Chemical Constituents from the Rice Straw of *Oryza sativa*

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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\(^3,^4\). They were later found in rice leaves and rice straw as phytoalexins\(^5,^6\).

Rice straw has been applied back in larger amounts into paddy and also upland fields, especially green house crops, 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\(^6\). 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\(^3\) 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 $d_6$-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 were dried in the Laboratoty temperature range (25-30°C) for 3 weeks. The voucher specimen (reference code ILPUM) 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 extract (11.2 g) was subjected to normal-phase column chromatography over silica gel with hexane-chloroform mixture yielded three compounds 1-3 with chloroform:methanol:ethyl acetate:hexane (9:1:1:1) and 14.2 g n-butanol extract.

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**n-Hexacosanoyl palmitate (1):** Dark yellow crystals; Rf 0.45 (CHCl₃:MeOH; 9:9:0.1); ¹H NMR (CDCl₃; 600 MHz): δ 4.30 (2H, t, J = 6.6 Hz, H-2'), 2.39 (2H, t, J = 7.2 Hz, H-3'), 2.03 (2H, m, CH₂), 3.11 (2H, m, CH₂), 1.67 (2H, m, CH₂), 1.59 (4H, m, 2 × CH₂), 1.28 (4H, m, 2 × CH₂), 1.24 (58 H, br s, 29 × CH₂), 0.86 (3H, t, J = 6.0 Hz, Me-16); ¹³C NMR (CDCl₃; 150 MHz): δ 167.73 (C-1), 65.53 (C-1'), 44.10 (CH₂), 39.31 (CH₃), 39.01 (CH₃), 38.65 (CH₂), 37.39 (CH₃), 37.22 (CH₃), 36.42 (CH₃), 34.05 (CH₃), 32.73 (CH₃), 31.87 (CH₃), 29.65 (17 × CH₃), 29.43 (CH₃), 29.31 (CH₃), 29.10 (CH₃), 28.87 (CH₃), 27.92 (CH₂), 27.14 (CH₂), 26.70 (CH₃), 25.57 (CH₃), 24.41 (CH.), 22.64 (CH₃), 19.70 (Me-26), 15.99 (CH₃), 14.09 (Me-16); IR (KBr, vₑᵤₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑ廨��), 9873

**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 ¹³C NMR of compound has been established its molecular formula C₂₉H₄₀O₂. The ion fragments arising at m/z 365 [C₁₅H₂₂O₂]⁺, 381 [O₂HCH(OH)₂CH₂]⁺ and 239 [CH₃(C₆H₅)₂CO]⁺. 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 ¹H NMR spectrum of 1 showed two protons two triplets at 6.430 (J = 6.6 Hz) and 2.39 (J = 7.2 Hz) were assigned to oxygenated methylene H-1' and H-2', respectively. The remaining methylenes as multiplets resonated between δ 2.03-1.28. A broad signal at δ 1.24 was assigned for multiplets methylenes. Two 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 ¹³C NMR spectrum of 1 displayed signals for ester carbon at δ 167.73 (C-1), oxygenated methylene 65.53 (C-1'), 44.10

![Chemical Constituents from the Rice Straw of Orzwa sativa](https://example.com/chemical-constituents.png)
methylenes as multiplets resonated between $\delta$ 1.94-1.22. Two three proton triplets at $\delta$ 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 $\delta$ 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 $\delta$ 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_{53}H_{89}O_{3}. The ion fragments arising at $m/z$ 279 [CH$_3$-(CH$_2$)$_{13}$-CH=CHCH$_2$CH=CH(CH$_3$)CO-O] and 423 [-OCH$_2$-(CH$_3$)$_2$-CH$_2$]. 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 $\delta$ 3.65 ($J = 6.8$ Hz) and 2.78 ($J = 7.2$ Hz) were assigned to oxygenated methylene H-1' and H-2', respectively. The vinylic protons at $\delta$ 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 $\delta$ 2.30, 2.07 and 2.02 were assigned for H-8, H-11 and H-14 protons. The remaining methylenes as multiplets resonated between $\delta$ 1.56-1.24. Two three proton triplets at $\delta$ 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 $\delta$ 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 $\delta$ 34.10-22.67. On the basis of these evidences the structure of 3 was established as n-non-acosanyl linoleate.

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