Oligosaccharides from *Hoya carnosa*

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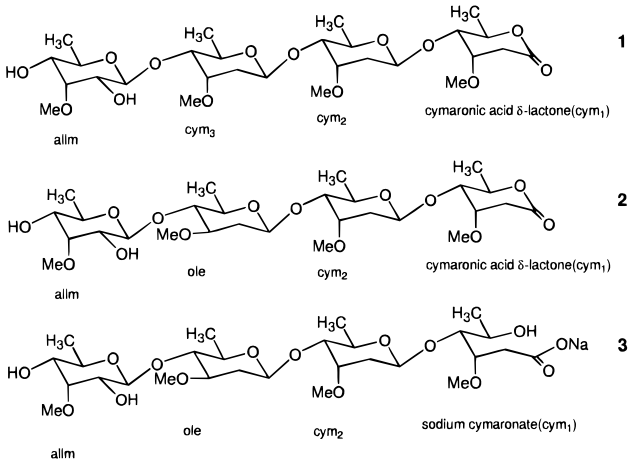
Oligosaccharides A, B, and C (**1**−**3**, respectively) were isolated from the stem of *Hoya carnosa*. Their structures were established by NMR and chemical methods. Compounds **1** and **2** were 6-deoxy-3-*O*-methyl-*β*-allopyranosyl(1→4)-*β*-cymaropyranosyl(1→4)-*β*-cymaropyranosyl(1→4)-*β*-cymaronic acid *δ*-lactone and 6-deoxy-3-*O*-methyl-*β*-allopyranosyl(1→4)-*β*-oleandropyranosyl(1→4)-*β*-cymaropyranosyl(1→4)-*β*-cymaronic acid *δ*-lactone, respectively. Compound **3** was the sodium salt of **2**.

Hoya carnosa R. Br. (Asclepiadaceae) is a perennial plant, indigenous to the southern part of Japan, China, and Australia.[1] The isolation of cyclitols was reported by Kindl et al. in 1966.[2] In continuation of our research for natural anti-sweet compounds from the Asclepiadaceae family,[3−5] we have examined the active constituents of *H. carnosa*. Two oligosaccharides, A and B (**1** and **2**) with lactone rings, and the sodium salt of the latter, C (**3**), were isolated; none was an anti-sweet compound. We describe here the isolation and structure elucidation of **1**−**3** by various NMR techniques, including COSY, HMQC, HMBC, and ROESY experiments and by chemical degradation.



The ethanolic extract of *H. carnosa* (from Formosa) was partitioned between EtOAc and H_2O. The H_2O layer was subjected to Amberlite XAD-2 column chromatography to give a fraction eluted by MeOH. Separation of the MeOH eluate using Si gel and reversed-phase HPLC gave oligosaccharides A and B (**1** and **2**) and a sodium salt, C (**3**).

Oligosaccharide A (**1**) was obtained as colorless needles. The molecular formula, $C_{28}H_{48}O_{14}$, is based on a quasi-molecular ion peak at *m*/*z* 607 $[M - H]^-$ in the FABMS and on the carbon content observed in the ^{13}C NMR spectrum. The IR absorption maxima at 1730 cm^{-1} and the signal at *δ* 168.8 in the ^{13}C NMR spectrum suggested the presence of a lactone ring. The ^1H NMR spectrum of **1** exhibited three anomeric protons at *δ* 5.14, 5.11, and 5.09; four methoxy signals at *δ* 3.86, 3.59, 3.57, and 3.49; and four methyl doublets at *δ* 1.55, 1.50, 1.33, and 1.32, indicating that **1** might consist of four deoxyhexoses with methoxy groups. Acidic hydrolysis of **1** afforded cymnarose (cym) and 6-deoxy-3-*O*-methylallose (allm) confirmed by TLC analysis.[6,7] In the ^{13}C NMR data, the corresponding three anomeric carbons were observed at *δ* 104.2, 100.4, and 99.7. Furthermore, from their coupling constants, the sugars all have *β*-glycosidic linkages. The remaining unassignable signals, except for those due to one 6-deoxy-3-*O*-methyl-*β*-allopyranose and two *β*-cymaropyranoses, were observed at *δ* 168.8, 79.0, 75.3, 74.3, 58.0, 35.7, and 19.1. COSY, HMQC, and HMBC spectra led to the identification of cymaronic acid *δ*-lactone as the fourth sugar. Further evidence for the sugar sequence and their linkage sites was derived from the HMBC experiment, which showed significant correlation peaks between anomeric protons and the glycosylated carbons. In the ^{13}C NMR spectrum, the signals shifted downfield by glycosylation[8,9] were observed at *δ* 83.2, 82.8, and 79.0. These were assigned to C-4 carbons of each sugar, indicating the probable points of glycosidic linkages in the oligosaccharide to be at C-4. The HMBC experiment revealed long-range couplings from H-1 of allm to C-4 of cym_3, H-1 of cym_3 to C-4 of cym_2, and H-1 of cym_2 to C-4 of cymaronic acid *δ*-lactone. Consequently, it was concluded that the structure of **1** was 6-deoxy-3-*O*-methyl-*β*-allopyranosyl(1→4)-*β*-cymaropyranosyl(1→4)-*β*-cymaropyranosyl(1→4)-cymaronic acid *δ*-lactone.

Oligosaccharide B (**2**) gave a $[M - H]^-$ peak at *m*/*z* 607 in the FABMS, consistent with a molecular formula of $C_{28}H_{48}O_{14}$, identical with that of **1**. The ^1H and ^{13}C NMR spectra of **2** were essentially identical to those of **1**. Comparison of the ^{13}C NMR spectra of **2** and **1** showed that the signals of the third cymarose unit in **1** were missing in **2** and signals due to a glycosylated *β*-oleandropyranosyl unit were seen at *δ* 101.8, 82.9, 79.2, 72.0, 57.3, 37.5, and 18.9 (Table 1). Indeed, the acid hydrolysis of **2** under the same conditions as **1** afforded oleandrose, in addition to 6-deoxy-3-*O*-methylallose and cymarose. The sugar linkages were confirmed by HMBC long-range correlations in the same way as for **1**. Consequently, it was concluded that the structure of **2** was 6-deoxy-3-*O*-methyl-*β*-allopyranosyl-

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Table 1. ^{1}H NMR Data of **1**−**3** in C_5D_5N

position	**1** δ_H	**2** δ_H	**3** δ_H
cym$_1$ 2	2.93 (dd, J = 17.5, 3.5)	2.94 (dd, J = 17.5, 3.5)	3.11 (m)
	3.04 (dd, J = 17.5, 4.0)	3.06 (dd, J = 17.5, 4.0)	3.50 (m)
3	4.10 (ddd, J = 4.0, 3.5, 2.0)	4.11 (ddd, J = 4.0, 3.5, 2.0)	4.46 (m)
4	3.90 (dd, J = 8.5, 2.0)	3.91 (dd, J = 8.5, 2.0)	4.27 (m)
5	4.82 (dq, J = 8.5, 6.5)	4.83 (dq, J = 8.5, 6.5)	4.50 (m)
6	1.33 (d, J = 6.5)	1.34 (d, J = 6.5)	1.52 (d, J = 6.0)
OMe	3.49 s	3.50 s	3.60 s
cym$_2$ 1	5.11 (dd, J = 9.5, 2.0)	5.12 (dd, J = 9.5, 2.0)	5.52 (dd, J = 9.5, 2.0)
2	1.78 (ddd, J = 13.5, 9.5, 3.5)	1.78 (ddd, J = 13.5, 9.5, 3.5)	1.79 (ddd, J = 13.5, 9.5, 3.5)
	2.26 (ddd, J = 13.5, 3.5, 2.0)	2.26 (ddd, J = 13.5, 3.5, 2.0)	2.39 (ddd, J = 13.5, 3.5, 2.0)
3	4.00 (dt, J = 3.0, 3.5)	3.99 (dt, J = 3.0, 3.5)	3.97 (dt, J = 3.0, 3.5)
4	3.41 (dd, J = 9.5, 3.0)	3.42 (dd, J = 9.5, 3.0)	3.42 (dd, J = 9.5, 3.0)
5	4.13 (dq, J = 9.5, 6.5)	4.15 (dq, J = 9.5, 6.5)	4.14 (dq, J = 9.5, 6.5)
6	1.32 (d, J = 6.5)	1.37 (d, J = 6.5)	1.34 (d, J = 6.5)
OMe	3.57 s	3.53 s	3.47 s
cym$_3$ 1	5.09 (dd, J = 9.5, 2.0)	4.68 (dd, J = 9.5, 2.0)	4.64 (dd, J = 9.5, 2.0)
or 2	1.82 (ddd, J = 12.5, 9.5, 2.0)	1.73 (ddd, J = 12.5, 9.5, 2.0)	1.79 (ddd, J = 12.5, 9.5, 2.0)
ole	2.34 (ddd, J = 12.5, 3.5, 2.0)	2.48 (ddd, J = 12.5, 3.5, 2.0)	2.43 (ddd, J = 12.5, 3.5, 2.0)
3	4.10 (ddd, J = 3.5, 3.0, 2.0)	3.59 (m)	3.56 (m)
4	3.54 (dd, J = 10.0, 3.0)	3.60 (m)	3.58 (m)
5	4.20 (dq, J = 10.0, 6.5)	3.56 (m)	3.52 (m)
6	1.50 (d, J = 6.5)	1.64 (d, J = 6.0)	1.63 (d, J = 6.0)
OMe	3.59 s	3.53 s	3.50 s
allm 1	5.14 (d, J = 8.0)	5.31 (d, J = 8.0)	5.29 (d, J = 8.0)
2	3.90 (dd, J = 8.0, 3.0)	3.90 (dd, J = 8.0, 2.5)	3.89 (dd, J = 8.0, 3.0)
3	4.08 (dd, J = 3.0, 2.5)	4.09 (dd, J = 3.0, 2.5)	4.08 (dd, J = 3.0, 2.5)
4	3.62 (dd, J = 9.5, 2.5)	3.62 (dd, J = 8.5, 3.0)	3.62 (dd, J = 9.5, 3.0)
5	4.17 (dq, J = 9.5, 6.5)	4.18 (dq, J = 8.5, 6.5)	4.17 (dq, J = 9.5, 6.5)
6	1.55 (d, J = 6.5)	1.56 (d, J = 6.5)	1.55 (d, J = 6.5)
OMe	3.86 s	3.84 s	3.83 s

(1→4)-β-oleandropyranosyl(1→4)-β-cymaropyranosyl(1→4)-β-cymaronic acid δ-lactone.

Oligosaccharide C (**3**) obtained in a more polar fraction, exhibited a quasi-molecular ion peak at m/z 648 [M − H]$^-$ in the FABMS, 40 mass units more than that of **2**. The IR absorption maxima at 1570 and 1410 cm^{-1} and the signal at δ 177.0 (br) in the ^{13}C NMR spectrum suggested the presence of carboxylate. Acid hydrolysis of **3** allowed identification of the same sugar components as **2**, that is, 6-deoxy-3-*O*-methylallose, cymarose, and oleandrose. On comparison of the ^{13}C NMR spectra of **3** and **2**, the signals for 6-deoxy-3-*O*-methyl-β-allopyranose, β-cymaropyranose, and β-oleandropyranose units were identical with both compounds. The major differences were the absence of signals at δ 168.8 and 74.3 in cymaronic acid δ-lactone and the appearance of signals at δ 177.0, 67.5, and 82.8, 79.3, 67.5, 57.7, 38.8, and 19.0. The corresponding protons in the ^{1}H NMR spectrum exhibited broad signals appearing at δ 4.46, 4.50, 4.27, 3.60, 3.50 and 3.11, and 1.52. These findings and the observation of the quasi-molecular ion peak at m/z 647 [M ($C_{28}H_{49}NaO_{15}$) − H]$^-$ in the FABMS indicated the presence of sodium cymaronate. The sugar linkages were confirmed by HMBC long-range correlations in the same way as for **1** and **2**. Consequently, the structure of **3** was concluded to be the sodium carboxylate of **2**.

Table 2. ^{13}C NMR Data of **1**−**3** in C_5D_5N

position	**1** δ_C	**2** δ_C	**3** δ_C
cym$_1$ 1	168.8 (s)	168.8 (s)	177.0 (s)
2	35.7 (t)	35.6 (t)	38.8 (t)
3	75.3 (d)	75.3 (d)	79.3 (d)
4	79.0 (d)	79.0 (d)	82.8 (d)
5	74.3 (d)	74.3 (d)	67.5 (d)
6	19.1 (q)	19.0 (q)	19.0 (q)
OMe	58.0 (q)	57.9 (q)	57.7 (q)
cym$_2$ 1	99.7 (d)	99.7 (d)	99.2 (d)
2	36.7 (t)	36.7 (t)	36.9 (t)
3	77.7 (d)	77.6 (d)	78.1 (d)
4	82.8 (d)	82.9 (d)	83.4 (d)
5	69.1 (d)	69.1 (d)	69.2 (d)
6	18.3 (q)	18.4 (q)	18.8 (q)
OMe	58.9 (q)	58.9 (q)	58.9 (q)
cym$_3$ 1	100.4 (d)	101.8 (d)	102.0 (d)
or 2	36.8 (t)	37.5 (t)	37.8 (t)
ole 3	78.1 (d)	79.2 (d)	79.4 (d)
4	83.2 (d)	82.9 (d)	83.0 (d)
5	69.4 (d)	72.0 (d)	72.2 (d)
6	18.6 (q)	18.9 (q)	19.2 (q)
OMe	58.9 (q)	57.3 (q)	57.4 (q)
allm 1	104.2 (d)	102.1 (d)	102.1 (d)
2	73.1 (d)	73.2 (d)	73.4 (d)
3	84.0 (d)	84.0 (d)	84.2 (d)
4	74.4 (d)	74.6 (d)	74.8 (d)
5	70.7 (d)	71.0 (d)	71.2 (d)
6	18.6 (q)	18.6 (q)	19.0 (q)
OMe	62.2 (q)	62.1 (q)	62.3 (q)

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-360 polarimeter. IR spectra were recorded on a JASCO FT−IR-5300, NMR spectra on Varian UNITY 600 spectrometer in pyridine-d_5 solutions using TMS as internal standard. NMR experiments included ^{1}H−^{1}H COSY, HMQC, HMBC, and ROESY. Coupling constants (J values) are given in hertz. The FABMS (Xe gun, 10 kV, triethylene glycol as the matrix) was measured on a JEOL JMS−PX303 mass spectrometer.

Plant Material. Stems of *Hoya carnosa* were collected in Formosa, in June 1997. A voucher specimen (TB 5423) is deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan.

Extraction and Isolation. Fresh stems (10 kg) of *H. carnosa*, collected in Taipei, Formosa, in June 1997, were extracted with absolute EtOH at room temperature for 6 weeks. The ethanolic extract was partitioned between H_2O and EtOAc. The H_2O layer was passed through an Amberlite XAD-2 column and eluted with 100% MeOH. The MeOH eluate (17 g) was chromatographed on Si gel with CH_2Cl_2−MeOH−H_2O (25:6:0.1−60:40:10) to give five fractions (1−5). Fraction 3 (2.0 g) was subjected to HPLC (ODS, CH_3CN−0.3% HOAc 1:4, pH 3.1) to afford oligosaccharides A (**1**, 5.0 mg) and B (**2**,

10 mg). Fraction 5 (1.0 g) was further subjected to chromatography on Si gel with CH_2Cl_2−MeOH−EtOAc−H_2O (6:2:4:1, lower layer) to give oligosaccharide C (**3**, 15.0 mg).

Oligosaccharide A (1): colorless needles, mp 191−193 °C; $[\alpha]^{25}_D$ +15.8° (*c* 0.4, MeOH); FT−IR (dry film) 3430 (br), 1730, 1450, 1375, 1165, 1095 cm^{-1}; ^1H and ^{13}C NMR, Tables 1 and 2, respectively; FABMS *m*/*z* 607 $[M - H]^-$; *anal.* C 55.05%, H 8.00%, calcd for $C_{28}H_{48}O_{14}$, C 55.25%, H 7.95%.

Oligosaccharide B (2): colorless needles, mp 193−195 °C; $[\alpha]^{25}_D$ +2.3° (*c* 0.6, MeOH); FT−IR (dry film) 3420 (br), 1735, 1450, 1370, 1160, 1090 cm^{-1}; ^1H and ^{13}C NMR, Tables 1 and 2, respectively; FABMS *m*/*z* 607 $[M - H]^-$; *anal.* C 55.10%, H 8.05%, calcd for $C_{28}H_{48}O_{14}$, C 55.25%, H 7.95%.

Oligosaccharide (3): amorphous solid; $[\alpha]^{25}_D$ −1.2° (*c* 1.4, MeOH); FT−IR (dry film) 3415 (br), 1570, 1410, 1375, 1165, and 1055 cm^{-1}; ^1H and ^{13}C NMR, Tables 1 and 2, respectively; FABMS *m*/*z* 647 $[M - H]^-$; *anal.* C 51.70%, H 7.80%, calcd for $C_{28}H_{49}NaO_{15}$, C 51.85%, H 7.61%.

Acid Hydrolysis of Oligosaccharides A−C (1−3). A solution of **1**−**3** (each 2 mg) in 1.0 mL of dioxane was treated with 0.5 mL of 1% H_2SO_4 and stirred at 100 °C for 60 min. After cooling, the reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. The H_2O layer was neutralized with Amberlite IRA-45 and evaporated under reduced pressure to give the sugar portion. Monosaccharides were identified by TLC−TLC solvent [CH_2Cl_2−EtOH (9:1)] R_f: cymarose, 0.47; oleandrose, 0.36; 6-deoxy-3-*O*-methylallose, 0.20. Allm and cym from **1** and allm, cym, and ole from **2** and **3** were detected, respectively.[6,7]

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