L-(-)-**Butane-1,2,4-triol** (L-**5**) was recovered from the supernatant of the precipitation of barium D-1,2,4-butanetriol-1-phosphate on a 25-mmol scale (1.1 L). The solution was concentrated to ~400 mL by evaporation and 500 mL of 95% ethanol was added to the solution. After the solution was concentrated to ~200 mL, another 800 mL of 95% ethanol was added and the solid (mainly sodium pyruvate) was removed by filtration. The solid was washed with ethanol and the combined solution concentrated. The removal of sodium pyruvate by filtration was continued until no further precipitate formed by evaporation and a slightly yellow oil remained. The crude product (0.77 g) corresponded to a yield of ~29%: ^{1}H NMR (D_2O) δ (DSS) 3.5-3.8 (3 H, m), 3.2-3.5 (2 H, m), 1.4-1.6 (2 H, m); ^{13}C NMR (D_2O) δ (DSS) 73.3 (s), 70.0 (s), 62.8 (s), 39.2 (s); $[\alpha]^{22}_D$ -26.6° (*c* 5, H_2O).[26]

Acknowledgment. We thank Dr. Chi-Huey Wong and Dr. Chris Roberts for stimulating discussions.

^{1}H NMR Analyses, Shielding Mechanisms, Coupling Constants, and Conformations in Steroids Bearing Halogen, Hydroxy, Oxo Groups, and Double Bonds

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Abstract: The ^{1}H NMR analyses of 16 5αH-androstanes and one progesterone analogue furnish shifts and coupling constants for the basic steroid skeleton and substituent-induced shifts (SIS) for oxo, hydroxy, and halogen groups as well as for a Δ^5 double bond. It is shown how a single 2D experiment complemented by a NOE difference spectrum can lead to complete assignments even with the most complicated spin systems comprising, e.g., 29 strongly coupled protons within only 1 ppm; the accuracy of information from 2D techniques is evaluated by comparison to some 1D and computer-simulated spectra. On the basis of up to six simultaneously observable couplings, a special approach is used to scan the conformational space of particularly flexible parts. Intermediate conformations between half-chair and twist are obtained with a torsional C14-C15-C16-C17 angle of $\phi \cong 20°$ for the D ring with a sp^2 (17-oxo) carbon and of $\phi \cong 10°$ with only sp^3 carbon atoms; the observed flat profiles, however, allow also for mixtures of different conformations, which is supported by MM2 calculations. For the Δ^4-3-oxo A ring, a sofa conformation is favored compared to a half-chair geometry. The observed shielding effects of heterosubstituents are partially at variance with the few earlier observations, which were mostly based on polysubstituted compounds. Classical shielding mechanisms were evaluated with the program SHIFT, based on force-field-minimized structures. Steric-induced shielding dominates in the hydrocarbon, leading to upfield shifts increasing with the number of 1,3-diaxial interactions. Linear electric-field effects predict, e.g., the shielding difference between equatorial and axial protons vicinal to C-Hal bonds and the deshielding observed for diaxial C-Hal/C-H bond arrangements. A combination of anisotropy and electric-field effects explains all shifts observed in the ketones with the exception of protons vicinal to C=O; a multilinear regression analysis leads to $\Delta\chi_1^{C=O}$ = -36 (-27) and $\Delta\chi_2^{C=O}$ = -24 (-21) (10^{-3} cm^3/molecule, old ApSimon values in parentheses); it is, however, demonstrated, that an analysis on the basis of NMR shifts alone leads to broad ranges of parameters. Parallels between ^{1}H and ^{13}C NMR shifts are drawn, particularly at γ and ϑ positions to C-Hal bonds.

Several aspects make steroids a particularly attractive challenge for the application of modern ^{1}H NMR methods: (i) the technical problems to be surmounted with these probably most complicated spin systems; (ii) the largely undiscovered wealth of information regarding the relation between chemical shifts, coupling constants, and molecular structure; (iii) the change of biological activity with structural variation. The combination of high magnetic fields, computer-aided PFT, and in particular 2D NMR spectroscopy has already been used by several workers for the ^{1}H assignments of steroids, in which the presence of functionalities and double bonds has led to spectral simplification.[1,2] Trying to find the most economical approach with these techniques, we analyzed 17 steroids including the basic skeleton androstane, which comprises 29 nonequivalent and strongly coupled protons over a range of only 1 ppm. The spectacular progress of spectral techniques finds the chemists rather unprepared for the intelligent digestion of the many newly accessible data. There has been very little progress in the quantitative analysis of NMR shielding parameters after the classical studies of Zürcher,[3] ApSimon,[4] and co-workers, who were forced to limit themselves largely to the observation of few time-averaged methyl signals in steroids. A very useful number of ^{1}H-shielding effects in cyclohexanes have been isolated by Anteunis and other workers;[5] the scrutinization in terms of shielding mechanisms is largely lacking, and the unique ability of steroids to provide many experimental data in a geometrically

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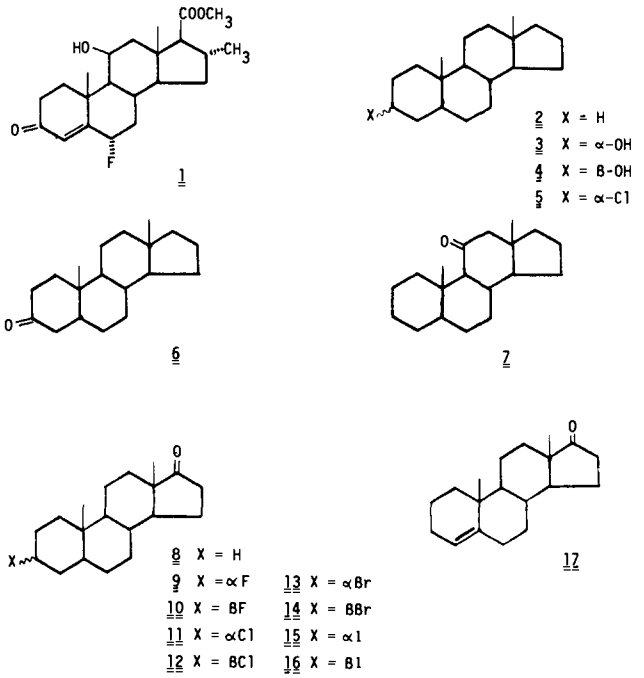
Scheme I



well-defined environment has not yet been exploited. In the present study, an effort is also made to analyze the ^{1}H NMR shifts on the basis of sound physical principles[3,4,6] and to apply ^{1}H–^{1}H coupling constants for signal assignments, for the calculation of vicinal couplings as proposed by Altona and co-workers[7] and for conformational analysis, e.g., of the flexible D ring.[8] We also addressed ourselves to errors involved with the extraction of coupling constants and shifts from 2D spectra and from the alternatively first-order-analyzed or computer-simulated conventional 1D spectra (Scheme I).

Strategies for Measurements and Assignments. Principles and applications of modern pulse[9] and 2D NMR[10] techniques have been reviewed recently, and were illustrated also with some steroids.[1,2] The progesterone analogue **1** is an example for functionalized terpenes which can be tackled by conventional decoupling alone, although 2D methods simplify the procedure. Signals H-4, H-6, H-11, H-20, and H-21 in **1** are assigned directly by comparison of multiplicities and literature data; irradiation at the corresponding positions partially leads only to small effects due, e.g., to allylic (1.9 Hz) or ^4J coupling (such as ^4J–"W" coupling to angular methyl groups)[11] but helps to establish the connectivity in the framework unambigously (Table A in the supplementary material). Axial protons in the cyclohexanoid parts are recognized by the large effects upon saturation of corre-

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(9) See, e.g.: Turner, C. J. *Progr. NMR Spectrosc.* **1984**, *16*, 311.

(10) See, e.g.: Bax, A. "Two-Dimensional Nuclear Magnetic Resonance in Liquids"; Delft University Press: Dordrecht, Holland, 1984. (b) Benn, R.; Günther, H. *Angew. Chem.* **1983**, *95*, 381; *Angew Chem., Int. Ed. Engl.* **1983**, *22*, 390.

(11) See, e.g.: Bhacca, N. S.; Williams, D. H. "Applications of NMR Spectroscopy in Organic Chemistry"; Holden-Day: San Francisco, London, Amsterdam 1964; p 108*ff*.

Table I. ^{1}H NMR Shifts in 6α-Fluoro-11β-hydroxy-16α-methyl-17β-(carboxymethyl)androst-4-en-3-one (**1**)[a]

proton	I[b]	II[c]	III[d]
1α	1.95	1.94	1.94
1β	2.20	2.20	2.20
2α	2.40[e]	2.36[e]	2.38
2β	2.47	2.47	2.49
4	6.02	*f*	6.02
6β	5.20	*f*	5.20[g]
7α	1.33[e]	1.26	1.26[g]
7β	2.39	2.39	2.39[g]
8	2.04	2.03	2.04[g]
9	1.06	1.05	1.06
11α	4.39	*f*	4.39
12α	1.56	1.55	1.55
12β	2.13	2.13	2.13
14	1.28[e]	1.30	1.30
15α	1.35	1.37	1.36
15β	1.74	1.74	1.74
16β	2.65	2.65	2.65
17α	1.89	1.88	1.89
18	0.98	0.98	0.98
19	1.43	1.42	1.42
20	1.04	1.03	1.04
21	3.70		

[a] In ppm, ±0.01, from internal Me_4Si; measured with (3 ± 1)% $CDCl_3$ solution at 25 °C. [b] Values from first-order analysis. [c] From ^{13}C–^{1}H shift-correlated 2D spectra. [d] By iterative spin system analysis. [e] Lines overlapping. [f] See 1D spectra values for I. [g] Signals not accessible to iteration; these values agree however with the simulated spectrum (Figure 1).

Table II. ^{1}H–^{1}H Coupling Constants (J, Hz) and Torsional Angles (ϕ, deg) in **1**

protons	J_{expl}[a]	J_{exptl}[b]	J_{calcd}[c]	ϕ_{MM2}[d]	ϕ_{calcd}[j]
1α,1β	13.4	13.1			
1α,2α	4.6	4.4	4.6	−51.9	53
1α,2β	13.3	13.4	13.3	−169.8	172
1β,2α	4.9	4.7	2.5	63.2	51.5
1β,2β	5.0	4.8	4.0	−54.7	51
2α,2β	16.7	16.8			
2α,4	0.9	0.9			
4,6β	1.9	1.9			
4,F	0.9	0.9			
6β,F	48.0	47.8[f]			
6β,7	12.4[i]	12.7[f]	10.0	176.1	
6β,7β	6.2[i]	5.9[f]	3.0	59.1	(36.5)[k]
7α,7β	13.7[h]	14.1[f]			
7α,8	11–13[e,i]	12.7[f]	13.1	−177.1	(171)[k]
7β,8	4–5[e,i]	4.4[f]	3.3	−58.5	(52.5)[k]
7α,F		1.6[f]			
7β,F		2.5[f]			
8,9	10.9[i]	10.8[f]	12.4	−177.3	(157)[k]
8,14	11[e,i]	10.8[f]	12.1	178.9	(160)[k]
9,11α	3.1	2.9	3.4	55.9	60
11α,12α	3.1	3.1	4.0	−52.4	59
11α,12β	3.0	3.0	2.6	62.9	59.5
12α,12β	14.6	14.5			
14,15α	7.4[l]	7.4[k]	5.6	−45.8	36.5
14,15β	11.7[l]	11.9[k]	12.1	−165.1	163.5
15α,15β	11.8	11.9			
15α,16β	3.0	2.9	1.9	−108.3	115
15β,16β	11.2	11.1	10.6	12.2	0
16β,17α	8.8	8.7	8.9	146.0	145
20CH_3,16β	6.9	6.8			

[a] From first-order analysis, ±0.2 Hz. [b] From iterative analysis, ±0.2 Hz, starting with the first-order values (a). [c] Calculated with the Altona equation from ϕ values. [d] From MM2 calculations. [e] Overlapping lines. [f] See footnote g to Table I; error ±0.2 Hz for $J < 4$ Hz, ±4 Hz for $J > 7$ Hz. [g] Estimated value. [h] From 2D ^{13}C–^{1}H shift-correlated spectra, ±1.5 Hz. [i] Estimated from the signals of only one proton. [j] Angle calculated from J_{exptl}[b] using eq 1. [k] Inaccurate because of experimental errors, see footnote f. [l] For deviation, see text.

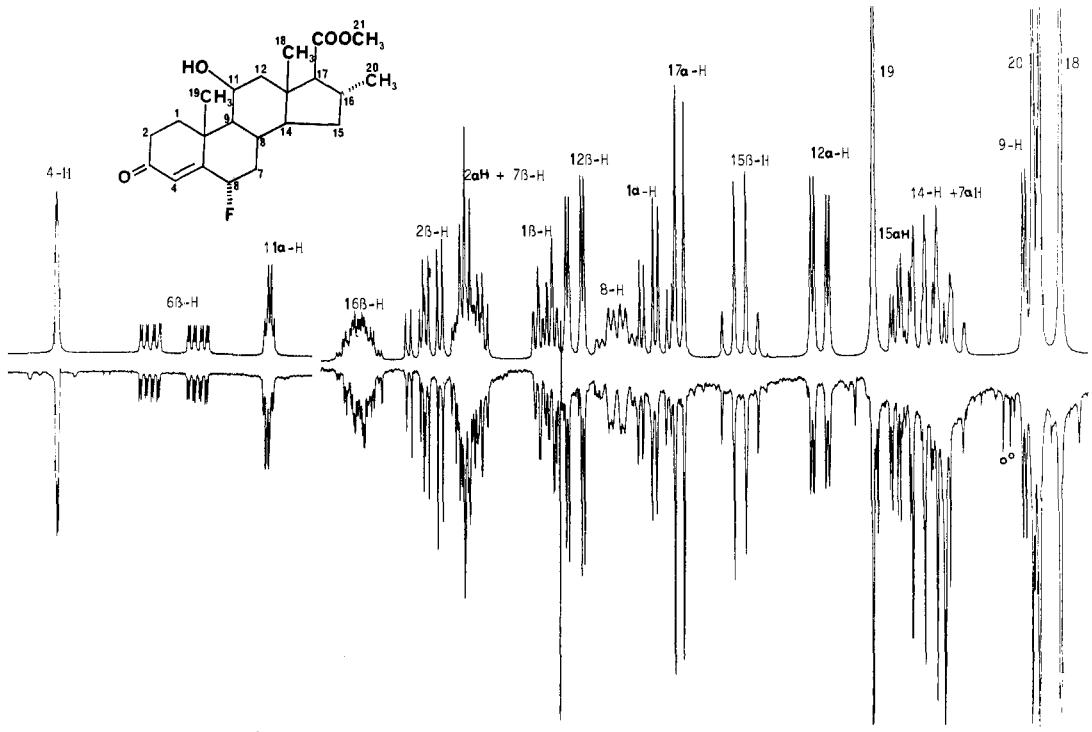

Figure 1. Experimental and theoretical ^1H NMR spectrum of **1** at 400 MHz; o denotes impurities.

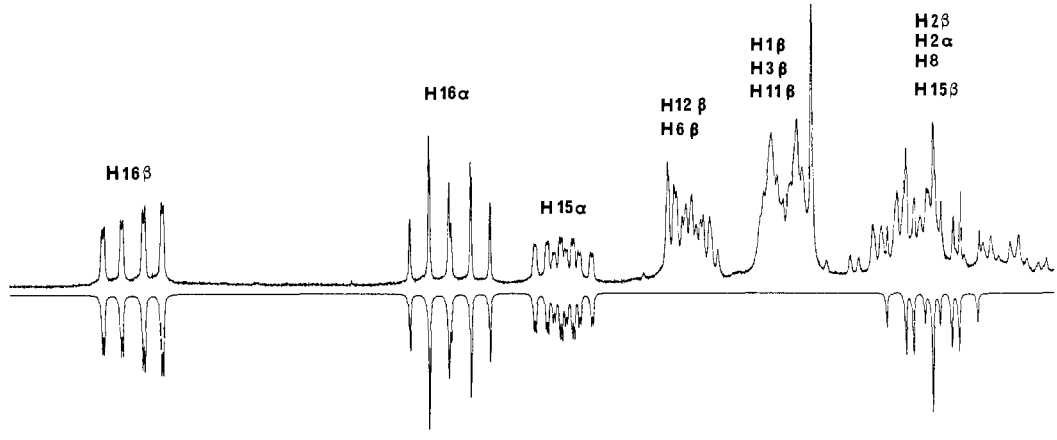

Figure 2. Experimental and theoretical ^1H NMR spectrum of the D-ring protons of **8** at 400 MHz.

sponding trans-vicinal nuclei transitions, geminal protons by similarly large effects. The epimeric protons at C-15 can be differentiated on the basis of the larger coupling between 15 β-H and 16 β-H (torsional angle according to MM2[12] calculation $\cong$12°) compared to 15 α-H/16 β-H ($\cong$108°); similar experiments with H-14 irradiation confirm this assignment. Only H-12α/β are undistinguishable by decoupling but are unambigously assigned on the basis of the regular e/a shift difference or of NOE experiments.

An iterative spin system analysis was possible with all protons in **1**, except H6–H9, which were not sufficiently separated in the 1D spectrum. Nevertheless, simulation of these parts with the values given in Tables I and II together with the iteration-obtained values for all other signals furnished a well-matching theoretical spectrum (Figure 1) and shift as well as coupling parameters, which showed little differences (<0.01 ppm and <0.3 Hz) to a first-order analysis (see Tables I and II). Similar agreement was found with the D-ring protons in 17-oxoandrostane (**8**), which also were accessible to iterative analysis (Figure 2, Table III).

2D Methods. Steroids posessing no or only few functionalities in remote positions require the application of 2D methods.[10] By far the most useful technique is *^{13}C–^1H shift correlated spectroscopy*,[10,13a,b] for which also the measuring parameter adjustment is less critical than with other 2D methods and the time requirements are moderate, provided the amount of material is not limiting. Accumulation of 16 transients per FID (due to phase cycling, the required minimum is 8 scans) with 25 mg of steroid, e.g., during 5 h on a 400-MHz instrument furnishes an average

Table III. ^1H–^1H Coupling Constants in the D Ring of 5αH-Androstan-17-one (**8**) and the Parent Δ^5 Compound (**17**)

	8		**17**	
H–H	J_{expt}[a]	J_{expt}[b]	J_{expt}[b]	$\Delta\phi$[c]
14,15α	5.5	5.8	6.3	+0.5
14,15β	12.0	12.5	12.6	+0.4
15α,15β	11.8	12.3	12.2	0.0
15α,16α	8.5	9.0	8.8	0.0
15α,16β	1.0	0.8	1.3	+0.2
15β,16α	8.5	9.1	9.3	+0.1
15β,16β	8.5	9.1	9.1	
16α,16β	18.3	19.3	19.3	

[a,b] See footnotes a and b to Table II. [c] Torsional angle change $\Delta\phi$ upon double bond introduction (MM2 calculated).

(12) Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127.

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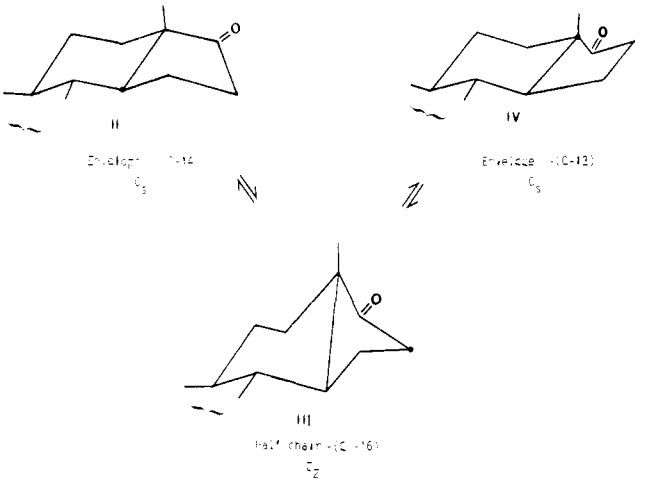
signal/noise ratio of ~10 in the 2D cross sections, if 4K data points are used for ^{13}C (digital resolution, e.g., 2.4 Hz at 50 ppm ≅ 5000-Hz sweep width) and 256 FID's in the ^1H dimension (digital resolution, e.g., 1.5 Hz at 2 ppm ≅ 800-Hz sweep width, with zero filling); the additional transform time for a 512 × 4K matrix, e.g., on an ASPECT-3000 is only ~15 min. Phase-sensitive representation of the cross sections (Figures 3 and I*) was preferred to obtain less distorted lines, although power or magnitude calculations require smaller S/N ratios and less careful optimization. The recently proposed spectral simplification and signal enhancement by decoupling in the proton dimension[14] is not practical for nonequivalent methylene protons, which cannot be decoupled simultaneously.

The first step in ^{13}C–^1H-shift correlation analysis is to determine the connectivity of protons to the carbon atoms on the basis of a known ^{13}C assignment,[15] using mostly the one-bond ^{13}C–^1H coupling by choosing a suitable delay in the pulse sequence ($\Delta = 1/^1J_{CH}$).[10] The method allows accurate ^1H shift determinations (±0.01 ppm), depending on the time spent on FID accumulation (see above), as long as the ^{13}C and particularly the ^1H shift difference is large enough. Even with the nonfunctionalized steroid **2**, we encountered only one signal pair (at C4 and C6) where the protons were overlapping in the cross section but could still be localized within ±0.1 ppm. The stereochemical assignment is greatly facilitated by inspection of the different cross sections; in cyclohexane, chairlike parts due to vicinal trans-coupling axial nuclei are invariably characterized by a large splitting (~12 Hz) in addition to the common splitting by the geminal coupling (~12 Hz) (see Figure I* in the supplementary material). Assignments in non-chair forms, such as in the D ring, require additional arguments (see below). The multiplets in the cross sections appear less distorted than with many other 2D techniques and often allow the extraction of moderately accurate coupling constants, eventually with the aid of simulated spectra (Figures 3b and I*). A comparison with some multiplets in the 1D spectra of **1** showed a deviation in the corresponding cross-sectional signals of <1 Hz, which is below the resolution in the f_1 range.

^1H–^1H shift correlated 2D spectra such as COSY-45[10,16] can be taken from as little as, e.g., 1 mg of steroid with a high-field instrument, which, due to the mostly used 16 scan-phase cycle and the necessary resolution in two dimensions, still requires then 5–10-h accumulation time. If there are many strongly coupled protons in a narrow shift range, the COSY contour plots (Figure 4) are interpretable only *after* assignments have been made with the aid of ^{13}C–^1H shift-correlated 2D spectra. That seemingly spurious cross peaks in COSY spectra were not the result of f_1 noise[10] was confirmed in the case of 5αH-androstan-17-one (**8**), where deuteration at C-16 removed all the corresponding cross peaks (Figures IIa*,b in the supplementary material). The differentiation between smaller and larger couplings is easier in the stacked plot representation (Figure 5); the relative intensities can be judged in these, although they not only depend on J and the pulse sequence used but also on T_2 relaxation times.[10] Under conditions emphasizing smaller couplings[10] (Figures 4 and 5), however, e.g., the long-range coupling of the angular methyl groups is easily recognized; the clearly visible coupling of 18-CH_3 to H17α and its absence at H17β was important for the D-ring assignment, since only the 17α proton is geometrically suited for the known W-coupling[11] path.

***J*-resolved spectra**[10,17] require little compound quantity and measuring time but are of little use for steroids with small shift differences. Even at high magnetic fields, the spin systems are too far away from first order ($\Delta\nu \sim J$) and only few couplings, such as ^4J, to angular methyl groups in **2** become sufficiently visible (Figure III*a+b in the supplementary material).

Scheme II



NOE Difference Spectroscopy. The pronounced dependence of the dipole–dipole relaxation on the through-space distance between protons makes nuclear Overhauser effects an invaluable aid for stereochemical assignments,[18] although at higher fields chemical shift anisotropy contributions to the relaxation process may diminish the observed effects. The high dispersion or spectral resolution needed for steroids and similar compounds necessarily results in much sharper lines than obtained in moderate magnetic fields; in consequence, a satisfactory nulling of the NOE difference spectra is sometimes difficult and requires a digital resolution of, e.g., 0.01 Hz.[18b,c] The accumulation time is further increased—to 10–50 h for a single irradiation spectrum, see Figure 6—by the need of high signal noise ratios: the NOE is larger than a few percent only for methine protons, which cannot relax with geminal protons, but small just for methylene protons where this additional assignment method really is needed.[1] Nevertheless, irradiation on the angular methyl protons in androstane (**2**) not only confirmed our earlier assignments of the β protons 1, 3, 6, 8, and 11 in the rings A–C but also of the D-ring β protons (Figure 6), whereas H17α could also be located by COSY-45 (see above), and H15α/β by comparison of the coupling to H14 (Table H* in the supplementary material). The only signal pair not resolved in the fully saturated D ring was H16α/β, which, however, differ by only ~0.15 ppm (Tables IV and V).

The above assignments were based on a selection of 1D and 2D techniques but not on chemical shift arguments. Although we investigated a range of similar compounds with planned variations at selected substituent sites, we refrained in view of this so far largely unknown territory from the use of substituent-induced shifts (SIS) for the proof of assignments. After having established the shifts in the basic steroid skeleton and the shielding variation by substituents (with **3**–**6**), or a double bond (with **17**), it is expected that SIS arguments will be helpful in the future of high-field ^1H NMR analysis of steroids and related compounds (see Table VII).

Coupling Constants and Conformational Analysis. (A) D Ring. The availability of accurate coupling constants from iterative spin system simulations for the D-ring protons in **1** and **8** (Tables II and III) allows for the first time a full analysis using *all* occuring signals of the cyclopentane moiety. The geometry of the D ring as the most flexible part of the skeleton is assumed to influence the biological activity;[19] the relevant envelope and half-chair forms

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(18) (a) Noggle, J. H.; Schirmer, R. F. "The Nuclear Overhauser Effect"; Academic Press: New York, 1971. (b) Sanders, J. K. M.; Mersh, J. D. *Progr. NMR Spectrosc.* **1982**, *13*, 353 and references cited therein. (c) For solutions of the problems due to high larmor frequencies and large correlation times, see: Bothner-By, A. A.; Stephens, R. L.; Lee, J.-M.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811.

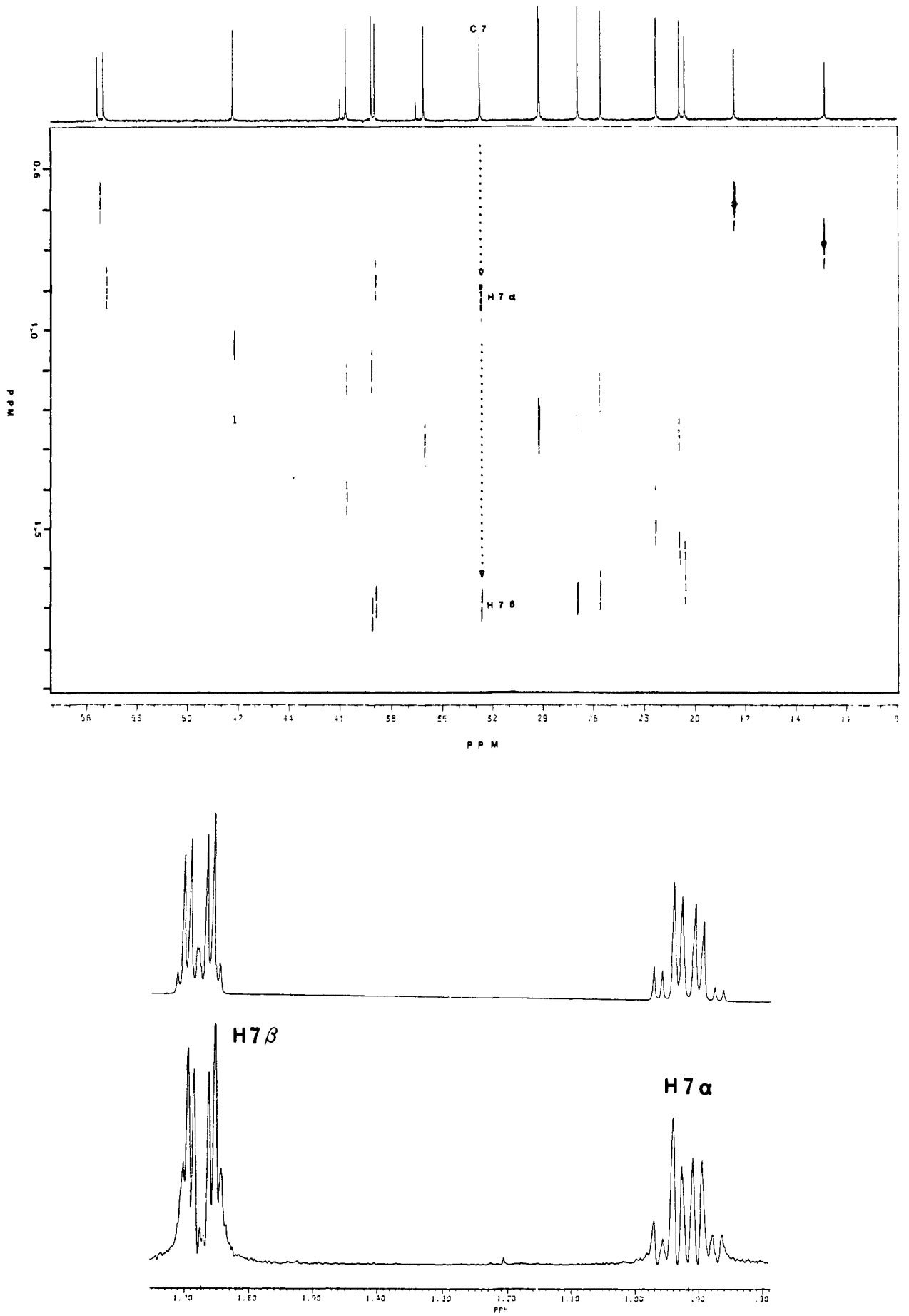


Figure 3. ^{13}C–^1H shift-correlated 2D spectrum of 5αH-androstane (**2**); (a, top) contour plot; (b, bottom) example for a cross section, with simulated spectrum for Hα, Hβ at C-7.

Table IV. ^{1}H NMR Shifts in 5α-Androstanes (2–8)[a]

	2, X = H	6, 3-oxo[b]	7, 11-oxo[b]	8, 17-oxo[b]	3, 3α-OH[b]	4, 3β-OH[b]	5, 3α-Cl[b]
1α	0.89	0.45	−0.13	0.00	0.44	0.07	0.23[c]
1β	1.66	0.35	0.74	0.01	−0.18	0.07	0.23[c]
2α	1.50	0.77	0.00	0.00	0.13	0.28	0.36
2β	1.41	0.96	0.00	0.00	0.27	−0.01	0.45
3α	1.23		−0.04	−0.01		2.34	
3β	1.67		−0.02	−0.01	2.38		
4α	1.22[c]	0.84[c]	0.01[d]	0.07[d]	0.16[c]	0.31[c]	0.37[c]
4β	1.22[c] ± 0.04	1.02[c]	0.01[d]	0.07[d]	0.30[c]	0.04[c]	0.51[c]
5	1.06	0.45	−0.07	0.01	0.50	0.04	0.64
6α	1.22[c]	0.11[d]	0.01[c]	0.03	−0.02[d]	0.04[d]	−0.02[d]
6β	1.22[c] ± 0.04	0.11[d]	0.01[c]	0.03	−0.02[d]	0.04[d]	−0.02[d]
7α	0.93	0.03	0.19	0.04	0.05	−0.02	0.06
7β	1.69	0.04	0.10	0.09	0.01	−0.02	0.01
8	1.29	0.05	0.36	0.26	0.01	−0.02	0.00
9	0.69	0.07	1.00	0.03	0.10	−0.03	0.06
11α	1.55	0.02[e]		0.12	0.02	−0.05	0.00
11β	1.26	0.13[e]		0.01	0.02	0.02	−0.01
12α	1.10	0.02	1.15	0.13	0.01	−0.01	0.02
12β	1.71	0.02	0.54	0.08	0.01	0.00	0.00
14	0.90	0.02	0.64	0.37	0.02	−0.01	0.02
15α	1.65	0.00	0.12	0.27	0.02	−0.03	0.00
15β	1.15	0.01	0.08	0.35	0.00	−0.01	0.00
16α	1.56[c]	0.03[d]	0.16[d]	0.49[c]	0.05[d]	0.00[d]	0.04[d]
16β	1.56[c] ± 0.16	0.03[d]	0.16[d]	0.89[c]	0.05[d]	0.00[d]	0.04[d]
17α	1.13	0.00	0.22		0.01	0.00	0.01
17β	1.42	−0.02	0.03		0.01	−0.01	0.01
18	0.69	0.03	−0.03	0.17	0.00	0.00	0.00
19	0.79	0.23	0.22	0.02	−0.01	0.02	0.00

[a] In ppm, ±0.01, unless noted otherwise; 0.3–1.0 M solution in $CDCl_3$, 25 °C. [b] Substituent induced shifts (SIS); ±0.02 ppm. [c] Signals overlapping either in the measured or the reference compound. SIS: value from averaged signals; error ±0.05 ppm (except H16: ±0.12 ppm). [d] Signals overlapping both in measured *and* reference compound. SIS: value from averaged signals. [e] α/β assignment exchangeable. Alternative SIS values: 11α, −0.28 ppm; 11β, 0.30 ppm.

are illustrated with the 17-oxo compound in Scheme II. Force-field calculations as well as some X-ray crystal studies indicate the occurrence of conformations intermediate between half-chair III and envelope II (Table I*).[20]

For the analyses of the vicinal coupling constants, we made use of the Karplus analogues equation (1), which was derived by Altona and his co-workers[7] and found to be particularly useful for cyclopentanoid structures.[21]

$$^3\mathcal{J} = P_1 \cos^2\phi + P_2 \cos\phi + P_3 + \sum \Delta\chi_i[P_4 + P_5 \cos^2\phi(\xi\phi + P_6|\Delta\chi_i|)] \quad (1)$$

Here P_1 to P_6 are parameters obtained from iterative analyses of more than 300 coupling constants; $\Delta\chi_i$ represents group electronegativities and ξ the substituent orientation.

Application of eq 1 on the basis of MM2 calculated half-chair III or envelope II conformations (Scheme II) showed vicinal coupling constants, which agreed with the experimental data almost within the standard deviation of ±0.5 Hz stated by Altona et al.[7] (Figure 7). Cautioned by our earlier observations on the very small energy differences between different cyclopentane geometries[20f] and suspecting that equally well matching ^3J values might be obtained for a number of other conformations, we decided to use another approach and plotted calculated coupling constants vs. the torsional angle ϕ (C14–C15–C16–C17), which serves to quantify the different geometries. Figure 8 shows that indeed all

Chart I

conformations between half-chair III and envelope II (Scheme II) are possible by the experimental coupling constants; the errors allowed for are those from Table III and are smaller than the variation of ±0.5 Hz[7] observed over systems with different substitution patterns. Thus, even though six different coupling constants can be used simultaneously, the range of permissible conformations is quite broad, as is the corresponding strain energy profile (Figure 8). These results underline the precautions necessary in the interpretation of X-ray analyses and of structure activity relations with flexible steroids.[19b,22]

With Δ^5-dehydroandrostan-17-one (**17**) we tried to analyze the possible conformational transmission of the double bond introduction. MM2 calculations predict torsional angle changes in the D ring—compared to the saturated skeleton (**2**)—between 0.0° and 0.5° (Table III); the H14–H15 coupling shows an increase in the range predicted by application of eq 1 (1.4 ± 0.6 Hz compared to the experimental ΔJ = 0.5 Hz). The double bond effects on the C ring amount to $\Delta\phi$ ±5° (MM2); they are too small to be analyzed on the basis of the larger coupling error involved with the 2D analysis (±1.5 Hz, see above) of the C-ring protons. The rapid decrease of conformational distortion[20b,22] is also reflected in the small shielding variations both with ^{1}H (Table V) as with ^{13}C (see Table B* in the supplementary material).

The progesterone analogue **1** allowed a full analysis of a D ring without trigonal carbon, since again *all* corresponding protons were accessible to spin system simulation with the 1D spectrum (Tables I and II). Exploration of the conformational space with the same approach as used for the ketone indicates a twist conformation with ϕ (C14–C15–C16–C17) = 6.5 ± 1°, which agreed with the

(19) (a) See, e.g.; Duax, W. L.; Griffin, J. F.; Rohrer, D. C.; Weeks, C. M.; Ebright, R. In "Biochemical Actions of Hormones"; Academic Press: New York, 1984; Vol. XI, p 187. (b) Schneider, H.-J.; Buchheit, U.; Gschwendtner, W.; Lonsdorfer, M. In "Molecular Structure and Biological Activity"; Griffin, J. F.; Duax, W. L., Eds.; Elsevier: New York, 1982; p 165*ff* and references cited therein.

(20) See, e.g.: (a) Fuchs, B. *Top. Stereochem.* **1978**, *10*, 1. (b) Romers, C.; Altona, C.; Jacobs, H. J. C.; De Graaf, R. A. G. *Chem. Soc. London, Spec. Publ.* **1974**, *4*, 531. (c) Duax, W. L.; Weeks, C. M.; Rohrer, D. C. *Top. Stereochem.* **1976**, *9*, 271. (d) Norton, D. A.; Duax, W. L. "Atlas of Steroid Structure"; Plenum Press: New York, 1975; *Vol. 1*. (e) Altona, C.; Geise, H.-J.; Romers, C. *Tetrahedron* **1968**, *24*, 13. (f) Schneider, H.-J.; Nguyen-Ba, Nge; Thomas, F. *Tetrahedron* **1982**, *38*, 2327.

(21) For a recent applications see: Leeuw, F. A. A. M. de; Altona, C.; Kessler, H.; Bermel, W.; Friederich, A.; Krack, G.; Hull, W. W. *J. Am. Chem. Soc.* **1983**, *105*, 2237.

(22) Cf.: Schneider, H.-J.; Gschwendtner, W.; Weigand, E. F. *J. Am. Chem. Soc.* **1979**, *101*, 7195.

Table V. ^1H NMR Shifts in 3X-Substituted 5αH-Androstan-17-ones (**8–16**) and in **17**[a]

	8, X = H[a]	**9**, 3α-F[b]	**10**, 3βF[b]	**11**, 3α-Cl[b]	**12**, 3β-Cl[b]
1α	0.89	0.43	0.09	0.60[c]	0.13
1β	1.67	−0.14	0.10	−0.18[c]	0.09
2α	1.46[c]	0.31	0.47	0.42[d]	0.57[c]
2β	1.46[c] ± 0.04	0.10	0.11	0.42[d]	0.31[c]
3α	1.22		3.24		2.63
3β	1.68	3.13		2.83	
4α	1.29[c]	0.34	0.45	0.38	0.51
4β	1.29[c] ± 0.06	0.12	0.22	0.46	0.36
5	1.07	0.50	0.04	0.67	0.10
6α	1.25[c] ± 0.04	−0.01*	0.10[d]	0.03[d]	0.06[d]
6β	1.25[c]	−0.09*	0.10[d]	0.03[d]	0.06[d]
7α	0.97	0.05	0.01	0.08	−0.01
7β	1.78	0.03	0.03	0.02	0.01
8	1.55	0.01	−0.02	0.01	−0.01
9	0.72	0.10	−0.04	0.15	−0.03
11α	1.67	0.01	−0.02	0.01	−0.05
11β	1.27	0.02	0.05	0.01	0.03
12α	1.23	0.01	0.00	0.01	−0.01
12β	1.79	0.01	−0.01	0.01	0.00
14	1.27	0.03	0.00	0.02	−0.03
15α	1.92	0.02	0.00	0.02	−0.01
15β	1.50	0.01	−0.02	0.01	−0.01
16α	2.05	0.02	0.01	0.02	0.00
16β	2.45	−0.03	−0.03	−0.02	−0.02
18	0.86	0.00	0.00	0.00	−0.01
19	0.81	0.01	0.05	0.00	0.05

	13, 3α-Br	**14**, 3β-Br	**15**, 3α-I	**16**, 3β-I	**17**, Δ⁵-en
1α	0.65[e]	0.16	0.64[c]	0.16	0.15
1β	−0.13[e]	0.06	−0.14[c]	−0.09	0.19
2α	0.49[d]	0.69[c]	0.47[c]	0.68[c]	0.08[d]
2β	0.49[d]	0.51[c]	0.24[c]	0.79[c]	0.08[d]
3α		2.80		2.93	−0.01
3β	3.05		3.26		0.08
4α	0.43[d]	0.58[d]	0.48*	0.72[d]	0.73
4β	0.43[d]	0.58[d]	0.21*	0.72[d]	0.97
5	0.72	0.12	0.68	0.11	
6α	0.03[d]	0.08[d]	0.06[d]	0.05[d]	*f*
6β	0.03[d]	0.08[d]	0.06[d]	0.05[d]	*f*
7α	0.09	−0.01	0.12	−0.01	0.70
7β	0.02	0.01	0.03	0.01	0.32
8	0.02	0.00	0.03	−0.01	0.10
9	0.18	−0.02	0.18	−0.03	0.35
11α	0.02	−0.05	0.03	−0.07	0.01
11β	0.02	0.05	0.02	0.03	0.23
12α	0.04	0.00	0.04	−0.02	0.07
12β	0.03	0.01	0.02	0.00	0.06
14	0.04	−0.02	0.05	−0.02	0.03
15α	0.03	0.00	0.04	−0.01	0.04
15β	0.00	0.00	0.01	−0.01	0.06
16α	0.04	0.02	0.03	0.01	0.04
16β	−0.02	−0.02	−0.01	−0.03	0.01
18	0.00	0.00	0.00	−0.01	0.04
19	0.01	0.07	0.02	0.07	0.22

[a] See Footnote a to Table I. [b–e] See footnotes to Table IV. (*): Interchangeable. [f] ν_{TMS} (H6) = 5.31 ppm.

observed five couplings with ±0.25 Hz (Figure 9). Again, the observed profiles are rather flat and, together with the MM2 calculated broad strain energy minimum, do not allow exclusion of the presence of pseudorotational conformers within the limits shown in Figure 9.

(B) A Ring in 1. The A ring in Δ^4-3-oxosteroids is believed to provide the most important binding site for many corticoids and progestins; these have been the subject of several X-ray investigations.[19a,20c,d] In crystals, both sofa and half-chair conformations (*S* and *HC*, Chart I) are observed; for the progesterone compound **1**, we obtained by MM2 a sofa geometry as the energy minimum, showing excellent agreeement ($\Delta\phi$, $\leq \pm 1°$) with X-ray-derived data (Table D* in the supplementary material). Spin system iteration of the A-ring protons in **1** provided accurate coupling constants, which, by application of eq 1 indicate clearly the presence of a sofa form in solution (Table II). The only significant disagreement between calculated and observed values

Chart II

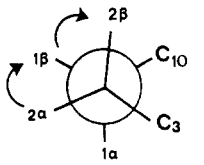


Table VI. ^1H–^1H Coupling Constants in Cyclohexanoid Parts[a]

type		J_{expt}, Hz	φ,[b] deg	J_{calcd}[c] for φ	J_{calcd}[c] for ±5°	example
^2J		−12–15				
^3J sec	a–e	5–7	55	4.0	±1.0	1α–2α
sec	a–a	13–14	171	12.2	0.2	1α–2β
sec	e–e	2–5	60	2.9	0.8	1β–2α
sec	e–a	3.5–6	57	3.4	1.0	1β–2β
tert	a–a	11–12	180	10.1	0.1	8–9

[a] From cross sections of ^{13}C–^1H shift-correlated 2D spectra (checked by spin system simulation); ΔJ = ±1.5 Hz digital resolution. [b] Torsional angle in androstane for the example given, from MM2 calculations. [c] Calculated from φ by using eq 1. [d] Calculated *J* change upon φ variation by ±5°.

(Table II) is visible for the H1β/H2 coupling, which leads to a φ value of 12° smaller than expected. Comparison with the complementary H1β/H2β angle, however, demonstrates that this deviation must be due to a failure of parameters in eq 1, as this angle seems not to be larger, as expected, but smaller as judged from the observed ^3J constant. The B-ring protons furnished experimental coupling constants which are not accurate enough (see above) for quantitative φ evaluations, but the C-ring values agree within ±2° with the calculation (Table II); again, one exception (^3J(11α/12α)) indicates limitations of eq 1, since a complementary angle (H11α/H12β) changes to a much lesser extent than suggested by the H11α/H12α value (Table II).

(C) Cyclohexanoid Parts. The protons in the rings A–C of the androstanes **2–17** could be analyzed in the cross sections of ^{13}C–^1H shift-correlated 2D spectra; within the resolution of ±1.5 Hz, all ^2J and ^3J values were in the range exemplified in Table VI. The observed ^3J values again show agreement with the coupling constants obtained with eq 1 and MM2-generated geometries. Geminal coupling constants usually are −13 ± 1.5 Hz but increase in the vicinity of carbonyl groups to up to 19 Hz (H16; H2, 15 Hz; H4, 14 Hz; H11, 14 Hz). Coupling over four bonds was directly measurable only in **1** between H4 and H2α (^4J = 0.9 Hz, from iteration). The angular methyl signals[11] are characterized by ^4J coupling to two or mostly three nonequivalent axial α protons and therefore only show line broadening of ~0.5 Hz or splittings of ~0.6 Hz in *J*-resolved 2D or in some 1D spectra, which is insufficient for the extraction of the corresponding coupling constants.

Shielding Effects and Mechanisms. The observation of substituent-induced shifts (SIS) in saturated steroids has been hitherto limited to "functional" X protons and to angular methyl groups.[3,4,6a,11] Comparable results are known so far only from studies with some cyclohexanes;[5] the data in Chart III and in Tables IV, V, and VII demonstrate that the SIS values are as similar and symmetrical as expected in cyclohexanoid systems, which allows them to be transferred to other frameworks in the future. It is noticeable, and reminiscent of some related ^{13}C NMR shifts, that the stereochemical shielding differences are often larger at the β and γ positions as compared to the shifts at the substituent site. The only available oxo group SIS values in cyclohexane stem from studies of α-halogen-substituted 4-*tert*-butylcyclohexanone,[5f] in which the presence of two strongly polar vicinal bonds can mask the influence of a single substituent. While the SIS values in steroids (Table IV and V) are in general agreement with the few available data on substituted cyclohexanes,[5] they do not support the notion of a general "syn-upfield, anti-downfield" effect[5d] of heterosubstituents on vicinal protons. Rather, the β effect is always deshielding and in more cases *stronger* for the syn than for the anti proton. Equatorial substituents deshield the equatorial and

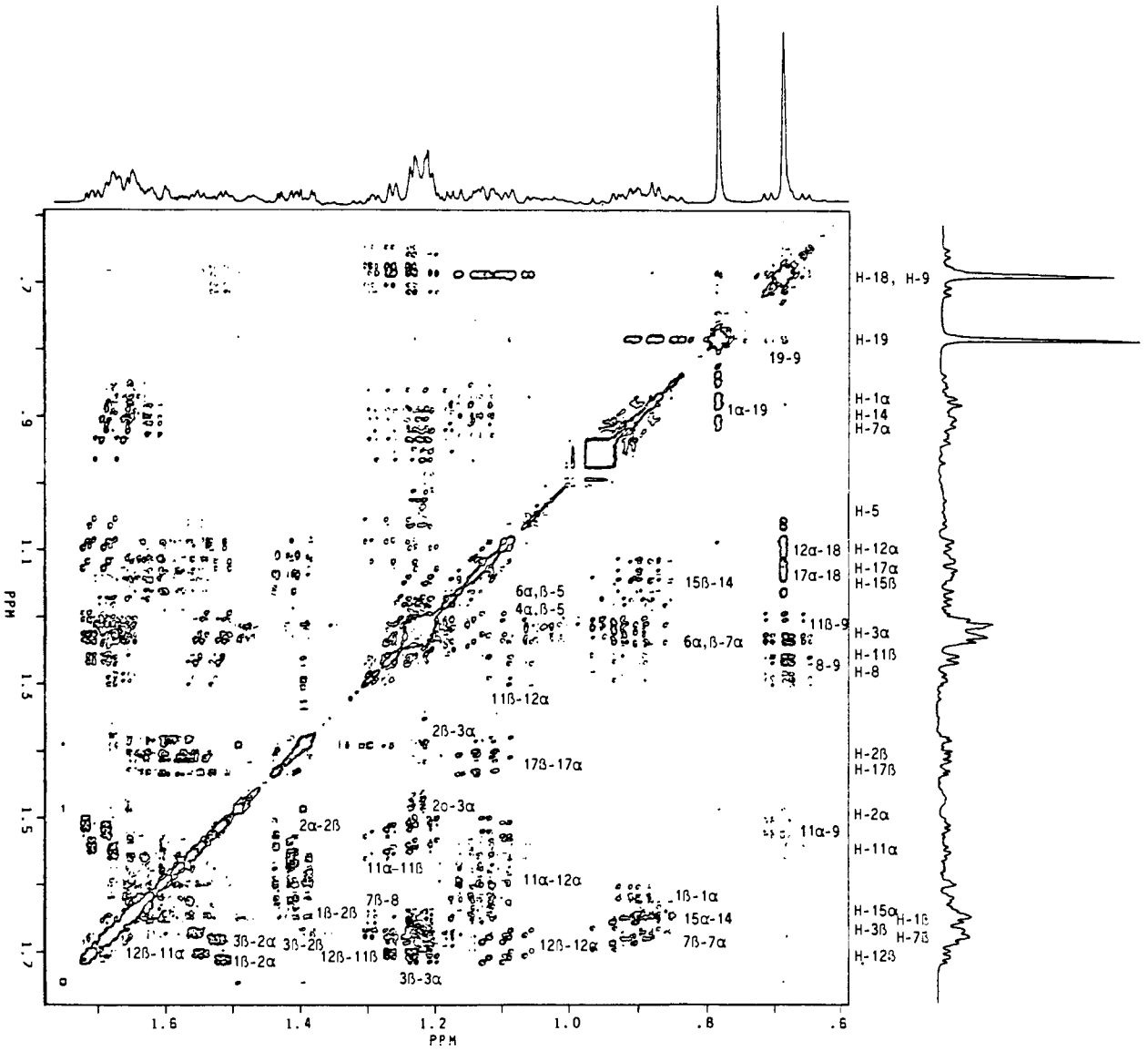
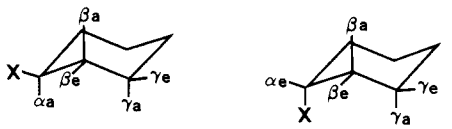


Figure 4. COSY-45 contour plot for 5αH-androstane (2), with emphasis on longer range coupling.

Table VII. Substituent X-induced ^{1}H NMR Shifts in Cyclohexanoid Systems[a]



X	H	X = F	Cl	Br	I	OH	=O(oxo)
e	α-a	3.24	2.63	2.80	2.93	2.34	
a	α-e	3.13	2.83	3.05	3.26	2.38	
e	β-e	0.46	0.53	0.65[b]	0.70	0.29	0.80
e	β-a	0.16[b]	0.33	0.63[b]	0.70	0.00	0.98
a	β-e	0.32	0.40	0.45	0.47	0.14	
a	β-a	0.11	0.45	0.46	0.23	0.28	
e	γ-e	0.10	0.10	0.06	−0.09	0.07	0.35
e	γ-a	0.10	0.12	0.14	0.15	0.05	0.45
a	γ-e	−0.14	−0.18	−0.13	−0.14	−0.18	
a	γ-a	0.46	0.65	0.68	0.66	0.47	

[a] In ppm from the parent hydrocarbon; ±0.03 ppm unless noted otherwise; mean values from observable steroid protons; 0.3 to 1.0 M solutions in $CDCl_3$, 25 °C. [b] ±0.05 ppm.

axial vicinal protons to an unexpectedly differing degree (Chart III, Tables IV and V, and J*), although the affected C–H bonds have the same syn relation to the C–X bond (ϕ difference according to MM2-calculated structures < 4° (Table C*)). Comparable SIS variations have been observed on ^{13}C NMR shifts in vicinally dimethylated cyclohexanes; there they are in accord with corresponding geometry differences.[23]

The most reliable substituent effect for stereochemical assignments is the pronounced syn-γ-H deshielding, which varies for all heterosubstituents between +0.43 and +0.68 ppm; this observation strongly suggests that a charge polarization along axial

(23) Schneider, H.-J.; Freitag, F. *Chem. Ber.* **1979**, *112*, 16. The MM1-calculated differences between *gauche*-X–C–Cβ–C angles amount here to 7°.

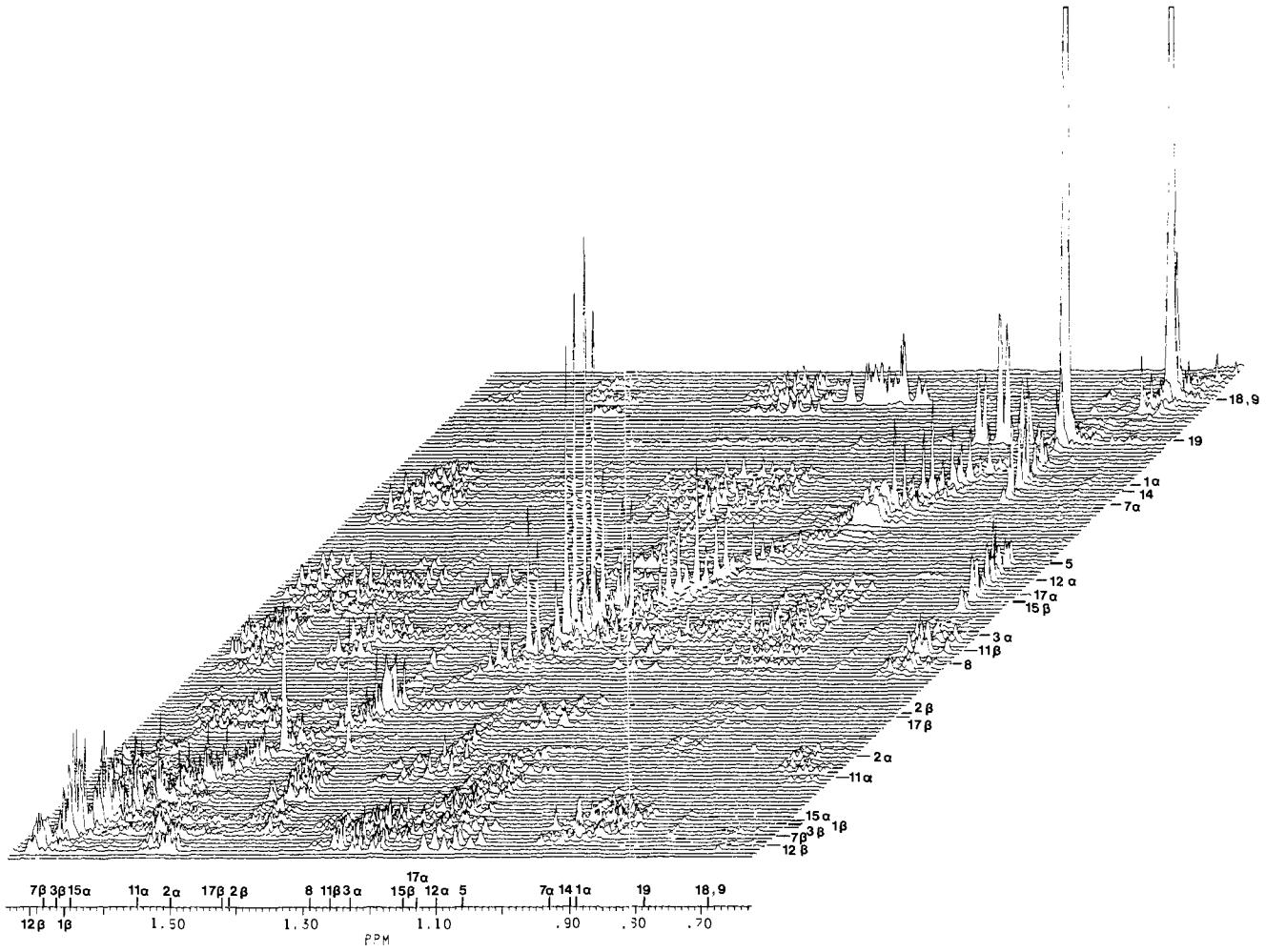


Figure 5. Stacked-plot representation of the COSY-45 experiment with 5α-androstane (**2**); long-range coupling emphasized.

C–H bonds is mainly responsible for both ^1H and ^{13}C NMR syn γ effects of heterosubstituents: for ^{13}C shifts, the corresponding SIS values vary between −4.5 and −7 ppm and thus show the expected sign and magnitude in view of the ~10 times higher sensitivity of ^{13}C shielding against charge density variation. Similar observations are made with the ϑ effects, which amount to +0.1 ppm for ^1H and to −1 to −2.5 ppm for ^{13}C shifts. The predominance of linear electric-field effects on ϑ-^{13}C shifts has been demonstrated earlier.[6c] The variation with different heterosubstituents is similar for ^1H and ^{13}C, although no quantitative correlation is visible, and there is no reason to invoke special effects, e.g., for iodine. The observed shielding variations provide a firm basis for a quantitative analysis of the relevant screening mechanisms. The shielding contributions were evaluated on the basis of realistic MM2-calculated geometries[19b] and were obtained with the program SHIFT[6e] which allows summation over different bonds and the use of different points of action along bonds or dipoles. The effects due to C–H bonds replaced by C–X bonds were always subtracted.

Hydrocarbon Shifts. The proton shifts in the basic steroid skeleton show a regular pattern, with the exception of the stereochemically not comparable D ring. All equatorial protons resonate at 1.5 ± 0.2 ppm, the axial protons, however, in a range of 0.7–1.2 ppm. The shielding of the axial protons increases with the number Z of other axial C–H bonds (Figure IV* of the supplementary material) as had been observed for some other compounds by Boaz.[24] The electric-field effect of the diaxial C–H dipoles, which earlier was considered to be the origin of such shielding variations,[24] was dismissed recently[6e] in favor of sterically induced charge polarizations, for which a sensitivity of ~0.05 ppm/μdyn was observed.[6e,25] A calculation of the steric forces F[6d,25,26] on the axial protons in the A, **B**, and C rings of androstane using eq 2,[6d] the parameters ϵ, r^*, and r as described for MM2,[12] and a similar procedure as described recently for F calculations in cyclohexanes[6e] furnish only a very rough correlation (Figure 10) (linear correlation coefficient $r = 0.74$). As demonstrated

$$F = k\,\frac{18\epsilon}{r^*}\left[\left(\frac{r^*}{r}\right)^{10} - \left(\frac{r^*}{r}\right)^{7}\right]\cos\theta \qquad (2)$$

earlier,[6d,e] the calculated shielding contributions are extremely dependent on the chosen parametrization and calculational procedure; the observed sensitivity for the androstane, however, is in the expected[6d,e] range of $k \cong 0.1$ (ppm/μdyn), if high-order effects of the atoms closer to the observed proton (geminal and vicinal atoms) are neglected (Table K* of the supplemental material).

Carbonyl Group Effects. The introduction of polar bonds can lead to steric distortions, which, however, even with the change from a sp^3 hybridization to sp^2 in a ketone seem to be of less significance compared to other screening variations.[6a,c] High-order or, e.g., square electric-field effects are particularly sensitive to small geometry variations and are moreover difficult to parameterize;[6a] they are, however, significant only for nuclei in the direct vicinity of the fluctuating dipole, e.g., for β-hydrogen atoms. We wanted to see to which degree the proton shifts in the extended steroid frameworks can be described by classical screening mechanisms for polar and polarizable bonds.

The linear electric-field effect σ_{EI} induced by a charge q at a bond with the polarizability P and the length l at a distance r from

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(25) See also: Cheney, T. *J. Am. Chem. Soc.* **1968**, *90*, 3386.

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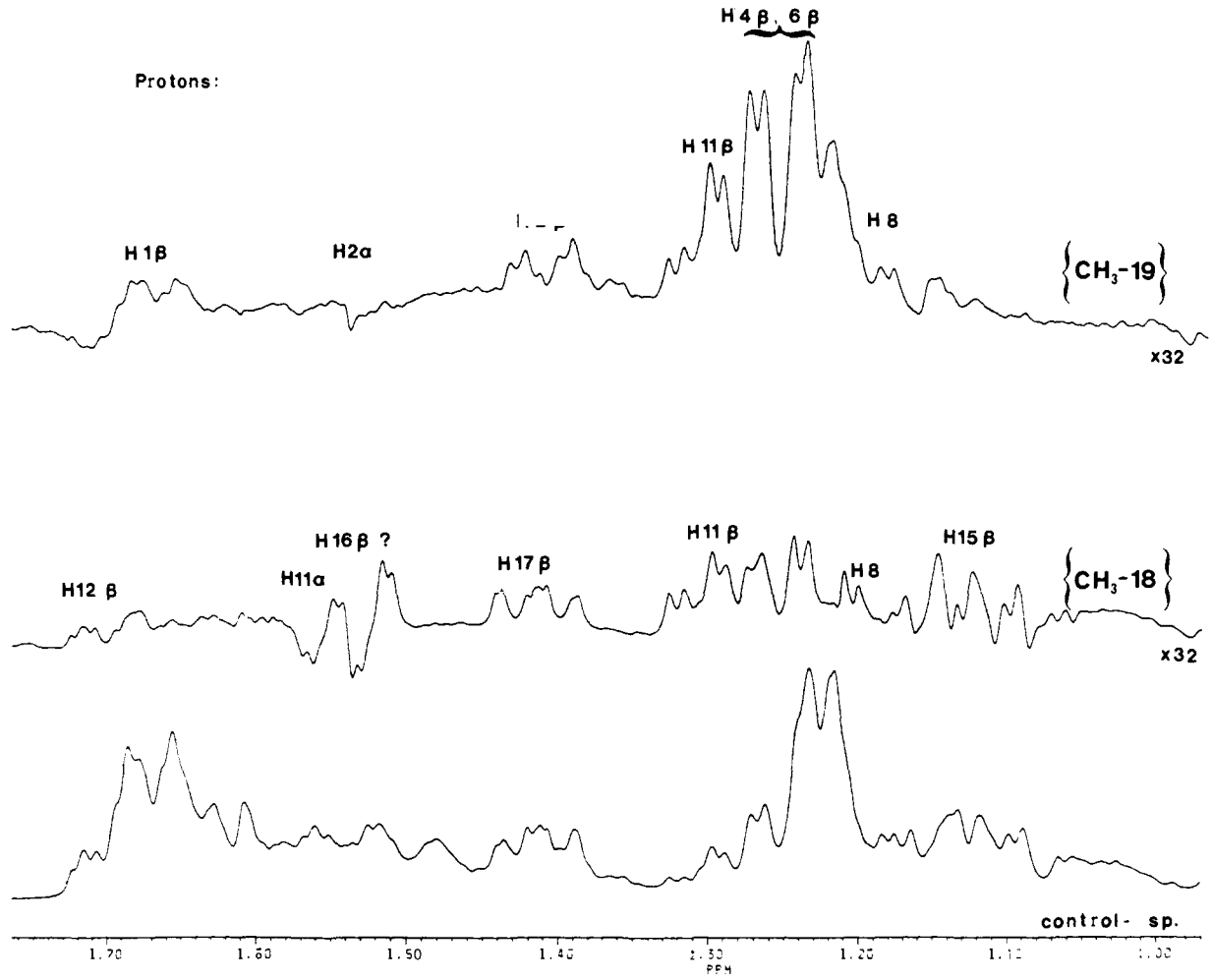


Figure 6. NOE Difference spectra with 5α-androstane (2).

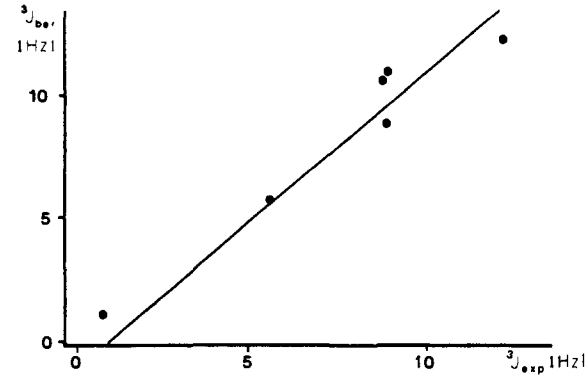


Figure 7. Comparison of calculated and experimental vicinal coupling constants for the D-ring in 5α-androstan-17-one (8) (geometry by MM2 calculation; see Table I*).

q and an angle θ between l and the field vector is represented by the Buckingham equation[27a] (3). The anisotropy effect σ_{An}

$$\sigma_{El} = \sum_i Pl^{-1} r_i^{-2} \cos(\theta_i) q_i \quad (3)$$

generated in a magnetic field by a difference $\Delta\chi$ between susceptibilities of bonds in different directions is given by the McConnell equation (4), where r represents the distance from the

$$\sigma_{An} = \Delta\chi(1 - 3\cos^2\theta)/3r^3 \quad (4a)$$

dipole to the proton and θ the angle between the symmetry axis

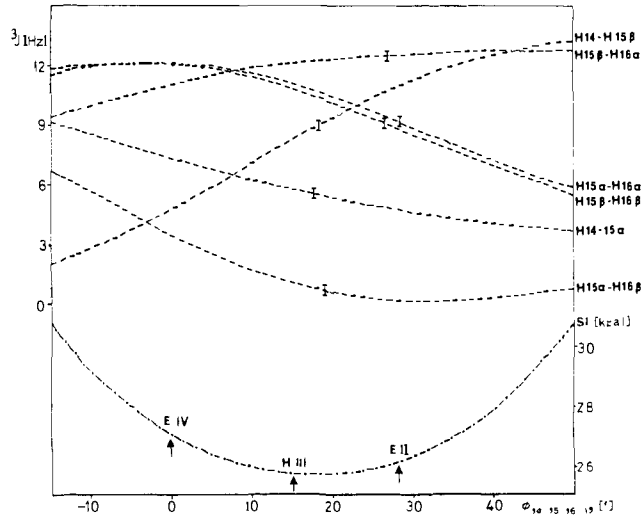


Figure 8. Calculated vicinal coupling constants (^3J) and strain energies (SI, MM2) vs. torsional angle (ϕ) in the D ring of 8. E IV, H III, E II, see Scheme II; II, experimental J values.

of the dipole and the distance vector. For dipoles of nonaxial symmetry, such as C=O, it is necessary to use $\Delta\chi_1$ and $\Delta\chi_2$ for susceptibility differences:[4a,c]

$$\sigma_{An} = [\Delta\chi_1^{C=O}(1-3\cos^2\theta_1) + \Delta\chi_2^{C=O}(1-3\cos^2\theta_2)]/3r^3 \quad (4b)$$

If we use a parametrization derived from bond dipole moments (Table L*) and for protons and the theoretically expected sensitivity of 17.8 ppm/charge unit[28] for σ_{EL} and calculate σ_{An} values

(27) (a) Buckingham, A. D. *Can. J. Chem.* **1960**, *38*, 300. (b) For our procedure, see: Schneider, H.-J.; Freitag, W.; Geschwendtner, W.; Maldener, G. *J. Magn. Reson.* **1979**, *36*, 273 and ref 6c.

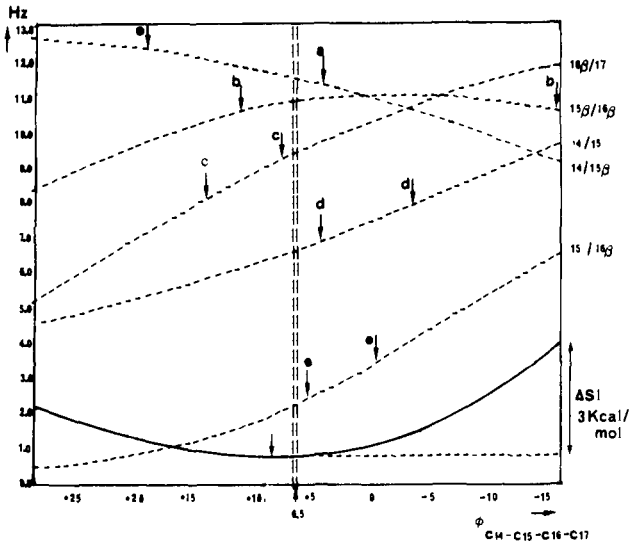
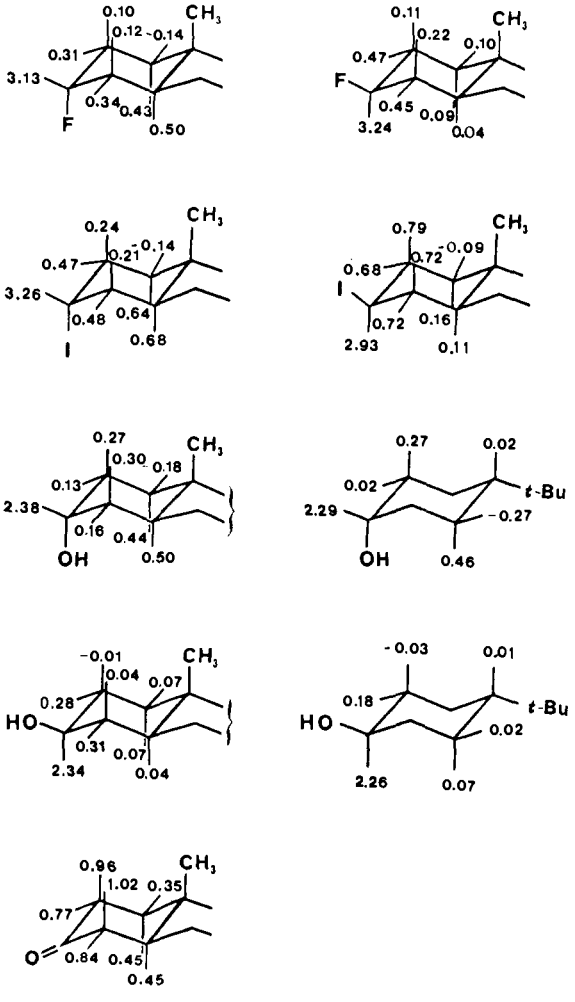


Figure 9. ^3J and SI as a function of ϕ (see legend to Figure 8) in the D ring of **1**; the arrows (↓) denote possible ϕ ranges corresponding to errors in ^3J.

Chart III



on the basis of the ApSimon parameters,[4a] we arrive at sizeable contributions from *both* mechanisms (Tables M1*–M3*), which represent, e.g., all proton SIS values in the 3-oxosteroid **6** remarkably well, with the only exception being the β protons (Figure 11). A similarly linear correlation is obtained for the 11-ketone **7** (Table N*); here not only the vicinal protons but particularly

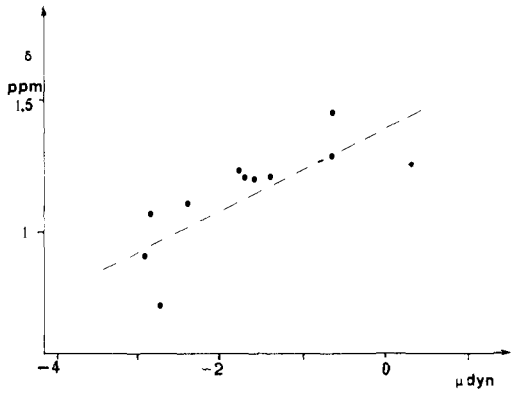


Figure 10. Steric forces F (eq 2) and ^1H NMR shifts at axial C–H bonds in 5αH-androstane (**2**) (data in Table K*).

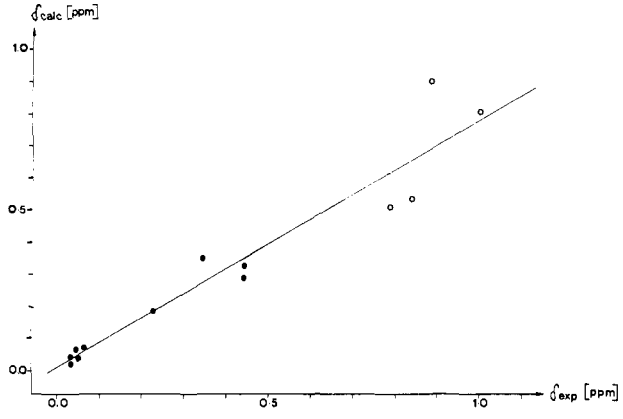


Figure 11. Experimental SIS in 5αH-androstan-3-one **6** vs. electric-field effects (parametrization see Table L*) and anisotropy effects (ApSimon parametrization); vicinal protons (O) induced.

the 1β-proton shift deviates substantially (by 0.6 ppm) from the calculated value (Table M2*). The strong deviation observed with protons approaching the carbonyl group closely was attributed already to a breakdown of eq 4, which should be used only for $r > 3$ Å.[4c] Similar difficulties were encountered in more recent approaches to carbonyl screening effects in peptides.[29] In view of the expected large contribution of high-order effects from the low ionization potentials and high polarizability of the oxo group and the absence of suitable parameters for an improved σ_{An} model,[29] the vicinal proton shifts in ketones are barely amenable to reliable calculations at the present time. More remote protons, in particular CH_3-18 and CH_3-19 in **6** and **7** agree well with calculated ($\sigma_{EI} + \sigma_{An}$) values; deviations for the 11-oxosteroid obtained by earlier workers are—in the light of MM2 calculated structures (Table C*)—not due to geometry distortions[4c] but likely due to the averaging procedure used for the methyl groups and to the use of unrealistic conformations. Ill-defined geometries, however, may be responsible for the somewhat larger deviations observed with **17** (Table M3*), which bears the oxo group in the flexible D ring.

Omitting the vicinal protons, our data for the ketones **6**, **7**, and **8** provide 25 SIS significant values (≥0.03 ppm); they include only six time averaged spins and lend themselves to an evaluation of the parameters P, l, q, and $\Delta\chi$ in eq 3 and 4 by multilinear least-square analysis. Similarly to ApSimon et al., who applied this procedure to methyl group shifts in a large range of oxosteroids,[4] we separate for the description of the carbonyl C=O effect on a C–H bond the adjustable variables in eq 3 and 4 from the geometry factors (g) which are derived on the basis of MM2-calculated geometries from eq 3 and 4 (cf. Table O*):

$$\Delta\vartheta_{calcd} = (\sigma_{EI}^{C-X} + \sigma_{An}^{C-X}) - (\sigma_{EI}^{C-H} - \sigma_{An}^{C-H}) \quad (5)$$

If all adjustable parameters are allowed to vary, we obtain excellent

(28) See, e.g.: Farnum, D. G. *Adv. Phys. Org. Chem.* **1975**, *11*, 123.

(29) Clayden, N. J.; Williams, R. J. P. *J. Magn. Reson.* **1982**, *49*, 383.

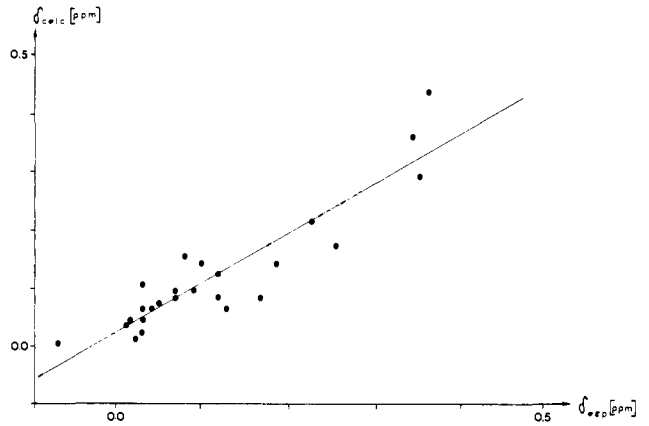


Figure 12. Experimental SIS in the ketones **6**, **7**, and **8** vs. the sum of electric-field and anisotropy effects (both optimized by multiple least-squares regression; vicinal protons omitted).

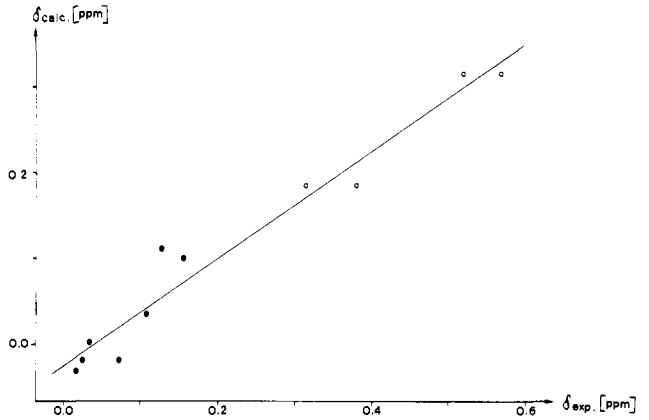


Figure 13. Experimental SIS in 3β-chloroandrostane (**12**) vs. linear electric-field effects.

correlations (multiple regression coefficient $R = 0.90$) but completely unreasonable parameters (Table O*). If only $\Delta\chi^{C=O}$ and $k = qP_{CH}$ are treated as variable coefficients, the regression analysis (Figure 11, Tables N* and O*) furnishes new values for $\Delta\chi_1^{C=O}$ −35.9 (−27.4), $\Delta\chi_2^{C=O}$ −24.4 (−21.0), and $k = 0.34$ (0.28) (old values[4a] in parentheses; see also Table L* and Figure 12). With $q_{C=O} = 0.4308$ (Table L*), a polarizability of $P_{CH} = 0.79$ is obtained; literature values for P_{CH} vary between 0.65 and 0.79 (Table L*). It should be emphasized that a linear correlation of our SIS values with the old χ and k parameters is almost as significant as the new one with optimized parameters (Table N*); this underlines the conclusion[6e] that, even with a large experimental basis set in a geometrically well-defined environment, a reliable parametrization of NMR screening factors is severely limited and needs to be complemented by independent physical methods.

Halide Effects. There are relatively few shielding calculations on anisotropy and field effects of carbon–heteroatom single bonds. Evaluations with nonmonovalent substituents are furthermore complicated by the presence of variable conformers and were for this reason not pursued for OH groups in the present work. Davies et al.[30] carried out a multilinear regression analysis based on methyl group shifts in several bromo- and chloroandrostanes and concluded that, except for the effect on C–H bonds in 1,3-diaxial position to C–Hal, electric-field effects were sufficient for the description. We came to the same conclusion in an earlier study[6a] which also included fluoro and iodo effect on steroid methyl group shifts. Nevertheless, based on ~50 SIS values from the halides **9**–**16**, multilinear regression analyses were carried out, again leading to unrealistic and k parameters (Table P*). If anisotropy contributions are calculated based on literature $\Delta\chi$ values, which partially stem from independent measurements (Table L*), and the resulting σ_{An} *together* with σ_{El} was compared to experimental SIS, the correlations usually were less satisfactory than those obtained with σ_{El} *alone* (Table Q*). In contrast to the ketones, vicinal protons are accommodated by the correlations with σ_{El}; although they are surprisingly linear, particularly with equatorially substituted halides (Figure 13), the slopes decrease from $m = 0.86$ to 0.37 in the order, F, Cl, Br, and I (Table Q*). The deviation from $m = 1.0$ can be due to errors in the charge-shielding relation (17.8 ppm/unit) and/or in the point charges q for the C–Hal bonds, which are largely derived from bond dipole moments (Table L*). The calculated contributions of σ_{An} increase from F to I (Tables M*1–M10*) and inclusion of σ_{An} does increase the slope of the correlations. The results confirm earlier conclusions[6a] that the shielding effects of carbon–halogen bonds can be understood largely on the basis of linear electric-field gradients; even stereochemical differences between vicinal (β) protons, or the substantial deshielding effects of axial halogen on syn γ protons are predicted by σ_{El}. There is no correlation between electronegativity parameters for X = Hal or OR and the observed shifts, not even for "functional" protons at C3.

Experimental Section

NMR Methods. Most measurements were performed with a Bruker AM 400 instrument, some with a WM 400 spectrometer at ambient temperature (298 ± 2 K). Sample concentrations in $CDCl_3$ were 0.05–0.1 M for most 1D experiments for NOE and 2D measurements similar as specified below with the example of 5αH-androstane (**2**).

The *homonuclear ^1H-shift-correlated 2D NMR* spectrum of **2** was obtained with the standard acquisition sequence[10] with a 45° mixing pulse, using phase cycling and *N*-type peak selection; a delay of 0.13 s was applied before and after the mixing pulse to emphasize long-range couplings. Data processing was carried out with the standard BRUKER software for ASPECT-3000. A f_2-spectral width of 700 Hz (1.75 ppm) over 2K data points gave a digital resolution of 0.7 Hz. A number of 512 spectra, each of 16 transients, gave, with appropriate incrementing of the evolution delay, a f_1 width of 700 Hz and a digital resolution of 0.7 Hz (with zero filling). Data were handled in absolute value mode and a sine bell window in both dimensions. The sample concentration was 0.1 *m* in $CDCl_3$; total acquisition time was about 6 h, processing time ca. 15 min.

The *^{13}C–^1H shift-correlated 2D NMR* spectrum of **2** was obtained with the usual pulse sequence[10] with phase cycling. Data processing was performed with standard BRUKER software. The spectral widths were 540 Hz in f_1 and 5400 Hz in f_2, giving digital resolutions of 0.53 and 1.3 Hz with a 512 × 4096 data point matrix; the transform size was 1K × 8K. Data were handled in power mode and the sine bell window in both dimensions in order to obtain better resolution and S/N ratio. The sample concentration was 2.0 *m* in $CDCl_3$. Acquisition of 16 transients for each of the 512 FID's required 5 h; processing took about 1 h.

The *^1H-J-resolved 2D NMR* spectrum of **2** was measured with the standard sequence[10] with phase cycling. The spectral widths were 700 Hz over 4K, giving a digital resolution of 0.17 Hz in f_2, and 44 Hz over 128 FID's in f_1, giving a digital resolution of 0.17 Hz with zero filling. The transform size was 256 × 8192 data points. The sine bell window was used in f_2 dimension, and a Gauss function with negative line broadening of −2.5 Hz in f_1. Data were handled in absolute value mode. Acquisition of 16 transients for each FID required 2 h, processing ca. 15 min.

NOE difference spectra of **2** were measured at 297 K with a 0.5 M solution in $CDCl_3$ with a spectral width of 520 Hz on 64K time domain points, giving with zero filling up to 128 K a digital resolution of 0.008 Hz. A total of 896 transients were recorded for each of the two NOE spectra and one control spectrum (off resonance), thus giving a total acquisition time of about 50 h. The decoupling power applied was about 1 mW, irradiation time was 5 s; a line broadening of 1.5 Hz was applied before spectrum subtraction.

Materials. The following compounds were either commercially available or gifts from the Schering AG or prepared by following literature procedures: 6α-fluoro; 11β-hydroxy, 16α-methyl-, and 17β-(carboxymethyl)androst-4-en-3-one (**1**); 5αH-androstan (**2**); 3α- and 3β-hydroxy-5αH-androstan-17-one (**18**, **19**); 17β-hydroxy-5α-H-androstan-3-one (**20**); 3β-hydroxyandros-5-en-17-one (**21**); cortisone (**22**); 5αH-androstan-17-one (**23**);[31] 5αH-androstan-17-ones; 3β-fluoro-**10**;[43]

(30) Davis, A. K.; Mathieson, D. W.; Nicklin, P. D.; Bell, J. R.; Toyne, K. J. *Tetrahedron Lett.* **1973**, 413.

(31) Burgstrahler, A. W.; Bager, D. L.; Naik, N. C. *Tetrahedron* **1976**, *32*, 309.

3α-chloro-**11**,[40] 3β-chloro-**12**.[40] ^{13}C NMR data are either available in the literature[15] or will be published in another context.

3α-Hydroxy-5αH-androstane (3). **18** (1 g, 3.4 mmol) was reduced with 0.6 g (10 mmol) of hydrazine hydrate (85%) and 0.8 g (14 mmol) of potassium hydroxide in 5 mL of diethylene glycol to yield 0.76 g (80%) of **3**, after extraction with dichloromethane and recrystallization in methanol: mp 145.5–146 °C [lit.[32] mp 145–146 °C].

3β-Hydroxy-5αH-androstane (4) was obtained similarly to **3** in 94% yield: mp 150.5–151.5 °C [lit.[33] mp 151–152 °C].

5αH-Androstan-3-one 6. **4** (1 g, 3.6 mmol) was reacted under ice-chilling in 100 mL of acetone with 1 mL of Jones reagent[34] (0.27 g of chromium trioxide, 0.23 mL of sulfuric acid with 1 mL of water). After 10 min, 200 mL of water was added, the solution extracted with dichloromethane and neutralized, and the solvent removed. Recrystallization in acetone/water yielded 0.75 g (76%) of **6**; mp 103.5–104 °C [lit.[35] mp 104.5 °C]. **5αH-Androstan-11-one (7).** Side-chain oxidation of cortisone yielded androst-4-en-3,11,17-trione (**24**)[36] (65%). Similar to literature procedures,[35] 1.2 g (4 mmol) of **24** in 50 mL of dioxane was added dropwise to a solution of 0.8 g (115 mmol) of lithium in 150 mL of liquid ammonia during 15 min. After 30 min of stirring, ammonium chloride was added, the ammonia was evaporated, and the residue after adding 50 mL of 1 N hydrochloric acid was extracted with dichloromethane. The organic phase was neutralized and dried over sodium sulfate, the solvent was removed, and 5 mL of Jones reagent[34] (see above, **6**) was added. After 10 min, 100 mL of water was added; after extraction with dichloromethane, neutralization, and recrystallization of the residue in acetone/hexane, 0.75 g (62%), of 5αH-androstan-3,11,17-trione (**25**) was obtained: mp 171 °C [lit.[36] mp 175 °C]. Huang-Minlon reduction as described for **3** yielded 0.44 g (70%) of **7**.[36]

Androst-5-en-17-one (17). **21** (5 g, 17.4 mmol) with 4.6 g (24 mmol) yielded 6.2 g (80%) of tosylate (**26**); 3 g (6.8 mmol) of **26** was refluxed for 3 h with 3 g (20 mmol) of sodium iodide, 3 g (46 mmol) of zinc dust, 3 mL of water, and 50 mL of dimethylethylene glycol.[37] The solution was filtered after addition of ether, washed with dilute hydrochloric acid, neutralized, and dried. Recrystallization of the residue in methanol yielded 1.25 g (68)% of **17**: mp 103–104 °C [lit.[38] mp 105–107 °C].

16,16-Dideuterio-5αH-androstan-17-one (27). Similar to literature procedures,[39] the ketone **8** was treated with sodium deuteriohydroxide in deuteriooxide; the exchange rate was ~95% (by NMR).

3α-Chloro-5αH-androstane (5). Following similar procedures,[40] 1 mL (14.4 mmol) of sulfuryl chloride was added dropwise to a stirred ice-chilled solution of 0.9 g (3.4 mmol) of **4** in 20 mL of pyridine. After 1 h at 10–25 °C, the mixture was poured into ice-chilled 5% aqueous hydrochloric acid; the residue was washed with dilute acid and water and recrystallized in methanol after drying to yield 500 mg (50%) of **5**: mp 109–110 °C. Anal. Calcd for $C_{19}H_{31}Cl$: C, 77.42% H, 10.53. Found: C, 77.0; H 10.45. ^{13}C NMR: Table B*.

3α-Fluoro-5αH-androstan-17-one (9). 3β-(Tosyloxy)-5αH-androstan-17-one[41] (0.5 g, 0.45 mmol) and 0.4 g (2.7 mmol) of tetraethylammonium fluoride were warmed to reflux in 15 mL of acetone for 3 days. After aqueous workup, column chromatography over silica gel with ethyl acetate–hexane (1:9 v/v), 25 mg (20%) of 5αH-androst-2-en-17-one and 100 mg (78%) of **9** were obtained: mp 114–115 °C. Anal. Calcd for $C_{19}H_{29}FO$: C, 78.08; H, 9.93. Found: C, 78.10; H, 9.94.

3α-Bromo-5αH-androstan-17-one (13). Following literature procedures,[41] 0.2 g (0.45 mmol) of 3β-(tosyloxy)-5αH-androstan-17-one was warmed to reflux with 0.18 g (2.1 mmol) of lithium bromide in 15 mL of acetone for 3 days. After purification as described for **9**, one obtained some 5αH-androst-2-en-17-one and 0.1 g (65%) of **13**: mp 130–132 °C. Anal. Calcd for $C_{19}H_{29}OBr$: C, 64.59; H, 8.22. Found: C, 64.37, H 8.14.

3β-Bromo-5αH-androstan-17-one (14). The same procedure as described for **13** yielded largely the Δ^2-elimination product and 20% **14**: mp 147 °C. Anal. Calcd for $C_{19}H_{29}OBr$: C, 64.59; H, 8.22. Found: C, 64.52; H, 8.24.

3α-Iodo-5αH-androstan-17-one (15). Similarly to literature procedures[42] 1 g (2 mmol) of 3β-(tosyloxy)-5αH-androstan-17-one was stirred with 1 g (4 mmol) of freshly prepared magnesium iodide in 5 mL of ether for 1 h at reflux temperature. Ice–water (5 mL) was added, the ether solution was washed with water and dried over sodium sulfate, and the solvent was removed under reduced pressure to yield after recrystallization in acetone/methanol 0.62 g (75%) of **15**: mp 123–124 °C. Anal. Calcd for $C_{19}H_{29}OI$: C, 57.14; H, 7.27. Found: C, 56.93; H, 7.20.

3β-Iodo-5αH-androstan-17-one (16). The compound was obtained in the same way as **15** in 80% yield: mp 151–153 °C. Anal. Calcd for $C_{19}H_{29}OI$: C, 57.14; H 7.27. Found: C, 57.02; H, 7.27.

Acknowledgment. This work was financially supported by the "Deutsche Forschungsgemeinschaft" and the "Fonds der Chemischen Industrie". Prof. M. Ashworth is thanked for linguistic help, B. Dohmen for technical assistance, the Schering AG, Berlin, and Prof. R. Wiechert for the gift of materials, and Dr. W. Hull (Bruker) and Dr. R. Kolshorn for preliminary measurements.

Registry No. 1, 98651-91-3; **2**, 438-22-2; **3**, 7657-50-3; **4**, 1224-92-6; **5**, 54155-81-6; **6**, 1224-95-9; **7**, 1755-32-4; **8**, 963-74-6; **9**, 1156-86-1; **10**, 361-80-8; **11**, 51104-91-7; **12**, 20612-47-9; **13**, 53512-66-6; **14**, 58507-05-4; **15**, 75980-93-7; **16**, 98635-28-0; **17**, 5225-35-4; **18**, 53-41-8; **19**, 20120-08-5; **21**, 53-43-0; **24**, 382-45-6; **25**, 1482-70-8; 3β-(tosyloxy)-5α*H*-androstan-17-one, 10429-07-9.

Supplementary Material Available: Table of decoupling effects in **1**, ^{13}C NMR shifts of **17** and **5**, MM2-derived geometries for **1**, **2**, **6**, **7**, **8**, **13**, **14**, and **17**, A ring conformation in Δ^4-3-oxosteroids, X-proton coupling constants in 3βX-substituted 5αH-steroids, X-proton coupling constants in 3αX compounds, approximate vicinal coupling constants with the X proton, and comparison to cyclohexane data, observed and estimated coupling constants with H15α/β, D-ring conformations in 5α-androstan-17-one (torsional angles), summary of SIS values in the 3-halogen skeleton, steric forces *F* on axial protons in **2**, number *Z* of diaxial C–H bonds, parametrization used for linear electric field and anisotropy effects, shielding contributions σ_{El} and σ_{An}, regression analysis for the ketones, based on σ_{El} and σ_{An}, multiple linear regression analysis with the ketones (with additional equations), multiple linear regression analysis with the halides, regression analysis and correlations for the halides, and figures of cross sections from ^{13}C–^1H shift-correlated 2D spectra of 5αH-androstane with corresponding computer simulations, COSY-45 contour plots of 5αH-androstan-17-one (**8**) *with* and *without* deuteration at C-16, *J*-resolved ^1H 2D spectrum of 5αH-androstane contour plot, cross section, comparison of axial proton shifts in 5αH-androstane with the number *Z* of diaxial interactions (see Table K*), linear correlations coefficient $r = 0.871$ (31 pages). Ordering information is given on any current masthead page.

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