Triterpenoid Saponins from *Schefflera divaricata*

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Ten new triterpenoid saponins (**3**−**12**), along with two known triterpenoid saponins (**1**, **2**), were isolated from the aerial parts of *Schefflera divaricata* Merill (Araliaceae). Their structures were determined by $^1H-^1H$ correlation spectroscopy (COSY, HOHAHA) and $^1H-^{13}C$ heteronuclear correlation (HETCOR, COLOC) NMR experiments, FABMS, and chemical data. The aglycon moieties are oleanolic acid for **1**−**4** and betulinic acid for **5**−**12**, respectively.

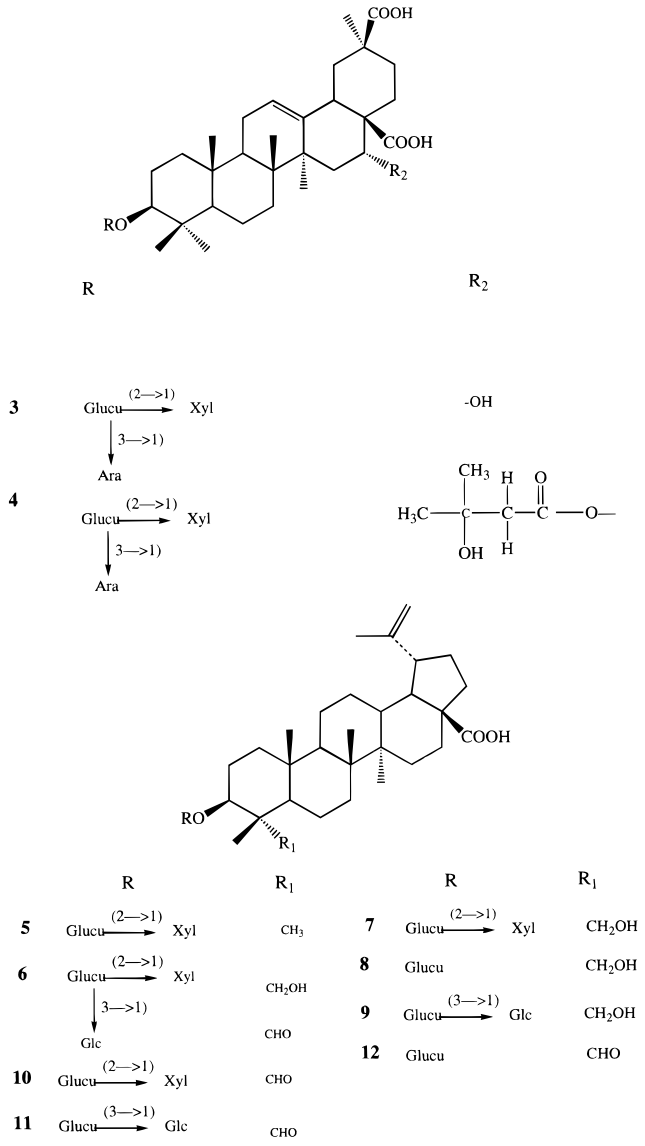
The Araliaceae family is one of the most medicinally important plant families. Perhaps the most famous member of this family is ginseng (*Panax ginseng* C.A. Meyer), the roots of which have been used in traditional oriental medicine for more than 5000 years.[1] Chemical and pharmacological investigations have indicated that triterpenoid saponins are important bioactive components existing in plants of the Araliaceae family.[2] 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.[3]

Schefflera divaricata Merill (Araliaceae) is a plant native of Java and is used as a folk medicine for rheumatism and as a general tonic. No chemical work, however, has been reported on this plant. In a search for novel bioactive natural products from medicinal plants, we have studied the polar extract obtained from both flowers and leaves of *S. divaricata*. The present paper deals with the isolation and structure determination of oleanolic acids **3** and **4** and betulinic acid-based saponins **5**−**12** from the aerial parts of *S. divaricata*.

Results and Discussion

The dried leaves of *Schefflera divaricata* were extracted successively with petroleum ether and MeOH. The MeOH extract was fractioned in H_2O−*n*-BuOH, and the *n*-BuOH extract was subjected to Sephadex LH-20 column chromatography followed by DCCC to give saponins **1**-**12**. **1** and **2** were known compounds identified as (**1**) 3*β*-*O*-*β*-D-galactopyranosyl-(1→3)-[*β*-D-glucopyranosyl-(1→4)]-*β*-D-glucopyranosylolean-12-en-28-oic acid and (**2**) 3*β*-*O*-*β*-D-galactopyranosyl-(1→3)-[*β*-D-glucopyranosyl-(1→4)]-*β*-D-glucuronopyranosylolean-12-en-28-oic acid by spectral data and direct comparison of their physical properties with those reported previously for these compounds.[4]

The molecular formulas for all compounds were determined by ^{13}C, ^{13}C-DEPT NMR data, and FABMS in negative ion mode. ^1H and ^{13}C NMR spectra indicated that saponins **3** and **4** had identical saccharide chains but differed in the aglycon portion. The FABMS of **3** ($C_{46}H_{72}O_{19}$) showed the $[M - H]^-$ ion at *m*/*z* 927 with prominent fragments at *m*/*z* 795 $[(M - H) - 132]^-$ and 779 $[(M - H) - 148]^-$ (cleavage of a pentose unit with or without the glycosidic oxygen) and at *m*/*z* 485 $[(M - H) - (132 + 176 + 132)]^-$ due to the subsequent loss of two pentose and a hexose units. Also observed was a fragment at *m*/*z* 883 $[(M - H) - 44]^-$ corresponding to loss of a carboxylic group from the *m*/*z* 927



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peak. The ^{13}C and ^{13}C-DEPT NMR spectra of **3** showed 46 signals of which 16 were assigned to the saccharide portion and 30 to a triterpene moiety. The ^{1}H NMR spectrum of **3** showed six singlets assignable to tertiary methyl groups in the range δ 0.85−1.35. The 3β-OH substitution was evident from the chemical shift and the J value of the proton assigned to C-3 centered at δ 3.20 (1H, dd, J = 11, 5.0 Hz). The signal at δ 3. 95 (br s) indicated the presence of a C-16α hydroxyl group, a conclusion that was further supported by ^{13}C NMR data (see the Experimental Section) and by the C-27 methyl, which resonated at δ 1.35, downfield from its usual position.[5] The ^{13}C NMR of **3** also showed a quaternary signal at δ 180.00, indicative of a carboxylic group, which was assigned to C-30 on the basis of upfield shifts exhibited by C-19, C-21, and C-29 and the downfield shift experienced by C-20.[6,7] Thus, the aglycon of **3** was 3β,16α-dihydroxyolean-12-ene-28,30-dioic acid.

^{13}C NMR signals assigned to the pentacyclic nucleus of the aglycon of compound **4** (see the Experimental Section) were similar to those reported for **3**. The FABMS of **4** ($C_{51}H_{80}O_{21}$) showed an $[M - H]^-$ ion at m/z 1027 that was 100 mass units higher than that of **3** and was compatible with an additional 3-hydroxy-3-methylbutanoyl moiety. Analysis of its NMR spectral data indicated that **4** possessed the same basic structure of **3** but that it was esterified at C-16, with a 3-hydroxy-3-methylbutanoyl group. The ^{1}H NMR spectrum was very similar to that of **3** with the main differences being a downfield shift of H-16 (δ 4.80 in **4** versus 3.95 in **3**), a signal at δ 2.25 (2H, s) for one methylene group, and a signal at δ 1.37 (6H, s) assigned to additional methyl groups. The ^{13}C NMR of **4** showed five additional signals characteristic of a 3-hydroxy-3-methylbutanoyl moiety, and differences were observed in the chemical shifts of C-16 (δ 74.20 in **3** versus δ 76.00 in **4**) and C-15 (δ 35.20 in **3** versus δ 33.00 in **4**) that indicated that **4** was esterified at C-16.[8]

Attachment of the glycosidic chain at C-3 was indicated by the significant downfield shift (δ 90.60) observed for this carbon in **3** and **4** relative to the corresponding signal in similar compounds,[4] e.g., oleanolic acid (δ 78.00), and was subsequently comfirmed by 2D-NMR experiments.[9] Structural elucidation of the glycosidic portion of these compounds began with the most abundant **3**. Acid methanolisis of the saponins gave glucuronic acid, xylose, and arabinose in a 1:1:1 ratio. This indicated that the sugar moiety linked to C-3 in **3** and **4** was a trisaccharide formed by glucuronic acid, xylose, and arabinose. The identity and sequence of sugar units in saponins **3** and **4** was deduced by a combination of 2D-$^{1}H-^{1}H$ correlation spectroscopy COSY, HOHAHA,[10] and 2D $^{1}H-^{13}C$ correlation J^1 (HETCOR and J long-range (COLOC)[11] (Table 1). COSY and HOHAHA permitted assignments of all spin correlations from H-1 to H-5 of the glucuronic acid unit and H-1 to H-5 of the xylose and arabinose units. These data, together with results obtained from HETCOR and literature data,[12,13] established that the xylose and arabinose units were terminal and bonded to C-2 and C-3 of glucuronic acid (the sugar directly linked to the aglycon). These data left two possible sequences for the triglycoside chains of compounds **3** and **4**: a COLOC[11] experiment differentiated the two structures. A key correlation peak was obtained between the anomeric proton of xylose (δ 4.49, d, J = 7.5 Hz) and C-2 (δ 82.90) of the glucuronic acid unit. Chemical shift, multiplicity, absolute values of the coupling constant, and magnitude in the ^{1}H NMR spectrum as well as ^{13}C NMR data indicated the β-configuration at the anomeric positions for the xylose and glucose units and the α-configuration for arabinose.[14]

Table 1. ^{1}H NMR Data (J in Hz) for the Oligosaccharide Moieties of Compound **3** in CD_3OD[a]

	3	
position	δC	δH
Glucu 1	105.60	4.51 d, J = 7.5
2	82.90	3.35 s.o.
3	83.50	3.42 t, J = 9.0
4	70.20	3.50 t, J = 9.0
5	76.50	3.68 d, J = 9.0
6	172.00	
Xyl 1	105.30	4.49 d, J = 7.5
	75.50	3.20 dd, J = 7.5, 9.5
3	77.60	3.30 t, J = 9.5
4	72.80	3.52 m
5	67.10	3.90 dd, J = 10.0, 5.0
		3.18 dd, J = 10.0, 2.0
Ara 1	105.20	4.37 d, J = 7.0
2	73.90	3.80 dd, J = 7.0, 8.5
3	75.00	3.84 dd, J = 8.5, 3.0
4	69.60	4.00 m
5	65.20	4.02 dd, J = 12.0, 2.0
		3.63 dd, J=12.0, 3.5

[a] Assignments were confirmed by $^{1}H-^{1}H$ COSY and HETCOR experiments.

Thus, the structures of **3** and **4** were determined to be 3β-*O*-α-L-arabinopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-β-D-glucuronopyranosyl-16α-hydroxyolean-12-ene-28,30-dioic acid **3**; 3β-*O*-α-L-arabinopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-β-D-glucuronopyranosyl-16-*O*-[3-hydroxy-3-methylbutanoyl]olean-12-ene-28,30-dioic acid **4**.

Compound **6** had the molecular formula $C_{47}H_{74}O_{19}$. The FABMS of **6** showed the $[M - H]^-$ ion at m/z 941 and fragments at m/z 779 $[(M - H) - 162]^-$, m/z 763 $[(M - H) - 178]^-$ (cleavage of a hexose unit with or without the glycosidic oxygen), and m/z 647 $[(M - H) - (162 + 132)]^-$ due to the loss of a pentose unit, and m/z 471 $[(M - H) - (132 + 162 + 176)]^-$. The ^{13}C and DEPT ^{13}C NMR spectra showed 47 signals of which 17 were assigned to the saccharide portion and 30 to the triterpene moiety.

The ^{1}H NMR spectrum of **6** showed, in addition to four methyl singlets assignable to tertiary methyls between δ 0.70−1.05, signals for an isopropylene function (δ 4.63 and 4.74, 1H each, br s and 1.73 3H, s) and signals for geminal protons at δ 3.30 and 3.70 (1H each, d, J = 12 Hz, H_2-23) for the tritepenic skeleton. A 3β-OH substitution was evident from the chemical shift and the J values of the C-3 proton at δ 3.21 (1H, dd, J = 10.5, 4.0 Hz). The ^{13}C NMR spectrum of **6** suggested a lupene-type triterpene skeleton.[15] A signal at δ 64.70 (C-23) (CH_2 by DEPT) and δ 13.20 (C-24) (CH_3 by ^{13}C-DEPT) indicated a $-CH_2OH$ group at C-4. Glycosidation of the alcoholic function at C-3 was indicated by the significant downfield shift observed for this carbon in **6** (see the Experimental Section) relative to the corresponding signals in model compounds.[15]

The oligosaccharide structure was determined by 2D-NMR. Even at high field (500 MHz), the 1D sugar spectral region of **6** was complex as most of the shifts

Table 2. ^{13}C NMR Data for the Oligosaccharide Moieties of Compounds **5**, **6**, **8**, and **9** in CD_3OD[a]

	5 δC	**6** δC	**8** δC	**9** δC
Glucur 1	104.8	104.9	105.3	105.1
2	83.0	81.5	75.1	83.4
3	77.8	85.6	78.1	78.0
4	71.1	70.2	73.6	71.1
5	78.1	78.3	78.1	78.3
6	177.5	177.5	177.9	177.3
Xyl 1	106.3	106.0		
2	76.2	76.2		
3	78.1	78.0		
4	72.4	72.2		
5	67.1	66.5		
Glc 1		106.2		106.0
2		75.3		75.4
3		78.5		78.5
4		71.7		71.7
5		78.7		78.7
6		62.8		62.7

[a] Assignments were confirmed by $^1H-^1H$ COSY and HETCOR experiments.

were found between δ 5.00 and 3.00 and were overlapped by the aglycon signals. 2D-HOHAHA spectroscopy experiments allowed resolution of the overlapped spectra of oligosaccharides into a subset of individual monosaccharide spectra. In the 2D-HOHAHA spectrum of **6** the anomeric proton signal ascribable to an β-D-glucuronopyranose (H-1′, δ 4.45, J = 7.0 Hz) showed connectivities to four methines (δ 3.68, 3.50, 3.42, 3.35). This together with the 2D-COSY-90 spectrum established the proton sequence within this sugar fragment as H-1 (δ 4.45), H-2 (δ 3.35), H-3 (δ 3.42), H-4 (δ 3.50), and H-5 (δ 3.68) (Table 3).

Similar observations of the HOHAHA and COSY experiments for all the other sugar residues (Tables 2 and 3) allowed complete sequential assignments for all proton resonances starting from the anomeric proton signals. HETCOR experiments that correlated all proton resonances with those of each corresponding carbon (Tables 2 and 3) 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 xylopyranosyl and glucopyranosyl residues suggested that these sugars were terminal units, while the glycosidation shifts on C-2 (∼+7 ppm) and C-3 (∼+7) of the glucuronopyranosyl unit indicated the presence of a glucuronopyranosyl residue glycosylated at C-2 and C-3. The position of each sugar unit was deduced from a 2D-ROESY[14] experiment that showed a cross peak between the signal at δ 4.45 (H-1 of the glucuronic acid) and the signal at δ 3.20 (H-3 of the aglycone moiety) and other key correlation peaks between the signal at δ 4.66 (H-1 of glucose) and the signal at δ 3.42 (H-3 of the glucuronic acid moiety). The ^1H NMR and ^{13}C NMR data indicated the β configuration at the anomeric positions for all sugar units.[14] Therefore, the structure 3β-*O*-β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-β-D-glucuronopyranosyl-23-hydroxylup-20(29)-en-28-oic acid was assigned to **6**.

Compound **7** had the molecular formula $C_{41}H_{64}O_{14}$. The FABMS spectrum of **7** showed the $[M - H]^-$ ion at m/z 779 and prominent fragments at m/z 647 $[(M - H) - 132]^-$; m/z 631 $[(M - H) - 148]^-$ (cleavage of a pentose unit with or without the glycosidic oxygen); and m/z 471 $[(M - H) - (132 + 176)]^-$ due to the subsequent loss of an uronic acid unit. The ^{13}C and DEPT ^{13}C NMR spectra showed 41 signals of which 11 were assigned to the saccharide portion and 30 to a triterpenic moiety. Analysis of NMR data of compound **7** and comparison with those of **6** showed **7** to differ from **6** only in the absence of the terminal glucopyranosyl unit (Tables 2 and 3). Therefore, the structure 3β-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucuronopyranosyl-23-hydroxylup-20(29)-en-28-oic acid was assigned to **7**.

The FABMS spectrum of **8** ($C_{36}H_{56}O_{10}$) showed the $[M - H]^-$ ion at m/z 647 and prominent fragments at m/z 471 $[(M - H) - 176]^-$ due to the loss of a glucuronic acid unit. The NMR spectra of compound **8** exhibited all resonances of an unsubstituted β-D-glucuronopyranosyl residue and of the same pentacyclic ring system as compound **6** (Tables 2 and 3). Thus, compound **8** was determined to be 3β-*O*-β-D-glucuronopyranosyl-23-hydroxylup-20(29)-en-28-oic acid.

Compound **9** had the molecular formula $C_{42}H_{66}O_{15}$. The FABMS spectrum of **9** showed the $[M - H]^-$ ion at m/z 809 and prominent fragments at m/z 631 $[(M - H) - 178]^-$, m/z 647 $[(M - H) - 162]^-$ (cleavage of a hexose unit with or without the glycosidic oxygen), and m/z 471 $[(M - H) - (162 + 176)]^-$ due to the loss of a glucuronic acid unit. The ^{13}C and DEPT ^{13}C NMR spectra showed 42 signals of which 12 were assigned to the saccharide portion and 30 to a triterpene moiety. The analysis of NMR data of compound **9** and comparison with those of **6** showed **9** to differ from **6** only for the absence of the terminal xylopyranosyl unit (Tables 2 and 3). Thus, compound **9** was determined to be 3β-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucuronopyranosyl-23-hydroxylup-20(29)-en-28-oic acid.

Compound **5** ($C_{41}H_{64}O_{13}$) exhibited a quasimolecular ion at m/z 763 in its FAB-MS spectrum, as well as signals at m/z 631 $[(M - H) - 132]^-$ and m/z 455 $[(M - H) - (132 + 176)]$ corresponding to the sequential losses of pentose and uronic acid units. The ^{13}C NMR spectrum showed 41 carbon signals of which 11 were assigned to the saccharide portion and 30 to a triterpene moiety. Acid hydrolysis of **5** yielded betulinic acid,[18,19] xylose, and glucuronic acid (GC) in the molar ratio 1:1:1. The spectral data for the aglycon moiety of **5** were identical with those of betulinic acid.[18,19] Comparison of NMR data of **5** with those of **7** indicated that both had the same glycosidic unit. Thus, compound **5** was determined to be 3β-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucuronopyranosyllup-20(29)-en-28-oic acid.

Compound **10** had the molecular formula $C_{41}H_{64}O_{13}$. Comparison of the NMR spectral data of **10** with those of **5** indicated that **10** was a 3β-hydroxylup-20(29)-en-28-oic acid derivative. In particular, hydrogens and carbons due to the C, D, E ring of **10** resonated near the same frequencies as the corresponding signals in betulinic acid, while the A- and B-ring ^1H and ^{13}C signals were shifted somewhat. The NMR spectra of **10** contained one less methyl and one more signal [^1H NMR δ 9.50 (1H, s); ^{13}C NMR δ 209.10 (CH)] than those of **5**, suggesting that one of the Me groups was replaced by a formyl group in **10**. The ^1H spectrum of **10** showed that the H-3 (δ 3.66) signals were shifted downfield by 0.5 ppm in comparison with those of **5**. In addition, one of the methyl signals was shifted downfield to δ 1.40 in **10** due to the formyl group. Therefore, the −CHO group

Table 3. ^{1}H NMR Data (J in Hz) for the Oligosaccharide Moieties of Compounds **5**, **6**, **8**, and **9** in CD_3OD[a]

	5 δH	**6** δH	**8** δH	**9** δH
Glucur 1	4.47 d, J = 7.0	4.45 d, J = 7.0	4.48 d, J = 7.0	4.48 d, J = 7.0
2	3.35 dd, J = 7.0, 9.0	3.35 dd, J = 7.0, 9.0	3.32 dd, J = 7.0, 9.0	3.33 dd, J = 7.0, 9.0
3	3.40 t, J = 9.0	3.42 t, J = 9.0	3.40 t, J = 9.0	3.40 t, J = 9.0
4	3.45 dd, J = 9.0, 10.0	3.50 dd, J = 9.0, 10.0	3.47 dd, J = 9.0, 10.0	3.48 dd, J = 9.0, 10.0
5	3.65 d, J = 10.0	3.68 d, J = 10.0	3.63 d, J = 10.0	3.64 d, J = 10.0
Xyl 1	4.55 d, J =7.6	4.52 d, J = 7.5		
2	3.20 dd, J = 7.6, 9.0	3.24 dd, J = 7.5, 9.0		
3	3.30 t, J = 9.0	3.45 J = 9.0		
4	3.50 ddd, J = 10.0, 9.0, 5.0	3.53 ddd, J = 10.0, 9.0, 5.0		
5a	3.89 dd, J = 10.0, 12.0	3.86 dd, J = 10.0, 11.0		
5b	3.12 dd, J = 5.0, 12.0	3.20 dd, J = 5.0, 11.0		
Glc 1		4.66 d, J = 7.5		4.62 d, J = 7.5
2		3.28 dd, J = 9.0, 7.5		3.28 dd, J = 9.0, 7.5
3		3.32 t, J = 9.0		3.31 t, J = 9.0
4		3.37 t, J = 9.0		3.38 t, J = 9.0
5		3.40 m		3.34 m
6a		3.85 dd, J = 12.0, 2.5		3.81 dd, J = 12.0, 2.5
6b		3.64 dd, J = 12.0, 6.0		3.62 dd, J = 12.0, 6.0

[a] Assignments are confirmed by ^{1}H−^{1}H COSY, HOHAHA, and HETCOR experiments.

could only be at C-23 or C-24.[20] The most significant features of the ^{13}C NMR spectrum of **10**, which suggested placement of the formyl group at C-23, were the downfield shifts exhibited by C-4 and C-6 and the upfield shifts experienced by C-3, C-5, and Me-24. The aglycon of **10** was elucidated to be 3β-hydroxy-23-oxolup-20(29)-en-28-oic acid and has not been reported previously. Analysis of NMR data of **10** for resonances of the glycosidic moiety indicated that **10** had the same glycosidic chain as **7**. Thus, **10** was determined to be 3β-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucuronopyranosyl-23-oxolup-20(29)-en-28-oic acid.

The FABMS of compound **11** ($C_{42}H_{64}O_{15}$) displayed a molecular ion peak at m/z 807 $(M - H)^-$ and prominent fragments at m/z 645 $[(M - H) - 162]^-$ and m/z 469 $[(M - H) - (162 + 176)]^-$ due to the subsequential losses of a hexose and glucuronic acid units. The ^{13}C aglycon signals were similar to those of **10** allowing identification of the aglycon of **11** as 3β-hydroxy-23-oxolup-20(29)-en-28-oic acid. Analysis of the spectral data for **11** revealed that this compound had the same glycosidic chain as compound **9**. Therefore, compound **11** was determined to be 3β-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucuronopyranosyl-23-oxolup-20(29)-en-28-oic acid.

The FABMS spectrum of compound **12** ($C_{36}H_{54}O_{10}$) displayed a molecular ion peak at m/z 645 $[M - H]^-$ and a fragment at m/z 469 $[(M - H) - 176]^-$ corresponding to the loss of a glucuronic acid residue. The NMR spectra of compound **12** exhibited all resonances of unsubstituted β-D-glucuronopyranose and the same pentacyclic ring system as compound **10**. Thus, **12** was concluded to be 3β-*O*-β-D-glucuronopyranosyl-23-oxolup-20(29)-en-28-oic acid.

Experimental Section

General Experimental Procedures. A Bruker AMX-500 spectrometer equipped with a Bruker X-32 computer using the UXNMR software package was used for NMR measurements in CD_3OD solutions. 2D-homonuclear proton chemical shift correlation (COSY), 2D-HOHAHA, ROESY, ^{1}H−^{13}C HETCOR, and COLOC experiments were obtained as described previously.[10,11] 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). DCCC separations were performed on a Buchi apparatus, equipped with 300 tubes. HPLC separations were performed using a Waters Model 6000A pump equipped with a U6K injector and a Model 401 refractive index detector.

Plant Material. The plant *S. divaricata* (Araliaceae) was collected in Palermo, Italy, in the spring of 1994; a sample has been deposited in the Herbarium of the Botanical Garden of Palermo.

Extraction and Isolation. Dried flowers of *S. divaricata* (500 g) were defatted with petroleum ether then extracted with MeOH to give (50 g) of residue. The methanolic extract was dissolved in H_2O. The H_2O extract (40 g) was partitioned between AcOEt and *n*-BuOH to afford an *n*-BuOH-soluble portion (6 g) and an AcOEt portion (1 g). The *n*-BuOH extract 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 16−23 (540 mg) from Sephadex containing a mixture of oleanolic glycosides **1** and **2** were separated by RPHPLC 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, 22.5 mg), **2** (t_R = 17 min, 16 mg). The AcOEt residue (1 g) contained pure compound **1**.

The air-dried leaves of *S. divaricata* (300 g) were defatted with petroleum and then extracted with MeOH to give (27 g) of residue. The methanolic extract was dissolved in H_2O. The H_2O extract (13 g) was partitioned between AcOEt and *n*-BuOH to afford an *n*-BuOH-soluble portion (3.6 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 250 fractions (4 mL) were collected. DCCC fractions 60−158 (250 mg) containing a mixture of oleanolic glycosides **1**−**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 (1:1) to yield pure compounds **1**

(t_R = 25 min, 22.5 mg), **2** (t_R = 17 min, 16 mg) , **3** ((t_R = 10 min, 28 mg), and **4** (t_R = 12.5 min, 21 mg).

Fractions 28−34 (1g) from Sephadex were purified by DCCC [$CHCl_3$−H_2O−EtOH−MeOH−*n*-PrOH (9:8:8:6:1), ascending mode, flow 10 mL/h]. DCCC fractions 110−145 (150 mg) and 160−195 (550 mg) containing a mixture of lupene glycosides were further 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 (7:3) to yield the following: from fractions 110−145, compounds **5** (t_R = 25 min, 40 mg), **8** (t_R = 17 min, 19 mg), **12** (t_R = 21 min, 12 mg); with MeOH−H_2O (65:25): from fractions 160−195, compounds **6** (t_R = 8 min, 38 mg), **7** (t_R = 13 min, 28 mg), **9** (t_R = 11 min, 20 mg), **10** (t_R = 16 min, 18 mg), and **11** (t_R = 14.5 min, 12 mg).

Methanolysis of Compounds 3−12, Carbohydrate Constituents. A solution of 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 evaporated to dryness under N_2. The residue was reacted with TRISIL-Z (Pierce) and analyzed by GLC. Retention times were identical to those of autentic methyl sugars.

Compound 1 was identified as 3-*O*-[β-D-galactopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl]olean-12-en-28-oic acid, previously isolated from *Calendula arvensis*, by spectral data.[4]

Compound 2 was identified as 3-*O*-[β-D-galactopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→4)]-β-D-glucuronopyranosyl]olean-12-en-28-oic acid, previously isolated from *Calendula arvensis*, by spectral data.[4]

Compound 3: $[\alpha]^{25}_D$ = +9.0° (*c* 1, MeOH); $C_{46}H_{72}O_{19}$, negative FABMS *m*/*z* [M − H]$^-$ 927, [(M − H) − 132]$^-$ 795, [(M − H) − 148]$^-$ 779, [(M − H) − (132 + 176 + 132)]$^-$ 485; ^{1}H NMR for aglycon (CD_3OD, 500 MHz) δ 0.92 (3H, s, Me-24), 0.99 (3H, s, Me-25), 1.09 (3H, s, Me-23), 1.19 (3H, s, Me-26), 1.18 (3H, s, Me-29), 1.35 (3H, s, Me-27), 3.20 (1H, dd, *J*=11.0, 5.0 Hz), 3.95 (1H,br s, H-16), 2.88 (1H, dd, *J* = 12.5, 5.0 Hz, H-18), 3.20 (1H, dd, *J* = 11.0, 5.0 Hz, H-3); ^{13}C NMR for aglycon (CD_3OD, 500 MHz) δ 16.1 (C-24), 16.7 (C-25), 17.9 (C-26), 19.4 (C-6), 24.5 (C-11), 26.3 (C-2), 26.4 (C-27), 27.4 (C-23), 27.4 (C-21), 28.3 (C-29), 31.1 (C-7), 32.4 (C-22), 35.2 (C-15), 36.6 (C-20), 37.3 (C-10), 38.9 (C-8), 39.36 (C-4), 40.3 (C-14), 42.3 (C-18), 42.7 (C-19), 52.2 (C-17), 57.2 (C-5), 74.2 (C-16), 90.6 (C-3), 123.5 (C-12), 145.0 (C-13), 181.9 (C-28), 180.0 (C-30). For NMR data of the sugar moiety see Table 1.

Compound 4: $[\alpha]^{25}_D$ = +3.4° (*c* 1, MeOH); $C_{51}H_{80}O_{21}$, negative FABMS *m*/*z* [M − H] − H]$^-$ 1027, [(M − H) − 132]$^-$ 895; ^{1}H NMR for aglycon (CD_3OD, 500 MHz) δ 0.90 (3H, s, Me-24), 0.99 (3H, s, Me-25), 1.12 (3H, s, Me-23), 1.15 (3H, s, Me-26), 1.16 (3H, s, Me-29), 1.36 (3H, s, Me-27), 1.47 (6H, s, Me-4′ and Me-5′) 2.25 (2H, br s, H-2′), 2.90 (1H, dd, *J* = 12.5, 5.0 Hz, H-18), 3.20 (1H, dd, *J* = 11.0, 5.0 Hz, H-3), 4.80 (1H, br s, H-16); ^{13}C NMR for aglycon (CD_3OD, 500 MHz) δ 16.1 (C-24), 16.6 (C-25), 17.9 (C-26), 19.1 (C-6), 24.4 (C-11), 26.5 (C-2), 26.5 (C-27), 27.2 (C-23), 27.8 (C-21), 28.2 (C-29), 32.0 (C-7), 32.5 (C-22), 33.0 (C-15), 36.7 (C-20), 37.4 (C-10), 38.8 (C-8), 39.0 (C-4), 40.4 (C-14), 42.5 (C-18), 42.7 (C-19), 50.0 (C-17), 57.0 (C-5), 76.0 (C-16), 90.0 (C-3), 123.5 (C-12), 144.9 (C-13), 176.7 (C-28), 180.1 (C-30), 21.2 (C-4'), 23.4 (C-5'), 70.2 (C-3'), 42.0 (C-2'), 175.0 (C-1'). NMR data of the sugar moiety are identical to those reported for compound **3**.

Compound 5: $[\alpha]^{25}_D$ +112° (*c* 1, MeOH); $C_{41}H_{64}O_{13}$, negative FABMS *m*/*z* [M − H]$^-$ 763, [(M − H) − 132]$^-$ 631, [(M − H) − 148]$^-$ 647, [(M − H) − (132 + 176)]$^-$ 455; ^{1}H NMR for aglycon (CD_3OD, 500 MHz) δ 0.72 (1H, dd, *J* = 11.0, 2.5 Hz, H-5), 0.76 (3H, s, Me-24), 0.81 (3H, s, Me-25), 0.90 (3H, s, Me-26), 0.95 (3H, s, Me-23), 1.00 (3H, s, Me-27), 1.73 (1H, s, Me-30), 1.86 (1H, dd, *J* = 8.0, 11.5 Hz, H-18), 2.82 (1H, br ddd, *J* = 11.5, 11.5, 5.0 Hz, H-19), 3.20 (1H, dd, *J* = 11.5, 5.0 Hz, H-3), 4.62 (1H, br s, H-29), 4.74 (1H, br s, H-29); ^{13}C NMR for aglycon (CD_3OD, 500 MHz) δ 15.1 (C-27), 16.0 (C-25), 16.4 (C-26), 16.0 (C-25), 16.5 (C-24), 18.1 (C-6), 20.0 (C-30), 21.9 (C-11), 26.5 (C-2), 26.9 (C-12), 28.0 (C-23), 28.2 (C-15), 30.2 (C-21), 33.1 (C-7), 32.7 (C-16), 37.4 (C-10), 37.9 (C-13), 38.0 (C-22), 40.2 (C-8), 39.9 (C-1), 39.0 (C-4), 42.8 (C-14), 47.4 (C-18), 57.0 (C-5), 51.0 (C-19), 50.3 (C-9), 56.3 (C-17), 90.0 (C-3), 111.0 (C-29), 151.8 (C-20), 182.0 (C-28). For NMR data of the sugar moiety see tables 2 and 3.

Compound 6: $[\alpha]^{25}_D$ +95° (*c* 1, MeOH); $C_{47}H_{74}O_{19}$, negative FABMS *m*/*z* [M − H]$^-$ 941, [(M − H) − 162]$^-$ 779, [(M − H) − (162 + 132)]$^-$, 647, [(M − H) − (162 + 148)]$^-$ 663, [(M − H) − (162 + 132 + 176)]$^-$ 471; ^{1}H NMR for aglycon (CD_3OD, 500 MHz) δ 0.71 (3H, s, Me-24), 0.91 (3H, s, Me-25), 1.05 (3H, s, Me-26), 1.00 (3H, s, Me-27), 1.73 (1H, s, Me-30), 1.86 (1H, dd, *J* = 8.0, 11.5 Hz, H-18), 2.26 (1H, br ddd, *J* = 11.5, 11.5, 5.0 Hz, H-19), 2.32 (1H, br ddd, *J* = 11.0, 11.5, 3.0 Hz, H-13), 3.21 (1H, dd, *J* = 10.5, 4.0 Hz, H-3), 3.30 (1H, d, *J* = 12.0 Hz, Ha-23), 3.70 (1H, d, *J* = 12.0 Hz, Hb-23), 4.63 (1H, br s, Ha-29), 4.74 (1H, br s, Hb-29); ^{13}C NMR for aglycone (CD_3OD, 500 MHz) δ 13.20 (C-24), 15.1 (C-27), 16.1 (C-25), 16.2 (C-26), 18.9 (C-6), 19.60 (C-30), 22.2 (C-11), 26.4 (C-2), 26.9 (C-12), 28.6 (C-15), 30.1 (C-21), 31.1 (C-7), 32.9 (C-16), 37.1 (C-10), 38.0 (C-13), 38.2 (C-22), 39.7 (C-8), 39.8 (C-1), 43.7 (C-4), 44.0 (C-14), 48.1 (C-18), 48.4 (C-5), 50.2 (C-19), 52.0 (C-9), 57.5 (C-17), 64.7 (C-24), 82.4 (C-3), 110.3 (C-29), 150.3 (C-20), 181.7 (C-28). For ^{13}C NMR data of the sugar moiety see Tables 2 and 3.

Compound 7: $[\alpha]^{25}_D$ +105° (*c* 1, MeOH); $C_{41}H_{64}O_{14}$, negative FABMS *m*/*z* [M − H]$^-$ 779, [(M − H) − 132)]$^-$ 647, [(M − H) − (132 + 176)]$^-$ 471. NMR data for the aglycon moiety are identical to those for **6**; for the sugar moiety signals are superimposable on those reported for **5**.

Compound 8: $[\alpha]^{25}_D$ +60° (*c* 1, MeOH); $C_{36}H_{56}O_{10}$, negative FABMS *m*/*z* [M − H]$^-$ 647, [(M − H) − 176)]$^-$ 471. NMR data for the aglycon moiety are identical to those for compound **6**; for the sugar moiety see Tables 2 and 3.

Compound 9: $[\alpha]^{25}_D$ +101° (*c* 1, MeOH); $C_{42}H_{66}O_{15}$, negative FABMS *m*/*z* [M − H]$^-$ 809, [(M − H) − 162]$^-$ 647, [(M − H) − (162 + 176)]$^-$ 471. NMR data for the aglycon moiety are identical to those for **6**; for the sugar moiety see Tables 2 and 3.

Compound 10: $[\alpha]^{25}_D$ +120 (*c* 1, MeOH); $C_{41}H_{64}O_{13}$, negative FABMS *m*/*z* [M − H]$^-$ 777, [(M − H) − 132]$^-$ 645, [(M − H) − (132 + 176)]$^-$ 469; ^{1}H NMR for aglycon (CD_3OD, 500 MHz) δ 0.94 (3H, s, Me-25), 1.06 (3H, s, Me-26), 1.00 (3H, s, Me-27), 1.40 (3H, s, Me-24), 1.75 (1H, s, Me-30), 1.89 (1H, dd, *J* = 8.0, 11.5 Hz, H-18), 2.28 (1H, br ddd, *J* = 11.5, 11.5, 5.0 Hz, H-19), 2.33 (1H,

br ddd, J = 11.0, 11.5, 3.0 Hz, H-13), 3.66 (1H, dd, J = 11.5, 5.0 Hz, H-3), 4.63 (1H, br s, Ha-29), 4.74 (1H, br s, Hb-29), 9.50 (1H, s, H-23); ^{13}C NMR for aglycon (CD_3OD, 500 MHz) δ 11.0 (C-24), 15.0 (C-27), 15.8 (C-25), 16.0 (C-26), 19.60 (C-30), 20.4 (C-6), 22.0 (C-11), 25.2 (C-2), 26.0 (C-12), 29.8 (C-21) 30.5 (C-15), 32.5 (C-7), 32.0 (C-16), 36.8 (C-10), 38.7 (C-13), 37.0 (C-22), 40.4 (C-8), 38.3 (C-1), 48.5 (C-5), 42.4 (C-14), 46.9 (C-18), 49.9 (C-19), 49.8 (C-9), 55.1 (C-4), 56.3 (C-17), 84.2 (C-3), 110.6 (C-29), 151.0 (C-20), 182.0 (C-28), 209.1 (C-23). ^{13}C NMR data for the sugar moiety signals were superimposable on those reported for compound **5**.

Compound 11: $[\alpha]^{25}_D$ +98° (*c* 1, MeOH); $C_{42}H_{64}O_{15}$, negative FABMS *m/z* $[M - H]^-$ 807, $[(M - H) - 162]^-$ 645, $[(M - H) - (162 + 176)]^-$ 469. NMR data for the aglycon moiety are superimposable on those for **10**; for the sugar moiety see Tables 2 and 3.

Compound 12: $[\alpha]^{25}_D$ +62 (*c* 1, MeOH); $C_{36}H_{54}O_{10}$, negative FABMS *m/z* $[M - H]^-$ 645, $[(M - H) - 176]^-$ 469; NMR data for the aglycon moiety are identical to those for **10**; for the sugar moiety signals are superimposable on those reported for **8**.

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