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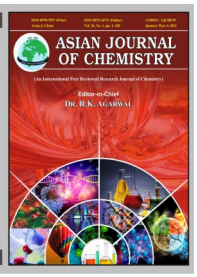

Glycerol Derivatives of Fatty Acid and Other Constituents from Straw of *Oryza sativa*

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Four compounds glyceryl-dioleo-linoleate (**1**), *n*-heptadecanyl oleate (**2**), *n*-Tridecanyl *n*-octadec-9, 12-dienoate (**3**), *n*-hexacos-14-enoic acid (**4**) were isolated from the methanol extract of *Oryza sativa* of rice straw. The structures have been elucidated with the help 600 MHz NMR using 1D spectral methods *viz*., ^{1}H and ^{13}C, aided by ESI/FTMS and IR spectroscopy. The compounds **1-4** are reported for the first time in rice straw of *O. sativa*.

Keywords: *Oryza sativa* L., Gramineae, Rice straw composition, Chemical constituents.

INTRODUCTION

Rice (*Oryza sativa* L.) is the major staple food in Asia and generally exists in two types, white hulled and colored hulled. The most common type (85 %) is white hulled rice. The germination of rice is of great agricultural importance and has long been known to be influenced by compounds present in the seed coat (hull)[1,2]. The compounds momilactone A and B from rice hulls cause germination and growth inhibition in the roots of rice[3-5]. They were later found in rice leaves and rice straw as phytoalexins[6,7].

Rice straw has been applied back in larger amounts into paddy and also upland fields, especially green house croppings, as an organic material mainly for soil improvement. The degradation products of rice straw in the soils may influence the growth of crops in both nutritional and physiological aspects. The elucidation of mechanism of humus formation from rice straw is also of importance for understanding its influence on plant growth[8]. Phenolic substances are widely distributed in various plants, including the different parts of rice plant. Some of the substances, which enter into soils from plants, cause dieback disease or other abnormal growth as inhibitors against plant growth. It was reported that *p*-coumaric acid and other phenolic acids, for instance, inhibited the growth of the upland rice plant. Kuwatsuka and Oshima[9] isolated and or identified *p*-hydroxybenzoic acid, vanillic, *p*-coumaric and ferulic acids from rice leaves. Inamatsu[10] also found *p*-coumaric acid in a methanol extracts of rice straw and recognized that the amount of the acid decreased during the heaping of rice straw.

Phenolic compounds were reported from rice straw on the basis of HPLC or GC analysis[8,11]. Identification of allelopathic compounds including momilactones A and B from rice straw and their biological activity have also been reported[11-17].

Previously reported compounds from rice straw were mostly studied on the basis of HPLC or GC analysis. Moreover, there are not many reports in the literature on the isolation of compounds through extraction and separation by column chromatography of rice straw. The identification of constituents from rice straw by spectroscopic analysis like IR, NMR and MS is rarely reported although they could be powerful tools to identify bioactive constituents. To our best of knowledge, this is the first report of the isolation of four compounds from the rice straw of *O. sativa*. All of the known compounds were identified by comparison with standards and literature data[13,18].

EXPERIMENTAL

Melting points were determined using a model IA9100 melting point apparatus (Electrochemical Engineering, Seoul, South Korea). Optical rotation was measured with an Instrument Ltd. (Seoul, Korea) model AA-10 polarimeter. UV spectra were measured with a TU-1800_{PC} UV-visible spectrophotometer. IR spectra were recorded on a Thermo Scientific FT-IR model Nicolet 6700 (USA) spectrophotometer at the Korea Institute of Science and Technology (KIST) Seoul, South Korea. ^{1}H NMR and ^{13}C NMR spectra were obtained at 600 and 150 MHz, respectively, using a Bruker Avance-600 spectrometer available at National Instrumentation Centre for Environmental Management (NICEM), college of Agriculture and Life Science, Seoul National University (SNU), Seoul, South Korea. NMR spectra were obtained in deuterated chloroform, d_5-pyridine and d_4-methanol using tetramethylsilane

(TMS) as an internal standard, with chemical shifts expressed in ppm (δ) and coupling constants (*J*) in Hz. High resolution ESI/FT mass spectra were recorded on a Thermo-Finnigan LTQ-Orbitrap instrument (Thermo Scientific, USA) equipped with Dionex U 3000 HPLC system (NICEM, Seoul National University). All chemicals were of an analytical grade. *n*-Hexane, ethyl acetate, methanol, ethanol, sulphuric acid and vanillin were purchased from Daejung Chemicals and Metals (Seoul, South Korea). Thin layer chromatography was performed on precoated silica gel 60 F_{254} plates (Merck). Visualization of the TLC plates was performed using a 5 % H_2SO_4 in ethanol spray reagent. Column chromatography was performed using silica gel (70-230 mesh) and LiChroprep RP-18 (40-63 μm; ODS silica gel) from Merck. Authentic standards of β-sitosterol, stearic acid, palmitic acid, oleic acid and D-xylose were purchased from Sigma-Aldrich (St. Louis, MO, USA).

The straw of *O. sativa* was collected from the Konkuk University experimental farm, Seoul, Korea in October 2010. After harvesting, the samples were dried in the laboratory temperature range (25-30 °C) for 3 weeks. The voucher specimen (reference code ILPUM variety) has been dried and deposited in the herbarium of the Department of Applied Life Science, Konkuk University, Korea.

Extraction of rice straw: Dried straws of *O. sativa* (10 kg) were immersed in methanol (55 L) for one week at room temperature and then the supernatant was concentrated under vacuum to yield 78 g extract. This material was suspended in water and extracted with hexane, ethyl acetate and *n*-butanol successively and evaporated to produce hexane (9.4 g), ethyl acetate (11.2 g) and *n*-butanol (14.2 g) extracts.

Isolation of compounds from hexane extract: The hexane extract (9.4 g) was column chromatographed over silica gel (70-230 mesh, 200 g, 800 mm × 25 cm) with hexane and ethyl acetate solvents and yielded 30 fractions (each fraction 200 mL) with the following eluants: fraction 1-2 in hexane, fractions 3-5 in hexane:EtOAc (9.5:0.5), fractions 6-11 in hexane: EtOAc (9:1), fractions 12-15 in hexane:EtOAc (8.5:1.5), fractions 16-20 in hexane:EtOAc (8:2), fractions 21-22 in hexane-EtOAc (7:3), fractions 23-26 in hexane-EtOAc (6:4), fractions 27-30 in hexane-EtOAc (1:1). Fraction 11, which was further purified by column chromatography over silica gel with methylene dichloride and methanol, produced two pure compounds **1** (32 mg) and **2** (29 mg). Fraction 16-20, after column chromatography over silica gel with hexane-chloroform and yielded two more compounds **3** (18 mg) and **4** (21 mg).

Glyceryl dioleo linoleate (1) (Fig. 1): Semi solid; R_f 0.41 (hexane: EtOAc; 7:3); IR (KBr, ν_{max}, cm^{-1}): 2925, 2854, 2363, 1736, 1722, 1635, 1461, 1377, 1239, 1163, 1042, 705; ^{1}H NMR ($CDCl_3$): δ 5.32 (4H, m, H-9', H-10', H-9''', H-10'''), 5.29 (2H, m, H-9'', H-10''), 5.18 (2H, m, H-13'', H-14''), 4.10 (1H, m, H-2), 4.02 (2H, m, H_2-1), 3.96 (2H, m, H_2-3), 2,75 (2H, m, H_2-12''), 2.28 (6H, m, H_2-2', H_2-2'', H_2-2'''), 2.05 (4H, m, H_2-8', H_2-8'''), 2.01 (4H, m, m, H_2-11', H_2-11'''), 1.98 (4H, m, 4H, m, H_2-8'', H_2-15''), 1.58 (8H, m, 4 × CH_2), 1.27 (14 H, br s, 7 × CH_2), 1.24 (40 H, br s, 20 × CH_2), 0.86 (6H, br s, Me-18', Me-18'''), 0.83 (3H, t, *J* = 6.5 Hz, Me-18''); ^{13}C NMR ($CDCl_3$): δ 173.59 (C-1'), 171.24 (C-1''), 168.69 (C-1'''), 130.40 (C-9'), 130.16 (C-9'''), 129.87 (C-10'), 129.71 (C-10'''), 129.58 (C-9''), 128.55 (C-10''), 128.02 (C-13''), 127.81 (C-14''), 70.29 (C-2), 66.21 (C-1), 64.97 (C-3), 54.36 (3 × CH_2), 39.66 to 22.58 (37 × CH_2), 14.06 (Me-18'), 11.70 (Me-18''), 10.81 (Me-18'''): ESI MS *m/z* (rel. int.) 882 $[M]^+$ ($C_{57}H_{102}O_6$ (1.3), 281 (14.2), 261 (23.8).

***n*-Heptadecanyl oleate (2) (Fig. 1):** Semi solid; R_f 0.32 (hexane: EtOAc; 7:3); IR (KBr, ν_{max}, cm^{-1}): 2922, 2852, 1742, 1642, 1464, 1260, 1170, 1097, 1029, 794, 765; ^{1}H NMR ($CDCl_3$): δ 5.35 (1H, m, H-9), 5.33 (1H, m, H-10), 4.57 (2H, d, *J* = 7.2 Hz, H_2-1'), 2.30 (2H, t, *J* = 7.5 Hz, H_2-2), 2.01 (2H, m, H_2-8), 1.62 (2H, m, H_2-11), 1.35 (4H, m, 2 × CH_2), 1.28 (18 H, br s, 9 × CH_2), 0.87 (3H, t, *J* = 6.5 Hz, Me-18), 0.84 (3H, t, *J* = 6.3 Hz, Me-17'); ^{13}C NMR ($CDCl_3$): δ 173.84 (C-1), 129.94 (C-9), 118.20 (C-10), 61.14 (C-1'), 51.33 (C-2), 40.49 (CH_2), 39.71 (CH_2), 37.34 (CH_2), 38.99 (CH_2), 36.60 (CH_2), 34.36 (CH_2), 32.46 (CH_2), 31.91 (CH_2), 29.44 (3 × CH_2), 25.01 (CH_2), 22.68 (CH_2), 19.71 (Me-18), 14.08 (Me-17'), ESI /FTMS *m/z* : 520 $[M]^+$ ($C_{35}H_{68}O_2$ (3.5), 281 (8.9), 239 (13.2).

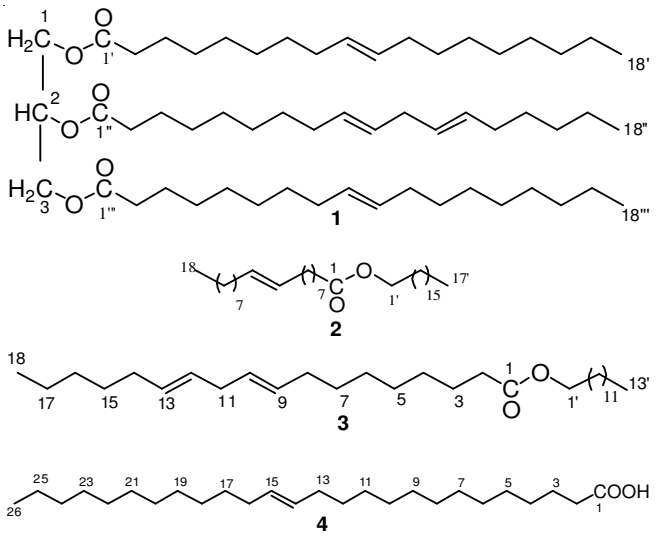

Fig. 1. Structure of chemical constituents of **1-4**

***n*-Tridecanyl *n*-octadec-9, 12-dienoate (3) (Fig. 1):** Semi solid; R_f 0.34 (hexane:EtOAc; 7:3); IR (KBr, ν_{max}, cm^{-1}): 924, 2854, 1734, 1642, 1428, 1378, 1163, 1075, 721; ^{1}H NMR ($CDCl_3$): δ 5.36 (2H, m, H-9, H-10), 5.31 (2H, m, H-12, H-13), 4.12 (2H, d, *J* = 6.6 Hz, H_2-1'), 2.31 (2H, t, *J* = 7.2 Hz, H_2-2), 2.28 (2H, m, H_2-11), 2.03 (2H, m, H_2-8), 1.98 (2H, m, H_2-14), 1.66 (4H, m, 2 × CH_2), 1.28 (4H, m, 2 × CH_2), 1.24 (30 H, br s, 15 × CH_2), 0.88 (3H, t, *J* = 6.5 Hz), Me-18), 0.83 (3H, t, *J* = 6.2 Hz, Me-13'); ^{13}C NMR ($CDCl_3$): δ 167.68 (C-1), 139.41 (C-9), 129.94 (C-10), 128.74 (C-12), 123.04 (C-13), 61.37 (C-1'), 59.30 (C-11), 52.26 (CH_2), 39.81 (CH_2), 39.30 (CH_2), 37.30 (CH_2), 37.22 (CH_2), 32.72 (CH_2), 29.64 (CH_2), 28.15 (CH_2), 24.40 (CH_2), 22.66 (CH_2), 14.06 (C-18), 13.99 (C-13'),; ESI/FTMS *m/z* 462 $[M]^+$ ($C_{31}H_{58}O_2$) (16.3), 279 (26.5).

***n*-Hexacos-14-enoic acid (4) (Fig. 1):** Semi solid; R_f 0.38 (hexane:-EtOAc; 7:3); IR (KBr, ν_{max}, cm^{-1}): 3110, 2918, 2863, 1705, 1642, 1439, 1271, 1062, 865, 721; ^{1}H NMR ($CDCl_3$): δ 5.33 (1H, m, H-14), 5.31 (1H, m, H-15), 2.32 (2H, t, *J* = 7.3 Hz, H_2-2), 2.04 (2H, m, CH_2), 1.99 (2H, m, CH_2), 1.61 (2H, m, CH_2), 1.52 (2H, m, CH_2), 1.25 (4H, m, 2 × CH_2), 1.21 (30H, br s, 15 × CH_2), 0.85 (3H, t, *J* = 6.5 Hz, Me-26); ^{13}C

NMR($CDCl_3$): δ 180.21 (C-1), 130.22 (C-14), 128.06 (C-15), 39.18 ((CH_2), 37.14 (CH_2), 33.98 (CH_2), 32.56 (CH_2), 31.83 (CH_2), 29.16 (CH_2), 28.79 (CH_2), 27.11 (CH_2), 25.38 (CH_2), 24.55 (CH_2), 22.68 (CH_2), 14.15 (C-26); ESI/FTMS: 394 $[M+H]^+$ ($C_{26}H_{50}O_2$) (18.2), 181 (97.2).

RESULTS AND DISCUSSION

The hexane extract of rice straw of *O. sativa* was chromatographed on a silica gel column using hexane-EtOAc and to yield four compounds **1-4**. All the compounds are reported for the first time in *O. sativa*. Compounds **1-4** and similar compounds were reported in lietrature[19-21].

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