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A New Pregnane-type Alkaloid from *Sarcococca saligna*

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Abstract: A new alkaloid, named sracosalgmine [(20S)-20-(dimethylamino)-16β, 3β-dimethoxy-pregn-5-ene] 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.

Key words: *Sarcococca saligna,* Buxaceae, steroidal alkaloids, sracosalgmine

INTRODUCTION

Sarcococca saligna Muel. (syn. *Sarcococca pruniformis* Lindl.) is an evergreen shrub abundantly found in the northwest region of Pakistan[1]. The leaves of this plant are commonly used locally for the treatment of fever and rheumatism[2,3]. A number of steroidal alkaloids have been reported from the leaves and from the aerial parts of this plant some of them showing cholin estrase inhibition[2-18].The steroidal alkaloids isolated from this plant also show presence of antispasmodic, antidiarrheal, antisecretory and calcium antagonist properties[19,20]. Some terpenoids have also been isolated from this plant[21]. A number of compounds have been identified by GC-MS technique from aerial parts of *Sarcococca saligna*[22]. The present study describes the isolation of one new pregnane-type alkaloid, sracosalgmine and its structure determination on the basis of spectroscopic techniques.

MATERIALS AND METHODS

General experimental procedure: IR spectra: JASCO 302-A spectrophotometer; UV spectra: Hitachi U3200 spectrophotometer; El, FD and HREI MS: JMS 11x100 (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 (precoated silica G-25 plates UV 254); 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. Forty grams were collected from Kuldana Murree Hills, Pakistan, in October 2003.

Extraction and isolation: The ethanolic extract of the air-dried aerial plants (17 kg) was evaporated to a gum (1.8 kg) and extracted with pet.ether to remove non-polar constituents. Total alkaloids (810 g) were obtained by extraction into 10% acetic acid. Partial separation of the alkaloids was achieved by extraction with $CHCl_3$ at different pH values (3.5, 8.5). The fraction obtained at pH 3.5 (74 g) was subjected to CC on silica gel and eluted with $CHCl_3$ and then with $CHCl_3$ - MeOH to obtain several fractions. A fraction obtained by CC elution with $CHCl_3$: MeOH (43:7) yielded a solid which was further purified by preparative TLC using *n*-hexane: Ethylacetate: Diethyamine (8.5:1.3:0.2) as eluent to afford a new compound named Sracosalgmine (5.0 mg).

Sracosalgmine: White solid m.p. 212-217° C; $[\alpha]_D^{27}$ +77 (c 0.44, $CHCl_3$); UV λmax (MeOH) inconclusive; IR ν_{max} KBr: 3550, 2950, 1665 (cm^{-1}); MS *m/z* (%) 389 (M^+, 3), 375 (4), 360 (2.7) 149 (1.5), 105 (0.8), 84 (1.8), 72 (100%), 73 (7.0), 58 (7.8); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ : 0.73 (3H, s, CH_3–18), 0.98 (3H, *s*, CH_3–19), 1.33 (3H, *d*, J = 6.4 Hz, CH_3– 21), 2.17 / 2.38 (2H, *m*, H – 7), 2.85 (3H, *d*, J = 2.4 Hz, NCH_3) 2.65 (3H, *d*, J = 2.4 Hz, NCH_3), 3.04 (1H, *m,* H-3), 3.19 (1H, *q,* J = 6.4 Hz, H-20), 5.34 (1 H, *b.s*, H-6) 2.98 (1H, *m*, H-16), 3.11 (3H, *b.s*, OCH_3), 3.34 (3H, *s*, OCH_3) (Table 1).

RESULTS AND DISCUSSION

An ethanolic extract of aerial parts of *Sarcococca saligna* after evaporation was triturated

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Table 1: ^{13}C NMR data of Sracosalgmine (in $CDCl_3$)

Carbon	Chemical shift (δ)	Multiplicity	Carbon	Chemical shift (δ)	Multiplicity
1	37.2	CH_2	13	43.2	C
2	28.0	CH	14	56.4	CH
3	80.4	CH	15	39.5	CH_2
4	38.7	CH_2	16	65.3	CH
5	140.9	C	17	55.3	CH
6	121.3	CH	18	12.2	CH_3
7	31.8	CH_2	19	19.3	CH_3
8	31.7	CH	20	52.5	CH
9	49.9	CH	21	13.0	CH_3
10	36.1	C	22	36.0	N CH_3
11	21.0	CH_2	23	43.3	N CH_3
12	30.8	CH_2	24	55.6	OCH_3
			25	54.8	OCH_3

with *n*-hexane to remove non-poplar compounds. The insoluble residue was then partitioned between chloroform and aqueous acid solution at various pH values. The chloroform fraction was subjected to repeated column chromatography to afford compound 1 and two known alkaloids *Saracosanaene* 2 and *Saracodine* 3 identified on the basis of reported spectral data[12].

Compound 1 was isolated as a white solid. The HREI mass spectrum of compound 1 revealed a molecular ion peak. at *m/z* 389 suggesting the molecular formula of the compound 1 as $C_{25}H_{43}NO_2$. Hence the compound 1 possessed five degrees of unsaturation. Four of these were accounted for a tetracyclic pregnane type structure and one for a double bond. The compound 1 showed a base peak at *m/z* 72.0835(C_4H_9N), which is characteristic of 20α- dimethyl amino group[23]. The IR spectrum ($CHCl_3$) showed absorptions at 3350 (NH) and 1664 cm^{-1} characteristic of amino and methoxy functions, respectively.

The ^1H NMR spectrum of compound 1 displayed two three–proton singlets at δ 3.34 and 3.11 indicating the presence of two methoxy groups. 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 = 6.4 Hz) was due to C-21 methyl group showing COSY 45° interaction with H-20 proton (δ 3.19). While two doublets (J = 2.4 Hz) at 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. In the ^1H NMR spectrum H-20 and H-17 methine protons also resonated comparatively downfield at δ 3.19 and 1.50, respectively. The de-shielding and splitting of N-methyl signals was atributed to the vicinity of an OCH_3 group at C-16, which was supported by NOESY experiment indicating long range coupling between methoxy function at C-16 and N-methyls resonating at δ 2.85 and 2.65 (J = 2.4 Hz). The H–6 olefinic proton (δ 5.34, *br.s*) showed interactions with

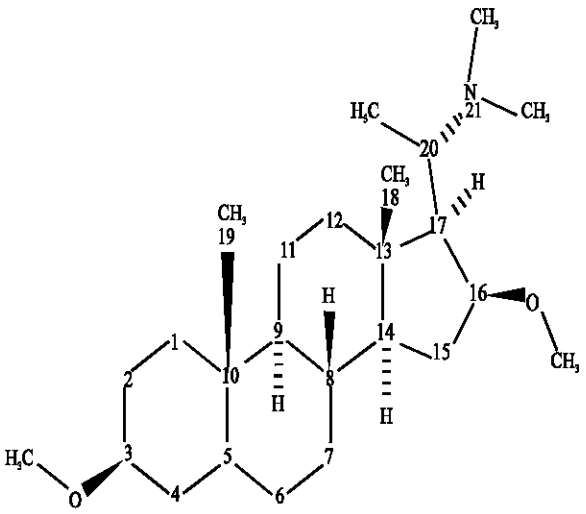

Fig. 1: Structure of compound 1 (Sracosalgmine)

H–7 protons resonating at δ 2.01. The H-3 proton resonated at δ 3.06 and showed COSY interactions 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, HMBC and DEPT spectroscopic techniques. On the basis of above evidences, compound 1 was inferred to be a new alkaloid isolated from *Sarcococca saligna* and named sracosalgmine [(20S)-20-(dimethylamino)-16β, 3β-dimethoxy-pregn-5-ene].

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