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Two novel cycloartane-type triterpene glycosides from the roots of *Astragalus prusianus*

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Abstract—Two novel cycloartane-type glycosides (**1**–**2**), 16-*O*-β-D-glucopyranosyl-20(*S*),24(*R*)-5(α),9-diepoxy-2α,3β,16β,25-tetrahydroxy-9,10-seco-cycloarta-1(10),6(7)-diene and 3-*O*-β-D-xylopyranosyl-16-*O*-β-D-glucopyranosyl-20(*S*),24(*R*)-epoxy-3β,16β,25-trihydroxycycloartane, were isolated from the roots of *Astragalus prusianus*. The 5α,9-epoxy structural feature in **1** is encountered for the first time.

1. Introduction

Astragalus L., the largest genus in the family Leguminosae, is represented by 380 species in the flora of Turkey.[1] The roots of *Astragalus* are used in traditional medicine as an antiperspirant, diuretic and tonic drug. It has also been used in the treatment of diabetes mellitus, nephritis, leukemia and uterine cancer.[2] In the district of Anatolia, located in South Eastern Turkey, an aqueous extract of the roots of *Astragalus* is traditionally used against leukemia and for its wound-healing properties. Known biologically active constituents of *Astragalus* roots represent two major classes of chemical compounds, polysaccharides and saponins.[2] *Astragalus* polysaccharides are known to have anticancer and immune enhancing properties in both in vitro and in vivo experiments.[3–6]

Chemical studies on *Astragalus* saponins have indicated the presence of cycloartane-type triterpenoid glycosides which were found to exert biological activities, e.g. anti-inflammatory, analgesic, diuretic, hypotensive and sedative effects.[6]

Our earlier investigations on *Astragalus* species resulted in the isolation of a series of cycloartane-type triterpenoidal saponins.[7–15] Continuing our studies on the constituents of *Astragalus* species, we investigated the roots of *A. prusianus*. This paper describes the isolation and structure elucidation of two novel cycloartane-type glycosides.

2. Results and discussion

The IR spectrum of compound **1** indicated the presence of hydroxyl (3396 cm^{-1}) and olefinic (1593 cm^{-1}) functionalities. The HRESI-MS of **1** provided $[M+Na]^+$ at *m/z* 687.2429 indicating the molecular formula $C_{36}H_{56}O_{11}$.

Inspection of the ^1H NMR of **1** (Table 1) showed **7** tertiary methyl groups at δ 0.92, 0.97, 1.09, 1.16 (×2), 1.28 and 1.44 (each s; respectively H_3-29, H_3-30, H_3-28 H_3-26, H_3-27, H_3-18 and H_3-21), a trisubstituted olefinic proton at δ 5.12 (1H, br.s, H-1), the protons of one disubstituted double bond at δ 6.43 dd (1H, *J*=1.4, 10.0 Hz, H-6) and 5.43 dd (1H, *J*=4.4, 10.0 Hz, H-7), as well as one anomeric proton signal at δ 4.23 d (1H, *J*=7.8 Hz, H-1′), indicative of the presence of a β-linked sugar unit.

The ^{13}C NMR spectrum of **1** displayed 36 signals, 6 of which were in good accordance with the presence of a β-D-glucopyranose moiety.[15] The 30 resonances of the aglycon moiety were consistent with a $C_{30}H_{46}O_6$ triterpene framework, indicating the presence of 8 degrees of unsaturation, i.e. two double bonds and six ring systems.

Keywords: *Astragalus prusianus*; leguminosae; cycloartanes.

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Table 1. ^{1}H and ^{13}C Assignments of **1** (in CD_3OD) and **2** (in C_5D_5N)

C/H	**1** (δ_C)	**2** (δ_C)	**1** (δ_H, *J* Hz)	**2** (δ_H, *J* Hz)
1	112.9 d	32.2 t	5.12 br.s	1.27 m, 1.53 m
2	73.8 d	30.2 t	4.11 br.s	1.91 m, 2.30 m
3	81.6 d	88.7 d	3.51 br.s	3.47 dd (4.0, 11.5)
4	37.8 s	41.5 s		
5	84.9 s	47.7 d		1.29[a]
6	135.5 d	21.0 t	6.43 dd (1.4, 10.0)	0.59 m, 1.47 m
7	127.1 d	26.2 t	5.43 dd (4.4, 10.0)	0.95 m, 1.17 m
8	49.5 d	47.8 d	2.25[a]	1.55[a]
9	80.0 s	20.0 s		
10	147.5 s	25.9 s		
11	34.7 t	26.8 t	1.64 ddd (3.3, 4.6, 14.2), 1.98[a]	1.10 m, 1.92 m
12	31.8 t	33.1 t	1.72 m, 1.86 m	1.90 m
13	46.7 s	46.9 s		
14	47.4 s	47.0 s		
15	46.5 t	48.0 t	1.77 dd (6.6, 13.0), 2.06 dd (7.2, 13.0),	2.02[a], 2.25[a]
16	84.8 d	83.7 d	4.30 ddd (5.2, 6.7, 7.8)	4.54[a]
17	60.0 d	60.1 d	2.30 d (8.1)	2.45 d (7.9)
18	19.3 q	21.7 q	1.28 s	1.60 s
19	46.5 t	30.2 t	2.25[a], 2.58 dt (1.9, 16.3)	0.43 (4.5), 0.19 (4.5)
20	88.3 s	87.2 s		
21	25.5 q	26.3 q	1.44 s	1.75 s
22	39.8 t	39.0 t	1.90 m, 2.04 m	2.13 m, 2.28 m
23	26.4 t	26.4 t	1.84 m, 1.96 m	2.04 m, 2.10 m
24	85.3 d	84.5 d	3.77 dd (5.8, 7.5)	4.09 dd (8.0, 5.0)
25	73.0 s	71.4 s		
26	26.3 q	26.3 q	1.16 s	1.42 s
27	27.1 q	27.7 q	1.16 s	1.48 s
28	22.1 q	26.3 q	1.09 s	1.24 s
29	22.6 q	15.6 q	0.92 s	1.03 s
30	21.7 q	20.7 q	0.97 s	0.88 s
1′	106.7 d	107.6 d	4.23 d (7.8)	4.85 d (7.3)
2′	75.6 d	75.7 d	3.16 dd (8.0, 8.7)	3.99 dd (7.4, 8.5)
3′	78.6 d	78.7 d	3.33 t (8.3)	4.21[a]
4′	71.8 d	71.6 d	3.26 t (8.6)	4.21[a]
5′	77.8 d	67.2 t	3.24 ddd (2.1, 5.4, 9.4)	4.35[a], 3.75 t (11.0)
(6′)	62.9 t	–	3.84 dd (2.1, 11.8), 3.66 dd (5.4, 11.8)	–
1″	–	106.7	–	4.78 d (7.6)
2″	–	75.7	–	4.02 dd (7.9, 9.0)
3″	–	79.0	–	4.26 t (8.9)
4″	–	72.0	–	4.20[a]
5″	–	78.5	–	3.96[a]
6″	–	63.1	–	4.32[a], 4.53[a]

Assignments confirmed by COSY, TOCSY, HMQC and HMBC experiments.
[a] Signal pattern was unclear due to overlapping.

The ^{13}C and DEPT spectra showed that the resonances assigned to the aglycon moiety comprise 7 methyl, 6 methylene, 9 methine, four of which are oxygen-bearing (δ 73.8, 81.6, 84.8 and 85.3) and three olefinic (δ 112.9, 127.1 and 135.5), and 8 quaternary carbons, four of which are oxygen-bearing (δ 73.0, 80.0, 84.9 and 88.3). The presence of a

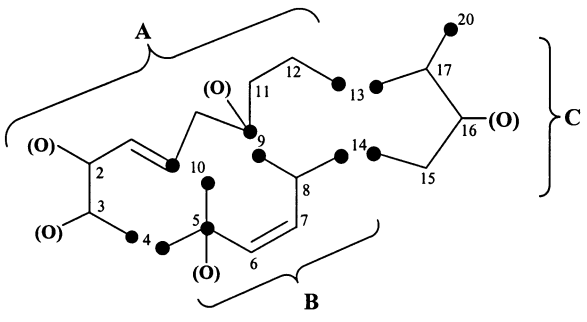


Figure 1. Fragments A–C deduced from 2D-NMR measurements of **1**.

20,24-tetrahydrofuran ring in compound **1**, a common feature of cycloartane-type triterpenoids isolated from *Astragalus* species, was evident from the proton and carbon chemical shifts (Table 1). Taking into account of the results of comprehensive 1D- and 2D NMR studies and previously reported spectroscopic data of related metabolites isolated from the genus *Astragalus*, it was inferred that **1** possesses a highly oxidized cycloartane-type nucleus.

The combined use of G-DQF-COSY, G-TOCSY and G-HMQC spectra of **1**, allowed the assignment of three major spin systems (Fig. 1) excluding the signals ascribable to the 20,24-tetrahydrofuran and the β-D-glucopyranose moiety: 'A' (H-3→H_2-12), 'B' (H-6→H-8) and 'C' (H_2-15→H-17). The spin system A starts with the H-3 resonance (δ 3.51, br.s) which showed cross peaks with H-2 (δ 4.11, br.s) in the G-TOCSY spectrum. H-2 couples with an olefinic proton at δ 5.12, (br.s, H-1) which showed allylic coupling with H_a-19 (δ 2.25). H_a-19 showed correlation with H_b-19 (δ 2.58 dt, J=1.9, 16.3 Hz), while the latter

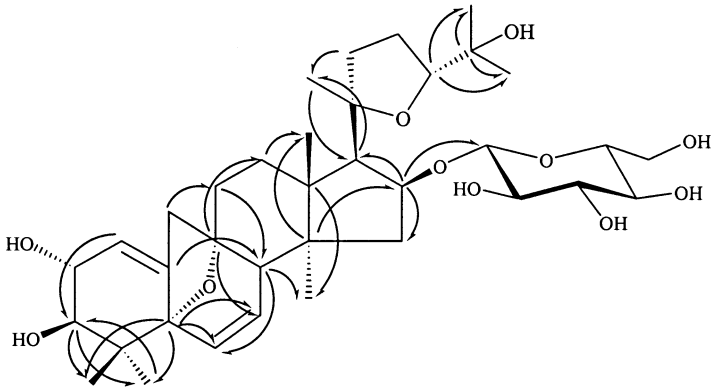


Figure 2. Key HMBC correlations of **1** (arrows from C to H).

proton showed cross peaks with both H_2-11 (δ 1.64 ddd, J=3.3, 4.6, 14.2 Hz and δ 1.98; 'W' coupling) which, in turn, coupled with H_2-12 (δ 1.72 m and 1.86 m) in the G-DQF-COSY and G-TOCSY spectra. The second spin system (B) could be traced from the olefinic methine proton H-6 (δ 6.43 dd, J=1.4, 10.0 Hz) to H-7 (δ 5.43 dd, J=4.4, 10.0 Hz) and from there to a methine proton at δ 2.25 (H-8). The spin system C commences with the characteristic H-17 (δ 2.30 d, J=8.1 Hz) which showed correlations with the oxymethine proton at C-16 (δ 4.30 ddd, J=5.6, 6.7, 7.8 Hz) which, in turn, exhibited cross peaks with the H_2-15 methylene protons (δ 2.06 dd, J=7.2, 13.0 Hz; δ 1.77 dd, J=6.6, 13.0 Hz).

In order to establish the interfragment relationship, a gradient heteronuclear multiple-bond correlation experiment (G-HMBC) was performed (Fig. 2), which not only connected the fragments but also helped in locating the glycosidic linkage and oxygen bridge. Thus, the anomeric proton of the β-D-glucopyranose moiety (δ 4.23, d, J=7.8 Hz, H-1′) displayed a long-range correlation to C-16 (δ 84.8, d). The quaternary carbon signal at δ 84.9 showed HMBC connectivities to H_3-28, H_3-29, H-3, H-6 and H-7, allowing it to be assigned unambiguously to C-5. In a similar fashion, the quaternary carbon signal at δ 80.0 was readily assigned to C-9 on the basis of long-range connectivities to H-8, H-12_a and H-7. An acetylation experiment was necessary in order to locate oxygen bridge. Acetylation of **1** yielded a hexa-acetate, **1a**. From the HRESIFTMS of **1a**, which displayed an $[M+Cl]^-$ ion at m/z 951.4306, a molecular formula of $C_{48}H_{68}O_{17}$ was proposed. The IR spectrum of **1a** still displayed a free hydroxyl absorption band after acetylation (3400 cm^{-1}), indicating the presence of the tertiary hydroxyl group (C-25). Initial ^{1}H NMR, and G-DQF-COSY studies of **1a**, were distinguished H-2 and H-3 at δ 5.04 (d, J=1.8 Hz), and 4.82 (s), respectively, showing the expected downfield

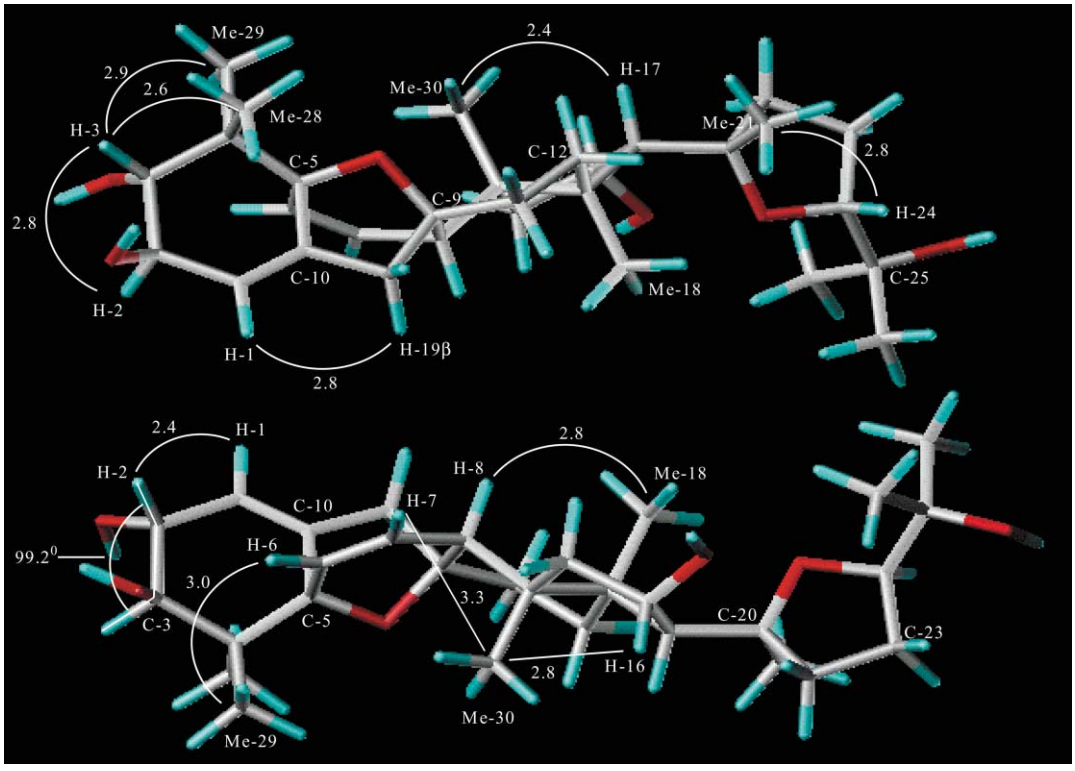
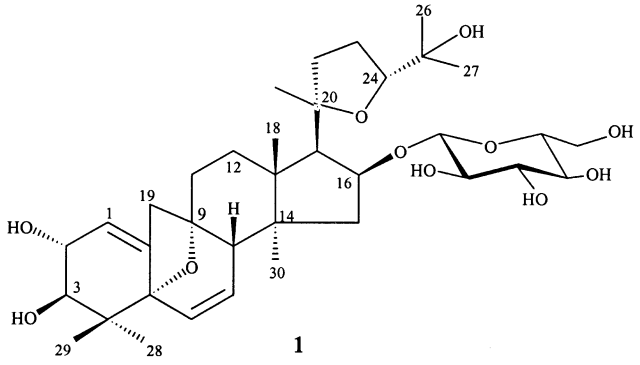


Figure 3. Interatomic distances and key nOe correlations on Conformer A obtained by Sybyl.

shift in comparison to **1**. Complete assignments of each acetylated proton were achieved by considering G-DQF-COSY, and NOESY, while the carbons were assigned from G-HMQC, G-HMBC spectra of **1a**. Since the carbon signals assigned to C-5 and C-9 did not show any acylation shift, the location of the oxo bridge could be determined unambiguously, leaving one solution: the C(5),C(9)-oxo bridge.

The relative stereochemistry at C-2(OH), C-3(OH) and C-16(O) was assigned by a combination of 2D-NOESY data, analysis of coupling constants, and molecular modeling studies performed on **1**. In regard to the stereochemistry of C-2(OH) and C-3(OH), three factors were taken into consideration: (i) We favour β-configuration of C-3(OH), consistent with all other naturally occurring cycloartanes present in the genus *Astragalus*. This assumption is supported by nOe correlations between H-3 and Me-28, Me-29 which points out that H-3 must be equatorial and α-oriented, leaving C-3(OH) axial and β-oriented. (ii) The strong nOe correlation between H-2 and H-3 implied that these protons are in close proximity. (iii) H-2 and H-3 were observed as broad singlets, indicating that the dihedral angel between H-2 and H-3 is near 90°, such small coupling indicates an axial/equatorial or equatorial/equatorial arrangement of these protons. Molecular modeling studies of **1** suggested the existence of the H-2 pseudoaxial and H-3 pseudoequatorial arrangement for conformational minimum. In the case of the forementioned arrangement, a dihedral angle between H-2 and H-3 was predicted to be 44.0° which requires a reasonable coupling. However, in the case of equatorial/equatorial arrangement, α-oriented C-2(O)–β-oriented C-3(O), the same dihedral angle was measured 99.2°, which is in agreement with the observed lack of significant coupling, while the H-2 and H-3 interatomic distance remains reasonable for nOe correlation (2.83 Å). Therefore, the configuration of C-2(OH), and C-3(OH) were assigned as α and β, respectively. NOESY networks from H-16 to H-17 and H_3-30, provided evidence for the β-orientation of the C-16 oxygen. The chemical shifts of C-20 and C-24 are comparable to those reported for analogous compounds having 20(*S*),24(*R*) configuration.[17,18] In the case of 20(*S*),24(*R*) configuration C-20 and C-24 resonate at δ 86.5–87.5 and 84.5–85.5, respectively,[16,17] while for the 20(*R*),24(*S*) configuration these carbons resonate at δ 87.0–88.0 and 81.5–82.5 ppm, respectively.[18,19] The relative stereochemistry of the C(5),C(9)-oxo bridge could not be inferred from 1D- and 2D NMR data. Thus, Drieding models and 3D-molecular modeling studies were undertaken to determine the relative stereochemistry. The coexistence of the C-1(10) and C-6(7) double bonds and the 5,9-oxo bridge in rings A and B causes ring B to adopt one of two possible conformations: (i) C-6, -7, -8, -19 and -10 are above the plane of C-5 and -9 (conformer A, Fig. 3). (ii) C-5, -8 and -9 are above the plane of C-6, -7, -19 and -10 (conformer B). While conformer A holds for α-oriented C(5),C(9)-oxo bridge, conformer B holds only for β-oriented C(5),C(9)-oxo bridge. In case of conformer B, C-19 and C-30 are forced in close proximity in **1**. The minimum interatomic distances between C-30 methyl protons and H-19$_\beta$ measured from the 3-D model was 2.6 Å, while the minimum distance for H-19$_\alpha$ and C-30 methyl protons was 2.2 Å, respectively, for conformer B. The interatomic distances for these protons were larger than 4.5 Å for conformer A. Thus, in case of conformer B, a strong nOe correlation between CH_3-30 and H-19$_\alpha$ must be expected. Owing to the conspicuous absence of the forementioned correlations, conformer B was not further considered. On the basis of these facts, it is possible to establish the dominant conformation and the α-oriented C(5),C(9)-oxo bridge.

Consequently, the structure of **1** was established as 16-*O*-β-D-glucopyranosyl-20(*S*),24(*R*)-5α,9-diepoxy-2α,3β,16β,25-tetrahydroxy-9,10-seco-cycloarta-1(10),6(7)-diene. For this compound we propose the trivial name of prusianoside A. This compound thus represents the first entry of the series of cycloartane-type compound possessing a 5α,9-oxo bridge.



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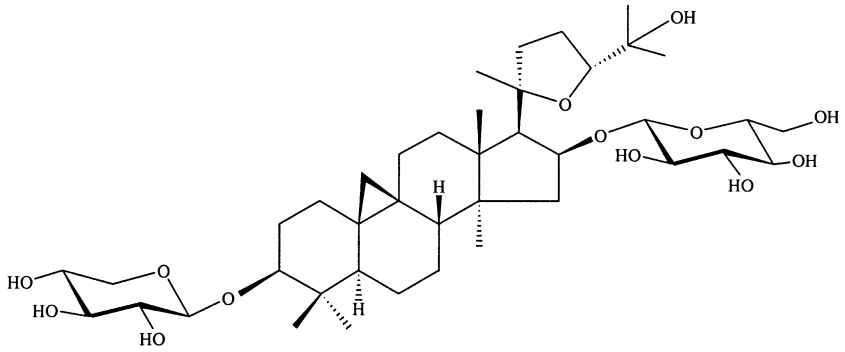
Compound **2** gave a quasi molecular ion peak $[M+Na]^+$ at *m/z* 791.4842 corresponding to a molecular formula of $C_{41}H_{68}O_{13}$. The NMR spectral data of **2** also revealed features of a cycloartane glycoside. The ^1H NMR spectrum of **2** displayed, for the aglycon moiety, characteristic signals due to the cyclopropane-methylene protons as an AX system (δ 0.19 and 0.43, J_{AX}=4.5 Hz, H_2-19) and 7 tertiary methyl groups (δ 0.88, 1.03, 1.24, 1.42, 1.48, 1.60 and 1.75). Additionally the resonances of two anomeric protons, indicative of the presence of two β-linked sugar units, were observed in the low-field region at δ 4.85 (d, *J*=7.5 Hz), and 4.78 (d, *J*=7.6 Hz).

The full assignments of the proton and carbon signals of the aglycon part of **2**, secured by G-DQF-COSY and G-HMQC spectra and the comparison of these data with those of quisquagenin [20(*R*),24(*S*)-epoxy-3β,16β,25-trihydroxycycloartane],[18] indicated the similarities of these compounds. The significant differences between the two compounds were the glycosylation shifts for C-3 (δ 88.7) and C-16 (δ 83.7), as well as the chemical shift of C-24 (δ 84.5). The chemical shift differences at C-24 could be attributed to a change of the absolute configuration at this center. The position of each sugar residue was unambigously determined by the G-HMBC experiment which showed long-range correlations between C-3 (δ 88.7) of the aglycon and H-1$_{xyl}$ (δ 4.85), as well as between C-16 (δ 83.7) of the aglycon and H-1$_{glu}$ (δ 4.78).

On the basis of this evidence, the structure of compound **2**



2

was established as 3-*O*-β-D-xylopyranosyl-16-*O*-β-D-glucopyranosyl-20(*S*),24(*R*)-epoxy-3β,16β,25-trihydroxy-cycloartane.

3. Experimental

3.1. General experimental

The IR spectra were recorded with an ATI Mattson Genesis Series FTIR spectrophotometer. The 1D- and 2D NMR spectra were obtained on a Bruker® Avance DRX 500 FT spectrometer operating at 500 (^{1}H) and 125 (^{13}C) MHz. The chemical shift values are reported as parts per million (ppm) relative to tetramethylsilane (TMS) for ^{1}H- and ^{13}C-, and the coupling constants are in Hz (in parentheses). For the ^{13}C NMR spectra, multiplicities were determined by a DEPT experiment. HRESIFTMS (High Resolution Electrospray Ionization Fourier Transformation Mass Spectrometry) were obtained using a Bruker BioApex FT-MS in ESI(+) or (−) mode.

3.2. Conformational analysis

The initial 3D structures of **1** were built in their neutral form (pH 7.4) using SYBYL Ver. 6.5 (Tripos, Inc, 1699 S, Hanley Road, Suite 303, St.Louis, MO 63144) on a Silicon Graphics Indigo® workstation. Structural attributes such as the atom types, bond types, the configuration of chiral centers, and the charge allocation were noted. The electrostatic point charges for the atoms were calculated within the program using two empirical methods.[23] A constant dielectric constant was used for all electrostatic interactions. The energy minimization was performed until the root-mean square energy gradient was lower than 0.005 kcal (mol×Å). Simulated annealing was used in order to reach the global minimum conformation. As a part of the process 5 cycles of heating the molecule to 500 K and then cooling it to 300 K were carried out. The resulting structure was energy minimized. The structures were solvated in water using the droplet method of solvation and following which the whole system was minimized. Tripos force field was applied to perform the energy minimization. Later the solution conformation was extracted and used for further calculations. It was used as an input for the measurement of distances between atoms and also to measure the dihedral bond angles. These measurements were made in order to ascertain the findings obtained using the NMR.

3.3. Chromatographic conditions

TLC: precoated Si 250F plates (Baker); developing system: $CHCl_3$/MeOH/H_2O mixtures (80:20:1, 80:20:2, 70:30:3 and 61:32:7); visualization: 30% H_2SO_4. Column chromatography: silica gel 230–400 mesh, RP (C-18, 40 mμ) (Merck).

3.4. Plant Material

A. prusianus DC. was collected from Kale, Mugla, South-West Anatolia, Turkey in September 1998. Voucher specimens have been deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

3.5. Extraction and isolation

Air-dried, powdered roots of *A. prusianus* (180 g) were extracted with MeOH under reflux, and then filtered. The filtrate was concentrated to dryness in vacuo (26.25 g). The extract was suspended in H_2O and subjected to VLC using reversed phase material (Sepralyte 40μm; 250 g), employing H_2O (500 mL), H_2O/MeOH (90:10→10:90, each 250 mL) and MeOH (500 mL) as the eluents. Fractions rich in saponins were combined (9.5 g). An aliquot of this fraction (5.6 g) was further subjected to column chromatography (silica gel, 130 g) and eluted with $CHCl_3$/MeOH (90:10; 85:15, each 250 mL) and $CHCl_3$/MeOH/H_2O mixtures (80:20:1, 500 mL; 80:20:2, 1 L; 70:30:3 1.25 L and 61:32:7, 1.5 L) to give six main fractions (Frs. A–F). Fr. A (150 mg) was chromatographed on a silica gel column (30 g), eluted with EtOAc/MeOH (45:5, 150 mL and 46:4, 400 mL) to yield compounds **1** (9.5 mg), **2** (8 mg) and astragaloside I[20] (7 mg). Fractionation of other fractions by open column chromatography (silica gel) using $CHCl_3$, $CHCl_3$/MeOH, $CHCl_3$/MeOH/H_2O mixtures led to the isolation of the other two compounds (cyclocanthoside D;[21] 41 mg and askendoside G;[22] 41 mg).

3.5.1. Prusianoside A (1). White powder, $[\alpha]_D^{25}=-112.5°$ (*c* 0.004, MeOH); IR (KBr) ν_{max} 3396, 2927, 2395, 2358, 2339, 1738, 1593, 1382, 1256, 1165, 1073, 1032 cm^{-1}. ^{1}H- and ^{13}C NMR (CD_3OD, 500 and 125 MHz, respectively): see Table 1. HRESIFTMS, $[M+Na]^+$ at *m/z* 687.2429 (calcd for $C_{36}H_{56}O_{11}$).

3.5.2. Prusianoside B (2). White powder, $[\alpha]_D^{25}=+20.0°$ (*c* 0.004, MeOH); IR (KBr) ν_{max} 3376, 2933, 2870, 2363,

1726, 1459, 1381, 1166, 1071, 1045 cm^{-1}. ^1H- and ^{13}C NMR (C_5D_5N, 500 and 125 MHz, respectively): see Table 1. HRESIFTMS, *m/z* 1049.5611 $[M]^+$, 1072.5305. $[M+Na]^+$ (calcd for $C_{51}H_{85}O_{22}$).

3.5.3. Acetylation of 1. Treatment of **1** (2 mg) with Ac_2O (1 mL) and C_5H_5N (1 mL) at room temperature overnight followed by the usual workup yielded **1a** (1.5 mg).

3.5.4. Compound 1a. White powder, IR (KBr) ν_{max} 3400 (OH), 1758 (ester) cm^{-1}. ^1H NMR (CD_3OD, 500 and 125 MHz, respectively); δ 6.25 (1H, dd, *J*=1.1, 9.9 Hz, H-6), 5.52 (1H, dd, *J*=4.4, 9.9 Hz, H-7), 5.18 (1H, t, *J*=9.5 Hz, H_{glu}-3), 5.09 (1H, d, *J*=1.8 Hz, H-1), **5.04** (1H, d, *J*=1.8 Hz, H-2), 4.96 (1H, t, *J*=9.6 Hz, H_{glu}-4), 4.90 (1H, dd, *J*=7.9, 9.6 Hz, H_{glu}-2), **4.82** (1H, br.s, H-3), 4.64 (1H, d, *J*=7.9 Hz, H_{glu}-1), 4.25 (2H, H-16 and H_{glu}-6a, overlapping), 4.15 (1H, dd, *J*=2.3, 11.7 Hz, H_{glu}-6b), 3.82 (1H, ddd, *J*=2.7, 5.2, 10.1 Hz, H_{glu}-5), 3.76 (1H, dd, *J*=5.3, 7.9 Hz, H-24), 2.09, 2.04, 2.01, 2.00, 1.98 and 1.93 (each 3H and s, $COCH_3$ ×6), 1.27, 1.21, 1.13, 1.11, 0.99, 0.98, and 0.94 (each 3H and s, Me-21, -18, -27, -26, -28, -29 and Me-30, respectively. HRESIFTMS, *m/z* 951.4306 $[M+Cl]^-$ (calcd for $C_{48}H_{68}O_{17}$).

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