The Correlation of ^{13}C Chemical Shifts of Steroids with Atomic Charges Calculated by the Extended Hückel Molecular Orbital Method*

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^{13}C chemical shifts of several of the sterols and steroidal hormones which have been investigated by Reich et al. (J. Amer. Chem. Soc. **91**, 7445 [1969]) are compared with the atomic charges derived from extended Hückel molecular orbital calculations for these molecules. The linear regression analysis of 126 observations for aliphatic carbons gives a correlation coefficient of 0.81, and a standard error of estimate of 8.2 ppm. The largest discrepancies arise for carbons in the neighbourhood of methyl groups, which cannot be attributed to deficiencies of the extended Hückel theory. The corrections for carbons in the α, β, and γ positions to a methyl group amount to +11.75, −6.44, and +4.45 ppm, respectively. The results are discussed in relation to the structural analysis of steroids.

Introduction

The knowledge of the charge distributions of biomolecules and drugs is extremely important in relation to their characteristic biological effects. The steroid hormones, that is the sexual and adrenal cortex hormones, have been investigated in this respect experimentally and by quantum mechanical methods. Cooper, Norton and co-workers[1] studied the electron density of a number of steroid hormones and related compounds in the solid state by X-rays. Reich et al.[2] determined the ^{13}C nuclear magnetic resonance spectra of a variety of sterols and steroidal hormones. Quantum mechanical calculations of the charge distributions of isolated molecules of steroid hormones have been carried out by Carbò and Pardillos[3], using the Del Re method, and by Repmann[4,5] using the Del Re-Hückel and the extended Hückel method. Janoscheck[6] calculated the electron densities of testosterone by means of the diagonalisation of the overlap matrix of a minimal basis set. In using different quantum mechanical methods of calculation the question arises as to the reliability of the calculated charge distributions. A quantity which is very sensitive to any changes of the overall charge distribution is the dipole moment of the molecule. Observed and calculated dipole moments of steroid hormones are compared in the papers referred to above[4,5]. To test the reliability of the calculated charges attributed to the individual carbon nuclei the observed ^{13}C chemical shifts of these nuclei may be used, because of the charge dependence of the local paramagnetic terms of the carbon shifts. According to Pople's theory[7,8] the local paramagnetic contribution to the ^{13}C chemical shift depends primarily on the local electron density on the carbon atom. But the electron density on the other hand is correlated with the atomic charge on the atom, for if Slater atomic orbitals are used and if a linear dependence of the screening constant on the atomic charge is assumed, a linear charge dependence of the paramagnetic contribution can be derived[8,9]. Concerning the correlation of the electron densities with the atomic charges the results found by Janoscheck[10] may also be mentioned. Although chemical shifts depend on other factors besides electron densities or atomic charges respectively, they should nevertheless be the dominant factor determining chemical shifts.

In this paper the atomic charges calculated by the extended Hückel molecular orbital method for estrone(1), progesterone(2), 11α-hydroxyprogesterone(3), androst-4-ene-3,17-dione(4), 19-norandrost-4-ene-3,17-dione(5), testosterone(6), 19-nortestosterone(7), 7α-methyltestosterone(8), nor-7α-methyltestosterone(9) (Fig. 1) are correlated with the corresponding ^{13}C chemical shifts observed by Reich et alias[2].

Methods and Molecular Geometries

The extended Hückel molecular orbital method (EHMO) has been proposed and described in de-

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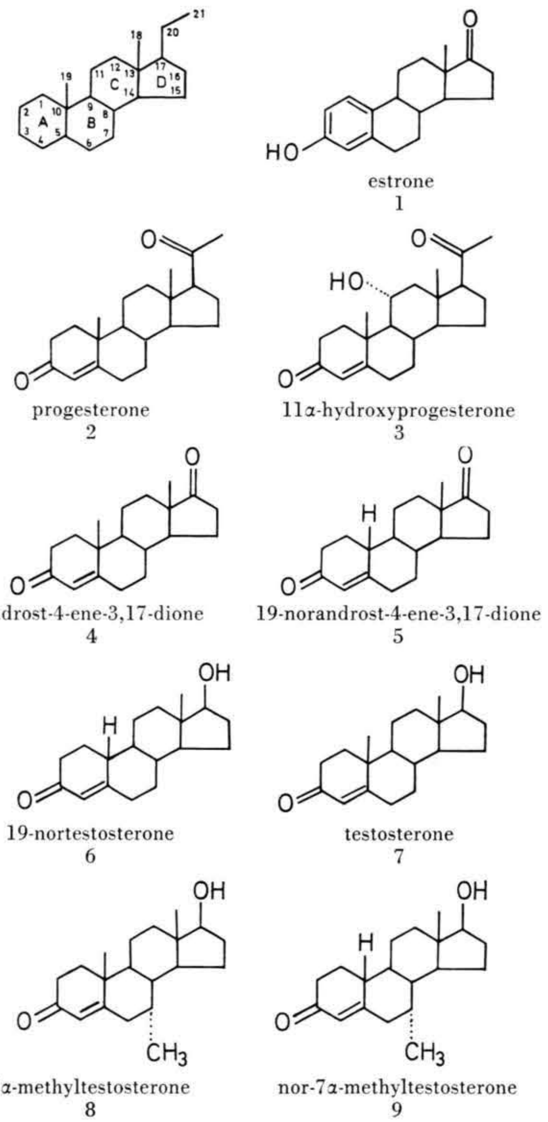


estrone
1

progesterone
2

11α-hydroxyprogesterone
3

androst-4-ene-3,17-dione
4

19-norandrost-4-ene-3,17-dione
5

19-nortestosterone
6

testosterone
7

7α-methyltestosterone
8

nor-7α-methyltestosterone
9

Fig. 1.

tail by Hoffmann[11]. In the linear combinations of atomic orbitals of the steroid molecules investigated here only the 1s Slater orbital for hydrogen (orbital exponent, $\zeta = 1.00$) and the 2s and 2p Slater orbitals for carbon ($\zeta = 1.625$) and for oxygen ($\zeta = 2.275$) are included. Minimization of the total energy leads to the secular equation $(H - S\varepsilon) \cdot C = 0$. S is the overlap matrix and C the matrix of the MO coefficients. The diagonal elements of the EHMO matrix H are given by the negative of the valence state ionization potentials[12]:
$H(1s) = -13.60$ eV, $C(2s) = -21.40$ eV, $C(2p) = -11.40$ eV, $O(2s) = -35.30$ eV, $O(2p) = -17.76$ eV, and the relationship of Wolfsberg and Helmholz[13] $H_{ij} = 0.5\,K(H_{ii} + H_{jj})\,S_{ij}$ is utilized for the off-diagonal elements. S_{ij} is the overlap integral between orbitals i and j, and $K = 1.75$[11]. The atomic charges are obtained via a Mulliken population analysis[14]. All calculations have been carried out using the QCPE program No. 64[15].

The calculations of correlation coefficients, standard errors, and estimates have been performed with the aid of the IBM standard program REGRE for multiple linear regression analysis[16].

The Cartesian coordinates of the carbon and oxygen atoms of the fundamental ring structures have been derived from X-ray crystal and molecular structure analysis. 4-Bromoestradiol[1a] has been taken as a basis for estrone, 12α-bromo-11β-hydroxyprogesterone[17] for all the other molecules. The coordinates of the hydrogen atoms, of the carbonyl oxygen of estrone, and of the atoms of the groups that change from molecule to molecule have been calculated using standard bond lengths and angles[18].

Results and Discussion

The observed ^{13}C chemical shifts in the assignement made by Reich et al.[2] and the corresponding calculated carbon charges of the molecules investigated in this study are given in Table 1. In Fig. 2 the spectroscopic results of progesterone are compared with the charges on the carbon atoms (numbered from 1 to 21) calculated by the EHMO and the "Del Re-HMO"[4] methods, respectively. A certain overall parallelism of shifts and electronic charges can be noticed for the EHMO as well as for the "Del Re-HMO" results. An increase of the chemical shift, that is an increase of the shielding

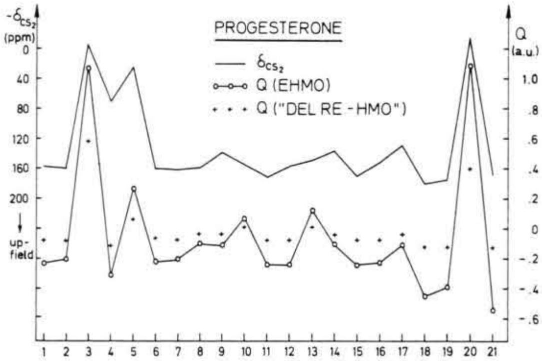


Fig. 2. Comparison of the ^{13}C chemical shifts with the atomic charges calculated by the extended Hückel molecular orbital method and by the Del Re method for progesterone.

Table 1. ^{13}C chemical shifts

Carbon	δ, 1[a]	Q, 1[b]	δ, 2	Q, 2	δ, 3	Q, 3	δ, 4	Q, 4
1	66,3	−1509	156,8	−2305	157,4	−2505	157,8	−2307
2	79,7	−1986	159,9	−2051	159,0	−2050	159,4	−2054
3	37,4	6124	−4,6	10735	−6,0	10734	−5,1	10735
4	77,4	−2212	68,5	−3094	68,1	−3092	68,5	−3097
5	55,1	579	23,2	2729	22,1	2725	23,1	2727
6			160,2	−2215	160,5	−2215	160,8	−2215
7			161,8	−2017	161,9	−2021	161,6	−2014
8			158,6	−961	158,3	−882	157,9	−955
9			138,3	−1089	133,3	−1346	138,4	−1089
10	63,1	−175	153,9	729	152,4	762	154,5	722
11			171,3	−2406	124,3	5180	172,1	−2446
12			156,5	−2435	142,1	−2730	162,2	−2317
13			148,8	1224	148,5	1335	145,3	673
14			136,3	−1043	136,8	−1046	141,6	−809
15			169,5	−2381	169,5	−2378	170,8	−2288
16			153,7	−2306	154,8	−2303	157,4	−2143
17			129,2	−1084	129,4	−1086	−25,9	11710
18			179,5	−4515	178,3	−4515	179,1	−4056
19			175,5	−3878	174,5	−3880	175,5	−3880
20			−14,7	10863	−14,9	10859		
21			168,1	−5341	168,2	−5342		
7α-CH								

[a] ^{13}C chemical shifts in ppm, upfield relative to CS_2. Data from Reich et al.[1]. The numbers 1, 2, ... refer to the numbering of the molecules in Figure 1.
[b] EHMO atomic charges in (atomic units) × 10^4.

of the nucleus, corresponds to an increase of the electronic charge. Larger discrepancies are found in the neighbourhood of the methyl groups attached to C-10 and C-13. Both methods show the effect that in going from C-9 to C-10 and/or from C-14 to C-13 the electronic charges decrease while the chemical shifts increase. This result for atoms in the neighbourhood of a methyl group will be discussed later. The chemical shifts of testosterone are plotted versus the atomic charges in Figure 3. Compared to the aliphatic carbons the carbons of the unsaturated part of ring A of testosterone show smaller chemical shifts. This fact is well known from the investigation of saturated and unsaturated compounds, and finds an explanation in Pople's theory of carbon shifts[8]. Carbon chemical shifts will therefore indicate charge density trends for carbons of the saturated and the unsaturated parts of a molecule seperately. The smallest shielding is found with the carbonyl carbon correlating to the smallest value of electronic charge. The points in the diagram corresponding to aliphatic carbons are grouped according to their binding character[19, 20] as quaternary, methine, methylene, methyl carbons, and the carbon to which the hydroxy group is attached. Carrying out the linear regression analyses (cf. Janoscheck[10]) gives straight lines of the form

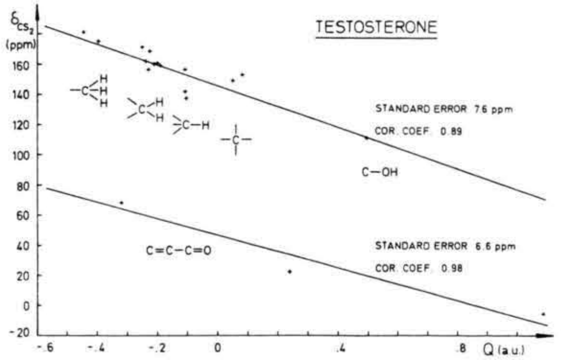


Fig. 3. The ^{13}C chemical shifts of testosterone plotted versus the atomic charges.

$$\delta_{\text{calc}} = a\,Q + b \tag{1}$$

with a correlation coefficient of 0.89 and a standard error of estimate of 7.6 ppm for the aliphatic carbons of testosterone, and of 0.98 and 6.6 ppm for the carbons of the unsaturated part. The correlation coefficients and the standard errors of estimate for the molecules studied in this paper are given in Table 2. Taking into account all 126 observations for aliphatic carbons an overall correlation coefficient of 0.81 is found. This is quite a satisfactory correlation, showing that the charge dependence plays the dominant role in the local and EHMO atomic charges.

δ, 5	Q, 5	δ, 6	Q, 6	δ, 7	Q, 7	δ, 8	Q, 8	δ, 9	Q, 9
165,7	−2121	165,8	−2121	156,7	−2294	156,3	−2306	165,7	−2120
156,1	−2045	156,2	−2046	158,7	−1902	158,4	−2052	156,0	−2046
−5,2	10740	−5,8	10729	−5,2	10966		10733	−5,2	10739
67,8	−3115	68,0	−3116	68,6	−3199		−3092	66,1	−3111
27,4	2883	26,6	2883	22,4	2450		2765	28,5	2922
157,2	−2137	157,2	−2139	160,0	−2119	153,6	−2240	149,5	−2164
160.8	−2016	161,5	−2022	160,6	−2014	161,2	−472	161,6	−473
152,5	−919	151,8	−942	156,7	−1084	151,8	−997	149,4	−962
142,7	−865	143,6	−866	138,2	−1043	145,3	−1149	149,3	−925
150,1	−815	149,9	−815	153,8	817	154,0	725	149,9	−813
166,7	−2396	166,2	−2396	171,6	−2510	171,5	−2439	165,7	−2389
162,3	−2324	155,7	−2399	155,7	−2318	155,7	−2393	155,7	−2400
145.1	672	149,4	541	149,6	491	149,4	552	149,3	552
142,3	−809	143,9	−1029	141,7	−1072	145,9	−1369	145,7	−1370
170,9	−2288	169,2	−2334	169,0	−2263	169,5	−2376	169,9	−2377
157,4	−2144	162,1	−2462	162,1	−2386	162,0	2458	162,2	−2459
−25,8	11719	111,4	4912	111,5	4950		4912	111,5	4912
179,0	−4056	181,4	−4467	181,5	−4448	181,5	4473	181,5	−4474
				175,5	−3973	174,8	−3873		
						179,9	−4611	179,9	−4603

paramagnetic term of the carbon shifts of steroid molecules.

A careful analysis of the calculated [according to Eq. (1)] and observed chemical shifts shows characteristic differences for carbons in the α, β, or γ positions[21] to an axial methyl group. The calculated shifts are systematically too small by 11.75 ppm in the mean in the α positions, and by 4.45 ppm in the γ positions. On the other side the calculated shifts in the β positions are always too large by 6.44 ppm in the mean. The same effect is found qualitatively if the charges calculated by the "Del Re-HMO" method[4] are used. These deviations could be due to defects of the semi-empirical methods used to calculate the charge distributions. But it is more probable that at least the greater part of the differences points to contributions to the chemical shifts which are not related directly to the electronic charges.

Dalling and Grant[22] observed a downfield shift of − 1.08 ppm for axial methyl-substituted carbons of methylcyclohexanes. But an upfield shift of

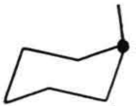
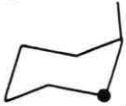
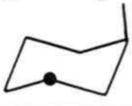
Table 2. Linear regression analyses.

	$\delta_{\text{calc}} = a\,Q + b$		$\delta'_{\text{calc}} = A\,Q + B\,\delta_{\text{c}} + C$		
Aliphatic carbons	Correlation coefficient	Standard error	Correlation coefficient	Standard error	
Progesterone	0.65	10.8	0.90	6.2	
11α-Hydroxyprogesterone	0.70	11.6	0.93	6.4	
Androst-4-ene-3,17-dione	0.78	7.7	0.94	4.4	
19-Norandrost-4-ene-3,17-dione	0.88	5.3	0.95	3.8	
19-Nortestosterone	0.94	5.5	0.97	4.3	
Testosterone	0.89	7.6	0.97	4.5	
7α-Methyltestosterone	0.78	7.4	0.97	3.2	
Nor-7α-methyltestosterone	0.92	6.7	0.97	4.5	
Overall (126 observations)	0.81 $a = -62.2$	8.2 $b = 146.6$	0.93 $A = -70.2$	5.3 $B = 1.1$	 $C = 143.8$
Carbons of groups $\left\{\begin{array}{l}\mathrm{C{=}C{-}C{=}O}\\ \mathrm{C{=}O}\end{array}\right.$	0.98 $a = -54.5$	6.6 $b = 47.0$	0.99 $A = -53.7$	4.4 $B = 1.6$	 $C = 48.8$
Aromatic carbons (estrone)	0.95 $a = -47.2$	5.6 $b = 63.5$			

4.10 ppm in the mean has been found by Reich et al.[2] on the introduction of the C-19 methyl group which is located on the front side (β-side) of the molecule.

Introduction of the axial 7α-methyl group which is located on the back side (α-side) of the molecule results in a small downfield shift of -0.17 ppm in the mean. Calculation of the chemical shift differences from the atomic charges [Eq. (1)] gives practically the same values on substitution of the C-19 and 7α-methyl group (see Table 3). The observed shift differences must arise therefore from contributions to the paramagnetic term which are not related to the electronic charges.

Table 3. Methyl substituent parameters.

Methylcyclohexanes			Steroids		
		$\Delta\delta_{obs}$ (ppm)[a]		$\Delta\delta_{obs}$ (ppm)[b]	δ_c (ppm)
α		-1.08	α	4.10 (C-10) -0.17 (C-7)	$+11.75$
β		-5.17	β	-5.89	-6.44
γ		$+5.39$	γ	$+4.97$	$+4.45$

[a] D. K. Dalling and D. M. Grant: J. Amer. Chem Soc. **89**, 6612 [1967].

[b] H. J. Reich et al.: J. Amer. Chem. Soc. **91**, 7445 [1969].

In β positions the chemical shift change on methyl substitution is observed to be -5.17 ppm and -5.89 ppm downfield for methylcyclohexanes and steroids, respectively. The chemical shift changes calculated from the atomic charges predict a small upfield shift of 0.47 ppm in the mean. Neither sign nor magnitude corresponds to the large shift changes observed in β positions.

In γ positions the atomic charges predict a small upfield shift of 0.52 ppm on methyl substitution while the observed chemical shifts are much larger and amount to $+5.39$ ppm for methylcyclohexanes and to $+4.97$ ppm for steroids. The differences between the mean values of observed and calculated [Eq. (1)] chemical shifts of atoms in β and γ positions to a methyl group are -6.34 ppm and $+4.45$ ppm, respectively (Table 3). Although the EHMO method fails to predict the inductive effect as Sichel and Whitehead[23] have shown, these corrections are too large to be attributed only to the failure of the EHMO method to describe induction along the carbon chain. Cheney and Grant[24, 25] have tried to explain the major parts of the β- and γ-methyl substituent parameters by some type of steric interaction.

Taking into account the corrections δ_c (Table 3) for carbons in the α, β, or γ position to a methyl group in a multiple linear regression analysis gives a linear relationship of the form

$$\delta_{calc} = A\,Q + B\,\delta_c + C\,. \qquad (2)$$

The correlation coefficient rises from 0.81 to 0.93 and the standard error of fit is lowered from 8.2 to 5.3 ppm for aliphatic carbons (Table 2). For carbons of unsaturated groups the values are 0.99 and 4.4 ppm for the correlation coefficient and the standard error, respectively.

The fact that the chemical shift depends only in some part on the atomic charges and that the correction for carbons in the neighbourhood of methyl groups plays a great role may also be seen from Fig. 4 which shows the variation of ^{13}C chemical shifts for quaternary, methine, methylene, and methyl carbons. The breadth of variation is comparatively small if in calculating the chemical shifts only the atomic charges are taken account of according to Equation (1). In this case the shifts of the carbon atoms with different bonding characteristics do not overlap. If the correction δ_c is included the breadth of variation is larger and corresponds more closely to what is observed.

In Fig. 5 examples are given which demonstrate observed and calculated chemical shift differences

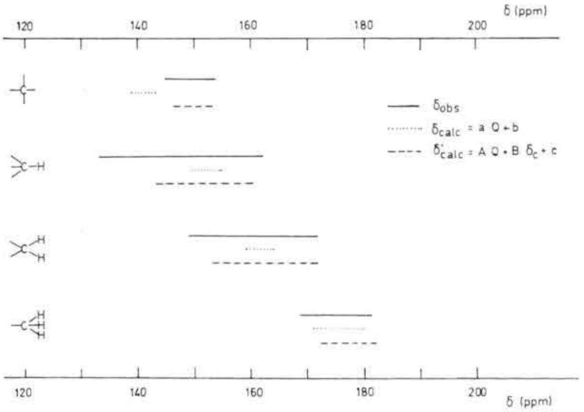


Fig. 4. The variation of ^{13}C chemical shifts for quaternary, methine, methylene, and methyl carbons.

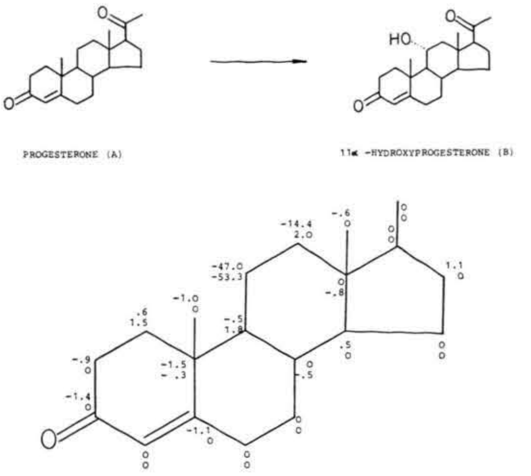


Fig. 5 a. progesterone (A) → 11α-hydroxyprogesterone (B).

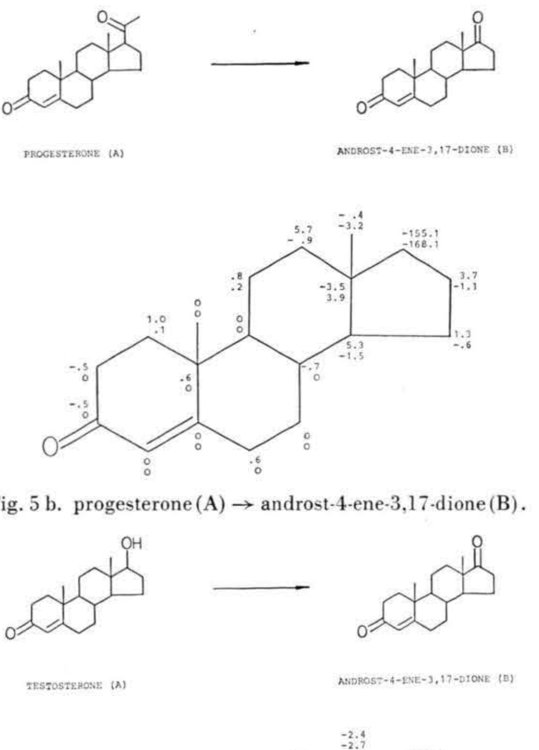


Fig. 5 b. progesterone (A) → androst-4-ene-3,17-dione (B).

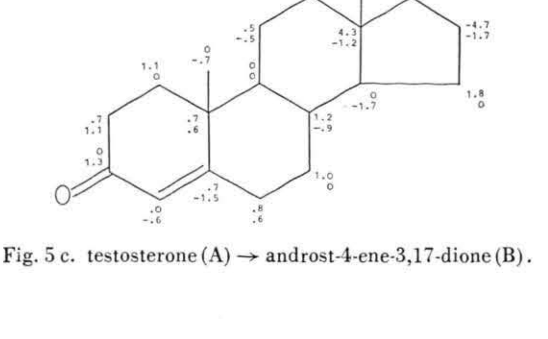


Fig. 5 c. testosterone (A) → androst-4-ene-3,17-dione (B).

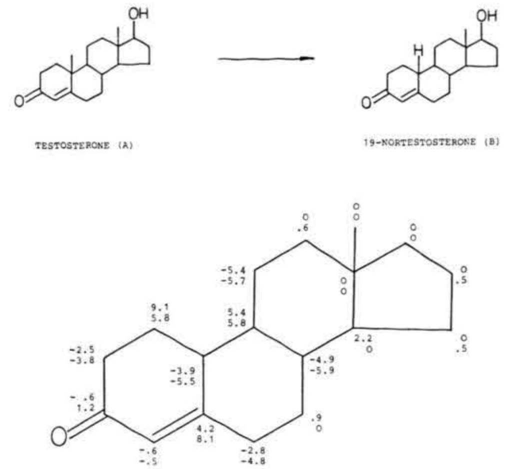


Fig. 5 d. testosterone (A) → 19-nortestosterone (B).

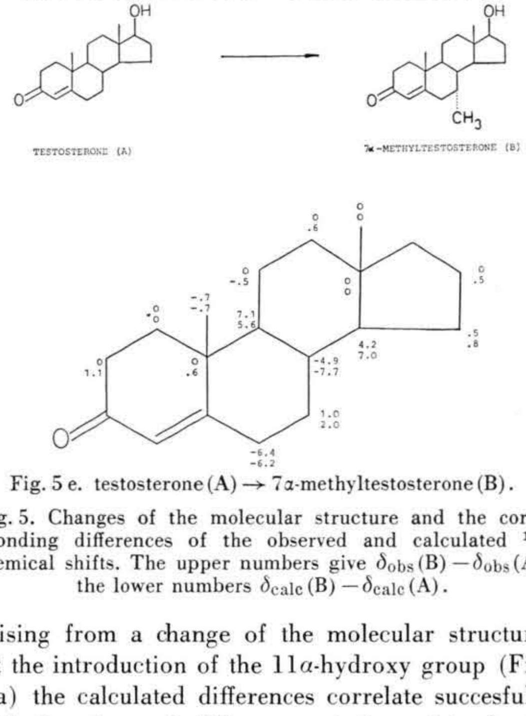


Fig. 5 e. testosterone (A) → 7α-methyltestosterone (B).

Fig. 5. Changes of the molecular structure and the corresponding differences of the observed and calculated ¹³C chemical shifts. The upper numbers give $\delta_{\mathrm{obs}}(\mathrm{B}) - \delta_{\mathrm{obs}}(\mathrm{A})$, the lower numbers $\delta_{\mathrm{calc}}(\mathrm{B}) - \delta_{\mathrm{calc}}(\mathrm{A})$.

arising from a change of the molecular structure. At the introduction of the 11α-hydroxy group (Fig. 5 a) the calculated differences correlate succesfully with the observed differences at the point of substitution, but they fail to correlate at the β positions, especially at C-12. Most of the chemical shift trends at the second atom from the substituent can be explained by the chemical concept of the inductive effect. The failure to predict the inductive effect must be ascribed, according to Sichel and Whitehead[23], to the assumptions of the extended Hückel theory.

Progesterone is turned into androst-4-ene-3.17-dione (Fig. 5 b) in exchanging the $COCH_3$ group at C-17 for a keto group. At the α position the right sign and magnitude of the calculated shift difference are found, but generally not at the β and γ positions. Apart from the failure of the EHMO method to predict the inductive effect the magnetic anisotropy of the keto group for which no correction has been made, may be responsible for this. The same trends may be noticed when the hydroxy group of testosterone is exchanged for the keto group at C-17 of androst-4-ene-3,17-dione (Fig. 5 c), although the right sign and magnitude of calculated shift differences are found at C-16 and C-18. Magnetic anisotropy of the keto group will play a role also in this case. It is remarkable that small chemical shift changes are observed and calculated even in remote positions of rings A and B, e. g. at C-2, C-10, C-6. This fact is of interest for the explanation of certain long-range effects in steroids.

Removal of the methyl group of testosterone (Fig. 5 d) results in larger changes of the chemical shifts in rings A and B and at C-11. Although only mean values for the correction of carbons in α, β, and γ positions to methyl groups are used the agreement between observed and calculated chemical shift differences is good. The same is true when the 7α-methyl group is introduced to testosterone (Figure 5 e).

Herrn Prof. Dr. H. Preuss danke ich für einige sachdienliche Hinweise.

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