Sulfated Triterpene Derivatives from *Fagonia arabica*

Angela Perrone,[†] Milena Masullo,[†] Carla Bassarello,[†] Arafa I. Hamed,[‡] Maria Antonietta Belisario,[†] Cosimo Pizza,[†] and Sonia Piacente*,[†]

Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, Via Ponte Don Melillo, 84084 Fisciano, Salerno, Italy, and Faculty of Science, South Valley University, Aswan 81528, Egypt

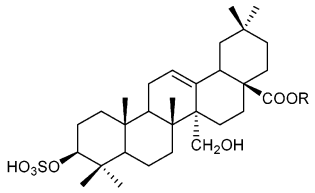
Received October 27, 2006

Two new sulfated triterpenes (**1**, **6**) and four new sulfated triterpene glycosides (**2**−**5**) have been isolated from the aerial parts of *Fagonia arabica*. Their structures were established by spectroscopic data analysis. Compounds **1**/**2** and **3**/**4** are sulfated derivatives of the rare sapogenins 3β,27-dihydroxyolean-12-en-28-oic acid and 3β,27-dihydroxyurs-12-en-28-oic acid, respectively. Compound **5** is an unusual disulfated oleanene derivative characterized by the occurrence of a 13,18-double bond, while compound **6** is the first reported naturally occurring saturated and sulfated pentacyclic triterpene of the taraxastane series with a C-20,28 lactone unit.

Fagonia arabica L. (Zygophyllaceae) is an annual and sometimes perennial shrub growing in Egypt and is used in traditional medicine.[1] The genus *Fagonia* is represented by 18 species in the flora of Egypt.[2] These species are often used in folk medicine, mainly as a popular remedy for the treatment of various skin lesions;[3,4] extracts of these plants have been reported to exhibit anti-inflammatory, analgesic, and antipyretic activities.[5] Additionally, *F. indica* is claimed to be a remedy for cancer in its early stages.[3,4] Several flavonol glycosides[6,7] and saponins or triterpenoid glycosides[8−14] have been isolated from various *Fagonia* species.

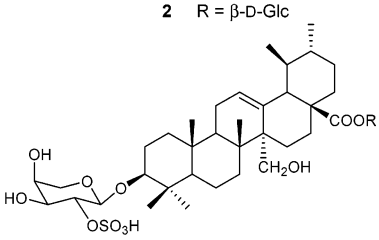
As a part of our ongoing research on new bioactive compounds from medicinal plants of the Egyptian desert, we have studied the aerial parts of *F. arabica*. In the present paper, we describe the isolation of two new sulfated triterpenes (**1**, **6**) and four new sulfated triterpene glycosides (**2**−**5**). Their structures were elucidated by extensive spectroscopic methods including 1D- (^{1}H and ^{13}C) and 2D-NMR (DQF-COSY, HSQC, HMBC, and TOCSY) experiments as well as ESIMS analysis.

On the basis of biological activities reported for *Fagonia* species,[3,4] the cytotoxic activity of compounds **1**−**6** was tested in human monocytic leukemia U937 cells.



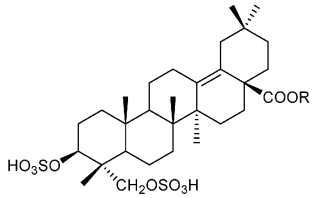
1 R = H

2 R = β-D-Glc

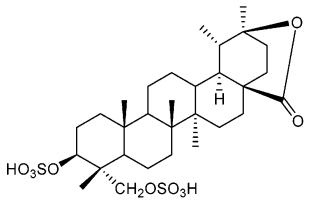


3 R = H

4 R = β-D-Glc



5 R = β-D-Glc



6

Results and Discussion

The aerial parts of *F. arabica* were extracted with 70% EtOH. The EtOH extract was fractionated over Sephadex LH-20 to yield compound **5**. The fractions obtained were chromatographed by reversed-phase HPLC to yield five new compounds, **1**−**4** and **6** (see Experimental Section).

The HRMALDITOFMS of **1** showed a major ion peak at *m*/*z* 551.3054 [M − H]$^-$, ascribable to the molecular formula $C_{30}H_{48}O_7S$ (calcd for $C_{30}H_{47}O_7S$, 551.3048), suggesting the presence of a sulfate group in the molecule. Acid hydrolysis of **1**, followed by treatment with $BaCl_2$, gave a white precipitate, thus demonstrating the presence of a sulfate residue. The ESIMS of **1** gave the highest mass ion peak at *m*/*z* 551, which was assigned to the [M − H]$^-$ ion. The MS/MS analysis of the ion at *m*/*z* 551 showed an ion peak at *m*/*z* 521 [M − H − 30]$^-$, corresponding to the neutral loss of formaldehyde, characteristic for aglycons possessing a hydroxymethyl residue.[15] A further ion peak was observed at *m*/*z* 471 [M − H − 80]$^-$, ascribable to the loss of a sulfate group. The ^{1}H NMR spectrum of compound **1** showed signals corresponding to six tertiary methyls at δ 0.82, 0.86, 0.93, 0.98, 0.99, and 1.07 and a typical signal of H-3ax at δ 3.98 (1H, dd, J = 12.1, 4.4 Hz), due to the presence of a β-OH group at C-3.[16] The downfield shift observed for H-3 (δ 3.98) was indicative of the substitution on the hydroxyl group. Further features were a signal at δ 5.65 (1H, t, J = 3.5 Hz), ascribable to an olefinic proton, and two signals at δ 3.79 (1H, d, J = 12.3 Hz) and 3.51 (1H, d, J = 12.3 Hz), ascribable to the protons of a primary alcoholic function. The ^{13}C NMR

* Author to whom correspondence should be addressed. Tel: ++39089-969763. Fax: ++39089969602. E-mail: piacente@unisa.it.
[†] Università degli Studi di Salerno.
[‡] South Valley University, Aswan.

10.1021/np060531m CCC: $37.00
Published on Web 03/06/2007

chemical shifts of all the hydrogenated carbons could be assigned unambiguously by the HSQC spectrum. These data suggested an oleanolic acid derivative with one of the methyl groups substituted by a $-CH_2OH$ function (δ 64.7).[17] Significant modifications of the chemical shift values of some carbons of rings C and D were observed by comparison with oleanolic acid.[17] The HMBC correlations indicated the placement of the $-CH_2OH$ group at C-14, suggesting for the aglycon structure 3β,27-dihydroxyolean-12-en-28-oic acid, a rare sapogenin previously reported from *Tetrapleura tetraptera*[18] and from various *Fagonia* species, in particular *F. cretica*[8,10] and *F. glutinosa*,[9] and also from *F. arabica*.[1] The position of the sulfate group was assigned to C-3 on the basis of the downfield shifts of the H-3 (δ 3.98) and C-3 (δ 87.4) signals, consistent with the presence of a sulfate group.[19,20] On the basis of these data, compound **1** was identified as the new 3-sulfate ester of 3β,27-dihydroxyolean-12-en-28-oic acid.

The molecular formula of compound **2** was established unequivocally to be $C_{36}H_{58}O_{12}S$ by HRMALDITOFMS (*m/z* 713.3582 $[M - H]^-$, calcd for $C_{36}H_{57}O_{12}S$, 713.3576). Acid hydrolysis of **2**, followed by treatment with $BaCl_2$, again demonstrated the presence of a sulfate residue. The ESIMS of **2** showed a major ion peak at *m/z* 713 $[M - H]^-$. Its MS/MS fragmentation showed two intense peaks at *m/z* 683 $[M - H - 30]^-$, due to the loss of a CH_2O unit,[15] and at *m/z* 551 $[M - H - 162]^-$, ascribable to the loss of a hexose unit. The NMR data (^{1}H, ^{13}C, 2D-TOCSY, DQF-COSY, HSQC, HMBC) of **2** in comparison to those of **1** revealed that compound **2** differs from **1** only in the presence of a β-glucopyranosyl unit (δ 5.42, 1H, d, $J = 7.5$ Hz), which was located at C-28 on the basis of the HMBC correlation between the proton signal at δ 5.42 (H-1_{glc}) and the carbon resonance at δ 178.2 (C-28). The configuration of the sugar unit was assigned after hydrolysis of **2** with 1 N HCl. The sugar unit of **2** was determined to be D-glucose. Thus, the structure of **2** was identified as the new 3-sulfate ester of 3β,27-dihydroxyolean-12-en-28-oic acid 28-*O*-β-D-glucopyranoside.

Compound **3** showed in the negative ESIMS a major ion peak at *m/z* 683 $[M - H]^-$ and significant fragments in MS/MS analysis at *m/z* 653 $[M + Na - 30]^+$ and 471 $[M - H - 212]^-$, ascribable to the loss of a sulfate-pentose unit. Its molecular formula was established unequivocally as $C_{35}H_{56}O_{11}S$ by HRMALDITOFMS (*m/z* 683.3479 $[M - H]^-$, calcd for $C_{35}H_{55}O_{11}S$, 683.3471). The ^{1}H NMR spectrum of compound **3** showed signals corresponding to four tertiary methyls at δ 0.87 × 2, 0.99, and 1.08, two secondary methyls at δ 0.94 (1H, d, $J = 6.2$ Hz) and 0.99 (1H, d, $J = 6.2$ Hz), characteristic of an ursane derivative, a typical signal of H-3ax at δ 3.18 (1H, dd, $J = 11.5$, 4.1 Hz), an olefinic proton at δ 5.49 (1H, t, $J = 3.5$ Hz), and a signal at δ 3.68 (2H), ascribable to the protons of a primary alcoholic function. These data also suggested an ursolic acid derivative with one of the methyl groups substituted by a $-CH_2OH$ function.[17] On the basis of the NMR data (^{1}H, ^{13}C, 2D-TOCSY, DQF-COSY, HSQC, HMBC) of **3** the aglycon was identified as 3β,27-dihydroxyurs-12-en-28-oic acid.[21] This rare sapogenin also has been previously isolated from *Fagonia* species, in particular *F. glutinosa*[9] and *F. arabica*.[1] The ^{1}H NMR spectrum for the sugar portion of compound **3** showed one anomeric proton signal at δ 4.75 (1H, d, $J = 3.7$ Hz). The chemical shifts of all the individual protons of the sugar unit were ascertained from a combination of 2D-TOCSY and DQF-COSY spectroscopic analysis, and the ^{13}C NMR chemical shifts of their relative attached carbons were assigned unambiguously from the HSQC spectrum (see Experimental Section). These data showed the presence of an unusual 2-sulfo-α-arabinopyranosyl unit (δ 4.75), as indicated by the downfield shifts of the H-2_{ara} (δ 4.46) and C-2_{ara} (δ 77.3) signals. An unambiguous determination of the linkage site was obtained from the HMBC spectrum, which showed key correlation peaks between the proton signal at δ 4.75 (H-1_{ara}) and the carbon resonance at δ 91.3 (C-3). The configuration of the sugar unit was assigned after hydrolysis of **3** with 1 N HCl. The sugar unit of **3** was determined to be L-arabinose. Thus, compound **3** was identified as the new 3β-(2-*O*-sulfo-α-L-arabinopyranosyl)-27-dihydroxyurs-12-en-28-oic acid.

The HRMALDITOFMS of **4** showed a major ion peak at *m/z* 845.4008 $[M - H]^-$, ascribable to the molecular formula $C_{41}H_{66}O_{16}S$ (calcd for $C_{41}H_{65}O_{16}S$, 845.3999). The ESIMS of **4** showed the highest mass ion peak at *m/z* 845, which was assigned to the $[M - H]^-$ ion. The MS/MS analysis of the ion at *m/z* 845 showed the most intense ion at *m/z* 683 $[M - H - 162]^-$, ascribable to the loss of a hexose unit. The MS^3 fragmentation of this ion showed an intense ion at *m/z* 653 $[M - H - 162 - 30]^-$, corresponding to the loss of a CH_2O unit.[15] A detailed analysis of the NMR data (^{1}H, ^{13}C, 2D-TOCSY, DQF-COSY, HSQC) showed that **4** differs from **3** only in the presence of a β-glucopyranosyl unit (δ 5.39, 1H, d, $J = 7.5$ Hz) linked to C-28, as deduced from the cross-peak observed in the HMBC spectrum between the proton signal at δ 5.39 (H-1_{glc}) and the carbon resonance at δ 178.3 (C-28). The configurations of the sugar units were assigned after hydrolysis of **4** with 1 N HCl. The sugar units of **4** were determined to be D-glucose and L-arabinose in the ratio 1:1. Thus, the structure of **4** was identified as the new 3β-(2-*O*-sulfo-α-L-arabinopyranosyl)-27-dihydroxyurs-12-en-28-oic acid 28-*O*-β-D-glucopyranoside.

The HRMALDITOFMS of **5** (*m/z* 793.3150 $[M - H]^-$, calcd for $C_{36}H_{57}O_{15}S_2$, 793.3144) supported the molecular formula $C_{36}H_{58}O_{15}S_2$, suggesting the presence of two sulfate groups in the molecule. The ESIMS of **5** showed the highest mass ion peak at *m/z* 793 and was assigned to the $[M - H]^-$ ion. The MS/MS analysis of the ion at *m/z* 793 showed the most intense ion at *m/z* 713 $[M - H - 80]^-$, ascribable to the loss of a sulfate group. The MS^3 fragmentation of this ion showed an intense ion at *m/z* 551 $[M - H - 80 - 162]^-$, corresponding to the loss of a hexose unit. The ^{1}H NMR spectrum of the aglycon portion of compound **5** showed signals corresponding to six tertiary methyls at δ 0.79, 0.80, 0.94, 0.98, 1.00, and 1.23, two signals at δ 3.98 (1H, d, $J = 9.4$ Hz) and 3.81 (1H, d, $J = 9.4$ Hz), ascribable to the protons of a primary alcoholic function, and a typical signal of H-3ax at δ 4.42 (1H, dd, $J = 11.9$, 4.9 Hz).[16] The downfield shifts observed for the H-3 (δ 4.42), H-23a (δ 3.81), and H-23b (δ 3.98) signals were indicative of the substitution on the hydroxyl groups. The ^{1}H and ^{13}C NMR data, in comparison with those of hederagenin,[22] indicated that **5** differs from hederagenin by the absence of the H-12 olefinic proton, the presence of a methylene signal at C-12, and the lack of a methine signal at C-18. These data suggested the presence of an unusual 13,18-double bond instead of the typical 12,13-double bond of a Δ^{12}-oleanene skeleton.[23,24] The positions of the sulfate groups were assigned to C-3 and C-23 on the basis of the downfield chemical shifts of the H-3 (δ 4.42) and C-3 (δ 80.7) signals and the H-23a (δ 3.81), H-23b (δ 3.98), and C-23 (δ 69.9) signals. Thus, the aglycon of **5** was identified as the 3,23-disulfate ester of 3β,23-dihydroxyolean-13(18)-en-28-oic acid. On the basis of the NMR data, the sugar unit was identified as a β-D-glucopyranosyl unit (δ 5.48). An unambiguous determination of the linkage site was obtained from the HMBC spectrum, which showed a key correlation peak between the proton signal at δ 5.48 (H-1_{glc}) and the carbon resonance at δ 177.7 (C-28). The configuration of the sugar unit was assigned after hydrolysis of **5** with 1 N HCl. The sugar unit of **5** was determined to be D-glucose. Thus, the structure of **5** was identified as the new 3,23-disulfate ester of 3β,23-dihydroxyolean-13(18)-en-28-oic acid 28-*O*-β-D-glucopyranoside. This is the first report of an oleanene derivative with an unusual 13,18-double bond as a sulfate ester.

The HRMALDITOFMS of **6** showed a major ion peak at *m/z* 631.2623 $[M - H]^-$, ascribable to the molecular formula $C_{30}H_{48}O_{10}S_2$ (calcd for $C_{30}H_{47}O_{10}S_2$, 631.2616), suggesting again the presence of two sulfate groups in the molecule. Compound **6** showed the highest mass ion peak at *m/z* 631 $[M - H]^-$ in the negative ESIMS. The MS/MS analysis of this ion exhibited the most intense ion at

m/*z* 551 [M − H − 80]$^-$, ascribable to the loss of a sulfate group. A further fragmentation of the ion at *m*/*z* 551 produced an intense ion at *m*/*z* 521 [M − H − 80 − 30]$^-$, corresponding to the loss of a CH_2O unit.[15] The ^{1}H NMR spectrum of **6** demonstrated signals corresponding to four tertiary methyls at δ 0.80, 0.95, 0.98, and 1.00, a downfield tertiary methyl at δ 1.34, and a secondary methyl at δ 1.06 (1H, d, J = 6.8 Hz), indicating a pentacyclic triterpene skeleton of the ursane or the taraxastane series.[25,26] Additionally, the spectrum showed two signals at δ 3.99 (1H, d, J = 9.7 Hz) and 3.83 (1H, d, J = 9.7 Hz), ascribable to the protons of a primary alcoholic function, and a typical signal of H-3ax at δ 4.40 (1H, dd, J = 11.7, 4.4 Hz).[16] The downfield shifts observed for the H-3 (δ 4.40), H-23a (δ 3.83), and H-23b (δ 3.99) signals were indicative of substitution on the hydroxyl groups. A detailed analysis of its NMR data (^{1}H, ^{13}C, DQF-COSY, HSQC, HMBC) indicated for **6** a 3β-hydroxytaraxastane skeleton with a −COOR (δ 179.8) group at C-28, and an −OR (δ 86.1) group at C-20, forming a δ-lactone. The presence of a δ-lactone ring was supported by the absorption peak in the IR spectrum at 1740 cm^{-1} and a signal at δ 179.8 in the ^{13}C NMR spectrum. The HMBC spectrum displayed cross-peaks between C-28 (δ 179.8) and the H-18 (δ 1.22), H-16 (δ 1.84), and H-22 (δ 1.67) signals, and also between C-20 (δ 86.1) and the Me-29 (δ 1.06), Me-30 (δ 1.34), and H-19 (δ 1.58) signals, providing confirmation for this δ-lactone ring between C-20 and C-28. The configurational assignment at C-17,18 (*trans* linkage of D/E rings) was derived by the ROESY spectrum, which showed key correlation peaks between the Me-27α (δ 1.00) and H-18α (δ 1.22) signals and between H-13β (δ 1.21) and the Me-26β (δ 0.98) and H-19β (δ 1.58) signals. Other diagnostic ROEs were recorded between the Me-29α (δ 1.06) and Me-30α (δ 1.34) signals. Therefore, the structure of **6** was established as the 3,23-disulfate ester of 3β,23-dihydroxytaraxastane-28,20β-lactone. While similar compounds of the taraxastane and ursane series have been previously described,[27,28] nevertheless this is the first report of a naturally occurring saturated and sulfated pentacyclic triterpene of the taraxastane series with a C-20,28 lactone functionality.

The cytotoxic activity of compounds **1**−**6** was tested in human monocytic leukemia U937 cells. In a range of concentrations between 0.1 and 100 μM, none of the tested compounds caused a significant reduction of the cell number as compared to controls (data not shown).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP 1000 polarimeter. IR measurements were obtained on a Bruker IFS-48 spectrometer. NMR experiments were performed on a Bruker DRX-600 spectrometer at 300 K. All 2D-NMR spectra were acquired in CD_3OD. Standard pulse sequence and phase cycling were used for DQF-COSY, 2D-TOCSY, HSQC, and HMBC spectra. Exact masses were measured by a Voyager DE mass spectrometer. Samples were analyzed by matrix-assisted laser desorption ionization time-of-flight (MALDITOF) mass spectrometry. A mixture of analyte solution and α-cyano-4-hydroxycinnamic acid (Sigma) was applied to the metallic sample plate and dried. Mass calibration was performed with the ions from ACTH (fragment 18-39) at 2465.1989 Da and angiotensin III at 931.5154 Da as internal standard. ESIMS analyses were performed using a ThermoFinnigan LCQ Deca XP Max ion-trap mass spectrometer equipped with Xcalibur software. Column chromatography was performed over Sephadex LH-20 (Pharmacia). HPLC separations were carried out on a Waters 590 system equipped with a Waters R401 refractive index detector, a Waters XTerra Prep MSC_{18} column (300 × 7.8 mm i.d.), and a Rheodyne injector. TLC was performed on silica gel F254 (Merck) plates, and reagent grade chemicals (Carlo Erba) were used throughout.

Plant Material. Fresh samples of *F. arabica* aerial parts were collected at Allaqi (southeast of Aswan, Egypt) in March 2004. A voucher specimen (No. 10966) was deposited at the Botany Department Herbarium, Faculty of Science of Aswan, Egypt.

Extraction and Isolation. The plant material (800 g) was extracted with 70% EtOH (3 × 1.5 L) for 20 days, yielding 19.3 g of extract. Part of the extract (2.7 g) was fractionated on Sephadex LH-20 (100 × 5 cm) using MeOH as the mobile phase. Ninety fractions (8 mL) were obtained. Fractions 61−63 (5.8 mg) corresponded to compound **5**. Fractions 42−43 (94.0 mg) were chromatographed by semipreparative HPLC using MeOH−H_2O (43:57) as mobile phase (flow rate 2.5 mL/min) to yield compound **2** (4.2 mg, t_R = 12.5 min). Fraction 47 (32.6 mg) was chromatographed by semipreparative HPLC using MeOH−H_2O (47:53) as mobile phase (flow rate 2.5 mL/min) to yield compounds **4** (3.4 mg, t_R = 13.8 min) and **1** (3.5 mg, t_R = 27.0 min). Fraction 49 (25.9 mg) was chromatographed by semipreparative HPLC using MeOH−H_2O (2:3) as mobile phase (flow rate 2.5 mL/min) to yield compound **3** (3.2 mg, t_R = 58.3 min). Fractions 76−80 (15.3 mg) were chromatographed by semipreparative HPLC using MeOH−H_2O (7:13) as mobile phase (flow rate 2.5 mL/min) to yield compound **6** (3.0 mg, t_R = 16.0 min).

Compound 1: white, amorphous powder; $[\alpha]^{25}_D$ +16.4 (*c* 0.20, MeOH); IR (KBr) ν_{max} 3450, 2946, 1698, 1648 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), see Table 1; ESIMS *m*/*z* 551 [M − H]$^-$; ESIMS/MS *m*/*z* 521 [M − H − 30]$^-$, 471 [M − H − 80]$^-$; HRMALDITOFMS *m*/*z* 551.3054 [M − H]$^-$ (calcd for $C_{30}H_{47}O_7S$, 551.3048).

Compound 2: white, amorphous powder; $[\alpha]^{25}_D$ +21.8 (*c* 0.25, MeOH); IR (KBr) ν_{max} 3472, 2932, 1734, 1685, 1642 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) (aglycon moiety), superimposable on those reported for compound **1**, excepted for C-28 at δ 178.2; ^{1}H NMR (CD_3OD, 600 MHz) (sugar portion) δ 5.42 (d, J = 7.5 Hz, H-1 Glc), 3.36 (dd, J = 9.0, 7.5 Hz, H-2 Glc), 3.38 (dd, J = 9.0, 9.0 Hz, H-3 Glc), 3.38 (dd, J = 9.0, 9.0 Hz, H-4 Glc), 3.44 (m, H-5 Glc), 3.85 (dd, J = 12.1, 2.5 Hz, H-6a Glc), 3.72 (dd, J = 12.1, 4.4 Hz, H-6b Glc); ^{13}C NMR (CD_3OD, 150 MHz) (sugar portion) δ 95.4 (C-1 Glc), 73.5 (C-2 Glc), 78.4 (C-3 Glc), 70.7 (C-4 Glc), 78.1 (C-5 Glc), 62.1 (C-6 Glc); ESIMS *m*/*z* 713 [M − Na]$^-$; ESIMS/MS *m*/*z* 683 [M − H − 30]$^-$, 551 [M − H − 162]$^-$; HRMALDITOFMS *m*/*z* 713.3582 [M − H]$^-$ (calcd for $C_{36}H_{57}O_{12}S$, 713.3576).

Compound 3: white, amorphous powder; $[\alpha]^{25}_D$ +60.4 (*c* 0.30, MeOH); IR (KBr) ν_{max} 3462, 2940, 1687, 1646 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) (aglycon moiety), see Table 1; ^{1}H NMR (CD_3OD, 600 MHz) (sugar portion) δ 4.75 (d, J = 3.7 Hz, H-1 Ara), 4.46 (dd, J = 8.5, 3.7 Hz, H-2 Ara), 3.97 (dd, J = 8.5, 3.0 Hz, H-3 Ara), 3.89 (m, H-4 Ara), 3.88 (dd, J = 11.9, 2.0 Hz, H-5a Ara), 3.50 (dd, J = 11.9, 3.0 Hz, H-5b Ara); ^{13}C NMR (CD_3OD, 150 MHz) (sugar portion) δ 103.3 (C-1 Ara), 77.3 (C-2 Ara), 71.9 (C-3 Ara), 66.6 (C-4 Ara), 62.1 (C-5 Ara); ESIMS *m*/*z* 683 [M − H]$^-$; ESIMS/MS *m*/*z* 653 [M − H − 30]$^-$, 471 [M − H − 212]$^-$; HRMALDITOFMS *m*/*z* 683.3479 [M − H]$^-$ (calcd for $C_{35}H_{55}O_{11}S$, 683.3471).

Compound 4: white, amorphous powder; $[\alpha]^{25}_D$ +55.0 (*c* 0.27, MeOH); IR (KBr) ν_{max} 3407, 2938, 1734, 1702, 1648 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) (aglycon moiety), superimposable on those reported for compound **3**, except for C-28 at δ 178.3; ^{1}H NMR (CD_3OD, 600 MHz) (sugar portion) δ 5.39 (d, J = 7.5 Hz, H-1 Glc), 3.35 (dd, J = 9.0, 7.5 Hz, H-2 Glc), 3.36 (dd, J = 9.0, 9.0 Hz, H-3 Glc), 3.40 (dd, J = 9.0, 9.0 Hz, H-4 Glc), 3.43 (m, H-5 Glc), 3.88 (dd, J = 12.1, 2.5 Hz, H-6a Glc), 3.71 (dd, J = 12.1, 4.4 Hz, H-6b Glc), 4.74 (d, J = 3.7 Hz, H-1 Ara), 4.46 (dd, J = 8.5, 3.7 Hz, H-2 Ara), 3.97 (dd, J = 8.5, 3.0 Hz, H-3 Ara), 3.89 (m, H-4 Ara), 3.83 (dd, J = 11.9, 2.0 Hz, H-5a Ara), 3.50 (dd, J = 11.9, 3.0 Hz, H-5b Ara); ^{13}C NMR (CD_3OD, 150 MHz) (sugar portion) δ 95.6 (C-1 Glc), 73.7 (C-2 Glc), 78.5 (C-3 Glc), 71.0 (C-4 Glc), 78.2 (C-5 Glc), 62.2 (C-6 Glc), 103.2 (C-1 Ara), 77.5 (C-2 Ara), 72.1 (C-3 Ara), 66.8 (C-4 Ara), 62.2 (C-5 Ara); ESIMS *m*/*z* 845 [M − H]$^-$; ESIMS/MS *m*/*z* 683 [M − H − 162]$^-$, 653 [M − H − 162 − 30]$^-$; HRMALDITOFMS *m*/*z* 845.4008 [M − H]$^-$ (calcd for $C_{41}H_{65}O_{16}S$, 845.3999).

Compound 5: white, amorphous powder; $[\alpha]^{25}_D$ +62.0 (*c* 0.29, MeOH); IR (KBr) ν_{max} 3446, 2950, 1735, 1689, 1641 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) (aglycon moiety), see Table 1; ^{1}H NMR (CD_3OD, 600 MHz) (sugar portion) δ 5.48 (d, J = 7.5 Hz, H-1 Glc), 3.34 (dd, J = 9.0, 7.5 Hz, H-2 Glc), 3.36 (dd, J = 9.0, 9.0 Hz, H-3 Glc), 3.36 (dd, J = 9.0, 9.0 Hz, H-4 Glc), 3.42 (m, H-5 Glc), 3.88 (dd, J = 12.1, 2.5 Hz, H-6a Glc), 3.72 (dd, J = 12.1, 4.4 Hz, H-6b Glc); ^{13}C NMR (CD_3OD, 150 MHz) (sugar

Table 1. ^{13}C and ^{1}H NMR Spectroscopic Data of the Aglycon Portions of Compounds **1**, **3**, **5**, and **6** (CD_3OD)

	1		**3**		**5**		**6**	
position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	39.4	1.64, 1.08, m	39.8	1.67, 1.08, m	39.3	1.82, 1.05, m	39.2	1.76, 1.01, m
2	25.3	2.11, 1.75, m	26.6	1.87, 1.77, m	25.0	2.19, 1.84, m	25.0	2.22, 1.82, m
3	87.4	3.98, dd (12.1, 4.4)	91.3	3.18, dd (11.5, 4.1)	80.7	4.42, dd (11.9, 4.9)	80.6	4.40, dd (11.7, 4.4)
4	39.5		39.8		42.7		42.1	
5	57.0	0.96, m	56.6	0.93, m	48.5	1.39, m	49.8	1.39, m
6	19.6	1.61, 1.46, m	19.3	1.56, 1.40, m	18.5	1.62, 1.39, m	18.2	1.69, 1.42, m
7	34.2	1.70, 1.35, m	34.8	1.80, 1.41, m	35.3	1.62, 1.40, m	34.6	1.57, 1.39, m
8	40.9		41.2		42.4		41.3	
9	49.6	1.93, m	49.5	1.78, m	52.0	1.63, m	51.9	1.48, dd (12.9, 2.9)
10	37.9		37.8		37.4		37.8	
11	24.7	1.93, 1.75, m	24.6	1.94, 1.78, m	23.0	1.60, 1.42, m	21.9	1.62, 1.32, m
12	128.9	5.65, t (3.5)	131.0	5.49 t (3.5)	26.2	2.82, 1.94, m	25.9	1.73, 1.31, m
13	140.3		135.6		140.3		44.3	1.21, m
14	48.2		48.4		45.2		42.6	
15	24.3	1.60, 1.20, m	23.1	1.80, 1.58, m	28.0	1.84, 1.14, m	28.3	1.84, 1.15, m
16	24.7	2.02, 1.84, m	25.0	2.05, 1.77, m	34.0	2.01, 1.59, m	28.6	2.02, 1.84, m
17	47.6		48.8		49.7		42.8	
18	42.6	2.97, m	54.8	2.33, d (11.5)	128.7		49.1	1.22, m
19	46.6	1.57, 1.20 m	40.2	1.30, m	42.0	2.49, d (13.9) 1.79, d (13.9)	43.1	1.58, m
20	31.3		40.7	0.99, m	33.4		86.1	
21	35.1	1.41, 1.21, m	31.7	1.47, 1.34, m	37.7	1.37, 1.23, m	27.5	2.06, 1.62, m
22	33.6	1.79, 1.54, m	38.0	1.68 (2H), m	36.1	2.26, 1.37, m	32.7	1.67 (2H), m
23	28.8	1.07, s	28.5	1.08, s	69.9	3.98, d (9.4) 3.81, d (9.4)	69.9	3.99, d (9.7) 3.83, d (9.7)
24	16.9	0.86, s	16.6	0.87, s	12.9	0.80, s	12.8	0.80, s
25	16.1	0.98, s	16.3	0.99, s	16.8	1.00, s	16.5	0.95, s
26	19.1	0.82, s	18.7	0.87, s	16.6	0.98, s	16.3	0.98, s
27	64.7	3.79, d (12.3) 3.51, d (12.3)	64.7	3.68 (2H), br s	21.2	1.23, s	14.7	1.00, s
28	180.0		176.7		177.7		179.8	
29	33.5	0.93, s	18.0	0.94, d (6.2)	32.4	0.94, s	18.5	1.06, d (6.8)
30	24.1	0.99, s	21.6	0.99, d (6.2)	24.4	0.79, s	24.1	1.34, s

portion) δ 95.8 (C-1 Glc), 73.7 (C-2 Glc), 78.5 (C-3 Glc), 71.0 (C-4 Glc), 78.4 (C-5 Glc), 62.2 (C-6 Glc); ESIMS *m/z* 793 $[M - H]^-$; ESIMS/MS *m/z* 713 $[M - H - 80]^-$, 551 $[M - H - 80 - 162]^-$; HRMALDITOFMS *m/z* 793.3150 $[M - H]^-$ (calcd for $C_{36}H_{57}O_{15}S_2$, 793.3144).

Compound 6: white, amorphous powder; $[\alpha]^{25}_D$ +111.0 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3482, 2940, 1740, 1693 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), see Table 1; ESIMS *m/z* 631 $[M - H]^-$; ESIMS/MS *m/z* 551 $[M - H - 80]^-$, 521 $[M - H - 80 - 30]^-$; HRMALDITOFMS *m/z* 631.2623 $[M - H]^-$ (calcd for $C_{30}H_{47}O_{10}S_2$, 631.2616).

Acid Hydrolysis. A solution (0.8 mg each) of **2**−**5** in 1 N HCl (0.25 mL) was stirred at 80 °C for 4 h. After cooling, the solution was concentrated by blowing with N_2. The residue was dissolved in 1-(trimethylsilyl)imidazole and pyridine (0.1 mL), and the solution was stirred at 60 °C for 5 min. After drying the solution with a stream of N_2, the residue was partitioned between H_2O and CH_2Cl_2 (1 mL, 1:1 v/v). The CH_2Cl_2 layer was analyzed by GC using an L-Chirasil-Val column (0.32 mm × 25 m). Temperatures of the injector and detector were 200 °C for both. A temperature gradient system was used for the oven, starting at 100 °C for 1 min and increasing up to 180 °C at a rate of 5 °C/min. The peak of the hydrolysate of **2** was detected at 14.72 min (D-glucose). L-Arabinose (8.93 and 9.82 min) was identified in the hydrolysate of **3**. The peaks of L-arabinose (8.90 and 9.79 min) and D-glucose (14.73 min) were detected in the hydrolysate of **4**. The peak of the hydrolysate of **5** was detected at 14.74 min (D-glucose). Retention times for authentic samples after being treated in the same manner with 1-(trimethylsilyl)imidazole in pyridine were detected at 14.71 min (D-glucose), 8.80 and 9.75 min (D-arabinose), 8.92 and 9.80 min (L-arabinose), and 14.66 min (L-glucose).

Detection of the Sulfate Group. A 1−2 mg aliquot of each sample was refluxed with 10% HCl (4 mL) for 4 h and then extracted with Et_2O. An aliquot of the aqueous layer of each was treated with 70% $BaCl_2$ to give a white precipitate ($BaSO_4$).[29]

Supporting Information Available: Experimental procedures of the biological assay of tested compounds. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Miyase, T.; Melek, F. R.; El-Gindi, O. D.; Abdel-Khalik, S. M.; El-Gindi, M. R.; Haggag, M. Y.; Hilal, S. H. *Phytochemistry* **1996**, *41*, 1175−1179.
(2) Tackholm, V. *Student's Flora of Egypt*; Cairo University Press: Cairo, 1974; p 302.
(3) Chopra, R. N.; Handa, K. L.; Kapur, L. D.; Chopra I. C. *Indigenous Drugs of India*; U. N. Dhur & Sons: Calcutta, 1982; p 507.
(4) Chopra, R. N.; Nayar, S. L.; Chopra I. C. *Glossary of Indian Medicinal Plants*; Council of Scientific & Industrial Research: New Delhi, 1956; p 116.
(5) El-Shabrawy, O. A.; El-Gindi, O. D.; Melek, F. R.; Abdel-Khalik, S. M.; Haggag, M. Y. *Fitoterapia* **1997**, *68*, 219−222.
(6) Al-Wakeel, Shahnaz A. M. *Biochem. Syst. Ecol*. **1992**, *20*, 259−264.
(7) El-Negoumy, S. I.; Al-Wakeel, S. A. M.; El-Hadidi, M. N.; Saleh, N. A. M. *Phytochemistry* **1986**, *25*, 2423−2434.
(8) Abdel-Khalik, S. M.; Miyase, T.; Melek, F. R.; El-Ashaal, H. A. *Pharmazie* **2001**, *56*, 247−250.
(9) Melek, F. R.; Miyase, T.; El-Gindy, M. R.; Abdel-Khalik, S. M.; Ghaly, N. D.; El-Kady, M. *Pharmazie* **2000**, *55*, 772−776.
(10) Abdel-Khalik, S. M.; Miyase, T.; El-Ashaal, H. A.; Melek, F. R. *Phytochemistry* **2000**, *54*, 853−859.
(11) Ansari, A. A.; Kenne, L.; Atta-ur-Rahman; Wehler, T. *Phytochemistry* **1988**, *27*, 3979−3982.
(12) Ansari, A. A.; Kenne, L.; Atta-ur-Rahman. *Phytochemistry* **1987**, *26*, 1487−1490.
(13) Atta-ur-Rahman; Ansari, A. A.; Kenne, L. *J. Nat. Prod*. **1984**, *47*, 186−187.
(14) Atta-ur-Rahman; Ansari, A. A.; Drexlex, S. A.; Clardy, J. *Heterocycles* **1982**, *19*, 217−220.
(15) Madl, T.; Sterk, H.; Mittelbach, M.; Rechberger, G. N. *J. Am. Soc. Mass Spectrom.* **2006**, *17*, 795−806.
(16) De Tommasi, N.; Piacente, S.; De Simone, F.; Pizza, C. *J. Nat. Prod.* **1993**, *56*, 1669−1675.
(17) Mahato, S. B.; Kundu, A. P. *Phytochemistry* **1994**, *37*, 1517−1575.
(18) Maillard, M.; Adewunmi, C. O.; Hostettmann, K. *Phytochemistry* **1992**, *31*, 1321−1323.

(19) Govindan, M.; Abbas, S. A.; Schimtz, F. J.; Lee, R. H.; Papkoff, J. S.; Slate, D. L. *J. Nat. Prod.* **1994**, *57*, 74−78.
(20) Patil, A. D.; Freyer, A. J.; Killmer, L.; Breen, A.; Johnson, R. K. *Nat. Prod. Lett.* **1997**, *9*, 209−215.
(21) Budzikiewicz, H.; Thomas, H. *Z. Naturforsch.* **1980**, *35B*, 226−232.
(22) Tori, K.; Seo, S.; Shimaoka, A.; Tomita, Y. *Tetrahedron Lett.* **1974**, *48*, 4227−4230.
(23) Yoshikawa, M.; Shimada, H.; Morikawa, T.; Yoshizumi, S.; Matsumura, N.; Muratami, T.; Matsuda, H.; Hori, K.; Yamahara, J. *Chem. Pharm. Bull.* **1997**, *45*, 1300−1305.
(24) Caputo, R.; Mangoni, L.; Monaco, P.; Previtera, L. *Phytochemistry* **1974**, *13*, 2825−2827.
(25) Yahara, S.; Ding, N.; Nohara, T.; Masuda, K.; Ageta, H. *Phytochemistry* **1997**, *44*, 131−135.
(26) Khan, A. Q.; Ahmed, Z.; Kazmi, S. N. U. H.; Malik, A. *J. Nat. Prod.* **1998**, *51*, 925−928.
(27) Aquino, R.; Tortora, S.; Fkih-Tetouani, S.; Capasso, A. *Phytochemistry* **2001**, *56*, 393−398.
(28) Das, M. C.; Mahato, S. B. *Phytochemistry* **1982**, *21*, 2069−2073.
(29) Akai, E.; Takeda, T.; Kobayashi, Y.; Ogihara, Y. *Chem. Pharm. Bull.* **1985**, *33*, 3715−3723.

NP060531M