Secondary Metabolites from the Roots of *Astragalus zahlbruckneri*

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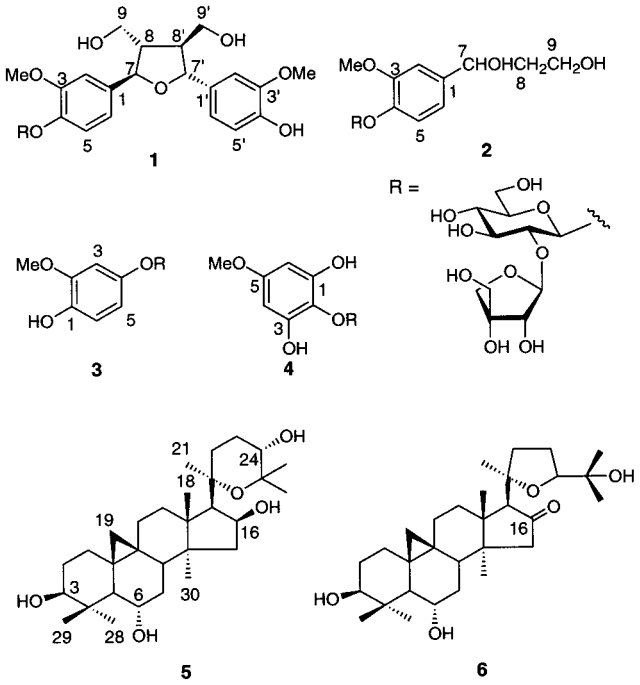
Four new phenolic glycosides, β-apiofuranosyl-(1→2)-β-glucopyranosides (**1**−**4**), along with the cycloartane triterpenes 20(*R*),25-epoxy-3β,6α,16β,24α-tetrahydroxycycloartane (**5**) and 20(*R*),24(*S*)-epoxy-3β,6α,25-trihydroxycycloartan-16-one (**6**) were isolated from roots of *Astragalus zahlbruckneri*. The structure elucidation of all compounds was based on their ^{1}H and ^{13}C NMR spectral data including 1D-TOCSY, DQF-COSY, HSQC, and HMBC experiments.

In the flora of Turkey, the genus *Astragalus* (Leguminosae) is represented by approximately 380 species,[1] among which *A. microcephalus* is used primarily for the production of the economically important gum, tragacanth.[2] Roots of these plants are used in Turkish folkloric medicine as an antiperspirant, diuretic, and tonic drug and for the treatment of diabetes mellitus, nephritis, leukemia, and uterine cancer.[1] Our research program on the constituents of Turkish *Astragalus* species has led to the discovery of a number of cycloartane-type glycosides from *A. melanophrurius*,[3] *A. oleifolius*,[4] *A. microcephalus*,[5] *A. brachypterus*,[6] and *A. trojanus*.[7,8] We report here the isolation and characterization of four new phenolic glycosides from the aqueous ethanolic extract from the roots of *A. zahlbruckneri*, along with two cycloartane derivatives.



Results and Discussion

The roots of *A. zahlbruckneri* were extracted successively with aqueous EtOH. The polar fractions of the ethanolic extract were chromatographed on silica gel to give four new phenolic glycosides (**1**−**4**) together with coniferyl alcohol 4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside.[9]

The molecular formula ($C_{31}H_{42}O_{16}$) of compound **1** was determined by ^{13}C NMR, ^{13}C DEPT NMR, and FABMS analysis in the negative ion mode. In the FABMS spectrum of **1** we observed the $[M - H]^-$ ion at *m/z* 669 and prominent fragments at *m/z* 537 $[(M - H) - 132]^-$ and *m/z* 375 $[(M - H) - (132 + 162)]^-$ due to the subsequent losses of one pentose and one hexose unit. The ^{13}C NMR spectrum displayed 31 signals, 11 of which were assigned to the saccharide portion. Carbon signals due to the aglycon moiety revealed two methoxy, two hydroxymethylene, two hydroxymethine, and eight methine carbons. The ^{1}H NMR spectrum of **1** for the aglycon moiety exhibited six aromatic proton signals, typical of two 1,3,4-trisubstituted aromatic rings. Further features were two signals ascribable to oxymethine groups at δ 5.00 and 4.98, along with two signals typical of two hydroxymethylene groups at δ 3.73 and 3.64, two singlets due to methoxy groups at δ 3.90 and 3.88, and a signal at δ 2.33. The DQF-COSY showed two $HOCH_2-CH-CH-O-$ sequences. The ^{1}H and ^{13}C NMR data matched very closely those reported for (+)-neoolivil;[10] hence it is a symmetric molecule with an all-trans configuration, and ^{1}H and ^{13}C NMR data of the two halves are the same. Due to the sugar chain, the chemical shifts of the two portions of **1** exhibited small differences. Thus, a ROESY experiment was performed to confirm the all-trans configuration. As reported by Schottner et al.,[10] the ROESY cross-peaks observed between H-2/6 and H-8, H-2′/H-6′ and H-8′, H-9 and H-7, and H-9′ and H-7′ were in agreement with the all-trans configuration. The ^{1}H NMR spectrum of **1** showed two anomeric proton signals. The 1D-TOCSY spectrum obtained by irradiating the signal at δ 5.02 clearly showed the spin system typical of a β-glucopyranosyl unit, while the 1D-TOCSY spectrum obtained by irradiating the signal at δ 5.58 revealed only another signal at δ 4.01 (1H, d, J = 2.0). The DQF-COSY experiment allowed complete sequential assignments of all sugar proton resonances, which were correlated by the HSQC experiment to the corresponding carbon signals. Thus, the disaccharide chain contained one β-glucose unit and one β-apiose unit,[11] and the interglycosidic linkage was established at C-2 of the glucose unit on the basis of the downfield shift exhibited by this carbon resonance (δ 77.9) when compared to the respective shift in unglycosylated models.[7] The sugar chain was placed at C-4 of the aglycon on the basis of the HMBC correlation between the anomeric proton signal at δ 5.02 and the carbon resonance at δ 147.6

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(C-4). Thus, compound **1** was determined to be (+)-neo-olivil 4-*O*-β-apiofuranosyl-(1→2)-β-glucopyranoside.

Compounds **2**−**4** showed FABMS fragmentation patterns similar to that of **1**. Comparison of ^{1}H and ^{13}C NMR data of **2**−**4** to those of **1** indicated an identical saccharide portion.

Analysis of ^{1}H and ^{13}C NMR data of **2** suggested a phenylpropanoid aglycon.[12] The ^{13}C NMR spectrum of the aglycon exhibited 10 signals, which were assigned to one methoxy, one hydroxymethylene, one hydroxymethine, one methylene as well as three aromatic CH groups, one aromatic quaternary carbon, and two phenolic functions. The ^{1}H NMR displayed three aromatic signals typical of a 1,3,4-trisubstituted ring along with signals at δ 4.78 (1H, dd, J = 5.2 and 8.3 Hz), 3.71 (1H, m), 3.62 (1H, m), 2.02 (1H, m), and 1.90 (1H, m). A further feature was the signal for a methoxy group at δ 3.87. The DQF-COSY experiment defined the sequence −CHOH−CH_2−CH_2OH. The HMBC experiment showed a correlation between the methoxy group at δ 3.87 and C-3 (δ 151.1) and the anomeric proton signal at δ 4.99 (H-1glu) and C-4 (δ 147.2), placing the methoxy group at C-3 and the disaccharide chain at C-4. On the basis of these data, **2** was identified as 7,8-dihydro-7-hydroxyconiferyl alcohol 4-*O*-β-apiofuranosyl-(1→2)-β-glucopyranoside.

The ^{13}C NMR spectrum of **3** showed, for the aglycon portion, seven signals. These were assigned to a methoxy group, three to aromatic CH, and three to phenolic functions. The ^{1}H NMR spectrum showed three aromatic signals along with a signal for a methoxy group at δ 3.86, which correlated in the HMBC spectrum with a signal at δ 149.6. Analysis of ^{13}C NMR spectrum and HMBC correlations allowed us to deduce the structure 2-methoxy-phenol-4-*O*-β-apiofuranosyl-(1→2)-β-glucopyranoside for **3**.

The ^{13}C NMR spectrum of **4** exhibited, for the aglycon moiety, five signals. These were indicative of a methoxy group, two aromatic CH, and four phenolic functions. In the ^{1}H NMR spectrum, the only features for the aglycon moiety were a signal at δ 6.13 (2H, s) and a signal for a methoxy group at δ 3.81. Analysis of ^{1}H and ^{13}C NMR resonances of **4** suggested a 1,2,3,5-tetrahydroxy-substituted phenyl ring. The location of the methoxy group at C-5 and of the disaccharide chain at C-2 were deduced from the HMBC correlation between the proton signal at δ 3.81 (OCH_3) and the carbon resonance at δ 155.7 (C-5), and between the anomeric signal of the glucose unit at δ 4.92 and the carbon resonance at δ 128.9 (C-2). Thus, the structure 3-hydroxy-5-methoxyphenol-2-*O*-β-apiofuranosyl-(1→2)-β-glucopyranoside was assigned to **4**.

The apolar fractions of the ethanolic extract of the roots of *A. zahlbruckneri*, submitted to silica gel column chromatography, afforded the cycloartane derivatives **5** and **6** along with cycloastragenol.[13−15]

Compound **5** ($C_{30}H_{50}O_5$) gave a quasi-molecular ion peak in its negative ion FABMS at *m*/*z* 489. The ^{1}H NMR spectrum of **5** displayed diagnostic signals due to the cyclopropane−methylene protons of a cycloartane derivative at δ 0.38 and 0.50 (each d, J = 4.5 Hz, H_2-19) and seven tertiary methyl groups.[5] Additionally, signals due to methine protons on oxygen-bearing carbon atoms (H-3, H-6, H-24, and H-16) appeared. The resonances for the oxygenated carbons also indicated the presence of four oxymethine carbons (δ 78.4, 73.6, 69.6, 69.3; C-3, C-16, C-6, C-24, respectively) and two oxygenated quaternary carbons (δ 79.3 and 75.0; C-20 and C-25, respectively). Full assignments of the proton and carbon signals were secured by ^{1}H−^{1}H DQF COSY, HSQC, and HMBC spectra. To clarify the intermolecular connectivities of the partial structure, the HMBC experiment was performed. With the help of this experiment, the occurrence of a side chain including a monohydroxypyrane portion was deduced. Thus the structure of **5** was established as 20(*R*),25-epoxy-3β,6α,16β,24α-tetrahydroxycycloartane, previously reported only as an aglycon of glycosides isolated from *Astragalus* spp.[5,16,17]

Compound **6** ($C_{30}H_{48}O_5$) gave a quasi-molecular ion peak in its negative ion FABMS at *m*/*z* 487. Analysis of ^{1}H and ^{13}C NMR data of **6** in comparison with those of cycloastragenol[7] clearly indicated that the difference between the two compounds should be confined to the occurrence of a keto group instead of a secondary alcoholic function. Carbon resonances of ring D and Me-18 in **6** suggested that the keto group was located at C-16. This hypothesis was confirmed by the HMBC experiment, which showed diagnostic long-range correlations between the signal at δ 218.3 and the proton signals at δ 2.92 (H-17) and 2.09 (H-15a and H-15b) and between the proton signal at δ 1.21 (Me-18) and the carbon resonance at δ 65.2 (C-17). Thus the structure 20(*R*),24(*S*)-epoxy-3β,6α,25-trihydroxycycloartan-16-one was assigned to compound **6**. This compound has been previously reported only as a cycloartane derivative obtained by chemical oxidation of cycloastragenol.[13−15]

(+)-Neo-olivil, previously isolated from the roots of *Urtica dioica*,[10] showed affinity to human sex hormone binding globulin (SHBG). For this reason this plant metabolite has been considered for its potential beneficial effects on benign prostatic hyperplasia (BPH).[18] Furthermore, tetrahydrofuran lignans are reported to possess various biological activities including antioxidant properties.[19]

Experimental Section

General Experimental Procedures. A Bruker DRX-600 spectrometer operating at 599.19 MHz for ^{1}H and 150.86 for ^{13}C using the UXNMR software package was used for NMR measurements in CD_3OD solutions. 2D experiments: ^{1}H−^{1}H DQF-COSY, inverse-detected ^{1}H−^{13}C HSQC and HMBC, and ROESY were obtained by employing the conventional pulse sequences as described previously.[7] The selective excitation spectra, 1D TOCSY[20] were acquired using waveform generator-based GAUSS-shaped pulses, mixing time ranging from 100 to 120 ms and a MLEV-17 spin-lock field of 10 kHz preceded by a 2.5 ms trim pulse. 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. 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).

Plant Material. *Astragalus zahlbruckneri* Hand.-Mazz. (Leguminosae) was collected from Sivrice, 28 km southeast of Elazig, East Anatolia, Turkey, in June 1999. A voucher specimen has been deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 99-047).

Extraction and Isolation. The air-dried powdered roots (450 g) were extracted with 80% aqueous EtOH (2 × 3 L) under reflux. The EtOH extracts were combined and evaporated to dryness in vacuo to yield 54 g of crude extract (yield 12%). An aliquot of ethanolic extract (40 g) was fractionated by open column chromatography by silica gel (600 g) employing gradient CH_2Cl_2−MeOH−H_2O mixtures (90:10:1, 1500 mL, 80:20:2, 1000 mL, 70:30:3, 1000 mL, 60:40:4, 1700 mL, and 50:50:5, 1000 mL), yielding 12 fractions (fractions A−L), A (500 mg), B (500 mg), C (733 mg), D (1860 mg), E (1640 mg), F (737 mg), G (1300 mg), H (4730 mg), I (4700 mg), J (4500 mg), K (4700 mg), and L (5800 mg). Repeated chromatography of fraction C (733 mg) on silica gel using *n*-hexane−diethyl ether−MeOH (10:10:1) as solvent system afforded **6** (17 mg), cycloastragenol (130 mg), and **5** (346 mg). Fraction L (5800 mg) was subjected to C_{18} MPLC (26 × 400 mm, i.d., Lichroprep

C-18; fraction volume 25−30 mL) using MeOH−H_2O gradients (0% to 40% MeOH) to give 64 fractions. Fractions 10−13 (85.7 mg) were rich in compound **3**, which was further purified on silica gel (30 g) column chromatography using CH_2Cl_2−MeOH−H_2O mixtures with increasing polarity (80:20:2 to 75:25:2.5) to yield the compound **3** (27.5 mg). Fractions 17−18 (60.5 mg) and 19−20 (33.5 mg) were combined and applied to a silica gel (30 g) column employing CH_2Cl_2−MeOH−H_2O mixtures with increasing polarity (80:20:2 and 75:25:2.5) to yield the compound **4** (11.5 mg) and compound **2** (21.5 mg). Fractions 29−31 (103 mg) were applied to a silica gel (30 g) column using CH_2Cl_2−MeOH−H_2O (80:20:2) as eluent to yield the coniferyl alcohol 4-*O*-*β*-D-apiofuranosyl-(1→2)-*β*-D-glucopyranoside (10 mg) and compound **1** (35.5 mg).

Compound 1: $[\alpha]^{25}_D$ −69.9°(*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 218 (5.00); ^{1}H NMR (CD_3OD, 600 MHz) δ 7.13 (1H, d, J = 8.0 Hz, H-5′), 7.10 (1H, d, J = 1.5 Hz, 5.0, H-2′), 7.05 (1H, d, J = 1.5, H-2), 6.98 (1H, dd, J = 8.0, 1.5 Hz, H-6′), 6.90 (1H, dd, J = 8.0, 1.5 Hz, H-6), 6.81 (1H, d, J = 8.0 Hz, H-5), 5.58 (1H, d, J = 2.0 Hz, H-1_{api}), 5.02 (1H, d, J = 7.5 Hz, H-1_{glu}), 5.00 (1H, d, J = 8.0 Hz, H-7′), 4.98 (1H, d, J = 8.0 Hz, H-7), 4.21 (1H, d, J = 10.0 Hz, H-4b$_{api}$), 4.01 (1H, d, J = 2.0 Hz, H-2_{api}), 3.90 (3H, s, OCH_3-3) 3.89 (1H, dd, J = 2.5, 11.5 Hz, H-6b$_{glu}$), 3.88 (3H, s, OCH_3-3′) 3.77 (1H, d, J = 10.0 Hz, H-4a$_{api}$), 3.75 (1H, dd, J = 7.5, 9.0 Hz, H-2_{glu}), 3.73 (2H, dd, J = 4.0, 11.0 Hz, H-9b, H-9′b), 3.71 (1H, dd, J = 4.5, 11.5 Hz, H-6a$_{glu}$), 3.64 (2H, dd, J = 5.5, 11.0 Hz, H-9a, H-9′a), 3.63 (1H, dd, J = 9.0, 9.0 Hz, H-3_{glu}), 3.60 (1H, d, J = 10.0 Hz, H-5b$_{api}$), 3.56 (1H, d, J = 10.0 Hz, H-5a$_{api}$), 3.43 (1H, dd, J = 9.0, 9.0 Hz, H-4$_{glu}$), 3.42 (1H, m, H-5$_{glu}$), 2.33 (2H, m, H-8, H-8′); ^{13}C NMR (CD_3OD, 150 MHz) δ 150.9 (C-3′), 149.1 (C-3), 147.9 (C-4′), 147.6 (C-4), 138.2 (C-1′), 134.8 (C-1), 120.5 (C-6), 119.9 (C-6′), 117.1 (C-5′), 116.0 (C-5), 111.7 (C-2′), 111.2 (C-2), 110.2 (C-1_{api}), 101.0 (C-1_{glu}), 84.5 (C-7), 84.1 (C-7′), 80.8 (C-3_{api}), 78.8 (C-3_{glu}), 78.0 (C-5_{glu}), 77.9 (C-2_{glu}), 77.5 (C-2_{api}), 75.5 (C-4_{api}), 71.4 (C-4_{glu}), 66.2 (C-5_{api}), 62.5 (C-6_{glu}), 61.8 (C-9, C-9′), 56.3 (O*C* H_3-3,3′) 55.4 (C-8, C-8′); FABMS *m*/*z* 669 [M − H]$^-$, 654 [(M − H) − 15]$^-$, 537 [(M − H) − 132]$^-$, 375 [(M − H) − (132 + 162)]$^-$.

Compound 2: $[\alpha]^{25}_D$ −56.0° (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 226 (5.00), 213.4 (4.94); ^{1}H NMR (CD_3OD, 600 MHz) δ 7.11 (1H, d, J = 8.0 Hz, H-5), 7.03 (1H, d, J = 1.5, H-2), 6.89 (1H, dd, J = 1.5, 8.0 Hz, H-6), 5.58 (1H, d, J = 2.0 Hz, H-1_{api}), 4.99 (1H, d, J = 7.5 Hz, H-1_{glu}), 4.78 (1H, dd, J = 5.2 and 8.3 Hz, H-7), 4.20 (1H, d, J = 10.0 Hz, H-4b$_{api}$), 4.01 (1H, d, J = 2.0 Hz, H-2_{api}), 3.88 (1H, dd, J = 2.5, 11.5 Hz, H-6b$_{glu}$), 3.87 (3H, s, OCH_3-3) 3.77 (1H, d, J = 10.0 Hz, H-4a$_{api}$), 3.73 (1H, dd, J = 7.5, 9.0 Hz, H-2_{glu}), 3.70 (1H, m, H-9b), 3.68 (1H, dd, J = 4.0, 11.5 Hz, H-6a$_{glu}$), 3.62 (1H, dd, J = 9.0, 9.0 Hz, H-3_{glu}), 3.61 (1H, m, H-9a), 3.58 (2H, br s, H-5a$_{api}$, H-5b$_{api}$), 3.42 (2H, m, H-4 $_{glu}$, H-5$_{glu}$) 2.02 (1H, m, H-8b), 1.90 (1H, m, H-8a); ^{13}C NMR (CD_3OD, 150 MHz) δ 151.1 (C-3), 147.2 (C-4), 140.9 (C-1), 119.2 (C-6), 116.8 (C-5), 111.1 (C-2), 110.8 (C-1_{api}), 100.8 (C-1_{glu}), 81.0 (C-3_{api}), 78.4 (C-3_{glu}), 77.9 (C-2_{glu}), 77.6 (C-2_{api}, C-5_{glu}), 74.9 (C-4_{api}), 72.0 (C-7), 71.1 (C-4_{glu}), 66.0 (C-5_{api}), 62.2 (C-6_{glu}), 60.1 (C-9), 56.3 (O*C*H_3-3) 42.5 (C-8); FABMS *m*/*z* 491 [M − H]$^-$, 476 [(M − H) − 15]$^-$, 344 [(M − H) − (15 + 132)]$^-$, 182 [(M − H) − (15 + 132 + 162)]$^-$.

Compound 3: $[\alpha]^{25}_D$ −59.0° (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 219 (5.00); ^{1}H NMR (CD_3OD, 600 MHz) δ 6.79 (1H, d, J = 1.5 Hz, H-3), 6.71 (1H, d, J = 8.0, H-6), 6.59 (1H, dd, J = 1.5, 8.0 Hz, H-5), 5.49 (1H, d, J = 2.0 Hz, H-1_{api}), 4.82 (1H, d, J = 7.5 Hz, H-1_{glu}), 4.13 (1H, d, J = 10.0 Hz, H-4b$_{api}$), 4.00 (1H, d, J = 2.0 Hz, H-2_{api}), 3.91 (1H, dd, J = 2.5, 11.5 Hz, H-6b$_{glu}$), 3.86 (3H, s, OCH_3-2′) 3.81 (1H, d, J = 10.0 Hz, H-4a$_{api}$), 3.71 (1H, dd, J = 4.0, 11.5 Hz, H-6a$_{glu}$), 3.62 (2H, br s, H-5b$_{api}$, H-5a$_{api}$), 3.61 (1H, d, J = 7.5 Hz, H-2_{glu}) 3.50 (1H, dd, J = 9.0, 9.0 Hz, H-3_{glu}) 3.41 (1H, m, H-5$_{glu}$), 3.39 (1H, dd, J = 9.0, 9.0 Hz, H-4$_{glu}$); ^{13}C NMR (CD_3OD, 150 MHz) δ 152.7 (C-4), 149.6 (C-2), 142.7 (C-1), 115.9 (C-6), 111.0 (C-1_{api}), 109.4 (C-5), 103.3 (C-3), 102.2 (C-1_{glu}), 80.0 (C-3_{api}), 78.8 (C-2_{glu}, C-3_{glu}), 78.0 (C-5_{glu}), 77.7 (C-2_{api}), 75.3 (C-4_{api}), 71.4 (C-4_{glu}), 65.7 (C-5_{api}), 62.2 (C-6_{glu}), 56.0 (O*C* H_3-2); FABMS *m*/*z* 433 [M − H]$^-$, 418 [(M − H) − 15]$^-$, 301 [(M − H) − 132)]$^-$, 139 [(M − H) − (132 + 162)]$^-$.

Compound 4: $[\alpha]^{25}_D$ −40.5° (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 215 (5.00); ^{1}H NMR (CD_3OD, 600 MHz) δ 6.13 (2H, s, H-4, H-6), 5.49 (1H, d, J = 2.0 Hz, H-1_{api}), 4.92 (1H, d, J = 7.5 Hz, H-1_{glu}), 4.08 (1H, d, J = 10.0 Hz, H-4b$_{api}$), 4.03 (1H, d, J = 2.0 Hz, H-2_{api}), 3.81 (3H, s, OCH_3-3′) 3.76 (1H, d, J = 10.0 Hz, H-5b$_{api}$), 3.75 (1H, dd, J = 2.5, 11.5 Hz, H-6b$_{glu}$), 3.71 (1H, d, J = 10.0 Hz, H-4a$_{api}$), 3.69 (1H, dd, J = 7.5, 9.0 Hz, H-2_{glu}), 3.67 (1H, dd, J = 4.0, 11.5 Hz, H-6a$_{glu}$), 3.63 (1H, d, J = 10.0 Hz, H-5a$_{api}$), 3.55 (1H, dd, J = 9.0, 9.0 Hz, H-3_{glu}), 3.49 (1H, dd, J = 9.0, 9.0 Hz, H-4$_{glu}$), 3.18 (1H, ddd, J = 2.5, 4.0, 9.0 Hz, H-5$_{glu}$); ^{13}C NMR (CD_3OD, 150 MHz) δ 155.7 (C-5), 155.0 (C-1, C-3), 128.9 (C-2), 110.4 (C-1_{api}), 103.3 (C-1_{glu}), 94.6 (C-4, C-6), 80.9 (C-3_{api}), 78.7 (C-2_{glu}, C-3_{glu}), 78.1 (C-2_{api}) 77.9 (C-5_{glu}), 75.6 (C-4_{api}), 71.4 (C-4_{glu}), 66.4 (C-5_{api}), 62.6 (C-6_{glu}), 56.8 (O*C* H_3-5); FABMS *m*/*z* 449 [M − H]$^-$, 302 [(M − H) − (132 + 15)]$^-$, 240 [(M − H) − (132 + 15 +162)]$^-$.

Compound 5: $[\alpha]^{D}_{25}$ +23.3° (*c* 0.5, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.60 (1H, ddd, J = 8.0, 8.2, 5.2 Hz, H-16), 3.53 (1H, m, H-6) 3.53 (1H, br s, H-24), 3.30 (1H, dd, J = 11.2, 4.5 Hz, H-3), 2.52 (1H, m, H-22b), 2.16 (1H, m, H-23b), 1.98 (2H, m, H-11b, H-15b), 1.86 (1H, d, J = 8.0, H-17), 1.82 (2H, m, H-2b, H-12b), 1.80 (1H, m, H-8), 1.72 (1H, m, H-23a), 1.68 (1H, m, H-12a), 1.58 (2H, m, H-1b, H-2a), 1.53 (3H, s, Me-21), 1.48 (1H, m, H-7b), 1.43 (3H, s, Me-18), 1.38 (1H, m, H-15a), 1.32 (1H, d, J = 8.0 Hz, H-5), 1.32 (1H, m, H-7a), 1.30 (3H, s Me-27), 1.26 (3H, s, Me-28), 1.22 (3H, s, Me-26), 1.19 (1H, m, H-1a), 1.15 (2H, m, H-11a, H-22a), 0.97 (3H, s, Me-29), 0.90 (3H, s, Me-30), 0.51 (1H, d, J = 4.5 Hz, H-19b), 0.38 (1H, d, J = 4.5 Hz, H-19a); ^{13}C NMR (150 MHz, $CDCl_3$) δ 79.3 (s, C-20), 78.4 (d, C-3), 75.0 (d, C-25), 73.6 (d, C-16), 69.6 (d, C-24), 69.3 (d, C-6), 60.2 (d, C-17), 53.8 (d, C-5), 47.8 (t, C-15), 46.9 (t, C-8), 46.5 (s, C-13), 45.9 (s, C-14), 41.5 (s, C-4), 38.0 (t, C-7), 34.0 (t, C-12), 32.1 (t, C-1), 31.5 (t, C-19), 30.3 (C-2), 29.4 (s, C-10), 28.2 (q, C-28), 27.9 (q, C-27), 27.7 (q, C-21), 27.6 (q, C-26), 26.0 (t, C-22), 25.8 (t, C-11), 23.0 (t, C-23), 20.9 (q, C-18), 20.8 (s, C-9), 20.2 (q, C-30), 15.3 (q, C-29); FABMS *m*/*z* 489 [M − H]$^-$.

Compound 6: $[\alpha]^{D}_{25}$ +10.0° (*c* 0.5, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.73 (1H, dd, J = 8.0, 5.0 Hz, H-24), 3.55 (1H, ddd, H-6, J = 10.0, 8.0, 4.5 Hz, H-6), 3.33 (1H, dd, J = 11.2, 4.5 Hz, H-3), 2.92 (1H, s, H-17), 2.12 (1H, m, H-23b), 2.09 (2H, s, H-15a, H-15b), 2.05 (1H, m, H-11b), 1.98 (1H, m, H-23a), 1.88 (1H, m, H-12b), 1.82 (1H, m, H-2b) 1.80 (2H, m, H-8, H-22b), 1.69 (1H, m, H-22a), 1.62 (1H, m, H-12a), 1.58 (2H, m, H-1b, H-2a), 1.44 (1H, m, H-7b), 1.38 (1H, m, H-7a), 1.37 (1H, d, J = 8.0 Hz, H-5), 1.30 (1H, m, H-11a), 1.29 (3H, s, Me-28), 1.27 (3H, s, Me-27), 1.21 (3H, s, Me-21), 1.19 (1H, m, H-1a), 1.19 (3H, s, Me-18), 1.16 (3H, s, Me-30), 1.12 (3H, s, Me-26), 0.98 (3H, s, Me-29), 0.55 (1H, d, J = 4.5 Hz, H-19b), 0.42 (1H, d, J = 4.5 Hz, H-19a); ^{13}C NMR (150 MHz, $CDCl_3$) δ 218.3 (s, C-16), 84.4 (s, C-20), 82.0 (d, C-24), 78.2 (d, C-3), 70.8 (d, C-25), 68.6 (d, C-6), 65.2 (d, C-17), 53.5 (d, C-5), 51.0 (t, C-15), 45.9 (t, C-8), 44.5 (s, C-13), 42.1 (s, C-14), 41.8 (s, C-4), 37.7 (t, C-7), 31.9 (t, C-12, C-22), 31.7 (t, C-1), 30.9 (t, C-19), 30.2 (t, C-2), 29.6 (s, C-10), 28.0 (q, C-27, C-28), 26.3 (t, C-23), 25.6 (t, C-11), 25.2 (q, C-21), 25.1 (q, C-26), 20.3 (s, C-9), 20.0 (q, C-18), 19.6 (q, C-30) 15.2 (q, C-29); FABMS *m*/*z* 487 [(M − H]$^-$.

Cycloastragenol[13−15] and coniferyl alcohol 4-*O*-*β*-D-apiofuranosyl-(1→2)-*β*-D-glucopyranoside[9] were identified by comparison of their spectral data with those reported in the literature.

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