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A and B from rice hulls of *Oryza sativa*

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Two new compounds orizalanasterolide A and B identified as lanast-1,11, 22-trien-3*β*, 9*α*-diol-18-al-21(24)-olido-3*β*-D-glucopyranoside **1**, lanast-1, 11, 22-trien-3*β*, 9*α*-diol-18-al-21(24)-olido-3*β*-D-glucopyranosyl-(1'-4')-*β*-D-glucopyranoside **2**, have been isolated from the rice hulls of *Oryza sativa*. Their structures have been elucidated with the help of 500/125 MHz NMR using 1D and 2D spectral methods *viz*: ^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HETCOR and DEPT aided by EIMS, FABMS and IR spectroscopy.

Keywords: *Oryza sativa***,** Poaceae**,** rice hull compounds, orizalanasterolide A and B

IPC: Int.Cl.[8]C07C

Rice (*Oryza sativa* L.) is the principal cereal food in Asia and the major staple food for the majority of the population. It generally consists of two types: white and coloured 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 inhibit growth and germination 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 B[8] and it was recently reported that rice seedlings release momilactone B into the environment[9]. The antioxidant activity of methanol extracts[10] and *C*-glycosylflavonoid 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.

In continuation of the study on rice hulls of *Oryza sativa* constituents, are now reported new and known compounds possessing inhibitory and cytotoxic activities[13-18]. Herein is reported the isolation and structural elucidation of additional two new compounds (**1** and **2**) (**Figure 1**)in minor quantities on the basis of spectral methods *viz*: ^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HETCOR, DEPT and aided by EI-MS, FAB-MS and IR spectroscopy.

The methanol extract of the *O. sativa* hulls was suspended in water and extracted with ethyl acetate followed by *n*-butanol. The ethyl acetate and *n*-butanol extracts were separated by a combination of column chromatography over silica gel and Lichroprep RP-18 (ODS Silica gel) and two new compounds were obtained from the ethyl acetate extract. For all the molecules studied, relative configurations were suggested on the basis of biogenetic considerations.

Results and Discussion

Compound **1** named orizalanasterolide A was obtained as a colourless semi-solid mass after repeated column chromatographic separation over silica gel and ODS silica gel. It responded positively to triterpenic glycoside tests. Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3510, 3447 and 3390 cm^{-1}), δ-lactone (1736 cm^{-1}), aldehydic group (1703 cm^{-1}), and unsaturation (1640 cm^{-1}). On the basis of FAB and EI mass spectra and ^{13}C NMR spectrum the molecular weight of **1** has

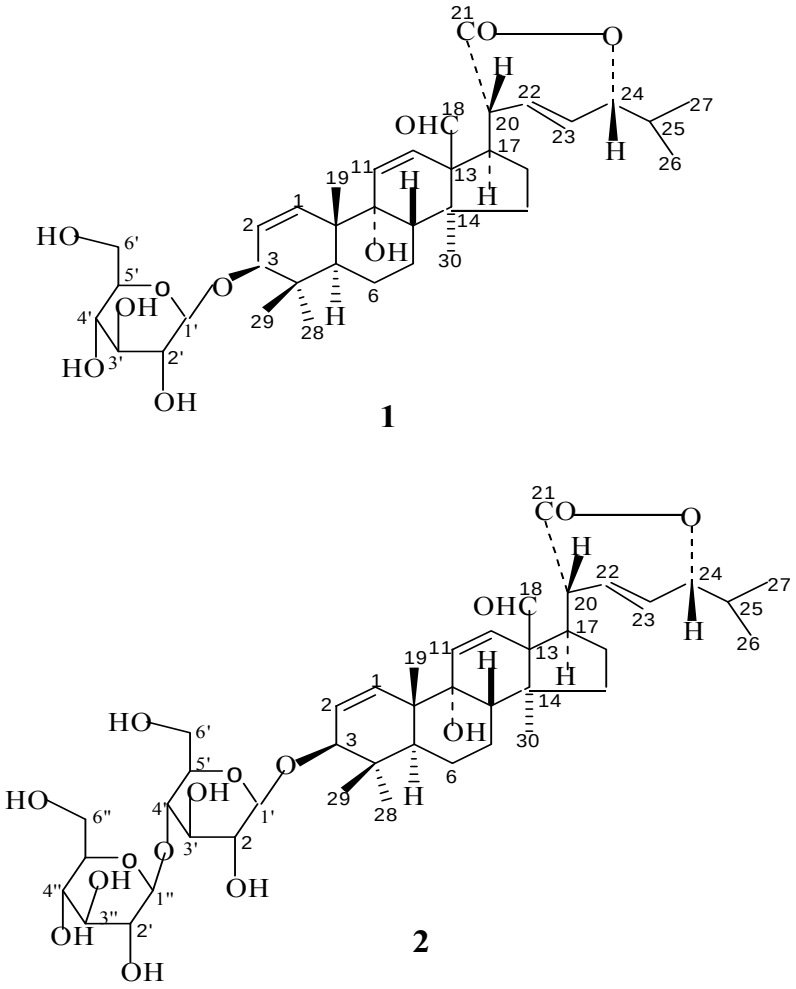

Figure 1 — Chemical structures of orizalanasterolide A (**1**) and B (**2**)

been established as *m/z* 644 which corresponded to tetracyclic glycoside $C_{36}H_{52}O_{10}$. It indicated eleven degrees of unsaturation; four of them were adjusted in the tetracyclic carbon framework, three in the olefinic linkage, two in δ-ring, one in aldehydic group and one in glucose moiety. The mass spectrum displayed important ion fragments at *m/z* 482 [M - 162]$^{+,}$ 315 [M-$C_8H_{11}O_7$ side chain-CO]$^+$, 303 [M-$C_8H_{11}O_2$ -ring D]$^{+,}$ 288 [303-Me]$^{+,}$ 273 [288-Me]$^{+,}$ 258 [273-Me]$^+$, 285 [303-H_2O]$^{+,}$ 270 [285-Me]$^{+,}$ 255 [270-Me]$^+$ and 240 [255-Me]$^{+.}$ The prominent ion peak generated at *m/z* 427, 55 [$C_{3,4}$-$C_{5,10}$-$C_{5,6}$ fission]$^+$, 69, 413 [$C_{3,4}$-$C_{5,10}$-$C_{6,7}$ fission]$^+$, 399, 83 [$C_{3,4}$-$C_{5,10}$-$C_{7,8}$ fission]$^+$, 100 [$C_{1,10}$-$C_{4,5}$ fission]$^+$, 138 [$C_{5,6}$-$C_{9,10}$ fission]$^+$, 166, 318 [$C_{7,8}$- $C_{9,10}$ fission]$^+$, 85 [$C_{2,3}$-$C_{5,10}$-$C_{5,6}$ fission]$^+$, 99 [$C_{2,3}$- $C_{5,10}$-$C_{6,7}$ fission]$^+$, 113 [$C_{2,3}$-$C_{5,10}$-$C_{7,8}$ fission]$^+$, 125 [$C_{1,2}$- $C_{5,10}$-$C_{7,8}$ fission]$^+$ and 139 [$C_{1,2}$-$C_{5,10}$-$C_{7,8}$ fission]$^+$, suggested the presence of one unsaturated linkage at C-1, one hydroxyl group in ring A placed at C-3 on the basis of biogenetic consideration and the saturated nature of ring B. The ion fragments at *m/z* 208, 274 [$C_{8,14}$-$C_{9,11}$ fission]$^+$, 135 [274-side chain]$^+$, 219 [$C_{9,11}$-$C_{13,14}$-$C_{16,17}$ fission]$^+$ and 109 [$C_{8,14}$-$C_{12,13}$ fission-side chain]$^+$, supported the existence of one hydroxyl group at C-9 and olefinic linkage at C-11. The mass fragmentation pattern of aglycone part is shown in **Figure 2**.

The ^{1}H NMR spectrum of **1** displayed three one-proton doublets at δ 5.85 (J = 10.5 Hz), 6.95 (J = 8.4 Hz), and 7.80 (J = 8.4 Hz), assigned to vinylic H-1, H-11 and H-12 respectively. A one-proton double doublet at δ 5.81 (J = 10.5, 6.0 Hz), and two one-proton multiplets at δ 6.62 and 6.31 were ascribed to the vinylic protons H-2, H-22 and H-23, respectively. A one-proton doublet at δ 4.94 (J = 10.5 Hz) and a one-proton double doublet at δ 4.97 (J = 6.0, 5.95) were attributed to 3α- and 24β-carbinol protons.

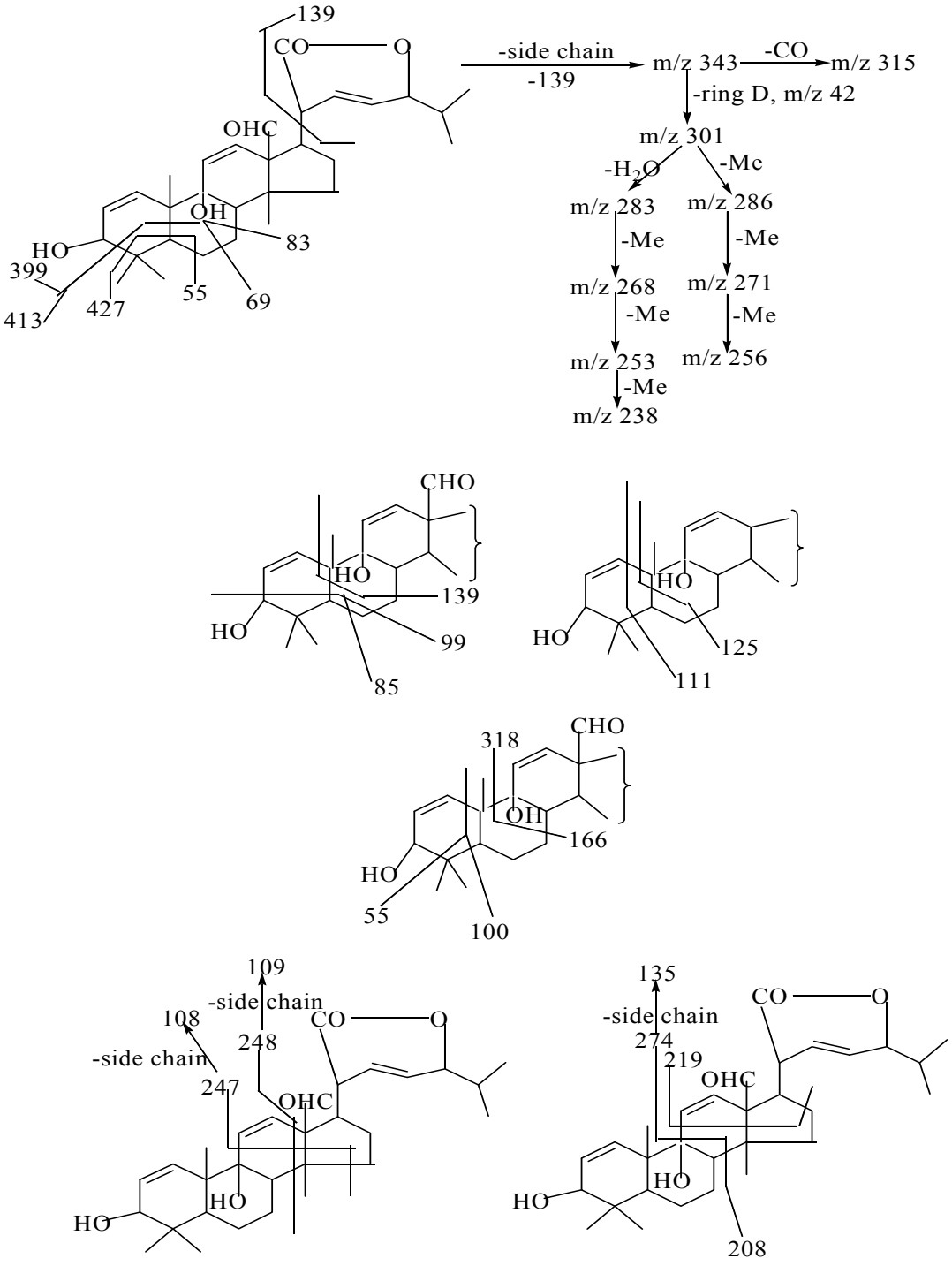

Figure 2 — Fragmentation pattern of aglycone portion of orizalanasterolide A

A one-proton singlet at δ 9.86 was assigned to C-18 aldehydic proton. Two three-proton doublets at δ 0.83 (J = 6.8 Hz), and 0.87 (J = 7.0 Hz) were ascribed to secondary C-26 and C-27 methyl protons. Four three-proton broad signals at δ 1.25, 0.87, 0.92, 0.81 and 0.90 were associated with tertiary C-19, C-27, C-28, C-29 and C-30 methyl protons. The presence of all methyl signals in the range δ 1.25-0.81 suggested that these functionalities were attached to the saturated carbons. A one-proton doublet at δ 5.68 (J = 4.5 Hz) was due to anomeric H-1' protons. One-proton doublets at δ 4.06 (J = 6.5 Hz) and 4.04 (J = 6.5 Hz)

were attributed to the oxygenated methylene H_2-6' protons. The other sugar protons appeared as one-proton doublet at δ 4.15 (H-2') and as one-proton multiplet at δ 4.08 (H-3'), 4.11 (H-4'), and 4.98 (H-5'). The remaining methylene and methine protons resonated between δ 2.33-1.01.

The ^{13}C NMR spectrum of **1** showed the existence of 36 carbon signals. Two deshielded signals at δ 190.95 and 173.31 were assigned to C-18 aldehydic carbon and δ-lactone C-21 carbons, respectively. The vinylic carbons appeared at δ 130.96 (C-1), 114.21 (C-2), 113.33 (C-11), 135.32 (C-12), 135.67 (C-22) and 132.52 (C-23). The C-3, C-9 and C-24 carbinol carbons resonated at δ 73.97, 84.90 and 72.93 respectively. The methyl carbons appeared at δ 34.65 (C-19), 22.91 (C-26), 25.15 (C-27), 25.11 (C-28), 26.65 (C-29) and 14.33 (C-30). Anomeric C-1' carbon was present at δ 110.44. The remaining carbons of the sugar moiety were determined in the range δ 64.65-70.71. The other methine and methylene carbons of the molecule resonated between δ 50.83-19.85. The multiplicity of each carbon was determined by DEPT spectrum which showed the presence of six methyl, five methylene and nineteen methine carbons. The number of quarternary carbons were found out by substracting the number of methyl, methylene and methine carbons from the ^{13}C NMR spectrum of the compound. The ^{1}H-^{1}H COSY spectrum displayed correlation of H-11 with H-12, H-2 with H-1 and H-3, H-22 with H-20, H-23 and H-24, H-24 with H-23, H-25 and H_3-26/H_3-27. ^{1}H-^{13}C HETCOR spectrum of **1** showed correlation of C-18 with H-12, C-21 with H-20, C-3 with H-2, H-3 and H_3-29. Acid hydrolysis of **1** yielded D-glucose (TLC comparison) and an aglycone. On the basis of the foregoing the structure of **1** (**Figure 1**) has been established as lanast-1, 11, 22-trien-3β, 9α-diol-18-al-21(24)-olido-3β-D-glucopyranoside.

Compound **2** named orizalanasterolide B, was obtained as a colourless semi-solid mass after repeated column chromatographic separation over silica gel and ODS silica gel. It responded positively to triterpenic glycosidic tests. Its IR spectrum exhibited characteristic absorption bands for hydroxyl group (3490, 3450, 3385 cm^{-1}), δ-lactone (1738 cm^{-1}), aldehydic group (1701 cm^{-1}), and unsaturation (1635 cm^{-1}). The FAB mass spectrum of **2** displayed a molecular ion peak at *m/z* 806 which corresponded to a diglycoside of lanosterol-type triterpene $C_{42}H_{62}O_{15}$. The study of mass fragmentation pattern and other spectral data (^{1}H and ^{13}C NMR) indicated that the molecule contained the aglycone indentical to the one determined in **1**.

In the ^{1}H NMR spectrum of **2** the two anomeric signals appeared as one-proton doublets at δ 5.23 (J = 7.5 Hz), and 5.18 (J = 7.9 Hz). The other hydroxy methine and oxygenated methylene protons resonated between δ 4.94-3.65. The ^{13}C NMR spectrum of **2** showed anomeric carbon signals at δ 110.20 (C-1') and 110.18 (C-1") shifting of C-4' signal from δ 65.27 to 78.13 suggested the attachment of second glycoside moiety at C-4'. In ^{1}H-^{13}C HETCOR spectrum a correlation H-4' was observed with C-1". Acid hydrolysis of **2** produced sugar and an aglycone identical to that obtained from **1** (TLC comparison). On the basis of spectral data analysis and chemical reactions the structure of **2** (**Figure 1**) has been elucidated as lanast-1, 11, 22-trien-3β, 9α-diol-18-al-21(24)-olido-3β-D-glucopyranosyl-(1'-4')-β-D-glucopyranoside.

Materials and Methods

All chemicals used were of analytical grade: hexane, ethyl acetate, methanol, ethanol, sulfuric acid and vanillin were purchased from Daejung Chemicals and Metals Co., Ltd, Korea. Precoated TLC plates (layer thickness 0.5 mm) and silica gel for column chromatography (70-230 mesh ASTM) and LiChroprep RP-18 (40-63 μm, reverse phase silica gel) were from Merck (Damstadt, Germany). Authentic samples were purchased from Sigma-Aldrich (USA). Optical rotation was measured on an AA-10 model polarimeter. Both ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained with a Brucker Avance (DRX-500) spectrometer operating at 500 and 125 MHz, respectively. NMR spectra were obtained in deuterated methanol using tetramethylsilane (TMS) as internal standard, with chemical shifts expressed in parts per million (δ) and coupling constants (J) in Hertz. Electron impact (EI 70 eV) mass spectra were recorded on a JEOL JMS-SX 102 A spectrometer and fast atom bombardment mass spectra (FABMS) on a JEOL JMS-AX 505 WA. Infrared spectra were recorded on a Thermo Mattson 60-AR spectrometer in KBr.

Experimental Section

Plant Material. The cultivar of *O. sativa* (*Ilpum byeo*; rice is called *byeo* in Korean) grown at Konkuk University experimental field in Korea, were harvested and separated in October, 2002. The

harvested plants were dried at RT (25°C), and then their separated body parts (hull, leaf and straw). The voucher specimen (No. KKU 96, HOCHOKJINDO) of hulls has been deposited in the herbarium of the Department of Applied Life Science.

Extraction of rice hulls. The dried hulls of *O. sativa* (10 kg) were immersed in MeOH (60 litre) for a week at RT 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 ethyl acetate (35 g) and butanol extracts (19 g) prepared.

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) and yielded 40 fractions with the following eluants (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 29-32 in EtOAc-MeOH (9:1), fractions 31-36 in EtOAc-MeOH (7:3), fractions 37-40 in MeOH. Fraction 1 on being subjected to further column chromatography 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 identified on TLC, after mixing (1.2 g) which was further subjected to column chromatography over silica gel (100 g, each fraction 200 mL) and TLC by using CH_2Cl_2, CH_2Cl_2/MeOH (99.8:0.2, 99.6:0.4, 99.4:0.6, 99.2:0.8, 99:1) as eluants to yield six fractions, 1-tetratriacontanol (50 mg) from the initial fraction. Fraction 6 (2.8 g) was crystallized and after purification by column chromatography yielded *β*-sitosterol (200 mg), confirmed by comparison to an authentic sample from Sigma by Co-TLC and spectroscopic data. Fraction 11 (2.1 g) was further purified by column chromatography over silica gel (100 g, each fraction 200 mL) with CH_2Cl_2, CH_2Cl_2/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) and momilactone B (70 mg). Fraction 12 (0.4 g) after column chromatography over silica gel (80 g; each fraction 150 mL) using dichloromethane and methanol as eluants, yielded a mixture of two compounds having very close R_f values. Further separation of this mixture over Lichroprep RP-18 (ODS silica gel; 50 mg; each fraction 50 mL) using sequential mixtures of MeOH and H_2O as eluants (elution order 80%, 60%, 40%, 20%, 10% aqueous methanol, 100% methanol) to yield two new compounds in a minor quantity orizalanasterolide A (**1**, 18 mg), and B (**2**, 16 mg) from fraction with the composition 40% water in MeOH respectively. Fraction 23 (1.2 g) after column chromatography 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).

Lanast-1, 11, 22-trien-3β, 9α-diol-18-al-21(24)-olido-3β-D-glucopyranoside, 1: Colourless semi-solid, $[\alpha]_D^{25}$ +14.6° (C 0.8 MeOH); IR (KBr): 3510, 3390, 2922, 2853, 1736, 1703, 1640, 1461, 1379, 1260, 1096, 1031, 803 cm^{-1}; ^1H and ^{13}C NMR, see **Tables I** and **II**; EI-MS: *m/z* (%) 482 $[M-162]^+$ (4.6), 429 (4.6), 427 (5.7), 413 (9.0), 399 (11.2), 369 (22.7), 359 (8.1), 355 (9.4), 345 (8.5), 315 (82.8), 301 (63.8), 286 (21.1), 283 (26.2), 274 (23.9), 271 (18.2), 268 (20.4), 256 (29.5), 253 (19.0), 238 (29.1), 234 (14.6), 221 (51.2), 152 (41.1), 147 (60.6), 138 (34.4), 135 (57.4), 125 (55.5), 111(66.9), 109 (75.4), 108 (44.8), 83 (86.6), 69 (94.5), 57 (100), 55 (91.7); FAB-MS (positive mode): *m/z* 645 $[M+H]^+$ ($C_{36}H_{53}O_{10}$).

Acid Hydrolysis of 1: Compound **1** (3 mg) was refluxed with 1 mol/L HCl-dioxane (1:1, v/v, 2 mL) on a water bath for 4 h. The reaction mixture was evaporated to dryness, then partitioned between $CHCl_3$ and H_2O four times. The $CHCl_3$ extract was concentrated and contained aglycone portion water extract as *β*-D-glucose as identified by TLC.

Lanast-1, 11, 22-trien-3β, 9α-diol-18-al-21(24)-olido-3β-D-glucopyranosyl-(1'-4')-β-D-glucopyranoside, 2: Colourless semisolid, $[\alpha]_D^{25}$ +24.6° (C 0.12, MeOH); IR (KBr): 3490, 3450, 3385, 2926, 2855, 1738, 1701, 1635, 1461, 1377, 1260, 1156, 1096, 1010, 803 cm^{-1}; ^1H and ^{13}C NMR, see **Tables 1** and **2**; EI-MS: *m/z* (%) 482 $[M-162\times2]^+$ (2.4), 429 (4.2), 427 (6.2), 413 (3.3), 399 (3.0), 359 (3.5), 345 (5.4), 315 (100), 301 (41.8), 286 (28.0), 283 (24.4), 274 (20.8), 271 (21.6), 268 (13.9), 256 (27.1), 253 (16.6), 238 (42.0), 234 (10.2), 221 (15.5), 219 (17.3), 208 (12.5), 192 (25.4), 152 (44.7), 149 (68.9), 138 (77.5), 135 (63.6), 125 (43.8), 123 (58.2), 111 (37.4), 109 (61.8), 108 (50.1), 95 (62.5), 91 (48.0), 83 (58.8), 69 (69.4), 57 (53.4), 55 (73.0); FAB-MS (positive mode): *m/z* 807 $[M+H]^+$ ($C_{42}H_{63}O_{15}$).

Acid Hydrolysis of 2. Compound **2** (2 mg) was refluxed with 1 mol/L HCl-dioxane (1:1, v/v, 1 mL) on a water bath for 4 h. The reaction mixture was evaporated to dryness, then partitioned between

Table I — [1]H NMR (500 MHz) spectroscopic data[a] in CD_3OD for compounds **1** and **2**

Position	**1**		**2**	
	α	β	α	β
1	5.85 d (10.5)	-	5.83 d (10.5)	-
2	5.81 dd (10.5, 6.0)	-	5.42 dd (5.5, 10.5)	
3	4.94 d (10.5)	-	4.94 dd (10.5)	-
4	-	-	-	-
5	2.33 dd (3.6, 4.5)	-	2.13 dd (4.5, 8.0)	-
6	1.35 m	1.05 m	1.35 m	1.05 m
7	1.25 m	1.02 m	1.27 m	1.08 m
8	-	2.28 m	-	2.33 m
9	-	-	-	-
10	-	-	-	-
11	6.95 d (8.4)	-	6.94 d (8.6)	-
12	7.80 d (8.4)	-	7.78 d (8.6)	
13	-	-	-	-
14	-	-	-	-
15	1.52 m	1.69 m	1.56 m	1.69 m
16	1.41 m	1.16 m	1.35 m	1.11 m
17	1.59 m	-	1.58 m	-
18	9.86 m	-	9.86 s	-
19	1.25 s	-	1.25 s	-
20	2.28 m	-	2.41 m	-
21	-	-	-	-
22	6.62 m	-	6.51 m	-
23	6.31 m	-	6.27 m	-
24	-	4.97 dd (6.0, 5.95)	4.91 m	-
25	1.35 m	-	1.33 m	-
26	0.83 d (6.8)	-	0.83 d (7.5)	-
27	0.87 d (7.0)	-	0.88 d (6.4)	-
28	0.92 br s	-	1.25 br s	-
29	0.81 br s	-	0.86 br s	-
30	0.90 br s	-	0.83 br s	-
1'	5.68 d (4.5)	-	5.23 d (7.5)	-
2'	4.15 d (10.0)	-	4.15 m	-
3'	4.08 m	-	4.08 m	-
4'	4.11 m	-	4.11 m	-
5'	4.98 m	-	4.94 m	-
6'	4.06 d (6.5)	4.04 d (6.5)	4.08 d (9.0)	4.06 d (9.0)
1"			5.15 d (7.9)	-
2"			4.12 m	-
3"			4.09 m	-
4"			4.11 m	-
5"			4.91 m	-
6"			3.65 d (6.5)	3.62 d (6.5)

[a]Spectra recorded at 500 MHz with compounds dissolved in CD_3OD. Coupling constant in Hertz are provided in parenthesis.

Table II — ^{13}C NMR (125 MHz) spectroscopic data[a] in CD_3OD for compounds **1** and **2**

Position	**1**	**2**
1	130.96	132.65
2	114.21	119.94
3	73.97	74.93
4	37.50	41.40
5	47.63	47.86
6	19.85	19.95
7	32.14	32.14
8	42.97	42.98
9	84.90	82.93
10	37.43	39.59
11	113.33	116.14
12	135.32	135.63
13	44.11	44.92
14	48.38	48.39
15	29.49	29.57
16	29.92	29.91
17	50.83	51.39
18	190.95	190.94
19	34.65	33.28
20	33.02	34.51
21	173.31	174.16
22	135.67	135.67
23	132.52	135.33
24	72.93	68.60
25	29.34	29.48
26	22.91	22.90
27	25.15	25.01
28	25.11	25.11
29	26.65	26.54
30	14.33	14.33
1″	110.44	110.20
2'	68.61	66.70
3'	66.57	65.27
4'	65.27	78.13
5'	70.71	67.29
6'	64.65	63.33
1'		110.18
2"		66.32
3"		65.27
4"		66.56
5"		72.95
6"		61.76

[a]Spectra recorded at 125 MHz with compounds dissolved in CD_3OD.

$CHCl_3$ and H_2O four times. The chloroform extract was concentrated and contained the aglycone portion and water extract as sugar portion as identified by TLC.

Conclusion

The two new compounds as triterpene glycoside (Lanast-1, 11, 22-trien-3β, 9α-diol-18-al-21(24)-olido-3β-D-glucopyranoside; Lanast-1, 11, 22-trien-3β, 9α-diol-18-al-21(24)-olido-3β-D-glucopyranosyl-(1'- 4')-β-D-glucopyranoside) have been reported from rice hulls of *Oriza sativa* as a natural product.

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