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MACROPHYLLICININ, A SAPONIN FROM *PRIMULA MACROPHYLLA*

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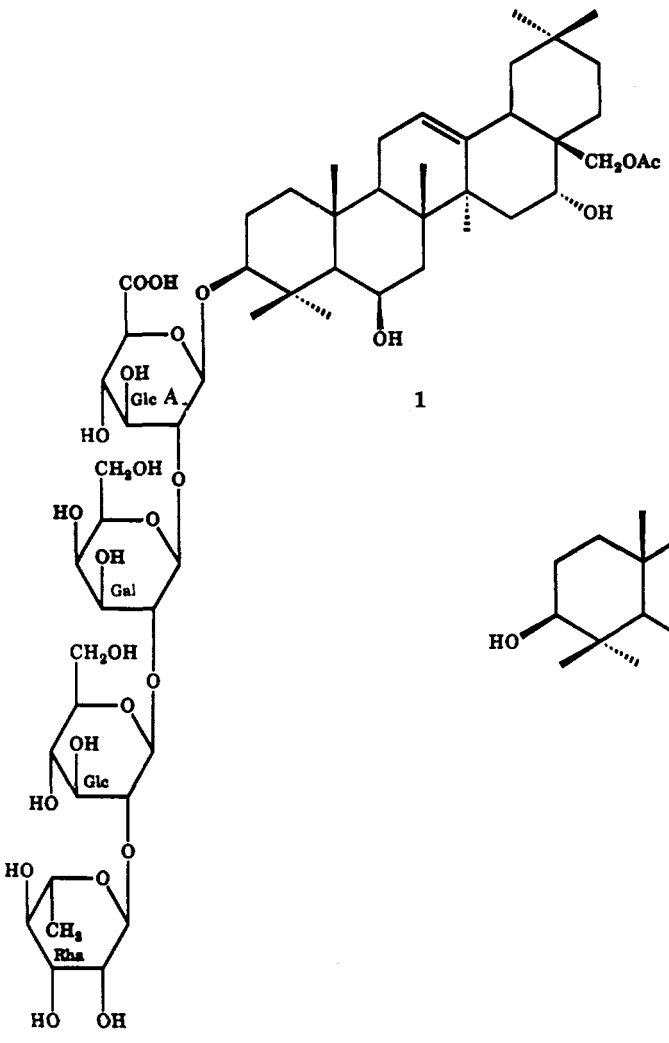
ABSTRACT.—A new triterpenoid saponin, designated macrophyllicinin [**1**], has been isolated from the MeOH extract of the whole plant of *Primula macrophylla*. Its structure was established by chemical and spectroscopic means as 3β-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl]-6β,16α-dihydroxyolean-12-en-28-acetate. The aglycone **2** of this saponin is also a new compound.

Primula macrophylla D. Don. (Primulaceae), syn. *Primula stuartii* Wall., is a perennial herb found in the KaraKorum and Kuram valley in Pakistan at an altitude of 3600–4900 meters (1). The farina on the leaves is locally used in Pakistan as well as in Afghanistan for the treatment of eye diseases. Our systematic phytochemical investigation of the whole plant of *P. macrophylla* has resulted in the isolation of 3,3′-dihydroxychalcone, 3′-methoxyflavone, sitosterol, flavone glucoside, and a new saponin, macrophyllicin (2–4). In continuation of our work on the chemical constituents of *P. macrophylla* we report here the isolation and structure elucidation of a new triterpenoid saponin designated macrophyllicinin [**1**].

RESULTS AND DISCUSSION

The *n*-BuOH-soluble fraction of the MeOH extract from the whole plant was subjected to cc over Si gel. The fraction eluted with $CHCl_3$-MeOH (4:1) yielded macrophyllicinin [**1**] and was purified on a flash chromatography column which was eluted with $CHCl_3$-MeOH (9:1). Macrophyllicinin [**1**], mp 314–315°, $[\alpha]^{27}D$ −9° (*c*=0.4, MeOH), was obtained as colorless needles from MeOH. Its ir spectrum exhibited characteristic absorption bands at 3350 cm^{-1} (OH), 1720 cm^{-1} (C=O), 1620 cm^{-1} (C=C), and 1100–1000 cm^{-1} (C-O-C). Acid hydrolysis (HCl, MeOH) of **1** afforded an aglycone **2** along with four sugar moieties which were identified as L-rhamnose, D-galactose, D-glucose, and D-glucuronic acid by co-tlc with authentic samples. The negative ion fabms of compound **1** showed a pseudomolecular ion peak at *m/z* 1161 $[M-H]^-$ and fragment ions at *m/z* 1119 $[M-H-42]^-$, 1015 $[M-H-146]^-$, 853 $[M-H-(146+162)]^-$, 691 $[M-H-(146+2\times162)]^-$, and 515 $[M-H-(146+2\times162+176)]^-$. These fragment ions suggested the sequential loss of four hexose units. It further indicated that the terminal deoxyhexose is rhamnose and the innermost monosaccharide unit is glucuronic acid. The negative ion fabms together with ^1H- and ^{13}C-nmr data (Table 1) allowed us to propose the formula $C_{56}H_{90}O_{25}$ for compound **1**.

The ^1H-*nmr spectrum* (400.13 MHz, DMSO-d_6) showed the existence of seven tertiary methyl groups characterized by the singlets at δ 0.79, 0.89, 0.90, 0.91, 0.92, 0.98, and 1.29. A doublet at δ 1.08 (J=6.0 Hz) was due to the methyl group of rhamnose. A sharp singlet at δ 1.94 was assigned to the methyl group of OAc. A triplet at δ 2.99 (J=8.10 Hz) was assigned to H-3 while a doublet at δ 0.67 (J=11.0 Hz) was assigned to H-5. Two carbinylic proton signals at δ 3.90 (ddd, J=6.8, 9.7, 12.7 Hz) and δ 3.08 (dd, J=10.5, 9.10 Hz) were assigned to H-6 and H-16, respectively. The downfield value due to H-28, at δ 3.45, indicated attachment of OAc at this position. The hydroxyl group at C-6 was assigned β configuration as there were no downfield chemical shifts of H-23, H-24, H-25, or



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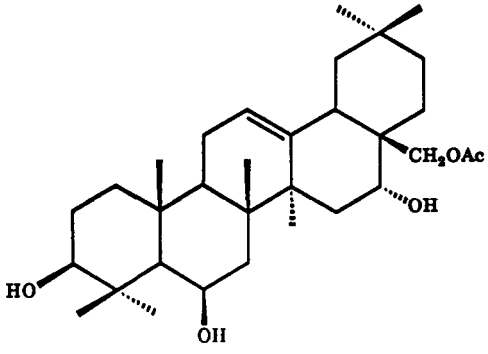

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H-26, which is characteristic of a 6α-OH group (5). On the other hand, the hydroxyl function at C-16 was assigned the α configuration because of a downfield shift (ca. +0.16) of the Me-27 protons at δ 1.29 (5,6). The distorted triplet at δ 5.24, characteristic of a vinylic proton, was ascribed to H-12.

Four anomeric protons were also observed in the ^{1}H-nmr spectrum. The three doublets at δ 4.20 (J=7.6 Hz), 4.80 (J=7.2 Hz), and 5.20 (J=6.0 Hz) were assigned to the anomeric protons of the D-glucuronic acid, D-galactose, and D-glucose, respectively. Their coupling constant values confirmed the β-glycosidic linkages. A broad singlet at δ 5.06 was attributed to the anomeric proton of the L-rhamnose indicating the α linkage.

The ^{13}C-nmr (75.43 MHz, DMSO-d_6) spectra showed 56 carbon resonances. The DEPT technique suggested 27 methine, 11 methylene, 9 methyl groups, and, by difference from the broad band (BB) spectrum, 9 quaternary carbon atoms (Table 1). Seven methyls were assigned to the aglycone; a methyl resonating at δ 21.8 was assigned to the OAc group while another methyl group at δ 17.8 was attributed to the C-6 methyl of rhamnose.

The downfield signal at δ 88.8 was assigned to C-3, which indicated the sugar linkage at this position, and a signal at δ 55.1 was assigned to C-5 (7,8).

TABLE 1. ^{1}H- and ^{13}C-nmr Assignments of Macrophyllicinin [**1**].

Position	δ ^{13}C	DEPT	δ ^{1}H	$^{1}H/^{1}H$ connectivity (COSY-45°)
1	38.4	CH_2	1.50 (m)	
2	27.5	CH_2	1.23 (m))	
3	88.8	CH	2.99 (t, J=8.1 Hz)	
4	42.8	—		
5	55.1	CH	0.67 (d, J=11.0 Hz)	H-6 (3.90)
6	68.0	CH	3.90 (ddd, J=6.8, 9.7, 12.7 Hz)	H-5 (0.67), H-7 (1.30)
7	44.0	CH_2	1.30 (unresolved)	H-6 (3.90)
8	40.8	—		
9	46.1	CH	1.50 (dd, J=8.0, 13.0 Hz)	
10	36.1	—		
11	23.0	CH_2	1.80 (unresolved)	H-12 (5.24)
12	123.0	CH	5.24 (distorted t)	H-11 (1.80)
13	142.0	—		
14	43.0	—		
15	32.3	CH_2	1.33 (unresolved)	H-16 (3.08)
16	67.0	CH	3.08 (dd, J=10.5, 9.1 Hz)	H-15 (1.33)
17	40.8	—		
18	40.4	CH	2.10 (br t, J=12.0, 13.0 Hz)	
19	46.1	CH_2	1.05 (overlapped)	
20	30.9	—		
21	33.4	CH_2	1.47 (m)	
22	30.0	CH_2	1.64 (unresolved)	
23	27.5	Me	0.98 (s)	
24	16.1	Me	0.79 (s)	
25	15.2	Me	0.91 (s)	
26	16.3	Me	0.90 (s)	
27	26.5	Me	1.29 (s)	
28	66.0	CH_2	3.45 (overlapped)	
29	33.3	Me	0.89 (s)	
30	24.9	Me	0.92 (s)	
$OCOCH_3$	171.9	—		
$OCOCH_3$	21.8	Me	1.94 (s)	
Glc A				
1	103.7	CH	4.20 (d, J=7.6 Hz)	H-2 (3.77)
2	79.7	CH	3.77 (overlapped)	H-1 (4.20)
3	73.6	CH		
4	70.9	CH		
5	74.2	CH		
6	174.6	—		
Gal				
1	100.1	CH	4.80 (d, J=7.2 Hz)	H-2 (3.16)
2	76.5	CH	3.16 (overlapped)	H-1 (4.80)
3	71.2	CH		
4	69.2	CH		
5	74.8	CH		
6	62.1	CH_2		
Glc				
1	99.0	CH	5.20 (d, J=6.0 Hz)	H-2 (3.60)
2	78.4	CH	3.60 (overlapped)	H-1 (5.20)
3	74.5	CH		
4	70.3	CH		
5	75.9	CH		
6	60.1	CH_2		
Rha				
1	100.7	CH	5.06 (br s)	H-2 (3.78)
2	70.7	CH	3.78 (overlapped)	H-1 (5.06)
3	71.7	CH		
4	71.9	CH		
5	69.1	CH	3.92 (m)	
6	17.8	Me	1.08 (d, J=6.0 Hz)	

The signals resonating at δ 68.0 and 67.0 were ascribed to C-6 and C-16. The downfield chemical shifts (ca. +48.5 and +45.2) of these carbons confirmed the hydroxyls at C-6 and C-16 (7). The signal at δ 66.0 was assigned to C-28. Unsaturation between C-12 and C-13 was revealed by the presence of signals at δ 123.0 and 142.0 (9). These ^{13}C-nmr assignments were further confirmed by an HMQC eqperiment. The signal at δ 55.1 assigned to C-5 showed correlation with H-5 at δ 0.67, while the signal at δ 68.0 assigned to C-6 showed connectivity with H-6 at δ 3.90 and this in turn showed connectivity in a COSY-45° experiment with δ 0.67 and 1.30 assigned to H-5 and H-7, respectively. Similarly the value at δ 67.0 assigned to C-16 exhibited correlation with δ 3.08 due to H-16. In the COSY-45° experiment the δ 3.08 signal showed connectivity with H-15 at δ 1.33. The signal at δ 123.0 showed connectivity with the proton H-12 at δ 5.24, which in turn showed connectivity with δ 1.80 due to H-11. The protons resonating at δ 3.45 were coupled with C-28 at δ 66.00. Thus the structure of the new aglycone macrophyllogenin acetate [**1**] was elucidated to be 3β,6β,16α-trihydroxy-olean-12-en-28-acetate.

The ^{13}C-nmr spectra of macrophyllicinin confirmed the presence of four sugar moieties by exhibiting four anomeric signals at δ 103.7, 100.1, 99.0, and 100.7 assigned to glucuronic acid, galactose, glucose, and rhamnose, respectively. In the HMQC experiment these signals showed coupling with their respective anomeric protons at δ 4.20, 4.80, 5.20, and 5.06. Similarly the signals at δ 79.7 (glc A C-2), 76.5 (gal C-2), 78.4 (glc C-2), and 70.7 (rha C-2) showed connectivity with their respective protons at δ 3.77, 3.16, 3.60, and 3.78. In the COSY-45° experiment, the H-2 of the four sugar moieties showed correlation with their respective anomeric protons also, thus establishing their assignments. The glycosidic linkages of the tetrasaccharide were similar to those of macrophyllicin and were established as follows (4). The fabms spectrum indicated that all hexose units were unbranched, rhamnose was the terminal and glucuronic acid was the innermost. In the ^{13}C-nmr spectrum of compound **1** the terminal rhamnose unit and innermost glucuronic acid were clearly observed (Table 1). All the carbon signals due to the sugar moieties were in good agreement with published data for similarly linked sugar moieties (4, 10–14). The interglycosidic linkages were determined by the ^{13}C-nmr glycosidation shifts. The C-2 of Glc A, Gal, and Glc showed peaks at δ 79.7, 76.5, and 78.4, respectively, corresponding to the glycosidation shifts of ca. +5.9, +4.7, +4.2 as compared with the methyl-glucuronoside, methylgalactoside, and methylglucoside, respectively (13). The upfield shifts of C-1 (−0.6, −4.8, −5.3) and C-3 (−2.9, −2.4, −2.7) of the glucuronic acid, galactose, and glucose also supported the (1↦2) linkage of these sugar moieties (13,15). The (1↦2) linkage of galactose with glucuronic acid was supported by comparing the ^{13}C-nmr data given in the literature for similarly linked sugar moieties (4,6,9,10). Thus the structure of macrophyllicinin [**1**] is 3β-*O*-[α-L-rhamnopyranosyl-(1↦2)-β-D-glucopyranosyl-(1↦2)-β-D-galactopyranosyl-(1↦2)-β-D-glucuronopyranosyl]6β,16α-dihydroxyolean-12-en-28-acetate. Macrophyllicin has a hydroxyl group at C-28 instead of an acetate group (4). The eims fragmentation also supported the above structure. The molecular ion peak was observed at *m/z* 516. The *m/z* 480 appeared due to the loss of water molecule $[M-H_2O]^+$. Other peaks at *m/z* 462 and 420 were attributed to the successive loss of water molecules from the $[M-H_2O]^+$. Fragment ions at *m/z* 201 and 187 are diagnostic for retro-Diels-Alder fragmentation (16).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were obtained on H_2SO_4 bath (capillary) and are uncorrected. A Flash Column Chromatograph model EF-10 using Si gel 60, 230–400 mesh size (E. Merck) was used for purification. Precoated Kieselgel 60, F_{254} cards (thickness 0.25 mm, Riedel de Haën, Art. No. 37360) were used for tlc. Purity was checked on hptlc cards (E. Merck, Art. No. 4628); chromatograms were sprayed with 0.1% $Ce(SO_4)_2$ in 2 N H_2SO_4 and heated at 80° for 5 min. Fabms spectra were obtained in the negative ion mode on a JEOL JMS-110 mass spectrometer. Eims were determined on a Finnigan-312 varian MAT-112 double focusing mass spectrometer connected to PDP 11/34 (DEC.) computer system. ^{1}H-nmr spectra were scanned on a Bruker AM-400, 400.13 MHz (DMSO-d_6). ^{13}C-nmr spectra were measured at 75.43 MHz (DMSO-d_6). Optical rotation was measured at 27° on a JASCO model DLP-360 digital polarimeter.

PLANT MATERIAL.—*P. macrophylla* was collected in August from "Sora Lasht" on the Pakistan Afghanistan border in Chitral (N.W.F.P.) Pakistan. The plant was identified by Mr. Saeed and Dr. A.R. Beg of the Pakistan Forest Institute, Peshawar, N.W.F.P., Pakistan and was confirmed by Dr. Yasin J. Nasir of the National Herbarium (Stewart Collections), Pakistan Agricultural Research Council, Islamabad. A voucher specimen no. 1085 (BM, E, RAW) is available for inspection in this herbarium.

EXTRACTION AND ISOLATION OF MACROPHYLLICININ [**1**].—The dried and powdered whole plant (15 kg) of *P. macrophylla* was extracted with MeOH. The resulting extract was concentrated under reduced pressure to 60° and fractionated with EtOAc-H_2O (1:1). The aqueous layer was partitioned with *n*-BuOH–H_2O (1:1). The *n*-BuOH layer was evaporated under reduced pressure at 70°. The residue obtained was dissolved in a minimum quantity of MeOH, and Et_2O was added dropwise. The resulting precipitate contained crude saponins which was dissolved in MeOH and chromatographed on a column with Si gel (70–230 mesh). Elution with $CHCl_3$-MeOH (4:1) afforded impure macrophyllicinin [**1**]. Pure saponin (30 mg) was obtained by repeated cc on a flash column by eluting with $CHCl_3$-MeOH (9:1) and crystallized from MeOH. The purity of the compound was checked on hptlc plates using *n*-BuOH–HOAc–H_2O (12:3:5).

Macrophyllicinin [**1**].—Colorless hygroscopic crystals; mp 314–315°; $[\alpha]^{27}D$ −9° (*c*=0.4, MeOH); ir ν max (KBr) cm^{-1} 3350, 1720, 1620, 1100–1000; negative ion fabms *m/z* 1161, 1119, 1015, 853, 691, 515; ^{1}H nmr (DMSO-d_6) and ^{13}C nmr see Table 1.

ACID HYDROLYSIS OF **1**.—Pure saponin **1** (10 mg) was refluxed with 10 ml HCl (18%) in aqueous MeOH (5 ml) for 5 h. MeOH was evaporated under reduced pressure. The mixture was then diluted with H_2O (1–2 ml) and extracted 3× with EtOAc. The aqueous layer was neutralized with Ag_2CO_3, filtered, and concentrated under reduced pressure. The residue was compared with standard sugars on Si gel plates (E. Merck, Art. No. 5554) by using solvent system *n*-BuOH–iPrOH–EtOAc–HOAc–H_2O (35:60:100:35:30). The spots were detected with aniline phthalate sugar reagent, which indicated that the sugars were D-glucuronic acid, D-galactose, D-glucose, and L-rhamnose.

Macrophylogenin acetate [**2**].—The EtOAc layer was concentrated and the aglycone, macrophyllogenin acetate, was obtained as a colorless semisolid substance which was compared with the original saponin macrophyllocinin by hptlc using $CHCl_3$-EtOAc (1:1). Spots were detected using the $Ce(SO_4)_2$ reagent and heating: mp 61–62° on a Gallenkamp microscopic melting point apparatus (uncorrected); ir ν max (KBr) cm^{-1} 3450, 2900, 1730, 1240; eims *m/z* (%) $[M]^+$ 516 (5), 480 (21), 462 (100), 420 (70), 388 (35), 280 (90), 224 (45), 201 (100), 187 (100), 173 (63), 149 (100); ^{1}H nmr (300.13 MHz, $CDCl_3$) δ 0.79 (3H, s, H-24), 0.88 (3H, s, H-29), 0.90 (3H, s, H-26), 0.93 (3H, s, H-25), 0.96 (3H, s, H-30), 1.00 (3H, s, H-23), 1.34 (3H, s, H-27), 2.10 (3H, s, $OCOCH_3$), 3.25 (1H, m, H-16), 3.77 (1H, m, H-6), 5.27 (1H, distorted t, H-12).

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LITERATURE CITED

1. S.I. Ali and E. Nasir, "Flora of West Pakistan," Fakhri Printing Press, Karachi, Pakistan, 1972, p. 539.
2. V.U. Ahmad, M.G. Shah, F.V. Mohammad, N. Ismail, and M. Noorwala, *Phytochemistry*, **30**, 4206 (1991).
3. V.U. Ahmad, M.G. Shah, F.V. Mohammad, and M. Noorwala, *J. Nat. Prod.*, **55**, 956 (1992).
4. V.U. Ahmad, M.G. Shah, F.V. Mohammad, and F.T. Baqai, *Phytochemistry*, **32**, 1543 (1993).
5. S. Ito, M. Kodama, M. Sunagawa, T. Oba, and H. Hikino, *Tetrahedron Lett.*, **34**, 2905 (1969).

6. I. Kitagawa, M. Yoshikawa, and I. Yosioka, *Tetrahedron Lett.*, **5**, 469 (1974).
7. V.U. Ahmad, V. Sultana, S. Arif, and Q.N. Saqib, *Phytochemistry*, **27**, 304 (1988).
8. V.I Grishkovets, A.A. Loloiko, A.S. Shashkov, and V.Ya. Chirva, *Khim. Prir. Soedin.*, 230 (1990).
9. I. Kitagawa, T. Taniyama, W.W. Hong, and M. Yoshikawa, *Yakugaku Zasshi*, **108**, 538 (1988).
10. T. Taniyama, Y. Nagahama, M. Yoshikawa, and I. Kitagawa, *Chem. Pharm. Bull.*, **36**, 2829 (1988).
11. I. Kitagawa, T. Taniyama, Y. Nagahama, K. Okubo, F. Yamauchi, and M. Yoshikawa, *Chem. Pharm. Bull.*, **36**, 2819 (1988).
12. S. Yahara, S. Emura, H. Feng, and T. Nohara, *Chem. Pharm. Bull.*, **37**, 2136 (1989).
13. P.A.J. Gorin and M. Mazurek, *Can. J. Chem.*, **53**, 1212 (1975).
14. S.B. Mahato and B.C. Pal, *J. Chem. Soc., Perkin Trans. 1*, 629 (1987).
15. C.J. Shao, R. Kasai, J.Da. Xu, and O. Tanaka, *Chem. Pharm. Bull.*, **37**, 311 (1989).
16. H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Structure Elucidation of Natural Products of Mass Spectrometry," Holden-Day, San Francisco, 1964, Vol. 2, p. 122.

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