

New Stigmastane Steroids Constituents from Rice Hulls of *Oryza sativa* and Inhibitory Activity on Radish seed

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Two new compounds 1'-(stigmast-11,20(21),25-trien-3 α ,9 α -diolyl)-3'-(pimara-11,15-dien-3 α -olyl) glycerol (**1**) and stigmast-5-en-3 α ,26-diol (**2**) along with known fatty acids *n*-hexacosanoic acid (**3**) and hexadecanoic acid (**4**), have been isolated from the methanol extract of rice hulls of *Oryza sativa*. The structures of the compounds were elucidated using 1D and 2D NMR spectral methods, viz; ¹H, ¹³C, ¹³C-DEPT, ¹H-¹H COSY, ¹H-¹³C HETCOR, HSQC and HMBC aided by IR, EIMS, FABMS and HRFABMS. Compound (**1**) showed inhibition to radish germination, growth of shoot and root length.

Key Words : *Oryza sativa* L., Poaceae, Rice hull compounds, 1'-(Stigmast-11,20(21),25-trien-3 α ,9 α -diolyl)-3'-(pimara-11,15-dien-3 α -olyl) glycerol, Stigmast-5-en-3 α ,26-diol

Introduction

Rice (*Oryza sativa* L.) is the principal cereal food in Asia and the major staple food of the majority of the population. It generally consists of two types: white and colored hulled, but the most common is white (85%). The germination of rice is of great agricultural importance and it has long been known that it is influenced by compounds present in the seed coat (hull).^{1,2} The diterpenoids (momilactones A and B) from the rice hulls are reported to growth and germination inhibition in the roots of rice.³⁻⁵ They were later found in rice leaves and straw as phytoalexins.^{6,7} A putative growth inhibitor was isolated from rice root exudates and identified as momilactone B⁸ and it was recently reported that rice seedlings release momilactone B into the environment.⁹ The antioxidant activity of methanol extracts¹⁰ and C-glycosyl-flavonoid from rice hulls have been reported.¹¹ The growth inhibitor sakuranetin, a flavonone phytoalexin from ultraviolet-irradiated rice leaves, has also been isolated.¹² However, it has been suggested that rice hulls, the most abundant agricultural by-product in rice growing areas, possess allelopathic substances that could serve as natural herbicides by inhibiting seed germination and the growth of weeds. The spectral data of known and new compounds of rice hulls and their activity were reported.¹³⁻¹⁷ This paper deals with the isolation and structural elucidation of two new steroids (**1-2**) on the basis of spectral data analysis and chemical reactions and inhibitory activity of compound (**1**) against radish germination, growth of shoot and root length.

Results and Discussion

Compound **1**, named sativasterolide, was obtained as a colourless crystalline mass from methanol extract of rice hulls. It gave positive tests with Liebermann-Burchard and

Salkowski reagents for steroids and a yellow colour with tetranitromethane solution in chloroform indicating unsaturated nature of the molecule. Its IR spectrum displayed characteristic absorption bands for hydroxyl group (3485 cm⁻¹) and unsaturation (1640 cm⁻¹). Its molecular formula (C₅₂H₈₂O₄) was established by positive ion HRFABMS at m/z 770 consistent with diterpenoid sterol glycerol derivative, C₅₂H₈₂O₄. The prominent ion peaks at m/z 425 corresponding to sterol containing three vinylic linkages and two hydroxyl groups, C₂₉H₄₅O₂ and the ion peak at m/z 287 tricyclic diterpene alcohol, C₂₀H₃₁O₂ were located to oxygenated methylene groups of the glycerol moiety. The ion fragments at m/z 410 [425 – Me]⁺, 407 [425 – H₂O]⁺, 392 [407 – Me]⁺, 288 [425 – C₁₀H₁₇, side chain]⁺, 273 [288 – Me]⁺, 258 [273 – Me]⁺, 255 [273 – H₂O]⁺, and 179 [C_{14,15} – C_{13,17} fission]⁺, suggested the attachment of C₁₀ diunsaturated side chain in the steroidal moiety. The ion fragments at m/z 269 [287 – H₂O]⁺, 242 [269 – CHCH₂]⁺, 227 [242 – Me]⁺, 272 [287 – Me]⁺, 254 [272 – H₂O]⁺, 227 [257 – CHCH₂]⁺, 257 [272 – Me]⁺, 188 [C_{4,5} – C_{1,10} fission]⁺, 148, 139 [C_{5,6} – C_{9,10} fission]⁺, 134, 153 [C_{6,7} – C_{9,10} fission]⁺, 120, 167 [C_{7,8} – C_{9,10} fission]⁺, indicated pimarane type carbon framework with saturated rings A and B and hydroxyl groups in ring A placed at C-3 on the basis of biogenetic consideration. The fragmentation pattern of compound **1** also are shown in Figure 2.

The ¹H-NMR spectrum of **1** displayed two one-proton doublets at δ 5.79 (*J* = 10.5 Hz), and 5.74 (*J* = 10.5 Hz) assigned to vinylic H-11 and H-12, respectively, interacting with each other and indicated tetrasubstituted C-9 and C-13 adjacent to the vinylic bonds. Two broad signals at δ 4.87 and 4.89 integrated for two protons each, were accounted C-21 and C-26 methylene protons. A one-proton broad multiplet at δ 3.19 was attributed to H-3 carbinol proton and its β -orientation was determined with its half-width of 11.7

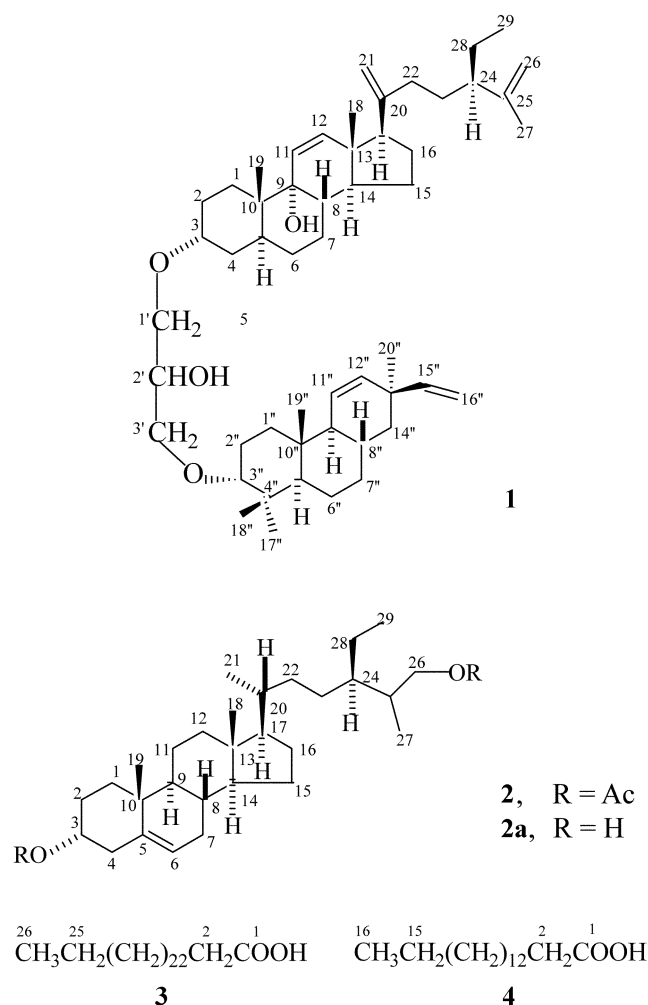


Figure 1. Chemical structures 1-4.

Hz. H-3 with α -orientation has half-width of more than 16.0 Hz. A three-proton broad signal at δ 1.69 was associated with C-27 methyl functionality attached to C-25 olefinic carbon. A three-proton triplet at δ 0.99 (J = 7.4 Hz), was ascribed to C-29 primary methyl protons. Two three-protons broad signals at δ 0.76 and 0.97 were assigned correspondingly to C-18 and C-19 tertiary methyl protons. Two one-proton doublets at 3.65 (J = 7.5 Hz) and 3.62 (J = 7.5 Hz), two one proton broad signals at δ 3.87 and 3.87 and one broad multiplet at δ 4.73 (1H) were assigned carbinol protons of glycerol moiety. Two one-proton broad signals at δ 5.23 and 5.04 were accounted to H-11" and H-12" vinylic protons of the diterpenic moiety and these signals were observed slightly upfield in comparison to H-11 and H-12 due to absence of the hydroxyl group on the adjacent carbon of the olefinic linkage. A two proton doublet at δ 4.90 (J = 6.0) was due to unsaturated methylene protons. A one-proton double doublet at δ 4.93 was ascribed to H-15" vinylic proton. A one-proton double doublet at δ 3.27 (J = 4.0, 4.5 Hz) was associated with H-3" β -oriented (equatorial) carbinol proton. The H-3" (equatorial) interacted with H-2 (equatorial) and H-2 (axial) forming J values of 4.0 and 4.5 Hz, respectively. Four three-proton broad signals at δ 0.77,

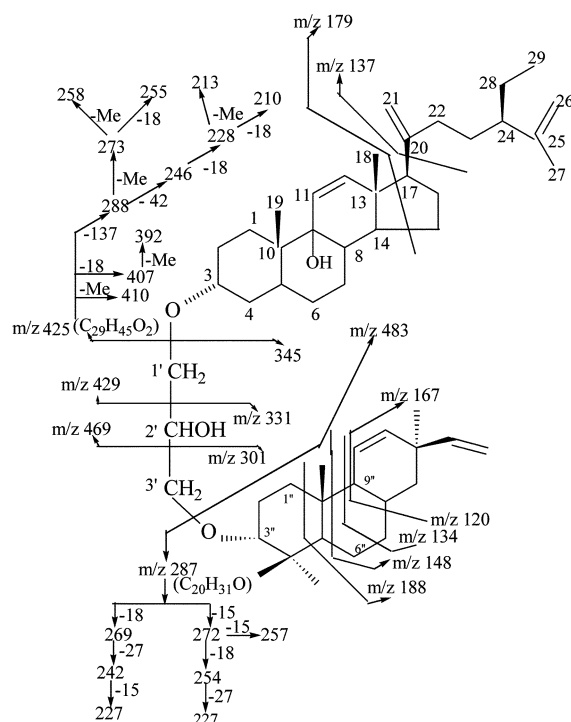


Figure 2. Fragmentation pattern of compound 1.

0.82, 0.97 and 1.25 were ascribed to C-17", C-18", C-19" and C-20" tertiary methyl protons. The remaining methylene and methine protons resonated between δ 2.63–0.99 (Table 1). The ^{13}C -NMR spectrum of **1** exhibited signals for vinylic carbons between δ 157.12–103.23, carbinol carbons between δ 79.37–63.20 and methyl carbons between δ 27.44 and 15.18. The multiplicity of each carbon was determined by DEPT experiments which showed the presence three C-21, C-26 and C-16" unsaturated methylene carbons, one C-9 quaternary carbinol carbon, five vinylic carbons at C-11, C-12, C-11", C-12" and C-15", three tertiary carbinol carbons at C-3, C-2' and C-3' and two oxygenated methylene carbons C-1' and C-3'. The ^1H - ^{13}C HETCOR spectrum of **1** exhibited hydrogen-carbon correlation of H-11 with C-9, C-12; H-12 with C-11, C-13 and C-18; H-12" with C-11", C-13" C-15" and C-20"; H-3" with C-2", C-4" and C-18'. The H-2' of glycerol moiety exhibited co-relation with C-1 and C-3' in HMBC spectrum of **1**. In HSQC, the H-C long range couplings were observed between H₃-27 with C-17, C-14, C-5, C-15, H₃-20" with C-5", C-7", C-9" and C-17"; and C-18 with H-20, H₃-19, H-3 and H-8. In ^1H - ^1H COSY spectrum H-11 with H-12; H-12, H-11" with H-12" and H-3 with H₂-2, H₂-1 and H₂-4 were observed. The NMR values of the steroidal moiety with compared with the related known compounds like erianol and spathodol.¹⁸ On the basis of these evidences, the structure of **1** has been established as, 1'-(stigmast-11,20(21),25-trien-3 α ,9 α -diolyl)-3'-(pimarane-11, 15-dien-3 α -olyl) glycerol.

Compound **2** was obtained as a colourless crystalline product was obtained from hexane-ethyl acetate (8 : 2) eluants. It responded positively to Liebermann-Burchard and Salkovaski tests for steroids. Its acetyl derivative showed

Table 1. ^1H and ^{13}C -NMR spectroscopic data in CDCl_3 of **1**

position	^1H -NMR		^{13}C -NMR
	alpha	beta	
1	1.47 m	2.30 ddd (2.5, 2.5, 12.1)	39.94
2	1.84 m	1.82 m	27.85
3	—	3.19 br m ($W_{1/2}$ 11.7)	79.37
4	1.31 m	1.28	44.02
5	1.61 m	—	45.11
6	1.33 m	1.28 m	18.73
7	1.69 m	1.22 m	38.91
8	—	1.16 m	55.14
9	—	—	79.29
10	—	—	37.88
11	5.79 d (10.5)	—	149.19
12	5.74 d (10.5)	—	135.59
13	—	—	44.45
14	1.15 m	—	59.11
15	0.92 m	1.49 m	33.43
16	1.63 m	1.54 m	31.53
17	1.42 m	—	55.42
18	0.76 br s	—	15.18
19	0.97 br s	—	22.37
20	—	—	157.12
21	4.87 br s	4.87 br s	110.31
22	2.31 m	2.26 br s	36.06
23	1.17 m	1.69 m	26.22
24	2.03	—	49.22
25	—	—	156.03
26	4.89 br s	4.89 br s	106.15
27	1.69 br s	—	27.44
28	1.15 m	1.69	22.44
29	0.99 t (7.4)	—	15.57
1'	3.65 d (7.5)	3.62 d (7.5)	63.20
2'	4.73 br m	—	76.44
3'	3.87 br s	3.84 br s	65.55
1''	1.49 m	2.05 ddd (7.5, 2, 8)	39.77
2''	2.01 m	1.84	25.23
3''	—	3.27 dd (4, 4.5)	76.79
4''	—	—	39.41
5''	1.63 m	—	44.16
6''	1.35 m	1.15 m	37.51
7''	1.57 m	1.31 m	39.04
8''	—	1.21 m	50.59
9''	2.63 m	—	54.39
10''	—	—	33.43
11''	5.23 br s	—	140.14
12''	5.04 br s	—	136.86
13''	—	—	39.24
14''	1.62 d (3.0)	1.56 d (8.5)	41.40
15''	4.93 dd (6.0, 6.0)	—	129.07
16''	4.90 d (6.0)	4.92 d (6.0)	103.23
17''	0.77 br s	—	17.79
18''	0.82 br s	—	15.94
19''	0.97 br s	—	19.29
20''	1.25 br s	—	20.21

 J values (in Hz) are in parenthesis

characteristic IR absorption bands for ester group (1732 cm^{-1}). Its electron impact mass spectrum and positive ion HRFABMS spectrum was established the molecular formula ($\text{C}_{29}\text{H}_{50}\text{O}_2$) at m/z 430 corresponding to a steroidal molecule, $\text{C}_{29}\text{H}_{50}\text{O}_2$. It indicated five double bond equivalents; four of them were adjusted in a tetracyclic carbon framework and the remaining one in the vinylic linkage. The ion fragments at m/z 415 $[\text{M} - \text{Me}]^+$, 400 $[\text{M} - 2 \times \text{Me}]^+$, 397 $[415 - \text{H}_2\text{O}]^+$, 382 $[397 - \text{Me}]^+$, 412 $[\text{M} - \text{H}_2\text{O}]^+$, 273 $[\text{M} - \text{C}_{10}\text{H}_{21}\text{O}]^+$, 255 $[272 - \text{H}_2\text{O}]^+$, 213 $[255 - \text{ring D fission}]^+$, and 199 $[213 - \text{Me}]^+$, suggested the presence of saturated side chain $\text{C}_{10}\text{H}_{21}\text{O}$ and one of the hydroxyl group in the steroidal skeleton. The ion fragments at m/z 124 $[\text{C}_{6,7} - \text{C}_{9,10} \text{ fission}]^+$, 138 $[\text{C}_{7,8} - \text{C}_{9,10} \text{ fission}]^+$, 178 $[\text{C}_{8,14} - \text{C}_{9,11} \text{ fission}]^+$, 192 $[\text{C}_{8,14} - \text{C}_{11,12} \text{ fission}]^+$, and 206 $[\text{C}_{8,14} - \text{C}_{12,13} \text{ fission}]^+$, suggested the presence of the hydroxyl group in ring A, placed at C-3 on the basis of biogenetic consideration, olefinic linkage at C-5 and saturated nature of ring C.

The ^1H -NMR spectrum of acetylated of **2** exhibited a one-proton doublet at δ 5.37 ($J = 4.5$ Hz), assigned to vinylic H-6. A one-proton broad multiplet at δ 4.60 with half width of 8.5 Hz was ascribed to β -oriented H-3. The H-3 equatorial interact with H_2 equatorial, H_2 axial, and H_{4a} , H_4 equatorial and when the H-3axial interact with H_2 and H_4 protons, then the $W_{1/2}$ would be expected near 16.0 Hz. Two one proton doublets at δ 4.06 ($J = 7.0$ Hz), and 4.03 ($J = 7.0$ Hz) was accounted oxygenated methylene H_2 -26. Two three-proton doublets at δ 2.04 and 2.02 were associated with the two acetyl protons at C-3 and C-26. Two-one proton broad signals at δ 1.01 and 0.67 were attributed to tertiary C-19 and C-18 methyl protons, respectively. Three doublets at δ 0.91 ($J = 6.5$ Hz), 0.84 ($J = 7.0$ Hz), 0.80 ($J = 6.5$ Hz), all integrated for three protons, were assigned to C-21, C-29 and C-27 methyl protons respectively. The presence of all the methyl signals in the range δ 1.01–0.67 supported their attachment on the saturated carbons. The remaining methylene and methine protons resonated between δ 2.32–1.01. The ^{13}C -NMR spectrum of the acetylated of **2a** displayed carbon signals for steroidal molecule and four carbons signals for the acetyl functions. The deshielded signals at δ 139.88 and 122.85 were assigned to vinylic C-5 and C-6 carbons. The signals at δ 74.19 and 64.87 were accounted C-3 carbinol and C-26 oxygenated methylene carbons, respectively. The methyl carbons appeared at δ 12.21 (C-18), 19.52 (C-19), 18.92 (C-21), 19.26 (C-27), and 12.07 (C-29). The upfield resonances of C-18 and C-29 at δ 12.21 and 12.07 suggested the location of the hydroxyl group at C-26. The DEPT spectrum of acetylated showed the presence of five primary, twelve secondary, nine tertiary, three quaternary and four acetyl (δ 21.21, 21.43, 171.40, 170.70) carbons. In HMBC spectrum, there were co-relations of C-5 with H-3 and C-19, C-3 with H_2 -2, H_2 -4, and H_2 -1, C-26 with H-25, C-19 with H_2 -1, H-9 and H_2 -11, and C-18 with H-17, H-14 and H_2 -12. In HSQC spectrum correlation observed were C-5 with H-5, C-3 with H-3 and C-26 with H-26. In ^1H - ^1H COSY spectrum there were correlation of H-6 with H_2 -7, H_2 -26 with H-25 and H-3 with H_2 -2, and H_2 -4. The ^1H and

Table 2. Inhibitory effects of compound (1) on germination and growth of radish

Concentration (ppm)	Germination (%)	Shoot length (mm)	Root length (mm)
0.0 control	80.5a (0.0)	27.5a (0.0)	23.7a (0.0)
50.0	76.0a (5.6)	30.2a (-9.8)	23.8a (-0.4)
100.0	30.8b (61.7)	26.1a (5.1)	16.1b (32.1)
200.0	12.9c (83.9)	12.8c (53.5)	12.3c (48.1)
500.0	10.6c (86.8)	8.92c (67.6)	5.5c (76.8)

Means with the same letters in a column are not significantly at $P = 0.05$. Values in the parentheses are inhibition percentages over the control. Values in the parentheses with (-) are estimation percentage over the control

^{13}C NMR values of 2a were compared with β -sitosterol,¹⁹ lawsaritol²⁰ and other related steroids.¹⁸ On the basis of spectral data analysis and chemical reactions the structure of compound 2a was established as stigmast-5-en-3 α ,26-diol which on acetylation with acetic anhydride and pyridine yielded compound 2 as stigmast-5-en-3 α ,26-diacetate.

Compounds 3 and 4 were identified as *n*-hexacosanoic acid and *n*-hexadecanoic acid, respectively, on the basis of spectral data analysis and chemical reactions.

Inhibitory effects of compound on radish. Compound (1, sativasterolide) had strong inhibition to radish on germination, growth of shoot and root length at 500 ppm. However, germination was slightly inhibited at 50 ppm, shoot and root length were stimulated, but at 100 ppm, germination and root length were significantly inhibited as compare with control. Increasing concentration of test compound showed to be generally correlated with inhibitory effects on germination, growth of shoot and root. At 200 ppm, shoot and root were reduced by 48.1 to 53.3%, respectively. It appears that the acetone dilution may contain phytotoxic substances that cause inhibition on growth of radish (Table 2).

There is no available report in the literature for either growth or germination inhibition of this compound. Numerous rice allelochemicals belonging to chemical classes of resorcinol, flavone, hydroxamic acid, phenols, phenolic acids, indoles, terpenic acid, fatty acids, as well as momilactones (A and B) were identified and were responsible for rice's allelopathic activity.^{13,16,17} Allelochemicals exudates from rice root of rice varieties at different growth stages exhibited variation and caused inhibition effects on barnyard grass.²¹ These compounds may be utilized for reducing weed interference and diminishing the dependency on synthetic agricultural chemicals, labor cost and environmental contamination. The inhibitory effects of this compound on radish were significantly inhibited at 100 ppm. However, decreased concentration at 50 ppm, growth of shoot and root were slightly stimulated. It needs further test with different paddy weeds with various concentrations for more understanding inhibitory activity of this compound.

Experimental Section

Chemicals. All chemicals were used of analytical grade:

hexane, ethyl acetate, methanol, ethanol, sulfuric acid, vanillin were purchased from Daejung Chemicals & Metals Co., Ltd, Korea. Precoated TLC plates (layer thickness 0.25 mm) and silica gel column chromatography (70-230 mesh ASTM) and LiChroprep RP-18 (40-63 μm) were from Merck (Darmstadt, Germany). Authentic samples of β -sitosterol, *n*-hexadecanoic acid, *n*-hexacosanoic acid were purchased from Sigma-aldrich (USA).

Instruments. Melting points were determined on Electrochemical Eng. melting point apparatus. Optical rotation was measured on an AA-10 model polarimeter. Both ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained with a Bruker Avance (DRX-500) spectrometer operating at 500 and 125 MHz, respectively. NMR spectra were obtained in deuterated chloroform, using tetramethylsilane (TMS) as internal standard, with chemical shifts expressed in parts per million (δ) and coupling constants (J) in hertz. EI-Mass spectra were recorded on a JEOL JMS-SX 102 A spectrometer and FABMS on a JEOL JMS-AX 505 WA. IR spectra were recorded on a Thermo Mattson 60-AR spectrophotometer.

Plant material. The cultivar of *O. sativa* grown at Konkuk University experimental field in Korea, were harvested in October, 2002. The hulls from harvested plants were separated by milling machine and dried at room temperature (25 $^\circ\text{C}$) for 7d, and then dried rice hulls were ground or powdered with a Wiley mill through a 40-mesh screen. The voucher specimen of hulls (No. KKKU 121, Ilpumbyeo) has been deposited in the herbarium of our department.

Extraction of rice hulls. The powered hulls of *O. sativa* (10 kg) were immersed in MeOH (60 litre) for a week at room temperature and then concentrated *in vacuo* to yield extract (150 g), which was suspended in H_2O and extracted with EtOAc and *n*-BuOH successively and prepared ethyl acetate extract (35 g) and butanol extract (19 g).

Isolation of compounds from ethyl acetate extract. The EtOAc extract (35 g) was subjected to normal phase column chromatography (CC) over silica gel (800 g), yielded 40 fractions with the following eluents (each fraction 500 mL): fraction 1 in hexane, fractions 2–5 in hexane-EtOAc (9 : 1), fractions 6–11 in hexane-EtOAc (8 : 2), fractions 12–15 in hexane-EtOAc (7 : 3), fractions 16–20 in hexane-EtOAc (1 : 1), fractions 21–22 in EtOAc, fractions 23–28 in EtOAc – MeOH (9.5 : 0.5), fractions 29–30 in EtOAc–MeOH (9 : 1), fractions 31–36 in EtOAc–MeOH (7 : 3), fractions 37–40 in MeOH. Fraction 1 with further CC and TLC over silica gel (50 g; each fraction 100 mL) with *n*-hexane-EtOAc yielded one pure compound: hentriacontane (50 mg). Fractions 2–5 showed similar on TLC, after mixing (1.2 g) which was further CC over silica gel (100 g, each fraction 200 mL) by using CH_2Cl_2 , $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (99.8 : 0.2, 99.6 : 0.4, 99.4 : 0.6, 99.2 : 0.8, 99 : 1) as eluents to yield six frs., 1-tetratriacontanol (50 mg) from the initial fraction. Fraction 6 (2.8 g) was crystallized and after purification by CC yielded β -sitosterol (200 mg), confirmed by comparison to an authentic sample from sigma by Co-TLC and spectroscopic

data. Fractions 7-10 after column chromatographed over silica gel (200 g; each fr. 200 mL) with Hexane-EtOAc (9.5 : 0.5; 9 : 1; 8.5 : 1.5; 8 : 2; 7 : 3) yielded ten fractions. The fraction 5 after purification through small column was obtained compound **1** (15 mg). The compound **2a** (30 mg) was obtained from fraction 7, compound **2a** due to insolubility in several solvents, prepared the acetylated product **2** for NMR analysis. The other compounds **3** (30 mg) and **4** (40 mg) were obtained from fractions 8-9. Fraction 11 (2.1 g) was further purified by CC over silica gel (100 g, each fraction 200 mL) with CH_2Cl_2 , $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (99.8 : 0.2, 99.6 : 0.4, 99.4 : 0.6, 99.2 : 0.8, 99 : 1), afforded two pure compounds, momilactone A (80 mg), momilactone B (70 mg). Fraction 12 (3.4 g) after CC over silica gel (80 g; each fraction 150 mL) using dichloromethane and methanol as eluants, yielded a yellow powder identified as tricin (10 mg), β -sitosterol-3-*O*- β -glucuronoside (50 mg) and 3,7-dimethyln-octan-1-yl benzoate (15 mg). Fraction 23 (1.2 g) after CC over silica gel (100 g; each fraction 100 mL) with chloroform and methanol yielded one pure compound β -sitosterol-3-*O*- β -D glucoside (50 mg).

Inhibitory activity determination method. The isolated compound from rice hulls was used in this experiment. Radish seeds were selected to detect any inhibitory activity of this compound. Empty and undeveloped seeds were discarded by floating in distilled water. The seeds were stored at -35°C until used for bioassays. In germination test, germination ratio of the used radish seeds was shown to be greater 80%. The isolated rice hulls compound was dissolved in acetone with 1%, and water 99% to obtain the following concentrations: 50, 100, 200, and 500 ppm. Fifty seeds of radish were placed on Petri dish (9 cm in diameter) lined with filter paper (Whatman) and added 10 mL of diluted solvent in each, respectively. Treatments with distilled water in acetone were as the control. Petri dishes were transferred into growth chamber under following condition: 25°C , humidity of 75%, lighted time of 9.00-17.00. After seven days, percentage germination, growth of shoot and root length were recorded.

Statistical Analysis. Data were analyzed using SAS version 6.12²² (SAS Institute, 1997). All of the treatments were replicated thrice with completely randomized design. The pooled mean values were separated based on the least significant difference (LSD) at the 0.05 probability level.

Sativasterolide (1). Colorless solid; mp $140-42^\circ$; $[\alpha]_{\text{D}}^{25} +23.6^\circ$ (c 0.15, CHCl_3), IR (KBr) ν_{max} : 3485, 2929, 2863, 1640, 1449, 1310, 1080, 1010, 995, 780 cm^{-1} ; FABMS (positive mode) m/z 770 ($\text{C}_{52}\text{H}_{82}\text{O}_4$), 425 ($\text{C}_{29}\text{H}_{45}\text{O}_2$) (7.4), 410 (18.0), 407 (16.7), 288 (100), 287 (91.2), 273 (30.2), 269 (31.8), 258 (11.3), 257 (52.2), 255 (48.5), 242 (6.6), 227 (22.2), 213 (6.4), 210 (2.2), 188 (8.8), 179 (13.0), 167 (11.3), 153 (14.1), 148 (28.7), 139 (15.2), 134 (24.8), 120 (37.1); HRFABMS (positive ion mode) m/z 771.2334 $[\text{M} + \text{H}]^+$, (calc. for $\text{C}_{52}\text{H}_{82}\text{O}_4$ -2.4 ppm error); ^1H and ^{13}C -NMR (Table 1).

Stigmast-5-en-3 α ,26-diacetate (2). Colorless solid; mp $112-15^\circ$ (acetate); $[\alpha]_{\text{D}}^{25} +12.3^\circ$ (c 0.15, CHCl_3 ; acetate); IR

(KBr) ν_{max} : 2916, 2848, 1732, 1466, 1370, 1248, 1185, 1039, 758 cm^{-1} ; ^1H -NMR: (CDCl_3): δ 5.37 (1H, d, $J = 4.5$ Hz, H-6), 4.60 (1H, br m, $W_{1/2}$ 8.5 Hz, H-3 β), 4.06 (1H, d, $J = 7.0$ Hz, H₂ - 26a), 4.03 (1H, d, $J = 7.0$ Hz, H₂ - 26b), 2.04 (3H, br s, COCH_3), 2.02 (3H, br s, COCH_3), 1.01 (3H, br s, Me - 29), 0.91 (3H, d, $J = 6.5$ Hz, Me - 21), 0.84 (3H, t, $J = 7.0$ Hz, Me - 29), 0.80 (3H, d, $J = 6.5$ Hz, Me - 27), 0.67 (3H, br s, Me - 18); ^{13}C NMR (CDCl_3): δ 38.35 (C - 1), 32.10 (C - 2), 74.19 (C - 3), 42.43 (C - 4), 139.88 (C - 5), 122.85 (C - 6), 32.12 (C - 7), 32.15 (C - 8), 50.27 (C - 9), 36.38 (C - 10), 21.25 (C - 11), 39.96 (C - 12), 42.54 (C - 13), 56.92 (C - 14), 24.51 (C - 15), 28.01 (C - 16), 56.21 (C - 17), 12.21 (C - 18), 19.52 (C - 19), 37.23 (C - 20), 18.92 (C - 21), 34.17 (C - 22), 26.14 (C - 23), 46.08 (C - 24), 29.88 (C - 25), 64.87 (C - 26), 19.26 (C - 27), 22.91 (C - 28), 12.07 (C - 29), 171.40, 170.70 (2 acetate ketone), 21.43, 21.21 (2 acetate Me); EIMS m/z (rel. int. %): 430 $[\text{M}]^+$ ($\text{C}_{29}\text{H}_{50}\text{O}_2$) (1.0), 415 (100), 412 (37.8), 400 (46.5), 397 (34.0), 381 (15.2), 367 (7.0), 351 (6.7), 329 (21.3), 315 (11.0), 303 (18.7), 273 (20.1), 255 (27.5), 231 (12.7), 213 (16.6), 206 (3.5), 199 (6.2), 192 (4.3), 178 (8.4), 159 (13.2), 145 (14.6), 138 (8.3), 124 (11.8), 111 (16.5), 97 (26.6), 83 (26.2), 69 (20.2), 57 (23.1), 55 (19.9); FABMS (positive mode) m/z 431 $[\text{M} + \text{H}]^+$; HRFABMS (positive ion mode) m/z 431.2312 $[\text{M} + \text{H}]^+$ (calc. for $\text{C}_{29}\text{H}_{50}\text{O}_2$ -3.5 ppm error).

n-Hexacosanoic acid (3): Colorless solid; IR (KBr) ν_{max} : 3290, 2914, 2846, 1700, 1450, 1390, 1310, 920, 725 cm^{-1} ; ^1H -NMR (CDCl_3): δ : 2.35 (1H, d, $J = 7.5$ Hz, H₂ - 2a), 2.32 (1H, d, $J = 7.5$ Hz, H₂ - 2b), 1.63 (2H, m, H₂ - 3), 1.25 (44 H, br s, 22 x CH_2), 0.89 (3H, t, $J = 7.0$ Hz, Me - 26); ^{13}C -NMR (CDCl_3): δ : 180.05 (C - 1), 34.23 (C - 3), 32.15 (C - 4), 29.93 (10 x CH_2)*, 29.87 (4 x CH_2)*, 29.82 (CH_2)*, 29.66 (CH_2)*, 29.59 (CH_2)*, 29.46 (CH_2)*, 29.29 (CH_2)*, 24.91 (CH_2)*, 22.91 (C - 25), 14.32 (C - 2); (*values may be interchanged); EIMS m/z (rel. int.): 396 $[\text{M}]^+$ ($\text{C}_{26}\text{H}_{52}\text{O}_2$) (7.4), 381 (12.9), 367 (100), 353 (40.1), 339 (28.5), 325 (11.5), 311 (7.4), 297 (7.7), 283 (6.7), 269 (9.1), 255 (6.3), 241 (9.1), 227 (7.1), 213 (6.3), 199 (5.5), 185 (15.5), 171 (9.0), 157 (4.7), 143 (6.0), 129 (33.6), 115 (9.5), 111 (12.2), 99 (11.6), 85 (21.8), 73 (38.3), 71 (31.7), 59 (24.3), 57 (48.5); FABMS (positive mode) m/z 397 $[\text{M} + \text{H}]^+$.

n-Hexadecanoic acid (4): Colorless solid; IR (KBr) ν_{max} : 3210, 2924, 2853, 1708, 1490, 1225, 1180, 1155, 759 cm^{-1} ; ^1H -NMR (CDCl_3): δ : 2.35 (1H, d, $J = 7.5$ Hz, H₂ - 2a), 2.32 (1H, d, $J = 7.5$ Hz, H₂ - 2b), 1.63 (2H, m, H₂ - 3), 1.25 (24 H, br s, 12 x CH_2), 0.88 (3H, t, $J = 6.5$ Hz, Me - 16); ^{13}C -NMR (CDCl_3): δ : 180.03 (C - 1), 34.25 (C - 2), 32.16 (C - 4), 29.91 (CH_2)*, 29.88 (CH_2)*, 29.86 (3 x CH_2)*, 29.80 (2 x CH_2)*, 29.65 (CH_2)*, 29.57 (CH_2)*, 29.45 (CH_2)*, 29.28 (CH_2)*, 24.90 (CH_2)*, 14.32 (C - 26); (*values may be interchanged); EIMS m/z (rel. int., %): 256 $[\text{M}]^+$ ($\text{C}_{16}\text{H}_{32}\text{O}_2$) (100), 241 (4.3), 227 (7.3), 213 (23.0), 185 (17.3), 171 (15.1), 157 (15.8), 143 (9.1), 129 (33.9), 99 (13.2), 97 (16.3), 85 (19.1), 73 (44.3), 59 (30.7), 57 (39.1); FABMS (positive mode) m/z 257 $[\text{M} + \text{H}]^+$.

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