3-Acetoxy-6-benzoyloxyapangamide from *Achyranthes aspera*

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ABSTRACT: 3-Acetoxy-6-benzoyloxyapangamide (**1**) has been isolated from an ethyl acetate extract of the stem of *Achyranthes aspera*. The structure of the isolated compound was established by modern spectroscopic techniques. The extract was found to show mild antibacterial activity against *Bacillus cereus*.

Key words: *Achyranthes aspera*, 3-acetoxy-6-benzoyloxyapangamide, Antibacterial activity.

INTRODUCTION

Achyranthes aspera L., locally known as Apang, is an annual, biennial, lower portion perennial erect under shrub or rather stiff herb growing up to 0.3 to 1.0 meter in height. It grows throughout the world in tropical and warmer regions and it is also found all over Bangladesh[1-3]. Ayurvedi, Yunani doctors and local Kabiraj[4,5] use the stems, leaves and fruits as a remedy for piles, renal dropsy, pneumonia, cough, kidney stone, skin eruptions, snakebite, gonorrhea, dysentery etc. 27-Cyclohexylheptacosan-7-ol, 16-hydroxy-26-methylheptacosan-2-one[6], a long chain alcohol[7], 17-pentatriacontanol[7], alkaloid[8], β-sitosterol[9] and spinasterol[9] were obtained during investigation of various extracts of this plant. In this paper, the isolation and characterization of 3-acetoxy-6-benzoyloxyapangamide and the antibacterial activity of ethyl acetate extract of the stem of this plant are reported.

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MATERIALS AND METHODS

General Experimental Procedures. Freshly distilled solvents were used for extraction, isolation and purification. Evaporation was performed under reduced pressure on a Buchii rotary evaporator. Melting point was determined on an electrothermal micro melting point apparatus. The IR (KBr) spectrum was recorded on a Shimadzu IR-470A spectrophotometer. The ^{1}H-NMR and ^{13}C-NMR, ^{1}H-^{1}H COSY, HSQC and DEPT spectra were taken in duterated chloroform ($CDCl_3$) with TMS as an internal standard in a 400 MHz or 100 MHz instrument. The mass spectrum was recorded in EI ionization mode.

Extraction of Plant Material and Isolation of Compound 1. The whole plants of *A. aspera* were collected from the Curzon Hall campus of the University of Dhaka. A voucher specimen for this collection has been deposited in the Herbarium of the Department of Botany, University of Dhaka. Its leaves and stems were separated and dried under mild sunlight and then at 45° C in an oven. The dried stems were powdered by a Cyclotec grinder (200 mesh) and the powder was used throughout this investigation.

The stem powder (750 g) was successively and exhaustively extracted in a Soxhlet apparatus, with petroleum ether (40-60ºC) and ethyl acetate. The ethyl acetate extract was concentrated and subjected to vacuum liquid chromatography (VLC) over (silica gel 60). The column was eluted with petroleum ether, mixture of petroleum ether with increasing amount of ethyl acetate, then net ethyl acetate and mixture of ethyl acetate with increasing amount of methanol. These eluates were collected in a series of test tubes and each test tube was examined by TLC. Based on similar TLC behavior, the eluates were combined to yield fractions F_1, F_2, F_3 and F_4. The fractions (F_1-F_4) were separately concentrated to a small volume (5 ml) and small amount of $CHCl_3$ was added to each fraction for crystallization. All the fractions are allowed to stand undisturbed for several days at room temperature. Then white crystalline powder (3.0 mg) was obtained from F_2, which was marked as **1**.

Physical and Spectral Data of Compound 1. White crystalline powder, m.p. 172-173ºC; R_f value = 0.74 (petroleum ether-ethyl acetate, 3 : 1); freely soluble in chloroform; IR $\nu_{max}^{KBr}cm^{-1}$: 3300, 3040, 2900, 2850, 1725, 1650, 1600, 1525, 1450, 1270, 700; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.24 (3H, s), 1.56 (6H, s), 2.01 (3H, s), 2.74 (2H, ddd, *J* = 12, 8, 4 Hz), 3.05 (1H, dd, *J* = 12, 8 Hz), 3.20 (1H, dd, *J* = 8, 6 Hz), 3.80 (1H, dd, *J* = 12, 4 Hz), 3.90 (1H, dd, *J* =12, 5 Hz), 4.34 (1H, m, *J* = 4 Hz), 4.74 (1H, q, *J* = 8 Hz), 5.92 (1H, d, *J* = 8 Hz), 6.76 (1H, d, *J* = 8 Hz), 7.07 (1 H, d, *J* = 6 Hz), 7.17(2H, q, *J* = 8, 7, 6 Hz), 7.45 (1H, t, *J* = 8, 7 Hz), 7.71 (1H, d, *J* = 8 Hz) ; ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 20.8, 29.7, 37.5 38.4, 49.5, 55.0, 64.6, 71.0, 126.8, 127.0, 127.2, 128.6, 128.7, 128.8, 129.1, 129.3, 131.9, 133.7, 136.6, 167.1, 170.2, 170.8; EIMS *m/z* (rel. int.): $[M]^+$ 413.25 (0.33), 323.20 (0.55), 311.25 (0.91), 269.20 (2.62), 252.15 (11.97), 232.20 (1.65), 172.20 (5.35), 133.15 (4.42), 131.15 (5.91), 106.10 (7.51), 105.10 (100.00), 91.10 (8.81), 77.10 (26.76), 51.05 (3.79).

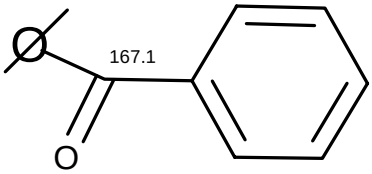
Antibacterial Screening. The antibacterial activitiy of ethyl acetate extract of the bark of *Achyranthes aspera* L. was determined against two gram-positive and five gram-negative bacteria. For the determination of antibacterial activity, the disc diffusion method described by A.W. Bauer[10] was employed. Nutrient Agar (NA) was used as a basal medium for test bacteria. Ethyl acetate was used as the solvent to prepare desired solution (1%). The plates were incubated at 37ºC for 24 hours.

RESULTS AND DISCUSSION

The EI mass spectrum of compound **1** showed the molecular ion peak at *m/z* 413.25 which revealed the molecular formula, $C_{23}H_{27}NO_6$ for this compound.

The IR spectrum of **1** showed a sharp peak at 3300 cm^{-1} indicative of the presence of a –NH– group. A strong band at 1725 cm^{-1} was attributable to a carbonyl group. Bands at 1600 and 1525 cm^{-1} revealed the presence of an aromatic skeleton.

The ^{1}H-NMR spectrum of **1** displayed signals for 5 protons in the chemical shift range of δ 7.07 − 7.71 with typical coupling constants of 6.0 Hz − 8.0 Hz are appropriate for aromatic protons, indicating a monosubstituted benzene ring[11]. The ^{13}C signal at δ 167.1 indicated the presence of a carbonyl group, which was attached to a benzene ring[11]. In addition the base peak at *m/z* 105 in the mass spectrum confirmed the presence of a benzoyl group 'A'.



A

In the ^{1}H-^{1}H COSY it was possible to take the peripheral ^{1}H signal at δ 6.76 in order to trace out the connectivities B_1 of the H atoms as

B_1 δ $6.76 \leftrightarrow 4.74 \leftrightarrow 3.20 \leftrightarrow 3.05$

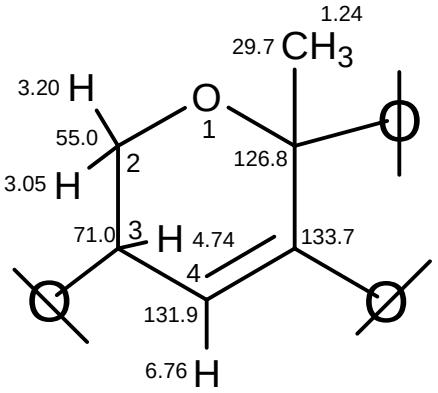
The HSQC spectral data confirmed the attachment of the protons of fragment B_1 as shown below in B_2

B_2	δ_H	6.76	4.74	3.20	3.05
		|	|	|	|

δ_C	131.9	71.0	55.0	55.0

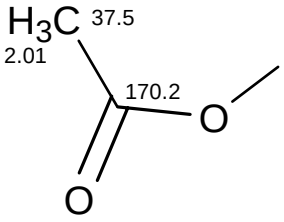
The ^{1}H-NMR signal at δ 1.24 and ^{13}C-NMR resonance at δ 29.7 were assigned to a methyl group linked to a quaternary carbon.

In ^{1}H-NMR doublet peak at δ 6.76 with 8 Hz coupling constant for the asterisk proton (C=C*H-CH-) along with information from $B_1$and B_2 yielded 'B'.

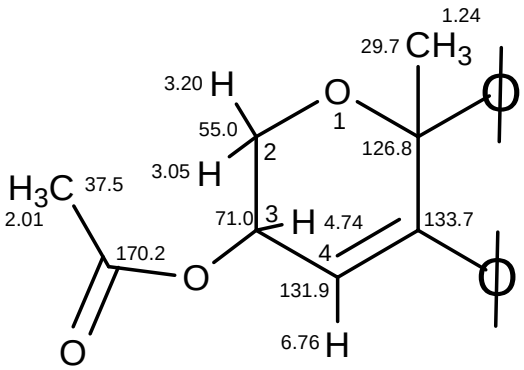


B

The singlet at δ 2.01 in the ^{1}H NMR spectrum and ^{13}C-NMR shift at δ 170.2 indicated an acetoxy group probably linked to a six membered ring[11] as shown by 'C'. Combination of partial structures B and C gave an extended fragment, D.



C



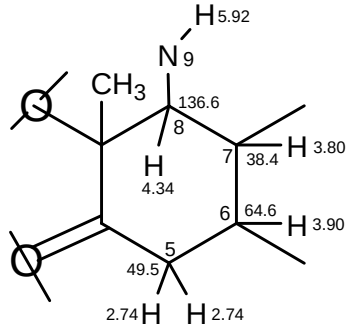
D

From the ^{1}H-^{1}H COSY, it was also possible to take the peripheral ^{1}H signal at δ 5.92 as a starting point in order to trace out the connectivities E of the H atoms. E_1 δ 5.92 ↔ 4.34 ↔ 3.90 ↔ 3.80 ↔ 2.74

The HSQC spectrum allowed to establish the linkage of the proton to their corresponding carbon as shown by E_2.

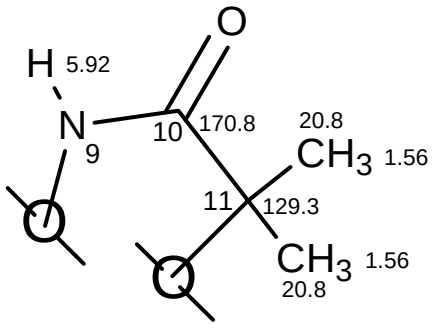
E_2	δ_H	5.92	4.34	3.80	3.90	2.74
			|	|	|	|
	δ_C		136.6	38.4	64.6	49.5

The ^{13}C-NMR signal at δ 64.6 along with the ^{1}H-NMR signal at δ 3.90 indicated a ring proton which was attached to a ring carbon bearing benzoyloxy group[11]. Combination of E_1 and E_2 gave structure 'E'.



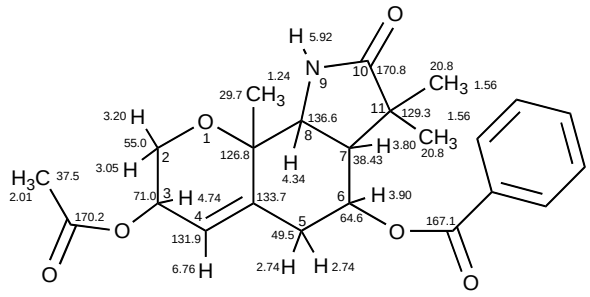
E

A sharp IR peak at 3300 cm^{-1}, ^{1}H-NMR signal at δ 5.92 and ^{13}C spectral shift at δ 170.8 indicated a monosubstituted amide proton[12]. According to the ^{1}H-^{1}H COSY, this amide group was linked to a methine carbon; the proton of which resonated at δ 4.34. The ^{1}H-NMR signal at δ 1.56 along with ^{13}C-NMR signal at δ 20.81 suggested the presence of two methyl groups, both of which were attached to the same carbon as shown in fragment F.



F

Consideration of the molecular formula of compound **1** along with combination of partition structures A to F established the identity of compound **1** as 3-acetoxy-6-benzoxyapangamide, which appears to be novel.



Compound **1**

In the antibacterial sensitivity screening the ethyl acetate extract revealed a mild inhibition of growth of a gram positive bacteria, *Bacillus coreus*.

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