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# STRUCTURES OF NEW TRITERPENE GLYCOSIDES, MALBRANCHEOSIDES A - D, FROM *MALBRANCHEA FILAMENTOSA*

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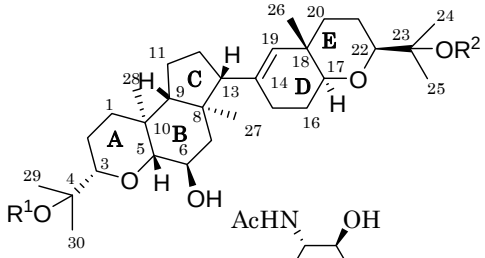
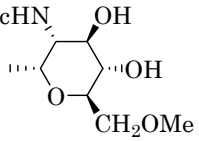
**Abstract** – In the course of searching for new biologically active metabolites in *Malbranchea filamentosa*, four new triterpene glycosides, malbrancheosides A (1) - D (4), were isolated. The structures of 1- 4 were confirmed by the chemical and spectroscopic investigation. Malbrancheosides A (1) - D (4) are the first example of triterpenoidal glycosides having D-glucosamine derivatives from the fungal sources.

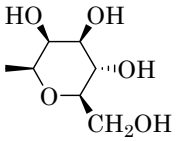
## INTRODUCTION

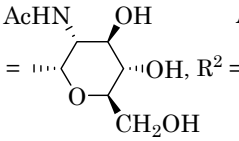
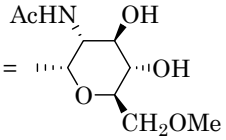
The causative fungi of severe mycosis, *e.g.* coccidioidomycosis caused by *Coccidioides immitis* RIXFORD & GILCHRIST, paracoccidioidomycosis by *Paracoccidioides brasiliensis* (SPLENDORE) ALMEIDA, blastomycosis by *Brastomyces dermatitidis* GILCHRIST & STOKES (teleomorph: *Ajellomyces dermatitidis* MCDONOUGH & LEWIS), and histoplasmosis by *Histoplasma capsulatum* DAHLING [teleomorph: *Ajellomyces capsulatus* (KWON-CHANG) MCGINNIS & KATZ], are all dimorphic hyphomycetes, the teleomorph of which should belong to the family Onygenaceae, in the order Onygenales.[1] The fungi of genus *Malbranchea* are also belong to the Onygenaceae as the perfect state and are taxonomically close to the above pathogenic fungi. This fact promted us to investigate the chemical constituents of *Malbranchea* fungi. From the preliminary antifungal test of the chloroform-methanol (1:1) extract of 15 species of *Malbranchea* fungi, cultivated on rice at 25°C for 21 days, against pathogenic yeasts, *Candida albicans* (ROBIN) BERKHOUT ATCC 90028 and *Cryptococcus neoformans* (SANFELICE) VUILLEMIN ATCC 90112, and pathogenic filamentous fungi, *Aspergillus fumigatus* FRESENIUS IFM 41362 and *Aspergillus niger* VAN TIEGHEM IFM 41398, *M. filamentosa* SIGLER & CARMICHAEL IFM41300 showed the characteristical antifungal activity against *C. neoformans*, which causes one of the deep-seated

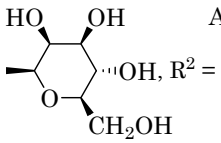
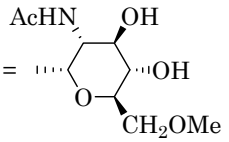
mycoses, cryptococcosis.[2] Recent study of metabolites of *M. filamentosa* IFM41300 resulted in the isolation of a new furanone derivative, 4-benzyl-3-phenyl-5*H*-furan-2-one,[3] and two new furanone glycosides, malfilamemtosides A and B.[2] Further investigation of the extract of the above fungus gave us the isolation of four new triterpene glycosides designated malbrancheosides A (**1**), B (**2**), C (**3**), and D (**4**). This paper deals mainly with the structural determination of **1** - **4**.



**1**: $R^1 = R^2 =$ 

**2**: $R^1$ = H, $R^2 =$ 

**3**: $R^1 =$  , $R^2 =$ 

**4**: $R^1 =$  , $R^2 =$ 

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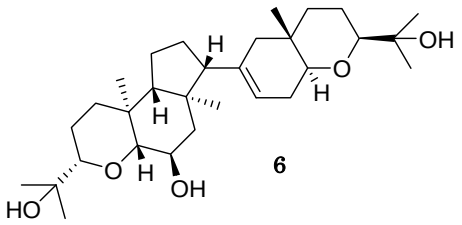

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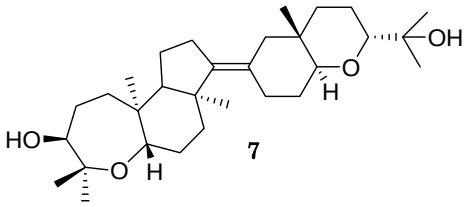

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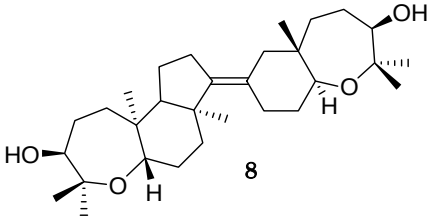

**8**

## RESULTS AND DISCUSSION

The molecular formula of malbrancheoside A (**1**), colorless crystalline powder, mp 185°C (from AcOEt-$CH_2Cl_2$), $[\alpha]_D^{20}$ +120.5° (MeOH), was confirmed as $C_{48}H_{80}N_2O_{15}$ by FAB mass spectrometry (MS) [*m/z* 947.5 $(M+Na)^+$] and elemental analysis. Since **1** showed greenish brown coloration by anisaldehyde-sulfuric acid reagent and two anomeric protons [δ 5.10 (d), 5.18 (d)] in the $^1$H-NMR spectrum (Table 1), malbrancheoside A (**1**) should be a glycoside having two sugar residues. Acid hydrolysis of **1** gave two triterpenoidal genins, malbrancheogenin (**5**), colorless crystalline powder, mp 183-184 °C (from AcOEt-$CH_2Cl_2$), $[\alpha]_D^{20}$ +49.5° ($CHCl_3$), and pseudomalbrancheogenin (**6**), colorless crystalline powder, m.p. 227-228 °C (from AcOEt), $[\alpha]_D^{20}$ +15.1° ($CHCl_3$), in addition with 6-*O*-methyl-D-glucosamine hydrochloride, $[\alpha]_D^{20}$ +62.9° ($H_2O$),[4] as a sugar moiety. The molecular formulae of **5** and **6** were determined as both $C_{30}H_{50}O_5$ by high resolution EI-MS. The $^{13}$C-NMR spectrum of **6** (Table 2) showed totally 30 carbons: 7 methyl carbons, 9 $sp^3$ methylene carbons, 7 $sp^3$

Table 1. $^{1}H$- and $^{13}C$-NMR Assignments for Malbrancheosides A (**1**) - D (**4**) in $CD_3OD$

| Carbon | **1** | | **2** | | **3** | | **4** | |
|---|---|---|---|---|---|---|---|---|
| No. | δC | δH (Hz) | δC | δH (Hz) | δC | δH (Hz) | δC | δH (Hz) |
| 1 | 39.4 | 1.19(m) | 39.2 | 1.20(m) | 39.4 | 1.19(m) | 39.4 | 1.19(m) |
| | | 1.60(m) | | 1.58(m) | | 1.60(m) | | 1.60(m) |
| 2 | 22.7 | 1.53(m) | 23.3 | 1.39(m) | 22.7 | 1.53(m) | 22.5 | 1.54(m) |
| | | 1.69(m) | | 1.57(m) | | 1.68(m) | | 1.76(m) |
| 3 | 86.6 | 3.30(m) | 87.5 | 3.24(m) | 86.6 | 3.31(m) | 86.5 | 3.31(m) |
| 4 | 80.3 | | 73.9 | | 80.2 | | 80.2 | |
| 5 | 92.7 | 2.78(d,10) | 92.5 | 2.78(d,10) | 92.6 | 2.78(d,10) | 92.6 | 2.79(d,10) |
| 6 | 68.5 | 3.72(m) | 68.6 | 3.76(m) | 68.5 | 3.73(m) | 68.5 | 3.74(m) |
| 7 | 48.3 | 1.20(m) | 47.6 | 1.20(m) | 48.3 | 1.21(m) | 48.2 | 1.21(m) |
| | | 2.00(dd,11,4) | | 2.00(dd,12,5) | | 2.01(m) | | 2.02(m) |
| 8 | 45.7 | | 45.5 | | 45.6 | | 45.6 | |
| 9 | 59.4 | 1.32(m) | 59.3 | 1.32(dd,12,5) | 59.4 | 1.32(m) | 59.3 | 1.32(m) |
| 10 | 36.7 | | 36.5 | | 36.7 | | 36.7 | |
| 11 | 20.2 | 1.40(m) | 20.1 | 1.40(m) | 20.2 | 1.40(m) | 20.2 | 1.41(m) |
| | | 1.54(m) | | 1.52(m) | | 1.54(m) | | 1.54(m) |
| 12 | 26.0 | 1.63(m) | 25.9 | 1.65(m) | 25.7 | 1.64(m) | 26.0 | 1.65(m) |
| | | 1.80(m) | | 1.80(m) | | 1.80(m) | | 1.80(m) |
| 13 | 59.0 | 1.96(m) | 59.0 | 1.95(m) | 58.9 | 1.95(m) | 58.9 | 1.96(m) |
| 14 | 135.5 | | 135.4 | | 135.5 | | 135.4 | |
| 15 | 32.4 | 1.98(m) | 32.3 | 1.97(m) | 32.4 | 1.99(m) | 32.2 | 1.98(m) |
| | | 2.18(m) | | 2.20(m) | | 2.18(m) | | 2.20(m) |
| 16 | 25.9 | 1.63(m) | 26.0 | 1.65(m) | 26.0 | 1.60(m) | 25.9 | 1.60(m) |
| | | 1.63(m) | | 1.65(m) | | 1.60(m) | | 1.60(m) |
| 17 | 83.7 | 3.18(dd,11,4) | 83.9 | 3.13(m) | 83.6 | 3.17(dd,12,4) | 83.9 | 3.20(dd,11,5) |
| 18 | 35.7 | | 35.6 | | 35.6 | | 35.6 | |
| 19 | 134.1 | 5.16(s) | 134.1 | 5.15(s) | 134.1 | 5.15(s)) | 134.0 | 5.16(brs) |
| 20 | 38.5 | 1.36(m) | 38.4 | 1.36(m) | 38.5 | 1.36(m) | 38.4 | 1.37(m) |
| | | 1.61(m) | | 1.59(m) | | 1.59(m) | | 1.60(m) |
| 21 | 23.9 | 1.54(m) | 23.4 | 1.60(m) | 23.8 | 1.54(m) | 23.1 | 1.60(m) |
| | | 1.60(m) | | 1.70(m) | | 1.62(m) | | 1.70(m) |
| 22 | 86.6 | 3.41(dd,11,3) | 86.4 | 3.38(m) | 86.6 | 3.41(m) | 86.3 | 3.37(m) |
| 23 | 80.2 | | 80.3 | | 80.1 | | 80.2 | |
| 24 | 23.4 | 1.15(s) | 23.2 | 1.24(s) | 22.7 | 1.14(s) | 22.7 | 1.21(s) |
| 25 | 23.4 | 1.20(s) | 24.8 | 1.24(s) | 23.4 | 1.20(s) | 23.4 | 1.21(s) |
| 26 | 20.8 | 0.94(s) | 20.9 | 0.94(s) | 20.8 | 0.94(s) | 20.9 | 0.95(s) |
| 27 | 17.1 | 0.75(s) | 17.1 | 0.75(s) | 17.0 | 0.74(s) | 17.0 | 0.75(s) |
| 28 | 14.4 | 0.90(s) | 14.2 | 0.88(s) | 14.4 | 0.89(s) | 14.4 | 0.91(s) |
| 29 | 22.7 | 1.20(s) | 23.9 | 1.18(s) | 22.6 | 1.20(s) | 23.1 | 1.25(s) |
| 30 | 22.7 | 1.20(s) | 26.2 | 1.08(s) | 23.4 | 1.20(s) | 24.8 | 1.25(s) |
| 1' | 91.9 | 5.16(d,4) | | | 92.9 | 5.17(d,3) | 96.6 | 4.87(brs) |
| 2' | 56.3[a)] | 3.76(m) | | | 56.2 | 3.77(m) | 74.0 | 3.76(m) |
| 3' | 72.8 | 3.59(m) | | | 72.8 | 3.63(m) | 75.8 | 3.41(m) |
| 4' | 71.9 | 3.28(m) | | | 72.9 | 3.29(m) | 68.8 | 3.54(m) |
| 5' | 72,7[b)] | 3.86(m) | | | 72.7 | 3.86(m) | 78.1 | 3.15(m) |
| 6' | 73.6[c)] | 3.55(m) | | | 73.7 | 3.56(m) | 63.2 | 3.65(m) |
| | | 3.55(m) | | | | 3.56(m) | | 3.78(m) |
| 6'-*O*-Me | 59.9 | 3.33(s) | | | | | | |
| CO-$CH_3$ | 173.9 | | | | 173.8 | | | |
| CO-$CH_3$ | 23.0 | 1.93(s) | | | 23.0 | 1.93(s) | | |
| 1" | 93.5 | 5.24(d,4) | 96.7 | 4.84(brs) | 93.5 | 5.24(d,4) | 92.9 | 5.12(d,4) |
| 2" | 56.1[a)] | 3.76(m) | 74.0 | 3.75(m) | 56.2 | 3.76(m) | 56.2 | 3.76(m) |
| 3" | 72.8 | 3.59(m) | 75.8 | 3.40(dd,10,4) | 72.7 | 3.58(m) | 72.8 | 3.61(m) |
| 4" | 72.9 | 3.28(m) | 68.9 | 3.51(dd,10,10) | 72.8 | 3.29(m) | 72.9 | 3.29(m) |
| 5" | 72,6[b)] | 3.87(m) | 78.2 | 3.12(ddd,10,5,2) | 72.6 | 3.87(m) | 72.7 | 3.88(m) |
| 6" | 73.7[c)] | 3.55(m) | 63.3 | 3.65(dd,11,5) | 73.6 | 3.54(m) | 73.3 | 3.57(m) |
| | | 3.55(m) | | 3.77(dd,11,2) | | 3.54(m) | | 3.57(m) |
| 6"-OMe | 59.9 | 3.33(s) | | | 59.9 | 3.33(s) | 59.9 | 3.34(s) |
| CO-$CH_3$ | 173.6 | | | | 173.8 | | 173.8 | |
| CO-$CH_3$ | 23.0 | 1.88(s) | | | 22.9 | 1.93(s) | 23.0 | 1.91(s) |

[a-c)] The assignments may be reversed.

methine carbons, five of which were bearing oxygen functions (δ 67.3, 79.2, 84.4, 85.1, 90.9), a $sp^2$ methine carbon (δ 119.9), 5 $sp^3$ quarternary carbons, two of which were bearing oxygen functions (δ 71.9, 72.2), and a $sp^2$ quarternary carbon (δ 134.9). Therefore pseudomalbrancheogenin (**6**) should be a pentacyclic triterpene. The planar structure of **6** was confirmed from the detailed analysis of $^1$H-$^1$H COSY and HMBC spectra (Figure 1). The $^1$H- and $^{13}$C-NMR spectra of malbrancheogenin (**5**) were closely similar with those of **6**, except for the chemical shifts of the olefinic carbons (δ 132.7 and 133.7 for **5**; δ 117.5 and 134.9 for **6**) and the coupling pattern of the olefinic proton [δ 5.16 (s) for **5**; δ 5.32 (brt, *J*=2 Hz) for **6**] (Table 2). Compound **5** was easily converted to **6** on acidic condition. These facts suggested that the structure of **5** was the same as that of **6**, except for the position of the double bond. The planar structure of malbrancheogenin (**5**) was consequently determined from the detailed analysis of $^1$H-$^1$H

Table 2. $^1$H- and $^{13}$C-NMR Assignments for Malbrancheogenin (**5**) and Pseudomalbrancheogenin (**6**)

| Carbon | **5** | | **6** | | (*S*)-MTPA ester of **6** | (*R*)-MTPA ester of **6** |
|---|---|---|---|---|---|---|
| No. | $\delta_C$ | $\delta_H$ (Hz) | $\delta_C$ | $\delta_H$ (Hz) | $\delta_H$ (Hz) | $\delta_H$ (Hz) |
| 1 | 37.5 | 1.63(m) | 37.5 | 1.66(m) | 1.63(m) | 1.64(m) |
| | | 1.19(m) | | 1.20(m) | 1.19(m) | 1.20(m) |
| 2 | 21.1 | 1.75(m) | 21.1 | 1.75(m) | 1.66(m) | 1.69(m) |
| | | 1.44(m) | | 1.43(m) | 1.39(m) | 1.42(m) |
| 3 | 85.1 | 3.22(m) | 85.1 | 3.27(dd,11,3) | 3.18(m) | 3.24(dd,12,3) |
| 4 | 72.0 | | 72.2 | | | |
| 5 | 90.9 | 2.87(d,10) | 90.9 | 2.86(d, 10) | 3.16(d, 10) | 3.17(d, 10) |
| 6 | 67.3 | 3.84(ddd,11,10,5) | 67.3 | 3.84(ddd,10,10,5) | 5.32(m) | 5.31(m) |
| 7 | 45.4 | 2.11(dd,12, 5) | 45.7 | 2.08(dd,12,5) | 2.11(dd,11,5) | 2.01(dd,12,5) |
| | | 1.23(m) | | 1.27(m) | 1.34(m) | 1.29(m) |
| 8 | 44.1 | | 43.8 | | | |
| 9 | 57.8 | 1.31(m) | 58.1 | 1.35(m) | 1.38(m) | 1.33(m) |
| 10 | 35.0 | | 35.0 | | | |
| 11 | 18.9 | 1.56(m) | 18.7 | 1.53(m) | 1.55(m) | 1.54(m) |
| | | 1.35(m) | | 1.34(m) | 1.41(m) | 1.40(m) |
| 12 | 24.5 | 1.68[c](m) | 25.3 | 1.80(m) | 1.79(m) | 1.77(m) |
| | | 1.68(m) | | 1.66(m) | 1.71(m) | 1.69(m) |
| 13 | 57.2 | 1.97(m) | 56.7 | 1.92(dd,11,10) | 1.95(m) | 1.89(m) |
| 14 | 133.7 | | 134.9 | | | |
| 15 | 30.6 | 2.23(dt,10,9) | 119.9 | 5.32(brt,2) | 5.33(m) | 5.30(m) |
| | | 1.97(m) | | | | |
| 16 | 24.4 | 1.80[c](m) | 29.0 | 2.21(ddd,13,7) | 2.21(m) | 2.21(m) |
| | | 1.68(m) | | 1.97(brdd,13,11) | 1.96(m) | 1.96(m) |
| 17 | 81.8 | 3.22(m) | 79.2 | 3.30(dd,11,6) | 3.31(dd,10,5) | 3.30(dd,10,6) |
| 18 | 34.3 | | 32.2 | | | |
| 19 | 132.7 | 5.16(s) | 45.1 | 1.88(d,18) | 1.74(m) | 1.68(m) |
| | | | | 1.68(m) | 1.74(m) | 1.68(m) |
| 20 | 36.8 | 1.63(m) | 38.0 | 1.60(m) | 1.55(m) | 1.55(m) |
| | | 1.35(m) | | 1.23(m) | 1.28(m) | 1.28(m) |
| 21 | 22.2 | 1.65(m) | 22.3 | 1.60(m) | 1.59(m) | 1.59(m) |
| | | 1.44(m) | | 1.42(m) | 1.45(m) | 1.45(m) |
| 22 | 85.1 | 3.22(m) | 84.4 | 3.17(dd,11,2) | 3.18(m) | 3.18(m) |
| 23 | 72.0 | | 71.9 | | | |
| 24 | 23.8 | 1.18[d](s) | 23.7 | 1.17(s) | 1.18(s) | 1.18(s) |
| 25 | 26.1 | 1.19[d](s) | 26.0 | 1.17(s) | 1.18(s) | 1.18(s) |
| 26 | 20.0 | 0.96(s) | 16.3 | 0.88(s) | 0.84(s) | 0.84(s) |
| 27 | 16.3 | 0.75(s) | 16.7 | 0.80(s) | 0.89(s) | 0.87(s) |
| 28 | 13.3 | 0.90(s) | 13.3 | 0.91(s) | 0.98(s) | 0.99(s) |
| 29 | 24.2 | 1.19[d](s) | 24.2 | 1.19(s) | 1.10(s) | 1.16(s) |
| 30 | 26.6 | 1.24(s) | 26.6 | 1.24(s) | 1.05(s) | 1.13(s) |

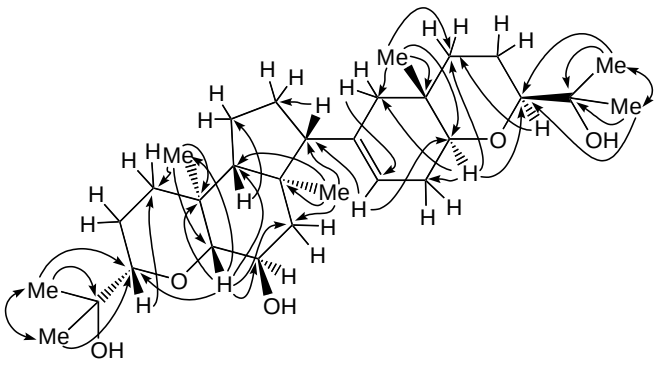

Figure 1 HMBC Correlations of pseudomalbrancheogenin (**6**)

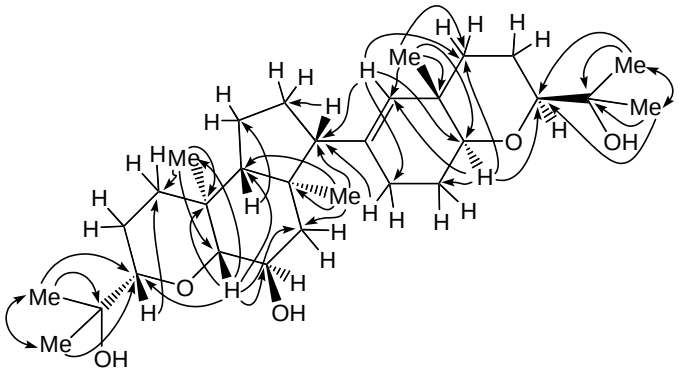

Figure 2 HMBC Correlations of malbrancheogenin (**5**)

COSY and HMBC (Figure 2) spectra.

The relative stereochemistry of malbrancheogenin (**5**) and pseudomalbrancheogenin (**6**) was confirmed from the analyses of the difference nuclear Overhauser enhancement (NOE) experiments and the NOESY spectrum of **6** (Figure 3). NOE correlations were observed from the methyl protons at C-10 (δ 0.91) to the methyl protons at C-8 (δ 0.80) and the methine proton at C-6 (δ 3.84). On the other hand, NOE correlations were observed from the methine proton at C-9 (δ 1.35) to the methine proton at C-5 (δ 2.86) and the methine proton at C-13 (δ 1.92). These results indicated that A/B/C rings were *trans*, *anti*-junction. Moreover, 5% of NOE was observed on the methine proton at C-5 (δ 2.86) when the methine proton at C-3 (δ 3.27) was irradiated. This result indicated that isopropyl residue at C-3 was oriented on the same side as the methyl group at C-10.

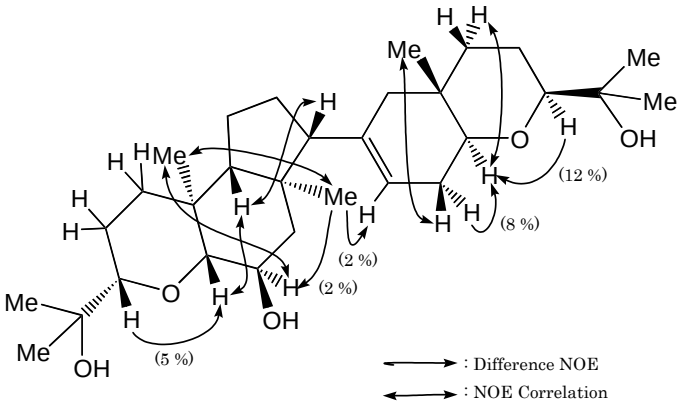


Figure 3 Difference NOE and NOE correlations of pseudomalbrancheogenin (**6**)

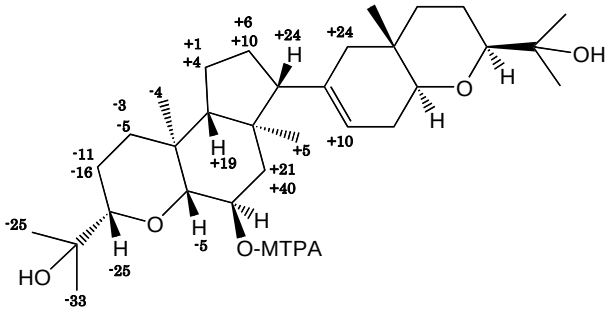


Figure 4 Chemical shift differences between (*S*)- and (*R*)-MTPA ester of pseudomalbrancheogenin (**6**)

An NOE correlation was observed between the methyl protons at C-26 (δ 0.88) and one of the methylene protons at C-16 (δ 1.97). On the other hand, 8% of NOE was observed on the methine proton at C-17 (δ 3.30) when the other methylene proton at C-16 (δ 2.21) was irradiated. These results indicated that D/E rings were *trans*-junction. From 12% of NOE on the above proton at C-17 (δ 3.30) irradiating the methine

proton at C-22 (δ 3.17), the isopropyl residue at C-22 was oriented on the same side as the methyl group at C-18. Therefore, the relative structure of pseudomalbrancheogenin (**6**) was determined except for the stereochemistry between A/B/C rings and D/E rings. The relative structure of malbrancheogenin (**5**) was also determined as same as **6**, which was correlated with the chemical conversion from **5** to **6** by the shift of the double bond.

In order to confirm the absolute configuration of the secondary alcohol at C-6 in the ring B of malbrancheogenin (**5**) and pseudomalbrancheogenin (**6**), the advanced Mosher's method[5] was applied to **6**. The (*R*)- and (*S*)-2-methoxy-2-phenyl-2-(trifluoromethyl)acetic acid (MTPA) esters of **6** were synthesized and the values of the chemical shift differences between the (*S*)- and (*R*)-MTPA esters [$\Delta\delta = \delta_S - \delta_R$ in Hz] were calculated. From the results (Figure 4), the secondary hydroxyl group at C-6 in **6** was assigned as *R*-configuration. Therefore, the absolute configuration of **5** and **6** was determined, except for the absolute stereochemistry of D/E rings.

The genuine genin of malbrancheoside A (**1**) should be malbrancheogenin (**5**), by the detailed analysis of the HMBC correlations of **1**, compared with those of **5** and **6** (Figure 5). Since acid hydrolysis of **1** gave 6-*O*-methyl-D-glucosamine hydrochloride,[4] as sugar moiety in addition with two triterpenoidal genins, **5** and **6**, and **1** showed two anomeric protons [δ 5.16 (d), 5.24 (d)] in the $^1$H-NMR spectrum (Table 1), as described above, **1** was triterpenoidal glycosides having two glucosamine derivatives. The sugar moieties were determined as two 6-*O*-methyl-*N*-acetyl-D-glucosamines from the analysis of HMBC correlations of **1** (Fig. 5) from 2-H of sugar moieties (2H, δ 3.76) to two acetyl carbonyls (δ 173.6 and 173.9) and those from 6-H of sugar moieties (4H, δ 3.55) to two methyl carbons (δ 59.9). The position of sugar moieties connecting to the aglycon part was also determined at C-4 and C-23, since the HMBC correlation between an anomeric proton (δ 5.16) and C-4 (δ 80.3) and that between another anomeric proton (δ 5.24) and C-23 (δ 80.2) were observed. The glycoside linkanges were determined as α-configuration, from the coupling constants of the anomeric protons (4 Hz for 1'-H and 1"-H). Therefore, the structure of malbrancheoside A (**1**) was confirmed as malbrancheogenin 4,23-bis-*O*-(2-acetylamino-2-deoxy-6- *O*-methyl-α-D-glucopyranoside), except for the stereochemistry of D/E rings (Figure 5).

The molecular formula of malbrancheoside B (**2**), colorless crystalline powder, mp 152°C, $[\alpha]_D^{20}$ +37.4° (MeOH), were confirmed as $C_{36}H_{60}O_{10}$ by CI-MS [*m/z* 653.4271 $(M+H)^+$]. Since **2** showed greenish brown coloration by anisaldehyde-sulfuric acid reagent and an anomeric protons [δ 4.84 (bs)] in the $^1$H-NMR spectrum (Table 1), malbrancheosides B (**2**) should be monoglycoside. Acid hydrolysis of **2** gave two triterpenoidal genins, malbrancheogenin (**5**) and pseudomalbrancheogenin (**6**), and D-mannose. D-Mannosyl moiety was also determined from the analysis of the NOESY spectrum of **2** (Figure 7). The genuine genin of **2** was determined as **5**, from the analysis of the HMBC correlations in **2** (Figure 6). The

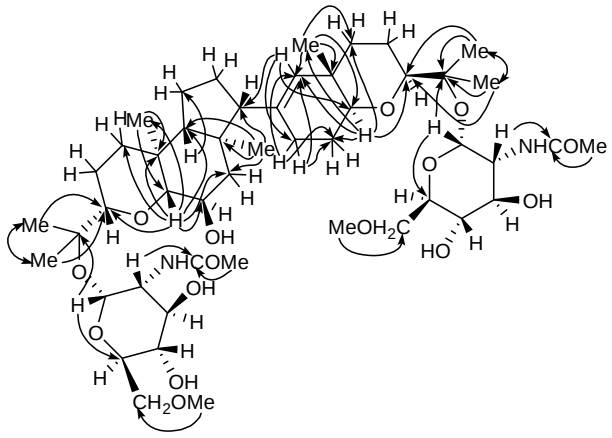

Figure 5 HMBC Correlations of malbrancheoside A (**1**)

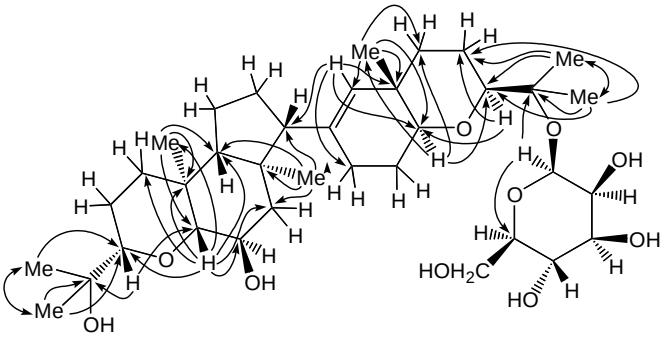

Figure 6 HMBC Correlations of malbrancheoside B (**2**)

$^1$H-NMR spectrum of the aglycon part in **2** was similar to that in **1**, except for the upfield shift of C-4 (δ 80.3 for **1**, δ 73.9 for **2**). The HMBC correlation from the anomeric proton of D-mannose [δ 4.84 (brs)] to C-23 (δ 80.3) was observed. These results revealed that the connecting position of the sugar moiety was at C-23. The glycoside linkanges (α or β) could not be determined from the coupling constants of the anomeric proton, since the coupling constant of the anomeric proton is usually around 1 Hz for both α- and β-D-mannose residues. The chemical shift of the anomeric proton and the $^1J_{C,H}$ of the anomeric carbon are δ 5.10-5.56 and 164-166 Hz, respectively, for α-glycoside and δ 4.62-5.00 and 153-156 Hz, respectively, for β-glycoside in the case of D-mannose.[6] From the chemical shift of the anomeric proton (δ 4.84) and the $^1J_{C,H}$ of the anomeric carbon (155 Hz) for the sugar moiety in **2**, the glycosidic bond of **2** was determined as β-configuration. The structure of malbrancheoside B (**2**) was consequently confirmed as malbrancheogenin 23-*O*-β-D-mannopyranoside, except for the stereochemistry of D/E rings (Figure 6).

The molecular formulae of C (**3**), colorless crystalline powder, mp 168°C, $[\alpha]_D^{20}$ +120.8° (MeOH) and D (**4**), colorless crystalline powder, mp 157°C, $[\alpha]_D^{20}$ +110.5° (MeOH), were confirmed as $C_{47}H_{78}N_2O_{15}$ and $C_{45}H_{75}NO_{15}$, respectively, by CI and positive FT-ESI-MS [*m/z* 933.5280 $(M+Na)^+$ for **3**, and *m/z* 892.5028 $(M+Na)^+$ and *m/z* 870.5210 $(M+H)^+$ for **4**]. Since **3** and **4** showed greenish brown coloration by anisaldehyde-sulfuric acid reagent and two anomeric protons each [δ 5.17 (d) and 5.24 (d) for **3**, δ 4.87 (brs) and 5.12 (d) for **4**] in the $^1$H-NMR spectrum (Table 1), malbrancheosides C (**3**) and D (**4**) should be diglycosides. Acid hydrolysis of **3** and **4** gave two genins, malbrancheogenin (**5**) and pseudomalbrancheogenin (**6**), which were identical with the genins derived from malbrancheosides A (**1**) and B (**2**). Since the $^1$H-NMR signals (Table 1) of the genin part in **3** and **4** were almost superimposable to those in **1** and **2**, the genin of **3** and **4** should be the same genin, malbrancheogenin (**5**), as that of **1** and **2**. This result was also consistent with the analysis of the HMBC correlations of **3** and **4** (Figures 8 and 9). The molecular formula ($C_{47}H_{78}N_2O_{15}$) of malbrancheoside C (**3**) suggested the loss of a methyl group

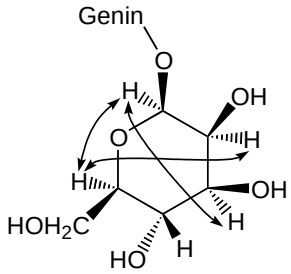


Figure 7 NOE Correlations of sugar moiety in malbrancheoside B (**2**)

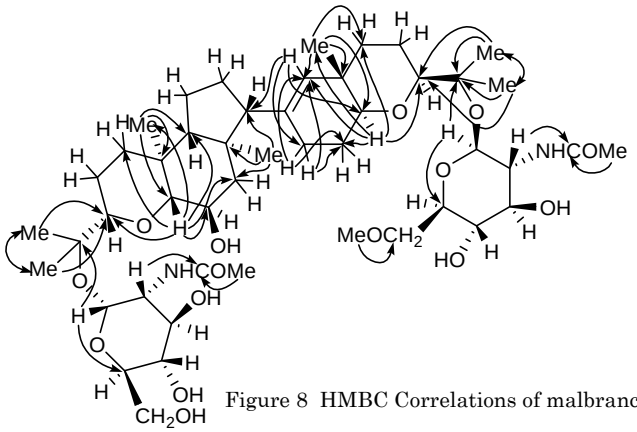


Figure 8 HMBC Correlations of malbrancheoside C (**3**)

from that of **1** ($C_{48}H_{80}N_2O_{15}$). The $^1$H-NMR spectra (Table 1) showed only one methyl signal ($\delta_C$ 59.9, $\delta_H$ 3.33) in **3** instead of two methyl signals ($\delta_C$ 59.9, $\delta_H$ 3.33) in **1**. It is assumed that **3** would be a monodemethyl compound of one of 6-O-methyl-*N*-acetylglucosamine moieties of **1**. The sugar residues were detertemined as *N*-acetyl-D-glucosamine and 6-*O*-methyl-*N*-acetyl-D-glucosamine from the $^1$H- and $^{13}$C-NMR spectra and the analysis of the NOESY spectrum of **3** (Figure 10). The position of sugar moieties was connected at C-4 and C-23 of the aglycon, as same as the case of **1**, from the HMBC correlations (Figure 8) between the anomeric proton [δ 5.17 (d)] of *N*-acetylglucosamine and C-4 (δ 80.2), and another anomeric proton [δ 5.24 (d)] of 6-*O*-methyl-*N*-acetylglucosamine and C-23 (δ 80.1) were observed. The glycoside linkanges were determined as an α-configuration, from the coupling constants of the anomeric protons (3 Hz for 1'-H, 4 Hz for 1"-H). The structure of malbrancheoside C (**3**) was consequently confirmed as malbrancheogenin 4-*O*-(2-acetylamino-2-deoxy-α-D-glucopyranosyl)-23-*O*-(2- acetylamino-2-deoxy-6-*O*-methyl-α-D-glucopyranoside), except for the stereochemistry of D/E rings.

The molecular formula ($C_{45}H_{75}NO_{15}$) of malbrancheoside D (**4**) suggested the loss of a methyl group and the replacement of an acetylamino residue to a hydroxyl group from that of **1** ($C_{48}H_{80}N_2O_{15}$). The $^1$H-NMR spectra (Table 1) showed only one methoxy signal ($\delta_C$ 59.9, $\delta_H$ 3.34) and one acetyl methyl signal ($\delta_C$ 20.3, $\delta_H$ 1.91) in **4** instead of two methoxy signals ($\delta_C$ 59.9, $\delta_H$ 3.33) and acetyl methyl signals ($\delta_C$ 23.0, $\delta_H$ 1.88 and 1.93) in **1**. The sugar moieties was determined as D-mannose and 6-*O*-methyl-*N*-acetyl-D-glucosamine from the $^1$H- and $^{13}$C-NMR spectra and the analyses of the HMBC and NOESY spectra of **4** (Figures 9 and 11). The position of sugar moieties was connected at C-4 and C-23 of the aglycon, as same as the case of **1**, from the HMBC correlations (Figure 9) between the anomeric proton [δ 4.87 (brd)] of D-mannose and C-4 (δ 80.2), and the anomeric proton [δ 5.12 (d)] of6-*O*-methyl-*N*-acetyl-D-glucosamine and C-23 (δ 80.2) were observed. The glycoside linkange of 6-*O*-methyl-*N*-acetyl-D-glucosamine was determined as an α-configuration, from the coupling constant of

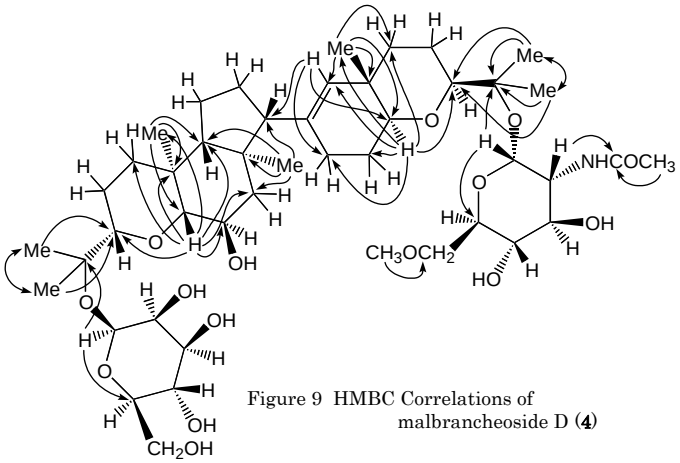

Figure 9 HMBC Correlations of malbrancheoside D (**4**)

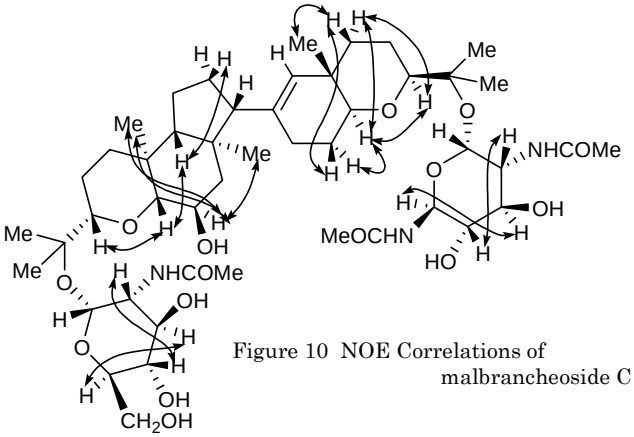

Figure 10 NOE Correlations of malbrancheoside C (**3**)

the anomeric proton (4 Hz for 1”-H), whereas the glycoside linkange of D-mannose was determined as β-configuration from the chemical shift of the anomeric proton (δ 4.87) and the $^{1}J_{C,H}$ of the anomeric carbon (154 Hz),[6] as same as that of **2**. The structures of D (**4**) was consequently confirmed as malbrancheogenin 4-*O*-β-D-mannopyranosyl)-23-*O*-(2-acetylamino-2-deoxy-6-*O*-methyl-α-D-glucopyranoside), except for the stereochemistry of D/E rings.

The incorporated pattern (Figure12) of pseudomalbrancheogenin (**6**) from the incorporation experiments of 2-$^{13}$C-acetate suggested that malbrancheosides A (**1**) – D (**4**) might be triterpenoidal glycosides.

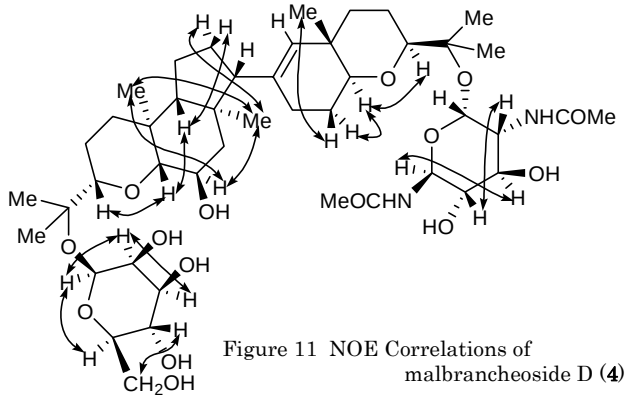

Figure 11 NOE Correlations of malbrancheoside D (**4**)

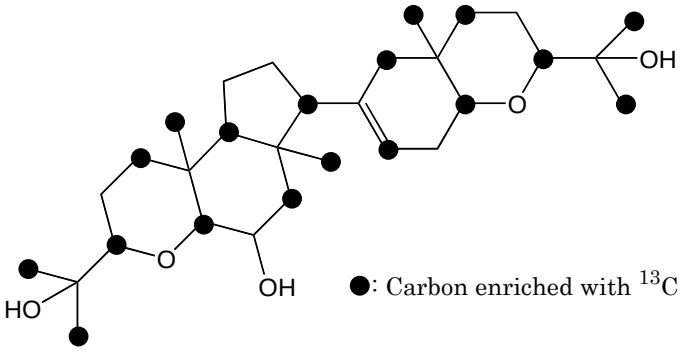


Figure 12 Incorporation of sodium 2-$^{13}$C-acetate in pseudomalbrancheogenin (**6**)

The acetone extract of *M. filamentosa* IFM41300 showed characterisitically the antifungal activity against *C. neoformans*, but the metabolites ever isolated from this fungus showed no antifungal activity. Since the activity was localized in the water extract after the partition with water and ethyl acetate, the isolation of the active compounds is now undergoing.

Malbrancheosides A (**1**) – D (**4**) are the first examples of triterpenoidal glycosides, which have 6-*O*-mehtyl-*N*-acetyl-D-glucosamine residue and/or D-mannose residue, from the fungal sources. Triterpenes having the same carbon skeleton as the genin of malbrancheosides were isolated as abdinols A (**7**) and B (**8**), originally isolated from marine sponge *Ptilocaulis spiculifer* of the family Axinellidae,[7,8]

as a new type of triterpene. It is interesting that triterpenes having the same new carbon skeleton were isolated from both fungi (**1** – **4**) and marine sponge (**7**, **8**).

## EXPERIMENTAL

**General.** Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-1000 spectrometer. EI- or CI- and FAB-MS were taken with a JEOL JMS-600W spectrometer and JEOL JMS-SX 102 spectrometer, respectively. ESI- and FT-ESI-MS were taken with a Waters Micromass ZQ spectrometer and a Thermo Fisher LTQ Orbitrap spectrometer, respectively. UV and IR spectra were recorded on a Hitachi U-3210 spectrophotometer or Amersham Bioscience Ultraspec2100pro spectrophotometer and a JASCO IR-810 spectrophotometer, respectively. $^{1}$H- and $^{13}$C-NMR spectra were recorded on a JEOL JNM Lambda-500 spectrometer (500.00 MHz for $^{1}$H, 125.43 MHz for $^{13}$C) and/or Bruker AV-400 spectrometer (400.13 MHz for $^{1}$H, 100.61 MHz for $^{13}$C), using tetramethylsilane as an internal standard. Coupling patterns are indicated as follows: singlet = s, doublet = d, triplet = t, quartet = q, multiplet = m, and broad = br. *J*-Values are in Hz. Centrifuged partition chromatography (CPC) was performed on a SANKI CPC LLB-M system with a SHODEX DS-4 pump in the ascending method (800 rpm, 5 ml/min at a flow rate). Low pressure liquid chromatography (LPLC) was performed on a Superior Elecronic SS50-1296 pump using a glass column (25 i.d.×300 mm) packed with Wako CQ-3 (30-50 μm) or Yamazen SI-40B (26 i.d. ×300 mm) prepacked column. High-performance liquid chromatography (HPLC) was performed on a Senshu SSC-3160 pump or Biotech Intelligent 302 pump with the flow rate of 4 ml/min using a Senshu Pak PEGASIL Silica 60-5 (10 i.d.×300 mm) or GL Inertsil SIL100A (10 i.d.×250 mm) prepacked column and using Senshu Pak PEGASIL ODS (10 i.d.×250 mm) or GL Inertsil ODS-P (10 i.d.×250 mm) prepacked column, equipped with a Shimamura YRU-883 RI-UV monitor for preparative purpose, whereas HPLC was performed on a JASCO PU-2080plus pump with the flow rate of 1 ml/min using GL Inertsil ODS-3 (4.6 i.d.×250 mm) prepacked column, equipped with a JASCO MD-2010 plus multiwave detector for analytic purpose. TLC was conducted on pre-coated Kieselgel 60 $F_{254}$ plates (Art. 5715; Merck). Spots on TLC were detected by their absorption under UV light, and/or by spraying anisaldehyde-sulfuric acid reagent and then heating.[9]

**Isolation of Malbrancheosides A (1) – D (4) from *Malbranchea filamentosa.*** *M. filamentosa*, strain IFM 41300 was cultivated on moistured rice (560 g) using 4 Roux flasks at 25 °C for 21 d. The cultivated rice was extracted with acetone and the solvent was evaporated *in vacuo* . The residue was suspended in $H_2O$ and extracted with AcOEt. The evaporated residue was extracted with heaxane, benzene, $CH_2Cl_2$,

MeOH, in turn, and each fraction was evaporated *in vacuo*. The hexane fraction was chromatographed on silica gel [hexane-acetone (5:2)] followed by the repeated purification of LPLC [hexane-acetone (5:2)] and then HPLC [hexane-acetone (5:1)] to give depsipeptide, bassidinolide (80 mg).[10] The MeOH fraction (1.5 g) was chromatographed on DIANION HP20 with $H_2O$, 20% MeOH, 40% MeOH, 80% MeOH, MeOH, acetone, in turn. The evaporated acetone eluate was purified by CPC using the solvent system of $CH_2Cl_2$-MeOH-$H_2O$ (5:6:4) to obtain 7 fractions. The fifth fraction (130 mg) was purified by HPLC on ODS (85% MeOH) to obtain malbrancheosides A (**1**) (36 mg) and B (**2**) (30 mg). The third fraction (100 mg) was repeatedly purified by HPLC on ODS [80 % MeOH and MeCN-MeOH- $H_2O$ (7:7:6)] to obtain malbrancheosides C (**3**) (18 mg) and D (**4**) (20 mg).

Malbrancheoside A (**1**): Colorless crystalline powder, mp 185 °C (from AcOEt-$CH_2Cl_2$). Anisaldehyde-sulfuric acid reagent: greenish brown. $[\alpha]_D^{20}$ +120.5° (*c* 1.02, MeOH). Positive FAB-MS *m/z*: 947.5 [$(M+Na)^+$]. *Anal*. Calcd for $C_{48}H_{80}N_2O_{15}$: C, 61.26; H, 8.76; N, 2.97. Found: C, 61.10; H, 8.52; N, 3.07. UV $\lambda_{max}^{EtOH}$ nm (log ε): end absorption. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400 (OH), 1660 (CONH). The $^1H$- and $^{13}C$-NMR data were summarized in Table 1.

Malbrancheoside B (**2**): Colorless crystalline powder, mp 152 °C (from MeOH). Anisaldehyde-sulfuric acid reagent: greenish brown. $[\alpha]_D^{20}$ +37.4° (*c* 0.50, MeOH). Positive ESI-MS *m/z*: 653 [$(M+H)^+$, 100]. CI-MS *m/z*: 653.4271 [$(M+H)^+$, 653.4264 for $C_{36}H_{61}O_{10}$, 47]. UV $\lambda_{max}^{EtOH}$ nm (log ε): end absorption. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400 (OH). The $^1H$- and $^{13}C$-NMR data were summarized in Table 1.

Malbrancheoside C (**3**): Colorless crystalline powder, mp 168 °C (from MeOH). Anisaldehyde-sulfuric acid reagent: greenish brown. $[\alpha]_D^{20}$ +120.8° (*c* 0.50, MeOH). Positive ESI-MS *m/z*: 933 [$(M+Na)^+$, 100]. Positive ESI-TOF-MS *m/z* : 933.5280 [$(M+Na)^+$, 933.5300 for $C_{47}H_{78}N_2O_{15}Na$, 100]. UV $\lambda_{max}^{EtOH}$ nm (log ε): end absorption. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400 (OH), 1650 (CONH). The $^1H$- and $^{13}C$-NMR data were summarized in Table 1.

Malbrancheoside D (**4**): Colorless crystalline powder, mp 157 °C (from MeOH). Anisaldehyde-sulfuric acid reagent: greenish brown. $[\alpha]_D^{20}$ +110.5° (*c* 0.50, MeOH). Positive ESI-MS *m/z* : 892 [$(M+Na)^+$, 100], 870 [$(M+H)^+$, 30]. Positive ESI-TOF-MS *m/z*: 892.5028 [$(M+Na)^+$, 892.5034 for $C_{45}H_{75}NO_{15}Na$, 100], 870.5210 [$(M+H)^+$, 870.5215 for $C_{45}H_{76}NO_{15}$, 29]. UV $\lambda_{max}^{EtOH}$ nm (log ε): end absorption. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400 (OH), 1650 (CONH). The $^1H$- and $^{13}C$-NMR data were summarized in Table 1.

**Acid Hydrolysis of Malbrancheoside A (1).** Malbrancheoside A (**1**) (80 mg) was dissolved in 4 M HCl (5 mL) and the solution was stirred at 90°C for 3 h. After cooling, the reaction mixture was extracted with $CHCl_3$ and the organic layer was dried over $Na_2SO_4$ and then concentrated *in vacuo*. The residue was purified by LPLC on silica gel with $CHCl_3$-acetone (3:1) to give two genins, malbrancheogenin (**5**) (21

mg) and pseudomalbrancheogenin A (**6**) (13 mg). The aqueous layer obtained after extraction of the reaction mixture was chromatographed by HPLC on ODS with 5% MeCN to give 6-*O*-methyl-D-glucosamine hydrochloride (12 mg).

Malbrancheogenin (**5**): Colorless crystalline powder, mp 183-184 °C (from AcOEt-$CH_2Cl_2$). $[\alpha]_D^{20}$ +49.5° (*c* 1.15, $CHCl_3$). EI-MS *m/z* (%): 490.3661 ($M^+$, 490.3658 for $C_{30}H_{50}O_5$, 34), 472 [$(M-H_2O)^+$, 13], 454 [$(M-2H_2O)^+$, 13], 431 [$(M-3H_2O-CH_3)^+$, 47], 413 (31), 395 (17). UV $\lambda_{max}^{EtOH}$ nm (log ε): end absorption. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3360 (OH). The $^1$H- and $^{13}$C-NMR data were summarized in Table 2.

Pseudomalbrancheogenin (**6**): Colorless crystalline powder, mp 227-228°C (from AcOEt); $[\alpha]_D^{20}$ +15.1° (*c* 0.80, $CHCl_3$); EI-MS *m/z* (%): 490.3677 ($M^+$, 490.3658 for $C_{30}H_{50}O_5$, 47), 472 [$(M-H_2O)^+$, 87], 454 [$(M-2H_2O)^+$, 24], 439 [$(M-2H_2O-CH_3)^+$, 16], 431 [$(M-3H_2O-CH_3)^+$, 56], 413 (39), 395 (33). UV $\lambda_{max}^{EtOH}$ nm (log ε): end absorption. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3420 (OH). The $^1$H- and $^{13}$C-NMR data were summarized in Table 2.

6-*O*-Methylglucosamine Hydrochloride: Colorless amorphous powder. Ninhydrin reagent: pink. $[\alpha]_D^{20}$ +62.9$^o$ (*c* 0.83, $H_2O$). Positive ESI-MS *m/z* (%) 194 [$(M-Cl)^+$, 77], 176 [$(M-Cl-H_2O)^+$, 100]. $^1$H-NMR δ ($CD_3OD$): α-anomer: 3.05 (1H, dd, *J* = 9.8, 3.4 Hz, 2-H), 3.38 (1H, dd, *J* = 9.8, 9.8 Hz, 4-H), 3.40 (3H, s, 6-*O*-Me), 3.81 (1H, dd, *J* = 9.8, 9.8 Hz, 3-H), 3.65 (2H, m, 6-$H_2$), 3.91 (1H, m, 5-H), 5.30 (1H, d, *J* = 3.4 Hz, 1-H);β-anomer: 2.78 (1H, dd, *J* = 9.4, 8.2 Hz, 2-H), 3.71 (1H, m, 4-H), 3.40 (3H, s, 6-*O*-Me), 3.50 (1H, dd, *J* = 9.4, 9.4 Hz, 3-H), 3.60 (2H, m, 6-$H_2$), 3.43 (1H, m, 5-H), 4.74 (1H, d, *J* = 8.2 Hz, 1-H). $^{13}$C NMR δ ($CD_3OD$): α-anomer: 56.2 (2-C), 59.6 (6-*O*-Me), 71.4 (3-C), 71.9 (4-C), 72.1 (5-C), 72.7 (6-C), 90.8 (1-C): β-anomer: 58.7 (2-C), 59.5 (6-*O*-Me), 72.0 (6-C), 72.8 (4-C), 74.1 (3-C), 77.1 (5-C), 94.7 (1-C).

**Acid Treatment of Malbrancheogenin (5) and Pseudomalbrancheo-genin (6).** Each of malbrancheogenin A (**5**) (5 mg) and pseudomalbrancheogenin (**6**) (5 mg) was dissolved in 10% aqueous HCl containing 50% EtOH (1 mL) and the solution was stirred at 90-100 $^o$C for 1 h. After cooling, the reaction mixture was extracted with $CHCl_3$ and the organic layer was dried over $Na_2SO_4$ and then concentrated *in vacuo*. The residue was analyzed by TLC with $CHCl_3$-acetone (3:1). Compounds **5** and **6** were obtained from **5**, whereas compound **6** was recovered in the above reaction.

**Synthesis of (*S*)- and (*R*)-MTPA Esters of Pseudomalbrancheogenin (6)**. (*S*)- or (*R*)-MTPA chloride (8 mg each) was added to a solution of pseudomalbrancheogenin (**6**) (25 mg) in pyidine (1 mL). The reaction mixture was kept at room temperature for 24 h, and then evaporated the solvent. The residue was purified by HPLC on silica gel [hexane-acetone (3:1)] to afford the (*R*)- or (*S*)-MTPA ester of **6** [10 mg for (*S*), 11 mg for (*R*)].

(*S*)-MTPA ester of **6**: Colorless amorphous powder. $^1$H-NMR ($CDCl_3$,) data were summarized in Table 2.
(*R*)-MTPA ester of **6**: Colorless amorphous powder. $^1$H-NMR ($CDCl_3$,) data were summarized in Table 2.

**Acid Hydrolysis of Malbrancheoside B (2).** Malbrancheoside B (**2**) (20 mg) was dissolved in 4 M HCl (10 mL) and the solution was stirred at 90 °C for 3 h. After cooling, the reaction mixture was extracted with $CHCl_3$ and the organic layer was dried over $Na_2SO_4$ and then concentrated *in vacuo*. The residue was purified by LPLC on silica gel with $CHCl_3$-acetone (5:1) to give two genins, malbrancheogenin (**5**) (5 mg) and pseudomalbrancheogenin (**6**) (2 mg). The aqueous layer obtained after extraction of the reaction mixture was chromatographed by HPLC on ODS with 5% MeCN to give D-mannose (2 mg).
D-Mannose: Colorless amorphous powder. $[\alpha]_D^{20}$ +15.2° (*c* 0.21, $H_2O$). Positive ESI-MS *m/z* (%) 181 [$(M+H)^+$, 100]. $^1$H-NMR δ ($CD_3OD$): an anomer: 3.27 (1H, m, 5-H), 3.44 (1H, dd, *J* = 10, 10 Hz, 4-H), 3.48 (1H, m, 3-H), 3.50 (1H, brd, *J* = 4 Hz, 2-H), 3.54 (2H, m, 6-$H_2$), 4.87 (1H, brs, 1-H); another anomer: 3.02 (1H, m, 5-H), 3.36 (1H, dd, *J* = 10, 9 Hz, 4-H), 3.48 (1H, brd, *J* = 4 Hz,, 2-H), 3.52 (1H, m, 3-H), 3.55 (2H, m, 6-$H_2$), 4.68 (1H, brs, 1-H).

**Acid Hydrolysis of Malbrancheosides C (3) and D (4).** Malbrancheosides C (**3**) and D (**4**) (20 mg) were each dissolved in 4 M HCl (10 mL) and the solution was stirred at 90 °C for 3 h. After cooling, the reaction mixture was extracted with $CHCl_3$ and the organic layer was dried over $Na_2SO_4$ and then concentrated *in vacuo*. The residue was purified by LPLC on silica gel with $CHCl_3$-acetone (5:1) to give two genins, malbrancheogenin (**5**) (5 mg for **3**, 3 mg for **4**) and pseudomalbrancheogenin (**6**) (2 mg for **3**, 4 mg for **4**).

**Incorporation of Sodium 2-$^{13}$C-Acetate in Pseudomalbrancheogenin (5).** *M. filamentosa*, strain IFM 41300 was cultivated on shaking (150 rpm) in 3% powdered rice broth (5 L) using 10 flasks at 25° for 8 d. After the addition of $^{13}CH_3CO_2Na$ (1.5 g) dissolved in 100 ml $H_2O$, this fungus was cultivated on shaking (150 rpm) at 25° for further 4 d. The cultured mixture was freezed drying and then extracted with MeOH. The evaporated extract (500 mg) was hydrolysed by 4 M HCl at 90° for 5 h and then extracted with $CHCl_3$. The evaporated extract (280 mg) was purified by LPLC [$CHCl_3$-acetone (3:1)] and then by HPLC [$CHCl_3$-acetone (5:1)] to give $^{13}$C enriched pseudomalbrancheogenin (**6**) (2 mg) in addition of trace of malbrancheogenin (**5**). The results were summarized in Figure 12.

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