Conformational Analysis via Heteronuclear Correlation NMR Spectroscopy

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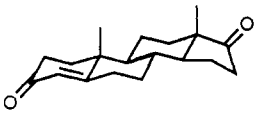
Heteronuclear multiple bond correlation (HMBC) spectroscopy has been used in concert with other modern two-dimensional NMR methods (COSY, NOESY, and ^1H–^{13}C HMQC) to determine the solution conformation of 4-androstene-3,17-dione. HMBC spectra, which provide qualitative H–C–C–C torsion angle information, are used to both confirm conformational assignments made using classical NMR methods (i.e. ^1H–^1H scalar and dipolar couplings) *and* to make additional conformational assignments from crowded regions of ^1H spectra where application of ^1H–^1H homonuclear correlated methods fail. The approach described here should have wide applicability for other organic compounds whose spectra are not amenable to conformational analysis using classical NMR methods.

The development of modern high-resolution NMR spectroscopy has enabled the complete or nearly complete assignment of previously uninterpretable ^1H NMR spectra to be made for a variety of molecules.[1] In addition to the obvious utility of these methods for structural elucidation, they have been important for the analysis of stereochemistry and of solution-state conformation. Although the determination of structural aspects of complex molecules by 1D NMR is frequently frustrated by signal overlap in their ^1H spectra, a variety of techniques has been developed that allow conformational and configurational assignments to be made.[2] Particularly useful in this regard are methods that involve detailed conformational analysis using scalar and dipolar ^1H–^1H homonuclear interactions at high magnetic fields, as well as those utilizing two-dimensional correlation methods.

Ring conformations of some steroids have been determined previously by an analysis of the three bond (vicinal) coupling constants between hydrogens on adjacent carbon atoms, along with NOE measurements to determine which hydrogens are α and which β.[3] These vicinal coupling constants are fit to a Karplus,[4] or modified Karplus,[5] equation to extract dihedral angles. As pointed out by Marat et al.,[3b] however, these coupling constants reflect a weighted average of two or more conformations in solution; thus, quantitative analysis in terms of a single conformation with specified bond angles is dangerous without further information.

We report here the application of the recently developed HMBC (heteronuclear multiple bond correlation) NMR spectroscopic method[6] to the conformational analysis of 4-androstene-3,17-dione (1). The ^1H NMR spectra of steroids exhibit very little chemical shift dispersion even at 500-MHz field strength, precluding complete conformation analysis using classical NMR methods. The HMBC technique was found to be particularly useful for both confirming partial conformational assignments made using ^1H–^1H correlation and for making new conformational assignments. In addition, the sensitivity of the ^1H-detected heteronuclear correlation methods allows small (1-mg) sample quantities to be used, in contrast to other methods that require an order of magnitude more sample.[6] This approach enables a self-consistent conformational assignment to be made for 1 in its entirety.



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NMR Signal Assignments. Since signal overlap in the ^1H NMR spectrum of 1 precludes unambiguous assignment of the chemical shifts from a 500-MHz COSY spectrum alone (supplemental figure S1a–d), ^1H–^{13}C heteronuclear multiple quantum coherence (HMQC) spectroscopy[7] was used to identify geminal proton resonances and to correlate proton signals with directly attached carbons. The HMQC spectrum (Figure 1) exhibits 1-bond coupling between carbons and directly bonded hydrogens; thus it provides an attractive means to overcome overlap problems by enabling the identification of hydrogens that are coupled to the same carbon atom. 4-Androstene-3,17-dione contains two methyl groups (δ 0.93 and 1.25) and one vinyl hydrogen (δ 5.71); signals due to these hydrogens are readily identifiable in the 1D ^1H spectrum (top of Figure 2). The remaining signals can be ascribed to geminal or methine protons based on the 2D HMQC spectrum. For example, proton signals at ca. δ 1.0 and 1.4 correlate with carbons whose resonances are δ 54.8 and 51.7, respectively; these must be methine protons since no other protons correlate with these carbon atoms. Obvious geminal protons include those at δ 1.6 and 2.0, both of which correlate with a carbon resonance at δ 22.1. Once signals were grouped according to proton type, protons on adjacent carbon atoms could be correlated using COSY data, and all protons were assigned.

Conformational Analysis. Analysis of ring conformations was accomplished using both 2D NOESY and

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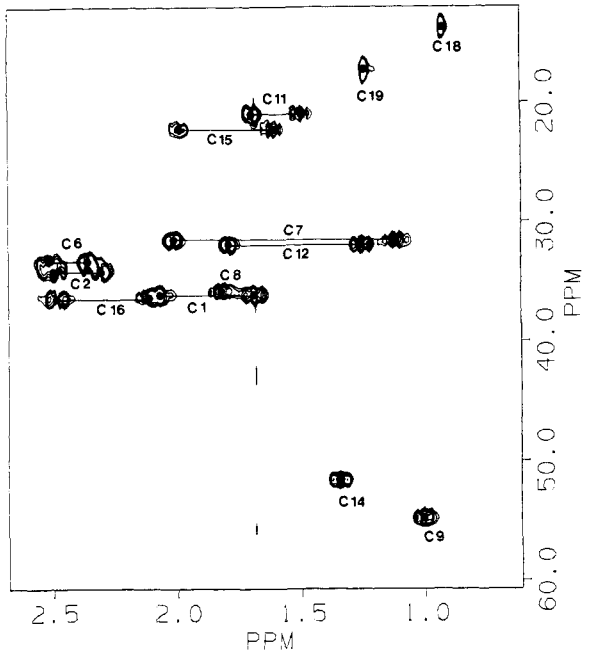

Figure 1. ^1H–^{13}C HMQC spectrum of 1. Horizontal lines connect signals due to geminal protons. NMR data were obtained with a GE GN-500 spectrometer (500.11 MHz, ^1H; 125.76 MHz, ^{13}C) and were transferred to a VAX 8600 computer for processing (FTNMR, Hare Research Inc.). All experimental data were obtained using a 0.4-mL sample containing 1.0 mg of 1 in 80% CD_3OD/20% D_2O.

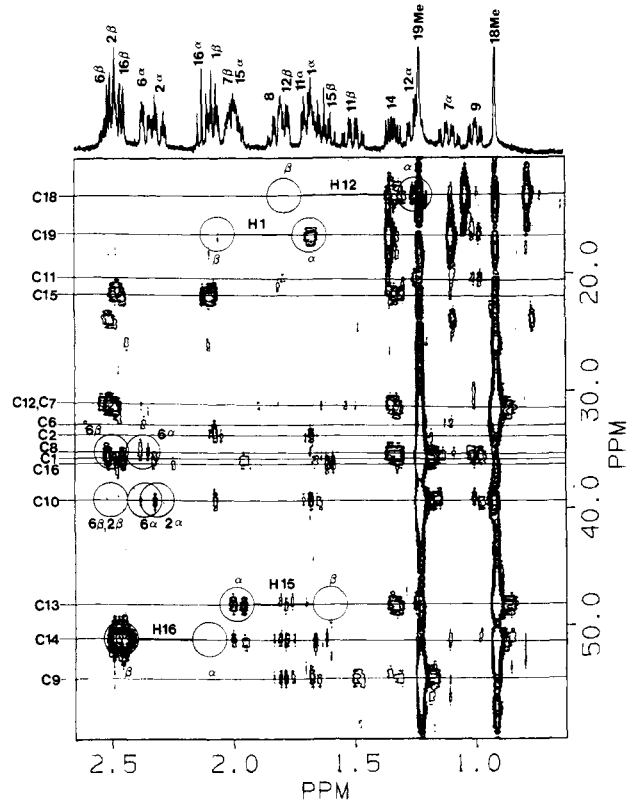

Figure 2. ^1H–^{13}C HMBC spectrum of 1. The sample conditions are those of Figure 1. A high-resolution ^1H spectrum (−0.2-Hz exponential line narrowing) is also shown.

^1H–^{13}C HMBC data. Moderately intense signals in the NOESY spectrum (supplemental figure S2) are found between the C-19 methyl protons and one of the two protons at both C-1 and C-2, enabling the two protons exhibiting cross peaks to be assigned to the β protons. Overlap of the signals of the 2β, 6β, and 16β hydrogens at δ 2.5 makes assignment of the conformation by the traditional method of analysis of coupling constants extremely difficult. However, use of a Karplus-type relationship to analyze the HMBC spectrum (Figure 2) allows the assignment of a half-chair conformation to the A ring. In HMBC spectra, cross peaks may be observed for two- and three-bond carbon–hydrogen couplings. Three-bond couplings (and, in general, correlation peak intensities) correlate with dihedral angles via a Karplus type relationship. The fact that H-1α gives a strong HMBC signal to C-19 and H-1β does not provide good evidence that the 1α hydrogen is axial. In addition, the HMBC cross peak for H-2α, but not H-2β, with C-10 is consistent with an equatorial 2α hydrogen.[8] The doublet of triplets for H-2α ($^3J_{H2\alpha-H2\beta}$ = 17 Hz, $^3J_{H2\alpha-H1}$ = 3 Hz) is expected for a half-chair conformation of ring A confirming this assignment.

Chair conformations for the B and C rings are consistent with the observed HMBC spectrum, with H-6α showing cross peaks with C-10 (weak) and C-8, as expected for an equatorial hydrogen. In addition, H-6α, but not H-6β, shows a cross peak with H-4 in the NOESY spectrum. H-12α gives an HMBC correlation with C-18, whereas H-12β does not, in agreement with a chair conformation of ring C with H-12β equatorial and H-12α axial.

Analysis of the D-ring spectrum suggests an envelope conformation. The HMBC spectrum exhibits a strong cross peak from C-13 to H-15α but not to H-15β, showing that H15α is pseudoequatorial and H-15β pseudoaxial. Similarly, C-14 exhibits strong coupling to H-16β but not H-16α, in agreement with a pseudoaxial H-16α and pseudoequatorial H-16β. The moderate NOESY cross peak of the C-18 methyl protons to H-15β and weak cross peak to H-16β are also consistent with an envelope structure.[9]

It should be noted that, in our approach, conformational assignments using HMBC are based solely on comparisons of intensities of cross peaks for *geminal* protons, where differences in T_2 relaxation, carbon hybridization, and other electronic effects are expected to be small. Comparisons of HMBC intensities of protons on different carbon atoms is inherently more risky due to the possibility of differences in T_2 relaxation rates. Differences in ^1H multiplet patterns must also be accounted for when evaluating relative HMBC signal intensities. Although quantitative coupling constants (and dihedral angles) are not obtained, the simple comparison of relative intensities (i.e. strong vs very weak or lacking) for geminal protons appears sufficient to make assignments of axial versus equatorial protons.

Conclusions

The determination of the conformation of 1 by a combination of NOESY (8 h), COSY (30 min), HMQC (2 h), and HMBC (12 h) spectroscopies was accomplished using approximately 1 mg of material and a total data acquisition time of less than 24 h. The rapid acquisition time, the minimal sample requirement, and the simplicity of analysis suggests that this method can be a powerful tool for the qualitative assignment of conformation. Despite the limitations described above, the HMBC method is clearly useful for steroids and should be applicable to conforma-

(8) Due to strong coupling effects for the geminal protons on C-2 and C-6, the asymmetric multiplets for these hydrogens in the 1D spectrum give asymmetric HMBC signals.

(9) The conformation of 1 is in agreement with that expected on the basis of the crystal structure (Busetta, B.; Comberton, G.; Courseille, C.; Hospital, M. *Cryst. Struct. Commun.* **1972**, *1*, 129).

tional analysis of natural products and compounds that are available in limited quantity and/or exhibit significant ^1H NMR signal overlap.[10] The development of new methods to obtain quantitative ^1H-C-C-^{13}C scalar coupling constants from HMBC spectra should increase the power of this tool.[11]

Experimental Section

4-Androstene-3,17-dione (1) was purchased from Sigma and purified by column chromatography (silica, ethyl acetate/hexane, 1:1), followed by recrystallization from ethyl acetate. The sample (1.25 mg) was dissolved in 0.5 mL of 80% CD_3OD/D_2O (Aldrich, 99.5 and 99.8 atom % D, respectively). All of the 2D NMR experiments were performed on a GE GN-500 spectrometer. The temperature was 25 °C, and all experiments were carried out without sample spinning. Proton and carbon chemical shifts were referenced to solvent CD_3OD.

The parameters used in this work are as follows. HMQC: 2 × 128 × 1024 data matrix size (two separate sets of data, with 1024 data points in t_2 and 128 data points in t_1); 32 scans (preceded by one dummy scan) per t_1 value; recycle delay = 2.5 s, 800 ms "weft" delay period; broad band (16 W) ^{13}C-decoupling during the acquisition period; 6-Hz Gaussian and 90°-shifted sine bell filtering in t_2 and t_1, respectively. HMBC: 128 × 512 data matrix size; 128 scans (preceded by 2 dummy scans) per t_1 value; recycle delay = 2.5 s; 36 μs 90° ^{13}C pulse width; Δ_1 and Δ_2 durations of 3.5 and 55 ms, respectively (see ref 6 for definitions); sine bell filter and 30-Hz Gaussian filtering in t_2 and t_1, respectively. COSY: 1 × 256 × 1024 data matrix size; 200-ms repitition delay; 32 scans per t_1 increment; sine bell filtering in t_2 and t_1, followed by application of a magnitude calculation.

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Registry No. 1, 63-05-8.

Supplementary Material Available: 2D NOESY and COSY spectra showing connectivities used to assign signals and perform conformational analyses (5 pages). Ordering information is given on any current masthead page.

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New Catalytic Activity of Polymer-Supported Quaternary Onium Salts. Regioselective Addition Reaction of Oxiranes with Active Esters Catalyzed by Insoluble Polystyrene-Bound Quaternary Ammonium and Phosphonium Salts

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A new regioselective addition reaction of oxiranes **15–17** with active esters **18** and **19** was carried out to give the adducts **20a–f** using quaternary onium salts on cross-linked polystyrene beads. The catalytic activity of the polystyrene beads was evalulated from rates of the reaction and yields of reaction products. In this reaction, the beads containing pendant benzyltripropyl- or benzyltributylphosphonium chlorides showed the highest catalytic activity among the polymeric catalysts. The beads containing pendant quaternary phosphonium salts also had higher catalytic activity than the beads containing pendant quaternary ammonium salts and the corresponding low molecular weight benzyltrialkylphosphonium salts. Furthermore, it was found from detailed kinetic studies that the rate of addition reaction of oxirane with active ester using polymer-supported quaternary onium salt as a catalyst was proportional to the initial catalyst concentration $[C]_0$ and the oxirane concentration [O], and was not dependent on the active ester concentration as follows: $d[P]/dt = k_2[C]_0[O]$, where [P] is the product concentration, and k_2 is the second-order rate constant. Given these kinetic results, the reaction mechanism of oxirane with active ester catalyzed by the polymer-supported quaternary onium salts is also assumed.

Quaternary ammonium salts bound to the cross-linked polystyrene beads have been widely used for anion exchange resins, which are typical functional polymers, and very important for the purification of water and various ionic chemicals. Insoluble polymer-supported phase-transfer catalysts were first utilized by Regen,[1] Montanari et al.,[2a] and Brown et al.,[3] independently. A variety of these catalysts containing either pendant quaternary ammonium or phosphonium salts[1-10] or pendant crown ethers[2a,11-14] have been extensively synthesized, and have been widely used in the fields of organic synthesis and organic reactions so far. This insoluble catalyst can be easily separated at the end of a reaction by filtration and can be reused for another run. However, catalytic activities of the polymer-supported phase-transfer catalysts are es-

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