RSC Advances

Cytotoxic Saponin Poliusaposides from Teucrium polium

Journal:	*RSC Advances*
Manuscript ID:	RA-ART-02-2015-002713.R1
Article Type:	Paper
Date Submitted by the Author:	06-Mar-2015
Complete List of Authors:	Elmasri, Wael; Texas Tech University, Chemistry and Biochemistry Hegazy, Mohamed Elamir; national Research Center, Mechref, Yehia; Texas Tech University, Chemistry and Biochemistry Pare, P. W.; Texas Tech University, Chemistry; Texas Tech University,

RSC Advances

RSCPublishing

ARTICLE

Cytotoxic Saponin Poliusaposide from *Teucrium polium*

Wael A. Elmasri,[a] Mohamed-Elamir F. Hegazy,[b] Yehia Mechref,[a] and Paul W. Paré [a†]

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Three saponin glycosides have been isolated and characterized from *Teucrium polium* L. (*Lamiaceae*). Compounds were isolated from a MeOH aerial plant extract. Structures were elucidated based on spectroscopic methods including UV, IR, 1D and 2D NMR, and HRESIMS data analyses. Identified compounds were evaluated for anticancer against 60 human tumor cell lines (NCI). The triterpene glycoside, poliusaposide C completely inhibited growth of a breast and colon cancer cell line and partially inhibited growth of a colon, renal and melanoma cell line. Structure-anticancer activity relationships are discussed.

Introduction

Teucrium polium is a member of family *Lamiaceae* with more than 300 species included in the genus *Teucrium*. Members of the genus are rich in sterols, saponins, polyphenol metabolites.[1,2] *Teucrium* have shown wide range of therapeutic activities including antibacterial[3] and anticancer agents.[4] *Teucrium polium* has many pharmacological actions such as antibacterial, antioxidant,[5] and anticancer.[4] The alcoholic extract inhibits proliferation and colonization of human carcinomas such as breast (BT20), lung (A549), and adenocarcinoma (MCF-7) cell lines.[4] Although *T. polium* contains many pharmacologically active metabolites including phenylpropanoid glycosides, iridoid glycosides, flavonoids,[6,7,8] and terpenoids[9,10] chemical investigations have yet to report on the presence and/or biological activity of *T. polium* saponins.

Saponins are a structurally related heterosides consisting of a steroid or triterpenoid backbone linked to a sugar moiety via one or multiple glycosidic linkages. The carbohydrate moiety consists of one or more hexoses, pentoses and/or uronic acids.[11] According to their aglycone skeleton, saponins can be classified as either steroidal saponins, non-steroidal saponins or steroidal amines.[11] Saponin glycosides have many traditional uses and industrial applications.[12,13] These glycosylated dervatives are responsible for many pharmacological actions including anthelmintic, antidiabetic, anticancer,[14] antileishmanial,[15-18] nematocidal,[19] antibacterial,[20] anti-inflammatory,[21] antioxidant,[22] and cytotoxic[11] activities. Saponins have shown cytotoxicity against a variety of human tumor lines including leukemia, esophageal, liver, gastric, lung, and colon.[23-29] Since several effective anticancer agents including paclitaxel, camptothecin,[4] vinblastine, and vincristine[23] have been discovered through phytochemical screening of medicinal herbs, a similar strategy is being employed here to mine for new anticancer agents.

The US National Cancer Institute (NCI) has developed a 60 tumor cell line screen to assay potential anticancer drugs. The nine panels represent tumor cell lines including: leukemia, melanoma, ovarian, breast, colon, lung, CNS, renal, and prostate. This screen has identified drug leads in the development of anticancer therapies.[30] Here are reported three saponin glycosides isolated and chemically characterized from *T. polium*; chemical analysis was performed by NMR- and mass-spectroscopy with cell cytotoxicity reported.

Results and discussion

T. polium aerial parts were extracted with CH_2Cl_2-MeOH and partitioned using a gradient of *n*-hexane, CH_2Cl_2, and MeOH. Compounds from the eluted fractions were purified using a combination of Sephadex LH-20 and silica gel CC as well as RP-HLPC. Compounds **1-3** were fully characterized.

Poliusaposide A (**1**) exhibited a $[M+Na]^+$ quasi-molecular ion peak at *m/z* 1595.6715 in HRESIMS

(calc. 1595.6724), which in conjunction with ^{13}C NMR data suggested a molecular formula of $C_{71}H_{112}O_{38}Na$. The IR spectrum showed a hydroxyl and an ester band at 3399 cm^{-1} and 1700 cm^{-1}, respectively. Physicochemical properties and spectral features indicated a triterpenoid saponin. From a total of 71 carbons, 30 were assigned to the triterpenoid aglycone and 41 to the oligosaccharide moieties (Tables 1 and 2). On acid hydrolysis, **1** gave D-apiose (Api), L-rhamnose (Rha), L-arabinose (Ara), D-xylose (Xyl), and D-glucose (Glc), as sugars component identified by TLC and GC analyses. The ^{1}H and ^{13}C NMR spectra exhibited signals for an olefinic proton at δ 5.36 (br*s*, H-12) and six methyl singlets, with six methyl groups at δ_H 1.26 (*s*, H_3-24), 1.23 (*s*, H_3-25), 0.76 (*s*, H_3-26), 1.39 (*s*, H_3-27), 0.87 (*s*, H_3-29) and 0.95 (*s*, H_3-30) showing correlations in the HMQC with their corresponding carbons at δ_C 13.6 (C-24), 17.2 (C-25), 17.7 (C-26), 27.2 (C-27), 33.3 (C-29), and 25.0 (C-30), respectively. The combined spectral data was consistent with $^{\Delta}12$ oleanene skeleton (Table 1).[31] Other prominent functional groups identified included signals of three oxygen-bearing methine protons at δ_H 4.32 (br*s*, H-2), 4.01 (br*d*, H-3), and 4.01 (br*d*, H-18) as well as two carbonyls at δ_C 180.8 and 176.9. The downfield chemical shift at δ_C 180.8 is indicative of an unsubstituted carboxylic group. Overall, NMR data was indicative of zahnic acid as the aglycone,[31] which is supported by HRESIMS ion peak at *m/z* 517.3133 in negative ion mode. The chemical shift values at δ_C 87.1 (C-3) and 176.9 (C-28) suggested that the saponin was a bisdesmosidic glycoside with saccharide units attached to listed positions. The presence of seven sugar residues was deduced from signals for seven anomeric carbons at δ_C 95.5, 101.1, 103.3, 103.7, 104.4, 105.6, and 107.5 correlated with δ_H 5.41,5.42,4.48, 4.51, 4.59, 4.61 and 4.45, respectively, in the HMQC spectrum. Two 6-deoxyhexoses were proposed based on two methyl carbon at δ_C 17.9 and 18.1, and five hexoses and/or pentoses were proposed based on five carbon signals between δ_C 61.2 and 66.9. The ring protons of the seven sugars were assigned starting from the readily identifiable anomeric protons by means of ^{1}H-^{1}H COSY, TOCSY, HMQC, and HMBC experiments. Units of one *β*-D-glucopyranoside (Glc-1), one *β*-D-glucopyranoside-2-Ac (Glc-2), one *α*-L-arabinopyranoside (Ara), two *α*-L-rhamnopyranoside (Rha-1 and Rha-2), one *β*-D-xylopyranoside (Xyl), and *β*-D-apiofuranoside (Api) were identified based on acid hydrolysis followed by TLC and GC analyses. Sugar

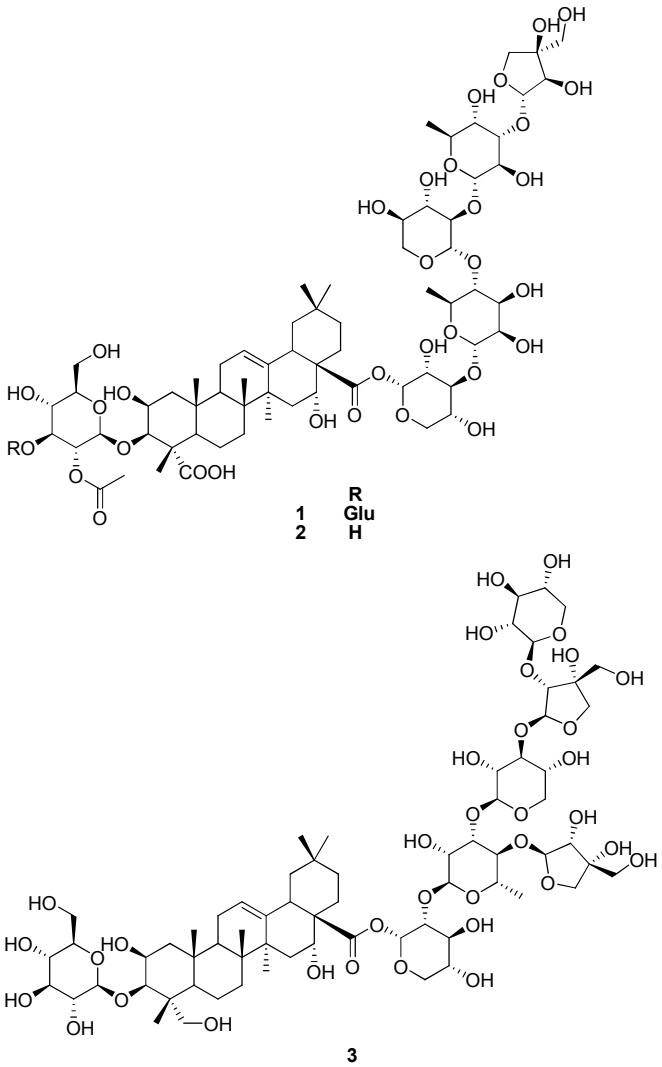
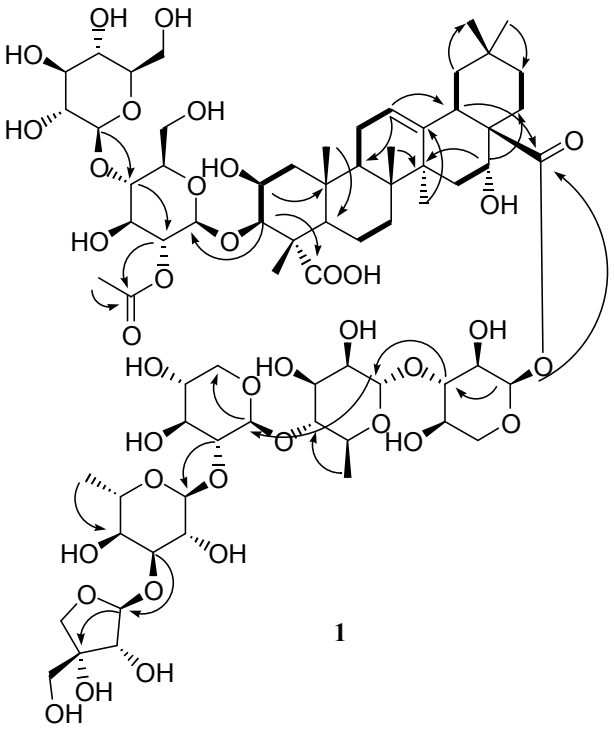
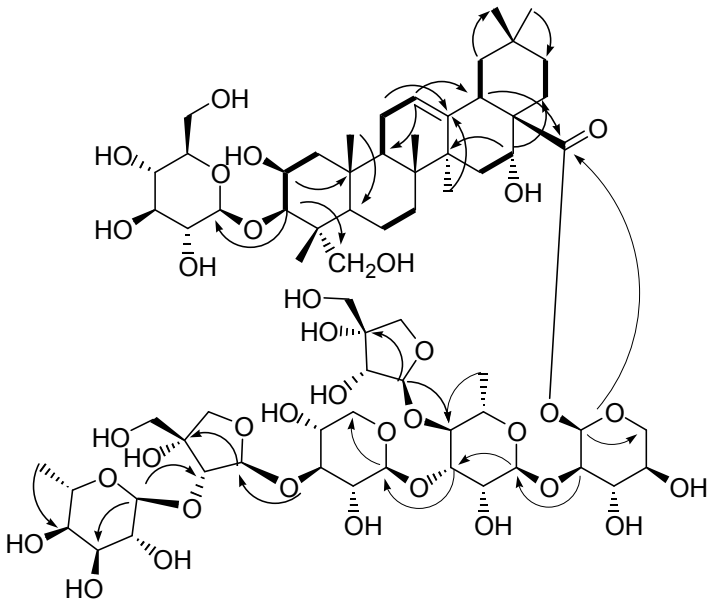


Figure 1: Chemically identified metabolites **1-3**

sequencing was established by analysis of HMBC and NOESY experiments as previously reported.[32] Cross-peaks between C-28 (δ_C 176.9) of the zahnic acid aglycone and H-1of Ara (δ_H 5.59), indicated that the Ara residue was linked at C-28 through an ester linkage. HMBC correlations between δ_H 5.59 (Ara H-1) and δ_C 75.4 (Ara C-3), which in turn correlated with δ_H 5.02 (C-1 of Rha-1) indicated that Rha-1 was linked to Ara by a (1→3) linkage. Correlations between δ_H 4.51 (Xyl H-1) and δ_C 83.1 (Rha-1 C-4) that in turn correlated with δ_H 1.23 (Rha-1 H-6) indicated a Xyl(1→4)-Rha-1 linkage. The ^{1}H-^{1}H COSY cross peak between δ_H 4.51 (Xyl H-1) and δ_H 3.82 (Xyl H-2) as well as HMQC established (Xyl C-2). HMBC correlations between (Xyl C-2) and δ_C 101.3 (Rha-2 H-1) established a Rha-2(1→2)-Xyl linkage. HMBC correlations from δ_H 5.26 (Rha-2 H-1) to δ_C 79.9 (Rha-2 C-3) and from (Rha-2 C-3) to δ_H 5.1 (Api H-1) indicated a Api(1→3)-Rha-2 linkage. The partial



1



3

Figure 2: HMBC and COSY correlations, shown as arrows and thick lines respectively, for **1** and **3**.

sequence of the glycosyl ester chain at C-28 was characterized as Api(1→3)-Rha-2(1→2)-Xyl(1→4)-Rha-1(1→3)-Ara(1→28)-Agly. HMBC cross-peaks between C-3 of the aglycone and δ_H 4.54 (Glc-1 H-1) indicated a Glc(1→3)-Agly linkage. HMBC correlations between δ_H 3.67 and δ_C 61.2 (Glc-1 C-6) and 74.0 (Glc-1 C-2), established (Glc-1 H-4) and a Glc-2(1→4)-Glc-1 linkage was established based on a correlation between (Glc-1 C-4) and 104.3 suggesting a Glc-2(1→4)-Glc-1(1→3)-Agly linkage. Thus the structure was elucidated as 3-*O*-[*β*-D-glucopyranosyl-2-acetate(1→4)-*β*-D-glucopyranosyl]-28-*O*-[*β*-D-apiofuranosyl(1→3)-*α*-L-rhamnopyranosyl(1→2)-*β*-D-xylopyranosyl(1→4)-*α*-L-rhamnopyranosyl(1→3)-*α*-L-arabinopyranosyl]zanhic acid ester.

Poliusaposide B (**2**) showed a $[M+Na]^+$ quasimolecular ion peak at *m/z* 1433.6102 in HRESIMS (calc. 1433.6195), which in conjunction with ^{13}C NMR data suggested a molecular formula of $C_{65}H_{102}O_{33}Na$. The IR spectrum showed hydroxyl and ester bands at 3391 cm^{-1} and 1730 cm^{-1}, respectively. Physicochemical properties and spectral features indicated a triterpenoid saponin. On acid hydrolysis, **2** gave D-apiose (Api), L-rhamnose (Rha), L-arabinose (Ara), D-xylose (Xyl), and D-glucose (Glc) as component sugars by TLC and GC analyses. ^1H and ^{13}C NMR showed signals for an olefinic proton at δ 5.34 (br*s*, H-12) and six methyl groups at δ_H 1.26 (*s*, H_3-24), 1.23 (*s*, H_3-25), 0.75 (*s*, H_3-26), 1.39 (*s*, H_3-27), 0.86 (*s*, H_3-29) and 0.94 (*s*, H_3-30). In addition to three oxygen-bearing methine protons at δ_H 4.32 (br*s*, H-2), 4.01 (br*d*, H-3), and 4.01 (br*d*, H-18), two carbonyls were observed at δ_C 180.8 and 176.9. Overall, NMR data of **2** was similar to poliusaposide A except for an absence of a terminal glucose in **2**. Based on that, the structure was elucidated as 3-*O*-[*β*-D-glucopyranosyl-2-acetate]-28-*O*-[*β*-D-apiofuranosyl(1→3)-*α*-L-rhamnopyranosyl(1→2)-*β*-D-xylopyranosyl(1→4)-*α*-L-rhamnopyranosyl(1→3)-*α*-L-arabinopyranosyl]zanhic acid ester.

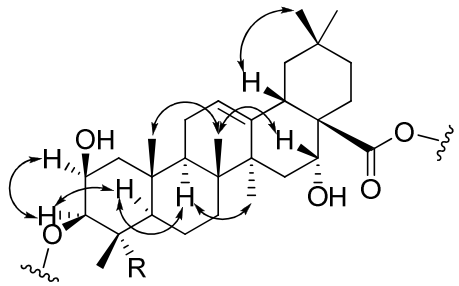


Figure 3: NOESY correlations for the triterpenoid aglycone unit

Table 1: ^{1}H and ^{13}C NMR data of **1-3** (δ in ppm, J in Hz) (400 MHz, methanol-d_4); signals were assigned on the basis of DEPT, ^{1}H-^{1}H COSY, HMQC, HMQC-TOCSY and HMBC experiments.

Position	**1**		**2**		**3**	
	^{1}H	**^{13}C**	**^{1}H**	**^{13}C**	**^{1}H**	**^{13}C**
1	1.26	44.4	1.26	44.7	1.15	44.4
	2.12		2.10		2.08	
2	4.32, br*s*	71.3	4.33, br*s*	71.0	4.28, br*s*	71.0
3	4.01	87.1	4.04	86.9	3.68	83.8
4		42.7		42.7		43.1
5	1.51	53.2	1.53	53.1	1.48	48.1
6	1.14	21.5	1.14	21.5	1.15	18.8
7	1.89	31.8	1.89	31.9	1.91	31.9
	1.72		1.73		1.68	
8		41.0		41.0		40.7
9	1.62	48.5	1.60	48.7	1.61	48.3
10		37.3		37.2		37.4
11	1.98	24.5	1.98	24.5	1.91	24.6
12	5.36, br*s*	123.5	5.34, br*s*	123.4	5.29, br*s*	123.5
13		144.5		144.5		144.7
14		41.0		40.7		42.9
15	1.14	36.3	1.14	36.3	1.15	36.5
	1.90		1.89		1.91	
16	4.47, *s*	74.4	4.47, *s*	74.4	4.43, *s*	74.6
17		53.3		53.3		51.0
18	3.02, br*d*	41.9	3.03, br*d*	41.9	2.92, br*d*	42.1
19	1.14	47.6	1.14	47.5	1.15	47.9
	2.25		2.25		2.26	
20		31.2		31.2		31.2
21	1.39	36.1	1.39	36.1	1.42	36.3
	1.72		1.73		1.72	
22	1.49	33.7	1.50	33.7	1.48	33.6
	1.26		1.26		1.26	
23		180.8		180.8		65.5
24	1.26, *s*	13.6	1.26, *s*	13.6	0.95, *s*	15.0
25	1.22, *s*	17.2	1.23, *s*	17.2	1.30, *s*	17.7
26	0.76, *s*	17.7	0.75, *s*	17.7	0.78, *s*	17.9
27	1.39, *s*	27.2	1.39, *s*	27.2	1.39, *s*	27.3
28		176.9		176.9		177.0
29	0.87, *s*	33.3	0.86, *s*	33.3	0.86, *s*	33.3
30	0.95, *s*	25.0	0.94, *s*	25.0	0.93, *s*	24.9

Poliusaposide C (**3**) displayed a $[M+Na]^+$ ion at *m*/*z* 1495.6527 (calc. 1495.6563), which in conjunction with ^{13}C NMR data suggested a molecular formula of $C_{68}H_{109}O_{36}Na$. The composition of the sugar moieties are D-apiose, L-rhamnose, L-arabinose, D-xylose, and D-glucose resulted from acid hydrolysis followed by GC analysis. Physicochemical properties and spectral features also indicated a triterpenoid saponin. A comparison of NMR spectra for **3** with a previously reported saponin glycoside, conyzasaponin F,[33] revealed that most structural features were the same for the two compounds except for the presence

Table 2: ^{1}H and ^{13}C NMR data of **1-3** (δ in ppm, J in Hz) (400 MHz, methanol-d_4); signals were assigned on the basis of DEPT, ^{1}H-^{1}H COSY, HMQC, HMQC-TOCSY and HMBC experiments.

Position	**1**		**2**		**3**	
	^{1}H	**^{13}C**	**^{1}H**	**^{13}C**	**^{1}H**	**^{13}C**
C3-Glc1						
1	4.54, *d* (7.33)	103.4	4.52, *d* (7.33)	103.5	4.42, *d* (7.79)	105.4
2	4.47, *t* (8.70)	74.4	4.67, *t* (8.70)	74.4	3.28	75.3
3	3.67	79.5	3.97	77.6	3.90	78
4	3.33	74.2	3.33	74.2	3.54	71.0
5	3.96	78.0	3.97	78.1	3.36	77.6
6	3.83	61.2	3.82	61.9	3.68	62.2
	3.91		3.91		3.85	
Glc2						
1	4.41, *d* (7.33)	104.3				
2	3.71	74.8				
3	3.29	77.8				
4	3.26	71.3				
5	3.97	78.1				
6	3.59	62.4				
	3.85					
OAC		172.1				
	2.10, *s*	21.2				
C28-Ara						
1	5.57, *d* (3.21)	93.9	5.57, *d* (3.66)	93.8	5.46, *d* (5.50)	95.4
2	3.80	71.0	3.80	70.7	3.33	77
3	3.75	75.4	3.75	75.5	3.68	69.0
4	3.84	66.9	3.85	66.7	3.54	70.8
5	3.51	63.6	3.50	63.4	3.16	66.5
	3.84		3.84		3.90	
Rha1						
1	5.02, br*s*	101.1	5.02, br*s*	101.1	5.18, br*s*	101.2
2	3.80	71.9	3.80	71.9	3.54	71.6
3	3.54	83.1	3.54	72.1	3.85	81.4
4	3.91	72.2	3.91	83.0	3.68	78.5
5	3.67	68.8	3.67	68.8	3.68	69.0
6	1.22, *s*	18.1	1.22, *s*	18.1	1.26, *s*	18.6
Xyl						
1	4.51,*d* (7.33)	106.2	4.51,*d* (7.33)	106.0	4.65, *d* (6.87)	104.7
2	3.31	75.8	3.29	75.7	3.36	74.6
3	3.42	84.1	3.42	84.0	3.33	85.9
4	3.52	69.7	3.52	69.7	3.54	71.0
5	3.20	66.9	3.20	66.9	3.16	66.5
	3.84		3.85		3.90	

	Rha2		Rha2		Api	
1	5.26, br*s*	101.3	5.26, br*s*	101.3	5.36, br*s*	110.2
2	3.26	71.3	3.23	71.0	4.12	85.3
3	3.91	79.9	3.91	80.1		81.0
4	3.86	71.6	3.83	71.5	4.06	74.8
5	3.96	69.9	3.97	69.9	3.60, *s*	64.9
6	1.23	17.9	1.23	17.9		
	Api		Api		Xyl	
1	5.11, br*s*	112.1	5.09, br*s*	112.2	4.38, *d* (6.87)	105.7
2	3.40	77.6	3.41	77.6	3.19	75.3
3		80.5		80.5	3.90	78.1
4	3.71	74.9	3.71	74.9	3.36	70.8
5	3.59, *s*	65.5	3.60, *s*	65.5	3.16	67.1
					3.90	
					Api	
					5.25, br*s*	111.9
					4.03	78.0
						80.1
					4.06	74.8
					3.60, *s*	64.7

Overlapped signals are reported without designating multiplicity.

of a terminal β-D-xylopyranosyl moiety off the C-28 oligosaccharide chain in **3** instead of a β-D-galactopyranosyl unit in conyzasaponin F. Hence, the structure was established as 3-*O*-[*β*-D-glucopyranosyl]-28-*O*-[*β*-D-xylopyranosyl-(1→2)-*β*-D-apiofuranosyl-(1→3)-*β*-D-xylopyranosyl-(1→3)-[*β*-D-apiofuranosyl-(1→4)]-*α*-l-rhamnopyranosyl-(1→2)-*α*-l-arabinopyranosyl]polygalacic acid ester.

Compounds **1-3** were assayed by a NCI 60 cell panel screen, at a single concentration of 10 μM. Cell growth inhibition percent (GIP) compared to a no-drug control and relative to the time zero number of cells is reported (Table 3). This assay allows for detection of both GIP (values between 0 and 100) and lethality (values less than 0). The highest-sensitivity cancer cell lines were found for compound **3**. This saponin completely inhibited cell growth for a breast (MDA-MB-468) and colon cancer line (HCC-2998) and lethality was 24 and 2%, respectively. In addition, GIP of 98, 94 and 91% were observed for a cell line for colon (COLO 205), renal (A498) and melanoma cancer (SK-MEL-498), respectively.

Chemical features that distinguish **1-2** from **3** are present in both the oligosaccharide derivatization and triterpenoid backbone as has been previously reported for other biologically active saponins.[31] The oligosaccharide moiety attached to C-28 contains different sugar types and linkages for the three isolated metabolites. Specifically, **1-2** have a linear, unbranched monosaccharide chain with a single apiose compared with an apiose branch linked to Rha-1 and a terminal apiose that has exchanged with the neighboring Rha-2 present in **3**. This branching link with increased biological activity is consistent with the previously reported bidesmosidic saponins in which the glycan branching at C-3 appears to be linked with increased biological activity.[34] In addition, the higher cytotoxic activity of **3** may be linked to the presence of multiple apiose units which is also consistent with a previous report for conyzasaponins D and F in which two apiose units were found to be necessary for conferring biological activity against HL-60 cells.[35] Compound **1** has the same number of sugars as **3** but there is a variation in the polarity balance between these saponins due to differences in the glycan arrangement for the two compounds. Specifically, **1** has a disaccharide at C-3 and a pentasaccharide at C-28 while **3** has a monosaccharide at C-3 and a hexosaccharide at C-28; these alterations generate a polarity difference across the aglycone unit. This difference in glycan chain polarity linked with biological activity is consistent with previous reports for bidesmosidic saponins that also exhibit a polarity balance correlation with cytotoxicity.[34]

Another structural feature that has been proposed to affect bioactivity is a reduction of the triterpenoid carboxylic acid group to a primary alcohol as shown in **3** compared with **1-2**. Specifically, cytotoxicity for bidesmosidic saponins increases with aglycone hydroxylation.[35]

Experimental

General experimental procedures- Optical rotations were measured in MeOH on an Autopal IV automatic polarimeter (Rudolph Research Analytical) equipped with a 10 cm microcell and a sodium lamp (λmax = 589 nm). UV data were obtained on a Genesys 20 spectrophotometer. IR (KBr) spectra were recorded on a ThermoNicolet model IR 100 spectrophotometer. NMR spectra were obtained on a Varian (Palo Alto, CA) Unity Inova 500 NMR spectrometer (^{1}H at 500 MHz and ^{13}C at 125 MHz) equipped with VNMR 6.1C software and Sun hardware; chemical shifts were reported in δ (ppm) and J coupling in Hz. The ^{13}C NMR multiplicities were determined by DEPT experiments. NOE measurements were obtained from 2D NOESY experiments. One-bond heteronuclear ^{1}H-^{13}C connectivities were determined by HMQC, and two- and three-bond ^{1}H-^{13}C connectivities were determined by HMBC experimentation. HRESIMS was performed on an LTQ Orbitrap Velos (Thermo Scientific, Pittsburgh, PA, USA) mass spectrometer. Data were processed using Xcalibur Qual browser software (Thermo Scientific, Pittsburgh, PA, USA). GCMS analysis was performed on an ISQ QD Single Quadrupole GC-MS system and data were processed using Xcalibur software (Thermo Scientific, Pittsburgh, PA, USA). HPLC was performed using a prep-C_{18} column (21.2 x 250 mm, 10 μm) on an Agilent 1100 apparatus equipped with a Rheodyne injector and with UV detectors. Column chromatography was carried out using EMD silica gel 60 (70-230 mesh). Analytical TLC was performed on EMD Millipore silica gel 60 F_{254} sheets, 0.25 mm thick.

Plant material- Teucrium polium aerial parts were collected from North Sinai, Egypt, in June 2010. A voucher specimen (SK-105) has deposited in the Herbarium of St. Katherine protectorate, Egypt.

Extraction and isolation- Aerial parts of *T. polium* (2 kg) were air-dried then crushed and extracted with CH_2Cl_2–MeOH (1:1) at room temperature. Solvent was removed and the residue (210 g) was fractionated using column chromatography (CC) silica gel eluting with *n*-hexanes, CH_2Cl_2 and MeOH in increasing order of polarity up to 15% MeOH. Based on TLC similarities, fractions (266-283) were combined (14 g) concentrated *in vacuo* and fractionated by successive silica gel CC, eluted with gradient CH_2Cl_2–MeOH (70:30) up to 100 % MeOH. Fractions were monitored by TLC eluting with CH_2Cl_2-MeOH-H_2O (7:3:0.5); 30 fractions were afforded. Sub-fraction 12-30 (4.5 g) was subjected to Sephadex LH-20 gel CC eluted with MeOH; 37 sub-fractions were obtained. Sub-fractions 2-8 (1.8 g), rich in saponins, were pooled and purified by RP-HPLC eluted with MeOH-H_2O (0.1 % HCHO) (56:44) system. Compounds 1 (45 mg), 2 (23 mg), and 3 (18 mg) were afforded.

Poliusaposide A (**1**) A white glassy powder; $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{25}}$= -30.17 (c 0.58, MeOH). UV_{max} 283; IR (KBr) cm^{-1}: 3391, 2929, 1731, 1633, 1381, 1254, 1046; ESI-MS *m/z*: $[M+Na]^+$ 1595.6715 (calc. 1595.6724) for $C_{71}H_{112}O_{38}$; elemental analysis (found C, 54.2; H, 7.2; O, 38.6 for $C_{71}H_{112}O_{38}$). For ^{1}H and ^{13}C NMR spectroscopic data, see Table 1 and 2.

Table 3: Growth inhibition percent (GIP) for **1-3** against 60 human cancer cell lines at 10 μM.[a]

Panel name	Cell line	GIP 1	2	3
Breast Cancer	MDA-MB-468			-24
	T-47D	4.1		50
	BT-549			26
	MCF7	1.7	6.3	31
	HS 578T	3.1		2.1
CNS Cancer	U251	7.4		58
	SF-295		0.1	38
	SF-539		2.9	14
	SNB-19			19
	SNB-75	11	13	7.4
	SF-268	8.7		14
Colon Cancer	HCC-2998		3.4	-1.8
	COLO 205	2.7		98
	HCT-116	0.2		54
	HCT-15			14
	HT29	7.8	3.5	40
	KM12	5.8		34
	SW-620			25
Leukemia	HL-60(TB)	4.5	2.3	79
	CCRF-CEM			14
	K-562	8.9		42
	MOLT-4	4.9		13
	RPMI-8226	2.4	5.4	36
	SR	17	17	32
Melanoma	SK-MEL-2	6.6		82
	SK-MEL-28			91
	M14			24
	MDA-MB-435			48
	LOX IMVI			19
	MALME-3M	6.7	1.1	10
	SK-MEL-5	3.1		12
	UACC-257	6.8		44
	UACC-62	1.7		21
Non-Small Cell Lung Cancer	EKVX			60
	NCI-H23	0.2		51
	NCI-H522	21	4.7	70
	HOP-92		21	
	NCI-H226			0.8
	A549/ATCC	11	0.9	50
	NCI-H322M	2.6	2.0	44
	HOP-62	15	11	26
Ovarian Cancer	OVCAR-4	4.3	8.5	57
	OVCAR-3			10
	IGROV1	3.1	4.0	14
	OVCAR-5	1.5	0.9	22
	OVCAR-8			1.2
	NCI/ADR-RES			20
	SK-OV-3		1.5	6.1
Prostate Cancer	PC-3	13		34
	DU-145			22
Renal Cancer	786-0	11	17	53
	A498		18	94
	SN12C	1.2	0.4	75
	UO-31	28	12	71
	RXF 393	10	16	29
	ACHN			22
	TK-10	6.8		26
	CAKI-1	5.9		28

[a] Negative values indicate lethality; blanks growth above 100%.

Poliusaposide B (**2**) A pale yellow glassy powder; $[\alpha]_D^{25}$= -25.50 (c 0.13, MeOH); UV_{max} 286. IR (KBr) cm^{-1}: 3391, 2929, 1730, 1644, 1591, 1381, 1256, 1045; ESI-MS *m/z*: $[M+Na]^+$ 1433.6190 (calc.1433.6195) for $C_{65}H_{102}O_{33}$; elemental analysis (found C, 55.3; H, 7.3; O, 37.4 for $C_{65}H_{102}O_{33}$). For ^1H and ^{13}C NMR spectroscopic data, see Table 1 and 2.

Poliusaposide C (**3**) A white glassy powder; $[\alpha]_D^{25}$= -33.00 (c 0.13, MeOH); UV_{max} 286. IR (KBr) cm^{-1}: 3391, 2931, 1729, 1292, 1381, 1257, 1079, 1040; ESI-MS *m/z*: $[M+Na]^+$ 1495.6527 (calc. 1495.6563) for $C_{67}H_{108}O_{35}$; elemental analysis (found C, 54.6; H, 7.4; O, 38.0 for $C_{67}H_{108}O_{35}$). For ^1H and ^{13}C NMR spectroscopic data, see Table 1 and 2.

*Sugar identification of **1-3** Acid hydrolysis*- A solution of **1-3** each 2 mg in 1 N HCl in (H_2O: dioxane, 1:1) (1 mL) was heated in a water bath to 80 ^oC for 2 hrs. After cooling, the reaction mixture was neutralized with Amberlite IRA-68 and the resin was removed by filtration; the filtrate was extracted with EtOAc (2 x 2 mL). The aqueous layer was concentrated *in vacuo*. Sugars were identified by TLC eluting with *n*-hexane:EtOAc:MeOH:HOAc:H_2O (1:4:2:0.5:0.5) by comparison with authentic standards.[36]

Gas chromatography analysis- For the sugar identification, an aqueous aliquot was dissolved in pyridine (0.2 mL) and trimethylsilylated with *N*-trimethylsilylimidazole (TMSI) (0.2 mL) at room temperature for 2 hrs. After addition of distilled H_2O to end the reaction, the mixture was partitioned with *n*-hexane (2 x 1 mL) and the organic layer was analyzed by GCMS.[37] Apiose, arabinose, rhamnose, xylose, and glucose were detected at 17.64, 17.82, 18.03, 18.97 and 21.76 min respectively, based on retention time comparisons with derivatized authentic standards. For identifying the sugar configuration, L-cysteine methyl ester hydrochloride (0.06 M) in 0.2 mL of pyridine was added to the aqueous layer. The mixture was stirred at 60 ^0C for 1 hr and then TMSI (0.2 mL) was added to the mixture and kept at room temperature for 2 hrs. The reaction mixture was partitioned with *n*-hexane and dist. The water and *n*-hexane layers were analyzed by GCMS. GCMS conditions[8] were as follows: injection temperature 290 ^oC; initial column oven temperature 40 ^oC then raised to 260 at 10 ^oC /min with a final temperature maintained for 7 min. He was used as the carrier gas (split ratio, 1/17) and detector temperature was 250 ^oC. For **1-3**, D-apiose, L-arabinose, D-xylose, L-rhamnose, and D-glucose were detected based on retention time matches of 23.8, 23.9, 24.0, 24.6, 26.2 min, respectively, with derivatized authentic standards.

In vitro anticancer screening- Cell toxicity screening was performed for compounds **1-3** at a single concentration (10 μM) by NCI according to a standard procedure[30, 38-40] for NCI-60 DTP human tumor cell screen (http://dtp.nci.nih.gov/branches/btb/ivclsp.html).

Conclusions

Saponin glycosides were reported from *T. polium* for the first time. Poliusaposide C showed potential in the treating breast (MDA-MB-468), colon (HCC-2998), colon (COLO 205), renal (A498) and melanoma (SK-MEL-498) cancers. *In vivo* assays using animal models will provide greater accuracy in determining cancer toxicity as well as begin to probe cytotoxic specificity.[41] Previous studies with select saponins have exhibited lower cancer cytotoxicity *in vitro* than with solid tumors in vivo.[42] Future chemical-derivatization and molecular-modeling studies are expected to provide additional insight into structure-anticancer relationships. Formulation also appears to be a driver in conferring biological activity with for example, a micronized oral form of the anti-tumor saponin 20(*R*)-ginsenoside Rh2 exhibits almost a two-fold higher cancer cytotoxicity than that of the native form.[43]

Acknowledgements

The authors are grateful to Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute (Bethesda, MD) (http://dtp.cancer.gov) for the *in vitro* screening. This research was supported in part by the Robert Welch Foundation (D-1478), NSF equipment grant CHE-1048553 and NSF CRIF program.

Notes and references

[a] Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, Texas 79409-1061, USA.

[b] Chemistry of Medicinal Plants Department, Center of Excellence for Advanced Sciences, National Research Centre, Giza, 12622, Egypt.

† Corresponding author e-mail: Paul.Pare@ttu.edu; FAX: 806 742 1289

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

1. M. J. Perez-Alonso, A. Velasco-Negueruela and J. A. Lopez-Saez, *J. Essent. Oil Res.*, 1993, **5**, 397-402.
2. A. Kamel and P. Sandra, *Biochem. Syst. Ecol.*, 1994, **22**, 529-532.
3. N. Belmekki, N. Bendimerad, C. Bekhechi and X. Fernandez, *J. Med. Plants Res.*, 2013, **7**, 897-902.
4. S. N. Nematollahi-Mahani, M. Rezazadeh-Kermani, M. Mehrabani and N. Nakhaee, *Pharm. Biol. (N. Y., NY, U. S.)*, 2007, **45**, 295-298.
5. F. Sharififar, G. Dehghn-Nudeh and M. Mirtajaldini, *Food Chem.*, 2008, **112**, 885-888.
6. S. De Marino, C. Festa, F. Zollo, F. Incollingo, G. Raimo, G. Evangelista and M. Iorizzi, *Food Chem.*, 2012, **133**, 21-28.
7. W. A. Elmasri, M.-E. F. Hegazy, M. Aziz, E. Koksal, W. Amor, Y. Mechref, A. N. Hamood, D. B. Cordes and P. W. Pare, *Phytochemistry (Elsevier)*, 2014, Ahead of Print.
8. W. A. Elmasri, *T.* Yang, P. Tran, M.-*E.* Hegazy, A. N. Hamood, *Y.* Me*chref* and P. W. *P*aré, *J. Nat. Prod.*, Editon edn., 2014, vol. Submitted, p. Submitted.
9. G. Cefarelli, B. D'Abrosca, A. Fiorentino, A. Izzo, C. Mastellone, S. Pacifico and V. Piscopo, *J. Agric. Food Chem.*, 2006, **54**, 803-809.
10. G. M. Wassel and S. S. Ahmed, *Pharmazie*, 1974, **29**, 540-541.
11. N. Ali, S. S. W. A. Shah, I. Shah, G. Ahmed, M. Ghias and I. Khan, *BMC Complementary Altern. Med.*, 2011, **11**, 106.
12. K. R. Price, I. T. Johnson and G. R. Fenwick, *CRC Crit. Rev. Food Sci. Nutr.*, 1987, **26**, 27-135.
13. R. San Martin and R. Briones, *Econ. Bot.*, 1999, **53**, 302-311.
14. C.-S. Yuan, C.-Z. Wang, S. M. Wicks and L.-W. Qi, *J. Ginseng Res.*, 2010, **34**, 160-167.
15. D. Mandal, N. Panda, S. Kumar, S. Banerjee, N. B. Mandal and N. P. Sahu, *Phytochemistry (Elsevier)*, 2006, **67**, 183-190.
16. M. Vermeersch, K. Foubert, R. I. Da Luz, L. Van Puyvelde, L. Pieters, P. Cos and L. Maes, *Phytother. Res.*, 2009, **23**, 1404-1410.
17. N. Germonprez, L. Maes, L. Van Puyvelde, M. Van Tri, D. A. Tuan and N. De Kimpe, *J Med Chem*, 2005, **48**, 32-37.
18. L. Maes, D. Vanden Berghe, N. Germonprez, L. Quirijnen, P. Cos, N. De Kimpe and L. Van Puyvelde, *Antimicrobial Agents Chemother*, 2004, **48**, 130-136.
19. M. A. R. Ibrahim and H. A. M. Srour, *Nat. Prod. Chem. Res.*, 2014, **2**, 1000123/1000121-1000123/1000124, 1000124 pp.

20. I. L. Acebey-Castellon, L. Voutquenne-Nazabadioko, D. T. M. Huong, N. Roseau, N. Bouthagane, D. Muhammad, E. Le Magrex Debar, S. C. Gangloff, M. Litaudon, T. Sevenet, V. H. Nguyen and C. Lavaud, *J. Nat. Prod.*, 2011, **74**, 163-168.
21. K. Takagi, E. H. Park and H. Kato, *Chem Pharm Bull (Tokyo)*, 1980, **28**, 1183-1188.
22. X. Lu, S. Qiu, X. Sun and Z. Li, *Shipin Kexue (Beijing, China)*, 2005, **26**, 86-90.
23. W. H. Talib and A. M. Mahasneh, *Sci. Pharm.*, 2010, **78**, 33-45.
24. Y. Li, J.-F. Gu, X. Zou, J. Wu, M.-H. Zhang, J. Jiang, D. Qin, J.-Y. Zhou, B.-X.-Z. Liu, Y.-T. Zhu, X.-B. Jia, L. Feng and R.-P. Wang, *Molecules*, 2013, **18**, 12916-12936.
25. S. Man, W. Gao, Y. Zhang, C. Ma, Y. Liu and Y. Li, *Arch. Pharmacal Res.*, 2011, **34**, 43-50.
26. M. Kong, J. Fan, A. Dong, H. Cheng and R. Xu, *Acta Biochim. Biophys. Sin.*, 2010, **42**, 827-833.
27. D. D. Ma, H. X. Lu, L. S. Xu and W. Xiao, *J. Int. Med. Res.*, 2009, **37**, 631-640.
28. J. Sun, B.-R. Liu, W.-J. Hu, L.-X. Yu and X.-P. Qian, *Phytother. Res.*, 2007, **21**, 1102-1104.
29. Y. Tong, F. Yang, G. Dai, Z. Ren and B. Wang, *Zhonghua Zhongyiyao Xuekan*, 2013, **31**, 2181-2183, 2181 plate.
30. R. H. Shoemaker, *Nat. Rev. Cancer*, 2006, **6**, 813-823.
31. C. Lavaud, L. Voutquenne, G. Massiot, L. Le Men-Olivier, B. C. Das, O. Laprevote, L. Serani, C. Delaude and M. Becchi, *Phytochemistry*, 1997, **47**, 441-449.
32. N. Germonprez, L. V. Puyvelde, L. Maes, M. V. Tri and N. D. Kimpe, *Tetrahedron*, 2004, **60**, 219-228.
33. Y. Su, K. Koike, D. Guo, T. Satou, J. Liu, J. Zheng and T. Nikaido, *Tetrahedron*, 2001, **57**, 6721-6726.
34. M. Takechi and T. Yasuo, *Phytochemistry*, 1990, **29**, 451-452.
35. H. C. Huang, M. D. Wu, W. J. Tsai, S. C. Liao, C. C. Liaw, L. C. Hsu, Y. C. Wu and Y. H. Kuo, *Phytochemistry*, 2008, **69**, 1609-1616.
36. H. Wang, Y. Sun, W. C. Ye, F. Xiong, H. J. Wu, C. H. Yang and S. X. Zhao, *Chem Pharm Bull*, 2004, **52**, 615-617.
37. K. Sakai, T. Nagao and H. Okabe, *Phytochemistry*, 1999, **51**, 309-318.
38. M. C. Alley, D. A. Scudiero, A. Monks, M. L. M. J. Hursey Czerwinski, D. L. Fine, B. J. Abbott, J. G. Mayo, R. H. Shoemaker and M. R. Boyd, *Cancer Research*, 1988, **48**, 589-601.
39. M. R. Grever, S. A. Schepartz and B. A. Chabner, *Seminars in Oncology*, 1992, **19**, 622-638.
40. M. R. Boyd and K. D. Paull, *Drug Dev. Res.*, 1995, **34**, 91-109.
41. S. C. Bang, J. H. Lee, G. Y. Song, D. H. Kim, M. Y. Yoon and B. Z. Ahn, *Chem Pharm Bull*, 2005, **53**, 1451-1454.
42. Y. Kim, S. C. Bang, J. H. Lee and B. Z. Ahn, *Arch Pharm Res*, 2004, **27**, 915-918.
43. Y. Gu, G. Wang, J. Sun, H. Xie and Y. Jia, *Int. J. Mass Spectrom.*, 2006, **252**, 11-19.