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TWO NONTERPENOIDAL BENZENOID CONSTITUENTS FROM LEAVES OF *AZADIRACHTA INDICA*

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Key Word Index—*Azadirachta indica*; Meliaceae; neem; leaves; nimbothalin; *n*-tridecyl benzene.

Abstract—Two novel nonterpenoidal constituents, nimbothalin and n-tridecyl benzene, have been isolated from leaves of *Azadirachta indica* and characterized spectroscopically. The structure of nimbothalin was established as 2-[(2,4-dicarboxy-3-methyl)-benzyl]-8,10-dimethyl-1,3,5,7,9-undeca-pentaene.

INTRODUCTION

Azadirachta indica popularly known as 'neem' or 'nimba', is indigenous to the Indo-Pakistan subcontinent and almost every part of the tree offers tremendous potential for medicinal, agricultural and industrial exploitation [1–8]. Its well known pesticidal properties [9–12] have been attributed to a wide variety of terpenoidal (e.g., azadirachtin) [13] and nonterpenoidal constituents. The hydrocarbon fractions isolated from fresh, as well as dried, neem leaves have been found to be toxic against the mosquitoes, *Aedes aegypti* and *Culex pipiens* [14, 15]. Moreover, long-chain fatty acids in neem oil form part of neem oil-based pesticides. Our present investigation of the petrol extract of fresh neem leaves has yielded two novel nonterpenoidal constituents, *n*-tridecyl benzene (**1**) and 2-[(2,4-dicarboxy-3-methyl)benzyl]-8,10-dimethyl-1,3,5,7,9-undecapentaene (nimbothalin) (**2**). Compound (**1**) is unique on account of its arylalkane nature, as earlier investigations of the neem hydrocarbon fraction have reported only aliphatic constituents. Nimbothalin (**2**) contains an isophthalic acid moiety bearing a substituted undecapentaenyl chain.

RESULTS AND DISCUSSION

Compound **1** had the molecular formula $C_{19}H_{32}$. The IR spectrum of the compound was simple, showing the absence of any characteristic functional group absorptions. Peaks at 3000 cm^{-1} (aromatic C–H stretch) and 700 cm^{-1} (out of plane C=C bend) indicated a partial aromatic structure in the molecule. The aliphatic portion of the molecule was shown by the peaks at 3850 cm^{-1} (C–H stretch), 1425, 1345, 1010 and 750 cm^{-1} (C–H bend).

The ^1H NMR spectrum of **1** showed a five-proton multiplet in the aromatic region, indicating a monosubstituted benzene ring. The terminal methyl protons appeared at the lowest frequency followed by 22 methylene protons at higher δ value. The higher frequency shift of the methylene group directly attached to the phenyl ring was attributed to the anisotropic deshielding by the benzene system.

The mass spectrum of **1** showed the $[M]^+$ peak at m/z 260. The fragmentation pattern was characteristic of monoalkyl benzenes, the base peak being observed at m/z 91, corresponding to the formation of benzyl cation, or more appropriately, a resonance stabilized tropylium ion which on elimination of a neutral acetylene molecule gave the cyclopentadienyl fragment ion at m/z 65. A characteristic cluster of ions at m/z 77, 78 and 79 was observed, which corresponded to the fragment ions $[C_6H_5]^+$, $[C_6H_6]^+$ and $[C_6H_7]^+$, respectively. The presence of a straight-chain saturated tridecyl chain directly attached to the benzene ring was confirmed by the typical fragmentation pattern characterized by the clusters of peaks, the corresponding peaks of each cluster being 14 (CH_2) mu apart, i.e. m/z 231, 217,

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203, 189, 175, 161, 147, 133, 119 and 105 representing the fragments $[M - Et]^+$; $[M - Pr]^+$; and so on.

Compound **2** had the molecular formula $C_{23}H_{26}O_4$. The IR spectrum displayed a broad intense band from 3100 cm^{-1} to 3500 cm^{-1} (O–H stretch). Other characteristic bands were observed at 1725 cm^{-1} (dimerized carboxylic C=O stretch), 1640 and 1600 cm^{-1} (symmetric and asymmetric coupled C=C–C=C stretch), 1380 and 1365 cm^{-1} (*gem*-dimethyl group bending vibrations), 3000 and 2950 cm^{-1} (aromatic and aliphatic C–H stretch respectively), 1245 and 1175 cm^{-1} (C–O stretch).

The ^{1}H NMR spectrum of **2** showed the presence two carboxylic protons in the compound. The four methyl groups were observed at δ 1.00 in the 60 MHz spectrum and the two methylene protons directly joined to the phenyl ring appeared at higher frequency (δ 1.80). Amongst the vinylic protons, those nearest to the isophthalic acid system (H-1 and H-3) were assigned the highest frequency. Accordingly, the two vinylic protons (H-4 and H-5) and the last three vinylic protons (H-6, H-7 and H-9) were assigned their respective frequency shifts in decreasing order. The two aromatic protons appeared at different frequency values in accordance with their chemical environments.

The mass spectrum of **2** gave a $[M]^+$ peak at *m/z* 366. The mass fragmentation data proved to be extremely useful in deducing the substitution pattern on the benzene ring, as well as the nature of the side-chain. A characteristically significant peak was noted at *m/z* 167, arising from the elimination of a neutral acetylene molecule from the substituted tropylium ion (*m/z* 193) generated in the first step through cleavage of the bond β– to the aromatic ring. Elimination of a water molecule from the ionic fragment corresponding to *m/z* 167 accounted for the base peak at *m/z* 149. Fragmentation of the side-chain gave other low intensity peaks at *m/z* 173 $(M^+ - 193)^+$, *m/z* 147 (*m/z* 173-acetylene), *m/z* 121 (*m/z* 147-acetylene) and *m/z* 95 (*m/z* 121-acetylene). On the basis of this fragmentation pattern, the possibility of methyl substitution at positions 3,4,5,6 and 7 in the side-chain was ruled out, because in the latter case, ionic fragments corresponding to elimination of substituted acetylene molecules would be expected in the mass spectrum contrary to our observations. The conspicuous absence of the fragment *m/z* 69 (*m/z* 95-acetylene) in the spectrum indicated methyl substitution at position 8. The assignment of the two terminal methyl groups was additionally supported by the IR data for geminal dimethyl substitution.

It is pertinent to note that, in addition to their novelty, compounds **1** and **2** bear significant structural resemblance to hydrocarbons and fatty acids respectively, both of which have known pesticidal properties. This factor coupled with their aromatic nature could contribute towards making them potential pesticidal agents.

EXPERIMENTAL

General

IR: KBr. ^{1}HNMR: in $CDCl_3$ at 60 MHz employing TMS as int. standard. MS: direct inlet, 70 eV. Prep. TLC: silica gel GF254 (Merck); visualization of the bands was done under UV.

Plant material

Fresh leaves of *A. indica* A. Juss were collected from the Botanical Garden, Punjabi University, Patiala, in August 1996. A voucher specimen (Acc. No. PUN 44788) is deposited in the Department of Botany, Punjabi University, Patiala.

Extraction

Fresh leaves (900 g) were cut into pieces and extracted with 95% EtOH (3 l) in a Soxhlet extractor. Removal of solvent *in vacuo* yielded a viscous dark brown mass A (13.6 g), which was extracted with petrol (40–60°) to yield an insol. residue (a_1). Removal of petrol *in vacuo* from the sol. portion yielded fr. A_1 (5.7 g). The insol. residue was further extracted with $CHCl_3$ and removal of solvent yielded a $CHCl_3$-sol. portion A_2 (4.5 g) and an insol. portion a_2 (3.4 g). The TLC chromatograms of the frs A_1 and A_2 in $CHCl_3$–MeOH (199:1) were exactly superimposable, each showing the presence of 14 components. Fr. A_1 was redissolved in petrol, (60–80°) and subjected to prep. TLC in $CHCl_3$–MeOH (199:1). The bands corresponding to the components **1** ($R_f = 0.963$) and **2** ($R_f = 0.628$) were removed and the individual constituents extracted out with petrol. Purity and authenticity of constituents was ascertained using TLC and spectroscopic methods.

n-Tridecyl benzene (**1**)

Creamish white crystalline powder (20.5 mg). IR $\nu_{\max}^{CHCl_3}$ cm^{-1}: 3000 and 700 (phenyl ring), 2850, 1425, 1345, 1010 and 750 (C–H). ^{1}H NMR ($CDCl_3$): δ 7.2 (5H, *m*, Ph), 2.3 (2H, *m*, H-1), 1.6 (22H, *m*, H-2 to H-12), 0.9 (3H, *t*, H-13). EIMS (probe) 70 eV, *m/z* (rel. int.): 260 $[M]^+$ (9), 231 $[M - Et]^+$ (3), 217 (5), 203 (7), 189 (9), 182 $[M - 78]^+$ (10), 175 (10), 161 (3), 147 (15), 133 (17), 119 (23), 105 (53), 92 $[M{-}CH_2{=}CHR]^+$ (12), 91 $[M - C_{11}H_{25}]^+$ (100), 79 $[C_6H_7]^+$ (7), 78 $[C_6H_6]^+$ (5), 77 $[C_6H_5]^+$ (8).

Nimbothalin (**2**)

Creamish white crystals (22.5 mg). IR $\nu_{\max}^{CHCl_3}$cm^{-1}: 3500–3100 (OH), 3000 and 2950 (CH), 1725 (C=O), 1640 and 1600 (C=C–C=C), 1380 and 1365 (*gem*-dimethyl), 1245 and 1175 (O–C–C). ^{1}H NMR ($CDCl_3$): δ 10.3 (2H, *s*, COOH), 7.8 (1H, *d*, H-5′), 7.6 (1H, *d*, H-6′), 5.79 (3H, *m*, H-1 and H-3), 5.27 (2H, *s*, H-4 and H-5), 4.32 (3H, *d*, H-6, H-7 and H-9), 1.80 (2H, *s*, Ph – CH_2), 1.00 (12H, *s*, 4 × CH_3). EIMS (probe) 70 eV *m/z* (rel. int.): 366 $[M]^+$ (4), 348 $[M-H_2O]^+$ (3), 193 $[M-C_{13H19}]^+$ (5), 173 $[M-C_{10}H_9O_4]^+$ (2), 167 $[173-C_2H_2]^+$ (4), 149 $[167-H_2O]^+$ (100), 147 $[173-C_2H_2]^+$ (5), 121 $[147-C_2H_2]^+$ (8), 95 $[121-C_2H_2]^+$ (28).

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