Separation and characterization of a new Alkaloid from Sarcococca saligna

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

Key Words: Sarcococca saligna ,Buxaceae,steroidal alkaloids,Sracosalgmionl.

1 INTRODUCTION

Sarcococca saligna Muel (syn. Sarcococca pruniformis Lindl.) grows abundantly in Kashmir Ceylon, Afghanistan and Sumatra [1]. Locally it is extensively used as a febrifuge and in the treatment of rheumatism [2],[3]. A characteristic feature of family Buxaceae is its high contents of steroidal alkaloids [4].Taxonomically it comprises of different genera Pachysandra, Sarcococca, simmonsia and Buxus.A number of alkaloids have been isolated from genus Buxus. The alkaloids found in genus Sarcococca and Pachysandra is simple pregnane derivatives lacking methyl substitution at C-4 and C-14, structurally they are very close to steroidal alkaloids of Apocynaceae [5]. Examples are sarcocine and pachysandra-A. The Pachyxadra alkaloids have been found to be active against gastric ulcers in mice [6]. A crystalline base isolated form leaves of S. saligna was tested for biological activity and its effect on neuromuscular transmissions were found to be remarkable [7].The extracts of various species of this genus have been used for the treatment of a variety of ailment and skin diseases etc. in folk medicine [8]. 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] [12] [13]. . Three tri-terpenes were also isolated from S. saligna [14] and a number of other compounds were identified by GC MS analysis. [15]

2 MATERIALS AND METHOD: General experimental procedure : IR spectra : JASCO 302-A spectrophotometer; UV spectra :Hitachi U 3200 spectrophotometer ; EI,FD and HREI MS : JMS 11×100 (with data system) and JMS-DA 500 mass spectrometers; 1H and 13C 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.

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3 CHROMATOGRAPHIC CONDITIONS: TLC (pre coated silica G-25 plates UV254) ; CC : Silica gel, (60_230 mesh, merck).Detection of the spots : 254 and 336 nm by UV and Dragendorff's spray reagent.

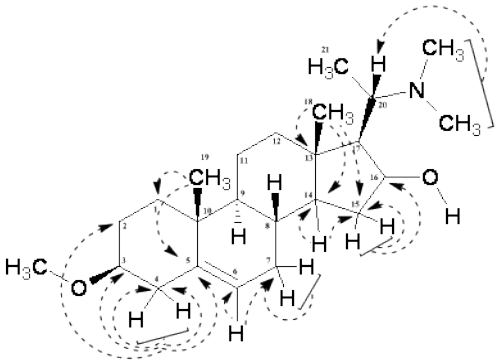
4 PLANT MATERIAL: Aerial parts of Sarcococca saligna Muel.(40kg) were collected from Kuldana Murree Hills,Pakistan,in October 2004.

5 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 separation 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 dichloromethane:MeOH (21:4) yielded a fraction containing alkaloids which are rechromatographed on silica gel column to afford a white impure solid. It was purified by prep.TLC to give pure compound Sracosalgminol (5.5 mg).

Sracosalgminol: White solid m.p.242-247 ° C; [α]D27 :+37 c 0.40,$CHCL_3$; UV λmax (MeOH) inconclusive ; IR vmax (KBr) : 3600,3550,2950,1665 cm-1 ; MS m/z(%)359 (M+_ H_2O , 3), 344(4), 84(1.9), 72(100%), 58(7.8) ^{1}H – NMR: ($CDCl_3$, 500 MHz at RT) δ : 1.82/1.01 (2H, m, 2H-1), 1.37/1.87m (2H, m, 2H-2), 3.04 (^{1}H, dddd, J= 15.0,15.0, 9.0, 4.5 Hz, H-3), 2.35/2.13 (2H, m, 2H-4), 5.34 (1H, t, H-6), 1.51/1.91 (2H, m, 2H-7) 1.45 (1H, m, H-8), 0.91 (1H, m, H-9), 1.40/1.52 (2H, m, 2H-11),1.16/1.93 (2H, m, 2H-12), 1.05 (1H, m, H-14), 1.08/1.59 (2H, m, 2H-15), 3.15 (1H, m, H-16), 1.50 (1H, m, H-17), 0.73 (3H, s, CH_3–18), 0.98 (3H, s, CH_3-19), 2.46 (1H, q, J = 6.4 Hz, H-20), 1.33 (3H, d, J = 6.4 Hz, CH_3–21), 2.65 (3H, br.s, N(CH_3)), 2.85 (3H, bs, NCH_3), 3.34 (3H, s, OCH_3) **(Table)**

6 RESULTS AND DISCUSSION:

Compound was isolated as a white solid. The HREI mass spectrum of compound revealed no molecular ion peak. An ion peak observed at m/z 359 was formed by the elimination of one water molecule from the molecular ion thus suggesting the molecular formula of the compound as $C_{24}H_{43}NO_2$ corresponding to the molecular ion m/z 377.



Structure of compound (*Sracosalgminol*) Showing HMBC connectivity's

Hence the compound showed five degrees of Hydrogen (H_2) deficiency. Four of these were accounted for a tetracyclic pregnane type structure and one for a double bond. The compound showed a base peak at m/z 72.0835 (C_4H_9N), which is characteristic of 20α-dimethyl amino group. The IR spectrum ($CHCl_3$) showed absorptions at 3400 (OH), 3350 (NH) and 1664 cm^{-1} characteristic of hydroxy, amino and methoxy functions respectively.

The ^1H NMR spectrum of compound displayed a three–proton singlet at δ 3.34 indicating the presence of a methoxy group. Two three–proton singlets at δ 0.73 and 0.98 were assigned to two angular methyl groups. A doublet at δ 1.33 (J = 6.5 Hz) was due to C-21 methyl group showing COSY 45° interaction with H-20 proton (δ 2.46, q). While broad singlets δ 2.65 and 2.85 were due to dimethylamino group at C-20, which was supported by the presence of a base peak m/z 72 in the mass spectrum. The de-shielding and splitting of N-methyl signals was suggesting the vicinity of a polar (OH, OCH_3) may have something to do with it. The H-16 proton resonated at δ 3.19 and was not acetylated indicating hydroxy group at C-16 is β-oriented and sterically hindered. The H–6 olefinic proton (δ 5.34, m) showed interactions with H–7 protons resonating at δ 2.01. The H-3 proton resonated at δ 3.06 and showed connectivity's with H-4 methylene protons resonating as multiplets at δ 2.17 and 2.38. The assignment of chemical shifts was further confirmed by HMQC which showed direct one bond correlation of all the protons **(Table)**.

Table: 3D NMR assignments of (Sracosalgminol)

No	Multipli -city	13C shift(δ)	1H Shift(δ)	Important HMBC
1	CH_2	37.2	1.82/1.01(2H,m)	C1,C3
2	CH_2	28.0	1.37/1.87(2H,m)	_
3	CH	65.3	3.04 (1H,m)	_
4	CH_2	38.7	2.35/2.13(2H,m)	C2,C3,C4,C5,C6
5	C	140.9	_	_
6	CH	121.3	5.34(1H,t)	C4,C7,C10
7	CH_2	31.8	1.51/1.91(2H,m)	C7
8	CH	31.7	1.45(1H,m,H-8)	_
9	CH	49.9	0.91(1H,m,H-9)	C8,C10
10	C	36.1	_	_
11	CH_2	21.0	1.40/1.51(2H,m)	_
12	CH_2	30.8	1.61/1.93(2H,m)	C9,C12,C14
13	C	43.2	_	_
14	CH	56.4	1.05(1H,m)	C14,C15
15	CH_2	39.5	1.08/1.59(2H,m)	C15,C16
16	CH	80.4	3.19(1H,m)	_
17	CH	55.3	1.50(1H,m)	_
18	CH_3	12.2	0.73(3H,s)	C13,C14,C17
19	CH_3	19.3	0.98(3H,s)	C1,C5
20	CH	52.5	2.46(1H,q,J=6.4HZ)	NCH3
21	CH_3	13.0	1.33(3H,d,J=6.4HZ)	C17,C20,C16
22	NCH_3	36.0	2.65(3H,bs)	C20
23	NCH_3	43.3	2.85(3H,bs)	C20
24	OCH_3	55.6	3.34(3H,s)	C3

HMBC connectivities of compound were particularly informative and indicated the position of hydroxyl group at C-16 (δ 80.4) **(Table)** and suggesting it a methylated product of compound . On the basis of above evidences, compound was inferred to be a new alkaloid isolated from S. saligna and named sarcosalgminol [(20S)-20-(dimethylamino)-16β-hydroxy-3β-methoxy-pregn-5-ene].

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