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FOURIER TRANSFORM ^{13}C NUCLEAR MAGNETIC RESONANCE STUDIES OF STEROIDS—II

DERIVATIVES OF 17β-(2,5-DIHYDRO-5-OXO-3-FURYL)-3β,5α,6-TRIHYDROXYANDROSTANE

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Abstract—The natural abundance ^{13}C NMR spectra of derivatives of 17β-(2,5-dihydro-5-oxo-3-furyl)-3β,5α,6-trihydroxy androstane have been measured and completely assigned. The substituent chemical shifts (S.C.S's) for 11α- and 17α-OH substitution are evaluated. The magnitude and sign of the S.C.S.'s are discussed and compared with previous results from the literature.

Previously we have reported the effects of OH substitution at various positions in the steroid nucleus[1] for systems related to either 5α,6α-epoxyandrostan-3β-ol, Δ^4-androsten-3-one and Δ^5-androsten-7-one. We were able to show that the one- and two-bond effects of OH substitution were similar in the different systems, provided the substituent change was remote from the skeletal change in the molecule. It was evident that longer-range effects were dependent upon the actual steroid system studied.

Recent work by Wolff *et al.*[2] on hydroxylated progesterone derivatives and halocortisol derivatives, has shown some of the problems encountered in the use of current ^{13}C NMR theory, especially that of attributing the upfield 3-bond shifts to the steric effect of the substituents. Similar difficulties are to be expected for OH group substitution.

Here we present the complete ^{13}C NMR assignment for systems **1** and **2**. The substituent chemical shifts (S.C.S.'s) for OH substituents at positions 11α and 17α on the steroid nucleus are discussed and correlated with previous data from the literature.

EXPERIMENTAL

The steroids **1a-c** and **2a-c** were prepared synthetically; while **1d**, **1e**, **2d** and **2e** were produced as fungal metabolites. Experimental details will appear elsewhere. The structure of each compound was confirmed by mass spectrometry, IR spectroscopy, ^1H and ^{13}C NMR spectroscopy, and elemental analysis.

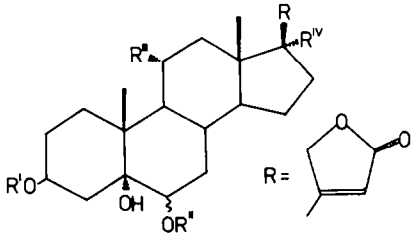
All NMR spectra were recorded on a Varian XL-100-12 spectrometer; experimental procedures have been detailed previously.[1] Spectra of **1c-e**, **2c** and **2d** were recorded in a mixed solvent of deuteriochloroform and methanol-d_4 (70%: 30%, I), while **1e**, **2e** and **2e** were recorded in pyridine-d_5 (II). Spectra of **1a**, **1b**, **2a** and **2b** have been recorded previously[1] in I.

RESULTS AND ASSIGNMENTS

The shifts and assignments of **1c-e** and **2c-e** are shown in Table 1. In a previous paper we assigned the resonance of **1a**, **1b**, **2a** and **2b** in I. These data are found in Table 1 (ref. 1). The use of off-resonance spectra, shift considerations and comparisons with literature data allowed the resonances of carbons 3, 5, 6, 9, 10, 11, 13, 14, 15, 17, 18, 19, 21 and 22 in all compound to be unambiguously assigned. The resonances of C6 (except for **1e**) and C16 (except for **1e** and **2e**) were assigned by the same methods (Fig. 1).

The remaining resonances were assigned as follows. The low field resonances that were unaffected by substituent change were assigned to C23 and the carbonyl resonance of the acetate group attached to C6. The resonance of 172·0 ppm in **1b** and **2b** reported previously can now be assigned unambiguously to the CO resonance of the acetate group attached to C3. The remaining low-field resonances were assigned to C20. In each spectrum the resonance of C23 was considerably smaller in intensity than that of C20 and that of the resonance of the acetate carbonyl attached to C6. This confirmed the assignments for **1e** and **2e** in II and indicated that the resonance of **1e** in I at 175·45 ppm, of greater intensity than the acetate CO resonance, belonged to C20. A separate resonance for C23 was not detected and it was assumed to be coincident with that of C20.

Only the resonances of carbons, 1, 2, 4, 7, 12, 8 (in **1e**) and 16 (in **1e** and **2e**) remained to be assigned. Comparison of **1c-e** and **2c-e** with the previous assignments of **1a**, **1b**, **2a** and **2b** allowed unambiguous assignment of C4 togehter with carbons 1, 2, 7, 12 and 16 of **1c**, **1d**, **2e** and **2d** and carbon 1 of **1e**. The remaining resonances of **1e** at 31·72, 30·58 and 30·50 ppm in I appeared as triplets in the



	R^I	R^{II}	R^{III}	R^{IV}
a	H	H	H	H
b	CH_3CO	CH_3CO	H	H
c	H	CH_3CO	H	H
d	H	CH_3CO	OH	H
e	H	CH_3CO	H	OH

System 1 β-OR^I
2 α-OR^I

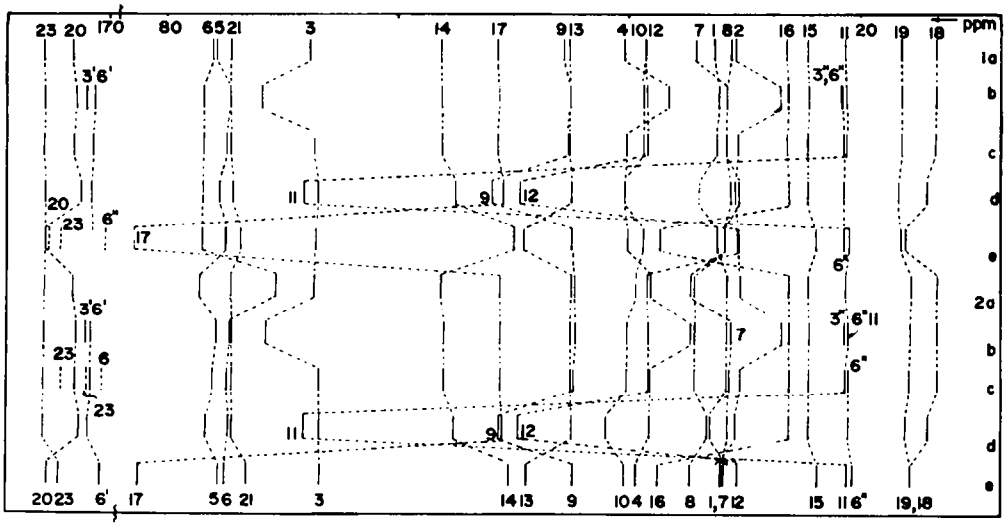


Fig. 1.

Table 1. ^{13}C chemical shifts of compounds 1c-e and 2c-e (ppm)

Compound Carbon	1c	1d	1e	1e	2c	2c	2d	2e
1	32.37	34.05	32.39	32.90	31.51	31.87	32.94	31.87
2	30.50	30.82	30.50+	32.08+	30.42	31.87	30.80	31.87
3	67.01	66.75	66.99	66.72	66.82	66.57	66.64	66.59
4	40.14	40.40	40.11	41.69	38.21	39.53	38.35	39.51
5	74.67	75.43	74.78	74.54	75.96	75.49	76.65	75.53
6	76.81	76.84	76.86	76.97	74.72	74.81	74.65	74.90
7	31.54	31.52	31.72	32.29+	31.33	31.87	31.38	32.19+
8	31.54	30.48	31.72	32.08+	34.37	34.49	33.17	34.84
9	45.18	51.80	44.96	45.34	44.75	44.84	51.32	44.95
10	38.78	40.40	38.77	39.10	40.14	40.48	41.93	40.50
11	21.22	68.01	21.03	21.27	21.29	21.38	68.03	21.31
12	38.48	49.43	30.58+	30.85	38.35	38.17	49.48	30.73
13	45.08	45.01	48.96	48.86	45.07	44.67	44.96	48.88
14	56.06	55.00	49.93	50.36	56.04	55.86	55.14	50.37
15	24.58	24.58	23.85	23.99	24.44	24.31	24.47	23.84
16	26.26	26.21	37.30	37.44	26.20	25.97	26.24	37.40
17	51.19	50.84	82.70	82.38	51.11	50.84	50.87	82.32
18	13.66	14.41	16.25	15.93	13.52	13.24	14.36	15.89
19	16.57	16.79	16.62	16.68	15.90	15.82	16.27	15.89
20	173.03	172.43	175.45	175.19+	172.86	171.93	172.50	175.22
21	74.35	74.22	73.46	73.02	74.30	75.49	74.28	73.03
22	115.95	116.08	115.67	115.91	116.01	116.10	116.13	115.92
23	175.66	175.50	175.45	174.12+	175.59	174.12	175.60	174.18+
3'	171.40	171.26	171.36	170.26	171.59	170.53	171.70	170.58
3"	21.51	21.48	21.48	21.27	21.06	20.85	21.06	20.82
Solvent	I	I	I	II	I	II	I	II

+ Assignments may be reversed in vertical column.

off-resonance experiment although the resonance at 31·72 ppm was of comparable height to the other resonances, whereas in the decoupled spectrum this resonance was considerably higher (indicating two overlapping resonances). Thus this resonance was assigned to C8. Comparison with previous data allowed the resonance at 30·73 ppm in **2e** and one of the resonances at 30·5 ppm **1e** to be assigned to C12. The other resonance of **1e** at 30·5 ppm was assigned to C2 by comparison with **1a** while the remaining overlapping resonance at 31·72 ppm must be assigned to C7. The resonances of **1e** at 32·08 and 32·29 ppm in II were assigned to C2, C8 and C7 although not unambiguously. Similarly C7 in **2e** could not be distinguished from those of C1 and C2, and these were arbitrarily assigned as shown. The fact that OH substitution at C17 should cause a substantial downfield shift for C16 and an upfield shift for C12, that is similar to that for C14, confirmed the assignment of these resonances in **1e** and **2e**.

DISCUSSION

The S.C.S's produced by substitution of an OH group in the 11α and 17α positions in the two systems are shown in Table 2. In order to minimize solvent effects, compounds are compared in the same solvent. The insolubility of **2e** in the mixed solvent (I) necessitated the use of pyridine as solvent. In order to compare the 17α-hydroxyl S.C.S's for this series with those of the

Table 2. Substituent chemical shifts for hydroxyl-group substitution in systems 1 and 2

Compound		1	2	3	4	5	6	7	8	9	10	11	12
11α-hydroxyl-substitution													
1d-1c	I	1.7	0.3	-0.3	0.3	0.8	0	0	-1.1	6.6	1.6	46.8	10.9
2d-2c	I	1.4	0.4	-0.2	0.1	0.7	-0.1	0.1	-1.2	6.6	1.8	46.7	11.1
17α-hydroxyl-substitution													
1e-1c	I	0	0	0	0	0.1	0	0.2	0.2	-0.2	0	-0.2	-7.9
2e-2c	II	0	0	0	0	0	0.1	0.3	0.4	0.1	0	-0.1	-7.4
								(0.5	0.3	-0.2	0	-0.2	-7.8

13	14	15	16	17	18	19	20	21	22	23
-0.1	-1.0	0	0	-0.3	0.8	0.2	-0.6	-0.1	0.1	-0.2
-0.1	-0.1	0	0	-0.2	0.8	0.4	-0.4	0	0.1	0
-6.1	3.9	-0.7	11.0	31.5	2.6	0	2.4	-0.9	-0.3	-0.2
-5.5	4.2	-0.5	11.4	31.5	2.7	0.1	3.3	-0.7	-0.2	0.1
-6.1	3.9	-0.7	11.2	31.3	2.6	0	-	-1.0	-0.4	-)+

+ corrected values : see text

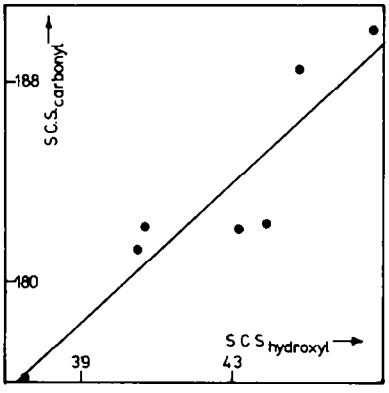


Fig. 2.

6β-acetoxy series it was necessary to correct them with the aid of the differences found previously between the two solvents for the 5α,6α-epoxy-17β-(2,5-dihydro-5-oxo-3-furyl)-androstane system[1]. The corrected values are shown in parentheses in Table 2. 2. Inspection of Table 2 indicates that the orientation of the 6-acetoxy group has little effect upon the S.C.S's found for either 11α- or 17α-hydroxyl substitution.

Conceptually the magnitude of a S.C.S. may be thought of as arising from two effects, a primary effect associated with the electronic, field and steric requirements of the substituent, and a secondary effect associated with the time-independent electronic and field effects of the molecule and the time-dependent steric effects arising from modifications necessary to accommodate the substituent. For a particular substituent the primary effect is a constant while the variation in the magnitude of a S.C.S. arises from the secondary effect. Thus the dependence of the magnitude of a S.C.S. upon the degree of substitution of the carbon undergoing substitution clearly arises from the latter effect.

The magnitude of the one-bond S.C.S. for equatorial hydroxyl substitution at C11 is larger than the same S.C.S. for other positions in the steroid nucleus,[3-5] at C4 of 1-t-butylcyclohexane[6] and at the various positions around the periphery of 10-methyl-*trans*-decalin (Table 3).[4] Even positions that have the same stereochemistry at the β-carbons have quite different S.C.S.'s thus the S.C.S.'s for C4 in 10-methyl-*trans*-decalin[4] and C6 in cholestan-3β-ol[3,4] of 40·7 and 40·5 ppm respectively, are considerably smaller than that of C11 found here. This difference in behaviour of C11 to that of other positions around the steroid nucleus should be apparent in other data. The only data available in the literature are those for the shieldings of the carbon in all the possible androstanones.[7,8] The various S.C.S.'s for the introduction of a CO group,[8] at positions for which hydroxyl data are available, are given in Table 3. The carbonyl S.C.S.'s were evaluated with the aid of shifts for androstane (carbons 1–4 and 6)[4] and those interpolated from Wehrli's data[8] (carbon 11). The latter were taken as an average of the shifts for C11 in compounds with the ketone function in rings A, B or D. The S.C.S.'s for OH substitution at C1, C2 and C4 of the steroid nucleus has been assumed to be the same as that for the same carbons in 10-methyl-*trans*-decalin. The largest S.C.S. for CO introduction is shown by C11, and a correlation exists between the carbonyl S.C.S.'s and the hydroxyl S.C.S.'s (Fig. 2).

The two-bond effects for 11α-hydroxyl substitution

Table 3. One bond S.C.S.'s for hydroxyl substitution and carbonyl introduction at various positions around the steroid nucleus

Position	Hydroxyl S.C.S.	Carbonyl S.C.S.†
1‡	37.5	176.1
2‡	44.8	188.5
3	43.9	182.3
4‡	40.7	182.2
6	40.5	181.3
11	46.7_5	190.0
4*	43.2	182.1

†see text.

‡Hydroxyl S.C.S.'s are for the relevant positions in 10-methyl-trans-decalin.

*1-t-butylcyclohexane system.

upon the tertiary carbon C9 are similar to those produced upon C5 for OH substitution at C4 and C6 (6·6 ppm in both cases),[4] while the effect upon C12 of 11·0 ± 0·1 ppm is larger than the same effects upon secondardy carbons observed for C2-hydroxylation (8·8, C1; 9·2, C3), C3-hydroxylation (9·7, C2; 9·4, C4), C4-hydroxylation (9·4, C3) and C6-hydroxylation (9·6, C7). Again a parallel exists with the results of CO substitution where the S.C.S.'s for the secondary carbons upon CO substitution at C2, C3, C4 and C6 range from 14·3 to 15·5 ppm while that upon substitution at C11 is 18·8 ± 0·5 ppm.[10]

These results suggest that the variation in magnitude of the one- and two-bond effects is closely associated with the particular carbon undergoing substitution, i.e. there is an inherent susceptibility for each carbon around the steroid nucleus to a particular substituent change.

The longer-range effects found here for 11α-hydroxyl substitution confirm those found previously. The magnitude of the four-bond effects is clearly not dependent on the proximity of the atom to the C–O bond as the effect at C5 (4·1 Å) is greater than the effect at C19 (3·1 Å). Similarly, although C14 and C18 are equidistant and have the same angular disposition with respect to the C–O dipole, the three-bond S.C.S.'s are equal but of opposite sign.

The magnitude and sign of the S.C.S.'s for 17α-hydroxyl substitution are similar to those found previously. Only small long-range effects (over more than three bonds) are apparent at C7 and C9. The comparatively large upfield shifts of C7 found by Wolff *et al.*[11] for substitution in progesterone and 21-hydroxyprogesterone, of 1·2 and 1·4 ppm respectively, are not encountered here nor in our previous work. The same authors, however, found only small S.C.S.'s for C7 in the hydroxyprogesterone derivatives (Table 4). Similarly the upfield three-bond S.C.S.'s found for the progesterone and 21-hydroxyprogesterone systems are not consistent either with their results for the 11β-hydroxyprogesterone systems, or with our present or previous work. We suggest that the previous assignment of C7 and C12 is incorrest in 17α-hydroxyprogesterone and **11β**, 17α-dihydroxyprogesterone.[11] A more self-consistent set of results, which is in agreement with our results (and see below), is obtained if the assignment of C7 and C12 are reversed for the former, and C7, C12 and C16 are interchanged in the latter (C12 and C16 are reversible in the original). These assignments are shown in parentheses in Table 4. Thus it is unlikely that the downfield shifts for C7 are caused by the buttressing effect suggested previously.[10]

The magnitude of the one- and two-bond effects have been shown previously to be dependent upon the nature of the 17β substituent. Wolff *et al.*[2] have shown that the magnitude of the three-bond upfield shift for halogen substitution at C9 in cortisol derivatives was not dependent upon the halogen size although for a particular halogenated derivative the three-bond (γ) upfield shifts did correlate qualtitatively with the halogen-Hγ distance. We have no X-ray data for compounds of the present study, although the corrected magnitudes of the S.C.S.'s for the progesterone system (Table 4) for C12 and C14 are in qualitative agreement with the results of an X-ray study on 17α-hydroxyprogesterone, which showed that the O_{17}--H_{14} distance was larger than the O_{17}—H_{12} distance (2·56 and 2·40 Å respectively).[12] There is also a qualitative correlation between all the three-bond S.C.S.'s (present work) and the dihedral angle between the Cγ–Cα and Cα–O bonds (Table 5). However, the three-bond S.C.S.'s in 11α-hydroxyl substitution show the opposite angular dependence, C19 which has a gauche dihedral angle, has a positive S.C.S. while the trans-dihedral angle carbons have a negative S.C.S.

Table 5. Correlation between dihedral angle and three-bond S.C.S.

Carbon	Dihedral angle	S.C.S.
12	40.8	-7.8
14	77.2	-6.1
15	94.7	-0.7
18	164.5	+2.6

In summary we have shown that the differences found in the S.C.S.'s for hydroxyl substitution at the various secondary-carbon atoms around the steroid nucleus are also apparent in other data from the literature; while our results for 17α-hydroxyl substitution have allowed us to correct inconsistencies in literature work. We must conclude, from the long-range effects found here and from the recent demonstration of the importance of electric field effects upon ^{13}C chemical shifts,[13,14] that simple explanations of longer-range effects in terms of either through-space or through-bond effects are inadequate. An assessment of the importance of each of these factors must await further detailed X-ray work on these systems.

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REFERENCES

[1]S. Lang, D. N. Lincoln and V. Wray, *J. Chem. Soc. Perkin II* 344 (1975).
[2]D. D. Giannini, P. A. Kollman, N. S. Bhacca and M. E. Wolff, *J. Am. Chem. Soc.* **96**, 5462 (1974).
[3]H. J. Reich, M. Jautelat, M. T. Messe, F. J. Weigert and J. D. Roberts, *Ibid.* **91**, 7445 (1969).
[4]S. H. Grover and J. B. Stothers, *Can. J. Chem.* **52**, 870 (1974).
[5]D. Leibfritz and J. D. Roberts, *J. Am. Chem. Soc.* **95**, 4996 (1973).
[6]J. D. Roberts, F. J. Weigert, J. I. Kroschwitz and H. J. Reich, *Ibid.* **92**, 1338 (1970).

Table 4. Literature values of the S.C.S.'s for 17α-hydroxyl substitution

System	7	8	9	10	11	12	13	14	15	16	17	18
Progesterone	-1.2(0.5)	0.2	0.0	-0.1	-0.2	-5.6(-7.9)	3.1	-5.5	0.8	8.3	26.7	1.6
11β-Hydroxyprogesterone	0.1	0.1	0.0	0.0	0.3	-7.5	2.9	-5.4	1.4	8.0	26.2	1.5
21-Hydroxyprogesterone	-1.4(0.4)	0.4	0.1	0.1	-0.1	-4.1(-7.5)	3.5	-5.4	0.9	8.2(9.6)	31.0	1.4
11β,21-Dihydroxyprogesterone	-0.2	-0.1	-0.2	-0.1	0.1	-7.8	3.0	-5.6	1.3	8.7	30.1	1.1

[7]N. S. Bhacca, Reported in *Carbon-13 NMR Spectroscopy* p. 291. by J. B. Stothers, Academic Press, London (1972).
[8]F. W. Wehrli, Reported in *Nuclear Magnetic Resonance Spectroscopy of Nuclei other than Protons* (Edited by T. Axenrod and G. A. Webb), p. 175. Wiley, London (1974).
[9]F. J. Weigert and J. D. Roberts, *J. Am. Chem. Soc.* **92**, 1347 (1970).
[10]R. J. Abraham and J. R. Monasterios, *J. Chem. Soc. Perkin II* 662 (1974).
[11]N. S. Bhacca, D. P. Giannini, W. S. Jankowski and M. E. Wolff, *J. Am. Chem. Soc.* **95**, 8421 (1973).
[12]J. P. Declercq, G. Germain and M. van Meerssche, *Cryst. Struct. Comm.*, **1**, 9 (1972).
[13]J. G. Batchelor, J. H. Prestegard, R. J. Cushley and S. R. Lipsky, *J. Am. Chem. Soc.* **95**, 6358 (1973).
[14]J. G. Batchelor, R. J. Cushley and J. H. Prestegard, *Ibid.* **39**, 1698 (1974).