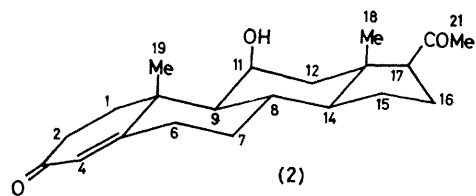
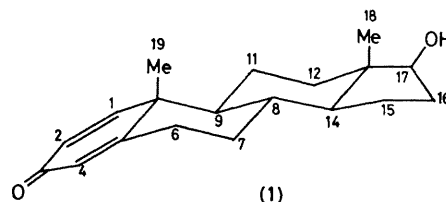


Measurement of the Vicinal and Geminal Proton Coupling Constants of Steroids using Proton Two-dimensional J Spectroscopy

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Summary Proton two-dimensional J spectroscopy at 270 and 400 MHz has been used to measure all the geminal and vicinal n.m.r. scalar couplings of 1-dehydrotestosterone and most of those of 11 β -hydroxyprogesterone.



PROTON two-dimensional (2D) J spectroscopy is a technique which greatly enhances n.m.r. spectral dispersion both by spreading spin-coupled multiplets into an extra dimension and by suppressing inhomogeneity broadening.¹⁻³ It has already proved valuable in the n.m.r. analysis of polypeptides² and oligo-saccharides⁴ where the spectra contain many overlapping but isolated spin systems, but it has not previously been applied to 'natural products' such as steroids which present the very different problem of highly extended spin systems. We now report the use of absolute value¹⁻³ 2D J spectroscopy at 270 and 400 MHz and of a phase sensitive variant⁵ at 270 MHz to determine all the proton chemical shifts and geminal and vicinal coupling constants of 1-dehydrotestosterone (1). In each case, data were acquired using the conventional¹⁻³ two pulse sequence [$90^\circ-t_1-180^\circ-t_2$ -F.I.D. (free induction decay)].

Figure (a) shows a portion of the absolute value 400 MHz 2D J spectrum of (1) 'tilted' so that only chemical shift

information appears in the f_2 dimension [Figure (b)] and coupled multiplets appear in the f_1 dimension. Almost every proton is well resolved, allowing measurement of all the chemical shifts and approximate geminal and vicinal coupling constants. 'Tails' can extend from the prominent methyl singlets into nearby multiplets but have been suppressed in these spectra by sine-bell resolution enhancement.

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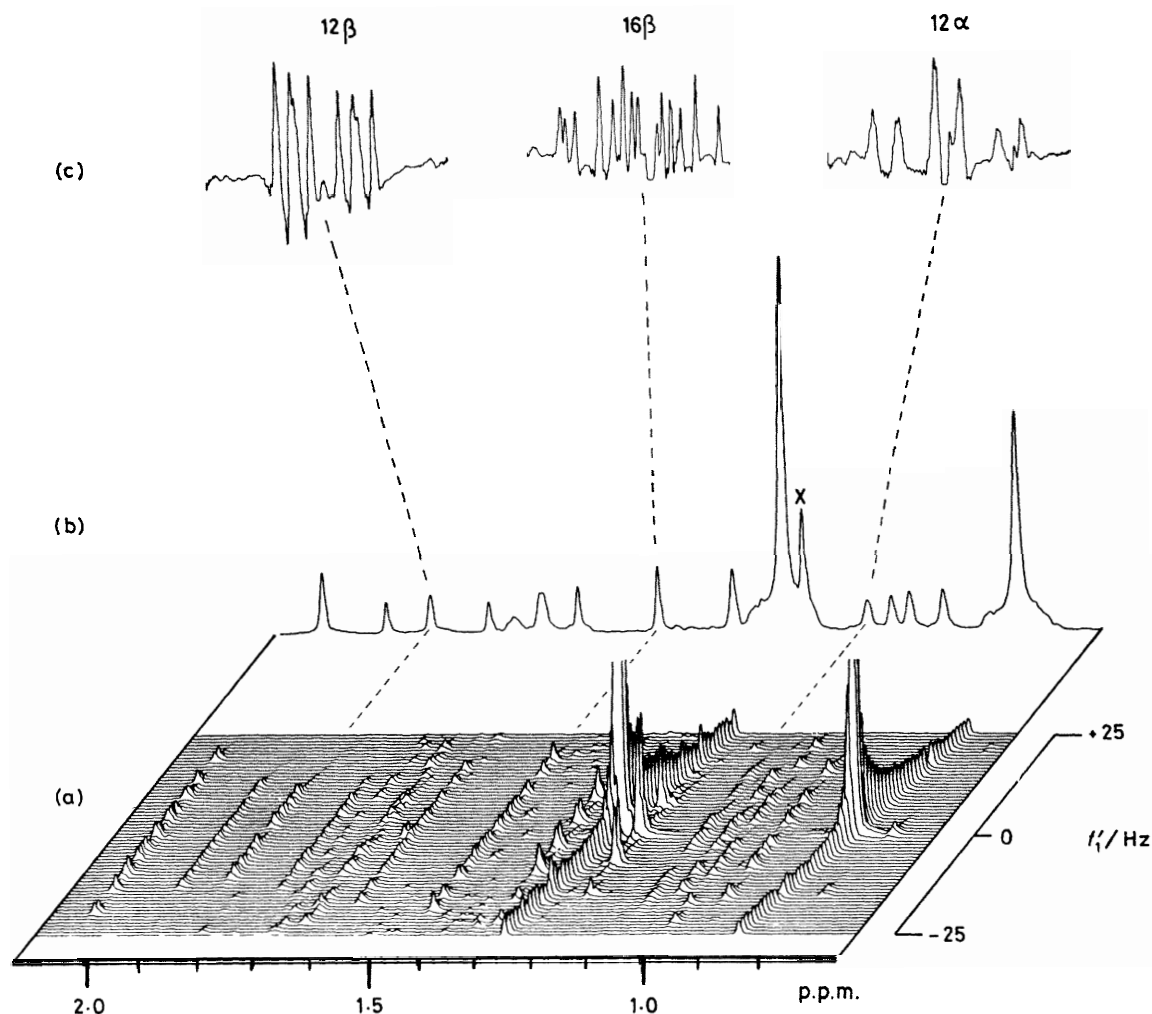


FIGURE. 'Tilted' partial 2D J spectra of (1) (0.06 M in CDCl_3) acquired with a spectral width and digital resolution of 1600 Hz and 0.78 Hz in the f_2 dimension (δ) and 50 Hz and 0.39 Hz in the f_1 dimension (J) (a) Stacked plot of the absolute value 400 MHz spectrum, (b) the 'proton-decoupled spectrum derived from (a) by projection on the f_2 axis, and (c) zero-filled individual proton cross sections from the phase-sensitive 270 MHz spectrum. In (b), 'X' marks the methyl resonance of added ethanol.

Figure (c) shows cross sections of some protons in the phase-sensitive mode;⁵ line shapes in these selected traces have been improved by inverse transformation, zero filling twice, and Fourier transformation, and the resulting line-widths (down to 0.4 Hz) are close to the limit imposed by T_2 .^{1,2} Although both phase and baseline distortions are visible, they do not prevent accurate measurement of splittings; as a bonus the methyl tails are usually less prominent. In the phase-sensitive mode, numerous long range couplings are also resolved but these are as yet unassigned.†

It is ironic that although 2D J spectroscopy readily enables measurement of coupling contrasts (Tables) in the well dispersed spectrum of (1), the large number of similar

TABLE I. Geminal coupling constants in (1) and (2)

Proton pair	$J/\text{Hz} (\pm 0.3 \text{ Hz})$	
	(1)	(2)
1	—	13.9
2	—	17.3
6	13.4	(14) ^a
7	12.8	n.m. ^b
11	13.3	—
12	12.7	14.3
15	12.1	11.4
16	13.2	13.3

^a Approximate value (± 1 Hz) determined in nuclear Overhauser enhancement difference spectrum (following communication). ^b Not measured because of signal overlap.

† $J_{4,\beta}$ 1.5 Hz is a trivial exception.

TABLE 2. Vicinal coupling constants in (1) and (2).

Proton pair	$J/\text{Hz} (\pm 0.3 \text{ Hz})$	
	(1)	(2)
ax.-ax.		
6 β ,7 α	13.4	(14) ^a
7 α ,8	11.5	n.m. ^b
8,9	12.3	11.3
9,11 β	10.5	—
11 β ,12 α	12.8	—
8,14	11.0	10.4
ax.-eq.		
6 α ,7 α	4.3	n.m. ^b
6 β ,7 β	4.8	(4.5) ^a
7 β ,8	4.0	n.m. ^b
9,11 α	4.4	3.6
11 α ,12 α	4.6	4.0
11 β ,12 β	3.9	—
eq.-eq.		
6 α ,7 β	2.6	n.m. ^b
11 α ,12 β	3.0	3.0
Ring A		
1 α ,2 α	—	4.9
1 α ,2 β	—	14.0
1 β ,2 α	—	4.8
1 β ,2 β	—	5.2
Ring D		
14,15 α	7.4	6.9
14,15 β	12.1	12.9
15 α ,16 α	9.4	9.9
15 α ,16 β	3.2	n.m. ^b
15 β ,16 α	5.6	5.6
15 β ,16 β	11.8	11.4
16 α ,17	9.0	9.4
16 β ,17	7.8	9.4

^a Approximate value (± 1 Hz) determined in nuclear Overhauser enhancement difference spectrum (following communication). ^b Not measured because of signal overlap.

couplings do not allow easy assignment, and the assignments depend on methods described in the following communication. 11 β -Hydroxyprogesterone (2) in the normal 400 MHz spectrum, has only three protons sufficiently resolved in the δ 0.8—2.6 region for direct measurement of coupling constants. The exact (<0.01 p.p.m.) degeneracy of several protons has allowed us to measure only 21 of the 30 two- and three-bond J 's by 2D J spectroscopy but we have determined all the chemical shifts and assigned them (following communication). The similarity of couplings in (1) and (2) is remarkable, particularly in ring D, and no doubt indicates many useful but hitherto inaccessible correlations.

The 270 MHz experiments using a home-built spectrometer controlled by a Nicolet 1180/293A computer system required in each case 7.7 h for data acquisition and 4 h for data processing. The corresponding 400 MHz experiments required 0.9 and 1.5 h respectively.

Two points have become clear to us during this work. First, that 2D J spectroscopy is a rapid and almost ideal method for resolving and measuring scalar couplings in spectra which consist of extended, weakly coupled but overlapping spin systems. Secondly, that powerful methods are required for assigning the resulting resolved resonances; this point is discussed further in the following communication.

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