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New phenolic methyl esters and arachidonyl arabinoside from the roots of *Ricinus communis* L.

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ABSTRACT

Two new phenolic acid esters characterized as 2′–(2-hydroxy-3,4- dioxymethylenephenyl) methyl acetate and 4′-(2-hydroxy-3,4-dioxymethylenephenyl) methyl n- butyrate and a fatty acid glycoside, n-eicosanoyl-β-D-arabinopyranoside, have been isolated as the new phytoconstituents from the roots ofRicinus communis L.(Euphorbiaceae) along with the known compounds n-hexatriacont-14-ene, n-heptacosanyl octadec-9-oate, stigmast-5, 22-dien-3β-olyl n-octadecanoate,stigmast-5, 22- dien-3β-olyl n-octadec-9′-enoate and stigmast -5, 22-dien- 3β-olyl n-eicosanoate. The structures of all these phytoconstituent, isolated for the first time from the roots, have been established by spectral data analysis and chemical reactions.

Key words: *Ricinus communis;* roots; phenolic acid esters; arachidoyl arabinoside; steroidal esters

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INTRODUCTION

Ricinus communis L. (Euphorbiaceae), known as castor plant, is an annual or perennial soft wooded small tree up to 6 m in height. It is widespread throughout tropics and warm temperate regions of the world and cultivated in India and other countries up to 2,000 m altitude [1,2]. It is an important oil seed crop which produces an oil rich in ricinoleic acid conferring unique properties of the oil [3,4,5]. The castor oil is cathartic and is official in some pharmacopoeias. It is used to prepare some liquid disinfectants like phenyls, hair oils, fixers, aromatic perfumes, lipsticks, hair lotions and tonics [2]. An emulsion of the oil and soap is effective against some crop pests. The oil is a source of N-isobutyl undecylate amide, a valuable synergist which can be used with pyrethrum. It is applied as a preservative to food grains and pulses. The plant leaves are prescribed to treat boils, sores, swellings, stomachache, jaundice, teeth carries, flatulence in children, guinea worm, sores and as a lactagogue. A decoction of the root is administered to relieve lumbago, and a root paste is applied to alleviate toothache. The efficacy of the seeds as a contraceptive drug has been studied with several traditional applications [6,7] . They have been used with arguable success in the treatment of warts, cold, tumours and indurations of the mammary glands, corns and moles [8,9,10]. Its extracts were found to cause proportional increase in mean wheal diameter in skin tests in castor bean allergic workers [11]. The anti-inflammatory and the free radical scavenging activities were well documented [12]. Nowadays, there is an increasing interest in the use of naturally occurring substances for the preservation of food. Plant essential oils and their components have been known to exhibit biological properties. The root bark is a powerful purgative [2, 13], anti-inflammatory, antioxidant [12], antifertility, contraceptive [14],antibacterial [15],

and yields 3-O-benzoyl-stigmast-5,22-dien-3β, 21-diol and 3α-hydroxy-pentatriacont-14-en-26-one [16].The leaves afforded ricinine, n-demethylricinine and flavonoid glycosides [17,18]. The seeds possessed gallotannins, lupeol and 30-nor lupan-3β-ol-20-one [2, 19 20]. An essential oil of the aerial parts is consisted of α-pinene, 1,8-cineole, α-thujone, camphor and camphene [21]. The present paper describes the isolation and characterization of aliphatic compounds, phenolic acid esters, steroidal esters and acyl arabinoside from the roots of *R. communis* of arid region.

MATERIALS AND METHODS

General experimental procedures

Melting points were measured on a thermoelectrically operated Perfit apparatus and are uncorrected. UV spectra were determined on Shimadzu-120 double beam spectrophotometer with methanol as a solvent. IR spectra were recorded in KBr pellet on Shimadzu FTIR-8400 spectrophotometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were scanned by Bruker spectrospin NMR instrument, using TMS as internal standard. FAB mass spectra were recorded on a Jeol D-300 spectrometer. Silica gel (60-120 mesh, Merck, Mumbai, India) was used for column chromatographyand thin-layer chromatography was performed on silica gel G coated TLC plates (Merck, Mumbai, India). The percentage yields of the isolated compounds were calculated on the basis of dried plant material taken for extraction.

Plant material

The fresh roots of *R. communis*were collected from the arid waste land of Jaipur (Rajasthan). The plant material was identified on the basis of exomorphic characters and reviews of literature by Prof. M. P. Sharma, taxonomist, Department of Botany, Jamia Hamdard, New Delhi. A voucher specimen No. PRL/ JH / 09 / 12 is deposited in the herbarium of the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi.

Extraction and isolation

The air-dried roots (2 kg) of *R. communis* were coarsely powdered and extracted exhaustively in a Soxhlet apparatus with methanol for 72 hr. The methanolic extract was concentrated under reduced pressure to obtain a dark brown viscous mass (234 g). Small portion of the extract was analyzed chemically to determine the presence of different chemical constituents. The viscous brown mass was dissolved in minimum amount of methanol and adsorbed on silica gel (60-120 mesh) for preparation of slurry. The slurry (200 g) was air-dried and chromatographed over silica gel column packed in petroleum ether. 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), pure chloroform and finally the mixture of chloroform and methanol (99:1, 97:3, 95:5, 92:8, 9:1, 3:1, 1:1, 1:3, v/v). Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having same R_f values were combined and crystallized. The isolated compounds were recrystallized to get the following pure compounds:

***n*-Hexatriacont-14-ene (1**)

Elution of the column with petroleum ether yielded pale yellow powder of **1**, recrystallized from chloroform-methanol (1:1), 117 mg (0.0058% yield); R_f **:** 0.7 (petroleum ether); m.p. : 81-83 ^{o} C; +ve FAB MS *m/z* (rel.int) : 504 $[M]^+$ ($C_{36}H_{72}$) (16.7) , 295 (10.3), 209 (21.3), 183 (100).

***n*-Heptacosanyl oleate (2)**

Elution of the column with petroleum ether-chloroform (9:1) gave colourless crystals of **2**, recrystallized from chloroform-methanol (1:1), 137 mg (0.0068%yield); m.p.**:** 85-86 ^{o} C ; FAB MS *m/z* (rel. int.) **:** 660 $[M]^+$($C_{45}H_{88}O_2$) (9.3), 395 (42.6), 379 (18.3), 281 (11.5), 265 (10.2).

Methyl communisoate (3)

Elution of the column with petroleum ether- chloroform (3:1) afforded colourless crystals of **3**,recrystallized from acetone-methanol (1:1), 135 mg (0.006% yield); R_f **:** 0.3 (petroleum ether- chloroform, 3:1); m.p.: 105-106 ^{o} C; UV λ_{max} (MeOH) : 205, 284 (log ε 4.2,1.8); IR ν_{max} (KBr) : 3446, 2917, 2849, 1721, 1636, 1516, 1462, 1272, 1162, 1033 cm^{-1}; ^{1}H NMR ($CDCl_3$) : δ 7.70 (1H, d, J =7.5 Hz, H-5), 6.22 (1H, d, J =7.5, Hz, H-6), 4.02 (2H, brs, O-CH_2-O), 3.68 (3H, brs, OMe), 3.55 (2H, brs, H_2-2′); ^{13}C NMR ($CDCl_3$) : δ 144.09 (C-1), 161.67 (C-2), 159.87 (C-3), 158.62 (C-4), 113.47 (C-5), 111.89 (C-6), 172.57 (C-1′), 37.32 (C-2′), 93.95 (O-CH_2-O), 57.05 (OMe); +ve FAB Ms *m/z* (rel. int.)**:**210 $[M]^+$ ($C_{10}H_{10}O_5$) (5.6), 195 (18.2), 179 (13.8), 178 (30.5), 162 (17.9), 151 (21.3), 134 (38.1).

***n*–Butyl ricicommunioate (4)**

Elution of the column with chloroform furnished colourless solid mass of **4**, recrystallized from chloroform-methanol (1:1), 163 mg (0.0081% yield); R_f**. :** 0.68 (chloroform-methanol, 9:1); m.p.: 68-69 ^{o}C; UV λ_{max}(MeOH) **:** 220 nm (log ε 4.2); IR ν_{max}(KBr) **:** 3436, 2918, 2851, 1721, 1637, 1535, 1496, 1458, 1360, 1255, 953 cm.$^{-1}$; ^{1}H NMR ($CDCl_3$) **:** δ 7.52 (1H, d, J =7.8 Hz, H-5), 6.07 (1H, d, J =7.8Hz, H-6), 3.99 (2H, brs, O-CH_2-O), 3.55 (3H, brs, OMe),2.33 (1H, m, H_2-2′a), 2.12 (1H, m, H_2-2′b), 1.25 (2H, brs, H_2 -3′), 2.80 (2H, brs, H_2-4′); ^{13}C NMR ($CDCl_3$): δ 143.73 (C-1), 160.77 (C-2), 158.89 (C-3), 157.67 (C-4), 113.41 (C-5), 112.52 (C-6),171.93 (C-1′),36,89 (C-2′), 29.10 (C-3′), 39.27 (C-4′), 93.04 (O-CH_2-O), 56.65 (OMe); +ve FAB MS *m/z***:** 226 $[M]^+(C_{11}H_{14}O_5)$ (5.1), 165 (100), 151 (12.8), 136 (70.41), 123 (15.2), 207 (18.1), 137 (47.3).

Stigmasterol stearate (5)

Further elution of the column with chloroform furnished colourless crystals of **5**, recrystallized from acetone-methanol (1.1); 245 mg (0.012% yield); m.p**. :** 118-120 ^{o} C ; R_f **:** 0.4 (petroleum ether-chloroform, 3:1);+ve FAB Ms *m/z* (rel.int) **:** 679 $[M+H]^+$ ($C_{47}H_{83}O_2$) (15.2), 411 (24.6), 396 (61.2), 394 (31.7), 284 (8.6), 271 (12.5), 267 (10.6), 255 (26.8), 240 (14.3), 213 (22.0), 198 (25.3).

Stigmasterol oleate (6)

Elution of the column with chloroform-methanol (99:1) mixture produced colourless crystals of **6**, recrystallized from acetone-methanol (1:1); 151 mg (0.0075 % yield); R_f **:** 0.4 (petroleum ether-chloroform, 1:1) ; m.p. **:** 180-190 $^{\circ}$ C; +ve FAB MS *m/z* (rel.int) **:** 676 $[M]^+$ ($C_{47}H_{80}O_2$) (2.5), 411 (20.6), 396 (29.1), 394(25.0), 381 (19.3), 282 10.2), 271 (15.8), 265 (12.3), 256 (13.1), 255 (20.1), 240 (21.4), 213 (14.5), 198 (10.9).

Stigmasterol arachidate (7)

Elution of the column with chloroform-methanol (97:3) afforded pale yellow crystals of **7**,recrystallized from acetone-methanol (1:1); 115 mg (0.0057% yield); R_f : 0.3 (petroleum ether- chloroform, 1:1); m.p. **:** 112-114 ^{o} C; +ve FAB MS *m/z* (rel. int.) **:** 706 $[M]^+$ ($C_{49}H_{86}O_2$) (2.5) , 411 (15.8), 396 (26.0), 394 (21.5), 312 (10.3), 255 (12.2).

Arachidoyl arabinoside (8)

Elution of the column with chloroform-methanol (19:1) gave colourless crystals of **8**, recrystallized from chloroform- methanol (1:1), 103 mg (0.0051% yield); R_f **:** 0.4 (chloroform); m.p**:** 121-123 $^{\circ}$C; UV λ_{max} (MeOH) **:** 206 nm (log ε 4.1); IRν_{max} (KBr) **:** 3459, 3350, 2917, 2849, 1722, 1638, 1445, 1381, 1264, 1127, 720 cm.$^{-1}$; ^{1}H NMR (DMSO-d_6) : δ 5.83 (1H, d, J = 7.0 Hz, H-1′), 4.35 (1H, m, H-2′), 4.27 (1H, m, H-3′), 4.07 (1H, m, H-4), 3.69 (2H, brs, H_2-5′), 2.77 (1H, d, J = 8.9 Hz, H_2-2a), 2.67 (1H, d, J = 8.7 Hz, H_2-2b), 2.27 (2H, m, CH_2), 2.17 (2H, m, CH_2), 1.77 (2H, m, CH_2), 1.65 (2H, m, CH_2), 1.25 (26H, brs, 13xCH_2), 0.88 (3H, t, J = 6.3 Hz, Me -20); ^{13}C NMR (DMSO-d_6): δ 170.11 (C-1), 34.06 (C-2), 33.89 (CH_2), 32.46 (CH_2), 29.23 (13 x CH_2), 28.60 (CH_2), 22.18 (CH_2), 14.17 (C-20), 104.15 (C-1′), 75.42 (C-2′), 71.86 (C-3′), 69.39 (C-4′), 65.48 (C-5′); +ve FAB MS *m/z* (rel. int.)**:** 444 $[M]^+(C_{25}H_{48}O_6)$ (1.9), 311 (19.6), 150 (26.7), 133 (22.2).

Hydrolysis of 8

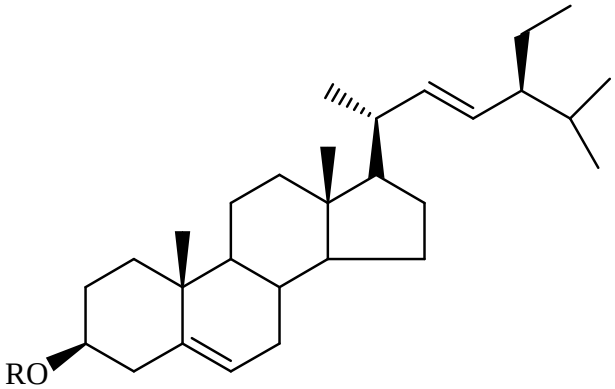
Compound **8** (12 mg) was heated with a mixture of ethanol (5 ml) and dil. HCl (2 ml) on a steam bath for 1 hour. The solvent was evaporated under reduced pressure and the residue was dissolved in chloroform to separate arachidic acid, m.p. 74-75 $^{\circ}$C, co-TLC comparable. The residue was redissolved in water and chromatographed over silica gel TLC along with standard samples of sugars using *n*-BuOH-AcOH-H_2O (4:1:5 v/v) as a developing solvent. The sugar was identified as D-arabinose.R_f. 0.18; $[\alpha]_D$ + 52.7° .

RESULTS AND DISCUSSION

Compound **1**, **2**, **5**, **6** and **7** were the known phytoconstituents characterized as n-hexatriacont-14-ene**,** *n*-heptacosanyl octadec-9-enoate, stigmast-5, 22-dien-3β-olyl *n*-octadecanoate,stigmast-5,22-dien-3β-olyl *n*-octadec-9′-enoate andstigmast -5, 22- dien- 3β-olyl *n*-eicosanoate,respectively, on the basis of spectral data analysis [22,23,24].

$CH_3\ (CH_2)_{12}\ CH{=}CH\ (CH_2)_{20}\ CH_3CH_3\ (CH_2)_7\ CH{=}CH\ (CH_2)_7\ COO\ CH_2\ (CH_2)_{25}\ CH_3$

1 **2**

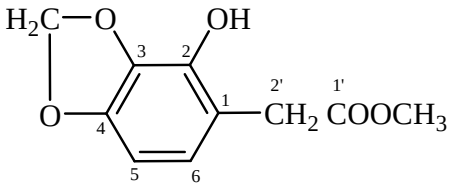


5. R = CO $(CH_2)_{16}$ CH_3

6. R = CO $(CH_2)_7$ CH=CH $(CH_2)_7$ CH_3

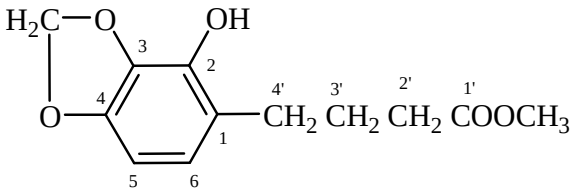
7. R = CO $(CH_2)_{18}$ CH_3

Compound **3**, named methyl communisoate, was obtained as a colourless crystalline mass from petroleum ether-chloroform (3:1) eluants. It responded positively to phenolic tests. Its IR spectrum showed characteristic absorption bands for hydroxyl group (3446 cm^{-1}), ester function (1721 cm^{-1}) and aromaticity (1636, 1516 cm^{-1}). On the basis of FAB mass and ^{13}C NMR spectra, the molecular ion peak of **3** was determined at *m/z* 210 consistent to the molecular formula of a dioxymethylene substituted phenylacetic acid ester $C_{10}H_{10}$ O_5. The ion peaks arising at *m/z* 151 $[M\text{-}COOCH_3]^+$, 134 $[151\text{-}OH]^+$, 179 $[M\text{-} OCH_3]^+$, 162 $[179\text{-}OH]^+$, 195 $[M\text{-}Me]^+$ and 178 $[195\text{-}OH]^+$ suggested the presence of methyl ester in the molecule. The ^1H NMR spectrum of **3**exhibited two AB-type doublets at δ 7.70 (J=7.5 Hz) and 6.22 (J =7.5 Hz) assigned to aromatic *ortho*-coupled H-5 and H-6, respectively. Two broad singlet at δ 4.02 and 3.55 integrating for two protons each and a three- proton broad singlet at δ 3.68 were associated correspondingly with dioxymethylene, methylene H_2-2′ and carboxy methyl protons, respectively. The ^{13}C NMR spectrum of **3** showed signals for an ester carbon at δ 172.57 (C-1′), aromatic carbons from δ 161.67 to 113.47, dioxymethylene carbon at δ 93.94, methoxy carbon at δ 57.05 and methylene carbon at δ 37.32 (C-2′). On the basis of the foregoing account, the structure of **3** has been elucidated as 2′– (2-hydroxy -3, 4- dioxymethylenephenyl) methyl acetate. This is a new phenolic acid ester.



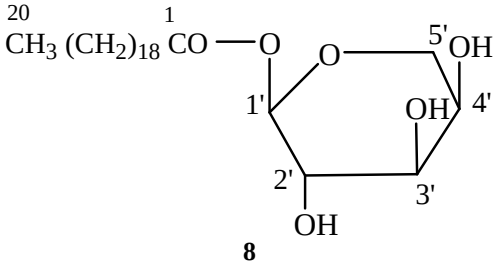
3

Compound **4,** named *n*-butyl ricicommunisoate, was obtained as a colourless crystalline solid mass from chloroform eluants. It yielded green colour with ferric chloride solution due to phenolic nature. The IR spectrum of **4** showed characteristic absorption bands for hydroxyl group (3436 cm^{-1}), ester function (1721 cm^{-1}) and aromatic ring (1637, 1535 cm^{-1}). On the basis of FAB mass and ^{13}C NMR spectral data, the molecular ion peak of **4** was determined at *m/z* 238 corresponding to the molecular formula of a dioxymethylene phenyl substituted butyric acid ester $C_{12}H_{14}O_5$. The ion peaks arising at m/z 223 $[M\text{-}Me]^+$, 207 $[M\text{-}OMe]^+$ and 137 $[M - (CH_2)_3COOMe]^+$ indicated the presence of carboxymethyl group linked to butyric acid. The ^1H NMR spectrum of **4** exhibited two one-proton doublets at δ 7.52 (J =7.8 Hz) and 6.07 (J=7.8 Hz) assigned to ortho-coupled H-5 and H-6, respectively. A two-proton broad singlet at δ 3.99 was ascribed to dioxomethylene protons. A three-proton broad singlet at δ 3.55 was accounted to methoxy protons. The methylene protons appeared as two- proton broad signals at δ 2.80 and 1.25 and as one-proton multiplets at δ 2.33 and 2.12. The ^{13}C NMR spectrum of **4** displayed signals for ester carbon at δ 171.93 (C-1′), aromatic carbons between δ 160.77-113.41, dioxymethylene carbon at δ 93.04, methoxy carbon at 56.65 and methylene carbons at δ 39.27, 36.84 and 29.10. On the basis of these results the structure of **4** has been characterized as 4′-(2- hydroxy-3,4-dioxymethylenephenyl) methyl *n*- butyrate. This is a new aromatic ester.



4

Compound **8,** named arachidoyl arabinoside, was obtained as a colourless crystalline mass from chloroform-methanol (19:1) eluants. It gave positive tests for glycosides. Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3459, 3350 cm^{-1}), ester function (1722cm^{-1}) and long aliphatic chain (720 cm^{-1}). On the basis of its FAB mass and ^{13}C NMR spectra the molecular ion peak of **8** was determined at *m/z* 444 consistent with the molecular formula of a fatty acid glycoside $C_{26}H_{48}O_6$. The ion peaks arising at *m/z* 133 $[C_5H_9O_4]^+$, 150 $[C_5H_{10}O_5]^+$ and 311 $[M-133]^+$ indicated that C_{20} fatty acid was linked with C_5 sugar unit. The ^1H NMR spectrum of **8** displayed a one-proton doublet at δ 5.83 (J =7.0 Hz) assigned to anomeric H-1′ proton. Three one-proton multiplets at δ 4.35, 4.27 and 4.07 were ascribed to hydroxymethine H-2′, H-3′ and H-4′ protons, respectively. A two-proton broad singlet at δ 3.69 and two one-proton doublets at δ 2.77 (J=8.9 Hz) and 2.67 (J= 8.7 Hz) were attributed to oxygenated methylene H_2-5′ suggesting arabinose unit and to methylene H_2-2 protons adjacent to the ester group. The other methylene protons appeared between δ 2.27-1.25. A three-proton triplet at δ 0.88 (J=6.3 Hz) was accounted to terminal C-20 primary methyl protons. The ^{13}C NMR spectrum of **8** displayed signals for ester carbon at δ 170.11 (C-1), anomeric carbon at δ 104.15 (C-1′), other sugar carbons between δ 75.42 – 65.48, methylene carbons from δ 34.06 to 22.18 and methyl carbon at δ 14.17 (C-20). Acid hydrolysis of **8** yielded arachidic acid and D-arabinose. On the basis of spectral data analysis and chemical reactions the structure of **8** has been characterized as *n*-eicosanoyl-β-D-arabinopyranoside. It is a new acyl arabinoside.



8

CONCLUSION

The present work characterized phenolic acid esters and an acyl glycoside as new phytoconstituents from the roots of *R. communis* which may be used as chromatographic fingerprinting markers for quality control of the drug of arid region and may be responsible for medicinal uses of the roots.

Acknowledgement

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