Isolation of A New Sterol from *Limonium stocksii* and Antimicrobial Activities of Crude Extract

Rahat A. Ali[a,*], Shaheen Inam[a] and Shaista Perveen[b]

[a]*Department of Chemistry, University of Karachi, Karachi 75270, Pakistan*

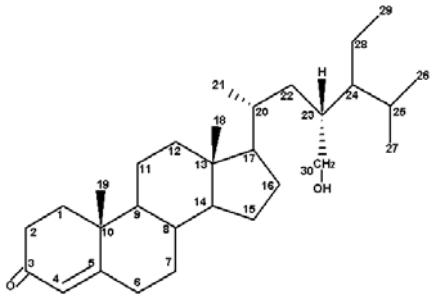
[b]*NED University of Engineering & Technology, Karachi 75270, Pakistan*

Abstract: A new sterol was isolated from the leaves of *Limonium stocksii,* together with two other known sterols. The structure of new sterol was determined to be 3-Oxo-23-α-hydroxy methyl stigmasta-4-ene on the basis of spectroscopic evidences.

Keywords: *Limonium stocksii*, sterols, 3-Oxo-23-α-hydroxy methyl stigmasta-4-ene.

1. INTRODUCTION

Limonium (Sea Lavender) commonly known as statice belongs to the family Plumbaginaceae (Leadwort) comprises 120 to 150 species of herbs and shrubs, found in different climatic regions from arctic to tropical, particularly with salt rich steppes, marshy places and sea coasts. In Pakistan, it is found in Baluchistan, Sindh and Hawksbay Karachi. Phytochemical studies of this plant showed that chemical constituents of *Limonium* are very complex. Species are rich in flavonoids [1-4], carbonyl compounds, hydrocarbons [5], fatty acids [6], naphthoquinone, tannins, alkaloids and amino acids [7, 8]. The pharmacological research showed that *Limonium* species have antipyretic, hemostatic, and depurative [9], antiviral [10, 11] and antitumoral [12, 13] activities. Specie also possesses skin conditioning effect [14], and lipase inhibitor for pharmaceutical cosmetics and food preparations [15].



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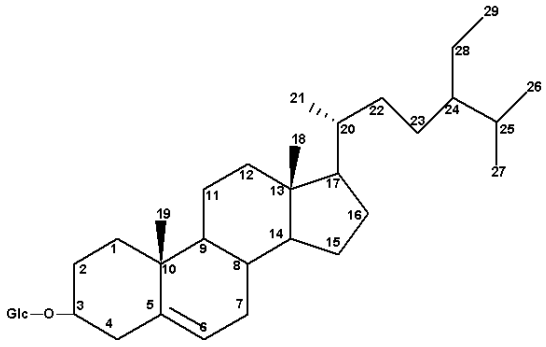
Keeping into account the phytochemical and pharmacological importance of genus *Limonium* studies on chemical constituents of *Limonium stocksii* was done. It showed the presence of a new compound *Limoniol* (**1**) *3-Oxo-23-α-hydroxy methyl stigmasta-4-ene* and two known compounds 3-O-β-D-glucopyranosyle-β-sitosterol (**2**) and3-Hydroxy-7-oxo-stigmasta-5-ene (**3**). Our studies also showed that crude ethanolic extracts of stems of *L. stocksii* showed antifungal and antibacterial activities.



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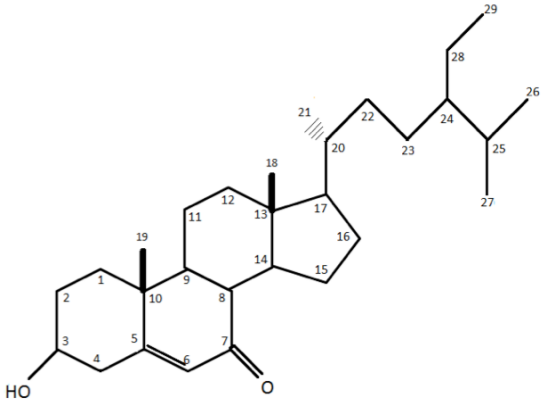

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2. EXPERIMENTAL

2.1. Plant Material

Plant was collected from Bhambore (Sindh) and Hawksbay Karachi and was identified by the plant taxonomist, Department of Botany University of Karachi

*Address correspondence to this author at the Department of Chemistry, University of Karachi, Karachi 75270, Pakistan,
E-mail: rahat_sultana786@yahoo.com

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Pakistan, where voucher specimen (G. H. NO.86448) was deposited.

2.2. Extraction and Isolation

The air dried leaves (2kg) and stems (60kg) were ground and soaked separately in 95% ethanol for fifteen days. The ethanolic extract was evaporated under vacuum to give a dark brown residue of leaves and reddish brown residue of stems extract. Leaves residue (60g) diluted with 500ml distilled water and extracted in succession, with hexane, $CHCl_3$ and EtOAc. The stem gum (200g) was also extracted separately with hexane, $CHCl_3$ and EtOAc.

The $CHCl_3$ extract of leaves (6.5g) was subjected to Si-gel column chromatography with hexane/$CHCl_3$ 5:5. The elusion was carried out with $CHCl_3$ and MeOH in increasing polarity. The fractions were combined by monitoring with TLC. Fraction obtained from hexane/$CHCl_3$ 5:5, detected on TLC plate with hexane/$CHCl_3$ 2.2:7.8 gave compound 1 (30mg). EtOAc (7.0g) fraction of leaves was subjected to Si-gel CC with $CHCl_3$, MeOH mixture, in increasing polarity. Fraction obtained from $CHCl_3$/MeOH 9.4:0.6, crystallized with acetone gave compound 2 (19.5mg). The $CHCl_3$ extract of stem (2.8g) was subjected to Si-gel CC using hexane, $CHCl_3$ and MeOH mixtures by increasing polarities. The fraction eluted with hexane/$CHCl_3$ 5:5, after further CC separation gave compound 3 (17.5mg) and 4(15.2mg) with $CHCl_3$/EtOAc 9.6:0.4 and 9.4:0.6 respectively.

Limoniol 1 white amorphous solid, UV; Max (EtOH) 243nm, IR bands ($CHCl_3$)3300 cm^{-1} (OH), 1668 cm^{-1} (α,β unsaturated C==O), 1662 cm^{-1} (C==C), ^1H and ^{13}C- NMR spectra; see Table **1**. HRMS; m/z 442 (Calculated 442.381061 for $C_{30}H_{50}O_2$), EIMS m/z 412 $(M\text{-}CH_2O)^+$ (95), 398 $(M\text{-}C_2H_4\text{-}H_2O)^+$ (25), 397 $(M\text{-}C_2H_5,H_2O)^+$ (30), 271 (M-side chain) (35), 124 $(M\text{-}C_8H_{15},H_2O)^+$ (100), 229 $(M\text{-}C_{16}H_{22}O)^+$ (55).

3. RESULTS AND DISCUSSION

HRMS of compound **1** showed molecular ion peak at m/z 442.381061 corresponds to molecular formula $C_{30}H_{50}O_2$.EIMS showed intense peak at m/z 412 due to the loss of CH_2O, 124 due to the loss of C_8H_{15} and loss of water other peak appearing in EIMS are explained in Figure **1** which showed the presence of steroidal skeleton. The IR spectrum showed the presence of hydroxyl and α-β unsaturated carbonyl group. ^{13}C-NMR spectra indicatedthe presence of six methyl, ten methylene, nine methine and four quaternary carbons. Methyl signals appeared at δ(11.96) C-18, (17.31) C-19, (18.69)C-21, (19.81) C-26, (19.02) C-27, and (11.97) C-29. The signal due to olefinic methine appeared at (δ123.71) C-4 and quaternary carbon associated to this, appeared at (δ171.84) C-5, carbon bearing oxo group appeared at (δ198.94) C-3. Comparison with the reported data [16, 17] showed that oxo group is present at C-3 and double bond is at C4/C5 position. The presence of conjugated carbonyl was confirmed from UV absorption (243nm). C^{13}NMR further showed the presence of a carbinylic methylene group at δ63.11 which was assigned to C-30. In ^1H-NMR the presence of methylenic proton attached to C-23 was confirmed by two doublets at δ3.60(J, 6.6Hz) and 3.63(J, 6.8Hz) which indicates the presence of

Table 1: NMR Data for Compound 1 ($CDCl_3$) (400 MHz)

Position	δ_H	δ_C
1	1.57m	35.64
2	2.4m	33.88
3		198.94
4	5.702s	123.01
5		171.84
6	2.39m	32.96
7		32.80
8	1.67t	35.62
9		53.81
10		36.61
11		21.02
12		39.64
13		42.38
14		55.87
15		24.18
16		28.19
17		56.00
18	0.690s	11.96
19	1.161s	17.31
20	1.3	36.11
21	0.905d (J, 6.2Hz)	18.69
22		33.97
23	1.54	26.06
24		45.80
25		29.14
26	0.876d (J, 6.4Hz)	19.81
27	0.860d(J, 6.4Hz)	19.02
28		23.06
29	0.825t(J, 7.6Hz)	11.97
30	3.602d(J, 6.6Hz), 3.63(J, 6.8Hz)	63.11

CH_2-OH group [19]. The ^{1}H-NMR study also showed olefinic proton as broad singlet at (δ5.702) H-4. More over the ^{1}H-NMR spectrum exhibited a proton signal at δ5.326 as broad singlet due to the presence of hydroxyl group at H-30. Similarly two singlets appeared at δ0.690 and 1.161 due to Me-18 and Me-19 respectively. The three doublets appeared at δ0.905 (J, 6.2Hz) and 0.876 (J, 6.4Hz), 0.860 (J, 6.4Hz), due to H-21, H-26 and H-27 respectively. A triplet appeared at δ0.825 (J, 7.6Hz) due to H-29.

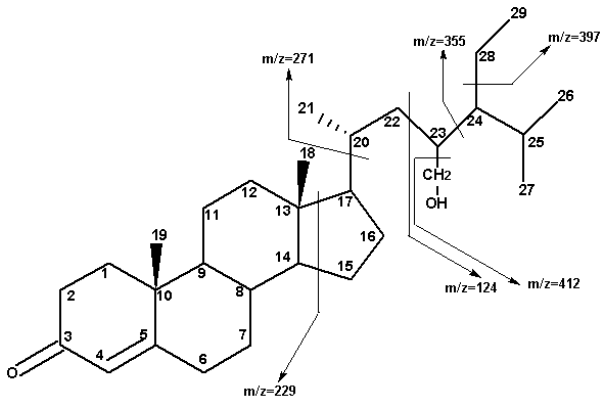

Figure 1: EIMS Fragments of *Limoniol* (**1**).

In ^{1}H-COSY spectrum of this compound showed no coupling of H-4 (δ5.6) proton which justified that the vicinal positionsare quaternary. Methylenic proton H-30 at (δ3.63) showed vicinal coupling with methine proton H-23 at (δ1.54), which is in turn correlated with C-25 (δ29.14) ($^3J_{CH}$ coupling), in the HMBC spectrum.

In HMQC spectrum of this compound methylenic carbon at (δ63.11) C-30 was correlated with two methylene protons at δ3.60 and 3.63. These two geminal protons were observed as dd, J, 6.6Hz, 6.8Hz, are the characteristic of the presence of CH_2-OH group [19].

A long range heteronuclear correlation observed by HMBC (Figure **2**), showed cross-peak between H-23 (δ1.54) to C-23 (26.06) ($^1J_{CH}$ coupling), C-25 (29.14) ($^3J_{CH}$ coupling) and C-30 (63.11) ($^3J_{CH}$ coupling), while H-30 (3.63) showed correlation with C-22 (33.97) ($^3J_{CH}$ coupling) and C-23 (26.06) ($^2J_{CH}$ coupling). All these correlations suggested the position of hydroxy methylene group at C-23 in the side chain.

The NOESY spectrum showed strong interaction of (δ1.54) H-23 proton with (δ1.161) 3H-19methyl proton and (δ1.3) H-20 proton, indicated the presence of α-hydroxy methyl substituent at C-23 position.

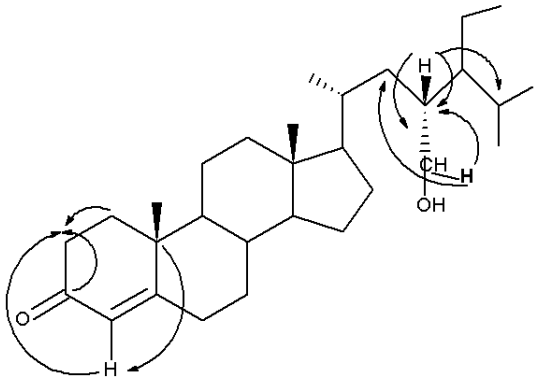

Figure 2: Selective HMBC correlation of *Limoniol* (**1**).

Beside the new compound, other isolated compounds from this plant were identified as 3-O-β-D-glucopyranosyle-β-stiosterol (**2**) [20-22], 3-hydroxy-7-oxo-stigmasta-5-ene (**3**) [16, 18], on the basis of spectral evidence and comparisons of physical data with literature values. Compounds 2 and 3 were isolated and reported from this specie for the first time.

Biological activities of crude extract of stems of the plant were also carried out. The data on antifungal activity in Table **2**, revealed that the crude extract of stems having inhibitory activity against Aspergillusflavus and Microsporumcanis [23, 24].

The mode of action of crude extract of stems on microbial growth showed low activity against Pseudomonas aeruginos, Table **3** [25-27].

Table 2: Antifungal Activity

Name of Fungus	Linear growth(mm) Sample growth		% Inhibition	Std. Drug MIC µg/ml
Trichphyton longifusus	80	100	20	Miconazole 70
Candida albicans	100	100	0	Miconazole 110.8
Aspergillus flavus	50	100	50	Amphotericin B20
Microsporum canis	50	100	50	Miconazole 98.4
Fusarimu solam	100	100	0	Miconazole73.25
Candida glabrata	100	100	0	Miconazole 110.8

Key: Conc. of sample 200 µg/ml in DMSO incubation Time 27($28^{o\pm}$ 1^{o}C), incubation period 7 days (7-10 days).

Table 3: Antibacterial Activity

Name of Bacteria	Zone of Inhibition Of Sample (mm)	Zone of Inhibition of Std. Drug (mm)
Eschericha coli	11	24
Bacillus subtilis	-	23
Shigella flexenari	-	28
Staphylococcus aureu	11	27
Pseudomonas aeruginosa	13	20
Salmonella typhi	-	26

Key: Conc. of sample 3 mg/ml of DMSO side of well 6mm (diameter) Std. Imipenum 10µg/disc.

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