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APPLICATION OF CARBON-13 N.M.R. SPECTROSCOPY TO STRUCTURAL DETERMINATION OF STEROIDAL TRISACCHARIDES, THEVETIN A AND B(1).

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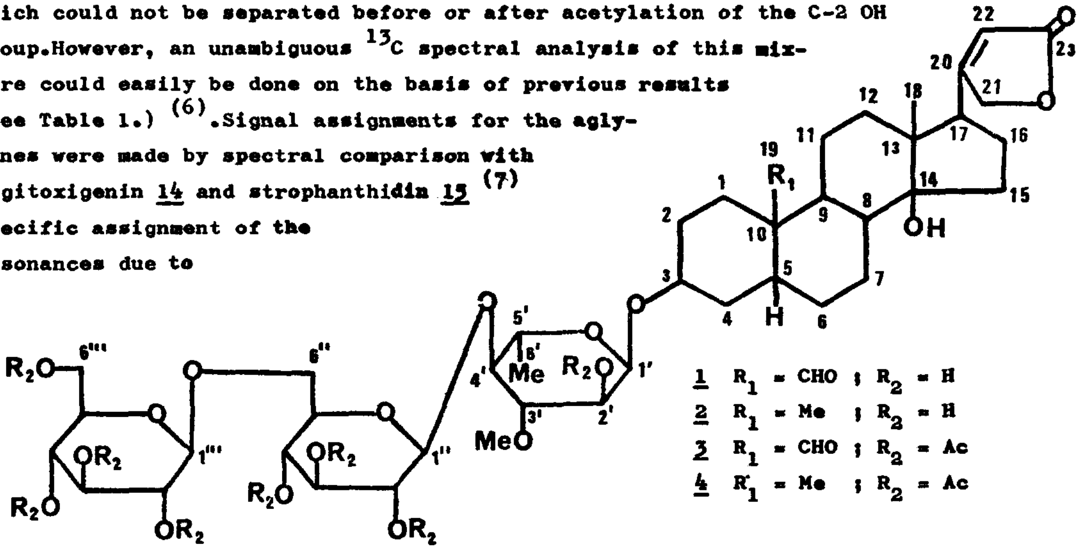
The medically important thevetin is a mixture of two compounds: thevetin A 1 and thevetin B (Cerberoside) 2. Information from previous studies do not indicate whether gentiobiose is attached to C-2' or C-4' (as shown here) of the thevetose residue in the steroidal trisaccharides 1 and 2 (2). Therefore, we have used ^{13}C N.M.R. spectroscopy to solve this problem and report here our unambiguous results.

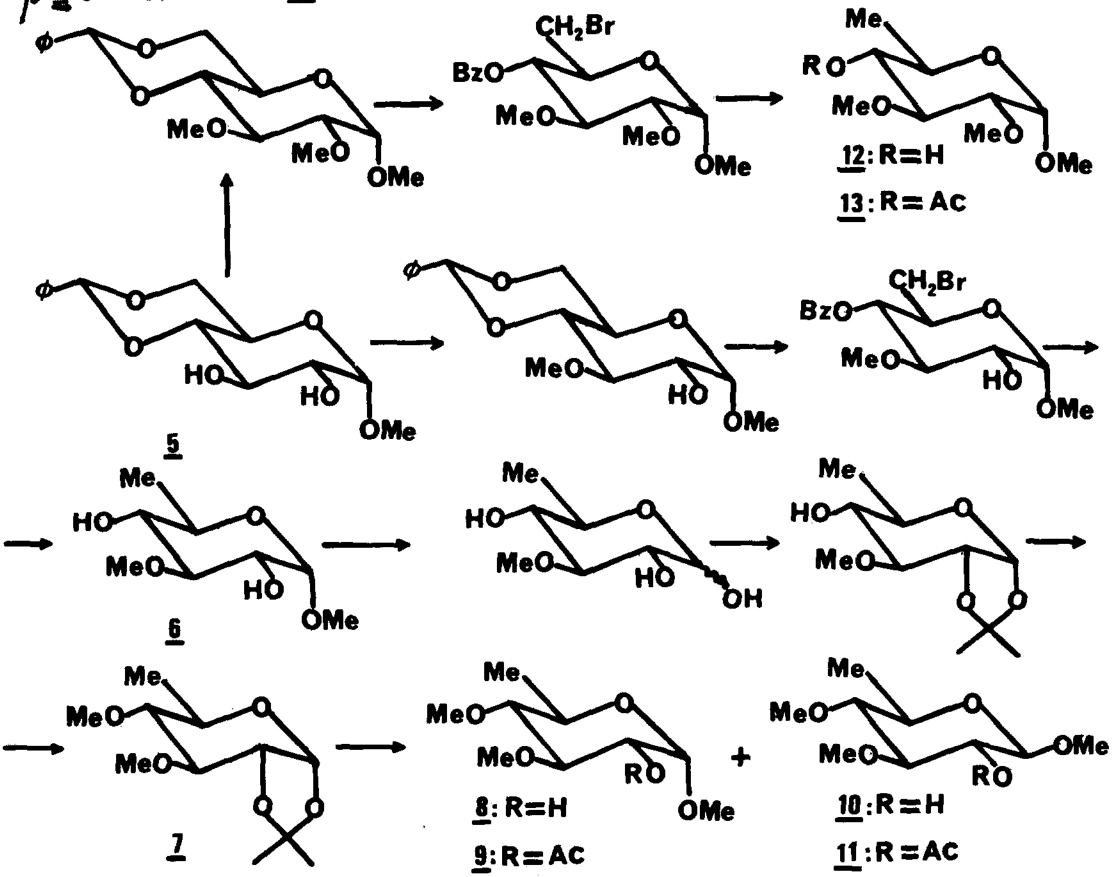
^{13}C N.M.R. spectra were recorded in $CDCl_3$ and /or pyridine-d_5 for the commercially available thevetin mixture (3), for peracetylated thevetins A 3 and B 4 obtained after chromatographic separation of the peracetylated thevetin mixture, and for several specially synthesized model compounds (see Table 1.).

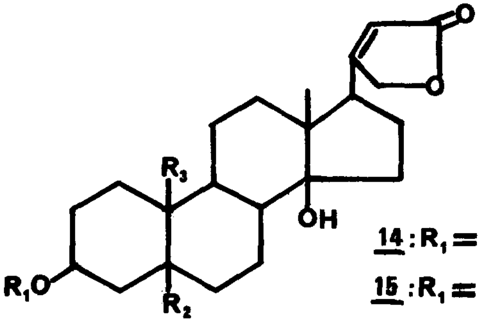
Methyl 4-O-methyl-α- 8 and -β-D-thevetosides 10, their acetates 9 and 11, methyl 2-O-methyl-α-D-thevetoside 12, and its acetate 13 were prepared from 4,6-O-benzylidene-α-D-glucopyranoside 5 according to the Scheme (4), using known reactions. Acid hydrolysis of intermediate 7 yielded a 1:1 mixture of two compounds 8 and 10 which could not be separated before or after acetylation of the C-2 OH group. However, an unambiguous ^{13}C spectral analysis of this mixture could easily be done on the basis of previous results (see Table 1.) (6). Signal assignments for the aglycones were made by spectral comparison with digitoxigenin 14 and strophanthidin 15 (7). Specific assignment of the resonances due to



1 R_1 = CHO ; R_2 = H
2 R_1 = Me ; R_2 = H
3 R_1 = CHO ; R_2 = Ac
4 R_1 = Me ; R_2 = Ac

the peracetyl β-gentiobiose moiety of the trisaccharides was made by comparison with those of methyl hepta-*O*-acetyl-β-gentiobioside 16 and methyl tetra-*O*-acetyl-β-*D*-glucopyranoside 17 (8).





14: $R_1 = R_2 = H$, $R_3 = Me$

15: $R_1 = H$, $R_2 = OH$, $R_3 = CHO$

TABLE 1. ^{13}C Chemical Shift Data ^{a}

carbon No.	3	4	14	15				
C-1	28.0 (28.3)	29.4 (29.6)	29.7 (30.3)	24.2 (24.6)				
C-2	25.2 (25.4)	26.6 (27.0)	27.9 (28.6)	26.9^{b} (27.2)				
C-3	73.0^{b} (72.4)	72.9 (72.5)	66.8 (66.1)	66.8 (66.6)				
C-4	29.4 (30.0)	30.4 (30.4)	33.3 (34.2)	37.3 (38.2)				
C-5	32.2^{c} (32.6)^{b}	36.5 (36.9)	36.0 (36.6)	74.6 (74.5)				
C-6	28.0 (28.3)	26.6 (27.0)	26.5 (27.3)	36.6 (37.5)				
C-7	20.6 (21.9)^{c}	20.6 (21.8)	21.4^{b} (21.6)^{b}	21.8 (22.6)^{b}				
C-8	42.2 (42.5)	41.7 (41.9)	41.8 (41.9)	41.9 (41.8)				
C-9	34.8^{c} (34.9)^{b}	35.6^{b} (35.4)	35.5 (35.9)	39.7^{c} (39.4)^{c}				
C-10	50.8 (51.1)	35.2^{b} (35.4)	35.5 (35.9)	54.7 (55.4)				
C-11	20.6 (20.4)^{c}	20.6 (21.8)	21.2^{b} (21.9)^{b}	17.7 (18.1)^{b}				
C-12	39.8 (39.7)	39.9 (39.8)	40.1 (39.9)	39.2^{c} (39.4)^{c}				
C-13	49.7 (50.0)	49.7 (50.1)	49.7 (50.1)	49.5 (49.8)				
C-14	84.8 (84.2)	85.3 (84.6)	85.5 (84.8)	84.9 (84.4)				
C-15	32.2 (32.6)	33.0 (33.1)	33.1 (33.2)	32.1 (32.1)				
C-16	26.8 (27.2)	26.6 (27.0)	26.9 (27.3)	27.0^{b} (27.2)				
C-17	50.8 (51.1)	51.0 (51.4)	51.0 (51.5)	50.6 (51.0)				
C-18	15.7 (16.0)	15.8 (16.2)	15.8 (16.2)	15.7 (16.0)				
C-19	206.0 (206.4)	24.0 (24.0)	23.8 (24.0)	208.9 (208.8)				
C-20	174.6 (175.7)	175.0 (175.9)	174.8 (175.9)	174.9 (175.7)				
C-21	73.0^{b} (73.4)	72.9 (73.4)	73.5 (73.6)	73.7 (73.7)				
C-22	117.6 (117.7)	117.5 (117.6)	117.6 (117.5)	117.8 (117.7)				
C-23	174.4 (174.4)	174.6 (174.4)	174.7 (174.4)	174.8 (174.5)				
			9	8	11	10	13	12
C-1'	93.2 (93.5)	93.2 (93.7)	97.0	99.3	101.6	103.6	97.6	97.3
C-2'	74.4 (74.9)	74.5 (74.9)	73.2	72.8	73.7	74.7	81.8	82.1
C-3'	79.9^{d} (80.7)^{d}	80.0^{c} (80.7)^{c}	81.3	84.4	84.6	86.0	80.7	83.0
C-4'	81.4^{d} (81.9)^{d}	81.4^{c} (81.8)^{c}	85.2	85.5	85.8	85.7	75.3	75.3
C-5'	66.1 (66.7)	65.9 (66.5)	66.6	66.9	71.4	71.5	65.3	67.0
C-6'	17.6 (18.2)	17.6 (18.2)	17.6	17.6	17.6	17.6	17.4	17.0
OMe (3')	60.9 (61.0)	60.8 (60.9)	60.8	60.9	60.2	60.9	60.7	61.0
OMe			60.8	60.8	60.8	60.8	59.1	58.4
OMe			55.0	55.1	56.5	57.1	55.2	55.0
			16^{f}					
C-1"	100.4^{e} (100.9)	100.4 (100.9)	101.5					
C-2"	70.4 (71.0)	71.9 (72.5)	71.3^{b}					
C-3"	72.8^{b} (73.4)	72.9 (73.4)	73.3^{c}					
C-4"	67.8 (68.2)	67.9 (68.3)	68.4					
C-5"	71.9 (73.4)	71.9 (73.4)	72.8^{c}					
C-6"	69.0 (69.5)	69.0 (69.5)	69.2					
				17^{f}				
C-1'''	100.2^{e} (100.9)	100.4 (100.9)	100.8	101.5 (101.9)				
C-2'''	71.0 (71.7)	71.1 (71.7)	71.2^{b}	71.3 (71.9)				
C-3'''	72.8^{b} (73.4)	72.9 (73.4)	72.8^{c}	72.9 (73.5)				
C-4'''	68.4 (69.1)	68.4 (69.1)	68.4	68.5 (69.2)				
C-5'''	71.9 (72.4)	71.9 (72.5)	72.0	71.8 (72.2)				
C-6'''	61.9 (62.3)	61.9 (62.4)	61.9	62.0 (62.4)				

[a] ^{13}C FT. NMR spectra were recorded at 22.63 or 15.08 MHz in CDCl$_3$ and/or pyridine-d_5 (data in parenthesis) containing TMS (δ_C 0) at ambient temperature. Accuracies of δ_C about ± 0.1. The δ_C for OAc groups are not listed. [b-e] these signal assignments may be reversed within a vertical column. [f] the OMe shifts for 16 and 17 are the following : respectively 56.9 , 56.8 (56.6).

To confirm the signal assignments of the aglycone moiety of 4, we examined the glycosidation shifts (9), *i.e.*, signal shifts from aglycone and saccharide to glycoside. As seen from Table 1. the α-L-thevetoside formation of 14 caused the following signal shifts: -1.3, +6.1, -2.9 and -4.4 ppm in $CDCl_3$ for the C-2, C-3, C-4 and C-1' signals respectively. These values are respectively similar to those for C-4 (-1.6), C-3 (+6.5), C-2 (-3.8) and C-1' (-3.3 ppm) by the tetra-O-acetyl-α-D-glucoside formation of (25R)-5β-spirestan-3β-ol (9). The reversed shift values for C-2 and C-4 are caused by the difference in the L- and D-series of the saccharides (9).

Inspection of Table 1. thus indicates an excellent shift similarity of the signals for the model compound 9 with those characterizing the α-L-thevetose moiety in 3 and 4 except for the C-1' and C-4' signals. Confirmatory evidence for the glycosylation position of β-gentiobiose was further provided by the fact that the spectrum of a thevetin mixture (3) in $CDCl_3$-CD_3OD (1:1) showed the anomeric C-1', C-1" and C-1''' signals at δ_C 97.9, 103.9 and 103.9 respectively, whereas that of 4 in the same solvent system exhibited them at δ_C 93.7, 100.8 and 100.8 respectively. These upfield acetylation shifts demonstrated the β-positions of these carbons from the OH groups.

From the ^{13}C spectral comparisons we conclude that the C-2' OH group of the naturally occurring thevetins is free and the C-4' OH group of α-L-thevetose must be involved in the glycosidic linkage with β-gentiobioside.

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