Hydrogen Bonds between Sexual Hormones and Nucleobases: NMR Investigations * §

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The hydrogen-bonded complexes of 17α-ethinyl-estradiol, progesterone, and testosterone with adenine- and uracil-derivatives have been investigated by means of nuclear magnetic resonance (NMR) spectroscopy using deutero-chloroform as a solvent. The thermodynamic and NMR-parameters (equilibrium constant K, enthalpy ΔH, entropy ΔS, and relative chemical shift of the complexes $\Delta\delta_c$) for selfassociation and mixed association have been determined. The strongest complex was formed by the phenolic hydroxyl group of estradiol with dimethyl-adenine ($K=14$ mole^{-1}, $-\Delta H=4$ kcal/mole, $-\Delta S=8$ cal/mole·grad). The values were considerably less for the OH-17 group of estradiol and testosterone. The interaction of the keto groups of progesterone and testosterone with nucleobases was very weak: $K<1.0$ mole^{-1}. The biological importance of these results is discussed.

During the last few years many investigations have shown that the sexual hormones enter the nucleus of their target cells and induce changes at the transcriptional and/or translational level [1–3]. It is, however, completely unknown, whether the hormones interact directly with nucleic acids or bind to proteins only. Furthermore, no clear evidence is yet available if their action is mediated by cyclic AMP [4]. For these reasons, several authors [5–9] have studied the molecular interaction of steroid hormones with cellular constituents. It has been suggested that the binding energy results mainly from hydrophobic forces. Moreover, other noncovalent interactions, such as hydrogen bonding and charge transfer, might play an important role for the specific action of each hormone. Kidson, Cohen, and Chin [7], for instance, have demonstrated associations of progesterone and, to a lesser extent, of testosterone and estradiol with PolyG in aqueous solutions. The authors believe, that this interaction is due, mainly, to hydrogen bonding.

In order to gain a more detailed understanding of the molecular mechanism of the steroid action, our recent studies have centered on the interaction of the sex steroids with nucleic acids and their constituents. In the present study the hydrogen bonds between sex steroids and several nucleobases in chloroform have been investigated by means of nuclear magnetic resonance (NMR) spectroscopy. These experiments promise to provide useful information on the nature of steroid-nucleic acid association.

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* Dedicated to Professor Dr. O. Hug on the occasion of his 60th birthday.

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Material and Methods

The following derivatives of sexual hormones and nucleobases have been used 17α-Ethinyl-estradiol ('estradiol'), 17α-ethinyl-estradiol-3-methylester ('mestranol'), progesterone, and testosterone as hormones; 6-amino-9-ethylpurine ('adenine'), 6-dimethylamino-9-ethylpurine ('dimethyladenine'), 1-cyclohexyluracil ('uracil'), and 1,3-dimethyluracil as nucleobases. All the hormones used have been purchased from Schering AG, Berlin, and the nucleobases from Cyclo-Chemical Co., Los Angeles. 1,3-Dimethyluracil has been obtained from Fluka AG, Buchs. The substances have been used without further purification.

Deuterochloroform (Merck, Sharp & Dohme, Munich) has been used as a solvent and stored over molecular sieves (Merck 4 Å) to remove traces of water.

All spectra have been taken on a Varian-HA 100-spectrometer equipped with a variable temperature system and operating in field and frequency sweep mode.

The apparent equilibrium constants K_S and the relative chemical shifts of the selfassociations $\Delta\delta_s = \delta_s$ (complex) $-\delta_M$ (monomer, extrapolated to zero

concentration) have been determined by the method of Lippert [10].

In investigating mixed associations, the concentration of the proton donors has been held constant (0.02 M except for testosterone, in which case it has been 0.1 M). The relative chemical shifts of the donor-protons $\Delta\delta$(donor group) = δ(donor group with acceptor) − δ_0(donor group without acceptor) have been plotted *vs* the variable acceptor concentrations at four different temperatures T. The apparent equilibrium constants K and the relative chemical shifts of the mixed associations $\Delta\delta_c = \delta_c$(complex) − δ_0(without acceptor) have been determined by the method of Benesi and Hildebrand, adapted to NMR spectroscopy [11, 12]. The iterative method of Nakano, Nakano, and Higuchi [13] was used in the case of testosterone. In both methods, the concentrations have been corrected for self-association.

The enthalpies ΔH and the entropies ΔS have been obtained from the van't Hoff plots.

Results

The ligands at C-3 and C-17 of the sexual hormones might both be involved in hydrogen bonding: Two hydroxyl groups in the case of estradiol, two keto groups in the case of progesterone, and one of each, hydroxyl and keto group, in the case of testosterone. Since also the nucleobases possess several hydrogen-bonding groups, various complexes of self-association and mixed association should be expected.

Except for progesterone, dimethyladenine, and dimethyluracil, all the other hormones and nucleobases studied dimerize within the limits of experimental accuracy (s. Table I). The existence of self-associations of a higher order cannot be excluded but does not influence these measurements. In general, the results obtained for the nucleobases are in good agreement with those ones reported previously [14, 15]. The selfassociation constant of mestranol is very small and may be neglected, therefore.

For the interactions of hormones with nucleobases (mixed association), the two active groups of the hormones may be studied independently. Their separation is so large (>10 Å) that they will form "isomeric dimers" [12]. Therefore, the termodynamic and NMR parameters could be calculated indenpen-

		Estradiol	Testosterone	Adenine	Uracil
K_s*	[mole^{-1}]	2.3 ± 1.0	0.4 ± 0.1	2.0 ± 0.2	6.5 ± 1.3
$\Delta\delta_c$	[ppm]	1.5 ± 0.6	4.4 ± 1.1	5.8 ± 0.6[a]	3.3 ± 0.7
$-\Delta H_s\left[\frac{\text{kcal}}{\text{mole}}\right]$		1.2 ± 0.6	3.7 ± 1.1	3.0 ± 0.5	4.1 ± 1.2
$-\Delta S_s\left[\frac{\text{cal}}{\text{mole}\cdot\text{deg}}\right]$		2.3 ± 1.2	14.3 ± 4.3	8.4 ± 1.3	9.9 ± 3.0

Table I. The thermodynamic and NMR parameters of selfassociation. Abbreviations: "Estradiol", 17α-ethinyl-estradiol; "adenine", 6-amino-9-ethylpurine; "uracil", 1-cyclohexyluracil.

[a] Theoretical value of the hydrogen bonding proton of the 6-NH_2-group; experimental value of the 6-NH_2-peak: (2.9 ± 0.3) ppm.

* 25 °C.

Table II. The thermodynamic and NMR parameters of the complexes of the OH-3 group of "estradiol" with nucleobases, calculated from the chemical shifts of the OH-3 and CH-4 prootons. Abbreviations: See Table I; "dimethyladenine", 6-dimethylamino-9-ethylpurine.

Observed proton	Nucleobase		Adenine	Dimethyl-adenine	Uracil	Dimethyl-uracil
OH-3	K*	[mole^{-1}]	exchange	13.5 ± 2.0	exchange	5.1 ± 0.8
	$\Delta\delta_c$	[ppm]	with	5.8 ± 0.3	with	3.8 ± 0.3
	$-\Delta H\left[\frac{\text{kcal}}{\text{mole}}\right]$		$^6NH_2$	4.0 ± 0.8	^3NH	3.6 ± 0.7
	$-\Delta S\left[\frac{\text{cal}}{\text{mole}\cdot\text{deg}}\right]$			8.3 ± 1.7		8.9 ± 1.8
CH-4	K*	[mole^{-1}]	17.0 ± 7.0	16.0 ± 5.0	3.1 ± 1.2	7.3 ± 2.2
	$\Delta\delta_c$	[ppm]	0.13 ± 0.02	0.06 ± 0.01	0.21 ± 0.09	0.06 ± 0.01
	$-\Delta H\left[\frac{\text{kcal}}{\text{mole}}\right]$		2.0 ± 0.8	2.2 ± 0.9	4.5 ± 2.3	3.4 ± 1.4
	$-\Delta S\left[\frac{\text{cal}}{\text{mole}\cdot\text{deg}}\right]$		1.0 ± 0.4	1.3 ± 0.5	12.9 ± 6.5	7.6 ± 3.0

* 25 °C.

dently for each of the two groups of estradiol and testosterone as well.

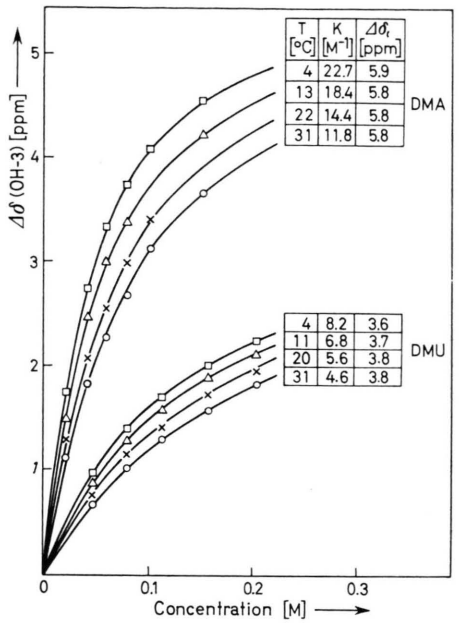

Fig. 1. The relative chemical shift of the phenolic proton of estradiol $\Delta\delta$(OH-3) *vs* the concentration of 6-dimethylamino-9-ethylpurine (DMA) and 1,3-dimethyluracil (DMU), resp. □ △ × ○ Observed values; —— calculated values using the tabelled. Parameters: Temperature T, apparent equilibrium constant K, and relative chemical shift of the complex $\Delta\delta_c$.

The strongest hydrogen bonds have been observed to exist between the phenolic hydroxyl group located at C-3 of estradiol and adenine derivatives (Table II). Since the JOB-plots [16] of the interactions between estradiol and dimethyladenine or dimethyluracil exhibited a maximum at a concentration ratio of 1:1, 1:1 complexes could be assumed for calculating the K-values. In Fig. 1, the experimental and "theoretical" values of $\Delta\delta$(OH-3) are plotted *vs* the concentration of the nucleobases used. The "theoretical" curves were obtained by using the parameters K and $\Delta\delta_c$ calculated as described earlier. As can be seen, the experimental values agree very well with the "theoretical" curves. It was not possible to determine the acceptor site of the nucleobases (N-1, N-3 or N-7).

Due to the rapid exchange of the phenolic proton of estradiol with the aminoprotons of adenine and uracil, the parameters of these complexes could not be determined by means of the chemical shift of the OH-3 proton. Therefore, these parameters have been calculated using the chemical shift of the aromatic H-4 proton of estradiol. This and the H-2 proton as well have been moved downfield. There is a fair agreement in the results obtained for the

Table III. The thermodynamic and NMR parameters of the complexes of the OH-17 group of "estradiol", "mestranol", and testosterone with nucleobasis. Abbreviations: See Tables I and II; "mestranol", 17α-ethinyl-estradiol-3-methylester.

OH-17	Nucleobase		Adenine	Dimethyl-adenine	Uracil	Dimethyl-uracil
estradiol	K*	[mole^{-1}]	exchange	1.3 ± 0.5	0.7 ± 0.5	0.8 ± 0.3
	$\Delta\delta_c$	[ppm]	with	5.5 ± 2.2	9.1 ± 5.7	3.3 ± 1.0
	$-\Delta H\left[\frac{\text{kcal}}{\text{mole}}\right]$		$^6NH_2$	3.9 ± 2.0	3.6 ± 2.5	3.5 ± 1.4
	$-\Delta S\left[\frac{\text{cal}}{\text{mole} \cdot \text{deg}}\right]$			12.0 ± 6.0	12.8 ± 9.0	12.1 ± 4.8
mestranol	K*	[mole^{-1}]	exchange	1.9 ± 0.8	0.9 ± 0.7	0.8 ± 0.3
	$\Delta\delta_c$	[ppm]	with	3.3 ± 1.0	7.6 ± 5.3	3.0 ± 0.9
	$-\Delta H\left[\frac{\text{kcal}}{\text{mole}}\right]$		$^6NH_2$	4.0 ± 2.0	3.4 ± 2.7	3.3 ± 1.7
	$-\Delta S\left[\frac{\text{cal}}{\text{mole} \cdot \text{deg}}\right]$			14.0 ± 7.0	11.8 ± 9.4	11.4 ± 5.7
testosterone	K*	[mole^{-1}]	1.9 ± 0.4	2.6 ± 0.5	0.9 ± 0.4	0.5 ± 0.2
	$\Delta\delta_c$	[ppm]	9.0 ± 1.0	2.0 ± 0.2	5.9 ± 3.0	3.8 ± 0.4
	$-\Delta H\left[\frac{\text{kcal}}{\text{mole}}\right]$		3.4 ± 1.0	3.8 ± 1.1	5.3 ± 3.2	4.3 ± 2.6
	$-\Delta S\left[\frac{\text{cal}}{\text{mole} \cdot \text{deg}}\right]$		9.6 ± 2.9	11.0 ± 3.3	18.0 ± 10.8	15.9 ± 9.5

* 25 °C.

phenolic and aromatic proton as well (s. Table II). From the K-values calculated it might be concluded that the complexes of estradiol with adenine derivatives are essentially stronger than those one with uracil derivatives.

In comparison to these results, the hydrogen bonds of OH-17 of estradiol and testosterone as well are considerably weaker (Table III).

In order to verify the low K-values of OH-17 of estradiol, additional experiments were conducted with mestranol, the 3-methylester of estradiol. As has been expected, the values obtained were of about the same order of magnitude, perhaps slightly larger.

The different strengths of the hydrogen bonds of these two hydroxyl groups is demonstrated clearly by the van't Hoff-plots (Fig. 2; only the interactions with nucleobases. The hydroxyl groups, especially because of clarity).

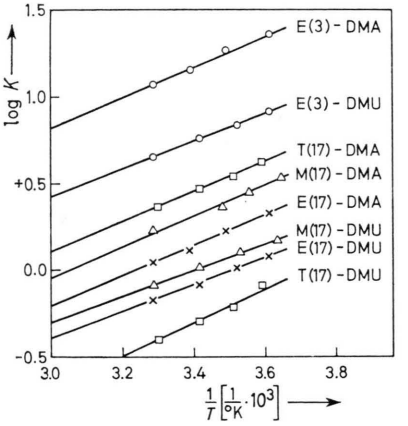

Fig. 2. Temperature dependency of some apparent equilibrium constants K (van't Hoff-plots). Abbreviations: E, 17α-ethinyl-estradiol; M, 17α-ethinyl-estradiol-3-methylester; T, testosterone; DMA, DMU: See Fig. 1.

The weakest hydrogen bonds have been observed to occur between the nucleobases and the keto groups of either progesterone or testosterone. In general, the K-values were less than 1.0 mole^{-1}.

Discussion

From the results obtained it might be concluded that all of the sexual hormones form hydrogen bonds with nucleobases. The hydroxyl groups, sepecially the OH-3 of estradiol, form considerably stronger complexes than the keto groups.

Although most of the substances and the solvent used are not normally present in biological systems, they resemble, to a large extent, physiological conditions. Thus, 17α-ethinyl-estradiol, a synthetic estrogen used widely in contraceptive pills, exhibits, at least, the estrogenic potency of the "physiological" estradiol [17]. The OH-17 group of 17α-ethinyl-estradiol may be slightly more acidic than the one of estradiol due to the ethinyl group. This difference, however, can be neglected in regard to hydrogen bonding.

Deuterochloroform was used as a solvent, since it is not possible to demonstrate hydrogen bonding under physiological conditions (aqueous solution, low concentration) with NMR technique. Nevertheless, the rel. strength of the functional groups mentioned above remains probably unchanged.

Despite of these limitations, the following conclusions might be drawn: The results obtained in regard to the hydrogen bonding capability of the OH-groups support the well-known suggestion, that, relative to estradiol, the higher estrogenic potency of diethylstilbestrol is due to its second phenolic group while the lower potency of estrone is due to the CO-17 group. The importance of hydrogen bonds between the OH-groups of estrogen analogs and a receptor protein has also been described recently by Terenius [18].

The keto groups of progesterone form very weak complexes with the nucleobases investigated. Similar results were obtained when this interaction was studied in aqueous solution using equilibrium dialysis technique [7]. Only in the case of guanine analogs the authors observed a strong interaction. They assumed that hydrogen bonding is predominantly involved. This association, however, may not be due to hydrogen bonding but rather to other noncovalent interactions. A charge transfer interaction seems to be possible since guanine is an excellent electron-donor and progesterone may act as an electron-acceptor.

At present, further experiments are conducted in order to elucidate the mechanism of action of the sexual hormones.

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[1] E. E. Baulieu, A. Alberga, C. Raynaud-Jammet, and C. R. Wira, Nature [London] New Biol. **236**, 236 [1972].

[2] B. W. O'Malley, T. C. Spelsberg, W. T. Schrader, F. Chytil, and A. W. Steggles, Nature [London] **235**, 141 [1972].

[3] J. L. Tymoczko and S. Liao, Biochim. biophysica Acta [Amsterdam] **252**, 607 [1971].
[4] M. Szego, The Sex Steroids, K. W. McKerns, ed., p. 1, Appleton-Century-Crofts, New York 1971.
[5] P. O. P. Ts'o and P. Lu, Proc. nat. Acad. Sci. USA **51**, 17 [1964].
[6] M. L. Goldberg and W. A. Atchley, Proc. nat. Acad. Sci. USA **55**, 989 [1966].
[7] C. Kidson, P. Cohen, and R. Chin, The Sex Steroids, K. W. McKerns ed., Appleton-Century-Crofts, p. 421, New York 1971.
[8] R. Chin and C. Kidson, Proc. nat. Acad. Sci. USA **68**, 2448 [1971].
[9] D. J. Ellis and H. J. Ringold, The Sex Steroids, K. W. Mc Kerns ed., Appleton-Century-Crofts, p. 73, New York 1971.
[10] E. Lippert, Ber. Bunsenges. physik. Chem. **67**, 267 [1963].
[11] J. J. Mieyal, J. Suchy, J. E. Biaglow, and H. Z. Sable, J. biol. Chemistry **244**, 4063 [1969].
[12] I. D. Kuntz, Jr. and M. D. Johnston, Jr., J. Amer. chem. Soc. **89**, 6008 [1967].
[13] M. Nakano, N. I. Nakano, and T. Higuchi, J. physic. Chem. **71**, 3954 [1967].
[14] G. Nagel and S. Hanlon, Biochemistry **11**, 816 [1972].
[15] Y. Kyogoku, R. C. Lord, and A. Rich, J. Amer. chem. Soc. **89**, 496 [1967].
[16] J. L. Dimicoli and C. Helene, Biochimie **53**, 331 [1971].
[17] M. H. Briggs and J. Brotherton, Steroid Biochemistry and Pharmacology, p. 102, Academic Press, New York 1970.
[18] L. Terenius, Acta pharmacol. toxicol. [Köbenhavn] **31**, 441 [1972].