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Isolation and Structure Elucidation of Two Triterpene Saponins from the Roots of Phytolacca Americana

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Purification of the n-BuOH fraction of the aqueous alcoholic extract from the roots of *Phytolacca americana* resulted in the isolation of two triterpene saponins named esculentoside B (**1**), and esculentoside A (**2**). The structures of isolated compounds were characterized on the basis of extensive spectral data (1D and 2D NMR; and MS) and in comparison with their Nuclear Magnetic Resonance (NMR) and Mass (MS) spectral data reported earlier.

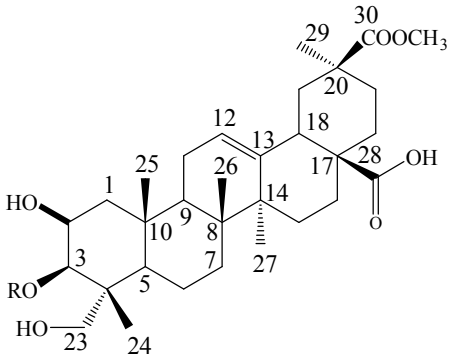
Keyword: *Phytolacca americana,* Triterpene saponins, Isolation and purification, NMR, MS, Structure elucidation.

1. Introduction

Phytolacca americana L. Van Houtt. (syn.: *Phytolacca acinosa* Roxb.) (Phytolaccaceae) and *Phytolacca americana* L. (Phytolaccaceae) are the plants native to the south of mainland China and the eastern United States respectively[1]. The dried roots of the above plants are used as a traditional herbal medicine and a folk medicine in China and are called "Shang-Lu" and "Chui-Xu Shang-Lu", respectively, which are used for the treatment of tumors, edema, bronchitis, and abscesses. *Phytolacca americana* is a toxic plant in the United States and causes vomiting when the fruits, young leaves, and roots are consumed[2]. Its rhizome has been used as a traditional crude diuretic drug in spite of having strong toxicity. A number of triterpenes, their glycosides, and neolignans have been isolated from the roots of *P. americana* and characterized their structures based on extensive spectroscopic studies[1-2].

As a part of our research to discover natural sweeteners, we have recently reported several diterpene glycosides from *S. rebaudiana* and *R. suavissimus*; triterpene glycosides from *Siraitia grosvenorii*; phenolic glycosides and sterols from *R. suavissimus*[3-11].

This paper describes the isolation and structure elucidation of two triterpene saponins namely esculentoside B (**1**), and esculentoside A (**2**) (Figure 1) from the roots of *P. americana* on the basis of extensive NMR and mass spectroscopic data and in comparison of their spectral properties (NMR and MS) reported from the literature.



1: R = Xylβ1; Esulentoside B

2: R = Glcβ1-4Xylβ1; Esulentoside A

Fig 1: Structures of Esculentoside B (1), and Esculentoside A (2)

2. Materials and Methods

2.1 General Instrumentation

Melting points were measured using a SRS Optimelt MPA 100 instrument and are uncorrected. Optical rotations were recorded using a Rudolph Autopol V at 25°C and NMR spectra were acquired on a Varian Unity Plus 600 MHz instrument using standard pulse sequences at ambient temperature. Chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. HRMS data was generated with a Thermo LTQ Orbitrap Discovery mass spectrometer in the positive positive ion mode electrospray. Instrument was mass calibrated with a mixture of Ultramark 1621, MRFA [a peptide], and caffeine immediately prior to accurate mass measurements of the samples. Samples were diluted with water:acetonitrile:methanol (1:2:2) and prepared a stock solution of 50 ul concentration for each sample. Each sample (25 ul) was introduced via infusion using the onboard syringe pump at a flow injection rate of 120 ul/min. Low pressure chromatography was performed on a Biotage Flash system using a C-18 cartridge (40+ M, 35-70 µm). TLC was performed on Baker Si-C_{18}F plates and identification of the spots on the TLC plate was carried out by spraying 10% H_2SO_4 in EtOH and heating the plate at about 80 °C.

2.2 Isolation and purification of Triterpene saponins (1-2)

The aqueous extract of the leaves of *P. americana* (10 g) was suspended in 200 ml water and extracted successively with *n*-hexane (3 x 200 ml), CH_2Cl_2 (3 x 150 ml) and *n*-BuOH (2 x 150 ml). The *n*-BuOH layer was concentrated under vacuum furnished a residue (1.4 g) which was purified on a Biotage flash chromatography system using C-18 (100 g) column (solvent system: gradient from MeOH-water (40:60) to 100% MeOH at 50 ml/min, detection at UV at 210 nm for 60 min by collecting 60 fractions. Fractions 42-48 were combined to get a residue 0.24 g, which on repeated purification using the gradient 90% MeOH in water to 100% MeOH on a C-18 (25 g) column at 10 ml/min for 40 min resulted esculentoside B (**1**, 42 mg), and esculentoside A (**2**, 32 mg), respectively.

2.3 Identification of Esculentoside B (1), and Esculentoside A (2)

2.3.1 Esculentoside B (1): White powder; ESI-MS (m/z): 665 $[M+H]^+$; ^{1}H NMR (600 MHz, C_5D_5N, ppm): 0.71 (3H, s, CH_3), 0.83 (3H, s, CH_3), 1.15 (3H, s, CH_3), 1.21 (3H, s, CH_3), 1.27 (3H, s, CH_3), 3.64 (3H, s, OCH_3), 4.25 (1H, d, *J* =6.5 Hz, anomeric proton of xylose), 5.15 (1H, m, C12-H); ^{13}C NMR (150 MHz, C_5D_5N, ppm): 44.1 (C-1), 70.4 (C-2), 80.8 (C-3), 43.8 (C-4), 47.9 (C-5), 17.6 (C-6), 32.8 (C-7), 42.2 (C-8), 47.9 (C-9), 36.6 (C-10), 23.4 (C-11), 123.4 (C-12), 145.1 (C-13), 42.2 (C-14), 27.7 (C-15), 23.4 (C-16), 46.6 (C-17), 42.8 (C-18), 42.9 (C-19), 45.7 (C-20), 30.4 (C-21), 33.8 (C-22), 63.6 (C-23), 13.8 (C-24), 16.9 (C-25), 17.4 (C-26), 25.8 (C-27), 178.8 (C-28), 28.5 (C-29), 177.6 (C-30), 51.9 (C-31), 105.1 (C-1 of Xyl), 73.5 (C-2 of Xyl), 76.7 (C-3 of Xyl), 70.4 (C-4 of Xyl), 65.5 (C-5 of Xyl).

2.3.2 Esculentoside A (2): White powder; ESI-MS (m/z): 827 $[M+H]^+$; ^{1}H NMR (600 MHz, C_5D_5N, ppm): 0.73 (3H, s, CH_3), 0.84 (3H, s, CH_3), 1.16 (3H, s, CH_3),1.22 (3H, s, CH_3), 1.29 (3H, s, CH_3), 3.72 (3H, s, OCH_3), 4.26 (1H, d, *J* = 7.4 Hz, anomeric proton of xylose), 4.34 (1H, d, *J* = 7.6 Hz, anomeric proton of glucose), 5.23 (1H,

m, C12-H); ^{13}C NMR (150 MHz, C_5D_5N, ppm): 44.0 (C-1), 70.6 (C-2), 80.8 (C-3), 43.8 (C-4), 48.1 (C-5), 17.0 (C-6), 32.7 (C-7), 41.9 (C-8), 49.2 (C-9), 36.6 (C-10), 23.8 (C-11), 124.0 (C-12), 145.2 (C-13), 41.9 (C-14), 27.8 (C-15), 23.6 (C-16), 46.8 (C-17), 42.7 (C-18), 42.8 (C-19), 45.5 (C-20), 30.6 (C-21), 33.9 (C-22), 63.7 (C-23), 14.0 (C-24), 16.8 (C-25), 17.5 (C-26), 25.9 (C-27), 178.6 (C-28), 28.4 (C-29), 177.5 (C-30), 52.1 (C-31), 105.2 (C-1 of Xyl), 73.7 (C-2 of Xyl), 75.1 (C-3 of Xyl), 77.5 (C-4 of Xyl), 63.6 (C-5 of Xyl), 101.3 (C-1 of Glc), 73.1 (C-2 of Glc), 76.8 (C-3 of Glc), 70.6 (C-4 of Glc), 76.7 (C-5 of Glc), 61.5 (C-6 of Glc).

2.4 Acid Hydrolysis of 1 and 2. To a solution of each compound **1** and **2** (1 mg) in MeOH (5 ml) was added 10 ml of 5% H_2SO_4 and the mixture was refluxed for 8 hours. The reaction mixture was then neutralized with saturated sodium carbonate and extracted with ethyl acetate (EtOAc) (2 x 5 ml) to give an aqueous fraction containing sugars and an EtOAc fraction containing the aglycone part. The aqueous phase was concentrated and compared with standard sugars using the TLC systems EtOAc/*n*-butanol/water (2:7:1) and CH_2Cl_2/MeOH/water (10:6:1); the sugar was identified as D-xylose in **1**; and the two sugars were identified as D-xylose and D-glucose in **2**[12-13].

3. Results and Discussion

Compound **1** was isolated as a white powder. The mass spectral data of compound **1** gave a molecular ion peak at m/z 665 corresponding to its $(M+H)^+$ ion suggesting the molecular formula as $C_{36}H_{56}O_{11}$, which was supported by the ^{13}C NMR spectral data. Liebermann-Burchard reaction indicated compound **1** is having a terpenoid skeleton[14-15]. The ^1H NMR spectra of compound **1** showed the presence of five methyl signals at δ 0.71, 0.83, 1.15, 1.21 and 1.27; one methoxyl group at δ 3.64 and a trisubstituted olefinic proton at δ 5.15. The ^1H NMR spectra of compound **1** also showed the presence of an anomeric protons at δ 4.25 (d, J = 6.5 Hz), indicating the presence of a sugar in its structure. The ^{13}C NMR and distortionless enhancement by polarization transfer (DEPT) spectra displayed the 36 carbons which consisted of two carbonyl groups, two carbons of a trisubstituted double bond, one anomeric carbon, five methyl groups, nine methylenes, three methines, six quaternary carbons and eight oxygen-bearing carbons. The presence of five methyl singlets and two carboxylic acids suggested that compound **1** belongs to oleanane type triterpenoid having a secondary hydroxyl and a primary hydroxyl groups along with a trisubstituted double bond between C-12/C-13. Acid hydrolysis of **1** afforded D-xylose, which was identified by comparison with the authentic sugars by using by comparative TLC detection[12-13]. Additionally, the coupling constants for the anomeric proton of xylose in **1** indicated that the anomeric configurations of D-xylose as β. The above spectral data indicated that **1** was a phytolaccinic acid triterpene glycoside linked with D-xylose. The ^{13}C NMR values for all the protons and carbons were assigned on the basis of HMQC and HMBC correlations and were given in materials and methods. In the ^{13}C NMR spectrum, the aglycone part of **1** was similar to phytolaccinic acid[1-2] except for C-3 which was shifted down-field, thus indicating that the sugar part was attached at the C-3 position. In order to confirm the linkages of the xylose sugar part and the structure of the aglycone, heteronuclear multi-bond correlation (HMBC) experiments were carried out, suggested the structure as shown in Figure 2. Thus the structure of **1** was assigned as 3-O-β-D-xylopyranosy phytolaccagenin (esculentoside B), supported by comparison with the reported literature data[1-2].

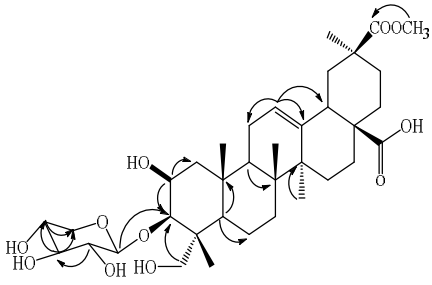


Fig 2: Key HMBC correlations of Esculentoside B (1)

Compound **2** was also isolated as a white powder. The mass spectral data of compound **2** gave a molecular ion peak at m/z 827 corresponding to its $(M+H)^+$ ion suggesting the molecular formula as $C_{42}H_{66}O_{16}$, which was supported by the ^{13}C NMR spectral data. Liebermann-Burchard reaction indicated compound **2** is also having a terpenoid skeleton as in **1**[14-15]. The ^1H NMR spectra of compound **2** also showed the presence of five methyl signals; one methoxyl group and a trisubstituted olefinic proton, same as **1**. Further, the ^1H NMR spectra of compound **2** also showed the presence of two anomeric protons at δ 4.26 (d, J = 7.4 Hz) and 4.34 (d, J = 7.6 Hz), indicating the presence of two sugar moieties in its structure. The ^{13}C NMR and DEPT spectra of **2** showed the 42 carbons which consisted of two carbonyl groups, two carbons of a trisubstituted double bond, two anomeric carbons, five methyl groups, nine methylenes, three methines, six quaternary carbons and thirteen oxygen-bearing carbons. Acid hydrolysis of **2** afforded D-xylose and D-glucose, which were identified with authentic sugars by comparative TLC detection[12-13]. Additionally, the coupling constants for the anomeric protons of xylose and glucose in **2** indicated that the anomeric configurations of D-xylose and D-glucose are β. These results indicated that **2** was also an oleane triterpene like **1** having phytolaccinic acid skeleton linked with D-xylose and D-glucose sugar units. The linkages of the two sugar parts and the structure of the aglycone, were confirmed on the basis of HMBC correlations as shown in Figure 3 suggested the structure of compound **2** as 3-O-[β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl] phytolaccagenin(esculentoside A); showed a good agreement with reported data[1-2].

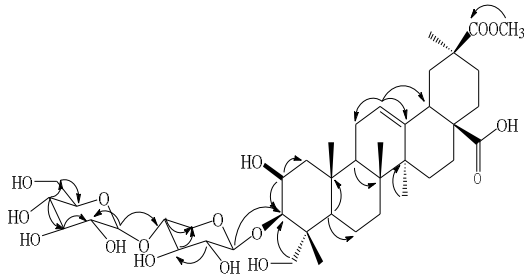


Fig 3: Key COSY and HMBC correlations of Esculentoside A (2)

4. Conclusion

Two triterpene saponins namely esculentoside B (**1**), and esculentoside A (**2**) were isolated from the commercial aqueous alcoholic extract of the roots of *P. americana* on the basis of extensive NMR and mass spectroscopic data and in comparison of their NMR and MS spectral properties reported from the literature.

5. References

1. Hiranobu T, Yuki N, Masami T, Yoshiyasu F. Triterpene Glycosides from the Cultures of *Phytolacca Americana*, Chemical and Pharmaceutical Bulletin 2001; 49: 246-248.
2. Fukuyama Y, Hasegawa T, Toda M, Kodama M, Okazaki H. Structures of americanol A and isoamericanol A having a neurotrophic property from the *Phytolacca Americana*, Chemical and Pharmaceutical Bulletin 1992; 40: 252-254.
3. Chaturvedula VSP, Mani U, Prakash I. Diterpene glycosides from *Stevia rebaudiana*, Molecules 2011; 16: 3552-3562.
4. Chaturvedula VSP, Prakash I. A new diterpenoid glycoside from *Stevia rebaudiana*, Molecules 2011; 16: 2937-2943.
5. Chaturvedula VSP, Prakash I. Structures of the novel diterpene glycosides from *Stevia rebaudiana*, Carbohydrate Research 2011; 346: 1057-1060.
6. Chaturvedula VSP, Rhea J, Milanowski D, Mocek U, Prakash I., Two minor diterpene glycosides from the leaves of *Stevia rebaudiana*, Natural Product Communications 2011; 6: 175-178.
7. Chaturvedula VSP, Clos JF, Rhea J, Milanowski D, Mocek U, DuBois GE, Prakash I. Minor diterpene glycosides from the leaves of *Stevia rebaudiana*, Phytochemistry Letters 2011; 4: 209-212.
8. Chaturvedula VSP, Prakash I. Additional minor diterpene glycosides from *Stevia rebaudiana*, Natural Product Communications 2011; 6: 1059-1062.
9. Chaturvedula VSP, Mani U, Prakash I. Structures of the novel α-glucosyl linked diterpene glycosides from *Stevia rebaudiana*, Carbohydrate Research 2011; 346: 2034-2038.
10. Chaturvedula VSP, Prakash I. Cucurbitane glycosides from *Sitaitia grosvenorii*, Journal of Carbohydrate Chemistry 2011; 30: 16-26.

11. Chaturvedula VSP, Prakash I. Isolation and structure elucidation of two triterpene acids from the Leaves of *Perilla Frutescens*. Journal of Pharmacognosy and Phytochemistry 2013; 1: 49-52.
12. Bedir E, Toyang NJ, Khan IA, Walker LA, Clark AM. A new dammarane type triterpene glycoside from *Polyscias fulva*. Journal of Natural Products 2001; 64: 95-97.
13. Huan VD, Yamamura S, Ohtani K, Kasai R, Yamasaki K, Nham NT. Oleanane saponins from *Polyscias fructicosa*. Phytochemistry 1998; 47: 451-457.
14. An RB, Kim HC, Jeong GS, Oh SH, Oh H, Kim, Y.-C. (2005), Constituents of the aerial parts of Agrimonia pilosa. Nat. Prod. Sci. 11, 196-198.
15. Kandati V, Govardhan P, Reddy CS, Nath AR, Reddy RR. *In-vitro* and *in-vivo* anti-inflammatory activity of *Andrographis serpyllifolia (Rottl. Ex Vahl.) Wt.*, International Current Pharmaceutical Journal 2012; 1: 199-204.