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PHYTOCHEMICAL STUDIES OF THE PETROLEUM ETHER EXTRACT OF THE LEAVES OF *Lagerstroemia speciosa Linn*

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ABSTRACT

Two new compounds, normal alcohol containing of higher carbons and isomer of β-sitosterol were isolated for the first time from the petroleum extract of the leaves of Lagerstroemia speciosa. The structure of the compound has been established on the basis of UV, IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra and identified as nonanol and isomer of β-sitosterol.

Keywords: *Lagerstroemia speciosa***,** petroleum ether extracts, isolation, 1-nonanol, 2 β-sitostero, spectral analyses.

INTRODUCTION

Lagerstroemia speciosa, locally known as Jarul is a good timber tree with lilac flowers and winged seed. It is widely distributed in most part of the world including Bangladesh and the sub continents [1]. Different parts of the plant are used for the treatment of various diseases [2-6]. Its, anti-diabetic, anti-HIV and antimicrobial activity have also been reported [6-8]. The isolation of *n*-pentanol and 2,3,8-tri-*O*-methyl ether of ellagic acid have been reported [8] from the petroleum ether and chloroform extracts of the leaves. In this paper, we describe the isolation and structure elucidation of nonanol and isomer of β-sitosterol from two different fractions of petroleum ether extracts of the leaves of *Lagerstroemia speciosa* for the first time. The isolated two new compounds were identified on the basis of UV, IR, ^{1}H- NMR, COSY, NOSEY, DEPT analyses.

EXPERIMENTAL SECTION

General

Melting point was determined on an electrochemical micro-melting point apparatus. IR spectra were recorded (KBr discs) on a Shimadzu IR-470A spectrophotometer, validation (ν_{max} in cm^{-1}). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker R-32 (400 MHz) instrument in $CDCl_3$ and DMSO-d_6 with TMS as an internal standard (chemical shifts in δ, ppm). UV spectra were recorded on HATACHI, U-2000 spectrophotometer Ultrospeck in methanol (λ_{max} in nm). Mass spectra were recorded on a Varian 3800 mass spectrophotometer. TLC was performed using silica gel GF_{254}. Freshly distilled solvents were used for extraction, isolation and purification. Evaporations were performed under reduced pressure on a Buchii rotary evaporator.

Plant Material

The leaves (2.5 Kg) of *Lagerstroemia speciosa* was collected from the Carzon Hall Campus of the University of Dhaka, Bangladesh, March 2006. The plant was identified and voucher specimen was deposited in the Department of Botany, Dhaka University, Dhaka, Bangladesh.

Procedure

Extraction of the plant material

The leaves (2.5 Kg) of *Lagerstroemia speciosa* were collected and dried either in an electrical oven below at 40 °C or sunlight. The dried leaves of this plant (2.5 Kg) were milled into powdered by a cyclotec grinder (200 mesh) and the powder is used in this investigation. The leaf powder (600 g) was exhaustively extracted in a Soxhlet apparatus for 36 h with petroleum ether (40-60 °C). The extract was evaporated in a rotatory evaporator and dried by vacuum pump. The concentrated crude extract (55.8 g) was subjected to vacuum liquid chromatography (VLC) over TLC grade silica gel (GF_{254}). The column was eluted initially with petroleum ether (40-60 °C) followed by the mixture of petroleum ether and ethyl acetate with increasing quantities of ethyl acetate. These elutes were collected in a series of test tubes with 15 mL in each fraction. Each fraction was examined by TLC. Based on the similar TLC behavior, this elutes were combined to yield thirteen fractions (F_1-F_{13}). The fractions (F_1-F_{13}) were concentrated separately and allowed to stand at room temperature for several weeks.

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White amorphous substance (10.0 mg) settled out from F_5 and white needle shaped crystals (6.50 mg) deposited from F_8 and were marked as L_1 and L_2, respectively. All attempts to obtain solid compounds from the other fractions met with failure.

Compound L_1

Obtained from column chromatography was further purified by preparative TLC over silica gel GF_{254} using chloroform-ethyl acetate (3:2) as developing solvent to give compound **L_1** (10 mg). The amorphous solid substance was further washed thrice with petroleum ether to removed impure compounds. It was soluble in a mixture of equal proportion of chloroform and methanol. The solution was applied in TLC micro glass slide, and viewed in an iodine chamber where a single brown spot were detected. Upon spraying the developed TLC plate with vanillin sulphuric acid reagent followed by heating at 110 °C for about 10 min, a round single violet spot was observed.

It could not be crystallized from any solvent. It was a colourless amorphous solid (9.7 mg). R_f 0.69 (chloroform-ethyl acetate; 3:2); MS: (M^+, 202); IR (KBr) : 3400, 2900, 2850, 1450, 1365, 1120, 1050, 740, 720 cm^{-1}; ^{1}H-NMR($CDCl_3$, δ values): 3.45 (t, J=7.0 Hz, H-1), 3.25 (s, -OH), 1.42 (m, J=8.0 Hz, H-8), 1.14 (dm, H-2, H-3, H-4, H-5, H-6, H-7), and 0.76 (t, J=7.0 Hz, H-9); ^{13}C-NMR ($DMSO-d_6$): 62.3 (C-1), 32.3 (C-2), 31.7 (C-3), 25.6 (C-7), 22.5 (C-8), 13.7 (C-9) and 29.5, 29.3, 29.1 (C-4, C-5, C-6).

Compound L_2

Fraction F_8 was passed through the column chromatography was further purified by preparative TLC over silica gel GF_{254} using chloroform-ethyl acetate (3:2) as developing solvent to give compound **L_1** (6.50 mg**).** It was crystallized from dilute methanol to give white needles (6.10 mg). The fine needle shaped crystal was further washed with petroleum ether to remove any impurities and finally the amount was found to be 6.0 mg. It was completely soluble in chlororoform. The solution was applied in TLC glass plate, and viewed in an iodine chamber where a single brown spots were detected whereas a violet spot was observed upon spraying with vanillin sulphuric acid reagents followed by heating at 110 °C for about 10 min on the separate sample applied TLC glass plate, which revealed that it was a single compound. White needles (6.0 mg), m.p.100-102 °C; R_f 0.53 (chloroform-ethyl acetate; 3:2); MS (m/z): (M^+, 426); UV: 232 nm; IR (KBr): 3500, 2900, 1660-1600, 1460, 1380, 1330, 1190, 1060, 980, 800 cm^{-1}.

The values of the ^{1}H-NMR and ^{13}C-NMR of the isolated compound **L_2** are given in Table 1.

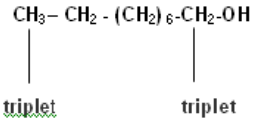
Table 1. ^{13}CNMR and ^{1}HNMR spectral data of compound **L_1**

Position	δ_C	δ_H	Position	δ_C	δ_H
1	37.3	0.84 (s)	16	28.2	4.09 (d)
2	31.3	1.24 (m)	17	61.2	
3	71.8	3.48 (t)	18	49.5	1.80 (m)
4	42.4		19	48.6	3.52 (m)
5	55.8	0.83 (m)	20	140.7	
6	56.8	1.26 (m)	21	32.0	0.96 (s)
7	31.6		22	36.2	1.80 (m)
8	45.8		23	28.5	1.21 (s)
9	42.3	1.28 (t)	24	16.3	1.01 (s)
10	36.2		25	16.4	0.83 (s)
11	20.9	1.18 (m)	26	16.4	1.13 (s)
12	28.1	1.15 (m)	27	16.2	1.12 (s)
13	36.1	2.46 (m)	28	171.7	
14	56.0		29	123.7	4.48 (s)
15	35.6	1.80 (m)			

Assignments are based on DEPT 90 and DEPT 135, ^{1}HNMR, ^{13}CNMR, ^{1}Hcosy

RESULT AND DISCUSSION

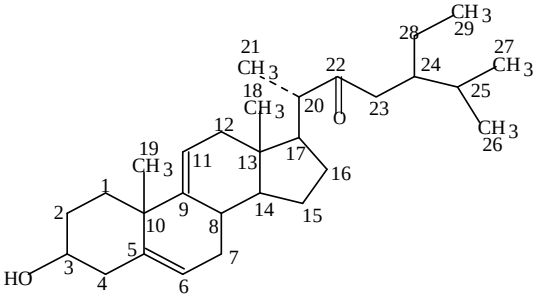
The thin layer chromatographic examination of the isolated compound **L_1** from the petroleum ether (40-60 °C). extract of the leaves of *Lagerstroemia speciosa* showed a single brown spot upon exposure to iodine chamber and a round violet spot was observed on the thin layer chromatographic plate when the vanillin-sulphuric acid reagents was sprayed on it followed by heating at 110 °C for about 10 min. The compound was obtained as white amorphous crystals and it could not be crystallized from any solvent. It was readily soluble in the mixture of chloroform and methanol and other organic solvents. Its IR spectrum showed [9,10] an absorption peak at 3300-3400 cm^{-1} and the absorption peak at 2900-2850 cm^{-1} indicating the presence of hydroxyl group and –CH- of aliphatic asymmetric stretching, respectively. The absorption band at 1450 and 1365 cm^{-1} indicating the presence of –C-H bending for $-CH_2-$ and $-CH_3$ group, respectively. A sharp absorption peak at 1120 cm^{-1} was demonstrated the presence of –C-O bond stretching while the other at 1050 cm^{-1} was the indicative of the presence of –C-O stretching of primary alcohol. Absorption band at 740 and at 720 cm^{-1} were suggested the presence of –C–H stretching for $(-CH_2-)_n$ groups. The ^{1}H-NMR spectrum showed an upfield triplet at δ 0.76 suggestive of the presence of $–CH_3$ group at position 9 attached to a methylene carbon. The other downfield triplet at δ 3.45 was indicative of the presence of a methylene group attached to the carbon at position one. The singlet at δ 3.25 was assigned for the alcoholic proton. The chemical shift at δ 1.42 with multiplet was characteristics of the methylene group of C-8. The distorted multiplet of the spectrum at chemical shift



Compound L_1

δ 1.14 ppm was assigned for the protons at carbon 2, 3, 4, 5, 6, and 7. The ^{13}C-NMR spectrum of the compound L_1 revealed the presence of 9 carbons. The chemical shift at δ 62.3 was assigned to the presence of methylene carbon of C-1 attached to alcoholic -OH group. Chemical shift at 13.7 ppm was attributable to methyl group at position 9 attached to a methylene group. The chemical shifts at δ 22.4, 25.2, 29.1, 29.2, and 29.4 were assigned for the presence of five-methylene carbon, which existed in the normal carbon chain of primary alcohol [11]. From the above analyses of the IR, ^{1}H-NMR and ^{13}C-NMR data it was established of the compound L_1 as 1-nonanol.

The thin layer chromatographic examination of the isolated compound **L_2** from the petroleum ether (40-60 °C) extract of the leaves of *Lagerstroemia speciosa* was visualized as brown spot and a violet spot was observed on the thin layer chromatographic plate when the vanillin-sulphuric acid reagents was sprayed on it followed by heating at 110 °C for about 10 min. It was completely soluble in chloroform. The steroidal nature of the compound was observed from the Salkowski and Liebermann Burchard's test [12]. Isomer of β-sitosterol was obtained as white needle shaped crystals m.p. 100-102 °C. It had the molecular formula $C_{29}H_{46}O_2$ as determined by HREIMS and confirmed by ^{1}H-NMR and ^{13}C-NMR (Table-1) and DEPT analysis. The UV spectra displayed characteristic absorption bands for a conjugated double bond at 232 nm. Its IR spectrum showed absorption band at 3510 cm^{-1} was an indicative of -OH group where as the absorption band at 2900 cm^{-1} was assigned to -CH of aliphatic asymmetric stretching. The absorption peaks at 1660 cm^{-1} and 1600 cm^{-1} were indicating the presence of >C=C< skeletal unit whereas the absorption band at 1460 cm^{-1} and 1340 cm^{-1} were suggested the -CH_2- and -CH_3 bending vibration, respectively. The other two absorption bands at 1250 cm^{-1} and 1190 cm^{-1} indicated the presence of -CH bending for -CH_3 group. The absorption peak at 1060 cm^{-1} was demonstrated the presence of -C-O stretching of secondary alcohol while the band at 980 cm^{-1} supported the steroidal nature [12] and the other absorption band at 800 cm^{-1} indicated the presence of proton attached to C=C. The ^{1}H-NMR spectrum showed three singlets at δ 1.13, 1.12 and 0.91 for the methyl protons at positions C-27, C-26 and C-21, respectively. A broad singlet at δ 4.48 was accounted for methyl protons at position C-29 that attached through methylene group. Two different singlets at δ 1.80 and



Compound L_2

3.52 were suggested due to the presence of methyl protons of C-18 and C-19. The ^{13}C-NMR spectrum of the compound L_2 revealed the presence of 29 carbons for a steroidal skeleton.

From the proton, carbon thirteen and DEPT ^{1}H-NMR chemical shift value it was assigned that there were two double bonds in the in the structural position at C-5 and C-9.

All the ^{13}C-NMR signals were in agreement with the data for sterol with the exception of ^{13}C chemical shift value at δ 171.7 ppm. This value is usually considered for the presence of carbonyl carbon. But there was no absorption band was monitored in the IR spectral analysis of the compound L_2 between the region 1680 cm^{-1} to 1850 cm^{-1} typically for >C=O group. Comparing the ^{13}C-NMR spectroscopic data with the reported data of the identical steroid compound along with the IR, ^{1}H-NMR and DEPT NMR spectral analysis and physical properties the identity of the isolated compound **L_2** was established as the isomer of β-sitosterol.

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