

simple problem it nonetheless represents a starting point for demonstrating the potential power inherent in computer interpretation of experimental data. Even when no unambiguous answers can be obtained it is impressive to note that the number of possible candidates is reduced drastically (e.g., 10 candidates out of 989 theoretical possibilities in examples 15 and 16 in Table I). In the case of mass spectra taken directly from gas chromatography effluents the program would not be able to utilize nmr input data. Thus multiple solutions would be possible for a particular problem. However, as stated above, a significant degree of truncation considering all possible aliphatic ethers would be achieved. Clearly one can program other physical data (for instance ir and uv spectral parameters) to supplement the mass spectral and nmr data currently used. With added experimental data and sophisticated programming the computer should be able to solve more complex problems and it is to this end that future research in our laboratories is being directed.

Experimental Section

The computer program described here, named Heuristic DENDRAL, runs on the PDP-10 time-sharing computer at the Stanford University Artificial Intelligence Laboratory. It is written in the LISP programming language in three large parts each requiring approximately 40K of core memory (with an estimated 15K of overlap between the parts). Although many factors influence the length of time the program takes from the time it receives the initial spectrum and molecular ion composition to the time it outputs its ordered list of explanatory structures, 4 or 5 min at the teletype will usually suffice for examples of the complexity described here.

The program is now confined to monofunctional aliphatic structures. However, we are currently working on the removal of these limitations as well as adding more mass spectrometry theory to the program such that more complex problems will be within the program's capability. Details of the computer program itself have been described elsewhere.²⁰

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Nuclear Magnetic Resonance Spectroscopy. Carbon-13 Spectra of Steroids¹

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Abstract: The natural abundance ¹³C resonance spectra of a variety of sterols and steroidal hormones have been determined at 15.1 MHz. The chemical shifts of the carbons in these substances were found to span on the order of 200 ppm and for most steroids with the aid of complete proton decoupling it was possible to resolve all of the carbon resonances one from the other. It has also been possible by using specific single-frequency and off-resonance proton decoupling, hydroxyl acetylation effects on chemical shifts, deuteration, and substituent influences in analogous compounds to make self-consistent and unambiguous assignments of nearly all of the resonances encountered. The carbon resonances are in general far more informative than proton resonances for structural analysis of steroids.

Instrumentation is now available for relatively routine determination of high-resolution nmr spectra of ¹³C in natural abundance in organic compounds.³⁻⁶ Noise-modulated proton decoupling⁷ is of special utility for organic structural analysis because it permits measurement of fully proton-decoupled spectra consisting of

sharp singlets when other nuclei with nonzero spin are either absent or undergo rapid quadrupole relaxation.

Proton nmr spectra have found extensive application in structural and conformational studies of steroids, although often few resonances other than methyl groups are easily assignable. The ¹³C nmr spectra of steroids promise to be considerably more useful, both because of the enormous sensitivity of ¹³C chemical shifts to structural changes^{4,8} and because each carbon atom of the skeleton and any attached groups can usually be examined individually. With the enhanced sensitivity expected from future instrumental improvements, the ¹³C nmr should also find wide application in biosynthetic tracer experiments.

We have measured the ¹³C spectra of a series of structurally related steroids and have been able to make satisfactory assignments of almost all of the resonances. Chemical-shift measurements were made with full proton noise decoupling.⁷ Under these conditions, spectra

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(3) J. B. Stothers, *Quart. Rev. (London)*, **19**, 144 (1965).

(4) (a) D. M. Grant and E. G. Paul, *J. Amer. Chem. Soc.*, **86**, 2984 (1964); (b) D. K. Dalling and D. M. Grant, *ibid.*, **89**, 6612 (1967).

(5) (a) F. J. Weigert and J. D. Roberts, *ibid.*, **89**, 2967 (1967); **90**, 3543 (1968); (b) F. J. Weigert, M. Winokur, and J. D. Roberts, *ibid.*, **90**, 1566 (1968).

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(7) (a) F. J. Weigert, M. Jautelat, and J. D. Roberts, *Proc. Nat. Acad. Sci. U. S.*, **60**, 1152 (1968); (b) L. F. Johnson and M. E. Tate, *Can. J. Chem.*, **47**, 63 (1969); (c) R. R. Ernst, *J. Chem. Phys.*, **45**, 3845 (1966).

(8) G. W. Buchanan, D. A. Ross, and J. B. Stothers, *J. Amer. Chem. Soc.*, **88**, 4301 (1966).

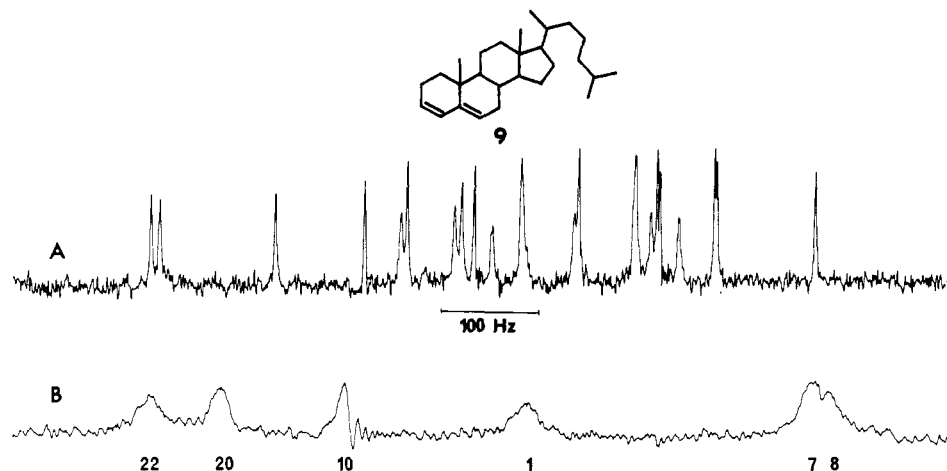


Figure 1. Noise-decoupled ^{13}C nmr spectra of the saturated region of a 1.5 M solution of cholesta-3,5-diene (9); (A) wide sweep (1000 Hz) spectrum, 28 scans at 250 sec/scan; (B) narrow sweep (100 Hz) spectrum used for chemical-shift measurements, 12 scans, 50 sec/scan. The barely resolved signals for C-7 and C-8 are separated by 0.1 ppm.

could be obtained on 0.2 M solutions, although 1.0 to 1.5 M solutions were used when possible. Figure 1 shows sample spectra, curve A being a wide-sweep spectrum showing all of the resonances of the saturated carbons of cholesta-3,5-diene, while curve B is a narrow-sweep (100 Hz) spectrum used for precise chemical-shift measurements.

In concentrated solutions of lower molecular weight molecules, exact proton-decoupling frequencies corresponding to each carbon can be readily determined, since only a single scan is required to observe a signal. If the proton spectrum can be assigned, the decoupling frequencies will aid in the assignment of the resonances in the carbon spectrum. This technique is most useful

Table I. ^{13}C Chemical Shifts of *cis*- and *trans*-4-*t*-Butylcyclohexanols and Their Acetates

Carbon	<i>trans</i>			<i>cis</i>		
	$\delta(\text{alcohol})^{a,b}$	$\delta(\text{ester})^a$	Δ	$\delta(\text{alcohol})^{a,b}$	$\delta(\text{ester})^a$	Δ
1	122.4	119.5	-2.9	127.8	123.9	-3.9
2	157.1	160.2	3.1	159.5	161.9	2.4
3	167.1	166.9	-0.2	171.8	170.7	-1.1
4	145.5	145.1	-0.4	144.6	144.7	0.1
Quaternary	160.7	160.2	-0.5	160.4	160.1	-0.3
Methyl ^c	165.3	165.0	-0.3	165.4	165.1	-0.3
Methyl ^d		171.8			171.8	
Carbonyl ^e		23.5			23.5	

^a In ppm, upfield relative to CS_2 . ^b Data from J. D. Roberts, F. J. Weigert, J. I. Kroschwitz, and H. J. Reich, *J. Amer. Chem. Soc.*, in press. ^c Methyl of *t*-butyl group. ^d Methyl of acetoxy group. ^e Acetoxy carbonyl.

Undecoupled spectra were not useful in this work because of their great complexity and the extensive scanning times needed to obtain sufficiently strong signals.^{7a} Off-resonance, single-frequency decoupled spectra, in which the sample is irradiated strongly at a frequency several hundred hertz off from the region of proton-resonance frequencies, have been found very useful. No long-range couplings are observed in such spectra and direct ^{13}C - ^1H couplings are reduced to 20-40 Hz while still providing some favorable Overhauser enhancement^{4,9} of signal intensity. In the steroids, off-resonance decoupling does not usually yield the clean doublets, triplets, and quartets for methine, methylene, and methyl carbons which may be seen in less complex molecules, but quaternary and methyl carbons can be readily identified (see Figures 2 and 3). In favorable cases, methine and methylene resonances can be distinguished from one another by the absence of a peak at the position of the methine resonance in the off-resonance decoupled spectrum (methylene carbons will generally show a peak at the resonance position).

(9) K. F. Kuhlman and D. M. Grant, *J. Amer. Chem. Soc.*, **90**, 7355 (1968).

in identification of carbons near functional groups where the proton resonances are most widely separated. Only limited use of this method was made in the steroids because determination of the exact decoupling frequencies is very time consuming, the two protons of methylene groups are nonequivalent, and most of the proton shifts are not known. In the case of the exceptionally soluble cholesteryl methyl ether, the exact decoupling frequencies of the methyl groups were determined and an assignment could be made on this basis.

It has been found that the carbons near hydroxyl groups can be readily identified from chemical-shift changes attendant to acetylation. Table I shows the characteristic shifts which occur on acetylation of the axial (*cis*) and equatorial (*trans*) 4-*t*-butylcyclohexanols. The downfield shift of the carbinol carbon (C-1) is apparently the result of a greater electron-withdrawing power of the acetoxy group than the hydroxyl group, while the positive shift of C-2 (and C-6) results from steric interaction with the acyl group. For axial cyclohexanols, there is also a 1 ppm downfield shift of C-3, possibly because of the smaller 1,3 interaction of the

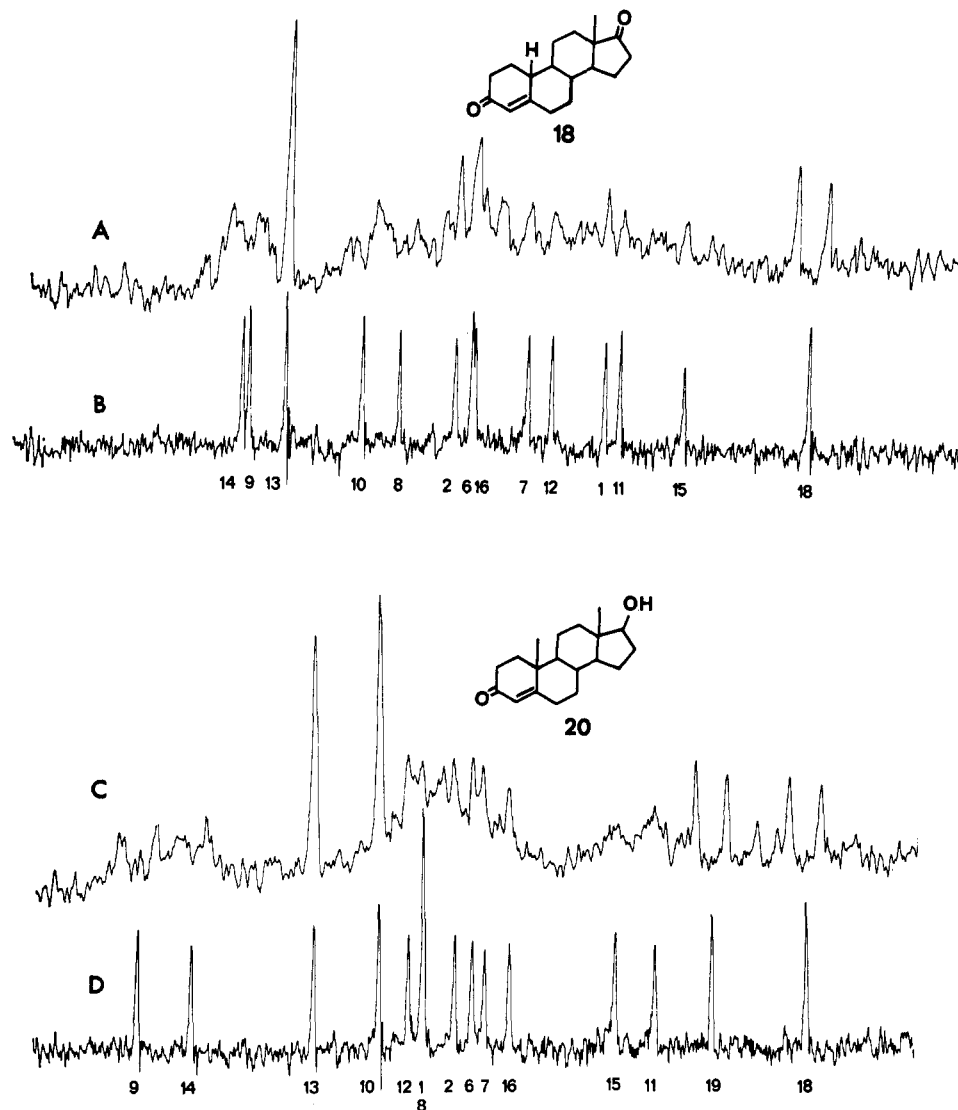


Figure 2. (A) ^{13}C spectrum of 19-norandrost-4-ene-3,17-dione (18) with off-resonance decoupling (550 Hz from center of aliphatic proton resonances); 202 scans, 50 sec/scan; (B) noise-decoupled spectrum of 18; 29 scans at 100 sec/scan; (C) off-resonance decoupled spectrum of testosterone (20); 160 scans, 50 sec/scan; (D) noise-decoupled spectrum of 20; 26 scans, 100 sec/scan.

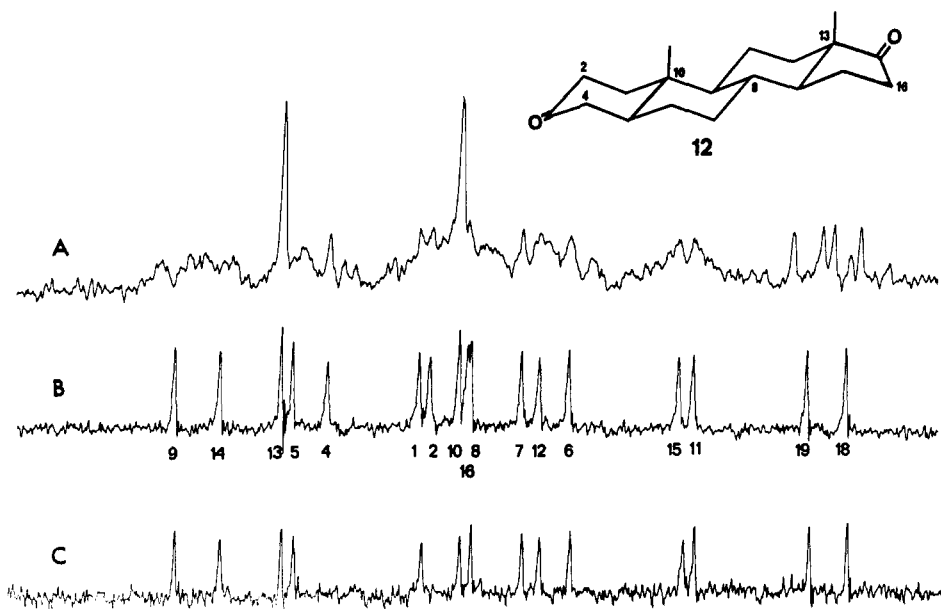


Figure 3. (A) Off-resonance decoupled ^{13}C nmr spectrum of androstane-3,17-dione (12); 138 scans; (B) noise-decoupled spectrum of 12; 54 scans; (C) noise-decoupled spectrum of 2,2,4,4,16,16- d_6 -androstane-3,17-dione.

Table II. The Chemical Shifts^a and Assignments for the ¹³C Spectra of Steroids^b

Carbon	1	2	3	3a	4a	6	6a	6b	7a	8	9	10	11a	12
1	153.6	153.9	155.2	155.6	159.4	155.0	155.2	155.1	154.4	153.9	158.5	159.5	156.4	154.0
2	170.2	154.6	160.7	164.9	166.3	160.9	164.3	164.2	164.3	160.3	169.4	169.0	165.0	154.7
3	165.5	-16.6	122.1	119.3	123.0	121.2	118.8	112.2	119.9	122.8	62.9	56.4	120.2	-16.5
4	163.2	148.0	153.9	158.3	159.4	150.1	154.1	153.5	155.7	151.4	68.2	68.1	154.8	148.1
5	145.2	145.8	147.3	147.7	152.2	51.3	52.6	51.5	51.5	51.8	51.1	32.5	29.3	145.8
6	163.2	163.4	163.5	163.7	164.1	71.2	69.9	71.2	72.1	73.1	69.6	64.6	65.8	163.7
7	160.2	160.6	160.2	160.4	160.4	160.5	160.3	160.5	75.8	75.8	160.6	-7.9	-7.6	160.6
8	156.7	156.8	156.7	156.8	156.8	160.5	160.3	160.5	54.0	51.9	160.5	146.5	147.2	157.4
9	137.3	138.4	137.7	137.9	137.9	142.0	142.1	142.0	146.3	146.0	143.9	142.7	142.6	138.2
10	156.0	156.9	156.9	156.9	156.7	156.0	155.8	155.6	155.3	155.3	157.3	156.3	154.2	156.6
11	171.4	170.9	171.1	171.1	171.5	171.3	171.2	171.3	171.4	171.3	171.3	171.1	171.2	171.7
12	164.2	164.2	164.2	164.3	164.1	164.2	160.0	164.3	164.3	164.2	164.2	163.8	163.9	161.8
13	149.7	149.8	149.7	149.8	149.8	150.1	150.0	150.0	149.5	149.5	149.9	148.9	149.3	145.1
14	135.6	136.0	135.8	135.8	135.7	135.6	135.5	135.5	138.0	137.9	135.3	141.6	142.3	141.1
15	168.2	168.2	168.2	168.3	168.3	168.2	167.9	168.2	169.5	169.4	168.3	166.0	166.1	170.7
16	152.1	152.3	152.1	152.3	152.2	152.5	152.4	152.4	153.1	153.1	152.3	153.3	153.5	157.1
17	135.7	135.9	135.8	135.8	135.7	136.0	135.9	135.9	136.3	136.5	135.9	137.2	137.3	-25.9
18	180.3	180.5	180.4	180.5	180.5	180.5	180.5	180.6	180.7	180.7	180.5	180.5	180.7	179.0
19	180.4	181.3	180.5	180.5	181.3	173.1	173.2	173.2	176.5	176.5	173.9	176.0	175.6	181.5
20	156.5	156.6	156.6	156.6	156.5	156.7	156.4	156.5	156.2	152.0	156.5	156.6	156.6	
21	173.7	173.8	173.9	173.8	173.8	173.7	173.6	173.7	173.6	173.1	173.7	173.5	173.6	
22	156.1	156.1	156.1	156.1	156.1	156.1	155.8	156.1	156.2	60.3	156.0	156.1	156.1	
23	168.3	168.5	168.4	168.4	168.4	168.4	168.2	168.4	168.5	56.5	168.3	168.4	168.4	
24	152.8	152.9	152.8	152.9	152.8	152.9	152.7	152.8	152.9	149.5	152.7	152.9	152.9	
25	164.4	164.5	164.5	164.5	164.5	164.5	164.3	164.5	164.5	159.3	164.4	164.5	164.5	
26	170.0	170.0	170.1	170.1	170.0	170.0	169.8	170.0	170.0	172.8	170.0	169.9	170.1	
27	169.8	169.8	169.9	169.9	169.8	169.7	169.6	169.8	169.8	171.5	169.8	169.7	169.9	
28										175.1				
CH ₃ ^d				171.9 ^d	171.8 ^d		171.6 ^d	137.4 ^e	171.6 ^d				172.0 ^d	
CO ^d				23.1 ^d	23.2 ^d				22.9 ^d				23.2 ^d	

^a In ppm from carbon disulfide. ^b The solvent was dioxane with more or less chloroform added to achieve complete solution. The uncertainty introduced by variations in the solvent composition is believed to be 0.1 ppm or less. The temperature with complete proton de-

acetoxy group with the 3-hydrogen relative to the hydroxyl group.

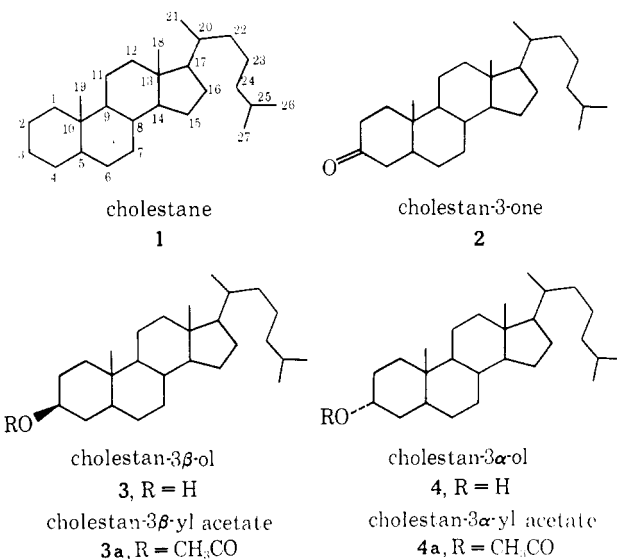
Specific deuteration is also an effective tool for identification of particular carbons. The signals for carbons α to carbonyl groups in several steroid ketones were identified in this way (see Figure 3). The signal for the deuterated carbon essentially disappears at the attainable signal-to-noise ratios because of quadrupole broadening, spin-spin splitting, and decreased Overhauser enhancement.

Previous work on ¹³C nmr spectroscopy has established the characteristic chemical shifts of various types of carbonyl carbons,¹⁰⁻¹² of unsaturated carbons,^{3,6b,12b-14} and of aliphatic carbons.^{4,15} Systematic studies of alkanes,^{4a} methylcyclohexanes,^{4b} and cyclohexanols^{8,15} have established much about the detailed nature of chemical-shift effects in these open-chain and cyclic systems and have been very helpful in making the steroid shift assignments.

It should be noted that in some of the assignments reported below, resonances separated by 0.5 ppm or less were assigned to particular carbons, although a reversed assignment could be equally valid. Unless one of a group of such lines was unambiguously identified by deuteration, as a quaternary carbon by off-resonance decoupling, or because one of the lines was a highly in-

variable one (such as C-22 to C-26 in cholestane), the assignments must be considered to some degree uncertain. In all such cases, the assignments given are the ones considered to be the most probable from chemical-shift considerations.

Cholestane (1), Cholestan-3-one (2), and Cholestan-3-ols 3 and 4. The resonances of the cholestane side chain (C-21 to C-27) can readily be identified by comparison with 2,6-dimethyloctane (5) as a model compound, the peaks of which could be assigned by the substituent parameters of Grant and Paul.^{4a}



The spectra of a series of diverse cholestane-type steroids having the same side chain show seven carbons whose resonances are as expected from the spectrum of

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(15) See Table I, footnote b.

13	13a	14	15	15a	16	17	18	19	20	20a	21	22	23	23a	24 ^c
155.0	155.5	156.8	157.4	157.4	157.6	157.8	165.7	165.8	156.7	156.7	156.3	165.7	66.3	66.4	155.4
160.7	164.7	159.9	159.0	159.3	158.7	159.4	156.1	156.2	158.7	158.6	158.4	156.0	79.7	73.6	157.8
121.5	119.0	-4.6	-6.0	-5.3	-4.8	-5.1	-5.2	-5.8	-5.2	-5.0		-5.2	37.4	43.3	-4.5
149.9	154.3	68.5	68.1	67.8	68.4	68.5	67.8	68.0	68.6	68.5		66.1	77.4	70.9	68.2
50.4	52.4	23.2	22.1	23.0	23.1	23.1	27.4	26.6	22.4	22.4		28.5	55.1	55.0	24.0
72.1	70.7	160.2	160.5	160.5	160.3	160.8	157.2	157.2	160.0	159.9	153.6	149.5		163.1	161.6
160.7	160.9	161.8	161.9	161.7	160.5	161.6	160.8	161.5	160.6	160.8	161.2	161.6		166.0	161.3
160.7	160.9	158.6	158.3	158.5	158.4	157.9	152.5	151.8	156.7	157.0	151.8	149.4		154.2	57.4
141.7	142.2	138.3	133.3	137.5	138.0	138.4	142.7	143.6	138.2	138.5	145.3	149.3		148.1	48.0
155.6	155.7	153.9	152.4	152.7	153.8	154.5	150.1	149.9	153.8	153.9	154.0	149.9	61.3	54.6	153.2
171.9	172.1	171.3	124.3	121.9	171.6	172.1	166.7	166.2	171.6	171.8	171.5	165.7		166.7	65.4
161.6	161.6	156.5	142.1	147.2	156.8	162.2	162.3	155.7	155.7	155.7	155.7	155.7		160.6	66.2
145.3	145.3	148.8	148.5	148.9	146.2	145.3	145.1	149.4	149.6	149.9	149.4	149.3		144.9	66.3
140.6	140.8	136.3	136.8	136.8	136.5	141.6	142.3	143.9	141.7	142.0	145.9	145.7		142.0	63.8
170.6	170.7	169.5	169.5	169.4	160.0	170.8	170.9	169.2	169.0	169.0	169.5	169.9		171.0	
157.1	156.9	153.7	154.8	155.7	36.9	157.4	157.4	162.1	162.1	164.9	162.0	162.2		157.2	
-26.2		129.2	129.4	129.6	49.3	-25.9	-25.8	111.4	111.5	110.3		111.5		-25.9	
179.3	179.1	179.5	178.3	178.7	176.8	179.1	179.0	181.4	181.5	180.6	181.5	181.5		179.1	
173.3	173.3	175.5	174.5	174.5	175.7	175.5			175.5	175.2	174.8				165.1
		-14.7	-14.9	-14.6	-2.9										
		168.1	168.2	168.3	166.1										

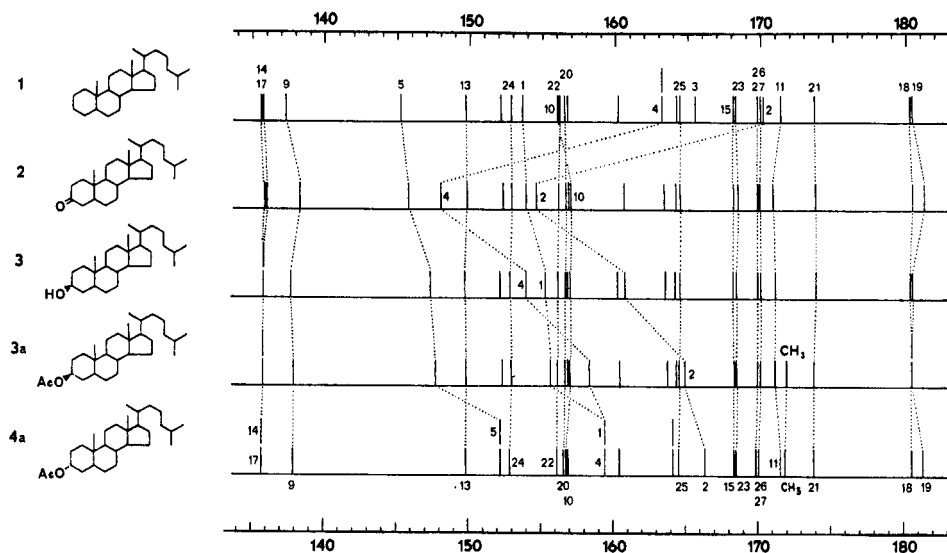


Figure 4. Correlation of ^{13}C chemical shifts for cholestane (1), cholestan-3-one (2), cholestan-3 β -yl acetate (3a), and cholestan-3 α -yl acetate (4a).

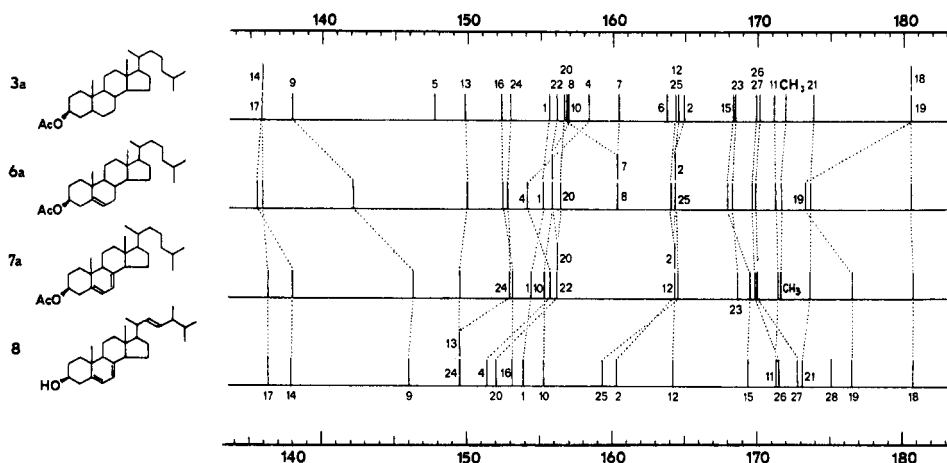


Figure 5. Correlation of ^{13}C chemical shifts for cholestan-3 β -ol (3a), cholesteryl acetate (6a), 7-dehydrocholesteryl acetate (7a), and ergosterol (8).

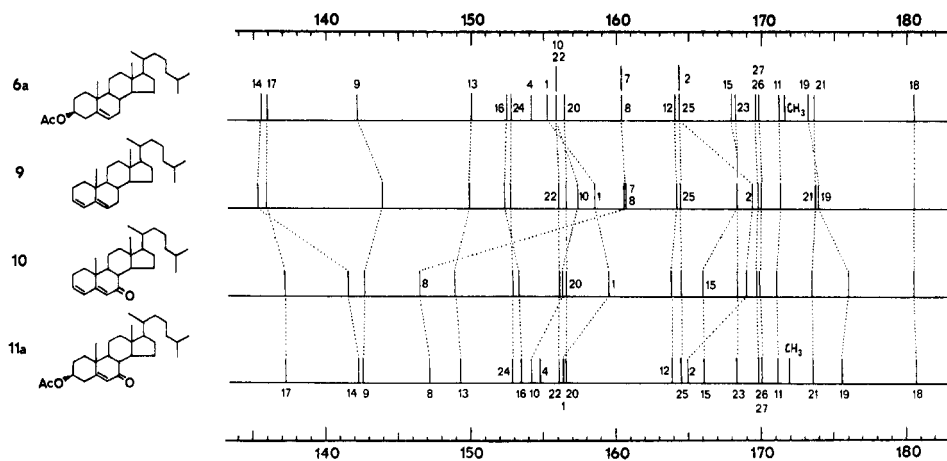


Figure 6. Chemical-shift correlations for cholesteryl acetate (6a), cholesta-3,5-diene (9), cholesta-3,5-dien-7-one (10), and cholest-5-en-7-on-3 β -yl acetate (11).

tions. Carbons 2 and 4 were assigned from acetylation shifts, and they can be distinguished in cholesterol, because only the allylic carbon C-4 changes between cholestanol and cholesterol.

Comparison of the ^{13}C chemical shifts of cyclohexane and cyclohexene shows that on introduction of a double bond into a six-membered ring the carbons α to the double bond come upfield 1.8 ppm, while those β come

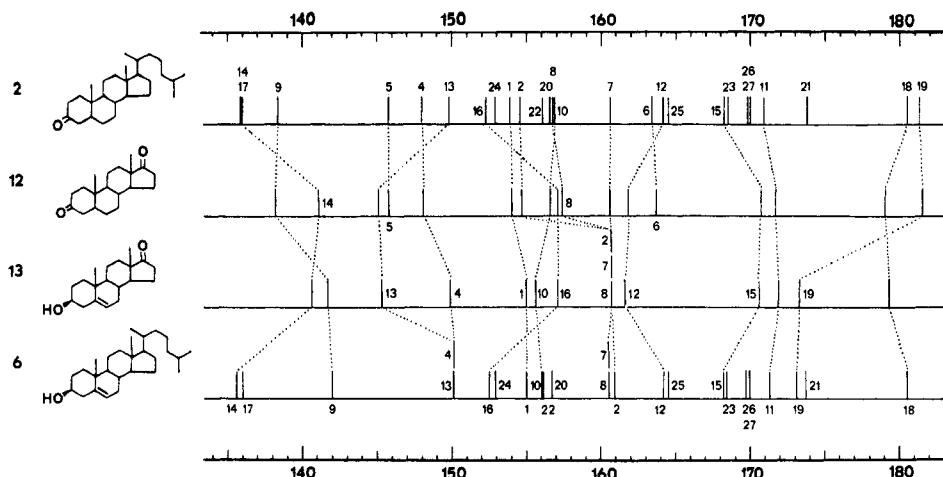
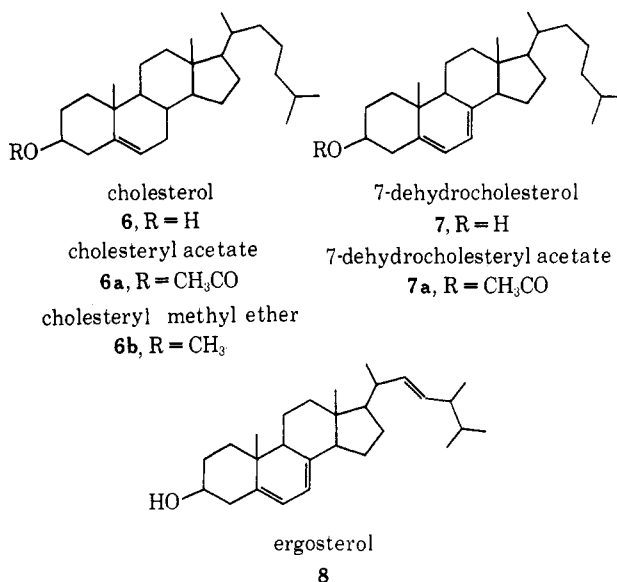


Figure 7. Chemical-shift correlations for cholestan-3-one (2), androstane-3,17-dione (12), 5-dehydroisoandrosterone (13), and cholesterol (6).

upfield 4.2 ppm.¹⁸ On this basis, one would expect C-9 and C-8 to move strongly upfield on going between compounds 3 and 6. In fact, one of the low-field resonances shifts 4.2 ppm upfield, and this can then be as-



signed to C-9. Disentanglement of the closely superimposed resonances at 152 to 162 ppm is less clear-cut, but one resonance which appears at 156.6 ppm in cholestanol 3 moves to 160.5 ppm in cholesterol, and this is assigned to C-8. The resonance for C-8 methine is expected at unusually high field as a result of strong steric interaction with C-18 and C-19. This assignment for C-8 is supported by off-resonance decoupled spectra of androstane-3,17-dione (Figure 3).

Introduction of a second double bond in ring B (compound 7a) eliminated two resonances at 160.3 ppm in 6 from the saturated alicyclic region, confirming their assignment to C-7 and C-8. Carbon 9 moves further upfield, as does one of the remaining methine resonances which can now be assigned to C-14. The other methine resonance at 136 ppm corresponds to C-17 and would not be expected to change as much as C-14. The resonance for C-15 moves upfield 1.6 ppm, differentiating it from the nearby C-23 peak. Carbons 2 and 4 were

again assigned from their acetylation shifts. Three resonances remain unassigned (164.2, 156.7, and 152.1 ppm) which correspond to C-12, C-20, and C-16.

The ring carbons of ergosterol (8) can be cleanly assigned by comparison with 7a, because these compounds differ only in the side chain. The close correspondence between the ring-carbon chemical shifts supports the assignments made above. The chemical shifts of the side-chain carbons for 8 can be estimated by application of the alkane substituent parameters of Grant and Paul^{4a} to introduction of a methyl group at C-24 of the cholestane side chain, and then using the following parameters¹⁸ to correct for the presence of the C-22,23 double bond: α , -3.0 ppm; β , 0; γ , 0.5 ppm. The unassigned resonances of ergosterol correspond to within 2 ppm of the estimated side-chain values. A unique assignment for C-21, C-26, and C-27 could not be achieved.

Cholesta-3,5-diene (9), Cholesta-3,5-dien-7-one (10), and Cholest-5-en-7-on-3 β -yl Acetate (11a). The assignment for the side chain and the carbons of rings B, C, and D of 9 was as for cholesterol (see Figure 6). Carbons 3 and 4 now appear in the unsaturated region. The two remaining resonances in the spectrum of 9 can be assigned to C-1 and C-2. Note that C-2 has essentially the same chemical shift as in cholestane, while C-1 is shifted upfield some 5 ppm. The introduction of a keto group at C-7 (10) results in a number of chemical-shift changes, but an assignment consistent with the above considerations can be readily made. The resonance for C-7 is shifted to the α,β -unsaturated ketone carbonyl region; C-8 moves downfield about 14 ppm, as expected. The signal for C-14 moves upfield 6.3 ppm from 9, apparently the result of a steric interaction with the carbonyl group. Carbons 12, 15, and 16 are significantly shifted, and this serves to distinguish these from the C-20, C-23, C-24, and C-25 resonances, which should remain unchanged. The chemical shifts of C-1 and C-2 are only slightly changed from 9.

In compound 11a, the resonances for the C and D ring carbons are unchanged from the dienone 10, while the ring A carbon signals are close to those for cholesteryl acetate, as expected.

Androstane-3,17-dione (12) and Dehydroisoandrosterone (13). The resonances of 12 and 13 (see Figure 7) serve to confirm the cholestane assignments and also

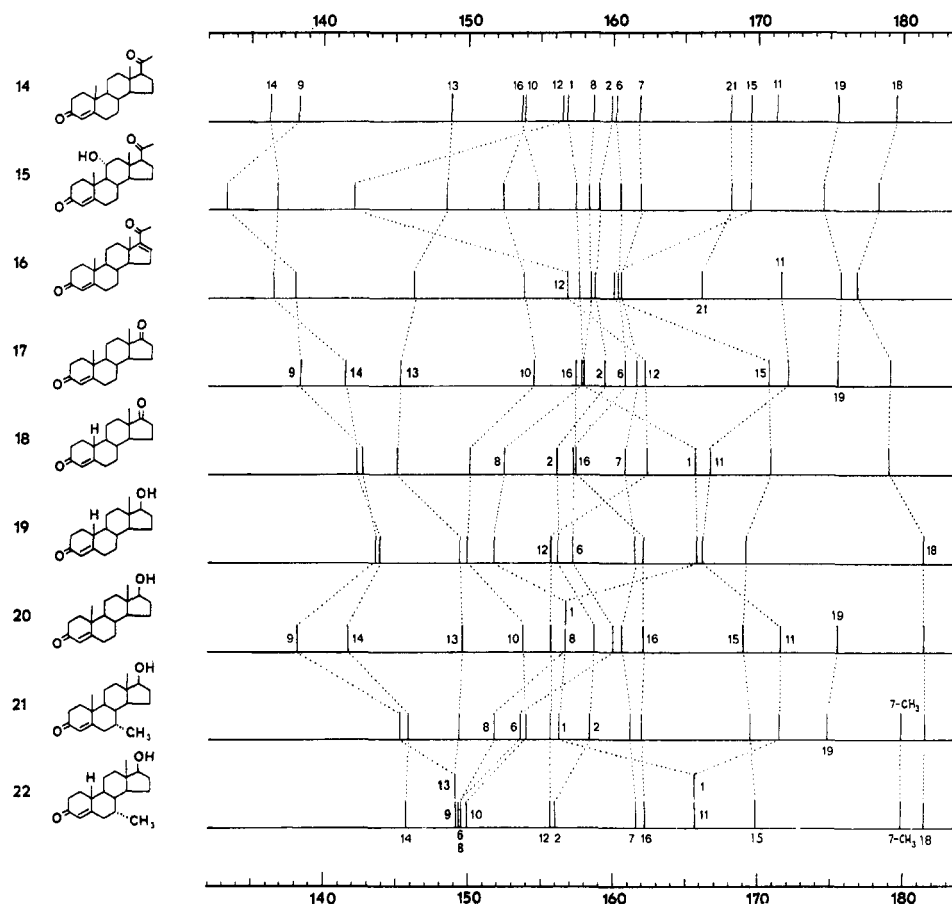
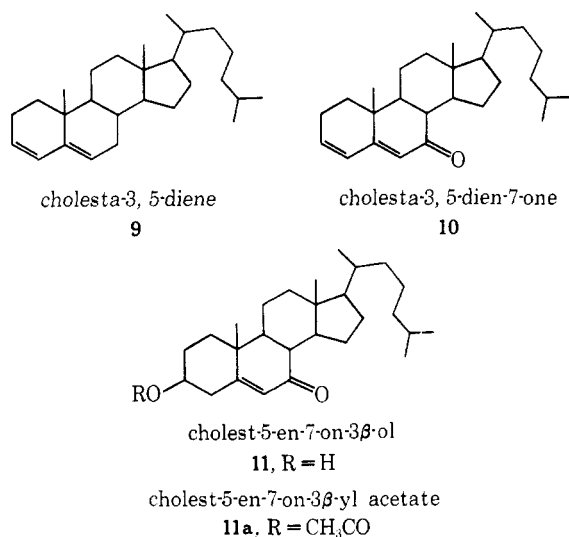


Figure 8. Chemical-shift correlations for changes in structure for progesterones **14–17**, androst-4-enedione **18–19**, and testosterone **19–22**.



provide a link in the assignments for testosterone, progesterone, and related steroids.

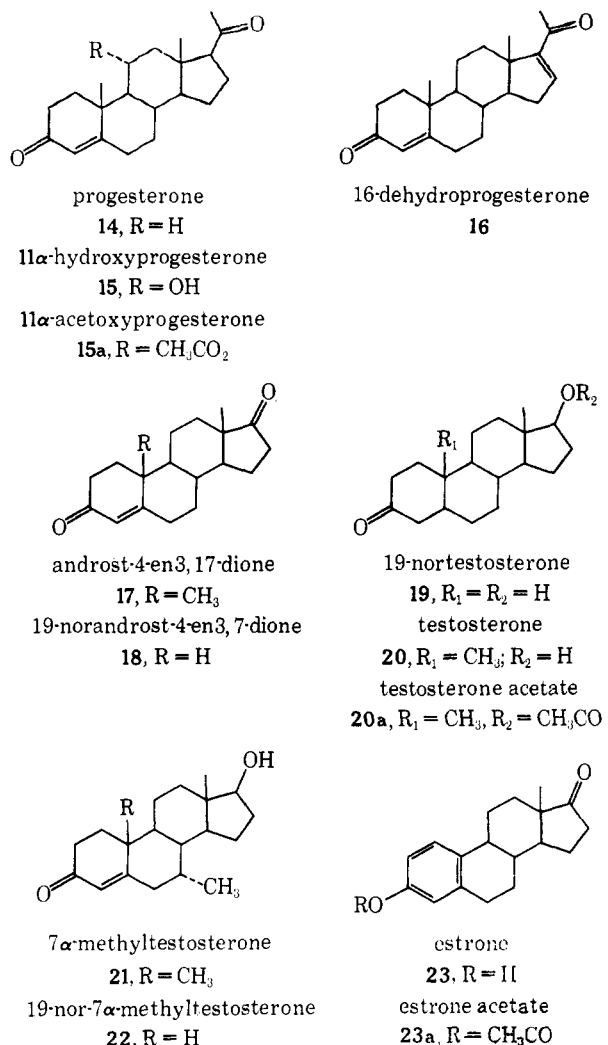
C-11 and C-19 in **2** are essentially matched by resonances in **12**. Base-catalyzed exchange gives the 2,2,4,4,16,16-*d*₆ derivative (see Figure 3), whose ¹³C spectrum has three fewer visible resonances than the protio compound, thus providing an independent assignment for these carbons. The off-resonance decoupled spectra of **12** (Figure 2) and of deuterated **12** allow identification of C-10, C-13, C-18, and, with a fair degree of certainty, C-8, strengthening a rather weak assignment in cholestan-3-one. The remaining carbons (C-11, C-12, C-14, and C-15) are identified by the usual chemical-shift considerations, C-11 and C-15 appearing at high field, C-14 at low field.

The signals for carbons of rings A and B in **13** can be identified by comparison with cholesterol, and those for rings C and D by comparison with **12**. All of these comparisons are consistent. Carbon 16 was independently identified from dideuterated **13**.

Progesterone (14), 11 α -Hydroxyprogesterone (15), 16-Dehydroprogesterone (16), Androst-4-ene-3,17-dione (17), 19-Norandrost-4-ene-3,17-dione (18), 19-Nortestosterone (19), Testosterone (20), 7 α -Methyltestosterone (21), 19-Nor-7 α -methyltestosterone (22), and Estrone (23). The assignments for this group of steroids (Figure 8) are more complex and somewhat more tentative than those of the compounds previously discussed. Changes in ring D appear to result in larger and less predictable shifts of distant carbons than did changes in rings A and B for compounds **1–4** and **6–13**, possibly as a result of strain associated with the C–D ring junction.

The off-resonance decoupled spectra allowed the usual assignments of methyl and quaternary carbons

The A and B ring carbons in **12** are as for cholestan-3-one (**2**), and, indeed, the chemical shifts for C-1 to



(except for **15**, **21**, and **23**, which were too insoluble to allow measurement of such spectra). In addition, a fairly confident assignment of the high-field methine resonance of C-8 (and C-10 for **18**) could be made for **18** and **20** (Figure 2) from off-resonance decoupled spectra. For example, in the spectrum of **20** (Figure 2c), the resonances for C-1 and C-8 are not resolved, but in the off-resonance decoupled spectrum this pair of signals has approximately the same intensity as the other methylene resonances, as expected for superimposed methine and methylene signals. On the other hand, in the spectra of **18** (Figure 2a) the two almost coincident methylene resonances C-6 and C-16 do give a more intense off-resonance decoupled signal. The signals for the C-8, C-9, C-10, and C-14 carbons in **18** show rough doublets when partially decoupled, confirming their assignment as methine resonances.

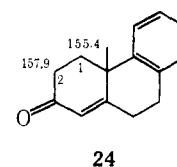
Base-catalyzed deuterium exchange of **16** led to changes in the resonances at 158.7, 160.0, and 166.1 ppm. These resonances are assigned to C-2, C-15, and C-21, respectively. The two angular methyl resonances (C-18 and C-19) could be distinguished both by comparison with previously assigned steroids **12** and **13** and by the absence of the C-19 signal in **18**, **19**, and **22**. The spectra of the 19-nor steroids similarly allowed distinction of the two quaternary resonances (C-10 and C-13).

As in the previous steroids, the two high-field methylenes of **14** and **17**–**23** correspond to C-11 and C-15.

They can be distinguished by the 5–6 ppm downfield shift of C-11 in the 19-nor steroids resulting from the absence of steric interaction with the C-19 methyl group. The large shifts of C-15 in **16** and C-11 in **15** confirm this assignment. Apart from C-17, the two lowest field, saturated alicyclic resonances are C-9 and C-14. The usual chemical-shift considerations clearly differentiate these for most cases. Thus the well-known effect of β -hydroxy (**15** compared to **14**) and β -methyl substitution (**18**, **19**, and **22** compared to **17**, **20**, and **21**) result in downfield shifts for C-9, while γ -gauche steric interactions with the 7 α -methyl group in **21** and **22** cause upfield shifts of carbons 5, 9, and 14 when compared with **20** and **19**.

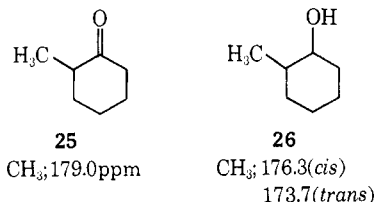
More intricate is the difficult problem of assigning the group of resonances from 150 to 162 ppm. These consist of the ring A and B (saturated) carbons which should remain unchanged in the normal steroids (**14**–**17**, **20**) and the C-12 and C-16 resonances, which are expected to vary considerably as ring D is changed. The signal at 153.7 ppm in **14** is downfield in **15**, and can be assigned to C-10, while the acetylation shift (**20**, **20a**) allows assignment of C-16 in the testosterone (**19** to **22**) at 162.1 ppm. The C-12 signal in the progesterones (**14**–**16**) can be located by the downfield shift of this peak in **15**. The chemical shifts of C-12, C-14, C-15, and C-16 in **17** and **18** should be unchanged from those in **12**, **13**, and **23**; they have been assigned on this basis.

There remain five relatively constant resonances in compounds **14**–**17** and **20** at *ca.* 157, 158, 159, 160, and 161 ppm; these must correspond to C-1, C-2, C-6, C-7, and C-8. Carbons 2 and 8 have been identified at 159 and 158 ppm by deuteration (**16**) and off-resonance decoupling, respectively. Comparisons with the model



compound **24** and other steroids suggest C-1 for the 157 ppm peak, and this is confirmed by the strong upfield shift of C-1 on removal of the C-19 methyl group in **18**, **19**, and **22**. Downfield shifts of C-6 and C-8 are expected both from removal of the C-19 methyl group (loss of γ -gauche steric effect) and introduction of the 7 α -methyl group (β effect). Consistent assignments can only be achieved if the resonances at 160 and 161 ppm are assigned to C-6 and C-7. The position of the C-7 absorption remains essentially unchanged in the series **14**–**22**, as befits its rather isolated location. Even introduction of the 7 α -methyl group has little effect, and this is in fact expected by comparison with the methylcyclohexanes (–1.1 ppm shift for axial methyl-substituted carbons^{4b}).

The position of the C-12 resonance in the testosterone can now be assigned to the invariant signal at 155.7 ppm. Good model compounds for the C-12 resonances are not available, but comparison of the methyl chemical shifts in **25** and **26** suggests that a downfield shift in the testosterone compared to the 17-keto steroids is expected. Removal of the C-19 methyl group in **18**, **19**, and **22** results in a downfield shift of the C-10 resonance opposite to the usual methyl α effect. This



is, however, expected from comparisons with 1,1-dimethylcyclohexanes.^{4b}

Estrone (**23**) resembles **18** in rings C and D, and also by the absence of the C-19 methyl. The resonances for C-11 to C-18 in **18** appear within a few tenths ppm of resonances in **23**. Carbons 6, 7, 8, and 9 were assigned to the remaining signals as shown in Table II.

Olefinic and Aromatic Carbons. Assignments for the sp² carbons are usually trivial, except for dienes, which can be rather difficult. The double bond carbons appear to be very sensitive even to minor changes in the molecule—both C-5 and C-6 change 1.3 ppm on acetylation of the C-3 hydroxyl group in cholesterol.

The low-field olefinic resonance in cholesterol at 51.3 ppm can be assigned to C-5 from the off-resonance decoupled spectrum. 7-Dehydrocholesteryl acetate (**7a**) shows two additional resonances of which the low-field signal has been assigned to C-8, the high-field one to C-7. Comparison of **7a** with **8** then allowed assignment of the C-22 and C-23 olefinic carbons in **8**. The same procedure was used for assignments of the olefinic carbons of cholesta-3,5-diene (**9**). Carbons 5 and 6 in **10** can be located by comparison with **11a**. The remaining resonances are assigned to C-3 and C-4. These assignments must be considered tentative until more is known about the chemical shift in cyclic olefins.

The aromatic carbon resonances in estrone acetate (**23a**) can be assigned by comparison with the spectrum of estrone. The acetylation shifts for phenol are as follows: C-1, 3.7 ppm; C-2, -6.2 ppm; C-3, 0.6 ppm; C-4, -4.4 ppm. Two of the aromatic resonances of **23a** are seen to be unchanged in **23**, and these are assigned to C-1 and C-5 (*meta* to hydroxyl). Three resonances are downfield 6 ppm in the acetate; the higher field ones were assigned to C-2 and C-4, the lower field one to C-10. The resonance for C-3 moves upfield 5.9 ppm in the acetate, as expected from the data for phenyl acetate and phenol.

Experimental Section

The steroids used in the present study were primarily commercial materials. Acetates were prepared by acetylation with acetic anhydride and pyridine (except for 7-dehydrocholesteryl acetate, cholesteryl acetate, and 3 β -acetoxycholest-5-en-7-one, which were commercial materials; cholestan-3 β -yl acetate, which was prepared by hydrogenation of cholesteryl acetate;¹⁸ and cholestan-3 α -yl acetate, which was prepared by buffered acetolysis of cholestan-3 β -yl tosylate¹⁹). Cholesteryl methyl ether was prepared by unbuffered methanolysis²⁰ of cholesteryl *p*-toluenesulfonate.²¹ Cholestan-3 was prepared by lithium aluminum hydride reduction of cholestan-3 β -yl tosylate. Cholesta-3,5-diene was prepared by copper sulfate catalyzed dehydration²² of cholesterol.

Chemical-shift measurements were made using the digital frequency sweep spectrometer^{5a} with pseudo-random noise-modulated proton decoupling^{7a} as previously described. Steroids were dissolved in dioxane or dioxane-chloroform mixtures; approximately 1.5 ml of 1 *M* solution was usually used, although measurements were performed on solutions as dilute as 0.12 *M*. Generally from 10 to 30 scans were averaged to obtain adequate signal-to-noise on the 1 *M* solutions. Sweep rates of 2 or 4 Hz/sec at 50 or 100 Hz sweep width were routinely used but slower sweep rates were employed when necessary to resolve closely spaced lines. Peak widths were generally 1 to 3 Hz. Several of the steroids (ergosterol, 7-methyltestosterone, estrone) were insufficiently soluble to allow measurements to be carried out as above, so that higher sweep rates and greater sweep widths were employed. The chemical shifts for these substances are slightly less accurate than for the remainder of the steroids.

Deuteration of Keto Steroids. A sample of steroid (1.0 g) was dissolved in 10 ml of dioxane, and 5–7 ml of deuterium oxide and 0.10 g (0.01 g for **16**) of sodium methoxide were added. After 14 hr at 80° (5 hr for **16**) the solution was poured into slightly acidified water, and the organic material extracted with ether. The ethereal solution was washed with saturated sodium chloride solution and dried, and the solvent evaporated. The residue was purified by crystallization from a suitable solvent (deuterated **16** was used crude). The ¹³C nmr spectra confirmed complete deuteration at the expected position(s).

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