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New Sesterterpene Diolyl Butanoate from Rice Straw of *Oryza sativa*

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One new compound 2,6,10,14-tetramethyl-18-butanecarboxymethylene-henecos-12-en-17β-ol (**1**) has been isolated for the first time from the ethyl acetate extract of *Oryza sativa* of rice straw. The structure of new compound was elucidated with the help of 600 MHz NMR using 1D and 2D spectral methods *viz*: ^{1}H and ^{13}C aided by ESI/MS and HR-ESI/FTMS and IR spectroscopy.

Keywords: *Oryza sativa* L, Gramnieae, Rice straw, Chemical constituent, Sesterterpene diolyl butanoate.

INTRODUCTION

Rice (*Oryza sativa* L.) is the major staple food in Asia and generally exists in two types, white hulled and coloured hulled. The most common type (85 %) is white hulled rice. The germination of rice is of great agricultural importance and has long been known to be influenced by compounds present in the seed coat (hull)[1,2]. The compounds momilactone A and B from rice hulls cause germination and growth inhibition in the roots of rice[3-5]. They were later found in rice leaves and rice straw as phytoalexins[6,7].

Rice straw has been applied back in larger amounts into paddy and also upland fields, especially green house croppings, as an organic material mainly for soil improvement. The degradation products of rice straw in the soils may influence the growth of crops in both nutritional and physiological aspects. The elucidation of mechanism of humus formation from rice straw is also of importance for understanding its influence on plant growth[8]. Phenolic substances are widely distributed in various plants, including the different parts of rice plant. Some of the substances, which enter into soils from plants, cause dieback disease or other abnormal growth as inhibitors against plant growth. It was reported that *p*-coumaric acid and other phenolic acids, for instance, inhibited the growth of the upland rice plant. Kuwatsuka and Oshima[9] isolated and or identified *p*-hydroxybenzoic acid, vanillic, *p*-coumaric acid and ferulic acid from rice leaves. Inamatsu[10] also found *p*-coumaric acid in a methanol extracts of rice straw and recognized that the amount of the acid decreased during the heaping of rice straw.

Phenolic compounds were reported from rice straw on the basis of HPLC or GC analysis[8,11]. Identification of allelopathic compounds including momilactones A and B from rice straw and their biological activity have been reported[11-17]. In continuation of our previous work[18-20], we have isolated one more new compound 2,6,10,14-tetramethyl-18-butanecarboxymethylene-henecos-12-en-17β-ol (**1**) from ethyl acetate extract of rice straw of *Oryza sativa*. This paper deals with isolation and structure elucidation of one new constituent (**1**), on the basis of ^{1}H and ^{13}C spectroscopic studies, including 2D-NMR, COSY, HSQC HMBC, ESI/MS, HR-ESI/FTMS, IR and chemical reaction. This is the first report of the isolation of new constituent (**1**) in the form of natural products.

EXPERIMENTAL

Melting points were determined using Electrochemical Engineering (Electrothermal, Seoul, South Korea) model IA9100 melting point apparatus. Optical rotation was measured with an Instrument Ltd. (Seoul, Korea) model AA-10 polarimeter. UV spectra were measured with a TU-1800PC UV-visible spectrophotometer. IR spectra were recorded on a Thermo Scientific FT-IR model Nicolet 6700 (USA) spectrophotometer at the Korea Institute of Science and Technology (KIST) Seoul, South Korea. ^{1}H- and ^{13}C NMR spectra were obtained at 600 and 150 MHz, respectively, using a Bruker Avance-600 spectrometer available at National Instrumentation Centre for Environmental Management (NICEM), College of Agriculture and Life Science, Seoul National University (SNU), Seoul, South Korea. NMR spectra were obtained in deuterated chloroform using tetramethylsilane (TMS) as an internal standard, with chemical shifts expressed in ppm (δ) and coupling constants (*J*) in Hz. High resolution ESI/FT mass spectra were recorded on a Thermo-Finnigan LTQ-Orbitrap instrument (Thermo Scientific, USA) equipped with Dionex

U 3000 HPLC system (NICEM, Seoul National University). All chemicals were of an analytical grade. *n*-Hexane, ethyl acetate, methanol, ethanol, sulphuric acid and vanillin were purchased from Daejung Chemicals and Metals (Seoul, South Korea). Thin layer chromatography was performed on precoated silica gel 60 F254 plates (Merck). Visualization of the TLC plates was performed using a 5 % H_2SO_4 in C_2H_5OH spray reagent. Column chromatography was performed using silica gel (70-230 mesh) and LiChroprep RP-18 (40-63 µm; ODS silica gel) from Merck. Authentic standards of known compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA).

The rice straw of *O. sativa* was collected from the Konkuk University experimental farm, Seoul, Korea in October 2010. After harvesting, the samples were dried in the laboratoty temperature range (25-30 °C) for 3 weeks. The voucher specimen (reference code ILPUM variety) has been dried and deposited in the herbarium of the Department of Applied Life Science, Konkuk University, Korea.

Extraction of rice straw: Dried straws of *O. sativa* (10 kg) were immersed in methanol (55 L) for one week at room temperature and then the supernatant was concentrated under vacuum to yield 78 g extract. This material was suspended in water and extracted with hexane, ethyl acetate and *n*-butanol successively to produce 9.4 g of hexane, ethyl acetate 11.2 g and 14.2 g *n*-butanol extract.

Isolation of compounds from ethyl acetate extract: The EtOAc extract (11.2 g) was subjected to normal-phase column chromatography over silica gel and yielded 30 fractions with the following eluants: 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 23-26 in EtOAc:MeOH (9.5:0.5), fractions 27-30 in EtOAc:MeOH (9:1). Fraction 6 was crystallized and, after purification by column chromatography, yielded β-sitosterol (23 mg). This was confirmed by comparison with an authentic sample from Sigma and previously isolated compound. Fraction 11, which was further purified by column chromatography over silica gel with methylene dichloride and methanol, produced one pure new compound (1, 26 mg). Fraction 23-26, after column chromatography over silica gel and Lichroprep RP-18 (octadecyl silica [ODS]), yielded one white compound in a powder form, this was identified as gallic acid. Fraction 27, after re-chromatography over silica gel column with chloroform and methanol, yielded one pure compound also in powder form as β-sitosterol-3-O-β-D-glucoside (12 mg).

2,6,10,14-Tetramethyl-18-butanecarboxymethylene-henecos-12-en-17β-ol. Yellow sold; m.p. 141-43 °C; R_f 0.34 ($CHCl_3$: MeOH; 9.5 : 0.2); $[\alpha]_D^{22}$: -23.7 (MeOH, c 0.2); IR (KBr, ν_{max}, cm^{-1}): 3419, 2964, 2860, 2361, 1722, 1601, 1408, 1378, 1280, 1120, 1060, 960, 741; ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) (Table-1); ESI MS *m/z* (rel. int.): 453 $[M + H]^+$ ($C_{29}H_{56}O_3$) (5.8), 309 (36.3), 279 (7.8), 223 (22.5), 197 (3.8), 113 (4.1); ESI/FTMS *m/z* 453.4314 (Calcd. for $C_{29}H_{56}O_3$; 453.4310).

Acetylation of compound 1: Compound **1** (5 mg) was refluxed with acetic anhydride (1.5 mL) and pyridine (0.5 mL) over a water bath at 80 °C for 4 h. After usual work up, compound **1** yielded as acetate derivative (**1a**, 3 mg, Fig. 1) solid. ^{1}H NMR spectrum gave an additional singlet at δ 2.07.

TABLE-1
^{1}H AND ^{13}C NMR SPECTRAL DATA OF COMPOUND **1**

Position	^{1}H NMR	^{13}C NMR
1	0.79 d (7.2)	13.99
2	1.48 m	37.98
3	1.31 m, 1.24 m	29.63
4	1.26 m, 1.18 br s	29.45
5	1.22 m, 1.28 m	29.30
6	1.33 m	31.86
7	1.23 m, 1.18 br s	29.09
8	1.18 br s, 1.23 m	29.03
9	1.18 br s, 1.23 m	28.86
10	1.65 m	30.31
11	1.96 m, 1.68 m	27.14
12	7.63 ddd (5.4, 3.6, 5.4)	128.78
13	7.45 dd (5.3, 3.6)	130.83
14	2.71 m	33.76
15	1.25 m,	1.28 m
16	1.60 m, 1.31 m	38.66
17	4.15 ddd (6.6, 6.0, 11.2)	68.09
18	1.63 m	30.50
19	1.23 m, 1.21 m	22.63
20	0.85 t (7.2)	10.90
21	0.81 d (7.8)	13.89
22	0.91 d (7.2)	13.67
23	0.94 d (7.2)	18.58
24	0.80 d (6.1)	19.12
25	4.23 d (6.6)	65.50
1'	-	167.70
2'	2.27 t (7.2)	39.30
3'	1.25 m, 1.18 br s	22.51
4'	0.88 t (7.1)	14.06

Coupling constants (*J*) in hertz (Hz) are provided in parenthesis

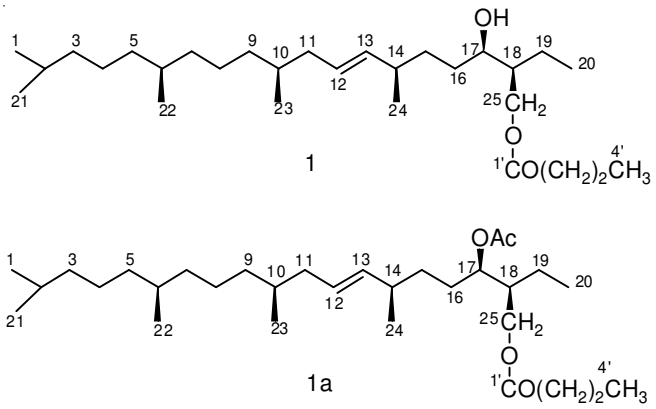

Fig. 1. Chemical structures of compound **1**, acetylated (**1a**)

RESULTS AND DISCUSSION

Compound (**1**) was obtained as yellow solid. It showed IR absorption bands for hydroxyl group (3419 cm^{-1}), ester group (1722 cm^{-1}) and unsaturation (1601 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, the molecular ion peak of **1** was determined at *m/z* 452 consistent to the molecular formula of a sesterterpenyl ester $C_{29}H_{56}O_3$. The ion fragments arising at *m/z* 113 [C_6-C_7 fission]$^+$, 197 [C_{11}-C_{12} fission]$^+$, 223 [C_{13}-C_{14} fission]$^+$, 279 [C_{16}-C_{17} fission]$^+$ and 309 [C_{17}-C_{18} fission]$^+$ indicated the existence of the vinylic linkage at C_{12} and hydroxyl group at C_{17}. The fragmentation pattern of compound **1** is shown in Fig. 2.

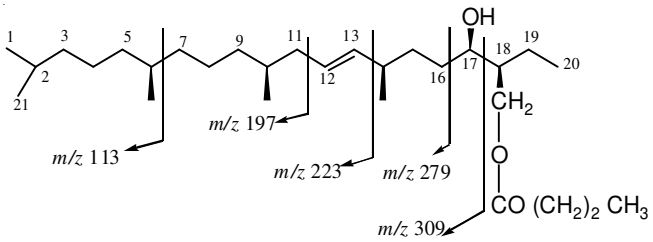

Fig. 2. Fragmentation patterns of compound **1**

^{1}H NMR spectrum of **1** displayed a one-proton triple doublet at δ 7.63 (*J* = 5.4, 3.6, 5.4) and a one-proton double doublet at δ 7.45 (*J* = 5.3, 3.6 Hz) was assigned to *cis*-oriented vinylic H-12 and H-13 protons, respectively. A one-proton triple doublet at δ 4.15 (*J* = 6.6, 6.0, 11.2 Hz) and a two-proton doublet at δ 4.23 (*J* = 6.6 Hz) were ascribed to α-oriented carbinol H-17 and oxygenated methylene H_2-25 protons, respectively. Five three-proton doublets at δ 0.79 (*J* = 7.2 Hz), 0.81 (*J* = 7.8 Hz), 0.91 (*J* =7.2 Hz), 0.94 (*J* = 7.2 Hz) and 0.80 (*J* = 6.1 Hz) and two three-proton triplets at δ 0.85 (*J* = 7.2 Hz) and 0.88 (*J* = 7.1 Hz) were accounted to secondary C-1, C-21, C-22 , C-23 and C-24 and primary C-20 and C-4' methyl protons, respectively, all attached to saturated carbons. The ^{13}C NMR spectrum of **1** exhibited signals for vinylic carbons at δ 128.78 (C-12) and 130.83 (C-13), carbinol carbon at δ 68.09 (C-17), oxygenated methylene carbon at δ 65.50 (C-25) and methyl carbons between δ 19.12-10.90. The ^{1}H-^{1}H COSY spectrum of **1** showed correlations of H-13 with H_2-11, H-12, H-14 and Me-24; H-17 with H_2-16, H-18 and H_2-25; and H_2-2' with H_2-3', Me-4' and H_2-25. The HMBC spectrum of **1** exhibited interactions of C-13 with H_2-11, H-12, H-14 and Me-24; C-17 with H_2-16, H-18 and H_2-25; and C-1' with H_2-25 and H_2-2'. The ^{1}H and ^{13}C NMR spectral data values of carbons were established by HSQC correlation values. On acetylation of compound **1** gave monoacetate derivative (**1a**) and signal of acetate proton at δ 2.01. On the basis of these evidences the structure of **1** was characterized as 2,6,10,14-tetramethyl-18-butanecarboxymethylene-henecos-12-en-17β-ol. This is a new sesterterpeneolyl ester.

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