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New flavanones from the seeds of *Rumex vesicarius* L.

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ABSTRACT

Rumex vesicarius L. (Polygonaceae) is an annual, monoecious glabrous, pale green herb cultivated as a leafy vegetable in many parts of India. The seeds are utilized as a refrigerant, to cure dysentery and as an antidote for scorpion venom. The seed powder is taken orally to treat liver diseases and as a laxative. Phytochemical investigation of the methanolic extract of seeds of R. vesicarius resulted in the isolation of two new flavanones characterized as 5,7,3'-trihydroxy-4'-methoxy-8-(3"-methyl-5"-methylene)hexenyl flavanone (vesicariaflavanone A, 4) and 5,7,3',4'-tetrahydroxy-6-methoxy-8-(3"-methylene)-hexenylflavanone (vesicariaflavone B, 5) along with the fatty acid glycerides identified as glyceryl-1-octadec-9'-enoate-3-phosphate (1), glyceryl-1,2-bis-hexadecanoate-3-phosphate (2) and glyceryl-1-octadec-9'-enoate-2-octahexanoate-3-phosphate (3). The structures of all the isolated phytoconstituents have been estabilished on the basis of spectral data analysis and chemical reactions.

KEYWORDS

Rumex vesicarius;
Polygonaceae;
Seeds;
Flavanones;
Structural elucidation.

INTRODUCTION

Rumex vesicarius L. (Polygonaceae), known as Chukra or Bladder dock, is an annual, monoecious glabrous, dichotomously branched, succulent pale green herb. It a native to south western Asia and northern Africa; cultivated as a leafy vegetable in many parts of India[1]. It is prescribed to treat tumors, hepatic diseases, indigestion, constipation, calculus, heart troubles, pains, spleen diseases, hiccough, flatulence, asthma, bronchitis, dyspepsia, piles, scabies, leucoderma, toothache and nausea[2-5]. It possesses diuretic, antiscorbutic, appetizer, astringent, carminative, laxative, stomachic and tonic properties. The leaves are eaten fresh and much appreciated for their acid taste; they can be added to salad and used as an antidote for snake venom. The plant is prescribed to reduce biliary disorders and to control cholesterol levels. The seeds are utilized as a refrigerant, to cure dysentery and as an antidote for scorpion venom. The seed powder is taken orally to treat liver diseases and as a laxative[2-4]. The plant contained bioactive substances such as flavonoids (vitexin, isovitexin, orientin and isorientin), anthraquinones particularly in roots (emodin and chrysophanol), quinones, carotenoids, vitamins, proteins, lipids, carbohydrates, reducing sugars, phenols, tannins, saponins, triterpenoids and organic acids[5-10]. The drug showed antidiarrheal and antidysenteric[10], antimicrobial[9,11,13], antioxidant[14] and diuretic[15] activities. The present paper describes the isolation and characterization of two new alkylated flavanones from the seeds of R. vesicarius.

MATERIAL AND METHODS

General experimental conditions

Melting points were determined on a Perfit melting apparatus (Ambala, Haryana, India) and are uncurrected. UV spectra were measured with a Lambda Bio 20 spectrophotometer (Perkin-Elmer-Rotkreuz, Switzerland) in methanol. Infra red spectra were recorded on Bio-Rad FTIR 5000 (FTS 135, Kawloon, Hong Hong) spectrophotometer using KBr pellets; γ_{max} values are given in cm^{-1}. ^{1}H and ^{13}C NMR spectra were screened on Advance DRX Bruker spectrospin 400 and 100 MHz, respectively, instruments (Karlesruthe, Germany) using $CDCl_3$ and TMS as an internal standard. Mass spectra were scanned by effecting FAB ionization at 70 eV on a JEOL-JMS-DX 303 spectrometer (Japan) equipped with direct inlet probe system. Column chromatography was performed on silica gel (60-120 mesh; Qualigen, Mumbai, India). TLC was run on silica gel G (Qualigen). Spots were visualised by exposing to iodine vapours, UV radiation and spraying with ceric sulphate solution.

Plant material

The seeds of *R. vesicarius* were procured from the Khari Baoli market of Delhi and identified by Prof. M.P. Sharma, Department of Botany, Jamia Hamdard, New Delhi. A voucher specimen is deposited in the herbarium of the Phytochemical Research Laboratory, Faculty of Pharmacy.

Extraction and isolation

The air-dried seeds (2.0 kg) was coarsely powdered, defatted with petroleum ether and extracted with methanol exhaustively in a Soxhlet apparatus. The combined extracts were filtered and concentrated under reduced pressure to get a dark brown viscous mass (125 g, 6.25%). The dried extract was dissolved in minimum quantity of methanol and adsorbed on silica gel (60-120 mesh) for preparation of slurry. It was dried in air and chromatographed over silica gel column (1.6 m × 16 mm × 2 mm) packed in petroleum ether. The column was eluted successively with different solvents in increasing order of polarity in various combinations, such as petroleum ether, petroleum ether- chloroform (9:1, 3:1, 1:1, 1:3 v/v), chloroform, chloroform-methanol (19.9:0.1, 99:1, 97:3, 19:1, 93:7, 9:1, 17:3, 3:1, 3:2, 2:3 v/v) and methanol. Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having the same R_f values were combined and crystallized. The isolated compounds were recrystallized to get pure compounds. The following compounds were isolated from the methanolic extract of the seeds of *R. vasicarius*:

Glyceryl-1-octadec-9'-enoate-3-phosphate (1)

Elution of the column with petroleum ether-$CHCl_3$ (1:3) gave colorless amorphous powder of 1, recrystallized from chloroform, 465 mg (0.0233% yield); R_f: 0.33 (petroleum ether-$CHCl_3$-MeOH, 1:4:1); m.p.: 62-63 °C; IR ν_{max} (KBr): 3450, 2919, 2851, 1740, 1645, 1410, 1043, 795, 765 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.28 (2H, m, H-9', H-10'), 4.87 (2H, dd, *J*= 11.6, 10.5 Hz, H_2-1), 4.53 (1H, m, H-2), 4.03 (2H, m, H_2-3), 2.22 (2H, t, *J*=7.2 Hz, H_2-2'), 2.02 (2H, m, H_2-11'), 1.96 (2H, brs, H_2-8'), 1.52 (2H, m, CH_2), 1.18 (20H, brs, 10 × CH_2), 0.77 (3H, t, *J*= 6.5 Hz, Me-18'); +ve ESI MS *m/z*: 436 $[M]^+$ ($C_{21}H_{41}O_7P$).

Glyceryl-1, 2-bis-hexadecanoate-3-phosphate (2)

Elution of the column with chloroform yielded pale yellow sticky mass of 2, 279 mg (0.014% yield); R_f: 0.54 ($CHCl_3$-MeOH, 4: 1); m.p.: 63-64°C; IR ν_{max} (KBr): 3480, 2919, 2851, 1738, 1467, 1310, 1250, 1190, 795, 773 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 4.18 (2H, m, H_2-1), 4.06 (1H, m, H-2), 3.58 (2H, brs, H_2-3), 2.34 (2H, t, *J*=6.1 Hz, H_2-2'), 2.23 (2H, t, *J*=7.2 Hz, H_2-2''), 1.63 (4H, brs, 2×CH_2), 1.25 (48 H, brs, 24 × CH_2), 0.87 (3H, t, *J*=6.3 Hz, Me-16'), 0.82 (3H, t, *J*=6.1 Hz, Me -16''); +ve ESI MS *m/z*: 650 $[M]^+$ ($C_{35}H_{71}O_8P$).

Glyceryl-1-octadec-9'-enoate-2-octahexanoate-3-phosphate (3)

Elution of the column with chloroform-methanol (99:1) afforded light brown sticky mass of 3, 465 mg (0.023 % yield); R_f: 0.60 ($CHCl_3$-MeOH, 4:1); m. p. 80-81°C; IR ν_{max} (KBr): 3450, 2920, 2845, 1738, 1640, 1360, 1285, 1120, 795 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.35 (2H, m, H-9', H-10'), 4.17 (2H, m, H_2-1), 4.02 (1H, m, H-2), 3.66 (2H, m, H_2-3), 263 (2H, t, *J*=7.5 Hz, H_2-2'), 2.32 (2H, t, *J*=7.2 Hz, H_2-2''), 1.61 (4H, m, H_2-8', H_2-11'), 1.56 (4H, m, 2 × CH_2), 1.25 (48H, brs, 24 × CH_2), 0.87 (3H, t, *J*=6.3 Hz, Me-18'), 0.84 (3H, t, *J*=6.1 Hz, Me-18''); +ve ESI MS *m/z*: 714 $[M]^+$ ($C_{39}H_{87}O_8P$).

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Vesicariaflavanone A (4)

Elution of the column with chloroform-methanol (97:3) furnished yellow amorphous powder of 4, recrystallized from methanol, 296 mg (0.1481% yield); R_f: 0.72 ($CHCl_3$-MeOH, 4:1); m.p.: 230-231°C; IR ν_{max} (KBr): 3360, 2920, 2855, 1705, 1640, 1440, 1390, 1325, 980, 955 cm^{-1}; UV λ_{max} (MeOH): 243, 287, 334 mm (log ε 2.1,5.9,0.8); ^1H NMR ($CDCl_3$): δ 6.85 (1H, brs, H-6), 6.81 (1H, d, *J*=2.8 Hz, H-2'), 6.54 (1H, d, *J*=8.1 Hz, H-5'), 6.48 (1H, dd, *J*=8.1, 2.8 Hz, H-6'), 5.66 (2H, brs, H_2-8'') 4.98 (1H, d, *J*=12.6 Hz, H-2), 3.55 (3H, brs, OCH_3), 2.76 (1H, d, *J*=12.6 Hz, H_2-3a), 2.70 (1H, d, *J*=12.6 Hz, H_2-3b), 2.44 (1H, brs, H_2-1''a), 2.38 (1H, brs, H_2-1''b), 2.33 (3H, brs, Me-6''), 2.00 (2H, m, H_2-4''), 1.72 (2H, m, H_2-2''), 1.30 (1H, m, H-3''), 0.96 (3H, d, *J*=6.6 Hz, Me-7''); ^{13}C NMR ($CDCl_3$): 78.16 (C-2), 42.20 (C-3), 194.98 (C-4), 163.28 (C-5), 95.69 (C-6), 166.20 (C-7), 94.72 (C-8), 162.31 (C-9), 101.50 (C-10), 126.98 (C-12), 113.13 (C-22), 144.39 (C-32), 144.81 (C-42), 114.78 (C-52), 117.29 (C-62), 40.06 (C-1'2), 38.90 (C-22 2), 50.88 (C-32 2), 39.45 (C-42 2), 129.15 (C-52 2), 28.76 (C-62 2), 13.83 (C-72 2), 108.54 (C-82 2), 55.15 (OMe); +ve ESI MS *m/z* (rel. int.): 412 $[M]^+$ $C_{24}H_{28}O_6$ (1.1), 397 $[M\text{-}Me]^+$ (6.1), 381 $[M\text{-}OMe]^+$ (5.7), 151 $[C_9H_{11}O_2]^+$ (20.1), 134 $[151\text{-}OH]^+$ (11.3), 108 $[C_8H_{12}]^+$ (2.3).

Vesicariaflavanone B (5)

Further elution of the column with chloroform-methanol (97:3) gave brown amorphous powder of 5, recrystallized from methanol, 50 mg (0.0025% yield); R_f: 0.65 ($CHCl_3$-MeOH, 4:1); m.p.: 180-181°C; IR ν_{max} (KBr): 3365, 2920, 2850, 1705, 1640, 1515, 1435, 1360, 1220, 1180, 990 cm^{-1}; UV λ_{max} (MeOH): 241, 287, 336 nm (log ε 2.1, 5.3, 0.7); ^1H NMR ($CDCl_3$): δ 6.87 (1H, d, *J*=2.6 Hz, H-2'), 6.77 (1H, d, *J*=7.8 Hz, H-5'), 6.68 (1H, dd, *J*=7.8, 2.6 Hz, H-6'), 5.88 (2H, brs, H_2-7''), 5.20 (1H, d, *J*=12.3 Hz, H-2), 3.78 (3H, brs, OMe), 2.98 (1H, d, *J*=12.3 Hz, H_2-3a), 2.52 (1H, d, *J*=10.8 Hz H_2-3b), 2.22 (2H, m, H_2-1''), 1.94 (2H, m, H_2-4''), 1.53 (2H, m, H_2-2''), 1.18 (2H, m, H_2-5''), 0.80 (3H, t, *J*=6.1 Hz, Me-6''); ^{13}C NMR ($CDCl_3$): 78.20 (C-2), 42.26.20 (C-3), 194.72 (C-4), 163.21 (C-5), 165.42 (C-6), 166.24 (C-7), 94.74 (C-8), 161.98 (C-9), 101.63 (C-10), 127.23 (C-12), 113.14 (C-22), 144.28 (C-32), 144.44 (C-42), 114.81 (C-52), 117.30 (C-62), 39.56 (C-1'2), 39.28 (C-22 2), 129.68 (C-32 2), 39.01 (C-42 2), 28.79 (C-52 2), 15.23 (C-62 2), 108.57 (C-72 2), 55.15 (OMe); +ve ESI MS *m/z* (rel. int.): 414 $[M]^+$ $C_{23}H_{26}O_7$ (72.1), 399 $[M\text{-}Me]^+$ (1.1), 383 $[M\text{-}OMe]^+$ (2.1), 136 $[C_8H_8O_2]^+$ (2.3).

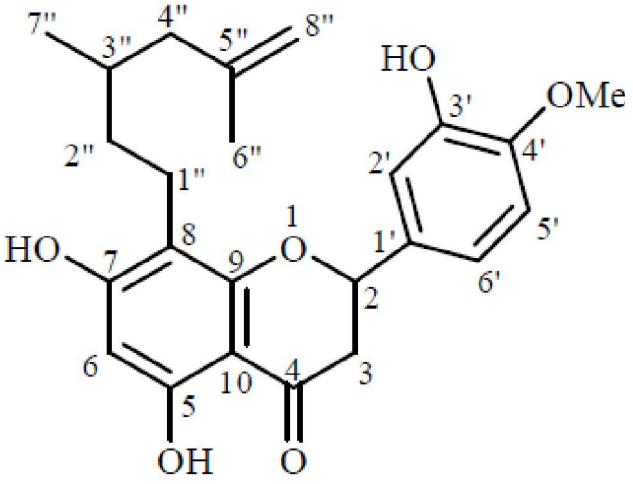
RESULTS AND DISCUSSION

The compounds 1, 2, 3 were the fatty acid glycerides identified as glyceryl-1-octadec-9'-enoate-3-phosphate, glyceryl-1, 2-bis-hexadecanoate-3-phosphate and glyceryl-1-octadec-9'-enoate-2-octahexanoate-3-phosphate, respectively.

Compound 4, named vesicariaflavanone A, was obtained as a yellow amorphous powder. Its UV absorption maxima at 287 and 334 nm indicated its flavanone nature[16]. On addition of aluminium chloride and aluminium chloride-HCl, a bathochromic shift of 40 nm was observed suggesting the presence of a chelated hydroxyl group at C-5. Its molecular ion peak was determined at *m/z* 412 consistent to an alkylated flavanone $C_{24}H_{28}O_6$ on the basis of mass and ^{13}C NMR spectra. Its IR spectrum exhibited absorption bands at 3360 cm^{-1} (OH group), 1705 cm^{-1} (keto group) and 1640, 1537 cm^{-1} (aromatic ring). The mass spectrum of 4 showed an RDA ion fragment at *m/z* 150 resulted from the B-ring cleavage and other ion peaks at *m/z* 397 $[M\text{-}Me]^+$, 381 $[M\text{-}OMe]^+$, 134 $[151\text{-}OH]^+$ and 108 $[C_8H_{12}$, side chain$]^+$ supporting the existence of a methoxy group in ring C and a C_8H_{12} side chain in ring A. Its ^1H NMR spectrum displayed a downfield one-proton broad singlet at δ 6.81 assigned to aromatic H-6 proton. Two one-proton doublets at δ 6.81 (*J*=2.8 Hz) and 6.54 (*J*=8.1 Hz) and a one-proton double doublet at 6.48 (*J*=8.1, 2.8 Hz) were ascribed to meta-coupled H-2', ortho-coupled H-5' and ortho-, meta-coupled H-6' aromatic protons, respectively. The vinylic H_2-8'' protons appeared as a two-proton signal at δ 5.66. The methoxy protons resonated as a three-proton singlet at δ 3.55. Three one-proton doublets at δ 4.98 (12.6 Hz), 2.76 (*J*=12.6 Hz) and 2.70 (*J*=12.6 Hz) were attributed to oxygenated methine H-2 proton

and methylene H_2-3 protons, respectively. The other methine and methylene protons of the side chain resonated between δ 2.76-1.72. A three-proton broad singlet at δ 2.33 and a three-proton doublet at δ 0.96 (*J*=6.6 Hz) were accounted to C-6'' methyl protons attached to the vinylic C-5 carbon and to secondary C-7'' methyl protons, respectively. The ^{13}C NMR spectrum of 4 displayed signals for carbonyl carbon at δ 194.98 (C-4), oxygenated methine carbon at δ 78.16 (C-2), flavanone aromatic carbons between δ 166.20-94.72, vinylic carbons at δ 129.15 (C-5'') and 108.54 (C-8'') and methoxy carbon at δ 55.15[17-19]. On the basis of above discussion the structure of 4 has been elucidated as 5,7,3'-trihydroxy-4'-methoxy-8-(3''-methyl-5''-methylene) hexenylflavanone.

Compound 5, designated as vesicariaflavanone B, was obtained as a brown amorphous powder from chloroform-methanol (97:3) eluants. It showed UV absorption maxima at 281 and 336 nm suggesting flavanone nature. On addition of aluminium chloride and aluminium chloride-HCl, a bathochromic shift of 40 nm was observed suggesting the presence of a chelated hydroxyl group at C-5[16]. Its IR spectrum exhibited absorption bands at 3365 cm^{-1} (OH group), 1705 cm^{-1} (keto group) and 1640, 1515 cm^{-1} (aromatic ring). Its molecular ion peak was determined at *m/z* 414 consistent to an alkylated flavanone $C_{23}H_{26}O_7$ on the basis of mass and ^{13}C NMR spectra. An RDA fragment ion arising at *m/z* 136 $[C_8H_8O_2]^+$ indicated the presence of two hydroxyl group in rings B/C. Other ion peaks appeared at *m/z* 399 and 383 due to elimination of a methyl [M-Me]$^+$ and a methoxy function from the molecular ion peak [M-OMe]$^+$. The ^{1}H NMR spectrum of 5 displayed two one-proton doublets at δ 6.87 (*J*=2.6 Hz) and 6.77 (*J*=7.8 Hz) and a one-proton double doublet at δ 6.68 (*J*=7.8, 2.6 Hz) assigned to meta-coupled H-2', ortho-coupled H-5' and ortho-, meta-coupled H-6' aromatic protons, respectively. The vinylic H_2-7'' protons appeared as a two-proton signal at δ 5.88. The methoxy protons resonated as a three-proton singlet at δ 3.78. Three one-proton doublets at δ 5.20 (12.3 Hz), 3.52 (*J*=12.3 Hz) and 2.98 (*J*=12.3 Hz) were ascribed to oxygenated methine H-2 and methylene H_2-3 protons, respectively. The other methylene protons of the side chain resonated between δ 2.22 -1.18. A three-proton triplet at δ 0.80 (*J*=6.1 Hz) was accounted to primary C-6'' methyl protons. The ^{13}C NMR spectrum of 4 displayed signals for carbonyl carbon at δ 194.72 (C-4), oxygenated methine carbon at δ 78.20 (C-2), flavanone aromatic carbons between δ 166.24-94.74, vinylic carbons at δ 129.68 (C-3'') and 108.57 (C-7''), methyl carbon at δ 15.23 and methoxy carbon at δ 57.41[17-19]. On the basis of above discussion the structure of 5 has been characterized as 5, 7, 3', 4'-tetrahydroxy-6-methoxy-8-(3''-methylene)-hexenylflavanone.



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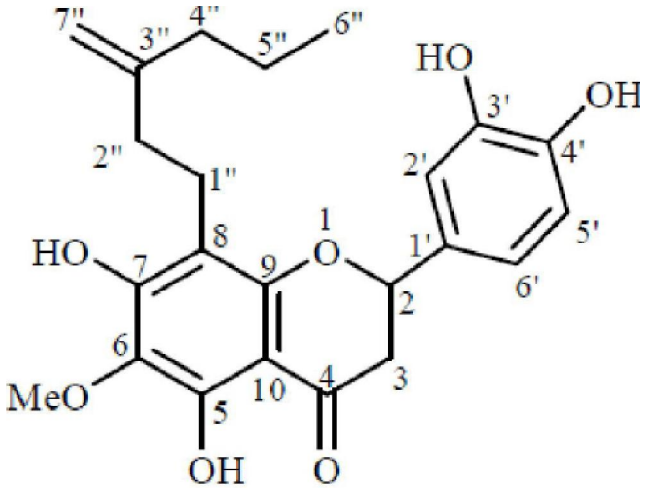

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CONCLUSION

Phytochemical investigation of the methanolic extract of seeds of *R. vesicarius* resulted in the isolation

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of two new flavanones. These phytoconstituents will increase the existing knowledge of traditionally used of *R. vesicarius* and may be used as chromatographic markers.

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