Terpenoids from *Atylosia scarabaeoides* and their antimicrobial activity

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ABSTRACT: Three terpenes-caryophyllene-4,5-oxide, α-amyrin and β-amyrin were isolated from the petroleum ether extract of *Atylosia scarabaeoides*. The structures of the compounds were confirmed by a series of 1D and 2D NMR and MS analyses. The minimum inhibitory concentration (MIC) of caryophyllene-4,5-oxide was found to be 50-200 μg/ml.

Key words: *Atylosia scarabaeoides*; Papilionaceae; Caryophyllene-4,5-oxide; Chemotaxonomy; Antimicrobial

INTRODUCTION

Atylosia scarabaeoides (L.) Benth (Fam. Papilionaceae), locally known as Banurkali or Thitkalai, is a slender, twining herb with densely grey-dowry stems[1] that is distributed throughout Bangladesh, India, Malaysia, China, Mauritius and Madagascar. [1,2] The plant is reported to be effective against diarrhoea in cattle.[1,2] Previous phytochemical investigations on this plant led to the isolation of hentriacontane, β-sitosteryl glucoside, D-(+)-pinitol, vitexin and atyloside.[3,4] There is no report of any biological work carried out on this plant before. [5] Here, we report, the isolation of caryophyllene-4,5-oxide, α-amyrin and β-amyrin from *Atylosia scarabaeoides* and their antimicrobial activity.

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MATERIALS AND METHODS

General Experimental Procedures. IR spectra were recorded as dry film on a Mattson Galaxy 5000 FT-IR spectrometer. HREIMS were obtained on a JEOL JMS-AX505HA double-focusing instrument at 70 eV. NMR spectra (both 1D and 2D) were acquired on a Bruker AMX-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer using the residual solvent peaks as internal standard. *J*-modulated ^{13}C spectra were acquired with a relaxation time (d_1) of 6 s. HMBC spectra were optimised for a long range $J_{H\text{-}C}$ of 7Hz (d_6=0.07s). Vacuum Liquid Chromatography (VLC) was carried out on short column packed with TLC grade silica gel (Kiselgel 60H, Merck) that was operated under reduced pressure. Column chromatography (CC) was carried out using Merck Si gel (mesh 70-230). PTLC was conducted by using Merck Si gel 60 PF_{254} on glass plates (20 cm X 20 cm) at a thickness of 0.5 mm. TLC was carried out on normal-phase Merck Si gel 60 PF_{254} plates. Spots on TLC and PTLC plates were visualized after spraying with 1% vanillin-H_2SO_4 followed by heating at 110° C for 5-10 min.

Plant Material. The plant *Atylosia scarabaeoides* was collected from the National Park, Rajendrapur, Gazipur, Bangladesh in March 2001. The plant was identified by the Bangladesh National Herbarium, Dhaka where a voucher specimen (Accession No. DACB-28141) for this collection has also been deposited.

Extraction and Isolation. The dried and powdered plant materials (240 gm) were extracted sequentially with petroleum ether (bp 60-80° C), $CHCl_3$ and methanol using a Soxhlet apparatus. The petroleum ether extract was subjected to VLC over silica gel using mobile phase petroleum ether, EtOAc and MeOH in order of increasing polarity. The eluates were combined together on the basis of TLC analysis. VLC fraction eluted with 6% EtOAc in petroleum ether was subjected to PTLC (mobile phase, 3% EtOAc in petroleum ether) to obtain compound **1** (12.4 mg; R_f = 0.46 in 5% EtOAc in petroleum ether). CC (mobile phase, 2-5% EtOAc in petroleum ether) of VLC fraction (6% EtOAc in petroleum ether) yielded a mixture (20.2 mg) of compounds **2** and **3**.

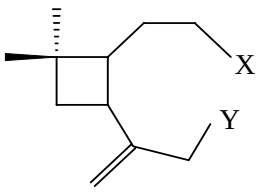
Antimicrobial Activity. Antimicrobial activity of compounds **1-3** was performed by microdilution titre assay[6] against Gram-positive bacteria (*Staphylococcus aureus* NCTC10788), Gram-negative bacteria (*Escherichia coli* NCTC9001, *Proteus vulgaris* NCTC4175 and *Klebsiella aerogenes* Welcome Res. Lab. CM345) and Fungi (*Aspergillus niger* NCPF3149 and *Candida albicans* IMI149007). This technique[6] uses 96 well plates offering the advantage of determining the minimum inhibitory concentration (MIC) at the same test. In this test, 100 µg/ml indicator solution (resazurin, 750 µg/ml) was first placed into the sterility control wells (11^{th} column) on the 96 well plates. 7.5 ml of indicator solution was then mixed with 5 ml test organism (10^8 cfu/ml) followed by transferring (100 µl each) into growth control wells (12^{th} column) and all test wells (1^{st}-10^{th} columns) on the plates. Sample solutions (100 µl each) were then applied onto the 1^{st} column of the plates. In a plate, up to 6 samples could be applied leaving two for controls (negative and positive). Once all samples and controls have been applied on 1^{st} column of the plate, half of the content (100 µl) from these wells was transferred to the 2^{nd} column of wells and each subsequent well was treated similarly (doubling dilution) up to 10^{th} column followed by discarding the last 100 µl contents. Finally, the plates were incubated at 37° C for around 5 hrs until growth control wells developed the growth (pink colour). In case of fungi, the incubation period was around 12 hrs. The activity was judged by observing the change of colour from pink to blue. As the process operates on doubling dilution of test materials, the lowest concentration at which change of colour occurred was considered as minimum inhibitory concentration (MIC) of a given compound.

RESULTS AND DISCUSSION

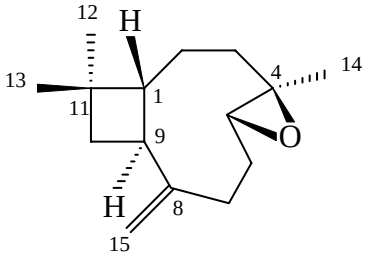
Compound **1** was isolated as oil from the petroleum ether extract of *Atylosia scarabaeoides*. The HREIMS established its molecular formula as $C_{15}H_{24}O$ indicating a mono-oxygenated sesquiterpene. The ^{1}H NMR (400 MHz, $CDCl_3$, Table 1) showed the presence of exomethylene protons (δ4.87, *br. s* and 4.98, *d*, *J*=1.5 Hz) and three methyls (0.99, 1.02 and 1.21). The last methyl group was deshielded supporting its attachment to an oxygenated carbon.

The *J*-modulated ^{13}C NMR (100 MHz, $CDCl_3$, Table 1) showed a total of 15 carbons. The identity of the compound was confirmed by combined studies of ^{1}H NMR, ^{13}C NMR, COSY, HC-COBIDEC and HMBC experiments. In the HMBC experiment, the methyls at δ0.99 (δ_C 30.1 from HC-COBIDEC) and 1.02 (δ_C 21.8 from HC-COBIDEC) exhibited a common ^2J correlation with a quaternary carbon at 34.4 and a ^3J correlation with a methine at δ51.0 (δ_H 1.77 from HC-COBIDEC). These two methyls were recognized as geminal methyls since the protons of one methyl showed ^3J correlation with the carbon of the other and *vice versa*. In the COSY-90 experiment, the proton at δ 1.77 was found to couple to proton at 2.61 (δ_C 49.0 from HC-COBIDEC) which further

coupled to proton at δ 1.63. In the HMBC experiment, the exomethylene protons showed ^3J correlation with methine carbon at δ 49.0 and methylene carbon at 29.9. The proton at 2.61 was connected to carbons at 113.0 (exomethylene) and 29.9 (methylene) by ^3J and to 152.1 (quaternary), 51.0 (methine) and 27.4 (methylene) by ^2J. The proton at 1.77 (H-1) revealed ^3J connectivities to carbons at δ152.1 (quaternary), 39.4 (methylene) and 21.8 (methyl). These findings supported the partial structure of the compound as **1a**. The downfield methyl (δ_H 1.21; δ_C 17.2 from HC-COBIDEC) protons showed ^3J connectivities to C-3 (δ_C 39.4, CH_2) and an oxygenated methine carbon at δ64.0 and ^2J connectivity to an oxygenated quaternary carbon at δ 60.1 in the HMBC experiment. So, this methyl could be placed at C-4. Since C-4 is an oxygenated quaternary, C-5 is an oxygenated methine and the compound has only one oxygen, the oxygen could be present as an epoxide between C-4 and C-5. In the HMBC experiment, the downfield proton at δ 2.89 showed ^3J correlation to C-3 (δ_C 39.4) and ^2J correlations to C-4 (60.1) and a methylene carbon at 30.4. Thus this proton was assigned as H-5, which appeared as a doublet of doublets due to the coupling with protons at δ 2.25 and 1.35 in the COSY. Thus the latter two protons must be C-6 methylene which further coupled to protons at δ 2.34 (H-7) in the COSY experiment. H-7 showed ^3J connectivity to methines at 64.0 (C-5) and 49.0 (C-9) and ^2J correlation to a quaternary (C-8) next to exomethylene. Thus, the compound 1 was characterized as caryophyllen-4,5-epoxide. Although this compound has been reported from various plants such as *Zingiber zerumbet*, [7] *Dacrydium cyspressium* [8] and *Annona reticulata*, [9] this is the first report from the genus *Atylosia*.



1a



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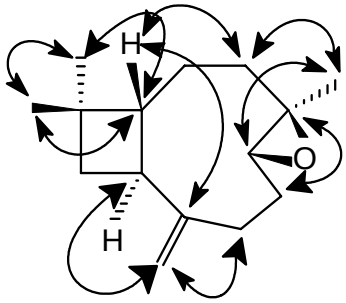

Figure 1. Major HMBC correlations in observed in **1**

Table 1. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) data of 1 in $CDCl_3$

Position	δ_H	δ_C	Position	δ_H	δ_C
1	1.77, *t*, *J*=9.9 Hz	51.0	8	-	152.1
2	1.41, *m*	40.0	9	2.61, *m*, *J*=9.6, 8.8 Hz	49.0
3	2.11, *m*	39.4	10	1.63, *m*	27.4
4	-	60.1	11	-	34.4
5	2.89, *dd*, *J*=10.6, 4.2 Hz	64.0	12	0.99, *s*	30.1
6	1.35, *m*	30.4	13	1.02, *s*	21.8
	2.25, *m*		14	1.21, *s*	17.2
7	2.34, *m*	29.9	15	4.87, *br*. *s*	113.0
				4.98, *d*, *J*=1.5 Hz	

Compounds **2** and **3** were isolated as white needles from the petroleum ether extract of *Atylosia scarabaeoides* as a mixture (1:1). By direct comparison of ^{1}H and ^{13}C NMR spectra with authentic samples, compounds **2** and **3** were identified as α-amyrin[10] and β-amyrin,[11] respectively.

The sesquiterpene, caryophyllene-4,5-oxide (**1**), was found to be active against all test organisms (MIC= 50-200 µg/ml) except the Gram-negative *E. coli*. The highest activity (MIC= 50µg/ml; 0.227 µmol) of this compound was found against *Proteus vulgaris* and *Klebsiella aerogenes* (Table 2). The mixture of α-amyrin and β-amyrin showed sensitivity against *Staphylococcus aureus*, *E. coli* and *Proteus vulgaris* but no sensitivity against Gram-negative *Klebsiella aerogenes* and the fungi tested.

Table 2. MIC values for compounds 1-3 against selected microorganisms

Compounds	*Staph. aureus*		*E. coli*		*Proteus vulgaris*		*Klebsiella aerogenes*		*Aspergillus niger*		*Candida albicans*	
	µg/ml	µmol	µg/ml	µmol	µg/ml	µmol	µg/ml	µmol	µg/ml	µmol	µg/ml	µmol
1	100	0.455	-	-	50	0.227	50	0.227	200	0.909	100	0.455
2+ 3	100	0.236	50	0.118	50	0.118	-	-	-	-	-	-

"-" indicates no activity

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