Measurement of Proton Geminal Coupling Constants *via* Selective Two Dimensional Indirect *J* Spectroscopy. Application to the Study of the Conformation of Steroids

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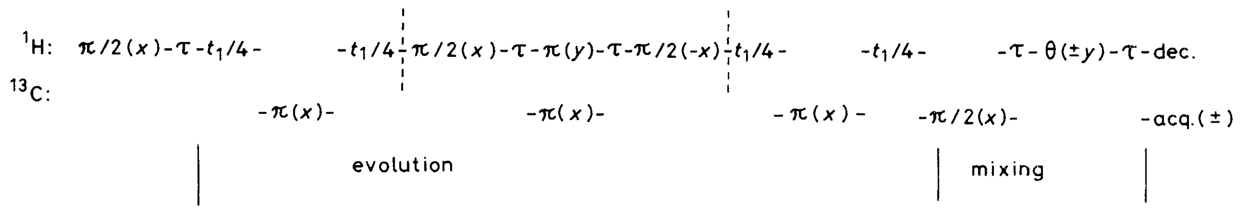
The geminal coupling constants for a series of steroids, measured by a new selective two-dimensional indirect *J* spectroscopy, provide direct information on the A-ring conformation and the ^{13}C assignments of these steroids.

The proton–proton geminal coupling constants ($^2J_{HH}$) have a conformational dependence whose potential utility has been recognized.[1,2] However, $^2J_{HH}$ is particularly difficult to measure in complex molecules because, in addition to the usual problems in resolving proton multiplets in complex molecules, $^2J_{HH}$ is frequently significant compared with the chemical shift difference between the geminal protons. Recent advances in two-dimensional (2-D) n.m.r. spectroscopy, such as proton 2-D *J* spectroscopy[3] and indirect *J* spectroscopy,[4] have substantially alleviated these difficulties. More recently, we proposed a selective indirect *J* spectroscopy specially designed to measure $^2J_{HH}$.[5] The pulse sequence [A][5] utilizes bilinear pulses[6] (marked between dotted vertical lines) to invert selectively only protons attached to the carbon being observed, resulting in decoupling between attached and non-attached protons.[6–8] Only $^2J_{HH}$ is thus observed.

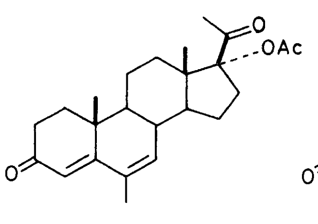


[A]

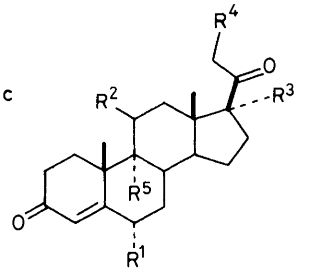
Table 1. Geminal coupling constants ($^2J_{HH}$) in Hz of several steroids as measured by selective indirect *J* spectroscopy and 2-D *J* spectroscopy.[a]

Carbon number	**(1)**[b]	**(2)**[c]	**(3)**[b]	**(4)**[b]	**(5)**[b]	**(6)**[d]	**(7)**[b]	**(8)**[b]
1	13.1	13.9	13.9	13.4	13.7	13.3	13.1	13.3
2	17.9	17.3	17.1	17.0	16.3	16.2	15.8	15.7
6		14.7	12.7[e]	14.4	12.7[e]		14.8	15.3
7		13.6	13.9[e]	12.7	14.4[e]	12.7	12.2	12.9
11	12.5			13.9		13.5		
12	12.2	14.3	13.8	12.3	13.6	12.6	13.5	13.4
15	11.9	11.4	10.7	12.0		12.2	11.9	11.8
16	15.2	13.3	13.8	13.7	16.0	16.1	13.7	12.9
21			19.0		16.9		19.1	17.3
ϕ(C-2–C-3) (°)	22[12]		27.6[9]	28.7[14]	35.6[15]	40.7[16]	40.6[9]	

[a] Uncertainty ±0.2 Hz. Only the magnitude of $^2J_{HH}$ is reported here. These couplings are known to be negative. [b] From selective indirect *J* spectroscopy in this work. [c] From 2-D *J* spectroscopy, ref. 10. [d] From 2-D *J* spectroscopy, ref. 11. [e] The ^{13}C assignments are suspected to have been reversed. See text.



(1)



	R¹	R²	R³	R⁴	R⁵
(2)	H	OH	H	H	H
(3)	H	OH	OH	OH	H
(4)	H	H	H	H	H
(5)	H	OH	H	OH	H
(6)	Me	H	OAc	H	H
(7)	H	OH	OH	OH	F
(8)	H	OH	OH	OAc	F

The conformation of the A ring of some steroids is intimately related to their physiological activity.[9] For pregn-4-en-3-ones, according to the model of Barfield *et al.*,[1] the A ring conformation can be diagnosed from the geminal coupling constant between the protons on C-2, which are adjacent to the carbonyl π-electrons at C-3. We have applied this indirect *J* selective technique to study the conformation of a series of substituted pregn-4-en-3,20-diones [(**2**)—(**8**)] and megestrol acetate (**1**).

The $^2J_{HH}$ obtained from pulse sequence [A] for steroids (**1**), (**3**)—(**5**), (**7**), and (**8**) are summarized in Table 1. The $^2J_{HH}$ obtained by 2-D *J* spectroscopy for compounds (**2**)[10] and (**6**)[11] are also included for comparison. For rings B, C, and D, the geminal coupling constants are quite consistent. Changes in their magnitudes can be attributed mainly to the effects of substitution[1] at adjacent positions. However, despite the lack of substitution on the A ring, the $^2J_{HH}$ of protons at C-2 span a range of 2.2 Hz (uncertainty ± 0.2 Hz). At one extreme, the conjugated double bonds at C-4 and C-6 of (**1**) cause the A ring to assume an almost planar conformation (the torsional angle along bond C-2–C-3 is *ca.* 22°[12]); the $^2J_{HH}$ of the protons on C-2 was found to be 17.9 Hz. At the other extreme, for 9α-fluorocortisol (**7**) in which the A ring was found to bend substantially below the average plane of the molecule in the crystal [ϕ(C-2–C-3) = 40.6°],[9] the $^2J_{HH}$ for protons on C-2 is 15.8 Hz. Similarly, for its 21-acetylated analogue (**8**), the corresponding $^2J_{HH}$ is 15.7 Hz. According to the theoretical model of Barfield *et al.*[1] the π-contribution to the $^2J_{HH}$ of protons adjacent to a C=O π-bond is given by equation (1), where ϕ is equivalent to the torsional angle along the bond between the carbon atom bearing the proton and the carbonyl carbon (in this case C-2–C-3) [a_i and A_i (i = 0, 1) are constants which have the physical meanings described in ref. 1].

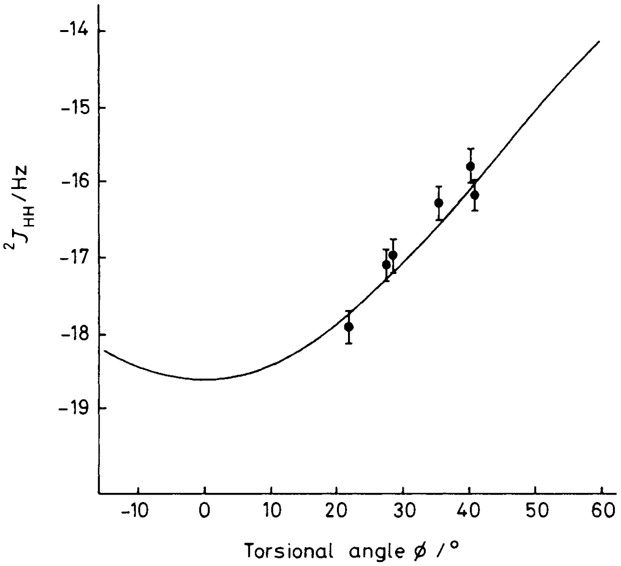


Figure 1. Comparison of the experimental $^2J_{HH}$ of protons on C-2 (●) and the calculated values (——) using equation (2) based on the C-2–C-3 torsional angle determined by *X*-ray diffraction in crystals. The error bars indicate only the uncertainties in the measurement of $^2J_{HH}$. Note that the angle is not defined in the same way as in ref. 1.

$$^2J^{\pi}_{HH} = a_0 + a_1 \cos^2\phi \qquad (1)$$

$$^2J_{HH} = A_0 + A_1 \cos^2\phi \qquad (2)$$

Thus experimentally, the measured $^2J_{HH}$ can be represented by equation (2). If ϕ(C-2–C-3) determined by *X*-ray diffraction and equation (2) are used to fit the experimental values of $^2J_{HH}$ of protons on C-2, an excellent agreement is found (Figure 1), with $A_0 = -12.6$ Hz and $A_1 = -6$ Hz.† The small deviations are within experimental error and may partly arise from a small change of the conformation between molecules in crystal and solution states. However, significant changes in the A ring conformation between the two states can be ruled out in most cases.‡ The present results show that $^2J_{HH}$ can often be used to determine the conformation of molecules in solution. Thus for (**2**), for which no *X*-ray diffraction data are available, φ(C-2–C-3) should be 26 ± 2°, according to the $^2J_{HH}$ measured.

The values of $^2J_{HH}$ can also be utilized as aids for assignment of ^{13}C spectra. For example, the protons on C-6, being adjacent to the π-bond on C-4 and C-5, are expected to have a relatively large $^2J_{HH}$, while for protons on C-7, $^2J_{HH}$ should be about 12—13 Hz, as is found in other cyclohexyl systems. This is indeed the case for (**2**), (**4**), (**6**), (**7**), and (**8**). However, if the previous ^{13}C assignment[13] is followed for (**3**) and (**5**), the relative magnitudes of $^2J_{HH}$ at C-6 and C-7 would be reversed. Therefore, our measurement of $^2J_{HH}$ strongly suggests that the previous assignment of C-6 and C-7 for these two compounds[13] should be interchanged.

The relative merits of the other 2-D techniques for proton multiplet determination have been discussed.[5] Although information obtained by this selective indirect *J* technique is more limited, it is also by far the least time-consuming. Since the spectral width in the F_1 dimension can be reduced to ±15 Hz, our experience indicates that only 16 blocks of data are sufficient to render good resolution. For samples not limited by solubility and availability, such as (**1**) and (**4**), the 2-D experiment can be completed in 10 minutes. Interpretation of the results is straightforward and requires no spectral simulation.

The NT-300 spectrometer was purchased partially through a grant from the National Science Foundation. We thank Dr. V. Rutar for helpful suggestions.

Received, 23rd May 1984: Com. 722

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† The only deviation from a monotonic relationship between $^2J_{HH}$ of H_2 and φ(C-2–C-3) occurs in (**6**). In ref. 11, it was reported that the n.m.r. results suggested that the structure of (**6**) in solution is different from that in crystal. The former is closer to having a normal A ring conformation, while the latter has an inverted A ring. This conclusion is perfectly consistent with the deviation observed in the present study.

‡ Although the sign of $^2J_{HH}$ cannot be determined by this technique, it is known to be negative (ref. 1). When comparing with theoretical predictions, the sign of $^2J_{HH}$ is taken into consideration.

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