

## Dicyclohexanyl Orizane Constituent from the Hulls of *Oryza sativa* and its Inhibitory Activity

ILL-MIN CHUNG, MOHD ALI† and ATEEQUE AHMAD\*

Department of Applied Life Science, Konkuk University, Seoul-143-701, South Korea

Fax: (82)(2)4467856; Tel.: (82)(2)4503730; E-mail: aahmadc@yahoo.com

From the hulls of *Oryza sativa* was isolated one new compound dicyclohexanyl orizane (1) along with several known compounds hentriacontane, 1-tetratriacontanol,  $\beta$ -sitosterol, momilactones A, B, tricin,  $\beta$ -sitosterol-3-*O*- $\beta$ -D-glucuronoside, 3,7-dimethyl-*n*-octan-1-yl benzoate and  $\beta$ -sitosterol-3-*O*- $\beta$ -D-glucoside. The structure of the new compound was elucidated by 500 MHz NMR using 1D and 2D spectral methods, viz.,  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^1\text{H}$ - $^1\text{H}$  COSY and HETCOR aided by EIMS, FABMS and IR. The compound 1 was found to have no inhibitory effects against duckweed (*Lemna paucicostata* Hegelm 381).

### 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)<sup>1,2</sup>. The diterpenoids (momilactones A and B) from the rice hulls are reported to possess growth and germination inhibitors against the roots of rice<sup>3-5</sup> and momilactone B was isolated also from rice root exudates<sup>6</sup>. Antioxidative activities of methanol extracts<sup>7</sup> and C-glycosyl flavonoid from the rice hulls<sup>8</sup> have been reported in the 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 rice hulls and isolated one new compound dicyclohexanyl orizane (1), along with known compounds,  $\beta$ -sitosterol, momilactones A, B, tricin, hentriacontane, 1-tetratriacontanol, 3,7-dimethyl-*n*-octan-1-yl benzoate,  $\beta$ -sitosterol-3-*O*- $\beta$ -D-glucuronoside and  $\beta$ -sitosterol-3-*O*- $\beta$ -D-glucoside. The later five compounds have been isolated from this plant for the first time. The paper deals with the isolation and structural elucidation of the new compound (1) based on  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, COSY and HETCOR aided by EIMS, FABMS, IR spectra and inhibitory activity of momilactones and the new compound.

†Faculty of Pharmacy, Hamdard University, New Delhi-110 062, India.

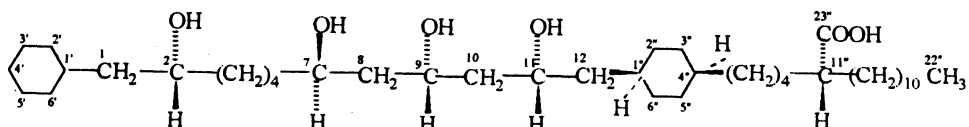


Fig. 1. Chemical structure of 1

## 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 a 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 an AA-10 model polarimeter. Both  $^1\text{H}$  and  $^{13}\text{C}$  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, 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 102A 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  $\text{H}_2\text{O}$  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,  $5.5 \times 90$  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 (7 : 3), frs. 37–40 in MeOH. Fr. 1 (500 mg) with further CC and TLC over silica gel with *n*-hexane/EtOAc to yield 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  $\text{CH}_2\text{Cl}_2$ ,  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  (99.8 : 0.2, 99.6 : 0.4, 99.4 : 0.6, 99.2 : 0.8, 99 : 1) as eluants to yield six frs., 1-tetratriacontanol (50 mg) from initial fr. 1. The fraction 6 (2.8 g) was crystallized and after purification through column chromatography with hexane/EtOAc obtained  $\beta$ -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  $\text{CH}_2\text{Cl}_2/\text{MeOH}$

(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 by using CH<sub>2</sub>Cl<sub>2</sub>/MeOH (99 : 1, 98 : 2, 97 : 3, 96 : 4, 95 : 5) as eluants to yield five frs. Two compounds were obtained from fr. 1, identified as triclin (10 mg) and  $\beta$ -sitosterol-3-*O*- $\beta$ -D-glucuronoside (50 mg) from fr. 3. The other impure frs. 2, 4 and 5 after mixing were rechromatographed over Lichroprep RP-18 (ODS silica gel) using sequential mixtures of H<sub>2</sub>O/MeOH as eluants (elution order 80%, 60%, 40%, 20%, 10% aqueous methanol, 100% methanol) to yield six frs. and obtained known 3,7-dimethyl-*n*-octan-1-yl benzoate (15 mg). The fraction 23 was subjected to silica gel with column chromatography eluting with CHCl<sub>3</sub>/MeOH to yield one new compound dicyclohexanyl orizane (35 mg, 1) and known  $\beta$ -sitosterol-3-*O*- $\beta$ -D-glucopyranoside (50 mg).

**Bioassay Phytotoxicity Determination (Lemna assay):** Test compound was dissolved in acetone including non-ionic surfactant Tween-20 and was mixed with 1/2 hunter medium. The final concentrations of acetone and Tween-20 were 1% and 0.01% respectively.

The technique to analyze the bioactivity with the identified compounds was based on the method of test solution<sup>9</sup> (2 mL) and one duckweed (*Lemna paucicostata* Hegelm 381) frond was added to each well with 24 wells in a plate. The plate was incubated in the growth chamber (26°C, 14 h photoperiod, 50  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) for 5 days. Herbicidal activity was then rated by visual scoring and by measuring chlorophyll content. The chlorophyll was extracted with dimethyl sulfoxide (DMSO) and analyzed using a UV-Vis spectrophotometric method<sup>10</sup>. Visual injury symptoms were recorded on day 5 on an injury symptoms scale of 0 to 100 (0 = no effect, 100 = complete death). Other measurements included: contents of chlorophyll ( $\mu$ g mL<sup>-1</sup>) = 2.367  $\times$  (absorbance)<sup>2</sup>  $\times$  6.299  $\times$  absorbance + 0.169, ( $r^2 = 0.999$ ); Inhibition of chlorophyll contents (%) = [(control - identified compound)/control]  $\times$  100.

**Statistical Analysis:** Analysis of variance for all data was undertaken using the general linear model (GLM) procedure of the SAS program<sup>11</sup>. All of the aforementioned experiments were replicated three times using a completely randomized design. The pooled mean values were separated based on least significant difference (LSD) at the 0.05 probability level.

**1-Cyclohexyl-12-(4''-*n*-hexadecan-11'' $\alpha$ -oic acid cyclohexyl)-*n*-dodecan-2 $\beta$ , 7 $\alpha$ ,9 $\beta$ ,11 $\beta$ -tetraol (1):**  $R_f$  0.42 (CHCl<sub>3</sub>) : MeOH, 9 : 1) m.p. 138–140°;  $[\alpha]_D^{22} + 14.2^\circ$  (pyridine); IR (KBr)  $\nu_{max}$ : 3339, 3150, 2919, 2855, 1680, 1620, 1544, 1466, 1130, 1069, 1005, 740, 724 cm<sup>-1</sup>; <sup>1</sup>H NMR (C<sub>5</sub>D<sub>5</sub>N):  $\delta$  10.12 (1H, br s, COOH-23''), 4.03 (1H, m,  $W_{1/2} = 3.75$  Hz, H-2 $\beta$ ), 3.76 (1H, br m,  $W_{1/2} = 11.17$  Hz; H-7 $\alpha$ ), 3.54 (1H, m,  $W_{1/2} = 7.02$  Hz, H-9 $\beta$ ), 3.34 (1H, m,  $W_{1/2} = 1.5$  Hz, H-11 $\beta$ ), 1.80 (1H, m,  $W_{1/2} = 5.9$  Hz, H-11'' $\beta$ ), 1.78 (1H, br m,  $W_{1/2} = 7.37$  Hz, H-1 $\beta$ ), 1.64 (1H, br m,  $W_{1/2} = 10.47$  Hz, H-1'' $\alpha$ ), 1.58 (1H, br m,  $W_{1/2} = 9.52$  Hz, H-4'' $\beta$ ), 1.42 (2H, m, H<sub>2</sub>-1), 1.27 (60H, br s, 30  $\times$  CH<sub>2</sub>), 0.89 (3H, t,  $J = 6.7$  Hz, Me-22''); <sup>13</sup>C-NMR (C<sub>5</sub>D<sub>5</sub>N):  $\delta$  175.75 (C-23''), 77.25 (C-2), 73.49 (C-7), 72.95 (C-9), 62.51 (C-11), 53.40 (C-11''), 36.19 (C-1''), 34.62 (C-4''), 32.60 (C-1'), 30.81 (C-8), 30.66 (C-10), 30.48 (23  $\times$  CH<sub>2</sub>), 30.39 (C-1), 30.10 (C-3), 30.08 (C-6), 27.30 (C-4), 26.30 (C-5), 23.41 (CH<sub>2</sub>), 14.75 (C-22'');

FABMS (negative mode)  $m/z$  665  $[M - H]^-$ , FABMS (positive mode)  $m/z$  667  $[M + H]^+$ ; EIMS  $m/z$ : (rel. int.), 666  $[M]^+$ , ( $C_{41}H_{78}O_6$ ) (28.5), 649 (12.1), 635 (7.4), 453 (10.7), 397 (27.3), 357 (100), 301 (11.4), 257 (10.4), 227 (16.3), 155 (9.2), 127 (9.0), 97 (43.7), 83 (35.5).

## RESULTS AND DISCUSSION

Compound (1) was obtained as a colourless amorphous powder from the EtOAc : MeOH (9.5 : 0.5) fraction named as dicyclohexanyl orizane. It gave effervescences with sodium bicarbonate solution. Its IR spectrum showed characteristic absorption bands for the hydroxyl group ( $3339\text{ cm}^{-1}$ ), the carboxylic group ( $3150, 1680\text{ cm}^{-1}$ ), and long aliphatic chain ( $740, 724\text{ cm}^{-1}$ ). The positive ion FAB mass spectrum of 1 displayed a molecular ion peak at  $m/z$  666 corresponding to an alkane tetraol,  $C_{41}H_{78}O_6$ . It indicated three double bond equivalents, which were adjusted in two cyclohexane rings and one carboxylic group. The prominent ion peaks appearing in EIMS at  $m/z$  83  $[C_1-C_1\text{ fission, }C_6H_{11}]^+$ , 97  $[C_1-C_2\text{ fission, }C_6H_{11}-CH_2]^+$ , 127  $[C_3-C_4\text{ fission, }C_6H_{11}-CH_2CHOH]^+$ , 227  $[C_8-C_9\text{ fission}]^+$ , 257  $[C_9-C_{10}\text{ fission}]^+$ , 301  $[C_{11}-C_{12}\text{ fission}]^+$ , 397  $[C_4-C_6]^+$ , 155  $[C_{11}'-C_{12}''\text{ fission}]^+$ , 453  $[M-C_{15}H_{31}]^+$ , and 357  $[453 - 97]^+$  suggested the location of one of the cyclohexanyl ring at one of the terminal carbon, a carboxylic group at C-11'', hydroxyl groups at C-2, C-7, C-9 and C-11 and another cyclohexanyl ring at C-12. The ion peak at  $m/z$  453 arose due to expulsion of the  $CH(COOH)-(CH_2)_{10}CH_3$  moiety from the molecular ion which was attached to C-8''. The fragmentation pattern of dicyclohexanyl orizane (1) are shown in Fig. 2.

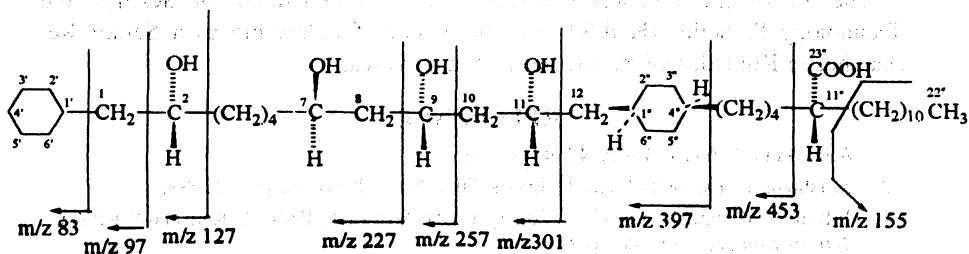


Fig. 2. Fragmentation pattern of 1

The  $^1H$ -NMR of 1 exhibited four one-proton multiplets at  $\delta$  4.03 ( $W_{1/2} = 3.75\text{ Hz}$ ), 3.76 ( $W_{1/2} = 11.17\text{ Hz}$ ), 3.54 ( $W_{1/2} = 7.0\text{ Hz}$ ) and 3.34 ( $W_{1/2} = 1.5\text{ Hz}$ ), which were assigned correspondingly to hydroxymethine H-2 $\beta$ , H-7 $\alpha$ , H-9 $\beta$  and H-11 $\beta$ , respectively. Three one-proton multiplets at  $\delta$  1.80 ( $W_{1/2} = 5.9\text{ Hz}$ ), 1.78 ( $W_{1/2} = 7.37\text{ Hz}$ ), 1.64 ( $W_{1/2} = 10.47\text{ Hz}$ ) and 1.58 ( $W_{1/2} = 9.52\text{ Hz}$ ) were ascribed to the methine protons H-9'' $\beta$ , H-1' $\beta$ , H-1' $\alpha$  and H-4'' $\beta$ . A three-proton triplet at  $\delta$  0.89 ( $J = 6.7\text{ Hz}$ ) was accounted to C-20'' primary methyl protons. The remaining methylene protons resonated as a two-proton multiplet at  $\delta$  1.42 and a 60-proton broad signal at  $\delta$  1.27. The  $^{13}C$ -NMR spectrum of 1 exhibited carbinol carbons at  $\delta$  77.25 (C-2), 73.49 (C-7), 72.95 (C-9) and 62.51 (C-11). The C-22'' methyl carbon appeared at  $\delta$  14.75. The

remaining methine and methylene carbons resonated between  $\delta$  53.46–23.41. A deshielded carbon signal at  $\delta$  175.75 was attributed to C-23'' carboxylic carbon. The  $^1\text{H}$ - $^{13}\text{C}$  HETCOR spectrum showed a correlation of C-7 with H<sub>2</sub>-8, H<sub>2</sub>-6, H-9, and H-11. The  $^1\text{H}$ - $^1\text{H}$  COSY spectrum displayed a correlation of H-9 with H-7 and H-11. Based on this evidence, the structure of **1** was established as 1-cyclohexyl-12-(4''-*n*-hexadecan-11'' $\alpha$ -oic acid-cyclohexyl)-*n*-dodecan-2 $\beta$ ,7 $\alpha$ ,9 $\beta$ ,11 $\beta$ -tetraol.

**Inhibitory Effects of Compounds on Tested Plants:** Momilactone A and B had high inhibitory activity against duckweed. Momilactone B was more active than momilactone A whereas the other compounds had lower or no inhibitory activity. Momilactone A and B showed reduction of chlorophyll content (85.8, 52.3 and 27.0%, and 98.3, 91.9 and 33.9% respectively) at the concentrations of 10, 3.3 and 1 ppm. At concentrations of 100 and 33 ppm, momilactone A reduced chlorophyll content by 98.9 and 95.6% respectively.

Visual injury due to momilactone A and B was 70, 50 and 20% and 98, 70 and 20% respectively at concentrations of 10, 3.3 and 1 ppm. The momilactone A showed visual injury of 98.9 and 95.6% at concentrations of 100 and 33 ppm respectively (Table-1). This result is similar to that of reference 4, which showed that momilactone A and B and their derivatives inhibit the germination of lettuce seeds.

The new compound dicyclohexanyl orizane (**1**) did not show any inhibition of chlorophyll nor any visual injury symptoms at concentrations of 10 and 100 ppm.

#### ACKNOWLEDGEMENT

The author (A. Ahmad) thankful to Korean Federation of Science and Technology Societies (KOFST) for the award of fellowship as a Senior Researcher in Konkuk University, Seoul, South Korea.

#### REFERENCES

1. A.K. Dutta, *Indian J. Agric.*, **42**, 984 (1973).
2. K. Ishizumi, Textbook of Rice Cultivars, Fului Nojyo Kankobu, p. 52 (1989).
3. T. Kato, C. Kabuto, N. Sasaki, M. Tsunagawa, H. Aizawa, K. Fujita, Y. Kato and Y. Kitahara, *Tetrahedron Lett.*, **39**, 3861 (1973).
4. T. Kato, M. Tsunakawa, N. Sasaki, H. Aizawa, K. Fujita, Y. Kitahara and N. Takahashi, *Phytochemistry*, **16**, 45 (1977).
5. N. Takahashi, T. Kato, M. Tsunagawa, N. Sasaki and Y. Kitahara, *Jap. J. Plant Breeding*, **26**, 91 (1976).
6. H. Kato-Naguchi, T. Ino, N. Sata and S. Yamamura, *Physiologia Plantarum*, **115**, 401 (2002).
7. N. Ramarathnam, T. Osawa, M. Namiki and S. Kawakishii, *J. Agric. Food Chem.*, **36**, 732 (1988).
8. ———, *J. Agric. Food Chem.*, **37**, 316 (1989).
9. K.S. Hong, B.H. Lee, K.H. Lee, I.T. Hwang, J.S. Kim and K.Y. Cho, *Korean J. Weed Sci.*, **20**, 225 (2000).
10. J.D. Hiscox and G.F. Israelstam, *Can. J. Bot.*, **57**, 1332 (1979).
11. SAS Institute. 2000 User's Guide: Basics. 5th Ed. SAS Institute Cary, North Carolina, USA.