Strophasterols⊠A to D with an Unprecedented Steroid Skeleton : From the Mushroom Stropharia rugosoannulata

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Strophasterols A to D with an Unprecedented Steroid Skeleton, from the Mushroom *Stropharia rugosoannulata***

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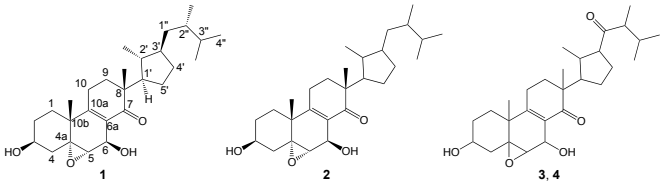
The mushroom *Stropharia rugosoannulata* is called saketsubatake in Japanese, and wine-cap stropharia in English. It belongs to the family Strophariaceae, which is widespread in northern temperate zones throughout the world. It is edible and is cultivated for food. During screening for anti-Endoplasmic reticulum (ER)-stress and anti-Methicillin-resistant *Staphylococcus aureus* (MRSA) effects of the extracts of various mushrooms, we found activity in the extract of this mushroom. ER-stress induces apoptotic pathways with a signaling between ER and mitochondria. By triggering apoptosis on neural cells, the stress is a major cause of degenerative diseases such as Alzheimer disease. [1, 2] MRSA has developed resistance to most antibiotics and is one of the most prevalent pathogen in nosocomial infections. Therefore, anti-ER and anti-MRSA substances are urgently required. Recently we reported that several active steroids were isolated from this mushroom. [3]

In further search for bioactive compounds from the mushroom, we discovered four novel steroids with a very unique and unprecedented carbon skeleton. Here, we describe the isolation, structure determination, and biological activity of the compounds from the mushroom.

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 hexane and H_2O, $CHCl_3$ and H_2O, and then EtOAc and H_2O. The hexane-soluble part was fractionated by repeated chromatography. As a result, four novel compounds (**1**-**4**), which were named strophasterols A to D, were purified (Scheme 1).



Scheme 1. Structures of strophastrerols A-D.

Strophasterol A (**1**) was obtained as white crystals. Its molecular formula was determined as $C_{28}H_{44}O_4$ by HRESIMS, *m/z* 467.3100 $[M+Na]^+$ (calcd for $C_{28}H_{44}NaO_4$ 467.3137), indicating the presence of seven degrees of unsaturation in the molecule. The planar structure of **1** was elucidated by interpretation of the NMR spectra including DEPT, COSY, HMBC and HMQC. The DEPT experiment indicated the presence of six methyls, eight methylenes, eight methines, and six quaternary carbons. In the NMR spectra of **1**, typical signals of a sterol, such as two hydroxymethines [C-3, δ_H 3.94 (m), δ_C 68.2; C-6, δ_H 4.84 (m), δ_C 63.0], four doublet methyls [C-2'CH_3, δ_H 0.97 (d, *J*=6.7 Hz), δ_C 20.8; C-4", δ_H 0.72 (d, *J*=6.7 Hz), δ_C 16.4; C-2"CH_3, δ_H 0.74 (d, *J*=6.7 Hz), δ_C 15.6; C-3"CH_3, δ_H 0.83 (d, *J*=7.0 Hz), δ_C 20.7], and two singlet methyls [C-8CH_3, δ_H 0.93 (s), δ_C 19.0; C-10bCH_3, δ_H1.31 (s), δ_C 22.3] were observed. However, this compound has an isolated five-membered ring instead of D ring of the ordinary steroid skeleton [C-1', δ_H 1.88 (m), δ_C 47.7; C-2', δ_H 1.25 (m), δ_C 41.5; C-3', δ_H 1.36 (m), δ_C 46.4; C-4', δ_H 1.03 (m), 1.73 (m), δ_C 31.8; C-5', δ_H 1.27 (m), 1.37 (m), δ_C 26.4; HMBC correlations, H-9, 8CH_3/C-1'; H-1'/C-7, 8, 8CH_3; H-5'/C-8]. The HMBC correlations indicated that

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

the new ring was attached to a side chain (H-2', 3', 4'/C-1"; H-1"/C-2', 3', 4', 2", 3", 2"CH_3; H-2"/C-3', 1", 3", 3"CH_3; H3"/C-1", 4", 2"CH_3, 3"CH_3; H4"/C-2", 3", 3"CH_3; H-2"CH_3/C-1", 2", 3"; H-3"CH3/C-2", 3", 4") (Table 1). All the HMBC correlations supported planar structure **1** for strophasterol A. Confirmation of the planar structure and determination of its absolute and relative configuration were performed by X-ray crystallography analysis of its bis(*p*-bromo)benzoate (Fig. 1). As a result, the structure of **1** was determined to be as shown in Scheme 1.

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

Position	**1**		
	δ_H (multiplicity, *J* in Hz)	δ_C	HMBC correlation
1	1.70 (m), 1.84 (m)	29.2	C-2, 3, 4a, 10b, 10b-CH_3
2	1.69 (m), 1.99 (m)	30.4	C-1, 3, 4, 10b
3	3.94 (m)	68.2	-
4	1.48 (m), 2.19 (m)	38.8	C-2, 3, 4a, 5, 10b
4a	-	62.5	-
5	3.21 (d, 2.7)	59.4	C-4, 4a, 6, 6a
6	4.84 (m)	63.0	C-4a, 5, 6a, 7, 10a
6a	-	128.1	-
7	-	205.5	-
8	-	45.6	-
9	1.65 (m), 2.00 (m)	32.1	C-7, 8,10, 10a, 8-CH_3, 1'
10	2.19 (m)	22.2	C-6a, 8, 9, 10a, 10b
	2.39 (ddd, 16.0, 10.0, 5.0)		
10a	-	159.5	-
10b	-	39.60	-
8-CH_3	0.93 (s)	19.0	C-7, 8, 9, 1'
10b-CH_3	1.31 (s)	22.3	C-1, 4a, 10a, 10b
1'	1.88 (m)	47.7	C-7, 8, 8-CH_3, 2', 4', 5', 2'-CH_3
2'	1.25 (m)	41.5	C-1', 3', 4', 2'-CH_3, 1"
3'	1.36 (m)	46.4	C-2'-CH_3, 1"
4'	1.03(m), 1.73 (m)	31.8	C-1', 2', 3', 5', 1"
5'	1.27 (m), 1.37 (m)	26.4	C-8, 1', 2', 3', 4'
2'-CH_3	0.97 (d, 6.7)	20.8	C-1', 2', 3'
1"	0.86 (m), 1.44 (m)	39.58	C-2', 3', 4', 2", 3", 2"-CH_3
2"	1.36 (m)	36.7	C-3', 1", 3", 4", 3"-CH_3
3"	1.60 (m)	30.2	C-1", 4", 2"-CH_3, 3"-CH_3
4"	0.72 (d, 6.7)	16.4	C-2", 3", 3"-CH_3
2"-CH_3	0.74 (d, 6.7)	15.6	C-1", 2", 3"
3"-CH_3	0.83 (d, 7.0)	20.7	C-2", 3", 4"

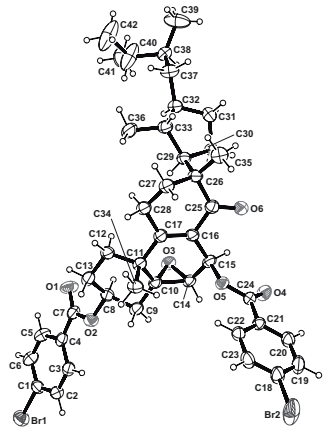


Figure 1. ORTEP drawing of bis(*p*-bromo)benzoate of **1** with ellipsoids at the 30% probability level. Hydrogen atoms are shown as small spheres of arbitrary radii.

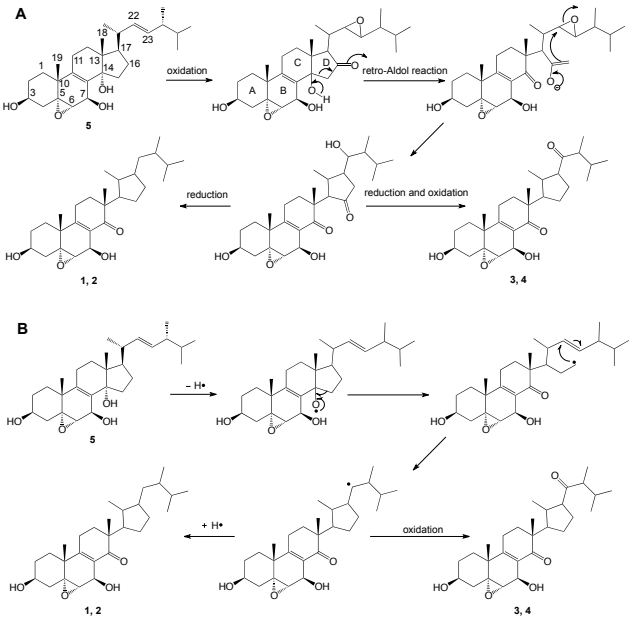
Pure strophasterol B (**2**) was obtained as white crystals. Its molecular formula was determined as $C_{28}H_{44}O_4$ by HRESIMS, *m/z* 467.2525 $[M+Na]^+$ (calcd for $C_{28}H_{44}NaO_4$ 467.2562), and the formula of the compound was the same as that of **1**. The NMR data of **2** were also very similar to those of **1** (Table S1 in the Supplemental Information). The planar structure elucidation was accomplished in the same manner as described for **1**. The molecular formula, the HMBC correlations and the other NMR data allowed us to conclude that the planar structure of **2** was the same as that of **1**. The difference between the ^{1}H- and ^{13}C-NMR chemical shifts of C-2', 3', 2'CH_3, 1" of **2** and those of **1** was larger than that between the other parts of **1** and those of **2**, suggesting that **2** might be a diastereomer of **1** at the five-membered ring.

Strophasterol C (**3**) was obtained as yellow amorphous compound. Its molecular formula was determined as $C_{28}H_{42}O_5$ by HRESIMS, *m/z* 481.2922 $[M+Na]^+$ (calcd for $C_{28}H_{42}NaO_5$ 481.2930), indicating the presence of eight degrees of unsaturation in the molecule. The structure of **3** was elucidated by interpretation of NMR spectra including DEPT, COSY, HMBC and HMQC. The DEPT experiment indicated the presence of six methyls, seven methylenes, eight methines, and seven quaternary carbons. Its NMR data, especially those of the A, B, and C rings were very similar to those of **1** and **2** (Table S1 in the Supplemental Information). This result and HMBC correlations of **3** suggested that this compound possessed the same carbon skeleton as **1** and **2**. However, **3** has a carbonyl (δ_C 216.5) instead of a methylene [**1**; δ_H 0.86 (m), 1.44 (m), δ_C 39.58: **2**; δ_H 1.05 (m), 1.17 (m), δ_C 34.5] of **1** and **2**. The position of the carbonyl was indicated by the HMBC correlations between H-2', 3', 4', 2", 3", 2"CH_3/C-1" (Table S1 in the Supplemental Information). As a result, **3** was determined as shown in Scheme 1.

Strophasterol D (**4**) was obtained as yellow amorphous compound. All the data showed that the planar structure of **4** was the same as **3** (Table S1 in the Supplemental Information).

The stereochemistry of **3** and **4** remains undetermined.

Our hypothetical biosynthesis of these steroids is illustrated in Scheme 2; compound **5** that has been already isolated from this mushroom may be a precursor of **1** to **4** [3]. The double bond at the positions of C-22 and 23 in the side chain of **5** is oxidized to be an epoxide and a ketone forms at



Scheme 2. Hypothetical biosynthesis of strophastrerols A-D.

C-16. Retro-Aldol reaction occurrs from the hydroxyl of C-14, then the five-membered ring forms. Finally, reduction and/or oxidation results in the formation of **1** to **4** (Scheme 2A). Cleavage of the C14-C15 bond with radical formation, followed by five-membered ring closure to C-22 at the double bond would be another possibility (Scheme 2B).

Strophasterols A-D (**1**-**4**) were evaluated by ER-stress suppression and anti-MRSA assay. In a protective activity assay against ER stress-dependent cell death caused by tunicamycin (TM) or thapsigargin (TG), none of the compounds showed any inhibitory effect on tunicamycin toxicity, but **1** showed an inhibitory effect on TG toxicity dose-dependently (increase in cell viability compared with control, 10.3 %, Figure S5 in the Supplemental Information). TM is an inhibitor of *N*-glycosylation of glycoproteins in the ER, and causes protein-misfolding there. TG, an inhibitor of the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase, also induces ER stress by disrupting the homeostatic balance of the Ca^{2+} concentration in the ER. This suggests that **1** can protect neuronal cells by attenuating the ER stress caused by the Ca^{2+}-ATPase inhibitor. In addition, **1** showed weak anti-MRSA activity (Figure S6 in the Supplemental Information).

Experimental Section

((Experimental Details))

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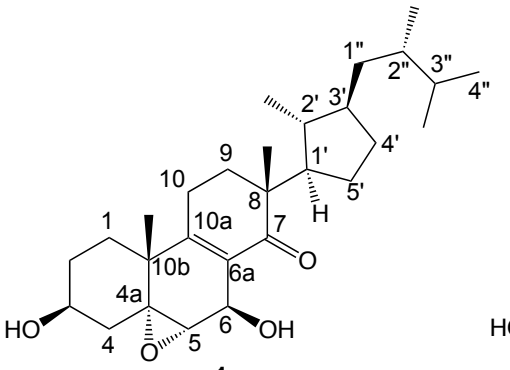
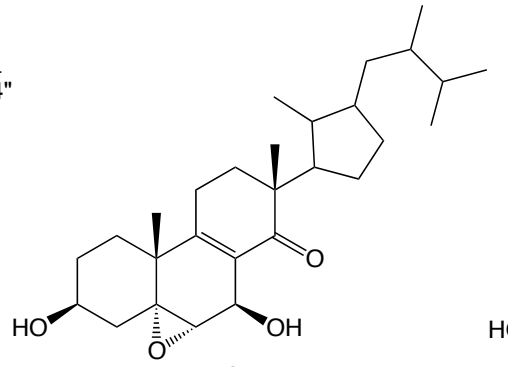
Keywords: anti-MRSA · endopasmic reticulum stress-suppression · mushroom · *Stropharia rugosoannulata* · unprecedented steroid skeleton

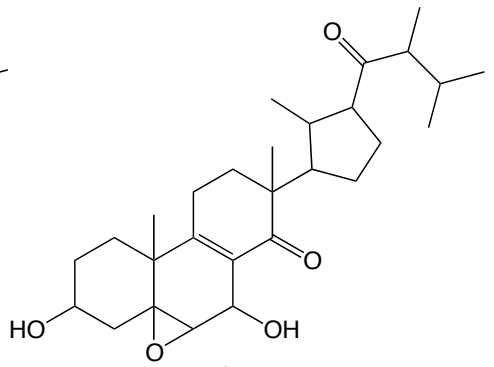
[1] T. Nakagawa, H. Zhu, N. Morishima, E. Li, J. Xu, B.A. Yankner, J. Yuan, *Nature* **2000**, *403*, 98-103
[2] T. Nakagawa, J. Yuan, *J. Cell Biol.* **2000**, *150*, 887-894
[3] J. Wu, K. Fushimi, S. Tokuyama, M. Ohno, T. Miwa, T. Koyama, K. Yazawa, K. Nagai, T. Matsumoto, H. Hirai, H. Kawagishi, *Biosci. Biotechnol. Biochem.* **2011**, *75*, 1631-1634



1

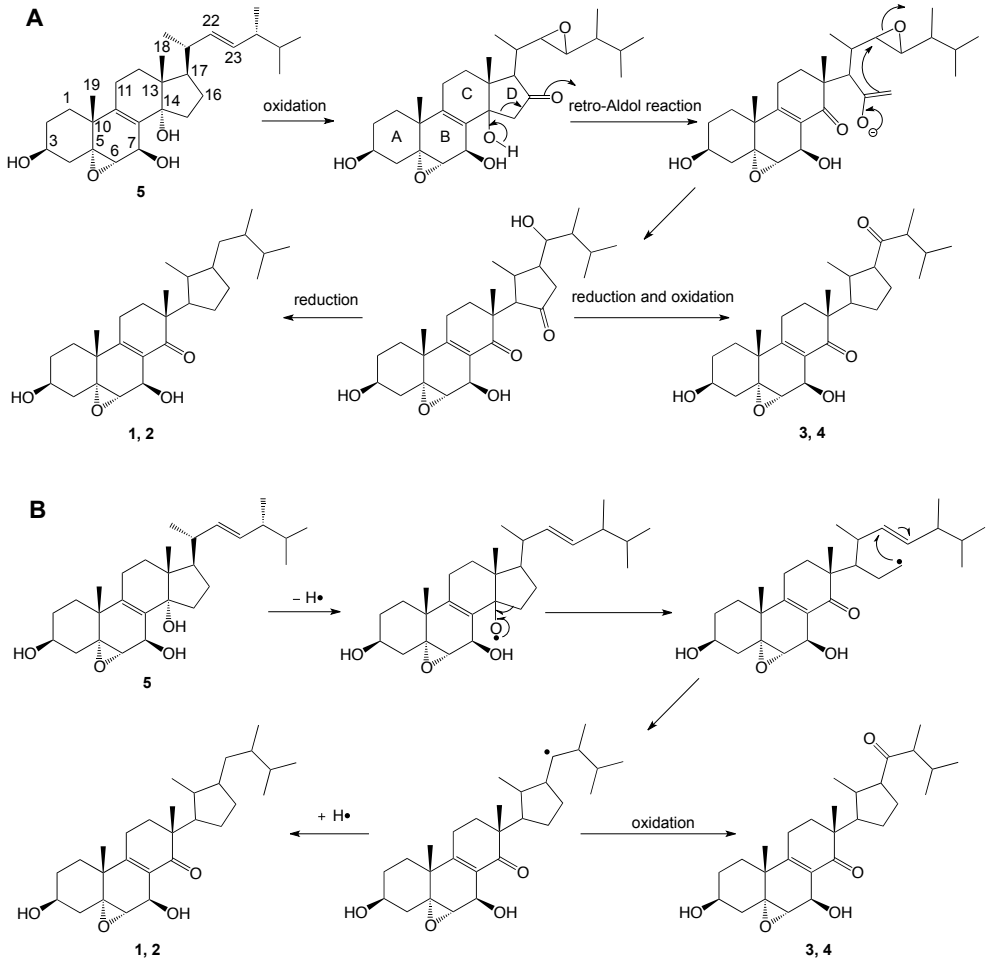


2



3, **4**

Scheme 1 Wu *et al.*



Scheme 2 Wu *et al*.

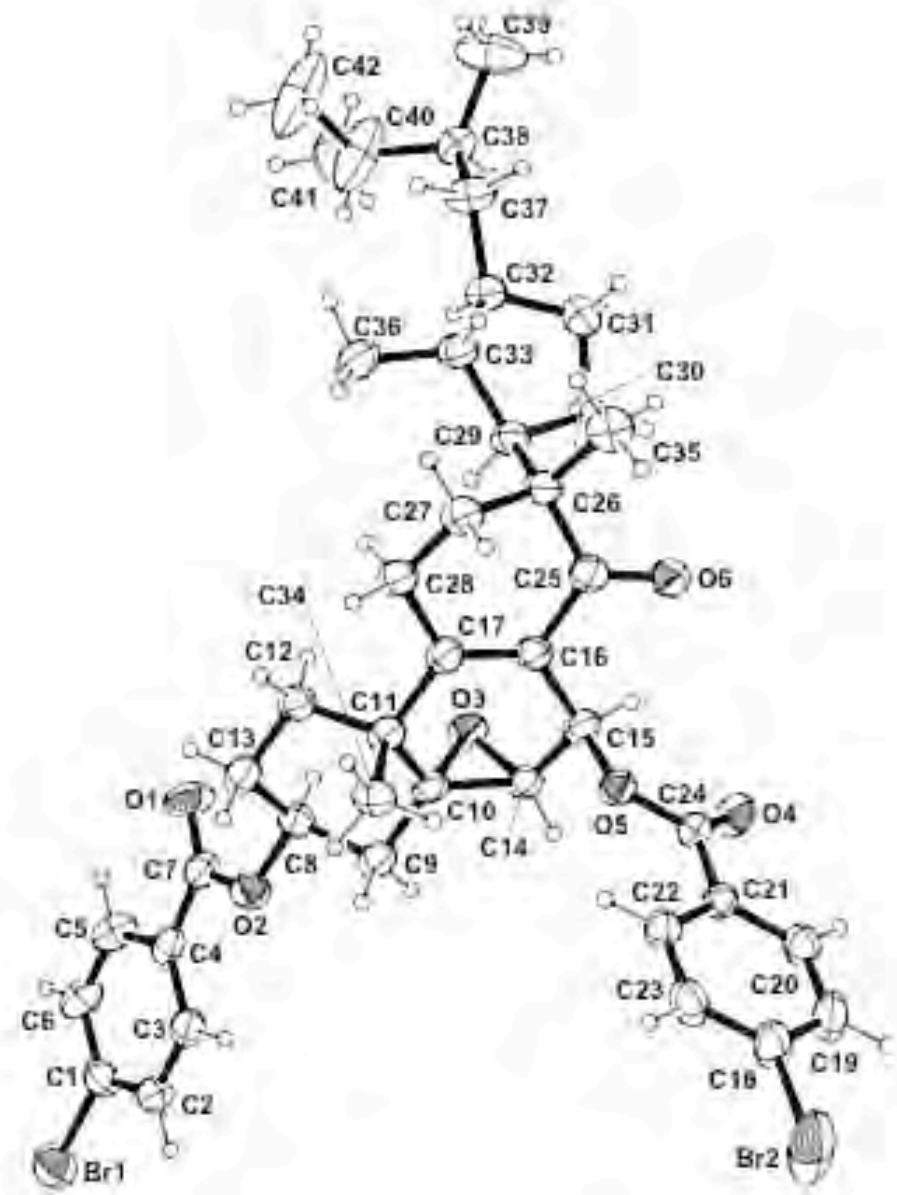

C39
C42
C40
C38
C41
C37
C32
C36
C31
C33
C30
C29
C35
C27
C26
C28
C25
O6
C34
C17
C12
C16
C11
O3
C15
C13
O1
C10
O5
C24
O4
C14
C7
C8
C9
C22
C21
O2
C5
C4
C23
C20
C6
C3
C19
C1
C2
C18
Br2
Br1

Fig. 1. Wu *et al.*

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	2.39 (ddd, 16.0, 10.0, 5.0)		
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3”	1.60 (m)	30.2	C-1”, 4”, 2”-CH_3, 3”-CH_3
4”	0.72 (d, 6.7)	16.4	C-2”, 3”, 3”-CH_3
2”-CH_3	0.74 (d, 6.7)	15.6	C-1”, 2”, 3”
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