methods described in the literature. *anti-trans*-1β-Hydroxy-8β-methyl-4,5-(4-oxo-1,2,3,4-tetrahydrobenzo)hydrindane (1) and 17β-hydroxyestra-5(10)-en-3-one (13) were either gifts or commercially available samples. 17β-Hydroxyestra-5(10),9(11)-dien-3-one (14) was available from another study.[5]

Deconjugated Enones (5a–8a).[29] The conjugated enone (0.2 g) was added to a solution of potassium (0.2 g) in *tert*-butyl alcohol (20 mL) (nitrogen atmosphere) and the mixture allowed to stir at 25 °C for 2 h. The resulting enolate anion was quenched with acetic acid (25 mL, 10%). Careful neutralization with aqueous potassium bicarbonate was followed by extraction with ether. The organic extract was washed with water and dried ($MgSO_4$) to give deconjugated enones. For physical data and analyses see Table III.

4,4-Dialkyl-Δ⁵-enones (5b–e through 8b–e). General Method. Enone (0.005 mol) was added in small portions to a stirred solution of potassium (0.78 g, 0.02 mol) in dry *tert*-butyl alcohol (50 mL) (nitrogen atmosphere). The mixture was refluxed for 15 min and cooled to room temperature. Alkyl halide (0.01 mol) was added and the mixture stirred for 15 min followed by 15 min reflux. Evaporation of *tert*-butyl alcohol under reduced pressure yielded a semisolid residue. This material was dissolved in methylene chloride and washed twice with 100-mL portions of sulfuric acid (5%) followed by water. The organic layer was dried ($MgSO_4$) and evaporated to yield the crystalline dialkyl derivative. The products from the reaction of 1,3-dibromopropane with enones 1–4, however, were viscous oils and were chromatographed on silica gel (100:1 ratio) using ethyl acetate–hexane mixtures as eluents. For the hydroxy derivatives (5, 6, and 8) 20% EtOAc–80% hexane and for cholestenes (7) 10% EtOAc–90% hexane mixtures were found suitable for elution. For physical data and analyses see Table III.

2,2-Dideuterio Derivatives.[33] Clean sodium (0.10 g) was dissolved in deuteriomethanol (10 mL) (nitrogen atmosphere). When the evolution of deuterium subsided, the 4,4-dialkyl derivative (0.10 g) was added and the mixture heated to boiling. After the addition of deuterium oxide (0.6 mL), heating was continued for an additional 1.5 h. Cooling afforded a crystalline deuterated compound which was washed with water and dried. Certain compounds did not form crystals and in such cases the mixture was evaporated and the residue obtained was extracted with ether. The ether extract was washed with a small amount of deuterium oxide, dried ($MgSO_4$), and evaporated to yield the 2,2-dideuterio compound in quantitative yield.

17β-Hydroxy-4α-trideuteriomethyl-4β-methylandrost-5-en-3-one (8b-d_3)[15] and 17β-Hydroxy-4β-trideuteriomethylestr-5-en-3-one (6b-d_3).[15] The appropriate 4-methyl 4-en-3-one **19** or **20** (0.01 mol) in *tert*-butyl alcohol (25 mL) was added to a solution of potassium (0.078 g, 0.02 mol) in *tert*-butyl alcohol (25 mL). After the mixture was allowed to stir at 25 °C for 30 min methyl-d_3 iodide (0.29 g, 0.02 mol) was added and stirring continued for 24 h. The usual workup (see general method) followed by chromatography on silica gel, using 30% Et_2O–70% hexane, afforded the methyl-d_3 derivatives **6b** and **8b** as colourless needles.

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Registry No. **1**, 76249-88-2; **2**, 434-22-0; **3**, 601-57-0; **4**, 58-22-0; **5a**, 76232-80-9; **5b**, 76249-89-3; **5c**, 76232-81-0; **5d**, 76232-82-1; **5e**, 76232-83-2; **6a**, 42028-18-2; **6b**, 31025-33-9; **6b-d_3**, 76232-84-3; **6c**, 76232-85-4; **6d**, 76232-86-5; **6e**, 76232-87-6; **7a**, 601-54-7; **7b**, 2220-42-0; **7c**, 1180-86-5; **7d**, 6916-08-1; **7e**, 76232-88-7; **8a**, 571-25-5; **8b**, 5062-44-2; **8b-d_3**, 76232-89-8; **8c**, 76232-90-1; **8d**, 76232-91-2; **8e**, 76232-92-3; **9**, 76232-93-4; **10**, 76232-94-5; **11**, 76232-95-6; **12**, 76232-96-7; **13**, 1089-78-7; **14**, 5218-51-9; **17a**, 1434-85-1; **17b**, 2059-40-7; **17c**, 19468-31-6; **18a**, 521-18-6; **18b**, 54550-06-0; **19**, 6959-54-2; **20**, 795-83-5.

Analysis of the Proton Nuclear Magnetic Resonance Spectrum of 11β-Hydroxyprogesterone by One- and Two-Dimensional Methods. Some Implications for Steroid and Terpenoid Chemistry

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By use of a recently proposed strategy for the total analysis of the proton NMR spectra of complex natural products, all the chemical shifts and virtually all geminal and vicinal coupling constants have been determined for 11β-hydroxyprogesterone. Two-dimensional *J* spectroscopy at 270 and 400 MHz has been used to resolve 11 virtually first-order multiplets between 1.0 and 2.5 ppm, allowing measurement of most geminal and vicinal coupling constants and some long-range couplings (notably $J_{1\alpha,19}$ and $J_{12\alpha,18}$). Methine protons were resolved and assigned by virtue of their slow spin-lattice relaxation. NOE difference spectroscopy was used to resolve and assign protons on the basis of spatial relationships, particularly between 1,3-diaxial neighbours (and their ring D equivalents). Both steady-state and transient methods of generating enhancements were investigated; the latter appears to be preferable in some circumstances. Decoupling-difference spectroscopy was used to resolve and assign protons by their scalar coupling relationships. Comparison of results for the title compound and the previously analyzed 1-dehydrotestosterone reveals the possibility of using many hitherto inaccessible shift and coupling constant structural correlations in future studies of steroids and related terpenoid substances.

The elucidation of complex natural product structures by using proton NMR spectroscopy has historically been hindered by three problems.

(a) Sensitivity. This has largely been overcome by the Fourier transform technique and, more recently, by the advent of high-field spectrometers.

(b) Resolution of Individual Resonances. High fields clearly help, but even at 400 MHz the spectra of most steroids and terpenoids are complex, containing many overlapping signals in the region above δ 3 and a few re-

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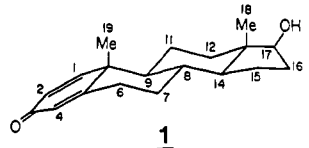
solved resonances spread over the remaining regions.

(c) **Assignment.** This problem in a sense becomes worse at high field: as more resonances with very similar chemical shifts and coupling constants are resolved, "classical" assignment methods based on correlations with related systems become almost useless. In addition, the large number of resonances to be assigned and measured places excessive demands on spectrometer time unless advantage is taken of new strategies for data acquisition and interpretation.

We[2] recently described a strategy, based on results with 1, for the solution of b and c above based on the use of



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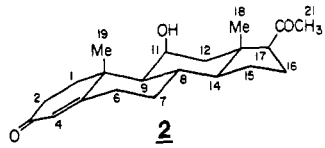

2

two-dimensional J spectroscopy for the resolution and analysis of individual proton resonances and on spin-lattice relaxation, NOE difference, and decoupling difference methods for assignments. In this paper we show how it may be implemented and extended for the more challenging case of 11β-hydroxyprogesterone (2) and discuss some chemical implications of the derived results. An important assumption in our approach is that even the apparently intractable methylene envelope of steroids is merely the superposition of many weakly coupled multiplets. This was almost correct in 1 but less so in 2: nevertheless, it has proved possible to assign all the proton chemical shifts and almost all the significant couplings in 2.[3] The same strategy has also been successfully applied to the analysis of vinblastine alkaloids[4a] and a modified enkephalin[4b] and appears to have considerable generality.

We only give here a brief summary of the techniques used in this work with the emphasis on their applicability to steroids in particular. Full details and references may be found in ref 2.

Spin-Lattice Relaxation Rates. The dominant relaxation mechanism for protons in medium-size organic molecules is the dipole–dipole interaction with neighboring protons. The relaxation rate $R_1(\mathrm{I})$ of a proton I as a result of this interaction with its neighbouring S protons is given by eq 1 where τ_c is a rotational correlation time, and r_{IS}

$$R_1(\mathrm{I}) \propto \tau_c \Sigma r_{IS}^{-6} \qquad (1)$$

are the distances from I to S. The protons directly attached to the steroid framework will all have effectively the same τ_c, so their relative relaxation rates will reflect distances from other protons. Thus methine protons will relax more slowly than methylene protons (and should therefore be resolved in partially relaxed spectra), and the methylene protons of ring D may relax slowly because they lack 1,3-diaxial relationships.

Nuclear Overhauser Enhancements. These are a manifestation of spin-lattice relaxation and also have an r^{-6} dependence. Thus although the largest NOE's are generally observed between geminal or vicinal protons, these relationships are often more easily established through scalar coupling. The most useful relationships to establish are those where there is spatial proximity but no coupling, 1,3-diaxial protons in steroids being an important example of this class. However, calculation[2] shows that these NOE's are unlikely to exceed at most 3%, and such enhancements are too small for routine observation with classical methods. NOE difference spectroscopy, in which a control spectrum is subtracted from the enhanced spectrum so that only *changes* in intensity appear, allows the routine measurement of enhancements of 0.5% or lower when computer control of the decoupling frequency enables the concurrent acquisition of a "control" and several different enhanced spectra to be carried out (see supplementary material). Either steady-state or transient NOE's may be generated, depending on whether continuous-wave saturation or a selective inversion is employed.

Clearly NOE difference spectroscopy should be a powerful assignment tool with several particularly appealing features for steroid chemistry: the proton to be enhanced need not be resolved, the protons which will be enhanced are largely predictable from geometrical considerations, their multiplicities are similarly predictable (facilitating recognition), and it is possible to observe enhancements of methyl groups when (in 5α,14α- or Δ4,14α-steroids) protons 6β, 8, and 11β are irradiated.[5]

Decoupling Difference Spectroscopy. If a decoupled spectrum is subtracted from a control spectrum, then only those protons which experience decoupling will appear in the difference spectrum. This is a very powerful way of detecting hidden protons, as its sensitivity relative to a normal spectrum approaches 100%, whereas the sensitivity of NOE-difference spectroscopy or FT INDOR[6] is 1–2 orders of magnitude lower.

Two-Dimensional J Spectroscopy. In weakly coupled systems, a tilted two-dimensional spectrum contains only chemical shift information along one frequency axis (f_2 or δ) and homonuclear scalar coupling along another axis (f_1 or J) perpendicular to the first. It is possible, therefore, to obtain a "proton-decoupled" proton spectrum containing only singlets at the chemical shifts and to obtain partial "J spectra" which give the scalar couplings for each multiplet, even when these multiplets are severely congested and overlapping in the normal one-dimensional spectrum. Because this is a spin-echo experiment, line widths are determined in principle by intrinsic T_2's rather than by magnet inhomogeneity. Strong coupling is easily recognized in two-dimensional J spectra by the appearance of unsymmetrical multiplets at the correct chemical shifts and additional intensity at a chemical shift midway between the coupled protons;[7] nevertheless, it appears to be possible to obtain reasonable values for coupling constants simply by inspection even when Δ/J is ca. 3.[2,8]

Results

One-Dimensional Spectroscopy. The 400-MHz

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(2) Hall, L. D.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1980**, *102*, 5703.
(3) Preliminary communications: (a) Hall, L. D.; Sanders, J. K. M.; Sukumar, S. *J. Chem. Soc., Chem. Commun.* **1980**, 366; (b) Hall, L. D.; Sanders, J. K. M. *Ibid.* **1980**, 368.
(4) (a) Hall, L. D.; Hunter, B. K.; Sanders, J. K. M., to be submitted for publication. (b) Gidley, M. J.; Hall, L. D.; Sanders, J. K. M.; Summers, M. C., submitted for publication.
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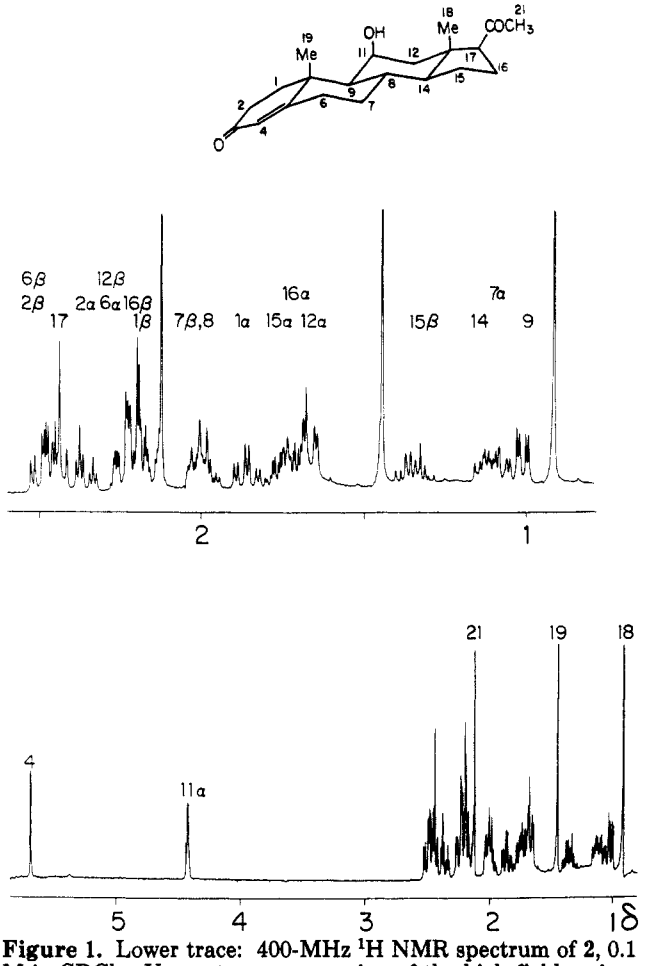

Figure 1. Lower trace: 400-MHz ^1H NMR spectrum of **2**, 0.1 M in $CDCl_3$. Upper trace: expansion of the high-field region.

Table I. Proton Chemical Shifts and Spin-Lattice Relaxation Rates for 11β-Hydroxyprogesterone

proton	shift, δ (±0.01)	R_1, s^{-1} (±10%)	proton	shift, δ (±0.01)	R_1, s^{-1} (±10%)
1α	1.84	1.7	12α	1.65	1.7
1β	2.18	(1.7)[a]	12β	2.21	(1.5)
2α	2.35	1.5	14	1.11	1.4
2β	2.47	(1.5)	15α	1.75	(1.6)
4	5.67	0.5	15β	1.33	1.7
6α	2.23	(1.7)	16α	1.69	(1.7)
6β	2.48	1.5	16β	2.17	(1.7)
7α	1.06	1.7	17	2.43	1.0
7β	2.0	(1.5)	18	0.90	1.5
8	1.98	(1.5)	19	1.44	1.1
9	1.00	1.2	21	2.11	0.6
11α	4.40	1.5			

[a] Figures in parentheses are only estimates as the proton is second order, overlapped, or both.

spectrum of **2** in $CDCl_3$ shows, in addition to H_4, $H_{11\alpha}$, and the readily assigned methyl singlets,[9] only four completely resolved single proton resonances (Figure 1). The remaining 15 carbon-bound protons occur in five groups of multiplets; in a carefully dried sample the hydroxyl resonance was found as a 3.3-Hz doublet at δ 1.12. It is clear from the appearance of the 1.33-ppm signal that at least part of the spin system is strongly coupled. Inspection of the spectrum allows *tentative* assignment of $H_{2\alpha}$ (doublet of triplets, δ 2.35) on the basis of a 17.3-Hz coupling which is characteristic of a specific orientation with respect to

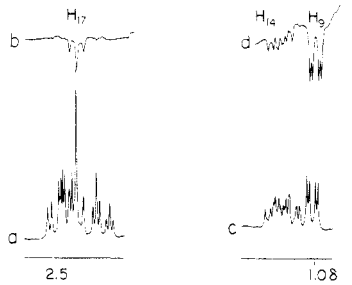

Figure 2. (a) 2.3–2.5-ppm region of the normal spectrum; (b) partially relaxed spectrum of the same region, monitored 0.4 s after a nonselective 180° pulse; (c) normal spectrum, 1.0–1.2 ppm; (d) 1.0–1.2 ppm, 0.45 s after a nonselective 180° pulse.

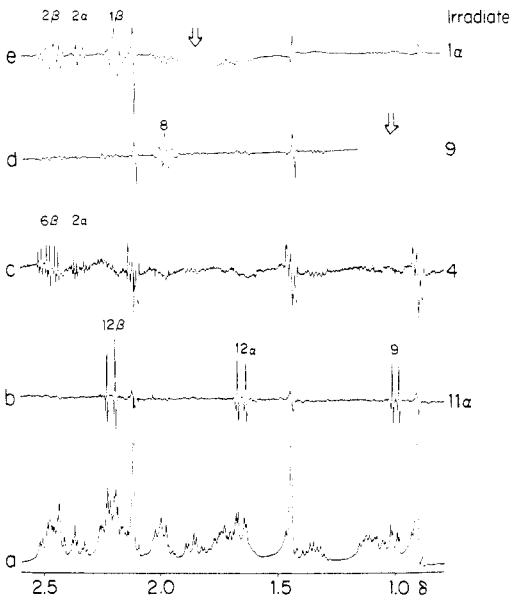

Figure 3. (a) δ 0.8–2.6 region of the normal spectrum; (b–e) decoupling difference spectra of the same region. Irradiated protons are indicated at the right side of each trace.

an adjacent π system,[10] but little further progress is possible without further experimentation. For ease of further discussion all the chemical shift assignments are collected in Table I together with those spin-lattice relaxation rates which could be measured.

As expected, partially relaxed spectra from inversion–recovery experiments readily revealed the position of some of the methine protons (Figure 2): H_{17} is seen to be a clean triplet at δ 2.43 when the methylene protons with which it overlaps are nulled, and H_{14} and H_9 are similarly revealed at high field, these assignments being based on expected multiplicities. The absence of an obvious candidate for the expected H_8 in the partially relaxed spectra is significant. In Table I, relaxation rates are quoted ±0.1 s^{-1}, but (except where extensive overlap creates problems) the relative order of relaxation rates is more reliably determined from inspection of partially relaxed spectra.

All spin-decoupling experiments were carried out as part of automated sequences (see supplementary material) and some of the most important are displayed as difference spectra in Figure 3. Irradiation of $H_{11\alpha}$ generates responses from the geminal pair at C_{12} and the already recognized H_9 (Figure 3). Note that in the normal spectrum $H_{12\beta}$ is completely buried. Decoupling H_4 removes a 1.5-Hz splitting from $H_{6\beta}$ and, near the limit of detect-

(9) Bhacca, N. S.; Williams, D. H. "Applications of NMR Spectroscopy in Organic Chemistry: Illustrations from the Steroid Field"; Holden-Day: San Francisco, 1964; pp 13–24.

(10) Jackman, L. M.; Sternhell, S. "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry"; Pergamon Press: Oxford, 1969; pp 270–275.

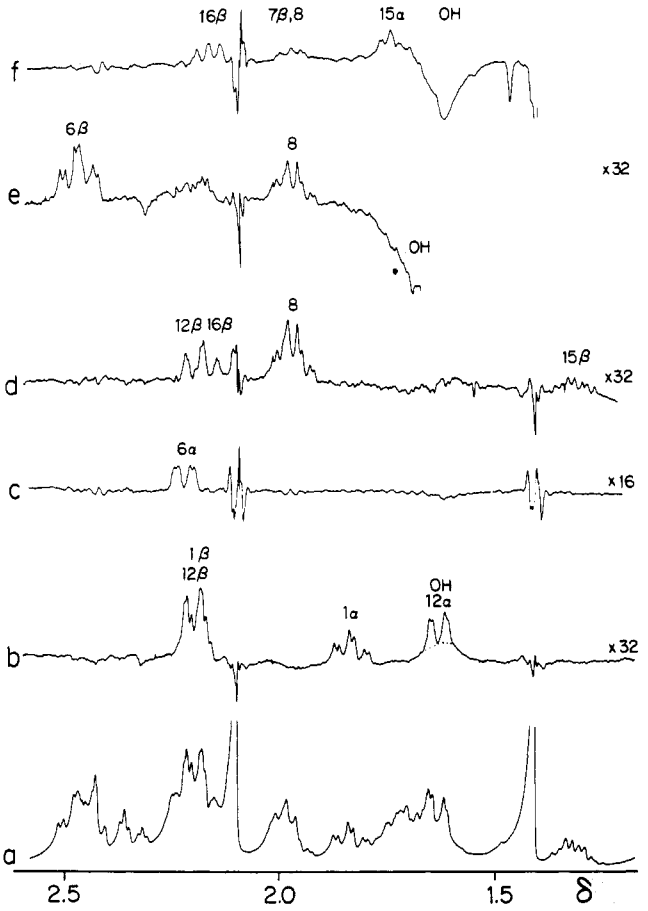

Figure 4. (a) δ 1.2–2.6 region of the normal spectrum; (b–f) NOE difference spectra obtained by preirradiation of $H_{11\alpha}$ (b), H_4 (c), CH_3-18 (d), CH_3-19 (e), and $H_{15\beta}$ (f).

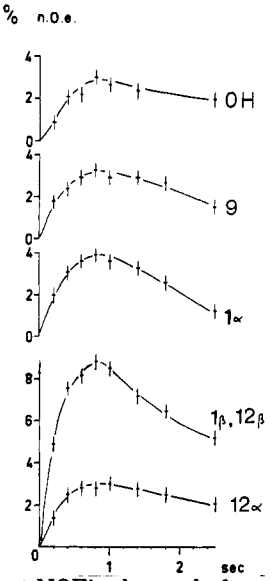

Figure 5. Transient NOE's observed after selective inversion of $H_{11\alpha}$; 700 FIDS were acquired for each point.

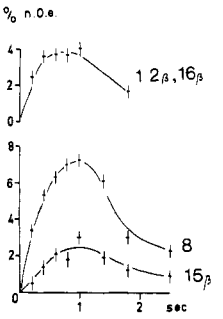

Figure 6. Transient NOE's observed after selective inversion of CH_3-18.

ability, a very small coupling to $H_{2\alpha}$ (Figure 3).

Many actual assignments rest on NOE difference spectra, some of which are displayed in Figure 4. All the connectivities established by decoupling and NOE methods are collected in Table II, but as the logic used in the interpretation of these results is complex, much of it is detailed in the following paragraphs. It should be stressed that some *individual* deductions have a rather low confidence level; however, the *combination* of these deductions leads to unambiguous conclusions via an overdetermined network of connectivities.

Except where stated otherwise, all NOE experiments were carried out by continuous-wave presaturation. The first set of NOE experiments was carried out by irradiation of the well-separated and assigned protons: $H_{11\alpha}$, H_4, $CH_3(18)$, and $CH_3(19)$. $H_{11\alpha}$ (Figure 4) gave enhancements to the expected C_{12} pair and H_9 (not shown) plus the hydroxyl proton and two further protons assigned as the C_1 pair. The only visible enhancement from H_4 was at a signal assigned to $H_{6\alpha}$ on the basis of the earlier experiments with 1. Both the angular methyls gave enhancement to H_8, although the multiplet appearance was rather second order in comparison with the clean doublet of quartets seen in 1. $CH_3(19)$ also enhanced $H_{6\beta}$, and $CH_3(18)$ enhanced $H_{12\beta}$ and two further signals assumed to be $H_{15\beta}$ and $H_{16\beta}$ although no attempt was made at this stage to distinguish between the latter pair.

The second-order appearance of H_8 from the two-proton multiplet at 2.00 ppm together with its nonappearance in partially relaxed spectra indicated that the other proton at that chemical shift was attached to C_7, H_9 having already been assigned. Irradiation of this two-proton multiplet led to enhancements of ca. 1.5% at each of the angular methyls, of at least 10% at a broad quartet (1.06 ppm) and of ca. 3% at the signal which can now be assigned to $H_{15\beta}$ as this would be expected to be much closer to $H_{7\beta}$ or H_8 than would $H_{16\beta}$. Irradiation of $H_{15\beta}$ either in the decoupling mode (Figure 3) or the NOE mode (Figure 4) confirmed the location of $H_{16\beta}$ and also gave responses in the 1.6–1.8-ppm region. As $H_{15\beta}$ is not first order in the normal spectrum (Figure 1), it appeared that the two remaining protons coupled to it, $H_{15\alpha}$ and $H_{16\alpha}$, were located together near δ 1.7, H_{14} having already been assigned in the partially relaxed spectra.

The irradiation of $H_{1\alpha}$ (δ 1.84) led to enhancement of $H_{1\beta}$ and H_9. This irradiation also led to slight saturation of the 1.75-ppm proton and was accompanied by a small but obvious enhancement of $H_{15\beta}$. Since this off-resonance effect would be expected only where large NOE's are operative, a very tentative assignment of $H_{15\alpha}$ may be made.

The final irradiation in both modes was of the three-proton multiplet at δ 2.3–2.5 containing H_{17}, $H_{6\beta}$, and $H_{2\beta}$. The NOE difference spectrum showed $H_{12\alpha}$, H_{14}, and $H_{16\alpha}$ enhanced by H_{17} (as in 1) and $CH_3(19)$ enhanced by $H_{6\beta}$. The NOE difference spectrum did not contain the 1.06-ppm H_7 signal, but the decoupling difference spectrum did, so this must be the α-proton, and the β-proton must be the one strongly coupled to H_8; the appearance of $H_{7\alpha}$ in the $\{H_{7\beta,8}\}$ spectrum also point to it being axial rather than equatorial.

The experiments described thus far allow the assignment of all proton chemical shifts in this compound with the minimal use of chemical shift or coupling information but with a heavy reliance on NOE arguments based on the precedent of a single compound, 1. Before describing

Table II. Connectivities Established by NOE Difference and Decoupling Difference Experiments[a]

proton irradiated	protons appearing in diff spectrum	
	NOE	decoupling
1α	1β, 9	1β, 2α, 2β
2α		1α, 1β, 2β
2β[b]		1α
4	6α	2α, 6β
6β[b]	19	7α, 7β
7β/8[c]	6β, 7α, 9, 15β, 18, 19	6α, 6β (7α/9/14)[d]
9	1α, 7β/8,[d] 12α	8, 11α
11α	1α, 1β, 9, 12α, 12β, OH	9, 12α, 12β, OH
15β	7β/8,[d] 15α/16α,[d] 16β, 18	15α, 16α, 16β
17[b]	12α, 14, 16α	16α
18	8, 15β, 12β/16β[d]	
19	2β/6β[d]	

[a] Absence of an expected entry is either because of signal proximity or because the experiment was not performed. Difference spectra were only inspected in the 0.8–2.6-ppm region. [b] These protons are effectively coincident and were irradiated together. [c] Irradiated together. [d] Too close to be safely distinguished.

concurrent two-dimensional work, we shall present results on transient NOE's which were acquired to allow comparison between them and more traditional continuous-wave presaturation.

In Figures 5 and 6 is plotted the evolution of transient NOE's generated after inversion of $H_{11\alpha}$ and of $CH_3(18)$ by weak 17-ms pulses from the decoupler (see supplementary material). It appears that enhancements build up to a maximum in approximately one T_1 and then die away slowly: only for H_9 and the hydroxyl are any enhancements visible 5 s after inversion, and then only near the limit of detectability. The maximum transient NOE values measured after inversion of $H_{11\alpha}$ were 8.8% for the coincident $H_{1\beta}$ plus $H_{12\beta}$, 3.9% for $H_{1\alpha}$, 3.0% for $H_{12\alpha}$, 3.3% for H_9, and 3.0% for the hydroxyl, each value being ± ~0.5%. The corresponding maximum enhancements after inversion of $CH_3(18)$ were 4.1% for $H_{12\beta}$ and $H_{16\beta}$ combined, 7.2% for H_8 and ca. 3% for $H_{15\beta}$. In a separate experiment, DANTE was used to invert $CH_3(18)$. The same protons appeared as in the gated decoupling and steady-state methods of generating enhancements.

Two-Dimensional Spectroscopy. Figure 7 shows, in addition to the normal 400-MHz spectrum of the high-field region, the "proton-decoupled" spectrum and all the partial J spectra derived from the 400-MHz, two-dimensional, J spectrum. The proton-decoupled spectrum shows, apart from some artifacts[11] marked with an X, a singlet for every proton in the molecule except the hydroxyl (which in this sample was too broad to be refocussed in the spin-echo experiment) and the coincident resonances around 2.47 and 2.19 ppm. Most of the corresponding J spectra above are symmetrical, first-order multiplets, but $H_{7\beta}$, H_8, and $H_{15\alpha}$ are too distorted to allow simple extraction of coupling constants. In the two-dimensional spectrum of the solution containing 2 drops of benzene, $H_{6\alpha}$ is sufficiently shifted from other signals that it is analyzable.

All the geminal and vicinal coupling constants except $J_{7\beta,8}$ and $J_{15\alpha,16\beta}$ were measured from the two-dimensional spectra or in NOE difference spectra and are collected in Tables III and IV. In addition, several long-range couplings were resolved and assigned: $J_{4,6\beta} = 1.5$ Hz, $J_{2\alpha,4} = 0.6$ Hz, $J_{1\alpha,19} = 0.4$ Hz, and $J_{12\alpha,18} = 0.5$ Hz. Assignment of some of the measured couplings was not trivial, even when all chemical shifts were known, as several protons were not visible in the two-dimensional spectra and were only seen under rather low resolution in one-dimensional difference spectra. For example, $H_{7\alpha}$ has, apart from the small coupling with $H_{6\alpha}$, couplings of 13.6, 13.6, and 12.1 Hz, but none of its three coupled partners is easily seen. $J_{6\beta,7\alpha}$ is known to be ca. 14 Hz from NOE difference spectra, and the remaining two assignments depend on comparison with compound 1. We are aware of the danger of a circular argument in deriving coupling constant correlations from such data. Assuming that $|J_{gem}|$ values for the protons on C_{15} and C_{16} are greater than 10 Hz, all the coupling assignments in ring D are unambiguous, despite $H_{15\alpha}$ and $H_{16\alpha}$ being absent or second order in the two-dimensional spectra.

Table III. Geminal and Vicinal Coupling Constants in Rings A-C[a]

proton pair	^2J	^3J		
		Ax-Ax	Ax-Eq	Eq-Eq
1α,1β	13.9			
1α,2α			4.9	
1α,2β		14.0		
1β,2α				4.8
1β,2β			5.2	
2α,2β	17.3			
6α,6β	14.7[b]			
6α,7α			4.7	
6α,7β				2.2[b]
6β,7α		13.6		
6β,7β			4.5[c]	
7α,7β	13.6[e]			
7α,8		12.1[e]		
7β,8			NM[d]	
8,9		11.3		
8,14		10.4		
9,11α			3.6	
11α,12α			4.0	
11α,12β				3.0
12α,12β	14.3			

[a] In hertz ±0.3 Hz. [b] Measured in solution containing 2 drops of benzene-d_6. [c] Estimated from the NOE difference spectrum. [d] Not measured. [e] Assigned by comparison with compound 1.

Table IV. Geminal and Vicinal Coupling Constants in Ring D[a]

proton	14	15β	16α	16β	17
15α	6.9	11.4	9.9	NM[b]	
15β	12.9		5.6	11.4	
16α				13.3	9.4
16β					9.4

[a] In hertz ±0.3 Hz. [b] Not measured.

Discussion

Spectroscopy and Assignments. We have discussed previously[2] many of the technical and practical aspects of these experiments and wish to make here only a few additional points.

The transient NOE appears to have some promise: we detect little selectivity in enhancement buildup rates, the maximum enhancement occurring around one T_1 period after selective inversion. This is faster than the buildup of steady-state NOE's. Transient methods have the additional advantage that frequency selectivity can be achieved without loss of NOE by using longer weaker

(11) That these are artifacts is apparent from their appearance as off-center singlets in the full two-dimensional spectrum and relevant partial J spectra. They may be ghosts, phantoms, or a new supernatural manifestation of spin alchemy: Bodenhausen, G.; Freeman, R.; Turner, D. L. *J. Magn. Reson.* **1977**, *27*, 511. Bachmann, P.; Aue, W. P.; Müller, L.; Ernst, R. R. *Ibid.* **1977**, *28*, 29.

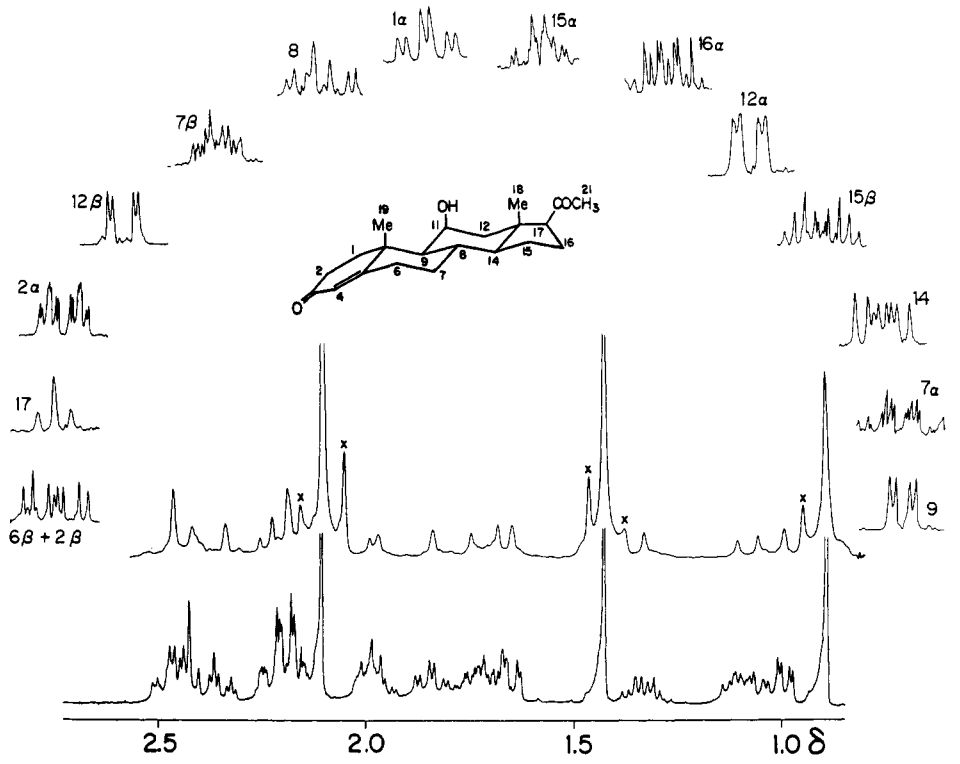

Figure 7. Lowest trace: the normal one-dimensional spectrum at 400 MHz, δ 0.6–2.6. Center trace: the 400-MHz "proton-decoupled" spectrum derived from the two-dimentional spectrum (X marks artifacts). Upper traces: partial J spectra of individual multiplets.

pulses. DANTE is as effective in generating NOE's but suffers from sideband problems[12] and, more seriously, is not well suited to use in automated sequences which cycle through many different irradiation frequencies in the course of a single run.

Acceptable "proton-decoupled" spectra of steroids and other natural products and partial J spectra free of "tails" from nearby singlets are only obtained, in our hands at least, when sine-bell resolution enhancement is used in the first fourier transformation. The cost of sine-bell enhancement in effective sensitivity is high, but when it is not used, partial J spectra are often dominated by the "tails" from other proton resonances. It is not clear to us whether this is intrinsic in the technique or merely a current experimental problem. Sine-bell enhancement can also suppress interference from very broad signals such as those from NH and OH, which may be a highly advantageous bonus.[4b]

Chemical Implications. We have in this work made severe demands on both hardware and software which are not yet widely available but have indicated elsewhere[2] that many of these experiments, particularly partially relaxed spectra and decoupling difference spectroscopy, may be carried out on almost any FT spectrometer. Thus it should no longer be necessary in structural studies of steroids using proton NMR to have to rely only on methyl signals and on shifted protons which are close to a functionality. Note, however, that our strategy is dependent on using connectivity with those characteristic signals as the entry point for analysis of the rest of the spectrum. Where two-dimensional J spectroscopy is not available, couplings can be estimated from decoupling difference spectra by suitable analysis, providing that complete decoupling is achieved.[2,13]

There are early examples of cross-ring NOE's being used in structure determinations of an ecdysone[14] and a podocarpane,[15] but in each case the enhanced proton was a *well-resolved methine* resonance which of necessity obtained its relaxation from distant protons. By use of difference techniques the enhanced protons are subject to neither restriction, making NOE difference spectroscopy perhaps the most powerful assignment tool available in steroid work: angular methyls are usually easily assigned and may be used to generate enhancements very selectively by using transient NOE methods. Not only are the enhancements generated by these protons (for a given gross geometry) almost entirely predictable but the appearance of enhanced signals also is predictable.

Two-dimensional J spectroscopy promises to be valuable in resolving overlapping first-order multiplets and allowing their analysis. In principle the two-dimensional J spectra of strongly coupled spin systems are analyzable, but their complexity is likely to render them of little value in the context of a steroid.

Finally, we point out what may be promising new correlations of chemical shifts and couplings in **1** and **2**. They can only be tentative, given their occurrence in these two rather similar molecules; in addition, $J_{7\alpha,7\beta}$ and $J_{7\alpha,8}$ in **2** are subject to the circular argument caveat mentioned in the Results. Nevertheless, the following observations are intriguing.

(a) $J_{ax,ax}$ involving bridgehead protons are in the range 10.4–12.3 Hz (average 11.3) while diaxial couplings involving only $J_{6\beta,7\alpha}$ and $J_{11\alpha,12\beta}$ are in the range 12.8–13.4 Hz.

(b) J_{gem} for the C_{15} pair is remarkably small in both molecules: 12.1 and 11.4 Hz in **1** and **2**, respectively.

(c) Couplings in ring D are virtually identical for the two molecules except for the reduced value of $J_{16\beta,17}$ in **1** expected from Booth's cis electronegativity effect.[16] Since the observed NOE's are also identical, this provides o-

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verwhelming evidence for very similar conformations.

(d) Protons $H_{7\alpha}$, H_8, H_9, and $H_{12\alpha}$ in **1** all fall within 0.1 ppm and clearly occupy positions symmetrically disposed with respect to a pseudosymmetry axis running from C_3 to C_{16}. In **2**, $H_{12\alpha}$ is shifted 0.5 ppm downfield by the adjacent hydroxyl group, but the other three protons are almost unshifted from the positions in **1**.

(e) $J_{12\alpha,18}$ of ca. 1 Hz are well-known in 11-keto steroids, and some $J_{1\alpha,19}$ have also been reported,[17] but our results suggest that these may in fact be general phenomena. The lack of similar couplings from $CH_3(18)$ to H_{14} is remarkable.

We do not offer detailed speculation as to the sources of these effects, except to note the substantial deviations from ideal geometry found in the crystal of **1**.[18]

Experimental Section

11β-Hydroxyprogesterone (**2**) was obtained from Sigma Chemical Co. and used without further purification. Solutions (0.1 M) were made up in $CDCl_3$ solution and, for reasons discussed previously,[2] were not degassed.

Details of most spectroscopic procedures were given in ref 2. A brief summary is presented here. All one-dimensional spectroscopy was carried out at 400 MHz, but two-dimensional spectra were obtained at both 270 and 400 MHz. One-dimensional difference spectra were obtained by irradiation at the first frequency for four transients, storage of the FID, repetition of the sequence for each of the remaining frequencies, and then repetition of the whole cycle, under computer control, up to 300 time for NOE experiments and up to 50 times for decoupling experiments. Detailed acquisition microprograms are given in the supplementary material.

Transient NOE's were generated either by a selective pulse from the decoupler or by DANTE.

After submission of the preliminary communications describing some of this work,[3] the original two-dimensional data sets were reprocessed with sine-bell resolution enhancement,[2] and a 270-MHz, two-dimensional, J spectrum was obtained from 0.4 mL of a $CDCl_3$ solution containing 2 drops of benzene-d_6. As a result, virtually all the missing[3] coupling constants were determined, and some chemical shifts have been refined by up to 0.02 ppm.

The total spectrometer time used in this study was 80–100 h, of which 25 h was needed for the transient NOE experiment shown in Figures 5 and 6. The two-dimensional acquisition, processing, and plotting required less than 10 h.

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Registry No. **2**, 600-57-7.

Supplementary Material Available: Appendix containing detailed aquisition microprograms (3 pages). Ordering information is given on any current masthead page.

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Antitumor Agents. 43. Conversion of Bruceoside-A into Bruceantin[1]

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Bruceoside-A (**1**) has been converted by two methods to bruceantin (**5**), a potent antileukemic agent. The first method involved the hydrolysis of **1** with potassium hydroxide followed by *p*-toluenesulfonic acid in methanol to afford 49% bruceolide (**2**). Esterification of **2** with 3,4-dimethyl-2-pentenoyl chloride (**9**) yielded the corresponding 3,15-diester (**3**) (47%) and the 3-monoester (**4**) (31%). Compound **4** was reconverted to **3** (77%) by further esterification. Selective hydrolysis of **3** with *p*-toluenesulfonic acid afforded **5** in 40% yield. The second method included the hydrolysis of **1** with potassium hydroxide to yield 57% 15-desenecioyl bruceoside-A (**6**). Boron trifluoride etherate hydrolysis of the 3,4-dimethyl-2-pentenoyl ester (**7**), prepared by esterification of **6** with the corresponding acid chloride, gave **5** in 58% yield.

Bruceantin (**5**), a potent antileukemic quassinoid isolated from the Ethiopian *Brucea antidysenterica*,[2] is currently under clinical trial as an anticancer agent by the National Cancer Institute.[3] It is important to establish an alternate source of **5** to ensure a continuing supply for clinical trials.[4] In connection with our recent isolation of novel antileukemic quassinoid glycosides, bruceoside-A (**1**) and -B from the Chinese *Brucea javanica* in good yield,[5,6] we report the first two methods (methods A and B) leading to the conversion of **1** to **5** in an overall yield of 14 or 33% (methods A and B, respectively). These two methods may be useful in planning the total synthesis of bruceantin and related active analogues. Attempted total synthesis of **5** is currently carried out by many laboratories.[7-11]

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