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Cyclocephaloside I: A Novel Cycloartane-Type Glycoside from *Astragalus microcephalus*

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A novel cycloartane-type glycoside, cyclocephaloside I (**1**) was isolated from the roots of *Astragalus microcephalus* in addition to known glycosides cyclocanthoside E (**2**) and astragaloside IV (**3**). The structure of **1** was determined by spectral (IR, ^{1}H and ^{13}C NMR, and FABMS) and chemical (acetylation) methods and established as 20,25-epoxy-3β-(β-D-xylopyranosyl)oxy-6α-(β-D-glucopyranosyl)oxy-cycloartane-16β,24α-diol.

In the flora of Turkey, the genus *Astragalus* (Leguminosae) is represented by approximately 380 species, which are listed under several sections.[1] *Astragalus* species, growing wild in Turkey, are of economical importance. Gum tragacanth is a very well-known foodstuff and pharmaceutical emulsifier, derived from *Astragalus* species. In Turkey, *A. microcephalus* is primarily used for the production of tragacanth.[2] Recently, we reported the isolation of eight cycloartane saponins from *Astragalus melanophrurius* Boiss., which were found to stimulate lymphocyte transfer in vitro,[3] and four novel cycloartane saponins from *Astragalus oleifolius* DC.[4] This paper describes the isolation and structure elucidation of a novel cycloartane-type glycoside, cyclocephaloside I (**1**), in addition to known glycosides cyclocanthoside E (**2**)[5] and astragaloside IV (**3**)[6] from the roots of *A. microcephalus* Willd.

The water-soluble part of the 80% aqueous ethanolic extract of the roots of *A. microcephalus* was first fractionated on Si gel. The fraction rich in saponins was subjected to medium-pressure liquid chromatography (MPLC) using LiChroprep C-18 as stationary phase and eluting with MeOH−H_2O mixtures (60−75% MeOH in H_2O) to yield the compounds **1**, **2**, and **3**, respectively.

The IR spectrum of **1** showed hydroxyl absorption bands. The FABMS of **1** exhibited quasimolecular ion peaks at *m/z* 807 $[M + Na]^+$ and at *m/z* 1569 $[2\ M + H]^+$, which are compatible with the molecular formula $C_{41}H_{68}O_{14}$. The ^{1}H NMR spectrum of **1** showed characteristic signals due to cyclopropane−methylene protons as an AX system (δ 0.16 and 0.54, AX system, J_{AX} = 4.0 Hz; H_2-19) and seven tertiary methyl groups. Additionally, the resonances for two anomeric protons were observed at δ 4.80 (d, J = 7.4 Hz) and 4.87 (d, J = 7.6 Hz). Thus, compound **1** was considered to be a cycloartane-type triterpene diglycoside. This observation was supported by the ^{13}C NMR spectral data of **1**. The NMR signals were analyzed by the use of COSY and TOCSY coupled with HMQC. The ^{1}H and ^{13}C NMR data supported the assignment of the sugar moieties in **1** as β-D-xylopyranose and β-D-glucopyranose.

The remaining carbon and proton resonances were consistent with $C_{30}H_{50}O_5$ for the aglycon moiety. This implied six saturated ring systems because there were no olefinic protons. Additional functionalities on the aglycon included four geminal methine protons on oxygen-bearing carbon atoms (H-3, H-6, H-16, and H-24). The resonances for the oxygenated carbons also indicated the presence of four oxymethine carbons (δ 88.6, 79.5, 74.0, 68.7; C-3, C-6, C-16, and C-24, respectively) and two oxygenated quaternary carbons (δ 78.9 and 75.2, C-20 and C-25, respectively). To clarify the intermolecular connectivities of the partial structures in **1** HMBC was used. By the help of this experiment, not only connectivities but also interglycosidic linkages were revealed. These data suggested the presence of the partial structure, a monohydroxypyran derivative. The carbon resonances assigned to the side chain consisting of a doublet (δ 68.7, C-24), two triplets (δ 26.3 and 26.7, C-22 and C-23, respectively), two singlets (δ 78.9 and 75.2, C-20 and C-25, respectively), and three quartets (δ 28.8, 28.6, and 28.0; C-21, C-26, and C-27, respectively). The HMBC correlations from C-17 (to Me-18 and Me-21), C-20 (to H-17 and Me-21), C-22 (to H-17 and Me-21), C-24 (to Me-26 and Me-27), and C-25 (to Me-26) confirm this proposal. On the other hand, the FABMS of **1** exhibited a common fragmentation peak at *m/z* 143 due to the side chain. Mild acetylation of **1** yielded an octaacetate, **1a**. From the FABMS of **1a**, which displayed a $[M + Na]^+$ ion at *m/z* 1143, a molecular formula of $C_{57}H_{84}O_{22}$ was proposed for **1a**. The IR of **1a** still exhibited a free OH absorption band after acetylation, together with the information from FABMS, indicating the presence of a free hydroxyl group on the sapogenol moiety. The peaks observed at *m/z* 259 and 331, corresponding to the triacetyl−xylose oxonium and the tetraacetyl−glucose oxonium ions, respectively, confirmed the terminal positions of these units. The remaining acetoxyl resonance in the ^{1}H NMR spectrum of **1a** was attributed to the sapogenol moiety. Indeed, the resonance due to the H-24 was observed at δ 4.77, showing the expected downfield shift in comparison to **1**. No downfield shifts were observed for H-3 and H-6, supporting the sites of glycosidations. Also, no downfield shift for H-16 was observed. In the COSY experiment performed with **1a**, the signal ob-

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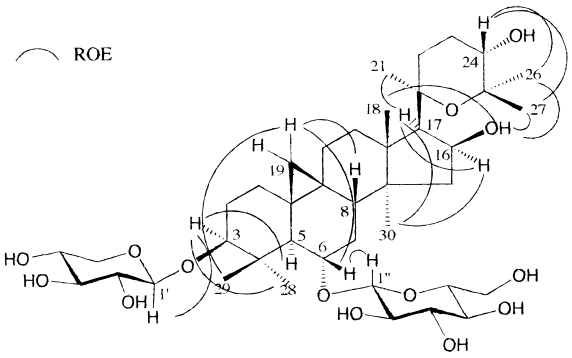
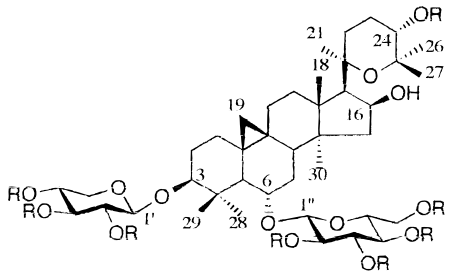


Figure 1. ROE correlations observed for compound **1**.

served at 4.60 ppm was assigned to H-16, which shows correlations to H_2-15 (δ 2.06 and 1.46), H-17 (δ 1.87), and a doublet signal at δ 5.29 (J = 3.9 Hz). The latter signal was assigned to a free hydroxyl group located at C-16, showing the site of the nonacetylated secondary hydroxyl group on the sapogenol moiety. This unexpected result can be explained by the steric hindrance of the side chain forming a pyran ring. HMQC of **1a** confirmed this deduction, because the signal assigned to C(16)-OH (δ 5.28) shows no correlation to any carbon resonance. The relative stereochemistry of **1** was established based on ROE data from a 500-ms ROESY experiment. The stereochemistry of the ring fusions and at the substituents could be unambiguously determined from ROE involving diaxial (1,3) correlations. Figure 1 displays the correlations that were used to elucidate the relative stereochemistry. Consequently, the structure of **1** was established as 20,25-epoxy-3β-(β-D-xylopyranosyl)oxy-6α-(β-D-glucopyranosyl)oxy-cycloartane-16β,24α-diol, for which the trivial name cyclocephaloside I is proposed.



(**1**) R = H
(**1a**) R = Ac

The carbon and proton resonances for the sugar moieties of **2** were similar to those in **1**. The remaining carbon and proton resonances for **2** were consistent with $C_{30}H_{52}O_5$ for the aglycon moiety. In the ^{1}H NMR spectrum of **2**, one secondary and six tertiary methyl resonances were observed. Thus, **2** appeared to have an acyclic side chain. The configuration of the C-24 chiral center was determined by comparing the ^{13}C NMR spectral data of compound **2** and of cyclocanthosides A, B, C, E, and G.[5] The C-24 atom, having the *S*-configuration,[5] resonates at δ 77.1. In contrast, the C-24 atom, having the *R*-configuration, resonates at δ 80.5.[7] Thus, the experimental results permit the conclusion that **2** has the 24*S*-configuration, and its structure was established as cyclocanthoside E.[5] The ^{1}H and ^{13}C NMR spectral data of **3** were identical with those of astragaloside IV.[6]

Experimental Section

General Experimental Procedures. See Çalis et al.[4]

Plant Material. *A. microcephalus* Willd. was collected from Mucur-Avanos, Nevsehir, Central Anatolia, in June 1995. Voucher specimens (95-017) have been deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

Extraction and Isolation. The air-dried, powdered roots (260 g) were extracted with 80% aqueous EtOH under reflux. The solvent was removed by rotary evaporation, yielding 30 g of extract. The H_2O-soluble part of the EtOH extract (14 g) was subjected to VLC using normal-phase Si gel 60 (150 g), employing $CHCl_3$−MeOH and $CHCl_3$−MeOH−H_2O mixtures with increasing polarity, yielding nine fractions (fractions A−I); fraction A (200 mL of $CHCl_3$−MeOH; 90:10, 150 mg), fraction B (200 mL of $CHCl_3$−MeOH; 87.5:12.5, 114 mg), fraction C (400 mL of $CHCl_3$−MeOH; 80:20, 117 mg), fraction D (200 mL of $CHCl_3$−MeOH−H_2O; 80:20:1, 1143 mg), fraction E (200 mL of $CHCl_3$−MeOH−H_2O; 80:20:2, 3181 mg), fraction F (200 mL of $CHCl_3$−MeOH−H_2O; 70:30:2, 1671 mg), fraction G (200 mL of $CHCl_3$−MeOH−H_2O; 70:30:3, 1100 mg), fraction H (200 mL of $CHCl_3$−MeOH−H_2O; 60:40:3, 614 mg), and fraction I (200 mL of $CHCl_3$−MeOH−H_2O; 60:40:4, 3870 mg). An aliquot (634 mg) of fraction G was subjected to MPLC, using LiChroprep C-18 as stationary phase, and eluted with MeOH−H_2O mixtures (60−75% MeOH in H_2O; 200 mL 60% MeOH, 200 mL 70% MeOH, and 300 mL 75% MeOH; fraction volume 12−14 mL) to yield the compounds **1** (fractions 25−26; 33 mg), **2** (fractions 30−34; 79 mg) and **3** (fractions 36−44; 140 mg), respectively.

Cyclocephaloside I (1): $[\alpha]^{20}_D$ +6.1° (*c* 0.42, MeOH); IR ν_{max} (KBr) 3400 (OH) cm^{-1}; ^{1}H NMR (C_5D_5N, 300 MHz) δ 4.87 (1H, d, J = 7.6 Hz, H-1″), 4.86 (1H, m, H-16), 4.80 (1H, d, J = 7.4 Hz, H-1′), 4.44 (1H, dd, J = 11.4, 2.3 Hz, H_a-6″), 4.35 (1H, overlapped, H_a-5′), 4.33 (1H, overlapped, H_b-6″), 4.22 (1H, overlapped, H-3″), 4.20 (2H, overlapped, H-4′ and H-4″), 4.10 (1H, dd, J = 8.4, 8.6 Hz, H-3′), 4.00 (2H, overlapped, H-2′ and H-2″), 3.88 (1H, overlapped H-5″), 3.76 (1H, dt, J = 9.5, 6.7 Hz, H-6), 3.68 (1H, overlapped, H_b-5′), 3.64 (1H, br s, H-24), 3.49 (1H, dd, J = 11.4, 4.2 Hz, H-3), 3.12 (1H, m, H_a-23), 2.35 (1H, m, H_a-2), 2.34 (1H, m, H_a-15), 2.27 (2H, m, H-7), 2.20 (1H, m, H_a-22), 2.15 (1H, m, H_a-11), 2.06 (1H, d, J = 7.6 Hz, H-17), 2.01 (3H, s, H_3-28), 1.98 (1H, m, H_b-2), 1.95 (1H, m, H-8), 1.87 (1H, d, J = 8.4 Hz, H-5), 1.86 (1H, m, H_b-15), 1.82 (2H, m, H_b-11, H_b-22), 1.78 (1H, m, H_a-12), 1.65 (1H, m, H_b-12), 1.64 (3H, s, H_3-18), 1.58 (1H, m, H_a-1), 1.51 (3H, s, H_3-21), 1.42 (3H, s, H_3-26), 1.34 (3H, s, H_3-29), 1.26 (3H, s, H_3-27), 1.22 (1H, m, H_b-23), 1.20 (1H, m, H_b-1), 0.88 (3H, s, H_3-30), 0.54 (1H, d, J = 4.0 Hz, H_a-19), 0.16 (1H, d, J = 4.0 Hz, H_b-19); ^{13}C NMR (C_5D_5N, 75.5 MHz) δ 107.7 (d, C-1′), 105.1 (d, C-1″), 88.6 (d, C-3), 79.5 (d, C-6), 79.2 (d, C-3″), 78.9 (s, C-20), 78.5 (d, C-3′), 78.1 (d, C-5″), 75.6 (d, C-2′), 75.6 (d, C-2″), 75.2 (s, C-25), 74.0 (d, C-16), 71.8 (d, C-4′), 71.3 (d, C-4″), 68.7 (d, C-24), 67.1 (t, C-5′),

63.1 (t, C-6″), 60.8 (d, C-17), 52.7 (d, C-5), 47.4 (t, C-15), 46.8 (s, C-14), 46.1 (d, C-8), 45.9 (s, C-13), 42.7 (s, C-4), 34.8 (t, C-7), 34.2 (t, C-12), 32.3 (t, C-1), 30.2 (t, C-2), 29.5 (t, C-19), 29.1 (s, C-10), 28.8 (q, C-21, C-28), 28.6 (q, C-26), 28.0 (q, C-27), 26.7 (t, C-22), 26.3 (t, C-23), 24.1 (t, C-11), 21.1 (s, C-9), 20.9 (q, C-18), 20.1 (q, C-30), 16.7 (q, C-29); FABMS *m/z* $[M + Na]^+$ 807 (47).

Cyclocanthoside E (2): IR υ_{max} (KBr) 3400 (OH) cm^{-1}; ^1H and ^{13}C NMR (^1H = 300 MHz; ^{13}C = 75.5 MHz, C_5D_5N) data identical to the literature.[5]

Astragaloside IV (3): IR υ_{max} (KBr) 3400 (OH) cm^{-1}; ^1H and ^{13}C NMR (^1H = 300 MHz; ^{13}C = 75.5 MHz, C_5D_5N) data identical to the literature.[6]

Acetylation of 1. Treatment of **1** (10 mg) with Ac_2O (1 mL) and pyridine (1 mL) at room temperature overnight followed by the usual workup yielded compound **1a**.

Cyclocephaloside I octaacetate (1a): IR υ_{max} (KBr) 3435 (OH), 1755 (ester) cm^{-1};^1H NMR ($CDCl_3$, 300 MHz) δ 5.29 [1H, d, J = 3.8 Hz, C(16)−OH], 5.18 (1H, t, J = 9.1 Hz, H-3′), 5.16 (1H, t, J = 9.3 Hz, H-3″), 5.04 (1H, t, J = 9.6 Hz, H-4″), 4.97 (1H, overlapped, H-2′), 4.94 (1H, overlapped, H-1″), 4.92 (1H, overlapped, H-4′), 4.77 (1H, br s, H-24), 4.60 (1H, d, J = 7.8 Hz, H-1″), 4.60 (1H, overlapped, H-16), 4.48 (1H, d, J = 7.3 Hz, H-1′), 4.17 (2H, m, H_2-6), 4.08 (1H, dd, J = 11.7, 5.3 Hz, H_a-5′), 3.67 (1H, ddd, J = 9.6, 5.1, 2.6 Hz, H-5″), 3.44 (1H, dt, J = 9.5, 6.7 Hz, H-6), 3.31 (1H, dd, J = 11.7, 9.6 Hz, H_b-5′), 3.09 (1H, dd, J = 11.2, 4.4 Hz, H-3), 2.36 (1H, m, H_a-22), 2.20 (1H, m, H_a-23), 2.06 (1H, m, H_a-15), 1.96 (1H, m, H_a-11), 1.92 (1H, m, H_a-2), 1.78 (2H, m, H-8, H_a-12), 1.75 (1H, m, H_b-23), 1.67 (1H, m, H_b-12), 1.66 (1H, d, J = 7.7 Hz, H-17), 1.65 (1H, m, H_b-2), 1.62 (1H, overlapped, H-5), 1.56 (3H, s, H_3-21), 1.52 (1H, m, H_a-1), 1.48 (2H, m, H-7), 1.46 (1H, m, H_b-15), 1.44 (3H, s, H_3-18), 1.35 (3H, s, H_3-26), 1.21 (1H, m, H_b-11), 1.19 (2H, m, H_b-1, H_b-22), 1.14 (3H, s, H_3-27), 0.99 (3H, s, H_3-28), 0.90 (3H, s, H_3-30), 0.89 (3H, s, H_3-29), 0.51 (1H, d, J = 4.2 Hz, H_a-19), 0.35 (1H, d, J = 4.2 Hz, H_b-19), additional signals: δ 2.13, 2.11, 2.00, 1.99 (each 3H, s), 2.04, 2.03 (each 6H, s) (aliphatic acetoxy × 8); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 103.1 (d, C-1′), 100.9 (d, C-1″), 88.9 (d, C-3), 80.6 (d, C-6), 79.6 (s, C-20), 75.0 (s, C-25), 73.5 (d, C-3″), 73.4 (d, C-16), 72.0 (d, C-3′), 72.0 (d, C-2″), 71.9 (d, C-5″), 71.6 (d, C-2′), 71.5 (d, C-24), 69.2 (d, C-4′), 68.2 (d, C-4″), 62.4 (t, C-6″), 62.2 (t, C-5′), 60.1 (d, C-17), 51.5 (d, C-5), 47.6 (t, C-15), 46.5 (s, C-14), 46.1 (d, C-8), 46.0 (s, C-13), 41.3 (s, C-4), 34.2 (t, C-12), 34.1 (t, C-7), 31.5 (t, C-1), 29.9 (t, C-19), 29.0 (t, C-2), 28.8 (s, C-10), 27.7 (q, C-26), 27.5 (q, C-27), 27.5 (q, C-28), 27.5 (q, C-21), 26.5 (t, C-22), 26.0 (t, C-11), 20.8 (s, C-9), 20.6 (t, C-23), 20.0 (q, C-18), 20.0 (q, C-30), 16.1 (q, C-29); additional signals 170.7−169.2 ($COCH_3$), 20.95−20.57 ($COCH_3$); FABMS *m/z* 1143 (100) $[M + Na]^+$ (calcd for $C_{57}H_{84}O_{22}$), 331 (13) [tetraacetyl−glucose oxonium]$^+$, 259 (17) [tetraacetyl−xylose oxonium]$^+$.

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