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Aliphatic esters and fatty acids from the fruits of *Lycium chinense* miller

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Abstract The fruits of *Lycium chinense* Miller (Solanaceae) possess emmenagogue, diuretic, antipyretic and hepatoprotective properties and are used to stimulate the immune system, to increase hormonal growth, to improve blood circulation and eye sight and to cure morning sickness. Phytochemical investigation of a chloroform extract of the fruits gave two new aliphatic esters, characterized as *n*-butanyl *n*-octadec-9,12-dienoate (*n*-butanyl linoleate,**1**) and *n*-tridecanyl *n*-octadec-9,12-dienoate (*n*-tridecanyl linoleate, **2**) and two new fatty acids identified as *cis,cis,cis* –*n*-octacos-5,8,11-trienoic acid (**4**) and *cis*-*n*-octacos -11-enoic acid (**5**) and along with a known saturated fatty acid, arachidic acid (**3**). The structures of these phytoconstituents have been elucidated on the basis of spectral analysis and chemical reactions.

Keywords *Lycium chinense*, fruits, aliphatic esters, fatty acids, structure elucidation.

Introduction

Lycium chinense Miller (Solanaceae), known as Goji berry or Gou-qu-zi, is a cold-hard, perennial shrub. Its origin is believed to be in the region of south-eastern Europe and south-western Asia, but now it is cultivated throughout the world. Today China is the largest producer of this fruit. The dried form of this fruit is emmenagogue, diuretic, antipyretic and hepatoprotective and marketed in Ayurvedic and herbal health shops as the Tibetan or Himalayan Goji berry. The red-orange ellipsoid fruits stimulate the immune system due to high vitamin C contents, increase human growth hormone and sperm production and improve blood circulation and eye sight. The berries are taken by pregnant females to prevent morning sickness and to alleviate hepatitis and insomnia, to improve memory, to provide longevity and as a tonic in traditional oriental medicine. The fruits exhibited hypotensive, hypoglycaemic and antipyretic activities [1, 2]. They have properties like nourishing the blood, enriching the yin, tonifying the kidney and the liver, and moistening the lungs [2, 3]. They are useful for reducing the risk of certain diseases such as arteriosclerosis, essential arterial hypertension, diabetes and night blindness [4-9]. The dried ripe fruits are an ingredient of herbal drugs and functional foods [10]. Goji berry contained sterols, polysaccharides, zeaxanthin and antioxidants like as lutein, β-carotene, vitamin C and lycopene. Potentially hepatoprotective glycolipid constituents and determination of betain in *L. chinense* fruits have been reported [11, 12]. Di- and tetraterpene glycosides and polyglycosides of fatty acids are published in recent reports from *L. chinense* fruits [13-16]. This paper describes isolation and characterization of aliphatic constituents from the berries of *L. chinense* available locally.

Materials and Methods

General

Melting points were determined on a Perfit apparatus without correction. The IR spectra were measured in KBr pellet on a Bio-Red FT-IR spectrometer. Ultraviolet (UV) spectra were obtained in methanol with a Lambda Bio 20 spectrometer. The ^{1}H (400 MHz), ^{13}C (100 MHz), COSY and HMBC NMR spectra were recorded on Bruker spectrospin spectrometer. $CDCl_3$ (Sigma-Aldrich, Bangalore, India) was used as a solvent and TMS as an internal standard. ESI MS analyses were performed on a Waters Q-TOF Premier (Micromass MS Technologies, Manchester, UK) Mass Spectrometer. Column chromatography separations were carried out on silica gel (Merck, 60–120 mesh, Mumbai, India). Precoated silica gel plates (Merck, Silica gel 60 F_{254}) were used for analytical thin layer chromatography and visualized by exposure to iodine vapours and UV radiations.

Plant material

The fruits of *L. chinense* were procured from a local market of Delhi and identified by Dr. H.B. Singh, Scientist F and Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. A voucher specimen of drug was deposited in the herbarium of Phytochemistry Research Laboratory, Jamia Hamdard, New Delhi.

Extraction and isolation

The fruits of *L. chinense* (500 g) were coarsely powdered and extracted exhaustively with chloroform using a Soxhlet apparatus for 18 h. The extract was concentrated under reduced pressure to get dark brown mass (103 g). The residue (100 g) was dissolved in minimum amount of methanol and adsorbed on silica gel column grade (60-120 mesh) to a obtain a slurry. The slurry was dried in air and chromatographed over silica gel column loaded in chloroform. The column was eluted with petroleum ether, petroleum ether-chloroform (9:1, 3:1, 1:1, v/v) mixtures and chloroform to obtain compounds **1 - 5**.

n-Butanyl linoleate (1)

Elution of the column with petroleum ether gave pale yellow gummy mass of **1**, IR ν_{max} (KBr): 2928, 2842, 1725, 1635, 1465, 1380, 1262, 1171, 722 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.33 (1H, m, H-9), 5.29 (1H, m, H-10), 5.25 (1H, m, H-12), 5.22 (1H, m, H-13), 4.13 (2H, t, *J*=6.9 Hz, H_2-1′), 2.75 (2H, m, H_2-11), 2.33 (2H, t, *J*=7.2 Hz, H_2-2), 2.02 (2H, m, CH_2), 1.98 (2H, m, CH_2), 1.62 (2H, m, CH_2), 1.29 (8H, brs, 4 × CH_2), 1.25 (10H, brs, 5×CH_2), 0.89 (3H, t, *J*=6.5 Hz, Me-18), 0.85 (3H, t, *J*=6.6 Hz, Me-4′); ^{13}C NMR ($CDCl_3$): δ 173.69 (C-1), 131.16 (C-9), 130.48 (C-10), 129.80 (C-12), 127.83 (C-13), 61.05 (C-1′), 34.31 (CH_2), 31.89 (CH_2), 29.79-29.11 (9×CH_2), 27.21 (CH_2), 24.93 (CH_2), 22.69 (CH_2), 14.18 (Me-18), 14.09 (Me-4′);+ve ion FAB MS *m/z* (*rel. int.*): 337 $[M+H]^+$ ($C_{22}H_{43}O_2$) (9.8),279 (13.1), 263 (15.3).

n-Tridecanyl linoleate (2)

Elution of the column with petroleum ether-chloroform (9:1) yielded a pale yellow gummy mass of **2**, IR ν_{max} (KBr): 2928, 2845, 1723, 1640, 1465, 1363, 1262, 1170, 723 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.35 (1H, m, H-9), 5.32 (1H, m, H-10), 5.29 (1H, m, H-12), 5.27 (1H, m, H-13), 4.15 (2H, t, *J*=7.2 Hz, H_2-1′), 2.75 (2H, m, H_2-11), 2.35 (2H, t, *J*=7.3 Hz, H_2-2), 2.01 (2H, m, CH_2), 1.98 (2H, m, CH_2), 1.62 (2H, m, CH_2), 1.56 (2H, m, CH_2), 1.28 (4H, brs, 4×CH_2), 1.25 (26H, brs, 13×CH_2), 0.86 (3H, t, *J*=6.6 Hz, Me-18), 0.83 (3H, t, *J*=6.5 Hz, Me-13′); ^{13}C NMR ($CDCl_3$): δ 173.67 (C-1), 130.32 (C-9), 127.81 (C-10), 129.52 (C-12), 127.91 (C-13), 60.83 (C-1′), 34.11 (CH_2), 33.93 (CH_2), 31.85 (CH_2), 29.71-29.03 (18 ×CH_2), 28.93 (CH_2), 27.15 (CH_2), 22.68 (CH_2), 14.15 (Me-18), 14.09 (Me-13′); +ve ion FAB MS *m/z* (*rel. int.*): 463 $[M+H]^+$ ($C_{31}H_{59}O_2$) (23.8), 279 (37.9), 263 (32.1).

Arachidic acid (3)

Elution of the column with petroleum ether-chloroform (3:1) afforded colourless crystals of **3**, IR ν_{max} (KBr): 3090, 2926, 2838, 1702, 1465, 1298, 725 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ2.33(2H, t, *J*=7.5 Hz, H_2-2), 1.66 (4H, m, 2×CH_2), 1.29 (10H, brs, 5×CH_2), 1.25 (20H, brs, 10×CH_2), 0.88 (3H, t, *J*=6.5 Hz, Me-20); ^{13}C NMR ($CDCl_3$): δ 179.73 (C-1), 33.98 (CH_2), 31.89 (CH_2), 29.68 (9 × CH_2), 29.58 (CH_2), 29.44 (CH_2), 29.35 (CH_2), 29.28 (CH_2), 29.06 (CH_2),

24.66 (CH_2), 22.68 (CH_2), 14.16 (Me-20); +ve ion FAB MS *m/z* (*rel. int.*): 313 $[M+H]^+$ ($C_{20}H_{41}O_2$) (12.4), 295 (18.8).

***n*-Octacos-5,8,11-trienoic acid (4)**

Elution of the column with petroleum ether-chloroform (1:1) furnished yellow semisolid mass of **4**, IR ν_{max} (KBr): 3210, 2917, 2850, 1709, 1640, 1463, 1410, 1295, 1187, 942, 722 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.40 (1H, m, $w_{1/2}$=7.1 Hz, H-5), 5.36 (1H, m, $w_{1/2}$=7.3 Hz, H-6), 5.33 (1H, m, $w_{1/2}$=6.9 Hz, H-8), 5.30 (1H, m, $w_{1/2}$=7.5 Hz, H-9), 5.29 (1H, m, $w_{1/2}$=6.8 Hz, H-11), 5.23 (1H, m, H-12), 2.77 (2H, t, *J*=6.5 Hz, H_2-2), 2.38 (2H, m, H_2-7), 2.32 (2H, m, H_2-10), 2.08 (2H, m, H_2-4), 2.04 (2H, m, H_2-13), 1.60 (4H, m, 2×CH_2), 1.29 (6H, m, 3×CH_2), 1.26 (6H, m, 3×CH_2), 1.23 (16H, brs, 8×CH_2), 0.86 (3H, t, *J*=6.6 Hz, Me-26); ^{13}C NMR ($CDCl_3$): δ 180.18 (C-1), 130.26 (C-5), 129.98 (C-6, C-8), 129.25 (C-9), 128.07 (C-11), 127.88 (C-12), 34.05 (CH_2), 31.76 (CH_2), 31.48 (CH_2), 29.70-29.12 (14×CH_2), 25.45 (CH_2), 24.60 (CH_2), 22.68 (CH_2), 14.16 (Me-28); +ve ion FAB MS *m/z* (*rel. int.*): 419 $[M+H]^+$ ($C_{28}H_{51}O_2$) (5.3), 331 (5.3), 305 (11.3), 291 (15.6), 265 (25.3), 251 (14.1), 225 (13.5).

***n*-Octacos-11-trienoic acid (5)**

Elution of the column with chloroform gave a colourless powder of **5**, m.p. 94-95 ºC; IR ν_{max} (KBr): 3199, 2927, 2848, 1706, 1638, 1465, 1419, 1298, 1225, 940, 727 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.33 (1H, m, $w_{1/2}$=8.1 Hz, H-11), 5.29 (1H, m, $w_{1/2}$=7.8 Hz, H-12), 2.38 (2H, t, *J*=7.2 Hz, H_2-2), 2.35 (2H, m, H_2-10), 2.03 (2H, m, H_2-13), 1.65 (4H, m, 2×CH_2), 1.33 (16H, brs, 8×CH_2), 1.28 (14H, brs, 7×CH_2), 1.25 (12H, brs,6×CH_2), 0.86 (3H, t, *J*=6.6 Hz, Me-30); ^{13}C NMR ($CDCl_3$): δ 180.61 (C-1), 129.93 (C-11), 129.72 (C-12), 34.16 (CH_2), 31.90 (CH_2), 29.68-29.05 (21×CH_2), 27.21 (CH_2), 24.66 (CH_2), 22.69 (CH_2), 14.15 (Me-28); +ve ion FAB MS *m/z* (*rel. int.*): 423 $[M+H]^+$ ($C_{28}H_{55}O_2$) (23.1), 279 (82.6).

Results and discussion

Compound **1** showed IR absorption bands for ester group (1725 cm^{-1}), unsaturation (1635 cm^{-1}) and long aliphatic chain (722 cm^{-1}). Its mass spectrum exhibited a molecular ion peak at *m/z* 337 ($C_{22}H_{43}O_2$) $[M+H]^+$. The ion peaks arising at *m/z* 279 $[CH_3(C1_6H_{28})COO]^+$and 263 $[CH_3(C_{16}H_{28})CO]^+$ indicated that linoleic acid was esterified with *n*-butanol. The ^1H NMR spectrum of **1** displayed four one-proton vinylic protons between δ 5.33-5.22, a two-proton triplet at δ 4.13 (*J*=6.9 Hz) due to oxygenated methylene H_2-1′ protons, other methylene protons from δ 2.75 to 1.25 and two three-proton triplets at δ 0.89 (*J*=6.5 Hz) and 0.85 (*J*=6.6 Hz) ascribed to terminal C-18 and C-4′ primary methyl protons, respectively. The ^{13}C NMR spectrum of **1** had signals for ester carbons at δ 173.69 (C-1), vinylic carbons between δ 131.16-127.83, oxygenated methylene carbon at δ 61.05 (C-1′) and methyl carbons at δ 14.18 (C-18) and 14.09 (C-4′). On the basis of these evidences the structure of **1** has been characterised as *n*-butanyl *n*-octadec-9,12-dienoate (Figure 1).

Compound **2**, $[M+H]^+$ at *m/z* 463 ($C_{31}H_{59}O_2$), showed IR absorption bonds for ester group (1723 cm^{-1}), unsaturation (1640 cm^{-1}) and long aliphatic chain (723 cm^{-1}).The mass ion peaks arising at *m/z* 279 and 263 similar to compound **1** suggested that linoleic acid was esterified with *n*-tridecanol. The ^1H NMR spectrum of **2** displayed four one-proton multiplets from δ 5.32 to 5.27 assigned to vinylic protons, a two-proton triplet at δ 4.15 (*J*=7.2 Hz) ascribed to oxygenated methylene H_2-1′ protons, other methylene protons between δ 2.75-1.25 and two three-proton triplets at δ 0.86 (*J*=6.6 Hz) and 0.83 (*J*=6.5 Hz) accounted to terminal C-18 and C-13′ primary methyl protons. The ^{13}C NMR spectrum of **2** exhibited signals for ester carbon at δ 173.67 (C-1), vinylic carbons from 130.32-127.91, oxygenated metthylene carbon at δ 60.83 (C-1′), other methylene carbons from δ 34.11 to 22.68 and methyl carbons at δ 14.15 (C-18) and 14.09 (C-13′). These data led to identify **2** as *n*-tridecanyl *n*-octadec-9,12-dienoate (Figure 1). Compound 3 was the known fatty acid identified as arachidic acid (Figure 1).

Compound **4** gave effevescences with sodium bicarbonate solution. Its IR spectrum showed absorption bands for carboxylic group (3210,1709 cm^{-1}), unsaturation (1640 cm^{-1}) and long aliphatic chain (722 cm^{-1}). The mass spectrum of **4** had a molecular ion peak at *m/z* 419 $[M+H]^+$ ($C_{28}H_{51}O_2$) and ion fragments generating at *m/z* 331 [C_4-

C_5 fission]$^+$, 305 [C_6-C_7 fission]$^+$, 291 [C_7-C_8 fission]$^+$, 265 [C_9-C_{10}fission]$^+$, 251 [C_{10}-C_{11}fission]$^+$ and 225 [C_{12}-C_{11}fission]$^+$ indicated the existence of three vinylic linkages at $C_{5(6)}$, $C_{8(9)}$ and $C_{11(12)}$ positions. The ^{1}H NMR spectrum of **4** displayed six deshielded one-proton multiplets from δ5.40 to 5.23 with half widths between 6.8 - 7.5 Hz due to all *cis*-oriented vinylic protons, methylene protons in the range of δ 2.77-1.23 and a three-proton triplet at δ 0.86 (*J*=6.6 Hz) accounted to terminal H_3-28 primary methyl protons. The ^{13}C NMR spectrum of **4** exhibited signals for carboxylic carbon at δ 180.18 (C-1), vinylic carbons between δ 130.26-127.88, methylene carbons from δ 34.05 to 22.68 and methyl carbon at 14.16 (C-28). On the basis of above discussion the structure of **4** has been elucidated as *cis,cis,cis-n*-octacos-5,8,11-trienoic acid (Figure 1). This is a new unsaturated carboxylic acid.

Compound **5** was obtained as a colourless powder and produced effervescences with sodium bicarbonate solution. Its IR spectrum showed absorption bands for carboxylic group (3199, 1706 cm^{-1}), unsaturation (1638 cm^{-1}) and long aliphatic chain (727 cm^{-1}). It had a molecular ion peak of *m/z* 423 [M+H]$^+$ corresponding to a molecular formula of an unsaturated fatty acid $C_{28}H_{55}O_2$. A prominent ion peak arising at *m/z* 279 [C_{10}-C_{11} fission, $CH_3(CH_2)_{17}CH{=}CH$] indicated the presence of a vinyl bond at $C_{11(12)}$-position. The ^{1}H NMR spectrum of **5** exhibited two one-proton multiplets at δ 5.33 and 5.29 with half width of 8.1 and 7.8 Hz, assigned to *cis*-oriented vinylic H-11 and H-12 protons, respectively, a two-proton triplets at δ 2.38 (*J*=7.2Hz) ascribed to methylene H_2-2 protons adjacent to the carboxylic group, other methylene protons between δ 2.35-1.25 and a three-proton triplet at δ 0.86 (*J*=6.6 Hz) accounted to terminal C-30 primary methyl protons. The ^{13}C NMR spectrum of **5** displayed signals for carboxylic carbon at δ 180.61 (C-1), vinylic carbons at δ 129.93 (C-11) and 129.72 (C-12), methelene carbons between δ 34.16-22.69 and methyl carbon at δ 14.15 (C-30). On the basis of these spectral data analysis the structure of **5** has been characterized as *cis-n*-octacos -11-enoic acid (Figure 1). This is a new unsaturated carboxylic acid.

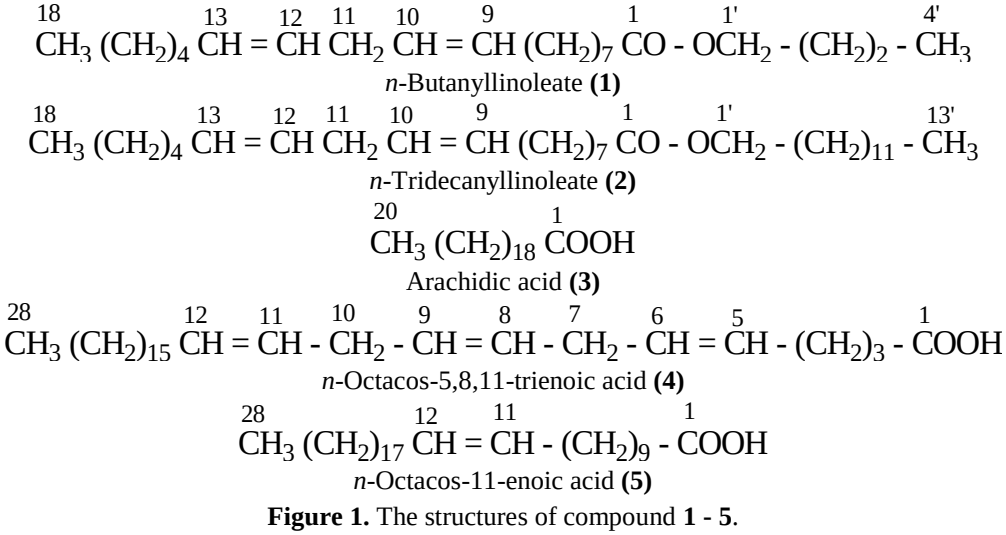

Figure 1. The structures of compound **1 - 5**.

References

1. Funayama S, Yoshida SK, Konno C, Hikino H. Structure of kukoamine A, a hypotensive principle of *Lycium chinense* root barks. *Tetrahedron Lett*er, 1980, 21: 1355-1356.
2. Potterat O. Goji (*Lycium barbarum* and *L. chinense*): Phytochemistry, pharmacology and safety in the perspective of traditional uses and recent popularity. *Planta Medica*, 2010, 76: 7-19.
3. Peng Y, Ma C, Li Y, Leung KSY, Jiang ZH, Zhao Z. Quantification of zeaxanthin dipalmitate and total carotenoids in *Lycium* fruits (*Fructus Lycii*). *Plant Foods Hum Nutr*, 2005, 60: 161–164.
4. Zargari A. *Medicinal Plants of the World: Chemical constituents, traditional and modern*. 5th (eds), Vol 3, Tehran University Publications, No. 1810/3, Tehran, Iran, 1992, 889.

5. Yahara S, Shigeyama C, Ura T, Wakamatsu K, Yashuhara T, Nohara T. Cyclic peptides, acyclic diterpene glycosides and other compounds from *Lycium chinense* Mill. *Chem Pharm Bull*, 1993, 41: 703-709.
6. Yang LL, Yen KY, Kiso Y, Kikino YH. Antihepatotoxic actions of formosan plant drugs. *J Ethnopharmacol*, 1987, 19: 103-110.
7. Dafini A, Yaniv Z. Solanaceae as medicinal plants in Israel. *J Ethnopharmacol*, 1994, 44: 11-18.
8. Rivera D, Obon C. The ethnopharmacology of Madeira and Porto Santo Islands, a review. *J Ethnopharmacol*, 1995, 46: 73-93.
9. Davis PH. *Flora of Turkish and east Aegean Islands*. 6th Edn., University Press, Edinburg, 1972, 445-449.
10. Chung IM, Nagella P, Ahn, YS, Kim SJ, Ahmad A. Composition of the essential oil and petroleum ether extract of *Lycium chinense* Miller fruits and antioxidant activity of its several extracts. *J Med Plants Res*, 2011, 5(25): 5973-5981.
11. Jung K, Chin YW, Kim YC, Kim J. Potentially hepatoprotective glycolipid constituents of *Lycium chinense* fruits. *Arch Pharm Res*, 2005, 28: 1381–1385.
12. Shin YG, Cho KH, Kim JM, Park MK, Park JH. Determination of betaine in *Lycium chinense* fruits by liquid chromatography–electrospray ionization mass spectrometry. *J Chromatogr A*, 1999, 857: 331–335.
13. Jung WS, Chung IM, Ali M, Ahmad A. New steroidal glycoside ester and aliphatic acid from the fruits of *Lycium chinense*. *J Asian Nat Prods Res*, 2012, 14: 301–307.
14. Chung IM, Ali M, Kim EH, Ahmad A. New tetraterpene glycosides from the fruits of *Lycium chinense*. *J Asian Nat Prod Res*, 2013, 15: 136–144.
15. Chung IM, Ali M, Nigella P, Siddiqui NA, Ahmad A. Evaluation of antioxidant activity of new constituents from the fruits of *Lycium chinense*. *Med Chem Res*, 2014, 23: 3852-3860.
16. Chung IM, Ali M, Parveen N, Kim SH, Ahmad A. New polyglucopyranosyl and polyarabinosyl of fatty acid derivatives from the fruits of *Lycium chinense* and its antioxidant activity. *Food Chem*, 2014, 151: 435-443.