 $[(M - H) - 178]^-$, 1071 $[(M - H) - (162 + 162]^-$, 925 $[(M - H) - (162 + 162 + 146)]^-$. NMR data for the aglycon moiety are identical to those for compound **1**, for the sugar moiety see Tables 1 and 2.

Compound 6: $[\alpha]^{25}_D$ = +13.5 (*c* 1, MeOH); $C_{41}H_{64}O_{13}$, negative FABMS *m/z* $[M - H]^-$ 1395, 1233 $[(M - H) - 162]^-$, 1217 $[(M - H) - 178]^-$, 1087 $[(M - H) - (162 + 146)]^-$, 925 $[(M - H) - (162 + 162 + 146)]^-$. NMR data for the aglycon moiety are identical to those for compound **1**; for the sugar moiety, see Tables 1 and 2.

References and Notes

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