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Original Article

Two Triterpenoid Saponins with α-glucosidase Inhibitory Activity from *Harpullia pendula* Seed Extract

Marian Nabil, Neveen S. Ghaly, Iman A.A. Kassem, Mary H. Grace and Farouk R. Melek*

ABSTRACT

Background: *Harpullia pendula* Planch (family Sapindaceae) is a small to medium rainforest tree native to Australia. **Objective**: This study aims to isolate triterpenoid saponins from *H. pendula* and test them as α-glucosidase inhibitors. **Materials and Methods:** The saponin compounds were obtained using variable chromatographic techniques and characterized by spectral analysis. **Results**: Two new triterpenoid saponins were obtained as an inseparable mixture from *H. pendula* methanolic seed extract. Their structures were determined as 3-*O*-β-D-glucopyranosyl-(1→2)-[α-L-arabinofuranosyl-(1→3)]-βD-glucuronopyranosyl22-*O*-angeloylA1-barrigenol and 3-*O*-β-D-galactopyranosyl-(1→2)-[α-L-arabinofuranosyl-(1→3)]-β-D-glucuronopyranosyl 22-*O*-(2-methylbutyroyl)-A1 barrigenol, respectively. The triterpene part 22-*O*-(2-methyl butyroyl) A1-barrigenol has never been characterized before. The α- glucosidase inhibitory activity of the two saponin mixture was evaluated *invitro* and proved to exhibit strong activity with IC_{50} value equals to 13.3 ± 5.0 ppm and IC_{90} value equals to 21.5 ± 8.0 ppm. **Conclusion**: Two new saponins were characterized from their mixture and found to exhibit α-glucosidase inhibitory activity.

Key words: *Harpullia pendula*, Sapindaceae, Triterpenoid saponins.

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INTRODUCTION

The plants of genus *Harpullia* (family Sapindaceae) are widely distributed from Malesia and Australia to the Pacific islands.[1] Previous investigations carried out on these plants proved them to be rich in polyhydroxylated triterpenoid saponins.[2-5] This type of saponins was reported to exhibit antimicrobial,[6-8] molluscicidal, miracidicidal and cercaricidal activities[9] as well as cytotoxic and Haemolytic properties.[4]

Harpullia pendula Planch known as tulipwood or tuliplance wood is a small to medium rainforest tree native to Australia. Phytochemical studies carried out on this species resulted in the isolation and characterization of the triterpenes A1-barrigenol, A1-barrigenol-22-*O*-angelate, camelliagenin A, camelliagenin-16 and 22-*O*-angelate, 22-α-hydroxyerthrodiol and 15α,16α,22α,28-tetrahydroxy-12-ene-3-one as well as quebrachitol and methyl-*p*-coumarate.[10,11] Three sapogenins characterized as angeloyl camelliagenin A, angeloyl A1- barrigenol and angeloyl A1-barrigenolmethyl ether, were also described from *H. pendula* and *H. cupanioides.*[12] Several acylated triterpenoid saponins with variable ester functions and sugar chains, were isolated and characterized from *H. cupanioides,*[2] *H. austro-caledonica,*[3] and *H. pendula.*[5] Flavonoids and phenolics from *H. pendula* were also reported.[5,13] Furthermore, two antimicrobial benzene acetic acid derivatives together with kaempferol glycosides were reported from *H. pendula.*[14]

As a part of our continuous investigations on bioactive saponins from plants cultivated in Egypt,[5] we described in this report the characterization of two further saponins from *H. pendula*.

RESULTS AND DISCUSSION

The butanol fraction from the defatted methanolic seed extract of *H. pendula*, was subjected to the polymer gel Diaion HP-20 and silica gel column chromatography to afford eight fractions. Previous examination of the major fraction by us, led to the isolation and characterization of the two saponins pendulaosides A and B.[5] In this report we described the characterization of two further new saponins **1** and **2** obtained as inseparable mixture from *H. pendula*. The negative high resolution LC-ESI-TOF-MS spectrum of **1** and **2** revealed the presence of two molecular ion peaks $[M-H]^-$ at *m/z* 1041-5229 ($C_{52}H_{81}O_{21}$) and at *m/z* 1043.5415 ($C_{52}H_{83}O_{21}$), respectively. The ^{1}H-NMR (700MHz) and ^{13}C-NMR (175MHz) spectra of **1** and **2** showed a set of major signals due to the common parts of the two molecules and minor ones corresponding to the other moieties with higher intensity for those of **1**. The ^{1}H-NMR spectrum (Table 1) showed the presence of seven methyl singlets at δ_H1.40, 1.08, 1.05, 1.03, 0.99, 0.91 and 0.88 ppm, four oxymethine proton signals at δ_H 3.21 (*dd*, *J*=12.0, 4.0 Hz), 3.77 (*m*), 3.92 (*d*, *J*=4.5 Hz) and 5.45 (*dd*, *J*=14.0, 6.3 Hz) together with oxymethylene proton signals at δ_H3.31 (*d*, *J*=11.0 Hz) and 3.13 (*d*, *J*=11.0 Hz). The spectrum also revealed the presence of an olefinic proton signal at δ_H5.43 (*brt*, *J*=3.0 Hz). These data suggested the presence of a triterpene moiety of oleanene skeleton. Extensive 1D (^{1}H, ^{13}C) and 2D (^{1}H-^{1}H COSY, TOCSY, HMQC, HMBC) NMR analysis, allowed complete assignments of the proton and carbon signals of a common triterpene part for **1** and **2** and confirmed

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Table 1: ^{1}H and ^{13}CNMR data of compounds 1 and 2 in CD_3OD.

	δ_c	δ_H		δ_c	δ_H	δ_c	δ_H
1	40.2	1.63(m), 1.00(m)			GlcUA		
2	27.2	1.90(m), 1.69(m)	1	105.0	4.54(d,7.5)	105.7	4.54(d,7.5)
3	92.3	3.21(dd,12.0,4.0)	2	78.7	3.70(m)	78.7	3.77(m)
4	40.6		3	87.5	3.70(m)	86.5	3.70(m)
5	56.8	0.79(d,15.0)	4	72.5	3.60(m)	72.5	3.60(m)
6	19.7	1.54(m), 1.4(m)	5	78.0	3.61(m)	78.0	3.60(m)
7	37.3	1.70(m)	6	178.5		178.5	
8	42.4				Glc		Gal
9	48.3	1.58(m)	1	104.0	4.78(d,9.0)	104.7	4.66(d,8.7)
10	38.0		2	75.5	3.18(dd,9.0,9.0)	73.3	3.56(m)
11	24.9	1.92(m)	3	78.0	3.38(m)	74.8	3.52(m)
12	126.4	5.43(brt,3.0)	4	70.0	3.53(m)	70.2	3.85(d,3.5)
13	144.6		5	78.2	3.22(m)	77.0	3.48(m)
14	48.5		6	61.0	3.80(m),3.77(m)	62.8	3.77(m),3.66(m)
15	68.7	3.77(m)			Ara(f)		
16	75.6	3.92(d,4.5)	1	111.1	5.25(d,2.0)	110.9	5.25(d,2.0)
17	45.8		2	83.6	4.11(m)	83.5	4.11(m)
18	42.6	2.52(dd,13.5,5.0)	3	77.9	3.86(m)	78.0	3.86(m)
19	47.7	2.43(t,13.0),1.05(m)	4	85.3	4.08(m)	85.4	4.08(m)
20	32.6		5	62.9	3.78(m),3.63(m)	63.0	3.78(m),3.63(m)
21	42.1	2.22(t,12.0),1.53(brd,12.0)			22-Angeloyl		22-methyl butyroyl
22	73.5	5.45(dd,14.0,6.3)	1'	169.7		177.5	
23	28.5	1.08(s)	2'	130.1		43.5	2.36(sextet,7.5,7.5)
24	17.0	0.88(s)	3'	138.2	6.06(q-like,7.5)	28.1	1.77(m),1.52(m)
25	16.4	0.99(s)	4'	16.0	1.97(dd,7.5,1.5)	12.5	0.96(t,7.0)
26	18.0	1.03(s)	5'	21.2	1.91(d-like,1.5)	17.0	1.14(d,7.0)
27	21.3	1.40(s)					
28	63.8	3.31(d,11.0),3.13(d,11.0)					
29	33.7	0.91(s)					
30	25.5	1.05(s)					

the existence of the five oxygenated carbons in this triterpene moiety. The proton signal at δ_H 3.21 correlated with a carbon signal at δ_C 92.3 in the HMQC spectrum and with C-23 (δ_C 28.5) and C-24 (δ_C17.0) signals in the HMBC spectrum, was assigned to the H-3α. The proton signal at δ_H3.77 was assigned to H-15β based on direct correlation with carbon signal at δ_C68.7 in the HMQC spectrum and coupling to H-16 signal (δ_H3.92, *d*, *J*=4.5 Hz) in the ^{1}H-^{1}H COSY and TOCSY experiments as well as HMBC correlations between 27-methyl signal at δ_H1.40 and C-15 signal at δ_C68.7. The proton signal at δ_H3.92 (*d*, *J*=4.5Hz) coupled to H-15 signal in the COSY and TOCSY and directly correlated with a carbon signal at δ_C75.6, was assigned to H-16β. The assignment was confirmed by the HMBC correlations between H-16 signal and signals due to C-15, C-17 (δ_C45.8), and C-18 (δ_C42.6). The deshielded signal at δ_H5.45 [*dd*, *J*=14, 6.3, δ_C73.5] was assigned to H-22β based on coupling constant values and HMBC correlations with C-16, C-17, C-21 (δ_C42.1) and C-28 (δ_C63.8). The observed downfield position of H-22β suggested acylation at this position. The oxymethylene proton signals (δ_H3.31, 3.13) appeared in the^{1}H-^{1}H COSY spectrum as an isolated AB system, were assigned to H_2-28 based on HMBC correlations with C-17 and C-18 signals. The olefinic proton signal at δ_H5.43 was assigned to H-12 on the basis of direct correlation with carbon signal at δ_C126.4 and coupling to H_2-11 signal at δ_H1.92 in the ^{1}H-^{1}H COSY spectrum. Based on the above information and comparison with literature data, both **1** and **2** had a triterpene part assigned as A1-barrigenol acylated at C-22 position. The 1D and 2D NMR analysis also revealed the presence of one set of signals due to an angeloyl residue and another set of lower intensity corresponding to a 2-methylbutyroyl group. The characteristic signals of the angeloyl residue were δ_H6.06 [(1H, *q-like*, *J*=7.5Hz, H-3`), $\delta_C$138.2], 1.97 [(3H, *dd*, *J*=7.5, 1.5, H-4`), δ_C16.0], 1.91 [(3H, *d-like*, *J*=1.5 Hz, H-5`), $\delta_C$21.1] along with signals at $\delta_C$169.7 (C-1`), and 130.1(C-2`). These data were very similar to the corresponding ones reported for other structurally related saponins bearing an angelate moiety.[2,5,15,16] The characteristic signals of the 2-methylbutyroyl group were $\delta_H$2.36 [(1H, *sextet*, *J*=7.5, 7.5 Hz, H-2`), δ_C 43.5], 1.52 and 1.77 (each 1H, *m*, H-3`), $\delta_c$28.1], 0.96 [3H, *t*, *J*= 7.0 Hz, H-4`, δ_C12.5] and 1.14 [(3H, *d*, *J*=7 Hz, H-5`), $\delta_C$17.0] together with $\delta_C$177.5 (C-1`).[5] The two ester moieties showed identical HMBC correlations to those previously reported by us for similar saponins bearing the same groups.[5] The HMBC correlations of the carbonyl carbons of the two ester groups at δ_C169.9 and 177.5 and the H-22β proton signal at δ_H5.45 verified acylation at C-22 position of the A1 barrigenol moiety. Consequently, the triterpene parts of **1** and **2** were defined as 22-*O*-angeloyl A1-barrigenol(Tri1) and 22-*O*-(2-methyl butyroyl) A1-barrigenol(Tri2), respectively. The triterpene part 22-*O*-(2-methylbutyroyl)-A1-barrigenol[22-*O*-(2-methylbutyroyl)3β,15α,16α,22α,28-pentahydroxyol] has never been characterized before.

Acid hydrolysis of mixture of **1** and **2** afforded the sugar components D-glucuronic acid, L-arabinose, D-glucose and D-galactose, indicating the glycosidic nature of **1** and **2**. The absolute configurations of these sugars were assigned based on their optical rotation values. The sugar parts of **1** and **2** revealed in the ^{1}H NMR spectrum (Table 1), the

occurrence of two major anomeric proton signals at δ_H5.25 (*d*, *J*=2.0 Hz) and 4.54 (*d*, *J*=7.5 Hz) as well two minor ones at δ_H4.78 (*d*, *J*=9.0 Hz) and 4.66 (*d*, *J*=8.7 Hz). In the HMQC spectrum the signal at δ_H5.25 was directly correlated with two anomeric carbon signals at δ_C111.1 and 110.9 (Table 1), while the signal at δ_H 4.54 was correlated with two anomeric carbon signals at δ_C105.7 and 105.0. The signals at δ_H4.78 and 4.66 were correlated with anomeric carbon signals at δ_C104.0 and 104.7, respectively. The combined use of the ^{1}H-^{1}H COSY and TOCSY spectra allowed the sequential assignments of the proton signals of all sugar units. Based on the assigned protons, the carbon signals of each monosaccharide were identified by the use of HMQC spectrum. Unambiguous assignments of closely related protons and carbons were made possible by the aid of HMBC experiment. The sugar with the anomeric proton signal at δ_H4.54 was assigned to a 2,3-disubstituted β-D-glucuronopyranosyl unit (GlcUA) while the proton signal at δ_H5.25 was assigned to a terminal α-L-arabinofuranosyl unit (Ara(f)). The two anomeric proton signals at δ_H4.78 and 4.66 were assigned to a β-D-glucopyranosyl (Glc) and a β-D-galactopyranosyl (Gal) units, respectively, with more intense signals of Glc compared to those of Gal. The configuration of the anomeric positions of GluUA, Glc and Gal were determined as β from the large coupling constant values between H-1 and H-2 (7.5-9.0 Hz) and their pyranose form was established based on their ^{13}CNMR data. The anomeric configuration of Ara(f) was assigned as α based on small coupling constant value between H-1 and H-2 (2.0 Hz) and its furanose form was deduced from the ^{13}CNMR chemical shift values and down field position of the anomeric proton signal.[5,17,18] The sugar pattern of **1** and **2** were established based on HMBC correlations. The observed correlations of **1** were GlcUAC-1 (δ_c105.0)/ Tri1H-3, GlcC-1(δ_c104.0)/GlcUAH-2(δ_H3.70) and Ara(f)-C1(δ_c111.1)/GlcUAH-3(δ_H3.70). The HMBC correlations of **2** were GlcUA C-1(δ_c105.7)/Tri2H-3, GalC-1(δ_c104.7)/GlcUAH-2(δ_H3.77) and Ara(f)C-1(110.9)/GlcUAH-3(δ_H3.70). These data indicated that both saponins **1** and **2** contained a trisaccharide unit at C-3 position of the triterpene moiety, different only in the nature of the terminal sugar attached to the C-2 of the inner β-D-glucuronic acid unit, being glucose in **1** and galactose in **2**. Therefore, the structures of the two sugar parts of **1** and **2** were elucidated as β-D-glucopyranosyl-(1→2)-[α-L-arabinofuranosyl-(1→3)]-β-D-glucuronopyranosyl and β-D-galactopyranosyl-(1→2)-[α-L-arabinofuranosyl-(1→3)]-β-D-glucuronopyranosyl, respectively. The structures of the triaccharide moieties of **1** and **2** were supported by the mass data. The negative ESIMS/MS spectra of the molecular ions showed fragmentation pattern in agreement with the presence of a trisaccharide chain composed of one pentose, one hexose and one uronic acid units. The first ion at *m/z*1041.5229 displayed two intense peaks at *m/z* 909.4804 [(M-132)-H]$^-$ and 571.4804 [(M-132-162-176)-H]$^-$ while the second ion at *m/z* 1043.5415 showed two corresponding peaks at *m/z* 911.4930 and *m/z* 573.4166

From the aforementioned information, the structures of **1** and **2** were concluded as 3-*O*-β-D-glucopyranosyl-(1→2)-[α-L-arabinofuranosyl-(1→3)]-β-D-glucuronopyranosyl22-*O*-angeloylA1-barrigenol and 3-*O*-β-D-galactopyranosyl-(1→2)-[α-L-arabinofuranosyl-(1→3)]-β-D-glucuronopyranosyl22-*O*-(2-methylbutyroyl)-A1barrigenol, respectively.

The α- glucosidase inhibitory activity of the two saponin mixture was evaluated *in vitro*.[19] The mixture proved to exhibit strong activity with IC_{50} value equals to 13.3 ± 5.0 ppm and IC_{90} value equals to 21.5 ± 8.0 ppm.

EXPERIMENTAL

General

Liquid chromatography-ion trap-top of flight mass spectrometry was performed using Shimadzu Liquid chromatography/ time-of-flight/ mass spectrometry (Shimadzu, Tokyo, Japan) with a Shim-pack XR-ODS column (50 mm x 3.0 mm x 2.2 μm). Solvent gradient consisted of 0.1% formic acid in H_2O (A) and acetonitrile (B). Compounds were eluted into the ESI ion source at the flow rate of 0.4 ml/min with a step gradient of B in A: 10-85% B (0-15 min), 85-10% B (15-18 min), isocratic at 10% B (2 min). Column was maintained at 40°C during the run. Nitrogen gas was used as nebulizer and drying gas with the flow rate set at 1.5 L/min. The ESI source voltage was set at 4.5 kV and the detector was set as 1.5 V. Ionization was performed using a conventional ESI source in the negative ionization mode. Shimadzu's LC-MS solution software was used for data analysis. NMR experiments were performed on a Bruker Avance 700 MHz spectrometer (Bruker BioSpin Corporation, Billerica, MA). ^{1}H, ^{13}C, HMQC, HMBC, COSY and TOCSY NMR spectra were acquired in CD_3OD at 700 MHz for proton and 175 MHz for ^{13}CNMR. Chemical shifts are given as δ values with TMS as internal standard. Optical rotations were measured with jasco p-2000 polarimeter. Preparative HPLC was carried out on Interchim4100 (Montlucon, France) (column, RP C-18 HQ; solvent system, CH_3CN-H_2O (33:67-70:30); Flow rate 20 ml/min; Detection, UV, 205 nm; temperature, 35^{0}C).

Plant material

H. pendula seeds were collected from the zoological garden in Giza, Egypt in September 2014. Plant identification was confirmed by Mrs. T. Labib, head specialist for plant identification in El-Orman public garden, Giza, Egypt. A Voucher specimen was deposited in the Herbarium of NRC (CAIRC).

Extraction and isolation

Air-dried seeds of *H. pendula* (100 g) were defatted with *n*-hexane then extracted with MeOH until exhaustion. The combined MeOH extract was evaporated under vacuum to dryness. The residue (4.5 g) was suspended in water and partitioned with EtOAc (5x100ml) then with water saturated *n*-BuOH (5x100 ml). Each combined fraction was individually evaporated under reduced pressure to yield 1.5 g EtOAc fraction and 2.1 g *n*-BuOH fraction. The *n*-BuOH fraction was dissolved in H_2O (0.002) and the H_2O solution was passed through a column chromatography packed with 100 g Diaion HP-20 polymer gel (Mitsubishi). After washing the column with distilled water, elution was carried out with 25%,50%, 75% aqueous MeOH and finally with 100% MeOH. The collected fractions were examined by silica gel TLC (Merck) using solvent systems *n*-BuOH-EtOH-NH_4OH (7: 2: 5) and $CHCl_3$-MeOH-H_2O (60: 30: 5) then visualized by spraying with 20% sulphuric acid in MeOH followed by heating at 110^{0}C. Based on TLC analysis, similar fractions were then combined. Fractions eluted with 75% and 100% MeOH were found similar and contained saponin constituents. The combined saponin fraction (1.35 g) was applied on a column chromatography packed with silica gel (60 g) and eluted with EtOAc -MeOH-H_2O with increasing polarity (30:2:1 - 5:2:1). A total of 50 fractions 50 ml each were collected. Similar fractions were combined after TLC analysis to give eight sub-fractions (A-H). The sub-fraction E (80 mg) eluted with EtOAc-MeOH-H_2O (15:2:1) was subjected to repeated HPLC to afford an inseparable mixture of **1** and **2** in the ratio 2:1.

Saponin 1

HR-ESI-MS m/z 1041.5229 [$C_{52}H_{82}O_{21}$-H]$^-$, calculated 1041.5276; ^{1}H and ^{13}CNMR (Table 1).

Saponin 2

HR-ESI-MS m/z 1043.5415 [$C_{52}H_{84}O_{21}$-H]$^-$, calculated 1043.5441; ^{1}H and ^{13}CNMR (Table 1).

Acid hydrolysis

The mixture of **1** and **2** (5 mg) in 1.5N HCl (5 ml) was heated at 100°C for 4h. After cooling, the aqueous layer was extracted with CH_2Cl_2 then neutralized by repeated addition of MeOH followed by evaporation. The sugars obtained were detected against authentic samples by TLC conducted on HPTLC using solvent system 1-propanol-EtOAc-H_2O (4.0, 0.5, 0.5). The chromatogram was visualized by spraying with aniline hydrogen phthalate reagent and heating at 110°C till the colour of the spots appeared. D-Glucuronic acid, L-arabinose, D-glucose and D-galactose were detected. The hydrolysis process was repeated using 40 mg of **1** and **2** and the obtained sugars were separated by preparative TLC using the solvent system mentioned above. The optical rotations of the isolated sugars were measured and confirmed by comparison with the literature values.

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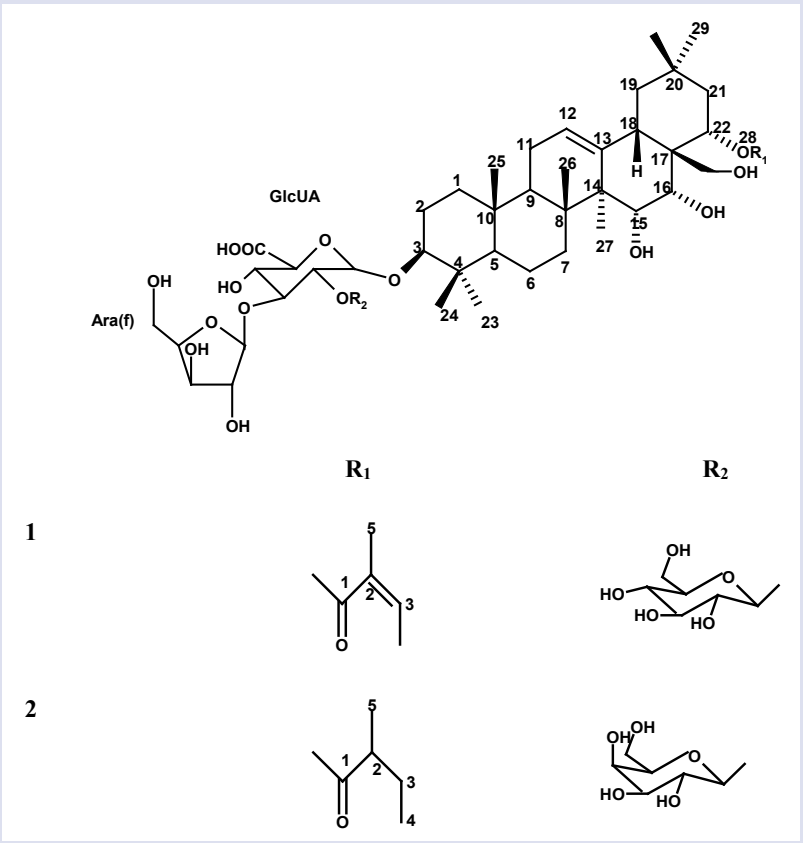
GRAPHICAL ABSTRACT



SUMMARY

Two new triterpenoid saponins named pendulaoside C and D, were obtained from *Harpullia pendula* seed methanolic extract. Their structures were established by spectral and chemical means.

ABOUT AUTHORS

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