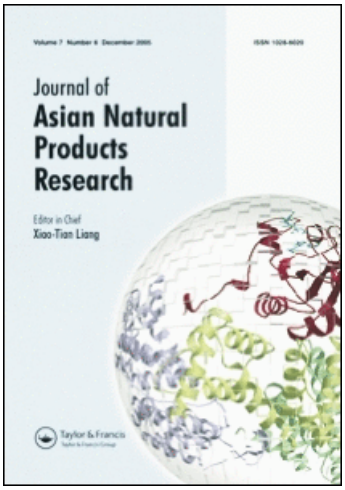


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# Triterpenoid Saponin from *Primula Elatior* Subsp. *Meyeri*

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# TRITERPENOID SAPONIN FROM *PRIMULA ELATIOR* SUBSP. *MEYERI*

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A new triterpenoid saponin, protoprimuloside B (**2**), has been isolated from the roots of *Primula elatior* subsp. *meyeri* and its structure deduced as 3β-O-{[α-L-rhamnopyranosyl-(1-2)-β-D-galactopyranosyl-(1-3)]-[β-D-glucopyranosyl-(1-2)]-β-D-glucopyranosyl}-protoprimuloside B by means of spectral data, especially NMR (400 MHz), including $^{1}H$, $^{13}C$, DEPT, COSY, HMBC, HMQC, NOESY techniques and (+)FAB-MS spectrum.

*Keywords*: *Primula elatior* subsp. *meyeri*; Primulaceae; Triterpene saponins; Protopriumuloside B

## INTRODUCTION

The genus *Primula* (Primulaceae) is represented by eight species in Turkey [1]. It has been reported that triterpenoidal saponins are the main chemical constituents in plants of Primulaceae family [2–4]. A previous study on *Primula elatior* subsp. *meyeri* has chemically identified sapogenin, protoprimulagenin A [2]. My ongoing study on the chemical constituents of this plant led to the isolation and structure elucidation of a new triterpenoid saponin, protoprimuloside B (**2**) by NMR and (+) FAB-mass spectra.

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## RESULTS AND DISCUSSION

The air-dried roots of *Primula elatior* subsp. *meyeri* was extracted by methanol and gave crude saponin fraction. Flash revers-phase silica gel RP-18 column chromatography of the crude saponin fraction afforded pure main triterpenoid saponin, protoprimuloside B (**2**).

Protoprimuloside B was obtained as an amorphous powder, mp 242–243°C, $[\alpha]_D$ −5.5. The positive-ion FAB mass spectral data (*m/z* 1182 $[M+Glycerol]^+$), in combination with the $^{13}C$ NMR spectral data (Tab. I), indicated a molecular formula of $C_{54}H_{90}O_{22}$. The FT-IR (KBr) spectrum revealed the presence of hydroxyl groups at 3442 (OH) and glycosidic linkage at 1082 (C—O) cm $^{-1}$. The $^{13}C$ NMR spectrum of compound **2** showed 54 resonances of which 30 were accounted for by the aglycone moiety and the rest (24) by the oligosaccharides moiety (4 × hexoses).

The $^1H$ NMR spectrum of **2** showed seven tertiary methyl groups at $\delta$ 0.90, 0.92, 0.93, 0.97, 1.07, 1.16 and 1.24 ppm. The $^{13}C$ resonances for the aglycone of **2** revealed three oxygenated $sp^3$ carbon resonances ($C_3$: $\delta$ 92.13, $C_{16}$: $\delta$ 77.87, $C_{28}$: $\delta$ 78.75 ppm) in the down field and the remaining oxygenated carbon resonances were accounted for by the four sugars. The thirty carbon aglycone was shown by DEPT spectra to have seven quaternary carbons ($\delta$ 32.41, 37.80, 40.73, 43.27, 45.28, 45.37, 88.37 ppm), five methines (51.36, 52.35, 56.80, 77.87, 92.13 ppm), eleven methylenes ($\delta$ 40.26, 27.06, 18.73, 35.17 19.86, 32.17, 37.37, 39.79, 37.05, 33.31, 78.75 ppm) and eight methyl groups ($\delta$ 16.77 (two peaks due to HETCOR and $^1H$ NMR), 17.93 (Rhamnose-$CH_3$) 18.82, 19.93, 24.94, 28.26, 33.92 ppm). A comparison of these findings with the well-established results in the literature [4–6] revealed that the aglycone has the characteristic carbons of a protoprimulagenin A triterpene.

The inspection of the NOESY and HMBC data for the aglycone part of the molecule (Fig. 1) revealed that the relative stereochemistry at the common centers were identical with those of reported triterpenes [3–6]. A NOESY experiment on compound **2** showed the presence of characteristic cross-peak correlation of 16-H ($\delta$ 3.88 ppm) with 15$\alpha$,$\beta$-H ($\delta$ 2.08, 1.78 ppm) and 28$\alpha$-H ($\delta$ 3.12 ppm). Thus, the hydroxyl group at $C_{16}$ was in the $\alpha$-configuration.

The appearance of four anomeric carbon resonances at $\delta$ 100.78, 102.05, 102.57 and 105.79 in the $^{13}C$ NMR spectrum and four proton resonances at $\delta$ 4.45, 4.90, 5.20 and 5.28 in the $^1H$ NMR spectrum, further confirmed the existence of a tetrasaccharide moiety in compound **2**. Additionally, in the $^{13}C$ NMR spectrum, there were 16 methine resonances between $\delta$ 70 and 82,

TABLE I $^{13}C$ and $^{1}H$ NMR spectral data for protoprimuloside B in $CD_3OD$

| *Aglycone of* **2**[a,b] | | | | *Sugar moiety* **2**[a,b] | | | |
|---|---|---|---|---|---|---|---|
| *C/H* | $^{13}C$ ($\delta$, *ppm*) | *DEPT* | $^{1}H$ ($\delta$, *ppm*) | *C/H* | $^{13}C$ ($\delta$, *ppm*) | *DEPT* | $^{1}H$ ($\delta$, *ppm*) |
| 1 | 40.26 | $CH_2$ | 1.12,1.48 | Glc I 1 | 105.79 | CH | 4.45 d, $J=7.2$ Hz |
| 2 | 27.06 | $CH_2$ | 1.50,1.75 | 2 | 79.30 | CH | 3.91 |
| 3 | 92.13 | CH | 3.18, t, $J=9.2$ Hz | 3 | 81.13 | CH | 4.08 |
| 4 | 40.73 | C | – | 4 | 71.80 | CH | 3.72 |
| 5 | 56.80 | CH | 0.72 | 5 | 78.09 | CH | 3.40 |
| 6 | 18.73 | $CH_2$ | 1.23, 1.46 | 6 | 62.74 | $CH_2$ | 3.84, 3.68 |
| 7 | 35.17 | $CH_2$ | 1.26, 1.98 | | | | |
| 8 | 43.27 | C | – | Galac 1 | 100.78 | CH | 5.20 d, $J=7.2$ Hz |
| 9 | 52.35 | CH | 1.49, m[c] | 2 | 76.14 | CH | 3.78 |
| 10 | 37.80 | C | – | 3 | 76.04 | CH | 3.70 |
| 11 | 19.86 | $CH_2$ | 1.20, 1.48 | 4 | 73.72 | CH | 3.40 |
| 12 | 32.17 | $CH_2$ | 1.52, 2.36 | 5 | 76.88 | CH | 3.52 |
| 13 | 88.37 | C | – | 6 | 63.54 | $CH_2$ | 3.90, 3.56 |
| 14 | 45.37 | C | – | | | | |
| 15 | 37.37 | $CH_2$ | 1.22, 2.08 | Rham 1 | 102.05 | CH | 5.28 br s |
| 16 | 77.87 | CH | 3.88, dd, $J=5.1$, 7.2 Hz | 2 | 72.60 | CH | 3.95 |
| | | | | 3 | 72.31 | CH | 3.70 |
| 17 | 45.28 | C | – | 4 | 73.72 | CH | 3.41 |
| 18 | 51.36 | CH | 1.28 | 5 | 70.24 | CH | 4.12 |
| 19 | 39.79 | $CH_2$ | 1.46, 1.60 | 6 | 17.92 | $CH_3$ | 1.21 |
| 20 | 32.41 | C | – | | | | |
| 21 | 37.05 | $CH_2$ | 2.10, 2.14 | Glc II 1 | 102.57 | CH | 4.90 d, $J=6.8$ Hz |
| 22 | 33.31 | $CH_2$ | 1.48, 2.06 | 2 | 75.95 | CH | 3.24 |
| 23 | 28.26 | $CH_3$ | 1.07, s | 3 | 78.09 | CH | 3.35 |
| 24 | 16.77 | $CH_3$ | 0.90, s | 4 | 72.60 | CH | 3.07 |
| 25 | 16.77 | $CH_3$ | 0.92, s | 5 | 77.87 | CH | 3.40 |
| 26 | 18.82 | $CH_3$ | 1.16, s | 6 | 62.74 | $CH_2$ | 3.84, 3.68 |
| 27 | 19.93 | $CH_3$ | 1.24, s | | | | |
| 28 | 78.75 | $CH_2$ | 3.12, 3.49 | | | | |
| 29 | 33.92 | $CH_3$ | 0.97, s | | | | |
| 30 | 24.94 | $CH_3$ | 0.93, s | | | | |

[a] Chemical shifts (ppm) are relative to $CD_3OD$.
[b] Assignments based on 2D-COSY, TOCSY, HMQC, HMBC and NOESY spectra.
[c] Overlapped.

three oxymethylenes at $\delta$ 62 and 64, and one methyl resonance at $\delta$ 17.92, supporting the existence of three hexopyranose and one deoxyhexopyranose residues [4]. The hexopyranose residues were identified as two glucopyranose and one galactopyranose, the 6-deoxyhexopyranose residue as rhamnopyranose by analysis of the COSY and TOCSY spectrum. The sugars were also analyzed on TLC after acid hydrolysis of compound **2** and were identified as rhamnose, galactose and glucose. The proton resonances

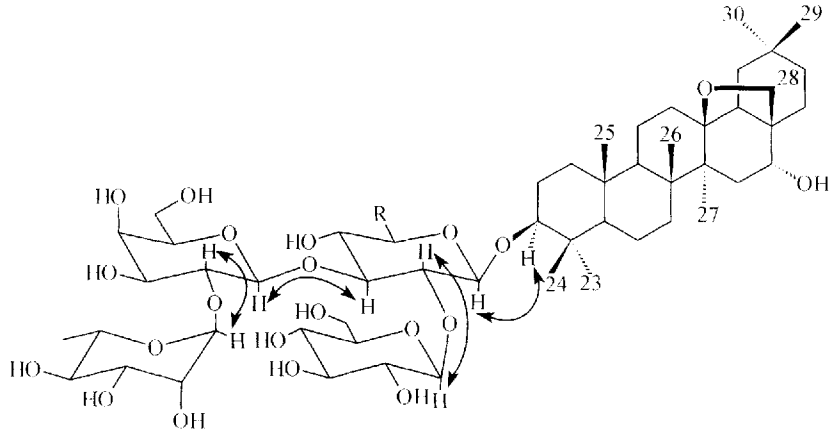


FIGURE 1 Interglycosidic NOE's of protoprimuloside B for sugar sequence and linkage site determination.



at $\delta$ 4.45 (d, $J = 7.2$ Hz), 4.90 (d, $J = 6.8$ Hz), 5.20 (d, $J = 7.2$ Hz) and 5.28 (br s) were assigned to anomeric protons of $\beta$-D-glucose I, $\beta$-D-glucose II, $\beta$-D-galactose, and $\alpha$-L-rhamnose, respectively. The $^{13}C$ NMR spectral data also indicated that the tetrasaccharidic sugar moiety should be attached at C-3 ($\delta$ 92.13) of the aglycone.

Three interglycosidic linkage and sequence in the sugar chain in **2** was established by 2D-NOESY, HMBC spectra as {$\alpha$-L-Rha-(1 → 2), -$\beta$-D-Galac-(1 → 3). [-$\beta$-D-Glc-II-(1 → 2)], -$\beta$-D-Glc-I} (Fig. 1). In the $^{13}C$ NMR spectra, interglycosidic carbon resonances were seen at $\delta$, 76.14, 79.30, 81.13 (CH) ppm in sugar moiety of compound **2** (Tab. I). The positions of the interglycosidic linkages between the monosaccharide units in **2** were found to be similar by comparison with the $^{13}C$ chemical shifts of compound **1** [4] and in view of the glycosylation effects observed in the $^{13}C$ NMR spectrum. The sugar moiety of compound **2** was remarkably similar to compound **1**. But, the only difference was glucuronic acid displaced by glucose in sugar chain of saponin **2**.

The sequence of the sugars were also established through positive ion FAB-MS which exhibited the molecular ion peak at m/z = 1182 $[M + Glycerol]^+$ and fragment ions m/z = 1150 $[M + Glycerol\text{-}CH_2OH\text{-}H]^+$, 1019 $[M + Glycerol\text{-}(Rham\text{-}O) + H]^+$, 1019 $[M + Glycerol\text{-}(Glc\ II)]^+$, 857 $[M + Glycerol\text{-}(Rham\text{-}O\text{-}Galac\text{-}O) + H]^+$, 685 $[M\text{-}Aglycone + 2H_2O\text{-}H]^+$, 679 $[M + Glycerol\text{-}(Rham\text{-}O\text{-}Galac\text{-}O)\text{-}(Glc\ II\text{-}O) + 2H]^+$, 345

$[(Rham\text{-}O\text{-}Galac\text{-}O)+H_2O+H]^+$, 163 $[Glc\ II]^+$, 150 $[Rham+2H]^+$ (Fig. 1).

Based upon the above observations, the structure of protoprimuloside B was established as 3$\beta$-O-{[$\alpha$-L-rhamnopyranosyl-(1-2)-$\beta$-D-galactopyranosyl-(1-3)]-[$\beta$-D-glucopyranosyl-(1-2)]-$\beta$-D-glucopyranosyl}-protoprimuloside B which is a novel natural product.

## EXPERIMENTAL SECTION

### General

NMR spectra were recorded on a Varian NMR at 400 MHz instrument in $CD_3OD$. (+) FAB was recorded on a Zabspec MS instrument. Melting point was obtained using a Kofler hot stage apparatus and are uncorrected. The optical rotation was measured with a Perkin-Elmer 241 polarimeter. Infrared spectrum was measured on a Perkin-Elmer 1600 FT-IR spectrometer. Flash column chromatography was performed on a revers-phase silica gel RP-18 and TLC was performed with precoated revers-phase silica gel RP-8 $F_{254}S$. A voucher specimen has been deposited in a deep freezer at the Department of Chemistry, Karadeniz Technical University, Turkey.



### Isolation of Compound 2

Specimens of *Primula elatior* subsp. *meyeri* were collected at Ovid Yaylası, in the northern part of Turkey in July, 1997. The root of *Primula elatior* subsp. *meyeri* were dried on a bench-top and air-dried roots (310 g) were extracted with cold MeOH (1 L × 3, 24 hour each). The total MeOH extract was filtered, and the filtrate was concentrated up to 50 ml on a rotary evaporator at 30°C. The $CH_3OH$ extract was precipitated and filtered off. The obtained crude mixture (1.2 g) was chromatographed on a flash revers-phase silica gel RP-18 (20 g) column eluted with $H_2O$ (30 ml) and discontinuous gradient of $H_2O$-$CH_3COCH_3$ (4:1, 50 ml; 4:2, 100 ml; 4:3, 100 ml; 1:1, 100 ml), then finally with $CH_3COCH_3$ (50 ml) to give 13 fractions ($\sim$30–40 ml each). The fractions 7–9 were combined after analyses of TLC to give compound **2** (372 mg). The TLC analysis with revers-phase silica gel RP-8 $F_{254}S$, $CH_3COCH_3$-$H_2O$, (5:7), of compound **2** showed that it was in pure form ($R_f$: 0.65). Colorless amorphous crystals, mp 242–243°C (dec); $[\alpha]_D$ −5.5 ($CH_3OH$; c 0.0049); $^1H$ NMR ($CD_3OD$, 400 MHz) and $^{13}C$ NMR ($CD_3OD$, 100 MHz) $\delta$ (ppm) see Table I; positive

FAB-MS (Glycerol) m/z (%); $m/z = 1182(21)$ $[M+Glycerol]^+$, 1166(30) $[M+Glycerol\text{-}H_2O+2H]^+$, 1150(15) $[M+Glycerol\text{-}CH_2OH\text{-}H]^+$, 1019(6) $[M+Glycerol\text{-}(Rham\text{-}O)+H]^+$, 1019(6) $[M+Glycerol\text{-}(Glc\ II)]^+$, 857(6) $[M+Glycerol\text{-}(Rham\text{-}O\text{-}Galac\text{-}O)+H]^+$, 685(3) $[M\text{-}Aglycone+2H_2O\text{-}H]^+$, 679(3) $[M+Glycerol\text{-}(Rham\text{-}O\text{-}Galac\text{-}O)\text{-}(Glc\ II\text{-}O)+2H]^+$, 668(4) $[M\text{-}Aglycone+H_2O]^+$, 566(2) $[M+Glycerol\text{-}Glycone+H_2O]^+$, 345(17) $[(Rham\text{-}O\text{-}Galac\text{-}O)+H_2O+H]^+$, 231(6), 192(100), 163(3) $[Glc\ II]^+$, 150(8) $[Rham+2H]^+$, 135(11), 121(10), 85(13).

## Acid Hydrolysis of 2

Compound **2** (15 mg) was refluxed with 2N HCl in aq. MeOH (5 ml) for 6 hr. The reaction mixture was then concentrated under reduced pressure to remove MeOH. It was then diluted with $H_2O$ (5 ml) and the aqueous layer was adjusted to pH 7 with $Ag_2CO_3$ and filtered. The supernatant was concentrated and compared with reference sugars on TLC (Silica gel, $H_2O$:MeOH:AcOH:EtOAc, 15:15:20:65). The sugars were detected by spraying the plate with a solution of aniline phthalate in BuOH which showed that the sugars in **2** were rhamnose, galactose and glucose.



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