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Isolation and Characterization of a New Compound from *Sarcococca saligna*

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Abstract: ***A New Alkaloid named Sracosalgnenone [(20S) -20- (dimethylamino) -3β-tigoloyl-pregn-5, 16-diene-4-one] was isolated from sarcococca saligna and its structure was established on the basis of spectroscopic techniques including^{1}H, ^{13}C-NMR and inverse 2D-NMR techniques (DEPT, HMQC and HMBC) UV, MS etc.***

Keywords: *Sarcococca saligna, Buxaceae, steroidal alkaloids, Sracosalgnenone.*

1. Introduction

Sarcococca saligna Muel (syn. *Sarcococca pruniformis* Lindl.) found in Pakistan [1] generally at an altitude form 4000-9000 ft. above sea level. By the local population it is used in the treatment of rheumatism [2, 3]. Many of steroidal alkaloids isolated from aerial parts of plant show cholinesterase inhibition [2-18]. Its alkaloids are antispasmodic, antidiarrheal, antisecretory and calcium antagonist properties. [19-20]. A characteristic feature of the family *Buxaceaes* is its high contents of steroidal alkaloids[21]. Taxonomically it comprises of different genera Pachysandra, *Sarcococca*, simmonsia and Buxus. A number of alkaloids have been isolated from genus Buxus. The extracts of various species of this genus have been used for the treatment of a variety of ailments and skin disease etc. in folk medicine. [22]]. A number of compounds have been identified by GCMS technique from ariel parts of *sarcococca saligna* [23]. The present study describes the isolation of one new pregnane type alkaloid *sracosalgnenone* and its structure determination on the basis of spectroscopic techniques.

2. Literature Survey

S. saligna Muel (syn. *S. pruniformis* Lindl.) is an evergreen shrub abundantly found in the northwest region of Pakistan [1]. Ismat Naeem et al, isolated a new alkaloid, sarcocenaene (3α-dimethylamino-20 α-*N*-methyl-*N*-acylamino-pregna-5, 16-diene), and two known alkaloids, pachyaximine-A and saracodine were isolated from *S. saligna* [9]. Recently Atta-ur-Rahman and his research group isolated a number of pregnane type steroidal alkaloids from *S. saligna* [10] [11] [16] [24]. . Three tri-terpenes were also isolated from *S. saligna* [14] and a number of other compounds were identified by GC MS analysis. [15]

3. Materials and Method

General experimental procedure: IR spectra: JASCO 302-A spectrophotometer; UV spectra: Hitachi U3200 spectrophotometer; EI, FD and HREI MS: JMS 11×100 (with data system) and JMS-DA 500 mass spectrometers; ^{1}H and ^{13}C NMR spectra: Bruker NMR spectrometer at 500 and 125 MHz, respectively at room temperature; chemical shift values (δ) in ppm, coupling constants (J) in Hz. Standard pulse sequences were used for COSY, HOHAHA, DEPT, HMQC and HMBC experiments.

Chromatographic conditions: TLC (pre coated silica G-25plates UV254) ; CC: Silica gel, 230-400 mesh. Detection of the spots: 254 and 336 nm by UV and Dragendorff's spray reagent.

Plant Material: Aerial parts of Sarcococca saligna Muel. (40kg) were collected from Kuldana Murree Hills, Pakistan, in October 2004.

Extraction and Isolation: The ethanolic extract of the air dried plant (20kg) was evaporated to a gum (2. 1kg) and extracted with petroleum ether to remove non polar constituents. Total alkaloids (900g) were obtained by extraction into 10% acetic acid. Partial separations of the alkaloids were achieved by extraction with $CHCL_3$ at different pH values (3. 5, 8. 5). The fraction obtained at pH 3. 5 (80g) was subjected to C. C on silica gel and eluted with $CHCL_3$ and then with $CHCL_3$-MeoH to obtain several fractions. A fraction obtained by VLC on elution with $CHCL_3$: MeOH (89: 11) was further purified by preparative TLC using n-hexane: ethylacetate: diethyl amine (8. 5: 1. 3: 0. 2) as eluent to yield pure compound *Sracosalgnenone* (5mg).

Sracosalgnenone: White solid m. p. 231-236 ˚C; UV λmax (MeOH) 278, 266, 204, 194; IRνmax ($CHCL_3$) : 3392, 2800, 1710 (α, β-unsaturated ketonic group), 1660, 1350 (cm^{-1}) ; MS *m/z (*%) 438 (M^+, 10), 423 (M^+_Me, 100), 83 (48), 72 (38), 55. ^{1}H-NMR ($CDCL_3$, 500 MHz at RT) δ: 1. 84/1. 92 (2H, m, 2H-1), 2. 17/1. 14 (2H, m, 2H-2), 3. 05 (1H, m), 6. 49 (1H, d, J=6. 9Hz, H-6), 2. 55 (1H, dd, J=18. 0, 6. 9Hz, H-7), 2. 34 (1H, *dd*, *J*=18. 0, 2. 5Hz, H-7), 1. 83 (1H, m, H-8), 2. 30 (1H, m, H-9), 1. 42/1. 60 (2H, m, 2H-11), 1. 87/1. 92 (2H, m, 2H-12), 1. 13 (1H, m, H-14), 1. 24 (2H, m, 2H-15), 5. 7 (1H, *s*, 1H-16), 0. 83 (3H, *s*, CH_3-18), 0. 90 (3H, *s*, CH_3-19) 2. 23 (1H, *q*, *J*=6. 5Hz, H-20), 0. 88 (3H, *d*, *J*=6. 5Hz, CH_3-21, 2. 30 (6H, *br. s*, *N* $(CH_3)_2$), 6. 50 (1H, *q*, 6. 9Hz, H-3'), 1. 7893H, *d*, *J*=6. 9Hz, CH_3-4'), 1. 87 (1H, *s*, CH_3-5'), 8. 17 (1H, br. s., *NH*) (**Table).**

4. Results and Discussion

An ethanolic extract of aerial parts of *Sarcococca saligna*

after evaporation was triturated with n-hexane to remove nonpolar compounds. The insoluble content was then partitioned between chloroform and aqueous acid solution at various pH values. The chloroform extract was subjected to repeated column chromatography to afford new alkaloid *Sracosalgnenone*. Compound was obtained as a white solid. The HREI mass spectrum of compound reveal a molecular ion peak [M^+] at *m/z* 438. 3226 analyzing for $C_{28}H_{42}N_2O_2$ with nine degrees of hydrogen (H_2) deficiency. The IR spectrum ($CHCL_3$) showed absorptions at 3392 (NH), 1620-1660 (C=O) and 1710cm^{-1}characteristic of an amide, and α, β-unsaturated ketone functions respectively. The ^{1}H NMR spectrum of compound showed characteristics chemical shifts of a tigloyl group at C-3 [10]. One proton resonating at δ 2. 35 showed direct correlation to carbon C-3 chemical shift at δ 57. 4 and HMBC connectivities with C-2 (δ 33. 0), a carbonyl carbon at C-4 (δ196) and C-5 (δ133. 0). One olefinic proton doublet resonating down field at δ 6. 49 showed COSY 45º interaction with C-7 protons and 1-3 bond removed correlation to carbon atoms with chemical shifts at δ 196. 6, 133. 0, 38. 9, 51. 0 suggesting a double bond at C-5, β to carbonyl function at C-4 [10]. Another one olefinic proton resonating at δ 5. 7 was directly connected to carbon atom with ^{13}C chemical shift at δ 127. 0 and showed HMBC connectivities to C-17, C-14 and C-13 (δ 156. 0, 22. 8 and 55. 1 respectively). Six proton signals of two methyl groups resonated at δ 0. 83 and 0. 90 and were due to C-18 and C-19 angular methyl groups of pregnane type skeleton.

A three proton doublet at δ 0. 89 (J = 6. 5 *Hz*) was assigned to secondary C-21 methyl group. The COSY 45° spectrum showed connectivity between C-21 methyl and C-20 methine proton (δ 2. 25). A broad six proton singlet at δ 2. 30 was assigned to a dimethyl amino group at C-20. On the basis of NMR (**Table**) and 2-D NMR studies (HMQC, HMBC, DEBT etc) the compound was assigned structure ([(20S) -20- (N, N-dimethylamino-3--tigloylamino) pregn-5, 16-diene-4-one]) and was found to be a new alkaloid named *sarcosalgnenone*.

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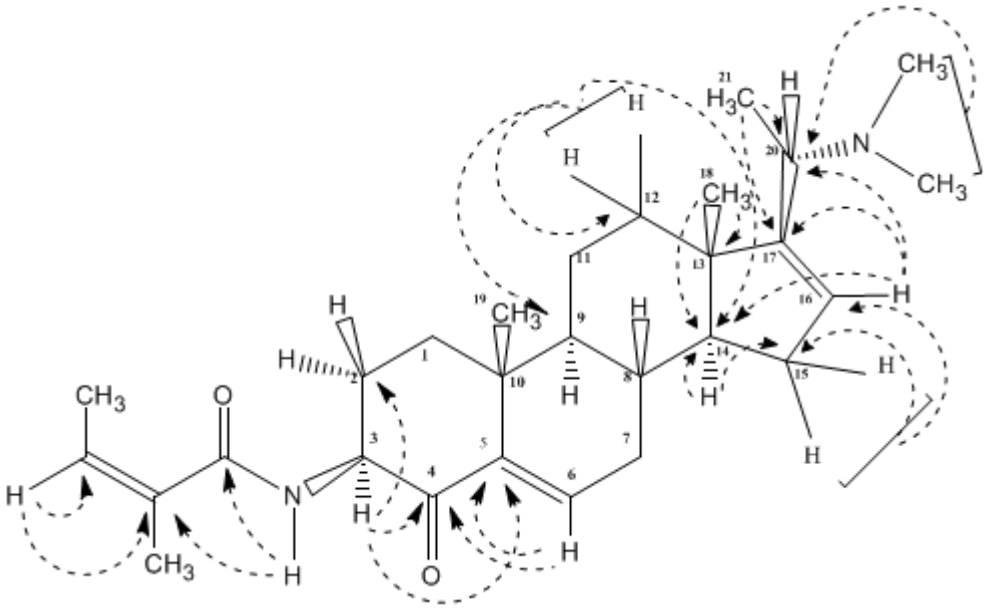
Table 3: D NMR assignments of compound (*Sracosalgnenone*)

No	Multiplicity	13C Shift (δ)	1H Shift (δ)	Important HMBC	No	Multiplicity	13C Shift (δ)	1H Shift (δ)	Important HMBC
1.	CH_2	34.3	1.84/1.92		14.	CH	54.4	1.13 (1H, *m*)	C14, C15
2.	CH_2	33.2	2.17/ 1.14 (1H, m)		15.	CH_2	20.8	1.24 (2H, *m*)	C15, C16
3.	CH	57.4	2.35 (1H, *m*),	C2, C4, C5	16.	CH	127.0	5.7 (1H, *s*)	C16, C17, C14, C13
4.	C	196.6	-		17.	C	156.0	-	
5.	C	133.0	-		18.	CH_3	12.2	0.83 (3H, *s*)	C13, C14, C17
6.	CH	131.8	6.49 (*d*, *J* = 6.9 *Hz*)	C4, C5,	19.	CH_3	13.3	0.90 (3H, *s*)	C1, C5
7.	CH_2	38.9	2.55 (*dd*, *J* = 18.0, 2.5 *Hz*) 2.34 (*dd*, *J* = 6.9, 2.5 *Hz*)		20.	CH	55.1	2.23 (1H, *q*, *J* = 6.5*Hz*)	-
8.	CH	24.1	1.83 (1H, *m*)		21.	CH_3	16.0	0.88 (3H, *d*, *J* = 6.5*Hz*)	C17, C20, C16
9.	CH	51.0	2.30 (1H, *m*)		22.	NCH_3	42.3	2.30 (3H, *bs*)	C20
10.	C	40.1	-		23.	NCH_3	42.3	2.30 (3H, *bs*)	C20
11.	CH_2	20.6	1.42/1.60 (2H, *m*)		24	C1'	167.7	-	
12.	CH_2	30.8	1.87/1.92 (2H, *m*)	C9, C12,	25.	C2'	131.8	-	
13.	C	46.0	-		26	C3'	133.5	6.50 (1H, *q*, 6.9 *Hz*)	
					27	C4'	12.0	1.78 (3H, *d*, *J* = 6.9 *Hz*)	
					28	C5'	14.0	1.87 (3H, *s*)	C2', C5'
						NH	-	8.17	C2', C1'



Structure of compound (*Sracosalgnenone*)
Showing some HMBC Connectivity's of Compound