CARDENOLIDE GLYCOSIDES FROM *Streptocaulon tomentosum* WIGHT & ARNOTT (မြင်းစုံနီ) (ASCLEPIADACEAE) in MYANMAR

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Abstract

This paper describes the isolation and comparative studies on NMR spectra of cardenolide glycosides from *Streptocaulon tomentosum* Wight & Arnott (Asclepiadaceae). Nine cardenolides were isolated from the roots of *Streptocaulon tomentosum*. by column chromatography and identified by NMR spectroscopy. They are 17α-H-periplogenin, 17α-H-periplogenin-β-*D* digitoxose,17α-H-periplogenin-β-*D* cymarose, 17α-H-periplogenin-β-glucosyl-(1-4)-2-*O*-acetyl-digitalose, 17β-H-periplogenin, 17β-H-periplogenin-β-*D* digitoxose, 17β-H-periplogenin-β-*D* cymarose, 17α -H-digitoxigenin, and 17 α-H-digitoxigenin-β-*D*-digitoxoside. Comparative studies on NMR spectra of cardenolide glycosides were carried out. Six cardenolides isolated from *Streptocaulon tomentosum* were tested for their antiproliferative activity *in vitro* against MCF-7 (human breast cancer cell line) and L 929 (mouse fibroblast cell line). Among these six cardenolides, 17α-H-periplogenin-3-*O*-β-*D*-digitoxoside and 17α-H-periplogenin-3-*O*-β-*D*-cymaroside exhibit significant antiproliferative activity (IC_{50} values, < 1μM) against MCF-7. Four cardenolides were examined for their cellular viability in the tumor cell and U 937 (human leukemic cell line) at concentrations 100 μM, 10 μM, and 1 μM. All these four cardenolides show the induction of apoptosis at 100 μM and 10 μM in both cell lines.

Keywords: *Streptocaulon tomentosum*, Asclepiadaceae, cardenolides, antiproliferative activity, cellular viability

Introduction

The genus *Streptocaulon* belongs to the family Asclepiadaceae and includes five species. Two species, *S. tomentosum* and *S. griffithii* J. D. Hooker grow in Myanmar. The roots of *Streptocaulon tomentosum* are used in Myanmar in traditional medicine for the treatment of anticancer, dysentery and stomachache, and the leaves are used externally for the treatment of snake poisoning and abscesses. In previous studies, nobody reported about the isolation of bioactive substances from S.*tomentosum*. However the isolation of cardenolides from the root of *S. juventas* (Lour.) Merr. and antiproliferative activity of cardenolides isolated from *S. juventas* have been reported (Ueda *et al*., 2003a; 2003b). Cardenolides occur in several plant families including the Asclepiadaceae, the Apocynaceae, the Scrophulariaceae, the Celastraceae, and the Tiliaceae. The cardiac glycosides, digitoxin and digoxin, have been used for the treatment of heart failure for hundreds of years. In 2005, digitoxin, digoxin, gitoxin and their corresponding aglycones were evaluated for growth inhibition activity in three human cancer cell lines TK-10 (renal), MCF-7 (breast), and UACC-62 (melanoma) at concentrations commonly found in cardiac patients. Digitoxin (IC_{50} 3.2-33.5 nM) and digoxin (IC_{50}14.6-29.5 nM) showed the highest level of growth inhibition in the three cell lines investigated (Lázaro *et al*., 2005). The above-mentioned reports suggest that digitalis may have an anticancer utilization.

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Materials and Methods

Sample Collection and Identification of Plant Samples

Streptocaulon tomentosum Wight and Arn. (Asclepiadaceae), roots collected in May 2002 from Mawlamyine Township, Myanmar by Dr Daw Hla Ngwe. The species was identified by Prof. Dr Aung Aung Min, Department of Botany, University of Yangon.

A voucher specimen of the clamberer (No. Y.H.V. 1004) is deposited in University of Yangon.

Spectral Studies

1D NMR spectra (^{1}H, ^{13}C) were recorded on a Varian Mercury 300 at 300.94 MHz for 1H, and at 100.57 MHz for ^{13}C NMR. 2DNMR spectra (HSQC, HMBC, COSY, ROESY) were recorded on a Varian Inova 500 at 499.81 MHz for ^{1}H. Chemical shifts in ppm were referenced to internal TMS (δ =0) for 1H and C_5D_5N (δ 149.81, 135.48, 123.50 ppm) for ^{13}C, respectively.

Isolation and Identification of Cardenolides from the Roots of *Streptocaulon tomentosum*

Dried powdered root of *S. tomentosum* (1000 g) was extracted with 80% aqueous EtOH. After evaporation of the solvent, the resulting crude extract was successively partitioned between organic solvents (*n*-hexane, ethylacetate, *n*-butanol) and water. The ethyl acetate crude extract (12 g) was chromatographically separated on silica gel 60 (70-230 mesh, Merck) with *n*-hexane, ethyl acetate, and methanol (increasing polarity) to afford 23 fractions. Fraction 12 and 15 were rechromatographed on silica gel 60 (230-400 mesh, Merck) using $CHCl_3$:MeOH (9.5:0.5, v/v) and silica RP18 using MeOH:H_2O (9:1, v/v) to give compound **1**. Compound **2** and **3** were obtained from fraction 14 (Kawaguchi *et al*., 1988). The subfractions 14 and 15 of the ethyl acetate extract provided, after silica gel and RP-18 column chromatography, the known compound **5** and the unknown compound **6**. Fraction 13 was rechromatographed on sephadex LH-20 eluting with MeOH to afford compound **4**. Fractions 5 to 11 were repurified on silica gel 60 (230-400 mesh, Merck) using $CHCl_3$:MeOH (9.5:0.5, v/v) to give compounds **8** and **9**.

The *n*-butanol fraction (18 g) was separated on silicagel 60 (230-400 mesh, Merck) using $CHCl_3$:MeOH (9.5:0.5, v/v) to give 28 subfractions. The subfraction 7 was purified by silica RP-18 using MeOH:H_2O (8:2, v/v) to give compound **7.**

Antiproliferative Activity of Cardenolides

Acid phosphatase assay: Cells were grown in 96-well plates at densities upto 100,000 cells per well. The culture medium was removed from these cells with a multichannel pipettor (Wheaton), and each well was washed once with 200 μl phosphate-buffered saline (PBS, pH 7.2). For nonadherent cells, to remove solution from plates, the 96-well plates were centrifuges at 2500 rpm for 10 min (Beckman GS-15R centrifuge). To each well, 100 μl of buffer containing 0.1 M sodium acetate (pH 5.0), 0.1 % Triton X-100, and 5 mM *p*-nitrophenyl phosphate was added. The plates were placed in a 37 °C incubator for 2 h. The reaction was stopped with the addition of 10 μl of 1 N NaOH, and colour development was assayed at 405 nm using a microplate reader (THERMOmax plate reader, Molecular Devices, Inc.). The nonenzymatic

hydrolysis of the pNPP substrate was determined for each assay by including wells that did not contain cells. This background value was typically 0.07-0.2 absorbance units.

Cellular Viability of Cardenolides

Cell culture and stimulation

Human U 937 myeloid leukemia cells were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 µg/ml penicillin, 100 µg/ml streptomycin, 2 mM L-glutamine (Gibco, Grand Island, N.V., USA). Human TUR myeloid leukemic cells (ATCC #2367) were grown in a similar medium supplemented with 400 µg/ml G418 (Sigma Chemical Co., St. Louis, MO/USA). The maintenance of the TUR cells in the presence of G418 was terminated one week before the appropriate experiments. U 937 and TUR cells were treated with the appropriate substances at a density of 2 x 10^5cells/ml for up to 72h, respectively. The cell number and viability of each culture was assessed by a Vi-Cell cell viability analyzer (Beckman Coulter) using an assay kit and the quantification software Vi-Cell version 1.01 according to the manufacturers protocol (Beckman Coulter).

Cell cycle analysis

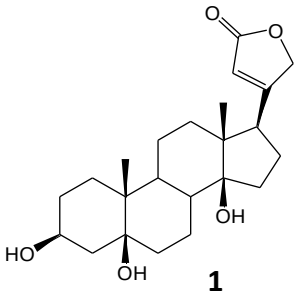
Following an appropriate incubation the cells were fixed in 70% (v/v) ice-cold ethanol at 4 °C for 24 h. The fixed cells were stained with CyStain DNA 2 step kit (Partec GmbH, Münster, Germany) and filtered through a 50 µm filter. The samples were then analyzed in a Galaxy flow cytometer (Dako-Cytomation GmbH, Hamburg, Germany) using FloMax analysis software (Partec) and the MultiCycle cell cycle software (Phoenix Flow Systems Inc., San Diego, CA.

Results and Discussion

Structure Elucidation of Cardenolides

(a) 17α-H-Periplogenin (1), 17α-H-periplogenin-β-*D* digitoxose (2), 17α-H-periplogenin-β-*D* cymarose (3)

Fraction 12 of the ethyl acetate extract gave compound **1**. Compound **2** and **3** were obtained from fraction 14 (Kawaguchi *et al*., 1988). The ^{1}H NMR (C_5D_5N) data of compound **1** (HR-ESI-MS: 413.23098 $[M+Na]^+$, calc. for $C_{23}H_{34}O_5Na$ 413.22984) agreed with the characteristic peaks of cardenolides. The signal of H-21 a and b in the butenolide ring showed at δ5.36 and 5.08 ppm (*dd*, *J* 18.1/1.4 Hz).



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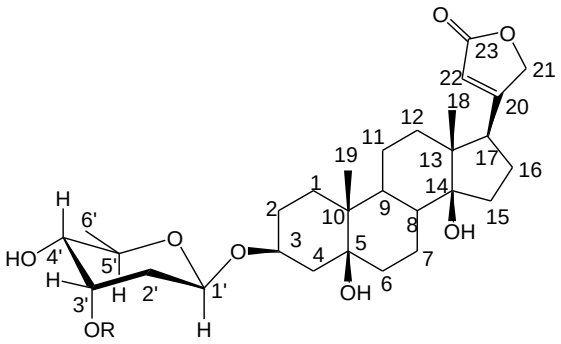


2 R = H

3 R = CH_3

The H-22 was observed as a singlet at δ 6.17 ppm. The H-3 signal appeared as a broad singlet at δ 4.46 ppm. The H-17 signal appeared at δ 2.84 as *dd* (*J* 9/3 Hz) and the H_3-18 and H_3-19 signals at 0.88 and 0.94 as singlet. According to EI-MS, the fragments at *m/z* 391 and 373 indicated the presence of a cardenolide aglycone.

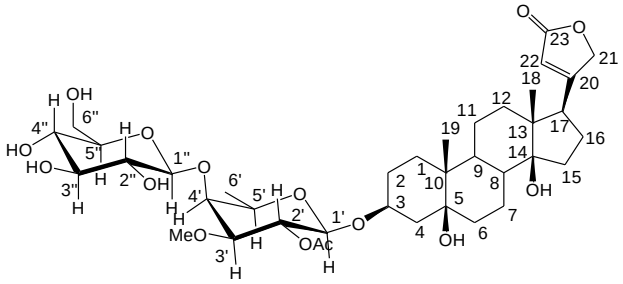
Compound **2** (HR-ESI-MS: 543.2924 $[M+Na]^+$, calc. for $C_{29}H_{44}O_8Na$ 543.2928) and compound **3** (557.3088 $[M+Na]^+$, calc. for $C_{30}H_{46}O_8Na$ 557.3084) exhibited a mass difference of 131 and 145 in comparison to compound **1**. Because this difference was derived from the sugar moiety, the molecular formula of these sugars was deduced to be $C_6H_{11}O_3$ and $C_7H_{13}O_3$, identified a digitoxose and cymarose by acid hydrolysis and GC-MS analysis required derivatization MSTFA (*N*-methyl-*N*-trimethylsilyltrifluoroacetamide) with authentic sugars sample. The data agree with 17α-H-periplogenin-β-*D*-digitoxose (**2**), and 17α-H-periplogenin-β-*D*-cymarose (**3**) (Ueda *et al*., 2003a).

(b) 17α-H-Periplogenin-β-glucosyl-(1-4)-2-*O*-acetyl-digitalose (4)

Fraction 13 of the ethyl acetate extract afforded the new compound **4** after column chromatography on sephadex LH 20 with MeOH.

The ESI-MS spectrum exhibited a $[M+Na]^+$ ion at *m/z* 777. Its molecular formula, $C_{38}H_{58}O_{15}$, (calcd. for $C_{38}H_{58}O_{15}Na$ 777.36679) was obtained through a combined application of ESI-MS, EI-MS, FT-ICR, ^{1}H NMR, and ^{13}C NMR. The ^{1}H NMR spectrum (in CD_3OD) of the aglycone was similar to compound **1**. The signal of H-21a and b also showed a pair of double doublets at δ 5.09 ppm and δ 4.91 ppm. The H-22 was observed as a singlet at δ 5.89 ppm. The H-3 signal appeared as a broad singlet at δ 4.12 ppm, revealing its α-configuration. The ^{1}H NMR and ^{13}C NMR data (in CD_3OD) demonstrated two molecules of sugar by two anomeric protons at δ 4.44 ppm for H 1′ (*dd*, *J* = 8, 3.8 Hz) and δ 4.57 ppm for H 1″ (*dd*, *J* = 8, 3.5 Hz). They were connected to the anomeric carbons at δ 102.1 and 104.6 ppm in the HSQC spectrum respectively. Their chemical shifts and coupling constants suggested β-linkage of the sugars.

The structural assignment was confirmed by carrying out 2D NMR techniques such as HSQC and H-H COSY. The HMBC spectral analysis displayed correlation peaks between H-3 and C-1′of the digitalosyl (3-*O*-methyl-β-fucopyranosyl) unit, the anomeric proton of the glucosyl residue and C-4′ of the digitalosyl unit, H-2′ of digitalosyl and OAc. The connectivity between H-H and H-C in the NOESY and HMBC were also presented in Table 1. According to these spectral data, the structure of compound **4** was assigned as 17α-H-periplogenin-3-*O*-β-glucopyranosyl-(1-4)-2-*O*-acetyl-3-*O*-methyl-β-fucopyranoside. It is a new combination of the known aglycone and sugar moieties.



Compound 4

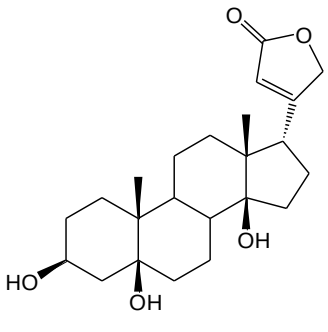
(c) 17*β*-H-Periplogenin (5), 17*β*-H-periplogenin-*β*-*D*-digitoxose (6), 17*β*-H-periplogenin-*β*-*D*-cymarose (7)

The subfractions 14 and 15 of the ethyl acetate extract provided, after silica gel and RP-18 column chromatography, the known compound **5** and the unknown compound **6**. Compound **7** was isolated from the *n*-butanol fraction. The compound **5** and **7** were identified as 17*β*-H-periplogenin **(5)** and 17*β*-H-periplogenin-*β*-*D*-cymarose **(7)** by different MS and 2D NMR experiments and by comparison with the spectral data from literature (Furuya *et al*., 1988).

Compound **5** had the composition $C_{23}H_{34}O_5$ on the basis of HR-MS. In the ^1H NMR (C_5D_5N) spectrum of **5**, the methyl proton signals H_3-18 and H_3-19 were shifted downfield to δ 1.227 and 1.186 ppm (each 3H, *s*) by comparison with that of compound **1**. The methine proton signal of H-17, coupling with the methylene protons of C-16, was also shifted downfield to δ 3.463 (1H, *t*, *J* 9.5 Hz). In the ROESY spectrum, a correlation peak between H-17 and H_3-18 was found. In the ^{13}C NMR spectrum (Table 3), the carbon C-12 and C-17 signals were shifted upfield to δ 31.1 and 48.8 ppm. Thereby the configuration of H-17 was revealed to be β (Furuya *et al*., 1988).

Compound **6** was isolated as a white powder. The molecular formula $C_{29}H_{44}O_8$ was deduced from positive ion ESI-FT-ICR-MS (*m/z* 543.29240 $[M+Na]^+$, calcd. for $C_{29}H_{44}O_8Na$ 543.29283). The ^1H and ^{13}C NMR data of the aglycone agree with the characteristic peaks of 17*β*-H-periplogenin (Furuya *et al*., 1988). The HMBC correlation between C-3 and the anomeric proton H-1′ proved the presence of the sugar moiety at C-3. This is further supported by the NOE of H-3 with H-1′. According to the EI-MS data, the fragments at *m/z* 391 and 373 indicated a aglycone moiety and *m/z* 131 and 113 fragments of a 2,6-deoxy sugar moiety. The 2D NMR correlation peaks of the sugar moiety and the vicinal ^1H, ^1H coupling constants confirmed the presence of digitoxose. After hydrolyses with HCl, the hydrolysed sugar moiety could be also identified as digitoxose by GC analysis as its trimethylsilylether.

According to the spectral data, compound **6** was identified as a new combination of the known aglycone and known sugar 17*β*-H-periplogenin-*β*-*D*-digitoxoside.



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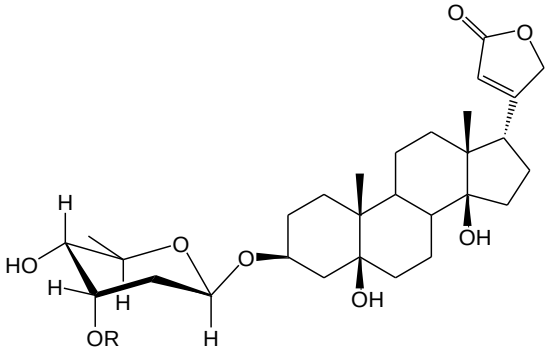
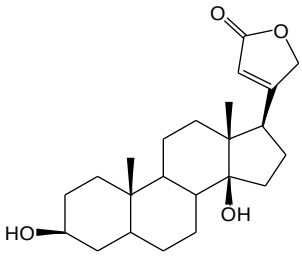


6 R = H
7 R = Me

(d) 17α-H-Digitoxigenin (8), 17α-H-digitoxigenin-β-*D*-digitoxoside (9)

Compounds **8** and **9** were isolated from fractions 10 and 11 of the ethyl acetate extract after silica gel chromatography. Their structures were confirmed by 2D NMR and comparison to reported data (Danieli *et al*., 1966).



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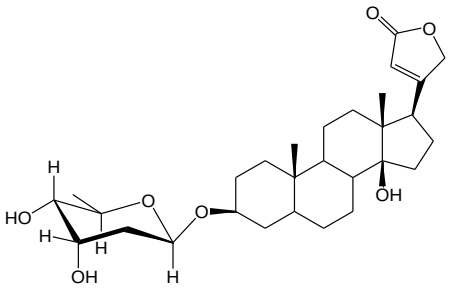

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Table 1 NMR Data of Compound 4 (500 MHz, CD_3OD)

Atom	δ_H [ppm]	δ_C [ppm]	$^nJ_{CH}$ coupling. δ_H [ppm], HMBC
1	1.36, 1.70 (*m*)	26.5	1.02 (19)
2	1.75, 1.64 (*m*)	26.9	
3	4.37 (*br s*)	78.7	4.84 (1′)
4	2.20, 1.65 (*m*)	36.1	1.70 (6A)
5		75.1	1.65 (4B), 1.70 (6A)
6	1.70, 1.50 (*m*)	35.6	1.02 (19)
7	2.30, 1.35 (*m*)	24.9	1.70 (6A), 1.85 (8)
8	1.85 (*m*)	41.6	2.30 (7A)
9	1.60 (*m*)	40.1	1.85 (8), 1.02 (19)
10		41.8	1.02 (19)
11	1.35, 1.25 (*m*)	22.7	1.60 (9)
12	1.36 (*m*)	40.9	1.00 (18), 2.81 (17)
13		50.9	1.00 (18), 2.81 (17)
14		86.3	1.00 (18), 2.81 (17), 2.10, 1.85 (15)
15	2.10, 1.85 (*m*)	33.3	
16	2.10, 2.00 (*m*)	28.0	2.81 (17)
17	2.818 (*m*)	51.9	2.00 (16B), 1.00(18)
18	1.001 (*s*)	16.3	1.36 (12)
19	1.029 (*s*)	17.3	
20		178.3	5.10 (21B), 6.16 (22), 2.81 (17)
21	5.10, 5.34 (*dd*, 8.4/1.7)	75.3	2.81 (17)
22	6.161 (*br s*)	117.9	2.81 (17)
23		177.2	6.16 (22)
1′	4.841 (*d*, 8.0)	102.1	3.73 (5′), 5.82 (2′)
2′	5.828 (*dd*, 10.2/8.0)	72.7	3.60 (3′), 4.44 (4′)
3′	3.608 (*dd*, 10.1/3.0)	83.5	5.82 (2′), 4.44 (4′), 3.45 (OMe)
4′	4.448 (*br d*, 3.0)	75.2	3.73 (5′)
5′	3.738 (*m*)	71.8	1.55 (6′)
6′	1.557 (*d*, 6.4)	17.3	3.73 (5′)
3′-OMe	3.457 (*s*)	58.5	3.60 (3′)
2′-OAc	2.225 (*s*)	21.1	
2′-OCOMe		172.2	5.82 (2′), 2.22 (COMe)
1″	5.149 (*d*, 7.7)	104.6	4.44 (4′), 3.99 (2″)
2″	3.99	75.9	5.14 (1″)
3″	4.248 (*dd*, 8.8/8.8)	77.9	4.19 (4″)
4″	4.190 (*dd*, 9.4/8.8)	71.8	3.96 (5″)
5″	3.96	78.2	4.36 (6″B), 4.19 (4″)
6″	4.36, 4.60 (*br d*, 11.5)	63.0	3.96 (5″)

Table 2 ^{1}H NMR Spectral Data of Cardenolides 1-3, 5-9 (300, 500 MHz, C_5D_5N)

H-Atom	δ_H [ppm]							
	1	**2**	**3**	**5**	**6**	**7**	**8**	**9**
1		1.44, 2.08 (*m*)	1.42, 2.04 (*m*)	1.50, 2.28 (*m*)	1.48, 2.12 (*m*)	1.48, 2.08 (*m*)		1.56, 1.76 (*m*)
2		1.72, 2.08 (*m*)	1.72, 2.04 (*m*)	1.84 (*m*)	1.76, 2.12 (*m*)	1.78, 2.08 (*m*)		1.22, 1.82 (*m*)
3	4.46 (*br s*)	4.397 (*br s*)	4.373 (*br s*)	4.483 (*br s*)	4.426 (*br s*)	4.424 (*br s*)	4.42 (*br s*)	4.335 (*br s*)
4		1.72, 2.18 (*m*)	1.74, 2.22 (*m*)	1.82, 2.34 (*m*)	1.76, 2.24 (*m*)	1.76, 2.24 (*m*)		1.60, 1.87 (*m*)
5								1.86 (*m*)
6		1.52, 1.90 (*m*)	1.52, 2.22 (*m*)	1.64, 1.98 (*m*)	1.56, 1.94 (*m*)	1.56, 1.96 (*m*)		1.82 (*m*)
7		1.30, 2.28 (*m*)	1.30, 2.28 (*m*)	1.34, 2.34 (*m*)	1.32, 2.34 (*m*)	1.34, 2.31 (*m*)		2.12 (*m*)
8		1.84 (*m*)	1.84 (*m*)	1.96 (*m*)	1.94 (*m*)	1.94 (*m*)		1.78 (*m*)
9		1.64(*m*)	1.64 (*m*)	1.66 (*m*)	1.62 (*m*)	1.62 (*m*)		1.76 (*m*)
11		1.24, 1.38 (*m*)	1.26, 1.40 (*m*)	1.24, 1.50 (*m*)	1.42 (*m*)	1.24, 1.48 (*m*)		1.38 (*m*)
12		1.40 (*m*)	1.40 (*m*)	1.12, 1.18 (*m*)	1.12, 1.18 (*m*)	1.12, 1.18 (*m*)		1.40 (*m*)
15		1.86, 2.08 (*m*)	1.86, 2.08 (*m*)	1.88, 2.18 (*m*)	1.86, 2.12 (*m*)	1.86, 2.10 (*m*)		1.90, 2.12 (*m*)
16		1.96, 2.10 (*m*)	1.96, 2.10 (*m*)	1.84, 2.13 (*m*)	1.78, 2.06 (*m*)	1.78, 2.08 (*m*)		1.98, 2.12 (*m*)
17	2.84 (*dd*, 9/3)	2.817 (*m*)	2.818 (*d*, 8.0)	3.463 (*t*, 9.5)	3.438 (*br dd*, 9.6/9.6)	3.447 (*br dd*, 9.6/9.6)	2.84 (*m*)	2.805 (*m*)
18	0.88 (*s*)	1.036 (*s*)	1.038 (*s*)	1.227 (*s*)	1.198 (*s*)	1.201 (*s*)	1.05 (*s*)	1.021 (*s*)
19	0.94 (*s*)	1.087 (*s*)	1.093 (*s*)	1.186 (*s*)	1.127 (*s*)	1.133 (*s*)	0.99 (*s*)	0.899 (*s*)
21	5.08, 5.36 (*dd*, 18.1/1.4)	5.06, 5.34 (*dd*, 18.1/1.4)	5.06, 5.34 (*dd*, 18.1/1.4)	4.85, 4.99 (*dd*, 17.5/1.4)	4.83, 4.99 (*dd*, 17.6/1.8)	4.83, 4.99 (*dd*, 17.6/1.8)	5.06, 5.36 (*dd*, 18.1/1.4)	5.06, 5.34 (*dd*, 18.1/1.4)
22	6.17 (*br s*)	6.158 (*br s*)	6.159 (*br s*)	6.162 (*br s*)	6.133 (*br s*)	6.131 (*br s*)	6.15 (*br s*)	6.156 (*br s*)
1′		5.465 (*dd*, 9.7/1.4)	5.177 (*dd*, 9.7/1.8)		5.489 (*dd*, 9.6/1.9)	5.186 (*dd*, 9.6/1.9)		5.494 (*dd*, 9.7/1.7)
2′		1.96 (*m*), 2.391 (*br d*, 13.2)	1.74, 2.30 (*m*)		1.99 (*ddd*, 3.2 /9.6/2.6) 2.41 (13.6 /3.6/1.9)	1.92-2.00 2.26-2.34 (*m*)		2.472
3′		4.426 (*d*, 2.7)	3.554 (*d*, 2.9)		4.439 (*d*, 2.6)	3.734 (*d*, 2.9)		4.498 (*d*, 2.9)
4′		3.623 (*dd*, .4/9.3)	3.536 (*dd*, .4/9.3)		3.633 (*m*)	3.562 (*m*)		3.669 (*dd*, 2.4/9.3)
5′		4.305 (*m*)	4.233 (*m*)		4.319 (*dq*, 9.4/6.2)	4.135 (*dq*, 9.4/6.2)		4.368 (*m*)
6′		1.597 (*d*, 6.1)	1.540 (*d*, 6.1)		1.602 (*d*, 6.2)	1.544 (*d*, 6.2)		1.634 (*d*, 6.3)
3′-OMe			3.417 (*s*)			3.417 (*s*)		

Table 3 ^{13}C NMR Spectral Data of Cardenolides 1-3, 5-9 (300, 500 MHz, C_5D_5N)

C-Atom	δ_C [ppm]							
	1	2	3	5	6	7	8	9
1	25.95	26.3	26.2	25.7	26.2	26.0	27.5	30.8
2	28.7	26.6	26.6	28.6	26.6	26.4	28.9	27.1
3	67.9	75.9	75.9	67.8	75.9	75.9	66.1	73.1
4	36.1	35.6	35.6	37.9	35.5	35.4	34.5	30.4
5	74.5	73.8	73.8	74.5	73.9	73.8	36.9	37.0
6	37.9	35.6	35.6	35.9	35.5	35.4	27.5	27.1
7	24.6	24.5	24.5	24.0	24.1	24.0	22.3	21.9
8	41.5	41.1	41.1	40.8	40.9	40.7	42.1	41.8
9	40.1	39.4	39.4	39.3	39.5	39.4	36.0	35.8
10	41.1	41.3	41.3	41.3	41.3	41.1	35.9	35.5
11	22.3	22.2	22.2	21.2	21.3	21.1	21.8	21.5
12	39.3	40.1	40.0	31.1	31.1	31.0	33.4	39.8
13	50.1	50.1	50.1	49.3	49.4	49.2	50.3	50.1
14	84.7	84.7	84.7	85.3	85.3	85.2	84.8	84.6
15	33.3	33.3	33.3	31.6	31.7	31.6	30.6	33.1
16	27.4	27.4	27.4	24.9	25.0	24.9	27.5	27.3
17	51.4	51.4	51.4	48.8	48.9	48.8	51.6	51.4
18	16.4	16.4	16.4	18.6	18.7	18.5	16.4	16.2
19	17.6	17.4	17.4	17.4	17.5	17.3	24.3	23.9
20	175.9	175.9	175.8	172.9	172.8	172.9	174.5	174.6
21	73.8	73.8	73.8	74.2	74.2	73.8	73.8	73.7
22	117.7	117.7	117.7	116.7	116.6	116.6	117.7	117.6
23	174.5	174.5	174.4	174.3	174.2	174.3	175.9	176.1
1′		97.6	97.4		97.5	97.4		96.7
2′		39.9	35.9		39.8	35.8		40.1
3′		68.6	78.8		68.6	78.7		68.7
4′		74.1	74.1		74.1	74.0		74.2
5′		70.7	71.2		70.6	71.1		70.3
6′		19.2	19.2		19.2	19.0		19.1
3′-OMe			58.1			58.0		

Antiproliferative Activity of Cardenolides

Six cardenolides isolated from *Streptocaulon tomentosum* were tested for their antiproliferative activity *in vitro* against MCF-7 (human breast cancer cell line) and L 929 (mouse fibroblast cell line) by acid phosphatase method (Yang *et al*., 1996). The antiproliferative activity of compounds **1, 2, 3, 4, 6** and **8** are summarized in Table 4. Cardenolides **1, 2, 3, 4, 6** and **8** show significant antiproliferative activity against MCF 7 cells (IC_{50} < 1 μM - 15,3 μM after 2 days; IC_{50} < 1 μM - 4,31 μM after 5 days incubation). However, cardenolides **2** and **3** possess considerable activity against L 929 (IC_{50} 24.2 and 32.1 μM after 5 days), while other cardenolides show no activity (IC_{50} > 100 μM). The antiproliferative activities of monoglycosidic cardenolides **2, 3** attached to digitoxose are stronger, while those of **4** attached to a disaccharide is weaker than the activity of the aglycone **1**. In addition, the antiproliferative activities of **2** and **3**

are also stronger than that of **5**. Therefore, the configuration of the γ-lactone ring is also significant. The 17α-configuration of the lactone ring correlates with a weaker effect than the 17β-configuration. Similarly, the induction of apoptosis by compounds **2** and **3** in tumor and U 937 cell lines is stronger in comparison to the other compounds.

Cellular Viability and Cell Cycle Analysis of Cardenolides

Four cardenolides (**2, 3, 6,** and **8**) were examined for cellular viability in the tumor cell line and U 937 (human leukemic cell line) at concentrations 100 μM, 10 μM, and 1 μM. All these four cardenolides show toxicity induction of apoptosis at high concentration (> 10 μM) (Table 5) in both cell lines. Compound **2** is the most detrimental at higher concentration in both of cell lines whereas compounds **6** and **8** show less activity. The most interesting observation is the higher activity of compound **2** against tumor cells vs U 937-cells at low concentration (1 μM). The same cardenolides (**2, 3, 6,** and **8**) were also analysed for the percentage of cells in G0, S, G2, G1 phases of the cell life cycle using flow cytometry. 2 cell lines were used, these are human U 937 myeloid leukemia cell line and tur cell line. Compounds **2** and **3** cause a block at the G_2/M-phase at 100 μM and 10 μM in both of cell lines whereas compounds **6** and **8** block at the G_2/M-phase at 100 μM.

Table 4 Antiproliferative Activities of Constituents Isolated from *S. tomentosum* in MCF-7 (Human Breast Cancer Cell Line) and L 929 (Mouse Fibroblast Cell Line)

No		IC_{50} (μM) (2 days incubation)		IC_{50} (μM) (5 days incubation)	
		MCF-7	**L 929**	**MCF-7**	**L 929**
1	17α-H-periplogenin	5.29	> 100	2.57	> 100
2	17α-H-periplogenin-3-*O*-β-*D*-digitoxoside	< 1	51.5	< 1	24.2
3	17α-H-periplogenin-3-*O*-β-*D*-cymaroside	< 1	64.0	< 1	32.1
4	17α-H-periplogenin-β-glucopyranosyl-(1→4)-2-*O*-acetyl-β-digitalopyranoside	15.3	> 100	4.31	> 100
6	17β-H-periplogenin-*3-O*-β-*D*-digitoxoside	7.19	> 100	3.73	> 100
8	17α-H-digitoxigenin	4.16	> 100	< 1	> 100
control	camptothecin	0,0804	0,179	0,0122	0,0285
control	doxorubicin	0,207	0,359	0,0049	0,0168

Table 5 Cell Viability of Constituents Isolated from *S. tomentosum* in U 937 (Human Leukemic Cell Line) and Tumor Cell in %.

compound	Cell Viability (%)					
	U 937			Tumor		
	100 μM	10 μM	1 μM	100 μM	10 μM	1 μM
2	14,60	26,10	82,80	21,50	11,10	69,50
3	26,90	28,90	68,10	42,30	11,50	27,60
6	28,60	93,90	97,10	28,00	70,20	91,20
8	26,70	77,50	97,30	28,90	32,10	89,80
control (1% DMSO)	98,20	98,40	98,70	94,30	94,30	96,10

Conclusion

Some cardenolides isolated from the roots of *Streptocaulon tomentosum* Wight show strong antiproliferative activity. Among six cardenolides, 17α-H-periplogenin-3-*O*-β-*D*-digitoxoside, and 17α-H-periplogenin-3-*O*-β-*D*-cymaroside exhibit significant antiproliferative activity (IC_{50} values, < 1μM) against MCF-7. Four cardenolides were examined for their cellular viability in the tumor cell and U 937 (human leukemic cell line) at concentrations 100 μM, 10 μM, and 1 μM. All these four cardenolides show the induction of apoptosis at 100 μM and 10 μM in both cell lines. So the roots of *Streptocaulon tomentosum* Wight may be useful for the treatment of anticancer.

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