



Research Article

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Nonpolar chemical constituents from the *Oryza sativa* L. bran

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Abstract

The rice bran comprises the germ, pericarp and aleurone layer. It serves as an important functional food that has cholesterol lowering properties, cardiovascular health benefits and anti-tumor activity, prevents high blood pressure, hyperlipidemia, hyperglycemia, diabetes mellitus, bone loss in women and is used in cosmetics and personal care products. Phytochemical investigation of a methanolic extract of *Oryza sativa* bran resulted in the isolation of natural phytoconstituents characterized as *n*-tetradecanyl linoleate (1), glyceryl-1,3-dioleoyl-2-linoleate (2), *n*-octadecanyl oleate (3), *n*-hexacosanyl oleate (4), *n*-nonacosanyl linoleate (5) and *n*-hexacosanyl stearate (6). The structures of these phytoconstituents have been elucidated on the basis of spectral data analysis and chemical reactions.

Keywords: *Oryza sativa*, Rice bran, Fatty esters, Glyceride, Characterization.

Introduction

Rice is the most important cereal product in Asia and is an overwhelming staple food in most populations of this region.^{1, 2} Milling of paddy yields 70 % of rice (endosperm) as the major product and by-products consisting of 20 % rice husk, 8 % rice bran and 2 % rice germ.^{1, 3-5} The bran serves as a valuable cattle feed. Fatty oil extracted from the bran is used for edible purpose.⁶ It comprises the germ, the pericarp, and aleurone layer and is often found mixed with varying quantities of husk. The bran is the hard outer layer of rice consisting of aleurone and pericarp. Rice bran contains an array of micronutrients like oryzanols, tocopherols, tocotrienols, phytosterols, fixed oil (20 %), proteins (15 %), carbohydrates (50 %, mainly starch) dietary fibers like beta-glucan, pectin, and gum.⁷⁻¹¹ It is suitable for niche markets like nutraceutical and pharmaceutical industry.

The presence of significant levels of minor-elements such as oryzanol, tocotrienol and phytosterols has a large nutraceutical application. They are used in the development of value-added healthy products³. γ -Oryzanol has been found to have higher antioxidant action in comparison with tocopherol. It comprises of ferulic acid esters of sterols and triterpene alcohols. The ferulic acid esters are campesterol, stigmasterol and β -cytosterol and the triterpene alcohols are cycloartenol, cycloartanol, 24-methylenecycloartanol and cyclobranol.¹²⁻¹³ Due to its antioxidant action, it is drawing immense interest in research world as a food additive. Stabilized rice bran contains large concentrations of several compounds and has the potential to prevent a range of chronic diseases. Rice bran serves as an important functional food that has cholesterol lowering properties, cardiovascular health benefits and anti-tumor activity.^{11, 14} Rice bran oil has shown immunostimulation effects. It is rich in phytosterols, sterolins and γ -oryzanol, and a compound with antioxidant property which may modulate the immune system.¹⁵ The γ -oryzanol of rice bran reduced a prominent amount of elevated serum levels in hypothyroid patients.¹⁶ Rice bran fraction derived from driselase treatment prevents high blood pressure, hyperlipidemia and hyperglycemia.¹⁷ The derivatives from the stabilized rice bran are rich in β -sitosterol which inhibit the growth and induce apoptosis in breast cancer cells.¹⁸

The nutraceuticals developed from the soluble and fiber fractions of rice bran control both type I and type II diabetes mellitus.¹⁹ Augmenting with rice bran health foods containing γ -oryzanol, reduced bone loss in women who suffered from postmenopausal osteoporosis.²⁰ Rice bran is also used in cosmetics and personal care products as an abrasive, absorbent, binder, bulking agent, hair and skin conditioning agents and surfactant. Modified arabinoxylan rice bran enhanced the activity of peritoneal natural killer cells in aged C57BL/6 and C3H mice.²¹ A lipoprotein fraction of rice bran induced apoptosis in human endometrial adenocarcinoma cells.²² The present paper describes the isolation and characterization of non-polar phytoconstituents from the rice bran.

Material and Methods

General

The IR spectra were recorded in KBr pellet on Win IR FTS 135 instrument (Biorad, USA). ¹H NMR (400 MHz) and ¹³C NMR (100 MHz) spectra were recorded by Bruker spectropin NMR instrument in CDCl₃ using TMS as internal standard. EIMS were scanned at 70 eV on a Jeol D-300 instrument (Jeol, USA). Column chromatography was performed on silica gel (Merck, 60-120 mesh) and thin layer chromatography on silica gel G-coated TLC plates (Merck).

Plant material

The rice bran was obtained from a Rice mill, Ghaziabad. It was dried in an oven at 45° C and kept in an airtight container.

Extraction and isolation of compounds

The rice bran (1.0 kg) was extracted with methanol in a Soxhlet apparatus. The solvent was evaporated under reduced pressure to obtain a dark brown mass (115.2 g). The dried extract was dissolved in minimum amount of methanol and adsorbed on silica gel to form a slurry. The slurry was air-dried and chromatographed over silica gel column prepared in petroleum ether. The column was eluted with n-hexane, petroleum ether and chloroform to isolate the following compounds:

n-Tetradecanyl linoleate (1)

Elution of the column with *n*-hexane-petroleum ether (1:1) gave a colourless semisolid mass of **1**; IR ν_{\max} (KBr): 1731, 1642, 1425, 1381, 1162, 1071, 722 cm⁻¹; ¹H NMR: δ 5.33 (1H, m, H-9), 5.29 (1H, m, H-10), 5.27 (2H, m, H-12, H-13), 4.25 (2H, t, *J*=6.8 Hz, H₂-1'), 2.23 (2H, t, *J*=7.3 Hz, H₂-2), 2.03-1.29 (12H, m, 6×CH₂), 1.23 (34H, brs, 17×CH₂), 0.87 (3H, t, *J*=6.6 Hz, Me-18), 0.84 (3H, t, *J*=6.3 Hz, Me-14'); ¹³C NMR: δ 169.28 (C-1), 139.32 (C-9), 129.97 (C-10), 128.74 (C-12), 123.11 (C-13), 61.41 (C-1'), 59.28-22.66 (C-24×CH₂), 14.13 (C-18), 14.03 (C-14'); EIMS *m/z* (*rel. Int.*): 476 [M]⁺ (C₃₂H₆₀O₂) (7.1), 279 (21.9).

Glyceryl-1,3-dioleo-2-linoleate (2)

Elution of column with *n*-hexane-petroleum ether (1:3) furnished a pale yellow semisolid of **2**; IR ν_{\max} (KBr): 1732, 1723, 1635, 1461, 1375, 1259, 1161, 1039, 720 cm⁻¹; ¹H NMR: δ 5.33 (2H, m, H-9', H-10'), 5.30 (2H, m, H-9''', H-10'''), 5.28 (2H, m, H-9'', H-10''), 5.15 (2H, m, H-13'', H-14''), 4.16 (1H, m, H-2), 4.05 (2H, m, H₂-1), 4.01 (2H, m, H₂-3), 2.75-1.58 (28H, m, 14×CH₂), 1.29 (14H, brs, 7×CH₂), 1.23 (38H, brs, 19×CH₂), 0.88 (6H, m, Me-18', Me-18'''), 0.83 (3H, t, *J*=6.6 Hz, Me-18''); ¹³C NMR: δ 171.69 (C-1'), 170.21 (C-1''), 169.81 (C-1'''), 130.42 (C-9'), 130.22 (C-9'''), 129.52 (C-10'), 129.68 (C-10'''), 129.37 (C-9''), 128.48 (C-10''), 128.07 (C-13'''), 127.95 (C-14''), 70.21 (C-2), 66.19 (C-1), 64.93 (C-3), 53.38 to 22.61 (40×CH₂), 14.13 (C-18'), 14.02 (C-18''), 11.92 (C-18'''); EIMS *m/z* (*rel. int.*): 882 [M]⁺ (C₅₇H₁₀₂O₆) (2.1).

n-Octadecanyl oleate (3)

Elution of the column with petroleum ether produced a pale yellow semisolid mass of **3**; IR ν_{\max} (KBr): 1739, 1640, 1461, 1259, 1173, 1095, 793, 723 cm⁻¹; ¹H NMR: δ 5.33 (1H, m, H-9), 5.30 (1H, m, H-10), 4.43 (2H, t, *J*=6.9 Hz, H₂-1'), 2.30-1.35 (24H, m, 12×CH₂), 1.28 (36H, brs, 18×CH₂), 0.86 (3H, t, *J*=6.3 Hz, Me-18), 0.83 (3H, t, *J*=6.1 Hz, Me-18'); ¹³C NMR: δ 171.81 (C-1), 129.87 (C-9), 123.05 (C-10), 64.15 (C-1'), 51.33-22.69 (30×CH₂), 19.68 (C-18), 14.06 (C-18'); EIMS *m/z* (*rel. int.*): 534 [M]⁺ (C₃₆H₇₀O₂) (2.9), 281 (8.6), 253 (12.9).

n-Hexacosanyl oleate (4)

Elution of the column with petroleum ether-chloroform (9:1) yielded a pale yellow semisolid mass of **4**; IR ν_{\max} (KBr): 1723, 1643, 1451, 1371, 1239, 1066, 751 cm⁻¹; ¹H NMR: δ 5.31 (1H, m, H-9), 5.16 (1H, m, H-10), 4.22 (2H, t, *J*=6.6 Hz, H₂-1'), 2.30-1.56 (16H, m, 8×CH₂), 1.23 (60H, brs, 30×CH₂), 0.85 (3H, t, *J*=6.5 Hz, Me-18), 0.82 (3H, t, *J*=6.3 Hz, Me-26'); ¹³C NMR: δ 169.71 (C-1), 130.03 (C-9), 124.97 (C-10), 64.11 (C-1'), 40.51-22.27 (38×CH₂), 16.41 (C-18), 14.47 (C-26'); EIMS *m/z* (*rel. int.*): 646 [M]⁺ (C₄₄H₈₆O₂) (6.8), 381 (100), 365 (5.9), 281 (12.3), 265 (8.1).

n-Nonacosanyl linoleate (5)

Elution of the column with petroleum ether-chloroform (4:1) yielded a yellow semisolid mass of **5**; IR ν_{\max} (KBr): 1729, 1645, 1451, 1359, 1215, 1161, 1063, 722 cm⁻¹; ¹H NMR: δ 5.39 (1H, m, H-9), 5.34 (1H, m, H-10), 5.30 (1H, m, H-12), 5.23 (1H, m, H-14), 4.10 (2H, t, *J*=6.7 Hz, H₂-1'), 2.78-1.28 (14H, m, 7×CH₂), 1.23 (32H, brs, 16×CH₂), 0.86 (3H, t, *J*=6.6 Hz, Me-18), 0.82 (3H, t, *J*=6.3 Hz, Me-29'); ¹³C NMR: δ 171.82 (C-1), 130.23 (C-9), 129.87 (C-10), 127.95 (C-12), 127.69 (C-13), 70.43 (C-1'), 34.10-22.69 (39×CH₂), 14.29 (Me-18), 14.15 (Me-29'); EIMS *m/z* (*rel. int.*): 686 [M]⁺ (C₄₇H₉₀O₂) (6.5), 423 (19.3), 279 (3.1).

***n*-Hexacosanyl stearate (6)**

Elution of the column with petroleum ether-chloroform (1:1) afforded a colourless amorphous powder of **6**; IR ν_{\max} (KBr): 1723, 1471, 1379, 1243, 1063, 971, 727 cm^{-1} ; ^1H NMR: δ 4.36 (2H, t, $J=6.8$ Hz, H_2-1'), 2.37 (2H, t, $J=7.2$ Hz, H_2-2), 2.35-1.28 (16H, m, $8\times\text{CH}_2$), 1.23 (62H, brs, $31\times\text{CH}_2$), 0.85 (3H, t, $J=6.2$ Hz, Me-18), 0.82 (3H, t, $J=6.4$ Hz, Me-26'); ^{13}C NMR: δ 169.71 (C-1), 66.08 (C-1'), 44.10 to 22.64 ($40\times\text{CH}_2$), 15.93 (C-26'), 14.11 (C-18); EIMS m/z (rel. int.): 648 $[\text{M}]^+$ ($\text{C}_{44}\text{H}_{88}\text{O}_2$) (8.1), 381 (11.2), 767 (5.1).

Results and Discussion

Compound **1** had a molecular ion peak at m/z 476 ($\text{C}_{32}\text{H}_{60}\text{O}_2$) in its mass spectrum relating to molecular formula of an aliphatic ester. An ion peak generated at m/z 279 $[\text{C}_5\text{H}_{11}\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COO}]^+$ indicated that linoleic acid was esterified with *n*-tetradecanol. Its IR spectrum exhibited absorption bands of ester group (1731 cm^{-1}), unsaturation (1642 cm^{-1}) and long aliphatic chain (722 cm^{-1}). The ^1H NMR spectrum of **1** displayed signals for vinylic protons as multiplets (δ 5.33-5.27), oxygenated methylene H_2-1' as a two-proton triplet at δ 4.25 ($J=6.8$ Hz), and primary methyl protons as three-proton triplets at δ 0.87 ($J=6.6$ Hz, Me-18) and 0.84 ($J=6.3$ Hz, Me-14'). The ^{13}C NMR spectrum of **1** showed signals for ester carbon at δ 169.28 (C-1), vinylic carbons from δ 139.32 to 123.11, oxygenated methylene carbon at δ 61.41 (C-1') and methyl carbons at δ 14.13 (C-18) and 14.03 (C-14'). On the basis of these evidences the compound **1** was identified as *n*-tetradecanyl linoleate.

Compound **2**, a glyceride, $[\text{M}]^+$ at m/z 882 ($\text{C}_{57}\text{H}_{102}\text{O}_6$), showed IR absorption bands for ester group (1732, 1723 cm^{-1}) and unsaturation (1635 cm^{-1}). Its ^1H NMR spectrum exhibited signals for eight vinylic protons between δ 5.33-5.15, oxygenated methine proton at δ 4.16, oxygenated methylene protons at δ 4.05 and 4.01, other methylene protons from δ 2.75 to 1.23 and methyl protons as a multiplet at δ 0.88 (6H) and as a three-proton triplet at δ 0.83 ($J=6.6$ Hz). The ^{13}C NMR spectrum of **2** displayed signals for ester carbons between δ 171.69 – 169.81, vinylic carbons from δ 130.42 to 127.95, oxygenated methine carbon at δ 70.21, oxygenated methylene carbons at δ 66.19 and 64.93 and methyl carbons at δ 14.13, 14.02 and 11.92. These spectral data led to characterized the structure of **2** as glyceryl-1,3-dioleoyl-3-linoleate.

Compound **3**, $[\text{M}]^+$ at m/z 534 ($\text{C}_{36}\text{H}_{70}\text{O}_2$), showed IR absorption bands for ester group (1739 cm^{-1}), unsaturation (1640 cm^{-1}) and long aliphatic chain (723 cm^{-1}). The ion fragments arising at m/z 281 $[\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COO}]^+$ and 253 $[\text{CH}_3(\text{CH}_2)_{17}]^+$ indicated that oleic acid was esterified with *n*-octadecanol. Its ^1H NMR spectrum of **3** exhibited signals for vinylic protons as one-proton multiplets at δ 5.33 and 5.30, oxygenated methylene as a two-proton triplet at δ 4.43 ($J=6.9$ Hz), methylene protons

between δ 2.30 – 1.28 and methyl protons as three-proton triplets at δ 0.86 ($J=6.3$ Hz, Me-18) and δ 0.83 ($J=6.1$ Hz, Me-18'). The ^{13}C NMR spectrum of **3** displayed signals for ester carbon at δ 171.81 (C-1), vinylic carbons at δ 129.87 (C-9) and 123.05 (C-10), oxygenated methylene carbon at δ 64.15 (C-1') and methyl carbons at δ 19.68 (C-18) and 14.06 (C-18'). On the basis of this discussion the structure of the compound **3** was determined as *n*-octadecanyl-*n*-octadec-9-enoate.

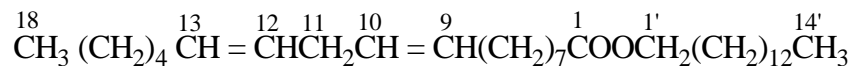
Compound **4**, $[\text{M}]^+$ at m/z 646 ($\text{C}_{44}\text{H}_{86}\text{O}_2$), showed IR absorption bands for ester group (1723 cm^{-1}), unsaturation (1643 cm^{-1}) and long aliphatic chain (751 cm^{-1}). The mass ion fragments arising at m/z 381 $[\text{CH}_3(\text{CH}_2)_{25}\text{O}]^+$, 365 $[\text{CH}_3(\text{CH}_2)_{25}]^+$, 281 $[\text{M}-365]^+$ and 265 $[\text{M}-381]^+$ suggested that oleic acid was esterified with *n*-hexacosanol. The ^1H NMR spectrum of **4** exhibited vinylic proton signals as one-proton multiplets at δ 5.31 (H-9) and 5.16 (H-10), oxygenated methylene protons as a two-proton triplet at δ 4.22 ($J=6.6$ Hz, H_2-1'), other methylene protons between δ 2.30 – 1.23 and primary methyl protons as three-proton triplets at δ 0.85 ($J=6.5$ Hz, Me-18) and δ 0.82 ($J=6.3$ Hz, Me-26'). The ^{13}C NMR spectrum of **4** displayed signals for ester carbon at δ 169.71 (C-1), vinylic carbons at δ 130.03 (C-9) and 124.97 (C-10), oxygenated methylene carbon at δ 64.11 (C-1') and methyl carbons at δ 16.41 (C-18) and 14.47 (C-26'). On the basis of these spectral data analysis, the structure of **4** has been elucidated as *n*-hexacosanyl-*n*-octadec-9-enoate.

Compound **5**, $[\text{M}]^+$ at m/z 686 ($\text{C}_{47}\text{H}_{90}\text{O}_2$), had IR absorption bands for ester group (1729 cm^{-1}), unsaturation (1645 cm^{-1}) and long aliphatic chain (722 cm^{-1}). The mass ion fragments arising at m/z 279 $[\text{CH}_3(\text{CH}_2)_3(\text{CH}_2\text{CH}=\text{CH})_2(\text{CH}_2)_7\text{COO}]^+$ and 423 $[\text{O}(\text{CH}_2)_{28}\text{CH}_3]^+$ indicated that linoleic acid was esterified with *n*-nonacosanol. Its ^1H NMR spectrum showed signals for vinylic protons as multiplets between δ 5.39 – 5.23, oxygenated methylene protons as a two-proton triplet at δ 4.10 ($J=6.7$ Hz, H_2-1'), other methylene protons between δ 2.78 – 1.23 and primary methyl protons as three-proton triplets at δ 0.86 ($J=6.6$ Hz, Me-18) and δ 0.82 ($J=6.3$ Hz, Me-29'). The ^{13}C NMR spectrum of **5** exhibited signals for an ester carbon at δ 171.82 (C-1), vinylic carbons between δ 130.23 – 127.69, oxygenated methylene carbon at δ 70.43 (C-1') and methyl carbons at δ 14.29 (C-18) and 14.15 (C-29'). This discussion led to establish the structure of **5** as *n*-nonacosanyl linoleate.

Compound **6**, $[\text{M}]^+$ at m/z 648 ($\text{C}_{44}\text{H}_{88}\text{O}_2$), showed IR absorption bands for ester group (1723 cm^{-1}) and long aliphatic chain (727 cm^{-1}). The mass ions arising at m/z 267 $[\text{CH}_3(\text{CH}_2)_{16}\text{CO}]^+$ and 381 $[\text{M}-267]^+$ suggested that stearic acid was esterified with *n*-hexacosanol. The ^1H NMR spectrum of **6** exhibited signals for oxygenated methylene protons as a three-proton triplet at δ 4.36 ($J=6.8$ Hz, H_2-1'), other methylene protons between δ 2.37 – 1.23 and primary methyl protons as three-proton triplets at δ 0.85 ($J=6.2$ Hz, Me-18) and δ 0.82 ($J=6.4$ Hz, Me-26'). The ^{13}C NMR spectrum of **6** displayed signals for ester carbon at δ

169.71 (C-1), oxygenated methylene carbon at δ 66.08 (C-1') and methyl carbons at δ 15.93 (C-26') and 14.11 (C-18). On the basis of above evidences, the structure of the compound **6** has been formulated as *n*-hexacosanyl stearate.

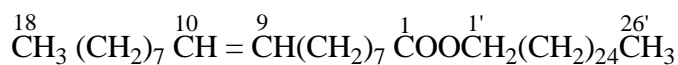
The structural formulae of compounds 1, 3-6.



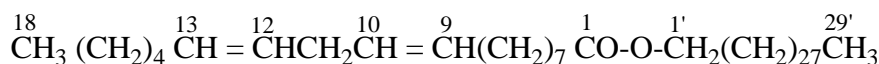
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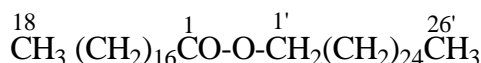
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4



5



6

Conclusion

Phytochemical investigation of rice bran furnished with the isolation of six neutral chemical constituents that may increase the existing knowledge of chemical constituents of rice bran. The isolated constituents may be useful in the treatment of hyperlipidaemia and obesity related disorders.

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