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TRITERPENE SAPONINS FROM *PRIMULA VERIS* SUBSP. *MACROCALYX* AND *PRIMULA ELATIOR* SUBSP. *MEYERI*[1]

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ABSTRACT.—The roots of *Primula veris* subsp. *macrocalyx* have yielded three new triterpenoid saponins which have been characterized as 3-*O*-{[α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)]-[β-D-glucopyranosyl-(1→2)]-β-D-glucuronopyranosyl}-priverogenin B 22-acetate (priverosaponin B 22-acetate) [**1**], 3-*O*-{[α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)]-[β-D-glucopyranosyl-(1→2)]-β-D-glucuronopyranosyl}-priverogenin B (priverosaponin B) [**2**], and 3-*O*-{[α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)]-[β-D-glucopyranosyl-(1→2)]-β-D-glucuronopyranosyl}-anagalligenin A (primacrosaponin) [**3**]. By contrast, the main saponin isolated from *Primula elatior* subsp. *meyeri* was 3-*O*-{[α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)]-[β-D-glucopyranosyl-(1→2)]-β-D-glucuronopyranosyl}-protoprimulagenin A [**4**], primulasaponin. Structure elucidations were carried out both chemically and spectroscopically.

The genus *Primula* (Primulaceae) is represented by eight species in Anatolia (Turkey). The main saponin of *Primula auriculata* Lam., *Primula megaseifolia* Boiss. and Bal. ex Boiss. and *Primula longipes* Freyn and Sint. has been previously reported (1,2) and its structure established as that formerly reported for primulasaponin from *Primula elatior* (3). In this communication three further saponins **1**, **2**, and **3** from *Primula veris* subsp. *macrocalyx* (Bunge) Ludi and the main saponin, primulasaponin [**4**], from *Primula elatior* subsp. *meyeri* (Rupr.) Valentine and Lamond are reported. Their structures and those of related derivatives, as well as those of degradation products, have been elucidated and ascertained spectroscopically (ir, ^{1}H nmr, ^{13}C nmr, 2D nmr, and fabms).

RESULTS AND DISCUSSION

The air-dried roots and rhizomes of *P. veris* subsp. *macrocalyx* and *P. elatior* subsp. *meyeri* were extracted with MeOH-H_2O (9:1). The crude saponin mixtures and their methyl esters were subjected to a series of chromatographic separations to yield the genuine saponins **1–3** from *P. veris* subsp. *macrocalyx*, **4** from *P. elatior* subsp. *meyeri*, and their respective methyl ester derivatives, **5–8**.

Saponin **1** was obtained as an amorphous powder. The ir spectrum showed absorptions at 3460 (OH), 1740 (>C=O of ester group), and 1710 cm^{-1}(>C=O of carboxylic acid group). Methylation of **1** with CH_2N_2 yielded **5**. The fabms of **5** exhibited a peak at *m/z* 1199 $[M+Na]^+$ compatible with the molecular formula $C_{57}H_{92}O_{25}$. Its ir spectrum exhibited absorptions at 3350, 1735, and 1710 cm^{-1}. The ^{1}H-nmr spectrum of **5** revealed seven tertiary methyl groups at δ 0.86, 0.89, 0.95, 0.98, 1.06, 1.14, and 1.27. Single proton resonances at δ 4.52 (J=7.5 Hz), 4.86 (J=7.4 Hz),

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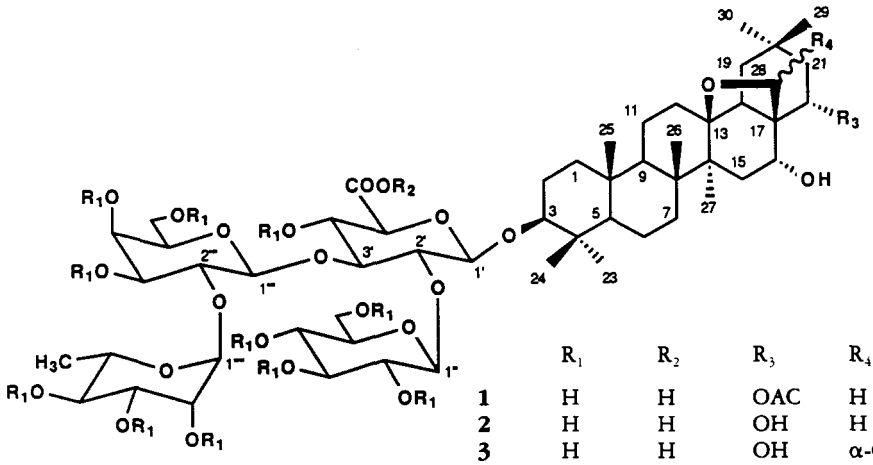
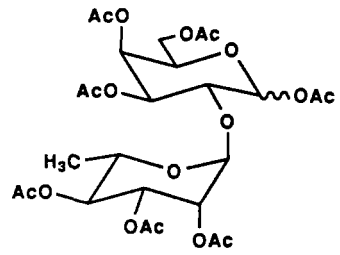


	R_1	R_2	R_3	R_4
1	H	H	OAC	H
2	H	H	OH	H
3	H	H	OH	α-OH
4	H	H	H	H
5	H	Me	OAc	H
6	H	Me	OH	H
7	H	Me	OH	α-OH
8	H	Me	H	H
16	Ac	Me	OAc	α-OAc



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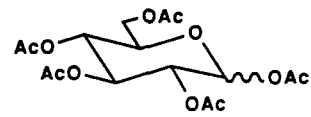

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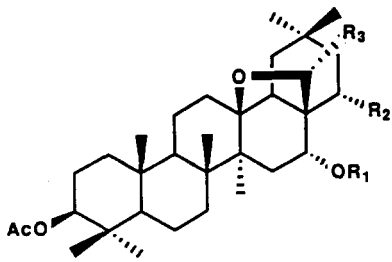

	R_1	R_2	R_3
11	H	OAc	H
12	Ac	OAc	H
13	H	OAc	OAc
14	Ac	H	H
15	H	H	H

5.16 (J=7.1 Hz) and 5.28 (J=1.4 Hz) were assigned to anomeric protons of β-D-glucuronic acid, β-D-glucose, β-D-galactose, and α-L-rhamnose, respectively. A doublet at δ 1.28 (J=6.2 Hz) and two three-proton singlets at δ 3.78 and 2.02 were evaluated, respectively, as a secondary methyl of rhamnose, a carbomethoxyl, and an acetoxy methyl group. Further, the resonance at δ 5.17 (1H, dd, J=11.1 and 7.1 Hz), assigned to H-22, indicated acylation at this position. The presence of four sugar residues was confirmed by the ^{13}C-nmr data of **5** (Table 1). The ^{13}C-nmr spectral data also indicated the tetrasaccharidic sugar moiety to be attached at C-3 (δ 92.3) of the aglycone.

Ordinary acid hydrolysis of saponins gives rise to some undesired secondary products of the sapogenol, e.g., opening of epoxides. To overcome this problem, the method of Kitagawa (4,5) was employed. Treatment of compound **1** with Ac_2O and pyridine (4,5) in a sealed tube yielded compounds **9**, **10**, **11**, and **12**. The structures of compound **9** [two anomers of heptaacetyl-2-*O*-(α-L-rhamnopyranosyl)-β-D-galactopyranoside] and **10** (two anomers of 1,2,3,4,6-pentaacetyl-β-glucopyranoside) were determined from their spectral data and chromatographic comparisons made with authentic samples of

TABLE 1. ^{13}C-nmr Spectral Data for the Methyl Ester Derivatives **5**, **6**, **7**, and **8** (CD_3OD, 75.5 MHz).

Carbon	Compound			
	5	**6**	**7**	**8**
C-1	40.2 t	40.2 t	40.2 t	40.2 t
C-2	27.2 t	27.2 t	27.2 t	27.2 t
C-3	92.3 d	92.3 d	92.4 d	92.3 d
C-4	40.7 s	40.7 s	40.7 s	40.7 s
C-5	56.8 d	56.8 d	56.7 d	56.8 d
C-6	18.7 t	18.8 t	18.8 t	18.7 t
C-7	35.1 t	35.2 t	35.1 t	35.2 t
C-8	43.5 s	43.5 s	43.6 s	43.3 s
C-9	52.1 d	52.1 d	51.3 d	52.4 d
C-10	37.8 s	37.8 s	37.9 s	37.8 s
C-11	19.8 t	19.9 t	20.0 t	19.9 t
C-12	33.5 t	33.7 t	33.9 t	32.2 t
C-13	88.3 s	88.3 s	89.0 s	88.3 s
C-14	45.8 s	44.6 s	44.7 s	45.4 s
C-15	36.8 t	36.8 t	36.8 t	37.4 t
C-16	70.2 d	69.2 d	69.3 d	77.9 d
C-17	49.5 s	53.2 s	53.2 s	45.3 s
C-18	51.3 d	51.4 d	47.7 d	51.4 d
C-19	38.9 t	39.0 t	39.1 t	39.8 t
C-20	33.8 s	33.6 s	33.7 s	32.4 s
C-21	42.6 t	46.2 t	46.2 t	37.1 t
C-22	76.3 d	75.9 d	70.3 d	33.3 t
C-23	28.3 q	28.3 q	28.3 q	28.3 q
C-24	16.7 q	16.7 q	16.8 q	16.7 q
C-25	16.7 q	16.7 q	16.8 q	16.7 q
C-26	18.8 q	18.9 q	18.9 q	18.8 q
C-27	20.1 q	20.2 q	20.0 q	19.9 q
C-28	77.6 t	78.0 t	98.6 d	78.7 t
C-29	33.4 q	33.7 q	33.8 q	33.9 q
C-30	25.7 q	26.0 q	26.0 q	25.0 q
C-1′	105.8 d	105.8 d	105.9 d	105.9 d
C-2′	79.0 d	79.0 d	79.0 d	78.9 d
C-3′	81.0 d	81.0 d	80.9 d	80.9 d
C-4′	72.6 d	72.6 d	72.6 d	72.6 d
C-5′	76.1 d	76.1 d	76.1 d	76.1 d
C-6′	170.8 s	170.8 s	170.9 s	170.8 s
C-1″	102.1 d	102.1 d	102.1 d	102.1 d
C-2″	76.0 d	75.9 d	76.0 d	75.9 d
C-3″	78.3 d	78.2 d	78.3 d	78.2 d
C-4″	71.6 d	71.6 d	71.6 d	71.6 d
C-5″	77.0 d	77.0 d	77.0 d	77.0 d
C-6″	62.8 t	62.8 t	62.8 t	62.8 t
C-1‴	102.6 d	102.6 d	102.6 d	102.6 d
C-2‴	78.0 d	78.0 d	78.0 d	78.1 d
C-3‴	73.8 d	73.8 d	73.8 d	73.7 d
C-4‴	71.6 d	71.6 d	71.6 d	71.6 d
C-5‴	76.4 d	76.4 d	76.4 d	76.4 d
C-6‴	63.6 t	63.6 t	63.6 t	63.5 t
C-1⁗	101.0 d	101.0 d	101.0 d	100.9 d
C-2⁗	72.6 d	72.6 d	72.6 d	72.6 d
C-3⁗	72.3 d	72.3 d	72.3 d	72.3 d
C-4⁗	75.9 d	75.9 d	75.8 d	75.8 d
C-5⁗	70.2 d	70.2 d	70.2 d	70.2 d
C-6⁗	17.9 q	17.9 q	17.9 q	17.9 q
$COOCH_3$	52.8 q	52.9 q	52.9 q	52.4 q
COMe	172.6 s			
$COCH_3$	21.0 q			

identical compounds obtained in the same way from primulasaponin (1,2). The ir and ^{1}H-nmr spectral data of **11** were identical to those reported (6,7) for priverogenin B 3,22-*O*-diacetate. We report ^{13}C-nmr data of **11** for the first time (Table 2).

TABLE 2. ^{13}C-nmr Spectral Data for Priverogenin B 3,22-*O*-diacetate [**11**], Anagalligenin A 3,22,28-*O*-triacetate [**13**], Protoprimulagenin A 3,16-*O*-diacetate [**14**], and Protoprimulagenin A 3-*O*-acetate [**15**] ($CDCl_3$, 75.5 MHz).

Carbon	Compound			
	11	**13**	**14**	**15**
C-1	38.8 t	38.7 t	38.6 t	38.6 t
C-2	23.8 t	23.7 t	23.8 t	23.7 t
C3	81.1 d	80.0 d	81.0 d	81.0 d
C-4	38.0 s	37.9 s	37.9 s	37.8 s
C-5	55.4 d	55.2 d	55.4 d	55.2 d
C-6	17.8 t	17.6 t	17.7 t	16.8 t
C-7	34.1 t	33.9 t	32.4 t	32.3 t
C-8	42.4 s	42.4 s	42.3 s	42.1 s
C-9	50.5 d	49.9 d	50.6 d	50.8 d
C-10	37.1 s	36.9 s	37.0 s	36.9 s
C-11	18.8 t	18.7 t	18.7 t	18.7 t
C-12	32.6 t	32.4 t	38.8 t	38.7 t
C-13	86.2 s	89.4 s	86.2 s	86.5 s
C-14	44.5 s	43.3 s	43.5 s	44.2 s
C-15	36.4 t	36.1 t	33.4 t	36.6 t
C-16	71.8 d	69.8 d	78.4 d	77.5 d
C-17	48.4 s	51.2 s	44.0 s	44.0 s
C-18	50.2 d	46.7 d	50.4 d	50.1 d
C-19	38.0 t	37.8 t	36.0 t	36.9 t
C-20	33.1 s	32.9 s	31.5 s	31.5 s
C-21	42.3 t	41.8 t	34.1 t	34.1 t
C-22	76.4 d	70.1 d	30.2 t	31.1 t
C-23	28.1 q	28.0 q	28.0 q	27.9 q
C-24	16.6 q	16.5 q	16.3 q	16.2 q
C-25	16.3 q	16.2 q	16.5 q	16.5 q
C-26	18.2 q	18.2 q	18.2 q	17.6 q
C-27	19.7 q	19.4 q	19.3 q	19.5 q
C-28	76.4 t	96.1 d	77.3 t	77.9 t
C-29	33.1 q	32.9 q	33.7 q	33.5 q
C-30	25.5 q	25.2 q	24.6 q	24.5 q
*C*OMe	171.0 s	170.9 s	170.0 s	171.1 s
	170.4 s	170.1 s	170.9 s	
		170.0 s		
$COCH_3$	21.4 q	21.4 q	21.9 q	21.3 q
	21.3 q	21.3 q	21.2 q	
		21.2 q		

Compound **12** showed an acetoxyl absorption band at 1735 cm^{-1} in its ir spectrum. The ^{1}H-nmr spectrum of **12** revealed the presence of seven tertiary methyl groups and signals for three acetoxy methyl groups. In addition, there were peaks at δ 3.49/3.62 (each 1H,d, AB system, J_{AB}=8.5 Hz) suggesting the presence of a 13β-28-epoxide, 4.48 (dd, J=8.1 Hz, H-3), 4.9 (dd, J=12 and 6 Hz, H-22) and 5.25 (d, J=5.4 Hz, H-16). These results were similar to those reported for priverogenin B 3,22-*O*-diacetate (6,7), the only major difference being the chemical shift value for H-16, indicating acetylation of 16-OH in **12**. The negative ion fabms exhibited a molecular ion peak at *m/z* 599

$[M-H]^-$ and fragment ions at *m/z* 541, 481, and 421 supporting this proposal. Thus, **12** was priverogenin B 3,16,22-*O*-triacetate.

Based upon the above, the structure of **1** was established as 3-*O*-{[α-L-rhamnopyranosyl-(1↦2)-β-D-galactopyranosyl-(1↦3)]-[β-D-glucopyranosyl-(1↦2)]-β-D-glucuronopyranosyl}-priverogenin B 22-acetate and named priverosaponin B 22-acetate.

Saponin **2** could not be obtained in a pure form and was eventually isolated as its methyl ester **6**. The ^{1}H-nmr spectrum of **6** (see Experimental) was similar to that of **5**, with the exception of an extra acetoxyl signal in **5**. The ^{13}C-nmr spectrum of **6** (Table 1) indicated it to have the same sugar moiety as **1** and to have priverogenin B as the aglycone. These suggestions were confirmed by negative fabms which exhibited peaks at *m/z* 1157 $[M+Na-H]^+$ αnd 1173 $[M+K-H]^-$ (calcd for $C_{55}H_{90}O_{24}$).

Alkaline hydrolysis of **6** afforded saponin **2** which upon, treatment with Ac_2O and pyridine, gave compounds **9**, **10**, **11**, and **12**. Thus, **2** was determined to be 3-*O*-{[α-L-rhamnopyranosyl-(1↦2)-β-D-galactopyranosyl-(1↦3)]-[β-D-glucopyranosyl-(1↦2)]-β-D-glucuronopyranosyl}-priverogenin B and named priverosaponin B.

Saponin **3**, gave $[\alpha]^{20}D$ −28.4° (*c*=0.44, MeOH). The fabms of **3** exhibited $[M+H]^+$ and $[M+Na]^+$ ions at *m/z* 1137.6 and 1159.6, consistent with the molecular formula $C_{54}H_{88}O_{25}$. Acid hydrolysis of **3** yielded glucuronic acid, galactose, glucose, and rhamnose, while treatment with CH_2N_2 yielded **7**. The fabms of **7** exhibited an $[M+Na+H]^+$ ion at *m/z* 1151.3 compatible with the molecular formula $C_{55}H_{90}O_{25}$. The ir spectrum showed strong absorptions at 3460 (OH), 1740 (>C=O, ester group), and 1700 cm^{-1} (>C=O, carboxylic acid group). The ^{1}H-nmr spectrum of **7** indicated the presence of seven tertiary methyl singlets at δ 0.86, 0.89, 0.95, 0.97, 1.06, 1.18, and 1.24 and four anomeric proton signals at δ 4.52 (d, *J*=7.6 Hz), 4.92 (d, *J*=7.6 Hz), 5.16 (d, *J*=7.3 Hz), and 5.28 (d, *J*=1.4 Hz) attributed to β-D-glucuronic acid, β-D-glucose, β-D-galactose, and α-L-rhamnose, respectively. The doublet at δ 1.27 (*J*=6.2 Hz) was attributed to the secondary methyl of the rhamnose moiety, whereas the singlets at δ 3.78 (3H) and δ 4.77 (1H) were assigned to an acyl group and H-28, respectively. The ^{13}C-nmr spectrum of **7** confirmed the presence of a tetrasaccharidic sugar moiety identical to that found in **5** and **6**. This was also consistent with anagalligenin A being the aglycone (8). Treatment of compound **3** with Ac_2O and pyridine (4,5) in a sealed tube resulted in the liberation of compounds **9**, **10**, and **13**.

The ir spectrum of **13** indicated hydroxyl (3500–3400 cm^{-1}) as well as acetoxyl functions (1730 cm^{-1}). The ^{1}H-nmr spectrum of **13** revealed the presence of three acetoxyl and seven tertiary methyl groups. The observation of signals attributable to H-3, H-16, and H-22 having chemical shift values similar to those of **11** suggested a common acetylation pattern. There was, however, no evidence for the methylene group of a 13β-28-epoxide linkage, and an additional one-proton singlet at δ 5.77 supported the presence of an acetylated secondary hydroxyl group at C-28. The negative ion fabms of **13** exhibited a molecular ion peak at *m/z* 615 $[M-H]^-$ and fragment ions at *m/z* 557, 497, 437, and 401, further supporting the deduction that **13** had four hydroxyl groups, three of which were acetylated. In its ^{13}C-nmr spectrum, there were no signals which could be assigned to an olean-12-ene structure, but the signal at 89.4 ppm (s) (Table 2), assigned to C-13, supported the presence of an epoxide between C-13 and C-28. The lack of C-28 methylene protons observed for **11** and **12** suggested a hemiacetal C atom at C-28. To verify the positions of acetylation, a series of 2D nmr experiments were performed. After assigning key proton and carbon (Table 2) resonances by homonuclear (COSY) (9) and heteronuclear (HMQC) (10) correlations, the long-range H,C-correlation (11) made clear that the proton at δ 5.77 (s) was bound to the carbon at δ 96.1 (d), and interactions

were shown between H-28 and C-16; H-22 and C-21, C-17, C-16, and C-28; and H-16 and C-14, C-18, and C-17. These results indicated that the 13β,28-epoxy linkage is hydroxylated at C-28; that is, **13** has a hemiacetal function instead of an epoxide moiety. A ROESY experiment (12) revealed close contacts between H-28, H-16, and H-15β consistent with an *R* configuration at C-28. Based on these results, **13** was determined to be anagalligenin A 3, 22,28-*O*-triacetate. Anagelligenin A was previously reported as the sapogenol moiety of a saponin from *Anagallis arvensis* (8).

Finally, **7** (the methyl ester derivative of **3**) was acetylated to yield **16**. The ^{1}H-nmr spectrum of **16** showed thirteen acetoxy methyl resonances, of which eleven were attributed to the sugar moiety. The signals for H-2′ (δ 4.01–4.03) and H-3′ (δ 3.99) of glucuronic acid as well as that of H-2‴ (δ 3.73–3.75) of galactose, whose assignments were based on the results of a TOCSY experiment (13), showed no down-field shifts upon acetylation, confirming the sites of glycosidations. The fabms of **16** also supported the proposed structure (*m/z* 1697 $[M+H]^+$, 1719.7 $[M+Na]^+$, calcd for $C_{81}H_{116}O_{38}$). Characteristic fragments resulting from the cleavage of the interglycosidic linkages were at *m/z* 1123 for the undecaacetyltetraglycosidic chain, *m/z* 561 [hexaacetylrhamnosyl-galactose]$^+$ for the diglycosidic chain, and *m/z* 273 [triacetylrhamnose]$^+$ and *m/z* 331 [tetraacetylglucose]$^+$ for the terminal sugars. Based on these results, it was evident that **3** is a new saponin 3-*O*-{[α-L-rhamnopyranosyl-(1↦2)-β-D-galactopyranosyl-(1↦3)]-[β-D-glucopyranosyl-(1↦2)]-[β-D-glucuronopyranosyl]}-anagalligenin A, for which we propose the trivial name primacrosaponin.

Although saponin **4** was identified as primulasaponin (1–3) by tlc comparisons with authentic samples, the structure was verified by the same methods used for compounds **1–3**. Thus, upon treatment by Ac_2O and pyridine, saponin **4** yielded **9**, **10**, **14**, and **15**. The spectra of **14** and **15** (Table 2) were the same as those reported for protoprimulagenin A 3,16-*O*-diacetate and protoprimulagenin A 3-*O*-acetate (1), respectively. ^{1}H- and ^{13}C-nmr spectral data of compound **8**, the methyl ester derivative of **4**, are also reported.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra (ν max cm^{-1}) were obtained on a Perkin-Elmer 257 spectrophotometer in KBr pellets. Optical rotations were measured with a Perkin-Elmer 141 polarimeter in MeOH. ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker AMX 300 and a Bruker AM 600 instrument (solvents and field strengths are given in the text). The fabms were recorded with a Kratos AEI-MS 50 spectrometer using NOBA as matrix. Isolations were carried out using medium-pressure liquid chromatography (mplc) with a LEWA pump, Rheodyne Injector, and Büchi glass column (18.5 mm×322 mm) (stationary phase: Sepralyte C18 40 μm, fraction collector: LKB 1700 minirac) as well as open cc [stationary phase: Si gel 60 (70–230 mesh, Merck)]. Si gel 60 F_{254} plates (Merck) and Si gel G plates, 0.5 mm thick were used for tlc and preparative tlc. Saponins were detected by spraying with 30% H_2SO_4 in H_2O and 1% vanillin in H_2SO_4 followed by heating at 110°, 5–10 min. Detection of acetylsapogenols was performed by spraying with distilled H_2O.

PLANT MATERIAL.—*P. veris* subsp. *macrocalyx* was collected from Kars (eastern Anatolia, Turkey), in June 1988. *P. elatior* subsp. *meyeri* was collected from Rize (northeastern Anatolia, Turkey) in July 1987. Voucher specimens have been deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University.

ISOLATION PROCEDURE.—The air-dried roots and rhizomes of the plants were powdered and extracted twice with MeOH-H_2O (9:1) in a Soxhlet. The MeOH extracts were combined and concentrated to dryness in vacuo. Residues were suspended in H_2O and partitioned with *n*-BuOH saturated with H_2O. The *n*-BuOH-soluble fractions were evaporated, and the residues, which were dissolved in the minimum amount of MeOH, were poured into Et_2O to precipitate the saponins. Crude saponin mixtures were separated chromatographically using either method 1 or 2 to yield pure saponins.

METHOD 1.—Saponin mixtures of both plants were separately subjected to mplc with 50–30% H_2O in MeOH. This procedure yielded pure **3** from *P. veris* and **4** from *P. elatior*. Compounds **1** and **2** from *P.*

veris were obtained as a mixture, which was further subjected to mplc using Si gel as stationary phase and $CHCl_3$-MeOH-H_2O (61:32:7) as eluent to yield **1**. Saponin **2** was isolated as its methyl ester **6**.

Priverosaponin B 22-acetate [**1**].—White, amorphous powder: ir ν max 3460, 1740, 1710 cm^{-1}.

Primacrosaponin [**3**].—White, amorphous powder: $[\alpha]^{20}D$ −28.4° (*c*=0.44, MeOH); ir ν max 3450, 1610 cm^{-1}; fabms *m/z* $[M+H]^+$ 1137.6, $[M+Na]^+$ 1159.6 (calcd for $C_{54}H_{88}O_{25}$, 1137.3).

Primulasaponin [**4**].—Identical data to those previously reported (1,2). White, amorphous powder: negative ion fabms *m/z*.$[M-H]^-$ 1103 (calcd for $C_{54}H_{88}O_{23}$, 1105.3).

METHOD 2.—Saponins were isolated as their methyl esters. Crude saponin mixtures of each plant (5 g) were dissolved in MeOH and treated with ethereal CH_2N_2. After methylation, solvents were evaporated and residues were separately chromatographed on a Si gel column eluted with $CHCl_3$-MeOH-H_2O (80:20:2, 70:30:3) to provide saponin esters **5**, **6**, and **7**, and eluted with $CHCl_3$-MeOH-H_2O (125:25:3) to yield saponin ester **8**.

Methyl ester **5** *of priverosaponin B 22-acetate.*—White, amorphous powder: ir ν max 3350, 1735–1710 cm^{-1}; fabms *m/z* $[M+Na]^+$ 1199 (calcd for $C_{57}H_{92}O_{25}$, 1177.4); ^1H nmr (300 MHz, CD_3OD) δ 0.86, 0.89, 0.95, 0.98, 1.06, 1.14, 1.27 (each 3H, s, 7×tert. Me), 1.28 (3H, d, *J*=6.2 Hz, sec. Me of rhamnose), 2.02 (3H, s, Ac), 3.78 (3H, s, COOMe), 4.52 (d, *J*=7.5 Hz, anomeric proton of glucuronic acid), 4.86 (d, *J*=7.4 Hz, anomeric proton of glucose), 5.16 (d, *J*=7.1 Hz, anomeric proton of galactose), 5.17 (dd, *J*=11.1 and 7.1 Hz, H-22), 5.28 (d, *J*=1.4 Hz, anomeric proton of rhamnose); ^{13}C nmr (75.5 MHz, CD_3OD) see Table 1.

Methyl ester **6** *of priverosaponin B.*—White, amorphous powder: ir ν max 3350, 1730 cm^{-1}; negative fabms *m/z* $[M+Na-H]^-$ 1157, $[M+K-H]^-$ 1173 (calcd for $C_{55}H_{90}O_{24}$, 1135.3); ^1H nmr (300 MHz, CD_3OD) δ 0.86, 0.90, 0.95, 0.96, 1.06, 1.15, 1.24 (each 3H, s, 7×tert. Me), 1.28 (3H, d, *J*=6.2 Hz, sec. Me of rhamnose), 3.78 (3H, s, COOMe), 4.52 (d, *J*=7.4 Hz, anomeric proton of glucuronic acid), 4.86 (d, *J*=7.5 Hz, anomeric proton of glucose), 5.18 (d, *J*=7.1 Hz, anomeric proton of galactose), 5.27 (d, *J*=1.3 Hz, anomeric proton of rhamnose); ^{13}C nmr see Table 1.

Methyl ester **7** *of primacrosaponin.*—White, amorphous powder: ir ν max 3460, 1740 cm^{-1}; fabms *m/z* $[M+Na+H]^+$ 1175 (calcd for $C_{55}H_{90}O_{25}$, 1151.3); ^1H nmr (300 MHz, CD_3OD) δ 0.86, 0.89, 0.95, 0.97, 1.06, 1.18, 1.24 (each 3H, s, 7×tert. Me), 1.27 (3H, d, *J*=6.2 Hz, sec. Me of rhamnose), 3.78 (3H, s, COOMe), 4.52 (d, *J*=7.6 Hz, anomeric proton of glucuronic acid), 4.92 (d, *J*=7.6 Hz, anomeric proton of glucose), 5.16 (d, *J*=7.3 Hz, anomeric proton of galactose), 5.28 (d, *J*=1.4 Hz, anomeric proton of rhamnose); ^{13}C nmr see Table 1.

Methyl ester **8** *of primulasaponin.*—White, amorphous powder: ir ν max 3450, 1730 cm^{-1}; ^1H nmr (300 MHz, CD_3OD) δ 0.91, 0.94, 0.95, 0.99, 1.10, 1.19, 1.27 (each 3H, s, 7×tert. Me), 1.32 (3H, d, *J*=6.2 Hz, sec. Me of rhamnose), 3.82 (3H, s, COOMe), 4.52 (d, *J*=7.6 Hz, anomeric proton of glucuronic acid), 4.92 (d, *J*=7.6 Hz, anomeric proton of glucose), 5.16 (d, *J*=7.3 Hz, anomeric proton of galactose), 5.28 (d, *J*=1.4 Hz, anomeric proton of rhamnose); ^{13}C nmr see Table 1.

ACID HYDROLYSIS.—Each saponin (10 mg) was refluxed with 5% HCl (5 ml) for 5 h. The reaction mixture was then concentrated under reduced pressure to remove MeOH, diluted with H_2O, and filtered. The filtrate was adjusted to pH 7 with Ag_2CO_3 and filtered. The supernatant was concentrated and compared with reference sugars on pc [descending method, *n*-BuOH–HOAc–H_2O (4:1:5)]. The sugars were detected by spraying a solution of aniline phthalate in *n*-BuOH saturated with H_2O.

SELECTIVE CLEAVAGE OF THE GLUCURONIDE LINKAGE IN SAPONINS BY Ac_2O AND PYRIDINE TREATMENT.—Solutions of each saponin **1**–**4** in Ac_2O and pyridine were separately heated in sealed tubes for 2 h at 100°, after which the reaction mixtures were poured into ice-H_2O. Precipitates were collected by filtration and subjected to cc over Si gel with hexane-Me_2CO (75:10) to give a mixture of acetyl sapogenols **11**–**15** and pure **9** and **10**. The fractions containing acetyl sapogenols **11**–**15** were purified by preparative tlc using cyclohexane-Me_2CO (5:1) as solvent system. From this chromatography, **11** and **12** were obtained from **1** and **2**, while **13** was derived from **3** and **14** and **15** from **4**.

3,22 Di-O-acetylpriverogenin B [**11**].—White, amorphous powder: ir ν max (KBr) 3500, 3400, 2940, 2860, 1725, 1240 cm^{-1}; negative fabms (NOBA) *m/z* $[M-H]^-$ 557 ($C_{34}H_{54}O_6$), $[M-H-H_2O]^-$ 539, $[M-HOAc]^-$ 499, $[M-HOAc-H_2O]^-$ 481, $[M-2\times HOAc]^-$ 437; ^1H nmr data of **11** identical with those previously reported (6,7); ^{13}C nmr see Table 2.

3,16,22-Tri-O-acetylpriverogenin B [**12**].—White, amorphous powder: ir ν max (KBr) 2940, 2865, 1735, 1240 cm^{-1}; negative fabms (NOBA) *m/z* $[M-H]^-$ 599 ($C_{36}H_{56}O_7$), $[M-HOAc]^-$ 541, $[M-2\times HOAc]^-$ 481, $[M-3\times HOAc]^-$ 421, ^1H nmr (300 MHz, $CDCl_3$) δ 0.84, 0.85, 0.89, 1.01, 1.05,

1.10, 1.13 (each 3H, all s, 7×tert. Me), 2.00, 2.05, 2.08 (each 3H, all s, 3×aliph. acetoxy), 3.49 and 3.62 (each 1H, AB system, J_{AB}=8.5 Hz, H_2-28), 4.48 (1H, H-3α), 4.90 (1H, dd, J=12 and 6 Hz, H-22β), 5.25 (1H, d, J=5.4 Hz, H-16β).

3,22,28-Tri-O-acetylanagalligenin A [**13**].—White, amorphous powder: ir ν max (KBr) 3500–3400, 2840, 1730, 1240 cm^{-1}; negative fabms (NOBA) *m/z* $[M-H]^-$615 ($C_{36}H_{56}O_8$), $[M-HOAc]^-$ 557, $[M-2\times HOAc]^-$ 497, $[M-(2\times HOAc)-H_2O]^-$ 479, $[M-3\times HOAc]^-$ 437; ^1H nmr (600 MHz, $CDCl_3$) δ 0.98 and 1.7 (each 1H, m, H-1), 1.6 (2H, m, H-2), 4,44 (1H, m, H-3), 0.75 (1H, dd, J=11.6 and 2.2 Hz, H-5), 1.43 (2H, m, H-6), 1.48 (1H, m, H-7), 1.17 (1H, m, H-9) 1.42 and 1.61 (each 1H, m, H-11), 1.97 and 1.4 (each 1H, m, H-12) 2.05 and 1.24 (each 1H, m, H-15), 4.38 (1H, br d, J=5.4 Hz, H-16), 1.7 (1H, dd, J=14.5 and 2.5 Hz, H-18), 2.3 and 1.2 (each 1H, dd and m, respectively, J=12.2 and 14.5 Hz, H-19), 1.54 and 1.95 (each 1H, m and ddd respectively, J=12.6 and 1.5 Hz, H-21), 5.16 (1H, dd, J=12 and 6 Hz, H-22), 0.85 (3H, s, H-23), 0.82 (3H, s, H-24), 0.81 (3H, s, H-25), 1.1 (3H, s, H-26), 1.21 (3H, s, H-27), 0.97 (3H, s, H-29), 0.98 (3H, s, H-30), 5.77 (1H, s, H-28), 2,03, 2.01, 1.96 (each 3H, all s, 3×aliph. acetoxyl); ^{13}C nmr see Table 2.

3,16-Di-O-acetylprotoprimulagenin A [**14**] *and 3-O-acetylprotoprimulagenin A* [**15**].—White, amorphous powders. The spectral data (ir, ^1H nmr, and ms) for **14** and **15** were the same as those previously reported (1). ^{13}C nmr see Table 2.

Heptaacetyl-2-O-(α-L-rhamnopyranosyl)-β-D-galactopyranoside [**9**] *and 1,2,3,4,6-penta-O-acetylglucopyranose* [**10**].—The structures of **9** and **10** were identified from chromatographic comparisons made with authentic samples obtained in the same way from primulasaponin (1,2).

Tridecaacetate **16** *of ester primacrosaponin.*—Compound **7** was treated with Ac_2O in pyridine at room temperature for 18 h and purified on a Si gel column eluted with C_6H_6-EtOH (9:1). The tridecaacetate of **7** was characterized by fabms and ^1H nmr. Fabms *m/z* $[M+Na]^+$ 1719.7, $[M+H]^+$ 1697 ($C_{81}O_{116}O_{38}$), 1123, 561, 331, 273; ^1H nmr (600 MHz, $CDCl_3$) δ (glucuronic acid) 4.4 (1H, d, J=7.3 Hz, H-1′), 4.1–4.03 (1H, H-2′), 3.99 (1H, d, J=9.4 Hz, H-3′), 5.19 (1H, t, J=8.2 Hz, H-4′), 3.92 (1H, d, J=9.2 Hz, H-5′), 3.72 (3H, s, COOMe), (glucose) 5.03 (1H, d, J=8.6 Hz, H-1″), 5.13–5.11 (1H, H-2″), 5.22 (1H, t, J=9.4 Hz, H-3″), 4.95–4.92 (1H, H-4″), 3.75–3.73 (1H, H-5″), 4.1–4.03 (1H, H-6″), 4.34 (1H, dd, J=12.4 and 3.3 Hz, H_b-6″), (galactose) 4.61 (1H, d, J=7.5 Hz, H-1‴), 3.75–3.73 (1H, H-2‴), 4.95–4.92 (2H, H-3‴ and H-4‴), 3.88 (1H, t, J=7 Hz, H-5‴), 4.1–4.03 (1H, Ha-6‴), 4.13 (1H, dd, J=11.2 and 7 Hz, H_b-6‴); rhamnose; 5.1–5.06 (2H, H-1⁗and H-2⁗), 5.13–5.11 (1H, H-3⁗), 5.36 (1H, d, J=3.1 Hz, H-4⁗), 4.1–4.03 (1H, H-5⁗), 1.22 (3H, d, J=6.2 Hz, H-6⁗), (aglycone moiety) 0.82, 0.84, 0.85, 0.98, 0.99, 1.10, 1.20 (each 3H, all s, 7×tert. Me), 4.5–4.4 (2H, H-3 and H-16), 5.16 (1H, dd, J=12 and 6 Hz, H-22), 5.8 (1H, s, H-28); 1.95, 1.96, 1.97, 1.98, 2.01, 2.039, 2.04, 2.05, 2.06, 2.07, 2.1, 2.12, 2.14 (each 3H, all s, 13×aliph. acetoxyl).

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