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SWEET AND BITTER CUCURBITANE GLYCOSIDES FROM *HEMSLEYA CARNOSIFLORA*

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Key Word Index—*Hemsleya carnosiflora*; Cucurbitaceae; Chinese folk medicine; cucurbitane glycoside; carnosifllosides I, II, III, IV, V, VI; carnosiflogenins A, B, C; sweet natural glycoside; bitter natural glycoside.

Abstract—From rhizomes of *Hemsleya carnosiflora*, collected in Yunnan, China, six cucurbitane glycosides were isolated; one named carnosifloside I was tasteless, three named carnosiflosides II, III and IV were bitter, and two named carnosiflosides V and VI were sweet. The structures of these glycosides were elucidated and the structure–taste relationships are described.

INTRODUCTION

The plants of the genus *Hemsleya* are abundant in Yunnan and Sichuan, China and have been used as herbal medicines by minor nationalities in these provinces. In our previous studies on Chinese cucurbitaceous medicinal plants, saponins of oleanane-type triterpenes from *H. macrosperma* C. Y., *H. chinensis* C. A. Meyer [1, 2] and *Bolbostemma paniculatum* (Maxim.) Franquet. [3] have been reported. The present paper deals with the isolation and structure determination of sweet and bitter principles of rhizomes of *H. carnosiflora* C. Y. Wu et Z. L. Chen, sp. nov. Some observations on the structure–taste relationships of cucurbitane glycosides are also reported.

RESULTS AND DISCUSSION

An ethanolic extract of the rhizomes was subjected to column chromatography on highly porous polymer followed by column chromatography on silica gel and then on silanized silica gel, affording eight compounds, numbered **1**–**8** in increasing order of polarity. Compounds **1** (yield 0.49%) and **2** (yield 0.28%) were identified as the known cucurbitane triterpenes 25-*O*-acetyl-dihydrocucurbitacin F [4] and dihydrocucurbitacin F [5], respectively.

A new sweet glycoside, **8** (yield 0.04%), was named carnosifloside VI. Hydrolysis of **8** with crude hesperidinase [6] yielded an aglycone (**9**) named carnosiflogenin C. Previously, Takemoto *et al.* [7–9] isolated the sweet cucurbitane glycosides mogrosides IV (**10**) and V (**11**) from a Chinese folk medicine, fruits of *Momordica grosvenori* Swingl. (Cucurbitaceae). The ^{13}C NMR signals of mogrol (**12**), the common aglycone of **10** and **11**, were assigned as shown in Table 2. Comparison of the electron-impact mass spectrum (Scheme 1), the ^{1}H NMR (Table 1) [8] and ^{13}C NMR (Table 2) spectra of compound **9** with those of **12** indicated that both compounds have a common ring structure, being different from each other in the structure of the side chain. The side chain of compound **9** has one trisubstituted double bond, one-methyl and one $-CH_2OH$ instead of the 24,25-diol system of **12**. As shown in Table 2, the carbon signals assigned to C-20 to C-27 of cycloart-24-ene-3β,26-diol (**13**) [10] occurred at almost the same positions as those in the spectrum of **9**. It follows that compound **9** is cucurbita-5,24-diene-3β,11α,26-triol.

D-Glucose was detected in the acid hydrolysate of compound **8** by Oshima's procedure [11] which allows for the identification of sugars, including the absolute configuration. Comparison of the ^{13}C NMR spectra (Table 2) of **9** with **8** showed the glycosylation shifts [12, 13] for C-2, C-3, C-24, C-25 and C-26, demonstrating that both the 3- and 26-hydroxyl groups of compound **8** were glycosylated. The electron impact mass spectrum of peracetylated compound **8** showed fragment ions at *m/z* 619 [(Glc–Glc)Ac_7] and 331 [(Glc)Ac_4]. Anomeric proton signals (in pyridine-d_5) of compound **8** at δ4.76, 4.82 and 5.02 (each 1H, *d*, J = 8.0 Hz) indicated the presence of three β-D-glucosyl units. The sequencing analysis [14–16] of permethylated **8** revealed the presence of terminal and 6-linked glucopyranosyl residues. Partial acid hydrolysis [8] of compound **8** yielded progenins (**14** and **15**). The ^{13}C NMR spectra of **14** and **15** were consistent with the formulation as the 26-*O*-β-gentiobioside and 26-*O*-β-D-glucopyranoside of compound **9**, respectively. Based on these results, the structure of **8** was established as the 3-*O*-β-D-glucopyranosido-26-*O*-β-gentiobioside of compound **9**.

Acid hydrolysis of another sweet glycoside, named carnosifloside V (**7**, yield 0.02%), produced D-glucose, while hydrolysis of compound **7** with crude hesperidinase gave **9**. In the electron-impact mass spectrum of peracetylated **7**, the fragment ions associated with terminal glucosyl (*m/z* 331) and glucobiosyl (*m/z* 619) units were

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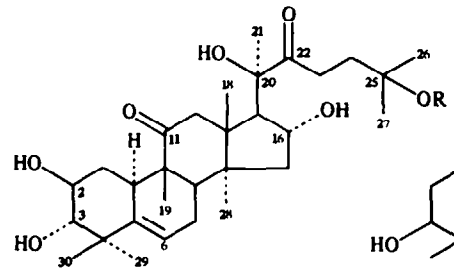
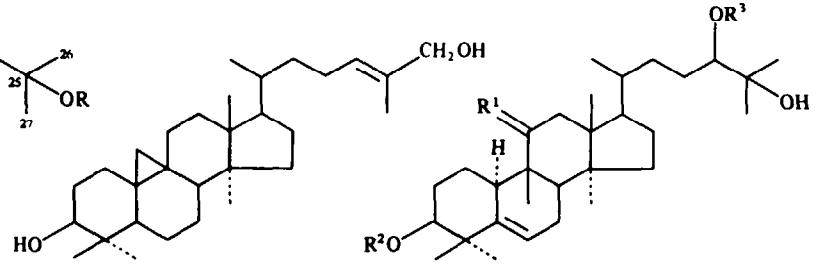


	R
1	Ac
2	H



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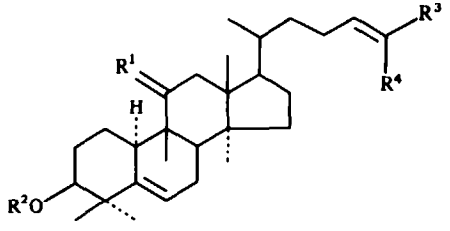
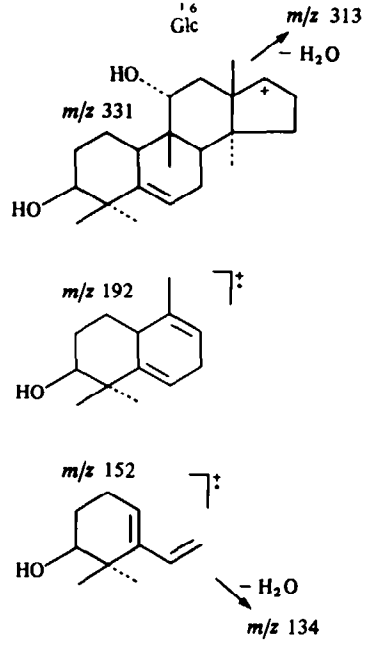


Glc: β-D-glucopyranosyl

	R^1	R^2	R^3	R^4	
9	αOH	H	CH_2OH	Me	tasteless
14	αOH	H	CH_2O-Glc^{6}Glc	Me	tasteless
15	αOH	H	CH_2O-Glc	Me	tasteless
7	αOH	Glc	CH_2O-Glc^{2}Glc	Me	sweet
8	αOH	Glc	CH_2O-Glc^{6}Glc	Me	sweet
16	=O	H	CH_2OH	Me	tasteless
3	=O	H	CH_2O-Glc^{6}Glc	Me	tasteless
18	=O	Glc	CH_2O-Glc	Me	tasteless
4	=O	Glc	CH_2O-Glc^{2}Glc	Me	bitter
5	=O	Glc	CH_2O-Glc^{6}Glc	Me	bitter
19	βOH	Glc	CH_2O-Glc^{6}Glc	Me	tasteless
20	=O	H	Me	CH_2OH	tasteless
21	=O	Glc	Me	CH_2O-Glc	tasteless
22	=O	H	Me	CH_2O-Glc^{6}Glc	tasteless
6	=O	Glc	Me	CH_2O-Glc^{6}Glc	bitter

	R^1	R^2	R^3
17	=O	H	H
12	αOH	H	H
10	αOH	Glc^{6}Glc	Glc^{2}Glc
11	αOH	Glc^{6}Glc	Glc^2(Glc^6)Glc



Scheme 1.

Table 1. ^{1}H NMR spectral data* for compounds **9**, **12**, **16**, **17** and **20** (270 MHz, $CDCl_3$, TMS as internal standard)

H	12†	9	17†	16	20
Me	0.85 *s*	0.82 *s*	0.76 *s*	0.74 *s*	0.74 *s*
	0.91 *s*	0.89 *s*	0.90 *d*	0.91 *d*	0.90 *d*
	0.93 *d*	0.92 *d*	(J = 5.5)	(J = 6.6)	(J = 6.6)
	(J = 6)	(J = 5.1)	1.04 *s*	1.03 *s*	1.03 *s*
	1.08 *s*	1.06 *s*	1.04 *s*	1.03 *s*	1.03 *s*
	1.18 *s*	1.13 *s*	1.12 *s*	1.12 *s*	1.12 *s*
	1.19 *s*	1.15 *s*	1.16 *s*	1.16 *s*	1.16 *s*
	1.19 *s*	1.67 *s*	1.16 *s*	1.66 *s*	1.79 *s*
	1.23 *s*		1.21 *s*		
3α-H	3.46 *m*	3.46 *t*-like	3.44 *m*	3.48 *t*-like	3.47 *t*-like
	($W_{1/2}$ = 6)	($W_{1/2}$ = 6.0)	($W_{1/2}$ = 6)	($W_{1/2}$ = 6.0)	($W_{1/2}$ = 6.0)
6-H	5.56 *br d*	5.57 *br d*	5.63 *br d*	5.66 *br d*	5.66
	(J = 6)	(J = 6.0)	(J = 6)	(J = 5.7)	(J = 5.9)
11β-H	3.92 *dd*	3.94 *dd*	—	—	—
	(J = 8, 8)	(J = 9.5, 7.3)			
12-H_2	1.84 *d*	‡	2.44 *d*	2.45 *d*	2.44 *d*
	(J = 8)		(J = 14)	(J = 14.3)	(J = 14.3)
			2.94 *d*	2.94 *d*	2.93 *d*
			(J = 14)	(J = 14.7)	(J = 14.3)
24-H	3.31 *m*	5.38 *t*	3.63 *m*	5.38 *t*	5.27 *t*
		(J = 7.0)		(J = 6.5)	(J = 7.0)

*Coupling constants (J in Hz) are in parentheses.
†Data taken from ref. [8], 100 MHz in $CDCl_3$–D_2O.
‡Obscured.

observed as in the case of compound **8**. Comparison of the ^{13}C NMR spectrum of compound **7** with that of **9** (Table 2) indicated that both the 3- and 26-hydroxyl groups were glycosylated. Anomeric proton signals (in pyridine-d_5) of compound **7** at δ4.83 (2H, *d*, J = 7.5 Hz) and 5.25 (1H, *d*, J = 7.5 Hz) indicated the presence of three β-glucosyl residues. The sequencing analysis of permethylated **7** revealed the presence of terminal and 2-linked glucopyranosyl units, indicating the presence of one β-sophorosyl group and one β-D-glucopyranosyl group. The allocation of both glycosyl moieties on two hydroxyl groups of the aglycone was elucidated by the chemical shifts of the anomeric carbon signals. It has been reported [12] that β-D-glucosides of secondary alcohols with (*S*)-chirality (regardless of axial or equatorial orientation), having alkyl substituents on their vicinal carbon, exhibit an anomeric carbon signal at remarkably low field near δ107. It was also reported [13] that the anomeric carbon signal of β-D-glucosides of allylic primary alcohols appears near δ103. Further, it is known that β-D-glucosylation at the 2-hydroxyl group of a glucosyl moiety (β-sophroside) results in an upfield shift of this anomeric carbon signal by about 2 ppm [17]. Based on these reports, the three carbon signals of compound **7** can be reasonably assigned as follows: δ101.7 to anomeric carbons of 26-*O*-β-sophorosyl linkage, δ106.2 to the terminal β-D-glucoside of the β-sophoroside moiety and δ107.2 to 3-*O*-β-D-glucoside, respectively. These assignments are consistent with the formulation of compound **7** as the 3-*O*-β-D-glucopyranosido-26-*O*-β-sophoroside of **9**.

The tasteless glycoside **3**, named carnosifloside I, and bitter glycosides **4** and **5**, named carnosiflosides II and III, were isolated in yields of 0.07, 0.05 and 0.35‰, respectively. Acid hydrolysis of these glycosides afforded D-glucose, while hydrolysis with crude hesperidinase yielded a common aglycone (**16**) named carnosiflogenin A, which showed an IR band due to a ketone at 1690 cm^{-1} (in chloroform). Comparison of the ^{13}C NMR spectrum of compound **16** with those of compound **9** and 11-oxo-mogrol (**17**) [8], a minor aglycone obtained from glycosides of fruits of *M. grosvenori* (Table 2), revealed that compound **16** has the same ring structure as that of **17** and the same side chain as that of **9**. The electron-impact mass spectrum of TMSi ether of compound **3** showed fragment ions at *m/z* 451 [Glc(TMSi)$_4$], 583 [Glc(TMSi)$_4$–O–CH_2–CH=O^+–TMSi, characteristic of hexose(1 → 6)hexose] and 829 [(Glc–Glc)(TMSi)$_7$]. The ^{1}H NMR spectrum of compound **3** showed two anomeric proton signals at δ4.80 and 5.06 (each 1H, *d*, J = 7 Hz) due to a β-glucosyl linkage, and the glycosylation shift in the ^{13}C NMR spectrum (Table 2) indicated the location of the glycosyl linkage at 26-OH. Based on these results, the structure of compound **3** was established as the 26-*O*-β-gentiobioside of **16**, which was supported by the ^{13}C NMR signals of the sugar moiety (Table 3).

The ^{13}C NMR signals of compound **5** due to the sugar moiety are almost superimposable over those in the spectrum of **8** (Table 3). The electron-impact mass spectrum of peracetylated **5** showed the same fragment ions due to the sugar moiety as those of **8**, and the sequencing analysis of permethylated **5** revealed the presence of terminal and 6-linked glucopyranosyl units. Partial hydrolysis of compound **5** gave **3** and a progenin (**18**), the latter of which was also obtained by hydrolysis with Takadiastase [18] (a crude mixture of glucoamylase). The ^{13}C NMR spectrum of compound **18** was consistent

Table 2. ^{13}C NMR spectral data of aglycone moieties of compounds **5**, **6**, **8**, **9**, **12**, **13**, **16**, **17**, **19** and **20** (25 MHz, C_5D_5N, TMS as internal standard)

C	13*†	12*	9*	9	8	19	17	16	5	20	6
1	32.0	24.1	24.1	25.8	26.2	23.9	21.2	21.2	22.1	21.2	22.1
2	60.4	28.4	28.4	30.8	29.5	29.3	29.7	29.7	28.3	29.8	28.3
3	78.8	76.5	76.5	76.3	87.9	87.5	75.5	75.5	87.1	75.5	87.2
4	40.5	42.0	42.0	42.3	42.3	41.8	41.8	41.9	41.9	41.9	41.9
5	47.5	142.0	142.0	144.2	144.1	142.9	141.3	141.1	141.2	141.1	141.2
6	21.1	121.0	121.1	119.2	118.4	119.3	118.9	118.9	118.4	118.9	118.4
7	28.2	25.4	25.4	24.6	24.6	25.0	24.2	24.2	24.0	24.2	24.0
8	48.0	43.1	43.1	43.6	43.4	41.9	44.0	44.1	43.9	44.1	43.9
9	20.0	39.8	39.8	40.2	40.0	40.7	49.1	49.1	49.0	49.1	49.0
10	26.1	35.9	35.9	36.6	36.5	40.0	35.9	35.9	35.9	35.9	35.9
11	26.1	78.7	78.8	77.8	77.9	72.1	213.8	213.8	213.7	213.7	213.7
12	35.6	40.2	40.2	41.1	41.0	39.3	48.7	48.7	48.6	48.7	48.8
13	45.4	47.2	47.2	47.4	47.3	45.7	49.1	49.1	49.5	49.6	49.5
14	48.8	49.3	49.3	49.8	49.7	49.9	49.6	49.4	49.5	49.6	49.5
15	33.0	34.1	34.1	34.5	34.6	35.2	34.5	34.5	34.5	34.5	34.5
16	26.5	29.1	29.2	28.4	28.3	28.2	28.1	28.0	28.3	28.0	28.0
17	48.8	50.4	50.2	50.7	50.5	51.2	49.8	49.6	49.5	49.6	49.7
18	18.1‡	16.7‡	16.7‡	17.0‡	17.0‡	18.0‡	16.9‡	16.8‡	16.8‡	16.9‡	16.9‡
19	29.9	25.1	25.8	26.7	26.6	22.9	20.1	20.1	20.2	20.2	20.2
20	36.0	35.7	35.9	36.2	36.1	36.3	35.9	35.9	35.9	36.0	35.9
21	18.3‡	18.5‡	18.6‡	18.8‡	18.8‡	18.8‡	18.2‡	18.2‡	18.1‡	18.3‡	18.2‡
22	36.0*	28.1	36.0	36.9	36.7	36.3	33.9	36.3	36.0	36.9	36.7
23	25.1	33.2	25.4	24.9	24.8	24.7	28.6	24.6	24.7	24.6	24.9
24	127.0	78.7	126.8	125.1	128.9	129.1	78.9	124.9	128.7	127.1	130.4
25	134.4	73.2	134.4	136.1	132.0	132.1	72.7	136.1	132.1	136.4	132.1
26	69.0	26.5	69.0	68.1	75.3	75.4	25.9	68.0	75.3	21.8	22.1
27	13.7	23.2	13.6	14.0	14.2	14.2	26.1	13.9	14.2	60.8	67.6
28	19.4‡	19.0‡	19.1‡	19.3‡	19.3‡	18.0‡	18.5‡	18.4‡	18.4‡	18.5‡	18.5‡
29	25.1	26.5	26 5	27.3	27.6	28.2	27.9	27.9	28.0	28.0	28.3
30	14.0	25.1‡	25.8‡	26.2‡	26.2‡	25.9‡	26.1‡	26.3‡	25.8‡	26.3‡	25.9‡

*Measured in $CDCl_3$.
†Data taken from ref. [10].
‡Signals may be interchangeable in each column.

with its formulation as the 3,26-di-*O*-*β*-D-glucopyranoside of **16**. Reduction of **5** with sodium borohydride [19] yielded **8** and its 11-epimer (**19**). Based on these results, the structure of compound **5** was established as the 3-*O*-*β*-D-glucopyranosido-26-*O*-*β*-gentiobioside of **16**.

The electron-impact mass spectrum of peracetylated **4** showed the same fragment ions due to the sugar moiety as those of **7** and the ^1H NMR spectrum exhibited three anomeric proton signals due to *β*-glucosyl linkages at *δ*4.82 (2H, *d*, *J* = 7.5 Hz) and 5.23 (1H, *d*, *J* = 7.5 Hz). The sequencing analysis of permethylated **4** revealed the presence of terminal and 2-linked glucopyranosyl units. In the ^{13}C NMR spectrum of compound **4**, carbon signals due to the aglycone moiety were almost superimposable over those in the spectrum of **5** and those associated with the sugar moiety appeared at almost the same positions as those of **7**. Consequently, compound **4** was formulated as the 3-*O*-*β*-D-glucopyranosido-26-*O*-*β*-sophoroside of **16**.

Another bitter glycoside **6**, named carnosifloside IV, was isolated in a yield of 0.05%. Hydrolysis of compound **6** with crude hesperidinase yielded an aglycone (**20**) named carnosiflogenin B, which exhibited an IR band due to a carbonyl group at 1690 cm^{-1} (in chloroform). As shown in Table 2, on going from compound **16** to **20**, the carbon signals due to C-24 and the methyl on the olefinic carbon were displaced downfield and the signal due to $-CH_2OH$ was shifted upfield, while other carbon signals remained almost unshifted. By reference [20] to the carbon signal difference between 1-hydroxylinalool and 9-hydroxylinalool, compound **20** must be the *Z*-isomer of **16** with respect to the double bond of the side chain, being formulated as shown (**20**).

In the ^{13}C NMR spectrum of compound **6**, the glycosylation shifts were observed for signals due to the carbons around both the 3- and 27-hydroxyl groups (Table 2) and carbon signals associated with the sugar moiety of compound **6** appeared at almost the same positions as those in the spectrum of **5** and **8** (Table 3). Partial hydrolysis of compound **6** afforded progenins **21** and **22**, the ^{13}C NMR spectra of which were in agreement with assignment of the structures of **21** and **22** as the 3,27-di-*O*-*β*-D-glucopyranoside and the 27-*O*-*β*-gentiobioside of **20**, respectively. The structure of compound **6** was thus established as the 3-*O*-*β*-D-glucopyranosido-27-*O*-*β*-gentiobioside of **20**.

Cucurbitane-type triterpenes are known as bitter principles of cucurbitaceous plants, while some of the

Table 3. ^{13}C NMR spectral data of the sugar moieties of compounds **7** and **8** (25 MHz, C_5D_5N, TMS as internal standard)

		7	8
3-Glc	1	107.2	107.2
	2	75.1	75.0
	3	78.5*	78.3*
	4	71.4†	71.4
	5	78.3*	77.9*
	6	62.8‡	62.8†
26-Glc	1	101.7	103.1
(inner)	2	83.8	75.0
	3	78.0*	78.3*
	4	71.7†	71.4
	5	78.0*	77.1
	6	62.9‡	69.9
Glc	1	106.2	105.2
(terminal)	2	76.7	75.0
	3	78.5*	78.3*
	4	71.3†	71.4
	5	78.0*	78.3*
	6	62.6‡	62.6†

*,†,‡Signals may be interchangeable in each column.

glycosides of triterpenes of this type such as compounds **10**, **11** and glycosides from *Bryonia dioica* Jacq [21] are sweet tasting. In view of the structure–taste relationship of these cucurbitane glycosides, it is noteworthy that the bisdesmosides of an 11α-hydroxy compound (**9**) are sweet and those of the 11-oxo aglycones (**16** and **20**) are bitter, while the monodesmosides of both the aglycones and the bisdesmoside (**19**) of the 11β-hydroxy compound are almost tasteless.

EXPERIMENTAL

^1H NMR spectra were measured at 100 and 270 MHz, ^{13}C NMR at 25 MHz in $CDCl_3$ or C_5D_5N; chemical shifts are given on the δ(ppm) scale with TMS as internal standard. MS were recorded at 75 eV. Acetylation or trimethylsilylation of glycosides for MS was carried out according to ref. [22]. GC/MS conditions: He carrier gas at 20 ml/min; 1.5 m × 2.6 mm column packed with 5% ECNSS-M; isothermal 170°; injection temp. 190°; separator temp. 190°; ionization voltage 70 eV. For silica gel CC, Kieselgel 60 (Merck) and for HPLC, a column of TSK-Gel ODS-120T (21.5 mm × 30 cm, detection, RI) were used. Acid hydrolysis of triterpene oligoglycosides [1] followed by identification of the resulting monosaccharides including absolute configuration [11] and the sequencing analysis of sugar moiety by GC/MS were carried out as described in previous papers [14–16].

Plant material was cultivated and harvested in the Botanical Garden of Kunming Institute of Botany, Yunnan, China and authenticated by Emeritus Professor Cheng-Yih Wu of this Institute. The specimen has been deposited in the Herbarium of this Institute.

Extraction and separation. Dried rhizomes of *H. carnosiflora* (300 g) were extracted with hot EtOH. After removal of the solvents by evaporation, the EtOH extracts (39 g) were chromatographed on highly porous polymer, DIAION HP-20 (Mitsubishi Chem. Ind. Tokyo, Japan) (H_2O, 80% aq. MeOH and MeOH successively and finally Me_2CO). The eluate (16 g) with 80% aq. MeOH was separated by CC on silica gel ($CHCl_3$–MeOH, 10:1; $CHCl_3$–MeOH–H_2O, 30:10:1; and then $CHCl_3$–MeOH–H_2O, 6:4:1, all homogeneous) to give four fractions, I–IV; fraction I was crystallized from MeOH–H_2O to give compound **1** (1.4 g, 0.5% yield). Fraction II was separated by HPLC (70% aq. MeOH) to give compound **2** (792 mg, 0.3% yield). These known compounds **1** and **2** were identified by comparison of the ^1H NMR and ^{13}C NMR spectra with those of corresponding authentic samples.

Fraction III was separated by repeated reverse-phase CC on LiChroprep RP-8 (Merck) (73% aq. MeOH) to give compound **3** (193 mg, 0.07% yield), and fraction IV was separated by repeated CC on silica gel ($CHCl_3$–MeOH–H_2O, 10:5:1, homogeneous) and then by reverse-phase CC on LiChroprep RP-8 (73% aq. MeOH) to give compounds **4** (145 mg, 0.05% yield), **5** (993 mg, 0.3% yield), **6** (150 mg, 0.05% yield), **7** (60 mg, 0.02% yield), and **8** (119 mg, 0.04% yield).

Carnosifloside I (**3**). White powder, $[\alpha]_D^{22}$ +59.6° (MeOH; *c* 1.42). (Found: C, 62.65: H, 8.82. $C_{42}H_{68}O_{13} \cdot 1\frac{1}{2}H_2O$ requires: C, 62.43;H, 8.86%.)

Carnosifloside II (**4**). White powder, $[\alpha]_D^{23}$ +55.9° (MeOH; *c* 0.95). (Found: C, 58.90; H, 8.42. $C_{48}H_{78}O_{18} \cdot 2H_2O$ requires: C, 58.88; H, 8.44%.)

Carnosifloside III (**5**). White powder, $[\alpha]_D^{14}$ +33.6° (MeOH; *c* 1.01). (Found: C, 58.36; H, 8.34. $C_{48}H_{78}O_{18} \cdot 2\frac{1}{2}H_2O$ requires: C, 58.34; H, 8.47%.)

Enzymatic hydrolysis of compound **5**. A soln of **5** (104 mg) and crude hesperidinase (100 mg; Tanabe Pharm. Ind. Co. Ltd., Osaka, Japan) in H_2O (10 ml) was incubated at 37° for 4 days. The hydrolysate was chromatographed on DIAION HP-20 (H_2O and then MeOH). The MeOH eluate was further purified by CC on silica gel ($CHCl_3$–MeOH, 10:1), affording **16** (9 mg). Compound **16**: Colourless needles ($CHCl_3$–C_6H_6), mp 170.5°, $[\alpha]_D^{16}$ +168.7° (MeOH; *c* 0.43). (Found: C, 78.83; H, 10.85. $C_{30}H_{48}O_3$ requires: C, 78.89; H, 10.59%.)

Partial hydrolysis of compound **5**. To a soln of **5** (150 mg) in dry MeOH (3 ml) was added 10% dry HCl–MeOH (3 ml) and the mixture was heated at 80° for 3 hr. The reaction mixture was neutralized with ion-exchange resin (Amberlite MB-3) and concentrated to dryness. The residue was separated by HPLC (80% aq. MeOH) to give **3** (26 mg) and **18** (16 mg). Compound **18**: White powder, $[\alpha]_D^{21}$ +66.5° (MeOH; *c* 0.95). (Found: C, 62.96; H, 8.99. $C_{48}H_{68}O_{13} \cdot H_2O$ requires: C, 63.14; H, 8.83%.)

Partial enzymatic hydrolysis of compound **5**. A soln of **5** (160 mg) and Takadiastase Y (160 mg; Sankyo Co. Ltd., Tokyo, Japan) in H_2O (9 ml) was incubated at 37° for 6 hr. The hydrolysate was chromatographed on DIAION HP-20 (H_2O and then MeOH). The MeOH eluate was further separated by CC on silica gel ($CHCl_3$–MeOH–H_2O, 30:10:1, homogeneous), affording **18** (42 mg).

Reduction of compound **5** *with* $NaBH_4$. To a soln of **5** (50 mg) in 50% aq. dioxane was added $NaBH_4$ (42 mg) and the mixture was heated at 50° for 3 days. The reaction mixture was acidified by passing it through a column of Dowex 50W-X8 (H^+ form) and was concentrated to dryness. Boric acid in the residue was removed by co-distillation with MeOH. The residue was separated by HPLC (33% MeCN) to give **8** (9 mg) and **19** (9 mg). Compound **19**: White powder, $[\alpha]_D^{16}$ −4.3° (MeOH; *c* 0.40). (Found: C, 58.99; H, 8.59. $C_{48}H_{80}O_{18} \cdot 2H_2O$ requires: C, 58.76; H, 8.63%.)

Carnosifloside IV (**6**). White powder, $[\alpha]_D^{18}$ +36.2° (MeOH; *c* 0.61). (Found: C, 57.68; H, 8.44. $C_{48}H_{78}O_{18} \cdot 3H_2O$ requires: C, 57.82; H, 8.49%.). Enzymatic hydrolysis of **6** (150 mg) with crude

hesperidinase under the same conditions as those of **5** yielded **20** (44 mg). Compound **20**: Colourless needles (MeOH–H_2O), mp 155–156°, $[\alpha]_D^{18}$ +159.3° (MeOH; *c* 0.54). (Found: C, 78.90; H, 10.92. $C_{30}H_{48}O_3$ requires: C, 78.89; H, 10.59%.) Partial hydrolysis of **6** (150 mg) with HCl under the same conditions as those of **5** yielded **21** (19 mg) and **22** (32 mg). Compound **21**: White powder, $[\alpha]_D^{13}$ +60.0° (C_5H_5N; *c* 0.39). (Found: C, 62.41; H, 9.03. $C_{42}H_{68}O_{13} \cdot 1\frac{1}{2}H_2O$ requires: C, 62.43; H, 8.86%.) Compound **22**: White powder, $[\alpha]_D^{13}$ +56.2° (C_5H_5N; *c* 0.66). (Found: C, 63.38; H, 8.94. $C_{42}H_{68}O_{13} \cdot H_2O$ requires: C, 63.14; H, 8.83%.)

Carnosifloside V (**7**). White powder, $[\alpha]_D^{26}$ +4.0° (MeOH; *c* 0.77). (Found: C, 59.34; H, 8.66. $C_{48}H_{80}O_{18} \cdot 1\frac{1}{2}H_2O$ requires: C, 59.30; H, 8.61%.)

Carnosifloside VI (**8**). White powder, $[\alpha]_D^{26}$ +6.0° (MeOH; *c* 0.60). (Found: C, 58.62; H, 8.74. $C_{48}H_{80}O_{18} \cdot 2H_2O$ requires: C, 58.76, H, 8.63%.). Enzymatic hydrolysis of **8** (100 mg) with crude hesperidinase under the same conditions as those of **5** yielded **9** (20 mg). Compound **9**: White powder, $[\alpha]_D^{29}$ +41.0° (MeOH; *c* 0.39). (Found: C, 78.64; H, 11.02. $C_{30}H_{50}O_3$ requires: C, 78.55; H, 10.99%.) Partial hydrolysis of **8** (150 mg) with HCl under the same conditions as those of **5** yielded **14** (33 mg) and **15** (21 mg). Compound **14**: White powder, $[\alpha]_D^{16}$ 0° (MeOH; *c* 2.1). (Found: C, 63.30; H, 9.05. $C_{42}H_{70}O_{13} \cdot H_2O$ requires: C, 62.98; H, 9.06%.) Compound **15**: White powder, $[\alpha]_D^{14}$ +22.0° (MeOH; *c* 1.27). (Found: C, 66.98; H, 9.60. $C_{36}H_{60}O_8 \cdot 1\frac{1}{2}H_2O$ requires: C, 66.74; H, 9.80%.)

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