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peak. Both tRNA$^{Gly}_{UCA}$-C$_{OH}$ and tRNA$^{Gly}_{UUA}$-C$_{OH}$ were produced in analogous fashion.[26]

**Method D: Total Chemical Synthesis and Purification of tRNA$^{Gly}_{CUA}$-C$_{OH}$.** The 5′-terminal DMT oligoribonucleotide was synthesized and purified as described above. The HPLC-purified oligoribonucleotide was concentrated by lyophilization and dissolved in 500 μL of distilled $H_2O$/HOAc (1:4) to remove the 5′-DMT group. The solution was allowed to stand at room temperature for 20 min, concentrated by lyophilization, and dissolved in TEAA (100 mM, pH 7.0) and injected onto a Vydac C-4 column equilibrated in TEAA (100 mM, pH 7.0) in $H_2O/CH_3CN$ (9:1). Gradient elution with $CH_3CN$ (10–20% $CH_3CN$ in 40 min) in TEAA (100 mM, pH 7.0) followed by ultraviolet detection at 254 nm resolved the desired peak.

**General Preparation of Chemically Misacylated tRNAs. Construction of L-3-Iodotyrosyl-tRNA$^{Gly}_{CUA}$-dCA (3).** Synthesis of L-3-iodotyrosyl-tRNA$^{Gly}_{CUA}$-dCA (**3**) was accomplished by ligation of tRNA$^{Gly}_{CUA}$-C$_{OH}$ (**1**; 20 μg) with 10 μg of 5′-*O*-phosphoryl-2′-deoxycytidylyl(3′-5′)-[2′-(3′)-*O*-(L-3-iodotyrosyl)adenosine] (**2**) in a 40-μL reaction containing 55 mM $Na^+$-Hepes, pH 7.5/15 mM $MgCl_2$/250 μM ATP/8 μg bovine serum albumin/10% DMSO, with 15 units of T4 RNA ligase. The mixture was incubated for 10 min at 37 °C and the reaction terminated by addition of 100 μL of a 250 mM NaOAc, pH 4.5/5 M NaCl/50 mM $MgCl_2$ buffer, followed by extraction once with phenol/$CHCl_3$/isoamyl alcohol (25:24:1) and once with $CHCl_3$/isoamyl alcohol (24:1) and precipitation with 2.5 volumes of EtOH. The precipitate was dissolved in 10 mM NaOAc, pH 4.5/1 mM EDTA/100 mM NaCl (50 μL) and filtered through a Sephadex G-25 Select D column equilibrated with the same buffer. The initial isotope peak was immediately absorbed onto a BD-cellulose column (2 × 0.5 cm) that had been equilibrated at 4 °C with 50 mM NaOAc, pH 4.5/10 mM $MgCl_2$/1.0 M NaCl. Unreacted tRNA$^{Gly}_{CUA}$-C$_{OH}$ (**1**) was removed by eluting the column with 10 mL of the buffer. The column was then eluted with the buffer containing 25% EtOH to effect elution of L-3-iodotyrosyl-tRNA$^{Gly}_{CUA}$-dCA (**3**).[4] The appropriate fractions were combined and the RNA EtOH-precipitated (2.5 volumes), washed with 70% EtOH, suspended in $H_2O$, lyophilized, and stored under argon at −80 °C as a fluffy white powder (7 μg). D-Phenylalanyl-tRNA$^{Gly}_{CUA}$-dCA (8 μg), *N*-methyl-L-phenylalanyl-tRNA$^{Gly}_{CUA}$-dCA (10 μg), L-phenyllactyl-tRNA$^{Gly}_{CUA}$-dCA (7 μg), and glycyl-tRNA$^{Gly}_{CUA}$-dCA (6 μg) wee produced in an analogous fashion.[25]

**Rapid Screening of Unlabeled Non-Natural Residues.** Translations with rabbit reticulocyte lysate were performed with a slightly modified procedure from manufacturer's instructions (Amersham). Magnesium ion and mRNA concentrations were determined as per instructions. A typical reaction (10 μL) contained lysate (9 μL), L-[$^{35}$S]methionine (15 μCi), L-[3,4,5-$^3$H]leucine (5 μCi), mRNA (2.0 μM), and chemically misacylated tRNA (20 μM). The mixture was incubated for 1 h at 30 °C followed by addition of synthetically prepared polypeptide standards, corresponding to the expected 8-mer and 16-mer products from the translation (10 μL of a 0.5 mM solution in 77% formic acid). The solution was immediately quenched with $H_2O$ (1.0 mL), and the resulting precipitate was centrifuged and the solvent decanted. A cycle of resuspension in 77% formic acid (10 μL) followed by precipitation with $H_2O$ (1.0 mL) was repeated twice. The resulting precipitate was dissolved in 77% formic acid (100 μL) followed by radioisotope detection by scintillation counting.

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# Conformational Analysis of Ring A and Total Assignment of 19-Functionalized 4-En-3-one Steroids. Applicability of 2D NOE as a Crucial Technique

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The conformational analysis of ring A of 4-en-3-one steroids with three kinds of functionalizations viz. the 19-hydroxy, 19-oxo, and the 6β,19-epoxy groups, is discussed using a simple protocol—COSY 90 + NOESY + HETCOR. The 2D NOE technique is found to be of immense importance in deducing the conformational preferences of these steroids. The conformations are derived by connectivity pattern analysis and independently by torsional angle calculations employing the Karplus equation. While steroids 1 and 2 show "normal" 1α,2β half-chair conformation, steroid 3 exhibits ring A in the 1β,2α "inverted" conformation. The conformational analysis highlights aspects like interactions of the 1,3-diaxial type and the effect of tilting the 19-carbon toward ring A or ring B. A comment is made to that effect.

With the advent of techniques of modern, multiple-pulse experiments in NMR methodology, the study of NMR of steroids has seen an explosive activity.[1] The olden-days analysis of steroids structure and conformation by the resonances of the 18-$H_3$ and 19-$H_3$ groups,[2] coupled with the analysis of deshielded region of the spectrum, has now been replaced by 2D NMR methodology and computer-aided simulation.[1a,d] While the horde of pulse experiments published to date[3] reveal that NMR techniques can provide solutions with varying degrees of rigorous execution, the effort should be to extract maximum information from minimum experimentation without jeopardizing authen-

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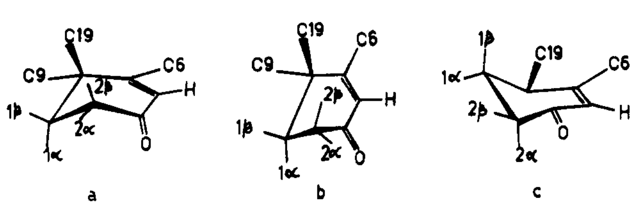


**Figure 1.** Three conformations of 4-en-3-one steroids: (a) normal 1α,2β conformation that has coplanar C3–C5 and C10 atoms, but C1 is "below" and C2 is "above" the plane; (b) 1α-sofa conformational which has coplanarity of C2–C10 atoms with C1 below the plane; (c) inverted 1β,2α conformation in which C1 is above and C2 is below the plane defined by the coplanarity of C3–C5 and C10 atoms, a and c represent half-chair geometry and form the extreme ends of conformational flexibility.

ticity. Numerous protocols have been reported for analyses of steroids, and considerable generalizations have been forthcoming by rigorous approaches.[1,4,5] Whereas Schneider et al.[1a] utilize a combination of MM2 calculations and $J$-correlated spectroscopy and 1D NOE measurements[6] for elucidation of a sofa conformation (Figure 1b) in preference to half-chair geometry (Figures 1a and 1c) for a $\Delta^4$-3-keto steroid, Marat et al.[1d] report an equilibrium conformation on the basis of studies of coupling constants at different temperatures. Hall and Sanders[4] advocate the use of NOE difference spectroscopy for the assignment of the β-face of steroid molecule due to the ease with which the identification of connectivities with 18-$H_3$ and 19-$H_3$ groups can be made, and this suggestion has been mostly followed.

Steady-state NOEs have, however, major disadvantages. These require high magnet stability over a prolonged time period, coupled with high digital resolution,[6b] necessitating an acquisition time of 10–50 h for a single irradiation. And yet enhancements as low as 0.1%,[7] which could lead to major conformational conclusion, might be obviated. In the extreme narrowing limit, the problem is even worse due to intrinsic low NOEs. Although transient NOEs can be advantageous in providing relatively more reliable estimates of distances than steady-state NOEs,[8] the experiment is time consuming due to the necessity of many growth-data-points as well as much longer relaxation delay needed for spins to return to equilibrium.

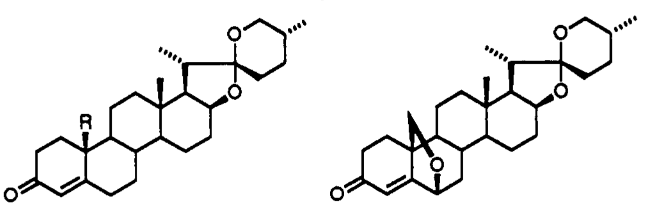
The 2D NOE technique[9] (NOESY) has been most often utilized for biomolecules like peptides and proteins,[10] ,polynucleotides,[11] and polysaccharides.[12] However, NOESY has not found central use in studies of rapidly tumbling molecules. A few scattered examples, e.g., cytochalasin B,[13a] sesquiterpenes,[13b] and imidazolines,[13c] illustrate the possibility of applicability of NOESY to small molecules especially of the type of steroids. For 4-en-3-one steroids, the A ring can exhibit any of the three conformations (Figure 1); however, as compared to saturated ring systems, the conformational flexibility is much reduced. Hence, following Bell and Saunders,[14] the the total NOE at nuclei i due to nuclei s, i.e., $\eta_i(s)$ as given by eq 1 *where*

$$\eta_i(s)\alpha[\tau_c/\rho^*]r^{-6} \quad (1)$$

*$\tau_c$ is the correlation time and $\rho^*$ is the total cross relaxation rate*, is a function only of the distance between the two nuclei ($r$) for the *same* molecule. Additionally, since NOESY is a transient NOE experiment, the estimates of distances by the intensities of cross-peaks would be more accurate. Hence for 4-en-3-one steroids, the possibility of arriving at correct conformational preference should be much greater with NOESY.

A simple protocol for elucidation of conformational preference of 4-en-3-one steroids **1**–**3** (Chart I) was envisaged. This would include analysis of the Dreiding model for an idea about the distances of protons of interest of A and B ring systems in various conformers and 1,3-diaxial interaction; analysis of $J$-correlated spectroscopy[15] experiment for identification of spin systems; homonuclear NOESY[16] experiment for relative orientation and approximate distances of protons of interest from known protons; and heteronuclear $J$-correlated spectroscopy[17] for confirmation of assignments made. The present paper discusses the analysis and the applicability of NOESY as a strategic technique, superceding the difficulties associated with it, for the analysis of conformational preferences of steroids **1**–**3**.

**Chart I. Structures of Steroids for Conformational Analysis**



**1**, R = $CH_2OH$
**2**, R = CHO

**3**

## Experimental Section

All 1D and 2D experiments on steroids **1**–**3** were conducted in a Varian VXR 300S spectrometer at ambient temperature. The $^1H$ NMR spectra were recorded as solutions in $CDCl_3$ with a digital resolution of 0.152 Hz. A pulse width of 45° and acquisition time of 4 s without any additional delay were used. An average of 10 mg of steroids was dissolved in 0.6 mL of $CDCl_3$. The $^{13}C$ NMR spectra were recorced at an operating frequency of 75 MHz with solutions of about 35 mg of steroid in 0.6 mL $CDCl_3$ with the central line of carbon signal of $CDCl_3$ being set to 77.00 ppm. A digital resolution of 0.26 Hz, pulse width of 53°, and relaxation delay of 2.0 s were used. An exponential multiplication factor of 2 was used for Fourier transformation.

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**Table I. Distances, Measured by the Use of Dreiding Model (in Å), between Protons of Interest in the Two Extreme Ends of Conformations. The Relative Distances Are of Importance for NOESY Analysis**

| | steroid 1 | | steroid 2 | | steroid 3 | |
|---|---|---|---|---|---|---|
| protons[b] | $1\alpha,2\beta$[a] | $1\beta,2\alpha$ | $1\alpha,2\beta$ | $1\beta,2\alpha$ | $1\alpha,2\beta$ | $1\beta,2\alpha$ |
| $1\alpha$-$H_A$ | 4.40 | 3.02 | 4.00 | 2.53 | 4.56 | 3.91 |
| $1\beta$-$H_A$ | 2.90 | 3.27 | 2.28 | 2.72 | 3.45 | 2.77 |
| $2\alpha$-$H_A$ | 4.53 | 5.48 | 3.94 | 5.24 | 4.68 | 5.73 |
| $2\beta$-$H_A$ | 3.23 | 5.67 | 2.16 | 5.05 | 2.80 | 5.61 |
| $6\beta$-$H_A$ | 2.90 | 2.37 | 2.34 | 1.85 | | |
| $7\beta$-$H_B$ | 4.71 | 4.13 | 4.50 | 4.00 | 4.56 | 4.44 |
| $8\beta$-$H_B$ | 2.77 | 2.40 | 2.46 | 2.19 | 2.56 | 2.46 |
| $11\alpha$-$1\alpha$ | 3.85 | 2.16 | 3.45 | 2.16 | 2.90 | 2.19 |
| $11\alpha$-$1\beta$ | 2.31 | 4.22 | 2.16 | 4.22 | 2.78 | 4.25 |

[a] $1\alpha,2\beta$ refers to normal $1\alpha,2\beta$ half-chair conformation, while $1\beta,2\alpha$ refers to inverted $1\beta,2\alpha$ half-chair conformation. [b] Protons $H_A$ and $H_B$ equal 19-H for steroid **2** and smallest distance between the protons is measured. Protons have been designated by their positions and nomenclature H is dropped.

The $^1H$–$^1H$ *J*-correlated spectroscopy[15] was performed using standard Varian software utilizing the method of double precision. A total of 256 $t_1$ increments and 1024W $t_2$ data points were employed. The FIDs were Fourier transformed onto a data matrix of 1024W × 1024W with sine-bell phase-shifted window functions followed by symmetrization of the two domains. The spectral width was restricted to 0.0–6.0 ppm for steroids **1** and **3**, while it was 0.0–10.0 ppm for steroid **2**. A typical COSY experiment took 60 min of spectrometer time with 20 mg of steroid in 0.6 mL of $CDCl_3$.

The homonuclear NOESY[16] experiments were performed under identical parameter conditions as the COSY experiments using standard Varian software. A mixing time of 0.9 or 1.0 s was used with a 10% variation of mixing time to eliminate *J*-coupling effects. The experiment was performed in phase-sensitive mode, and to achieve higher signal to noise ratio, 48 scans were accumulated for each FID. The Fourier transformations along both f1 and f2 dimensions were carried out with relatively stronger sine-bell phase-shifted window functions to enhance resolution. A typical experiment required 6–7 h of spectrometer time.

The heteronuclear *J*-correlated spectroscopy[17] was carried out on a solution of 50–75 mg of steroid in 0.6 mL $CDCl_3$. A total of 128 $t_1$ increments and 512 $t_2$ data points were utilized. The FIDs were Fourier transformed onto a data mztrix of 512W × 1024W after weighing. A relaxation delay of 1.5 s was used. The spectral width was restricted to 0–130 ppm. A typical HETCOR experiment needed 5 h of spectrometer time.

The double quantum filtered *J*-correlated spectroscopy[18] (DQF-COSY) experiments for steroids **1**–**3** were carried out in a Bruker AM500 spectrometer and processed on X32 peripheral. Quadrature detection with time-proportional phase increment method for detection of double quantum transitions was used. A total of 512 data points for the $t_1$ dimension of 2048*W* for the $t_2$ dimension were employed. The FIDs were Fourier transformed onto a data matrix of 4K × 4K size using sine-bell phase-shifted window functions to give a digital resolution of 1.20 Hz per point along both the dimensions. The spectral widths for all steroids were restricted to 0.4–4.8 ppm; i.e., the olefinic signal was folded to increase resolution. The relevant cross-peaks were blown up for coupling constant calculations. A typical DQF-COSY experiment required 7.5–9 h of spectrometer time with 75–100 mg of steroid in 0.7 mL of $CDCl_3$.

The synthesis and characterization of steroids **1**–**3** have been achieved as described by Sunthankar and Telang.[19]

## Results and Discussion

As a result of the observation that A-ring conformation[20] and conformational flexibility[21] of the steroid backbone play a significant role on the receptor binding ability, steroids with 10-$CH_3$ group[1a,d,4] or of 19-nor type[1c] have been mainly investigated. Rigorous experimentation has shown that steroids with no or 2α-substituent in the A ring exhibit "normal" and 1α-sofa forms, while Wong et al.[1c] conclude an equilibrium conformation for 19-nor 4-en-3-one steroids. Our investigations into the conformational preferences of spiro[cyclopropane-1,2′-steroids],[22] which possess a relatively rigid A ring due to the spirocyclopropane ring, required a crucial NOESY experiment for the elucidation of normal 1α,2β conformation. Having succeeded in employing 2D NOE for rapidly tumbling molecules, we thought of investigating the technique for steroids with 19-functionalized skeletons with a view to highlight the interactions that control conformational preferences. We had a few questions before us. (i) Is the greater van der Waals radii of the 19-group, and the consequent greater 1,3-diaxial interaction, solely responsible for the conformationally more biased molecule? (ii) Or is the tilting of the 19-carbon toward the A or B ring, as observed for a series of 2α-bromo 4-en-3-one steroids,[23] also responsible for conformational control.

The van der Waals radius of the 10-$CH_2OH$ group in 19-hydroxy derivative **1** is more than that of 10-$CH_3$ group of the usual steroids, and hence flagpole interactions with all axial protons are increased. The ring A can relieve these interactions by a relatively easy conformational flip; however, ring B cannot. The study of the Dreiding model indicates the possibility of a stronger hydrogen bond of the hydroxyl with the carbonyl oxygen in the 1α,2β normal than in the 1β,2α inverted conformation. Hydrogen bonding then puts 19-carbon over the A ring, more so in the 1α,2β normal conformation. For the 10-aldehyde derivative **2**, the van der Waals radius is greater than for the 10-$CH_3$ group, but the aldehydic group is not restrained in motion, as the 19-OH group, and the 19-carbon is not "biased" toward either of the rings. The derivative **2** then necessarily provides conformational preferences due to van der Waals interactions only. On the other hand, the 6β,19-epoxy derivative **3** presents a "clamping" of the 19-carbon over the B ring, thereby eliminating van der Waals interaction and offering a clean model for the question ii. The following sections are divided according to the steroids and the application of a simple protocol—COSY 90 + NOESY + HETCOR—to analysis of ring A conformational preferences of steroids **1**–**3** presented. The protocol is not rigorous in itself, with the possibility of many pitfalls arising from wrong assignments; however, with consider-

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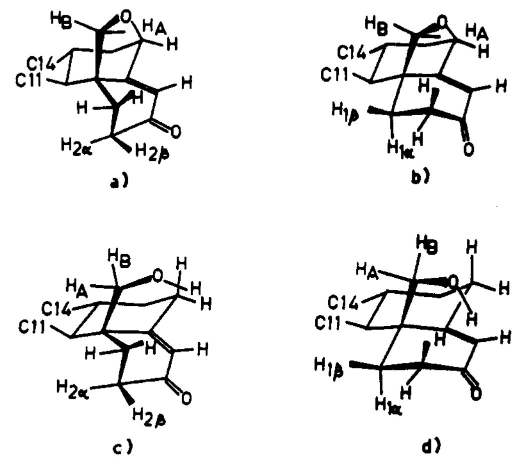

**Figure 2.** Disposition and nomenclature of 19-protons of steroids 1 and 3 in two conformations. The protons are named according to their vicinity to ring A or ring B: a and b represent the inverted $1\beta,2\alpha$ and normal $1\alpha,2\beta$ conformations for steroid 3, respectively, while c and d represent the same for steroid 1, respectively. The NOESY connectivity of these protons should be very crucial.

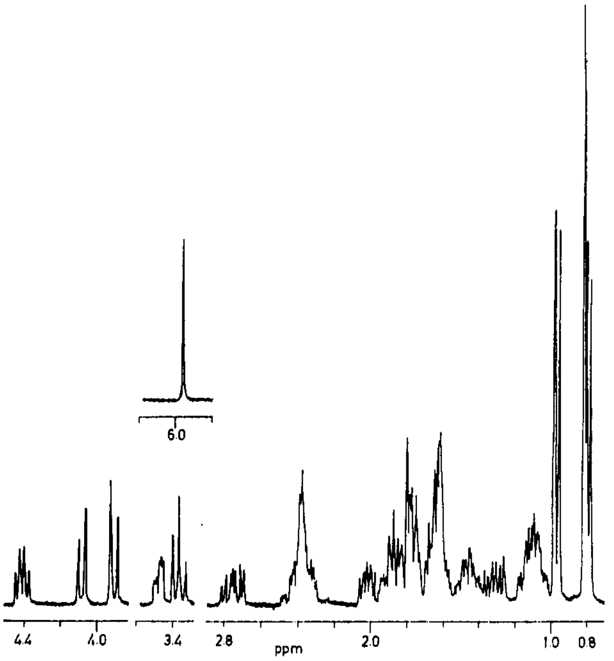

**Figure 3.** $^1H$ NMR spectrum of 19-hydroxy steroid 1 in $CDCl_3$ with a drop of $D_2O$ at ambient temperature. Note the crowding in the region $\delta$ 2.1–2.5.

able data accumulated by many workers, and with care, it can be safely applied with a fair degree of success. In addition, the protocol requires a total spectrometer time of 13–15 h, in contrast to 50 plus h for earlier rigorous strategy, and hence can be performed within 1 day. The results have been corroborated with torsional angle calculations that prove the applicability of the protocol.

**(25*R*)-19-Hydroxyspirost-4-en-3-one (1).** In the hydrogen-bonded conformers, the 19-$H_2$ do not lie over ring A; however, the relative orientation of these protons gets fixed. These then can be named as $H_A$ and $H_B$ depending upon their vicinity with either of the rings (Figure 2). The various calculated distances on the basis of study of Dreiding models for the two extreme ends of conformers are as tabulated in Table I. The distance clearly indicates the possibility of observing cross-peaks of $H_A$ with all protons of A ring in both conformers except perhaps 2β-H in the $1\beta,2\alpha$ conformation in a NOESY spectrum. The $^1H$ NMR spectrum (Figure 3) shows a complex multiplet pattern in the region $\delta$ 2.1–2.5, which is expected of protons at 1, 2, and 6 nuclear positions. The spin system of B ring can be identified by the connectivity of 4-H with 6β-H;[24] however, ring A protons cannot be identified. Thus, 6β-H, 6α-H, 7β-H, and 7α-H occur at $\delta$ 2.46, 2.31, 1.90 and 1.08, respectively. On repeated trials of performing NOESY experiments with different mixing times, it was found that the experiment with a mixing time of 1.0 s was best suited to differentiate the relative distances of protons of A ring. This was about 10% less than the known $T_1$ values of 1.2 s for the 19-$H_3$ in usual steroids.[4] Similar mixing times have been utilized for the other two steroids too and dipolar connectivities found to be a fair measure of distances. The NOESY experiment (Figure 4) shows a cross-peak between protons at $\delta$ 4.08 and 1.80. This proton could be 1β-H; however, COSY does not indicate any A ring proton to resonate at $\delta$ 1.80. In sharp contrast, hence, to the expected dipolar connectivity between $H_A$ and protons of the A ring, the NOESY experiment does not provide the crucial information. This is evidently due to the hydrogen-bonded nature of the hydroxyl group. This also indicates that a 1D NOE experiment would fail to provide the connection with ring A protons. However, the NOESY experiment does show intense cross-peaks between protons at $\delta$ 1.75 and 2.36 and between protons at $\delta$ 2.27 and 2.73, all known to belong to A ring. These are the geminal pairs of protons at 1 and 2 positions and based upon the expected anisotropic effect of the hydrogen-bonded hydroxyl on 2β-H; the proton at $\delta$ 2.73 is assigned 2β-configuration. The observation of cross-peak between 2β-H and a proton at $\delta$ 2.36 and the absence of cross-peak between 2β-H and proton at $\delta$ 1.75 indicates an equatorial 1β configuration for the proton at $\delta$ 2.36. Thus, the relative intensities of cross-peaks in the NOESY spectrum lead to a $1\alpha,2\beta$ normal conformation for the ring A of the 19-hydroxy steroid

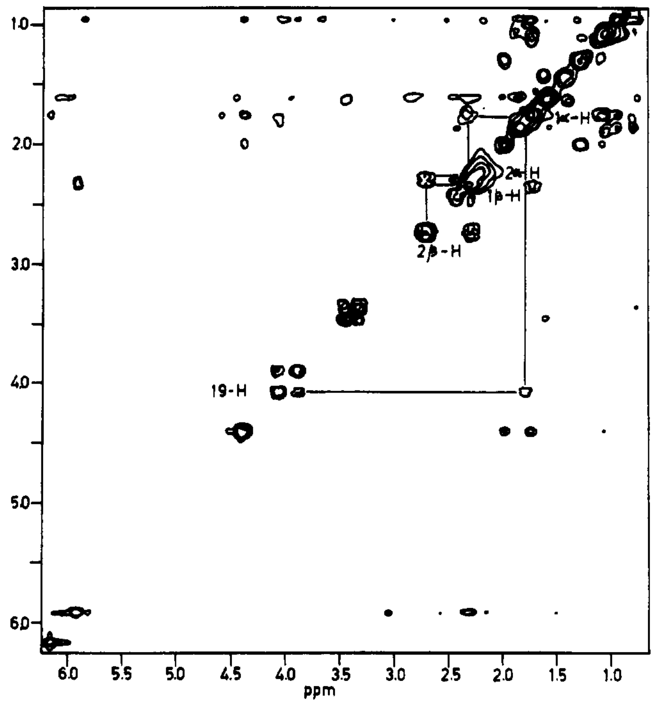

**Figure 4.** Homonuclear NOESY spectrum of 19-hydroxy steroid 1 at mixing time of 1.0 s. Negative NOEs are observed for all protons. The crucial connectivity between $H_A$ and ring A protons is not observed. Note the distinct intensity differences of cross-peaks of geminal and vicinal pair of protons.

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[24] Wittstruck, T. A.; Malhotra, S. K.; Ringold, H. J. *Tetrahedron Lett.* **1963**, 1699.

**Table II. Assignment (Chemical Shifts in ppm) of $^1H$ and $^{13}C$ NMR Spectra of Steroids 1–3**

| | steroid 1 | | | | steroid 2 | | | | steroid 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $^{13}C$ | $\alpha$ | $^1H$ | $\beta$ | $^{13}C$ | $\alpha$ | $^1H$ | $\beta$ | $^{13}C$ | $\alpha$ | $^1H$ | $\beta$ |
| 1 | 33.35 | 1.75 | | 2.36 | 29.38 | 1.75 | | 2.73 | 26.48 | 2.22 | | 1.80 |
| 2 | 34.94 | 2.27 | | 2.73 | 34.14 | 2.37 | | 2.32 | 33.17 | 2.37 | | 2.37 |
| 3 | 200.34 | | | | 197.74 | | | | 198.52 | | | |
| 4 | 126.58 | | 5.96 | | 127.21 | | 5.97 | | 114.83 | | 5.81 | |
| 5 | 167.48 | | | | 161.04 | | | | 171.67 | | | |
| 6 | 33.50 | 2.31 | | 2.46 | 33.82 | 2.50 | | 2.62 | 77.09 | 4.69 | | |
| 7 | 32.30 | 1.08 | | 1.90 | 31.57 | 1.14 | | 1.97 | 41.22 | 1.27 | | 2.08 |
| 8 | 35.84 | | | 1.80 | 36.53 | | | 1.84 | 33.42 | | | 1.58 |
| 9 | 53.93 | 1.01 | | | 53.48 | 1.20 | | | 50.29 | 1.65 | | |
| 10 | 43.91 | | | | 55.14 | | | | 45.89 | | | |
| 11 | 21.32 | 1.60 | | 1.49 | 21.56 | 1.73 | | 1.55 | 23.91 | 1.57 | | 1.49 |
| 12 | 39.94 | 1.20 | | 1.72 | 39.57 | 1.45 | | 1.77 | 39.41 | 1.24 | | 1.80 |
| 13 | 40.42 | | | | 40.17 | | | | 41.40 | | | |
| 14 | 55.90 | 1.05 | | | 55.69 | 1.06 | | | 54.44 | 1.25 | | |
| 15 | 31.59 | 1.30 | | 2.00 | 31.48 | 1.29 | | 2.04 | 31.45 | 1.34 | | 1.92 |
| 16 | 80.55 | 4.41 | | | 80.33 | 4.41 | | | 80.59 | 4.41 | | |
| 17 | 62.00 | 1.74 | | | 61.95 | 1.76 | | | 61.92 | 1.79 | | |
| 18 | 16.40 | | 0.82 | | 16.16 | | 0.80 | | 16.63 | | 0.87 | |
| 19 | 65.73 | 4.09 | | 3.91 | 201.00 | | 9.95 | | 75.59 | 4.24 | | 3.52 |
| 20 | 41.59 | | | 1.86 | 41.57 | | | 1.88 | 41.49 | | | 1.88 |
| 21 | 14.41 | | 0.96 | | 14.39 | | 0.96 | | 14.43 | | 0.97 | |
| 22 | 109.20 | | | | 109.16 | | | | 109.15 | | | |
| 23 | 31.32 | 1.58 | | 1.62 | 31.28 | 1.57 | | 1.65 | 31.29 | 1.58 | | 1.65 |
| 24 | 28.74 | 1.62 | | 1.49 | 28.71 | 1.62 | | 1.50 | 28.71 | 1.60 | | 1.48 |
| 25 | 30.20 | | | 1.60 | 30.17 | | | 1.59 | 30.18 | | | 1.60 |
| 26 | 66.81 | 3.48 | | 3.36 | 66.78 | 3.42 | | 3.36 | 66.78 | 3.48 | | 3.36 |
| 27 | 17.07 | | 0.79 | | 17.06 | | 0.79 | | 17.11 | | 0.79 | |

**Table III. Coupling Constants and Dihedral Angles Calculated on the Basis of DQF-COSY Spectrum for Steroids 1 and 2**

| | steroid 1 | | steroid 2 | |
|---|---|---|---|---|
| coupling constant | value (Hz) | torsion (deg) | value (Hz) | torsion (deg) |
| $^2J_{1\alpha,1\beta}$ | 13.61 | | 14.85 | |
| $^3J_{1\alpha,2\alpha}$ | 6.77 | 32 | 6.24 | 36 |
| $^3J_{1\alpha,2\beta}$ | 15.45 | 172 | *a* | |
| $^3J_{1\beta,2\alpha}$ | 2.42 | 58 | 7.10 | 30 |
| $^3J_{1\beta,2\beta}$ | 7.20 | 30 | 5.33 | 41 |
| $^2J_{2\alpha,2\beta}$ | 17.10 | | *a* | |

[a] Indicates not found.

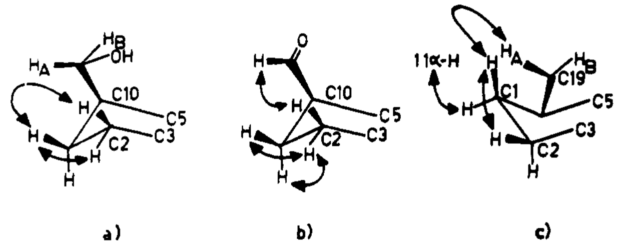

**Figure 5.** NOESY connectivity pattern as observed for steroids 1–3: (a) for steroid 1, 2β-H has to be axial to not show any NOE with 1α-H; (b) for steroid 2, 19-H shows dipolar correlation with 2β-H; (c) for steroid 3, 1α-H shows NOESY cross-peak with 11α-H.

1 (Figure 5). The various assignments were to be confirmed by means of a heteronuclear *J*-correlated spectroscopy experiment (Figure 6), and the total assignment of $^{13}C$ and $^1H$ resonances are as tabulated in Table II.

The above analysis, on the basis of connectivity pattern, was confirmed by means of double quantum filtered-COSY experiment. The DQF-COSY experiment produces enormous "clarity" in the spectrum, due to the reduction in intensity of dominant singlets and the resultant $T_1$ noise. The connectivities obtained from the COSY experiment are easy to visualize here. In order to calculate dihedral angles, coupling constants were necessary and these were obtained from the blowups of cross-peaks. Two cross-peaks were found to be good. The crosspeaks between 2β (ω1) and 1β (ω2) (Figure 7a) and between 1α (ω1) and 2α (ω2) (Figure 7b) yielded the required set of coupling constants (Table III). Utilizing the modified Karplus equation II[25] for calculation of vicinal coupling constants, rather than more complicated versions,[26] provided the torsional

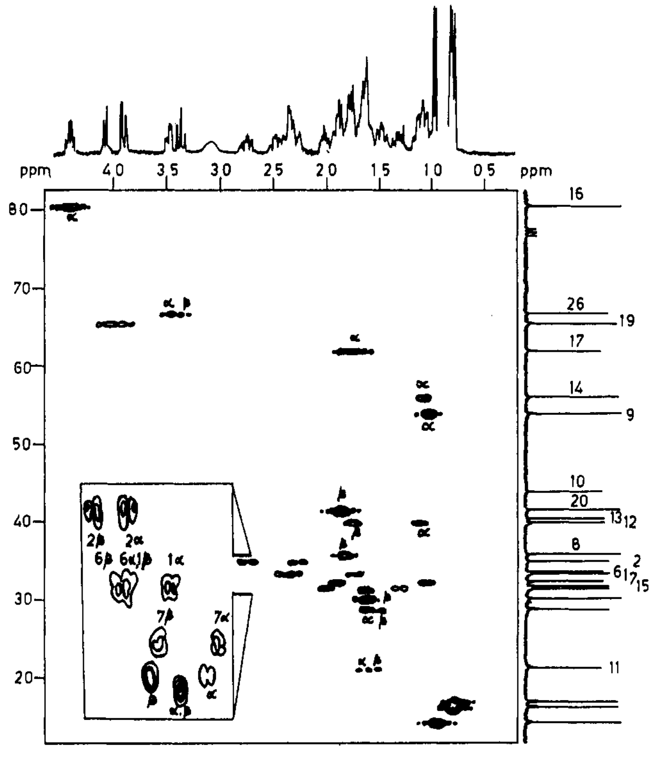

**Figure 6.** Heteronuclear *J*-correlated spectroscopy experiment for steroid 1. It confirms the assignment made and provides the total assignment of $^{13}C$ and $^1H$ resonances.

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(25) Williamson, K. L.; Johnson, W. S. *J. Am. Chem. Soc.* **1961**, *83*, 4623.

(26) (a) Haasnoot, C. A. G.; Deleeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783. (b) Colucci, W. J.; Jungk, S. J.; Gandour, R. D. *Magn. Reson. Chem.* **1985**, *23*, 335.

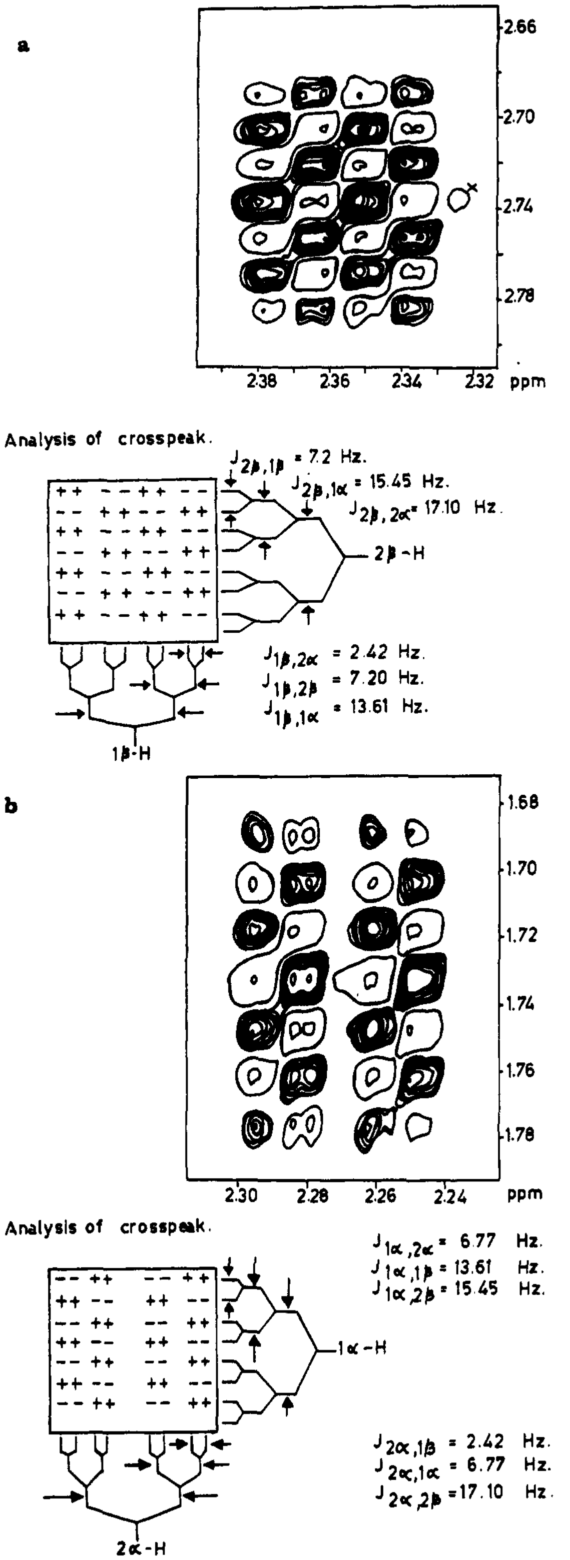

**Figure 7.** Blowups of the cross-peaks and the analysis for elucidation of coupling constants from double quantum filtered-COSY spectrum: (a) the cross-peak between 2β (ω1) and 1β (ω2); (b) the crosspeak between 1α (ω1) and 2α (ω2). Positive components of the peaks are indicated by only two levels— outermost and the innermost. x is an artifact in crosspeak a.

angles as in Table III. The torsional angles indicate a normal 1α,2β half-chair conformation for the steroid 1. Thus, the NOESY results have been corroborated by dihedral angle measurements.

$$^3J = \begin{cases} 10\cos^2\theta - 0.28 & 0 < \theta < 90° \\ 16\cos^2\theta - 0.28 & 90 < \theta < 180° \end{cases}$$

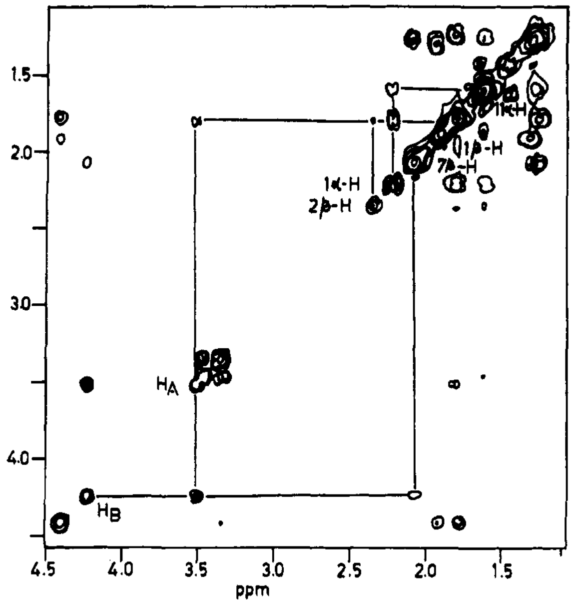

**Figure 8.** NOESY spectrum of 6β,19-epoxy steroid 3. Crucial dipolar connectivity between 1α-H and 11α-H is observed.

**(25*R*)-19-Oxospirost-4-en-3-one (2).** The protons of ring B can be assigned by their $^1H$ NMR spectrum and $^1H-^1H$ COSY spectrum; however, ring A protons cannot be assigned. As observed for steroid 1, the NOESY spectrum is most informative. In addition to the connectivities with other protons, the NOESY spectrum shows a dipolar coupling between 19-H and a proton of A ring system (δ 2.32) at $\tau_m$ = 1.0 s. The $^1H$ NMR and heteronuclear correlation experiment indicate that two protons belonging to the same carbon occur in the region of δ 2.35, i.e., at δ 2.32 and 2.37. The NOESY spectrum shows intense cross-peaks between protons at δ 2.73 and 1.75 indicative of geminal dipolar coupling. In addition, weaker couplings are observed for the pairs δ 2.73 and 2.37 and 2.37 and 1.75. Thus, the crucial assignment is provided by 19-H with 2β-H (δ 2.32), and the NOESY connectivity pattern (Figure 5b) indicates a 1α,2β normal conformation. However, it becomes difficult to differentiate normal and 1α-sofa forms only on the basis of NOESY, and we conjecture an equilibrium conformation biased toward the normal conformation. An unprecedented deshielded nature of 1β-H is observed due to long-range anisotropy of the carbonyl group.

The DQF-COSY experiment and the application of Karplus equation lead to torsions as depicted in Table III. The dihedral angle between 1α-H and 2α-H is unaffected by the electronegativity influence of the carbonyl group and hence a true measure of torsion. The value of 36° indicates a normal 1α,2β half-chair conformation.

**(25*R*)-6β,19-Epoxyspirost-4-en-3-one (3).** Although the epoxy ring formation reduces conformational flexibility, ring A can still exhibit normal and inverted conformations as revealed by model study. However, the probability of 1α-sofa is much reduced due to planarity of the A ring induced by the conformation that is not favored by 6β,19-bridge. The homonuclear COSY spectrum shows connectivities of 4-H with a proton at 19-position and 6α-H. This forms a simple starting point for assigning ring B protons. The NOESY spectrum (Figure 8) shows a dipolar coupling between 7β-H (δ 2.08) and a proton at δ 4.24, which is hence assigned $H_B$. Thus, $H_A$ occurs at δ 3.52 and shows dipolar coupling with only one ring A proton at δ 1.80. Since 2α-H and 2β-H are expected to resonate downfield, the proton at δ 1.80 is 1β-H. Further, 1β-H has an intense cross-peak with a proton at δ 2.20, which is then assigned 1α-H. The integration of $^1H$ NMR spectrum indicates two protons at δ 2.37; thus, 1β-H shows NOESY cross-peak with 2β-H too. Additionally, a NOESY cross-peak is observed between 1α-H and 11α-H, deduced from HETCOR, which proves the vicinity of the two protons. The normal conformation has 1β-H equatorial, which brings the 11α-H within the dipolar coupling distance, and hence should exhibit the NOESY cross-peak, while the inverted conformation requires dipolar coupling between 1α-H and 11α-H. Thus, the NOESY connectivity pattern (Figure 5c) suggests the 1β,2α inverted conformation.

It should be mentioned here that although the DQF-COSY experiment yielded the confirmation of various assignments, the cross-peaks were not analyzable due to intense strong coupling effects, making dihedral angle calculations difficult.

## Conclusions

The study of conformational preferences of these steroids highlights the following:

(i) It is possible to utilize the 2D NOE technique for qualitative distance estimates for steroids of 19-functionalized type, although the technique is known to be fraught with problems for small molecules. Quantitative distance calculations would require rigorous growth curves and are not attempted.

(ii) The protocol—COSY 90 + HETCOR + NOESY at 300 MHz—serves to provide conformational preferences for these steroids rather successfully. More experiments need to be performed to generalize its applicability. In addition, the protocol demands careful analysis due to its nonrigorous approach.

(iii) The conformational preferences of 4-en-3-one steroids are not only governed by 1,3-diaxial interactions but also by the "tilt" of 19-carbon toward either of the rings. A formal tilting of 19-carbon toward ring A induces greater bias for normal 1α,2β conformation, while that toward ring B produces inverted 1β,2α conformation.

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**Registry No.** 1, 23925-63-5; 2, 133930-37-7; 3, 133930-38-8.

**Supplementary Material Available:** $^1H$ NMR spectra of steroids 2 and 3; COSY spectra of steroids 1–3; NOESY spectrum of steroid 2 and HETCOR spectra of steroids 2 and 3 (8 pages). Ordering information is given on any current masthead page.

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# Flow Thermolysis Rearrangements in the Indole Alkaloid Series: 1,2-Dehydroaspidospermidine

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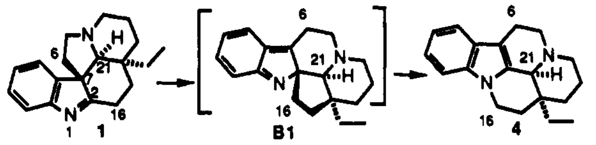
Flow thermolysis of 1,2-dehydroaspidospermidine (1) at various temperatures allowed isolation of all four predictable rearrangement products, namely indolenines 2 and 3 and indoles 4 and 5. The structures of the rearranged products were confirmed by chemical and spectroscopic means, particularly HMBC and HMQC NMR techniques.

Flow and flash thermolysis have scarcely ever been applied to alkaloidal materials,[1,2] although these methods combine several advantages: absence of extra reagent, easy separation of the products, and strictly unimolecular reactions. A limitation lies in the difficulty of rigid temperature control.

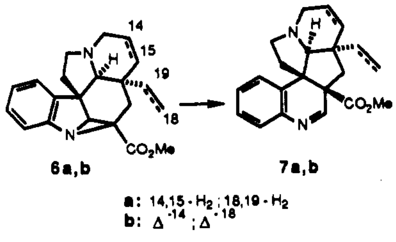
For several years, we have been interested in the flow thermolysis of indole alkaloids, after our attention had been drawn to the synthetic applications developed in Reims by Chuche et al.[3] In a preliminary study,[2] flow thermolysis of 1,2-dehydroaspidospermidine (1) at 580 °C was shown to yield vincane 4, which implied two successive [1,5] sigmatropic shifts via the o-quinonoid intermediate B1 (Scheme I).

Later, flow thermolysis of aziridines 6a,b to dihydroquinolines 7a,b was used as the key step in a synthetic entry into the *Melodinus* alkaloids[4,5] (Scheme II).

Scheme I



Scheme II



We have now thoroughly reexamined the flow thermolysis of 1,2-dehydroaspidospermidine (1) as a model for the thermal rearrangements of indolenines. Thermal rearrangements of indolenine $A_1$ (Scheme III) are expected to

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[3] (a) Manisse, N.; Chuche, J. *Bull. Soc. Chim. Fr.* **1972**, 2422. (b) Coqueret, X.; Bourelle-Wargnier, F.; Chuche, J. *Tetrahedron* **1986**, *42*, 2263.

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