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# NEW TRITERPENE GLYCOSIDE FROM *Cyclamen adzharicum* TUBERS

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*Nine pure glycosides were isolated from total saponins of* Cyclamen adzharicum *Pobed. (Primulaceae). The total chemical structure of cyclamen F, 3β-O-[β-D-Xylp(1→2)]-[β-D-Glcp(1→2)]-(β-D-Glcp(1→4)-α-L-Arap)-16α-hydroxy-13,28-epoxy-30,30-dibutoxyolean, was elucidated using modern physicochemical and spectral methods (NMR, $^1H$, $^{13}C$, HMBC, HMQC, DEPT, COSY, MS). A glycoside with the cyclamen F chemical structure has not been reported and, therefore, is a new organic compound.*

**Key words:** *Cyclamen adzharicum*, triterpene glycoside, NMR, MS.

The genus *Cyclamen* L. (Primulaceae) comprises perennial herbaceous plants. Five species are indigenous to the flora of Georgia [1]. *C. adzharicum* Pobed. is distributed in beech forests and bushes on mountain slopes [1]. Tubers containing a large quantity of triterpene saponins have been used in folk medicine to treat various diseases [2].

Comparative TLC of triterpene glycosides from fresh dried (air and microwave) tubers of *C. adzharicum* showed that the drying conditions did not affect the content of triterpene glycosides, represented by at least 17 compounds, that we named cyclamens A-Q.

Fresh peeled tubers were extracted with 70% MeOH. The solid contained the whole gamut of saponins and accompanying substances of the starting plant. Extraction of the MeOH extract with butanol-1 produced 8% of total saponins. According to HPLC analysis, the triterpene glycoside content of the extract was 52.73%.

Fractionation of the butanol total saponins by low-pressure liquid chromatography over reversed phase RP-18 with gradient elution (30:70→90:10 MeOH:$H_2O$) provided a crude separation of the total and produced two- and four-component fractions, multiple rechromatography of which over columns of silica gel 60 and polyamide MN SC6 using $CHCl_3$:$CH_3OH$:$H_2O$ (26:14:3) and $CH_3OH$:$H_2O$ (10→40% MeOH) mobile phases isolated nine pure compounds, cyclamens D, F, G, K, L, M, N, P, and Q.

Herein we describe elucidation of the chemical structure of cyclamen F. The structures of glycosides D, K, M, N, and Q were elucidated earlier [3–8]. The structures of glycosides L and P are being studied.

Cyclamen F, $C_{60}H_{102}O_{23}$, MW 1190.6812, MALDI-TOF MS 1213.6709 $[M + Na]^+$, was a white crystalline powder, insoluble in water, mp 195–197°C, $[\alpha]_D^{25}$ 5.3° (*c* 0.15, MeOH).

Acid hydrolysis produced up to 40% of the aglycon. TLC of the sugar part detected glucose, arabinose, and xylose. TLC of the progenin and monosaccharide fractions formed after basic hydrolysis of the glycoside proved the monodesmoside nature of the glycoside with monosaccharides on C-3 of the genin.

The full chemical structure of cyclamen F was established by NMR spectroscopy ($^1H$, $^{13}C$, HMBC, HMQC, DEPT, COSY, MS) and high-resolution mass spectral analysis.

COSY ($^1H$) spectra showed chemical shifts of anomeric H atoms at 4.38 ppm (d, J = 7.6 Hz), 4.51 (d, J = 7.8), 4.68 (d, J = 7.8), 4.49 (d, J = 7.5) and indicated that the two glucose and xylose units had the $\beta$-configuration; arabinose, $\alpha$-configuration.

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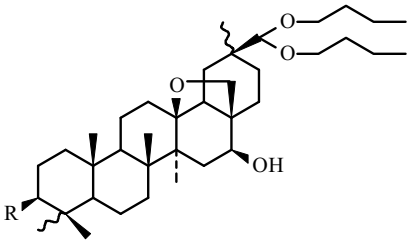
TABLE 1. $^{13}C$ and $^{1}H$ NMR Spectra of the Aglycon of Cyclamen F ($CD_3OD$, δ, ppm, J/Hz)

| C atom | $\delta_C$ | $\delta_H$ | C atom | $\delta_C$ | $\delta_H$ |
|---|---|---|---|---|---|
| 1 | 40.17 | 1.78; 1.01 | 20 | 41.45 | – |
| 2 | 27.22 | 1.85; 1.78 | 21 | 32.72 | 1.90; 1.62 |
| 3 | 91.35 | 3.15 | 22 | 31.91 | 1.81; 1.51 |
| 4 | 40.47 | – | 23 | 28.35 | 1.05 |
| 5 | 56.78 | 0.72 (d, J = 12.1) | 24 | 16.69 | 0.84 |
| 6 | 18.71 | 1.43 | 25 | 16.72 | 0.89 |
| 7 | 35.05 | 1.83; 1.22 | 26 | 18.77 | 1.14 |
| 8 | 43.32 | – | 27 | 19.87 | 1.22 |
| 9 | 51.29 | 1.21 | 28 | 78.54 | 3.55; 3.17; J = 9.3 |
| 10 | 37.72 | – | 29 | 24.18 | 0.89 |
| 11 | 19.87 | 1.51 | 30 | 107.17 | 4.60 |
| 12 | 33.66 | 1.80; 1.55 | $\alpha$-$CH_2$ | 72.05 | 3.79; 3.48 |
| 13 | 88.43 | – | | 71.39 | 3.78; 3.45 |
| 14 | 45.09 | – | $\beta$-$CH_2$ | 33.48 | 1.56 |
| 15 | 36.99 | 2.11; 1.20 | | 33.67 | |
| 16 | 77.83 | 3.88 | $\gamma$-$CH_2$ | 20.63 | 1.42 |
| 17 | 44.91 | – | | 20.48 | |
| 18 | 51.46 | 1.54 | $CH_3$ | 14.34 | 0.94; 0.93 (t, J = 7.4) |
| 19 | 35.10 | 2.20; 1.78 | | 14.22 | |

The $^{13}C$ NMR spectrum showed resonances of anomeric C atoms at 105.58 ppm, 104.8, 104.35, and 107.35 (Table 1) and confirmed that a tetrasaccharide moiety was bonded to the genin. Weak-field chemical shifts of C-2 and C-4 of the arabinose (79.37 and 80.20 ppm) and a weak-field shift of glucose C-2 (85.15) were consistent with 1→2-, 1→4-, and 1→2-bonds. This was confirmed in the HMBC spectrum as a clear correlation between resonances for arabinose C-2 and glucose H-1 (4.68 ppm), arabinose C-4 and H-1 of a second glucose (4.51), and glucose C-2 and xylose H-1 (4.49).

The chemical shift of the genin C-3 atom (91.35 ppm) showed that its hydroxyl was substituted by a monosaccharide unit. This was confirmed by the correlation peak for H-1 (Ara) and C-3 (Agl) $\delta_H/\delta_C$ 4.38/91.35 (Table 2). The $^{13}C$ NMR spectrum exhibited resonances for C-13 (88.43), C-28 (78.54), and C-16 (77.83) that suggested two butoxy groups were located on C-30. This was unambiguously proved by the presence in the HMBC spectrum of correlation peaks for H-30/C-αBu ($\delta_H/\delta_C$ 4.60/72.05 and 4.60/71.39). An aglycon of similar structure has not been reported. Therefore, it is a new genin.

Based on the chemical results and the $^{13}C$ NMR experiments, the chemical structure of cyclamen F was established as 3β-*O*-[β-D-Xyl*p*(1→2)]-[β-D-Glc*p*(1→2)]-(β-D-Glc*p*(1→4)-α-L-Ara*p*)-16α-hydroxy-13,28-epoxy-30,30-dibutoxyolean.




R = β-D-Xyl*p*(1→2)-β-D-Glc*p*(1→4)-α-L-Ara*p*-*O*
↑2
1
β-D-Glc*p*

A glycoside with the chemical structure of cyclamen F has not been reported. Therefore it is a new organic compound.

Total saponins and pure glycosides from tubers of *C. adzharicum* have been found to be highly cytotoxic and showed antiprotozoic activity. A sample of the total preparation named kochivardini has been proposed for treating acute and recidival paranasal sinusitis. It was prepared under clinical certification [3]. Therefore, the isolation and structure elucidation of pure glycosides from tubers of *C. adzharicum* is definitely interesting.

TABLE 2. $^{1}H$ and $^{13}C$ NMR Spectra of the Monosaccharide Unit of Cyclamen F ($CD_3OD$, δ, ppm, J/Hz)

| C atom | $\delta_C$ | $\delta_H$ | C atom | $\delta_C$ | $\delta_H$ |
|---|---|---|---|---|---|
| Glucose (Glc) | | | Arabinose (Ara) | | |
| 1 | 104.8 | 4.51 (d, J = 7.8) | 1 | 105.68 | 4.38 (d, J = 7.6) |
| 2 | 85.15 | 3.38 | 2 | 79.37 | 3.78 |
| 3 | 77.83 | 3.58 | 3 | 74.46 | 3.80 |
| 4 | 70.97 | 3.25 | 4 | 80.20 | 3.88 |
| 5 | 77.54 | 3.35 | 5 | 65.7 | 4.21 |
| 6 | 62.56 | 3.86 | | | 3.52 |
| | | 3.64 | Xylose (Xyl) | | |
| Glucose (Glc) | | | 1 | 107.35 | 4.49 (d, J = 7.5) |
| 1 | 104.35 | 4.68 (d, J = 7.8) | 2 | 75.91 | 3.25 |
| 2 | 76.02 | 3.18 | 3 | 78.02 | 3.26 |
| 3 | 77.64 | 3.38 | 4 | 71.11 | 3.51 |
| 4 | 71.99 | 3.31 | 5 | 67.42 | 3.98 |
| 5 | 77.90 | 3.35 | | | 3.31 |
| 6 | 63.30 | 3.83 | | | |
| | | 3.61 | | | |

## EXPERIMENTAL

Raw material (*C. adzharicum* tubers) was collected near Kobuleti, Adzhar AR (Georgia).

NMR spectra were recorded at room temperature on Bruker AMX-400 ($^{13}C$) and Bruker AMX-500 ($^{1}H$) apparatuses (Py-$d_5$, $CD_3OD$, δ, ppm) with TMS internal standard; high-resolution mass spectra, in a Jeol JMS-700 instrument (Jeol Ltd., Akishima, Tokyo, Japan). Melting points were measured on a Buchi Melting Point B-540 apparatus. Optical rotation $[\alpha]_D^{25}$ was measured on a Perkin–Elmer Model 341 Orot Polarimeter.

Extracts and fractions enriched in saponins were separated in a low-pressure liquid chromatograph (Jobin, Yvon Chromato Spac prep 100) using MeOH:$H_2O$ under gradient conditions.

TLC of sapogenins and monosaccharides was performed on silica gel plates (Silica gel 60, 0.02, Merck) using solvent systems $CHCl_3$:MeOH:$H_2O$ (26:14:3, 1), BuOH:HOAc:$H_2O$ (4:1:5, 2), $CHCl_3$:MeOH (20:1, 3), and $CH_2Cl_2$:MeOH:$H_2O$ (50:25:5, 4).

**Acid Hydrolysis of Cyclamen F.** Glycoside (3 mg) was placed in a flask, treated with HCl (10%, 3 mL), and heated at 100°C for 4 h. Sapogenins were extracted by $Et_2O$. The aqueous part was neutralized by *N*,*N*-dioctylmethylamine (10% $CHCl_3$) and dried. Sapogenins and sugars were identified by TLC with standards using systems 3 and 4.

**Basic Hydrolysis of Cyclamen F.** Glycoside (5 mg) was placed in a flask, treated with aqueous KOH (5%, 5 mL), heated at 100°C for 90 min, and neutralized by HCl (10%, pH 5). Sapogenin was extracted by butanol-1 and analyzed by TLC using systems 1 and 2.

Extracts and fractions enriched in saponins were separated in a low-pressure liquid chromatograph (Jobin, Yvon Chromato Spac prep 100) using MeOH:$H_2O$ under gradient conditions.

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