

TASTE-MODIFYING TRITERPENE GLYCOSIDES FROM *STAUROGYNE MERGUENSIS*

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Key Word Index—*Staurogyne merguensis*; Acanthaceae; oleanane-type triterpene glycosides; strogins 1–5; taste-modifying activity.**Abstract**—Five new oleanane-type triterpene glycosides named strogins 1–5 were isolated from leaves of *Staurogyne merguensis*. Their structures were determined on the basis of chemical and spectral evidence. After strogins 1, 2 and 4 were held in mouth, water elicited a sweet taste. On the other hand, strogins 3 and 5 had no activity. The structure–activity relationship is discussed. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

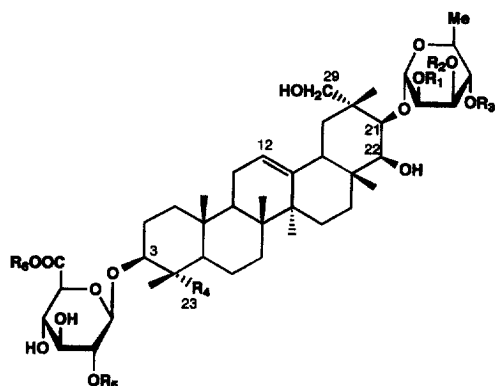
In previous studies, we have determined the structures of several taste modifiers such as the antisweet glycosides gymnemic acid [1] and ziziphin [2], and the taste-modifying proteins miraculin [3] and curculin [4]. In the course of these studies, we noticed a plant having a new type of taste-modifying activity on Penang island of Malaysia. After chewing the leaves of *Staurogyne merguensis* Wall, water elicits a sweet taste. The plant

grows wild and native people often cook rice with its leaves to give a sweet taste to the rice. In spite of this interesting property, no study on the active principle has been carried out. In the present study, we established a method for the isolation of the active principle, which we named strogin. Five strogins (1–5) were purified and their structures were determined.

RESULTS AND DISCUSSION

Strogin gave a positive Liebermann–Burchard colour reaction indicative of a triterpene saponin. Acid hydrolysis of strogins 1–5 gave D-glucuronic acid and L-rhamnose along with D-xylose in the case of strogins 1, 3, 5, and D-glucose in the case of strogin 4. These sugars were identified by GC-mass spectrometry of their TMSi derivatives. Strogin 1 was a main component. Alkaline hydrolysis of strogin 1 gave **1a** which on enzymatic hydrolysis with naringinase afforded an aglycone. The aglycone was analysed for $C_{30}H_{50}O_5$ by high resolution FAB-mass spectrometry. The 1H and ^{13}C NMR spectra indicated that the aglycone was an oleanane triterpene with two primary hydroxyl groups, three secondary hydroxyl groups and one trisubstituted double bond (Table 1). Further examination of the aglycone by means of COSY, HMBC and NOE led to the formulation of the aglycone as $3\beta,21\beta,22\beta,23,29$ -pentahydroxy-olean-12-ene, revealing that the aglycone was a novel compound.

Comparison of the ^{13}C NMR spectra of strogin 1 and its aglycone showed glycosylation shifts around C-3 and C-21 (Table 1), indicating that **1** was the 3,21-O-glycoside. The linkages between sugars and the positions of acetyl groups were determined by a combina-

**1:** $R_1=R_2=R_3=Ac$
 $R_4=CH_2OH$, $R_5=Xyl$, $R_6=H$ **2:** $R_1=R_2=R_3=Ac$
 $R_4=CH_2OH$, $R_5=R_6=H$ **3:** $R_1=R_2=R_3=Ac$
 $R_4=CH_3$, $R_5=Xyl$, $R_6=H$ **4:** $R_1=R_2=R_3=Ac$
 $R_4=CH_2OH$, $R_5=Glc$, $R_6=H$ **5:** $R_1=R_3=Ac$, $R_2=R_6=H$
 $R_4=CH_2OH$, $R_5=Xyl$ **1a:** $R_1=R_2=R_3=R_6=H$
 $R_4=CH_2OH$, $R_5=Xyl$ **1b:** $R_1=R_2=R_3=Ac$
 $R_4=CH_2OH$, $R_5=Xyl$, $R_6=CH_3$

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Table 1. ^{13}C NMR spectral data for compounds **1–5** and **1c** (100 MHz, pyridine- d_5)

C	1	2	3	4	5	1c*
1	38.9	38.7	38.9	38.8	38.9	38.6
2	26.3	25.6	26.4	26.0	26.3	27.3
3	81.9	80.0	89.3	82.9	81.9	73.5
4	43.7	43.3	39.5	43.6	43.7	42.5
5	47.3	47.4	55.8	48.0	47.3	48.5
6	18.1	18.2	18.4	18.3	18.1	18.3
7	32.6	32.5	32.8	32.6	32.6	32.3
8	40.4	40.3	40.2	40.3	40.4	40.0
9	48.0	47.9	47.8	48.1	48.0	47.7
10	36.7	36.7	36.7	36.8	36.7	36.8
11	23.9	23.9	23.8	23.9	23.9	23.9
12	122.9	122.9	122.9	122.9	122.9	122.3
13	144.4	144.4	144.3	144.4	144.5	144.3
14	42.2	42.1	42.1	42.2	42.2	41.8
15	26.7	26.6	26.6	26.8	26.7	26.3
16	27.6	29.8	29.8	27.6	27.6	27.1
17	39.8	39.6	39.6	39.8	39.6	38.6
18	43.0	43.0	43.0	43.0	43.0	42.9
19	41.0	40.9	41.0	41.1	41.1	40.8
20	41.4	41.3	41.3	41.5	41.5	40.5
21	79.0	78.8	78.7	79.0	77.7	70.6
22	79.0	78.8	78.7	79.0	79.1	79.4
23	63.6	64.4	27.8	65.1	63.6	68.3
24	13.2	13.4	16.3	13.4	13.2	12.5
25	16.3	16.2	15.6	16.3	16.3	15.8
26	17.1	17.1	17.0	17.1	17.1	16.8
27	26.7	26.7	26.6	26.7	26.7	26.3
28	22.1	22.0	22.0	22.2	22.1	21.8
29	68.7	68.6	68.6	68.7	68.7	71.7
30	18.0	17.9	17.9	17.9	18.0	17.0

*1c: aglycone of **1**, **2**, **4**, **5**.

tion of COSY, HMBC and NOESY (Table 2). On the basis of all these data, the chemical structure of strogin **1** was established as 3β -[β -D-xylopyranosyl(1 \rightarrow 2)- β -D-glucuronopyranosyloxy]-21 β -[(2,3,4-tri-O-acetyl)- α -L-rhamnopyranosyloxy]-22 β ,23,29-trihydroxyolean-12-ene.

The structures of strogins **2–5** were determined by similar means. The results established that they contained the same oleanene triterpene aglycone as strogin **1**.

Strogin **1** has a xylo-glucuronosyl residue at C-3 of the aglycone and triacetyl L-rhamnose at C-21. Strogin **2** lacks D-xylose. In strogin **4**, the D-xylose of strogin **1** is replaced by D-glucose. Strogin **5** has diacetyl L-rhamnose at C-21. In strogin **3**, the hydroxymethyl group at C-23 of strogin **1** is replaced by a methyl group.

The sweetness was evaluated by the psychometric method [5]. Strogin **1** itself showed a weak sweet taste. Strogin **1** (1 mM) elicited sweetness equivalent to that of 0.15 M sucrose (Fig. 1A). Water elicited sweetness equivalent to that of 0.3 M sucrose after 1 mM strogin **1** was held in the mouth for 3 min (Fig. 1B). Strogins **2** and **4** also showed the same taste-modifying activity but their activity was weaker than that of strogin **1**. Strogins **3** and **5** have no sweet and taste-modifying activities. This suggests that the hydrogen at C-23 of

the aglycone plays an important role in the expression of the activities.

Esterification of the glucuronic acid residue of strogin **1** (compound **1b**) led to a complete loss of the taste-modifying activity. In addition, selective removal of the acetyl groups of rhamnose by hydrolysis (to give compound **1a**) also led to a complete loss of the taste-modifying activity. These results suggest that the carboxyl group of glucuronic acid at C-3 and the acetyl groups of rhamnose at C-21 play an important role in the expression of the activity.

The structure of strogin **1** resembles that of gymnemic acid I [6] which has antisweet activity. Both have an oleanene skeleton and glucuronic acid at C-3 of the aglycone. The main structural differences between the two are found in the groups attached to the E-ring. Hence, the difference in activities may be due to the differences in the structures of the E-rings. This notion is consistent with the finding that the moiety at C-21 plays an important role in the activity of the strogins.

EXPERIMENTAL

General. Mps: uncorr; NMR: JEOL JNM A-400 spectrometer in $\text{C}_5\text{D}_5\text{N}$ soln; CC: silica gel (Kieselgel 60, 70–230 mesh, Merck), silanized silica gel (Kieselgel 60 silanisiert, 70–230 mesh, Merck) and

Table 2. ^{13}C NMR spectral data of the sugar moieties of compounds 1–5 (100 MHz, pyridine- d_5)

C	1	2	3	4	5
C-3-O-GlcA					
1	104.5	104.2	104.7	104.2	104.5
2	84.0	74.3	83.1	83.6	84.0
3	77.7	78.0	77.6	77.9	77.7
4	73.1	73.3	73.2	73.0	73.1
5	77.3	76.0	76.0	77.5	77.4
6	172.4	*	176.5	172.4	172.4
Xyl(1→2)					
1	107.1		106.5		107.1
2	76.5		76.1		76.5
3	78.2		77.7		78.2
4	71.1		70.8		71.1
5	67.5		67.2		67.5
Glc(1→2)					
1				106.0	
2				76.9	
3				78.1	
4				71.5	
5				78.3	
6				62.7	
C-21-O-Rha					
1	100.0	99.8	99.8	100.0	100.1
2	71.0	70.7	70.7	70.7	74.0
3	70.3	70.3	70.3	70.4	68.0
4	71.7	71.7	71.7	71.8	75.5
5	67.2	67.2	67.2	67.2	67.5
6	17.7	17.6	17.6	17.7	17.9
AcO-					
1	20.5	20.5	20.4	20.5	20.8
	20.6	20.5	20.5	20.6	20.1
	20.7	20.6	20.6	20.7	
2	170.0	170.2	170.2	170.0	170.5
	170.2	170.3	170.3	170.2	170.6
	170.2	170.3	170.3	170.2	

*Obscured.

highly porous polymer resin [Diaion HP-21 (Mitsubishi Chem. Ind. Co., Ltd)]; HPLC: TSK-gel ODS 80T (7.8 mm \times 30 cm, Tosoh Co., Ltd).

Extraction and sepn. Air-dried leaves of *S. merguensis* Kuntze (from Penang, Malaysia, 40 g) were extracted with hot water. The extract was chromatographed on a column of Diaion HP-21 (H_2O , 30, 60 and 80% aq. acetone, successively and finally acetone). The 60% aq. acetone eluate (800 mg) was sepd in five frs by CC on silica gel with CH_2Cl_2 -MeOH- H_2O (60:36:14, lower phase). Frs 1 and 2 were subjected to chromatography on a silica gel column (CHCl_3 -MeOH- H_2O , 18:8:1) to give strogins 2 and 3 in yields of 0.03, 0.10% from air-dried leaves, respectively. Chromatography of fr. 3 on a silanized silica gel column (CH_3CN - H_2O -AcOH 38:62:0.1) afforded strogin 1 in yields of 0.13%. Frs 4 and 5 were chromatographed by HPLC [TSK-gel ODS 80T, 7.8 mm \times 300 mm, gradient elution with MeOH (65–100%) in 0.05% aq. trifluoroacetic acid], affording strogins 4 and 5 in yields of 0.03 and 0.02%, respectively.

Strogin 1. Powder, mp 211–213°, $[\alpha]_{\text{D}} -5.04^\circ$

(MeOH; *c* 0.97). HR-FAB-MS: *m/z* 1069.5238, $\text{C}_{53}\text{H}_{82}\text{O}_{22}$ -H requires 1069.5220. ^1H NMR (400 MHz, pyridine- d_5) δ : 0.98, 1.05, 1.06, 1.27, 1.32, 1.39 (all 3H, *s*), 1.32 (3H, *d*, *J*=6.1 Hz rhamnose methyl), 1.92, 1.96, 2.03 (all 3H, *s*, acetyl methyl), 3.30, 3.87 (both 1H, ABq, *J*=10.4 Hz, 29-H), 3.94 (1H, *d*, *J*=2.9 Hz, 22-H), 4.26 (1H, *dd*, *J*=4.5, 11.8 Hz, 3 α -H), 4.61 (1H, *d*, *J*=2.9 Hz, 21-H), 5.24 (1H, *d*, *J*=6.8 Hz, H-1 of Xyl), 5.63 (1H, *s*, H-1 of Rha); ^{13}C NMR: Tables 1 and 2.

Strogin 2. Powder, mp 250–251°, $[\alpha]_{\text{D}} -4.67^\circ$ (MeOH; *c* 0.68). HR-FAB-MS: *m/z* 937.4769, $\text{C}_{48}\text{H}_{74}\text{O}_{18}$ -H requires 937.4798. ^1H NMR (400 MHz, pyridine- d_5) δ : 0.89, 0.94, 0.02, 1.27, 1.29, 1.35 (all 3H, *s*), 1.31 (3H, *d*, *J*=6.3 Hz, rhamnose methyl), 1.94, 1.97, 2.03 (all 3H, *s*, acetyl methyl), 3.29, 3.83 (both 1H, ABq, *J*=10.4 Hz, 29-H), 3.91 (1H, *d*, *J*=2.9 Hz, 22-H), 4.54 (1H, *d*, *J*=2.9 Hz, 21-H), 5.57 (1H, *s*, H-1 of Rha); ^{13}C NMR: Tables 1 and 2.

Strogin 3. Powder, mp 243–245°, $[\alpha]_{\text{D}} -8.20^\circ$ (MeOH; *c* 0.69). HR-FAB-MS: *m/z* 1053.5300, $\text{C}_{53}\text{H}_{82}\text{O}_{21}$ -H requires 1053.5271. ^1H NMR (400 MHz, pyridine- d_5) δ : 0.86, 1.00, 1.04, 1.21, 1.30, 1.30,

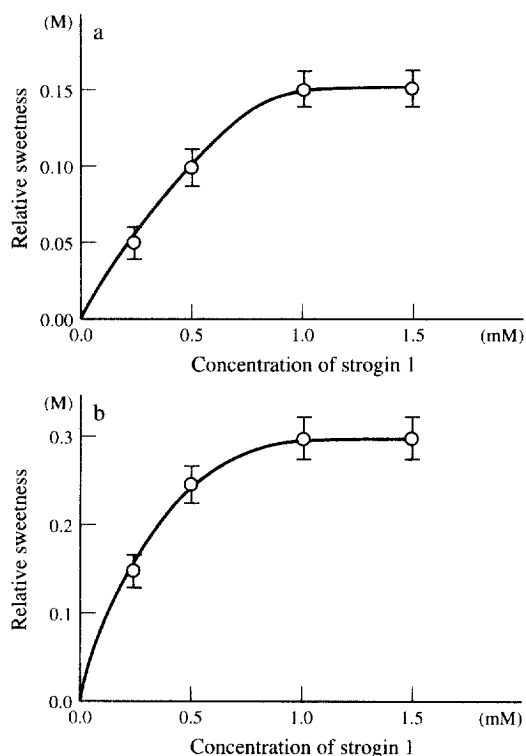
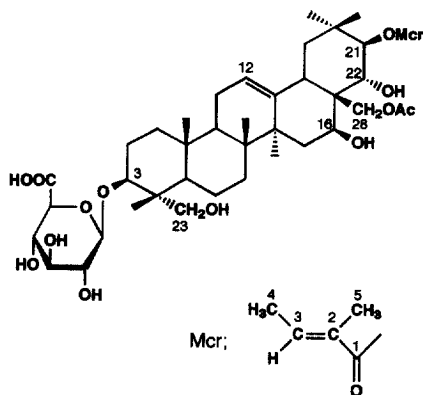


Fig. 1. (a) The sweetness of strogin 1 as a function of its concentration. The sweetness was evaluated by a psychophysical method. The sweetness in the ordinate represents sucrose concentration whose sweetness is equivalent to that of the test solution. (b) Taste-modifying activity of strogin 1 as a function of strogin 1 concentration. Strogin 1 solution was held in the mouth for 3 min and then the sweetness induced by water was evaluated. Each value in the figure represents mean \pm SE (4 subjects).

1.37 (all 3H, *s*), 1.32 (3H, *d*, $J=6.3$ Hz, rhamnose methyl), 1.94, 1.97, 2.03 (all 3H, *s*, acetyl methyl), 3.21 (1H, *dd*, $J=3.9, 11.5$ Hz, 3α -H), 3.31, 3.85 (both 1H, ABq, $J=10.4$ Hz, 29-H), 3.93 (1H, *d*, $J=2.9$ Hz,



Gymnemic acid I

22-H), 4.57 (1H, *d*, $J=2.7$ Hz, 21-H), 4.75 (1H, *d*, $J=7.6$ Hz, H-1 of GlcA), 5.09 (1H, *d*, $J=7.8$ Hz, H-1 of Xyl), 5.59 (1H, *s*, H-1 of Rha); ^{13}C NMR: Tables 1 and 2.

Strogin 4. Powder, mp 219–221°, $[\alpha]_{\text{D}} -3.68^\circ$ (MeOH; *c* 0.54). HR-FAB-MS: m/z 1099.5270, $\text{C}_{54}\text{H}_{84}\text{O}_{23}$ -H requires 1099.5326. ^1H NMR (400 MHz, pyridine- d_5) δ : 0.96, 1.03, 1.11, 1.26, 1.33, 1.40 (all 3H, *s*), 1.32 (3H, *d*, $J=6.1$ Hz, rhamnose methyl), 1.92, 1.96, 2.03 (all 3H, *s*, acetyl methyl), 3.31, 3.85 (both 1H, ABq, $J=10.5$ Hz, 29-H), 3.95 (1H, *d*, $J=2.9$ Hz, 22-H), 4.61 (1H, *d*, $J=2.9$ Hz, 21-H), 5.15 (1H, *d*, $J=7.3$ Hz, H-1 of GlcA), 5.39 (1H, *d*, $J=7.8$ Hz, H-1 of Glc), 5.64 (1H, *s*, H-1 of Rha); ^{13}C NMR: Tables 1 and 2.

Strogin 5. Powder, mp 231–232°, $[\alpha]_{\text{D}} -3.08^\circ$ (MeOH; *c* 0.49). HR-FAB-MS: m/z 1027.5190, $\text{C}_{51}\text{H}_{80}\text{O}_{21}$ -H requires 1027.5115. ^1H NMR (400 MHz, pyridine- d_5) δ : 0.99, 1.05, 1.07, 1.28, 1.32, 1.38 (all 3H, *s*), 1.32 (3H, *d*, $J=6.6$ Hz, rhamnose methyl), 1.80, 1.99 (each 3H, *s*, acetyl methyl), 3.35, 3.97 (both 1H, ABq, $J=10.2$ Hz, 29-H), 3.94 (1H, *d*, $J=2.7$ Hz, 22-H), 4.27 (1H, *dd*, $J=4.2, 11.7$ Hz, 3α -H), 4.62 (1H, *d*, $J=2.7$ Hz, 21-H), 5.21 (1H, *d*, $J=7.1$ Hz, H-1 of GlcA), 5.25 (1H, *d*, $J=6.8$ Hz, H-1 of Xyl), 5.66 (1H, *s*, H-1 of Rha); ^{13}C NMR: Tables 1 and 2.

Alkaline hydrolysis. A soln of 1 (50 mg) in 10 ml of 1.5% KOH was allowed to stand for 3 hr. The soln was neutralized with DOWEX 50W, and the reaction product was concentrated to dryness (1a, 42 mg).

Enzymatic hydrolysis. A soln of 1a (80 mg), and crude naringinase (90 mg, Sigma chemical Co., Ltd) in sodium acetate buffer (pH 4.5, 30 ml) was incubated at 37° for 10 days. The reaction mixt. was extracted with EtOAc, and the EtOAc layer was evapd to dryness. The residue (18 mg) was subjected to chromatography on silica gel (elution with CHCl_3 -MeOH, 12:1) to give aglycone (31 mg, mp 271–273°, HR-FAB-MS: m/z 489.3627, $\text{C}_{30}\text{H}_{50}\text{O}_5$ -H requires 469.3582).

Measurement of activity. The taste-modifying activity of strogin was assayed using four subjects as described previously [5]. A strogin soln (1 ml) was held in the mouth for 3 min and spat out. The mouth was rinsed with water and then water was tasted. The sweetness induced by water was evaluated by comparing its sweetness with that of a series of standard sucrose solutions (0.05–0.4 M). The sweet activity of strogins was also evaluated by comparing sweetness of the strogin soln with that of a series of the standard sucrose solutions.

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