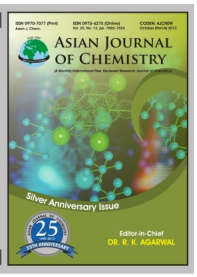
Asian Journal of Chemistry; Vol. 25, No. 17 (2013), 9872-9874

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.15553



Chemical Constituents from the Rice Straw of *Oryza sativa*

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(*Received*: 30 March 2013; *Accepted*: 25 October 2013) AJC-14294

Four compounds, *n*-hexacosanyl palmitate (**1**), *n*-hexacosanyl oleate (**2**), *n*-nonacosanyl linoleiate (**3**) and stigmasterol (**4**) were isolated from the methanol extract of *Oryza sativa* of rice straw. The structures of the compounds were elucidated using ^{1}H NMR, ^{13}C NMR in combination with IR, ESI/MS, ESI/FTMS.

Key Words: *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 coloured 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 identified *p*-hydroxybenzoic acid, vanillic, *p*-coumaric and ferulic acids from rice leaves. Inamatsu[10] also found *p*-coumaric acid in a methanol extract of rice straw and recognized that the amount of the acid decreased during the heaping of rice straw.

The 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 been reported.

Previously reported compounds from rice straw only on the basis of HPLC or GC analysis[11-13]. Because there are no reports in the literature on the isolation of compounds through extraction and separation by column chromatography of rice straw. Identification of constituents from rice straw by spectroscopic analysis like IR, NMR and mass is not reported, so far. Identification bioactive constituents with growth or germination inhibitory and algicidal properties is still required. To achieve these objectives, the aims of our research were to isolated and identified constituents from rice straw.

This paper deals with isolation and structure elucidation of three constituents, on the basis of ^{1}H and ^{13}C spectroscopic studies, ESI/FTMS and IR from rice straw. This is the first report of the isolation of four constituents *n*-hexacosanyl palmitate (**1**), *n*-hexacosanyl oleate (**2**), *n*-nonacosanyl linoleiate (**3**) and stigmasterol (**4**) from rice straw of *Oryza sativa*.

EXPERIMENTAL

Melting points were determined using Electrochemical Engineering (Electrothermal, Seoul, South Korea) model IA9100 melting point apparatus. Optical rotation was measured with an Instruments Ltd (Seoul, South Korea) model AA-10 polarimeter. ^{1}H 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). 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.

The straw of *O. sativa* were collected from the Konkuk University experimental farm, Seoul, Korea in October 2010. After harvesting, the samples was dried in the Laboratoty temperature range (25-30 ºC) 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.

Extraction of rice straw: Dried straws of *O. sativa* (10 kg) were immersed in methanol (55 L) for 1 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 to produce 9.4 g of hexane, ethyl acetate 11.2 g and 14.2 g *n*-butanol extract.

Isolation of compounds from ethyl acetate extract: The EtOAc extract (11.2 g) was subjected to normal-phase column chromatography over silica gel and yielded 30 fractions with the following eluants (each fraction 250 mL): fraction 1-4 in hexane, fractions 5-8 in hexane:EtOAc (9:1), fractions 9-12 in hexane:EtOAc (8:2), fractions 13-16 in hexane:EtOAc (7:3), fractions 17-20 in hexane:EtOAc (1:1), fractions 21-24 in hexane-EtOAc (3:7), fractions 25-30 in EtOAc. Further chromatography of fractions 17-18 and 21-22 rechromatographed over silica gel with hexane-chlorofom mixture yielded three compounds **1** (23 mg), **2** (21 mg) and **4** (18 mg).

***n*-Hexacosanyl palmitate (1):** Dark **y**ellow crystals; R_f 0.45 ($CHCl_3$: MeOH; 9.9:0.1); ^{1}H NMR ($CDCl_3$, 600 MHz): δ 4.30 (2H, t, *J* = 6.6 Hz, H_2-1'), 2.39 (2H, t, *J* = 7.2 Hz, H_2-2), 2.03 (2H, m, CH_2), 2.01 (2H, m, CH_2), 1.97 (2H, m, CH_2), 1.67 (2H, m, CH_2), 1.59 (4H, m, 2 × CH_2), 1.28 (4H, m, 2 × CH_2), 1.24 (58 H, br s, 29 x CH_2), 0.86 (3H, t, *J* = 6.0 Hz, Me-16), 0.83 (3H, t, *J* = 6.6 Hz, Me-26'); ^{13}C NMR ($CDCl_3$; 150 MHz): δ 167.73 (C-1), 65.53 (C-1'), 44.10 (CH_2), 39.31 (CH_2), 39.01 (CH_2), 38.65 (CH_2),), 37.39 (CH_2), 37.22 (CH_2), 36.42 (CH_2), 34.05 (CH_2), 32.73 (CH_2), 31.87 (CH_2), 29.65 (17 × CH_2), 29.43 (CH_2), 29.31 (CH_2), 29.10 (CH_2), 28.87 (CH_2), 27.92 (CH_2), 27.14 (CH_2), 26.70 (CH_2), 25.57 (CH_2), 24.41 (CH_2), 22.64 (CH_2), 19.70 (Me-26'), 15.99 (CH_2), 14.09 (Me-16); IR ((KBr, ν_{max} cm^{-1}): 2964, 2860, 1721, 1468, 1377, 1241, 1064, 970, 737; ESIFT MS *m/z* (rel. int.) 621 $[M+H]^+(C_{42}H_{85}O_2)$ (9.3), 381 (10.1), 365 (2.8), 239 (2.5).

***n*-Hexacosanyl oleate (2):** Yellow solid; R_f 0.42 ($CHCl_3$: MeOH; 99:0.1); ^{1}H NMR ($CDCl_3$; 600 MHz); δ 5.30 (1H, m, H-9), 5.07 (1H, m, H-10), 4.10 (2H, t, *J* = 6.8 Hz, H_2-1'), 2.30 (2H, t, *J* = 7.2 Hz, H_2-2), 2.03 (2H, m, H_2-8), 1.94 (2H, m, H_2-11), 1.63 (2H, m, CH_2), 1.56 (6H, m, 3 x CH_2), 1.22 (62 H, br s, 31 × CH_2), 0.83 (3H, t, *J* = 6.5 Hz, Me-18), 0.84 (3H, t, *J* = 6.5 Hz, Me-26'); ^{13}C NMR ($CDCl_3$;150 MHz): δ 169.25 (C-1), 130.12 (C-9), 125.06 (C-10), 64.17 (C-1'), 40.44 (CH_2), 40.09 (CH_2), 38.12 (CH_2), 37.97 (CH_2), 34.70 (CH_2), 33.48 (CH_2), 32.62 (CH_2), 30.36 (CH_2), 30.05 (CH_2), 30.01 (CH_2), 29.92 (CH_2), 28.67 (CH_2), 27.86 (CH_2), 27.34 (CH_2), 25.49 (CH_2), 25.14 (CH_2), 23.35 (CH_2), 23.16 (CH_2), 20.24 (CH_2), 16.44 (CH_2), 14.50 (Me-18); IR (KBr, ν_{max} cm^{-1}): 2964, 2818, 1722, 1645, 1453, 1374, 1240, 1067, 753; ESI FTMS *m/z* (rel. int.) 647 $[M+H]^+(C_{44}H_{87}O_2)$ (7.3), 381 (100), 365 (6.2).

Stigmasterol (4): Colourless soild; R_f 0.42 ($CHCl_3$: MeOH; 99:0.1); m.p 162-164 ºC; ESI /FTMS *m/z* (rel. int.) 413 $[M + H]^+(C_{29}H_{48}O)$ (27.3); ^{1}H and ^{13}C NMR data (compare with literature).

Isolation of compounds from *n*-butanol extract: The entire *n*-butanol extract (14.2 g) was subjected to normal-phase CC over silica gel (800 g) to yield 32 fractions (250 mL) with the following eluants: fraction 1-2 with chloroform, fractions 3-5 with chloroform:methanol (99:1), fractions 6-11 with chloroform:methanol (98:2), fractions 12-15 with chloroform: methanol (97:3), fractions 16-20 with chloroform:methanol (96:4), fractions 21-24 with chloroform:methanol (9.5:0.5), fractions 25-28 with chloroform:methanol (9:1), fractions 29-30 with chloroform:methanol (8.8:1.2), fractions 31-32 with chloroform:methanol (8.5:1.5). All fractions were examined by TLC. Fractions 2-3 were combined and re-chromatographed over silica gel again with dichloromethane:methanol to yield one compound **3** (19 mg).

***n*-Nonacosanyl linoleiate (3):** Yellow solid; R_f 0.38 ($CHCl_3$:MeOH; 99:0.1) ^{1}H NMR ($CDCl_3$): δ 5.36 (1H, m, H-9), 5.35 (1H, m, H-10), 5.32 (1H, m, H-12), 5.25 (1H, m, H-13), 3.65 (2H, t *J* = 6.8 Hz, H_2-1'), 2.78 (2H, t, *J* = 7.2 Hz, H_2-2), 2.30 (2H, m, H_2-11), 2.07 (2H, m, H_2-8), 2.02 (2H, m, H_2-14), 1.56 (4H, m, 2 × CH_2), 1.28 (2H, m, CH_2), 1.24 (12 H, br s, 6 × CH_2), 0.87 (3H, t, *J* = 6.5 Hz, Me-18), 0.81 (3H, t, *J* = 6.2 Hz, Me-29'); ^{13}C NMR ($CDCl_3$): δ 174.81 (C-1), 130.20 (C-9), 129.92 (C-10), 128.05 (C-12), 127.74 (C-13), 70.51 (C-1'), 34.10 (CH_2), 32.77 (CH_2), 31.91 (CH_2), 31.49 (CH_2), 29.68 (4 × CH_2), 29.32 (CH_2), 29.13 (CH_2), 27.16 (CH_2), 25.60 (CH_2), 22.67 (CH_2), 14.27 (Me-18), 14.10 (Me-29'); (IR, KBr, ν_{max} cm^{-1}): 2916, 2860, 1732, 1645, 1456, 1362, 1204, 1157, 1065, 720; ESI/FTMS *m/z* (rel. int.) 687 $[M + H]^+$ $(C_{47}H_{90}O_2$ (6.2), 423 (21.5), 279 (2.9).

RESULTS AND DISCUSSION

The chemical structures of compounds **1-4** are given in Fig. 1.

Compound **1**, was obtained as a dark yellow crystals. The ESI/FTMS and ^{13}C NMR of compound **1** has been established its molecular formula $C_{42}H_{84}O_2$. The ion fragments arising at *m/z* 365 $[CH_2$-$(CH_2)_{24}$-$CH_3]^+$, 381 [-O CH_2-$(CH_2)_{24}$-$CH_3]^+$ and 239 $[CH_3$-$(CH_2)_{14}$-$CO]^+$. Its IR spectrum exhibited characteristic absorption bands for ester functions (1721 cm^{-1}), long aliphatic chain (737 cm^{-1}). The fragmentation pattern of **1** is shown in Fig. 2.

The ^{1}H NMR spectrum of **1** showed two protons two triplets at δ 4.30 (*J* = 6.6 Hz) and 2.39 (*J* = 7.2 Hz) were assigned to oxygenated methylene H_2-1' and H_2-2, respectively. The remaining methylenes as multiplets resonated between δ 2.03-1.28. A broad signal at δ 1.24 was assigned for multiples methylenes. Two three proton triplets at δ 0.86 (*J* = 6.0 Hz) and 0.83 (*J* = 6.6 Hz) was integrating for Me-16 and Me-26'. The ^{13}C NMR spectrum of **1** displayed signals for ester carbon at δ 167.73 (C-1), oxygenated methylene 65.53 (C-1'), 44.10

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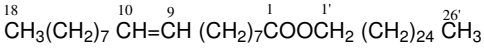

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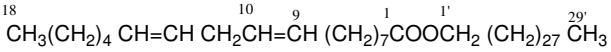

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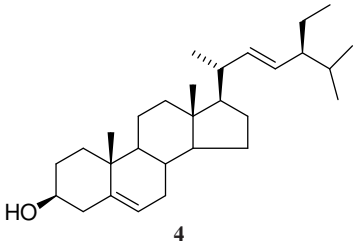

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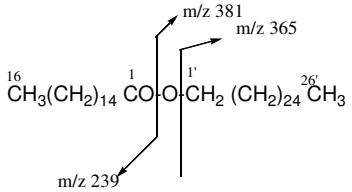
Fig. 1. Chemical constituents of compounds **1-4**



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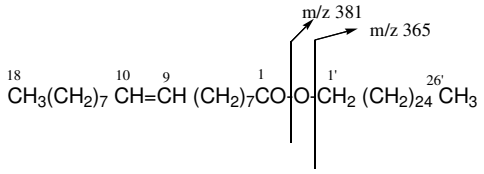

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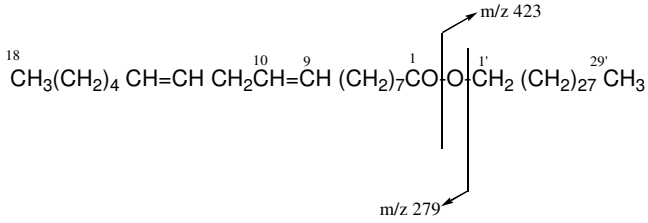

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Fig. 2. Fragmentation pattern of compounds **1, 2** and **3**

C-2) and two methyl 14.09 (C-16), 19.70 (C-26'). The other methylenes resonated between at δ 44.10-22.64. On the basis of these evidences the structure of **1** was established as *n*-hexacosanyl palmitate.

Compound **2**, was obtained as a yellow powder. The ESI/FTMS and ^{13}C NMR of compound **2** has been established its molecular formula $C_{44}H_{86}O_2$. The ion fragments arising at *m/z* 365 $[CH_2\text{-}(CH_2)_{24}\text{-}CH_3]^+$ and 381 $[\text{-O } CH_2\text{-}(CH_2)_2\text{-}CH_3]^+$. Its IR spectrum exhibited characteristic absorption bands for ester functions (1722 cm^{-1}), long aliphatic chain (753 cm^{-1}). The fragmentation pattern of **2** is shown in Fig. 2.

The ^{1}H NMR spectrum of **2** showed two protons two triplets at δ 4.10 (J = 6.8 Hz) and 2.30 (J = 7.2 Hz) were assigned to oxygenated methylene H_2-1' and H_2-2, respectively. The vinylic protons at δ 5.30 and 5.07 showed multiplets for H-9 and H-10 protons, respectively. Two multiplets showed at δ 2.03 and 1.94 was assigned for H_2-8 and H_2-11 protons. The remaining methylenes as multiplets resonated between δ 1.94-1.22. Two three proton triplets at δ 0.83 (J = 6.5 Hz) and 0.84 (J = 6.4 Hz) was integrating for Me-18 and Me-26'. The ^{13}C NMR spectrum of **2** displayed signals for ester carbon at δ 169.25 (C-1), oxygenated methylene 64.17 (C-1') and vinylic carbons 130.12 (C-9), 125.06 (C-10), methyls 14.50 (C-18), 18.31 (C-26'). The other methylenes resonated between at δ 40.44-20.24. On the basis of these evidences the structure of **2** was established as *n*-hexacosanyl oleate.

Compound **3**, was obtained as a yellow solid. The ESI/FTMS and ^{13}C NMR of compound **3** has been established its molecular formula $C_{47}H_{90}O_2$. The ion fragments arising at *m/z* 279 $[CH_2\text{-}(CH_2)_4\text{-}CH{=}CHCH_2CH{=}CH(CH_2)_7CO\text{-}O]^+$ and 423 $[\text{-O } CH_2\text{-}(CH_2)_{27}\text{-}CH_3]^+$. Its IR spectrum exhibited characteristic absorption bands for ester functions (1732 cm^{-1}), long aliphatic chain (720 cm^{-1}). The fragmentation pattern of **3** is shown in Fig. 2.

The ^{1}H NMR spectrum of **3** showed two protons two triplets at δ 3.65 (J = 6.8 Hz) and 2.78 (J = 7.2 Hz) were assigned to oxygenated methylene H_2-1' and H_2-2, respectively. The vinylic protons at δ 5.36, 5.35, 5.32 and 5.25 showed multiplets for H-9, H-10, H-11 and H-12 protons, respectively. Three multiplets showed at δ 2.30, 2.07 and 2.02 were assigned for H_2-8, H_2-11 and H_2-14 protons. The remaining methylenes as multiplets resonated between δ 1.56-1.24. Two three proton triplets at δ 0.87 (J = 6.5 Hz) and 0.81 (J = 6.2 Hz) was integrating for Me-18 and Me-29'. The ^{13}C NMR spectrum of **3** displayed signals for ester carbon at δ 174.81 (C-1) and vinylic carbons 130.20 (C-9), 129.92 (C-10), 128.05 (C-12) and 127.74 (C-13) and methyls 14.27 (C-18), 14.10 (C-29'), oxygenated methylene 70.51 (C-1'). The other methylenes resonated between at δ 34.10-22.67. On the basis of these evidences the structure of **3** was established as *n*-non-acosanyl linoleiate.

ACKNOWLEDGEMENTS

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011-0015691).

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