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Stigmasteryl Arachidate Constituent from the Straw of *Oryza sativa*

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One compound stigmasteryl-3β-arachidate (**1**) was isolated and identified from the rice straw of *Oryza sativa*. The structure of the compound was elucidated by 1D and 2D NMR (COSY, HSQC and HMBC) spectroscopic techniques aided by ESI-MS and IR spectra. To best of our knowledge, the compound **1** was identified for the first time from the rice straw of *Oryza sativa*.

Keywords: *Oryza sativa* L., Gramineae, Rice straw, Chemical constituent, Stigmasteryl-3β-arachidate.

INTRODUCTION

Rice (*Oryza sativa* L.) is the major staple food in Asia and generally exists as 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 momilactones A and B from rice hulls cause germination and growth inhibition in the rice roots[3-5]. They are also found in rice leaves and rice straw as phytoalexins.[6,7]. Rice straw is reapplied in large amounts to paddy and upland fields, especially greenhouse croppings, as an organic material for soil improvement. The degradation products of rice straw in the soil may influence the growth of crops in both nutritional and physiological terms. The elucidation of the mechanism of humus formation from rice straw is also important for understanding its influence on plant growth[8]. Phenolic substances are widely distributed in various plants, including the different parts of rice plants. Some of these substances, which enter the soil from plants, are plant growth inhibitors and cause dieback disease or other abnormal growth. It has been reported that *p*-coumaric acid and other phenolic acids, for instance, inhibited the growth of upland rice plants. Kuwatsuka and Oshima[9] isolated and/or identified *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid and ferulic acid from rice leaves. Inamatsu[10] also found *p*-coumaric acid in 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 activities have been reported[11-16]. In continuation of our previous work[17,18], we have isolated one more compound from rice straw of *O. sativa*. To our best of knowledge, the compound **1** (Fig. 1) was identified for the first time. Earlier the compound stigmasteryl-3β-arachidate was reported from roots of *Ricinus communis*[19].

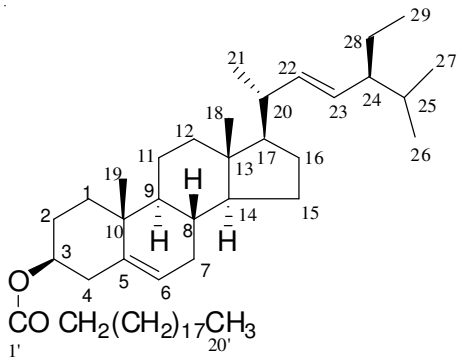

Fig. 1. Chemical structure of stigmasteryl-3β-arachidate (**1**)

EXPERIMENTAL

Melting points were determined using a model IA9100 melting point apparatus (Electrochemical Engineering, Seoul, South orea). 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 and ^{13}C NMR spectra were obtained at 600 and 150 MHz, respectively, using a Bruker Avance-600 spectrometer, available at the National Instrumentation Center for Environmental Management (NICEM), College of Agriculture and Life Science, Seoul National University (SNU), Seoul, South Korea. NMR spectra were obtained in deuterated chloroform,

using tetramethylsilane (TMS) as an internal standard, with chemical shifts expressed in parts per million (δ) and coupling constants (*J*) in hertz. Highresolution ESI/FT mass spectra were recorded on a Thermo-Finnigan LTQ-Orbitrap instrument (Thermo Scientific, USA) equipped with a Dionex U 3000 HPLC system (NICEM, Seoul National University). All chemicals were of analytical grade. *n*-Hexane, ethyl acetate, methanol, ethanol, sulfuric acid and vanillin were purchased from Daejung Chemicals and Metals (Seoul, South Korea). Thin-layer chromatography was performed on precoated silica gel 60 F254 plates (Merck). Visualization of the TLC plates was performed using 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; octadecyl silica (ODS) gel] from Merck. Authentic standards of stigmasterol and arachidic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA).

The rice (*O. sativa* L.) straw used in the present study was collected after harvesting of rice cereal at the Konkuk University experimental farm, Yeoju, South Korea, in October 2010. The collected samples were dried in the laboratory in the temperature range of 25-30 °C for 3 weeks with some modifications of a previous study[15]. A 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 *O. sativa* straw (10 kg) was immersed in methanol (55 L) for 1 week at room temperature and then the supernatant was concentrated under vacuum to yield 78 g of 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 (CC) over silica gel (70-230 mesh, 200 g, 800 × 25 mm) with hexane and ethyl acetate solvents and yielding 25 fractions (each frcation 250 mL): fractions 1-2 in hexane, fractions 3-4 in hexane/EtOAc (9.5:0.5), fractions 5-6 in hexane/EtOAc (9:1), fractions 7-8 in hexane/EtOAc (8:2), fractions 9-10 in hexane/EtOAc (7:3), fractions 11-12 in hexane/EtOAc (6:4), fractions 13-14 in hexane/EtOAc (1:1); fractions 15-16 in hexane/EtOAc (4:6) fractions 17-18 in hexane/EtOAc (3:7); fractions 19-20 in hexane/EtOAc (2:8), fractions 21-22 in hexane/EtOAc (1:9); fractions 23-25 in EtOAc. Fractions 19-20 combined and rechromatographed over silica gel with chloroform and methanol to afford compound **1** in crystalline form with eluants chloroform-methanol (97:3).

Stigmasteryl-3β-arachidate (1): Yellow crystals; R_f 0.34 (hexane:ethyl acetate; 2:8); m.p. 112-114°; IR (KBr, ν_{max}, cm^{-1}): 2932, 2845, 1723, 1637, 1401, 1379, 1240, 1192, 1064, 966, 930, 801, ; ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.33 (1H, m, H-6), 5.13 (1H, dd, *J* = 9.0, 9.0 Hz, H-22), 5.01 (1H, dd, *J* = 9.0, 8.4 Hz, H-23), 3.51 (1H, br m, $W_{1/2}$ = 15.6 Hz, H-3a), 2.32 (2H, t, *J* = 7.2 Hz, H2-2), 1.02 (3H, br s, Me-19), 0.92 (3H, d, *J* = 6.3 Hz, Me-21), 0.87 (3H, d *J* = 6.2 Hz, Me-26), 0.85 (3H, d, *J* = 6.2 Hz, Me-27), 0.83 (3H, t, *J* = 6.5 Hz, Me-20'), 0.79 (3H, t, 6.1 Hz, Me-29), 0.67 (3H, br s, Me-18), 2.80-1.04 (59 H, m, 26 x CH_2, 7 x CH); ^{13}C NMR (150 MHz; $CDCl_3$): δ 37.19 (C-1), 31.47 (C-2), 71.65 (C-3), 42.23 (C-4), 140.67 (C-5), 121.58 (C-6), 31.82 (C-7), 33.86 (C-8), 50.07 (C-9), 36.29 (C-10), 21.01 (C-11), 39.54 (C-12), 43.13 (C-13), 56.69 (C-14), 24.23 (C-15), 29.07 (C-16), 55.93 (C-17), 11.91 (C-18), 19.32 (C-19), 36.07 (C-20), 18.64 (C-21), 138.25 (C-22), 129.18 (C-23), 45.74 (C-24), 29.24 (C-25), 18.92 (C-26), 18.89 (C-27), 22.98 (C-28), 11.78 (C-29), 171. 99 (C-1'), 51.16 (C-2'), 40.44 (CH_2), 39.54 (CH_2), 39.61 (CH_2), 29.65 (6 x CH_2), 28.85 (CH_2), 28.67 (CH_2), 28.13 (CH_2), 26.01 (CH_2), 25.34 (CH_2), 24.76 (CH_2), 22.63 (CH_2), 14.06 (C-20'); ESIMS *m/z* rel. int. 707 $[M + H]^+$ ($C_{49}H_{87}O_2$) (2.2), 411 (45.3), 394 (32.8), 311 (6.7), 295 (35.1), 139 (37.6) (Fig. 2).

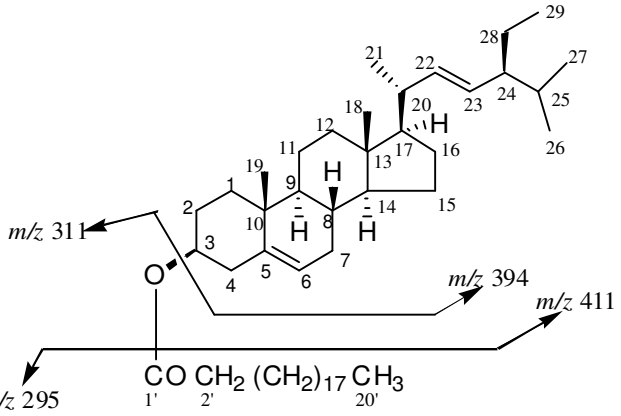

Fig. 2. Fragmentation patterns of stigmasteryl-3β-arachidate (**1**)

Acid hydrolysis of compound 1: Compound **1** (5 mg) was refluxed with 2 mL of 1M hydrochloric acid/dioxane (1:1) in a water bath for 4 h. The reaction mixture was evaporated to dryness and column chromatographed over silica gel with hexane and ethyl acetate to obtain the stigmasterol and arachidic acid and confirmed with authentic standards.

RESULTS AND DISCUSSION

Compound **1**, stigmasteryl-3β-arachidate, was obtained as a yellow crystals from hexane-ethyl acetate (2:8) eluants. Its IR spectrum showed characteristic absorption bands at 2932, 2845 and ester function (1723 cm^{-1}), double bonds (1637 cm^{-1}) On the basis of ESI mass and ^{13}C NMR spectra, the molecular ion peak of 1 was determined at *m/z* 706 consistent to the molecular formula of sterol attached with some long chain acid $C_{49}H_{86}O_2$.

The ^{1}H NMR spectrum of **1** indicated steroidal moiety attached arachidic acid moiety at C-3 position, as displayed one-proton multiplet at δ 5.33 and two double doublets at δ 5.01 (*J* = 9.0, 8.4 Hz), 5.13 (*J* = 9.0, 9.0) were assigned to H-6, H-22 and H-23 protons, respectively. A one-proton broad multiplet at δ 3.51 with half-width of 15.6 Hz was ascribed to oxygenated methane H-3α proton. Two proton triplet at δ 2.32 (*J* = 7.2 Hz) was due to methylene H_2-protons adjacent to ester group. Two three protons broad signals at δ 0.67 and 1.02 and three doublets at δ 1.02 (*J* = 6.3), 0.87 (*J* = 6.2) and 0.85 (*J* = 6.6) intergrating for three protons each were accounted to terteiary C-18 and C-19 and secondary C-21, C-26 and C-27

methyl protons, respectively. Two three protons triplets at δ 0.79 (J = 6.1 Hz) and 0.83 (J = 6.5 Hz) were due C-20 and C-20' primary methyl protons. The remaining methylene and methine protons appeared from δ 2.80 to 1.04. The ^{13}C NMR spectrum of **1** displayed signals for ester carbon at δ 171.99 (C-1'), vinylic carbons 140.67 (C-5), 121.58 (C-6), 138.25 (C-22) and 129.18 (C-23), oxygenated methine carbon δ 71.65 (C-3). The methyls carbons were appeared at δ 11.91 (C-18), 19.32 (C-19), 18.64 (C-21), 29.24 (C-25), 18.92 (C-26) and 18.98 (C-27). The ^{13}C NMR spectral data of the steroidal nucleus were compared with the reported values of sitosterol and its derivatives[19-22]. The ^{1}H ^{1}H COSY spectrum of **1** showed correlations of H-3 with H_2-1, H_2-2, H_2-4 and H_2-2'; H-6 with H_2-4, H_2-7 and H-8. The HMBC spectrum of **1** exhibited interactions of C-3 with H_2-2, H_2-4 and H-2'; C-5 with H_2-4, H-3 and H-6' and C-1' with H-3 and H_2-2' and H_2-3'. The HSQC spectrum of **1** showed correlations of C-3 at δ 71.65 with H-3 at δ 3.51; C-6 at δ 121.58 with H-6 at δ 5.33. Acid hydrolysis of **1** yielded stigmasterol and arachidic acid. On the basis of spectral data and chemical reactions, the structure of **1** has been established as stigmasteryl-3β-arachidate. This is a known steroidal compound and reported for the first time in rice straw of *O. sativa*.

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