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CHEMICAL CONSTITUENTS OF THE FUNGUS *HERICIUM ERINACEUS* SH1

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SUMMARY

*By combined chromatographies, cerebroside B (**1**), hericenone D (**2**), ergosterol (**3**), ergosterol peroxide (**4**) and β-adenosine (**5**) were isolated from the dried finished product of the medicinal mushroom Hericium erinaceus SH1. Their structures were determined from the physico-chemical and spectroscopic evidence. This is the first report of **1** from this fungus.*

Keywords: *Hericium erinaceus SH1, cerebroside B, hericenone D.*

I - INTRODUCTION

Recently, studies on medicinal fungi increase rapidly because of their high nutrition value and various biological activities. In our program, the investigation on bioactive compounds and bio-assay guided chemical fractionation of finished products of fungi as well as their preparations grown on the liquid media has been doing. The medical mushroom *Hericium erinaceus* has been known as a very interesting in the medicinal use with the bioactive compounds. This paper reports the isolation and structural determination of cerebroside B (**1**), hericenone D (**2**), ergosterol (**3**), ergosterol peroxide (**4**), and β-adenosine (**5**) from the methanol extract of the finished products of this fungus.

II - EXPERIMENT

1. General experimental procedures

The Electronspray Ionization (ESI) mass spectrum was obtained using an AGILENT 1100 LC-MSD Trap spectrometer. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded on Bruker AM500 FT-NMR spectrometer. Chemical shifts are referenced to δ using tetramethylsilan (TMS) as an internal standard. Column chromatography (CC) was performed on silica gel 230 - 400 mesh (0,040-0,063 mm, Merck) or YMC RP-18 resins (30 - 50 μm, FuJisilisa Chemical Ltd.). Thin layer chromatography (TLC) was performed on DC-Alufolien 60 F_{254} (Merck 1.05715) or RP_{18} F_{254s} (Merck) plates.

2. Fungus material

The finished products of *Hericium erinaceus* SH1 have been chosen for chemical study.

3. Extraction and isolation

The dried finished products of the fungus *Hericium erinaceus* SH1 (2 l) was boiled in distilled water for 1 hour, then extracted with methanol, filtered and concentrated in vacuum to give the methanol extract (15 g), which was suspended in water and then extracted in turn with chloroform and ethyl acetate to give the chloroform (5 g), ethyl acetate (7 g) and water

(3 g) extracts. The chloroform extract (5 g) was then chromatographed on a silica gel column eluted with chloroform/methanol as eluent increasing concentration of methanol to give five fractions C1 (1.0 g), C2 (0.5 g); C3 (0.7 g), C4 (0.6 g) and C5 (0.2 g). The C1 fraction (1.0 g) was repeatedly chromatographed on silica gel column to yield **2** (10 mg), **3** (20 mg) and **4** (7mg) as white crystals. The ethyl acetate extract (7 g) was separated by combination of normal and reverse phase column chromatographies to obtain **1** (15 mg) and **5** (30 mg).

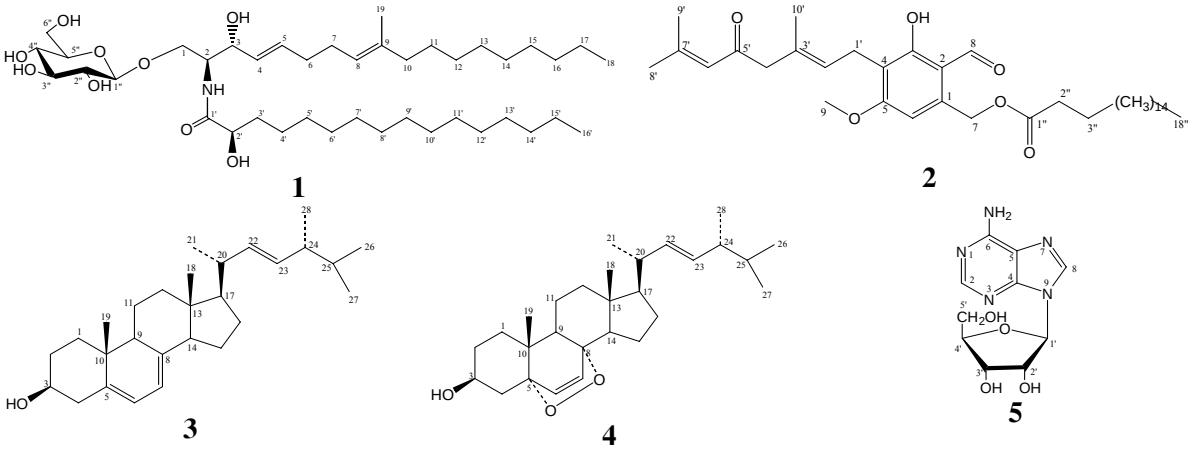

Fig. 1: The structures of **1 - 5**

Cerebroside B (**1**): White amorphous powder, mp 180 - 190ºC, negative ESI-MS *m/z*: 726.6 $[M-H]^-$. Positive ESI-MS *m/z*: 750.5 $[M+Na]^+$, 728.3 $[M+H]^+$, 710.5 $[M-H_2O+H]^+$, 548.3 $[M\text{-glucose}+H]^+$ ($C_{41}H_{77}NO_9$, M = 727); ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD), see table 1.

Hericenone D (**2**): White crystals, mp 41 - 43ºC, Positive ESI-MS *m/z*: 599.2 $[M + H]^+$. Negative ESI-MS *m/z*: 597.4 $[M - H]^-$ ($C_{37}H_{58}O_6$, M = 598); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.53 (1H, s, H-6), 5.32 (2H, s, H-7), 10.11 (1H, s, H-8), 3.91 (3H, s, H-9), 3.40 (2H, d, *J* = 7.5 Hz, H-1'), 5.32 (1H, d, *J* = 7.5 Hz, H-2'), 3.00 (2H, s, H-4'), 6.09 (1H, t, *J* = 1.0, 1.5 Hz, H-6'), 1.84 (3H, d, *J* = 1.5 Hz, H-8'), 2.12 (3H, d, *J* = 1.0 Hz, H-9'), 1.78 (3H, d, *J* = 0.5 Hz, H-10'), 2.33 (2H, d, *J* = 7.5 Hz, H-2"), 1.62 (2H, m, H-3"), 1.25 (26H, m, H-4"-17"), 0.88 (3H, m, H-18") and 12.37 (1H, s, OH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 138.70 (C-1), 112.91 (C-2), 162.94 (C-3), 117.33 (C-4), 163.49 (C-5), 105.55 (C-6), 62.90 (C-7), 193.10 (C-8), 55.93 (C-9), 21.62 (C-1'), 126.27 (C-2'), 130.35 (C-3'), 55.55 (C-4'), 199.54 (C-5'), 122.85 (C-6'), 155.42 (C-7'), 27.66 (C-8'), 20.67 (C-9'), 16.41 (C-10'), 173.20 (C-1"), 34.25 (C-2"), 24.89 (C-3"), 29.13-31.94 (C-4"-17"), and 14.12 (C-18").

Ergosterol (**3**): White needles, mp 168ºC, Positive ESI-MS *m/z*: 397.3 $[M + H]^+$ ($C_{28}H_{44}O$, M = 396); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.25 (1H, m, H-1_a), 1.75 (1H, m, H-1_b), 1.50 (1H, m, H-2_a), 1.78 (1H, m, H-2_b), 3.63 (1H, m, H-3), 2.28 (2H, t, *J* = 13.5 Hz, H-4), 5.56 (1H, dd, *J* = 2.0, 5.5 Hz, H-6), 5.38 (1H, m, H-7), 1.97(1H, m, H-9), 1.65 (1H, m, H-11), 1.34 (1H, m, H-12_a), 1.90 (1H, m, H-12_b), 1.92 (1H, m, H-14), 1.67 (1H, m, H-15), 1.28 (1H, m, H-16_a), 1.74 (1H, m, H-16_b), 1.25 (1H, m, H-17), 0.63 (3H, s, H-18), 0.95 (3H, s, H-19), 2.05 (1H, m, H-20), 1.03 (3H, d, *J* = 6.5 Hz, H-21), 5.17 (1H, dd, *J* = 8.5, 15.5 Hz, H-22), 5.22 (1H, dd, *J* = 8.5, 15.5 Hz, H-23), 1.76 (1H, m, H-24), 1.38 (1H, m, H-25), 0.84 (3H, d, *J* = 6.5 Hz, H-26), 0.82 (3H, d, *J* = 6.5 Hz, H-27) and 0.91 (3H, d, *J* = 6.5 Hz, H-28); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 38.39 (C-1), 32.02 (C-2), 70.47 (C-3), 40.83 (C-4), 141.36 (C-5), 119.60 (C-6), 116.30 (C-7),

Table 1: NMR data of **1** and reported cerebrosides

C	δ_C *,a	δ_C #,b	**1**		
			δ_C b,c	δ_H b,d (multiplicity, J in Hz)	HMBC (H→C)
1	70.1 t	70.3 t	70.13 t	3.73 (m)/4.13 (m)	C-2, 1’
2	54.5 d	55.1 d	55.01 d	4.01 (m)	C-1, 3
3	72.3 d	73.4 d	73.29 d	4.16 (m)	C-1, 2, 4, 5
4	131.8 d	131.6 d	131.51 d	5.50 (dd, 7.0, 15.5)	C-2, 3, 6
5	132.3 d	135.1 d	135.04 d	5,76 (dt, 6.0, 15.5)	C-3, 6, 7
6	33.0 t	34.9 t	34.02 t	2.07 (m)	C-5, 7
7	28.1 t	29.2 t	29.09 t	2.10 (m)	C-6, 8
8	124.1 d	125.3 d	125.22 d	5.17 (t, 7.0)	C-6, 7, 10, 19
9	136.0 s	137.3 s	137.18 s	-	
10	39.9 t	41.3 t	41.18 t	2.00 (t, 6.5)	C-8, 9, 11, 19
11	29.5-29.9 t	29.5 t	29.52 t	1.43 (m)	
12-15	29.5-29.9 t	30.8-31.4 t	30.79-31.24 t	1.31 (br s)	
16	32.0 t	33.6 t	33.49 t	1.31 (br s)	
17	22.9 t	24.2 t	24.15 t	1.33	
18	14.2 q	15.0 q	14.86 q	0.92 (t, 7.0)	C-16, 17
19	16.0 q	16.7 q	16.53 q	1.62 (s)	C-8, 9, 10
1’	-	177.7 s	177.60 s	-	
2’	72.4 d	73.6 d	73.49 d	4.01	C-1’, 3’, 4’
3’	35.6 t	36.4 t	36.28 t	1.57 (m)/1.74 (m)	C-1’
4’	25.8 t	26.7 t	26.57 t	1.44 (m)	
5’-13’	29.5-29.9 t	-	30.79-31.24 t	1.31 (br s)	
14’	32.0 t	33.6 t	33.49 t	1.31 (br s)	
15’	22.9 t	24.2 t	24.15 t	1.33	
16’	14.2 q	15.0 q	14.86 q	0.92 (t, 7.0)	C-14’, 15’
1”	105.6 d	105.2 d	105.13 d	4.29 (d, 7.5)	C-1
2”	75.0 d	75.5 d	75.39 d	3.22	C-1”
3”	78.4 d	78.5 d	78.31 d	3.37 (m)	
4”	71.4 d	72.0 d	71.97 d	3.31	
5”	78.5 d	78.5 d	78.38 d	3.30	
6”	62.6 t	63.2 t	63.08 t	3.69/3.89 (dd, 2.0, 1.5)	

*δ_C of cerebroside B [3], #δ_C of catacerebroside A [4], [a]recorded in pyridine-d_5, [b]recorded in CD_3OD-d_4, [c]125 MHz, [d]500 MHz.

139.80 (C-8), 46.27 (C-9), 37.04 (C-10), 21.13 (C-11), 39.11 (C-12), 42.41 (C-13), 54.57 (C-14), 23.01 (C-15), 28.29 (C-16), 55.76 (C-17), 12.06 (C-18), 16.30 (C-19), 40.82 (C-20), 21.11 (C-21), 135.60 (C-22), 131.99 (C-23), 42.85 (C-24), 33.10 (C-25), 19.66 (C-26), 19.96 (C-27) and 17.62 (C-28).

Ergosterol peroxide (**4**): White needles, mp 181 - 183ºC, Positive ESI-MS *m/z*: 429.3 $[M + H]^+$ ($C_{28}H_{44}O_3$, M = 426); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.56 (1H, m, H-1_a), 1.85 (1H, m, H-1_b), 1.72 (1H, m, H-2_a), 1.96 (1H, m, H-2_b), 3.97 (1H, m, H-3), 1.27 (1H, m, H-4_a), 1.97 (1H, m, H-4_b), 6.24 (1H, d, *J* = 8.5 Hz, H-6), 6.50 (1H, d, *J* = 8.5 Hz, H-7), 1.51 (1H, m, H-9), 1.41 (1H, m, H-11_a), 1.62 (1H, m, H-11_b), 1.55 (1H, m, H-12_a), 2.11 (1H, m, H-12_b), 1.58 (1H, m, H-14), 1.25 (1H, m, H-15_a), 1.53 (1H, m, H-15_b), 1.37 (1H, m, H-16_a), 1.78 (1H, m, H-16_b), 1.24 (1H, m, H-17), 0.86 (3H, s, H-18), 0.88 (3H, s, H-19), 2.03 (1H, m, H-20), 1.01 (3H, d, *J* = 7.0 Hz, H-21), 5.14 (1H, dd, *J* = 8.5, 15.5 Hz, H-22), 5.22 (1H, dd, *J* = 8.5, 15.5 Hz, H-23), 1.87 (1H, m, H-24), 1.50 (1H, m, H-25), 0.88 (3H, d, *J* = 6.6 Hz, H-26), 0.83 (3H, d, *J* = 6.5 Hz, H-27), and 0.91 (3H, d, *J* = 6.5 Hz, H-28); ^{13}C-NMR (125 MHz, $CDCl_3$): 30.09 (C-1), 34.71 (C-2), 66.49 (C-3), 39.37 (C-4), 82.17 (C-5), 135.22 (C-6), 130.75 (C-7), 79.44 (C-8), 51.12 (C-9), 36.99 (C-10), 20.64 (C-11), 39.91 (C-12), 44.58 (C-13), 51.70 (C-14), 23.42 (C-15), 28.65 (C-16), 56.23 (C-17), 12.88 (C-18), 18.18 (C-19), 39.73 (C-20), 20.89 (C-21), 135.44 (C-22), 132.33 (C-23), 42.79 (C-24), 33.08 (C-25), 19.65 (C-26), 19.96 (C-27), and 17.57 (C-28).

β-Adenosine (**5**): White needles, mp. 234-235ºC, Positive ESI-MS *m/z*: 268 $[M + H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 8.14 (1H, s, H-2), 8.34 (1H, s, H-8), 5.88 (1H, d, *J* = 6.5 Hz, H-1'), 4.61 (1H, dd, *J* = 4.0, 10.5, H-2'), 4.15 (1H, br s, H-3') 3.97 (1H, dd, *J* = 3.5, 6.5 Hz, H-4'), 3.57 (1H, m, H-5'$_a$), 3.68 (1H, m, H-5'$_b$), 7.32 (2H, s, NH_2), 5.42 (1H, OH-2'), 5.17 (1H, d, *J* = 3.0, OH-3'), and 5.43 (1H, OH-5'); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 152.41 (C-2), 149.08 (C-4), 119.37 (C-5), 156.17 (C-6), 139.95 (C-8), 87.95 (C-1'), 73.47 (C-2'), 70.68 (C-3'), 85.92 (C-4'), and 61.69 (C-5').

III - RESULTS AND DISCUSSION

Compound **1** was obtained as white amorphous powder. Its molecular formula was suggested as $C_{41}H_{77}NO_9$ from the ESI-MS (at *m/z* 750.5 $[M + Na]^+$ and *m/z* 728.3 $[M + H]^+$) and NMR spectral data.The ^{1}H-NMR spectrum showed typical signals of a glycosphingolipid with three methyl groups at δ_H 0.92 (6H, t, *J* = 7.0 Hz) and 1.62 (3H, s), overlap signals of two long hydrocarbon chains at δ_H 1.31 (44H, br s), one anomeric proton at δ_H 4.29 (1H, d, *J* = 7.5 Hz, H-1"). The ^{13}C-NMR spectrum showed signals of one carbon bearing to nitrogen atom at δ_C 55.01; three methyl groups at δ_C 16.53, 14.86, and 14.86; four olefinic carbons at δ_C 137.18, 135.04, 131.51 and 125.22; and two carbons connected to oxygen atoms at δ_C 73.49 and 73.29; six signals of a β-D-glucopyranoside unit at δ_C 105.13 (C-1"), 78.38 (C-5"), 78.31 (C-3"), 75.39 (C-2"), 71.97 (C-4") and 63.08 (C6").

The correlation of anomeric proton at δ 4.29 (1H, d, *J* = 7.5 Hz, H-1") with oximethine carbon at δ 70.13 (t, C-1) confirmed the attached position of the sugar unit at C-1. The down field of a singlet signal at δ_H 1.62 (3H) as well as the correlation between this proton to C-8 (δ 125.22) and C-9 (137.18) confirmed that this methyl group attached to one of two double bonds. *Trans* configuration of C-4/C-5 double bond was identified by large vicinal coupling constant (*J* = 15.5 Hz). According to the published papers [1, 2], cerebrosides from fungi usually contain: glucose, sphingoid base [(4*E*,8*E*)-9-methyl-4,8-sphingadienine] and fatty acids which contain 14-18 or 22-26 carbon atoms and a hydroxyl group at C-2 position. Detail analysis of 1D- and 2D-NMR spectra (table 1) confirmed above statement. Furthermore, the ^{13}C-NMR spectral data of **1** was compared to those of cerebroside B [3] and found to match. However, there is small difference of the C-5 chemical shift (changed from δ_C 135.04 to 132.3 ppm) because of the NMR recording solvent. To confirm this, the ^{13}C-NMR spectral data of **1** were recompared to those of a similar compound (catacerebroside A) recorded in the same solvent (CD_3OD) and

found to match completely [4]. Thus, compound **1** was identified as 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*E*)-2-[(2*R*)-2-hydroxyhexadecanoylamino]-9-methyl-4,8-octadecadiene-1,3-diol (cerebroside B), which has been isolated from *Phellinus linteus*, *Pachybasium* sp., *Clitocybe* spp., *Schizophyllum commune*... However, this is the first report of **1** from *Hericium erinaceus*.

Compound **2** was obtained as white crystals. The molecular formula was suggested as $C_{37}H_{58}O_6$ from ESI-MS (at *m/z* 599.2 $[M + H]^+$ (positive) and *m/z* 597.4 $[M - H]^-$ (negative)) and NMR spectral data. The ^{1}H-NMR spectrum exhibited typical signals of one aromatic proton at δ_H 6.53 (1H, s), one proton of hydroxyl group, one formyl group and one methoxyl group which are all attached to aromatic ring at δ_H 12.37 (1H, s), 10.11 (1H, s) and 3.92 (3H, s), two tri-substituted double bonds at δ_H 5.32 (1H, d, *J* = 7.5 Hz) and 6.09 (1H, t, *J* = 1.5 Hz), three methyl groups at δ_H 0.88 (3H, m), 1.84 (3H, d, *J* = 1.5 Hz) and 2.12 (3H, d, *J* = 1.0 Hz) and overlap signals of saturated long hydrocarbon chain at δ_H 1.25 (26H, m). The ^{13}C-NMR and DEPT spectra exhibited typical signals of one aromatic ring at δ_C 105.55, 112.91, 117.33, 138.70, 162.94 and 163.49, one formyl group at δ_C 193.10, one methoxyl group at δ_C 55.93, two tri-substituted double bonds at δ_C 126.27, 122.85, 130.35 and 155.42, one carbonyl group at δ_C 199.54, one carboxylate carbon at δ_C 173.20, three methyl groups at δ_C 14.12, 20.67 and 27.66 and signals of saturated long hydrocarbon chain at δ_C 22.70 - 31.94.

On the HMBC spectrum, the correlation of the hydroxyl proton (δ 12.37) with C-2 (δ_C 112.91)/C-3 (δ_C 162.94)/C-4 (δ_C 117.33) confirmed the location of the hydroxyl group at C-3, the H-9 (δ_H 3.91) correlated to C-5 (δ 163.49) confirmed the location of the methoxyl group at C-5. By similar analysis, overall structure of compound **2** was characterized as shown in Fig. 2, which was further confirmed by comparison and agreement of the ^{13}C-NMR data of **2** with those of Hericenone D [5]. Obviously, **2** was identified as 3-(3,7-Dimethyl-5-oxo-2,6-octadienyl)-2-hydroxy-6-(hydroxymethyl)-4-methoxybenzaldehyde-1'-O-octadecanoyl (hericenone D).

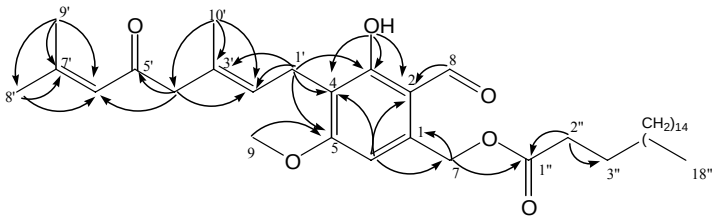

Fig. 2: Selected H-C long-range correlations in the HMBC spectrum of **2**

Compounds **3**, **4,** and **5** were identified as ergosterol [6], ergosterol peroxide [7] and β-adenosine [8], respectively by the comparisons of their NMR spectral data with the literature.

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