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# Oryzasesterpenolide from the Hulls of *Oryza sativa* and Complete NMR Assignments of Momilactones A, B and Tricin

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From the hulls of *Oryza sativa* was isolated one new compound oryzasesterpenolide (1) along with nine compounds hentriacontane, 1-tetratriacontanol, β-sitosterol, momilactones A, B, tricin, β-sitosterol-3-*O*-β-D-glucuronoside, 3,7-dimethyl-*n*-octan-1-yl benzoate and β-sitosterol-3-*O*-β-D-glucoside. The structure of the new compound is elucidated by 500 MHz NMR using 1D and 2D spectral methods *viz.*, $^1$H, $^{13}$C, $^1$H-$^1$H COSY and HETCOR aided by EIMS, FABMS and IR. The complete $^1$H and $^{13}$C NMR assignments of momilactones A, B, tricin and 3,7-dimethyl-n-octan-1-yl benzoate are reported.

## INTRODUCTION

Rice (*Oryza sativa* L.) is the principal cereal food in Asia and the major staple food of the majority of the population. Although there are two main types, white and coloured hulls, the most commonly used type is the white hull (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 possess growth and germination inhibitors against the roots of rice[3, 4, 5] and momilactone B was isolated also from rice root exudates[6]. Antioxidative activities of methanol extracts[7] and *C*-glycosyl flavonoid from the rice hulls[8] have been reported in literature. Because there are few reports in literature of the chemical constituents of rice hulls, identification of further constituents is still required. we have now examined the constituents of the rice hulls and isolated one new compound oryzasesterpenolide (1), along with nine known compounds, β-sitosterol, momilactones A, B, tricin, hentriacontane, 1-tetratriacontanol, 3,7-dimethyl-*n*-octan-1-yl benzoate, β-sitosterol-3-*O*-β-D-glucuronoside and β-sitosterol-3-*O*-β-D-glucoside. The latter five compounds have been isolated from this plant for the first time. This paper deals with the isolation and structural elucidation of a new compound based on $^1$H NMR, $^{13}$C NMR, COSY and HETCOR aided by EIMS, FABMS, IR spectra and the complete $^1$H and $^{13}$C NMR assignments of momilactones A, B, tricin and 3,7-dimethyl-*n*-octan-1-yl benzoate are discussed. In earlier published work, momilactones A, B ($^1$H NMR) and tricin ($^{13}$C NMR)

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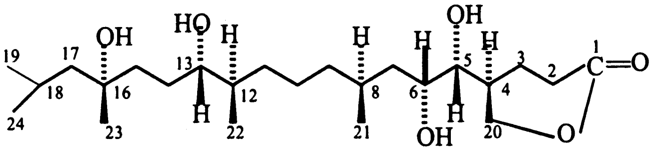
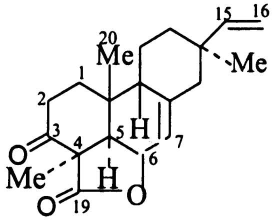
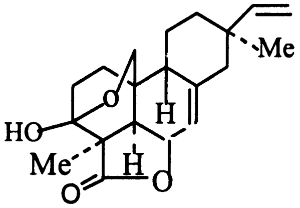
values were incompletely described[3, 4]. To the best of our knowledge for 3,7-dimethyl-*n*-octan-1-yl benzoate $^1H$ and $^{13}C$ NMR values are not reported in literature.



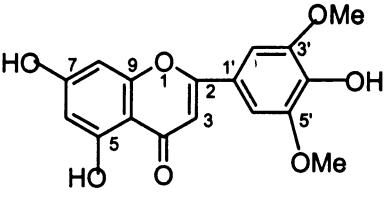
Oryzasesterpenolide (1)



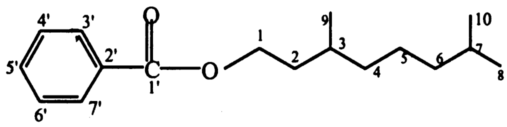
Momilactone A



Momilactone B



Tricin



3,7- dimethyl-n-octan-1-yl benzoate

Fig. 1. Chemical structures of oryzasesterpenolide, momilactones A, B, tricin and 3,7-dimethyl-n-octan-1-yl benzoate

## EXPERIMENTAL

Melting points were determined on Electrochemical Eng. melting point apparatus and TLC was carried out on precoated silica gel plates (Merck). Spots were detected under UV (254 and 366 nm) before and after dipping in chamber with 1% vanillin sulfuric acid (ethanol solution). TLC glass plates used precoated silica gel (Merck), layer thickness 0.25 mm and column chromatography was carried out on silica gel (70–230 mesh, Merck) and Lichroprep RP-18 (ODS silica gel, Merck). Optical rotation was measured on a 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 chloroform, methanol and pyridine using tetramethylsilane (TMS) as internal standard, with chemical shifts expressed in parts per million ($\delta$) 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 hulls of *O. sativa* were collected from Konkuk University (experimental farm), Seoul, South Korea in October, 2002. The voucher specimen (No. KKU 96, HOCHOKJINDO) was deposited in the herbarium of our department.

**Extraction and Isolation.** The dried hulls of *O. sativa* (10 kg) were immersed in MeOH for a week at room temperature and afterwards concentrated in vacuum to give an extract (150 g), which was suspended in $H_2O$ and extracted with EtOAc and *n*-BuOH successively. The EtOAc extract (35 g) was subjected to normal phase column chromatography over silica gel column (70–230 mesh, 800 g, 5.5 × 90 cm), yielded 40 fractions with the following eluants (each fraction 500 mL): fraction 1 in hexane, frs. 2–5 in hexane/EtOAc (9 : 1), frs. 6–11 in hexane/EtOAc (8 : 2), frs. 12–15 in hexane/EtOAc (7 : 3), frs. 16–20 in hexane/EtOAc (1 : 1), frs. 21–22 in EtOAc, frs. 23–28 in EtOAc/MeOH (9.5 : 0.5), frs. 29–32 in EtOAc/MeOH (9 : 1), frs. 33–36 in EtOAc/MeOH and (7 : 3), frs. 37–40 in MeOH. Fr. 1 (500 mg) with further CC and TLC over silica gel with n-hexane/EtOAc yielded one pure compound: hentriacontane (50 mg). Frs. 2–5 are same on TLC, after mixing (1.2 g) which was further CC and TLC over silica gel 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 eluents to yield six frs., 1-tetratriacontanol (50 mg) from intial fr. 1. The fraction 6 (2.8 g) was crystallized and after purification through column chromatography with hexane/EtOAc afforded β-sitosterol (200 mg) and confirmed by comparison to an authentic sample from Sigma. The fraction 11 (2.1 g) which was further purified by CC over silica gel with $CH_2Cl_2$/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 (3.4 g) after CC over silica gel by using $CH_2Cl_2$/MeOH (99 : 1, 98 : 2, 97 : 3, 96 : 4, 95 : 5) as eluants yielded five frs. Two compounds were obtained from fr. 1, identified as tricin (10 mg) and β-sitosterol-3-*O*-β-D-glucuronoside (50 mg) from fr. 3. The other impure frs. 2, 4 and 5 after mixing and rechromatographing over Lichroprep RP-18 (ODS silica gel) using sequential mixtures of $H_2O$/MeOH as eluants (elution order 80%, 60%, 40%, 20%, 10% acqueous methanol, 100% methanol) yielded six frs. and obtained one new oryzasesterpenolide (**1**, 10 mg) known as 3,7-dimethyl-*n*-octan-1-yl benzoate (15 mg). The fraction 23 was subjected to silica gel with chromatography eluting with $CHCl_3$/MeOH to yield one pure compound β-sitosterol-3-*O*-β-D-glucopyranoside (50 mg).

**8, 12, 16, 18-Tetramethyl-n-nonacosan-5α, 6α. 13α, 16α-tetraol-1,21-olide (1):** $R_f$ 0.32 ($CHCl_3$ : MeOH 9 : 1), gummy; $[\alpha]_D^{22}$: –24.6° (MeOH); IR $\nu_{max}$ (KBr): 3443, 3410, 3390, 2923, 2860, 1736, 1462, 1377, 1260, 1125, 1079 $cm^{-1}$; EIMS: 430 $[M]^+$ ($C_{24}H_{46}O_6$) (11.9), 396 (9.9), 382 (6.8), 368 (4.8), 265 (4.7), 239 (6.7), 201 (4.5), 187 (5.3), 173 (7.9), 159 (9.6), 129 (7.8), 115 (5.4), 111 (9.9), 109 (17.1), 97 (24.1), 95 (33.2), 83 (31.8), 57 (100); FABMS (positive mode) m/z 431 $[M + H]^+$. $^1H$ NMR (500 MHz; MeOD) and $^{13}C$ NMR (125 MHz; MeOD) δ (see Table-1).

TABLE-1

$^1H$ AND $^{13}C$-NMR SPECTRAL DATA OF ORIZASESTERPENOLIDE (1)

| Position | $^1H$ NMR |  | $^{13}C$ NMR |
|---|---|---|---|
|  | alpha | beta |  |
| 1 | — | — | 175.11 |
| 2 | 2.36 d (7.35) | 2.33 d (7.35) | 36.94 |
| 3 | 1.67 m | 1.62 m | 30.15 |
| 4 | 2.08 m | — | 52.86 |
| 5 | — | 3.74 dd (5, 4.9) | 76.03 |
| 6 |  | 4.22 ddd (5, 6.8, 5) | 74.42 |
| 7 | 1.67 m | 1.57 m | 30.01 |
| 8 | 2.04 | — | 46.73 |
| 9 | 1.27 br s | 1.04 m | 27.86 |
| 10 | 1.27 br s | 1.18 m | 26.83 |
| 11 | 1.27 br s | 1.27 br s | 26.62 |
| 12 | 2.02 m | — | 37.15 |
| 13 | — | 4.39 ddd (8.2, 7.5, 11.5) | 73.14 |
| 14 | 1.53 m | 1.48 m | 25.76 |
| 15 | 2.24 d (7.5) | 2.21 d (8.0) | 25.62 |
| 16 | — | — | 80.78 |
| 17 | 1.27 br s | 1.27 br s | 23.37 |
| 18 | 1.57 m | — | 32.66 |
| 19 | 1.07 d (5.0) | — | 12.39 |
| 20 | 3.56 d (4.5) | 3.53 d (5.5) | 61.92 |
| 21 | 0.89 d (6.65) | — | 19.47 |
| 22 | 0.86 d (7.5) | — | 19.86 |
| 23 | 1.31 br s | — | 20.23 |
| 24 | 0.94 d (5.4) | — | 14.46 |

Coupling constant (J) values in Hertz (Hz) are in parenthesis

**Momilactone A:** $R_f$ 0.52 ($CH_2Cl_2$ : MeOH 9.5 : 0.5); m.p. 235–236°C; $[\alpha]_D^{25}$ –277° ($CHCl_3$); IR, $\nu_{max}$: 2932, 1765, 1700, 1386, 1181 $cm^{-1}$; EI-MS (m/z): 314 $[M]^+$ (calc. for $C_{20}H_{26}O_3$) (100%), 299 (21.7), 286 (6.8), 272 (8.7), 271 (24.5), 270 (17.0), 256 (4.1), 255 (18.0), 243 (4.8), 232 (12.8), 229 (4.8), 227 (7.1), 213 (12.7), 201 (6.6), 200 (9.6), 189 (7.3), 157 (7.7), 133 (12.9), 105 (9.7), 91 (12.1), 81 (15.7), 55 (7.7); FAB-MS: (positive mode) m/z 315 $[M + H]^+$; (negative mode) m/z 313 $[M - H]^-$. $^1H$ NMR (500 MHz; $CDCl_3$) and $^{13}C$ NMR (125 MHz; $CDCl_3$) δ: (see Tables 2 and 3).

TABLE-2

$^1H$-NMR ASSIGNMENTS OF MOMILACTONE A

| Position | 1 ($CDCl_3$; 60 MHz) $\delta_H$ | 2 ($CDCl_3$; 100 MHz) $\delta_H$ | 3 ($CDCl_3$; 500 MHz) $\delta_H$ |
|---|---|---|---|
| 1 | — | — | *2.60–2.64, 2H, m* |
| 2 | — | — | *1.59–1.63, 1H, m*<br>*1.89–1.91, 1H, m* |
| 3 | — | — | — |
| 4 | | | — |
| 5 | 2.32, 1H, d (5.0 Hz) | 2.32.1H, d, (5.0 Hz) | 2.31, 1H, d (5.0 Hz) |
| 6 | 4.83, 1H, t (5.0 Hz) | 4.83, 1H, t (5.0 Hz) | 4.85, 1H, t (5.0 Hz) |
| 7 | 5.70, 1H, d (5.0 Hz) | 5.70, 1H, d (5.0 Hz) | 5.71, 1H, d (5.0 Hz) |
| 8 | — | — | — |
| 9 | — | — | *1.78–1.81, 1H, m* |
| 10 | — | — | — |
| 11 | —<br>— | —<br>— | *1.32 & 1.74, 1H, dd, each (1.22 & 3.12 Hz)* |
| 12 | — | — | *1.60, 2H, m* |
| 13 | — | — | — |
| 14 | 2.03 & 2.22, 2H, d (12.5 Hz) | 2.03 & 2.22, 2H, d (12.5 Hz) | 2.06 & 2.21, 2H, d (12.0 Hz) |
| 15 | 5.85, 1H, dd (17 & 10.8 Hz) | 5.85, 1H, dd (17 & 10.8 Hz) | 5.85, 1H, dd (17.5 & 10.7 Hz) |
| 16 | 4.93, 1H, dd (10.8 & 1.2 Hz)<br>4.95,1H, dd (17 & 1.2 Hz) | 4.93, 1H, dd (10.8 & 1.2 Hz)<br>4.95, 1H, dd (17 & 1.2 Hz) | 4.94, 1H, dd (10.7 & 7.5 Hz)<br>4.98, 1H, dd (17.5 & 7.5 Hz) |
| 17 | 0.90, 3H, s | 0.90, 3H, s | 0.89, 3H, s |
| 18 | 1.52, 3H, s | 1.52, 3H, s | 1.52, 3H, s |
| 19 | — | 1.00, 3H, s | — |
| 20 | 1.00, 3H, s | | 0.99, 3H, s |

Coupling constant (*J*) values in Hertz (Hz) are in parenthesis

Reference data from:

1. *Tetrahedron Lett.*, **39**, 3861–3864 (1973).
2. *Phytochemistry*, **16**, 45–48 (1977).
3. Current data reported in this report; italic values first time.

TABLE-3
$^{13}$C-NMR ASSIGNMENTS OF MOMILACTONE A

| Position | ($CDCl_3$, 15.35 MHz)[3]<br>$\delta_C$ | ($CDCl_3$, 25 MHz)[4]<br>$\delta_C$ | ($CDCl_3$, 125 MHz)†<br>$\delta_C$ |
|---|---|---|---|
| 1 | 34.89 | 34.90 | 35.08 |
| 2 | 31.25 | 31.30 | 31.41 |
| 3 | 205.00 | 205.00 | 205.37 |
| 4 | 53.57 | 53.80 | 53.76 |
| 5 | 46.67 | 46.70 | 46.66 |
| 6 | 73.17 | 73.20 | 73.36 |
| 7 | 114.06 | 114.10 | 114.23 |
| 8 | 147.98 | 148.00 | 148.22 |
| 9 | 50.24 | 50.20 | 50.39 |
| 10 | 32.52 | 32.50 | 32.66 |
| 11 | 24.03 | 24.00 | 24.19 |
| 12 | 37.25 | 37.30 | 37.44 |
| 13 | 40.16 | 40.20 | 40.32 |
| 14 | 47.57 | 47.60 | 47.73 |
| 15 | 148.95 | 149.00 | 149.15 |
| 16 | 110.12 | 110.10 | 110.36 |
| 17 | 21.97 | 22.00 | 22.16 |
| 18 | 21.42 | 21.42 | 21.66 |
| 19 | 174.25 | 174.30 | 174.51 |
| 20 | 21.78 | 21.80 | 21.99 |

†Current data reported in this paper.

**Momilactone B:** $R_f$ 0.50 ($CH_2Cl_2$ : MeOH 9.5 : 0.5); m.p. 240°C (decom.); $[\alpha]_D^{25}$ −185° ($CHCl_3$); IR, $\nu_{max}$: 2939, 1735, 1670, 1233, 1129, 1073, 932 cm$^{-1}$; EI-MS (m/z): 330 $[M]^+$ (calc. for $C_{20}H_{26}O_4$); FAB-MS (positive mode) m/z: 331 $[M + H]^+$ (100%), 312 $[M - H_2O]^+$ (25), 270 (18), 227 (12), 199 (10), 185 (20), 154 (70), 136 (56), 107 (28), 91 (32), 55 (35), 41 (27), 39 (16), 29 (9); (negative mode) m/z: 329 $[M - H]^-$ (7%), 305 (35), 304 (6), 199 (28), 153 (100), 152 (55), 40 (40), 26 (4). $^1$H NMR (500 MHz; $CDCl_3$) and $^{13}$C NMR (125 MHz; $CDCl_3$) $\delta$ (see Tables 4 and 5).

TABLE-4

$^1$H NMR ASSIGNMENTS OF MOMILACTONE B

| Position | (CDCl$_3$, 60 MHZ)[3] $\delta_H$ | (CDCl$_3$, 100 MHz)[4] $\delta_H$ | (CDCl$_3$, 500 MHz)† $\delta_H$ |
|---|---|---|---|
| 1 | — | — | *1.67–1.72, 2H, m* |
| 2 | — | — | *2.09.2H, dd* |
| 3 | — | — | *4.13, 1H, s (OH)* |
| 4 | — | — | — |
| 5 | 2.20, 1H, dd (7.1 & 2.1 Hz) | 2.20, 1H, dd (7.1 & 2.1 Hz) | 2.20, 1H, dd (6.7 & 1.6 Hz) |
| 6 | 4.94, 1H, dd (7.1 & 4.5 Hz) | 4.94, 1H, dd (7.1 & 4.5 Hz) | 4.94, 1H, dd (7.2 & 4.4 Hz) |
| 7 | 5.68, 1H, d (4.5 Hz) | 5.68, 1H, d (4.5 Hz) | 5.67, 1H, d (4.7 Hz) |
| 8 | — | — | — |
| 9 | — | — | *1.44, 1H, dd (3.4 Hz each)* |
| 10 | — | — | — |
| 11 | — | — | *1.22, 1H, dd (12.4 & 7.5 Hz)*<br>*1.66, 1H, m* |
| 12 | — | — | *1.55–1.56, 2H, m* |
| 13 | — | — | — |
| 14 | Overlapped with others | — | *2.01, 1H, d (12 & 1.8 Hz)*<br>*2.13, 1H, dd (12.0 Hz)* |
| 15 | 5.83, 1H, dd (17.6 & 10.5) | 5.83, 1H, dd (17.6 & 10.5) | 5.82, 1H, dd (17.4 & 10.7 Hz) |
| 16 | 4.92, 1H, dd (10.5 & 1.2)<br>4.95, 1H, dd (17.6 & 1.2) | 4.92, 1H, dd (10.5 & 1.2)<br>4.95, 1H, dd (17.6 & 1.2) | 4.92, 1H, dd (10.4 & 1.3 Hz)<br>4.94, 1H, dd (17.5 & 1.4 Hz) |
| 17 | 0.87, 3H, s | 0.87, 3H, s | 0.86, 3H, s |
| 18 | 1.40, 3H, s | 1.40, 3H, s | 1.40, 3H, s |
| 19 | — | — | — |
| 20 | 3.55, 1H, dd (9.0 & 2.1 Hz)<br>4.07, 1H, bd (9.0 Hz) | 3.55, 1H, dd (9.0 & 2.1)<br>4.07, 1H, bd (9.0 Hz) | 3.56, 1H, dd (9.1 & 3.2 Hz)<br>4.06, 1H, dd (9.1 & 1.9 Hz) |

Coupling constant (J) values in hertz (Hz) are in parentheses.

†Current data reported in this report; italic values first time.

TABLE-5

$^{13}$C NMR ASSIGNMENTS OF MOMILACTONE B

| Position | ($CDCl_3$, 15.35 MHz)[3] $\delta_C$ | ($CDCl_3$, 25 MHz)[4] $\delta_C$ | ($CDCl_3$·125 MHZ)† $\delta_C$ |
|---|---|---|---|
| 1 | 28.82 | 28.80 | 29.00 |
| 2 | 26.45 | 26.50 | 26.63 |
| 3 | 96.59 | 96.60 | 96.78 |
| 4 | 50.36 | 50.40 | 50.53 |
| 5 | 43.02 | 43.00 | 43.17 |
| 6 | 73.78 | 73.80 | 73.93 |
| 7 | 114.00 | 114.00 | 114.19 |
| 8 | 146.70 | 146.70 | 146.88 |
| 9 | 44.65 | 44.70 | 44.87 |
| 10 | 30.76 | 30.80 | 30.93 |
| 11 | 24.81 | 24.80 | 24.97 |
| 12 | 37.25 | 37.30 | 37.41 |
| 13 | 39.98 | 40.00 | 40.17 |
| 14 | 47.44 | 47.40 | 47.72 |
| 15 | 148.83 | 148.80 | 149.02 |
| 16 | 110.18 | 110.20 | 110.40 |
| 17 | 21.90 | 21.90 | 22.06 |
| 18 | 18.99 | 19.00 | 19.16 |
| 19 | 180.52 | 180.50 | 180.64 |
| 20 | 72.74 | 72.70 | 72.90 |

†Current data reported in this paper.

**Tricin:** $R_f$ 0.64 ($CHCl_3$ : MeOH 9 : 1); m.p. 286–289°C; UV $\lambda_{max}$ (MeOH): 241, 269 and 352 nm; IR, $\nu_{max}$: 3468, 2928, 1647, 1506, 1359, 1166, 830 cm$^{-1}$; $^1$H-NMR (500 MHz, MeOD): δ 7.27 (2H, s, H-2′ and 6′), 6.62 (1H, s, H-8), 6.16 (1H, s, H-6), 3.95 [6H, s, 2-(OMe)]; $^{13}$C-NMR (125 MHz, MeOD): δ 164.20 (C-2), 104.77 (C-3), 183.71 (ketone, C-4), 165.94 (C-5), 105.29 (C-6), 168.56 (C-7), 95.89 (C-8), 159.67 (C-9), 105.00 (C-10), 120.93 (C-1′), 122.74 (C-2′ and 6′), 149.81 (C-3′ and 5′), 141.47 (C-4′), 57.02 (2-OMe); EI-MS, m/z: 330 $[M]^+$ (calcd. for $C_{17}H_{14}O_7$) (100%), 315 $[M - Me]^+$ (3.8), 300 $[315 - Me]^+$ (3.2), 287 (7.0), 269 (2.2), 259 (4.5), 244 (2.3), 221 (2.6), 213 (4.7), 178 (4.8), 163 (3.3), 153 (80), 123 (2.2), 107 (3.2), 95 (2.2), 69 (3.2), 57 (3.1); FAB-MS (positive mode) m/z: 331 $[M + H]^+$ (58.9%), 330 (28.6), 289 (10.1); 176 (35.3), 154 (100), 136 (74.4), 107 (26.8), 77 (23.8), 43 (24.4), 23 (27.3); (negative mode) m/z: 329 $[M - H]^-$ (45.2%), 306 (37.7), 305 (35.08), 199 (29.7), 168 (29.4), 153 (100), 122 (16.8), 46 (41.5).

**3,7-dimethyl-*n*-octan-1-yl benzoate:** Oil; $R_f$ 0. 38 (Hex : EtOAc 1 : 1); UV $\lambda_{max}$ ($CHCl_3$): 271 nm; IR (KBr) $\nu_{max}$: 2927, 2850, 1723, 1550, 1461, 1360, 1272, 1210, 1125, 1020, 771 cm$^{-1}$; $^1$H-NMR ($CDCl_3$): δ 7.70 (2H, m, H-3′, H-7′), 7.53

(2H, m, H-4′, H-6′), 7.26 (1H, br s, H-5′), 4.23 (1H, d, J = 6.1 Hz, $H_2$-1a), 4.21 (1H, d, J = 6.5 Hz, $H_2$-1b), 2.34 (1H, m, H-3), 1.68 (1H, m, H-7), 1.54 (2H, m, $H_2$-3), 1.31 (4H, br s, $H_2$-4 and 5), 1.25 (2H, m, $H_2$-6), 0.93 (3H, d, J = 7.4 Hz, Me-9), 0.92 (3H, d, J = 7.6 Hz, Me-10); 0.90 ( 3H, t, *J* = 5.1 Hz, Me-8); $^{13}$C-NMR ($CDCl_3$): δ 68.38 (C-1), 28.80 (C-2), 38.96 (C-3), 29.91 (C-4), 23.97 (C-5), 23.19 (C-6), 30.58 (C-7), 11.13 (C-8), 14.25 (C-9), 11.17 (C-10), 167.97 (C-1′), 132.68 (C-2′), 131.09 (C-3′), 129.02 (C-4′), 129.02 (C-5′), 129.02 (C-6′), 131.09 (C-7′); EIMS m/z (rel. int.): 262 $[M]^+$ ($C_{17}H_{26}O_2$) (27.6).

## RESULTS AND DISCUSSION

The methanol extracts of rice hulls were separated as described in the experimental section to yield new and known compounds. The known compounds were identified by direct comparison with authentic samples, spectral data and published data[3, 4, 9–12].

**Oryzasesterpenolide (1)** was obtained from the ethyl acetate extract of rice hulls. Its IR spectrum showed characteristic absorption bands for hydroxyl group (3443, 3410, 3390 $cm^{-1}$) and δ-lactone ring (1736 $cm^{-1}$). Its mass spectrum displayed a molecular ion peak at m/z 430 corresponding to a norsesterpenolide molecule, $C_{24}H_{46}O_6$. It indicated one double bond equivalent adjusted lactone ring. The prominent ion fragments were generated at m/z 57 $[C_{16}$-$C_{17}$ fission$]^+$, 83 [101, $C_{15}$-$C_{16}$ fission—$H_2O]^+$, 115 $[C_{14}$-$C_{15}$ fission$]^+$, 129 $[C_5$-$C_6$ fission and $C_{13}$-$C_{14}$ fission$]^+$, 159 $[C_6$-$C_7$ and $C_{12}$-$C_{13}$ fission$]^+$, 187 $[C_{11}$-$C_{12}$ fission$]^+$, 173 $[C_7$-$C_8$ fission$]^+$ and 201 $[C_{10}$-$C_{11}]^+$; suggested location of the hydroxyl groups at C-5, C-6, C-13 and C-16 and lactone ring at one of the terminal position.

The $^1$H-NMR spectrum showed a one proton double doublet at δ 3.74 (J = 5.0, 4.9 Hz) and two one-proton doublet of double doublets at δ 4.22 (J = 5.0, 6.8, 5.0 Hz), 4.39 (J = 8.25, 7.5, 11.5 Hz) assigned to carbinol H-5β, H-6β and H-13 respectively. Two one-proton doublets δ 3.56 (J = 4.5 Hz) and 3.53 (J = 5.5 Hz) were ascribed oxygenated H-20 methyl protons. Four three-proton doublets at δ (J = 4.5 Hz), 0.89 (J = 6.6 Hz), 0.86 (J = 7.0 Hz) and 0.94 (J = 5.4 Hz), and a three-protons broad signal at δ 1.31 were attributed to C-19, C-21, C-22 and C-24. The remaining methine and methylene protons resonated between δ 2.36–1.27. The presence of all methyl signals up to δ 1.31 supported the attachment of the structure functionalities to saturated carbon. The $^{13}$C-NMR spectrum exhibited signals for carbinol carbons at 76.03 (C-5), 74.42 (C-6), 73.14 (C-13), 80.78 (C-16) and 61.92 (C-20), lactone carbons at δ 175.11 (C-1) and methyl carbons at δ 12.39 (C-19), 19.47 (C-21), 19.86 (C-22), 20.23 (C-23) and 14.46 (C-24). The remaining methine and methylene carbons appear between δ 52.86–23.37. The absence of any signal beyond δ 4.39 in the $^1$H-NMR spectrum and between 175.11–80.78 in the $^{13}$C-NMR ruled out the existence of a vinylic bond in the molecule. In the $^1$H-$^{13}$C HETCOR spectrum showed correlation of C-1 with $H_2$-2, C-20 with H-4 and H-5 and C-13 with H-12, H-14. The $^1$H-$^1$H COSY spectrum displayed correlation of $H_2$-20 with H-5, H-5 with H-6 and H-13 with H-12. On the basis of these evidences the structure has been elucidated as 8,12,16,18-tetramethyl-*n*-nonacosan-5α,6α,13α,16α-tetraol-1,21-olide.

**Momilactone A** was isolated as colourless crystals that exhibited an m/z of 314 on EI-MS. A FAB-MS of compound gave a positive-mode molecular ion peak $[M+H]^+$ at m/z 315 and a negative-mode at m/z 313 $[M-H]^-$. This suggested a molecular ion peak of m/z 314. The IR spectrum of compound **4** gave absorption bands at 2932, 1765, 1700, 1386 and 1181 $cm^{-1}$. The bands at 1765 and 1700 $cm^{-1}$ suggested lactone and ketone groups, respectively. The $^1$H-NMR ($CDCl_3$) spectrum displayed three singlets at δ 0.89, 1.52 and 0.99 for C-17, C-18 and C-20 tertiary methyl groups, respectively and two multiplets at δ 2.60–2.64 and δ 1.89–1.91 for C-1 and C-2 methylene groups, respectively. Those signals at δ 2.31 (d, J = 5.0 Hz), δ 4.85 (t, J = 5.0 Hz) and δ 5.71 (d, J = 5.0 Hz) were assigned to the C-5 to C-7 protons for two methine and one olefinic groups. The multiplets at δ 1.80 and 1.60 for C-9 and C-12 methine and methylene protons, and the double doublets at δ 1.32,1.74 and 2.06, 2.21 each for the C-11 and C-14 methylene protons. Finally, some other signals in the downfield region as double doublets at δ 5.85 (J = 17.5 Hz and 10.7 Hz) for C-15 and δ 4.94 (J = 10.8 Hz and 1.2 Hz), 4.98 (J = 17 Hz and 1.2 Hz) for C-16 were ascribed as an olefinic proton. The $^{13}$C NMR spectrum of the compound showed resonances for all 20 carbons (δ 35.08, 31.41, 205.37, 53.76, 46.66, 73.36, 114.23, 148.22, 50.39, 32.66, 24.19, 37.44, 40.32, 47.73, 149.15, 110.36, 22.16, 21.66, 174.51 and 21.99), which included three methyl, six methylene, five methine and six quaternary carbons. The $^{13}$C NMR spectrum clearly exhibits the presence of both a ketone and a lactone group at δ 205.37 and 174.51, respectively. All olefinic carbons were assigned to 149.15, 148.22, 114.23 and 110.36. Analysis of the compound by $^1$H-, $^{13}$C- and 2D-NMR (COSY, HETCOR) supported confirmation of the structure as momilactone A. $^1$H and $^{13}$C-NMR values are given in detail in Tables 2 and 3. On this basis all the data confirm that this compound is momilactone A and also confirm from reported values[3, 4].

**Momilactone B** was isolated as colourless crystals that exhibited an m/z of 330 on EI-MS. A FAB-MS of the compound gave a positive-mode molecular ion peak at m/z $[M+H]^+$ of 331 and a negative-mode m/z at 329 $[M-H]^-$. This suggested a molecular ion peak of m/z 330. The IR spectrum gave bands at 2939, 1735, 1670, 1233, 1129, 1037 and 932 $cm^{-1}$, the band at 1735 $cm^{-1}$ suggesting a lactone group. The $^1$H-NMR ($CDCl_3$) spectrum of the compound displayed two 3H singlets at δ 0.86, 1.40 for the C-17 and C-18 tertiary methyl groups. Two multiplets at δ 1.52–1.56 and δ 2.09 for the C-1 and C-2 methylene groups, respectively and at δ 4.13 exhibited a single peak for the hydroxyl proton. Those signals at δ 2.20 (dd, J = 7.1 Hz and 2.1 Hz), δ 4.94 (dd, J = 7.1 Hz and 4.5 Hz) and δ 5.67 (d, J = 4.5 Hz) were assigned to the C-5, C-6 and C-7 protons (all methine groups), respectively. Other double doublets were at δ 1.44 for C-9 (methine) and 1.22, 1.66 for C-11 methylene protons, respectively. A multiplet at δ 1.55 was for C-12 (methylene) and a doublet at δ 2.01 (J = 12.0 Hz) and double doublet 2.13 (J = 12.0 Hz and 1.8 Hz) was for the C-14 methylene protons. A double doublet at δ 5.82 (J = 17.6 Hz and 10.7 Hz), δ 4.92 (J = 10.5 Hz and 1.2 Hz) and 4.94 (J = 17.6 Hz and 1.2 Hz) were for the C-15 and C-16 olefinic protons. Two more double doublets at δ 3.56 (dd, J = 9.1 Hz and 3.2 Hz) and δ 4.06 (dd, J = 9.1 and 1.9 Hz) were for C-20 methylene protons. The $^{13}$C-NMR spectrum of this compound showed resonances for all 20 carbons (δ 29.00, 26.63, 96.78, 50.53, 43.17, 73.93, 114.19, 146.88, 44.87, 30.93, 24.97, 37.41, 40.17, 47.72, 149.02, 110.40, 22.06, 19.16, 180.64 and 72.90), which included two methyl, seven methylene, five methine and six quaternary carbons. The $^{13}$C-NMR spectrum clearly exhibits the presence of a ketone at δ 180.00 and the downfield hydroxyl carbon at δ 96.7 was attached directly with oxygen atom on both sides. The methylene carbon for C-20 position also showed downfield in its spectrum, which was attached with oxygen atom. Analysis of the compound by $^1$H-, $^{13}$C- and 2D-NMR (COSY, HETCOR) supported the confirmation of the structure as momilactone B. $^1$H and $^{13}$C-NMR values are given in detail in Tables 4 and 5. All these data confirm this compound to the momilactone B which is also confirmed from reported values[3, 4].

**Tricin** was isolated as a yellow powder that exhibited a molecular ion peak on EI-MS at an m/z of 330 $[M]^+$. A FAB-MS gave a positive-mode molecular ion peak at m/z $[M + H]^+$ of 331 and a negative-mode molecular ion peak $[M - H]^-$ at 329. This suggested a molecular ion peak of m/z 330. The IR spectrum of the compound gave intense absorption bands at 3468, 2928, 1647, 1506, 1359, 1166 and 830 cm$^{-1}$. The bands at 3468 and 1647 cm$^{-1}$ suggested hydroxyl and ketone groups, respectively. The $^1$H-NMR of the compound showed a singlet at δ 7.27, which was assigned to aromatic H-2′ and H-6′. Another two singlets at δ 6.16 and 6.62 were assigned to H-6 and H-8, respectively. Moreover, a singlet at δ 3.95 indicated the presence of two methoxy group protons. The $^{13}$C-NMR spectrum also showed 15 carbon signals in the molecule for 17 carbons. Analysis of the compound by $^1$H-, $^{13}$C- and 2D-NMR (COSY, HETCOR) supported confirmation of the structure as tricin. The results of 500 MHz $^1$H-NMR and 125 MHz $^{13}$C-NMR data are shown in the Results section. To the best of our knowledge, the $^{13}$C-NMR values for tricin are reported for the first time.

**3,7-Dimethyl-n-octan-1-yl benzoate** showed characteristic IR absorption bands for ester group 1723 cm$^{-1}$. Its electron impact mass spectrum displayed a molecular ion peak at m/z 262, which corresponded to a benzoate-type molecule, $C_{17}H_{26}O_2$. The $^1$H-NMR spectrum exhibited two de-shielded multiplets at δ 7.70 (2H) and 7.53 (2H), which were assigned to aromatic H-3′, H-7′ and H-4′, H-6′, respectively, and one proton broad singlet at 7.26 was assigned to H-5′. Two one-proton doublets at δ 4.23 (J = 6.5 Hz) and 4.21 (J = 6.5 Hz), two doublets at δ 0.93 (J = 7.4 Hz) and 0.92 (J = 7.6 Hz) and triplet at δ 0.90 (J = 5.1) each integrated for three protons were associated with C-9, C-10, C-8 methyl. The remaining methine and methylene protons appeared in the range of δ 1.25–2.34. The $^{13}$C-NMR spectrum displayed all the signals for the molecules. Based on this evidence, the structure was established as 3,7-dimethyl-n-octan-1-yl benzoate.

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