Phytochemical studies on the aerial parts of *Sarcococca hookeriana* (Baillon) of Nepalese origin.

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Abstract:

Two known pregnane type of alkaloids, axillaridine A and spiropachysine, and an unidentified pregane alkaloid along with oleanolic acid and stigmasteryl glucoside have been isolated from chloroform extract of the aerial parts of *Sarcococca hookeriana*. The structures of the alkaloids are established on the basis of spectral analysis of ^{1}H-NMR, ^{13}C-NMR, HMQC, COSY and HMBC spectrum. All compounds were isolated for the first time from *S. hookeriana*. So far, species of *Sarcococca* was not reported to contain spiropachysine which was initially reported as a novel alkaloid from *Pachysandra terminalis*[1] SIEB. et ZUCC.(Buxaceae).

Introduction:

The small green shrub *Sarcococca hookeriana* (Baillon)[2] is distributed in East, West and Central Nepal at altitude[3] 1800-3500 m. Plants of the genus *Sarcococca* have been extensively used in the indigenous system of medicine since ancient time for the treatment of various ailments especially malaria, rheumatism, skin infection[4]. The plants are reported to be used as an antipyretic, hypotensive[5] and found to have antiulcer[6], antitumor[6], ganglion-blocking[5,6], activities. Many steroidal alkaloids isolated from the genus *Sarcococca* was shown to possess anticholinesterase[5,7,8,9] antibacterial[10,11] and ganglion-blocking[5,6] properties. Four species of *Sarcococca*(*S. hookeriana, S. coriaceae, S. wallichi and S. saligna*) of Buxaceae family have been reported[3] from different parts of Nepal. But practically no literature about the phytochemical work on *Sarcococca hookeriana* (Baillon), is available so far. The extensive use of *Sarcococca* species in folk medicine and their various important biological activities inspired us to undertake the chemical investigation of *Sarcococca hookeriana* (Baillon). The present study on the aerial parts of the plant yielded three pregnane type steroidal alkaloids along with oleanolic acid and stigmasterylglucoside. The structures pf the isolated compounds were established by detail analysis of their spectra ^{1}H-NMR, ^{13}C-NMR, HMQC, COSY and HMBC.

General experimental procedure:

Melting points determined were uncorrected. IR spectra were recorded on Bio-Rad Win-IR Pro spectrophotometer. ^{1}HNMR, ^{13}CNMR, DEPT, COSY, HMQC and HMBC were recorded in $CDCl_3$ chemical shift (□) are given in ppm with TMS as internal standard. Medium Pressure Liquid Chromatography on silica-gel- 60 (particle size 0.040-0.063 mm, 230-400 mesh, ASTM) packed column (Buchi Borosilicate 3.3, code no. 17981) was used for column chromatography. The purity of the compound was checked on TLC (silica-gel G_{254} precoated plates).

Plant materials:

Aerial parts of *S. hookeriana* was collected from Daman (at 2322 m. altitude), Makawanpur district, Nepal in the month of August. The plant was identified by Dr. R. P. Choudhary, Central Department of Botany, T. U. Kirtipur.

Extraction and Isolation:

The air dried and powdered aerial parts (1.0 kg) of the *S. hookeriana* (Baillon) were extracted with ethanol (10 L.) at room temperature. The concentrated alcoholic extract was diluted with cold distilled water (1000 ml) and defatted with hexane (5x1000ml) and hexane extract was concentrated to afford hexane extract (18gm). After defatting the extract with hexane, it was extracted with chloroform (5x1000ml.) followed by extraction with ethyl acetate(5x1000ml).The extracts were concentrated under reduced pressure to yield chloroform extract (29.7gm.) and viscous ethyl acetate (90gm) respectively. Chloroform extract (25gm) was subjected to MPLC over silica gel -60 (particle size 0.040-0.063 mm, 230-400 mesh ASTM) packed Buchi borosilicate 3.3, code no. 17981 column (49x3.9 cm.). The column was eluted with 0.5% diethyl amine in hexane, acetone, methanol solvents system gradients in the order of increasing polarity and collecting 100ml effluent as one fraction to obtain (i) hexane fractions (1-12), (ii) 5% acetone in hexane fraction (13-29), (iii) 15% acetone in hexane (30-100), (iv) 25% acetone in hexane fractions (101-153), (v) 40% acetone in hexane fractions (154-214), (vi) 70% acetone in hexane fractions (215-275), (vii) acetone fractions (276-300), (viii) 5% methanol in acetone fractions (301-373), (ix) 15% methanol in acetone fractions (374-424), (x) 30% methanol in acetone fractions (425-500), (xi) 70% methanol in acetone fractions (501-526) and (xii) methanol fractions (527-542). .

Fractions (50-58**)** eluted with 15% acetone in hexane was concentrated under reduced pressure to give **Compound R1** (44 mg) The spot on TLC plate was visualized under UV and by spraying with Dragendorff's reagent gave orange color spot on the TLC plate.

Fractions (82-103) eluted with 15% and 25% acetone in hexane was evaporated to solid residue. The residue was crystallized from acetone to afford **Compound R2** (27mg). The spot on TLC plate was UV visible and gave orange colored spot with Dragendorff's reagent.

Fractions (139-151) were concentrated to greenish yellow colored solution afforded gelatinous precipitate. The precipitate was crystallized to afford **Compound R4** (23 mg.). It was UV inactive, visualized the spot spraying with 1% vanillin in conc. sulphuric acid and charring.

Fractions (170-180) eluted with 40% acetone in hexane was concentrated, cooled in freeze for about 24 hours yielded **Compound R3** (30 mg.). The spot on TLC plate was visible under UV, gave orange color spot on TLC plate with Dragendorff's reagent.

Fractions (231-238) eluted with 70% acetone in hexane gave gelatinous precipitate which was recrystallised from methanol: chloroform (1:1) to afford **Compound R5** (75 mg.).It was UV visible.

Compound R1 [20□-dimethylamino-3-benzoylamino-5□-pregn-2(3)-en-4-one] (axillaridine A):

White amorphous compound, $C_{30}H_{42}N_2O_2$, mp 226^{0}C (lit[12,]. mp. 223-224^{0}C), R_f =0.718 (acetone: hexane: diethyl amine=2: 3: 0.004), 0.87 (acetone: hexane: diethyl amine=2: 3: 0.04),

IR (KBr) □max: 3384 cm^{-1}(-NH), 2930 cm^{-1}, 2860 cm^{-1}, 2770 cm^{-1}, 1658 cm^{-1} (□,□-unsaturated cyclohexenone), 1532 cm^{-1}.

^{1}H-NMR($CDCl_3$) □H: 8.6 (1H,s,-NH-); 7.805 (1H,dd,J=2.5,6.9Hz,H-2); 2.59, 2.38 (2H,m,H-1); 2.44 (1H,m,H-20); 2.34 (1H,m,H-5); 2.18 (6H,s,-NMe_2); 2.09 (2H,m,H-6); 1.95, 1.19 (2H,m,H-12); 1.88, 1.48 (2H,m,H-16); 1.84, 0.9 (2H,m,H-7); 1.64, 1.11 (2H,m,H-15); 1.39 (1H,m,H-17); 1.38 (2H,m,H-11); 1.33 (1H,m,H-8); 1.08 (1H,m,H-14); 1.05 (1H,m,H-9); 0.92 (3H,s,H-19); 0.88 (3H,d,J=6.4Hz.,H-21); 0.67 (3H,s,H-18).Phenyl protons □H: 7.838 (1Hx2,brdd,J=7.0 Hz.,H-2',H-6'); 7.525 (1H,br dd,J=7,7 Hz.H-4'); 7.465 (1Hx2,br dd,J=7,7 Hz.,H-3',H-5').

^{13}C-NMR($CDCl_3$)□c: 39.08 (C-1), 126.68 (C-2), 131.42 (C-3), 196.43 (C-4), 54.85 (C-5), 20.51 (C-6), 30.55 (C-7), 34.67 (C-8), 53.98 (C-9), 40.04 (C-10), 20.82 (C-11), 39.44 (C-12), 41.66 (C-13), 56.25 (C-14), 23.94 (C-15), 27.62 (C-16), 54.90 (C-17), 12.28 (C-18), 13.35 (C-19), 61.05 (C-20), 9.88 (C-21), 39.88 (NMe_2), 165.7 (PhCO), 134.62 (C-1'), 126.97 (C-2'), 128.74 (C-3'), 131.84 (C-4'), 128.74 (C-5'), 126.97 (C-6').

EI-MS m/z 462 [M^+] $C_{30}H_{42}N_2O_2$, 447.36, 431.39, 270.20, 224.96, 209.03, 105.01, 72.0

Compound R2 (spiropachysine):

White amorphous compound, $C_{31}H_{46}N_2O$ mp 290^{0}C (lit[1,13] mp 290-292^0.C), R_f=0.61 (acetone: hexane: diethylamine=2: 3: 0.004), 0.77 (acetone: hexane: diethylamine=2: 3: 0.04),IR (KBr) □max: 3450.39 cm^{-1}, 2944.82 cm^{-1}, 1695.6 cm^{-1}, 1463.9 cm^{-1}.

^{1}H-NMR($CDCl_3$) □H: 3.38 (3H,s,-NMe-); 2.45 (1H,m,H-20); 2.32, 1.72 (2H,m,H-2); 2.18 (6H,s,-NMe_2); 2.08, 1.46 (2H,m,H-4); 1.95, 1.18 (2H,m,H-12); 1.87, 1.49 (2H,m,H-16); 1.83, 1.61 (2H,m,H-1); 1.8 (1H,m,H-5); 1.75, 0.95 (2H,m,H-7); 1.62, 1.1 (2H,m,H-15); 1.57, 1.35 (2H,m,H-11); 1.46 (1H,m,H-8); 1.4 (1H,m,H-17); 1.31, 1.18 (2H,m,H-6); 1.1 (1H,m,H-14); 1.03 (3H,s,H-19); 0.89 (3H,d,J=6.5Hz.,H-21); 0.83 (1H,m,H-9); 0.7 (3H,s,H-18).Phenyl protons □H: 7.77 (1H,d,J=7 Hz,H-3'); 7.525 (1H,dd,J=7,7Hz.,H-5'); 7.389 (1H,dd,J=7,7Hz.,H-4'); 7.375 (1H,d,J=7 Hz.,H-6').

^{13}C-NMR($CDCl_3$) □c: 35.54 (C-1), 32.21 (C-2), 64.07 (C-3), 39.7 (C-4), 42.4 (C-5), 28.73 (C-6), 31.79 (C-7), 35.48 (C-8), 54.54 (C-9), 35.06 (C-10), 20.92 (C-11), 39.7 (C-12), 41.67 (C-13), 56.62 (C-14), 23.97 (C-15), 27.64 (C-16), 54.86 (C-17), 12.38 (C-18), 11.48 (C-19), 61.07 (C-20), 9.84 (C-21), 39.88 (NMe_2), 29.48 (NMe), 152.63 (C-1'), 130.69 (C-2'), 123.3 (C-3'), 127.7 (C-4'), 131.56 (C-5'), 120.91 (C-6'), 167.91 (PhCO-).

EI-MS m/z: 462[M^+] $C_{31}H_{46}N_2O$, 447.39, 198.15, 172.08, 92.09, 77.00, 72.05.

Compound R3:

A grayish white crystalline, mp 196^{0}C, $R_{f\,=}$ 0.187 (acetone: diethylamine=5: 0.004), 0.1835 (acetone:hexane: diethylamine=2: 3: 0.04), IR (KBr) □max: 3425.8, 2930.7, 1694.8, 1385.8 cm$^{-1.}$

^{1}H-NMR ($CDCl_3$) □□□see table-III.

Compound R4 (Oleanolic acid):

White amorphous, $C_{30}H_{48}O_3$ mp 170^{0}C (lit[14]mp.288-290^{0}C), R_f = 0.87 (methanol: chloroform = 1:9), IR□$_{max}$ (KBr): 3450.39 cm^{-1} (hydroxyl group- OH) 1695.047 cm^{-1} (carbonyl group- COOH), 1463 cm^{-1}.

^{1}H-NMR ($CDCl_3$) □□□0.77 (s, 3H, H- 24), 0.775 (s, 3H, H- 26), 0.90 (s, 3H, H- 29), 0.908 (s, 3H, H- 25), 0.93 (s, 3H, H- 30), 0.98 (s, 3H, H- 23), 1.14 (s, 3H, H- 27), 2.8 (1H, dd, j=13.7, 4.0 Hz, □H-18), 3.22 (1H, dd, j=10, 4.5 Hz, □H-3), 5.27 (1H, dd, j=3.4, 3.4 Hz, H-12).

Compound R5 (Stigmasteryl glucoside):

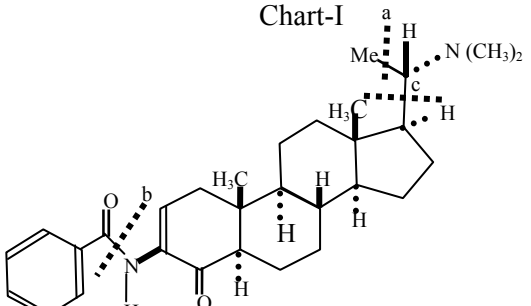
White amorphous compound, mp 274^{0}C (dec.), R_f= 0.54 (glass) (methanol: ethyl acetate= 1:4),IR □$_{max}$ (KBr): 3456 cm^{-1}, 2931.7 cm^{-1}, 1462.5 cm^{-1}, 1069.9 cm^{-1}, 1024 cm^{-1}.

Results and discussions:

Compound R1:

A white amorphous compound, $C_{30}H_{42}N_2O_2$(M^+, m/z: 462), melted at 226^{0}C and IR (KBr) spectrum showed absorption at 3384 cm^{-1}(-NH-), 1658 cm^{-1} (□, □- unsaturated cyclohexenone and benzamide). The mass spectrum of the compound exhibited the [M^+] m/z at 462 in agreement with the molecular formula $C_{30}H_{42}N_2O_2$. The peaks at m/z 447,105 and 72 displayed by its mass spectrum(Chart-I) are consistent with the 20 α –dimethylamino-3-benzoylamino -5α-pregn-2-en-4-one (axillaridine A) [12] .

Chart-I



a [M^+-Me], [$C_{29}H_{39}N_2O_2$, m/z=447]; b $PhCO_2$[C_7H_5O, m/z=105]; c $N(CH_3)_2$,[$C_4H_{10}N$,m/z=72]

The ^{1}H-NMR spectrum displayed two singlet for tertiary methyl protons at □0.67, □0.92 and a doublet for a secondary methyl protons at □0.88 (d, J=6.4 Hz.) were assigned to C- 18, C- 19 and C- 21 respectively. A singlet of 6H resonated at □2.18 was due to two methyl groups attached to the nitrogen atom at C- 20. A singlet peak of 1H at □8.64 was assigned to the amide NH where as the double doublets peak at □7.805 (H, dd, J=2.5, 6.9 Hz.) was assigned to the olefinic proton at C-2. The position of the olefinic proton at C- 2 was confirmed by COSY spectrum in which proton at C- 2 (□7.805) was found to be coupled with C- 1 (□2.59, 2.38) protons. The presence of five olefinic protons as three multiplets at □7.838 (2H, br dd, J=7, 7 Hz.), □7.465 (2H, br dd, J=7, 7 Hz.) and □7.525 (1H, br dd, J=7, 7 Hz.) which were assigned for (C- 2', C- 6'), (C- 3', C- 5') and C- 4' of benzene nucleus. The ^{13}C-NMR spectrum of R1 displayed resonance for 30 carbons. DEPT indicated that the presence of five methyl, seven methylene, twelve methine and six quaternary carbon atoms including two carbonyl groups at □165.7 and □196.40 in the compound. The ^{13}C-NMR values were found in close agreement with the reported literature value of axillaridine A[12]. These spectral evidences suggested the compound to be axillaridine A which was further confirmed by HMQC, COSY and HMBC correlations (Table I) and mp value.

Table- I ^{13}C-NMR (□c), ^{1}H-NMR (□H), DEPT, HMBC correlations of compound **R1**

Carbon	□c	□H(J= Hz)	DEPT	Observed connectivity in HMBC spectrum.
1	39.08	2.59, 2.38	CH_2	0.92 (H-19), 2.34(H-5))
2	126.68	7.805(H, dd, J=2.5, 6.9Hz)	CH	2.59 (H-1), 2.38 (H-1)
3	131.42		C	2.59 (H-1), 2.38 (H-1), 7.805(H-2)
4	196.43		C	8.64(NH), 7.805(H-2), 2.59(H-1), 2.38(H-1)
5	54.85	2.34	CH	0.92(H-19), 2.59(H-1), 2.09(H-6), 1.84(H-7)
6	20.51	2.09,	CH_2	1.84(H-7), 0.92(H-19), 2.34(H-5)
7	30.55	0.9, 1.84	CH_2	2.34(H-5), 2.09(H-6), 1.05(H-9)
8	34.67	1.33,	CH	2.09(H-6), 1.84(H-7), 0.92(H-19), 1.05(H-9)
9	53.98	1.05	CH	0.92(H-19), 2.34(H-5), 2.38(H-1)
10	40.04		C	0.92(H-19), 2.59(H-1)
11	20.82	1.38, 1.55	CH_2	1.19(H-12), 1.95(H-12), 1.05(H-9)
12	39.44	1.19, 1.95	CH_2	0.67(H-18), 1.38(H-11), 1.55(H-11)
13	41.66		C	0.67(H-18), 1.39(H-17), 1.19(H-12), 1.95(H-12)
14	56.25	1.08	CH	0.67(H-18),1.11(H-15)
15	23.94	1.11, 1.64	CH_2	1.08(H-14)
16	27.62	1.48, 1.83	CH_2	1.39(H-17)
17	54.90	1.39	CH	0.67(H-18), 0.88(H-21), 2.44(H-20)
18	12.28	0.67	CH_3	1.95(H-12),1.19(H-12),1.39(H-17), 1.08(H-14)
19	13.35	0.92	CH_3	2.59(H-1), 2.38(H-1), 2.34(H-5), 1.05(H-9)

20	61.05	2.44	CH	2.18(NMe_2), 0.88(H-21), 1.39(H-17), 0.67(H-18)
21	9.88	0.88(d, J=6.4Hz)	CH_3	1.39(H-17), 2.44(H-20)
NMe_2	39.88	2.18	2Me	2.18(NMe)**, 2.44(H-20)**
PhCO	165.7		C	8.64(NH), 7.838(H-2', 6')
1'	134.62		C	7.465(H-3', H-5')
2'	126.97	7.838(2H, br dd, J=7 Hz, H-2', H-6')	CH	7.525(H-4'), 7.465(H-3', H-5')
3'	128.74	7.465(2H, br dd, J= 7, 7 Hz , H-3', H-5')	CH	7.838(H-2', 6'), 7.525(H-4')
4'	131.84	7.525(1H, br dd, J= 7, 7 Hz, H-4')	CH	7.838(H-2', 6')
5'	128.74	7.465(2H, br dd, J= 7, 7 Hz, H-3', H-5')	CH	7.838(H-2', 6'), 7.525(H-4')
6'	126.97	7.838(2H, br dd, J= 7 Hz, H-2', H-6')	CH	7.525(H-4'), 7.465(H-3', H-5')
PhCONH		8.64		

Compound R2:

Compound R2 a white amorphous compound, $C_{31}H_{46}N_2O$ (M^+ m/z: 462), melted at 290^{0}C. Its IR spectrum displayed peaks at 3450.39cm^{-1} (-OH), 2944.82cm^{-1}(-C-H), 1695.6cm^{-1}(-CO-). ^{1}HNMR showed singlet for two tertiary methyl protons at 0.7, 1.03 and a doublet for secondary methyl protons at 0.89 (d, J= 6.5Hz). These methyl protons were assigned for C-18, C-19 and C-21 protons respectively. A singlet integrated for 6H protons was observed at 2.18 due to two methyl protons attached to a nitrogen atom at C-20. All these signals were found identical to those of the compound R1 except the slight difference in the value of C-19. protons. No olefin proton signal was observed in its ^{1}HNMR spectrum. The absence of-NH- proton signal of secondary benzamide as exhibited by the ^{1}HNMR of compound R1 at 8.64 but appearance of one more singlet for three protons at 3.38 in its ^{1}HNMR suggested the presence of one more-N-CH_3. Multiplets displayed between 7.37 to 7.77 were assignable to four aromatic protons suggesting the presence of disubstituted benzene ring. The ^{1}HNMR showed that compound R2 differs from compound R1 having one more methyl group and absence of secondary amino group. ^{13}CNMR spectrum displayed a total of 31 signals which were resolved into six methyl, nine methylene, ten methine and six quaternary carbons as indicated by its DEPT. These spectral data of compound R2 found in close agreement with spiropachysine[13]. The ^{13}CNMR values of C, D rings, C-20, C-21 and -NMe_2 were in absolute agreement with the values of compound R1. However the c values of benzene ring differ significantly. Thus the compound was assigned spiropachysine which was further supported by HMBC correlation (Table: II), HMQC and COSY spectrum. Moreover a strong support was provided by the mass spectrum, in which three peaks exhibited at m/z [M^+] 462,172.02,198.15, and 72 may be assigned to the fragment ions a, b, and c (Chart-II).Its spectral data and melting point were found similar to that of reported value of spiropachysine[1,13]. To our knowledge the alkaloid possessing a unique five membered spiro-lactam system structure have been isolated for the first time from the genus *Sarcococca.*

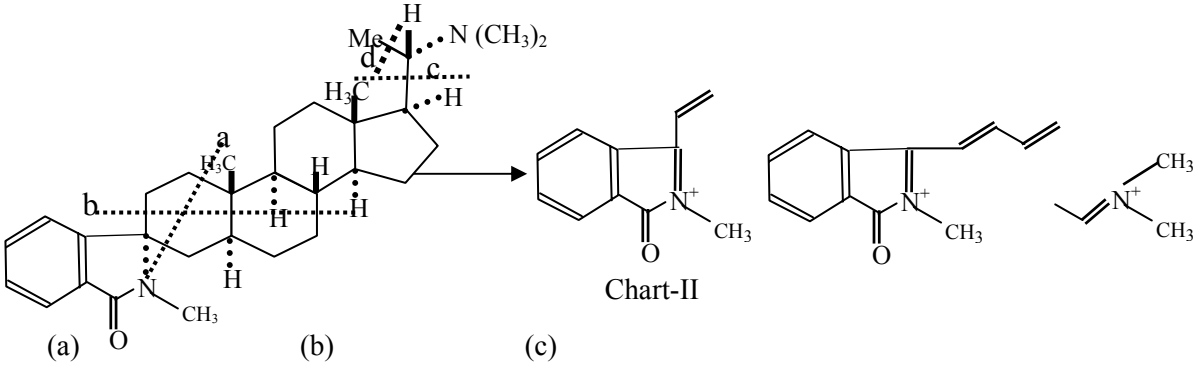


Chart-II

(a) [$C_{11}H_{10}ON$,m/z=172] (b) [$C_{13}H_{12}NO$,m/z=198]

(c)[$C_4H_{10}N$,m/z=72] (d) [M^+-Me], [$C_{30}H_{43}ON_2$,m/z=447]

Table- II. ^{13}C-NMR (□c), ^{1}H-NMR (□H), DEPT, HMBC correlations of compound **R2**

Carbon	□c	□H(J= Hz)	DEPT	Observed connectivity in HMBC spectrum.
1	35.54	1.83, 1.61	CH_2	2.32(H-2), 1.03(H-19)
2	32.21	2.32, 1.72	CH_2	1.83(H-1), 1.61(H-1)
3	64.07		C	2.32(H-2), 1.83(H-1), 2.08(H-4), 1.46(H-4), 3.38(-NMe), 7.375 (H-6'), 1.72(H-2)
4	39.70	2.08, 1.46	CH_2	1.80(H-5)
5	42.40	1.80	CH	2.08(H-4), 1.03(H-19)
6	28.73	1.31, 1.18	CH_2	0.95(H-7), 1.75(H-7), 2.08(H-4)
7	31.79	0.95, 1.75	CH_2	0.83(H-9), 1.10(H-14), 1.18(H-6)
8	35.48	1.46	CH	0.83(H-9)
9	54.54	0.83	CH	1.03(H-19), 1.95(H-12), 1.61(H-1)
10	35.06		C	1.03(H-19), 1.83(H-1), 1.61(H-1), 0.83(H-9), 2.08(H-4)
11	20.92	1.35, 1.57	CH_2	1.18(H-12), 1.95(H-12), 0.83(H-9)
12	39.70	1.18, 1.95	CH_2	0.70(H-18), 1.40(H-17)
13	41.67		C	0.70(H-18), 1.40(H-17),
14	56.62	1.10	CH	0.70(H-18), 1.10(H-15), 1.95(H-12), 1.49(H-16)
15	23.97	1.62, 1.10	CH_2	1.10(H-14), 1.87(H-16), 1.49(H-16)
16	27.64	1.87, 1.49	CH_2	2.45(H-20), 1.40(H-17), 1.10(H-15)
17	54.86	1.40	CH	2.45(H-20), 0.70(H-18), 0.89(H-21).
18	12.38	0.70	CH_3	1.10(H-14), 1.40(H-17), 1.95(H-12), 1.18(H-12)
19	11.48	1.03	CH_3	1.80(H-5), 0.83(H-9), 1.83(H-1), 1.61(H-1)
20	61.07	2.45	CH	1.40(H-17), 0.89(H-21), 2.18(NMe)
21	9.84	0.89, d, J=6.5Hz	CH_3	2.45(H-20), 1.40(H-17)
NMe_2	39.88	2.18	$2CH_3$	2.18(NMe), 2.45(H-20), 1.40(H-17).
-NMe	29.48	3.38	CH_3	
6'	120.91	7.375, d, 1H, J=7 Hz.	CH	7.389(H-4'), 7.77(H-3')
3'	123.30	7.778, d, J=7 Hz.	CH	7.525(H-5'), 7.389(H-4'), 7.375 (H-6')
4'	127.70	7.389, dd, J=7, 7 Hz.	CH	7.778(H-3'), 7.525(H-5'), 7.37(H-6')
2'	130.69		C	7.525(H-5'), 7.389(H-4'), 3.38(-NMe), 7.375(H-6')
5'	131.56	7.525, dd, J=7, 7Hz.	CH	7.778(H-3'), 7 389(H-4'), 7.375(H-6')
1'	152.63		C	7.778(H-3'), 7.525(H-5'), 7.389(H-4'), 2.32(H-2), 2.08(H-4).
PhCO-	167.91		CO	7.778(H-3'), 7.389(H-4'), 3.38 (-NMe-)

Compound R3:

A greyish white colored crystalline Compound R3 was found single spotted on TLC. The compound responded positively all the tests for alkaloids. Its IR spectrum displayed peaks at 3425.8cm^{-1} (-OH), 2930.7cm^{-1}(-C-H), 1694.8cm^{-1}(-CO-). However its ^{1}HNMR spectrum exhibited most of all the characteristic peaks those appeared in ^{1}HNMR spectrum of Compound R4 and many characteristic signals of the Compound R2 (see table III). Thus on the basis of its ^{1}HNMR it was assumed that the compound may be mixture of Compound R2 and Compound R4 or Compound R2 in which the Compound R4 is present as a part of the moiety. The compound R3 was found to be pure on its TLC. The doublet signal at □□□d□□due to H-21 of compound R2 has been shifted to downfield at □ıı□d□□ whereas the signal at □□ı□ being displayed for two methyl groups attached to the nitrogen atom of compound R2 was not observed in ^{1}HNMR of compound R3, at the same time the signal for methyl group was found to be shifted to downfield at □□□□ and was integrated for only one methyl group, showing missing one of the methyl group. The downfield shift of the H-21 methyl group signal in comparison to compound R2 from □□□to□ıı□has supported the absence of secondary amino proton (-NH-Me)From this observation it may be concluded that one of the methyl group

of N-dimethylaminoethane substituent at C-17 in Compound R2 might have been replaced by oleanolic acid (Compound R4) moiety most probably through C-28 carboxyl group thereby forming amide bond.

Table-III. ^{1}H-NMR chemical shifts ( ) of compounds **R3, R2** and **R4**.

S.no.	**R3**	**R2**	**R4**
1	0.7, s, 3H.	0.7, s, 3H, H-18.	
2	0.77, s, 3H.		0.77, s, 3H, H-24.
3	0.78, s, 3H.		0.78, s, 3H, H-26.
4	0.89, s, 3H.		0.89, s, 3H, H-29.
5	.	**0.89d, 3H, H-21***	
6	0.908, s, 3H.		0.908, s, 3H, H-25.
7	0.93, s., 3H.		0.93, s, 3H, H-30.
8	0.98 s, 3H.		0.98s, 3H, H-23.
9	1.03, s, 3H.	1.03, s, 3H, H-19.	
10	1.13, s, 3H.		1.14, s, 3H, H-27.
11	**1.18, d, 3H.***		
12		**2.18, s, 6H,-NMe$_2$**.**	
13	**2.42, s, 3H**.**		
14	2.84, 1H, dd.		, 2.8, 1H, dd, βH-18
15	3.22, 1H.		3.22, 1H, dd, αH-3.
16	3.38, s, 3H,	3.38, s, 3H, -NMe-	
17	5.27, 1H, dd.		5.27, 1H, dd, H-12.
18	7.375, 1H (Ar.), d, H-6'.	7.375, 1H (Ar.), d, H-6'.	
19	7.389, 1H (Ar.), dd, H-4'.	7.389, 1H (Ar.), dd, H-4'.	
20	7.525, 1H (Ar.), dd, H-5'.	7.525, 1H (Ar.), dd, H-5'.	
21	7.778, 1H (Ar), d, H-3'	7.778, 1H (Ar), d, H-3'	

The compound is tentatively assigned to be a oleanoyl derivative of spiropaschysamine. However, further spectral analysis for complete assignment is on progress.

Compound R4:

Compound R4 a white amorphous compound, IR(KBr) spectrum displayed peaks at 3450.39cm^{-1} and 1695.047 cm^{-1} indicating the presence of hydroxyl and carbonyl groups respectively. ^{1}H-NMR ($CDCl_3$) displayed seven quaternary methyl peaks and one olefinic proton at 5.28 (dd, J= 3.4 Hz., H- 12) in ^{1}H-NMR along with other four methine protons confirm the compound as oleanolic acid which was further supported for confirmation[15] by the peak of H- 18 exhibited at 2.8 in the ^{1}H-NMR. The compound was further confirmed by Co- TLC with the authentic sample.

Compound R5:

White amorphous compound, mp 275^{0}C (dec). By comparing R_f value, mp[16], IR (KBr) spectrum with that of authentic samples the Compound R5 determined to be stigmasteryl glucoside.

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