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Chemical Constituents from the Fruits of *Lycium chinense*

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Five compounds *viz.*, *n*-hexacos-5,8,11-trienoic acid (**1**), *n*-tridecanyl *n*-octadec-9,12-dienoate (**2**), *n*-triacont-11-enoic acid (**3**), *n*-eicosanoic acid (**4**), *n*-butyl octadec-9,12-dienoate (**5**) were isolated from the methanol extract of *Lycium chinense* fruits. Their structures were elucidated spectroscopically. To the best of our knowledge, all the compounds **1-5** were identified for the first time from the fruits of *L. chinense*.

Key Words: *Lycium chinense*, Solanaceae.

INTRODUCTION

Lycium chinense Miller fruits (Fructus Lycii) known as "Gou-Qi-Zi" in Chinese, has long history of application as a valuable tonic and health food supplement for improving vision and maintaining good health. It is reputed to have the properties of nourishing the blood, enriching the yin, tonifying the kidney and liver, moistening the lungs[1,2]. Fruits of *L. chinense* (Solanaceae), distributed in northeast Asia, specially China, Japan, Korea and Taiwan, have been widely used as a tonic in traditional medicine. Potentially isolated constituents were reported to exhibit hypertensive, hypoglycemic and antipyretic activities[3,4]. Several compounds like cerebrosides and lyciumamide in this plant are known to display various bioactivities[5,6]. Potentially hepatoprotective glycolipid constituents and determination of betain in *L. chinense* fruits were reported[7,8]. Antimicrobial compounds have also been reported from *L. chinense* roots[9]. Specific α-galactosidase inhibitors, N-methylcalystegines structure/activity relationship of calystegines from *L. chinense* were reported[10]. The plant is reported to possess antibacterial, anticancer, antioxidant properties[9,11,12] and antihepatotoxic activity[5,13].

Several compounds like cyclic peptides, acyclic diterpene glycosides and other compounds from *L. chinense*[14], glycoconjugates from *L. barbarum* were reported[15]. Variation in fruit sugar composition of *L. barbarum* and *L. chinense* of different regions and varieties were also reported[16]. Evaluation of antioxidant and other activities of compounds from *L. barbarum* and *L. chinense* was reported[17,18]. In continuation of our previous work[19-21] on *L. chinense* fruits, another five long chain compounds were isolated as a natural product. This paper deals with the isolation and structural elucidation of five compounds (**1-5**, Fig. 1) on the basis of spectral methods. To the best of our knowledge, all the compounds (**1-5**) were identified for the first time from the fruits of *L. chinense*.

EXPERIMENTAL

All chemicals used were of analytical grade. Hexane, ethyl acetate, chloroform, methanol, ethanol, water, sulphuric acid and vanillin were purchased from Daejung Chemicals and Metals Co. Ltd, Korea. Pre-coated thin layer chromatography (TLC) plates (layer thickness 0.25 mm), silica gel for column chromatography (70-230 mesh ASTM) and LiChroprep RP-18 (40-63 μm) were from Merck (Darmstadt, Germany). Authentic standards of β-sitosterol and D-glucose were purchased from Sigma-Aldrich, St. Louis, MO, USA. Optical rotation was measured with an instrument on an AA-10 model polarimeter (Instruments Ltd., Seoul, South Korea). IR spectra were recorded on a Infinity Gold FT-IR (Thermo Mattson, USA) spectrophotometer, which was available at Korea Institute of Science and Technology, Seoul, South Korea. Both ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance 600 high resolution spectrometer operating at 600 and 150 MHz, respectively. This NMR machine was available at Seoul National University (SNU), Seoul, South Korea and all NMR spectra were recorded at SNU (Instrument, Bruker, Germany). NMR spectra were obtained in deuterated methanol and chloroform using tetramethylsilane (TMS) as an internal standard, with chemical shifts expressed in ppm (β) and coupling constants (*J*) in Hz. FAB/MS data were recorded on a JMS-700 (Jeol,

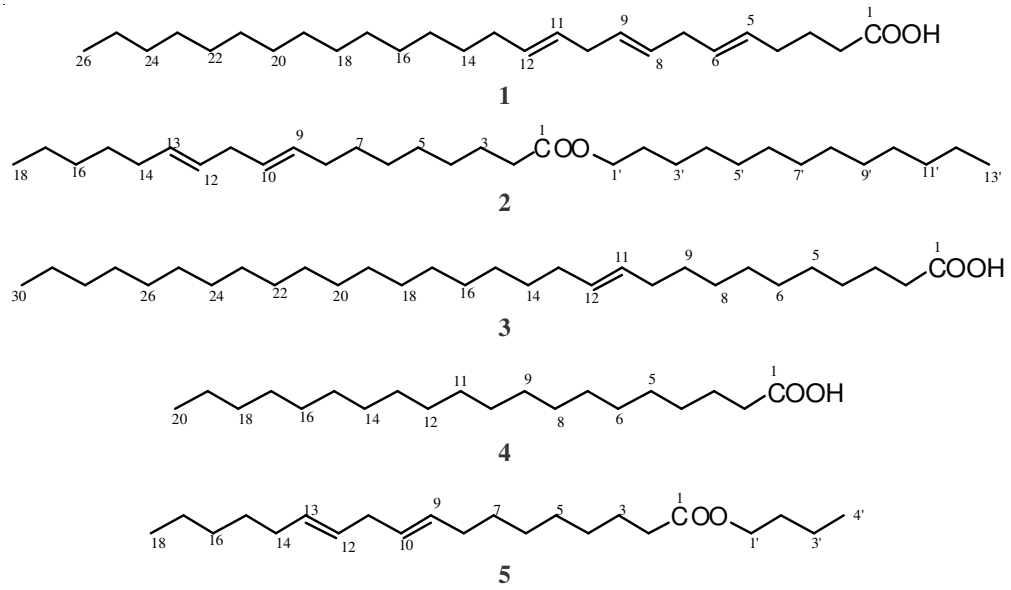

Fig. 1. Structures of compounds **1-5**

Japan) spectrometer instrument which was available at SNU, Seoul, South Korea.

Fruits of *Lycium chinense* were purchased from local medicinal plants shop market in Seoul, Korea and were identified by the Department of Pharmacognosy. Voucher specimen No. KU/LC/2010 has been deposited in Department of Applied Life Science, Konkuk University.

Extraction of fruits: The fruits of *L. chinense* (3.1 kg) were immersed in methanol (8 L) for 3 days at room temperature and then the supernatant was concentrated *in vacuo* to yield 230 g of extract. This material was suspended in water and extracted with hexane, ethyl acetate and *n*-butanol successively to produce 20.0 g, 10.1 g and 40 g extract, respectively.

Isolation of the compounds from hexane extract: The entire hexane extract (20 g) was subjected to normal phase CC over silica gel (500 g) to yield 28 fractions (each of 500 ml) with the following eluants: fractions 1-2 with hexane, fractions 3-4 with hexane:chloroform (9:1), fractions 5-6 with hexane:chloroform (8:2), fractions 7-8 with hexane:chloroform (7:3), fractions 9-10 with hexane:chloroform (6:4), fractions 11-12 with hexane:chloroform (1:1), fractions 13-14 with hexane:chloroform (4:6), fractions 15-16 with hexane: chloroform (3:7), fractions 17-18 with hexane:chloroform (2:8), fractions 19-22 with hexane:chloroform (1:9), fractions 23-28 with chloroform. All fractions were examined by TLC. Fractions 15-16 (0.8 g) and 17-18 (0.6 g) were further chromatographed over silica gel with hexane-chloroform and obtained five compounds **1** (29 mg), **2** (23 mg), **3** (21 mg), **4** (24 mg) and **5** (54 mg).

***n*-Hexacos-5,8,11-trienoic acid (1):** Colourless powder; IR (KBr, ν_{max}, cm^{-1}): 3410, 2917, 2850, 1708, 1645, 1463, 1410, 1295, 1187, 942, 723; ^{1}H NMR ($CDCl_3$): δ 5.41 (1H, m, H-5), 5.38 (1H, m, H-6), 5.35 (1H, m, H-8), 5.33 (1H, m, H-9), 5.31 (1H, m, H-11), 5.29 (1H, m, H-12), 2.77 (1H, d, *J* = 6.54 Hz, H_2-2a), 2.75 (1H, d, *J* = 6.8 Hz, H_2-2b), 2.35 (2H, m, H_2-7), 2.33 (2H, m, H_2-10), 2.05 (2H, m, H_2-4), 2.02 (2H, m, H_2-13), 1.61 (4H, m, 2 × CH_2), 1.30 (6H, m, 3 × CH_2), 1.28 (6H, m, 3 × CH_2), 1.23 (12 H, br s, 6 × CH_2), 0.88 (3H, t, *J* = 6.6 Hz, Me-26); ^{13}C NMR ($CDCl_3$): δ 180.16 (C- 1), 130.16 (C-5), 129.97 (C- 6, 8), 129.23 (C-9), 128.01(C-11), 127.84 (C-12), 34.07 (CH_2), 31.75 (CH_2), 31.49 (CH_2), 29.72 (CH_2), 29.64 (CH_2), 29.56 (CH_2), 29.49 (CH_2), 29.41 (CH_2), 29.34 (CH_2), 29.31 (CH_2), 29.22 (CH_2), 29.12 (CH_2), 29.03 (CH_2), 29.99 (CH_2), 27.16 (CH_2), 29.14 (CH_2), 25.48 (CH_2), 24.62 (CH_2), 22.66 (CH_2), 22.55 (CH_2), 14.10 (Me-26); FAB MS (positive mode) *m/z* (rel. int.): 391 $[M + H]^+$ ($C_{26}H_{47}O_2$) (11.8), 303 (100), 277 (67.2), 263 (28.7), 237 (14.5), 223 (13.6).

***n*-Tridecanyl *n*-octadec-9, 12-dienoate (2):** Colourless solid, R_f 0.48 ($CHCl_3$); IR (KBr, ν_{max}, cm^{-1}): 2923, 2852, 1742, 1641, 1463, 1367, 1260, 1171, 721; ^{1}H NMR ($CDCl_3$; 600 MHz): δ 5.36 (1H, m, H-9), 5.33 (1H, m, H-10), 5.31 (1H, m, H-12), 5.29 (1H, m, H-13), 4.12 (1H, d, *J* = 7.2 Hz, H_2-1'a), 4.10 (1H, d, *J* = 7.2 Hz, H_2-1'b), 2.31 (1H, d, *J* = 7.2 Hz, H_2-2a), 2.29 (1H, d, *J* = 7.2 Hz, H_2-2b), 1.99 (2H, m, H_2-11), 1.61 (2H, m, H_2-8), 1.58 (2H, m, H_2-14), 1.27 (8H, br s, 4 × CH_2), 1.26 (10 H, br s, 5 × CH_2), 1.23 (20 H, br s, 10 × CH_2), 0.87 (3H, t, *J* = 7.2 Hz, Me-18), 0.84 (3H, t, *J* = 7.1 Hz, Me-13′); ^{13}C NMR ($CDCl_3$; 150 MHz): δ 174.39 (C-1), 130.12 (C-9), 129.92 (C-10), 129.67 (C-12), 127.96 (C-13), 60.14 (C-1′), 51.41 (C-2), 34.05 (CH_2), 33.97 (CH_2), 31.88 (CH_2), 29.71 (3 × CH_2), 29.65 (4 × CH_2), 29.56 (CH_2), 29.48 (CH_2), 29.41 (CH_2), 29.33 (CH_2), 29.29 (CH_2), 29.22 (CH_2), 29.11 (CH_2), 29.07 (CH_2), 29.03 (CH_2), 28.95 (CH_2), 27.15 (CH_2), 27.10 (CH_2), 14.18 (Me-18), 14.07 (Me-13′); FABMS (positive ion mode) *m/z* (rel. int.): 463 $[M + H]^+$ ($C_{31}H_{59}O_2$) (25.6), 279 (38.2), 263 (31.8).

***n*-Triacont-11-enoic acid (3):** Yellow semi-solid; IR (KBr, ν_{max}, cm^{-1}): 3190, 2917, 2849, 1704, 1635, 1463, 1410, 1296, 1215, 940, 757; ^{1}H NMR ($CDCl_3$): δ 5.35 (1H, m, H-11), 5.33 (1H, m, H-12), 2.35 (2H, t, *J* = 7.2 Hz, , H_2-2), 2.37 (2H, m, H_2-10), 2.02 (2H, m, H_2-13), 1.63 (4H, m, 2 × CH_2), 1.31 (16 H, br s, 8 × CH_2), 1.29 (14H, br s, 7 × CH_2), 1.25 (12H, br s, 6 × CH_2), 0.87 (3H, t, *J* = 6.8 Hz, Me-30); ^{13}C NMR ($CDCl_3$); δ 180.53 (C-1), 129.68 (C-11), 129.69 (C-12), 34.10 (CH_2), 39.91 (CH_2), 29.66 (14 × CH_2), 29.58 (CH_2), 29.42 (CH_2), 29.36 (CH_2), 29.31 (CH_2), 29.23 (CH_2), 29.04

(CH_2), 27.18 (CH_2), 27.12 (CH_2), 24.64 (CH_2), 22.68 (CH_2), 14.12 (CH_2); FAB MS (positive mode) *m/z* (rel. int.): 451 $[M + H]^+$ ($C_{30}H_{59}O_2$) (22.5), 279 (83.4).

***n*-Eicosanoic acid (4):** White crystalline solid; IR (KBr, ν_{max}, cm^{-1}): 3490, 2916, 2848, 1707, 1463, 1297, 725; ^1H NMR ($CDCl_3$;600 MHz) δ 2.35 (2H, t, *J* = 7.5 Hz, H_2-2), 1.64 (4 H, m, 2 × CH_2), 1.29 (10 H, br s 5 × CH_2), 1.25 (20 H, br s, 10 × CH_2), 0.89 (3H, t, *J* = 6.6 Hz, Me-20); ^{13}C NMR ($CDCl_3$; 150 MHz): δ_C 179.72 (C-1), 33.96 (CH_2), 31.90 (CH_2), 29.68 (9 × CH_2), 29.58 (CH_2), 29.42 (CH_2), 29.35 (CH_2), 29.23 (CH_2), 29.03 (CH_2), 24.64 (CH_2), 22.68 (CH_2), 14.13 (Me-20); FAB MS (positive mode): *m/z* (rel.int.): 313 $[M]^+$ ($C_{20}H_{41}O_2$) (12.6), 295 (18.3).

***n*-Butyl octadec-9, 12-dienoate (5):** Colourless amorphous powder; R_f 0.45 ($CHCl_3$); ^1H NMR ($CDCl_3$; 600) δ 5.37 (1H, m, H-9), 5.34 (1H, m, H-10), 5.32 (1H, m, H-12), 5.30 (1H, m, H-13), 4.11 (2H, t, *J* = 7.14 Hz, H_2-1'), 2.30 (2H, t, *J* = 7.2 Hz, H_2-2), 2.01 (2H, m, H_2-11), 1.62 (4H, m, 2 × CH_2), 1.29 (8 H, br s, 4 × CH_2), 1.26 (12H, br s, 6 × CH_2), 0.88 (3 H, t, *J* = 6.6 Hz, Me-18), 0.85 (3H, t, *J* = 7.1 Hz, Me-4'); ^{13}C NMR ($CDCl_3$ 150 MHz) β 174.42 (C-1), 130.16 (C-9), 129.95 (C-10), 129.71 (C-12), 127.85 (C-13), 60.16 (C-1'), 34.36 (CH_2), 31.90 (CH_2), 29.81 (CH_2), 29.67 (CH_2), 29.57 (CH_2), 29.50 (CH_2), 29.42 (CH_2), 29.35 (CH_2), 29.23 (CH_2), 29.12 (CH_2), 29.09 (CH_2), 27.16 (CH_2), 24.95 (CH_2), 22.67 (CH_2), 14.21 (Me-18), 14.11 (Me-4'); IR (KBr, ν_{max}, cm^{-1}): 2916, 2849, 1737, 1641, 1462, 1378, 1260, 1170, 720; FAB MS (positive mode) *m/z* (rel. int.): 337 $[M + H]^+$ ($C_{22}H_{43}O_2$) (10.3), 279 (12.2), 263 (14.7).

RESULTS AND DISCUSSION

Compound **1,** was obtained as a colourless compound. The FAB mass and ^{13}C NMR spectral data led to established its molecular formula $C_{26}H_{46}O_2$ ion peak at *m/z* 391. Its IR spectrum exhibited characteristic absorption bands for carboxylic (3410 cm^{-1}), unsaturation (1645, 1463 cm^{-1}) and long aliphatic chain (723 cm^{-1}). The ^1H and ^{13}C NMR spectral are also supported the structure of compound **1**. The spectral details are given in experimental part. The mass fragmentation pattern of **1** is shown in Fig. 2. On the basis of spectroscopic analysis, the structure of **1** has been established as *n*-hexacos-5,8,11-trienoic acid.

Compound **2**, was obtained as a colourless compound and its molecular formula $C_{31}H_{58}O_2$ was established from its ^{13}C NMR and FAB MS. Its IR spectrum exhibited characteristic absorption bands for ester function (1725, 1641 cm^{-1}), unsaturation (1641 cm^{-1}) and long aliphatic chain (721 cm^{-1}). The mass fragmentation pattern of **2** is shown in Fig. 2. The ^1H NMR spectrum of **2** showed four one proton multiplets at δ 5.36, 5.33 5.21 and 5.29, assigned to vinylic protons H-9,

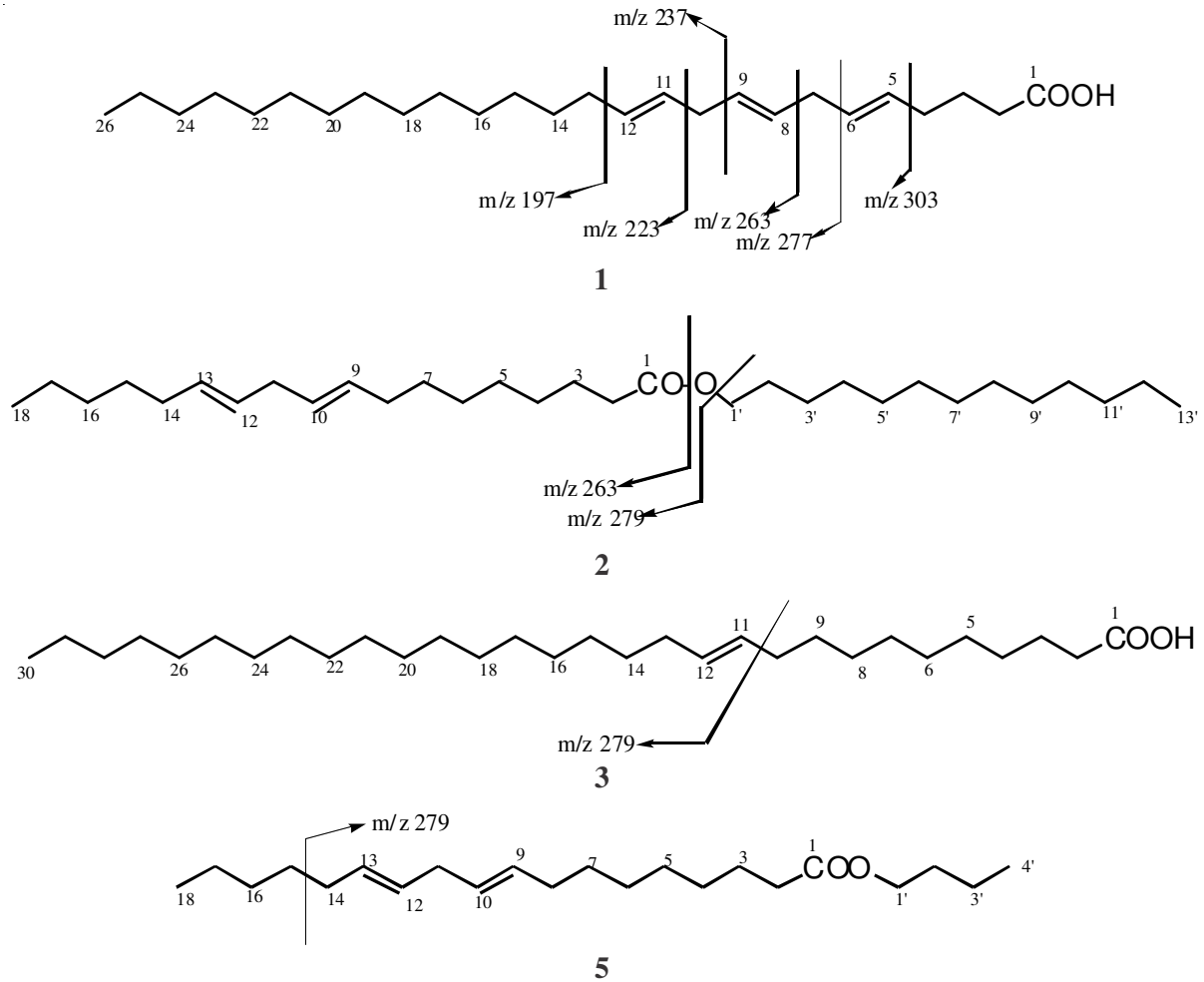

Fig. 2. Fragmentation pattern of compounds **1-3** and **5**

H-10, H-12 and H-13, respectively. Two one proton doublets at δ 2.31 (J = 7.2 Hz) and 2.29 (J = 7.2 Hz) were attributed to oxygenated methylene H_2-2. Two one-proton doublets at δ 4.10 (J = 7.2 Hz) and 4.12 (J = 7.2 Hz) were ascribed to C-1' methylene protons adjacent to ester group. Two multiplets at δ 1.61 and 1.99 integrated for two protons were accounted to C-8 and C-11 methylene protons attached to the vinylic carbons. Two protons multiplets at δ 1.58 were assigned for methylene protons attached to the vinylic carbon C-13. The remaining methylene protons resonated between δ 1.23 to 1.27. Two three protons triplets at δ 0.84 (J = 7.1 Hz) and 0.87 (J = 7.2 Hz) were associated to C-18 and C-13' primary methyl protons. The ^{13}C NMR spectrum of **2** displayed important signals for ester carbon at δ 174.39 (C-1), oxygenated methylene carbon at δ 60.14 (C-1'), methyl carbons at δ 14.18, 14.07 (C-18, C-13'), vinylic carbons δ 130.12 (C-9), 129.92 (C-10), 129.67 (C-12), 127.96 (C-13) and methylene carbons at δ 34.05 to 27.10. On the basis of this evidence the structure of **2** has been established as *n*-tridecanyl *n*-octandec-9,12-dienoate.

Compound **3**, was obtained as a yellow semi-solid compound. The FAB mass and ^{13}C NMR spectral data led to established its molecular formula $C_{30}H_{58}O_2$. Its IR spectrum exhibited characteristic absorption bands for carboxylic function (3190 cm^{-1}), ketone (1704 cm^{-1}), unsaturation (1635, 1463 cm^{-1}) and long aliphatic chain (757 cm^{-1}). The ^{1}H and ^{13}C NMR spectral data are also supported the structure of compound **3**. The Spectral details are given in experimental part. The mass fragmentation pattern of **3** is shown in Fig. 2. On the basis of spectroscopic analysis, the structure of **3** has been establisherd as *n*-triacont-11-enoic acid.

Compound **4**, was obtained as a colourless compound and its molecular formula $C_{20}H_{40}O_2$ was established from its ^{13}C NMR and FAB MS. Its IR spectrum exhibited characteristic absorption bands for carboxylic function (3490 cm^{-1}), ketone (1707 cm^{-1}) and long aliphatic chain (725 cm^{-1}). The ^{1}H and ^{13}C NMR spectral data are also supported the structure of compound **4**. The spectral details are given in the experimental part. The ^{1}H and ^{13}C NMR data of compound **4** are given experimental part. On the basis of spectroscopic analysis, the structure of **4** has been establisherd as *n*-eicosanoic acid.

Compound **5** was obtained as a colourless compound and its molecular formula $C_{22}H_{43}O_2$ was established from its ^{13}C NMR and FABMS *m/z* 337 $[M + H]^+$. Its IR spectrum exhibited characteristic absorption bands for ester function (1737 cm^{-1}), unsaturation (1641 cm^{-1}) and long aliphatic chain (774 cm^{-1}). The mass fragmentation pattern of **5** is shown in Fig. 2.

The ^{1}H NMR spectrum of **5** showed four one-proton multiplets at δ 5.37, 5.34, 5.32, 5.30 assigned to vinylic protons H-9, H-10, H-12 and H-13, respectively. Two protons triplet at δ 2.31 (J = 7.2 Hz) was attributed to oxygenated methylene H_2-2. Two protons triplet at δ 4.11 (J = 7.2 Hz) and was ascribed to H_2-1' methylene protons adjacent to ester group. Two three-proton triplets at δ 0.88 (J = 6.6 Hz) and 0.85 (J = 7.1 Hz) were associated to C-18 and C-4' primary methyl protons. The remaining methylene protons resonated between δ 2.01 to 1.26. The ^{13}C NMR spectrum of **5** displayed important signals for ester carbons at δ 174.42 (C-1), oxygenated methylene carbon at δ 60.16 (C-1'), methyl carbons at δ 14.21, 14.11 (C-18, C-4') and methylene carbons between δ 34.36 - 22.67. On the basis of these evidences the structure of **5** was established as *n*-butyl octadec-9, 12-dienoate.

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