

Two New Oxygenated Heterocyclic Constituents from *Phyllanthus fraternus* Roots

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Two new oxygenated heterocyclic constituents, named phyllanthusolactone and phyllanthodocosanyl ester, have been isolated from the alcoholic extract of the root of *Phyllanthus fraternus*. The structures of the compounds have been established as 16-(18, 22, 22-trimethyl cyclohex-17-enyl)-4-methyl-hexadec-16-ol-3, 17-diene-5, 23-olide and 10-(12, 16, 16-trimethyl cyclohex-11-ene)-11-yl-3, 7-dimethyl-deca-2,4,6-triene-1-yl-n-docosan-13-one-18, 22-olide-1-oate, respectively on the basis of chemical reactions and spectral data.

Phyllanthus fraternus Webster (syn. *P. niruri* auct. non.Linn., Family Euphorbiaceae) is a herb occurring as a rainy season weed throughout the hotter parts of India particularly on cultivated land. It is a well known folklore medicinal plant. The fresh roots are an excellent remedy for jaundice; an infusion is useful as tonic, diuretic, and in menorrhagia¹⁻⁴. Lignans⁵⁻¹⁰, flavonoid glycosides^{11,12}, alkaloids¹³, steroids¹⁴ and terpenoids^{7,15} have been isolated from the various parts of the plant. This manuscript deals with the isolation and structural elucidation of two new oxygenated heterocyclic constituents from the roots of *P. fraternus*.

EXPERIMENTAL

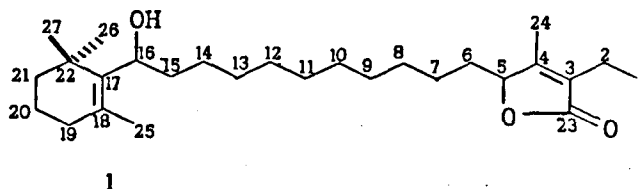
Extraction:

The roots (1.0 kg) of *P. fraternus* were collected, from the Jamia Hamdard Campus, air-dried, pulverised and extracted exhaustively with EtOH (95%) in a Soxhlet apparatus. The combined extracts were dried under reduced pressure to obtain a brown-coloured viscous residue (55 g, 5.5%). The dried ethanolic extract was subjected to silica gel column chromatography after formation of slurry. The column was eluted with petroleum ether, petroleum ether-CHCl₃ (9:1), 3:1, 1:1, 1:3 v/v), CHCl₃, CHCl₃-MeOH (99:1, 95:5, 9:1, 3:1, 1:1) and MeOH to isolate the following compounds.

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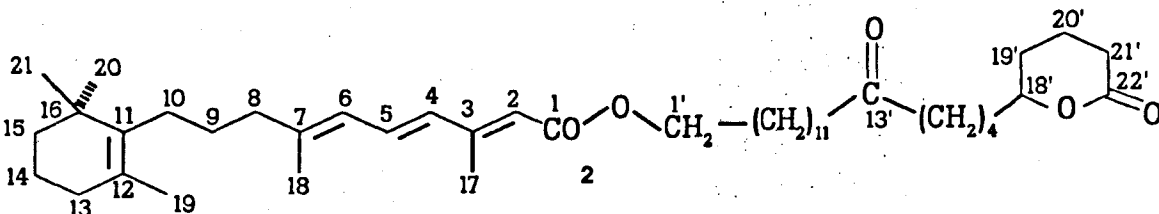
Compound 1:

Elution of the column with petroleum ether-CHCl₃ (3:1) afforded colourless waxy solid **1**, crystallized from CHCl₃-MeOH (1:1), 135 mg (0.0135 %), mp 75-76°, R_f 0.30 (C₆H₆-CHCl₃ 1:1), [α]_D²⁰ -65.21° (c 0.23, CHCl₃). UVλ_{max} (CHCl₃): 242, 263, 275 nm (log ε 9.3, 4.6, 4.5). IR ν_{max} (KBr): 3396, 3252, 2920, 2852, 1742, 1596, 1474, 1466, 1386, 1364, 1264, 1056, 804, 720 cm⁻¹. ¹H-NMR (400 MHz, CDCl₃): δ 3.67 (1H, m, H-5), 3.63 (1H, m, H-16), 2.08 (2H, m, H₂-19), 1.68(2H, m, H₂-2), 1.57(6H, brs, Me-24, Me-25), 1.28 (24H, brs, 12xH₂), 1.22(6H, brs, Me-26, Me-27), 0.89(3H, t, J=6.6 Hz, Me-1). EIMS m/z (rel. int.): 418 [M]⁺ (C₂₇H₄₆O₃) (1.4), 389(16.5), 334(1.7), 293(1.2), 279(1.3), 277(1-3), 265(2.2), 251(2.2), 237(3.2), 223(1.2), 209 (2.9), 195(4.2), 181(4.0), 167(5.4), 153(18.5), 135(2.8), 139(3.8), 125(5.8), 123(100), 110(2.4), 96(73.95), 84(20.5). Acetylation of **1** yielded a monoacetyl product, mp 77-79°, IR ν_{max} 1725 cm⁻¹.



Compound 2:

Elution of the column with CHCl₃-MeOH (95:5) afforded a light yellow powder of **2**, re-crystallized from



CHCl₃-MeOH (1:1), 110 mg (0.011%), mp 65-66°, *R_f* 0.56 (n-hexane-diethyl ether-EtOAc, 60:40:1), UVλ_{max} (CHCl₃): 252, 323, nm (log ε 2.3, 5.6). IR ν_{max} (KBr): 2960, 2850, 1745, 1735, 1710, 1605, 1515, 1460, 1375, 1260, 1160, 1020, 800, 715 cm⁻¹. ¹H-NMR (400 MHz, CDCl₃): δ 7.64(1H, d, J=13.2 Hz, H-4), 7.42(1H, d, J=6.6 Hz, H-6), 6.85(1H, brs, H-2), 5.36(1H, m, H-5), 4.22(1H, m, H-18'), 4.18(1H, brs, H₂-1'a), 3.93(1H, brs, H₂-1'b), 2.36(6H, m, H₂-12', H₂-14', H₂-2'), 2.04(4H, m, H₂-13, H₂-10), 1.73(3H, brs, Me-18), 1.70(3H, brs, Me-17), 1.65(3H, brs, Me-18), 1.62(3H, brs, Me-19), 1.23(36H, brs, 18xCH₂), 0.90(3H, brs, Me-20), 0.86(3H, brs, Me-21). EIMS *m/z* (rel.int): 666 [M]⁺(C₄₃H₇₀O₅) (1.9), 589(7.7), 553(2.8), 525(6.6), 511(38.0), 501(1.9), 483(44.4), 469(8.4), 461(1.8), 455(19.9), 441(14.2), 427(4.4), 413(9.4), 399(5.2), 395(1.8), 385(3.9), 371(2.9), 367(4.3), 357(9.2), 343(2.9), 341(14.4), 337(5.0), 329(3.2), 323(2.9), 315(11.0), 309(5.1), 299(6.7), 281(4.0), 271(5.5), 267(3.0), 253(4.0), 239(8.4), 231(3.5), 225(4.5), 211(5.6), 205(9.1), 197(9.5), 183(6.0), 165(10.0), 155(8.5), 151(12.7), 141(15.0), 137(18.3), 130(76.6), 123(20.1), 120(48.3), 113(36.3), 99(16.3), 83(25.2), 69(33.2), 57(87.1). Alkaline hydrolysis of **2** yielded diterpene acid, mp 105-107°, IR ν_{max} 3350, 1690 cm⁻¹ and a keto acid, IR ν_{max} 3450, 3290, 1710, 1690 cm⁻¹.

RESULTS AND DISCUSSION

Compound **1**, namely phyllanthusolactone, was obtained from petroleum ether-chloroform (3:1) eluants. Its IR spectrum exhibited characteristic absorption bands for hydroxyl group (3396 cm⁻¹) and 5-membered α, β-unsaturated (1742 cm⁻¹). It showed a molecular ion peak at *m/z* 418 in its mass spectrum corresponding to C₂₇H₄₆O₃ which indicated five degrees of unsaturation. The ion fragments at *m/z* 123 (C₁₆-C₁₇ fission), 277 [M-123-H₂O]⁺, 153, 265 (C₁₅-C₁₆ fission) and 135 [153-H₂O]⁺ reflected the presence of an unsaturated cyclohexene ring at one of the terminal possessing three methyl groups and a hydroxyl group at C-16. The presence of γ-lactone at C-5(23) was reduced from the ion peaks appearing at *m/z* 125, 293 (C₅-C₆ fission), and the fragments generated due to subsequent elimination of the methylene groups from the

293 at *m/z* 279, 265, 251, 237, 223, 209, 195, 181 and 167.

The ¹H-NMR of **1** displayed two one-proton each multiplets at δ 2.08 and 1.68 assignable to methylene protons at C-19 and C-2, respectively. Two one-proton each multiplets at δ 3.67 and 3.63 were attributed correspondingly to H-5 and H-16 carbinol protons. Two broad singlets at δ 1.57 and 1.22, integrated for six protons each were associated with C-24, C-25, C-26 and C-27 methyl functionalities. A three-protons triplet with coupling interaction of 6.6 Hz at δ 0.89 was ascribed to the terminal C-1 methyl group. The remaining twelve methylene protons resonated as a broad singlet at δ 1.28. Acetylation of **1** yielded a monoacetyl product **1a**.

On the basis of these accumulative evidences, the structure of phyllanthusolactone (**1**) has been formulated as 16-(18, 22, 22-trimethylcyclohex-17-enyl)-4-methylhexadec-16-ol-3, 17-diene-5, 23-olide. This novel homosesterterpene γ-lactone is being reported for the first time from any natural or synthetic source.

Compound **2**, named phyllanthodocosanyl ester, was obtained as a light yellow coloured crystalline product from chloroform-methanol (95:5) eluants. Its UV absorption maxima at 253 and 323 nm suggested a chromophore in the molecule. It has characteristic IR absorption bands for δ-lactone (1745 cm⁻¹), ester (1735 cm⁻¹), carbonyl group (1710 cm⁻¹), unsaturation (1605 cm⁻¹) and long aliphatic chain (800, 715 cm⁻¹). The mass spectrum of **2** exhibited a molecular ion peak at *m/z* 666 consistent with the molecular formula of a diterpene compound esterified with a long chain aliphatic δ-lactone, C₄₃H₇₀O₅. The base peak at *m/z* 165 (C₇-C₈ fission), appeared due to generation of C₁₂H₂₁ mass unit, suggested the presence of trimethyl substituted cyclohexenyl ring at one of the terminal carbon. The formation of prominent ion peak at *m/z* 205 (C₅-C₆ fission), 231 (C₃-C₄ fission), 271 (C₂-C₁ fission), 299 (O-C₁ fission), 501 [M-165]⁺, 461 [M-205]⁺ and 395 [M-271]⁺ indicated CH₃-C=CH-CH=CH-C(CH₃)₂-C=CH-CO]⁺, type chromophore in the diterpene moiety. The spectrum showed the presence C_nH_{2n+1}⁺, C_nH_{2n}⁺ ions reflecting

aliphatic nature of the molecule. Most of the fragments were separated by 14 mass units and decreased in ion intensities and abundance with increasing molecular weight of the long chain hydrocarbon^{16,17}. The ion fragments at m/z 299, 367 [M-299]⁺, 315 (O-C₁ fission) and the subsequent peaks formed due to removal of 14 mass units in between m/z 337 to m/z 99 indicated the esterification of the C₂₁H₃₁O homo-diterpenic moiety with C₂₂H₃₉O₄ unit. The intensities of the peaks suddenly intensified at m/z 469, 183 (C₁₃-C₁₂ fission) and at m/z 511, 155 (C₁₃-C₁₄ fission) indicating the presence of the carbonyl group at C-13'. The location of δ -lactone moiety at one of the terminal carbon of the aliphatic chain was inferred from the ion peak generating at m/z 99.

The ¹H-NMR spectrum displayed two one-proton each downfield doublets at δ 7.64 (J=13.2 Hz) and 7.42 (J=6.6 Hz) assigned to H-4 and H-6, respectively and five one-proton each signals at δ 6.85 (brs), 5.36 (m), 4.22 (m), 4.18 (brs) and 3.93 (brs), ascribed correspondingly to H-2, H-5, H-18', H₂-1'a and H₂-1'b protons. Three broad singlets at δ 1.70, 1.65 and 1.62, integrating three protons each, were associated with C-17, C-18 and C-19 methyls attached to olefinic carbons. Two three-protons, each broad singlets at δ 0.90 and 0.86 were accounted to C-20 and C-21 tertiary methyls, respectively. The remaining methine and methylene functionalities resonated at δ 2.36 (6H, m), 2.04 (4H, m) and 1.23 (36 H, brs). Alkaline hydrolysis of **2** yielded a diterpenic acid and a keto acid. Based on these reasons the structure of phyllanthodocosanyl ester (**2**) has been established as 10-(12, 16, 16-trimethyl cyclohex-11-ene)-11-yl-3,7-dimethyl-deca-2,4,6-triene-1-yl-n-docosan-13-one-18, 22-olide-1-oate. This is a new homo-diterpenic ester of docosanoic acid containing δ -lactone ring at one of the terminal carbon.

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