Asian Journal of Chemistry Vol. 21, No. 6 (2009), 4744-4748

New Unsaturated Fatty Acid from Roots of *Bougainvillea spectabilis* Willd.

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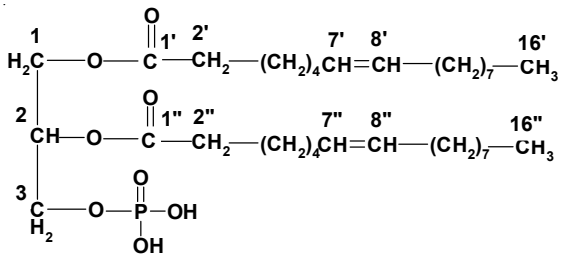
A rare phosphoglycerol, phytoalcohol and a new unsaturated fatty acid were isolated from ethanolic extract of roots of *Bougainvillea spectabilis* Willd (Nyctaginaceae). The structures of these compounds were established on the basis of the detailed chemical reaction and spectral analysis as 1,2-dipalmitoleoyl glyceryl phosphate (**1**), *n*-henetriacontanol (**2**) and *n*-octacos-9-enoic acid (**3**).

Key Words: *Bougainvillea spectabilis*, Nyctaginacaea, Unsaturated fatty acid, Phosphoglycerol.

INTRODUCTION

Bougainvillea spectabilis Willd. (Nyctaginaceae) is principally an ornamental plant[1]. It is a scandent or straggling shrub, vine or tree usually armed with spines and mainly distributed in tropical and subtropical regions of South America and cultivated throughout India[2-5]. It has been reported to possess antidiabetic, antibacterial, larvicidal, antiviral and antiinflammatory activities[6-19]. The fresh flower bearing ivory white bracts contains glycosides and quercetin and leaves contain pinitol, spinosterol and quercetin[20-22]. In the present paper, the isolation and structure elucidation of 1,2-dipalmitoleoyl glyceryl phosphate (**1**), *n*-henetriacontanol (**2**) and *n*-octacos-9-enoic acid (**3**) from root of the plant are described.



Compound **1**

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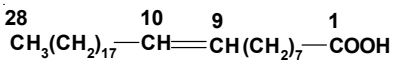
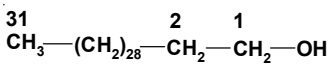


Compound **2**



Compound **3**

EXPERIMENTAL

Melting points, uncorrected are taken on a Complab melting point apparatus. Optical rotations were measured on JASCO-1020 Polarimeter, The UV spectra were measured with UV-Visible-NIR spectrophotometer (Cary 5000 Varian Australia Pvt. Ltd.). IR spectra were obtained using a Shimadzu (FTIR 8000 using KBr pellets). ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance II (400 MHz, USA). Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. EIMS were recorded on Jeol SX-102 mass spectrometer. Column chromatography was carried out using neutral alumina (Qualigens). Merk precoated silica gel 60 F_{254} plates were used for TLC.

The roots of *Bougainvillea spectabilis* Willd. were collected from the Campus of Guru Jambheshwar University of Science and Technology, Hisar, India during the month of July 2006. The plant was taxonomically identified and authenticated by Dr. H.B. Singh, Head, Raw Materials, Herbarium and Museum Division, National Institute of Science Communication and Information Resources (CSIR), New Delhi, vide reference no, NISCAIR/RHMD/CONSULT/06/771/88. A voucher specimen of the same has been retained in the Department for the further reference.

Extraction and isolation: The air dried powdered roots of *Bougainvillea spectabilis* (3 kg) were soxhlet extracted with 95 % ethanol for 72 h and the combined ethanolic extracts were collected and evaporated under reduced pressure and kept in desiccators. The dried ethanolic extract was dissolved in minimum amount of methanol and adsorbed on silica gel (for column 80-120) to form slurry. The air-dried slurry was chromatographed on neutral alumina column loaded in petroleum ether (b.p. 60-80 ºC). The column was eluted with gradient solvent system of petroleum-ether, petroleum ether-chloroform, chloroform, chloroform-methanol and methanol in increment of polarity to afford the following compounds:

1,2-Dipalmitoleoyl glyceryl phosphate (1): Elution of the column with chloroform furnished colourless crystals of compound **1**, recrystallized from methanol, 250 mg (0.85 % yield). m.p.: 65-66 °C, $[\alpha]_D^{30}$ + 26.5° ($CHCl_3$); UV λ_{max} (MeOH): 209 nm (log ε 4.9); IR (KBr, ν_{max}, cm^{-1}): 3430, 2919, 2840, 1732, 1644, 1456, 1373, 1242, 1171, 1037, 721; ^{1}H NMR ($CDCl_3$) δ: 5.38 (1H, m, H-7'), 5.32 (1H, m, H-7''), 5.14 (1H, m, H-8'), 5.11 (1H, m, H-8''), 4.16 (1H, m, H-2), 4.14 (1H, d, *J* = 7.42 Hz, H_2-3a), 4.13 (1H, d, *J* = 7.2 Hz, H_2-3b), 4.11 (1H, d, *J* = 6.4 Hz, H_2-1 a), 4.09 (1H, _d), *J* = 6.4 Hz, H_2-1b), 2.28 (1H, d, *J* = 7.6 Hz, H_2-2'a), 2.26 (1H, d, *J* = 7.6 Hz, H_2-2'b), 2.07 (1H, d, *J* = 6.8 Hz, H_2-2''a), 2.03 (1H, d, *J* = 6.8 Hz, H_2-2''b), 1.67 (2H, brs, H_2-6'), 1.65 (2H, m, H2-6''), 1.62 (2H, m, H_2-9'), 1.59 (2H, m, H_2-9''), 1.30 (6H, brs, 3 × CH_2), 1.25 (24H, brs, 12 × CH_2), 1.23 (6H, brs, 3 × CH_2), 0.86

(3H, t, *J* = 7.2 Hz, MeOH-16'), 0.84 (3H, t, *J* = 6.8 Hz, Me-16''); ^{13}C NMR ($CDCl_3$) δ: 173.95 (C-1'), 173.17 (C-1''), 130.25 (C-7'), 130.03 (C-7''), 129.81 (C-8'), 128.08 (C-8''), 72.11 (C-2), 65.09 (C-3), 60.20 (C-1), 56.10 (C-2'), 45.87 (C-2''), 34.44 (C-6'), 31.96 (C-6''), 31.57 (C-9'), 29.75 (C-9''), 29.58 (3 × CH_2), 29.52 (2 × CH_2), 29.38 (2 × CH_2), 29.32 (CH_2), 29.17 (CH_2), 27.26 (CH_2), 26.22 (CH_2), 25.67 (CH_2), 25.03 (CH_2), 23.49 (CH_2), 23.10 (CH_2), 27.63 (CH_2), 19.87 (CH_2), 19.08 (CH_2), 14.30 (Me-16), 14.17 (Me-16''). EIMS m/z (rel. int.): 644 $[M]^+$ ($C_{35}H_{65}O_8P$).

Hydrolysis of compound 1: Compound **1** (35 mg) was heated with alkaline ethanol (5 mL) on a steam bath for 1 h. After usual work up palmitoleic acid was obtained, Co-TLC comparable.

***n*-Henetriacontanol (2):** Elution of the column with methanol yielded colourless crystals of compound **2**, recrystallized from methanol, 300 mg (0.91 % yield). m.p.: 93-95 °C, $[\alpha]_D^{30}$ + 62° (CH_3OH); IR(KBr, ν_{max}, cm^{-1}): 3138, 1398, 700; ^{1}H NMR (DMSO-d_6) δ: 3.04 (1H, d, *J* = 5.6 Hz, H_2-1a), 3.01 (1H, d, *J* = 5.6 Hz, H_2-1b), 2.59 (2H, m, H_2-2), 1.25 (56H, brs, 28 × CH_2), 0.83 (3H, t, *J* = 6.2 Hz, Me-31); EIMS m/z (rel. int.): 452 $[M]^+$ ($C_{37}H_{64}O$) (5.9).

***n*-Octacos-9-enoic acid (3):** Elution of the column with ethyl acetate:methanol (1:1) afforded colourless amorphous powder of compound **3**, recrystallized from methanol, 305 mg (0.94 % yield). m.p.: 74-75 °C, $[\alpha]_D^{30}$ + 36° (CH_3OH), UV λ_{max} (MeOH): 212 nm (log ε 5.1); IR (KBr, ν_{max}, cm^{-1}): 3151, 1700, 1653, 1401, 1074, 829, 722; ^{1}H NMR ($CDCl_3$) δ: 5.40 (1H, m, H-9), 5.33 (1H, m, H-10), 2.60 (1H, d, *J* = 5.6, H_2-2a), 2.58 (1H, d, *J* = 5.6 Hz, H_2-2b), 2.03 (2H, m, H_2-8), 1.98 (2 H, m, H_2-11), 1.58 (2H, m, H_2-3), 1.29 (8H, brs, 4 × CH_2), 1.25 (32H, brs, 16 × CH_2), 0.85 (3 H, t, *J* = 7.2 Hz, Me-28); ^{13}C NMR (DMSO-d_6) δ: 172.82 (C-1), 137.81 (C-9), 132.69 (C-10), 55.98 (C-9), 45.37 (C-8), 37.81 (C-11), 29.13 (21 × CH_2), 20.77 (C-28); EIMS m/z (rel. int.): 422 $[M]^+$ ($C_{28}H_{54}O_2$) (5.3), 279 (11.2).

RESULTS AND DISCUSSION

Compound **1** a palmitoleonyl glyceryl phosphate, was obtained as a colourless mass from chloroform eluent of the ethanolic extract of *B. spectabilis*. Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3430 cm^{-1}) ester group (1732 cm^{-1}) and unsaturation (1644 cm^{-1}). The mass spectrum of **1** exhibited a molecular ion peak at m/z 644 corresponding to a molecular formula of dipalmitoleic acyl glyceryl phosphate, $C_{35}H_{65}O_8P$. It indicated the presence of four double bond equivalents. Two of them were adjusted in the vinylic linkage and two in the ester group. The ^{1}H NMR spectrum of **1** showed four one-proton multiplets at δ 5.38, 5.32, 5.14 and 5.11 assigned to vinylic protons. A one-proton multiplet at 4.16 was ascribed to carbinol H-2 proton. Four one-proton doublets at δ 4.14 (*J* = 7.2 Hz), 4.13 (*J* = 7.2 Hz), 4.11 (*J* = 6.4 Hz) and 4.09 (*J* = 6.4 Hz) were ascribed correspondingly to oxygenated methylene H_2-3 and H_2-1 protons. Four upfilled one-proton doublets at δ 2.28 (*J* = 7.6 Hz), 2.26 (*J* = 7.6 Hz), 2.07 (*J* = 6.8 Hz) and 2.03 (*J* = 6.8 Hz) were attributed to methylene H_2-2' and H_2-2'' protons adjacent to the ester groups.

The remaining methylene protons resonated between δ 1.67-1.23. Two three-proton triplets at δ 0.86 (*J* = 7.2 Hz) and 0.84 (*J* = 6.8 Hz) were associated with the primary C-16' and C-16" methyl protons. The ^{13}C NMR spectrum of **1** displayed signals for ester carbons at δ 173.95 (C-1') and 173.17 (C-1"), vinylic carbons between δ 130.75-128.08, carbinol carbon at δ 72.11 (C-2), oxygenated methylene carbons at δ 65.09 (C-3) and 60.20 (C-1), methyl carbons at δ 14.30 (C-16) and 14.17 (C-16") and the methylene carbons between δ 56.10-19.08. Alkaline hydrolysis of **1** yielded palmitoleic acid. On the basis of spectral data analysis and chemical reactions the structure of AM-I has been established as 1,2-dipalmitoleoyl glyceryl phosphate or glyceryl-1-hexadec-7'-enoyl-2-hexadec-7"-enoyl-3-phosphate.

The methanol eluent of the column furnished hemetriacontanol, colourless crystals. Its IR spectrum showed characteristic absorption bands for alcoholic group (3138 cm^{-1}) and long aliphatic chain (700 cm^{-1}). Its mass spectrum exhibited a molecular ion peak at m/z 454 corresponding to long chain saturated alcohol $C_{31}H_{64}O$. It has all saturated linkages. The ^{1}H NMR spectrum of **2** displayed two one-proton doublets at δ 3.04 (*J* = 5.6 Hz) and 3.01 (J= 5.6 Hz) assigned to hydroxyl-methylene H_2-1 protons. Two broad signals at δ 2.59 (2H) and 1.25 (56H) were attributed to methylene protons. A three-proton triplet at δ 0.83 (*J* = 6.2 Hz) was accounted to terminal C-31 primary methyl protons. The absence of any signal beyond δ 3.04 indicated saturated nature of the molecule. On the basis of the foregoing account the structure of AM-III has been formulated as *n*-henetriacontanol.

Compound **3** named spectabilinosoic acid was obtained as colourless crystalline mass from ethyl acetate:methanol (1:1) eluent. It gave effervescences with sodium bicarbonate solution and decolourlized bromine water indicating unsaturated carboxylic acid nature of the molecule. Its IR spectrum exhibited characteristic absorption bands for carboxylic group (3151, 1700 cm^{-1}), unsaturation (1653 cm^{-1}) and long aliphatic chain (829, 722 cm^{-1}). The mass spectrum of **3** displayed a molecular ion peak at m/z 422 corresponding to a molecular formula of a monounsaturated fatty acid, $C_{28}H_{54}O_2$. A prominent ion peak at m/z 279 generated due to cleavage of C_8-C_9 linkage indicated the existence of the vinylic linkage at C_9. The ^{1}H NMR spectrum of **3** showed two one-proton multiplets at 5.40 and 5.33 assigned to vinylic H-9 and H-10, respectively. Two one-proton doublets at δ 2.60 (*J* = 5.6 Hz) and 2.58 (*J* = 5.6 Hz) were ascribed to H_2-2 methylene protons adjacent to the carboxylic group. Three multiplets at δ 2.03, 1.98 and 1.58 all integrated for two protons each, were attributed to methylene H_2-8 and H_2-11 protons adjacent to the vinylic carbons and to methylene H_2-3 protons, respectively. An eight-proton broad signal at δ 1.28 and a 32-proton broad signal at δ 1.25 were associated with the remaining methylene protons. A three-proton triplet at δ 0.85 (*J* = 7.2 Hz) was accounted to primary C-28 methyl protons. The ^{13}C NMR spectrum of 3 presented signals for carboxylic carbon at δ 172.82 (C-1), vinylic carbons at δ 137.81 (C-9) and 132.69 (C-10), methylene carbons between δ 55.98-29.13 and methyl carbon at δ 20.77 (C-28). The absence of any signal between δ 2.60-5.33 in the ^{1}H NMR spectrum and between δ 132.69-

55.98 in the ^{13}C NMR spectrum ruled out the location of a carbinol carbon in the molecule. On the basis of spectral data analysis and chemical reactions the structure of **3** has been established as *n*-octacos-9-enoic acid. This is a new unsaturated fatty acid isolated from a natural or synthetic source for the first time.

ACKNOWLEDGEMENTS

The authors are thankful to the Head, SAIF, CDRI, Lucknow for scanning the various spectra.

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(*Received*: 19 September 2008; *Accepted*: 31 March 2009) AJC-7389