Acetals of Three New Cycloartane-Type Saponins from Egyptian Collections of *Astragalus tomentosus*

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Three new cycloartane-type saponin ethyl acetals, deacetyltomentoside I (**2**), tomentoside III (**3**), and tomentoside IV (**4**), were isolated along with the known acetal tomentoside I (**1**) from the aerial parts of *Astragalus tomentosus* of Egyptian origin. The saponins from which the acetals are most probably derived are also new compounds. The structures of the acetals were established as 6α-hydroxy-23α-ethoxy-16*β*,-23(*R*)-epoxy-24,25,26,27-tetranor-9,19-cyclolanosta-3-*O*-*β*-D-xyloside (**2**), 6α-acetoxy-23α-ethoxy-16*β*,23-(*R*)-epoxy-24,25,26,27-tetranor-9,19-cyclolanosta-3-*O*-[*β*-D-(4′-*trans*-2-butenoyl)]xyloside (**3**), and 6α-acetoxy-23α-ethoxy-16*β*,23(*R*)-epoxy-24,25,26,27-tetranor-9,19-cyclolanosta-3-*O*-[*β*-D-glucopyranosyl(1 → 2)]-*β*-D-xyloside (**4**), by detailed spectroscopic and chemical studies.

The genus *Astragalus* (Leguminosae) is one of the largest and most widely distributed genera, comprising 2000 species distributed mainly in the northern temperate regions and tropical African mountains;[1] 32 species of this genera have been identified in Egypt.[2] Some species of this genus have been documented to exhibit immunostimulant, cardiovascular, and antiviral effects.[3,4] Many saponins of the cycloartane and oleanene types have been reported from the genus *Astragalus*,[3,4] and phytochemical studies on Egyptian *Astragalus* species have resulted in the isolation of a series of cycloartane-type saponins.[5−7] Previous phytochemical studies on *Astragalus tomentosus* Lam. resulted in the isolation of only two saponins, tomentoside I and II,[6,7] and this plant was thus selected for phytochemical investigation. In the present work we report the isolation of three new triterpene acetals of the cycloartane type, deacetyltomentoside I (**2**), tomentoside III (**3**), and tomentoside IV (**4**), along with the known saponin tomentoside I (**1**).Purification of the CH_2Cl_2-soluble extract of the aerial parts of *A. tomentosus* by repeated column chromatography over Si gel and MCI gel, followed by reversed-phase PTLC and HPLC, yielded the known saponin tomentoside I (**1**) and the three new saponins **2**−**4**.

Compound **1** was identified as tomentoside I by comparison of its physical and spectroscopic data with the literature data.[6] Compound **2** was obtained as colorless needles. Its IR spectrum displayed a hydroxyl absorption at 3494 cm^{-1}, and its HRFABMS showed a molecular ion peak at *m*/*z* 578.3817, corresponding to the molecular formula $C_{33}H_{54}O_8$. The ^{1}H NMR and ^{13}C NMR spectra of **2** (Tables 1 and 2) were very similar to those of tomentoside I (**1**),[6] except for the lack of signals for an acetate group and a corresponding shift of the signals for H-6 and C-6 to *δ* 3.72 and 67.1, respectively. The presence of a hydroxyl group at C-6 was confirmed by a COSY correlation of H-6 (*δ* 3.72) with H-5 (*δ* 1.76) and an HMBC correlation of H-5 (*δ* 1.76) with C-6 (*δ* 67.1). The xylose group was assigned to C-3 by a low-field methine signal at *δ* 88.6,[8] and this was confirmed by the HMBC correlations of H-3 (*δ* 3.63) with the anomeric carbon at *δ* 107.7, and of H-1′ (*δ* 4.91) with C-3 (*δ* 88.6). Thus compound **2** was identified as deacetyltomentoside I, or 6α-hydroxy-23α-ethoxy-16*β*,23-(*R*)-epoxy-24,25,26,27-tetranor-9,19-cyclolanosta-3-*O*-*β*-D-xylopyranoside.

Compound **3**, designated as tomentoside III, was isolated as white needles whose molecular formula was established as $C_{39}H_{60}O_{10}$ from HRFABMS and ^{13}C NMR data. The IR spectrum displayed absorption bands at ν_{max} 1732, 1707, and 1655 cm^{-1}, and four characteristic signals in its ^{13}C NMR spectrum (Table 2) at 166.6, 170.2, 145.2, and 122.9 were diagnostic for two ester carbonyls and two olefinic carbons, respectively. The ^{1}H NMR spectrum (Table 1) showed two upfield signals at *δ* 0.19 and 0.50 due to cyclopropane methylene protons characteristic for cycloartane-type saponins. A signal for an anomeric proton at *δ* 4.87 (d, $J = 7.6$ Hz) correlated to the anomeric carbon at *δ* 107.4 in its HSQC spectrum indicated the presence of one sugar molecule. The sugar was fixed at C-3 due to its low-field shift at *δ* 87.6 (glycosylation shift).[8] Further evidence for the linkage site of the sugar at C-3 was derived from the HMBC correlation of H-3/C-1′ and H-1′/C-3. The sugar was identified as xylose by acid hydrolysis and TLC comparison with an authentic sample, and the glycoside as *β*-D-xylopyranoside by comparing the ^{13}C NMR data of the sugar part with literature data.[5,6] The ^{13}C NMR spectrum of **3** revealed the presence of two esters, one of which was identified as an acetate attached to the aglycone at C-6 by comparison of spectroscopic data with those of tomentoside I.[10] The other ester was assigned to position 4 of the sugar from the downfield shift of C-4′ (*δ* 72.9).[5,9] The acyl group was assigned as *trans*-2-butenoyl due to the presence of two olefinic protons at δ_H 6.79 (m) and δ_H 5.88 (dd, 1.6, 15.6) corresponding to carbons at δ_C 145.2 and 122.9 in the HSQC spectrum of **3**. This was further supported by COSY (H-1‴/H-2‴; H-2‴/H-3‴), HMBC (H-2‴/C-1‴, C-3‴, C=O; H-1‴/C-2‴, C=O; H-4′/C-3′, C-5′, C=O; H-3‴/C-1‴; Figure 1), and 2DTOCSY (H-1‴/H-2‴, H-3‴; H-3/H-1′, H-2′, H-3′, H-4′, H-5′) correlations. The location of the *trans*-2-butenoyl moiety at C-4′ was supported by an HMBC correlation between the ester carbonyl carbon (*δ* 166.1) and H-4′ (*δ* 5.48). Further evidence for the

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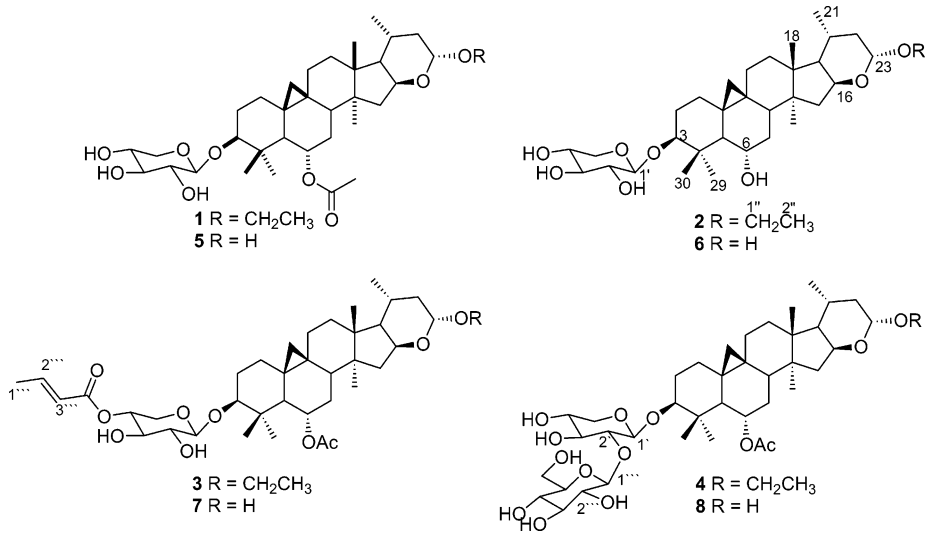
Scheme 1. Tomentoside I (**1**), Deacetyltomentoside I (**2**), Tomentoside III (**3**), and Tomentoside IV (**4**)



presence of a butenoyl ester attached to the sugar was derived from analysis of the EIMS fragments at *m*/*z* 201 [butenoyl + xylose, 73]$^+$ and 69 [butenoyl, 100]$^+$ and by comparison of the ^{1}H and ^{13}C NMR data of the esterified xylose with the reported data of kahiricoside I.[5] Thus the structure of tomentoside III (**3**) was established as 6α-acetoxy-23α-ethoxy-16*β*,23(*R*)-epoxy-24,25,26,27-tetranor-9,19-cyclolanosta-3-*O*-[*β*-D-(4′-*trans*-2-butenoyl)]xylopyranoside.

Compound **4**, designated as tomentoside IV, was isolated as an amorphous powder. Its HRFABMS and ^{13}C NMR data deduced the molecular formula as $C_{41}H_{66}O_{14}$. Its IR spectrum showed the presence of hydroxyl (3392 cm^{-1}) and ester carbonyl (1733 cm^{-1}) groups. The ^{1}H and ^{13}C NMR spectra showed that **4** is similar to tomentoside I (**1**) except for the presence of an extra sugar. The extra sugar was identified as glucose by acid hydrolysis and TLC comparison with an authentic sample. The *β*-D-glucoside was linked to C-2′ of the xylose as deduced from the deshielding of C-2′[8] and HMBC (H-1‴, C-2′; H-2′/C-1‴) correlations. The presence of a terminal glucose unit was further supported by analysis of the EIMS, which showed a fragment at *m*/*z* 578, corresponding to [M − (glc + Ac)]$^+$. The occurrence of a glucopyranosyl(1 → 2)xylopyranoside moiety was also supported by comparing the ^{13}C NMR data with the reported data for astragaloside VI isolated from *Astragalus membranaceus*.[10] Thus the structure of tomentoside IV (**4**) was established as 6α-acetoxy-23α-ethoxy-16*β*,23(*R*)-epoxy-24,25,26,27-tetranor-9,19-cyclolanosta-3-*O*-[*β*-D-glucopyranosyl(1→2)]-*β*-D-xylopyranoside.

It should be noted that all the tomentosides have ethoxy groups at the anomeric position in ring E. Since ethoxy groups are relatively rare in nature, it seems probable that these compounds are artifacts of the isolation process, which involved the use of ethanol as solvent. The actual natural products would then be the as yet unreported compounds **6**−**8**, with hydroxyl substituents in place of ethoxyl substituents. Support for this hypothesis is found in the isolation of both the ethyl acetal tomentoside I (**1**) and the corresponding hemiacetal tomentoside II (**5**) from *A. tomentosus*.[6,7]

Experimental Section

General Experimental Procedures. Melting points were recorded on an electrothermal digital apparatus. Optical rotations were taken on a Perkin-Elmer 241 polarimeter. IR spectra were measured on a MIDAC M-series FTIR instrument. NMR spectra were recorded in pyridine-d_5 at 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR using standard Varian pulse sequence programs. The HRFABMS were obtained on a JEOL HX-110 instrument. HPLC was performed on a Shimadzu LC-10AT instrument with an ODS C-18 column (250 × 10 mm).

Plant Material. *A. tomentosus* Lam. (Leguminosae) was collected from Rosetta 40 km east of Alexandria, Egypt, in April 2002. A voucher specimen was deposited at the herbarium of the Department of Botany, Faculty of Science, University of Alexandria, Egypt.

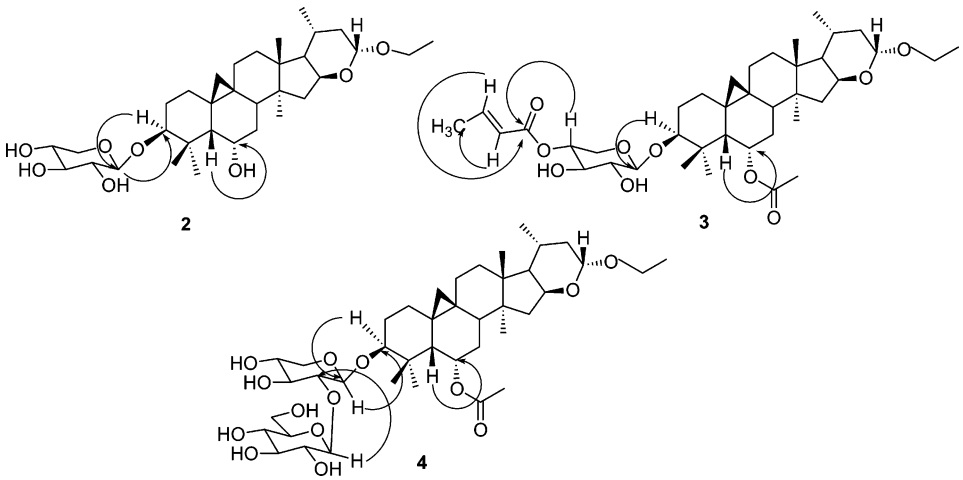


Figure 1. Selected HMBC correlations of compounds **2**−**4**.

Table 1. ^{1}H NMR Data for Compounds **2**−**4** [400 MHz pyridine-d_5][a]

position	2	3	4
1	1.24 m, 1.62 m	1.25 m, 1.62 m	1.23 m, 1.60 m
2	2.04 m, 2.42 m	1.93 m, 2.32 m	1.92 m, 2.32 m
3	3.63 dd (5.9, 11.3)	3.50 dd (6.0, 10.8)	3.43 dd (4.4, 11.6)
4			
5	1.76 dd (8.4, 11.1)	1.53 dd (7.6, 10.4)	1.52 m
6	3.72 m	4.97 m	4.93 m
7	1.82 m	1.29 m, 1.78 m	1.25 m, 1.82 m
8	1.76 m	1.78 m	1.76 m
9			
10			
11	1.65 m	1.75 m	1.74 m
12	1.48 m	1.46 m, 1.76 m	1.43 m, 1.78 m
13			
14			
15	1.57 m, 1.94 m	1.53 m, 1.81 m	1.48 m, 1.83 m
16	4.41 m	4.41 ddd (5.5, 8.6, 14.8)	4.40 m
17	1.90 m	1.80 m	1.91 m
18	1.55 s	1.13 s	1.08 s
19	0.22 d (4.8)	0.19 d (4.8)	0.16 d (4.4)
	0.55 d (4.8)	0.50 d (4.8)	0.47 d (4.4)
20	1.88 m	1.53 m	1.62 m
21	0.87 d (6.4)	0.87 d (6.4)	0.85 d (6.0)
22	1.52 m	1.54 m	1.49 m
23	4.92 m	4.93 m	4.93 m
28	0.98 s	0.94 s	0.93 s
29	1.97 s	1.39 s	1.34 s
30	1.31 s	1.09 s	1.26 s
xyl			
1′	4.91 d (7.4)	4.87 d (7.6)	4.85 d (6.8)
2′	4.07 t (8.4)	4.32 t (8.5)	4.22 t (6.8)
3′	4.16 t (8.4)	4.06 t (8.5)	4.23 m
4′	4.23 dt (5.2, 8.4)	5.48 dt (5.4, 8.6)	4.15 dt (5.2, 8.4)
5′	4.35 d (8.4)	3.68 d (8.6)	4.30 d (10)
	4.37 d (5.2)	4.40 d (5.4)	3.70 d (5.2)
1″	3.46 q (7.1)	3.49 q (7.0)	3.48 q (7.2)
	3.83 q (7.0)	3.87 q (7.2)	3.88 q (6.8)
2″	1.18 dd (1.6, 7.0)	1.20 t (7.2)	1.19 t (6.8)
glc			
1‴			5.34 d (7.6)
2‴			4.19 dd (7.6, 9.5)
3‴			3.93 t (9.5)
4‴			4.32 m
5‴			3.94 m
6‴			4.44 dd (12, 3.0)
			3.84 dd (12, 5)
O*CH*$_3$CO		2.04 s	2.00 s
OCH$_3$*CO*			
OCOCH=CH*CH*$_3$		1.58 dd (1.8, 6.8)	
OCOCH=*CH*CH$_3$		6.97 m	
OCO*CH*=CHCH$_3$		5.88 dd (1.6, 15.6)	

[a] Assignments confirmed by gHSQC, gCOSY, and 2DTOCSY experiments and by comparison with the literature data.[6]

Table 2. ^{13}C NMR Data for Compounds **2**−**4** [100 MHz pyridine-d_5][a]

position	2	3	4
1	32.4	31.9	31.8
2	30.3	29.9	30.0
3	88.6	87.6	87.4
4	42.7	42.2	42.3
5	56.7	56.6	56.5
6	67.1	70.3	70.2
7	38.2	38.1	38.1
8	53.9	49.9	49.8
9	21.2	21.0	21.0
10	29.2	28.1	28.0
11	26.2	25.9	26.0
12	33.3	33.0	32.9
13	44.8	44.8	44.2
14	46.1	46.0	46.0
15	43.5	43.3	43.2
16	70.6	70.5	70.5
17	46.2	44.5	44.8
18	20.2	19.8	19.8
19	29.6	27.8	27.5
20	25.6	25.6	25.6
21	20.6	20.6	20.6
22	33.3	33.2	33.2
23	99.1	99.1	99.1
28	19.5	19.3	19.3
29	28.7	26.7	26.8
30	16.6	16.4	16.3
xyl			
1	107.7	107.4	106.3
2	75.7	75.1	83.6
3	78.6	75.7	78.1
4	71.3	72.9	71.0
5	67.4	63.4	66.8
glc			
1			105.6
2			77.1
3			78.4
4			71.7
5			78.1
6			70.2
1″	62.7	62.7	62.7
2″	15.7	15.7	15.7
O*CH*$_3$CO		21.7	21.7
OCH$_3$*CO*		170.2	170.3
OCOCH=CH*CH*$_3$		17.6	
OCOCH=*CH*CH$_3$		145.2	
OCO*CH*=CHCH$_3$		122.9	
O*CO*CH=CHCH$_3$		166.1	

[a] Assignments confirmed by gHSQC and gHMBC experiments and by comparison with the literature data.[6]

Extraction and Isolation. The air-dried powdered aerial parts (1.5 kg) of *A. tomentosus* were extracted by maceration in EtOH/H_2O (8:2, 10 L). The solvent was removed under vacuum to yield 200 g of the crude extract, of which 100 g was suspended in MeOH/H_2O (6:4, 500 mL) and extracted with hexane (3 × 500 mL). The MeOH extract was diluted with H_2O to 50% aqueous MeOH and extracted with CH_2Cl_2 (4 × 500 mL). The CH_2Cl_2 extract (18 g) was chromatographed over an MCI gel column using H_2O/MeOH (5:6 to 0:10) and MeOH/CH_2Cl_2 (10:0 to 8:2) to yield six fractions (A−F). Fraction B (4 g) afforded **1** (2.5 g) upon crystallization with MeOH. Fraction D (3.5 g) was subjected to silica gel column chromatography eluting with hexane/EtOAc (9:1 to 0:10) to yield 12 fractions (D1−D12). Fraction D3 (164 mg) was purified on reversed-phase PTLC using MeOH/H_2O (8.5:1.5) to furnish **3** (25 mg). Fraction D7 (200 mg) was chromatographed on a silica gel column using CH_2Cl_2/MeOH (96:4) as an eluent to afford four fractions (D7-a to D7-d). Fraction D7-c (11 mg) was chromatographed on reversed -phase HPLC using MeOH/H_2O (8:2) to afford **2** (6.2 mg) and **4** (3 mg).

Acid Hydrolysis of 2−4. Methanolic solutions of **2**−**4** (1 mg each) were treated separately with 1 mL of 3% H_2SO_4 in dry MeOH under reflux for 8 h. The solutions were then neutralized by Na_2CO_3 and extracted with EtOAc to give an aqueous fraction containing sugar(s). The sugars were identified by TLC comparison with authentic samples using CH_2Cl_2/MeOH/H_2O (6:4:1) and *n*-BuOH/HOAc/H_2O (5:5:1) as mobile phases.

Deacetyltomentoside I [6α-hydroxy-23α-ethoxy 16*β*,23-(*R*)-epoxy-24,25,26,27-tetranor-9,19-cyclolanosta-3-*O*-*β*-D-xylopyranoside] (2): colorless needles; mp 283 °C; $[\alpha]^{25}_D$ −22.7° (*c* 0.092, MeOH); IR ν_{max} 3494, 1442, 1370, 1165 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; EIMS *m*/*z* (rel int) 533 (5), 428 (78), 381 (100), 311 (23); HRFABMS *m*/*z* 578.3817 $[M]^+$ (calcd for $C_{33}H_{54}O_8$, 578.3819).

Tomentoside III [6α-acetoxy-23α-ethoxy-16*β*,23(*R*)-epoxy-24,25,26,27-tetranor-9,19-cyclolanosta-3-*O*-[*β*-D-(4′-*trans*-2-butenoyl)]xylopyranoside] (3): white needles; mp 198−200 °C; $[\alpha]^{25}_D$ −29.7° (*c* 0.30, MeOH); IR ν_{max} 3475, 2917, 2849, 1732, 1707, 1655 1244 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m*/*z* (rel int) 442 (10.0), 428 (20), 410 (57), 381 (100), 382 (50), 365 (46), 349 (21); LRFABMS *m*/*z* 504 (15), 459 (17), 201 (100), 133 (57); HRFABMS *m*/*z* 687.4199 $[M - 1]^{+\bullet}$ (calcd for $C_{39}H_{59}O_{10}$, 687.4108).

Tomentoside V [6α-acetoxy-23α-ethoxy-16*β*,23(*S*)-epoxy-24,25,26,27-tetranor-9,19-cyclolanosta-3-*O*-[*β*-D-glucopyranosyl(1→2)]-*β*-D-xylopyranoside] (4): white amorphous powder; mp 178−180 °C; $[\alpha]^{25}_D$ −17.5° (*c* 0.29, MeOH); IR ν_{max} 3392, 2945, 1733, 1461, 1369, 1243 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRFABMS *m*/*z* 782.4466 $[M]^{+\bullet}$ (calcd for $C_{41}H_{66}O_{14}$ 782.4453); EIMS (rel int) *m*/*z* 604 (5.2), 578 (10.4), 382 (20.8), 369 (31.2), 340 (53.7), 314 (59.3), 265 (100).

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Supporting Information Available: ^1H NMR spectra for compounds **2**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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