^{13}C NMR Spectra of 5β,14β-Hydroxysteroids*

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The ^{13}C chemical shifts of 30 substituted 5β,14β-hydroxysteroids are described and discussed. Substituent chemical shifts for 12α- and 12β-hydroxy groups were evaluated, and interesting γ effects were observed. A correlation is shown to exist between the ^{1}H shifts of H-17 and the ^{13}C shifts of C-17 for various C-12 substituents. A relationship for the distance of the substituent at C-12 from H-17 in the 14β,20-lactones was demonstrated, where an increased distance between these atoms is associated with smaller shift differences.

INTRODUCTION

A vast amount of ^{13}C NMR data on steroids has been reported and reviewed.[1,2] However, for the 5β,14β-hydroxysteroids, substances related to cardioactive compounds such as digitoxigenin (**1**), digoxigenin (**13**) and their side-chain degradation products, only a few investigations have been published.[3–8] Shift assignments in these steroids have been based on the well documented 5β,14α-skeleton, in particular the hydroxylated bile-acid derivatives,[9] and the 5α,14β-hydroxysteroids such as the antitumour-active conduranogenins.[10,11] In this paper we report an investigation of the ^{13}C NMR spectra of 20 5β,14β-hydroxysteroids with different side-chain and ten related 5β,14β-hydroxysteroid-20-lactones.

RESULTS AND DISCUSSION

The assignments of the ^{13}C signals were made on the basis of single-frequency off-resonance decoupling (SFORD) spectra, 2D experiments (^{1}H–^{1}H and ^{13}C–^{1}H shift correlations), selective ^{1}H decoupled ^{13}C spectra, empirical correlations and comparison with earlier literature data.[1–11] The chemical shifts in the A ring were only very slightly influenced by the side-chain and by the substituent at C-12. These shift values are in the range found for the A ring of 5β-cholestanes.[1] Results are given in Table 1.

The different side-chains in **1–12** exhibited their main effects on the shifts of the D ring. The restricted rotation of the side-chain in **10** and **11**, caused by *cis* fusion of the C–D rings, allowed elucidation of the stereochemistry at C-20. The C-21 methyl group in the 20*S*-alcohol is closer on average, to the plane of the D ring than the corresponding 20*R*-alcohol, resulting in an upfield shift for C-16 ($\Delta\delta_{10-11} = -8.4$ ppm) and C-20 ($\Delta\delta_{10-11} = -5$ ppm). Apart from D ring effects, 14β,20-lactone formation causes a significant shielding of C-8 (*ca.* −7.2 ppm) and variable effects on C-9 and C-12.

Substituent chemical shifts (SCSs) for the introduction of 12α and 12β groups can be deduced from the present data, and are shown in Table 2. The γ SCSs values for C-14 and C-18 are relatively constant within each series, and those for C-18 are sufficiently different to afford a convenient stereochemical probe for the C-12 substituent geometry. The other γ-SCS values for C-9 and the unambiguously assigned C-17 show remarkable variability, and indicate the operation of additional effects that are system specific.

All the 12-substituted steroids showed large downfield shifts for the proton at C-17 compared with the unsubstituted compounds. Closer examination of the ^{1}H and ^{13}C data (Table 3) for these compounds indicated a linear correlation between the ^{1}H shift of H-17 and the ^{13}C shift of C-17 (Fig. 1; slope = −7.7, correlation coefficient $r = -0.977$). That the effect is dependent on the distance between the substituent at C-12 and the proton at C-17 was demonstrated by the data for the 14β,20-lactones (Table 3), where the magnitude of the individual shifts for similar substituents is considerably smaller. Thus, there is a ^{13}C shift difference of 11.5 ppm between **2** and **15**, while for the lactones **29** and **31** this is reduced to 3.7 ppm. In the lactones, the hydrogen at C-17 is twisted out of the plane of the D ring by lactone ring formation, which results in an increase in the distance between H-17 and the C-12 substituent. Although the effect is weaker in the lactones, a linear correlation of slope comparable to that of the cardenolides still exists (Fig. 1; slope = −6.4, $r = -0.966$).

Analogous effects were not observed for the shifts of H-18 and C-18, presumably owing to the free rotation of the methyl group.[12] A distinction between steric and electronic interactions to interpret the cause of the effects at C-17 is not possible. The ^{13}C shift differences were all in the range expected for γ_{gauche} effects. It is remarkable that **2**, as well as **15** and **18**, where the conformation of the rings is changed and the main interaction should result from anisotropic effects, should correlate with the 12-hydroxy and

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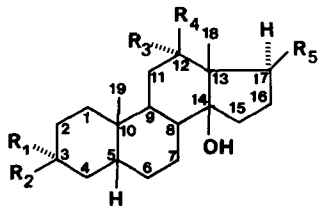
* Presented at the 4th North German NMR Meeting, Braünschweig, 10 May 1985.

CCC-0749–1581/85/0023–0959$02.50



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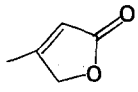
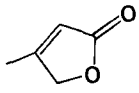
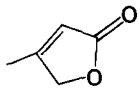
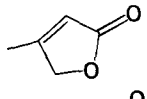
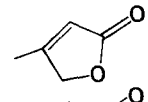
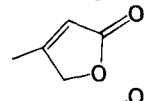
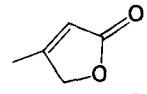
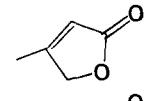
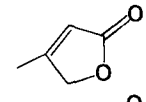
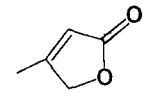
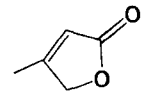
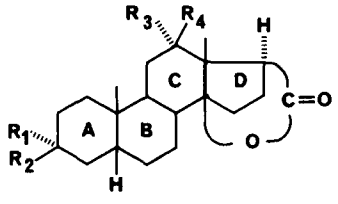
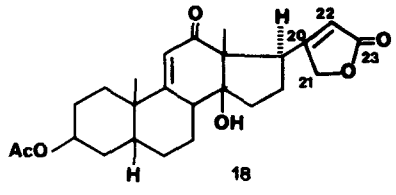
1	H	OH	H	H	
2	H	OAc	H	H	
3	O		H	H	
4	H	OH	H	H	CCH_3 (O)
5	H	OAc	H	H	CCH_3 (O)
6	O		H	H	CCH_3 (O)
7	H	OH	H	H	CH_2OH
8	H	OAc	H	H	CH_2OH
9	O		H	H	CH_2OH
10	H	OAc	H	H	(*S*)—$CHOHCH_3$
11	H	OAc	H	H	(*R*)—$CHOHCH_3$
12	H	OH	H	H	$COH(CH_3)_2$

Compound	R_1	R_2	R_3	R_4	R_5
13	H	OH	H	OH	
14	H	OH	O		
15	O		O		
16	H	OAc	H	OH	
17	H	OAc	OH	H	
19	O		H	OMs	
20	H	OSEM	H	OSEM	
21	H	OAc	H	OAc	

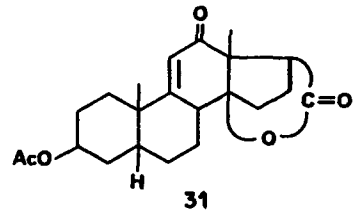


22	H	OAc	OH	H	
23	H	OH	H	OH	
24	H	OAc	H	OH	
25	O		H	OH	
26	H	OAc	O		

27	H	CH_3OCO (O)	H	OH
28	H	OAc	OMs	H
29	H	OAc	H	H
30	O		H	OMs



18



31

Ac = CH_3CO; Ms = CH_3SO_2; SEM = $(CH_3)_3SiCH_2CH_2OCH_2$

Scheme 1

12-ester compounds. For the α,β-unsaturated-12-oxo-14β,20 lactone **31** an increased effect was observed compared with the 12-oxo steroid **26,** as expected if electronic interactions were responsible for the shifts. In contrast, however, the highest shift difference in the cardenolides was observed for **15** and not **18**. Further, no significant solvent effects on H-17 and C-17 could be determined, which were expected if only polar interactions were responsible for the observed correlation.

Further studies in progress are necessary for 5β,14β-hydroxysteroids and 5β,14α-steroids with new side-chains in order to find a satisfactory explanation of these effects.

Table 1. ^{13}C NMR chemical shifts for 1–19 and 21–31[a]

Carbon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	29.6	30.5	37.1	29.8	30.6	37.1	30.1	30.6	37.2	30.6	30.6	30.2	30.7	29.4	36.9
2	27.8	25.0	36.6	27.9	25.1	36.8	27.0	25.2	36.9	25.1	25.1	28.0	27.9	27.9	36.4
3	66.7	70.5	212.9	66.9	70.5	212.7	67.0	70.6	212.5	70.6	70.6	67.0	66.6	66.5	211.5
4	33.1	30.5	42.1	33.3	30.6	43.2	33.6	30.6	42.3	30.6	30.6	33.7	33.5	33.7	41.8
5	35.9	36.8	43.6	36.2	37.0	43.8	36.6	37.0	43.9	37.0	37.0	36.6	36.8	36.1	43.6
6	26.4	26.4	26.5	26.6	26.4	26.6	27.9	26.5	26.7	26.4	26.3	27.2	26.6	26.2	26.2
7	21.2	21.2	21.0	20.9	20.9	20.9	21.3	21.0	21.1	21.0	20.6	20.8	21.9	21.9	21.4
8	41.6	41.7	41.6	39.9	40.1	40.0	40.9	40.8	40.9	40.7	40.7	40.2	41.3	41.4	41.1
9	35.3	35.6	36.6	39.2	35.2	35.4	35.8	35.7	37.7	35.6	35.6	35.7	32.6	33.3	34.2
10	35.3	35.6	35.2	35.2	35.2	35.0	35.7	35.2	35.3	35.2	35.2	35.8	35.5	35.7	35.4
11	21.2	21.2	21.2	21.1	21.5	21.6	21.7	21.3	21.2	21.3	21.4	21.8	30.0	37.4	37.3
12	39.9	39.9	39.8	39.1	39.3	39.4	40.1	39.8	39.8	40.1	41.6	42.2	74.8	211.4	211.1
13	49.6	49.6	49.6	49.2	49.3	49.3	47.8	47.4	47.5	47.6	47.7	49.2	56.4	64.0	64.1
14	85.4	85.3	85.3	84.7	84.9	85.0	84.8	84.5	84.2	84.8	85.4	85.6	85.8	86.5	86.2
15	32.9	33.1	33.1	34.0	34.0	34.0	32.7	32.7	32.8	32.6	32.2	30.5	33.0	33.1	33.0
16	26.9	26.9	26.9	24.9	24.9	24.9	22.3	21.9	21.9	18.1	26.5	23.9	27.9	26.9	27.0
17	50.9	51.3	50.8	62.3	62.5	62.5	51.7	51.5	51.5	56.3	56.6	60.3	46.1	39.9	39.8
18	15.7	15.8	15.8	15.3	15.4	15.4	15.0	14.8	14.8	14.9	16.4	18.1	9.4	16.5	16.5
19	23.7	23.7	22.6	22.6	23.8	23.8	24.0	23.8	22.7	23.8	23.3	24.0	23.8	23.3	22.2
20	175.3	175.1	175.4	217.6	217.5	217.6	62.5	62.6	62.7	65.6	70.6	73.1	177.1	174.5	174.7
21	73.6	73.6	73.4	33.3	33.5	33.3	—	—	—	22.1	23.8	31.5	74.6	73.7	73.9
22	117.3	117.5	117.8	—	—	—	—	—	—	—	—	31.9	117.0	118.6	118.6
23	174.9	174.4	174.5	—	—	—	—	—	—	—	—	—	176.3	173.5	170.6
CH_3(Ac)	—	21.2	—	—	21.4	—	—	21.4	—	21.4	21.4	—	—	—	—
COO(Ac)	—	170.8	—	—	170.6	—	—	170.6	—	170.7	170.7	—	—	—	—

Carbon	16	17	18	19	21	22	23	24	25	26	27	28	29	30	31
1	30.5	30.5	33.5	36.9	30.5	30.7	29.8	30.7	36.8	30.6	30.6	30.6	30.5	36.8	31.2
2	25.0	25.1	25.0	36.6	25.0	24.9	27.8	25.0	36.6	24.8	24.9	24.8	24.8	36.5	24.9
3	70.2	70.5	70.6	211.5	70.2	70.4	66.6	70.4	211.8	69.7	74.3	70.6	70.1	211.1	70.1
4	30.5	30.5	31.3	41.9	30.5	30.6	33.6	30.6	42.0	30.5	30.4	30.6	30.5	42.0	32.8
5	36.9	37.0	37.6	43.4	36.9	37.2	36.1	37.1	43.5	36.8	36.7	37.0	37.0	43.4	37.6
6	26.3	26.3	25.9	27.4	26.3	25.7	25.9	25.6	25.9	25.9	25.6	25.5	25.7	25.8	26.3
7	21.5	21.1	21.3	21.2	21.5	21.6	21.8	21.7	21.4	21.9	21.7	21.6	21.7	21.2	22.1
8	41.4	42.0	41.3	41.1	41.3	34.8	33.6	33.7	34.3	35.4	33.4	34.4	34.3	34.3	36.2
9	32.6	29.5	165.7	33.6	32.4	30.4	33.2	33.6	33.5	33.8	33.6	27.8	35.0	33.5	164.5
10	35.0	35.0	41.0	35.2	35.2	35.0	35.6	35.4	35.3	35.8	35.3	34.9	35.3	35.4	41.7
11	30.2	29.7	120.6	28.1	26.5	30.3	29.2	29.0	29.0	36.4	29.0	30.4	19.3	27.0	122.1
12	75.0	76.6	202.2	85.1	77.4	76.7	67.4	67.6	66.9	207.8	76.2	80.2	35.0	75.8	198.1
13	55.6	52.0	61.0	54.7	54.2	51.9	54.9	54.9	54.9	62.5	54.9	51.3	49.6	53.9	60.3
14	85.5	85.5	83.7	85.7	85.6	94.9	94.3	94.2	93.9	93.0	94.2	93.5	94.7	92.9	91.7
15	33.3	34.8	32.3	33.1	33.2	30.2	28.1	28.1	28.2	27.6	28.1	29.7	27.8	27.9	29.1
16	27.4	28.9	26.7	27.4	27.3	23.5	19.1	19.1	19.0	18.9	19.1	21.6	19.2	18.9	19.8
17	45.7	45.6	41.2	45.6	46.1	54.5	52.2	52.2	52.1	50.4	52.2	53.9	53.9	52.6	50.2
18	9.0	17.1	15.0	10.2	10.4	18.8	10.2	10.1	10.2	16.0	10.2	18.7	16.1	10.9	14.5
19	23.6	23.6	30.9	22.4	23.5	22.2	23.4	23.4	22.2	23.0	23.2	23.3	23.3	22.1	30.9
20	175.6	175.7	175.1	174.4	174.4	179.6	178.7	178.8	178.6	176.6	178.8	177.7	179.2	177.3	176.7
21	73.3	73.8	73.9	73.6	73.4	—	—	—	—	—	—	—	—	—	—
22	117.6	117.7	118.2	118.2	117.9	—	—	—	—	—	—	—	—	—	—
23	174.4	174.6	174.5	173.1	173.8	—	—	—	—	—	—	—	—	—	—
CH_3(Ac)	21.4	21.4	21.4	—	2 × 21.2[b]	21.4	—	21.4	—	21.4	—	21.4	21.4	—	21.4
*C*OO(Ac)	170.4	170.4	170.6	—	2 × 170.4[b]	170.4	—	170.4	—	170.4	—	170.4	170.4	—	170.4
CH_3OCO											54.5				
CH_3*O*CO											155.3				
CH_3SO_2				38.6								38.7		38.6	

[a] The ^{13}C chemical shifts of **20** have not been completely assigned because of overlap of signals from the substituents.
[b] The chemical shifts for both acetoxy groups at C-3 and C-12 are identical.

EXPERIMENTAL

^{13}C NMR spectra were recorded on a Bruker AM 300 NMR spectrometer at 75.5 MHz (sweep width 18 518 Hz, temperature 24 °C, pulse width 32°, pulse repetition 2.2 s, number of scans 400–1000, number of data points 32K, internal lock, 5 mm tube). ^{1}H NMR spectra were recorded on the same instrument at 300.1 MHz (sweep width 3311 Hz, pulse width 30°, number of scans 48, pulse repetition 0.3 s, number of data points 32K, internal lock, 5 mm tube). The solutions were 0.2–0.4 mmol ml^{-1} in $CDCl_3$. Chemical shifts are relative to TMS.

Table 2. SCS values for 12α- and 12β-hydroxy groups in 5β,14β-hydroxy-steroids

Compounds	
	12α SCS
17 – 1	α: 36.7. β: 8.5(11), 2.4(13). γ: −5.8(9), 0.1(14), −5.3(17), 1.4(18)
22 – 29	α: 41.7. β: 11.0(11), 2.3(13). γ: −4.6(9), 0.2(14), 0.6(17), 2.7(18)
	12β SCS
13 – 1	α: 34.9. β: 8.8(11), 6.8(13). γ: −2.7(9), 0.1(14), −4.8(17), −6.3(18)
16 – 2	α: 35.1. β: 9.0(11), 6.0(13). γ: −3.0(9), 0.2(14), −5.2(17), −6.8(18)

Table 3. ^{1}H chemical shifts of H-17 and H-18 and ^{13}C chemical shifts of C-17 and C-18 for the 12-substituted 5β,14β-hydroxysteroids and their lactone derivatives

		Chemical shift (ppm)			
Type	Compound No.	H-17	C-17	H-18	C-18
5β,14β-Hydroxysteroids	**2**	2.8	51.3	0.94	15.8
	21	2.9	46.1	0.90	10.4
	20	3.2	45.7	0.80	11.4
	19	3.4	45.6	0.9	10.2
	16	3.4	45.7	0.80	9.0
	17	3.4	45.6	0.85	17.1
	18	4.0	41.2	0.92	15.0
	15	4.2	39.8	1.10	16.5
Lactone derivatives	**29**	2.45	53.9	1.1	16.1
	25	2.8	52.1	1.0	10.2
	30	2.9	52.6	1.1	10.9
	22	2.8	54.5	1.1	18.8
	28	2.9	53.9	1.15	18.7
	26	3.0	50.4	1.3	16.0
	31	3.1	50.2	1.3	14.5

Digitoxigenin (**1**) and digoxigenin (**13**) were commercially available and were converted to their acetates **2** and **21** in the usual manner with acetic anhydride–pyridine. Compounds **4–6**,[13] **10**, **11**,[13] **14–17**,[14] **19**[15] and the 14β,20-lactones **22–29**[16] were synthesized as described in the literature. Alcohol **7** was obtained by reduction of the corresponding 17β-carboxylic acid[17] with $LiAlH_4$; *N*-bromosuccinimide oxidation[15] of **7** gave **9** and CrO_3 oxidation of **1** resulted in **3**. Compound **12** was obtained by reaction of **5** with methyllithium.[13] Reaction of **13** with SEMCl[18] gave **20** after 24 h.

3β-Acetoxy-14β-hydroxy-12-oxo-5β-car-9(11),20(22)-dienolide (18)

3β-Acetoxy-12-oxo-5β-card-20(22)-enolide (10.22 mol, 4.4 g) and pyridinium hydrobromide–perbromide (3 g) were stirred in CH_2Cl_2 (300 ml) for 1 h at 25 °C. After extraction with $NaHCO_3$ solution and water, the solvent was evaporated *in vacuo*. The residue was dissolved in DMF (200 ml) and refluxed with LiBr (750 mg) and Li_2CO_3 for 5 h. Dilution with water and extraction with diethyl ether, after drying with $NaSO_4$ and evaporation *in vacuo* resulted in a syrup. Crystallization from acetone–water gave 3.1 g (70%) of **18**, m.p. 211–212 °C.

3β-Acetoxy-12-oxo-5β-androst-9(11)-en-17β-carboxylic acid 14β-lactone (31)

Lactone **26** (200 mg) and selenium dioxide (120 mg) were refluxed for 18 h in acetic acid (15 ml). After neutralization with NaOH, extraction with $CHCl_3$ and evaporation to dryness, the residue was chromatographed on a short column of SiO_2 with ethyl acetate–hexane (2:1) as eluant. Evaporation and recrystallization from ethyl acetate–cyclohexane gave 120 mg (60%) of **31**, m.p. 212–214 °C.

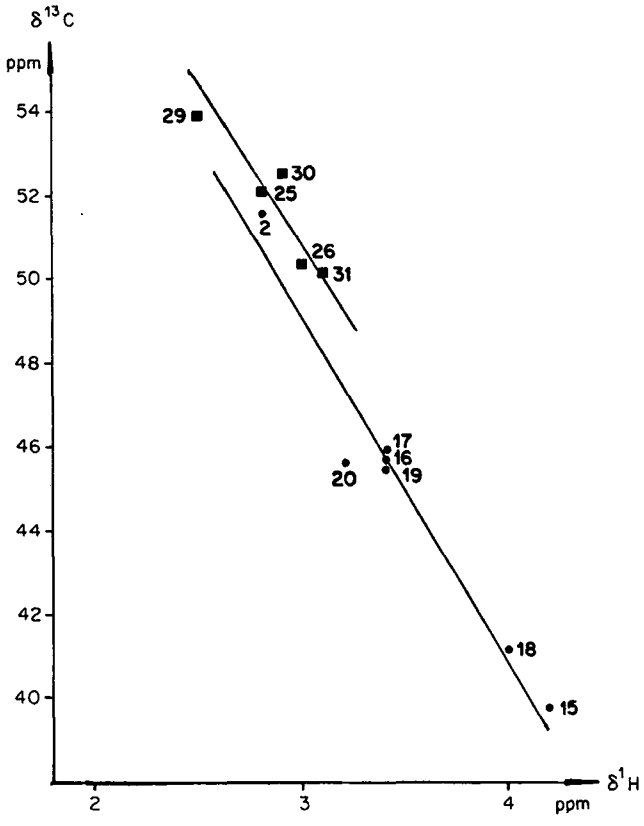


Figure 1. Plot of the chemical shifts of ^{1}H for H-17 against ^{13}C for C-17. ●, Cardenolides; ■, 14β,20-lactones.

3β-Acetoxy-14β,20-dihydroxy-21-nor-5β-pregnane (8)

3β-Acetoxy-14β-hydroxy-5β-androstane-17β-carboxylic acid (10 mmol, 3.2 g) and carbonyldiimidazole (2.2 g) were stirred in THF (180 ml) for 30 min. Stirring was continued for a further 1 h after addition of $NaBH_4$ (1 g). The reaction was terminated with acetic acid and the solution was concentrated *in vacuo*. The residue was diluted with water and extracted with $CHCl_3$. The extracts were evaporated to dryness to give a white residue, which on crystallization from acetone–water gave 2.8 g (85%) of **8**, m.p. 194–196 °C.

All the synthesized compounds were analysed for C and H and the results were within ±0.4% of the theoretical values.

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Received 7 May 1985; accepted 2 June 1985