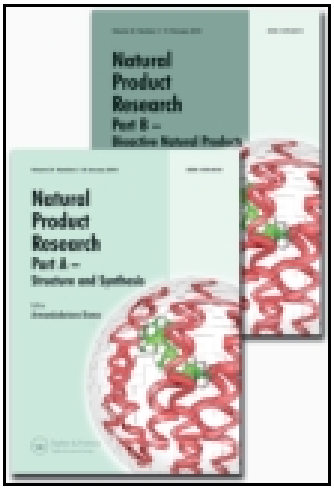

Natural Product Letters
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gnpl19

New Antibacterial Steroidal Alkaloids from Sarcococca Brevifolia

U. L.B. Jayasinghe [b] , M. Nadeem [a] , Atta Ur Rahman [a] , M. Iqbal Choudhary [a] , H. D. Ratnayake [c] & Zareen Amtul [a]

[a] H.E.J. Research Institute of Chemistry, University of Karachi , Karachi, 75270, Pakistan

[b] Institute of Fundamental Studies , Kandy, Sri Lanka

[c] Royal Botanical Gardan , Peradeniya, Sri Lanka

Published online: 04 Oct 2006.

To cite this article: U. L.B. Jayasinghe , M. Nadeem , Atta Ur Rahman , M. Iqbal Choudhary , H. D. Ratnayake & Zareen Amtul (1998) New Antibacterial Steroidal Alkaloids from Sarcococca Brevifolia , Natural Product Letters, 12:2, 103-109, DOI: 10.1080/10575639808048277

To link to this article: http://dx.doi.org/10.1080/10575639808048277

Natural Product Letters
Volume 12(2), pp. 103–109
Reprints available directly from the Publisher
Photocopying permitted by license only

NEW ANTIBACTERIAL STEROIDAL ALKALOIDS FROM *SARCOCOCCA BREVIFOLIA*

U.L.B. JAYASINGHE[2], M. NADEEM[1], ATTA-UR-RAHMAN[1], M. IQBAL CHOUDHARY[1], H.D. RATNAYAKE[3] AND ZAREEN AMTUL[1]

[1]H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan
[2]Institute of Fundamental Studies, Kandy, Sri Lanka
[3]Royal Botanical Gardan, Peradeniya, Sri Lanka

(Received 11th August 1997)

Abstract: Three new steroidal alkaloids, epipachysamine-*E*-5-ene-4-one (**1**), N_b-demethylepipachysamine-*E*-5-ene-4-one (**2**) and *iso*-*N*-formylchonemorphine (**3**) have been isolated from *Sarcococca brevifolia*. Structures of these compounds were determined by spectroscopic studies. Compounds **1** and **3** exhibited strong antibacterial activity against *Bacillus cereus, Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Proteus mirabilis, Corynebacterium diphtheriae* and *Pseudomonas aeruginosa*.

Key Words: Steroidal alkaloids; *Sarcococca brevifolia*; pregnane; epipachysamine-*E*-5-ene-4-one; N_b-demethylepipachysamine-*E*-5-ene-4-one; *iso*-*N*-formylchonemorphine; antibacterial.

INTRODUCTION

The plants of family Buxaceae are well known for producing steroidal alkaloids and a number of new steroidal alkaloids have been reported.[1-12] It comprises several genera including *Pachysandra, Sarcococca, Simondsia* and *Buxus* which are rich sources of steroidal alkaloids.[13] These plants have been extensively used for the treatment of malaria, rheumatism and skin diseases.[1-3, 14]

There are two *Sarcococca* species, *S. brevifolia* and *S. ceylanica* occuring in Sri Lanka and both are endemic.[15] *S. brevifolia* Muel.Arg. is an evergreen shrub, growing in the central of the country. No phytochemical work has reported on methanolic extract of this plant so far. Present studies on the ethanolic extract of the plant has yielded three new steroidal alkaloids epipachysamine-*E*-5-ene-4-one (**1**), N_b-demethylepipachysamine-*E*-5-ene-4-one (**2**) and *iso*-*N*-formylchonemorphine (**3**). Structures of these compounds were established through detailed spectroscopy such as 1D and 2D NMR experiments.[16]

RESULTS AND DISCUSSION

Compound (1) was isolated as an amorphous solid. The HREIMS of (1) exhibited the M^+ at *m/z* 440.3304 analyzing for $C_{28}H_{44}N_2O_2$ (calc.440.3403), hence the compound (1) possessed eight degrees of unsaturation, four of which were accounted for by the tetracyclic pregnane-type steroidal skeleton, two were due to carbonyl groups and the remaining two were due to olefinic bonds. Compound (1) showed IR absorptions at 3651 (NH), 1660 (amide), 1630 and 1490 (CO conj.sec.amide) cm-[1]. The ^{1}H-NMR spectrum of (1) showed two one-proton multiplets at δ 5.67 and 7.67 due to the C-2' and C-6 olefinic protons, respectively. A six-proton singlet at δ 2.16 was ascribed to $N_b(Me)_2$ subtituted at C-20, a three-proton doublet at δ 0.98 (J = 6.5 Hz) was due to the C-21 secondary methyl protons and a one-proton multiplet at δ 1.90 was due to the C-20 methine proton. Another one-proton multiplet at δ 3.50 was assigned to the C-3 methine proton while two (three-proton) methyl singlets at δ 0.65 and at δ 0.85were due to angular C-18 and C-19 methyl groups respectively.

The ^{13}C-NMR (broad-band decoupled and DEPT spectra) of (1) revealed 7 signals for CH_2, 8 signals for CH, 7 signals for CH_3 and 6 signals for quaternary carbons. The signal at δ 196.2 was assigned to α, β-unsaturated carbonyl carbon (C-4), while that at δ 165.5 was assigned to the amidic carbonyl carbon (C-1'). Other important signals were at δ 131.6 (C-5), 125.6 (C-6), 153.0 (C-3'), and 118.7 (C-2'). The complete ^{1}H- and ^{13}C-NMR chemical shift assignments are summarized in Table-1. The HMBC and COSY-45° techniques also confirmed these assignements.

The UV, IR and ^{1}H-NMR spectra of compound (2) ($C_{27}H_{42}N_2O_2$) were similar to those of (1) except that the ^{1}H-NMR spectrum of (2) exhibited only a 3H singlet at δ 2.16 due to the N_b-methyl group. The spectroscopic studies therefore showed that (2) is N_b-demethyl derivative of (1).

Compound (3) was also isolated as an amorphous solid. EIMS and FDMS showed the molecular ion at *m/z* 374. HREIMS exhibited the M^+ peak at *m/z* 374.3297 analyzing for $C_{24}H_{42}N_2O$ (calc. 374.3296), hence it possessed five degrees of unsaturation, four of which were due to the tetracyclic structure of the pregnane-type skeleton and one was due to the presence of a carbonyl group present in it. Compound (3) showed the base peak at *m/z* 72 representing fragment C_4H_6NO which arose by the cleavage of C-17/C-20 bond.

The ^{1}H-NMR spectrum of (3) showed a one-proton singlet at δ 8.07 was due to the aldehydic proton, another one-proton signal at δ 5.30 was due to the NH proton. A three-proton doublet at δ

1.17 (J = 6.5 Hz) was ascribed to the C-21 methyl and a one-proton multiplet at δ 4.10 was assigned to the C-20 methine proton. A one-proton multiplet at δ 3.50 was assigned to the C-3 methine proton and a six-proton singlet at δ 2.50 was due to $N_a(Me)_2$ group. The HMBC and COSY-45° connectivitie confirmed these assignments. The ^{13}C-NMR spectra showed 9 signals for CH_2, 7 signals for CH, 5 signals for CH_3 and 3 signals for quaternary carbon atoms. The signal at δ 159.0 was assigned to amidic carbonyl carbon atom. Compound (3) is the isomer of N_a-formyl chonemorphine which has N-formyl subtituent at C-3[17]. The complete ^{1}H- and ^{13}C-NMR chemical shift assignments have been summarized in Table-1.

Compounds (1) and (3) exhibited strong activity against a number of pathogenic bacteria. Antibacterial activity of (1) was against *Bacillus cereus*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. Similarly compound (3) has shown strong activity against *Bacillus cereus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*. Due to their strong antibacterial activities comparable to that of standard antibiotics such as amoxicillin and ampicillin, cephalexin etc. they can be likely candidates for *in vivo* evaluation. MIC values of both of these compounds are given in Table-2.

GENERAL EXPERIMENTAL

IR spectra were recorded on JASCO 302-A spectrophotometer. UV spectra were recorded on Hitachi U-3200 spectrophtometer. EI MS (70 ev), FD, HREI MS were measured on JMS-HX 100 and JMS-DA 500 mass spectrophotometers. ^{1}H-NMR and ^{13}C- NMR spectra were recorded on Bruker NMR spectrometers operating at 500 and 125 MHz respectively at room temperatures. The chemical shift values are reported in ppm (δ) units. Standard pulse sequences were used for COSY-45°, HOHAHA, DEPT, HMQC and HMBC experiments.

Merck silica gel GF 254 (0.25 mm) and silica PF 254 Mecrk (1 mm) were used for thin layer chromatographic separations. The eluent for compounds (1-3) was pure $CHCl_3$.Spots and bands were detected under UV radiations (254 and 366 nm). Spray reagent for TLC was Dragendorff's reagent.

EXTRACTION AND ISOLATION

The dried whole plant of *Sarcococca brevifolia* was extracted with methanol. Evaporation of the methanol extract gave a brown solid (275 g) which was dissolved in 2N HCl and the acidic extract was washed with CH_2Cl_2. Basification with 20% NH_4OH followed by extraction with CH_2Cl_2

gave the non-quaternary alkaloids (2 g) as a brown solid. Separation of the latter over silica gel (Merck Art 7734; eluent: hexane-ethyl acetate-methanol) followed by preparative thin-layer chromatography (eluent $CHCl_3$, chamber saturated with NH_3 vapour) gave three new steroidal alkaloids epipachysamine-*E*-5-ene-4-one (1), N_b-demethylepipachysamine-*E*-5-ene-4-one (2) and *.iso-N*-formylchonemorphine (3).

BACTERICIDAL BIOASSAY

Bactericidal activity was determine by agar well diffusion method. This test was performed by spreading 18-24 hour old pathogenic bacterial cultures containing approximately 10^4-10^6 colony forming units (CFU/mL) on the surface of nutrient agar (Bio M Laboratories, USA BMO 13-62-N) plates. Wells were dugged in the media with the help of a sterile metallic borer. Test samples of different concentrations prepared in dimethylsulfoxide (DMSO Merck) are added in their respective wells. Pure DMSO was used as a control. Other wells are supplemented with reference compounds *i.e.* amoxicillin.$3H_2O$, ampicillin.$3H_2O$ and cephalexin-Na^+, serving as positive controls.[18]

EXPERIMENTAL

Epipachysamine-*E*-5-ene-4-one (1) (30 mg, $1.5x10^{-3}$ %), $C_{28}H_{44}N_2O_2$; amorphous solid *m.p.*150-151°C; $[\alpha]_D^{25}$= 6° (*c* = 0.2, $CHCl_3$), *Rf* = 0.51; UV (MeOH) λ max; 194 (log ε = 4.11); IR ($CHCl_3$) *V* max; 3651, 3325, 1660, 1630, 1490 cm^{-1}, HREI MS *m/z*; 440.3403 (calc. $C_{28}H_{44}N_2O_2$); EI MS: *m/z* (rel. int., %); 440 (3), 425 (5), 84 (47), 72 (100), ^{1}H- and ^{13}C-NMR see Table **1.**

N_b-Demethylepipachysamine-*E*-5-ene-4-one (2) (10 mg, $5x10^{-4}$); $C_{27}H_{42}N_2O_2$; amorphous solid *m.p.* 145-147°C, $[\alpha]_D^{25}$ = -8° (*c* = 0.29, $CHCl_3$); *Rf* = 0.9; UV (MeOH) λ *max* 194 (log ε = 4.11); IR ($CHCl_3$) *V* max; 3651, 3325, 1660, 1630, 1490 cm^{-1}, HREI MS; *m/z* 426.3246 (calc. $C_{27}H_{42}N_2O_2$); EI MS: *m/z* (rel. int., %) 426 (7), 369 (15), 83 (100); ^{1}H-NMR ($CDCl_3$, 500 MHz), δ 3.50 (1H, m, H-3), 7.67 (1H, m, H-6), 0.98 (1H, d, *J* = 6.5 Hz, H-21), 2.16 (3H, s, N_bCH_3), 2.07 (1H, br.d., N_bH), 5.30 (1H, br.d., N_aH), 5.67 (1H, s, H-2'), 1.85 (6H, s , 2Me at C-3').

***Iso* -*N*-Formylchonemorphine (3)** (50 mg, $2.5x10^{-3}$%); $C_{24}H_{42}N_2O$; amorphous solid m.p.220-222°C, $[\alpha]_D^{25}$ = -14° (*c* = 0.2, $CHCl_3$), *Rf* = 0.36; UV (MeOH) λ *max* 192 (logε =3.80); IR ($CHCl_3$) *V* max; 3400, 1678, 1500 cm^{-1}; HREI MS; *m/z* 374.3297 (calc. $C_{24}H_{42}N_2O$); EI MS: *m/z* (rel. int.,%) 374 (18), 359(2), 317(2), 302(2), 147(1), 110 (49), 84(100), ^{1}H- and

^{13}C-NMR δ see Table 1.

Table-1: ^{1}H- and ^{13}C-NMR spectra for 1 and 3 ($CDCl_3$, 500 MHz).

	1		3	
Carbon	**^{13}C-NMR**	**^{1}H-NMR**	**^{13}C-NMR**	**^{1}H-NMR**
1	20.2 (CH_2)	0.97/2.15(m)	36.9 (CH_2)	1.06/1.87(m)
2	20.5 (CH_2)	1.32/2.05(m)	22.1 (CH_2)	2.02/1.65(m)
3	54.6 (CH)	3.50 (m)	65.4 (CH)	3.50 (m)
4	196.2 (C)	-	39.4 (CH_2)	1.14/2.0(m)
5	153.0 (C)	-	53.9 (CH0	2.02 (m)
6	125.6 (CH)	7.67 (m)	28.4 (CH_2)	1.32/1.75 (m)
7	39.0 (CH_2)	2.25/2.23 (m)	23.9 (CH_2)	1.09/1.59 (m)
8	50.1 (CH)	2.55 (m)	35.2 (CH)	1.35 (m)
9	31.8 (CH)	2.05 (m)	56.5 (CH)	1.06 (m)
10	34.8 (C)	-	35.9 (C)	-
11	24.0 (CH_2)	1.60/1.76 (m)	20.0 (CH_2)	1.15/1.20 (m)
12	39.4 (CH_2)	1.15/2.35 (m)	39.2 (CH_2)	1.12/1.90 (m)
13	39.9 (C)	-	42.1 (C)	-
14	34.6 (CH)	1.55 (m)	45.4 (CH)	1.15 (m)
15	30.5 (CH_2)	1.80/0.85 (m)	31.7 (CH_2)	1.68/0.88 (m)
16	27.8 (CH_2)	1.60/1.90 (m)	26.8 (CH_2)	1.20/1.15 (m)
17	54.8(CH)	1.30 (m)	56.4 (CH)	1.30 (m)
18	12.3 (CH_3)	0.65 (s)	12.3 (CH_3)	0.65 (s)
19	13.3 (CH_3)	0.85 (s)	12.3 (CH_3)	0.85 (s)
20	56.0 (CH)	1.90 (m)	46.7 (CH)	4.10 (m)
21	12.1 (CH_3)	0.98 (d)	21.7 (CH_3)	1.17 (d, J = 6.5 Hz)
$N(CH_3)_2$	40.0 (CH_3)	2.16 (s)	39.4 (CH_3)	2.50 (s)
NH	-		-	5.30 (br.d)
N-CHO	-		-	8.07 (s)
1'	165.5 (C)	-	159.0 (C)	-
2'	118.7 (CH)	5.67 (m)		
3'	131.6 (C)	-		
4'	18.0 (CH_3)	1.86 (s, 6H)		
5'	25.0 (CH_3)	-		

Table-2: Antibacterial Activity of Compounds **1** and **3** (Minimal inhibitory concentration in mg/ml).

	MIC			
Bacteria	**1 (mg/ml)**	**3 (mg/ml)**	**Standard Drugs**	**MIC**
B. cereus	0.0625	0.1250	Amoxicillin. $3H_2O$	0.2500
			Ampicillin. $3H_2O$	0.2500
			Cephalexin-Na^+	0.0078
C. diphtheriae	0.2500	0.2500	Amoxicillin. $3H_2O$	0.2500
			Ampicillin. $3H_2O$	0.1250
			Cephalexin-Na^+	0.1250
E. coli	0.2500	0.2500	Amoxicillin. $3H_2O$	0.2500
			Ampicillin. $3H_2O$	1.0000
			Cephalexin-Na^+	0.5000
K. pneumoniae	0.2500	0.2500	Amoxicillin. $3H_2O$	0.2500
			Ampicillin. $3H_2O$	0.2500
			Cephalexin-Na^+	0.2500
P. mirabilis	0.2500	0.2500	Amoxicillin. $3H_2O$	0.00781
			Ampicillin. $3H_2O$	0.00781
			Cephalexin-Na^+	-
P. aeruginosa	0.1250	0.1250	Amoxicillin. $3H_2O$	0.1250
			Ampicillin. $3H_2O$	0.0625
			Cephalexin-Na^+	-
S. aureus	0.03125	0.0312	Amoxicillin. $3H_2O$	0.2500
			Ampicillin. $3H_2O$	0.1250
			Cephalexin-Na^+	-

REFERENCES

1. J. M. Kohli, A. Zaman, and A. R. Kidwai, (1964). Separation and characterization of the alkaloids of *Sarcococca pruniformis*. *Tetrahedron Lett..*, 3309.
2. A. Chatterjee, B. Das, C. P. Dutta and K. S. Mukherjee (1965). Steroidal alkaloids of *Sarcococca pruniformis* Lindl. *Tetrahedron Lett.*, 67.
3. A. Chatterjee, B. Das., C. P.Dutta, and K. S. Mukherjee, (1966). Steroidal alkaloids of

Sarcococca pruniformis J. Ind. Chem. Soc., **34**, 285.

4. J. M. Kohli, A. Zaman and A. R. Kidwai (1967). Separation and characterization of alkaloids of *Sarcococca pruniforms. Tetrahedron*, **23**, 3829.
5. G. A. Miana and M. Kiamuddin (1969). Alkaloids of *Sarcococca saligna* Muel: salignine. *Pak. J. Sci. Ind. Res.*, **12**, 161.
6. M. Qui, R. Nie, X. Wang and J. Zhou (1989). Structure of sarcorucinine D and pachyaximine A, B. *Acta Bot. Sinica*, **31**, 535.
7. I. C. Chopra and K. L. Handa (1951). Chemical examination of *Sarcococca pruniformis* Lindl. *Ind. J. Pharm.*, **13**, 129.
8. A. Chatterjee. and K. S. Mukherjee (1966). Alkaloids of *Sarcococca pruniformis* Lindl.: salignine. *Chem. Ind.*, **19**, 769.
9. R. N. Chopra (1952). The Drug Research Laboratory, Jammu-Tawi, 1941-1651. *J. Sci. Ind. Res.*, **11A**, 241.
10. J. M. Kohli, A. Zaman, and A. R.Kidwai (1971). Alkaloids of *Sarcococca pruniformis. Phytochemistry*, **10**, 442.
11. M. Chiu, D. Cao, Z. Li, and R. Nie (1992) *Chinese Chem. Lett.*, **3**, 433.
12. M. Chiu, D. Cao, Z. Li, and R.Nie, (1993) *Zhiwu Xuebao*, **35**, 885.
13. M. I. Choudhary (1987). "Isolation and Structural Studies on Alkaloids of Some Indigeneous Medicinal Plants, *Buxus papillosa*, *Catharanthus roseus* and *Cissampelos pareira*". Ph.D., Dessertation, H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan.
14. M. Kiamuddin, . and H. K. M. A. Hye, (1970). Pharmacological activity of an Alkaloid from *Sarcococca saligna. Pak. J . Sci. Ind. Res.*, **13**, 59.
15. J. D. Hooker, , *Flora of British India*, (1982) B. S. M. P. Singh Publishers, Dehra Dun, India , **5**, 266.
16. Atta-ur-Rahman and M. I. Choudhary, "Solving Problems with NMR Spectroscopy", Academic Press, San Diego (1996).
17. J. Benerji and A. Chatterjee (1978). Constituents of *Chonemorpha macrophylla* G. Don: structure of *N*-formylchonemorphine and japindine, two new minor alkaloids. *Indian J. Chem.*, **16B** 346.
18. R. A. Carron, J. M. Maran, L. Montero, A. Fernandozlago and A. Dominguez (1987) *Plantes Medicinales et Phytotherapie*, **21**, 195.