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Saponins isolated from *Polyscias guilfoylei* F. Araliaceae.

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ABSTRACT

The saponins are chemical compounds present in several plants that have a different biological activity. This study based on the importance of these compounds in traditional medicine. Three saponins were isolated by chromatographic methods (CC, PC, PTLC and HPLC) from *Polyscias guilfoylei*. The isolated compounds identified by ^{1}HNMR, ^{13}CNMR and HMQC as: 3-O-[β-D-glucopyranosyl (1→3) β-D-glucuronopyranosyl-6`-methyl ester] oleanolic acid-28-O-methyl ester, 3-O- β-D- glucuronopyranosyl-6`-methyl ester oleanolic acid – 28 – O – β – D – glucopyranosyl and 3-O-[β-D-glucopyranosyl (1→3) β-D-glucuronopyranosyl-6`- methyl ester] oleanolic acid – 28 – O - β – D -glucopyranosyl ester. It is the first time to separate compounds 1, 2 and 3 from genus *Polyscias*.

Keywords: *Polyscias guilfoylei*, Araliaceae, triterpene, saponins.

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INTRODUCTION

Araliaceae is one of the most important plant families rich in saponins. Genus polyscias contains ornamental plants which usually grow as shrubs. Different *polyscias* species[1-9] were subjected to phytochemical study revealing the presence of saponins as the main constituents. Genus *Polyscias* was used in traditional medicine as anti-inflammatory [10], anti-toxin, anti-bacterial, diuretic and anti-dysentry[3].

The biological activity studied for *polyscias* species showed weak cytotoxicity [8], molluscicidal and schistosomicidal activities [11] as well as immuno-stimulant activity [12].

MATERIALS AND METHODS

Plant Material

Samples of the aerial parts of *Polyscias guilfoylei* were collected from Orman Garden, Dokki, Cairo, Egypt in January, 2007. The plant was identified by Dr. Trease Labib, the head specialist at the Orman Garden, Where a voucher specimen was deposited at Faculty of Pharmacy Helwan University.

Extraction and isolation

The defatted air dried powdered mixture of leaves and stems of *P. guilfoylei* (850 g) are extracted three times (3 x 2L) with methanol at room temperature. The concentrated combined extract (36 g) was dissolved at the least amount of methanol and added to a large amount of acetone to precipitate the crude saponin (10 g). The crude saponin mixture (10 g) is passed over porous polymer gel column (Misubishi Diaion HP-20). Elution is carried out using H_2O, MeOH: H_2O (1: 1) and finally by MeOH to afford saponin mixture (3.7 g). An aliquot of the saponin mixture (2 g) was chromatographed on a silica gel column eluted using a gradient of $CHCl_3$-MeOH (90:10) to $CHCl_3$-MeOH- (50:50).

Fractions eluted by $CHCl_3$-MeOH (70:30) (40mg) and fractions eluted by $CHCl_3$-MeOH-H_2O (70:30:3) (1.75 g) were collected to give fraction I and fraction II respectively. They were examined on TLC plates using solvent system chloroform: methanol: water (6.5: 3: 0.5) and spray reagent 10% sulphuric acid. Fraction (I) is subjected to preparative thin layer chromatography using chloroform: methanol: ethyl acetate: water (28:30:35:5) to give compound 1 (35 mg).

Compound 1 on mineral acid hydrolysis at 100ºC gave the sugar components glucuronic acid and glucose in the produced aqeous hydrolysate in addition to oleanolic acid as aglycone in the chloroform layer. While mild acid hydrolysis, using 0.1N HCl gave glucose only, thus establishing its position as the terminal sugar and glucuronic acid as the inner one in the ether chain. They were identified by PC using solvent system n-butanol: acetic acid: water (4:1:5) and aniline phthalate as spray reagent.

While fraction (II) gave two major spots with Rf = 0.14 and R_f = 0.10 is fractionated on a reversed phase ODS column using H_2O -MeOH (70:30) with gradient decrease in polarity. Fractions eluted with H_2O - MeOH 65:35 were collected and examined on silica gel TLC plate using solvent system chloroform: methanol: water (6.5: 3: 0.5) and 10% sulphuric acid as spray reagent. It showed nearly one spot with R_f value 0.14 which is compound 2 (55.0 mg).

Compound 2: was purified on a reversed phase ODS column after isolation from the saponin fraction by CC and finally purified by HPLC. On alkaline hydrolysis compound 2 gave only glucose unit indicating glycosylation on C-28 carboxylic group and on acid hydrolysis it yielded glucuronic acid and glucose. They were identified by PC using solvent system n-butanol: acetic acid: water (4:1:5) and aniline phthalate as spray reagent. While the other spot (120.0 mg) that obtained from the fraction II was purified on silica gel PTLC plate using solvent system chloroform: methanol: ethyl acetate: water (28:30:35:5) and spray reagent 10% sulphuric acid in methanol to give compound 3. Final purification of compound 3 was achieved by HPLC technique.

Compound 3**:** white amorphous powder, R_f = 0.10 on solvent system chloroform: methanol: water (6.5: 3: 0.5). Acid hydrolysis of this compound afforded the monosaccharides glucose and glucuronic acid.

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Alkaline hydrolysis yielded glucose as the sole sugar component, indicating that this sugar is linked to carboxylic function. Consequently, the remaining sugar must be connected through ether linkage. Mild acid hydrolysis, using 0.1N HCl furnished glucose, thus establishing its position as the terminal sugar and glucuronic acid as the inner in the ether chain.

RESULTS AND DISSCUSION

The saponin fraction obtained from the acetone precipted of the aqueous methanolic extract of the *Polyscias guilfoylei* after CC, repeated PTLC and HPLC.

Table 1: ^{13}C Chemical shifts of saponins in pyridine-d5

C	Compound 1	Compound 2	Compound 3	C	Compound 1	Compound 2	Compound 3
1	38.6	38.7	38.7	**16**	23.7	23.8	23.8
2	26.5	26.6	26.6	**17**	47.0	47.1	47.1
3	89.3	89.2	89.4	**18**	41.8	41.8	41.8
4	39.5	39.5	39.5	**19**	46.1	46.3	46.3
5	55.7	55.8	55.8	**20**	30.8	30.8	30.8
6	18.5	18.5	18.5	**21**	34.0	34.1	34.1
7	33.1	33.2	33.2	**22**	32.8	32.6	32.6
8	39.7	40.0	40.0	**23**	28.1	28.2	28.1
9	49.9	48.1	48.1	**24**	16.9	16.9	16.9
10	37.0	37.0	37.0	**25**	15.4	15.5	15.6
11	23.4	23.5	23.5	**26**	17.2	17.5	17.5
12	122.9	122.9	122.9	**27**	26.2	26.1	26.2
13	144.2	144.2	144.2	**28**	178.0	176.4	176.5
14	42.0	42.2	42.2	**29**	23.7	23.7	23.7
15	28.1	28.3	28.3	**30**	33.2	33.2	33.2
				Methyl	51.6	-	-
1`	106.7	107.3	105.9	**1```**	-	-	95.8
2`	75.6	75.4	74.2	**2```**	-	-	74.2
3`	87.5	78.0	87.5	**3```**	-	-	79.3
4`	71.5	73.2	71.7	**4```**	-	-	71.3
5`	78.3	77.3	76.6	**5```**	-	-	78.9
6`	170.2	170.8	170.2	**6```**	-	-	62.3
Methyl	52.1	52.0	52.2				
1``	105.9	95.8	106.7				
2``	74.1	74.2	75.6				
3``	78.7	79.3	78.7				
4``	71.7	71.3	71.5				
5``	76.6	78.9	78.3				
6 ``	62.5	62.3	62.6				

Compound 1: The ^{1}HNMR spectrum of compound 1 in pyridine d_5 showed, seven methyl singlet's at δ 0.83, 0.85, 0.92, 0.94, 0.98, 1.20, 1.29, a double of doublet at δ 3.36 (J= 4.0 Hz) typical for an axial oxymethine proton, a triplet at δ 5.37 (J=3.0 Hz); all are characteristic for a triterpene moiety of olean-12-ene type. The ^{13}CNMR spectrum of compound 1 (table 1) showed signals due to the aglycone moiety very similar to those of oleanolic acid [13-15] except the down field shift of C-3 at δ 89.3 and an additional signal at δ 51.6 due to the methyl ester group at C-28. This conclusion was further substantiated by the presence of two anomeric protons doublets at δ 5.31 (J=8.0 Hz) and δ 4.94 (J=7.4 Hz) assigned to a β-glucose and a β-glucuronic acid respectively in the ^{1}HNMR spectrum of compound 1. This assignment based on the multiplicity and coupling constant values of the proton signals extracted from the sugar area by the aid of ^{1}H-^{1}H COSY. Their corresponding carbons appeared in the HMQC spectrum at δ 105.9 and 106.7 for glucose and glucouronic acid respectively. The assigned ^{13}CNMR chemical shift values implied that the sugar units were in the pyranose

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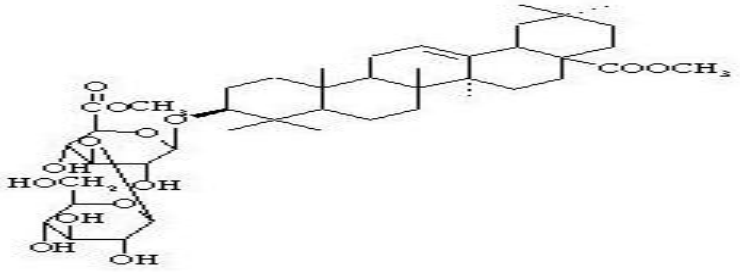
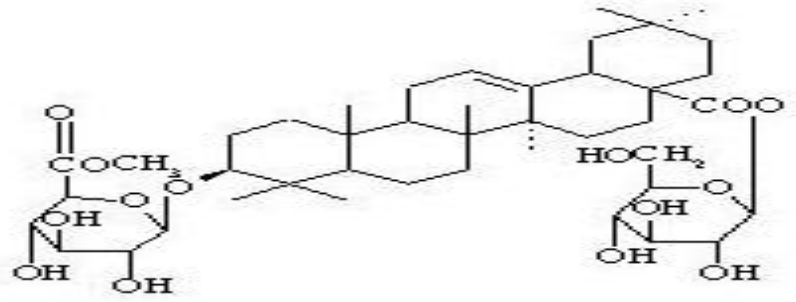
form with chemical shift signals for β-glucuronopyranose at δ 24.1, 87.5, 71.5, 76.6, 170.2 and for β-glucopyranose unit at δ 75.6, 78.7, 71.7, 78.3, 62.5. Comparison of the carbon signals in compound 1 with those reported in the literature for unsubstituted methyl glucuronic acid moiety [16] indicated glycosylation shift for C-3 (δ 87.5) of the β-glucuronopyranose unit and methylation of its carboxylic group(δ 52.1). Therefore the β-glucopyranose unit must be attached to C-3 of the inner β-glucuronopyranose one which was in turn connected to the aglycone C-3 position. The interglycosidic linkages were further confirmed by the long-range coupling in the HMBC spectrum between signals due to the aglycone C-3 (δ 89.3) and H-1 (δ 4.94) of the β-glucouronopyranose unit, C-3 (δ 87.5) of the β-glucouronopyranose and H-1 (δ 5.31) of the β-glucopyranose unit. The individual spin systems for the individual monosaccharide units were demonstrated from the HOHAHA spectrum and the sequqnce of the protons was then deduced from ^{1}H-^{1}H COSY spectrum. On the basis of the assigned protons, the ^{13}C resonances of each sugar unit were identified from the HMQC spectrum and confirmed by HMBC experiment. Compound 1 is assigned the structure of 3-O-[β-D-glucopyranosyl (1→3) β-D-glucuronopyranosyl-6`-methyl ester] oleanolic acid-28-O-methyl ester. The known compound 1 is identified by analysis of their spectral data of their methyl esters as spinasaponin A and by comparison with literature values[17,18].

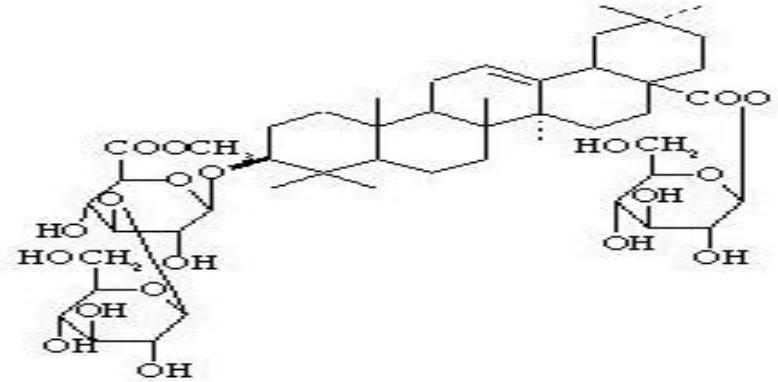


Compound 2 showed in its ^{1}HNMR spectrum, seven methyl signals at δ 0.84, 0.89, 0.91, 0.97, 1.09, 1.26, 1.29 and a double of doublet at δ 3.37 (J=12.0, 4.0Hz) typical for an axial oxymethine proton, a triplet at δ 5.42 (J=3.0Hz); all are characteristic for a triterpene moiety of olean-12-ene type [13-15]. In addition, two anomeric proton signals at δ 6.32 (d, J=7.8Hz) and 4.98 (d, J=9.0Hz), three protons singlet at δ 3.73 were observed suggesting the presence of β-D-glucose and β-D-glucuronic acid with methyl ester residue respectively. The ^{13}CNMR spectrum of saponin 2 (table 1) showed signals due to the aglycone moiety similar to those of the oleanolic acid [13-15] except the down field shift and the upfeild shift of the carbon signals due to C-3 (δ89.2) and C-28 (δ176.4) respectively indicating glycosylation at these positions. This conclusion was further substantiated by the presence of two anomeric carbon signals at δ 107.3 and 95.8 with their corresponding proton doublets at δ 4.98 and 6.32 in the ^{1}HNMR spectrum for glucuronic acid and glucose units respectively. The β-glucopyranose unit esterified the C-28 carboxylic acid group of the oleanolic acid based on the position of its anomeric center and the chemical shift values of their carbon signals at δ 95.8, 74.2, 79.3, 71.3, 78.9, 62.3. Therefore the other sugar signals must be related to the β-D-glucuronopyranosyl which was in turn connected to the aglycone C-3 position. The carbon chemical shift value of the β-D-glucuronic acid resonating at δ 107.3, 75.4, 78.0, 73.2, 77.3, 170.8 as well as a signal at δ 52.0 assigned to a carbomethoxy group (3.73 ppm in its ^{1}HNMR) confirming the presence of glucuronic acid methyl ester residue. In comparison of the ^{1}HNMR and the ^{13}CNMR spectra of compound 2 to the corresponding value in related compounds with a free methyl glucuronic acid moiety [19]. Therefore, the known compound 2 was assigned the structure of 3-O-β-D- glucuronopyranosyl-6'-methyl ester oleanolic acid – 28 – O – β – D - glucopyranosyl. Compound 2 was previously identified from the spectral data of their methyl esters as Chikusetsusaponin Iva[19].



Compound 3: The ^{1}HNMR spectrum of compound 3 in pyridine-d_5 indicated that it is a bidesmosidic triglycoside of oleanolic acid and exhibited seven singlet signals due to seven methyl groups at δ 0.82, 0.88,

0.90, 0.94, 1.06, 1.25, 1.26, a broad triplet vinyl proton at δ 5.40 (t, J=2.5Hz) and carbinylic proton at δ 3.32 (dd, J=3.5, 12 Hz). It is also showed three anomeric proton signals at δ 6.28 (d, J=8.0Hz), 5.27 (d, J= 7.8Hz) and 4.91 (d, J=7.4Hz). The position of ^{13}CNMR signals of the genin part in compound 3 (table 1) compared to that of compound 2, showed close similarity indicating it is a bidesmosidic saponin of oleanolic acid type with glycosidation at C-3 (δ 89.3) and C-28 (δ 176.4) of the aglycone. The ^{13}CNMR spectrum of compound 3 also showed signals due to seven Sp3 carbons at δ 15.5, 16.9, 17.5, 23.8, 28.1, 28.3 and 33.1 as well as two Sp2 carbons at δ 122.9 and 144.2. Also, in the ^{13}CNMR spectrum, the sugar signals linked to C-3 was identified for β-D-glucuronopyranosyl from the signals at δ 105.9, 74.2, 87.5, 71.7, 76.6, 170.2 and 52.2 for the carbomethoxyl group and for β-D-glucopyranosyl from the signals at δ 106.7, 75.6, 78.7, 71.5, 78.3, 62.6. The sugar unit at C-28 was identified as β-D-glucopyranosyl from the signals at δ 95.8, 74.2, 79.3, 71.3, 78.9, 62.3. Comparison of the ^{13}C NMR data of the sugar unit of compound **3** attached at C-3 with that of compound 1 revealed identical structure of the disaccharide chain at C-3 as β-D-glucopyranosyl (1→3) β-D-glucuronopyranosyl - 6'- methyl ester and with triglycosidic oleanolic acid compounds [20] with glucose unit at C-28. The above studies established the structure of the known compound 3 as: 3-O-[β-D-glucopyranosyl (1→3) β-D-glucuronopyranosyl-6'- methyl ester] oleanolic acid – 28 – O - β – D -glucopyranosyl ester. Compound 3 was previously separated and identified by means of their spectral analysis [21].



REFERENCES

[1] Paphassarang S, et al. Phytochem 1989; 28(5):1539-41.
[2] Paphassarang S, et al. J Nat Prod 1990; 53(1):163-166.
[3] Huan VD, et al. Phytochem 1998; 47(3): 451-457.
[4] Chaboud A, et al. Pharmazie 1995; 50(5): 371..
[5] Proliac J, et al. Pharmazie 1996; 51(8): 611-612.
[6] Mitaine-Offer AC, et al. Biochem Systemat Ecol 2004; 32: 607-610.
[7] Bedir E et al. J Nat Prod 2001; 64(1): 95-97.
[8] Chaturvedula VSP et al. Planta Medica 2003; 69(5): 440-444.
[9] Gopalsamy N, et al. Phytochem 1990; 29(3):793-795.
[10] Paphassarang S et al. J Nat Prod 1989; 52(2):239-42.
[11] Jacqueline E, et al. Egypt J Pharm Sci 2007; 48: 87-97.
[12] Divakar MC, et al. Indian J Nat Prod 2005; 21(3): 7-14.
[13] Kizu H, Tsuyoshi T. Chem Pharm Bull 1980; 28 (9): 2827-2830.
[14] Budzi KH, et al. J Am Chem Soc 1963; 85: 3688-3699.
[15] Shama M, et al. J Org Chem 1962; 27: 4512-4517.
[16] Warashina T, et al. Chem Pharm Bull 1991; 39: 388-396.
[17] Cong X, et al. Phytochem 1990; 29: 595-599.
[18] Chennlii R et al. Phytochem 1987; 26: 1785-1788.
[19] Nei RL, et al. Planta Med 1984; 50(4): 322-327.
[20] Back K, Pedersen C. Adv Carbohyd Chem Biochem 1983; 41: 27-66.
[21] Romussi G, et al. Arch Pharm 1987; 320: 153-158.