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# TRITERPENE GLYCOSIDES FROM *Astragalus* AND THEIR GENINS. LXXXV. STRUCTURE OF CYCLOMACROSIDE E

**D. A. Iskenderov, I. M. Isaev, and M. I. Isaev***

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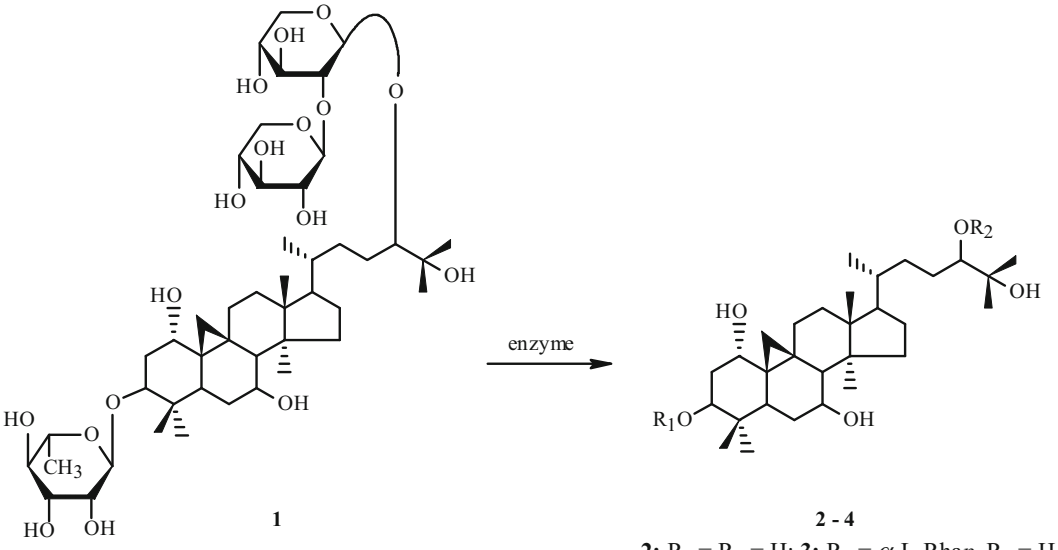
*The structure of a new cycloartane-type triterpene glycoside, cyclomacroside E, which was isolated from roots of* Astragalus macropus *Bunge (Leguminosae), was established as 3-*O*-α-L-rhamnopyranoside,24-*O*-[(β-D-xylopyranosyl)(1→2)-β-D-xylopyranoside]-24*R*-cycloartan-1α,3β,7β,24,25-pentaol.*

**Keywords:** *Astragalus macropus* Bunge, Leguminosae, cycloartane triterpenoids, cyclomacroside C, cyclomacroside D, cyclomacroside E, cyclomacrogenin B, PMR, $^{13}$C NMR, DEPT spectra.

We reported previously that ten compounds were isolated from roots of *Astragalus macropus* Bunge (Leguminosae) [1]. Three of these were identified as *β*-sitosterol, *β*-sitosterol *β*-D-glucopyranoside, and D-3-*O*-methyl-*chiro*-inositol. Structures of six new triterpenoids and glycosides were established (secomacrogenin B [2]; cyclomacrogenin B (**2**) [1]; and cyclomacrosides A [3], B [4], C (**3**) [5], and D (**4**) [6]). In continuation of this research, we determined the chemical structure of compound I [1], which we called cyclomacroside E (**1**).




**1** **2 - 4**

**2:** $R_1 = R_2 = H$; **3:** $R_1 = \alpha$-L-Rha*p*, $R_2 = H$

**4:** $R_1 = \alpha$-L-Rha*p*, $R_2 = \beta$-D-Xyl*p*

Examination of the PMR spectrum of the new compound **1** indicated that it was a glycoside and belonged to the cycloartane triterpenoid series [7-10].

Paper chromatography of the carbohydrate part of the acid hydrolysate of **1** in the presence of authentic samples and taking into account biogenetic principles detected D-xylose and L-rhamnose.

The PMR spectrum of cyclomacroside E exhibited resonances for three anomeric protons at δ 4.86 (d, $^3$J = 7.5 Hz), 5.13 (d, $^3$J = 7.3 Hz), and 5.22 (d, $^3$J = 1.5 Hz). The corresponding C atoms resonated in the $^{13}$C NMR spectrum of **1** at δ 102.85, 106.68, and 104.34. These facts indicated that cyclomacroside E was a trioside and contained D-xylose and L-rhamnose in a 2:1 ratio (Table 1).

S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax: (99871) 120 64 75, e-mail: m_isaev@rambler.ru. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 209–212, March–April, 2010. Original article submitted October 26, 2009.

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TABLE 1. Chemical Shifts of C Atoms and DEPT Parameters of **1**–**4** (100 MHz, $C_5D_5N$, δ, ppm)

| C atom | DEPT | **1** | **2** | **3** | **4** |
|---|---|---|---|---|---|
| 1 | CH | 72.31 | 72.67 | 72.44 | 72.44 |
| 2 | $CH_2$ | 37.18 | 39.02 | 37.12 | 37.11 |
| 3 | CH | 84.17 | 72.97 | 84.26 | 84.24 |
| 4 | C | 40.75 | 41.02 | 40.84 | 40.82 |
| 5 | CH | 39.15 | 39.40 | 39.22 | 39.20 |
| 6 | $CH_2$ | 32.02 | 32.37 | 32.13 | 32.12 |
| 7 | CH | 69.99 | 70.16 | 70.06 | 70.02 |
| 8 | CH | 55.37 | 55.25 | 55.37 | 55.31 |
| 9 | C | 20.94 | 21.01 | 21.03 | 21.02 |
| 10 | C | 30.92 | 31.35 | 31.00 | 30.97 |
| 11 | $CH_2$ | 26.31 | 26.49 | 26.42 | 26.41 |
| 12 | $CH_2$ | 33.17 | 33.33 | 33.27 | 33.22 |
| 13 | C | 45.97 | 46.02 | 46.03 | 46.02 |
| 14 | C | 48.94 | 49.16 | 49.10 | 49.07 |
| 15 | $CH_2$ | 37.98 | 37.91 | 37.96 | 37.99 |
| 16 | $CH_2$ | 29.10 | 29.00[a] | 29.00 | 29.58 |
| 17 | CH | 52.23 | 52.41 | 52.41 | 52.20 |
| 18 | $CH_3$ | 17.93 | 17.95 | 18.01 | 17.97 |
| 19 | $CH_2$ | 29.00 | 29.00[a] | 29.04 | 28.99 |
| 20 | CH | 36.97 | 36.45 | 36.46 | 36.81 |
| 21 | $CH_3$ | 18.96 | 18.71 | 18.70 | 19.03 |
| 22 | $CH_2$ | 34.01 | 34.24 | 34.24 | 33.58 |
| 23 | $CH_2$ | 28.38 | 28.24 | 28.32 | 28.25 |
| 24 | CH | 88.65 | 79.07 | 79.08 | 89.66 |
| 25 | C | 71.98 | 72.71 | 72.72 | 71.91 |
| 26 | $CH_3$ | 25.85 | 25.97 | 25.92 | 25.69[a] |
| 27 | $CH_3$ | 26.45 | 26.23 | 26.10 | 26.77 |
| 28 | $CH_3$ | 18.50 | 19.05 | 19.01 | 18.59 |
| 29 | $CH_3$ | 25.64 | 26.07 | 25.72 | 25.69[a] |
| 30 | $CH_3$ | 14.34 | 14.02 | 14.45 | 14.44 |
| | | *α*-L-Rha*p* | | | |
| 1 | CH | 104.34 | | 104.51 | 104.52 |
| 2 | CH | 72.31 | | 72.34 | 72.30 |
| 3 | CH | 72.78 | | 72.89 | 72.89 |
| 4 | CH | 74.00 | | 74.11 | 74.09 |
| 5 | CH | 69.66 | | 69.78 | 69.77 |
| 6 | $CH_3$ | 18.27 | | 18.40 | 18.40 |
| | | 24-*O*-*β*-D-Xyl*p* | | | |
| 1 | CH | 102.85 | | | 106.14 |
| 2 | CH | 83.39 | | | 75.14 |
| 3 | CH | 77.79 | | | 78.43 |
| 4 | CH | 70.55 | | | 70.91 |
| 5 | $CH_2$ | 67.33 | | | 67.26 |
| | | Terminal *β*-D-Xyl*p* | | | |
| 1 | CH | 106.68 | | | |
| 2 | CH | 75.88 | | | |
| 3 | CH | 77.88 | | | |
| 4 | CH | 70.96 | | | |
| 5 | $CH_2$ | 66.77 | | | |

Resonances denoted by the same letters are mutually overlapped within columns.

A detailed study of the PMR and $^{13}C$ NMR spectra indicated that this glycoside contained cyclomacrogenin B (**2**) as the genin and that it was glycosylated at C-3 and C-24. Therefore, cyclomacroside E was a bisdesmoside where one D-xylose and L-rhamnose were terminal monosaccharides.

Cyclomacroside E was hydrolyzed by a complex enzyme of *Helix pomatia* stomach juice. Cyclomacrogenin B (**2**) and two progenins, cyclomacrosides C (**3**) and D (**4**), were isolated from the enzymolysis products. Formation of **3** and **4** suggested that L-rhamnose and one D-xylose were bonded to the genin at C-3 and C-24 and that both monosaccharides had the pyranose form, L-rhamnose, the $^1C_4$-conformation and $\alpha$-configuration; D-xylose, the $^4C1$-conformation and $\beta$-configuration.

The location of the terminal D-xylose was found by comparing $^{13}C$ NMR spectra of cyclomacrosides D and E. The anomeric C atom of D-xylose in the $^{13}C$ NMR spectrum of cyclomacroside D was found at δ 106.14. The resonance of this atom in the $^{13}C$ NMR spectrum of cyclomacroside E underwent a strong-field shift and was observed at δ 102.85. As expected, C-2 of D-xylose on C-24 experienced a glycosylation effect and resonated at δ 83.39. Therefore, the terminal D-xylose was bonded to C-2 of the D-xylose on C-24. The chemical shifts of the terminal D-xylose C atoms and the SSCC of its anomeric proton were consistent with the pyranose form, $^4C_1$-conformation, and $\beta$-configuration.

Thus, cyclomacroside E had the structure 3-*O*-$\alpha$-L-rhamnopyranoside,24-*O*-[($\beta$-D-xylopyranosyl)(1→2)-$\beta$-D-xylopyranoside]-24*R*-cycloartan-1$\alpha$,3$\beta$,7$\beta$,24,25-pentaol.

## EXPERIMENTAL

**General comments** have been published [11]. We used solvent systems *n*-BuOH:Py:$H_2O$ (6:4:3, 1), $CHCl_3$:MeOH (15:1, 2), $CHCl_3$:MeOH:$H_2O$ (70:12:1, 3; 70:23:1, 4). Descending PC was performed on FN-1 paper. Monosaccharides in chromatograms were detected by spraying with anilinium phthalate with subsequent heating to 110°C. PMR and $^{13}C$ NMR spectra were recorded in deuteropyridine on a UNITYplus 400 spectrometer (Varian) with HMDS internal standard. $^{13}C$ NMR spectra were obtained with full C–H-decoupling and under DEPT conditions. Chemical shifts in $^{13}C$ NMR spectra were given relative to the resonance of the $\beta$-C atoms of deuteropyridine (δ 123.493 relative to TMS).

**Isolation and separation of isoprenoids from roots of *A. macropus* Bunge** has been reported [1].

**Cyclomacroside E (1)**, compound I [1], $C_{46}H_{78}O_{17}$, white amorphous powder.

PMR spectrum (400 MHz, $C_5D_5N$, δ, ppm, J/Hz, 0 = HMDS): 0.36 and 0.76 (d, $^2J$ = 4, 2H-19), 0.83, 0.88 (s, 2 × $CH_3$), 0.97 (d, $^3J$ = 6.4, $CH_3$-21), 1.01, 1.07, 1.32, 1.37 (s, 4 × $CH_3$), 1.45 (d, $^3J$ = 6, L-rhamnose $CH_3$), 3.53 (dd, $^2J$ = 11.1, $^3J$ = 9.6, terminal D-xylose H-5a), 3.58 (dd, $^2J$ = 11.3, $^3J$ = 9.8, C-24 D-xylose H-5a), 3.70 (t, $^3J_1 = {}^3J_2$ = 2.6, H-1), 3.74 (dd, $^3J_1$ = 7.8, $^3J_2$ = 1.6, H-24), 3.79 (td, $^3J_1 = {}^3J_2$ = 9, $^3J_3$ = 3.4, H-7), 3.93–4.23 (genin H-3; D-xylose H-2, H-3, H-4; C-24 D-xylose H-5e, L-rhamnose H-4, H-5), 4.28 (dd, $^2J$ = 11.3, $^3J$ = 5.1, terminal D-xylose H-5e), 4.36 (dd, $^2J$ = 9.1, $^3J$ = 3.3, L-rhamnose H-3), 4.45 (dd, $^3J_1$ = 3.5, $^3J_2$ = 1.6, L-rhamnose H-2), 4.86 (d, $^3J$ = 7.5, C-24 D-xylose H-1), 5.13 (d, $^3J$ = 7.3, terminal D-xylose H-1), 5.22 (d, $^3J$ = 1.5, L-rhamnose H-1).

Table 1 lists the $^{13}C$ NMR spectrum.

**Acid Hydrolysis of Cyclomacroside E (1).** Gllycoside **1** (50 mg) was dissolved in methanolic $H_2SO_4$ (5 mL, 0.25%), refluxed on a water bath for 4 h, cooled, and diluted with water. The MeOH was distilled off. The resulting precipitate was filtered off. As expected, the precipitate did not contain the native genin and consisted of its destruction products. Therefore, the precipitate was not analyzed. The filtrate was heated on the water bath for another hour to destroy methylglycosides. The mixture was cooled and neutralized with $BaCO_3$. The precipitate was removed. The solution was concentrated. Paper chromatography (PC) detected D-xylose and L-rhamnose by comparison with authentic samples. The PMR and $^{13}C$ NMR spectra showed that glycoside **1** contained these monosaccharides in a 2:1 ratio.

**Enzymatic Hydrolysis of Cyclomacroside E (1).** Glycoside **1** (500 mg) in water (100 mL) was treated with lyophilized dried *Helix pomatia* stomach juice (50 mg) in EtOH (10 mL), left at 35–37°C for two months, and treated with *n*-BuOH. The BuOH extract was evaporated to dryness. The solid was chromatographed over a column with elution by system 2 to afford **2** (12 mg), $C_{30}H_{52}O_5$, mp 209–211°C (MeOH), which was identified as cyclomacrogenin B by direct comparison with an authentic sample using TLC and PMR and $^{13}C$ NMR spectra.

PMR spectrum (400 MHz, $C_5D_5N$, δ, ppm, J/Hz, 0 = HMDS): 0.34 and 0.83 (d, $^2J$ = 4.5, 2H-19), 0.90 (d, $^3J$ = 6.3, $CH_3$-21), 1.03 (s, $CH_3$-18), 1.06 (s, $CH_3$-30), 1.15 (s, $CH_3$-28), 1.19 (s, $CH_3$-29), 1.40 (s, $CH_3$-26), 1.43 (s, $CH_3$-27), 1.85 (d,

$^3J$ = 9, H-8), 2.30 (ddd, $^2J$ = 12.9, $^3J_1$ = 4.2, $^3J_2$ = 3, H-2), 2.49 (ddd, $^2J$ = 14.4, $^3J_1$ = 9.8, $^3J_2$ = 6.2, H-11), 2.59 (dd, $^3J_1$ = 13.4, $^3J_2$ = 4.6, H-5), 3.67 (dd, $^3J_1$ = 8.9, $^3J_2$ = 2.5, H-24), 3.77 (t, $^3J_1$ = $^3J_2$ = 2.9, H-1), 3.90 (ddd, $^3J_1$ = 10.5, $^3J_2$ = 9.6, $^3J_3$ = 4, H-7), 4.30 (dd, $^3J_1$ = 12, $^3J_2$ = 4.5, H-3).

Table 1 lists the $^{13}C$ NMR spectrum.

Continued elution of the column by system 3 isolated progenin **3** (135 mg), $C_{36}H_{62}O_9$, mp 281–283°C (MeOH) that was identified as cyclomacroside C [5].

PMR spectrum (400 MHz, $C_5D_5N$, δ, ppm, J/Hz, 0 = HMDS): 0.36 and 0.77 (d, $^2J$ = 4.3, 2H-19), 0.83 (s, $CH_3$-30), 0.86 (s, $CH_3$-29), 0.90 (d, $^3J$ = 6, $CH_3$-21), 1.02 (s, $CH_3$-18), 1.12 (s, $CH_3$-28), 1.40 (s, $CH_3$-26), 1.43 (s, $CH_3$-27), 1.47 (d, $^3J$ = 5.6, L-rhamnose $CH_3$), 1.80 (d, $^3J$ = 9.2, H-8), 2.40 (m, H-2), 2.47 (m, H-11), 2.52 (dd, $^3J_1$ = 13, $^3J_2$ = 4.4, H-5), 3.67 (dd, $^3J_1$ = 8.9, $^3J_2$ = 2.7, H-24), 3.71 (t, $^3J_1$ = $^3J_2$ = 2.9, H-1), 3.85 (ddd, $^3J_1$ = 10.5, $^3J_2$ = 10, $^3J_3$ = 3.9, H-7), 4.05 (dd, $^3J_1$ = 11.7, $^3J_2$ = 4.4, H-3), 4.17 (t, $^3J_1$ = $^3J_2$ = 9.4, L-rhamnose H-4), 4.20 (m, L-rhamnose H-5), 4.39 (dd, $^3J_1$ = 8.9, $^3J_2$ = 3.4, L-rhamnose H-3), 4.47 (dd, $^3J_1$ = 3.4, $^3J_2$ = 1.7, L-rhamnose H-2), 5.25 (d, $^3J$ = 1.7, L-rhamnose H-1).

Table 1 lists the $^{13}C$ NMR spectrum.

Further elution of the column by the same system afforded progenin **4** (48 mg), $C_{41}H_{70}O_{13}$, mp 151–153°C (MeOH), identical to cyclomacroside D [6].

PMR spectrum (400 MHz, $C_5D_5N$, δ, ppm, J/Hz, 0 = HMDS): 0.35 and 0.78 (d, $^2J$ = 4.4, 2H-19), 0.83 (s, $CH_3$-30), 0.858 (d, $^3J$ = 6.3, $CH_3$-21), 0.86 (s, $CH_3$-29), 1.02 (s, $CH_3$-18), 1.08 (s, $CH_3$-28), 1.32 (s, $CH_3$-26), 1.38 (s, $CH_3$-27), 1.47 (d, $^3J$ = 5.9, L-rhamnose $CH_3$), 1.81 (d, $^3J$ = 9, H-8), 2.41 (m, H-2), 2.48 (m, H-11), 2.52 (dd, $^3J_1$ = 13, $^3J_2$ = 4, H-5), 3.61 (dd, $^2J$ = 11, $^3J$ = 9.7, D-xylose H-5a), 3.72 (t, $^3J_1$ = $^3J_2$ = 2.8, H-1), 3.75 (br.d, $^3J$ = 8, H-24), 3.85 (m, H-7), 3.90 (dd, $^3J_1$ = 8.7, $^3J_2$ = 7.8, D-xylose H-2), 4.04 (t, $^3J_1$ = $^3J_2$ = 8.7, D-xylose H-3), 4.06–4.22 (genin H-3; D-xylose H-4, H-5e; L-rhamnose H-4, H-5), 4.40 (dd, $^3J_1$ = 8.9, $^3J_2$ = 3.4, L-rhamnose H-3), 4.49 (dd, $^3J_1$ = 3.4, $^3J_2$ = 1.6, L-rhamnose H-2), 4.81 (d, $^3J$ = 7.6, D-xylose H-1), 5.26 (d, $^3J$ = 1.5, L-rhamnose H-1).

Table 1 lists the $^{13}C$ NMR spectrum.

Continued rinsing of the column by system 4 isolated starting glycoside **1** (170 mg).

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