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NEW SAPONINS FROM THE ROOTS OF *PHYTOLACCA POLYANDRA*

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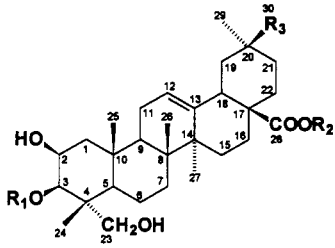
ABSTRACT.—Two new triterpenoid saponins were isolated from the roots of *Phytolacca polyandra*. The structures of these saponins, polyandrasides A [**1**] and B [**2**], were established as 3-*O*-β-D-glucopyranosyl-28-*O*-glucopyranosyl-esculentagenic acid 30-acetate and 3-*O*-β-D-glucopyranosyl-28-*O*-glucopyranosyl-phytolaccagenin, respectively, on the basis of spectroscopic and chemical evidence.

The roots of *Phytolacca esculenta* Van Houtte (Phytolaccaceae) have been used in traditional Chinese medicine for the treatment of tumors, edema, and bronchitis (1). Saponins from the roots of this plant have shown several types of biological activity in inducing immune interferon (IFN-γ), interleukin-2, lymphotoxin, and tumor necrosis factor, as well as exhibiting potent antiinflammatory activity (2–4). Thirteen new saponins from the roots of this plant have been reported previously (5–14). However, another species, *Phytolacca polyandra* Batalin, is also rich in saponins. Our interest in the biological activities of saponins led to further investigation of *P. polyandra*. This paper describes the structural elucidation of polyandrasides A [**1**] and B [**2**] isolated from this plant.

Chromatography of a MeOH extract of the roots of *Phytolacca polyandra* on Si gel and purification by hplc gave polyandrasides A [**1**] and B [**2**]. Compound **1** gave positive Liebermann-Burchard and Molish reactions. The ir spectrum of **1** indicated the presence of double-bond, carboxyl, hydroxyl, acetate, and methyl groups. After acid hydrolysis, **1** yielded a genin [**3**] and glucose as the sugar moiety. On basic hydrolysis, **3** gave esculentagenic acid [**4**], identified by direct comparison with an authentic sample (tlc, ir, ms) (11). The fabms showed pseudo-molecular ions $[M+Na]^+$ at *m/z* 893 and $[M+K]^+$ at *m/z* 909 together with the fragment ion



1 $R_1=R_2=$glc $R_3=CH_2OAc$
2 $R_1=R_2=$glc $R_3=COOCH_3$
3 $R_1=R_2=H$ $R_3=CH_2OAc$
4 $R_1=R_2=H$ $R_3=CH_2OH$

$[(M+Na)-162]^+$ at *m/z* 731, which corresponds to a loss of the terminal glucose. The eims of **1** exhibited fragment ions at *m/z* 529 [genin−OH]$^+$, 498 [*m/z* 529−CH_2OH]$^+$, 438 [*m/z* 498−OAc−H]$^+$, *m/z* 529 [genin−OH]$^+$, 498 [*m/z* 529−CH_2OH], 438 [*m/z* 498−OAc−H]$^+$, 306 [RDA fragment], 262 [*m/z* 306−CH_3CO−H]$^+$, and 246 [*m/z* 306−OAc−H]$^+$. The ^{1}H-nmr spectrum of **1** revealed five tertiary methyl signals between δ 0.94–1.37, an olefinic proton signal at δ 5.37, an acetyl signal at δ 2.10 (3H, s, OAc), and two anomeric proton signals at δ 5.12 (1H, d, J=7.9 Hz) and 6.28 (1H, d, J=7.9 Hz). The coupling constants of the two anomeric protons indicated a β- configuration for the C-1 of glucose in each case.

These results suggested that **1** was an acetylated bisdesmoside containing two molecules of glucose. The ^{13}C-nmr spectrum of **1** was very close to that of esculentoside J (11), except for the chemi-

cal shifts of C-30 and 3-*O*-glucose, indicating that one actetyl group was located at C-30. The ^{13}C-nmr spectrum of **1** showed significant glycosidation shifts for C-3 (+9.7 ppm) relative to the same carbon in the aglycone. A carbon signal at δ 95.7 ppm also indicated that one terminal glucose moiety was linked to the 28-COOH functionality of **1** in the β- configuration. The significant downfield shift for C-30 (+2.2 ppm) suggested that the acetyl group was linked to C-30. Thus, the structure of **1** (polyandraside A) was established as 3-*O*-β-D-glucopyranosyl-28-*O*-β-D-glucopyranosyl-esculentagenic acid-30-acetate.

Compound **2** also gave positive Liebermann-Burchard and Molish tests. After acid hydrolysis, **2** afforded phytolaccagenin (15), identified by direct comparison with an authentic sample (tlc, mp, ir, ms) and glucose. The ^{1}H-nmr spectrum of **2** showed five tertiary methyl signals (δ 1.13–1.70) together with one *O*-methyl signal at δ 3.56, an olefinic proton signal at δ 5.55, and two anomeric proton signals at δ 5.13 (1H, d, J=7.9 Hz) and 6.25 (1H, d, J=7.9 Hz). The coupling constants of the two anomeric protons of **2** indicated a β-configuration for C-1 of glucose. The fabms of **2** showed pseudomolecular ions $[M+Na]^+$ at *m/z* 879 and $[M+K]^+$ at *m/z* 895 together with a fragment ion $[(M+Na)-162]^+$ at *m/z* 717, which corresponds to the loss of the terminal glucose. The eims of **2** exhibited fragment ions at *m/z* 532 (genin), 486 [*m/z* 532−COOH−H]$^+$, 426 [*m/z* 532−COOH−$COOCH_3$−2H], 292 [RDA fragment], 246 [*m/z* 292−COOH−H], and 187 [*m/z* 292−COOH−$COOCH_3$]. The ^{13}C-nmr spectrum of **2** showed a significant glycosidation shift for C-3 (+9.7 ppm) relative to the same carbon in the aglycone. A carbon signal at δ 95.7 ppm also indicated that the terminal glucose moiety was linked to the 28-COOH group of **2** in the β- configuration. Based on these results the structure of **2** (polyandraside B) was established as 3-*O*-β-D-glucopyranosyl-28-*O*-β-D-glucopyranosyl-phytolaccagenin.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Boetius-Phmk 05 apparatus and uncorrected. Uv (MeOH) and ir (KBr) spectra were measured on Hitachi-UV-3000 and Hitachi 270-50 spectrophotometers, respectively. ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker MSL-300 instrument operating at 300 MHz and chemical shift values were given as δ (ppm) values with TMS as internal standard. Eims and fabms were recorded on a Varian-MAT 212 spectrometer. Hplc was performed on a Waters liquid chromatograph model 510, using a column (7.8 mm×300 mm) packed with μ-Bondapak C_{18} and eluted with 60% MeOH/H_2O. Qingdao Si gel was used for cc and tlc.

PLANT MATERIAL.—The roots of *P. polyandra* Bat. (200 g dry wt) were collected from Sichuan Province, People's Republic of China, in November, 1993. A voucher specimen is deposited in the Herbarium of the School of Pharmacy (SH 03172), Second Military Medical University, Shanghai, People's Republic of China.

EXTRACTION AND ISOLATION.—The saponin fraction was prepared from the roots of *P. polyandra* as described previously (6). The extract (50 g) was subjected to repeated cc on Si gel, eluting with $CHCl_3$-MeOH (8:2) to afford **1** (120 mg) and **2** (90 mg). Compounds **1** and **2** were then purified by hplc, eluting with 60% MeOH/H_2O to yield pure **1** (50 mg) and **2** (35 mg), respectively.

Polyandraside A [**1**].—White powder; mp 206.0–208.0°; molecular formula $C_{44}H_{70}O_{17}$; uv (MeOH) λ max 214 nm; ir (KBr) ν max 3450 (OH), 1740, 1730 (ester), 1640 (C=C), 1080, 1035 (C-O) cm^{-1}; ^{1}H nmr (C_5D_5N, 300 MHz) δ 0.94, 1.10, 1.21, 1.28, 1.37 (3H each, s, 5×tertiary Me), 2.10 (3H, s, MeCO), 5.37 (1H, m, H-12), 5.12 (1H, d, J=7.9 Hz, anomeric proton), 6.28 (1H, d, J=7.9 Hz, anomeric proton), 3.55–4.46 (sugar protons); ^{13}C-nmr (C_5D_5N, 300 MHz) data, see Table 1; fabms, see text.

Polyandraside B [**2**].—White powder; mp 205.5–206.5°; molecular formula $C_{43}H_{68}O_{17}$; uv (MeOH) λ max 214 nm; ir (KBr) ν max 3450 (OH), 1730 (ester), 1640 (C=C), 1080, 1035 (C-O) cm^{-1}; ^{1}H nmr (C_5D_5N, 300 MHz) δ 1.12, 1.13, 1.19, 1.27, 1.53 (3H each, s, 5×tertiary Me), 3.56 (3H, s, OMe), 5.55 (1H, s, H-12), 5.13 (1H, d, J=7.9 Hz, anomeric proton), 6.25 (1H, d, J=7.9 Hz, anomeric proton), 3.60–4.45 (sugar protons); ^{13}C-nmr (C_5D_5N, 300 MHz) data, see Table 1; fabms, see text.

ACID HYDROLYSIS OF **1**.—Compound **1** (20

TABLE 1. ^{13}C-Nmr Data (C_5D_5N, 300 MHz) for **1** and **2**.

Carbon	Compound	
	1	**2**
Aglycone		
C-1	44.0	44.0
C-2	71.4	71.5
C-3	82.9	82.9
C-4	41.1	41.1
C-5	48.4	48.4
C-6	17.9	18.0
C-7	32.8	32.8
C-8	39.8	40.0
C-9	47.6	47.6
C-10	37.0	36.9
C-11	23.8	23.9
C-12	123.8	123.8
C-13	143.5	143.7
C-14	42.7	42.7
C-15	28.0	28.2
C-16	23.5	23.4
C-17	46.4	46.4
C-18	43.1	43.1
C-19	42.1	42.3
C-20	43.9	43.9
C-21	29.5	30.5
C-22	34.0	34.0
C-23	65.3	65.4
C-24	14.9	15.0
C-25	17.4	17.5
C-26	17.2	17.2
C-27	26.1	26.1
C-28	176.8	176.8
C-29	28.1	28.0
C-30	67.5	176.0
C-31		51.6
Acetyl		
1′	170.8	
2′	20.3	
3-Glucosyl		
C-1	105.5	105.6
C-2	75.4	75.4
C-3	78.4	78.5
C-4	70.3	70.4
C-5	78.7	78.8
C-6	62.5	62.5
28-Glucosyl		
C-1	95.7	95.7
C-2	74.0	74.0
C-3	79.2	79.2
C-4	70.9	70.8
C-5	78.1	78.2
C-6	62.0	61.8

mg) in 5% HCl/EtOH (5 ml) was refluxed for 6 h, and concentrated under reduced pressure. The residue was diluted with 10 ml H_2O. The resulting precipitate was collected by filtration and chromatographed on Si gel, eluting with $CHCl_3$-MeOH (50:1) to afford a genin [**3**] (8 mg), which was crystallized from MeOH as needles; mp 256–258°, ir (KBr) ν max 3400 (OH), 1720 (ester), 1650 (C=C) cm^{-1}. The filtrate was neutralized with 201-7 ion exchange resin and evaporated to dryness. Glucose was detected by tlc ($CHCl_3$-MeOH, 1:1).

BASIC HYDROLYSIS OF **3**.—Compound **3** (5 mg) in 1 N NaOH (2 ml) were refluxed for 1 h. The reaction mixture was neutralized with 1 N HCl and the resulting precipitate was collected by filtration to afford esculentagenic acid [**4**] (2 mg), identified by direct comparison with an authentic sample of esculentagenic acid from our laboratory (11) (tlc, ir, ms).

ACID HYDROLYSIS OF **2**.—Compound **2** (20 mg) was treated by the above-mentioned method to afford phytolaccagenin (10 mg), identified by direct comparison with an authentic sample of phytolaccagenin (tlc, mp, ir, ms).

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