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Research Article

Fatty Acid Glucosides from Roots of *Cichorium Intybus* Linn.

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Abstract
Three new fatty acid glucosides, identified as n-octadec-9-enoyl-β-D-glucopyranoside (**2**), n-octadec-9-enoyl-β-D-glucopyranosylo (1→6)-β-D-glucopyranoside (**3**), n-octadec-9-en-12-ol-1-oyl- β-D-glucopyranoside (**4**) along with a diglyceride phosphate have been isolated from methanolic extract of the roots of *Cichorium intybus*. Their structures were established on the basis of spectral analysis and chemical reactions.

Keywords: Cichorium intybus; n-octadec-9-enoyl-β-D-glucopyranoside; n-octadec-9-enoyl-β-D-glucopyranosylo (1→6)-β-D-glucopyranoside; n-octadec-9-en-12-ol-1-oyl-β-D-glucopyranoside; diglyceride phosphate.

INTRODUCTION

Cichorium intybus L. (Asteraceae), commonly known as chicory, is a perennial herb distributed in the temperate parts of the world and found wild in Punjab and Andhra Pradesh regions [1]. It is used in Indian system of medicine as a cardiotonic, anti-inflammatory, digestive, stomachic, liver tonic and diuretic drug [2]. The main reported phytoconstituents of chicory roots are phenylacetic acid esters, cichoriosides [3], sonchuside A [4], ixerisoside, magnolialide [5] and endesmanolides [6-7]. In the present paper we now describe the isolation and characterization of two new glucosides, along with diglyceride phosphate (**1**) and oleoyl glucoside (**2**) from the roots of *Cichorium intybus*.

MATERIAL AND METHODS

Plant material

The roots of *C. intybus* were collected from the herbal garden of Jamia Hamdard, New Delhi and identified by Taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard (Hamdard University). A voucher specimen (No. PRL/JH/05/28) was deposited in the herbarium of the Faculty of Pharmacy, Jamia Hamdard, New Delhi.

Melting points were determined on a Perfit apparatus (uncorrected). The IR spectra were recorded in KBr pellet on Win IR FTS 135 instrument (Biorad, USA). ^{1}H (300 MHz), ^{13}C (75 MHz), and 2D NMR spectra were recorded by Bruker spectrospin NMR instrument in $CDCl_3$, using TMS as internal standard. FAB ionization at 70 eV was scanned on a Jeol D-300 instrument (Jeol, USA). Column chromatography was performed on silica gel (Merck, 60-120 mesh) and thin-layer chromatography on si lica gel G coated TLC plates (Merck).

Extraction and isolation

The air-dried roots (2 kg) of *C. intybus* were coarsely powdered and extracted exhaustively in a Soxhlet apparatus with methanol for 72 hours. The methanolic extract was concentrated under reduced pressure to obtain dark brown viscous mass. Small portion of the extract was analyzed chemically to determine the presence of different chemical constituents. The viscous dark brown mass was adsorbed on silica gel (60-120 mesh) for column after being dissolved in little quality of methanol for preparation of slurry. The slurry (200 g) was air dried and chromatographed over silica gel column packed in petroleum ether. The column was eluted successively with petroleum ether, mixture of petroleum ether and chloroform (9:1, 3:1, 1:1 and 1:3), pure chloroform and finally the mixture of chloroform and methanol (99:1, 98:2, 96:4, 95:5, 97:3, 9:1). Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions (having same R_f values) were combined and crystallized. The isolated compounds were recrystallized to get the pure compounds.

Compound 1: Elution of the column with chloroform-methanol (49 : 1) as colourless amorphous mass (methanol)**,** m.p.: 88-90 ºC; UV λ_{max} (MeOH): 206, 284 nm (log ε 4.9, 1.1); IR υ_{max} (KBr): 3399, 2909, 2843, 1721, 1718, 1627, 1489, 1443, 1377, 1250, 1163, 715 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.34 (3H, m, H-10′, H-13′, H-15′), 5.31 (3H, m, H-9′, H-12′, H-16′), 5.11 (2H, m, H-9″, H-10″), 4.17 (1H, m, H-2), 3.92 (1H, d, *J* = 12.6 Hz, H_2-3a), 3.90 (1H, d, *J* = 12.6 Hz, H_2-3b), 3.71 (1H, d, *J* = 12.6 Hz, H_2-1a), 3.69 (1H, d, *J* = 12.5 Hz, H_2-1b), 2.77 (2H, m, H_2-14′), 2.50 (2H, m, H_2- 11′), 2.36 (1H, d, *J* = 7.2 Hz, H_2-2′a), 2.34 (1H, d, *J* = 7.2 Hz, H_2-2′b), 2.32 (1H, d, *J* = 6.6 Hz, H_2-2″a), 2.30 (1H, d, *J* = 6.6 Hz, H_2-2″b), 2.03 (2H, m, H_2-8′), 1.75 (2H, m, H_2-17′), 1.67 (2H, m, H_2-8″), 1.62 (2H, m, H_2-11″), 1.25 (32H, brs, 16 × CH_2), 0.87 (3H, t, *J* = 6.1 Hz, Me-18′), 0.85 (3H, t, *J* = 6.2 Hz, Me-18″); ^{13}C NMR ($CDCl_3$): δ 173.26 (C-1′), 171.68 (C-1″), 144.68 (C-12′), 129.99 (C-13′), 127.79 (C-15′), 127.76 (C-16′), 125.01 (C-10′), 122.11 (C-9′), 115.55 (C-9″), 113.94 (C-10″), 70.26 (C-2), 65.11 (C-3),

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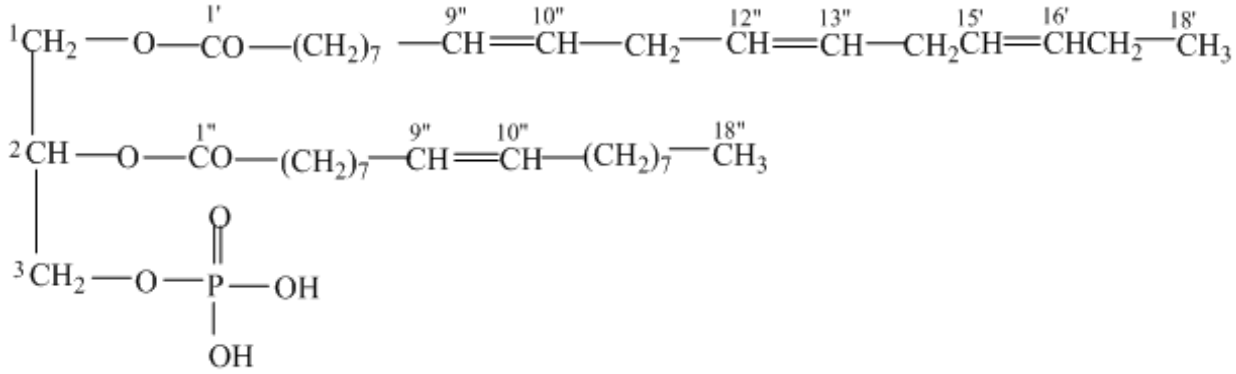


Compound 1

62.09 (C-1), 55.96 (C-2′), 50.73 (C-2″), 40.75 (C-11′), 34.10 (C-14′), 34.02 (C-8″), 32.16 (C-11″), 31.88 (C-17′), 29.65 (11 × CH_2), 27.17 (CH_2), 25.94 (CH_2), 24.86 (CH_2), 23.64 (CH_2), 21.02 (CH_2),19.72 (CH_2), 18.49 (CH_2), 14.06 (Me-18′),14.05 (Me-18″); +ve ion FAB MS *m/z* (rel. int.): 695 $[M]^+$ ($C_{39}H_{69}O_8P$) (6.8), 665 (24.3), 650 (18.7), 350 (31.6), 265 (15.6), 261 (14.2).

Alkaline hydrolysis of 1: Compound **1** (35 mg) was refluxed with ethanolic 1N KOH solution for thirty min on a steam bath. The reaction mixture was acidified with dil. HCl, extracted with $CHCl_3$ (3 × 10 ml), the chloroform layer washed with H_2O (2 × 10 ml) and dried over anhydrous Na_2SO_4. After evaporation of the solvent, a mixture of oleic acid and linolenic acid was obtained (TLC - comparable).

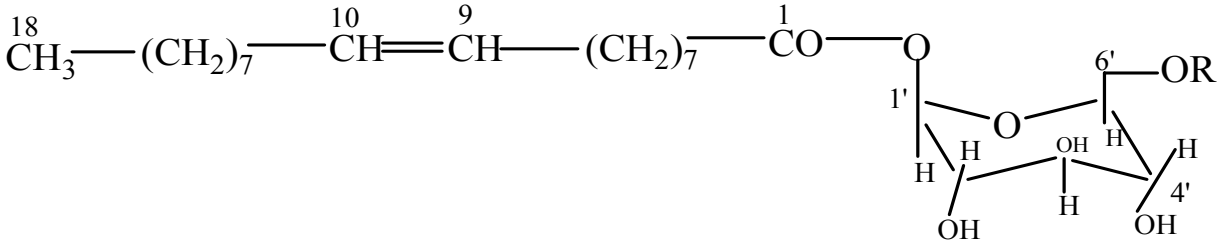
Compound 2: Elution of column with chloroform-methanol (24 :1) afforded as colourless crystals (methanol); m.p.: 113-115 ºC; UV λ_{max} (MeOH): 206 nm (log ε 4.2); IR υ_{max} (KBr): 3403, 3355, 2913, 2845, 1736, 1647, 1464, 1369, 1257, 717 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.39 (1H, m, H-9), 5.33 (1H, m, H-10), 4.28 (1H, d, *J* = 7.1 Hz, H-1′), 4.15 (1H, m, H-5′), 3.88 (1H, dd, *J* = 7.2, 6.5 Hz, H-2′), 3.77 (1H, dd, *J* = 6.5, 7.0 Hz, H-3′), 3.65 (1H, m, H-4′), 3.16 (2H, brs, H_2-6′), 2.44 (1H, d, *J* = 7.1Hz, H_2-2a), 2.33 (1H, d, *J* = 7.1Hz, H_2-2b), 2.02 (2H, m, H_2-8), 1.82 (2H, m, H_2-11), 1.61 (2H, m, H_2-3), 1.26 (20 H, brs, 10 × CH_2), 0.88 (3H, t, *J* = 6.5 Hz, Me-18); ^{13}C NMR ($CDCl_3$): δ 176.63 (C-1), 129.65 (C-9), 127.83 (C-10), 75.59 (C-1′), 74.12 (C-5′), 72.68 (C-2′), 71.93 (C-3′), 69.89 (C-4′), 61.12 (C-6′), 51.95 (C-2), 34.39 (C-8), 34.02 (C-11), 32.17 (C-3), 32.17 (CH_2), 31.44 (CH_2), 29.21 (3 × CH_2), 25.52 (CH_2), 25.18 (CH_2), 24.84 (CH_2), 24.57 (CH_2), 22.18 (CH_2), 13.61 (Me-18); +ve ion FAB MS *m/z* (rel. int.): 444 $[M]^+$ ($C_{24}H_{44}O_7$) 2.3, 163 (27.8).

Acid hydrolysis of 2: Compound **2** (35 mg) was dissolved in ethanol (5 ml), dil HCl (3 ml) added and the reaction mixture refluxed for 1 h. The solvent was evaporated under reduced pressure and the residue was dissolved in $CHCl_3$. It was chromatographed over silica gel TLC (petroleum ether : chloroform:: 1:1) with a standard solution of oleic acid. The fraction dissolved in water was concentrated and chromatographed over paper with a standard solution of D-glucose (*n*-BuOH : H_2O : AcOH ; 4:1:5) top layers, R_f 0.12.

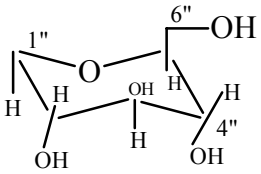
Compound 3: Futher elution of column with chloroform-methanol (24 : 1) yielded colourless crystals (methanol); m.p.: 56-58 ºC; UV λ_{max} (MeOH): 201 nm (log ε 3.2); IR υ_{max} (KBr): 3334, 3245, 2914, 2846, 1730, 1618, 1462, 1370, 1255, 1166, 1071, 1021, 721 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.36 (2H, m, H-9, H-10), 5.11 (1H, d, *J* = 7.1 Hz, H-1′), 5.03 (1H, d, *J* = 7.2 Hz, H-1″), 4.78 (2H, m, H-5′, H-5″), 4.39 (1H, dd, *J* = 7.1, 6.3 Hz, H-2′), 4.33 (1H, dd, *J* = 7.2, 6.5 Hz, H-2″), 4.10 (1H, m, H-3'), 4.07 (1H, m, H-3″), 3.78 (1H, m, H-4′), 3.70 (1H, m, H- 4″), 3.47 (2H, brs, H_2-6′), 3.21 (2H, brs, H_2-6″), 2.33 (1H, d, *J* = 7.2 Hz, H_2-2a), 2.31 (1H, d, *J* = 7.2 Hz, H_2-2b), 2.01 (2H, m, H_2-8), 1.83 (2H, m, H_2-11), 1.47 (2H, m, H_2-3), 1.25 (20H, brs, 10 × CH_2), 0.85 (3H, t, *J* = 6.6 Hz, Me-18); ^{13}C NMR($CDCl_3$): δ 175.61 (C-1), 129.64 (C-9), 121.42 (C-10), 101.04 (C-1′, C-1″), 79.01 (C-5′, C-5″), 75.41 (C-2′), 73.47 (C-2″), 73.17 (C-3′), 71.78 (C-3″), 71.76 (C-4′), 70.27 (C-4″), 63.47 (C-6″), 61.06 (C-6′), 51.59 (C-2), 49.61 (CH_2), 34.23 (CH_2), 33.93 (CH_2), 32.48 (CH_2), 32.21 (CH_2), 31.47 (CH_2), 30.50 (CH_2), 29.26 (CH_2), 28.91 (CH_2), 25.58 (CH_2), 24.91 (CH_2), 24.59 (CH_2), 22.24 (CH_2), 13.71 (CH_3); +ve ion FAB MS *m/z* (rel. int.): 606 $[M]^+$ ($C_{30}H_{54}O_{12}$) (5.3), 281 (41.6), 265 (53.2), 162 (37.8).

Acid hydrolysis of 3: Compound **3** (35 mg) was dissolved in ethanol (5 ml), dil HCl (3 ml) added and the reaction mixture refluxed for 1 h. The solvent was evaporated under reduced pressure and the residue was dissolved in $CHCl_3$. It was chromatographed over silica gel TLC (petroleum ether : chloroform:: 1:1) with a standard solution of oleic acid. The residue after separating the fatty acid fraction was dissolved in water and chromatographed over paper with a standard solution of D-glucose (*n*-BuOH:H_2O:AcOH::4:1:5) top layers, R_f 0.12.

Compound 4: Elution of the column with chloroform-methanol (23 : 2) mixture gave colourless crystals (methanol); m.p.: 83-85 ºC; UV λ_{max} (MeOH): 207 nm (log ε 4.3); IR υ_{max} (KBr): 3445, 2921, 2852, 1741, 1651, 1462, 1418, 1368, 1251, 1077 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.33 (2H, m, H-9, H-10), 4.76 (1H, d, *J* = 7.1 Hz, H-1′),



2. R=H



3. R=

4.10 (1H, m, H-5′), 3.77 (1H, dd, J = 6.5, 7.1 Hz, H-2′), 3.69 (1H, brm,$w_{1/2}$ = 9.5 Hz, H-12), 3.61 (1H, m, H-3′), 3.48 (1H, m, H-4′), 3.35 (1H, d, J = 9.5 Hz, H_2-6′a), 3.31 (1H, d, J = 9.5 Hz, H_2-6′b), 2.30 (1H, d, J = 7.1 Hz, H_2-2a), 2.28 (1H, d, J = 7.1 Hz, H_2-2b), 2.01 (2H, m, H_2 - 10), 1.99 (2H, m, H_2 -11), 1.52 (2H, m, H_2 -3), 1.26 (16 H, brs, 8 × CH_2), 0.85 (3H, t, J = 6.6 Hz, Me-18); ^{13}C NMR ($CDCl_3$): δ 176.35 (C-1), 130.62 (C-9), 129.87 (C-10), 103.69 (C-1′), 73.52 (C-5′), 72.43 (C-2′), 71.81 (C-3′), 69.89 (C-12), 63.67 (C-4′), 61.40 (C-6′), 56.19 (C-2), 34.72 (C-8), 32.68 (C-11), 31.92 (C-13), 29.76 (3 × CH_2), 27.76 (CH_2), 25.46 (CH_2), 22.66 (CH_2), 14.02 (C-18); +ve ion FAB MS *m/z* (rel. int.): 460 $[M]^+$ ($C_{24}H_{44}O_8$) (5.3), 281 (48.6), 163 (15.3).

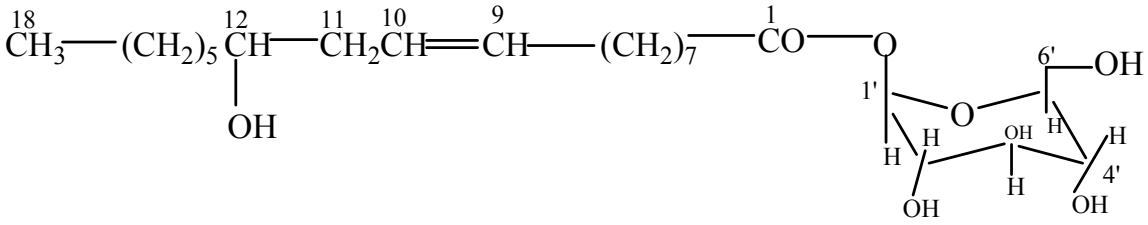
Acid hydrolysis of 4: Compound **4** (35 mg) dissolved in ethanol (5 ml), dil HCl (3 ml) added and the reaction mixture refluxed for 1 h. The solvent was evaporated under reduced pressure and the residue dissolved in $CHCl_3$. It was chromatographed over silica gel TLC (petroleum ether:chloroform::1:1) with a standard solution of ricinoleic acid. The residue after separating the fatty acid fraction was dissolved in water and chromatographed over paper with a standard solution of D-glucose (*n*–BuOH:H_2O:AcOH:: 4:1:5) top layers R_f 0.12.

RESULT AND DISCUSSION

Compound **1**, is a common glyceride phosphate identified as glyceryl-1-linolenyl-2-oleoyl-3-phosphate.

Compound **2,** obtained as colourless crystals, decolourized bromine water and gave positive tests for glycosides. Its IR spectrum showed characterstic absorption bands for hydroxyl group (3403, 3355 cm^{-1}), ester group (1736 cm^{-1}), unsaturation (1647 cm^{-1}) and long aliphatic chain (717 cm^{-1}). On the basis of mass and ^{13}C NMR spectra its molecular weight was established at *m/z* 444 consistent with a molecular formula $C_{24}H_{44}O_7$ of a C-18 fatty acid glycoside. The ion fragments arising at *m/z* 163 $[C_6H_{11}O_5]$ supported the location of the glucose moiety at the terminal side of the oleic acid chain. The ^{1}H NMR spectrum of **2** exhibited two one-proton multiplets at δ 5.39 and 5.33 assigned to vinylic H-9 and H-10 protons. A one-proton doublet at δ 4.28 (J = 7.1 Hz) was ascribed to anomeric proton H-1′ and a one-proton multiplet at δ 4.15 was accounted to carbinol H-5′. The remaining sugar protons resonated between δ 3.88 - 3.16. Two one-proton doublets at δ 2.44 and 2.33 (J = 7.1 Hz, each) were ascribed to H_2-2 methylene protons adjacent to the ester group. Two multiplets at δ 2.02 and 1.82, both integrating for two-protons each, were attributed to H_2-8 and H_2-11 methylene protons adjacent to vinylic carbons. The remaining methylene protons resonated as a broad singlet at δ 1.26 (20 H). A three-proton triplet at δ 0.88 (J = 6.5 Hz) was ascribed to C-18 primary methyl protons. The ^{13}C NMR spectrum of **2** showed important signals for ester carbon at δ 176.63 (C-1), vinylic carbons at δ 129.65 (C-9) and 127.83 (C-10), methyl carbon at δ 13.61(Me-18) and methylene carbons between δ 51.95 - 22.18. The hydroxyl methylene carbon appeared at δ 61.12 (C-6′) and the remaining carbon of sugar resonated between δ 75.59 - 69.89. The ^{13}C NMR values of the sugar were compared with ^{13}C NMR chemical shift of sugar parts [8]. The HMBC spectrum of **2** showed correlation of C-1 with H_2-2 and H-1′, C-9 with H-10 and H_2-8; and C- 6′ with H-5′and H-4′. Acid hydrolysis of **2** yielded oleic acid and β-D glucose (TLC comparable). On the basis of the above mention discussion the structure of compound **2** has been established as n-octadec-9-enoyl-β-D-glucopyranoside.

Compound **3**, obtained as colourless crystals, showed positive tests for glycosides and decolourized bromine water. Its IR spectrum showed characterstic absorption bands for hydroxyl groups (3334, 3245 cm^{-1}), ester group (1730 cm^{-1}), unsaturation (1618 cm^{-1}) and long aliphatic chain (721 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, its molecular weight was established at *m/z* 606 consistent with a molecular formula $C_{30}H_{54}O_{12}$ of a C-18 fatty acid diglycoside. The prominent ion peaks appearing at *m/z* 265 $[C_{17}H_{33}CO;\ CO{-}O\ fission]^+$ and 281 $[C_{17}H_{33}COO]^+$ indicated that oleic acid was attached to glycone moiety. The ion fragments arising at *m/z* 162 $[C_6H_{10}O_5]$ supported the location of the glucose moiety at the terminal side of the glycoside chain. The ^{1}H NMR spectrum of **3** exhibited one two-proton multiplet at δ 5.36 assigned to vinylic H-9 and H-10 protons. Two one-proton doublets at δ 5.11 (J = 7.1 Hz) and 5.03 (J = 7.2 Hz) were ascribed to anomeric protons H-1′ and H-1″. One two-proton multiplet at δ 4.78 was



accounted to carbinol H-5′ and H-5″ of sugar moieties. Two one-proton doublet at δ 4.39 (*J* = 7.1, 6.3 Hz) and 4.33 (*J* = 7.2, 6.5 Hz) were ascribed to carbinol H-2′ and H-2″ protons, respectively. Two one-proton doublets at δ 2.33 (*J* = 7.2 Hz) and 2.31 (*J* = 7.2 Hz) were associated with the H_2-2 methylene protons nearby the ester function. The remaining sugar protons resonated between δ 4.10 – 3.21. Two multiplets at δ 2.01 and 1.83, both integrated for two-protons each, were attributed to H_2-8 and H_2-11 methylene protons adjacent to vinylic C-9 and C-10 carbons. A three-proton triplet at δ 0.85 (*J* = 6.6 Hz) was ascribed to terminal C-18 primary methyl protons. The remaining methylene protons appeared at δ 1.47 (2H) and 1.25 (20 H). The ^{13}C NMR spectrum of **3** displayed important signals for ester carbon at δ 175.61 (C-1), vinylic carbon at δ 129.64 (C-9) and 121.42 (C-10), methyl carbon at δ 13.71 (Me-18) and methylene carbons between δ 51.59 - 22.24. The anomeric carbons resonated at 101.04, carbinol signals of the sugar residue appeared between δ 79.01 - 61.06. The appearance of C-6′ in the deshielding region at δ 3.47 in the ^{1}H NMR spectrum and at δ 6.47 in the ^{13}C NMR spectrum indicated the location of another sugar at this carbon. The HMBC spectrum of **3** exhibited correlation of C-1 with H_2-2 and H-1′, C-9 with H-10 and H_2-8; C- 6′ with H-5′and H-1′ and C-6″ with H-5″. Acid hydrolysis of compound **3** yielded oleic acid and β-D glucose (TLC comparable). On the basis of the above mention discussion the structure of **3** has been established as n-octadec-9-enoyl-β-D-glucopyranosylo (1→ 6)-β-D-glucopyranoside.

Compound **4**, obtained as a colourless crystalline mass, decolourized bromine water and gave positive test for glycosides. Its IR spectrum showed characterstic absorption bands for hydroxyl group (3445 cm^{-1}), ester group (1741 cm^{-1}) and unsaturation (1651 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, its molecular weight was established at *m/z* 460 $[M]^+$ consistent with a molecular formula $C_{24}H_{44}O_8$ of a C-18 fatty acid glycoside. A prominent ion peaks at *m/z* 281 [CO −O fission]$^+$ and *m/z* 163 $[C_6H_{11}O_5]$ supported the location of the glucose moiety at the terminal side of the ricinoleic acid chain. The ^{1}H NMR spectrum of **4** exhibited a two-proton multiplet at δ 5.33 assigned to vinylic H-9 and H-10 protons. A one-proton doublet at δ 4.76 (*J* = 7.1 Hz) was attributed to anomeric H-1′ proton. A one-proton multiplet at δ 4.10 were ascribed to carbinol H-5′ protons. Three one-proton multiplets at δ 4.10, 3.61 and 3.48 were ascribed to carbinol H-5′, H-3′ and H-4′ protons, respectively. A one-proton double doublet at δ 3.77 was accounted to carbinol H-2′ proton. Two one-proton doublets at δ 3.35 (*J* = 9.5 Hz) and 3.31 (*J* = 9.5 Hz) were accommodated to hydroxymethylene H_2-6′ protons. A one-proton multiplet at δ 3.69 with half width of 9.5 Hz was due to α-oriented H-12 carbinol proton. Two one-proton doublets at δ 2.30 (*J* = 7.1 Hz) and 2.28 (*J* = 7.1 Hz) were attributed to C-2 methylene protons adjacent to the ester group. A three-proton triplet at δ 0.85 (*J* = 6.6 Hz) was ascribed to terminal C-18 primary methyl protons. The remaining methylene protons appeared between δ 2.01-1.26. The ^{13}C NMR spectrum of **4** displayed important signals for ester carbon at δ 176.35 (C-1), vinylic carbons at δ 130.69 (C-9), 129.87 (C-10), methyl carbon at δ 14.02 (Me-18) and sugar carbons between δ 73.52-61.40. The anomeric carbon appeared at δ 103.62 indicating the presence of one sugar moiety in the glycosidic chain. The carbinol carbon appeared at δ 69.89 (C-12). The ^{13}C NMR values of the sugar were compared with ^{13}C NMR chemical shifts of sugar parts [8]. The HMBC spectrum of **4** showed correlation of C-1 with H-1′ and H_2-2; C-9′ with H-10 and H_2-8; C-12 with H_2-11 and H-10; and C- 6′ with H-5′. Acid hydrolysis of **4** yielded ricinoleic acid and β-D-glucose (TLC comparable). On the basis of the above discussion, the structure of **4** has been established as n-octadec-9-en-12-ol-1-oyl- β-D-glucopyranoside.

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