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CHEMICAL CONSTITUENTS FROM THE HULLS OF *Oryza sativa* WITH CYTOTOXIC ACTIVITY

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*Five new compounds, orizaterpenol (**1**), orizaterpenoid (**2**), orizaterpenyl benzoate (**3**), orizanor-diterpenyl benzoate (**4**), and orizaditerpenyl benzoate (**5**), along with nine known compounds, were isolated and identified from the rice hulls of* Oryza sativa. *Their structures were elucidated with the help of different spectroscopic techniques. Orizaterpenol (**1**) and the known momilactone A (**6**) and B (**7**) were found to have cytotoxic effects against P388 murine leukaemia cells, while the other new and known compounds exhibited weak cytotoxicity.*

Key words: *Oryza sativa* L, Poaceae, rice hull composition, orizaterpenol, orizaterpenoid, orizaterpenyl benzoate, orizanor-diterpenyl benzoate, orizaditerpenyl benzoate.

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 colored 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 [3–5] and momilactone B was isolated also from rice root exudates [6]. The antioxidative activities of methanol extracts [7] and C-glycosyl flavonoid from the rice hull [8] have been reported in the literature. Because there are few reports in the literature on the chemical constituents of rice hulls, identification of further bioactive constituents is still required. To achieve these objectives the aims of our research are to isolate, and identify their constituents as well as their activity, including momilactone A and B from the rice hull. We have now examined the constituents of the rice hull and isolated five new compounds (**1–5**), along with nine known compounds, momilactone A (**6**), momilactone B (**7**), β-sitosterol, tricin, 3,7-dimethyl-*n*-octan-1-yl benzoate, hentriacontane, 1-tetratriacontanol, β-sitosterol-3-O-β-*D*-glucuronoside, and b-sitosterol-3-O-β-*D*-glucopyranoside. The latter five compounds have been isolated from this plant for the first time. This paper deals with the isolation and structural elucidation of compounds (**1–5**) based on ^{1}H NMR, ^{13}C NMR, COSY, HETCOR, and HSQC aided by EIMS, FAB MS, HREIMS, and IR spectra. It also focuses on the cytotoxic activity of the new and known compounds.

The methanol extract of the *O. sativa* hulls was suspended in water and extracted with ethyl acetate and then *n*-butanol. The ethyl acetate extract was separated by a combination of column chromatography over silica gel and Lichroprep RP-18 (ODS Si gel) to yield five new and nine known compounds.

For all the molecules studied, relative configurations were suggested on the basis of biogenetic speculations.

Orizaterpenol (1). The EIMS of **1** showed a molecular ion peak at *m/z* 304 and its molecular formula was determined to be $C_{20}H_{32}O_2$ by HREIMS at *m/z* 304.2398 (calc. 304.2402). The positive-ion FAB mass spectrum of **1** also displayed a molecular ion peak at *m/z* 304. It indicated that there were five double bond equivalents; four were adjusted in a phyllocladane-type tetracyclic carbon framework and the fifth to the vinylic linkage. The ion peaks appearing in EI MS at *m/z* 289 $[M\text{-}Me]^+$,

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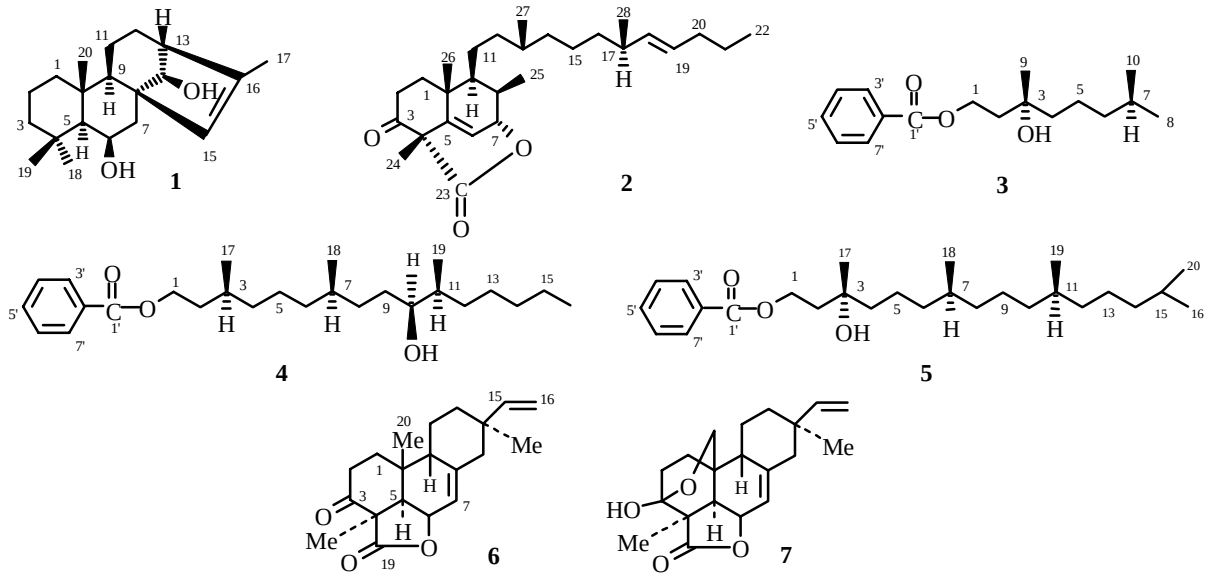

273 [289-Me]$^+$, 255 [273-H_2O]$^+$, 240 [255-Me]$^+$, 286 [M-H_2O]$^+$, 271 [286-Me]$^+$ and 222 [240-H_2O]$^+$ suggested the presence of two removable hydroxyl groups in the molecule. The ion fragments at *m/z* 97 [$C_{1,10}$–$C_{5,10}$–$C_{5,6}$ fission]$^+$, 83, 221 [$C_{1,3}$–$C_{5,6}$ fission]$^+$, 69 [$C_{2,3}$–$C_{5,6}$ fission]$^+$, 55 [$C_{3,4}$–$C_{5,6}$ fission]$^+$, 127, 177 [$C_{1,10}$–$C_{6,7}$ fission]$^+$, 162 [$C_{5,6}$–$C_{9,10}$ fission – H_2O]$^+$, 144 [162-H_2O]$^+$, 150 [$C_{6,7}$–$C_{9,10}$ fission]$^+$, 132 [150-H_2O]$^+$, and 136 [M-150 – H_2O]$^+$ showed the saturated nature of rings A and B and the location of the hydroxyl group at C-6. The MS fragmentation pattern is shown in Fig. 1. Its IR showed characteristic absorption bands for the hydroxyl group (3356 cm^{-1}) and for unsaturation (1635 cm^{-1}).

The ^{1}H NMR spectrum of **1** showed a one-proton broad signal at δ 4.95, which was assigned to vinylic H-15. A one-proton doublet at δ 3.68 (J = 10.7 Hz) and a one-proton broad multiplet at δ 3.31 with half-width of 14.2 Hz were attributed to α-oriented carbinol H-14α and H-6α protons, respectively. A three-proton broad signal at δ 1.62 was attributed to C-17 methyl protons located on the C-16 olefinic carbons. A nine-proton broad signal at δ 0.98 was assigned to C-18, C-19, and C-20 tertiary methyl protons. The remaining methylene and methine protons resonated in the range δ 1.10–2.21. The ^{13}C NMR spectrum displayed 20 carbon signals of the molecule. The carbon signals at δ 124.22 and 139.37 and at δ 65.35 and 64.94 were correspondingly assigned to vinylic C-15 and C-16 and to carbinol C-6 and C-14 carbons. The ^{1}H and ^{13}C NMR assignments were deduced by means of the combined use of ^{1}H–^{1}H COSY, ^{1}H-^{13}C HETCOR, and HSQC spectra in combination with standard 1D NMR techniques [9, 10]. The general strategy adopted for the spectral analysis of (**1**) required the assignment of the isolated ^{1}H NMR resonances, followed by tracing of the cross-peak connectivities in the ^{1}H–^{1}H COSY spectrum and their correlation in the ^{13}C-dimensions in the one-bond HETCOR spectrum to give the ^{13}C NMR assignments for the protonated carbon resonances. The HETCOR spectrum also led to the identification of the general proton partners in several cases, even though some were severely overlapped in the δ 1.1–2.1 range.

Based on the above evidence, the structure of **1** has been elucidated as phylloclad-15-ene-6β,14β-diol.

Orizaterpenoid (2). Its IR spectrum showed characteristic absorption bands for the ester group (1725 cm^{-1}), the keto group (1698 cm^{-1}), and for unsaturation (1620 cm^{-1}). Both EI MS and FAB MS (positive mode and negative mode) displayed a molecular ion peak at *m/z* 428, which corresponds to a bicyclic nor-triterpenic formula, $C_{28}H_{44}O_3$. This indicated that there were seven double bond equivalents, two each of which were adjusted in δ-lactone, olefinic linkages, and cyclic rings, and one to the carbonyl group. The prominent ion peaks in EI MS at *m/z* 413 [M-Me]$^+$, 398 [413-Me]$^+$, and 383 [398-Me]$^+$ were generated due to the subsequent elimination of the methyl group from the molecular ion peak. The ion peaks appearing at *m/z* 56 [$C_{1,10}$–$C_{3,4}$ fission]$^+$, 192 [$C_{7,8}$–$C_{9,10}$ fission]$^+$, and 233 [M-$C_{14}H_{27}$, side chain]$^+$ suggested the presence of the carbonyl group at C-3 and the lactone ring involving hydroxyl group at C-7 and the carbonyl group at C-23. The generation of the ion peak at *m/z* 43[C_{19}–C_{20} fission]$^+$ and the subsequent ion peaks at *m/z* 69, 97, 111 etc. supported one of the vinylic linkage at C-18. The mass fragmentation pattern is shown in Fig. 1.

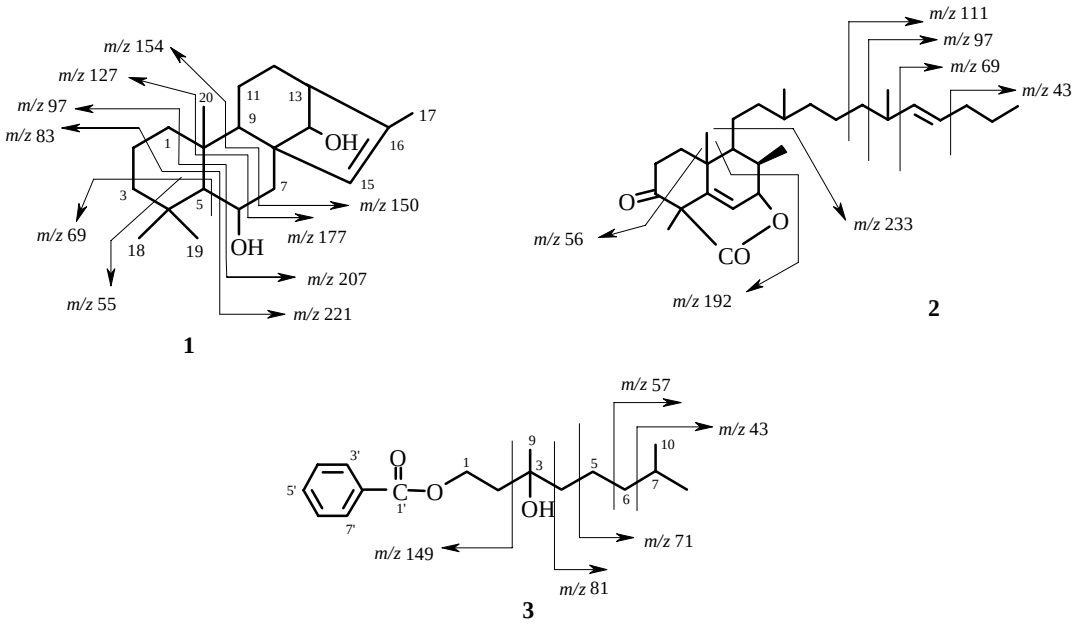

Fig. 1. MS fragmentation of compounds **1**–**3**.

The ^{1}H NMR of **2** displayed two one-proton double doublets at δ 5.15 (J = 8.6, 8.6 Hz) and 5.05 (J = 8.65, 8.69 Hz) and a one-proton broad signal at δ 5.80, which were assigned to vinylic H-18, H-19, and H-6, respectively. A one-proton double doublet at δ 4.34 (J = 3.0, 3.1 Hz) was attributed to carbinol H-7. A three-proton triplet at δ 0.84 (J = 5.45 Hz) was attributed to C-22 primary methyl protons. Two three-proton broad signals at δ 1.37 and 0.74 were attributed to C-24 and C-26 tertiary methyl protons. The C-25, C-27, and C-28 secondary methyl protons resonated as doublets that corresponded to δ 0.93 (J = 6.45 Hz), 0.82 (J = 4.4 Hz), and 0.79 (J = 7.9 Hz). The remaining methylene and methine protons appeared in the range of δ 1.03–2.50 (Table 1). The presence of all methyl signals between δ 0.74 and 1.37 supported their location on the saturated carbons. The ^{13}C NMR spectrum of **2** exhibited important carbon signals for the C-3 carbonyl carbon at δ 200.76, C-23 ester carbon at δ 168.91, and C-7 oxygenated methine carbon at δ 73.40. The vinylic carbon signals appeared at δ 129.68 (C-6), 126.46 (C-18), 138.31 (C-5), and 126.25 (C-19). The remaining carbon signals resonated in the range of δ 19.18–56.21 (Table 1). The ^{1}H–^{1}H COSY spectrum of **2** showed a correlation between H-7 with H-8 and H-9, and between H-18 with H-17, H-19, and H-20. The ^{1}H–^{13}C HETCOR spectrum of **2** exhibited correlations of C-3 with H-2 and H-1; C-6 with H-7, H-8, H-9 and H-25; C-23 with H-24; and C-9 with H-25 and H-26.

Based on this evidence, the structure of **2** has been elucidated as **4**, 8β,10β-trimethyl-9-(13,17-dimethyldodec-18-enyl)decalin-5-en-3-one-23,7α-olide.

Orizaterpenyl Benzoate (3). The IR spectrum of **3** showed characteristic absorption bands at 3445 cm^{-1} (OH) and 1728 cm^{-1} (ester group). Its electron impact mass spectrum displayed a molecular ion peak at *m/z* 278, which corresponded to a decanyl benzoate-type molecule, $C_{17}H_{26}O_3$. The prominent ion fragments appearing at *m/z* 43 [C_6–C_7 fission]$^+$, 57 [C_5–C_6 fission]$^+$, 71 [C_4–C_5 fission]$^+$, 85 [C_3–C_4 fission]$^+$, and 149 [C_2–C_3]$^+$ supported the presence of the hydroxyl group at C-3 and the 3,7-dimethyloctanyl-type moiety esterified with benzoic acid. The positive- and negative-ion FAB mass spectrum of **3** also showed a molecular ion peak at *m/z* 278. The mass fragmentation pattern is shown in Fig. 1.

The ^{1}H NMR spectrum of **3** exhibited two deshielded multiplets at δ 7.70 (2H) and 7.52 (3H), which were assigned to aromatic H-3′, H-7′, and H-4′, H-5′, H-6′, respectively. Two one-proton double doublets at δ 4.23 (J = 5.7, 5.7 Hz) and 4.21 (J = 6.2, 6.2 Hz) were attributed to oxygenated C-1 methylene protons. A three-proton broad signal at δ 1.35 was attributed to tertiary C-9 methyl protons attached to C-3. Two three-proton doublets at δ 0.92 (J = 7.5 Hz) and 0.88 (J = 6.8 Hz) were attributed to C-8 and C-10 secondary methyl protons. The remaining methine and methylene protons appeared in the range of δ 1.31–1.68. The ^{13}C NMR spectrum of **3** displayed aromatic carbon signals between δ 132.64–128.02, C-1′ ester carbon at δ 167.89, hydroxyl carbon at δ 67.21, and methyl carbons at 11.11 (C-8), 29.09 (C-9), and 14.19 (C-10). In the ^{1}H–^{13}C HETCOR spectrum, C-3 showed a correlation with H_2-2, H_2-4 and H-7, and C-7 with H_3-8, H_3-10, H_2-6, and H_2-5. The ^{1}H–^{1}H COSY spectrum showed a correlation of H_2-1 with H-7, and H-7 with H-4.

TABLE 1. ^{1}H and ^{13}C NMR Spectral Data of **2** (δ, ppm, 0-TMS, $CDCl_3$)

C atom	^{1}H		^{13}C
	α	β	
1	1.03 d (J = 6.5)	1.13 m	38.77
2	2.48 dd (J = 3.1)	2.50 dd (J = 2.1, 4.9)	40.67
3	-	-	200.76
4	-	-	56.21
5	-	-	138.31
6	5.80 br s	-	129.68
7	4.34 dd (J = 3.0, 3.1)	–	73.40
8	2.06 m	-	42.71
9	1.53 ddd (J = 3.8, 3.3, 3.5)	-	56.09
10	-	-	51.44
11	1.26 m	1.23 m	23.27
12	1.18 m	1.71 m	24.35
13	1.99 m	-	46.03
14	1.14 m	1.13 m	26.29
15	1.13 m	1.50 m	28.38
16	1.17 m	1.46 m	29.35
17	2.38 m	-	53.85
18	5.15 dd (J = 8.6, 8.6)	-	126.46
19	5.05 dd (J = 8.6, 8.6)	-	126.25
20	1.50 m	2.02 m	39.81
21	1.14 m	1.17 m	21.38
22	0.84 t (J = 5.4)	-	12.17
23	-	-	168.91
24	1.37 br s	-	20.01
25	0.93 d (J = 6.4)	-	19.69
26	0.74 br s	-	20.71
27	0.82 d (J = 4.4)	-	19.23
28	0.79 d (J = 7.9)	-	18.93

Coupling constants (Hz) are given in parentheses.

Based on this evidence, the structure of **3** was determined as 3,7-dimethyl-*n*-octan-3α-ol-1-yl benzoate.

Orizanor-diterpenyl Benzoate (4). The IR spectrum of **4** showed absorption bands for the hydroxyl (3456 cm^{-1}) and ester groups (1730 cm^{-1}). Its mass spectrum displayed a molecular weight at *m/z* 404, which corresponded to the structural formula of a nor-diterpenyl ester with benzoic acid, $C_{26}H_{44}O_3$. The base peak generated at *m/z* 129 suggested the location of the hydroxyl group at C-10, and the fragmentation pattern is shown in Fig. 2. The $_1$H-NMR spectrum of **4** exhibited aromatic proton signals as a two-proton multiplet at δ 7.72, assigned to H-3′ and H-7′, and a three-proton multiplet at δ 7.59, assigned to H-4′, H-5′, and H-6′. Two one-proton doublets at δ 4.34 (J = 7.05 Hz) and 4.31 (J = 7.9 Hz) were attributed to oxygenated C-1 methylene protons. A one-proton broad multiplet at δ 3.74 with a half-width of 9.8 Hz was attributed to α-oriented carbinol H-10. Three doublets at δ 0.90 (J = 4.2 Hz), 0.88 (J = 7.8 Hz), and 0.84 (J = 6.5 Hz), each integrated for three protons, were associated with C-17, C-18, and C-19 secondary methyl protons. A three-proton triplet at δ 0.79 (J = 6.75 Hz) was due to C-16 primary methyl protons. The remaining methylene and methine protons appeared in the range of δ 1.29–2.23. The presence of all methyl signals between δ 0.79 and 0.90 indicated the existence of all functions being on the saturated carbons.

The ^{13}C NMR spectrum of **4** showed aromatic carbon signals between δ 129.56 and 132.96, an ester carbon at δ 169.02 (C-1), hydroxymethine carbon at δ 76.02 (C-10), methyl carbons at δ 19.60 (C-17), 12.54 (C-18), 14.45 (C-19), and 11.75 (C-16), and an oxygenated methylene carbon at δ 66.80 (C-1). In the ^{1}H–^{13}C HETCOR spectrum, correlations were observed between C-1′ and H-3′, H-7′ and H_2-1; C-2′ and H-3′, H-7′, H-4′, H-5′ and H-6′; and C-10 with H_2-10, H-11 and H_3-19. In the ^{1}H–^{1}H COSY spectrum, ^{1}H–^{1}H correlations were observed between H-3′ and H-4′, H-7′ and H-6′, H_2-1 and H_2-2, and between H-3 and H_2-4 and H_2-5.

TABLE 2. 50% Growth Inhibition (IC_{50}) Values of the New and Known Compounds against P388 Murine Leukemia Cells

Compound	IC_{50} (μg/mL)
Momilactone A	0.85
Momilactone B	0.07
Orizaterpenol	4.2
Orizaterpenoid	60
Orizaterpenyl benzoate	22
Orizanor-diterpenyl benzoate	50
Tricin	15
1-Tetratriacontanol	45
β-Sitosterol	50
β-Sitosterol-*β*-glucuronoside	67
Hentriacontane	>100
β-Sitosterol-*β*-*D*-glucopyranoside	>100

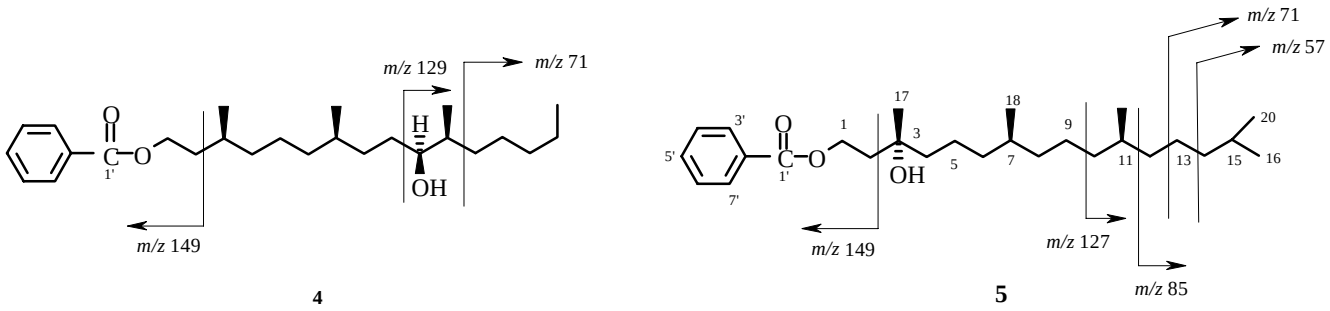

Fig. 2. MS fragmentation of compounds **4**, **5**.

Based on the above evidence, the structure of **4** was elucidated as 3,7,11-trimethyl-*n*-hexadecan-10*β*-ol-1-yl benzoate.

Orizaditerpenyl Benzoate (5). Its molecular formula was determined by HREIMS as $C_{27}H_{46}O_3$ at *m/z* 418.3084 (calc. 418.3083). Its molecular weight was also established at *m/z* 418 based on the EI mass spectral and ^{13}C NMR data. This was consistent with the molecular formula of an acyclic diterpenyl ester of benzoic acid, $C_{27}H_{46}O_3$. The prominent ion fragments at *m/z* 57 [C_{13}–C_{14} fission]$^+$, 71 [C_{12}–C_{13} fission]$^+$, 85 [C_{11}–C_{12} fission]$^+$, 127 [C_9–C_{10} fission]$^+$, and the base peak at *m/z* 149 [C_2–C_3 fission]$^+$ supported the acyclic saturated nature of the diterpene moiety, which possessed a hydroxyl group at quaternary carbon C-3. The positive ion FAB mass spectrum also showed a molecular ion peak at *m/z* 418. The mass fragmentation pattern is shown in Fig. 2. Its IR showed characteristic absorption bands for the hydroxyl (3445 cm^{-1}) and ester (1729 cm^{-1}) groups.

The ^1H NMR spectrum of **5** exhibited a two-proton multiplet at δ 7.72, assigned to aromatic H-3′ and H-7′, and a three-proton multiplet at δ 7.61, assigned to aromatic H-4′, H-5′, and H-6′. Two one-proton double doublets at δ 4.31 (J = 6.9, 4.0 Hz) and 4.27 (J = 6.9, 4.0 Hz) were attributed to oxygenated C-1 methylene protons. A broad three-proton signal at δ 1.29 was associated with C-17 tertiary methyl protons. Four three-proton doublets at δ 0.91 (J = 6.8 Hz), 0.88 (J = 6.8 Hz), 0.86 (J = 6.4 Hz), and 0.80 (J = 6.8 Hz) were attributed to secondary C-18, C-19, C-16, and C-20 methyl protons, respectively. The remaining methine and methylene protons resonated in the range of δ 1.16–1.74. The presence of all methyl proton signals between δ 0.80 and 1.29 indicated the presence of all methyl functions being attached to the saturated carbons. The ^{13}C NMR spectrum of **5** displayed important signals for the ester carbon at δ 169.45, aromatic carbons between δ 130.03 and 133.72, oxygenated methylene carbon at δ 65.55, and for methyl carbons at δ 11.88 (Me-16), 12.66 (Me-17), 14.86 (Me-18), 19.68 (Me-19), and 20.15 (Me-20). In the ^1H–^{13}C HETCOR spectrum, there were correlations between C-1′ and H-3′, H-7, and H_2-1. In the ^1H–^1H COSY spectrum, correlations of H-3′ with H-4′ and H-5′, and H_2-1 with H_2-2 were observed.

Based on spectral data analyses, the structure of 5 was elucidated as 3,7,11,15-tetramethyl-*n*-hexadecan-3*α*-ol-1-yl benzoate.

Diterpenoids and triterpenoids have been reported to show cytotoxic activity against leukemia cells [11, 12]. This suggested that the biological activity of the terpenoids is critically dependent on the olefinic system to show cytotoxic activity. Orizaterpenol (**1**) and momilactone A (**6**) and B (**7**) showed good cytotoxicity on P388 murine leukemia cells, while the other new and known compounds had weak cytotoxic activity. The results of new and known compounds are presented in Table 2.

EXPERIMENTAL

Melting points were determined on an Electrochemical Eng. melting point apparatus and are uncorrected. TLC was carried out on precoated Si gel plates (Merck). Spots were detected under UV light (254 and 366 nm) before and after dipping in a chamber with 1% vanillin sulfuric acid (ethanol solution). Preparative TLC was on precoated Si gel plates with a layer thickness of 0.5 mm (Merck) unless indicated, or else column chromatography was undertaken on Si gel (70–230 mesh, Merck). Optical rotations were measured on an AA-10 model polarimeter. Both ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were measured on a Brucker Avance (DRX-500) using $CDCl_3$ as the solvent. EI mass spectra were recorded on a JEOL JMS-SX 102 A spectrometer and FAB MS on a JEOL JMS-AX 505 WA. IR spectra were recorded on a Thermo Mattson 60-AR spectrophotometer. UV spectra were obtained on a UV-VIS spectrophotometer TU-1800_{PC}.

Plant Material. Hulls of *O. sativa* were collected from the Konkuk University experimental farm in Seoul, South Korea, in October 2002. The voucher specimen (No. KKU 96, HOCHOKJINDO) has been deposited in the Herbarium of our Department.

Extraction and Isolation. Dried hulls of *O. sativa* (10 kg) were immersed in MeOH for one week at room temperature and then concentrated in vacuum to give an extract (150 g) that was suspended in H_2O and successively extracted with EtOAc and *n*-BuOH. The EtOAc extract (35 g) was subjected to normal phase column chromatography over Si gel to yield 40 fractions with the following eluents: 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 29–34 in EtOAc–MeOH (9:1 and 7:3), and fractions 35–40 in MeOH. With further CC and TLC over Si gel with hexane–EtOAc, fractions 1 and 5 yielded two pure compounds: hentriacontane (60 mg) and 1-tetratriacontanol (50 mg). Fraction 6 crystallized, and after purification through column chromatography yielded *β*-sitosterol (500 mg), which was confirmed with an authentic sample from Sigma. Fraction 9 was further purified by CC over Si gel with methylene chloride and methanol to yield compound **2** (55 mg). Fraction 11 was further purified by CC over Si gel with methylene dichloride and methanol to form two pure compounds, momilactone A (6, 80 mg) and momilactone B (7, 70 mg). Fraction 12, after CC over Si gel using dichloromethane and methanol as eluents, yielded a yellow compound in powder form, which was identified as tricin (10 mg), and *β*-sitosterol-*β*-*D*-glucuronoside (50 mg). After mixing other impure fractions and rechromatographing over Lichroprep RP-18 (ODS silica gel) using sequential mixtures of MeOH and H_2O as eluents (elution order 80%, 60%, 40%, 20%, 10% aqueous methanol, 100% methanol), four compounds were obtained in minor quantities **1** (10 mg), **3** (10 mg), **4** (8 mg), **5** (10 mg), and 3,7-dimethyl-*n*-octan-1-yl benzoate (15 mg). Fraction 23, after CC over Si gel with chloroform and methanol, yielded one pure compound as *β*-sitosterol-3-O-*β*-*D*-glucopyranoside (50 mg).

Phylloclad-15-ene-6*β*,14*β*-diol (1). R_f 0.32 (Hex–EtOAc; 1:1), mp 161–163°C; $[\alpha]_D$ + 2.3° (MeOH); UV spectrum (MeOH, λ_{max}, nm): 241; IR spectrum (KBr, ν, cm^{-1}): 3356 (OH), 2936, 2850, 1630, 1451, 1360, 1235, 1030, 755.

PMR (500 MHz, MeOD, δ, ppm, J/Hz): 4.95 (1H, br s, H-15), 3.68 (1H, d, J = 10.7, H-14*α*), 3.31 (1H, br m, $w_{1/2}$ = 14.2, H-6*α*), 2.21 (1H, br s, H-13), 2.01 (1H, br s, H-9*α*), 1.91 (2H, br s, H_2-1), 1.78 (1H, br s, H-5), 1.75 (1H, br s, H_2-2a), 1.62 (3H, br s, Me-17), 1.52 (1H, br s, H_2-2b), 1.47 (1H, d, J = 8.25, H_2-3a), 1.37 (1H, d, J = 5.5, H_2-7a), 1.34 (1H, d, J = 10.05, H_2-7b), 1.32 (1H, m, H_2-11a), 1.29 (1H, br s, H_2-3b), 1.24 (1H, br s, H_2-11b), 1.21 (1H, br s, H_2-12a), 1.10 (1H, br s, H_2-12b), 0.98 (9H, br s, Me-18, Me-19, Me-20).

^{13}C NMR (125 MHz, MeOD): δ 41.98 (C-1), 32.36 (C-2), 33.78 (C-3), 44.50 (C-4), 50.05 (C-5), 65.35 (C-6), 41.03 (C-7), 43.81 (C-8), 51.65 (C-9), 40.97 (C-10), 32.40 (C-11), 32.36 (C-12), 48.63 (C-13), 64.94 (C-14), 124.22 (C-15), 139.37(C-16), 27.82 (C-17), 19.13 (C-18), 30.55 (C-19), 22.21 (C-20); FAB MS (positive mode) *m/z* 305 $[M+H]^+$; EI MS *m/z* (rel. int.): 304 $[M]^+$ ($C_{20}H_{32}O_2$) (56.3), 289 (12.9), 286 (24.5), 273 (47.7), 271 (38.9), 255 (100), 240 (7.6), 228 (16.2), 221 (2.7), 175 (11.1), 162 (15.6), 150 (13.9), 144 (19.9), 136 (8.5), 132 (22.9), 129 (9.3), 118 (22.7), 105 (35.8), 97 (5.2), 83 (8.2), 69 (8.4), 55 (11.4); HREIMS *m/z* 304.2398 (calc. 304.2402).

4,8β,10β-trimethyl-9-(13,17-dimethyldodec-18-enyl)decalin-5-en-3-one-23,7α-olide (2). R_f 0.37 (Hex–EtOAc; 7:3); mp 212–214°C, $[\alpha]_D$ + 24.0° ($CHCl_3$); UV spectrum ($CHCl_3$, λ_{max}, nm): 267; IR spectrum (KBr, ν, cm^{-1}): 2948, 2855, 1725, 1698, 1620, 1462, 1405, 1219, 960, 810, 757 cm^{-1}.

PMR (500MHz, $CDCl_3$, δ, ppm, J/Hz) and ^{13}C NMR (125 MHz, $CDCl_3$), see Table 1; FAB MS (negative mode) *m/z* 427 $[M–H]^-$; FAB MS (positive mode) *m/z* 429 $[M+H]^+$; EI MS *m/z* (rel. int.) 428 $[M]^+$ ($C_{28}H_{44}O_3$ (100), 413 (31.7), 398 (10.5), 383 (9.1), 365 (7.8), 345 (4.3), 335 (7.1), 329 (6.8), 315 (10.7), 313 (11.2), 287 (23.8), 269 (18.3), 259 (7.5), 245 (37.3), 233 (2.8), 192 (5.8), 167 (10.4), 151 (24.1), 149 (35.7), 139 (8.4), 136 (16.9), 123 (14.8), 111(9.0), 99 (14.1), 97 (16.8), 83 (18.3), 71 (11.9), 69 (18.1), 56 (17.3), 55 (28.3), 43 (100).

3,7-Dimethyl-*n*-octan-3α-ol-1-yl-benzoate (3). Oil; R_f 0.38 (Hex–EtOAc; 1:1); UV spectrum ($CHCl_3$, λ_{max}, nm): 270; $[\alpha]_D$ + 1.6° ($CHCl_3$); IR spectrum (KBr, ν, cm^{-1}): 3445, 2929, 2840, 1728, 1550, 1462, 1381, 1279, 1123, 1071.

PMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.70 (2H, m, H-3′, H-7′), 7.52 (3H, m, H-4′, H-5′, H-6′), 4.23 (1H, dd, J = 5.7, 5.7, H-1a), 4.21 (1H, dd, J = 6.2, 6.2, H-1b), 1.68 (1H, m, H-7), 1.44 (1H, m, H_2-2a), 1.41 (1H, m, H_2-2b), 1.38 (2H, m, H_2-4), 1.35 (3H, br s, Me-9), 1.32 (2H, m, H_2-5), 1.31 (2H, m, H_2-6), 0.92 (3H, d, J = 7.5, Me-8), 0.88 (3H, d, J = 6.8, Me-10).

^{13}C NMR (125 MHz, $CDCl_3$): δ 68.30 (C-1), 30.54 (C-2), 67.21 (C-3), 29.85 (C-4), 23.93 (C-5), 23.14 (C-6), 38.92 (C-7), 11.11 (C-8), 29.09 (C-9), 14.19 (C-10), 167.89 (C-1′), 132.64 (C-2′), 131.02 (C-3′), 128.96 (C-4′), 131.02 (C-5′), 128.02 (C-6′), 131.02 (C-7′); FAB MS (negative mode) *m/z* 277 $[M–H]^-$; FAB MS (positive mode) *m/z* 278 $[M+H]^+$; EI MS *m/z* (rel. int.) 278 $[M]^+$ ($C_{17}H_{26}O_3$) (10.6), 211 (12.0), 167 (22.0), 149 (57.3), 85 (14.2), 71 (27.1), 57 (32.2).

3,7,11-Trimethyl-*n*-hexadecan-10β-ol-1-yl-benzoate (4). R_f 0.43 ($CHCl_3$–MeOH; 9:1), mp 142–143°C; UV spectrum (MeOH, λ_{max}): 227; $[\alpha]_D$ + 4.6° (MeOH); IR spectrum (KBr, ν, cm^{-1}): 3456 (OH), 3320, 2960, 2850, 1730, 1570, 1461, 1360, 1281, 1126, 1073, 725.

PMR (500 MHz, MeOD, δ, ppm, J/Hz): 7.72 (2H, m, H-3′, H-7′), 7.59 (3H, m, H-4′, H-5′, H-6′), 4.34 (1H, d, J = 7.0, H_2-1a), 4.31 (1H, d, J = 7.9, H_2-1b), 4.10 (1H, br m, $w_{1/2}$ = 9.8, H-10a), 2.23 (1H, m, H-11), 1.78 (2H, m, H_2-2), 1.73 (2H, m, H_2-4), 1.70 (1H, m, H-3), 1.42 (1H, m, H-7), 1.39 (2H, m, H_2-5), 1.29 (14H, br s, 7 × CH_2), 0.90 (3H, d, J = 4.2, Me-17), 0.88 (3H, d, J = 7.8, Me-18), 0.84 (3H, d, J = 6.5, Me-19), 0.79 (3H, t, J = 6.75, Me-16).

^{13}C NMR (MeOD): δ 66.80 (C-1), 30.41 (C-2), 32.64 (C-3), 30.37 (C-4), 29.98 (C-5), 27.47 (C-6), 37.31 (C-7), 26.82 (C-8), 26.61 (C-9), 76.02 (C-10), 52.82 (C-11), 27.42 (C-12), 23.66 (C-13), 20.00 (C-14), 23.35 (C-15), 11.75 (C-16), 19.60 (C-17), 12.54 (C-18), 14.45 (C-19), 169.02 (C-1′), 132.96 (C-2′), 129.56 (C-3′), 131.93 (C-4′), 131.93 (C-5′), 131.93 (C-6′), 129.56 (C-7′); EI MS *m/z* (rel. int.): 404 $[M]^+$ ($C_{26}H_{44}O_3$) (11.2), 389 (11.3), 375 (46.1), 360 (16.2), 346 (16.3), 329 (13.5), 301 (10.2), 297 (14.1), 273 (7.9), 259 (6.1), 245 (15.3), 231(13.1), 187 (31.8), 171 (21.7), 149 (97.4), 129 (100), 97 (68.3), 85 (66.2), 73 (60.1).

3,7,11,15-Tetramethyl-*n*-hexadecan-3α-ol-1-yl-benzoate (5). R_f 0.61 ($CHCl_3$–MeOH; 9:1), viscous yellow solid; UV spectrum (λ_{max}, MeOH) 237 nm; $[\alpha]_D$ + 8.6° (MeOH); IR spectrum (KBr, ν, cm^{-1}): 3445, 2959, 2855, 1729, 1580, 1461, 1360, 1280, 1125, 1030, 742.

PMR (500 MHz, MeOD, δ, ppm, J/Hz): 7.72 (2H, m, H-3′, H-7′), 7.61 (3H, m, H-4′, H-5′, H-6′), 4.31 (1H, dd, J = 6.9, 4.0, H_2-1a), 4.27 (1H, dd, J = 6.9, 4.0, H_2-1b), 1.74 (2H, m, H_2-2), 1.71 (2H, m, H_2-4), 1.70 (1H, m, H-7), 1.44 (2H, m, H_2-5), 1.41 (2H, m, H_2-6), 1.39 (1H, m, H-11), 1.36 (1H, m, H-15), 1.36 (4H, m, H_2-8, H_2-9), 1.32 (4H, m, H_2-10, H_2-12), 1.29 (3H, m, Me-17), 1.16 (2H, m, H_2-14), 0.99 (2H, m, H_2-13), 0.91 (3H, d, J = 6.8, Me-18), 0.88 (3H, d, J = 6.8, Me-19), 0.86 (3H, d, J = 6.4, Me-16), 0.80 (3H, d, J = 6.8, Me-20).

^{13}C NMR (125 MHz, MeOD): δ 65.55 (C-1), 35.79 (C-2), 67.43 (C-3), 34.44 (C-4), 33.17 (C-5), 32.42 (C-6), 40.87 (C-7), 30.76 (C-8), 30.56 (C-9), 29.81 (C-10), 39.92 (C-11), 27.95 (C-12), 27.25 (C-13), 24.18 (C-14), 37.49 (C-15), 19.68 (C-16), 12.66 (C-17), 14.86 (C-18), 11.88 (C-19), 20.15 (C-20), 169.45 (C-1′), 133.72 (C-2′), 132.49 (C-3′), 130.03 (C-4′), 132.17 (C-5′), 130.03 (C-6′), 132.49 (C-7′); FAB MS (positive mode) *m/z* 419 $[M+H]^+$; EI MS *m/z* (rel.int.): 418 $[M]^+$ ($C_{27}H_{46}O_3$) (4.5), 293 (72.5), 275 (8.2), 167 (22.5), 149 (100), 127 (31.5), 99 (4.9), 85 (16.3), 71 (26.7), 57 (20.2); HREIMS *m/z* 418.3084 (calc. 418.3083).

Bioassays for Cytotoxic Activity. The cytotoxic assays were performed using the MTT assay method. The murine P388 leukemia cells were cultured in RPMI 1640 medium (Nissui) supplemented with 5% heat-inactivated fetal bovine serum (FBS) and kanamycin (5.3 mL/L) in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. The cell suspension (100 μL) was added to each well (3×10^3 cells/well) of a 96-microwell plate (Iwaki, flat bottom, treated polystyrene) and incubated for 24 h. Test compounds were dissolved in DMSO in various concentrations (100, 30, 10, 3, 1, 0.3 and 0.1 μg/mL) and 10 μL of the

test solution or DMSO (control) was added to each well. The plate was kept in an incubator for 48 h. After terminating cell culture by adding 20 μL MTT (5% in PBS) to each well, the plate was further incubated for 4 h. To each well, 100 μL of 10% SDS–0.01N HCl was added. The plate was read on a microplate reader (MPR A4i, Tosoh) at 550 nm. A dose response curve was plotted for each compound and the concentrations giving 50% inhibition of cell growth (IC_{50}) were recorded.

Statistical Analysis. All experiments were repeated three times with four replications in a completely randomized design. Analysis of variance for all data was accomplished using the general linear model (GLM) procedure of the statistical analysis system program (SAS Institute, 1996). The pooled mean values were separated based on the least significant difference (LSD) at the 0.05 probability level.

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