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Graphical Abstract

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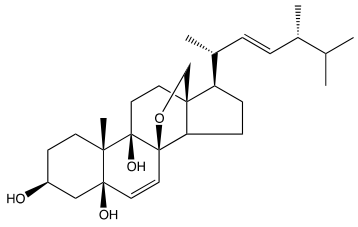

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An unusual sterol from the mushroom *Stropharia rugosoannulata*

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ABSTRACT

An unusual sterol having an unprecedented ether ring (**1**) along with another new sterol (**2**) were isolated from the fruiting bodies of *Stropharia rugosoannulata*. Their structures were determined by the interpretation of spectroscopic data. The relative stereochemistry of **1** was determined by X-ray crystallographic analysis.

Introduction

The mushroom *Stropharia rugosoannulata* is called saketsubatake in Japanese, and wine-cap stropharia in English. It belongs to the Strophariaceae family which is widespread in northern temperate zones throughout the world. It is edible and is cultivated for food in Japan. In the previous papers, we have reported the isolation of osteoclast formation-suppressing compounds, anti-fungal ones, endoplasmic reticulum (ER) stress suppressing ones.[1] Furthermore, strophasterols A to D with an unprecedented steroid skeleton have been isolated from this mushroom.[2] In further research, we discovered two novel sterols. Herein, we describe the isolation and structure determination of the compounds from the mushroom.

Results and discussion

Fresh fruiting bodies of *S. rugosoannulata* were extracted with EtOH and then with acetone. After the solutions were combined and concentrated, they were partitioned between *n*-hexane and H_2O, $CHCl_3$ and H_2O, and then EtOAc and H_2O. The hexane-soluble residue was fractionated by repeated chromatography. As a consequence, two novel compounds (**1** and **2**) were purified (Fig. 1).

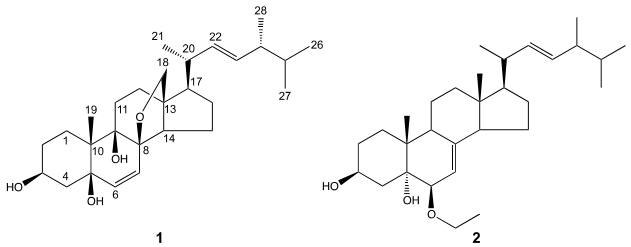

Figure 1. Structures of **1** and **2**.

Compound **1** was purified as white crystals. Its molecular formula was determined as $C_{28}H_{44}O_4$ by HR-ESI-MS *m/z* 467.3137 $[M+Na]^+$ (calcd for $C_{28}H_{44}NaO_4$, 467.3100), indicating the presence of seven degrees of unsaturation in the molecule. The structure of **1** was elucidated by interpretation of NMR spectra including DEPT, COSY, HMQC, and HMBC. The DEPT spectrum indicated the presence of five methyls, eight methylenes, ten methines, and five quaternary carbons. In the NMR spectra of **1**, typical signals of a sterol such as a side-chain possessing four doublet methyls and an olefine (from C-20 to 28) and a hydroxymethine (C-3) were observed. However, this compound has only a singlet methyl (C-19) unlike usual sterols having two singlet methyls at C-18 and C-19. The NMR data, especially HMBC collations indicated the presence of another double bond (C-6, 7) and four steroidal rings (Fig. 2). These data and the degree of the unsaturation of **1** suggested that this compound had an additional ring in the molecule. The lack of a singlet methyl and the HMBC correlation between C-8 and H-18 led us to conclude that an ether ring between C-8 and C-18

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existed in **1** (Fig. 2). The complete assignment of the proton and carbon signals as well as HMBC correlations were summarized in Fig. 2 and Table 1. The structure including relative stereochemistry of **1** was confirmed by X-ray crystallographic analysis (Fig. 3).[3,4] To our knowledge, the ether ring between C-8 and C-18 is unprecedented in the steroid skeleton.

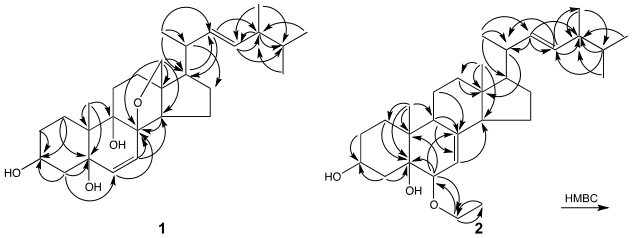

Figure 2. Crucial HMBC correlations in **1** and **2**.

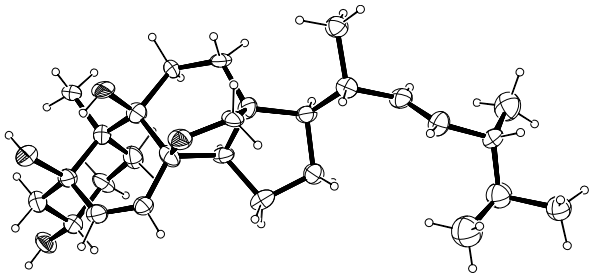

Figure 3. ORTEP drawings of **1** with ellipsoids at the 20% probability level. Hydrogen atoms are shown as small spheres of arbitrary radii.

Compound **2** was isolated as a white powder. Its molecular formula was determined as $C_{30}H_{50}O_3$ by HRESIMS *m/z* 481.3658 $[M+Na]^+$ (calcd for $C_{30}H_{50}O_3$, 481.3640) and the degree of unsaturation of the compound was six. This compound has two additional carbons compared with general steroids in mushrooms like ergosterol. The DEPT spectrum indicated the presence of seven methyls, eight methylenes, eleven methines and four quaternary carbons. The molecular formula, the HMBC correlations (Table 1, Fig. 2) and the chemical shifts indicated the presence of a hydroxylmethine (C-3), four doublet methyls (C-21, 26, 27, 28), two singlet methyls (C-18, 19), a double bond between C-7 and C-8 (C-7, 8). It was also suggested that the two additional carbons formed an ethyl ether linked to C-6 (C-6-$O\underline{CH_2}CH_3$, C-6-$OCH_2\underline{CH_3}$). The complete assignment of the proton and carbon signals as well as HMBC correlations were summarized in Fig. 2 and Table 1. ^{1}H-NMR and ^{13}C-NMR chemical shifts of **2** are very similar to those of 3β,5α-dihydroxy-6β-methoxyergosta-7,22-diene which has a methyl ether instead of the ethyl ether in **2** and whose absolute configuration has been known, suggesting that **2** had the same relative stereochemistry as the methylated one.[5,6] The absolute configuration of **2** was determined by comparison of its specific rotation value with that of the methylated one[5]; **2**, $[\alpha]_D^{25}$ = -50 (*c* = 0.12, in $CHCl_3$); the analogue, $[\alpha]_D^{20}$ = -61 (*c* = 1.19, in $CHCl_3$). As a result, the structure of **2** was determined as shown.

In summary, we isolated two novel sterols (**1** and **2**) from the hexane-soluble of the fresh fruiting bodies of *S. rugosoannulata*. The skeleton of **1** is unprecedented.

Table 1. ^{1}H and ^{13}C NMR data for **1** and **2** (in $CDCl_3$)

Position	**1**			**2**		
	δ_H (multiplicity, *J* in Hz)	δ_C	HMBC correlation	δ_H (multiplicity, *J* in Hz)	δ_C	HMBC correlation
1	1.45 (m), 1.52 (m)	31.3	C-2, 3, 5, 10, 19	1.52 (m), 1.58 (m)	32.8	C-5, 9
2	1.47 (m), 1.75 (m)	30.9	C-1, 3, 10	1.43 (m), 1.83 (m)	30.9	C-1, 3
3	3.50 (m)	67.8	-	4.03 (m)	67.9	-
4	1.74 (m), 1.81 (m)	44.7	C-2, 3, 5, 6, 10	1.70 (m), 2.13 (dd, 13.1, 11.6)	39.5	C-2, 3, 5, 10
5	-	71.6	-	-	76.4	-
6	5.76 (d, 10.1)	137.5	C-5, 8, 10	3.23 (d, 4.9)	80.4	C-5, 7, 8, 10, 6-$O\underline{CH_2}CH_3$
7	5.48 (d, 10.1)	125.3	C-5, 8, 9, 14	5.33 (m)	115.9	C-5, 6, 9, 14
8	-	83.8	-	-	143.0	-
9	-	80.1	-	1.87 (m)	43.93	C-1, 7, 8, 10, 11, 19
10	-	43.5	-	-	37.2	-
11	1.52 (m), 1.92 (m)	27.7	C-10, 12, 13	1.55 (m)	22.2	C-8, 12
12	1.82 (m), 1.90 (m)	33.6	C-9, 11, 13, 14, 17	1.27 (m), 2.02 (m)	39.4	C-9, 11, 13, 14, 18
13	-	54.3	-	-	43.87	-
14	2.21 (dd 8.9, 10.1)	54.0	C-8, 9, 12, 13, 15	1.86 (m)	54.9	C-9, 13, 18
15	1.53 (m), 1.58 (m)	21.5	C-13, 14, 16, 17	1.40 (m), 1.45 (m)	22.9	C-14
16	1.62 (m)	28.8	C-14, 15, 17, 20	1.27 (m), 1.70 (m)	27.9	C-13
17	1.77 (m)	52.7	C-12, 13, 14, 16, 18, 20, 22	1.26 (m)	56.0	C-13, 16, 18
18	3.45 (d, 7.9), 3.75 (d, 7.9)	74.3	C-8, 12, 13, 14, 17	0.58 (s)	12.3	C-12, 13, 14, 17
19	1.25 (s)	14.6	C-1, 5, 9, 10	1.00 (s)	18.4	C-1, 5, 9, 10
20	1.96 (m)	41.2	C-16, 21, 22, 23	2.01 (m)	40.4	C-13, 22, 23
21	0.98 (d, 6.7)	21.4	C-17, 20, 22	1.01 (d, 7.6)	21.1	C-17, 20, 22
22	5.14 (dd, 15.4, 8.2)	133.9	C-17, 20, 21, 23, 24	5.15 (dd, 15.3, 7.9)	135.5	C-20, 23, 24
23	5.22 (dd, 15.4, 7.9)	133.5	C-20, 22, 24, 25, 28	5.20 (dd, 15.3, 7.3)	132.1	C-20, 22, 24, 28
24	1.82 (m)	42.9	C-22, 23, 25, 26, 27, 28	1.83 (m)	42.8	C-22, 23, 25, 26, 27, 28
25	1.45 (m)	33.0	C-23, 24, 26, 27, 28	1.45 (m)	33.1	C-23, 24, 26, 27, 28
26	0.79 (d, 6.7) *	19.6*	C-24, 25, 27*	0.80 (d, 6.7) *	19.6*	C-24, 25, 27*
27	0.81 (d, 6.7) *	19.9*	C-24, 25, 26*	0.81 (d, 6.7) *	19.9*	C-24, 25, 26*
28	0.89 (d, 6.7)	17.5	C-23, 24, 25	0.90 (d, 6.7)	17.6	C-23, 24, 25
6-$O\underline{CH_2}CH_3$				3.44 (m), 3.62 (m)	65.6	C-6, 6-$OCH_2\underline{CH_3}$
6-$O\underline{CH_2}CH_3$				1.31 (dd, 7.0, 6.7)	15.7	C-6-$O\underline{CH_2}CH_3$

* Interchangeable between 26 and 27

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References and notes

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6. ^{1}H-NMR of 3β,5α-dihydroxy-6β-methoxyergosta-7,22-diene (400 MHz, $CDCl_3$); 0.59 (s, H18), 0.81 (d, 6.59, H26), 0.83 (d, 6.32, H27), 0.91 (d, 6.87, H28), 0.99 (s, H19), 1.01 (d, 6.59, H21), 3.16 (d, 4.95, H6), 3.38 (s, OCH_3), 4.03 (m, H3), 5.15 (dd, 15.38, 7.69, H22), 5.21 (dd, 15.38, 7.00, H23), 5.39 (dd, 4.95, 2.47, H7); ^{13}C-NMR (100 MHz, $CDCl_3$); 12.3 (C18), 17.6 (C28), 18.3 (C19), 19.7 (C21), 20.0 (C26), 21.1 (C27), 22.2 (C11), 22.9 (C15), 27.9 (C16), 30.8 (C2), 32.8 (C1), 33.5 (C25), 37.3 (C10), 39.4 (C4), 39.5 (C12), 40.4 (C20), 42.9 (C24), 43.9 (C9), 43.9 (C13), 55.0 (C14), 56.0 (C17), 58.3 (OCH_3), 67.9 (C3), 76.2 (C5), 82.4 (C6), 115.0 (C7), 132.2 (C22), 135.5 (C23), 143.7 (C8).

Supplementary Material

Supplementary data associated with this article can be found, in the online version, at

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