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Chemical Constituents of *Hemigraphis hirta* T.anders (Acanthaceae)

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Abstract: Phytochemical examination of the petroleum ether extract of *Hemigraphis hirta* has led to the isolation of squalene (1), lupeol (2) and β-sitosterol (3) by a combination of column and preparative thin layer chromatography. The structures of these compounds were determined by spectroscopic analysis (UV, IR, ^{1}H-NMR, ^{13}C-NMR) as well as by comparison of their spectral data with previously reported values.

Key words: *Hemigraphis hirta*, acanthaceae, squalene, lupeol, β-sitosterol

Introduction

The Acanthaceae is a large plant family consisting of shrubs, herbs and rarely trees (Trease and Evan, 1983). Modern research carried out on the Acanthaceous plants revealed that most of the plants belonging to this family are medicinally important as they contain biologically active compounds (Kirtikar and Basu, 1994). *Hemigraphis hirta*, a member of this family found to grow throughout Bangladesh, has also considerable reputation for its medicinal value as a folk medicine in the treatment of *Shigellosis*. The whole plant extract is used by the native physicians to cure shigellosis. It is also used in the treatment of abdominal pain, glossitis, stomatitis, acute wounds and anthelmentic (Nasir and Ali, 1974; Kirtikar and Basu, 1994). The miracle of this plant, in the treatment of diarrhoea and dysentery also have been described in the books of medicinal botany (Bhattacharya, 1980). The plant is also useful for the treatment of urolithiasis (Mariappan *et al.*, 1995). Previous phytochemical studies on *H. hirta* revealed the presence of stigmasterol and n-hentriacontanol (Mukherjee and Chakraborty, 1994).

As the significant antimicrobial activity are contained in the petroleum ether extract (Alam *et al.*, 2002) we have chosen the plant for further phytochemical investigation that led to the isolation of two terpenoids, squalene (1) and lupeol (2) and a steroid β-sitosterol. We, herein, report the isolation, purification and structure elucidation of these three compounds. Although these compounds are known natural products but this is the first report of the isolation of these three compounds from this plant.

Materials and Methods

Plant materials: The whole plant was collected when it became mature (July-August' 2000) from Elangi under Jhenaidah District, Bangladesh and was taxonomically identified by Professor A.T.M Naderuzzaman, Department of Botany, Rajshahi University, Bangladesh

Extraction: The experiment was carried out in Phytochemistry Research Laboratory, Department of Pharmacy, Rajshahi University, Bangladesh. The dried coarse powder (600 g) was extracted in a soxhlet apparatus with rectified spirit for 5 days at 70°C, filtered and concentrated with a rotary evaporator under reduced pressure at 50°C to yield a greenish semisolid mass (13 g). The ethanolic extract was diluted with distilled water and was successively extracted with petroleum ether, chloroform, ethyl acetate and methanol and the amounts of each extract obtained are 6.00, 0.55, 0.25 and 6.20 g respectively.

Isolation and characterization of compounds: A portion of petroleum ether extract (4 g) was subjected to column chromatography on silica gel of 60-120 mesh size (Beckett and Stenlake, 1986). The column was first eluted with n-hexane and then n-hexane with increasing portions of ethyl acetate, then with ethyl acetate and finally with methanol which gave 33 fractions. Fraction 4-9 was chromatographed on silica gel and eluted with cyclohexane which afforded compound 1(20 mg), while compound 2 (50 mg) was obtained from the fraction 10-15 by partial crystallization with n-hexane and acetone and recrystalization from chloroform-acetone. Fraction 16-21 was also chromatographed on silica gel and eluted with n-hexane: ethyl acetate (5:1) which yielded compound 3.

Spectroscopic instruments: ^{1}H and ^{13}C-NMR spectrums were recorded on the Jeol-Ex 500 MHZ and FT NMR spectrometers in $CDCl_3$ as solvent with tetramethylsilane (TMS) as an internal standard and the chemical shifts are given in δ-value.

The spectral study was performed at the NCI Frederick, Cancer Research and Development Centre, Frederick, Maryland, USA.

Physical and spectroscopic analysis of the isolated compounds

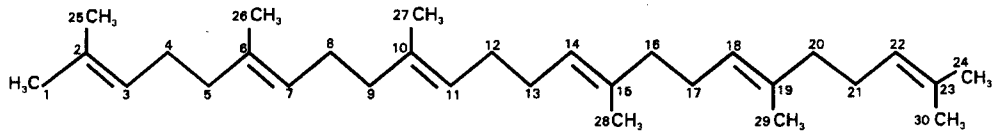
Compound 1: Yellow colored oil. R_f value: 0.625 (n-hexane:ethyl acetate 30:1). ^{1}H-NMR: δ_H 1.66 (s), 1.58 (bs), 5.10 (m), 2.05 (m), 5.10 (m), 1.58 (bs), 5.10 (m), 2.05 (m), 2.05 (m), 1.58 (bs), 5.10 (m), 2.05 (m); ^{13}C-NMR: δ_C: 135.08 (C-10, C-15) 134.88 (C-6, C-19), 131.22 (C-2, C-23), [methine carbons (CH)]: δ124.40 (C-3, C-22), 124.30 (C-11, C-14), 124.20 (C-7, C-18), [methylene carbons (CH_2)]: 39.75 (C-5, C-20), 39.72 (C-9, C-16), 28.27 (C-12, C-13), 26.77 (C-4, C-21), 26.66 (C-8, C-17), [methyl carbons (CH_3)]: 25.67 (C-24, C-1), 17.65 (C-30, C-25), 16.02 (C-29, C-26), 15.98 (C-27, C-28).

Compound 2: White needle shape crystals, R_f value: 0.59 (n-hexane: ethyl acetate, 7:1). IR spectrum exhibited strong absorption bands at 3351 cm^{-1} (due to hydroxyl group) and at 1639 cm^{-1} and 882 cm^{-1} (exomethylene group). ^{1}H-NMR: δ_H δ 0.90 (2H, t), 1.54 (2H, q), 3.20 (1H, dd), 0.68 (1H, d), 1.39 (2H, q), 1.47 (2H, m), 1.26 (1H, d), 1.24 (2H, q), 1.06 ((2H, q), 1.67 (1H, t), 1.69 (2H, t), 1.38 (2H, t), 1.37 (1H, t), 2.38 (1H, m) ; ^{13}C-NMR: δ_C 38.95, 27.66, 79.24, 39.09, 55.54, 18.56, 34.53, 41.08, 50.68, 37.41, 21.17, 25.39, 38.30, 43.07, 27.69, 35.82,43.27, 48.55, 48.22.

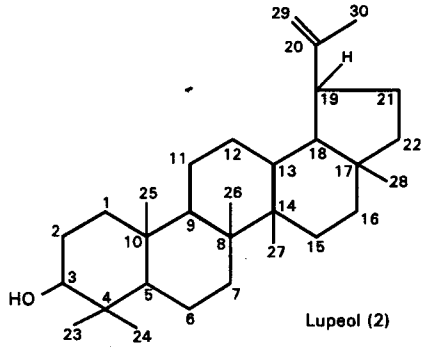
Compound 3: White needle shape crystals, R_f value: 0.50 (n-hexane: ethyl acetate, 5: 1). mp. 137°C. ^{1}H-NMR: δ_H 0.70 (3H, d, J = 7.2 Hz), 0.80 (3H, d, J = 7.2 Hz), 0.83 (3H, d, J=1.32 Hz), δ0.86 (3H, s), δ1.04 (3H, d, J=7.84 Hz), δ.69 (3H, s), 5.36 (1H, d, J = 5.1 Hz), 3.55 (1H, m), 0.9-δ2.4 (methylene and methine protons), 5.16 (1H, dd, J = 15.2, J = 8.6 Hz) and δ5.03 (1H, dd, J = 15.8, 8.6 Hz).

Results and Discussion

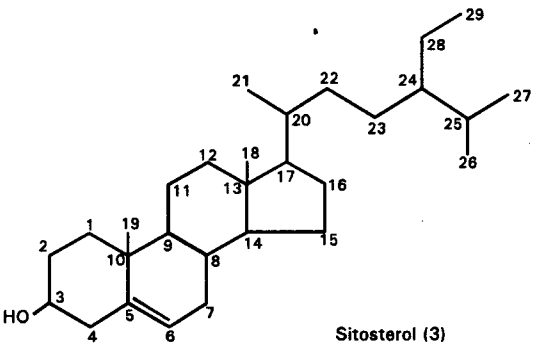
The petroleum ether extract of *Hemigraphis hirta* yielded three pure compounds that were identified as squalene (1), lupeol (2) and β-sitosterol (3) by spectroscopic analysis as well as by comparison of their spectral data with previously reported values. The ^{13}C-NMR spectrum of compound 1 (Table 2) displayed 15



Squalone (1)



Lupeol (2)



Sitosterol (3)

Table 1: Comparison of ^{1}H-NMR spectral data of the compound 1 and authentic squalene

Position of protons	Compound 1(δ value in ppm)	Squalene (δ value in ppm) (Mitsuo *et al.*, 1996)
1, 24-Me	δ1.66 (s)	δ1.68
25, 30-Me	δ1.58 (bs)	δ1.60
H-3, 22	δ5.10 (m)	δ5.12
H-4, 21	δ2.05 (m)	δ2.02
H-5, 20	δ5.10 (m)	δ2.09
26, 29-Me	δ1.58 (bs)	δ1.60
H-7, 18	δ5.10 (m)	δ5.12
H-8, 17	δ2.05 (m)	δ2.09
H-9, 16	δ2.05 (m)	δ2.02
27, 28-Me	δ1.58 (bs)	δ1.60
H-11, 14	δ5.10 (m)	δ5.12
H-12, 13	δ2.05 (m)	δ2.02

Table 2: ^{13}C- NMR spectral data of compound 1

Position of carbon	^{13}C-NMR spectral data (δ values in ppm) in $CDCl_3$	Nature of carbon
	δ135.08	C (quaternary).
	δ134.88	C (quaternary)
	δ131.22	C (quaternary)
	δ130.84	C (quaternary)
	δ128.79	C (quaternary)
7, 18	δ124.40	CH (tertiary)
11, 14	δ124.30	CH (tertiary)
3, 22	δ124.20	CH (tertiary)
5, 20	δ39.75	CH_2 (secondary)
9, 16	δ39.72	CH_2 (secondary)
12, 13	δ28.27	CH_2 (secondary)
4, 21	δ26.77	CH_2 (secondary)
8, 17	δ26.66	CH_2(secondary)
24, 1	δ25.67	CH_3 (primary)
30, 25	δ17.65	CH_3 (primary)
29, 26	δ16.02	CH_3 (primary)
27, 28	δ15.98	CH_3 (primary)

distinct resonances represents 30 carbons, while the DEPT experiment showed the presence of eight methyls, ten methylenes, six methines and six trisubstituted quarternary carbons. The ^{1}H-NMR spectral data (Table 1) indicated that the compound was squalene, an acyclic triterpenoid (Yumi *et al.*, 1996) thus the spectra showed six olefinic proton (m, δ 5.10, H-3, H-7, H-11, H-14, H-18 and H-22). This was further substantiated by the presence of three methine carbons resonating at δ124.28, δ124.31 and δ124.4, ten methylene proton (m δ 2.05, H-4, H-5, H-8, H-9, H-12, H-13, H-16 H-17, H-20 and H-21) and finally a singlet at δ1.66 (6H, s, H-1 and 24 Me) together with a broad singlet at δ1.58 (9H, bs) which corresponded respectively, to an in-chain allylic methyl group and three out-chain allylic groups of a polyprenoid system (Alejandro *et al.*, 1996). In the ^{13}C-NMR spectrum, the out of chain methyl groups resonating at δ17.65, δ16.02 and δ15.98 indicated the geometry of the six trisubstitutional double bonds, while signal appearing at δ25.67, confirmed its in-chain position (Mitsuo *et al.*, 1996). These NMR spectral data were almost the same as aqualene. On the basis of these ^{1}H and ^{13}C-NMR spectral features and by comparison with the authentic data (Mitsuo *et al.*, 1996) compound 1 was identified as squalene. This is the first report of its occurrence from the genus *Hemigraphis*.

The ^{1}H-NMR spectrum of compound 2 (400 MHZ, $CDCl_3$, Table 3) supported the presence of exomethylene protons at δ4.70 (1H, d, J = 2.4 Hz) and δ 4.58 (1H, m) for H-29. The ^{1}H-NMR spectrum also exhibited a vinylic methyl proton as singlet at δ1.69 for H-30 and six tertiary methyls at δ0.77, δ0.80, δ0.84, δ0.95, δ0.98 and δ1.04. These features indicated that the compound is a triterpene (Anowar, 1999). A J modulated ^{13}C-NMR spectrum (100 MHZ, $CDCl_3$) showed a total of 30 carbons. Among these the exomethylene carbon appeared at 109.54, the quaternary carbon attached to be exomethylene at 151.19 and the oxygenated methine at 79.24. The chemical shifts of all of the carbons were in very close agreement with that of lupeol reported in the literature (Sakakibara *et al.*, 1983). Moreover, Co-TLC identified the compound 2 as lupeol with an authentic sample. Although lupiol is a known compound but for the first time it is reported from the genus *Hemigraphis*.

The ^{1}H-NMR spectrum of compound 3 (400 MHZ, $CDCl_3$) exhibited a broad doublet at δ5.36 (1H, d, J = 5.1 Hz) attributed to be a double bonded proton typical for H-6 and a multiplet at δ3.55 (1H, m) integrated for one proton which could be H-3 of a steroidal skeleton. Other signals appeared between δ0.9-δ2.4 were due to the methylene and methine protons. These features were in close agreement with those of β-sitosterol reported in the literature (Hill *et al.*, 1990). It was further confirmed by a mixed melting point determination with an authentic sample of β-sitesterol. This is the first report from this plant.

Literature review on the plant *Hemigraphis hirta* suggests that there is a little chemical investigation on this plant and Mukherjee (1992) isolated stigmasterol and n-hentriacontanol from the petroleum ether extract. Thus the findings of this investigation

Table 3: Comparison of ^{1}H-NMR and ^{13}C-NMR spectral data of compound 2 with authentic Lupeol

Position of C/H	^{1}H-NMR data of compound 2	^{1}H-NMR data of lupeol (Sakakibara *et al.*, 1983)	^{13}C-NMR data of compound 2	^{13}C-NMR data of lupeol (Sakakibara *et al.*, 1983)
1	δ 0.90 (2H, t)	δ 0.91	38.95	38.67
2	δ 1.54 (2H, q)	δ 1.54	27.66	27.35
3	δ 3.20 (1H, dd)	δ 3.17	79.24	78.94
4			39.09	38.81
5	δ 0.68 (1H, d)	δ 0.69	55.54	55.25
6	δ 1.39 (2H, q)	δ 1.39	18.56	18.28
7	δ 1.47 (2H, m)	δ 1.41	34.53	34.23
8			41.08	40.78
9	δ 1.26 (1H, d)	δ 1.28	50.68	50.38
10			37.41	37.11
11	δ 1.24 (2H, q)	δ 1.25	21.17	20.89
12	δ 1.06 ((2H, q)	δ 1.07	25.39	25.08
13	δ 1.67 (1H, t)	δ 1.67	38.30	38.00
14			43.07	42.78
15	δ 1.69 (2H, t)	δ 1.71	27.69	27.41
16	δ 1.38 (2H, t)	δ 1.38	35.82	35.54
17			43.27	42.95
18	δ 1.37 (1H, t)	δ 1.37	48.55	48.24
19	δ 2.38 (1H, m)	δ 2.39	48.22	47.94
20			151.19	150.88
21	δ 1.33 (2H, m)	δ 1.33	30.09	29.80
22	δ 1.20 (2H, m)	δ 1.20	40.24	39.96
23	δ 0.95 (3H, s)	δ 0.98	28.22	27.95
24	δ 0.77 (3H, s)	δ 0.77	15.59	15.35
25	δ 0.84 (3H, s)	δ 0.84	16.34	16.09
26	δ 1.04 (3H, s)	δ 1.04	16.21	15.94
27	δ 0.97 (3H, s)	δ 0.97	14.78	14.51
28	δ 0.80 (3H, s)	δ 0.79	18.23	17.97
29	δ 4.58 (1H, m), 4.70 (1H,d)	δ 4.56 and δ4.69	109.54	109.31
30	δ 1.69 (3H, s)	δ 1.69	19.54	19.28

and previous investigations (Rashid, 2002; Rahman, 2000; Haque, 2000) would give valuable information about various new chemical compounds from Bangladeshi plants.

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