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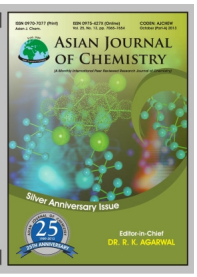

Aliphatic Alcohol, Acid, Ester and Other Constituents from Rice Straw of *Oryza sativa*

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Eight compounds, *n*-octacos-9-enyl propionate (**1**), 1-tetratriacontanol (**2**), β-sitosterol (**3**), *n*-tetracontan-15α-ol (**4**), tritriacontan-4,12-diene (**5**), *n*-tritetracontan-5α-ol (**6**), gallic acid (**7**) and β-sitosterol-3-O-β-D-glucoside (**8**) have been isolated and identified from methanol extract of rice straw of *Oryza sativa*. The structures of these known compounds were elucidated with the help different spectroscopic techniques.

Key Words: *Oryza sativa* L., Gramineae, Rice straw composition.

INTRODUCTION

Rice (*Oryza sativa* L.) is the principal cereal food in Asia and the major staple of the majority of the population. It generally occurs as two types, with white and coloured hulls, although the white hulled variety is more common (85 %). The germination of rice seed is of great agricultural importance and it has long been known to be influenced by compounds present in the seed coat (hull)[1]. The compounds momilactone A and B from rice hulls cause germination and growth inhibition in the roots of rice[2-4]. They were later found in rice leaves and straw as phytoalexins[5,6].

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 nutritrional and physiological aspects. The elucidation of mechanism of humus formation from rice straw is also of importance for understanding its influence on plant growth[7]. Phenolic substances are widely distributed in various plants, including the 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 compounds, for instance, inhibited the growth of the upland rice plant. On the other hand, Kuwatsuka and Oshima[8] isolated and or identified *p*-hydroxybenzoic acid, vanillic, *p*-coumaric acid and ferulic acid from rice leaves. Inamatsu[9] also found *p*-coumaric acid in a methanol extract of rice straw and recognized that the amount of the acid decreased during the heaping of rice straw.

The phenolic compounds were reported from rice straw on the basis of GC analysis[7]. Identification of allelopathic compounds including momilactones A and B from rice straw and their biological activity have been reported[10,11].

Previously reported compounds from rice straw only on the basis of HPLC or GC analysis. Because there are no reports in the literature on the isolated compounds through column chromatography of rice straw. Identification of constituents by spectroscopic analysis like IR, NMR and mass still not reported of rice straw. Identification bioactive constituents with growth or germination inhibitory properties is still required. To achieve these objectives, the aims of our research were to isolated and identified constituents from rice straw. This papers deals with the isolation and structure elucidation of known compounds from rice straw of *Oryza sativa* on the basis of IR, mass and NMR. Identification of other constituents of rice straw of *O. sativa* is in progress.

EXPERIMENTAL

All chemicals used were of analytical grade. Hexane, ethyl acetate, chloroform, methanol, ethanol, water, sulphuric acid and vanillin were purchased from Daejung Chemicals and Metals Co. Ltd, Shiheung (Gyeonggi-do) Korea. Pre-coated TLC plates (layer thickness 0.25 mm), silica gel for column chromatography (70-230 mesh ASTM) and LiChroprep RP-18 (40-63 μm) were from Merck, Darmstadt, Germany. An authentic standard of compounds were purchased from Sigma-Aldrich, St. Louis, Missourri, USA. Both ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance 600 high resolution spectrometer operating at 600 and 150 MHz, respectively. This

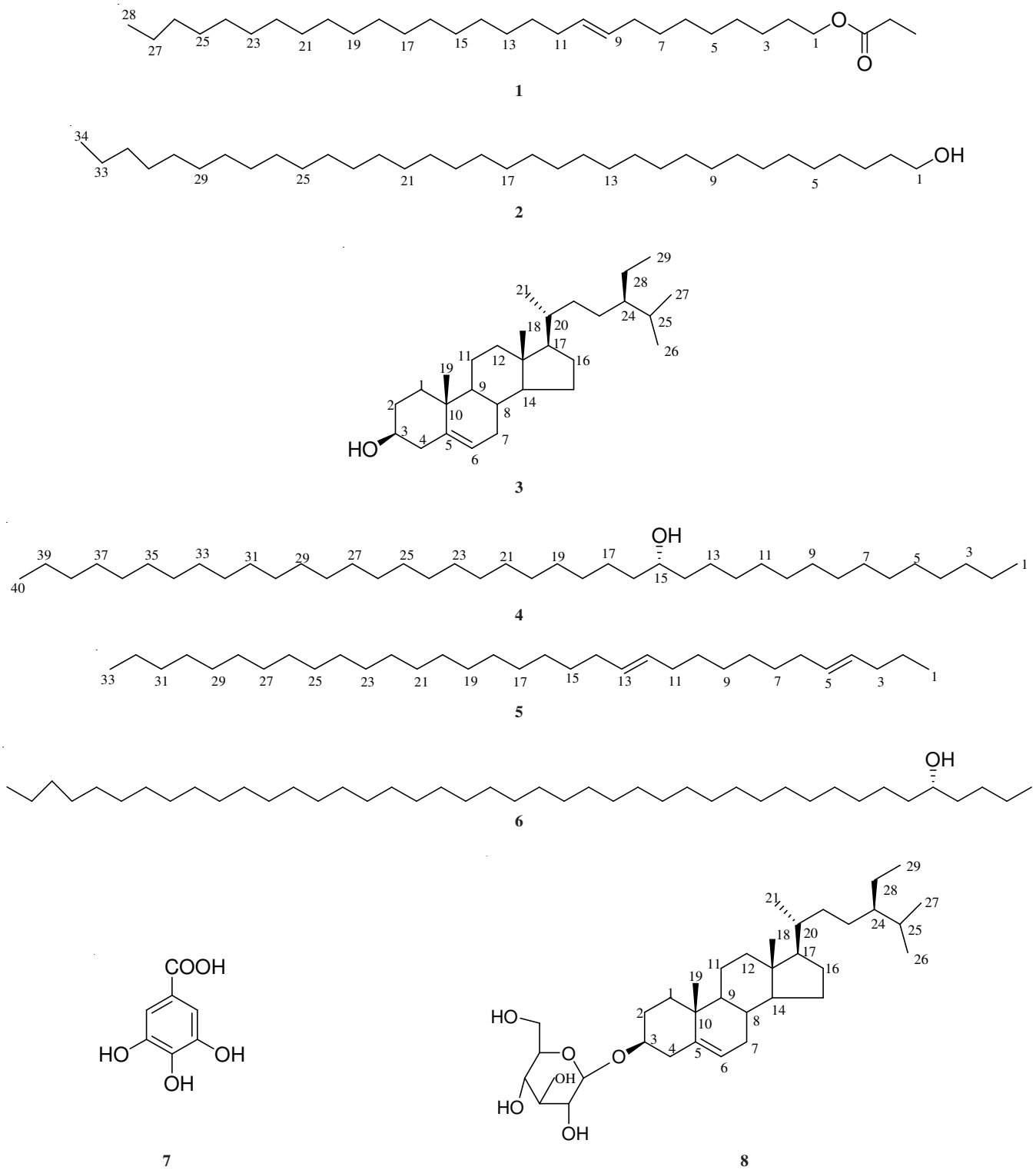

Fig. 1. Chemical structures of compounds **1-8**

NMR machine was available at Seoul National University (SNU), Seoul, South Korea and all NMR spectra were recorded at SNU (Instrument, Bruker, Germany). NMR spectra were obtained in deuterated chloroform and pyridine using tetramethylsilane (TMS) as an internal standard, with chemical shifts expressed in ppm (δ) and coupling constants (*J*) in Hz. FAB MS data were recorded on a JMS-700 (Jeol, Japan) spectrometer instrument which was available at SNU, Seoul, South Korea. IR spectra were recorded on an Infinity Gold FT-IR (Thermo Mattson, USA) spectrophotometer, which was available at Korea Institute of Science and Technology, Seoul, South Korea.

The straw of *O. sativa* were collected from the Konkuk University Experimental Farm, Seoul, Korea in October 2010. After harvesting, the samples was dried in the Laboratoty temperature range (25-30 ºC) for 3 weeks. The voucher specimen (ILPUM variety) has been dried and deposited in the herbarium of our department.

Preparation of extracts: Dried straws of *O. sativa* (10 kg) were immersed in methanol (MeOH) for 1 week at room temperature and concentrated under vacuum to produce an extract (78 g), which was suspended in water and extracted successively with hexane, ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH).

Isolation of compounds from hexane extract: The hexane extract was chromatographed over silica gel with hexane and ethyl acetate solvents and initially yielded three compounds *n*-octacos-9-enyl propionate (**1**, 11 mg) and 1-tetratriacontanol (**2**, 16 mg). Tritriacontan-4,12-diene (**5**, 7 mg). The separation of other fractions is in progress.

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 40 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 23-28 in EtOAc:MeOH (9.5:0.5), fractions 29-32 in EtOAc:MeOH (9:1), fractions 33-34 in EtOAc:MeOH (7:3) and fractions 35-40 in MeOH. Fraction 6 was crystallized and, after purification by column chromatography, yielded β-sitosterol (**3**, 23 mg). This was confirmed by comparison with an authentic sample from Sigma. Fraction 11 was further purified by column chromatography over silica gel with methylene dichloride and methanol, produced two pure compounds: *n*-tetracontan-15α-ol (4, 6 mg), *n*-tritetracontan-5α-ol (6, 9 mg) fraction 12, 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 (**7**, 3 mg). Fraction 23, after column chromatography over silica gel with chloroform and methanol, yielded one pure compound: β-sitosterol-3-O-β-D-glucoside (**8**, 12 mg).

***n*-Octacos-9-enyl propionate (1):** Colourless solid. R_f 0.32 ($CHCl_3$); IR ((KBr, ν_{max}, cm^{-1}): 2918, 2849, 1735, 1635, 1467, 1380, 1174, 724; ^1H NMR ($CDCl_3$) δ 5.34 (m, 1H, H-9), 5.32 (m, 1H, H-10), 4.06 (d, J = 6.5 Hz, 1H, H_2-1a), 4.04 (d, J = 6.5 Hz, 1H, H_2-1b), 2.29 (d, J = 7.5 Hz, 1H, H_2-2'a), 2.27 (d, J = 7.5 Hz, 1H, H_2-2'b), 2.01 (m, 2H, CH_2-11), 1.62 (m, 2H, H_2-8), 1.58 (m, 2H, CH_2), 1.55 (m, 2H, H_2-7), 1.25 (br s, 38 H, 19×CH_2), 0.89 (t, J = 6.0 Hz, 3H, Me-28), 0.86 (t, J = 7.0 Hz, 3H, Me-3'); ^{13}C NMR ($CDCl_3$) δ: 174.23 (C-1'), 130.13 (C-9, C-10), 64.62 (C-1), 34.62 (CH_2), 32.16 (CH_2), 30.01 (CH_2), 29.93 (3×CH_2), 29.89 (2×CH_2), 29.84 (CH_2), 29.82 (CH_2), 29.76 (CH_2), 29.71 (CH_2), 29.59 (CH_2), 29.55 (CH_2), 29.51 (CH_2), 29.49 (CH_2), 29.40 (CH_2), 28.90 (CH_2), 27.44 (CH_2), 26.18 (CH_2), 25.27 (CH_2), 22.92 (CH_2), 14.34 (CH_3-28, CH_3-3'); FAB-MS *m/z* (rel. int.) 464 $[M]^+$ ($C_{31}H_{60}O_2$) (7.4), 437 (20.2), 407 (33.9), 379 (68), 365 (8.3), 351 (9.6), 337 (9.7), 323 (9.7), 309 (9.5), 295 (11.2), 281 (10.3), 279 (15.6), 267 (10.4), 253 (13.2), 225 (11.8), 221 (9.5), 211 (12.9), 197 (13.7), 183 (15.2), 169 (15.3), 155 (16.4), 149 (28.3), 141 (18.8), 127 (20.8), 113 (29.1), 97 (24.9), 85 (62.1), 71 (85), 57 (100).

1-Tetratriacontanol (2): Colouless solid, R_f 0.65 (hexane: EtOAc 8.5:1.5); m.p. 83-86 ºC; $[\alpha]_D^{20}$-0.020º ($CHCl_3$); IR (KBr, ν_{max}, cm^{-1}): 3450, 2917, 1465, 1215, 757; ^1H NMR ($CDCl_3$): 0.88 (3H, t, J = 7.0 Hz, methyl), 1.55 (2H, m, CH_2CH_2-OH), 1.25-1.33 (all CH_2 protons), 3.64 (1H, t, OH); ^{13}C NMR ($CDCl_3$): 14.33 (methyl), 63.31 (C-OH), 22.90, 24.98, 25.95, 29.30, 29.47, 29.52, 29.60, 29.83, 29.80, 29.86, 29.91, 32.15, 33.04 (all methylene carbons assignments may be interchanged); EI-MS: *m/z* 494 $[M]^+$ (calcd. (%) for $C_{34}H_{70}O$), 476 $[M-H_2O]^+$ (4.7 %), 448 (23.4), 392 (11.5), 376 (4.2), 362 (5.6), 348 (5.3), 321 (5.7), 306 (10.5), 292 (6.8), 279 (7.4), 265 (7.6), 237 (9.5), 223 (10.5), 209 (12.2), 195 (14.0), 181 (16.6), 153 (24.6), 139 (32.0), 125 (48.3), 111 (74.4), 97 (100), 71 (61.7), 57 (87.7); FAB-MS: (positive mode) *m/z* 495 $[M + H]^+$; (negative mode) *m/z* 493 $[M-H]^-$.

β-Sitosterol (3): R_f 0.48 (Hex:EtOAc 8:2); m.p. 139-142 ºC; $[\alpha]_D^{14}$-37º ($CHCl_3$); IR (KBr, ν_{max}, cm^{-1}): 3429, 2936, 1644, 1462, 1376, 1057; EI-MS: *m/z* 414 $[M]^+$ (calcd. (%) for $C_{29}H_{50}O$), 396 $[M-H_2O]^+$ (70.4), 381 (37.9), 367 (10.2), 351 (8.7), 329 (38.5), 303 (31.1), 289 (11.4), 1 273 (26.2), 255 (48.5), 241 (7.6), 231 (21.7), 213 (36.2), 199 (13.7), 173 (16.1), 159 (27.6), 145 (33.0), 133 (23.2), 107 (25.2), 95 (26.7), 81 (27.0), 55 (22.9). (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 $[M + H]^+$; ^1H and ^{13}C NMR similar to reported literature values[12-14].

***n*-Tetracontan-15α-ol (4):** Colourless solid; R_f 0.34 ($CHCl_3$:MeOH); 9.8:0.2); IR (KBr, ν_{max}, cm^{-1}: 3420, 2920, 2851, 1731, 1465, 1239, 1072, 950, 830, 724; ^1H NMR ($CDCl_3$) δ: 3.36 (br m, W1/2 = 7.7 Hz, 1H, H-15β), 2.18 (m, 1H, CH_2), 1.89 (m, 2H, CH_2), 1.41 (m, 2H, CH_2), 1.17 (m, 2H, CH_2), 1.16 (m, 4H, 2 × CH_2), 1.13 (br s, 62 H, 31 × CH_2), 0.76 (t, J = 5.5 Hz, 3H, Me-1), 0.74 (t, J = 6.0 Hz, 3H, Me-40); ^{13}C NMR ($CDCl_3$) δ: 77.18 (C-15), 33.26 (CH_2), 31.90 (CH_2), 29.69 (29 × CH_2), 29.64 (CH_2), 29.61 (CH_2), 29.57 (CH_2), 29.34 (CH_2), 22.90 (CH_2), 22.67 (CH_2), 16.79 (CH_3-1), 14.10 (CH_3-40); FAB MS (positive ion mode) *m/z* 579 $[M+H]^+$ ($C_{40}H_{83}O$).

Tritriacontan-4,12-diene (5): Yellow semi-solid; IR (KBr, ν_{max}, cm^{-1}): 2924, 2854, 1590, 1460, 1365, 1215, 1130, 1090, 725; ^1H NMR: ($CDCl_3$, 500 MHz):δ: 5.37 (1H, br s, H-4), 5.33 (2H, br s, H-5, H-12), 5.30 (1H, br s, H-13), 2.81 (1H, br m, H_2-6a), 2.76 (1H, br s, H_2-6b), 2.29 (2H, br s, H_2-3), 2.03 (2H, br s, H_2-11), 2.01 (2H, br s, H_2-14), 1.57 (2H, br m, H_2-7), 1.28 (10 H, br s, 5 × CH_2), 1.25 (26 H, br s, 13 × CH_2), 1.22 (8 H, br s, 4 × CH_2), 0.87 (6H, t, J = 6.0- Hz, Me-1, Me-33); ^{13}C NMR: ($CDCl_3$, 500 MHz):δ: 130.44 (C-1), 130.15 (C-12), 128.23 (C-4), 128.11 (C-13), 32.02 (C-6), 30.41 (C-11), 29.92 (C-14), 29.57 (12 × CH_2), 29.21 (C-3), 28.96 (CH_2), 15.98 (Me-1), 14.32 (Me-28); FAB MS (positive mode) *m/z* 461 $[M + H]^+$; ($C_{33}H_{64}$).

***n*-Tritetracontan-5α-ol (6):** Colourless solid; R_f 0.30 ($CHCl_3$:MeOH; 9.8:0.2); IR (KBr, ν_{max}, cm^{-1}): 3421, 2919, 2850, 1468, 1260, 1067, 980, 870, 725; ^1H NMR ($CDCl_3$) δ: 3.48 (brm, $W_{1/2}$ = 6.5 Hz, 1H, H-5β), 1.58 (br s, 2H, CH_2), 1.32 (brs, 4H, 2×CH_2), 1.29 (br s, 6H, 3×CH_2), 1.25 (br s, 72 H, 36×CH_2), 0.89 (t, J = 5.5 Hz, 3H, Me-1), 0.86 (t, J = 6.0 Hz, 3H, Me-43); ^{13}C NMR ($CDCl_3$) δ: 77.17 (C-5), 33.2 (CH_2), 31.90 (CH_2), 29.69 (33×CH_2), 29.64 (CH_2), 29.57 (CH_2), 29.35 (CH_2), 22.88 (CH_2), 22.67 (CH_2), 16.78 (CH_3-1), 14.10 (CH_3-43); FAB-MS *m/z* $[M]^+$ ($C_{43}H_{88}O$) (1.5); FAB MS *m/z* 621 $[M+H]^+$ ($C_{43}H_{89}O$).

Gallic acid (7): Colourlees solid; R_f 0.45; ($CHCl_3$-MeOH (9:1); m.p. 248-250 ºC, yield in minor amount, M^+ 170, $C_7H_6O_5$, identical (IR, MS and NMR) with authentic sample.

β-Sitosterol-3-O-β-D-glucoside (8): R_f 0.47 ($CHCl_3$: MeOH 9:1); $[\alpha]_D^{20}$-0.09º (pyridine); IR: (KBr, ν_{max}, cm^{-1}): 3429, 2933, 1635, 1376, 1073; FAB-MS: *m/z* 414 [M-glucose]$^+$ (calcd. (%) for $C_{29}H_{50}O$) (25.7 %), 396 [M-H_2O]$^+$ (100), 382 (42.8), 367 (9.4), 354 (4.0), 329 (9.9), 303 (9.0), 288 (11.5), 275 (14.8), 255 (33.2), 279 (9.2), 213 (20.5), 199 (8.4), 173 (9.4), 159 (19.5), 147 (28.3), 133 (17.0), 107 (19.3), 95 (22.9), 81 (25.3), 55 (20.8); ^1H and ^{13}C NMR similar to reported literature values[14].

The chemical structure of all the eight compounds are given in Fig. 1.

RESULTS AND DISCUSSION

Compound **1** was obtained as a colourless compound and its molecular formula was deduced as $C_{31}H_{60}O_2$ from its ^{13}C NMR and FAB MS *m/z* 464. The ^1H NMR spectrum of **1** showed two one-proton multiplets at δ 5.34 and 5.32 assigned to vinylic H-9 and H-10, respectively. Two one-proton doublets at δ 4.06 (J = 6.5 Hz) and 4.04 (J = 6.5 Hz) were attributed to oxygenated methylene H_2-1. Two one-proton doublets at δ 2.29 (J = 7.5 Hz) and 2.27 (J = 7.5 Hz) were ascribed to C-2' methylene protons adjacent to ester groups. Two multiplets at δ 2.01 and 1.62 integrated for two protons were accounted to C-11 and C-8 methylene protons attached to the vinylic carbons. Two three-proton triplets at δ 0.89 (J = 6.0 Hz) and 0.86 (J = 7.0 Hz) were associated to C-28 and C-3' primary methyl protons, respectively. The remaining methylene protons resonated between δ 1.58-1.25. The ^{13}C NMR spectrum of **1** displayed important signals for ester carbon at δ 174.23 (C-1'), oxygenated methylene carbon at δ 64.62 (C-1), methylene carbons at δ 14.34 (C-28, C-3'), vinylic carbons at δ 130.13 (C-9, C-10) and methylene carbons between δ 34.62-22.92. On the basis of this evidence the structure of **1** has been established as *n*-octacos-9-enyl propionate. The values of spectral data were compared with the help of reported values[15].

Compound **2** was isolated as white granular crystals with a melting point of 83-86 ºC. It exhibited a peak at *m/z* 476 [M-H_2O]$^+$ on EI-MS, FAB-MS of compound **2** gave a positive-mode [M + H]$^+$ ion peak of 495 and a negative mode [M-H]$^-$ ion peak of 493. This suggested a molecular ion peak [M]$^+$ of 494. The IR spectrum displayed intense absorption bands at 3450, 2917, 1465, 1215 and 757 cm^{-1}, with the band at 3450 cm^{-1} indicating the presence of a hydroxyl group. ^1H NMR of compound **2** in $CDCl_3$ gave signals that appeared as one triplet at δ 0.88 (3H, t, J = 6.7 Hz) for methyl proton, a multiplet at δ 1.55 should be signal of methylene protons of CH_2CH_2OH and a triplet at δ 3.64 (CH_2OH) represented a hydroxyl proton. All remaining methylene protons appeared as a broad singlet at δ 1.25-1.33. ^{13}C NMR in $CDCl_3$ produced carbon signals in its spectrum at δ 63.34, 33.04, 32.15, 29.92, 29.88, 29.84, 29.83, 29.66, 29.58, 29.47, 29.30, 25.96, 24.98, 22.91 and 14.33 for methylene attached to hydroxyl group, all methylenes and one methyl carbon. Analysis of compound **2** by ^1H, ^{13}C and mass supported the assigned structure **2** as 1-tetratriacontanol[14].

Compound **3** was isolated as colourless needle-shaped crystals that exhibited an *m/z* of 414 [M]$^+$ (calculated for $C_{29}H_{50}O$), which suggests a molecular ion peak. The IR spectrum of compound **3** showed an absorption bands at 3429, 2936, 1644, 1462, 1376 and 1057 cm^{-1}, with the band at 3429 cm^{-1} characteristic of a hydroxyl group. The ^1H NMR of compound **3** displayed a one proton broad multiplet at δ 3.51, which was assigned to a carbinol 3α-proton. Two three-proton broad signals at δ 0.63 and 0.94 were ascribed to tertiary C-18 and C-19 protons, respectively. Three doublets at δ 0.92 (J = 7.42 Hz), 0.81 (J = 7.5 Hz) and 0.91 (J = 7.15 Hz), which integrated for three protons each, were attributed to secondary C-21, C-26 and C-27 methyl functionalities, respectively. Another triplet at δ 0.88 (J = 7.5 Hz) was attributed to C-29 methyl protons and a one-proton broad multiplet at δ 5.56 for vinylic proton was attributed to C-6. The remaining methine and methylene protons resonated between δ 2.29 and 1.13. The ^{13}C NMR spectrum of compound **3** showed the presence of 29 carbon signals in the molecule. The signals at δ 140.72 and 121.71 were ascribed to vinylic carbons at position C-5 and C-6. Carbon signals appeared at δ 37.31 (C-1), 31.57 (C-2), 71.80 (C-3), 42.19 (C-4), 11 31.87 (C-7, 8), 50.10 (C-9), 36.48 (C-10), 21.11(C-11), 39.81 (C-12), 42.33 (C-12 13), 56.79 (C-14), 24.32 (C-15), 28.26 (C-16), 56.11 (C-17), 11.87 (C-18),13 19.39 (C-19), 36.17 (C-20), 18.82 (C-21), 33.95 (C-22), 26.13 (C-23), 45.85 (C-24), 29.18 (C-25), 19.48 (C-26), 19.07 (C-27), 23.09 (C-28) and 12.32 (C-15 29). Based on the above results, the structure **3** was elucidated as β-sitosterol. The values of spectral data were compared with the help of reported values[12-14].

Compound **4**, an aliphatic alcohol was obtained as a colourless amorphous powder. The FAB-MS and ^{13}C NMR data showed to corresponding to molecular formula $C_{40}H_{82}O$, *m/z* 579 [M + H]$^+$. The ^1H NMR spectrum of **4** displayed a one-proton broad multiplet at δ 3.36 with half width of 7.75 Hz assigned to carbinol 15β. Two three-proton triplets at δ 0.76 (J = 5.5 Hz) and 0.74 (J = 6.0 Hz) were attributed to terminal C-1 and C-40 primary methyl protons. The remaining methylene protons appeared between δ 2.18-1.13. The ^{13}C NMR spectrum **4** showed important signals for carbinol carbon at δ 77.18 (C-15), methyl carbons at δ 16.79 (C-1) and 14.10 (C-40) and methylene carbons between δ 33.26 - 22.90. The absence of signals beyond δ 3.36 in the ^1H NMR spectrum and δ 77.18 in the ^{13}C NMR spectrum indicated saturated nature of the compound devoid of any carbonyl group. On the basis of the foregoing account the structure of **4** has been elucidated as *n*-tetracontan-15α-ol. The values of spectral data were compared with the help of reported values[15].

Compound **5** was obtained from the ethyl acetate extract of rice straw. as semi-solid compound. IR spectrum showed characteristic absorption bands for unsaturation (1590 cm^{-1}) and long aliphatic chain (725 cm^{-1}). Its mass spectrum displayed a molecular ion peak at *m/z* 460 corresponding to molecular formula of an alkene. The ^1H NMR spectrum of **5** exhibited two one-proton broad signals at δ 5.37 and 5.30 assigned to vinylic H-4 and H-13, respectively. The ^1H NMR signals at δ 2.81 (1H br m), 2.76 (1H, br s), 2.29 (2H, br s), 2.03 (2H, br s) and 2.01 (2H, br s), were associated with the methylene protons adjacent to the vinylic carbons. Two six-proton triplets at δ

0.87 (J = 6.0 Hz) were accounted to C-1 and C-33 primary methyl protons, respectively. The remaining methylene protons resonated at δ 1.57 (2H), 1.28 (10H), 1.25 (26 H) and 1.22 (8H). The ^{13}C NMR spectrum displayed signals for vinylic carbons at δ 130.44 (C-5), 130.15 (C-12), 128.23 (C-4) and 128.11 (C-13), methyl carbons at δ 15.98 (C-1) and 14.32 (C-33) and the remaining carbons signals between 34.33 - 22.80. On the basis of the foregoing account the structure of **5** has been elucidated as tritriaontan-4,12-diene. The values of spectral data were compared with the help of reported values[16].

Compound **6** was obtained as an amorphous powder. The FAB MS and ^{13}C NMR data of **6** showed a peak at *m/z* 621 $[M + H]^+$ corresponding to molecular formula $C_{43}H_{88}O$. The ^{1}H NMR spectrum of **6** exhibited a one-proton broad multiplet at δ 3.48 with half-width of 6.5 Hz assigned to carbinol proton H-5β. Two triplets at δ 0.89 (J = 5.5 Hz) and 0.86 (J = 6.0 Hz) integrated for three-protons each, were attri-buted to terminal C-1 and C-43 primary methyl protons. The remaining methylene protons resonated between δ 1.58-1.25. The ^{13}C NMR spectrum of **6** showed important signals for carbinol carbon (δ 77.17) and methyl carbons (δ 16.78, C-1; 14.10 (C-43) and methylene carbons between δ 33.21- 22.67. The absence of any signal beyond δ 3.48 in the ^{1}H NMR spectrum δ 77.17 in the ^{13}C NMR spectrum suggested the saturated nature of the molecule. On the basis of spectral data analysis and chemical reactions, the structure of **6** has been characterized as *n*-tritetracontan-5α-ol. The values of spectral data were compared with the help of reported values[15].

Compound **7** exhibited IR absorption bands for OH (3345 cm^{-1}), COOH (1689 cm^{-1}) and double bond (1620 cm^{-1}) functions. An $[M]^+$ ion at *m/z* 170 in its mass spectrum suggested the molecular formula as $C_7H_6O_5$. The ^{1}H and ^{13}C NMR data were compared with reported literature values[17]. On the basis of spectral data analysis and compared with authentic sample, the structure of **7** has been characterized as gallic acid[17].

Compound **8** was isolated as a colourless powder that exhibited a fragmentation ion peak at an *m/z* of 414 $[M\text{-glucose}]^+$. The IR spectrum gave an absorption bands at 3429, 2933, 1635, 1376 and 1073 cm^{-1}, with the absorption band at 3429 cm^{-1} characteristic of a hydroxyl group. Compound **8** is a glucoside of compound **3**; other than the glucose protons and carbons, the ^{1}H and ^{13}C NMR values were almost the same as those of **3**. A one-proton doublet at δ 4.97 (J = 11.1 Hz) was attributed to anomeric H-1. Four one proton doublets at δ 13 4.16 (J = 9.3 Hz), 3.92 (J = 4.45 Hz), 3.85 (J = 4.85 Hz) and 3.56 (J = 4.85 Hz) and two one-protons multiplets at δ 3.83 and 3.60, were assigned to the remaining glucose protons. In ^{13}C NMR, the H-1 anomeric carbon appeared at δ 103.09 and other glucose carbons appeared at δ 79.11, 78.80, 75.84, 72.22 and 65.58. Based on the above evidence, compound 8 was confirmed as β-sitosterol-3-O-β-D-glucoside. The values of spectral data were compared with the help of reported values[14].

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