Natural Products

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

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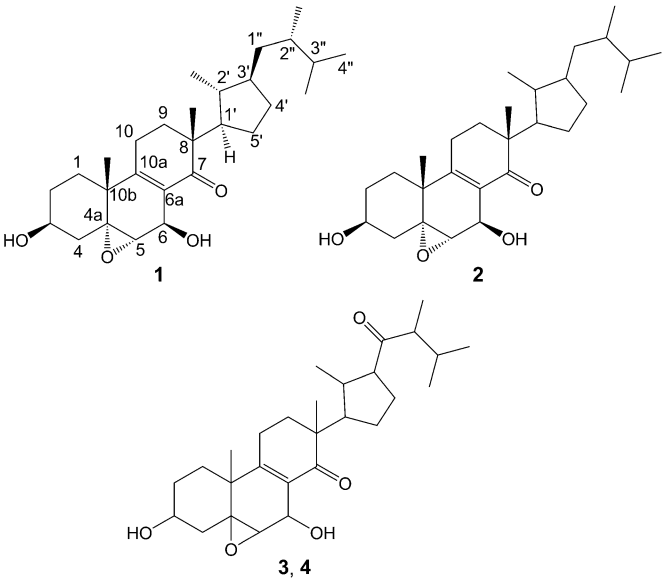
The mushroom *Stropharia rugosoannulata* is called saketsubatake in Japanese, and wine-cap stropharia in English. It belongs to the family Strophariaceae, which is widespread in northern temperate zones throughout the world. It is edible and is cultivated for food. During screening for anti-endoplasmic-reticulum (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 signaling between the ER and mitochondria. By triggering apoptosis on neural cells, the stress is a major cause of degenerative diseases such as Alzheimer's disease.[1,2] MRSA has developed resistance to most antibiotics and is one of the most prevalent pathogens in nosocomial infections. Therefore, anti-ER-stress 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 having a very unique and unprecedented carbon skeleton. Herein, 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 *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 result, four novel compounds (**1**–**4**), which were named strophasterols A, B, C, and D, were isolated (Scheme 1).



Scheme 1. Structures of strophastrerols A–D.

Strophasterol A (**1**) was obtained as white crystals. Its molecular formula was determined to be $C_{28}H_{44}O_4$ by HRESIMS with *m*/*z* 467.3100 $[M+Na]^+$ (calcd for $C_{28}H_{44}NaO_4$ 467.3137), thus 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 data. The DEPT experiment indicated the presence of six methyl, eight methylene, and eight methane groups, as well as six quaternary carbon atoms. In the NMR spectra of **1**, typical signals of a sterol corresponding to two hydroxymethines [C3: δ_H = 3.94 ppm (m), δ_C = 68.2 ppm; C6: δ_H = 4.84 ppm (m), δ_C = 63.0 ppm], four doublet methyls [C2′CH_3: δ_H = 0.97 ppm (d, J = 6.7 Hz), δ_C = 20.8 ppm; C4″CH_3: δ_H = 0.72 ppm (d, J = 6.7 Hz), δ_C = 16.4 ppm; C2″CH_3: δ_H = 0.74 ppm (d, J = 6.7 Hz), δ_C = 15.6 ppm; C3″CH_3: δ_H = 0.83 ppm (d, J =

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7.0 Hz), $\delta_C = 20.7$ ppm], and two singlet methyls [C8CH_3: $\delta_H = 0.93$ ppm (s), $\delta_C = 19.0$ ppm; C10bCH_3: $\delta_H = 1.31$ ppm (s), $\delta_C = 22.3$ ppm] were observed. However, this compound has an isolated five-membered ring instead of the D ring that is typical of a steroid skeleton [C1′: $\delta_H = 1.88$ ppm (m), $\delta_C =$ 47.7 ppm; C2′: $\delta_H = 1.25$ ppm (m), $\delta_C = 41.5$ ppm; C3′: $\delta_H =$ 1.36 ppm (m), $\delta_C = 46.4$ ppm; C4′: $\delta_H = 1.03$ (m), 1.73 ppm (m), $\delta_C = 31.8$ ppm; C5′: $\delta_H = 1.27$ (m), 1.37 ppm (m), $\delta_C =$ 26.4 ppm; HMBC correlations: H9, C8CH_3/C1′; H1′/C7, C8, C8CH_3; H5′/C8]. The HMBC correlations indicated that the new ring was attached to a side chain (H2′, H3′, H4′/C1″; H1″/ C2′, C3′, C4′, C2″, C3″, C2″CH_3; H2″/C3′, C1″, C3″, C3″CH_3; H3″/C1″, C4″, C2″CH_3, C3″CH_3; H4″/C2″, C3″, C3″CH_3; H2″CH_3/C1″, C2″, C3″; H3″CH3/C2″, C3″, C4″; Table 1). All the HMBC correlations provide support for the 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 derivative (Figure 1). As a result, the structure of **1** was determined to be that as shown in Scheme 1.

Pure strophasterol B (**2**) was obtained as white crystals. Its molecular formula was determined to be $C_{28}H_{44}O_4$ by HRESIMS with *m*/*z* 467.2525 $[M+Na]^+$ (calcd for $C_{28}H_{44}NaO_4$ 467.2562), and the formula of the compound

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

Position	**1**		
	δ_H [ppm] (multiplicity, *J* [Hz])	δ_C [ppm]	HMBC correlation
1	1.70 (m), 1.84 (m)	29.2	C2, C3, C4a, C10b, C10bCH_3
2	1.69 (m), 1.99 (m)	30.4	C1, C3, C4, C10b
3	3.94 (m)	68.2	–
4	1.48 (m), 2.19 (m)	38.8	C2, C3, C4a, C5, C10b
4a	–	62.5	–
5	3.21 (d, 2.7)	59.4	C4, C4a, C6, C6a
6	4.84 (m)	63.0	C4a, C5, C6a, C7, C10a
6a	–	128.1	–
7	–	205.5	–
8	–	45.6	–
9	1.65 (m), 2.00 (m)	32.1	C7, C8, C10, C10a, C8CH_3, C1′
10	2.19 (m) 2.39 (ddd, 16.0,10.0, 5.0)	22.2	C6a, C8, C9, C10a, C10b
10a	–	159.5	–
10b	–	39.60	–
8-CH_3	0.93 (s)	19.0	C7, C8, C9, C1′
10b-CH_3	1.31 (s)	22.3	C1, C4a, C10a, C10b
1′	1.88 (m)	47.7	C7, C8, C8CH_3, C2′, C4′, C5′, C2′CH_3
2′	1.25 (m)	41.5	C1′, C3′, C4′, C2′CH_3, C1″
3′	1.36 (m)	46.4	C2′-CH_3, C1“
4′	1.03(m), 1.73 (m)	31.8	C1′, C2′, C3′, C5′, C1″
5′	1.27 (m), 1.37 (m)	26.4	C8, C1′, C2′, C3′, C4′
2′-CH_3	0.97 (d, 6.7)	20.8	C1′, C2′, C3′
1″	0.86 (m), 1.44 (m)	39.58	C2′, C3′, C4′, C2″, C3″, C2″CH_3
2″	1.36 (m)	36.7	C3′, C1″, C3″, C4″, C3″CH_3
3″	1.60 (m)	30.2	C1″, C4″, C2″CH_3, C3″CH_3
4″	0.72 (d, 6.7)	16.4	C2″, C3″, C3″CH_3
2″-CH_3	0.74 (d, 6.7)	15.6	C1″, C2″, C3″
3″-CH_3	0.83 (d, 7.0)	20.7	C2″, C3″, C4″

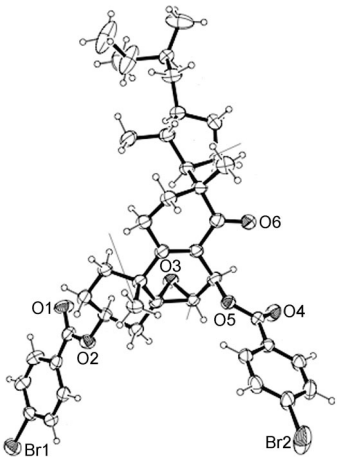


Figure 1. ORTEP drawing of the bis(*p*-bromo)benzoate derivative of **1**. Thermal ellipsoids are shown at the 30% probability level. Hydrogen atoms are shown as small spheres of arbitrary radii.

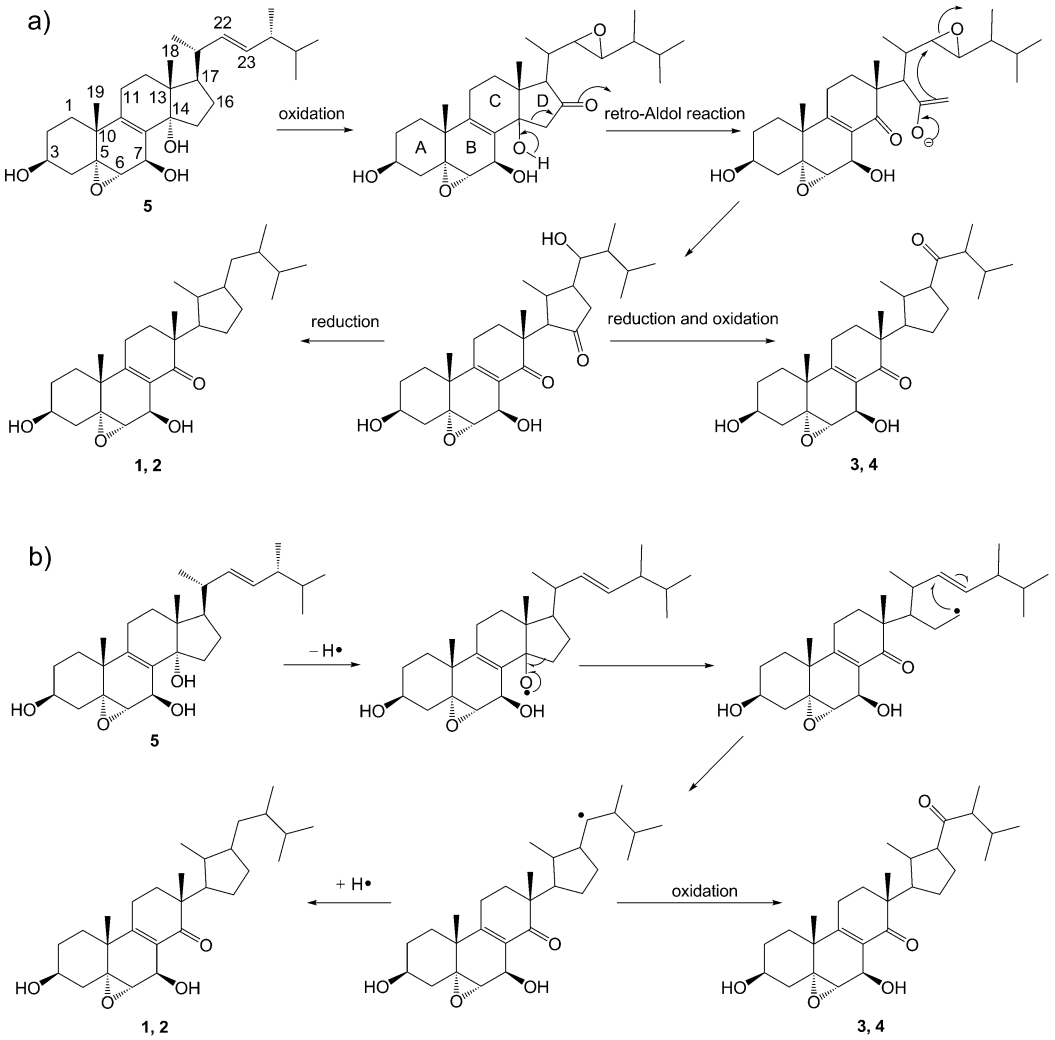
was the same as that of **1**. The NMR data of **2** were also very similar to those of **1** (see Table S1 in the Supporting Information). The planar structure was elucidated in the same manner as described for **1**. The molecular formula, the HMBC correlations, and the other NMR data allowed us to conclude that the planar structure of **2** was the same as that of **1**. The differences between the ^{1}H and ^{13}C NMR chemical shifts of C2′, C3′, C2′CH_3, and C1″ of **2** and those of **1** was larger than those between the other portions of **1** and those of **2**, thus suggesting that **2** might be a diastereomer of **1** at the five-membered ring.

Strophasterol C (**3**) was obtained as a yellow amorphous compound. Its molecular formula was determined to be $C_{28}H_{42}O_5$ by HRESIMS with *m*/*z* 481.2922 $[M+Na]^+$ (calcd for $C_{28}H_{42}NaO_5$ 481.2930), thus 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 data. The DEPT experiment indicated the presence of six methyl, seven methylene, and eight methane groups, as well as seven quaternary carbon atoms. The NMR data, especially those of the A, B, and C rings were very similar to those of **1** and **2** (see Table S1 in the Supporting 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 group ($\delta_C = 216.5$ ppm) instead of the methylene group [**1**: $\delta_H = 0.86$ (m), 1.44 ppm (m), $\delta_C = 39.58$ ppm; **2**: $\delta_H = 1.05$ (m), 1.17 ppm (m), $\delta_C = 34.5$ ppm] in **1** and **2**. The position of the carbonyl group was indicated by the HMBC correlations between H2′, H3′, H4′, H2″, H3″, H2″CH_3/C1″ (see Table S1). As a result, **3** was determined to be the structure as shown in Scheme 1.

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

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

Our hypothetical biosynthesis of these steroids is illustrated in Scheme 2. The compound **5**, which has been already



Scheme 2. Hypothetical biosynthesis of strophastrerols A–D.

isolated from this mushroom may be a precursor of compounds **1**–**4**.[3] The double bond at the C22 and C23 in the side chain of **5** is oxidized to an epoxide, and a ketone forms at C16. A retro-aldol reaction occurs from the hydroxy of C14, with subsequent formation of the five-membered ring. Finally, reduction and/or oxidation results in the formation of **1**–**4** (Scheme 2a). Cleavage of the C14-C15 bond through radical formation and subsequent five-membered ring closure to C22 at the double bond would be another possibility (Scheme 2a).[4]

Strophasterols A–D (**1**–**4**) were evaluated by ER-stress suppression and an 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 a dose-dependent inhibitory effect on TG toxicity (increase in cell viability compared with control, 10.3%, see Figure S5 in the Supporting Information). TM is an inhibitor of N-glycosylation of glycoproteins in the ER, and causes misfolding of proteins. 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 data 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 (see Figure S6 in the Supporting Information).

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[4] We thank a referee for suggesting this alternative route.

