Natural Abundance ^{13}C–^{13}C Coupling Constants in a Steroid

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Summary All the one-bond ^{13}C–^{13}C coupling constants have been determined at natural abundance for 1,2-didehydrotestosterone 17-*O*-acetate (**1**).

WE report here the first determination of all the one-bond carbon–carbon coupling constants for a derivative of a biologically active steroid.

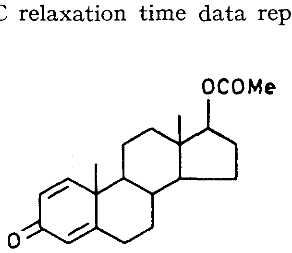
In addition to problems related to the preparation of solutions of very high concentration, the major difficulty in measuring ^{13}C–^{13}C couplings at natural abundance in steroids arises from the relatively broad resonance lines of proton-bearing carbon atoms. In our spectral investigations aimed at obtaining ^{13}C–^{13}C couplings without enrichment on mono-, sesqui-, and di-terpenic natural products at 60 °C, the width at 0·55% height of the strong central lines due to molecules containing only one ^{13}C isotope was only 4—5 Hz for methylene or methine signals.[1] This narrow line width could not be reproduced even at higher temperatures for steroids. The relatively broad signals, at the expense of the extremely high signal to noise ratio which is required, are a consequence of the large molecular size of the steroidal skeleton which is reflected by short spin–lattice relaxation

TABLE 1. ^{13}C N.m.r. spin–lattice relaxation time data for steroids.

Steroid	Solvent	Concentration/M	Temperature/°C	NT_1/s[a]
Estrone 3-*O*-acetate	$(CD_3)_2SO$	*ca.* 4	90	0·23
Estrone 3-*O*-acetate	$[^2H_8]$Dioxan	*ca.* 4	95	0·47
Estrone 3-*O*-acetate	$[^2H_{10}]$-*p*-Xylene	*ca.* 6	135	0·92
(**1**)	$[^2H_8]$Dioxan	*ca.* 7	120	1·06
Cholesteryl chloride[b]	CCl_4	1	42	0·52

[a] Average NT_1 value of proton-bearing carbon atoms of the backbone. [b] Ref. 3.

times. As a result, our efforts were first directed towards the reduction of the resonance line width of proton-bearing carbon atoms by obtaining longer T_1 values.[2] This was attempted in different solvents and by measuring the T_1 values at higher temperatures. Table 1 shows these results, together with ^{13}C relaxation time data reported for cholesteryl chloride.[3]



(1)

Based on the average NT_1 values of Table 1, it was decided to measure the natural abundance ^{13}C–^{13}C couplings for a 7M solution of 1,2-didehydrotestosterone 17-*O*-acetate (**1**) (m.p. 151 °C) in [$^2H_8$]dioxan at 120 °C. The proton-decoupled ^{13}C Fourier transform (F.t.) n.m.r. spectrum was recorded at 25·16 MHz with a Varian XL-100-15 spectrometer equipped with a Varian 620/L-100 computer operated with the MOS-E disk system (no quadrature detection was applied). A 12 mm external diameter tube was used containing 4 g of (**1**) and 1·7 ml of [$^2H_8$]dioxan, and the spectrum was accumulated for 32 h. The number of data points was 32k with a frequency range of 4800 Hz giving a digital resolution of 0·3 Hz/point. In order to avoid interference of the satellites with spinning side bands, the sample tube was rotated slowly (8—12 Hz); after every 6 h a slightly different speed was used. After resolution enhancement with the gaussian multiplication technique[4] the width at 0·55% height of the strong central lines, due to molecules containing only one ^{13}C isotope, was about 6·8 Hz for proton-bearing carbon atoms. The carbon signal assignments were based on literature data[5] and are in agreement with our work.

TABLE 2. ^{13}C N.m.r. spectral data for 1,2-didehydrotestosterone 17-*O*-acetate (**1**).

Carbon	Chemical shift/ p.p.m.[a]	Spin-lattice relaxation time/s	Coupled with carbon	One-bond coupling constant/ Hz
C-1	155·1	1·14	C-2	62·8
			C-10	41·4
C-2	128·4	1·11		
C-3	185·0	16·07	C-2	52·0
			C-4	53·8
C-4	124·8	1·13	C-5	64·5
C-5	168·0	9·70	C-6	39·0
			C-10	39·0
C-6	33·3	0·52	C-7[b]	33·0
C-7	34·0	0·55		
C-8	36·4	0·94	C-7	34·4
			C-9	32·4
C-9	53·7	1·02		
C-10	44·1	11·09	C-9	31·1
C-11	23·2	0·53	C-9	34·4
			C-12	33·0
C-12	37·7	0·54	C-13	35·5
C-13	43·8	8·20	C-17	38·1
C-14	51·1	1·02	C-8	36·1
			C-13	31·4
			C-15	32·9
C-15	24·3	0·55	C-16	32·6
C-16	28·4	0·55		
C-17	82·9	0·97	C-16	38·3
C-18	12·6	2·88	C-13	36·1
C-19	19·4	1·66	C-10	33·8
C=O	170·3	14·08		
CH_3CO	20·9	3·02	C=O	59·8

[a] Chemical shifts were measured with respect to [$^2H_8$]dioxan (66·8 p.p.m.) and are given for tetramethylsilane = 0. [b] This ^1J value was calculated from f_2 and f_3 using AB rules.[7] The one-bond coupling constants are shown only once for all coupling pairs at that nucleus where the ^1J values could be the more precisely determined from the expanded spectra.

Simple analysis of the spectral data, which concurred with results obtained using the computer program CABSA,[6] is given in Table 2. The determination of all the existing one-bond carbon–carbon coupling constants is based essentially on the unsymmetrical distribution of the satellite signals on each side of the strong central lines. As far as the coupling between C-6 and C-7 is concerned only the inner satellites of the strong AB system ($J/\Delta f$ 1·8111) were detected. In this case the coupling constant was calculated from the inner satellites f_2 and f_3 as described previously.[7] Simple calculations on the AX or AB systems afforded the isotope shifts.[8] They were of the order of $-0·012 \pm 0·008$ p.p.m. The spectral section containing the two sp^3 quaternary carbon resonances of (**1**) (C-10 and C-13) is shown in the Figure.

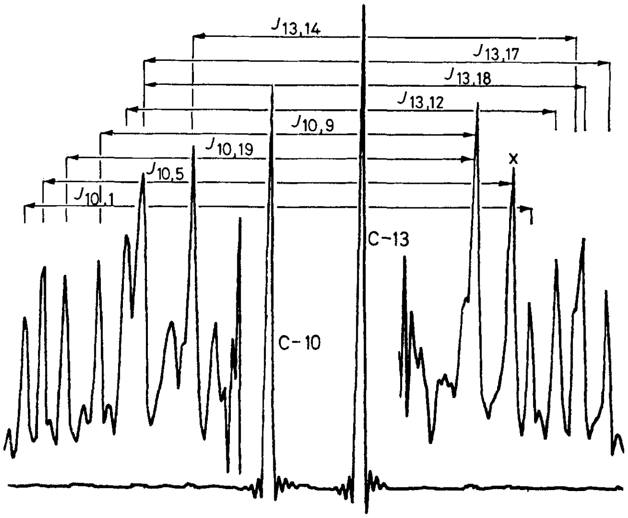


FIGURE. Section of the 25·16 MHz proton-decoupled ^{13}C F.t. n.m.r. spectrum of 1,2-didehydrotestosterone 17-*O*-acetate (**1**) in [$^2H_8$]dioxan at 120 °C (C-10 and C-13). Only the lines due to molecules containing only one ^{13}C atom are shown in the lower spectrum; for clarity, these lines are not included in the upper spectrum. The satellites due to species containing two ^{13}C isotopes are shown with increased amplitude in the upper spectrum (some spinning side-bands are also detectable). The signal x in the upper spectrum is due to a satellite signal and an impurity.

It is of interest to note the identity between $J_{6,7}$ and $J_{11,12}$ and between $J_{7,8}$ and $J_{9,11}$. One-bond coupling constants exhibited by methyl carbon atoms are known[9] to be larger ($J_{13,18}$ 36·1 Hz) than those shown by methylene or methine carbon atoms in similar environments. Consequently, the relatively low value of $J_{10,19}$ in (**1**) (33·8 Hz) is attributed to the influence of the sp^2 hybridized sites at C-1 and C-5. This interpretation is in agreement with the low value of $J_{13,18}$ (32·9 Hz) in estrone 3-*O*-acetate where C-17 is a carbonyl site.[1] The coupling constants $J_{12,13}$ and

$J_{8,14}$ are larger than expected and may be related to the presence of the five-membered D-ring in (**1**).

It is obvious that with high-field n.m.r. spectrometers, requiring reasonable amounts of material and much shorter accumulation times, these experiments will be extremely useful for the establishment of the carbon connectivity pattern of new substances. In the case of complex molecules whose ^{13}C n.m.r. spectra exhibit a number of closely spaced resonances the strong central lines may overlap even at higher field with some satellite signals of interest. In such situations for the determination of the coupling constants and the carbon connectivity pattern the application of Freeman's technique[10] is advisable.

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