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New phosphoglycerides from the seeds of *Lactuca sativa L.*

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Abstract The seeds of *Lactuca sativa* L. (Asteraceae) are regarded as an anodyne, galactagogue, demulcent, emollient and used to treat bronchitis, asthma and whooping cough. Phytochemical investigation of the methanolic extract of the seeds of *L. sativa* led to isolation of phytoconstituents characterized as glyceryl-1,2-dioleiyl-3-phosphate (**1**), glyceryl-1-octadec-9′-enoate-3-phosphate (**2**), glyceryl-1-octadec-9′-enoate-2-hexadecanoate-3-phosphate (**3**), glyceryl-1-octadec-9′-enoate-2-octadecanoate-3-phosphate (**4**) and *β*-sitosterol (**5**). The structures of all the isolated phytoconstituents have been estabilished on the basis of spectral data analysis and chemical reactions.

Keywords *Lactuca sativa*, Asteraceae, Seeds, Phosphoglycerides, Structures elucidation.

Introduction

Lactuca sativa L.; syn. *Lactuca scariola* L. var. *sativa* L. (Asteraceae), commonly known as kahu or garden lettuce, is an erect, glaucescent, annual or biennial herb, up to 2 m in height, with milky sap and hairless leaves. It grows wildly in the western Himalayan tract, Siberia, Mediterranean region and westwards to the Atlantic [1, 2]. It is mostly used for salad and cooked as a vegetable. The plant is a sedative and the dried milk of the cultivated forms is used as a calmant. Its seeds are regarded as an anodyne, galactagogue and emollient. The viscous milky juice of the wild forms is used as a substitute for opium [3]. The fresh plant is a mild sedative, purgative, diuretic, diaphoretic and antispasmodic. It has been found useful in the treatment of the coughs, phthisis, bronchitis, asthma and whooping cough. A decoction of the seeds is taken as a demulcent and to cure chronic bronchitis. A lettuce poultice is applied to treat burning sensation and scalding, and to painful and irritable ulcers [4]. It is effective as a depressant, analgesic and anti-inflammatory remedy [5]. The steroidal constituents, lactucaxanthin [6], hydrolactucin, lactucin and lactupicrin [7], lactuside A and macroliniside A [8-10] were the reported phytoconstituents from *L. sativa*. The present paper describes the isolation and characterization of new phosphoglycerides from the *L. sativa* seeds.

Materials and methods

General

Melting points were determined on a Perfit melting apparatus (Ambala, Haryana, India) and are uncorrected. UV spectra were measured with a Lambda Bio 20 spectrophotometer (Perkin-Elmer-Rotkreuz, Switzerland) in

methanol. Infrared spectra were recorded on Bio-Rad FTIR 5000 (FTS 135, Kawloon, Hong Hong) spectrophotometer using KBr pellets; λ_{max} values are given in cm^{-1}. The ^{1}H and ^{13}C NMR spectra were screened on Avance DRX 300, Bruker spectrospin 300 and 75 MHz instruments, respectively, (Karlesruthe, Germany) using $CDCl_3$ and TMS as an internal standard. EIMS were scanned at 70 eV on a Jeol D-300 instrument (Jeol, USA). Column chromatography was performed on silica gel (60-120 mesh; Qualigen, Mumbai, India). TLC was run on silica gel G (Qualigen). Spots were visualized by exposing to iodine vapours, UV radiation and with spraying ceric sulphate solution

Plant material

L. sativa seeds were procured from Khari Baoli market of Delhi and authenticated by Dr. M. P. Sharma, Taxonomist, Department of Botany, Jamia Hamdard, New Delhi. A voucher specimen is deposited in the herbarium of the Phytochemical Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi.

Extraction and isolation

The dried drug (2 kg) was coarsely powdered, defatted with petroleum ether and exhaustively extracted with methanol (95 %). The combined extracts were then concentrated on a steam bath and dried under reduced pressure to get 450 g (22.5 % yield) of dark brown mass. It was dissolved in minimum quantity of methanol and adsorbed on silica gel (60-120 mesh) for the preparation of slurry. The slurry was dried in air and chromatographed over silica gel column packed in petroleum ether. The column was eluted with petroleum ether, chloroform and methanol successively in the order of increasing polarity to isolate the following compounds:

Glyceryl-1, 2-dioleiyl-3-phosphate (1)

Elution of the column with petroleum ether-chloroform (3:1) eluants gave a buff white amorphous powder of **1**, recrystallized from chloroform-methanol (1:1), 228 mg (0.0015% yield); R_f: 0.43 (petroleum ether-chloroform, 1:1); m.p.: 69-70 °C; IR ν_{max} (KBr): 3500, 2925, 2854, 1735, 1640, 1462, 1378, 1247, 1215, 1025, 793, 771 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.27 (2H, m, H-9′, H-9″), 5.21 (2H, m, H-10′, H-10″), 4.79 (1H, m, H-2), 4.42 (2H, m, H_2-1), 3.58 (2H, m, H_2-3), 2.35 (2H, m, H_2-2′), 2.32 (2H, m, H_2-2″), 2.22 (2H, m, H_2-8′), 2.19 (2H, m, H_2-8″), 1.97 (2H, m, H_2-11′), 1.94 (2H, m, H_2-11″), 1.55 (4H, m, 2 x CH_2), 1.19 (36H, brs, 18 × CH_2), 0.85 (3H, t, *J*=6.1 Hz, Me-17′), 0.80 (3H, t, *J*=6.3 Hz, Me-17″); ^{13}C NMR ($CDCl_3$): δ 170.97 (C-1′), 169.76 (C-1″), 129.98 (C-9′), 129.71 (C-9″), 121.62 (C-10′), 118.85 (C-10″), 80.94 (C-2), 68.85 (C-1), 65.06 (C-3), 34.50 (CH_2), 34.15 (CH_2), 31.90 (CH_2), 29.68 (10×CH_2), 29.59 (CH_2), 29.51 (CH_2), 29.43 (CH_2), 29.32 (CH_2), 28.37 (CH_2), 25.4168 (CH_2), 22.68 (CH_2), 14.52 (Me-18′), 14.09 (Me-18″); +ve ESI MS *m/z*: 700 $[M]^+$ ($C_{39}H_{73}O_8P$) (2.5).

Glyceryl-1-octadec-9′-enoate-3-phosphate (2)

Further elution of the column with petroleum ether- chloroform (3:1) eluents afforded a yellow amorphous powder of **2**, recrystallized from chloroform-methanol, 351mg (0.0017% yield). R_f: 0.33 (petroleum ether-chloroform, 1:1); m.p.: 79-80 °C; IR ν_{max} (KBr): 3450, 2919, 2850, 1739, 1640, 1463, 1380, 1250, 1210, 1025, 793, 745 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.28 (1H, m, H-9′), 5.21 (2H, m, H-10′), 4.18 (1H, m, H-2), 4.09 (2H, m, H_2-1), 3.57 (2H, m, H_2-3), 2.69 (2H, m, H_2-2′), 2.24 (2H, m, H_2-8′), 2.09 (2H, m, H_2-11′), 1.52 (2H, brs, CH_2), 1.18 (20H, brs, 10×CH_2), 0.77 (3H, t, *J*=6.1 Hz, Me-18′); ^{13}C NMR ($CDCl_3$): δ 173.11 (C-1′), 127.83 (C-9′), 119.22 (C-10′), 78.91 (C-2), 67.86 (C-1), 65.14 (C-3), 45.53 (C-2′), 37.10 (C-8′), 31.92 (C-11′), 29.20 (7 × CH_2) 27.69 (CH_2), 24.85 (CH_2), 22.69 (CH_2), 20.37 (CH_2), 14.10 (Me-18′); +ve ESI MS *m/z*: 436 $[M]^+$ ($C_{21}H_{41}O_7P$) (2.5).

Glyceryl-1-oleiyl-2-palmityl-3-phosphate (3)

Elution of the column with chloroform eluants yielded a brown sticky mass of **3**, recrystallized from methanol, 331 mg (0.0022 % yield); R_f: 0.55 (chloroform: methanol, 4:1); m.p.: 60-61 °C; IR ν_{max} (KBr): 3410, 3370, 2923, 2853, 1745, 1738, 1640, 1468, 1378, 1247, 1215, 1025, 795, 725 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.27 (1H, m, H-9′), 5.19 (2H, m, H-10′), 4.20 (1H, m, H-2), 4.11 (2H, m, H_2-1), 3.59 (2H, m, H_2-3), 2.69 (2H, m, H_2-2′), 2.34 (2H, m, H_2-2″), 2.04

(2H, brs, H_2-8′), 1.97 (2H, m, H_2-11″), 1.54 (4H, brs, 2 × CH_2), 1.18 (44H, brs, 22 × CH_2), 0.84 (3H, t, *J*=6.5 Hz, Me-18″), 0.80 (3H, t, *J*=6.1 Hz, Me-16″); +ve ESI MS *m/z*: 674 $[M]^+$ ($C_{37}H_{71}O_8P$) (1.4).

Glyceryl-1-oleiyl-2-stearyl-3-phosphate (4)

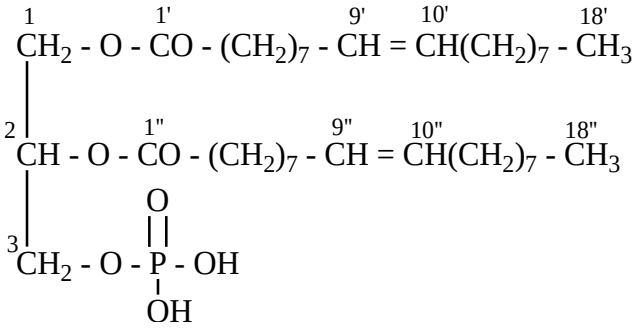
Further elution of the column with chloroform eluents furnished a colorless sticky mass of **4**, recrystallized from chloroform- methanol (1:1), 350 mg (0.0017 % yield); R_f: 0.53 (petroleum ether-chloroform-methanol, 1:4:1); m.p.: 86-87 °C; IR ν_{max} (KBr): 3374, 2923, 2854, 1743, 1728, 1643, 1375, 1245, 1027, 793, 735 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.35 (1H, m, H-9′), 5.29 (1H, m, H-10′), 4.16 (1H, m, H_2-2), 4.06 (2H, d, *J*=9.5 Hz, H_2-1), 3.46 (2H, d, *J*=10.5 Hz, H_2-3), 2.77 (2H, m, H_2-2′), 2.32 (2H, m, H_2-2″), 2.04 (2H, m, H_2-8′), 1.62 (2H, m, H_2-11′), 1.57 (4H, m, 2 x CH_2), 1.30 (18 H, brs, 9×CH_2), 1.26 (30 H, brs, 15×CH_2), 0.86 (3H, t, *J*=6.5 Hz, Me-18′) 0.82 (3H, t, *J*=6.3 Hz, Me-18″); EIMS *m/z*: 702 $[M]^+$ ($C_{39}H_{75}O_8P$) (2.3).

***β*-Sitosterol (5)**

Elution of the column with chloroform-methanol $CHCl_3$-MeOH (49:1) gave a colorless amorphous powder of **5**, recrystallized from acetone, 125 mg (0.0008 % yield); R_f: 0.43 (petroleum ether-chloroform-methanol, 1:4:1); m.p. and m.p.: 135-137 °C; IR ν_{max} (CCl_4): 3450, 1620 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.30 (d, *J*=5.5 Hz, H-6), 3.50 (1H, brm, $w_{½}$=18.5 Hz, H-3α), 1.01 (3H, brs, Me-19), 0.97 (3H, d, *J*=6.5 Hz, Me-21), 0.86 (3H, d, *J*=6.0 Hz, Me-26), 0.81 (3H, d, *J*=6.0 Hz, Me-27), 0.83 (3H, d, *J*=6.03 Hz, Me-29), 0.67 (3H, brs, Me-18) ; ESI MS *m/z*: 414 $[M]^+$ ($C_{29}H_{50}O$) (21.3).

Results and Discussion

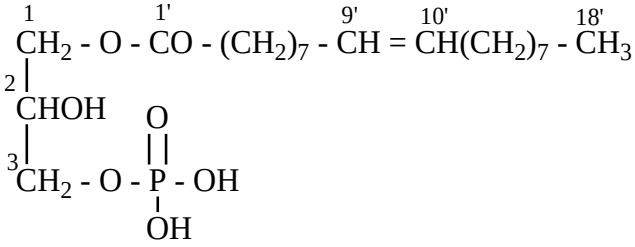
Compound **1** responded positively to bromine water test for unsaturation and showed IR absorption bands for hydroxyl group (3500 cm^{-1}), ester group (1735 cm^{-1}), unsaturation (1640 cm^{-1}) and long aliphatic chain (793, 791 cm^{-1}). Its mass spectrum displayed a molecular ion peak at *m/z* 700 ($C_{39}H_{73}O_8$ P). The ^1H NMR spectrum of **1** displayed two downfield multiplets, integrating for two-protons each, at δ 5.27 and 5.21 assigned to vinylic protons H-9′, H-9″ and H-10′ and H-10″. The methylene protons appeared between δ 2.35-1.19. Two three-proton triplets at δ 0.85 (*J*=6.1 Hz) and 0.80 (*J*=6.3 Hz) were assigned to terminal methyl protons Me-18′ and Me-18″, respectively. Its ^{13}C NMR spectrum exhibited signals for ester carbons (δ 170.97 for C-1′, 169.76 for C-1″), vinylic carbons between δ 129.98-118.85, oxygenated glycerol carbons at δ 80.94 (C-2), 68.85 (C-1) and 65.06 (C-3), methylene carbons from δ 34.50-22.68 and primary methyl carbons δ 14.52 (C-18′) and 14.09 (C-18″). On the basis of above discussion the structure of **1** has been elucidated as glyceryl-1, 2-dioleiyl-3-phosphate.



1. Glyceryl-1,2-dioleiyl-3-phosphate

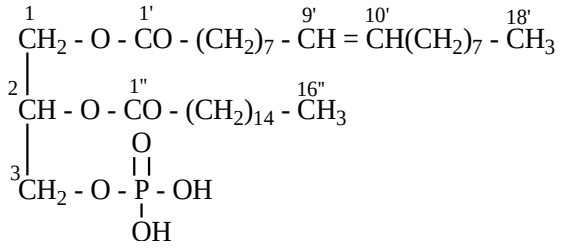
Compound **2** gave positive tests for unsaturation and displayed IR absorption bands for hydroxyl groups (3450 cm^{-1}), ester group (1739), unsaturation (1640 cm^{-1}) and long aliphatic chain (793, 745 cm^{-1}). Its mass spectrum exhibited a molecular ion peak relating to a molecular formula $C_{21}H_{41}O_7$ P (*m/z* 436). The ^1H NMR spectrum of **2** exhibited two one-proton multiplets at δ 5.28 and 5.21 assignable to vinylic H-9′ and H-10′ methine protons, respectively, oxygenated methylene protons as two-proton multiplets at δ 4.09 (H_2-1) and 3.57 (H_2-3),

hydroxymethine proton at δ H-2, methylene protons from δ 2.69 to 1.18 and a three-proton triplet at δ 0.77 (*J*=6.1 Hz) ascribed to terminal methyl C-18′ protons. The ^{13}C NMR spectrum of **2** displayed important signals for ester carbon at δ 173.11 (C-1′), vinylic carbons at δ 127.83 (C-9′) and 119.22 (C-10′), oxygenated methylene carbons at δ 67.86 (C-1) and 65.14 (C-3), carbinol carbon at δ 78.91 (C-2), methylene carbons between δ 45.53 -20.37 and methyl carbon at δ 14.10 (C-18′). On the basis of above discussion the structure of **2** has been characterized as glyceryl-1-octadec-9′-enoate-3-phosphate.



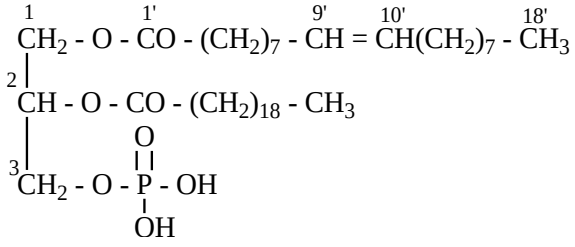
2. Glyceryl-1-octadec-9′-enoate-3-phosphate

Compound **3** gave positive tests for unsaturation and showed IR absorption bands for hydroxyl groups (3410, 3370 cm^{-1}), ester functions (1745, 1738 cm^{-1}), unsaturation (1640 cm^{-1}) and long chain aliphatic chains (795, 725 cm^{-1}). It had a molecular ion peak at *m/z* 674 ($C_{37}H_{71}O_8P$) in the mass spectrum. The ^1H NMR spectrum of **3** displayed two one-proton downfield multiplets at δ 5.27 and 5.19 assigned to vinylic H-9′ and H-10′ protons, respectively. A one-proton multiplet at δ 4.20 and two multiplets at δ 4.11 (2H) and 3.59 (2H) were ascribed to oxygenated methine H-2 and methylene H_2-1 and H_2-3 protons, respectively. The remaining methylene protons resonated from δ 2.69 to 1.18. Two three-proton triplets at δ 0.84 (*J*=6.5 Hz), 0.80 (*J*=6.1 Hz) were accounted correspondingly to primary C-18′ and C-16″ methyl protons. On the basis of foregoing discussion the structure of **3** has been formulated as glyceryl-1-octadec-9′-enoate-2-hexadecanoate-3-phosphate.



3. Glyceryl-1-oleiyl-2-palmityl-3-phosphate

Compound **4**, $[M]^+$ at *m/z* 702 ($C_{39}H_{75}O_8P$), responded positively to bromine water test for unsaturation and showed IR absorption bands for hydroxyl groups (3374 cm^{-1}), ester functions (1743, 1728 cm^{-1}), unsaturation (1643 cm^{-1}) and long aliphatic chain (793, 735 cm^{-1}). The ^1H NMR spectrum of **4** displayed two one-proton downfield multiplets at δ δ 5.35 and 5.29 assigned to vinylic H-9′ and H-10′ protons, respectively.



4. Glyceryl-1-oleiyl-2-stearyl-3-phosphate

A one-proton multiplet at δ 4.16 (1H, m, H_2-2) and two two-proton doublets at δ 4.06 (2H, d, *J*=9.5 Hz,), 3.46 (2H, d, *J*=10.5 Hz, H_2-3) were ascribed to oxygenated methine H-2 and methylene H_2-1 and H_2-3protons, respectively. The remaining methylene protons appeared between 2.77-1.26. Two three-proton triplets at δ 0.86 (*J*=6.5 Hz) and 0.82 (*J*=6.3 Hz) were accounted to primary C-18′ and C-18″ methyl protons, respectively. On the basis of these evidences the structure of **4** has been established as glyceryl-1-octadec-9′-enoate-2-octadecanoate-3-phosphate.

Compound **5** was a known phytosterol identified as β-sitosterol.

Aknowledgement

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