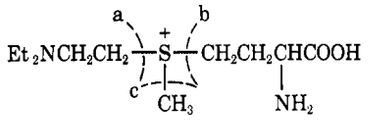
water. For example, the tenfold difference in competition factor for imidazole–aziridinium ion *vs.* imidazole–iodoacetate arises from the nearly tenfold difference in k_w for the alkylating agents. It is thus a serious question whether it is, in this case, more revealing to compare k_a's or competition factors. Since there is no compelling reason, other than convenience and custom, to pick water as the reference nucleophile, it is not the absolute magnitude of the nucleophilic constants which is significant but their relative order. Thus the significant change in relative rates for "hard" and "soft" nucleophiles with the iodoacetate alkylating agents, like the marked change in relative order for the thiosulfate ion in nucleophilic attack at sp^2 *vs.* sp^3 carbon, must signal major changes in the factors affecting the transition states involved.

The alkylation of methionine monopeptide provided a difficult problem in isolating the reaction product. The primary product is a sulfonium salt. This product can then undergo hydrolytic cleavage at each of the three C–S^+ bonds to regenerate methionine or to form homoserine (or its lactone) or alkylated homocysteine.[8,18] Our data indicate that the cleavage conditions we used favored removal of the diethylamino group (a, 12.1%) followed by the amino acid residue (b, 8.6%) and least of all the methyl group (c, 1.2%), or a:b:c 55:40:5, respectively. This ratio, which involves the assumption that the methionine sulfoxide measured by amino acid analyzer must have arisen from IIc, is supported by the fact that the sum of the



IIc

cleavage products observed (21.9%) is in good agreement with the extent of alkylation measured indirectly by colorimetry (23%).

Registry No.—Ia, 6367-14-2; Ib, 10061-65-1; Ic, 29744-03-4; Id, 6367-11-9; Ie, 6367-17-5; If, 38615-99-5; IIa, 38616-00-1; IIb, 38616-01-2; IId, 38616-02-3; IIf, 38616-03-4; L-homocystine, 626-72-2; L-homocystine dimethyl ester hydrochloride, 38616-04-5; *N,N'*-diacetyl-L-homocystine dimethyl ester, 38616-05-6; *N,N'*-diacetyl-L-homocystinmethylamide, 38616-06-7; alkylated L-homocysteine, 38616-07-8; diethylaziridinium ion, 18899-07-5; iodoacetic acid, 64-69-7; iodoacetamide, 144-48-9.

(18) W. B. Larson, E. Gross, C. M. Foltz, and B. Witkop, *J. Amer. Chem. Soc.*, **84**, 1715 (1962).

Carbon-13 Magnetic Resonance Spectroscopy of Steroids. Estra-1,3,5(10)-trienes[1]

THOMAS A. WITTSTRUCK* AND KENNETH I. H. WILLIAMS

The Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545

Received October 16, 1972

The natural abundance carbon-13 magnetic resonance spectra of estra-1,3,5(10)-triene and 17 derivatives are reported. Substituent effects upon the chemical shifts of each carbon atom are determined and discussed in terms of factors known to influence ^{13}C chemical shifts.

With the advent of instrumentation for the determination of high-resolution nuclear magnetic resonance spectra of ^{13}C in natural abundance, a number of papers have appeared describing its application to the structural elucidation of natural products. The techniques of ^{13}C magnetic resonance spectroscopy appear to promise to have as great an impact upon such studies as did the techniques of proton magnetic resonance in the last decade. Just as steroids provided model compounds from which much was learned with regard to the relationship between the observed nmr parameters (chemical shifts and spin–spin coupling constants) and molecular structure in proton spectroscopy,[2] so too this class of compounds, because of their well-defined structures, promises to aid in relating the ^{13}C magnetic resonance parameters to molecular structure.

In 1969, Reich, *et al.*, published the first extensive ^{13}C investigation of steroids, examining chiefly the spectra of cholestane derivatives.[3] These authors indicated that in general carbon resonances are far more informative than proton resonances for structural analysis of steroids.

Since we have available to us a large number of steroids of verified structure, incorporating a variety of the common functional groups, we have initiated a study of the carbon-13 magnetic resonance spectra of steroids. It is hoped that through these studies the nature of substituent effects upon ^{13}C chemical shifts may be better understood. In the present paper, the ^{13}C spectra of a number of derivatives of estra-1,3,5(10)-triene are reported, together with correlations of chemical shifts with carbon atoms of the steroids. Substituent effects upon the chemical shifts of the aromatic carbon of ring A are discussed, as well as a preliminary report of substituent effects upon the atoms of the nonaromatic portion of the molecule. A fuller discussion of these latter substituent effects will be presented in a later paper.

Experimental Section

The steroids used in this study, all known compounds, are listed in Table I. They were dissolved in dioxane, whose ^{13}C signal was used as the internal lock signal. Concentrations of steroids ranged from about 0.06 *M* to about 0.35 *M*. Three milliliters of solution was used in each case for the analysis, and was contained in a 12-mm o.d. sample tube. The tube was spun at 18 rps during the analysis, which was performed at room temperature.

Spectra were obtained at 25.1 MHz using a Varian Associates HA-100-15 spectrometer, together with a Varian Associates

(1) Supported by The Public Health Service, Research Grants No. GM16928 and AM13582.

(2) N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, San Francisco, Calif., 1964.

(3) H. J. Reich, M. Jautelat, M. T. Messe, F. J. Weigert, and J. D. Roberts, *J. Amer. Chem. Soc.*, **91**, 7445 (1969).

TABLE I
STEROIDS INVESTIGATED

Compd no.	Compd
I	Estra-1,3,5(10)-triene
II	3-Hydroxyestra-1,3,5(10)-triene
III	17β-Hydroxyestra-1,3,5(10)-triene
IV	Estra-1,3,5(10)-trien-17-one
V	3,17β-Dihydroxyestra-1,3,5(10)-triene
VI	3-Hydroxyestra-1,3,5(10)-trien-17-one
VII	3,17α-Dihydroxyestra-1,3,5(10)-triene
VIII	17β-Acetoxyestra-1,3,5(10)-triene
IX	3,17β-Diacetoxyestra-1,3,5(10)-triene
X	3,17α-Diacetoxyestra-1,3,5(10)-triene
XI	3-Acetoxyestra-1,3,5(10)-trien-17-one
XII	3-Iodo-17β-acetoxyestra-1,3,5(10)-triene
XIII	3-Methoxy-17β-hydroxyestra-1,3,5(10)-triene
XIV	3-Methoxyestra-1,3,5(10)-trien-17-one
XV	3-Methoxy-16β-hydroxyestra-1,3,5(10)-triene
XVI	3-Methoxyestra-1,3,5(10)-trien-16-one
XVII	3-Methoxy-16α,17α-dihydroxyestra-1,3,5(10)-triene
XVIII	3-Hydroxyestra-1,3,5(10)-trien-16,17-dione

V-3530 ^{13}C wide sweep accessory and a Varian Associates C-1024 time averaging computer. In addition, a Varian Associates V-3512 heteronuclear decoupler was used to provide both continuous wave and incoherent proton decoupled spectra.

The ^{13}C chemical shifts of steroids extend over a range of about 225 ppm. For this reason the spectra were obtained in segments. The segment occurring at lowest field contains peaks for carbonyl carbons (*ca.* 220–218 ppm downfield from TMS). The second segment contains the peaks for aromatic carbons and olefinic carbons when present, and extends from about 160 to 110 ppm.

The third segment contains peaks for carbinol carbons, in the vicinity of 80 ppm, while the fourth segment, extending from about 55 to 10 ppm, contains the peaks for the remaining nonfunctionalized carbon atoms of the steroid. The second and fourth segment were investigated for each compound, the others only when functional groups were present which indicated a need for their investigation.

Each segment was initially scanned over a range of 1500 Hz, at a sweep rate of 30 Hz/sec. The number of scans required to obtain a spectrum is primarily a function of concentration of steroid in solution, and ranged from about 100 to 1600 scans. In most cases relevant portions of each segment were then rescanned at slower sweep rates (*ca.* 10 Hz/sec) using narrower sweep widths (*e.g.*, 500 Hz). In this manner sufficient resolution was obtained for most steroids studied here to completely characterize each peak. In only a few instances did it prove impossible to resolve peaks for different carbon atoms.

The chemical shifts were measured in hertz from the lock signal (dioxane) and when feasible also from the peak for internal TMS. They are reported in parts per million downfield from TMS = 0 and are presented in Table II.

Results and Discussion

The unsubstituted estra-1,3,5(10)-triene (I) may be regarded as the parent of the steroids used in this study. Four steroids containing only one substituent each, as well as a number containing two substituents each, were available. By comparisons of the spectra of the steroids differing by only one substituent and an estimate of anticipated substituent effects, peak assignments could be made for each of the carbon atoms in a given steroid. As illustrative of the approach used in making peak assignments, the interpretation of the spectrum of I is described.

The initial assignments for the carbon atoms of the aromatic ring may be made by comparing the spectrum of I with that of 3-hydroxyestra-1,3,5(10)-triene (II). The peaks for the aromatic carbons of II are shifted relative to those of I by the influence of the

TABLE II
^{13}C CHEMICAL SHIFTS OF AROMATIC STEROIDS[a]

C	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII
1	125.96	126.88	126.12	126.31	126.90	126.90	127.17	126.19	127.02	126.91	127.07	128.51	126.69	127.32	126.95	126.53	126.81	126.91
2	126.22	113.40	126.44	126.69	113.51	113.45	113.71	126.43	119.56	119.52	119.68	135.55	112.02	112.49	112.34	112.25	112.26	113.81
3	126.22	155.59	126.44	126.69	155.64	155.76	155.73	126.43	149.78	149.76	149.90	91.63	158.26	158.92	158.70	158.71	158.44	156.06
4	129.73	115.76	129.76	129.86	115.92	115.86	116.05	129.72	122.33	122.28	122.44	138.57	114.25	114.74	114.57	114.49	114.37	115.98
5	137.40	138.42	137.50	137.49	138.43	138.20	138.66	137.35	138.46*	138.45*	138.25	140.36	138.10	138.43	138.60	138.22	138.29	138.20
6	39.48*	39.94*	39.69	39.06	39.78	39.33	40.13	39.27	39.14	39.49	38.93	38.90	39.80	39.36	39.78	39.08	39.90	38.31
7	26.87	27.25	26.98	27.33	27.10	27.41	27.05	28.21*	27.82*	28.49	27.07	27.62	27.12	27.30	27.03	27.05	28.98*	27.43
8	29.95	30.10	30.23	30.04	30.18	30.19	30.37	30.14	30.05	30.06	30.02	29.55	30.38	30.26	30.34	30.35	30.54	29.96
9	45.12	44.64	45.53	45.43	44.79	44.95	44.59	45.23	44.92	44.55	45.05	44.81	44.87	45.00	44.71	44.73	44.59	44.45
10	141.47	132.44	141.39	141.10	132.28	131.92	132.64	141.19	138.64*	138.70*	139.01	141.02	133.14	133.18	133.57	133.04	133.37	131.50
11	28.56	28.67	28.18	26.31	28.01	26.44	28.92	26.71	26.74	26.57	26.53	26.41	28.09	26.57	28.71	28.91	26.54*	26.23
12	40.89	40.96	37.88	32.56	37.61	32.51	33.14	37.71	37.70	32.64	32.48	37.57	37.75	32.43	40.98	39.08	35.94	31.64
13	41.44	41.47	44.08	48.37	43.88	48.33	46.22	43.66	43.66	45.57	48.23	43.54	43.90	48.32	39.41	39.86	46.26	48.71
14	54.19	54.05	51.25	51.26	50.77	51.11	48.39	50.64	51.66	49.96	52.02	50.54	50.90	51.10	52.22	51.34	47.10	43.20
15	25.54	25.56	23.90	22.16	23.66	22.17	24.88	23.88	23.79	24.82	22.14	23.76	23.74	22.04	37.53	39.08	17.65	36.09
16	20.88	20.90	31.25	35.93	30.99	35.86	32.36	28.09*	28.18*	30.48	35.85	28.16	31.17	35.87	71.31			204.58
17	39.24*	39.31*	81.86	218.92	81.93	219.28	79.70		82.96	82.22		83.07		219.08	53.73	56.15		204.58
18	17.56	17.64	11.61	13.92	11.47	13.92	17.53	12.46	12.35	16.77	13.84	12.37	11.55	13.76	19.27	18.27	9.72	13.73
								20.82[b]	20.78[b]	20.70[b]	20.70[b]	20.69[b]	55.13[c]	55.28[c]	55.05[c]	55.25[c]	55.20[c]	

[a] In parts per million, downfield relative to internal TMS; solvent dioxane; uncertainty ±0.05 ppm; assignments for signals marked with an asterisk are not certain; omitted values were not observed. [b] –OCOC*H$_3$. [c] –OCH$_3$.

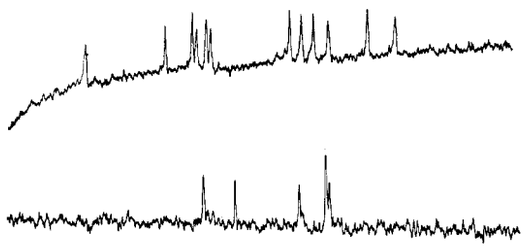


Figure 1.—Incoherent-noise decoupled spectrum of estra-1,3,5(10)-triene. Upper portion, aliphatic region (64.04–4.20 ppm downfield from TMS); lower portion, aromatic region (163.57–103.80 ppm downfield from TMS). The dip on the low-field side of the upper portion results from the proximity of the lock signal, dioxane.

phenolic hydroxyl at carbon-3. In order to estimate the substituent effect of this OH, use was made of phenolic shielding effects reported in the lieterature for a number of simpler molecules.

Comparison of the spectra of *m*- and *p*-cresol with that of toluene shows that the greatest substituent effect from the hydroxyl is observed for the carbon atom bearing this function. The signal for this carbon is shifted downfield 26.7 ppm in *m*-cresol and 27. 2 ppm in *p*-cresol from the value observed for toluene.[4-6] In both cresols the carbon atoms ortho to the OH are shielded by *ca.* 12.8 ppm, while the meta carbons are deshielded by 1–2 ppm and the para carbon is shielded by *ca.* 6.9 ppm.

The incoherent-noise decoupled spectrum of I, reproduced in Figure 1, is illustrative of the spectra obtained in this study. The aromatic segment of the spectrum consists of five peaks, one of which obviously results from the overlap of two unresolved peaks from two different carbon atoms. When the continuous wave decoupled spectrum of this region is obtained it is observed that the two lowest field peaks remain as singlets, whereas the others appear as doublets. Hence these two peaks (at 137.40 and 141.47 ppm) arise from the quaternary carbon atoms of ring A, namely, C-5 and C-10.

In similar fashion it was shown that for II the quaternary carbons give signals at 132.44 and 138.42 ppm. In II, carbon-5 is meta to the phenolic OH at carbon-3 and is expected to be deshielded in comparison to I, whereas carbon-10, which is para to the phenolic OH, is expected to be shielded relative to I. The only assignments consistent with this predicted behavior are those shown in Table II; *i.e.*, for I, δ (carbon-5) 137.40, δ (carbon-10) 141.47, and, for II, δ (carbon-5) 138.42 (deshielded 1.02 ppm), δ (carbon-10) 132.44 ppm (shielded 9.03 ppm).

The peak at 155.59 ppm in the spectrum of II must be assigned to carbon-3, since it is the only peak in this spectrum which is greatly displaced from those in the spectrum of I.

Carbon-1 of II is meta to the phenolic OH at carbon-3 and should be deshielded in comparison to I. The peak at 126.88 ppm of II is assigned to carbon-1, since it is the only unassigned peak of II which is downfield from any of the unassigned peaks of I. As a corollary to this assignment, carbon-1 of I must be assigned to either the peak at 125.96 or one of the overlapping peaks at 126.22 ppm. The peaks at 113.40 and 115.76 ppm of II must then arise from the carbon atoms ortho to the phenolic OH. Both of these carbons are symmetrically disposed with respect to carbon-9, but not with respect to carbon-6. From the data for *m*-cresol it is seen that the signal for the carbon atom between the phenolic and methyl functions occurs further downfield by about 2.4 ppm than that para to the methyl. Thus it seems reasonable to assign the peak at 115.76 ppm of II to carbon-4 and that at 113.40 ppm to carbon-2.

If a tentative assignment of the peak at 125.96 ppm of I is made for carbon-1, it is reasonable to assign one of the peaks at 126.22 ppm to carbon-2 and that at 129.73 ppm to carbon-4. The other of the unresolved peaks at 126.22 ppm of I is then assigned to carbon-3.

In order to corroborate these assignments, the spectra of 17β-hydroxyestra-1,3,5(10)-triene (III) and of 3,17β-dihydroxyestra-1,3,5(10)-triene (V) were compared, as well as those of estra-1,3,5(10)-trien-17-one (IV) and 3-hydroxyestra-1,3,5(10)-trien-17-one (VI). The aromatic segments of the spectra of III and IV are analogous to that of I, while those of V and VI are analogous to that of II, indicating that the effect of the substituent at carbon-17 is of minor importance to the chemical shifts of the aromatic ring A carbons (however, see below). The spectrum of VI has been reported by Reich, *et al.*,[3] and our assignments for VI agree with those obtained by these authors.

Assignments of peaks for the nonaromatic carbon atoms is perhaps best illustrated by reference to Figure 2, in which the appropriate spectral regions of I and III are schematically compared, followed by a comparison of I and II. The continuous wave decoupled spectrum of I shows a quartet centered at 17.56 ppm, which must arise from a methyl group, and is thus assigned to carbon-18. A similar quartet appears in the continuous wave decoupled spectrum of III, at 11.61 ppm. The peak at 41.44 ppm of I was the only peak remaining as a singlet in the continuous wave spectrum of this compound and is thus assigned to the quaternary atom, carbon-13, as, for the same reason, is the peak at 44.08 ppm in the spectrum of III.

By comparing the structures of I and III it is seen that the remaining unassigned carbon atoms may be classified into those expected to be relatively unperturbed by substitution at carbon-17, and those which might be expected to experience a greater effect from that substituent. In the former category are carbon-6, -7, and -9, and perhaps carbon-8 and -11. All other carbons are within three bonds of the substituent at carbon-17.

Using Figure 2, each unassigned peak of I is successively paired with one of the unassigned peaks of III, noting the difference in chemical shifts, $\Delta\delta$, between the pairs. These values of $\Delta\delta$ are the "substituent effects" of the 17β-OH. By this process it is seen that, at most, only six sets of peaks might possibly qualify as being relatively unperturbed by the substituent at carbon-17 of III ($\Delta\delta$ approximately 1 ppm or less). The sets, together with their values of $\Delta\delta$, are (those from III listed first) 45.53–45.12 (0.41), 39.69–39.48 (0.21), 39.69–39.24 (0.45), 30.23–29.95

(4) P. C. Lauterbur, *J. Amer. Chem. Soc.*, **88**, 1838, 1846 (1961).
(5) W. R. Woolfenden and D. M. Grant, *ibid.*, **88**, 1496 (1966).
(6) T. D. Alger, D. M. Grant, and E. G. Paul, *ibid.*, **88**, 5897 (1966).

(0.28), 28.18–28.56 (−0.38), and 26.98–26.87 (−0.11). Any other pairings result in larger values of $\Delta\delta$.

Because of the magnetic anisotropy of the aromatic ring A, it is to be expected that the signal for carbon-9 would be further downfield than that for carbon-8 or -11, while the signal for carbon-6 would be further downfield than that for carbon-7. In ethylbenzene, for instance, the methylene carbon signal occurs 13.5 ppm further downfield than the methyl carbon signal.[7] Furthermore, the secondary or tertiary nature of the carbon under consideration should influence its chemical shift. Thus, for example, the benzylic carbon signal of 2-phenylpropane occurs 5.5 ppm further downfield than the benzylic carbon signal of ethylbenzene.[7] By analogy, it would be expected that the signal from carbon-9 should occur further downfield than that from carbon-6. The signals at 45.12 ppm of I and at 45.53 of III are thus assigned to carbon-9, and the signals at 39.48 or 39.24 ppm of I and 39.69 of III are assigned to carbon-6.

Having made assignments for the benzylic carbons, tentative assignments may be made for carbon-7, -8, and -11, by analogy with ethylbenzene and 2-phenylpropane. The signal for the tertiary carbon-8 should occur further downfield than that for the secondary carbon-11. The signal at 26.87 ppm of I cannot belong to carbon-8, since it is the furthest upfield of the signals as yet unassigned. It must therefore arise from either carbon-7 or -11. The signal for the carbon atom β to the aromatic ring in ethylbenzene occurs 13.5 ppm to higher field than does the signal for the carbon α to the ring. In 2-phenylpropane these same signals are separated by 10.3 ppm.[7] Hence the signal at 26.87 ppm of I is assigned to carbon-7, since the difference between the chemical shift values for this signal and either of those to be assigned to carbon-6 is *ca.* 12.5 ppm. In contrast, had this peak been assigned to carbon-11, the difference between the signal at 26.87 ppm and that assigned to carbon-9 would be 18.25 ppm.

Assignments for carbon-8 and -11 are then made on the following basis. The signal at 29.95 ppm of I is assigned to the tertiary carbon-8, whose signal should occur at lower field than that for the secondary carbon-11, which is assigned the signal at 28.56 ppm.

Assignments for the signals of those carbon atoms of III categorized above as being most influenced by the effect of the substituent at carbon-17 were accomplished by comparing the spectrum of III with that of 19-nortestosterone, whose assignments were established by Reich, *et al.*[3] To a first approximation it may be assumed that the presence of the aromatic ring A in III will not significantly alter the chemical shifts of carbon-12, -14, -15, -16, and -17 (*i.e.*, those carbon atoms of III whose signals remain to be assigned) from the values observed in the spectrum of 19-nortestosterone. On this basis the signal at 81.86 ppm of III is assigned to carbon-17, the signal at 51.25 ppm of III to carbon-14, the signal at 37.88 ppm to carbon-12, the signal at 31.25 ppm to carbon-16, and that at 23.90 ppm to carbon-15. In general, the assumption used here appears to be valid, since the signals for carbon-12 and -16 occur at almost the same chemical shift values in III as in 19-nortestosterone. The signal for carbon-15 is shifted to slightly higher field in III, while

(7) G. B. Savitsky and K. Namikawa, *J. Phys. Chem.*, **67**, 2430 (1963).

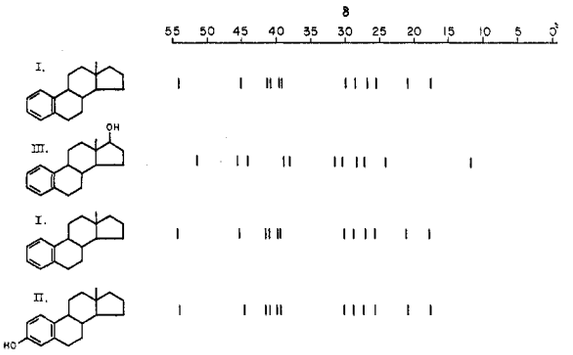


Figure 2.—Aliphatic regions of the ^{13}C spectra of compounds I, II, and III. The scale is parts per million downfield from internal TMS.

that for carbon-14 occurs about 1 ppm further downfield in III than in 19-nortestosterone.

Having made these assignments for III, the corresponding assignments for I may be attempted. This necessitates some knowledge of the effect of the 17β-hydroxy upon the chemical shifts, so that the spectra of I and III may be compared. According to Roberts, *et al.*, the effect of the hydroxyl in cyclopentanol, as compared to cyclopentane, is to shift the peak for the α carbon 48.0 ppm downfield, the peak for the β carbons 9.7 ppm downfield, and the peak for the γ carbons 1.9 ppm upfield.[8] In the present case, carbon-17 is the α carbon, carbon-13, and -16 are the β carbons, and carbon-12, -14, -15, and -18 are the γ carbons. The signals for carbon-13 and -18 have already been assigned, leaving six signals for I to be assigned. From Figure 2 it is obvious that the signal at 54.19 ppm of I must be assigned to carbon-14, and that at 25.54 ppm to carbon-15, since both of these signals should experience upfield shifts in going from I to III. This leaves four signals, at 40.89, 39.48, 39.24, and 20.28 ppm, in the spectrum of I to assign to carbons-6, -12, -16, and -17. Since carbon-16 is β to the hydroxyl at carbon-17 its signal should shift downfield in analogy with the β-carbon shift of cyclopentanol. From Figure 2 it is seen that the only assignment which will allow a downfield shift for the signal of carbon-16, once the above assignments have been made, is to assign the signal at 20.88 ppm of I to carbon-16.

Assignments of the remaining three signals (for carbon-6, -12, and -17) are tenuous, but since in general signals for carbon atoms in five-membered rings occur at higher field than those in six-membered rings, and since both carbon-12 and carbon-17 of I are methylene carbons symmetrically disposed with respect to carbon-13, the signal at 40.89 ppm of I is assigned to carbon-12. The signals for carbon-6 and -17 of I are not assigned with certainty.

Remaining assignments for II were made by comparison of its spectrum with that of I.

By a process comparable to the above, assignments were made for IV, using the comparison with the spectrum of 19-norandrostene-3,17-dione of Reich, *et al.*,[3] as well as the carbonyl substituents effects found in cyclopentanes.[9] The assignments for V and VI were made by comparing their spectra with those of III

(8) J. D. Roberts, F. J. Weigert, J. I. Kroschwitz, and H. J. Reich, *J. Amer. Chem. Soc.*, **92**, 1338 (1970).

(9) F. J. Weigert and J. D. Roberts, *ibid.*, **92**, 1347 (1970).

and IV, respectively. The spectrum of VII was assigned by comparison to those of II and V.

The spectrum of VIII was assigned by comparison with those of I and III, with the aid of the effects of the acetate function upon cyclopentyl chemical shifts reported by Christl, *et al.*[10] The spectra of IX, X, and XI were analyzed by comparison with that of VIII, and a knowledge of the acetate effect upon the chemical shifts of aromatic carbons.[11] The effect of the iodo substituent, needed for the interpretation of the spectrum of XII, was obtained from the paper of Lauterbur.[12] The interpretations of the spectra of XIII and XIV, both of which contain 3-methoxy substituents, were made in comparison with those of III and IV, using the methoxy substituent effect upon aromatic carbon chemical shifts reported by Maciel and Natterstad.[11]

Substituent Effects.—The substituent effects for carbon-13 magnetic resonance chemical shifts deduced for this study are presented in Table III. The uncertainty in these values is ±0.10 ppm. A number of these substituent effects are labeled "directly evaluated," meaning that they were obtained by subtraction of chemical shifts for compounds differing by a single substituent. For instance, the 17β-OH effects were obtained by subtracting chemical shifts for I from those for III, or of II from V. Other substituent effects listed in Table III were obtained by making use of these directly evaluated substituent effects to predict the spectrum of some compound *not* investigated here, whose values were then subtracted from those of a compound which was available. The substituent effects for 16-keto, 16β-OH, 16α,17α-di-OH, and 17α-OAc were evaluated in this manner.

In a few cases the substituent effect could be directly evaluated by subtraction of chemical shifts for I from those of a steroid bearing only the substituent of interest. For instance, the 3-OH effect can be directly evaluated from II − I. When these values are compared with those for the same substituent obtained by subtraction of chemical shifts of a monosubstituted steroid from those of a disubstituted steroid (*e.g.*, V − III, or VI − IV) it is seen that agreement is generally good, although small, but puzzling, discrepancies do occur. For example, the 3-OH effect upon the chemical shift of carbon-5 of VI appears to be 0.31 ppm less than that observed in II, which is outside the uncertainty limits of ±0.10 ppm. Similar small discrepancies are observed for the substituent effects at other carbons, and for other substituent effects. Whether this observation implies that the presence of a second, remote substituent has an influence upon the effect of the first must await the determination of substituent effects to a greater accuracy than observed here.

Ring A Substituents.—The effects of substituents upon the chemical shifts of carbon atoms in aromatic rings have been the topic of a number of investigations. The largest variations in $\Delta\delta$ have been found for the carbon bearing the substituent. This reflects the influence of inductive and resonance effects of the substituent on the electronic environment of this carbon, as well as purely magnetic effects of the substituent.[11]

(10) M. Christl, H. J. Reich, and J. D. Roberts, *J. Amer. Chem. Soc.*, **93**, 3463 (1971).
(11) G. E. Maciel and J. J. Natterstad, *J. Chem. Phys.*, **42**, 2427 (1965).
(12) P. C. Lauterbur, *ibid.*, **38**, 1606 (1963).

TABLE III
SUBSTITUENT EFFECTS[a]

C	3-OH[b] II–I	3-OH[b] V–III	3-OH[b] VI–IV	17β-OH[b] III–I	17β-OH[b] V–II	17α-OH[b] VII–II	16β-OH	16α,17α-diOH	17β-OAc[b] VIII–I	3-OAc[b] XI–IV	3-OAc[b] IX–VIII	17α-OAc	3-I XII–VIII	3-OMe[b] XIII–III	3-OMe[b] XIV–IV	17-Keto[b] IV–I	17-Keto[b] VI–II	16-Keto	16,17-diketo[b] XVIII–II
1	0.92	0.78	0.59	−0.16	0.02	0.29	0.10	−0.04	0.13	0.76	0.83	1.02	2.32	0.57	1.01	0.35	0.02	−0.30	0.03
2	−12.82	−12.93	−13.24	0.22	0.11	0.31	0.54	0.46	0.21	−7.01	−6.87	0.17	9.12	−14.42	−14.20	0.47	0.05	0.45	0.41
3	29.37	29.20	29.07	0.22	0.05	0.14	0.66	0.43	0.21	23.21	23.35	0.19	−34.80	31.82	32.23	0.47	0.17	0.67	0.47
4	−13.97	−13.84	−14.00	0.03	0.16	0.29	0.35	0.25	−0.01	−7.42	−7.39	−0.06	8.85	−15.51	−15.12	0.13	0.10	0.23	0.22
5	1.02	0.93	0.71	0.10	0.01	0.24	0.60	0.29	−0.05	0.76	1.11	−0.06	3.01	0.60	0.94	0.09	−0.22	0.22	−0.22
6	0.46	0.09	0.27	0.21	−0.16	0.19	0.19	0.31	−0.21	−0.13	−0.13	0.14	−0.37	0.11	−0.30	−0.42	−0.61	−0.50	−1.63
7	0.38	0.12	0.08	0.11	−0.15	−0.20	0.02	1.97	1.34	−0.26	−0.39	2.01	−0.59	0.14	−0.03	0.46	0.16	0.04	0.18
8	0.15	−0.05	0.15	0.28	0.08	0.27	0.24	0.44	0.19	−0.02	−0.09	0.20	−0.59	0.15	0.22	0.09	0.09	0.25	−0.14
9	−0.48	−0.74	−0.48	0.41	0.15	−0.05	0.25	0.12	0.11	−0.38	−0.31	−0.26	−0.42	−0.66	−0.43	0.31	0.31	0.27	−0.19
10	−9.03	−9.11	−9.18	−0.08	−0.16	−0.20	0.35	0.15	−0.28	−2.09	−2.55	−0.22	−0.17	−8.25	−7.92	−0.37	−0.52	−0.18	−0.94
11	0.11	−0.17	0.35	−0.38	−0.66	0.25	0.24	−1.93	−1.85	0.22	0.03	−2.02	−0.30	−0.09	−0.26	−2.25	−2.01	0.44	−2.44
12	0.07	−0.27	−0.05	−3.01	−3.35	−7.84	0.22	−4.84	−3.81	−0.08	0.01	−8.87	−0.14	−0.13	−0.13	−8.33	−8.45	−1.68	−9.32
13	0.03	−0.20	−0.04	2.64	2.41	4.75	−1.85	5.00	2.22	−0.14	0.00	4.13	−0.12	−0.18	−0.05	6.93	6.86	−1.40	7.24
14	−0.14	−0.48	−0.15	−2.94	−3.28	−5.66	−1.62	0.86	−3.55	0.76	1.02	−5.25	−0.10	−0.35	−0.16	−2.93	−2.94	−2.50	−10.85
15	0.02	−0.24	−0.01	−1.64	−1.90	−0.68	12.15	4.45	−1.66	−0.02	−0.09	−0.63	−0.12	−0.16	−0.12	−3.38	−3.39	13.70	10.53
16	0.02	−0.26	−0.07	10.37	10.09	11.46	50.51		7.21	−0.08	0.09	9.51	0.07	−0.08	−0.06	15.05	14.96		183.68
17	0.07	−0.03	−0.36	42.62	42.61	40.39									0.16	179.68	179.97		165.27
18	0.08	−0.14	0.00	−5.95	−6.17	−0.11	1.77	−7.78	−5.10	−0.08	−0.11	−0.68	−0.09	−0.06	−0.16	−3.64	−3.72	0.77	−3.91

[a] In parts per million; negative sign represents an upfield shift; uncertainty ±0.10 ppm. [b] Directly evaluated; see definition in text.

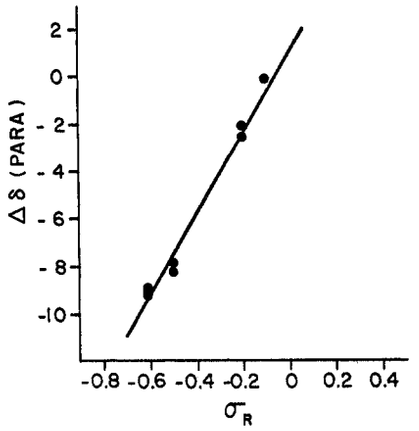


Figure 3.—Substituent effects at carbon-10 [Δδ(para)] *vs.* Taft's σ_R values. Δδ (para) = (17.94 σ_R + 1.42) ± 0.14.

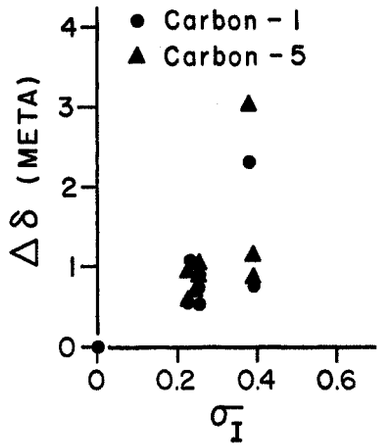


Figure 4.—Substituent effects at carbon-1 and -5 [Δδ(meta)] *vs.* Taft's δ_I values.

Spiesecke and Schneider pointed out that, although resonance effects, when possible, contribute substantially to the substituent effect at the ortho carbon, the magnetic field effects of the substituent appear to outweigh its inductive effects at this carbon.[13] These authors were unable to correlate the substituent effects at the meta carbon with electronegativity of the substituent, and concluded that inductive effects do not extend to these carbon atoms. Lauterbur, however, reported that Δδ of the meta carbons is inversely proportional to σ_I for certain substituents.[14] Maciel and Natterstad believed that the meta carbons are far enough away from the substituents to preclude appreciable magnetic field effects, but found no correlation between Δδ for these carbons and σ_I.[11]

There is general agreement that the changes in chemical shifts of the para carbon primarily reflect resonance effects of the substituent.

All of the ring A substituents reported in this work have been investigated as simple benzene substituents by others. Following the practice of other investigations an attempt has been made to relate the substituent effects determined here to the appropriate σ values.[15,16] Although the number of substituents available for this study was limited, the following correlations were determined. For the substituent effects at carbon-10 (*i.e.*, the para carbon) Taft's σ_R values have been used. Figure 3 shows the plot of Δδ (carbon-10) *vs.* σ_R, which indicates that in all probability resonance effects are indeed the chief contribution to substituent effects at the para position.

In the meta position carbon-1 and -5 are not equivalent owing to the presence of the rest of the steroid molecule attached to the aromatic A ring through carbon-5 and -10. The plots of Δδ (meta) for carbon-1 and -5 *vs.* Taft's σ_I values are shown in Figure 4. Although a general trend is recognizable, *i.e.*, a shift to lower field with increasing σ_I value, the correlation is not so good as that seen for carbon-10. It would appear that inductive effects are present at the meta carbon, but that in addition some other effect contributes to the observed values of Δδ(meta). This is especially obvious in the case of the iodo substituent, where

(13) H. Spiesecke and W. G. Schneider, *ibid.*, **35**, 731 (1961).
(14) P. C. Lauterbur, *J. Amer. Chem. Soc.*, **83**, 1846 (1961).
(15) R. W. Taft, Jr., *ibid.*, **79**, 1045 (1957).
(16) R. W. Taft, E. Price, I. R. Fox, D. C. Levin, K. K. Andersen, and C. T. Davis, *ibid.*, **85**, 709 (1963).

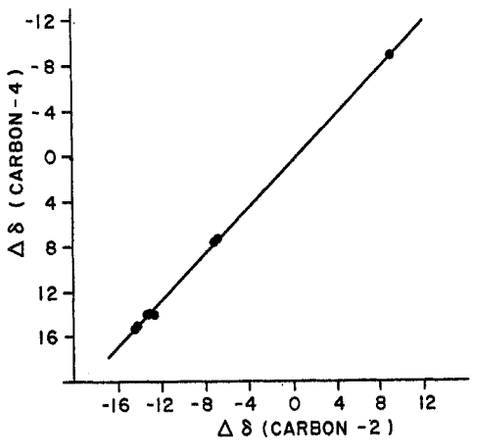


Figure 5.—Substituent effects at carbon-4 *vs.* those at carbon-2, both carbons ortho to the substituent. Δδ(C-4) = [1.03 Δδ (C-2) − 0.47] ± 0.18.

the signal for carbon-5 is deshielded 0.69 ppm more than that for carbon-1 by the iodine at carbon-3.

A similar situation is observed for the ortho carbon-2 and -4, which again are not equivalent to one another. In every case the signal for carbon-4 occurs downfield from that for carbon-2, apparently as a result of deshielding effects of ring B upon carbon-4. In addition, however, upon substitution at carbon-3, carbon-4 experiences a shielding effect which is not observed at carbon-2 (or alternatively, carbon-2 experiences a deshielding effect). This is illustrated in Figure 5, in which Δδ for carbon-4 is plotted *vs.* Δδ for carbon-2. The plot is quite linear, but does *not* pass through the origin, and the slope is not unity. One explanation of such behavior is that the substituent at carbon-3 causes some other site to exert a differential shielding upon carbon-2 and -4, possibly by a resonance interaction between the substituent and this site.[17] Since presumably resonance interactions cannot occur *via* the meta carbon-5, this restricts the site to the para carbon-10 or carbon-9 which is attached to it. However, unless the aromatic ring is asymmetric (*i.e.*, not a perfect hexagon) any effect arising at either carbon-9 or carbon-10 should be equally shared at carbon-2 and -4. It therefore follows that, if the origin of this effect arises from an

(17) T. A. Wittstruck and E. N. Trachtenberg, *ibid.*, **89**, 3510 (1967).

interaction of the substituent with either carbon-9 or -10, ring A cannot be symmetric.

It should be noted here that carbon-3 substituent effects are also observed at carbon atoms outside the aromatic ring, especially at carbon-9, which is para to the substituent.

Ring D Substituents.—In studies of substituent effects upon carbon chemical shifts in cyclohexanes and cyclopentanes, previous authors have largely explained conformational differences in terms of steric effects. Roberts, *et al.*, pointed out that the major influence upon carbon-13 chemical shifts in such compounds arise from inductive, resonance, and steric effects.[8] They further point out that, in such systems as under consideration here, inductive effects may be assumed to be independent of conformation, whereas resonance, and especially steric effects, should be sensitive to conformational changes. With regards to steric effects, the role of the γ carbons and their axial hydrogens appears to be particularly important. Thus, upon introduction of an *axial* hydroxyl into cyclohexane, the interaction between the substituent and the axial γ protons presumably is responsible for the additional 5.4-ppm shielding of the α carbon when compared to the α carbon of equatorial cyclohexanols. This same steric effect in the presence of an axial substituent presumably causes an elongation of the C-β–C-α bond. This results in a shielding of the β carbon, such that the *net* substituent effect at the β carbon is smaller deshielding from an axial substituent than from an equatorial substituent.

In steroids, ring D, like other cyclopentyl rings, assumes a puckered conformation, in which the substituents are never truly axial or equatorial. Thus 1–3 steric interactions should not be expected to be as severe as in the case of cyclohexyl rings. As an example, Christl, *et al.*, interpreted the large difference in substituent effects at the α carbon (for hydroxyl and methyl substituents) between cyclohexane and cyclopentane in terms of lesser steric hindrance in the five-membered rings.[10]

The essence of the above interpretation of carbon-13 shieldings seems to be that, whatever may be the "pure" substituent effect at the α carbon, the α carbon will also experience a shielding effect from the γ carbons and their hydrogens, *which will vary* depending upon the steric relationship between the α and γ carbons. The *net* (observed) substituent effect at the α carbon will thus be different for the *same* substituent, depending upon the stereochemical relationship to other atoms in the molecule.

The same should also be true of the β and γ carbons. What is observed, then, is not just the effect of the isolated substituent (which might be assumed to be constant), but that *plus* the effects of other portions of the molecule which change upon the introduction of the substituent. For this reason, there can be no meaning to an expression such as "the hydroxyl effect," but rather each point of substitution must be considered separately.

With the above in mind, the data of Table III may be examined. The ring D substituents included here have all been previously reported in terms of their effects on cyclopentanes. In general, the substituent effects of Table III agree, at least in sign, with those reported for similarly substituted cyclopentanes.

From proton spectra, it is deduced that for 17α-OH steroids the dihedral angle between the 16α proton and the 17β proton is nearly 90° (signal for 17β proton is a doublet, $J \cong 5$ Hz). From molecular models it may be shown that the only conformation of ring D in which this may be realized is that in which the 17α OH is almost purely axial. In such a conformation, steric interactions between the 17α-hydroxyl and the axial 12α and 14α protons should be nearly maximum.

In the 17β-OH steroids, a rather broadened triplet ($J \cong 8$ Hz) is observed for the proton signal of the 17α proton, which can be interpreted as indicating that the dihedral angle between the 17α proton and the 16α proton is approximately 25°, while that between the 17α proton and the 16β proton is approximately 145°. In this conformation, the 17β OH is tilted only slightly above the plane of the D ring (quasiequatorial), and the strongest steric interactions appear to be those with the 16β proton, the 18-methyl protons, and, to a very slight extent, the 12β proton. The 17-carbon signal of 17α-OH is *less* deshielded than that of 17β-OH by 2.22 pm, which, if the steric interpretation of substituent effects is correct, means that in the 17α-OH steroids the net hydroxyl group interactions with other groups is greater than in the 17β-OH steroids. The above consideration of ring D stereochemistry from proton spectroscopy evidence seems to bear this out. Furthermore, the 17-hydroxy effects at other carbons support this theory. In the 17α-OH case, the shielding effect is much greater at carbon-12 (a secondary γ carbon) than at carbon-16 (also a secondary γ carbon), in accord with the observation that steric interactions between the 17α-OH and the axial 12α and 14α proton were maximum. Thus, from this limited data, it appears that steric effects do play a major role in controlling substituent effects in carbon-13 magnetic resonance.

Because of the relatively small amount of data as yet available, further interpretation of substituent effects upon the nonaromatic carbon chemical shifts will be postponed.

Registry No.—I, 1217-09-0; II, 53-63-4; III, 2529-64-8; IV, 53-45-2; V, 50-28-2; VI, 53-16-7; VII, 57-91-0; VIII, 2755-14-8; IX, 3434-88-6; X, 1474-52-8; XI, 901-93-9; XII, 38605-46-8; XIII, 1035-77-4; XIV, 1624-62-0; XV, 1229-33-0; XVI, 6038-22-8; XVII, 7004-98-0; XVIII, 1228-73-5.