

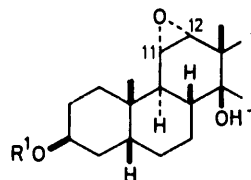
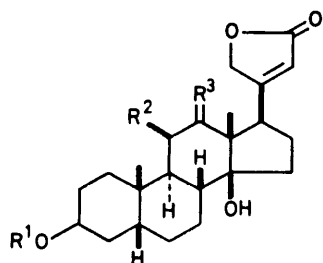
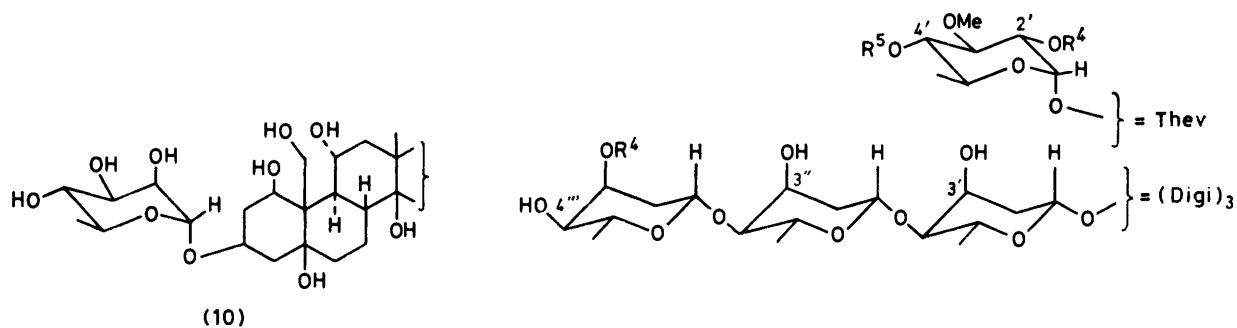
Carbon-13 N.m.r. Spectra of Cardenolide Glycosides. Configuration of the 11,12-Epoxyde in Cerbertin

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The ^{13}C n.m.r. data of the *Digitalis* and the *Cerbera* cardiac glycosides, and of ouabain and neriifolin, have been analysed. Both ^{13}C and ^1H n.m.r. results show that the 11,12-epoxyde in cerbertin has an α -configuration.

EARLY ^{13}C n.m.r. studies on the cardenolides were mainly on the genins.^{1,2} Natural glycosides examined in recent years include those with a 2,6-deoxy- or 6-deoxy-pyranose at position 3 β ,³ and those with a 2,6-deoxy-hexosulose doubly linked to positions 2 α and 3 β .⁴⁻⁶ There

also included. Measurements were made in CDCl_3 - CD_3SOCD_3 (1 : 2), a good solvent for both glycosides and genins, which yields chemical shifts within 1 p.p.m. of those recorded in CDCl_3 (except for oxygen-bearing carbons).⁸



	R ¹	R ²	R ³	R ⁴	R ⁵
(1)	(Digi) ₃	H	H ₂	H	
(2)	(Digi) ₃	H	β -OH, α -H	H	
(3)	(Digi) ₃	H	β -OH, α -H	Ac	
(4)	H	H	H ₂		
(5)	H	H	β -OH, α -H		
(6)	Thev	H	H ₂	H	H
(11)	Thev	Cl	O	Ac	Ac
(12)	Thev	Cl	α -OH, β -H	Ac	Ac

	R ¹	R ⁴	R ⁵
(7)	Thev	H	H
(8)	Thev	Ac	H
(9)	Thev	Ac	Ac

are also isolated examples of studies on cardenolide di- and tri-saccharides.⁷ In a previous paper we analysed the ^{13}C n.m.r. shieldings of a number of semi-synthetic cardenolide analogues.⁸ Here we present the ^{13}C chemical-shift data of a number of natural cardenolides and their derivatives, viz. the *Digitalis* tri- β -D-digitoxosides digitoxin (1), digoxin (2), and 3'''-acetyldigoxin (3); the α -L-thevetosides neriifolin (6), cerbertin (8),⁹ 4'-acetylcerberin (9), and deacetylcerberin (7);⁹ and the α -L-rhamnoside ouabain (10). For comparison, data of the genins digitoxigenin (4) and digoxigenin (5) are

RESULTS AND DISCUSSION

Carbohydrate Signals.—The carbohydrate carbons of the α -L-thevetosides (6)—(9) were assigned from consideration of the shift changes upon acetylation of the equatorial 4'- and 2'-hydroxy-groups (*ca.* +3 p.p.m. at the attached carbon and -2 to -5 p.p.m. at the α -carbons).[†] The same consideration applied to the tri- β -D-digitoxosides (1)—(3) enabled recognition of the signals of C-3''', C-2''', and C-4''' on the terminal carbohydrate. For 3'''-acetyldigoxin (3), the identity of the C-3''' signal (70.4 p.p.m.) is confirmed by the magnitude of the residual C-H coupling in the single-frequency off-

[†] Our assignments of the α -L-thevetoside carbons are in agreement with those given to neriifolin and its 2'- and 2',4'-acetates in pentadeuteriopyridine (ref. 3).

TABLE I
¹³C N.m.r. chemical shifts ^a

Carbon atom	Compounds									
	(5) ^b	(2)	(3)	(1)	(4) ^{b, c}	(6)	(7)	(8)	(9)	(10)
1	29.7	29.9	29.9	29.8	29.6	30.2	29.9	29.8	29.6 ^c	69.4 ^d
2	27.6	26.5	26.4	26.6	27.7	26.6	26.3 ^d	26.3 ^d	26.3 ^d	34.8 ^f
3	65.0	72.1	72.1	72.1	65.0	71.9	71.8	71.4	71.7	70.8 ^d
4	33.1	29.8	29.7	29.8	33.2	29.6	29.9	29.8	29.9 ^c	35.7 ^f
5	35.8	36.3	36.3	36.3	35.8	36.0	35.4	35.6 ^f	35.8 ^f	74.6
6	26.5	26.5	26.4	26.6	26.6	26.6	26.9 ^d	26.7 ^d	26.7 ^d	33.4 ^f
7	21.5	21.5	21.4	20.9	21.2	21.0	21.2	21.1	21.1	22.8
8	40.7	40.7	40.7	41.0	41.1	41.0	38.1	38.2	38.1	39.3
9	31.9	32.0	32.0	35.0	35.1	35.1	35.4	35.3 ^f	35.3 ^f	39.3
10	35.0	34.8	34.8	34.9	35.1	34.9	34.1	35.0	35.1	47.3
11	29.7	29.9	29.9	20.9	20.9	20.9	54.6	54.8	54.8	66.8
12	73.8	73.5	73.5	39.4	39.4	39.3	63.2	63.4	63.4	48.6
13	55.9	55.8	55.9	49.5	49.6	49.4	49.0	49.0	49.1	49.3
14	84.8	84.6	84.6	83.9	84.0	83.9	82.7	82.8	82.8	83.7
15	32.6	32.6	32.6	32.4	32.3	32.3	32.6	32.7	32.6	32.5
16	27.1	27.0	27.1	26.6	26.6	26.6	28.1	28.1	28.1	21.3
17	45.4	45.4	45.4	50.6	50.6	50.6	45.6	45.7	45.8	49.9
18	9.3	9.4	9.4	15.7	15.7	15.7	15.8	15.7	15.8	17.0
19	23.7	23.7	23.7	23.7	23.8	23.6	23.8	23.9	23.9	60.3
20	176.3	176.1	176.5	175.8	175.9	175.8	174.4	173.7	173.9	175.4
21	73.5	73.5	73.5	73.1	73.2	73.1	73.3	73.2	73.3	73.3
22	116.5	116.3	116.3	116.6	116.6	116.6	116.5	116.7	116.6	116.6
23	174.6	174.1	174.2	173.8	173.9	173.9	173.8	173.7	173.9	174.1
1'		95.3	95.3	95.3		97.3	97.2	93.2	92.9	97.3
1''		98.9	98.8	98.9						
1'''		98.9	98.8	98.9						
2'		38.1 ^d	38.0 ^d	38.1 ^d		72.6	72.1	73.7	73.3	70.8
2''		38.0 ^d	37.9 ^d	38.0 ^d						
2'''		38.2	36.0	38.2						
3'		66.3	66.2	66.3		83.5	83.5	80.4	74.7	70.8
3''		66.3	66.2	66.3						
3'''		67.3	70.4	67.3						
4'		81.9 ^f	81.9 ^f	81.9 ^f		75.2	75.2	75.0	78.0	72.5
4''		82.2 ^f	82.2 ^f	82.2 ^f						
4'''		72.8	70.8	72.8						
5'		67.7	67.7	67.7		67.6	67.6	67.3	65.1	68.3
5''		67.7	67.7	67.7						
5'''		69.4	70.2	69.4						
6'		18.1	18.1	18.1		17.7	17.7	17.4	17.0	17.8
6''		18.1	18.1	18.1						
6'''		18.3	18.1	18.3						
—OCOMe			21.1					20.6	20.5, 20.7	
—OCOMe			169.9					169.8	169.9, 169.7	
—OMe						59.8	59.8	60.1	59.9	

^a In p.p.m. downfield from tetramethylsilane in CDCl₃-CD₃SOCD₃ (1:2) with δ(CDCl₃) 78.8 p.p.m. ^b For assignments in CD₃OD-CDCl₃, see ref. 1. ^c Data from ref. 8. ^{d-f} Signals within a vertical column may be interchanged.

resonance decoupled spectrum. In digitoxin (1) and digoxin (2), C-4''' (with attached hydroxy-groups) is shielded by 9 p.p.m. compared with C-4' and C-4'' (with attached glycosyl groups). Likewise C-3''' and C-5''' are less shielded (by *ca.* 1 p.p.m.) than their counterparts on the other two residues. Assignment of the α-L-rhamnoside signals of ouabain (10) follows that of semi-synthetic cardenolide α-L-rhamnosides studied by us.⁸

Aglycone Carbons.—Carbon-3 in the aglycone resonates in the same region as the oxygen-bearing carbons of the carbohydrate, but its signal in all glycosides except ouabain (10) is recognised by its invariance (71.8 ± 0.4 p.p.m.).

For glycosides of digitoxigenin, *viz.* digitoxin (1) and neriifolin (6), the aglycone carbons were assigned * by comparison with our data on semi-synthetic digitoxi-

genin pyranosides,⁸ on digitoxigenin (4) (in CDCl₃ or CD₃-SOCD₃-CDCl₃),⁸ and on 3-acetyldigitoxigenin (in CDCl₃).⁶ Extension of the assignments to aglycone carbons of the 12β-hydroxycardenolide glycosides, digoxin (2) and 3'''-acetyldigoxin (3), demonstrated the expected substituent effects due to the 12β-hydroxy-group. These are γ-gauche shieldings on C-18 (−6.3) and C-17 (−5.2 p.p.m.), a periplanar heteroatom effect¹⁰ on C-9 (−3.1 p.p.m.), and equatorial β-effects on C-11 (*ca.* +9) and C-13 (+6.3 p.p.m.).

Position and Configuration of the Epoxide in Cerbertin.—¹H N.m.r. spectroscopy showed that the epoxide in cerbertin (8) and its carbohydrate-altered analogues (7) and (9) is of the type $\overline{\text{C}}\text{H}\cdot\text{O}\cdot\text{C}\text{H}$, and is thus at the 11,12- or 6,7-positions of the digitoxigenin skeleton.⁹ A 6,7-epoxide would have little effect on the shielding of C-12. This carbon in digitoxigenin and its glycosides neriifolin (6) and digitoxin (1) resonates at 39.5 p.p.m. Since a methylene signal within 5 p.p.m. of this position

* For assignment of the genins (4) and (5) in CDCl₃-CD₃OD, see ref. 1.

is not found in the spectra of cerbertin (8) and its analogues (7) and (9), the epoxide cannot be at the 6,7-position.

With the epoxide placed at the 11,12-position the assignments in Table 1 for the aglycone carbons of cerbertin (8) and its analogues (7) and (9) are given with the consideration that the ring-c epoxide will have minimal influence on the shieldings of carbons 3—7, 15, and 20—23.

In Table 2 the shieldings of C-18 and of methine carbons

TABLE 2

¹³C N.m.r. chemical shifts (to the nearest 0.5 p.p.m., except C-18)

	C-5	C-8	C-9	C-17	C-18
Digitoxigenin glycosides (1) and (6)	36	41	35	50.5	15.7
11 α ,12 α -Epoxydigitoxigenin glycosides (7)—(9)	35.5	38	35.5	45.5	15.8

in the range 35—51 p.p.m. are presented for the digitoxigenin glycosides (1) and (6) and for their 11,12-epoxy-analogues (7)—(9). Comparing the aglycone data for the two series [*e.g.* neriifolin (6) *vs.* deacetylcerbertin (7)], carbon shieldings are identical (± 1.5 p.p.m.), except for those shown in Table 2, and for the epoxy-carbons 11 and 12. An 11,12-epoxy-oxygen, if β , is expected to shield C-18 and C-8 strongly. Table 2 shows that there is no effect of any magnitude on C-18, but instead a strong shielding effect on C-17 (-5 p.p.m.). Both observations are compatible with an α -configuration for the epoxide.

Two ¹H n.m.r. results confirm the above conclusion. Firstly, the 11 β -chloro-12-ketone (11) obtained on oxidation of the chlorohydrin (12) from diaxial opening of the α -epoxide ring in 4'-acetylcerbertin (9)⁹ was re-examined at 100 MHz. The signal of CHClCO (δ 4.46) is a singlet of $W_{1/2}$ 2.7 Hz, the broadening being due to *eq-ax* coupling between 11-H and 9-H.* A sharp

* Other signals in CDCl₃; δ 0.85, 1.03 (d), and 1.28 (all Me), 2.07 and 2.12 (both Ac), 3.47 (OMe), 3.68 (t, *J* 9.5 Hz, 3'-H), 4.67 (dd, *J* 4, 9.5 Hz, 2'-H), 4.73 (t, *J* 9.5 Hz, 4'-H), 5.09 (d, *J* 4 Hz, 1'-H), 4.84 (21-H), and 5.99 (22-H).

† The doublet for 11-H is distinguished from that for 12-H (*J*_{11,12} 3.9 Hz) because it is broader (by 0.6 Hz) owing to coupling with 9-H (δ *ca.* 1.8). Saturation of 9-H caused sharpening of the signal of 11-H by 0.6 Hz. Other signals (in CDCl₃) are as for the chloro-ketone (11).

singlet would be expected for the corresponding signal from a 12-chloro-11-ketone derived from diaxial opening of a β -epoxide.

Secondly, 12-H (δ 2.88) in 4'-acetylcerbertin (9) showed a 12% nuclear Overhauser effect (n.O.e.) (relative to OMe) when the 13-methyl signal (δ 0.97) was saturated; none was observed for 11-H (δ 3.06). Also, no n.O.e. was observed when the 10-methyl (δ 1.06) was irradiated.† The results are compatible with the close proximity of the 13-methyl and 12 β -H in an 11 α ,12 α -epoxide.

EXPERIMENTAL

For conditions of the ¹³C n.m.r. and n.O.e. measurements, see ref. 8 and 4, respectively.

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