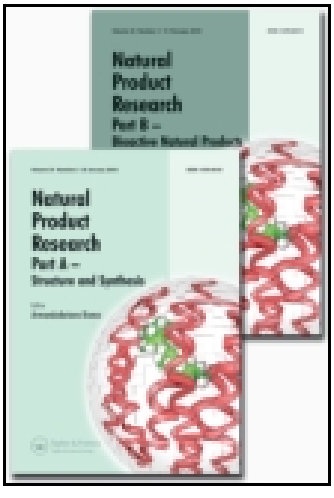

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11-HYDROXYEPIPACHYSAMINE-*E*, A NEW STEROIDAL ALKALOID FROM *SARCOCOCCA BREVIFOLIA*

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Abstract: A new steroidal alkaloid, 11-hydroxyepipachysamine-*E* has been isolated from the aerial parts of *Sarcococca brevifolia* Muell. Arg. of the family Buxaceae. The structure of this compound was elucidated on the basis of detailed spectral analysis.

Key Words: *Sarcococca brevifolia*, Buxaceae, 11-hydroxyepipachysamine-*E*, steroidal alkaloids.

INTRODUCTION

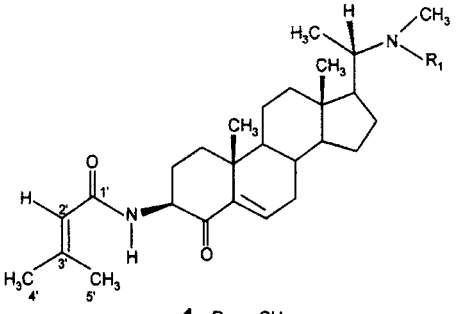
Plants of the family Buxaceae have been used in the indigenous system of medicine for the treatment of various disorders, especially malaria, rheumatism and skin infections[1]. The family Buxaceae is well known for steroidal alkaloids[2,3]. *Sarcococca brevifolia* of the family Buxaceae is an evergreen shrub, growing in mid island regions of Sri Lanka. There are two *Sarcococca* species, *S. brevifolia* and *S. zeylanica*, both being endemic to Sri Lanka[4]. We have previously reported three new steroidal alkaloids, epipachysamine-*E*-5-ene-4-one(**1**), N_b-demethyl epipachysamine-*E*-5-ene-4-one(**2**) and *iso*-*N*-formylchonemorphine(**3**) from the

aerial parts of *Sarcococca brevifolia*[5]. In this paper we report the isolation and structure elucidation of another new steroidal alkaloid, 11-hydroxyepipachysamine-*E* (**4**) from this plant.

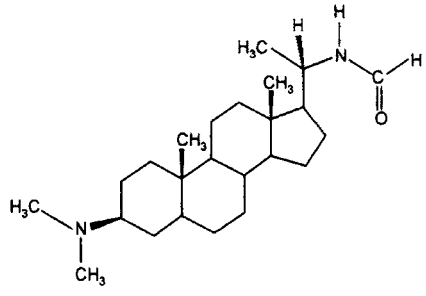
RESULTS AND DISCUSSION

The MeOH extract of the aerial parts of *S. brevifolia* were processed for nonquaternary alkaloids. Chromatographic separation of the alkaloidal fraction over a column of silica gel followed by PTLC gave compound **4** in a yield of 0.002%. The HREIMS of the compound **4** showed M^+ at *m/z* 444.3698, corresponding to the molecular formula $C_{28}H_{48}O_2N_2$, indicating the presence of six degrees of unsaturation in the molecule. Four of these were accounted for by the tetracyclic structure of the pregnane-type steroid, one by the carbonyl group and the remaining one by the olefinic double bond. The peak at *m/z* 429.3447 ($C_{27}H_{45}O_2N_2$) was due to the loss of a methyl group from the molecular ion. The base peak at *m/z* 72.0803 ($C_4H_{10}N$) was due to the trimethyliminium cation, $Me\text{-}CH{=}N^+(Me)_2$ formed by the cleavage of ring-D nitrogen-containing side chain[6]. The UV spectrum of compound **4** was closely related to those of compounds **1** and **2** reported previously[5]. The IR spectrum displayed absorptions at 3350 (OH), 1660 (α, β unsaturated amide carbonyl) and 1610 (C=C) cm^{-1}. The ^1H NMR spectrum of **4** showed two sharp singlets at δ 0.70 and 1.00 which were assigned to C-18 and C-19 methyl protons respectively. A three-proton doublet at δ 1.32 (J = 6.7 Hz) was assigned to C-21 secondary methyl protons. Two three-proton signals at δ 2.65 and 2.86 were assigned to the two Me groups of N_b. A one-proton multiplet at δ 3.20 was due to the C-20 methine proton, which showed coupling with the C-21 methyl group. A multiplet at δ4.00 was assigned to H-3α. In the ^{13}C NMR spectra a methine carbon signal resonated at δ65.0 was assigned to C-20 and substantiated

by the HMQC spectra. In the HMBC spectrum, the C-18 and C-19 methyl carbon signals resonated at δ 12.6 and 14.3 respectively, were correlated with the proton signal at δ3.90 (*m*) which was assigned to due to H-11 and the hydroxyl group was accordingly located at C-11 (δ69.5).



1 $R_1 = CH_3$
2 $R_1 = H$



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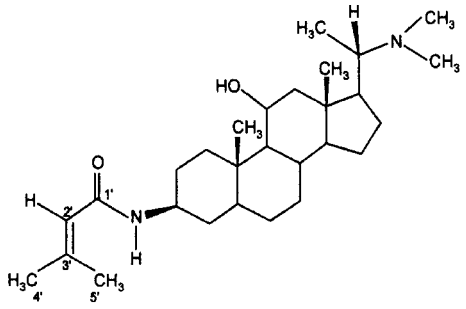

4

In the long-range ^1H-^1H COSY spectra, H-11 (δ 3.90) showed strong connectivity with the protons at δ 1.81 and 1.15 which were therefore assigned to the methylene carbon at C-12 (δ 40.4). Further in the HMBC spectra of **4**, strong correlations were observed for H-2'(δ 5.56) to C-1' (δ 163.0), C-4'(δ 19.8),

C-5' (δ 27.2); H-4' (δ2.14) to C-2' (δ118.5), C-3' (δ 153.0), C-5' (δ 27.2); H-5' (δ 1.84) to C-2' (δ 118.5), C-3' (δ 153.0), C-4' (δ 19.8). These correlations gave evidence for the presence of $(Me)_2C$=CHCO- group in the compound **4**. Thus the structure of **4**, a new natural product, was deduced as 11-hydroxyepipachysamine-*E*. The ^1H and ^{13}C NMR assignments are given in Table 1.

EXPERIMENTAL

IR spectra were obtained from JASCO 302-A spectrometer. UV spectra were obtained from Hitachi U 3200 spectrophotometer. Optical rotation was measured using a HORIBA Model SEPA-200 polarimeter at 22°. EI and HREIMS were recorded on JMS HX100 and JMS-DA 500 mass spectrometers. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR) in $CDCl_3$ solution with tetramethylsilane as an internal reference.

Plant Material: Aerial parts of *S. brevifolia* Muell. Arg. were collected from the Uva Province of Sri Lanka in August 1995 and identified by Mr. H.D. Ratnayake, Royal Botanical Gardens, Peradeniya. A voucher specimen was deposited at the Institute of Fundamental Studies, Kandy, Sri Lanka.

Extraction and Isolation: The dry ground mature aerial parts of *Sarcococca brevifolia* (2kg) were extracted with hot MeOH. Evaporation of the MeOH gave dark brown solid (275g), which was taken up 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 (2g) as a brown solid. Separation of the

Table 1 : ^{1}H and ^{13}C NMR of **4** (400MHz/100MHz, $CDCl_3$)

Carbon No	^{13}CNMR	^{1}HNMR
1	31.8 (CH_2)	1.67, 1.72*(m)*
2	20.8 (CH_2)	1.32, 1.51*(m)*
3	50.4 (CH)	4.00*(m)*
4	39.8 (CH_2)	1.86, 1.88*(m)*
5	41.4 (CH)	1.09*(m)*
6	28.0 (CH_2)	2.15, 1.95*(m)*
7	28.8 (CH_2)	1.23, 1.31*(m)*
8	34.7 (CH)	1.36*(m)*
9	55.2 (CH)	2.8*(m)*
10	36.1 (C)	
11	69.5 (CH)	3.90(*m*)
12	40.4 (CH_2)	1.81, 1.15*(m)*
13	43.5 (C)	
14	56.2 (CH)	1.10*(m)*
15	24.4 (CH_2)	1.22, 1.76*(m)*
16	27.8 (CH_2)	1.81, 1.86*(m)*
17	52.6 (CH)	1.50*(m)*
18	12.6 (CH_3)	0.70(*s*)
19	14.3 (CH_3)	1.00(*s*)
20	65.0 (CH)	3.20(*m*)
21	12.7 (CH_3)	1.32 (*d*, *J*=6.7Hz)
N_bMe_1	34.7 (CH_3)	2.65(*s*)
N_bMe_2	43.4 (CH_3)	2.86(*s*)
1'	163.0 (C)	
2'	118.5 (CH)	5.56(*m*)
3'	153.0 (C)	
4'	19.8 (CH_3)	2.14(*s*)
5'	27.2 (CH_3)	1.84(*s*)

latter over silica gel (eluent: *n*-hexane-EtOAc-MeOH) followed by preparative thin layer chromatography (eluent: $CHCl_3$, chamber saturated with NH_3 vapour) furnished compound **4** (30mg).

11-hydroxyepipachysamine-E (**4**): amorphous solid; $[\alpha]_D$ = ¯15° (*c* .25, $CHCl_3$); UV λ_{max}^{EtOH} : 199 nm ; IR $\nu_{max}^{CHCl_3}$: 3350, 2900, 2850, 2750, 1720, 1660, 1620, 1610, 1510, 1440, 1380, 1160, 1040 cm^{-1}; ^1H & ^{13}C NMR: see Table 1; HREIMS *m/z* (rel. int.): 444.3698 ($[M]^+$, $C_{28}H_{48}O_2N_2$, 9), 429.3447 ($[M\text{-}CH_3]^+$, $C_{27}H_{45}O_2N_2$, 12), 72.0803 ($[Me\text{-}CH{=}N(Me)_2]^+$, $C_4H_{10}N$, 100); EIMS *m/z* (rel. int.): 444 (5), 388 (5), 319 (5), 256 (5), 164 (8), 72 (100), 55 (10).

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