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A NEW TRITERPENE GLYCOSIDE FROM FRUIT OF *Phytolacca americana*

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Glycosides H and I, the structures of which were established by modern physicochemical analytical methods (PMR, ^{13}C NMR, COSY, TOCSY, HMBC, MS) and acid-base hydrolysis, were isolated from the purified total saponins from fruit of Phytolacca americana *containing at least 10 triterpene glycosides by rechromatography of enriched fractions over a column of silica gel. Glycoside H was a bidesmoside of phytolaccageninic acid, which was isolated earlier from cell culture of* Phytolacca acinosa*. Glycoside I was 3-O-(β-D-xylopyranosyl-(1→3)-β-D-galactopyranosyl-(1→3)-β-D-xylopyranosyl)-28-O-β-D-glucopyranosyl phytolaccagenin, which was isolated by us for the first time.*

Keywords: *Phytolacca americana*, American pokeweed, triterpene glycoside, sapogenin, phytolaccagenin.

The genus *Phytolacca* (pokeweed) (Phytolaccaceae) is represented in Georgia by one species, *Phytolacca americana* L., which was imported from North America. This is a perennial herbaceous weed of 1–3 m height. Its fruit is ~7–8 mm in diameter and is round without ribs when ripe [1].

American pokeweed is used in folk medicine to treat diseases such as rheumatism, syphilis, skin diseases, etc. [2]. Up to 10 triterpenes were previously isolated and characterized chemically from various organs of pokeweed [3–6].

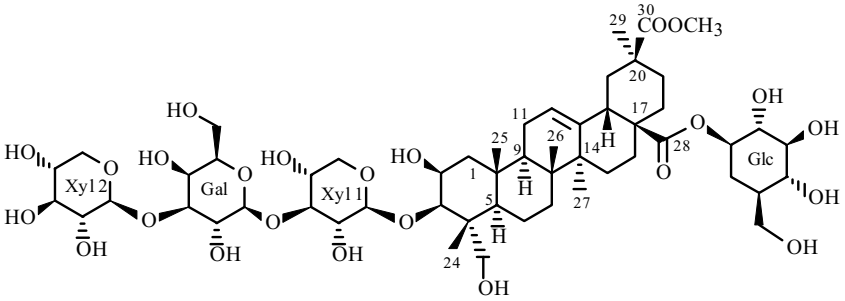
The isolation of triterpene saponins from ripe fruit of American pokeweed, the identification of a known glycoside, and the elucidation of the structure of one new glycoside are described herein.

A comparative qualitative analysis by TLC of triterpene glycosides isolated from fresh ripe American pokeweed fruit dried in air and in a microwave oven showed that the drying conditions did not affect the spectrum of these glycosides.

Rechromatography of enriched fractions of purified total saponins containing at least 10 triterpene glycosides designated by us by the letters A, B, C, D, E, F, G, H, I, and J isolated glycosides H and I.

The products of total acid hydrolysis contained according to TLC the monosaccharides xylose, galactose, and glucose. Glycosides H and I were not methylated by an ether solution of diazomethane. However, alkaline hydrolysis cleaved glucose.

The final structures of the isolated glycosides were elucidated using modern physicochemical analytical methods of PMR, ^{13}C NMR, and 1D and 2D experiments (Tables 1 and 2). According to the results, glycoside H was identified as a bidesmoside and a trioside of phytolaccageninic acid that was isolated earlier from *Phytolacca acinosa* cell culture [6].



Glycoside I

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TABLE 1. ^{13}C NMR and PMR Spectra of the Aglycon Part of Glycoside I (CD_3OD, δ, ppm, J/Hz)

C atom	δ_C	δ_H	C atom	δ_C	δ_H
1	44.5 t	2.07 (1H, m) 1.15 (1H, m)	17	47.4 s	
2	71.6 d	4.26 (1H, m)	18	43.9 d	2.70 (2H, dd, J = 13.2, 3.2)
3	83.6 d	3.61 (1H, m)	19	43.3 t	1.95 (1H, m) 1.69 (1H, m)
4	43.2 s		20	45.0 s	
5	48.2 d	1.31 (1H, m)	21	31.4 t	2.00 (1H, m) 1.38 (1H, m)
6	18.6 t	1.48 (2H, m)	22	34.4 t	1.72 (1H, m) 1.55 (1H, m)
7	33.4 t	1.60 (1H, m) 1.28 (1H, m)	23	65.5 t	3.60 (1H, m) 3.23 (1H, m)
8	40.7 s		24	14.7 q	0.92 (3H, s)
9	49.4 d	1.58 (1H, m)	25	17.6 q	1.28 (3H, s)
10	37.5 s		26	17.8 q	0.80 (3H, s)
11	24.7 t	1.98 (2H, m)	27	26.4 q	1.17 (3H, s)
12	124.4 d	5.32 (1H, m)	28	177.6 s	
13	144.6 s		29	28.7 q	1.14 (3H, s)
14	43.1 s		30	178.8 s	
15	28.9 t	1.79 (1H, m) 1.09 (1H, m)	OMe	52.4 q	3.70 (3H, s)
16	24.2 t	2.04 (1H, m) 1.78 (1H, m)			

TABLE 2. ^{13}C NMR and PMR Spectra of the Monosaccharide Part of Glycoside I (CD_3OD, δ, ppm, J/Hz)

C atom	δ_C	δ_H	C atom	δ_C	δ_H
	Glucose			Galactose	
1′	95.8 d	5.34 (1H, d, J = 7.4)	1‴	105.1 d	4.60 (1H, d, J = 7.7)
2′	73.9 d	3.30 (1H, m)	2‴	72.2 d	3.79 (1H, m)
3′	78.3 d	3.40 (1H, m)	3‴	84.2 d	3.64 (1H, m)
4′	71.1 d	3.34 (1H, m)	4‴	69.9 d	4.05 (1H, d, J = 2.9)
5′	78.8 d	3.34 (1H, m)	5‴	76.7 d	3.60 (1H, m)
6′	62.4 t	3.81 (1H, m) 3.67 (1H, m)	6‴	62.6 t	3.79 (1H, m) 3.69 (1H, m)
	Xylose 1			Xylose 2	
1″	106.2 d	4.43 (1H, d, J = 7.4)	1⁗	106.3 d	4.48 (1H, d, J = 7.1)
2″	74.6 d	3.47 (1H, t, J = 8.0)	2⁗	72.9 d	3.65 (1H, m)
3″	87.5 d	3.52 (1H, m)	3⁗	74.0 d	3.55 (1H, m)
4″	69.9 d	3.62 (1H, m)	4⁗	69.7 d	3.81 (1H, m)
5″	66.4 t	3.90 (1H, dd, J = 11.8, 5.2)	5⁗	67.1 t	3.86 (1H, dd, J = 11.6, 2.6)
		3.25 (1H, m)			3.57 (1H, m)

Glycoside I was a white amorphous powder. According to a peak for the pseudomolecular ion with *m*/*z* 1143.5202 for $[M + Na]^+$ (HR-ESI-MS high-resolution mass spectrum), the molecular formula corresponded with $C_{53}H_{84}O_{25}$. Analysis of the ^{13}C and DEPT-135 NMR spectra showed that the compound contained nine quaternary C atoms and 24 methine, 14 methylene, and 6 methyl groups. Resonances of C atoms containing protons including four anomeric protons (δ_H 5.34, 4.60, 4.48, and 4.43), five secondary C atoms (δ_C 67.1, 66.4, 65.5, 62.6, 62.4), two carbonyl groups (δ_C 178.8 and 177.6), and two tri-substituted double bonds (δ_C 144.6 and 124.4) were identified in the HSQC spectrum. HMBC and COSY spectra confirmed the presence in the molecule of the aglycon phytolaccagenin [7].

The sites of attachment of the sugar moieties to the aglycon were established by HMBC and ROESY experiments; the anomeric configurations, by coupling constants, the values of which were >7 Hz.

Based on the aforementioned, we proposed the structure of glycoside I as 3-*O*-(*β*-D-xylopyranosyl-(1→3)-*β*-D-galactopyranosyl-(1→3)-*β*-D-xylopyranosyl)-28-*O*-*β*-D-glucopyranosyl phytolaccagenin, which was a new compound.

EXPERIMENTAL

Isolation and Purification. Purified total triterpene saponins were obtained by first treating air-dried ground fruit of *P. americana* with $CHCl_3$ to remove fats and slightly polar substances from the raw material and then extracting with EtOH (96%). The EtOH was distilled off. The aqueous residue was extracted with EtOAc. The dried EtOAc extract was passed over a column of Diaion LH-20 to produce three 3-4-component low-polarity, medium-polarity, and polar fractions. The polar fraction was separated over a column of Kieselgel-60 (Merck) with elution by $CHCl_3$:CH_3OH:H_2O (26:14:3). The final purification of the glycosides from ballast impurities was carried out using preparative chromatography on an Agilent 1100 series prep instrument (20 × 250 mm, Intertsil prep-ODS column using UV detection, MWD, wavelength 254 nm, bandwidth 100, H_2O 45%, MeOH 55%). Two glycosides H and I were isolated. The purity of these was checked on an analytical HPLC (Agilent 1100, 6.0 × 250 mm, Intertsil prep-ODS column using DAD, UV-MSD, H_2O 45%, MeOH 55%).

NMR spectra were recorded on a Bruker Avance instrument at operating frequency 400 MHz for ^1H and 100 MHz for ^{13}C. The structures of the glycosides were elucidated by analyzing ^1H, ^{13}C, COSY, TOCSY, HMBC, HSQC, and DEPT-135 spectra. Chemical shifts (δ) are given in ppm relative to TMS.

TLC monitoring of sapogenins and monosaccharides was performed on silica gel plates (Silica gel 60 F_{254}, Merck) using solvent systems $CHCl_3$:MeOH:H_2O (1, 26:14:3), BuOH:HOAc:H_2O (2, 4:1:5), $CHCl_3$:MeOH (3, 20:1), CH_2Cl_2:MeOH:H_2O (4, 50:25:5). Aglycons were detected by phosphotungstic acid solution (25%) in EtOH; monosaccharides, by anilinium biphthalate. Chromatograms were heated to 100-150°C after treatment with the detecting reagent.

Total Acid Hydrolysis. Glycoside (3 mg) was placed into a flask, treated with HCl solution (3 mL, 10%), and heated at 100°C for 4 h. Sapogenins were extracted with Et_2O. The aqueous part was neutralized by *N*,*N*-dioctylmethylamine (10% in $CHCl_3$). Sugars, i.e., glucose, galactose, and xylose, were identified in both instances on TLC with standards using system 4; sapogenins, system 3.

Alkaline Hydrolysis. Glycoside (5 mg) was placed into a flask, treated with aqueous KOH (5 mL, 5%), heated at 100°C for 90 min, and neutralized with HCl (10%) to pH 5. Prosapogenins were extracted by 1-BuOH and then analyzed on TLC using systems 1 and 2.

Glycoside I, white amorphous powder, $C_{53}H_{84}O_{25}$, based on a peak for the pseudomolecular ion 1143.5202 $[M + Na]^+$ (HR-ESI-MS high-resolution mass spectrum), $[\alpha]_D^{25}$ +4.87° (*c* 1, MeOH). Tables 1 and 2 present the NMR spectral data.

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