ISOLATION AND CHARACTERIZATION OF URSANE AND OLEANANE TYPE TRITERPENES FROM THE ROOTS OF *STREPTOCAULON TOMENTOSUM*. WIGHT & ARNOTT (‖r i;p*e) (ASCLEPIADACEAE) IN MYANMAR

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Abstract

The aim of this paper is to study the NMR spectroscopic characterization of seven triterpenes from *Streptocaulon tomentosum* Wight & Arnott (Asclepiadaceae). Four ursane type triterpenes and three oleanane type triterpenes were isolated from the roots of *Streptocaulon tomentosum*. by column chromatography and identified by NMR spectroscopy. Comparative studies on NMR spectra of ursane type and oleanane type triterpenes were done. Four ursane type triterpenes were identified as α-amyrin acetate (compound 2),2α,3α,23-trihydroxy-urs-12-en-28-oic-acid (compound 3), 2α,3β-dihydroxy-urs-12-en-28-oic-acid (compound 4), and 2α,3β,23-trihydroxy-urs-12-en-28-oic-acid (compound 6). Three oleanane type triterpenes were identified as β-amyrin acetate (compound 1), 2α,3β-dihydroxy-olean-12-en-28-oic-acid (compound 5) and 2α,3β,23-trihydroxy-olean-12-en-28-oic-acid (compound 7).

Keywords: *Streptocaulon tomentosum*, Asclepiadaceae, ursane, oleanane, triterpenes

Introduction

The roots of *Streptocaulon tomentosum* are used in Myanmar in traditional medicine for the treatment of anticancer, dysentery and stomach-ache, and the leaves are used externally for the treatment of snake poisoning and abscesses. The triterpenoids, having a C_{30} skeleton, constitute a large, diverse group of natural products derived from squalene or, in the case of 3β-hydroxy triterpenoids, the 3*S*-isomer of squalene 2,3-epoxide. In excess of 4000 triterpenoids have been isolated so far and more than 40 skeletal types have been identified. Oleananes and ursanes often occur together and, in the past decade, have been reported from a wide range of families including the Araliaceae, Asclepiadaceae, Bignononiaceae, Cactaceae, Campanulaceae, Celastraceae, Compositae, Ericaceae, Fagaceae, Labiateae, Leguminosae, Phytolaccaceae, Primulaceae, Rosaceae, Rubiaceae, Sapotaceae, Theaceae, Umbelliferae and Urticaceae (Dey & Harborne, 1991). The biological activities of triterpenoids and triterpenoid saponins are immunostimulation (Press *et al*., 2000), anti-tumor-promoting activity (Konoshima & Takasaki, 2000), anti-inflammatory activity and anti-insect activity (Connolly & Hill, 2002). This paper focused on the NMR spectroscopic characterization of ursane and oleanane types triterpenes isolated from the roots of *Streptocaulon tomentosum* Wight and Arn. (Asclepiadaceae).

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.

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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. 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 of Triterpenes from the Roots of *Streptocaulon tomentosum*

Dried powdered root of *Streptocaulon tomentosum* (Asclepiadaceae) (1 kg) was extracted with 80% EtOH (1 L × 3) for one week. The solvent was evaporated to the remaining water layer. Then the water layer was successively partitioned between organic solvents (*n*-hexane, ethyl acetate, *n*-butanol 300 mL × 3) and water. The *n*-hexane fraction (48 g) was chromatographed over silica gel 60 (70-230 mesh, Merck), using a stepwise gradient of *n*-hexane: ethyl acetate (9.5:0.5, 9:1, ...2:1, increasing polarity) to give four fractions. Fraction 1 (8 g) and fraction 2 (4 g) were rechromatographed on silica gel 60 column (230-400 mesh, column size id 2 cm × 60 cm) with the solvent system *n*-hexane: chloroform (3:1) and (9:1-4:1) to give β-amyrin acetate (**1**), and α-amyrin acetate (**2**). The ethyl acetate fraction (12 g) was separated on a silicagel column (70-230 mesh, column size id 3 cm × 60 cm) and eluted with *n*-hexane: ethyl acetate: methanol (increasing polarity 9:1:0, 7:3:0.5, ...to pure MeOH) to give 23 fractions (each about 300 mg). Fractions 5-11 were rechromatographed on silica gel 60 (230-400 mesh, column size 1.5 cm × 40 cm) using $CHCl_3$: MeOH (9.5:0.5; 4.7:0.3) to give compounds **3-7.**

Identification of Ursane and Oleanane Types Triterpenes

The structures elucidations were determined by ^{1}H NMR, ^{13}C NMR, ESI-MS, FT-IR, GC-MS and confirmed by comparison with the literature values (Matsunaga *et al*., 1988; Chen *et al*., 1993; Hisham *et al*., 1993; De Pascual Teresa *et al*., 1987).

Results and Discussion

Structure Elucidation of Ursane and Oleanane Type Triterpenes

The *n*-hexane fraction was repeatedly chromatographed on a silica gel column and β-amyrin acetate (**1**), and α-amyrin acetate (**2**). The structures elucidations were determined by ^{1}H NMR, ^{13}C NMR, ESI-MS, GC-MS and confirmed by comparison with the literature values.

Compound 1: β-amyrin acetate

colourless needles Yield: 100 mg, 0.01%

mp.: 242-243 °C

$[\alpha]_D$: + 80.1 ° (c = 1.10, $CHCl_3$)

TLC: R_f = 0.47 (system T_1, violet colour with vanillin/H_2SO_4, inactive under UV)

IR: (KBr), ν_{max}= 1730, 1635, 1240, 812 cm^{-1}

^{1}H NMR: (300 MHz, $CDCl_3$): δ 0.84 (3H, *s*, H-28), 0.88 (12H, *s*, H-23, 24, 29, 30), 0.98 (6H, *s*, H-25, 26), 1,14 (3H, *s*, H-27), 2.07 (3H, *s*, OAc), 4.54 (1H, *dd*, *J* 11.6 Hz, H-3α), 5.21 (1H, *t*, *J* 3.5 Hz, H-12)

GC-MS: RT = 18.03 min, 468 $[M]^+$, 453 (33), 408 $[M\text{-}HOAc]^+$ (5), 393 (40), 281 (70), 218 (100), 203 (68), 69 (90)

EI-MS: (70 ev) *m/z* (rel. int): 468 $[M]^+$, 453 (2), 408 $[M\text{-}HOAc]^+$ (5), 218 (100), 203 (20)

Compound 2: α- amyrin acetate

Colourless needles, Yield: 1.5 g, 0.15%

mp.: 243 °C

$[\alpha]_D$: + 76 ° (c = 1.0, $CHCl_3$)

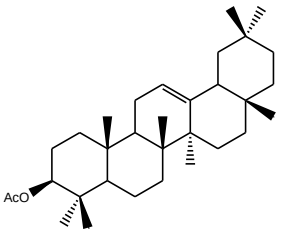
TLC: R_f = 0.47 (system T_1, violet colour with vanillin/H_2SO_4, inactive under UV)

IR: (KBr), ν_{max}= 1730, 1380, 1370, 1250, 1030, 1000, 985, 960 cm^{-1}

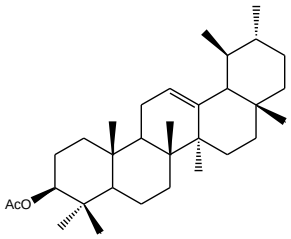
^1H NMR: (300 MHz, $CDCl_3$): δ0.79 (3H, *s*, H-28), 0.88 (12H, *s*, H-23, 24, 29, 30), 0.98 (3H, *s*, H-26), 1.01 (3H, *s*, H-25), 1,07 (3H, *s*, H-27), 2.05 (3H, *s*, OAc), 4.50 (1H, *dd*, *J* 9.7 Hz, H-3α), 5.12 (1H, *t*, *J* 3.6 Hz, H-12)

GC-MS: RT = 18.85 min, 468 $[M]^+$, 453 (45), 408 [M- HOAc]$^+$ (38), 393 (40), 281 (100), 218 (80), 203 (30), 69 (98)

EI-MS: (70 ev) *m/z* (rel. int): 468 $[M]^+$, 453 (10), 408 [M- HOAc]$^+$ (20), 218 (100), 203 (10)



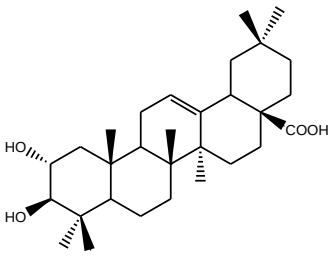
Compound 1
(Oleanane type)



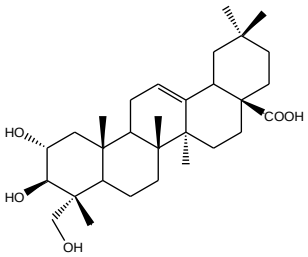
Compound 2
(Ursane type)

Compound **3-7** were isolated from fraction 5-11 of the ethyl acetate extract after repeated column chromatography on silica gel. Identification of these known compounds was based on 1D and 2D NMR, MS and comparison of their spectroscopic data with literature values (Sashida *et al*., 1992; Kojima & Ogura, 1986; Yaguchi, 1988).

In the compounds **3, 6** and **7** the molecular formula is $C_{30}H_{48}O_5$ by means of HR-ESI-MS. In compound **4** and **5** the molecular formula is $C_{30}H_{48}O_4$. Their mass spectra present ions at *m/z* 248 resulting from the retro-Diels-Alder fragmentation characteristic of the ursane and oleane skeletons. Furthermore they possess an ion at *m/z* 203 characteristic of Δ^{12}- triterpenoids (Budzikiewicz *et al*., 1963). In the ^1H NMR (C_5D_5N) spectra of these compounds, the signal of H-18 permitted the distinction between the oleane and ursane skeletons. The H-18 signal appears at δ2.6 ppm in the ursane skeleton and at δ3.3 ppm in the oleane skeleton. The proton signals of H-29 and H-30 in the ursane skeletons appears as a doublet, but in oleane as a singlet. The chemical shifts of C-12 and C-13 (δ125 and 139 ppm in ursane, δ122 and 144 ppm in oleane) and H-12 (δ5.20-5.4 ppm) suggests that these compounds are Δ^{12}-unsaturated triterpenoids. The ^{13}C NMR spectra (table. 1) clearly exhibited the difference in the chemical shifts of C-12, C-13, C-17, C-18, C-19, C-20, C-22, C-27, C-29 and C-30 between the ursane group (**3, 4, 6**) and the oleane group (**5, 7**). The coupling constant of H-3 (*J* 2.3 Hz) in **3** suggested that two OH groups at C-2 and 3 were at the *cis* position. Besides, the ROESY correlation between H-3 and H-2, H-23, H-24 also confirmed the β-configuration of H-2 and H-3. However, the coupling constant of H-3 (*J* 9.4 Hz) in **4-7** showed that the two OH groups at C-2 and C-3 were in *trans* position and there was no ROESY correlation between H-3 and H-2 (**see HMBC, ROESY in table 2**).

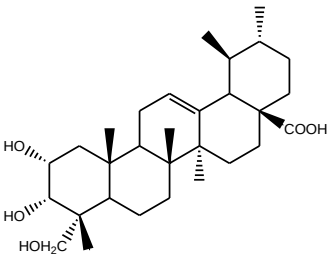


compound 5

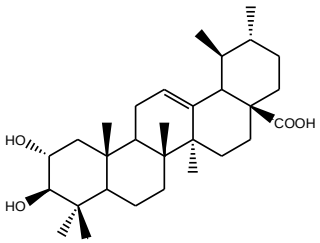


compound 7

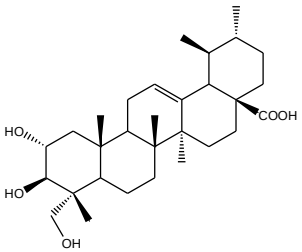
Figure 1 Oleanane type triterpenes



compound 3



compound 4



compound 6

Figure 2 Ursane type triterpenes

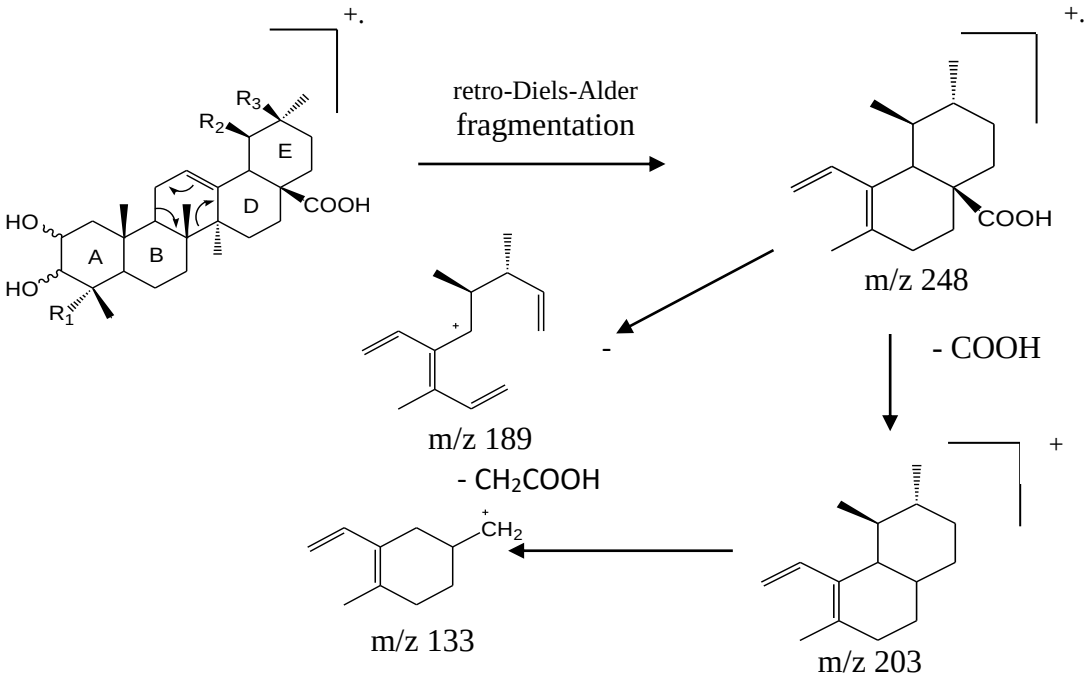


Figure 3 Proposed EI mass spectral fragmentation of triterpenoids **1-7**

Table 1 ^{13}C NMR spectral data of triterpenoid 2-7

(300, 500 MHz, 2 in $CDCl_3$; 3-5 in C_5D_5N; 6-7 in CD_3OD)

C-Atom	δ_C [ppm]					
	2	**3**	**4**	**5**	**6**	**7**
1	38.5	42.8	47.9	47.7	48.0	47.9
2	23.4	66.2	68.6	68.6	69.7	69.7
3	80.9	78.9	83.8	83.8	78.1	78.1
4	37.7	41.9	40.0	40.0	44.1	44.1
5	55.3	43.5	55.9	55.9	48.1	48.1
6	18.3	18.3	18.8	18.8	19.1	19.1
7	32.9	33.2	33.2	33.2	33.6	33.6
8	39.7	40.1	39.8	39.8	40.8	40.5
9	47.7	47.9	48.1	48.1	48.2	48.2
10	36.8	38.3	38.4	38.5	39.0	39.0
11	22.8	23.7	23.7	23.7	24.5	24.5
12	124.2	125.5	125.5	122.4	126.6	123.4
13	139.5	139.3	139.3	144.7	139.8	145.4
14	42.1	42.5	42.5	42.2	43.4	43.0
15	28.2	28.6	28.6	28.2	29.1	28.8
16	26.7	24.9	24.9	23.9	24.6	24.0
17	33.8	48.0	48.0	46.6	48.9	47.6
18	59.0	53.5	53.5	41.9	54.3	42.7
19	39.7	39.4	39.4	46.4	40.4	47.2
20	39.7	39.4	39.3	30.9	40.4	31.8
21	31.3	31.0	31.0	33.5	31.6	33.3
22	41.6	37.4	37.4	34.2	38.1	34.9
23	28.1	71.2	29.4	29.3	66.2	66.2
24	15.8	17.8	17.7	17.7	13.9	13.9
25	14.2	17.1	16.9	16.9	17.5	17.5
26	16.8	17.5	17.5	17.7	17.8	17.7
27	17.6	23.8	23.9	26.1	24.1	26.5
28	28.8	179.9	179.9	179.9	181.7	181.5
29	23.3	17.5	17.5	33.2	17.7	33.6
30	21.4	21.3	21.4	23.7	21.6	24.0
COMe	21.5					
CO	170.8					

Table 2 NMR data of triterpenoids 3-7 (500 MHz, 3-5 in C_5D_5N; 6-7 in CD_3OD)

H-Atom	δ_H [ppm]					HMBC	ROESY
	3	**4**	**5**	**6**	**7**	**(3-7)**	**(3-7)**
1	1.82, 1.94 (*m*)	1.28, 2.26 (*m*)	1.28, 2.26 (*m*)	0.88, 1.96 (*m*)	0.88, 1.96 (*m*)	C-2, 3, 25	
2	4.289 (*m*)	4.115 (*ddd*, 11/9.4/4.4)	4.115 (*ddd*, 11/9.4/4.4)	3.687 (*m*)	3.687 (*m*)	C-3	H-3 (in **3**), 25, 24
3	4.168 (*d*, 2.3)	3.420 (*d*, 9.4)	3.420 (*d*, 9.4)	3.350 (*dd*,9.7/2.4)	3.350 (*dd*,9.7/2.4)	C-24, 4, 1	H-2 (in **3**), 23, 24
5	2.02-2.08 (*m*)	1.04 (*m*)	1.04 (*m*)	1.28 (*m*)	1.28 (*m)*	C-25, 10, 4	
6	1.34, 1.60 (*m*)	1.36, 1.54 (*m*)	1.36, 1.54 (*m*)	1.38 (*m*)	1.38 (*m*)	C-25, 5	
7	1.34, 1.72 (*m*)	1.84, 2.04 (*m*)	1.84, 2.04 (*m*)	1.54, 1.74 (*m*)	1.54, 1.74 (*m*)	C-26, 8, 14	
9	1.94 (*m*)	1.76 (*m*)	1.76 (*m*)	1.66 (*m*)	1.66 (*m*)	C-25, 11, 10, 8, 5,	H-25, 26
11	1.96-2.08 (*m*)	1.98 (*m*)	1.98 (*m*)	1.94 (*m*)	1.94 (*m*)	C-12, 13, 9, 8, 10	
12	5.480 (*brs*)	5.476 (*m*)	5.476 (*m*)	5.242 (*m*)	5.242 (*m*)	C-11, 14, 9, 18, 13	H-18, 29
15	1.14-2.36 (*m*)	1.18, 2.36 (*m*)	1.18, 2.36 (*m*)	1.08 (*m*)	1.08 (*m*)	C-27, 26, 16, 8, 14	
16	1.98-2.06 (*m*)	2.00, 2.12 (*m*)	2.00, 2.12 (*m*)	1.94 (*m*)	1.94 (*m*)	C-15, 17	
18	2.626 (*br d*, 11.3)	2.641 (*br d*, 11.4)	3.314 (*dd*, 13.9, 4.0)	2.202 (*d*, 11.2)	2.849 (*dd*, 13.6, 3.9)	C-9, 16, 19, 20, 14, 17, 12, 13, 28	H-29, 20 (in ursane type), 12
19	1.42 (*m*)	1.46 (*m*)	1.28, 1.80 (*m*)	1.38 (*m*)	1.14, 1.70 (*m*)	C-29, 30, 22, 20, 17, 18	
20	1.00 (*m*)	1.04 (*m*)		0.98 (*m*)		C-30, 21 (in ursane type)	
21	1.34, 1.44 (*m*)	1.40 (*m*)	1.36, 1.56 (*m*)	1.36, 1.52 (*m*)	1.28, 1.66 (*m*)	C-29, 30, 22, 20, 17,	
22	1.96 (*m*)	1.98 (*m*)	1.18, 1.46 (*m*)	1.66 (*m*)	1.20, 1.40 (*m*)	C-17, 28	
23	3.77, 3.94 (*d*, 10.8)	1.291 (*s*)	1.291 (*s*)	3.261 (*d*, 11.0)	3.261 (*d*, 11.0)	C-24, 2, 3, 4, 5	H-3
24	0.87 (*s*)	1.092 (*s*)	1.092 (*s*)	0.692 (*s*)	0.690 (*s*)	C-25, 4, 5, 23, 3	H-25,2, 3, 23
25	1.00 (*s*)	0.991 (*s*)	0.991 (*s*)	1.042 (*s*)	1.028 (*s*)	C-24, 11, 10, 4, 5, 9, 1	H-24, 26, 3
26	1.07 (*s*)	1.060 (*s*)	1.032 (*s*)	0.846 (*s*)	0.813 (*s*)	C-25, 7, 8, 14, 9	
27	1.14 (*s*)	1.220 (*s*)	1.275 (*s*)	1.132 (*s*)	1.175 (*s*)	C-15, 8,14, 18, 12, 13	
29	0.965 (*d*, 6.4)	0.995 (*d*, 4.9)	0.954 (*s*)	0.865 (*d*, 6.4)	0.907 (*s*)	C-19, 20	
30	0.925 (*d*, 6.2)	0.960 (*d*, 5.9)	1.014 (*s*)	0.967 (*d*, 6.0)	0.941 (*s*)	C-21, 19, 20	

Conclusion

Oleanane and ursane type triterpenes are rich in the roots of *Streptocaulon tomentosum* Wight. Literature survey showed that oleanane and ursane typetriterpenes possess anti-tumor-promoting activity, anti-inflammatory activity and anti-insect activity. So the roots of *Streptocaulon tomentosum* Wight may be useful for the treatment of anticancer.

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