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A NEW DISACCHARIDE, GLAUCOBIOSE FROM CHINESE DRUG "PAI-CH'IEN": A COMPARISON OF ^{13}C NMR WITH ITS DIASTEREOMERIC ISOMER, STROPHANTHOBIOSE

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Abstract: The structure of a new disaccharide named glaucobiose (4) has been established, and the ^{13}C NMR of its methyl β- (5) and α-glycoside (6), and that of methyl β-strophanthobioside (2) were studied.

We have already reported[1] the structures of aglycones and glycosides obtained from "Pai-ch'ien"[2a] ("Bai-qian"[2b]), dried root of Cynanchum glaucescens Hand-Mazz (Asclepiadaceae). We wish to report now the structure of a new disaccharide named glaucobiose (4) and the ^{13}C NMR study on glycosidation shifts for its methyl β- (5) and α-glycoside (6), and methyl β-strophanthobioside (2).

The rather polar fractions of crude glycosides of this drug were hydrolyzed with 75% MeOH-0.05 N H_2SO_4 at 50°, 30 min, and the hydrolysates subjected to repeated silica gel column chromatography to yield 5 and 6 in the ratio 5:2 as colorless fine needles. Compound 5, mp 95-98°, $[\alpha]_D$ -32.1° (c=1.00, MeOH), and 6, mp 143-145°, $[\alpha]_D$ -156° (c=1.00, MeOH) possessed the same molecular formulae of $C_{14}H_{26}O_9$ on the bases of their elemental analyses and FD-MS (m/z: 339($M+H)^+$). Each of them was further hydrolyzed with 0.05 N H_2SO_4 at 50°, 30 min to give glaucobiose (4) as an amorphous hygroscopic white powder, $[\alpha]_D$ -72.6° (c=0.81, H_2O), $C_{13}H_{24}O_9$ (m/z: 325($M+H)^+$), which showed positive Keller-Kiliani reaction suggesting the presence of 2-deoxysugar. On hydrolysis using snail enzyme (β-glucosidase) 4 afforded cymarose and glucose. The ^1H NMR (Table I) of 5 and 6 showed that they are the anomeric isomers of methyl glycosides. This was further established by the J-values of $^{13}C-^1H$ coupling on the anomeric centers of them[3], 155.2 Hz for the glucose and 158.1 Hz for the cymarose in 5, and 158.8 Hz for the glucose and 166.2 Hz for the cymarose in 6.

On acetylation 4 gave penta-O-acetyl glaucobiose (7), 163-165.5°, $[\alpha]_D$ -33.2° (c=1.71, $CHCl_3$), whose ^1H NMR (400 MHz) in $CDCl_3$ revealed all the proton signals without overlapping. The assignments[4] are listed in Table II. This was supported by the prominent fragment peaks at m/z: 145 (base peak) and 163 in the FD-MS of 4 (Fig. 1). On the other hand, a well known disaccharide strophanthobiose (1) (4-O-β-D-glucopyranosyl-D-cymaropyranose)[5] was identified from C. caudatum Max (Asclepiadaceae).

Table I. ^1H NMR data for 5 and 6 (δ in pyridine-d_5) (200 MHz)

	5	6
6-CH_3	1.49 (3H, d, J=6.3 Hz)	1.41 (3H, d, J=6.6 Hz)
2-CHa	1.68 (1H, ddd, J=13.5, 9, 2 Hz)	1.69 (1H, ddd, J=14, 4, 3.5 Hz)
2-CHe	2.30 (1H, ddd, J=13.5, 4, 2 Hz)	2.30 (1H, ddd, J=14, 4, 2 Hz)
1,3-OCH_3	3.46, 3.56 (each 3H, s)	3.30, 3.47 (each 3H, s)
1-CH	4.90 (1H, dd, J=9, 2 Hz)	4.67 (1H, dd, J=4, 2 Hz)
1'-CHa	5.00 (1H, d, J=7.6 Hz)	4.99 (1H, d, J=7.6 Hz)

a=axial, e=equatorial

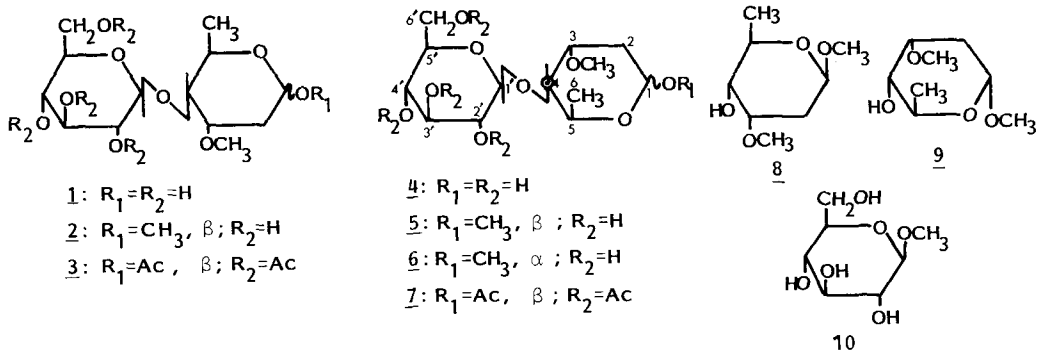


Chart 1

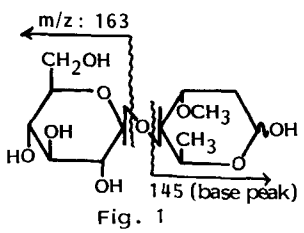


Fig. 1

Table II. ^1H NMR data for 7 (δ in $CDCl_3$) (400 MHz)

1-CHa	6.00 (1H, dd, J=9, 2.4 Hz)	1'-CHa	4.67 (1H, d, J=7.8 Hz)
2-CHa	1.66 (1H, ddd, J=13.7, 9, 3 Hz)	2'-CHa	4.98 (1H, dd, J=9.8, 7.8 Hz)
2-CHe	2.24 (1H, ddd, J=13.7, 5.4, 2.4 Hz)	3'-CHa	5.08 (1H, dd, J=9.8, 9.3 Hz)
3-CHe	3.75 (1H, ddd, J=5.4, 3, 3 Hz)	4'-CHa	5.22 (1H, dd, J=9.8, 9.3 Hz)
3-OCH_3	3.39 (3H, s)	5'-CHa	3.69 (1H, ddd, J=9.8, 5.4, 2.4 Hz)
4-CHa	3.51 (1H, dd, J=8.3, 3 Hz)	6'-CH_2	4.14 (1H, dd, J=12, 2.4 Hz)
5-CHa	4.08 (1H, dq, J=8.3, 6.4 Hz)		4.21 (1H, dd, J=12, 5.4 Hz)
5-CH_3	1.27 (3H, d, J=6.4 Hz)	-OAc	2.01, 2.03 x2, 2.08 x2 (each 3H, s)

a=axial, e=equatorial

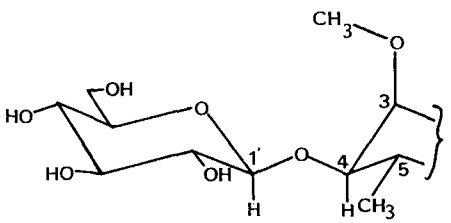
Tanaka et al.[6] and Tori et al.[7] reported the β-D-glucosidation effects on carbon chemical shifts (glucosidation shifts) of both glucose and aglycone moieties. According to their studies, when one of the β-carbons of aglycone is substituted, the magnitude of glucosidation shifts depends significantly on the position, syn or anti to the pyranose-ring oxygen atom in the most stable conformation averaged around the glycosidic linkage.

Table III. ^{13}C NMR Chemical Shifts for 2, 5, 6, 8, 9, and 10 (ppm in pyridine-d_5) (25 MHz)

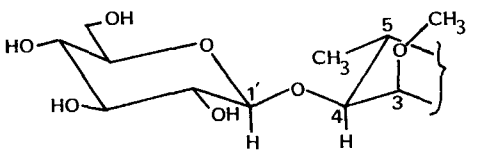
	8	10	2	5	9	6
C-1	99.4		99.3	99.2	97.6	97.5
-2	35.1		36.1	35.2	31.9	31.9
-3	78.5		77.8(-0.7)[b]	78.2[a]	76.5	73.2(-3.3)
-4	74.0		83.2(+9.2)	79.0(+5.0)	73.2	78.2(+5.0)
-5	71.0		69.4(-1.6)	69.3(-1.7)	65.2	64.2(-1.0)
-6	18.9		18.6	18.9	18.5	18.3
-OMe	56.0		56.0	55.8	56.7	56.7
	57.8		58.4	57.9	54.7	54.8
C-1′		105.4	106.5(+1.1)	101.8(-3.6)		101.7(-3.7)
-2′		74.8	75.4	74.9		75.1
-3′		78.1	78.4	78.4[a]		78.4
-4′		71.4	71.9	71.6		71.9
-5′		78.1	78.4	78.2[a]		78.4
-6′		62.5	63.1	62.7		62.8
-OMe		56.7				

a) Assignments may be interchanged.
b) Values (in parentheses) are $\Delta\delta = \delta_{\underline{2}} - \delta_{\underline{8}(\underline{10})}$.

Namely, alkyl-substitution on the syn-β-carbon in a secondary alcohol, the anomeric carbon chemical shift of glucose (C-1') changes ca. -4 ppm from that of methyl β-D-glucopyranoside (10)[8] and the α-carbon of aglycone (C-α) +5.5 (±1.5 ppm), while on the anti-β-carbon, the C-1' changes 0 (±1.5 ppm) from that of 10 and the C-α +10.4 (±1.5 ppm). The ^{13}C NMR for 2, 5, and 6 were measured and their carbon chemical shifts assigned (Table III) on the bases of methyl β-D-cymaropyranoside (8)[4a, 9], methyl α-L-cymaropyranoside (9)[1b, 4a], and 10[8].



2



5 and 6

Fig. 2

In the ^{13}C NMR of 2 (Table III), its glucosidation shifts pattern is evidently corresponding to the case of anti-β-substitution while of 5 and 6 to that of syn-β-substitution, which is illustrated in Fig.2. As previously reported[1b], the cymarose which constitutes the glycosides of this drug belongs to L-series. Combination of these knowledge led to the structure of 4 as 4-O-β-D-glucopyranosyl-L-cymaropyranose. Regarding to these three compounds, the axial-substituted C-3 methoxyl groups constributed little to the magnitude of glucosidation shifts.

From the plant taxonomical point of view, it is noteworthy that these two diastereomeric disaccharides were obtained from the same genus. To the best of our knowledge this is the first example that the glucosidation shifts could be compared between these two diastereomeric disaccarides

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