Complete Assignment of Proton N.M.R. Spectra of Steroids using Nuclear Overhauser Enhancement-difference and Decoupling-difference Techniques

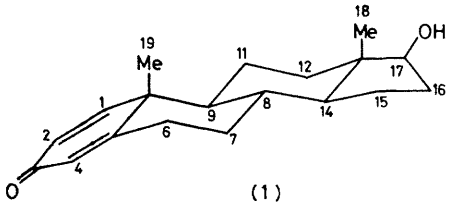
By LAURANCE D. HALL* and JEREMY K. M. SANDERS*†

(*Department of Chemistry, University of British Columbia, Vancouver, British Columbia*, V6T 1Y6 *Canada*)

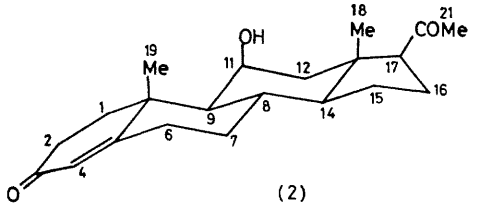
Summary A combination of n.O.e.-difference and spin decoupling-difference n.m.r. spectroscopy has been used to resolve and assign every proton resonance of 1-dehydrotestosterone and 11β-hydroxyprogesterone.

IT has been obvious for some time that modern high-field n.m.r. spectrometers will allow much greater detail to be obtained from the spectra of complex organic molecules, and that proton two-dimensional (2D) J spectroscopy can increase effective dispersion even further.[1,2] It is less clear how the formidable assignment problem arising from an array of similar chemical shifts and coupling constants can be solved; for example, using 2D J spectroscopy we have detected and analysed, but not assigned, all the proton resonances of (**1**) and most of (**2**).[2] Potentially useful 2D assignment techniques include spin decoupling

† On leave from University Chemical Laboratory, Cambridge CB2 1EW, August 1979—August 1980.



(1)



(2)

during the 2D J experiment,[3] a spin correlation pulse sequence,[4] and, when the ^{13}C spectrum is already assigned, ^{13}C–^{1}H 2D shift correlation spectroscopy.[5] We show here that a combination of one-dimensional proton–proton nuclear Overhauser enhancement (n.O.e.)-difference and proton–proton spin decoupling-difference techniques can provide unambiguous assignments for all the protons of both (**1**) and (**2**) through their spatial and scalar coupling relationships.

Although the traditional‡ n.O.e. experiment[6] is very powerful,[7] it is limited in scope both because the signal to be observed must be resolved and because the minimum credible effect using integration is *ca.* 5%. In n.O.e.-difference spectroscopy,[8,9] a control spectrum without n.O.e. is subtracted from the spectrum with n.O.e. so that only spectral changes should appear. The signal of interest need no longer be resolved and the lower limit of observable n.O.e. is determined only by instrument stability.

In the 400 MHz spectrum of (**1**) the methyl signals, 17-H and the olefinic protons of ring A are trivially assigned. Pre-irradiation of 17-H gives in the n.O.e.-difference spectrum [Figure (b)] only three resonances which were tentatively assigned as 12α-H, 14-H, and 16α-H on the basis of geometrical and multiplicity expectations. Table 1 lists the n.O.e.s observed in this way and shows that virtually all the expected axial–axial and cross ring connectivities can be determined. Observed n.O.e.s are in the range 0·5—5% with an attainable subtraction precision of better than 99·7% if some exponential line broadening is used. The necessary frequency selectivity is obtained using sub-saturating power levels for the irradiation. For methyl singlets a selective 180° pulse can be generated using either DANTE[10] or gated decoupling.[11]

TABLE 1. Proton–Proton nuclear Overhauser enhancement in (**1**) and (**2**).[a]

Proton irradiated	Observed n.O.e. (**1**)	Observed n.O.e. (**2**)
1	11α	—
1α	—	1β, 9
4	6α	6α
6β	7β, 8, 19	19
7β, 8	—	6β, 7α, 15β, 18, 19
11α	—	1α, 1β, 9, 12α, 12β
15β	—	7β/8, 16α, 16β, 18
16α	15α, 16β	—
17	12α, 14, 16α	12α, 14, 16α
18	8, 11β, 12β, 15β, 16β	8, 12β, 15β, 16β
19	6β, 8, 11β	6β, 8

[a] Absence of an expected n.O.e. is usually due to proximity of resonances. Only the δ 0·6—2·6 region was searched for n.O.e.s.

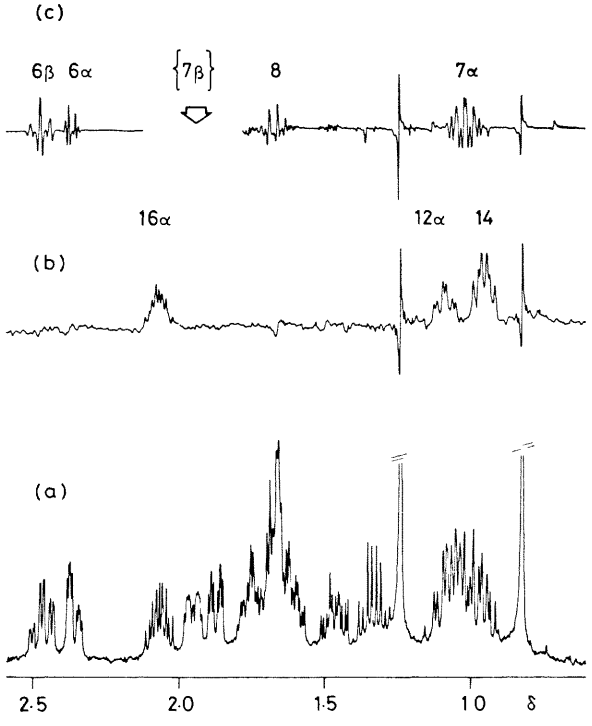


FIGURE. Partial 400 MHz spectra of (**1**) (0·06 M in $CDCl_3$). (a) Normal spectrum, 100 transients with resolution enhancement, (b) n.O.e. difference spectrum with pre-irradiation of 17-H (360 transients with 1·5 Hz line broadening), (c) decoupling-difference spectrum with irradiation of 7β-H (100 transients).

Tentative assignments were confirmed by spin decoupling-difference spectroscopy[9]§ in which a control spectrum is subtracted from a decoupled one; only those resonances directly coupled to the irradiated signal appear. Figure (c) shows the effect of irradiating 7β-H; responses occur only from 6α-H, 6β-H, 7αH, and 8-H. The latter two are hidden amongst other multiplets in the normal spectrum [Figure (a)]. The result of many such experiments is an over-determined, completely unambiguous assignment of all the proton chemical shifts of (**1**) (Table 2). 11β-Hydroxyprogesterone (**2**) presents (apart from methyl signals) only five completely resolved single proton resonances even at 400 MHz. Nevertheless, all the chemical shifts (Table 2)

‡ Measuring the change in intensity of a proton resonance when a nearby proton has been saturated by pre-irradiation.

§ Ref. 9 came to our attention during the preparation of this manuscript.

TABLE 2. Chemical shift assignments for aliphatic protons of (1) and (2).

Proton	(1)[a]	(2)[b]
1α	—	1·84
1β	—	2·18
2α	—	2·35
2β	—	2·47
6α	2·36	2·23
6β	2·47	2·48
7α	1·01	1·06
7β	1·96	2·00
8	1·67	2·00
9	1·04	1·00
11α	1·77	4·40
11β	1·68	—
12α	1·09	1·65
12β	1·87	2·19
14	0·95	1·11
15α	1·61	1·75
15β	1·33	1·33
16α	2·07	1·69
16β	1·47	2·19
17	3·64	2·43

[a] 0·06 M In $CDCl_3$; uncertainty ± 0·10 p.p.m. [b] 0·10 M In $CDCl_3$; uncertainty ± 0·01 p.p.m.

and most of the coupling constants have been obtained in conjunction with the 2D J experiments described in the preceding paper.[2] There are many similarities in the observed n.O.e. and chemical shifts of (**1**) and (**2**). Using computer control to carry out sequential experiments automatically, the spectrometer time required for the assignment of (**2**) was *ca.* 3 days.

It is apparent that the combination of n.O.e.-difference and decoupling-difference techniques is a powerful, independent method for analysing complex proton spectra, but it is in association with proton 2-dimensional J spectroscopy that the general solution to the assignment and hidden resonance problems appears to lie.

We thank the M.R.C. of Great Britain for a Travelling Fellowship (J. K. M. S.) and the N.S.E.R.C. of Canada for financial support.

(*Received, 13th December* 1979; *Com.* 1300.)

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