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CHEMICAL CONSTITUENTS OF BROWN RICE GRAIN (*Oryza sativa*)

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*One new compound 3,7,11,15,19-pentamethyl-9α,10α,11α,17α,18α-pentahydroxy-n-tetracosan-1-oxy-p-hydroxycaffeoate (oryzaterpenyl caffeoate) (**1**), together with three known fatty acids linoleic acid, stearic acid and myristic acid were isolated and identified from the rice grain of* Oryza sativa. *The structure of the new compound was elucidated by 1D and 2D NMR spectroscopic techniques (^1H–^1HCOSY, ^1H–^{13}C HETCOR) aided by EI-MS, and IR spectra.*

Key words: *Oryza sativa*, Poaceae, rice grains, 3,7,11,15,19-pentamethyl-9α,10α,11α,17α,18α-pentahydroxy-*n*-tetracosan-1-oxy-*p*-hydroxycaffeoate, fatty acids.

Rice (*Oryza sativa*) is the principal cereal food in Asia, the major staple food of the majority of the population, and is generally of two types; white hulled and colored hulls, but the most common is white (85%). On the variety front around the globe three major varieties are produced, of which Javanica, a medium grain variety, is grown only in Indonesia, and another two varieties; a long grain variety known as Indica, best suited for warm climate, is cultivated throughout South and Southeast Asia, and Central and South America. The other major variety the round grain Japonica; is well suited for cold the climate of East and Northeast Asia as well as some parts of North America like California. Color hulled rice, though not used by many, is still popular in China and Japan. One of the colored rice brown or red is believed to be rich in vitamin B and also has high fibre, and carbohydrate and protein contents, thus classifying it as a healthy food. The absence of gluten in this rice also makes it nonallergic to masses. This healthy rice is reported to have a marked effect on reducing the risk of coronary heart disease in both men and women, where a 36% reduction in heart attack is observed in men consuming such rice. Prolamin and glutelin compounds from mature and developing rice grains [1–2], which are polymeric procyanidins that are radical-scavenging components [3], have been reported. Several lipids from brown rice have been reported in the literature [4]. The antioxidant activity of brown rice, measured by DPPH and TBA methods, have also been described [5]. The literature does not contain much information even on the chemical constituents of its grain except for four reports [1–4]. This paper deals with the isolation and structural elucidation of a new compound **1** based on ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY, ^{1}H–^{13}C HETCOR aided by EIMS and IR spectra and three known fatty acids: linoleic, stearic, and myristic.

Oryzaterpenyl Caffeoate (1). Oryzaterpenyl caffeoate **1** was obtained as a yellow semi-solid in minor quantity from the aqueous methanol (80%) extract of the rice grains. Its IR spectrum exhibited characteristeric absorption bands for hydroxy (3465 cm^{-1}) and ester (1730 cm^{-1}) groups. The mass spectrum of **1** displayed a molecular ion peak at *m/z* 65 corresponding to an acyclic nor-triterpene esterified with hydroxycaffeoic acid moiety, $C_{38}H_{68}O_8$. The prominent ion peaks generated at *m/z* 149 [HO-C_6H_4-CH_2CH_2CO]$^+$, 503 [M-149]$^+$ and 165 [HO-$C_6H_4CH_2CH_2$COO]$^+$ supported the esterification of the hydroxycaffeoyl moiety with the triterpene alcohol. The prominent ion peaks at *m/z* 71, 581 [C_{19}–C_{20} fission]$^+$, 99 [C_{18}–C_{19} fission]$^+$, 129 [C_{17}–C_{18} fission]$^+$, 159, 493 [C_{16}–C_{17} fission]$^+$, 479 [C_{15}–C_{16} fission]$^+$, 201, 451[C_{14}–C_{15} fission]$^+$, 215 [C_{13}–C_{14} fission]$^+$,

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and 229 [C_{12}–C_{13} fission]$^+$ suggested the location of the two hydroxyl groups at C-18 and C-17. The other intensified ion peaks appearing at *m/z* 243 [C_{11}–C_{12} fission]$^+$, 287 [C_{10}–C_{11} fission]$^+$, 317 [C_9–C_{10} fission]$^+$, 347 [C_8–C_9 fission]$^+$, 361 [C_7–C_8 fission]$^+$, 389 [C_6–C_7 fission]$^+$, 403 [C_5–C_6 fission]$^+$, 417 [C_4–C_5 fission]$^+$ and 431 [C_3–C_4 fission]$^+$ indicated the location of the remaining three hydroxyl groups at C_{11}, C_{10} and C_9. The ^{1}H NMR spectrum of **1** contains four one-proton multiplets at δ 7.71, 7.69, 7.53, and 7.51 assigned to aromatic H-6′, H-8′, H-5′ and H-9′ respectively. A one-proton multiplet at δ 4.32 with a half -width of 6.9 Hz was ascribed to the β-oriented H-9 carbinol proton. Another one-proton multiplet at δ 4.26 with half-width of 6.8 Hz was attributed to the β-oriented H-18 carbinol proton. Two one-proton doublets at δ 4.25 (J = 5.9 Hz) and 4.23 (J = 5.9 Hz) were associated with C-1′ oxygenated methylene protons. Another one-proton doublet at δ 4.29 (J = 3.0 Hz) was attributed to the β-oriented H-10 hydroxymethine proton.

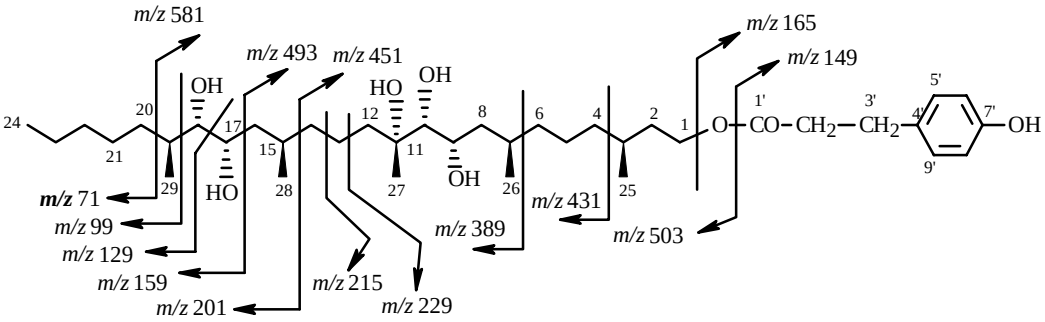

Fig. 1. Fragmentation pattern of **1**.

A one-proton double doublet at δ 4.21 with coupling interactions of 2.3, 6.3 and 4.6 was assigned to carbinol H-17β. A three-proton broad signal at δ 1.25 was due to C-27 tertiary methyl protons attached to the carbinol carbon. Four three-proton doublets at δ 0.92 (J = 7.55 Hz), 0.88 (J = 6.7 Hz), 0.84 (J = 6.5 Hz), and 0.82 (J = 8.0 Hz) were attributed to C-29, C-28, C-26, and C-25 secondary methyl protons, respectively. A three-proton triplet at δ 0.77 (J = 7.6 Hz) was ascribed to C-24 primary methyl protons. The remaining methine and methylene protons appeared between δ 1.25–0.77 suggesting their attachment at the saturated carbon atoms. The ^{13}C NMR spectrum of **1** exhibited signals for the ester carbon δ 167.96, benzene carbon signals between δ 167.91–129.02, and oxygenated carbons at δ 64.50 (C-1), 66.10 (C-9), 66.41 (C-10), 70.98 (C-11), 64.61 (C-18), and 68.37 (C-17). The methyl carbon signals appeared at δ 11.17 (C-24), 11.59 (C-25), 14.31 (C-26), 25.69 (C-27), 19.77 (C-28), and 22.87 (C-29). The remaining methylene and methine carbon signals resonated in the range δ 23.97 - 33.31. The ^{13}C–^{1}H HETCOR spectrum of **1** showed correlation of C-7′ with H-6′, H-5′, H-8′, and H-9′; C-9 with H-8, H-10, and H-7; C-10 with H-9 and H-8; C-17 with H-15, H-16, H-18, and H-19; C-18 with H-16, H-17, H-19, and H-20. The ^{1}H–^{1}H COSY spectrum of **1** showed ^{1}H–^{1}H correlation of H-5′ with H-6′ and H-8′ with H-9′; H_2-1 with H_2-2, H-1, and H_2-2′. On the basis of the foregoing account the structure of **1** has been formulated as 3,7,11,15,19-pentamethyl-9α,10α,11α,17α,18α-pentahydroxy-*n*-tetracosan-1-oxy-*p*-hydroxyl caffeoate. The spectroscopic and physical data of known fatty acids are described in the experimental section.

EXPERIMENTAL

Melting points were determined on an Electrochemical Eng. melting point apparatus and are uncorrected. Optical rotation was measured on an AA-10 model polarimeter. IR spectra were recorded on a Thermo Mattson 60-AR spectrophotometer. UV spectra were recorded using a UV-vis spectrometer TU-180PC.^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were obtained on a Brucker Avance (DRX-500) using $CDCl_3$ as solvent. EI-Mass spectra were recorded on a JEOL JMS-SX 102 A spectrometer. Column chromatography was performed over silica gel 70–230 (Merck). TLC analyses were performed on precoated silica gel glass plates 60 F_{254} (Merck) and visualized under UV light and by spraying with a vanillin 1 g/sulfuric acid 5 ml/ethanol 94 ml solution followed by heating (100–110°C).

Plant Material. The grains of *O. sativa* were collected from Konkuk University (experimental farm) Seoul, South Korea in October 2002.

Extraction and Isolation of Compounds. The dried grains of *O. sativa* (2 kg) were finely ground to powder form and extracted with 80% methanol at room temperature. The solvent was evaporated under reduced pressure, the extract was freeze dried, and a powder extract was obtained (26.1 g). The whole extract was subjected to normal phase silica gel (600 g) column chromatography and gave the following fractions. Fractions 1–2 in hexane, frs. 3–4 in hexane–EtOAc (9:1), frs. 5–6, hexane–EtOAc (8:2), frs. 7–10 hexane–EtOAc (7:3), frs. 11–12 in hexane–EtOAc (3:2), frs. 13–14 in hexane–EtOAc (1:1), frs. 15–16 in hexane–EtOAc (2:3), frs. 17–18 in hexane–EtOAc (3:7), frs. 19–20 in hexane–EtOAc (2:8), frs. 21–22 in hexane–EtOAc (1:9), frs. 23–24 in EtOAc, frs. 25–26 in EtOAc–MeOH (9:1), frs. 27–28 in EtOAc–MeOH (7.5:2.5), frs. 29–30 in EtOAc–MeOH (1:1), frs. 31 and 32 in EtOAc–MeOH (2.5:7.5), and frs. 33–34 in MeOH. Fraction 7 after CC and preparative TLC afforded a new compound in minor quantity (1.3 mg) and other known fatty acids (100 mg). Fractions 8–11 after TLC, and after mixing and further CC over silica gel column chromatography also afforded known fatty acids (130 mg). The identify of the compounds was confirmed by comparison with an authentic sample from Sigma. The other polar fraction contain only sugar molecules on the basis of NMR.

3,7,11,15,19-Pentamethyl-9α,10α,11α,17α,18α-pentahydroxy-*n*-tetracosan-1-oxy-*p*-hydroxycaffeoate (1). Yellow semi-solid; R_f 0.34 (Hexane–EtOAc, 7:3); UV λ_{max}($CHCl_3$) 238 nm; $[\alpha]_D^{22}$ + 29.8° (*c* 0.8, $CHCl_3$); IR (neat): ν_{max} 3465, 2950, 2855, 1730, 1470, 1360, 1235, 1150,1080, 725 cm^{-1}.

PMR spectrum (δ, ppm, $CDCl_3$, TMS, J/Hz): 7.71 (1H, m, H-6′), 7.69 (1H, m, H-8′), 7.53 (1H, m, H-5′), 7.51(1H, m, H-9′), 4.32 (1H, m, $W_{1/2}$ = 6.9, H-9β), 4.29 (1H, d, J = 3.0, H-10β), 4.26 (1H, m, $W_{1/2}$ = 6.8, H-18β), 4.25 (1H, d, J = 5.9, H_2-1′α), 4.23 (1H, d, J = 5.9, H_2-1′β), 4.21 (1H, ddd, J = 2.3, J = 6.3, J = 4.6, H-17β), 1.72 (1H, ddd, J = 7.15, J = 4.5, J = 7.0, H-19α), 1.69 (1H, ddd, J = 7.1, J = 6.0, J = 6.0, H-15α), 1.42 (1H, m, H-7α), 1.39 (2H, m, H_2-16), 1.37 (2H, m, H_2-12), 1.36 (1H, m, H_2-8), 1.35 (2H, m, H_2-2), 1.33 (1H, m, H-3α), 1.31 (10 H, br s, 5 × CH_2), 1.30 (8H, br s, 4 × CH_2), 1.25 (3H, br. s, Me-27), 0.92 (3H, d, J = 7.55, Me-29), 0.88 (3H, d, J = 6.7, Me-28), 0.84 (3H, d, J = 6.5, Me-26), 0.82 (3H, d, J = 8.0, Me-25), 0.77 (3H, t, J = 7.6, Me-24).

^{13}C NMR ($CDCl_3$): δ 64.50 (C-1), 30.58 (C-2), 33.31(C-3), 26.35 (C-4), 26.07 (C-5), 23.97 (C-6), 36.69 (C-7), 34.56 (C-8), 66.10 (C-9), 66.41(C-10), 70.98 (C-11), 32.98 (C-12), 29.31 (C-13), 29.14 (C-14), 36.79 (C-15), 32.71 (C-16), 68.37 (C-17), 64.61(C-18), 38.96 (C-19), 29.70 (C-20), 28.99 (C-21), 28.81(C-22), 26.98 (C-23), 11.17(C-24), 11.59 (C-25), 14.31 (C-26), 25.69 (C-27), 19.77 (C-28), 22.87 (C-29), 167.96 (C-1′), 26.95 (C-2′), 26.53(C-3′), 132.69 (C-4′), 131.08 (C-5′), 132.56 (C-6′), 167.91 (C-7′), 131.11 (C-8′), 129.02 (C-9′).

EIMS *m/z* (rel. int.): 652 $[M]^+$ ($C_{38}H_{68}O_8$) (6.1), 596 (34.6), 581 (34.2), 565 (82.6), 550 (45.3), 535 (46.7), 507 (24.2), 503 (12.5), 493 (12.6), 451 (5.6), 431 (20.9), 417 (16.1), 403 (19.7), 389 (18.5), 361 (18.7), 347 (17.4), 317 (53.6), 289 (22.1), 287 (14.6), 249 (10.7), 243 (70.1), 229 (18.1), 215 (27.3), 201 (6.3), 165 (100), 159 (7.3), 149 (47.8), 129 (12.3), 99 (12.3), 71 (60.5), 57 (100).

Fatty Acids. Colorless oil; R_f 0.32–0.41 (Hexane–EtOAc, 7:3).

Linoleic Acid (2). Colorless solid; R_f 0.32.

EIMS *m/z* (rel.int.): M^+ 280 ($C_{18}H_{32}O_2$, linoleic acid) (28.0), 284 ($C_{18}H_{36}O_2$, stearic acid) (5.2), 228 ($C_{14}H_{28}O_2$, myristic acid) (5.6), 256 (100), 238, 213, 185, 171, 129, 79, 55.

Stearic Acid (3). colorless solid; R_f 0.41, mp 69–70°C.

EIMS *m/z* (rel. int.) 284 $[M]^+$ ($C_{18}H_{36}O_2$)(5.2), 256 ($C_{16}H_{32}O_6$) (100), 238 (12), 213 (39), 185 (22), 129 (48), 57 (76).

Myristic Acid (4). Colorless solid; R_f 0.38; mp 58–59°C.

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