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Phytochemical investigation of the leaves of *Borago officinalis* L.

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ABSTRACT

*Borago officinalis L. (Boraginaceae), commonly known as bee bread or borage, is a native to Europe, North Africa and Asia minor and cultivated in some countries including Iran, Turkey, Spain and India as ornament. Its leaves are useful as diuretic, demulcent, emollient and expectorant. Phytochemical investigation of its leaves led to the isolation of a new acyclic triterpenic ester, two fatty acid ester and a steroidal ester characterized 3,7,11,15,19,23-hexamethyl-n-tetracos-cis-6,cis-12-dien-18β,23-diol-1dodecanoate (boragoterpenyl laurate) (**1**), 4'β-hydroxynon-cis-6'-enyl-1'-octadecanoate (hydroxynonenyl stearate) (**2**), n-nonacosanyl-n-octanoate (n-nonacosanylcaprylate) (**3**) and stigmast-5,22-dien-3β-olyl-n-octadec-9'-enoate (boragosteryl oleate) (**4**). The structures of all the isolated phytoconstituents have been elucidated on the basis of spectral data analysis and chemical reactions.*

Keywords: *Borago officinalis* L., Boraginaceae, leaves, esters.

INTRODUCTION

Borago officinalis L. (Boraginaceae), commonly known as bee bread or borage, is a course, hairy, erect, annual herb, 30-70 cm high. It is a native to Mediterranean region and North Africa and cultivated in Iran, Turkey, Spain, Asia Minor and India as an ornamental and to repel insects. The leaves are bristly hairy with large ovate stalked lower leaves, narrower, wavy-edged stalkless upper ones. The tender leaves and flowers are relished as pot herb and used in pickles and cakes. They import a pleasant flavour and cooling effect to beverages. The mature leaves are suspected to cause dermatititis [1]. It is mainly marketed in pharmaceutical sector in health foods and nutritional supplements. There is a small market for borage as a spice and for the young leaves in salad. Mucilage from the crushed foliage is useful to treat catarrh, rheumatism and some skin diseases. Borage has beneficial effect on the brain, being used to dispel melancholy and to induce euphorbia. The leaves and flowers are adrenal gland stimulant, restorative, galactogogue, diuretic, diaphoretic, expectorant, laxative, febrifuge, nervine and antidepressive [1]. Borage oil is taken orally in nutritional and clinical supplements where impaired or inadequate Δ-6 desaturase activity may be involved in the initiation and progression of several dicades [2]. The impairement may be alleviated by dietary supplementation with γ-linolenic acid. Pyrrolizidine alkaloids [3,4], γ-linolenic acid [5], cyanogenetic glycoside dhurrine [1], rosmarinic acid [6], essential oil composed mainly of nonadecane, tetracosane and heptacosane [7] and fatty acids consisting of α- and γ-linolenic, stearidonic and palmitic acids [7,8,2] have been reported from the borage leaves. This manuscript describes isolation and characterization of acyclic triterpenic, steroidal and fatty acid esters from the borage leaves.

MATERIALS AND METHODS

The melting points were determined on a Perfit apparatus and are uncorrected. The IR spectra were recorded on KBr pellet using a Jasco FT/IR-5000 instrument (FTS 135, Hongkong). The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were scanned on Avance DRX 400, Bruker spectrospin 400 MHz instrument (Rheinstetten, Germany) using $CDCl_3$ as solvent and TMS as internal standard. FAB-MS were measured using JEOL-JMS-DX 303

spectrometer (Peabody, MA, USA). Column (450×4×0.2 cm) chromatography was performed on silica gel (60-120 mesh, Qualigens, Mumbai, India) and thin layer chromatography on silica gel G-coated TLC plates (Merck). Spots were visualized by exposure to iodine vapours, UV radiation and by spraying with ceric sulphate solution.

Plant material

The leaves of *B. officinalis* were procurred from local market of Delhi, Khari Baoli and authenticated by Prof. M.P. Sharma, Taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard (Hamdard University). A voucher specimen No. PRL/JH/08/48 is deposited in the herbarium of the Phytochemistry Research Laboratory, Jamia Hamdard, New Delhi, India.

Extraction and isolation

The dried and pulverized leaves (3 kg) were extracted exhausted with ethanol (95 %). The ethanolic extract was concentrated under reduced pressure to yield brown, viscous, syrupy mass (150 g, 5.0 %). It was dissolved in minimum amount of methanol and adsorbed on silica (60-120 mesh) for the preparation of slurry. It was dried and chromatographed over silica gel column packed in petroleum ether (b.p. 60-80 ºC). The column was eluted successively with petroleum ether, mixture of petroleum ether and chloroform (9:1, 3:1, 1:1, and 1:3 *v/v*), chloroform and chloroform-methanol (99:1, 49:1, 19:1, 9:1 *v/v*) in order of increasing polarity to isolate the following compounds:

Boragoterpenyl laurate (1)

Elution of column with petroleum ether furnished colourless crystals of **1**, recrystallized from chloroform:acetone (1:1), 250 mg (0.0069 % yield), R_f: 0.4 (petroleum ether : chloroform, 9:1); m.p. 98-99 ºC; UV λ_{max} (MeOH): 210 nm (log ε 5.1); IR ν_{max} (KBr): 3368, 2920, 2851, 1733, 1635, 1461, 1376, 1166, 1009, 793, 721 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.34 (1H, dd, *J*= 5.5, 5.3 Hz), 5.25 (1H, m, $w_{1/2}$=8.3 Hz, H-13), 5.11 (1H, dd, *J*=6.5, 5.3 Hz, H-12), 4.50 (2H, t, *J*=8.5 Hz, H-2′), 3.63 (1H, brm, $w_{1/2}$=17.1 Hz, H-18a), 2.86 (1H, m, H-11), 2.80 (2H, m, H_2-8), 2.29 (2H, t, *J*=7.2Hz, H_2-2′), 2.04 (1H, m, H-19), 1.85 (2H, m, H_2-14), 1.83 (2H, m, H_2-5), 1.68 (4H, brs, H_2-22, H_2-17), 1.60 (3H, brs, Me-26), 1.52 (4H, m, 2×CH_2), 1.29 (6H, brs, 3×CH_2), 1.25 (8H, brs, 4×CH_2), 1.25 (14H, brs, 7×CH_2), 1.22 (6H, brs, Me-24, Me-30), 1.01 (3H, d, *J*=6.1 Hz, Me-29), 0.96 (3H, d, *J*=6.3 Hz, Me-28), 0.87 (3H, d, *J*=6.5 Hz, Me-27), 0.77 (3H, d, *J*=7.1Hz, Me-25), 0.65 (3H, t, *J*=6.1 Hz, Me-12′); ^{13}C NMR ($CDCl_3$): δ 173.61 (C-1′), 140.75 (C-7), 130.16 (C-12), 129.72 (C-13), 117.51 (C-6), 80.49 (C-23), 72.36 (C-18), 62.65 (C-1), 50.66 (C-11), 48.72 (C-19), 22.23 (C-15), 39.67 (C-3), 32.78 (C-5), 36.94 (C-8), 36.82 (C-14), 35.89 (C-2′), 34.78 (C-22), 32.14 (CH_2), 31.53 (CH_2), 31.34 (CH_2), 30.98 (CH_2), 30.15 (CH_2), 29.66 (CH_2), 29.42 (CH_2), 29.32 (CH_2), 29.22 (CH_2), 29.11 (4×CH_2), 28.10 (CH_2), 27.91 (CH_2), 27.53 (CH_2), 25.68 (CH_2), 24.77 (Me-24, Me-30), 23.30 (Me-26), 23.30 (Me-25), 22.60 (Me-29), 21.0 (Me-26), 18.1 (Me-27), 15.8 (Me-28), 14.0 (Me-12′); +ve FAB MS *m/z* (rel. int): 649 $[M+H]^+$ ($C_{42}H_{81}O_4$) (29.3), 589 (4.3), 489 (7.8), 419 (17.6), 406 (70.1), 393 (41.7), 390 (31.6), 365 (18.9), 325 (23.5), 323 (22.6), 283 (18.2), 255 (46.7), 229 (27.5), 227 (30.2), 199 (31.6), 183 (36.5), 159 (35.2), 129 (23.6).

Hydroxynonenyl stearate (2)

Elution of column with petroleum ether: chloroform (1:1) yielded colourless crystalline crystals of **2**, recrystallized from chloroform 310 mg (0.0086 % yield); R_f: 0.6 (chloroform:methanol, 1:1); m.p. 41-42 ºC; UV λ_{max} (MeOH): 207 nm (log ε 4.1); IR ν_{max} (KBr): 3510, 2919, 2850, 1725, 1640, 1460, 1015, 725 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.36 (1H, m, $w_{1/2}$= 8.5 Hz, H-6′), 5.33 (1H, m, $w_{1/2}$=8.1 Hz, H-7′), 4.47 (2H, t, *J*=4.8 Hz, H_2-1′), 3.63 (1H, brm, $w_{1/2}$=16.6 Hz, H-5*α*), 2.33 (2H, t, *J*=7.5 Hz, H_2-2), 2.27 (2H, m, H_2-5′), 2.24 (2H, m, H_2-8′), 2.19 (2H, m, CH_2), 2.13 (2H, m, CH_2), 1.54 (2H, m, CH_2), 1.29 (12H, brs, 6×CH_2), 1.25 (6H, brs, 3×CH_2), 1.17 (10H, brs, 5×CH_2), 0.88 (3H, t, *J*=6.5 Hz, Me-9′), 0.84 (3H, t, *J*=6.3Hz, Me-18); ^{13}C NMR ($CDCl_3$): δ 173.71 (C-1), 130.10 (C-6′), 129.93 (C-7′), 80.62 (C-4′), 63.11 (C-1′), 34.38 (C-5), 33.85 (C-2), 32.75 (C-8′), 31.90 (C-3′), 29.67 (C-3), 29.44 (2×CH_2), 29.33 (2×CH_2), 29.14 (5×CH_2), 27.94 (CH_2), 27.56 (CH_2), 27.18 (CH_2), 25.13 (CH_2), 24.21 (CH_2), 22.66 (CH_2), 21.94 (C-9′), 14.29 (C-18); +ve FAB MS *m/z* (rel. int): 425 $[M+H]^+$ ($C_{27}H_{53}O_3$) (20.3), 409 (27.8), 406 (30.9), 395 (16.8), 369 (10.1), 355 (11.6), 325 (12.2), 283 (12.7), 255 (22.6), 169 (38.1), 141 (25.3).

***n*-Nonacosanyl caprylate (3)**

Elution of the column with petroleum ether-chloroform (1:3) gave colourless crystals of **3**, recrystallized from chloroform-methanol (1:1), 210 mg (0.0058 % yield); R_f: 0.7 (chloroform:methanol, 1:1); m.p. 71-72 ºC; UV λ_{max} (MeOH): 260 nm (log ε 5.3); IR ν_{max} (KBr): 2917, 2850, 1732, 1615, 1465, 794, 723 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.99 (1H, t, *J*=6.9 Hz, H_2-1′), 2.37 (1H, t, *J*=7.5 Hz, H_2-2), 1.63 (2H, m, H_2-2), 1.52 (2H, m, CH_2), 1.29 (50H, brs, 25×CH_2), 1.25 (10H, brs, 5×CH_2), 0.93 (3H, t, *J*=6.3 Hz, Me-8), 0.85 (3H, t, *J*=6.9 Hz, Me-29′); ^{13}C NMR ($CDCl_3$): δ 171.65 (C-1), 64.30 (C-1′), 31.92 (C-2), 29.69 (2×CH_2), 29.43 (26×CH_2), 29.35 (2×CH_2), 29.24 (CH_2), 22.68 (CH_2), 14.10 (C-8, C-29′); +ve FAB MS *m/z* (rel. int): 551 $[M+H]^+$ ($C_{37}H_{75}O_2$) (7.1), 423 (8.2), 143 (17.9), 127 (13.1).

Boragosteryl oleate (4)

Elution of the column with chloroform afforded colourless crystals of **4**, recrystallized from chloroform, 330 mg (0.00916 % yield); R_f: 0.5 (chloroform:methanol, 19:1); m.p. 115-116 ºC; UV λ_{max} (MeOH): 240 nm (log ε 4.5); IR ν_{max} (KBr): 3381, 2919, 2851, 1732, 1698, 1640, 1470, 1365, 1365, 1308, 1035, 723 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.34 (1H, d, *J*=5.5 Hz, H-6), 5.25 (1H, m, H-22), 5.21 (1H, m, H-23), 5.05 (1H, m, H-9′), 4.93 (1H, m, H-10′), 4.02 (1H, brm, $w_{1/2}$=16.5 Hz, H-3α), 2.36 (2H, t, *J*=7.3 Hz, H_2-2′), 2.29-1.16 (51H, m, 22×CH_2, 7×CH_2), 1.01 (3H, brs, Me-19), 0.96 (3H, d, *J*=6.3 Hz, Me-21), 0.87 (3H, d, *J*=6.6 Hz, Me-26), 0.85 (3H, d, *J*=6.0 Hz, Me-27), 0.82 (3H, t, *J*=7.1 Hz, Me-29), 0.78 (3H, t, *J*=6.3 Hz, Me-18′) ; ^{13}C NMR ($CDCl_3$): δ 37.24 (C-1), 31.59 (C-2), 71.83 (C-3), 40.48 (C-4), 140.76 (C-5), 121.69 (C-6), 31.15 (C-7), 31.90 (C-8), 51.23 (C-9), 36.49 (C-10), 21.07 (C-11), 39.76 (C-12), 42.25 (C-13), 56.85 (C-14), 24.29 (C-15), 128.23 (C-16), 56.04 (C-17), 178.73 (C-18), 19.38 (C-19), 36.13 (C-20), 18.77 (C-21), 138.31 (C-22), 130.04 (C-23), 45.82 (C-24), 29.25 (C-25), 19.80 (C-26), 19.02 (C-27), 23.73 (C-28), 11.84 (C-29), 173.72 (C-1′), 34.37 (C-2′), 33.63-22.68 (13×CH_2), 129.75 (C-9′), 129.36 (C-10′), 14.16 (C-18′); +ve FAB MS *m/z* (rel. int): 707 $[M+H]^+$ ($C_{47}H_{79}O_4$) (3.1), 425 (21.8), 286 (18.9), 281 (12.3), 271 (29.2), 265 (55.1), 241 (21.7).

RESULTS AND DISCUSSION

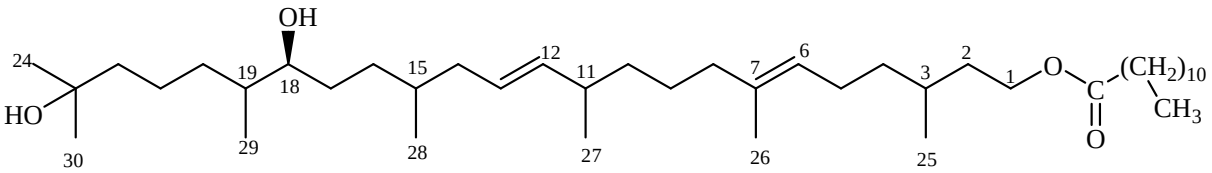
Compound **1**, named boragoterpenyl laurate, was obtained as a colourless crystalline mass from petroleum ether eluents. Its IR spectrum showed characteriristic absorption bands for hydroxyl groups (3368 cm^{-1}), ester function (1733 cm^{-1}), unsaturation (1635 cm^{-1}) and long aliphatic chain (793, 721 cm^{-1}). It had molecular ion peak at *m/z* 649 $[M+H]^+$ on the basis of FAB mass and ^{13}C NMR spectra consistent with the molecular formula of a triterpenic ester $C_{48}H_{81}O_4$ with three double bond equivalents. The ion peaks arising at *m/z* 183 $[CH_3\ (CH_2)_{10}CO]^+$, 199 $[CH_3(CH_2)_{10}COO]^+$, 589 $[M\text{-}Me_2COH]^+$, 406 [589-183]$^+$ and 390 [589-199]$^+$ indicated that tertiary hydroxyl was located at one terminal of the molecule and laurate group at other end of the chain. The ion fragments generating at *m/z* 129 [C_{18}-C_{19} fission, $Me_2C(OH)(CH_2)_3CHMe]^+$, 519 $[M\text{-}129]^+$, 159 [C_{17}-C_{18} fission, $Me_2C(OH)(CH_2)_3CH(OH)Me]^+$, 489 $[M\text{-}159]^+$, 229 [C_{13}-C_{14} fission]$^+$, 419 $[M\text{-}229]^+$, 255 [C_{11}-C_{12} fission]$^+$, 393 $[M\text{-}255]^+$, 325 [C_7-C_8 fission]$^+$, 323 $[M\text{-}325]^+$, 365 [C_5-C_6 fission]$^+$ and 283 $[M\text{-}365]^+$ suggested the existence of one of the hydroxyl group at C-18 and vinylic linkages at C-12 and C-6. The ^1H NMR spectrum of **1** showed three one-proton signals as double doublets at δ 5.34 (*J*=5.5, 5.3 Hz) and 5.11 (6.5, 5.3 Hz) and as a multiplet at δ 5.25 ($w_{1/2}$=8.3 Hz) assigned to *cis*-oriented H-6, H-12 and H-13 vinylic protons, respectively. A two-proton triplet at δ 4.50 (*J*=8.5 Hz) and a one-proton multiplet at δ 3.63 with half-width of 17.1 Hz were attributed to oxygenated methylene H_2-1 and carbinol H-18α protons, respectively. A three-proton broad signal δ 1.60, a six-proton broad singlet at δ 1.22, four three-proton doublets at δ 1.01 (*J*=6.1 Hz), 0.96 (*J*=6.3 Hz), 0.87 (6.5 Hz), 0.77 (*J*=7.1 Hz) and a three-proton triplet at δ 0.65 (*J*=6.1 Hz) were ascribed to C-26 methyl protons attached to the vinylic carbon, tertiary C-24 and C-30 methyl protons, secondary C-29, C-28, C-27 and C-25 methyl protons, and primary C-12′ methyl protons, respectively. The remaining methylene and methine protons appeared between δ 2.86-1.25. The ^{13}C NMR spectrum of **1** showed signals for ester carbon at δ 173.61 (C-1′), vinylic carbons at δ 140.75 (C-7), 130.16 (C-12), 129.72 (C-13) and 117.51 (C-6), carbinol quaternary carbon at δ 80.49 (C-23), carbinol secondary carbon at δ 72.36 (C-18), oxygenated methylene carbon at δ 62.65 (C-1), methine and methylene carbons from δ 50.66 to 25.68 and methyl carbons between δ 24.77-14.0. Alkaline hydrolysis of **1** yielded lauric acid as one of the component, m.p. 43-44 ºC; TLC comparable. On the basis of these evidences, the structure of **1** has been elucidated as 3,7,11,15,19,23-hexamethyl-*n*-tetracos-cis-6,cis-12-dien-18β,23-diol-1-dodecanoate. It is a new acyclic triterpenic ester.

Compound **2**, named hydroxynonenyl stearate, was obtained from petroleum ether-chloroform (1:1) eluents. Its IR spectrum displayed distinctive absorption bands for hydroxyl group (3510 cm^{-1}), ester function (1725 cm^{-1}), unsatuaration (1640 cm^{-1}) and long aliphatic chain (725 cm^{-1}). The molecular ion peak of **2** was determined at *m/z* 425 $[M+H]^+$ ($C_{27}H_{53}O_3$) on the basis of mass and ^{13}C NMR spectra. The ion fragments arising at *m/z* 283 $[CH_3(CH_2)_{16}COO]^+$ and 141 [M-283, $(CH_2)_3CHOHCH_2$-CH=$CHCH_2CH_3]^+$ suggested that stearic acid was esterified with nonendiol. The ion peaks generating at *m/z* 325 [$C_{3'}$-$C_{4'}$, fission, $CH_3(CH_2)_{16}COO(CH_2)_3]^+$, 355 [$C_{4'}$-$C_{5'}$ fission; $CH_3(CH_2)_{16}$ $COO(CH_2)_3CHOH]^+$ and 369 [$C_{5''}$-$C_{6'}$ fission, $CH_3(CH_2)_{16}COO(CH_2)_3CHOHCH_2]^+$ indicated location of the hydroxyl group at C-4′ and vinylic linkage at C-6′. The ^1H NMR spectrum of **2** showed two one-proton multiplets at δ 5.36 ($w_{1/2}$=8.5 Hz) and 5.33 ($w_{1/2}$=8.1 Hz) assigned to *cis*-oriented vinylic H-6′ and H-7′ protons, respectively, a two-proton triplet at δ 4.47 (*J*=4.8 Hz) ascribed oxygenated methylene H_2-1′ protons, one-proton broad multiplet at δ 3.63 with half-width of 16.6 Hz attributed to α-oriented carbinol H-5 proton, two three-proton triplets at δ 0.88 (*J*=6.5 Hz) and 0.84 (*J*=6.3 Hz) accounted to primary C-9′ and C-18 primary methyl protons, respectively, and methylene protons between δ 2.33-1.17. The ^{13}C NMR spectrum of **2** exhibited signals for ester carbon at δ 173.71 (C-1), vinylic carbons at δ 130.10 (C-6′) and 129.93 (C-7′), hydroxyl methine carbon at δ 80.62 (C-4′), oxygenated methylene carbon at δ 63.11 (C-1′), methylene carbons between δ 34.38-22.66 and methyl carbons at δ 21.94 (C-9′) and 14.29 (C-18). Alkaline hydrolysis of **2** yielded stearic acid, m.p. 68-69 ºC; Co-TLC

comparable. On the basis of foregoing discussion, the structure of **2** has been characterized as 4′*β*-hydroxy-non-cis-6′-enyl-1′-octadecanoate. This is a new fatty acid ester.

Compound **3** designated as *n*-nonacosanyl caprylate, was obtained as a colourless crystalline mass from petroleum ether-chloroform (1:3) eluents. Its IR spectrum showed characteristic absorption bands for ester group (1732 cm^{-1}) and long aliphatic chain (723 cm^{-1}) and had a molecular formula $C_{37}H_{75}O_2$, *m/z* 551 $[M+H]^+$ as determined by FAB-MS and ^{13}C NMR spectra, indicating one degree of unsaturation. The ion peaks forming at *m/z* 127 $[CH_3(CH2)_6CO]^+$, 143 $[CH_3(CH_2)_6COO]^+$ and 423 [M-127; $O(CH_2)_{28}CH_3]^+$ indicated that capric acid was esterified by *n*-nonacosanol. The ^1H NMR of **3** exhibited a one-proton triplet at δ 3.99 (*J*=6.9 Hz) assigned to oxygenated methylene H_2-1′ protons, other methylene protons between δ 2.37-1.25 and two three-proton triplets at δ 0.93 (*J*=6.3 Hz) and 0.85 (*J*=6.9 Hz) ascribed to C-8 and C-29′ primary methyl protons, respectively. The ^{13}C NMR spectrum of **3** showed signals for ester carbon at δ 171.65 (C-1), oxygenated methylene carbon at δ 64.30 (C-1′), other methylene carbons between δ 31.92-22.68 and methyl carbons at δ 14.10 (C-8, C-29′). Alkaline hydrolysis of **3** yielded caprylic acid as one of the constituent, Co-TLC comparable. On the basis of these evidences, the structure of **3** has been identified as *n*-nonacosanyl-*n*-octanoate.

Compound **4**, named boragosteryl oleate, was obtained as a colourless crystalline mass from chloroform eluant. Its IR spectrum showed characteristic absorption bands for carboxylic group (3381, 1698 cm^{-1}), ester function (1732 cm^{-1}), unsaturation (1640 cm^{-1}) and long aliphatic chain (723 cm^{-1}). Its molecular formula was deduced as $C_{47}H_{79}O_4$ by FAB mass and ^{13}C NMR spectra, *m/z* 707 $[M+H]^+$, indicating nine degree of unsaturation. The ion peaks arising at *m/z* 265 $[CH_3(CH_2)_7CH=CH(CH_2)_7CO]^+$, 281 $[CH_3(CH_2)_7CH=CH(CH_2)_7COO]^+$, 425 $[M-281]^+$, 271 $[286-Me]^+$ and 241 $[286-COOH]^+$ indicated that the sterol unit was esterified with oleic acid and the sterol processed one each vinylic linkage in the carboxylic skeleton and side chain and one carboxylic group in the steroidal mass. The ^1H NMR spectrum of **4** exhibited five one-proton signal as a doublet at δ 5.34 (*J*=5.5 Hz) and as multiplets from 5.25 to 4.93 assigned to vinylic H-6, H-22, H-23 and H-9′ and H-10′ protons. A one-proton broad multiplet at δ 4.02 with half-width of 16.5 Hz was ascribed to oxygenated methine H-3*α* proton. A two-proton triplet at δ 2.36 (*J*=7.3 Hz) was attributed to methylene H_2-2′ adjacent to the ester group. Six three proton signals as a broad signal at δ 1.01, as three doublets at δ 0.96 (*J*=6.3 Hz), 0.87 (*J*=6.6 Hz) and 0.85 (*J*=6.0 Hz) and as triplets at δ 0.82 (*J*=7.1 Hz) and 0.78 (*J*=6.3 Hz) were associated correspondingly to tertiary C-19, secondary C-21, C-26 and C-27 and primary C-29 and C-18′ methyl protons. The remaining methine and methylene protons resonated from δ 2.29 to 1.16. The ^{13}C NMR spectrum of **4** displayed signals for ester carbon at δ 173.72 (C-1′), vinylic carbons in the range of δ 140.76 to 121.69, oxygenated methine carbon at δ 71.83 (C-3), carboxylic carbon at δ 178.73 (C-18) and methyl carbons at δ 19.38 (C-19), 18.77 (C-21), 19.80 (C-26), 19.02 (C-27), 11.84 (C-29) and 14.16 (C-18′). The absence of a ^1H NMR signal near δ 0.67 for C-18 methyl carbon suggested location of the carboxylic group at C-18. The ^1H and ^{13}C NMR spectral data of the steroidal unit of **4** were compared with the reported data of the similar compounds. Alkaline hydrolysis of **4** yielded oleic acid as one of the component, Co-TLC comparable. On the basis of the above discussion, the structure of **4** has been elucidated as stigmast-5,22-dien-3*β*-olyl-n-octadec-9′-enoate. This is a new steroidal ester.



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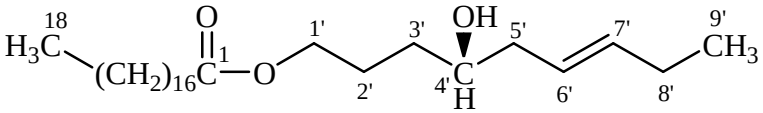

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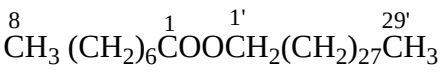

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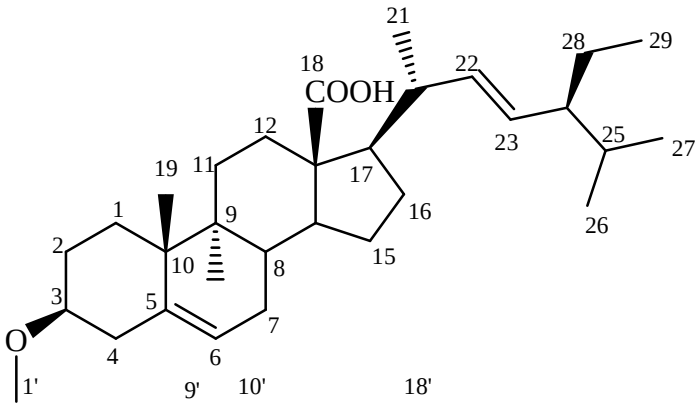

4

CONCLUSION

The leaves of *B. officinalis* contained esters of squalene-type triterpene, fatty acids and a sterol which might be responsible for the medicinal properties of the plant.

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