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SHIELDING EFFECTS AT 17α-SUBSTITUTED ESTROGENS. A TENTATIVE EXPLANATION FOR THE LOW BIOLOGICAL ACTIVITY OF 17α-ETHYL-ESTRADIOL BASED ON I.R. AND NMR SPECTROSCOPIC STUDIES

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Summary—Infrared data of the hydroxyl stretching band and NMR data of the hydroxyl proton for 7 different 17-substituted estradiol-3-methyl-ether compounds have been recorded. The band positions can be related to the extent of shielding effects or intramolecular interactions of hydrogen bonding type. The splitting of several i.r. bands can be explained on the basis of rotamers and restrictions in the free rotation of the hydroxyl group. This holds especially for 17α-ethyl-estradiol, in which the access to the free electron pairs of the OH group is hindered by the 17α-ethyl group. This may explain the very low receptor binding and reduced biological activity of 17α-ethyl-estradiol in contrast to the stronger binding of 17α-methyl-, 17α-vinyl or 17α-ethinyl-estradiol.

INTRODUCTION

Optimal binding of estrogens to the cytosolic receptor from uteri of immature rats requires a phenolic hydroxyl group at the C-3-position of the aromatic A-ring and a further substituent with a free electron pair in the 17-position [1]. If this substituent is a 17β-hydroxyl group—as in the case of estradiol (**1**)—a further substitution in the 17α-position by a methyl (**2**), vinyl (**4**) or ethinyl group (**5**) does not influence remarkably the receptor binding. Surprisingly the introduction of a 17α-ethyl group (**3**) causes a pronounced decrease in receptor binding, which also is reflected by an increase in the Allen–Doisy threshold value by two orders of magnitude [1, 2] and the results of the uterus growth test [3] (Table 1). Up to now, this striking behavior of 17α-ethyl-estradiol remains unexplained. It is not convincing to explain this effect solely through the size of this group during the binding process of the steroid to its receptor, since this should hold true also for the vinyl- (**4**) and the ethinyl compound (**5**).

In order to get more detailed information about the possible reasons of the exceptional behaviour of 17α-ethyl-estradiol as compared to similarly substituted compounds we investigated the OH stretching frequencies of the i.r. spectra and the proton resonance signal of the 17-hydroxyl group in NMR, using the 3-methyl ethers (**1a–6a**) of the steroids under investigation. The compound (**7a**) was introduced into the investigation, because Hoerhold *et al.*[4] found, that 17α-azidomethyl-estradiol (**7**) shows a receptor binding comparable to 17α-methyl-estradiol (**2**).

EXPERIMENTAL

The steroids were obtained from Schering AG, Berlin. Spectroscopic quality CCl_4 and d_6-DMSO were used as solvents. The i.r.-spectra were recorded on a Perkin–Elmer 580 spectrophotometer, using a cuvette with KBr windows and variable path length. i.r. Measurements were done with 10 mM solutions in CCl_4 at 1.5 mm pathlength. Comparison with 3.3 mM solutions and 4.5 mm pathlength yielded identical

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Table 1. Biological activities of different 17α-substituted estradiol and estrone derivatives

	17β-R	17α-R′				
	R	H	CH_3	CH_2–CH_3	CH–CH_2	C–CH
Receptor binding test, RCF_{50} values*	–OH	1	1.5	16	1.6	1
Allen–Doisy threshold value, s.c., μg*	–OH	0.25–0.5	0.3–1	300–1000	1	0.3
Vaginal smear assay, relative activities†	–OAc		1000	2	250	1000
Uterine growth test, relative activities‡	–OH	370		1	100	770

*Determined by the authors, see [1]. †Data from [2]. ‡Data from [3].

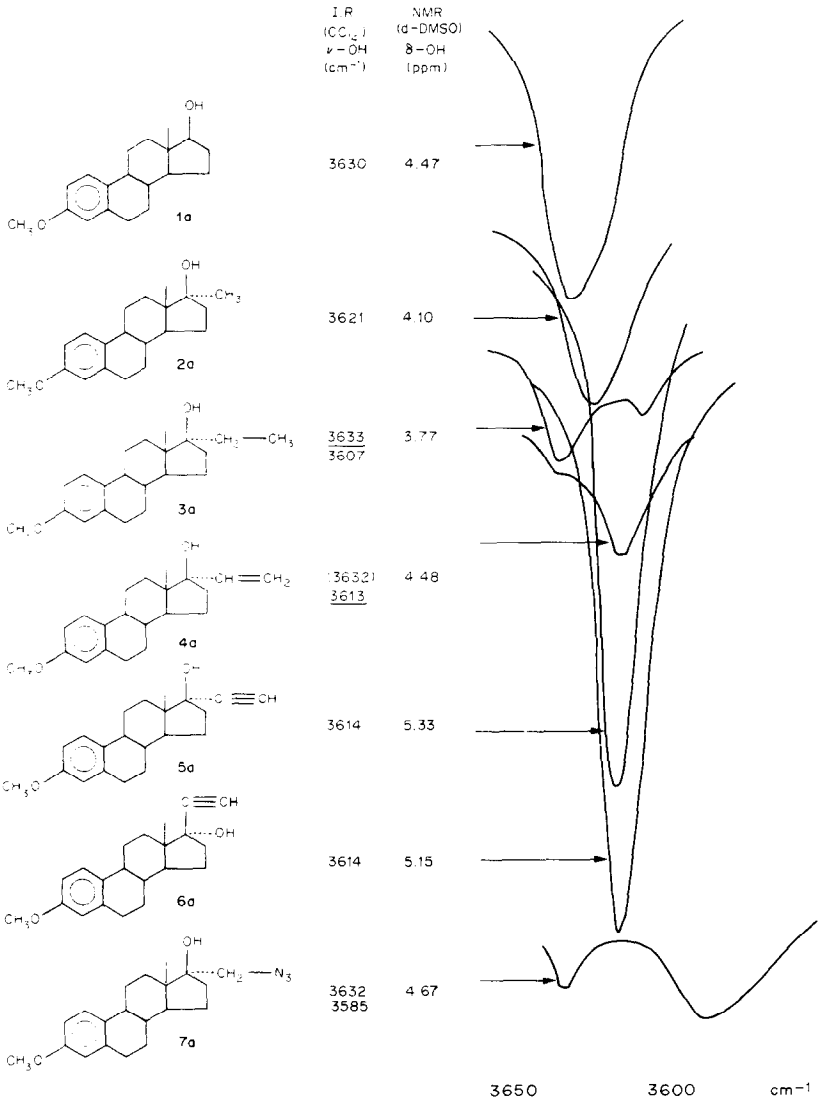

Table 2

absorption spectra, thus intermolecular interactions could be excluded at these concentrations. The NMR-spectra were recorded on a HR 90 spectrometer from Bruker or a HX 100 spectrometer from Varian. NMR-data are expressed on the δ-scale. The receptor binding test is described in details elsewhere [1].

RESULTS AND DISCUSSION

i.r.-Spectroscopic investigations on the OH stretching band

Normally, highly diluted primary aliphatic alcohols in CCl_4 show an absorption band at 3640–3635 cm^{-1}, together with a mostly covered band at 3626 cm^{-1}. Secondary aliphatic alcohols usually absorb at 3627–3625 cm^{-1} with a side band or shoulder at 3617 cm^{-1}, while tertiary aliphatic alcohols show an absorption band at about 3617 cm^{-1} [5–10]. The observed absorption values of the compounds investigated by us are presented in Table 2. Comparison of our results with the above mentioned standard values allow the following conclusions:

(1) Besides the ethinyl compounds (**5a**) and (**6a**) all steroids under investigation showed absorption bands at higher frequencies than one would expect from the standard values. Van der Maas and Lutz [5] found that cyclopentanol has an absorption maximum at 3625,5 cm^{-1} and 1-ethylcyclopentanol absorbs at 3617,6 cm^{-1} [5]. Furthermore they observed, that β-methyl groups exert a strong frequency raising effect and they explained this phenomenon with a "shielding effect", resulting in a reduced interaction of the OH-group with the solvent CCl_4. They assume, that in general the O–H–Cl interaction, similar to the

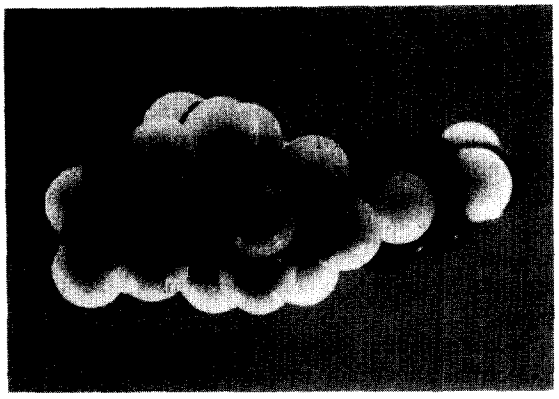

Fig. 1. Estradiol.

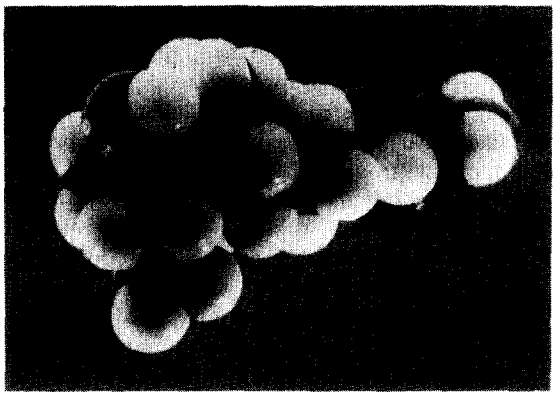

Fig. 3. 17α-Ethyl-estradiol, ethyl group in "axial" orientation.

hydrogen bonding, causes a frequency lowering effect, which in the presence of shielding substituents is less pronounced. In fact cyclopentanol in the gas phase shows an absorption maximum at 3653 cm^{-1} [11], which is lowered in CCl_4 solution to 3625,5 cm^{-1} [5]. Thus a reduced frequency lowering effect due to shielding by the angular methyl group at C-13 may account for the frequency shift of about 5 cm^{-1} in the case of compound (**1a**) and (**2a**).

(2) The spectrum of compound (**3a**) clearly shows two absorption maxima at 3633 and 3607 cm^{-1}. The spectrum of compound (**4a**) has a maximum at 3613 cm^{-1} with a pronounced shoulder at 3632 cm^{-1}. All three compounds are tertiary alcohols which normally should absorb at 3617 cm^{-1}. The band splitting of (**3a**) in comparison with (**2a**) is surprising, since (**3a**) differs from (**2a**) only by the extension of the 17α-methyl substituent by a further methylen group. However, analysis by means of a molecular model of (**3a**) reveals, that the rotation of the ethyl group around the bond to C-17 could create two completely different situations for the 17β-hydroxyl group. In the case of an "axial" orientation of the ethyl substituent (terminal methyl group of the ethyl substituent directed towards the α-side of the steroid, see Fig. 3), no major interaction between the 17β-OH group and the 17α-substituent should occur. This situation would be similar as in the case of the 17α-methyl compound (**2a**).

On the other hand, such an "axial" orientation is not probable because of sterical hindrance exerted by the α-protons at C-12, C-14 and C-16. More likely would be an "equatorial" orientation of the ethyl group (terminal methyl group of the ethyl substituent directed toward the β-side of the steroid, see Fig. 4). In this case we expect a much higher shielding effect with a shift to higher frequencies and a limitation of the free rotation of the 17β-OH group around the C-17-O-axis. In the case of the 17α-methyl compound (**2a**, Fig. 2) and the unsubstituted steroid (**1a**, Fig. 1) such a hindrance does not occur. Therefore we think that the band splitting and the appearance of the band at 3633 cm^{-1} is caused by the "equatorial" rotamer.

Band splitting in case of simple aliphatic alcohols in CCl_4 are also reported by van der Maas and Lutz [5]. They measured a single peak absorption maximum for 3-hydroxy-3-methyl-pentane at 3618,1 cm^{-1}, whereas the signal for 3-hydroxy-2,3,4-trimethyl-pentane is split into two peaks at 3627.6 cm^{-1} and 3613.5 cm^{-1}.

The same considerations should also hold for the band splitting and the weak band at 3632 cm^{-1} of the 17α-vinyl-estradiol derivative (**4a**). However, in this

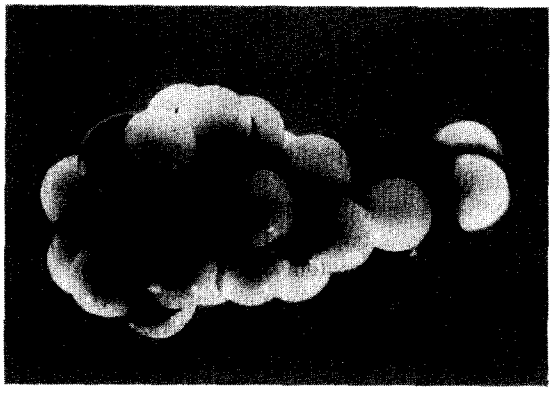

Fig. 2. 17α-Methyl-estradiol.

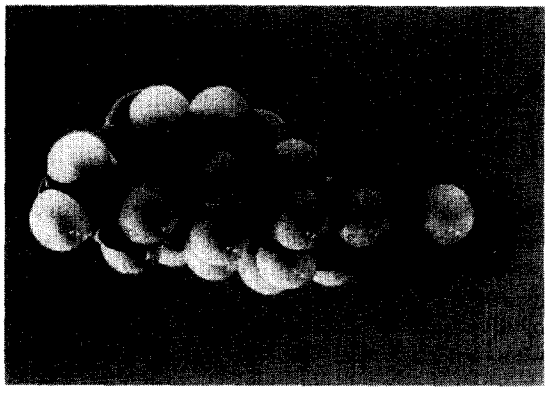

Fig. 4. 17α-Ethyl-estradiol, ethyl group in "equatorial" orientation.

case a slightly lower interference should be expected due to the larger angle within the sp^2-hybridized vinyl substituent. A different approach to explain the frequency shift can be based on the assumption of an intramolecular interaction between the OH proton and the π-electrons of the vinyl group. In this way, Schleyer *et al.*[12] and Oki and Iwamura[13] tried to explain the band splitting of allyl alcohol CH_2–CH–CH_2–OH with a main band at 3618 resp. 3619 cm^{-1} and a shoulder at 3631 resp. 3635 cm^{-1}. Also Arnaud and Armand[14] interpreted the asymmetric absorption bands of β-unsaturated alcohols by intramolecular hydrogen bonding.

Comparison of the band intensities from the ethyl compound (**3a**) and the vinyl compound (**4a**) reveals that in the case of (**3a**), the band at higher frequency is more intensive, whereas in the case of (**4a**) the band at lower frequency is more pronounced. If one supposes similar dipol moments for the different rotamers of the OH group, the relationship of the band intensities should correspond to the relative concentrations of the rotamers. On this basis one could explain the weaker receptor binding of the ethyl compound (**3**) as compared to the vinyl compound (**4**) by the assumption that the absorption band at higher frequency indicates a situation unfavorable for receptor binding, whereas the band at lower frequency indicates the more favorable orientation.

(3) In either of both the C-17 epimeric ethinyl compounds (**5a**) and (**6a**), an interaction of the linear 17α-substituent with the OH group is not possible. No shielding effect can be expected. Consequently only one OH stretching band shows up. Surprisingly, the OH band of (**5a**) and (**6a**) (3614 cm^{-1} in both cases) is lower as expected for aliphatic tertiary alcohols, whereas the OH stretching band of the previously discussed compounds (**1a**) and (**2a**) showed a shift to higher frequencies due to the shielding by the C-13 methyl group (identical with shielding by the C-13 methyl group. Furthermore the equal position of the bands for the epimeric compounds (**5a**) and (**6a**) is striking, because in (**6a**) the OH group cannot be shielded by the C-13 methyl group. One should explain the band position by a frequency lowering effect of the ethinyl substituent, caused probably by an interaction of the OH-proton with the π-system. Schleyer *et al.*[12] also observed a shift to lower frequencies between the OH band of 1-propanol (3638 cm^{-1}) and propargyl alcohol HC–C–CH_2–OH (3620 cm^{-1}).

In compound (**6a**) with a 17α-OH group in quasi-axial position shielding by the C-13 methyl group is not possible, but a similar shielding effect may be exerted by the axial resp. quasi axial α-positioned hydrogen atoms at C-12 and C-16. This is supported by the results of Weinman[15] and Allsop *et al.*[16], who observed that the OH group of steroids or triterpenoids in axial positions show stretching frequencies at higher wave numbers than OH groups in equatorial positions (approx 6 cm^{-1}). This shielding exerted either by the C-13 methyl group or the hydrogens at C-12 and C-16 partially compensates the frequency lowering effect of the ethinyl group in (**5a**) and (**6a**).

(4) The azidomethyl substituted compound (**7a**) also shows a split absorption band. In this case, the high wave number difference between the two absorption maxima (47 cm^{-1}) suggests stronger intramolecular hydrogen bonding between the hydroxyl proton and the free *n*- or π-electrons of the azido group.

Such interactions between the hydroxyl proton and π-electrons are already well investigated: Schleyer *et al.*[12] and Oki and Iwamura[13] observed double bands in case of 3-buten-1-ol(3634 cm^{-1} and 3594 cm^{-1} [12], resp. 3635.2 cm^{-1} and 3596.1 cm^{-1} [13]) and 3-butin-1-ol (3640 cm^{-1} and 3598 cm^{-1} [12]), which were interpreted as effects of intramolecular hydrogen bonding. Trifan *et al.*[17] explained the double band of α-hydroxyethylferrocene (3617 cm^{-1} and 3574 cm^{-1}) in the same way. Similar effects were observed by Goldman and Crisler[18] in the case of 2-phenylethanol for the first overtone vibration and by Baker and Shulgin[19] in the case of the fundamental vibration of 2-allylphenol (3605 cm^{-1} and 3546 cm^{-1}). Furthermore the latter authors also demonstrated, that intramolecular hydrogen bonding may also occur between OH groups and other proton accepting groups beside π-electrons [19]. The nitrogen atom showed an especially pronounced frequency lowering capacity. For example, in the case of 2-dimethylaminophenol, a wave number difference of 244 cm^{-1} could be observed. Therefore the band of (**7a**) at 3585 cm^{-1} should belong to a species with intramolecular hydrogen bonding, while the band at 3632 cm^{-1} should be assigned to a form with a free OH group, influenced by the shielding effect of the C-13 methyl group and a further shielding effect caused by the "equatorial" oriented azidomethyl group. An orientation of the azidomethyl group without any shielding effect on the OH group is hardly possible because of the steric hindrance of the α-positioned hydrogen atoms at C-12 and C-16. The relatively high intensity of the 3585 cm^{-1} bands suggests that the OH group is encountered preferentially in the bound form.

NMR spectroscopic investigations on the OH proton resonance signal

Table 2 shows the positions of the NMR resonance signals for the OH proton of compound (**1a–7a**) in 0.05 M solution in d_6-DMSO. In all cases the signal showed up as a broadened singlet. A diamagnetic shift of the OH proton signal, concomitant with increasing alkyl substitution in compound (**1a**), (**2a**) and (**3a**), can be observed. In this series two effects have to be considered: an inductive effect on the one hand, shifting the signal to higher field and an effect from the solvent, leading to a low field shift. The low field shift should be reduced, if the access of the

solvent is blocked by adjacent groups. The latter effect could account for the relatively far shift to higher field in the ethyl substituted compound (**3a**).

Shoolery and Rogers[20] observed also a diamagnetic shift of the signal for the 17β-OH group of testosterone in $CDCl_3$ after substitution by a 17α-methyl group. In compound (**4a**) the anisotropic effect of the vinyl group should cause a significant shift of the OH resonance to lower field, as seen in the signal difference between ethanol (2.58 ppm) resp. propanol (2.28 ppm) and 2-propenol (3.58 ppm). Such a shift of the OH proton signal in (**4a**) is only detectable when compared with compound (**3a**), but not with (**1a**). This indicates, that the paramagnetic shift of the vinyl group is diminished by a diamagnetic shift, which might originate from a reduced solvent interaction as in the ethyl substituted compound (**3a**).

Although an ethinyl substituent should exert a lower anisotropic effect than a vinyl substituent, the observed paramagnetic shift for (**5a**) and (**6a**) compared with (**1a**) is higher than for (**4a**). This should be due to the fact, that the solvent interaction with the OH group is less influenced by the ethinyl substituents of (**5a**) and (**6a**) than by the vinyl substituent of (**4a**), a conclusion supported also by our i.r. data. The greater paramagnetic shift of (**5a**) as compared to (**6a**) may be explained by van der Waals interactions with the C-13-methyl group in compound (**5a**). Also compound (**7a**) shows a paramagnetic shift relative to (**1a**), (**2a**) and (**3a**), caused by the azidomethyl substituent.

CONCLUSIONS

The analysis of the i.r. and NMR spectra of different 17-alkyl-estradiol-3-methylether compounds revealed that the low receptor binding capacity of 17α-ethyl-estradiol (**3**), compared with 17α-methyl- (**2**), 17α-vinyl- (**4**) and 17α-ethinyl-estradiol (**5**), is also reflected by spectroscopic peculiarities. The i.r. spectrum of (**3a**) shows a main band at 3633 cm^{-1}, which is a surprisingly high wavenumber for a tertiary aliphatic alcohol. Also the NMR spectrum of (**3a**) is striking in the fact that none of the compounds in comparison shows the signal for the OH proton at such a high field. Both observations can only be explained by a strongly reduced interaction between the solvent and the OH group, caused by the 17α-ethyl substituent. This effect is much less pronounced in the case of the 17α-methyl, 17α-vinyl- or a 17α-ethinyl-substituent.

The models demonstrate that only the preferred equatorial rotamer of 17α-ethyl-estradiol is able to shield the 17β-OH group and to hinder the free rotation around the C–O axis. Both factors reduce the interaction with the solvent CCl_4 which is proton acceptor for the proton of the OH group. On the other hand an interaction of the OH group with a protonating group of the receptor—probably a SH group, as discussed by Ikeda[22]—requires the access to the orbital of one of the two electron pairs of the oxygen. The C-13 methyl group limits the rotation of the OH-group and thus the sector accessible to the orbitals of the free electron pairs.

Figure 1 shows the position of the orbital of the free pro-S electron pair, indicated by an arrow. Rotation of the OH group around the C–O bond by 240° places the orbital of the pro-R electron pair in the same position. One might assume that the presumptive interaction of the SH-group requires the orbital in this position. Also in the case of the 17-keto compound estrone, where the orbitals of both free electron pairs of the oxygen are strongly located, the indicated position should be favored, because the position of the second electron pair again is shielded by the C-13 methyl group.

In the equatorial rotamer of (**2a**) the 17α-ethyl group should cover a sector of about 180° (Fig. 4) and hinders the access to the OH-group and its rotation. This should be the reason for the weak biological activity of 17α-ethyl estradiol as compared to 17α-vinyl estradiol. In the latter compound the axial position should be preferred, as indicated by i.r. spectra. In this case, the access to the orbitals of both free electron pairs of the oxygen is not hindered. The 17α-hydrogen (Fig. 1), the methyl (Fig. 2) and the ethinyl group (not shown) do not interfere with the OH-group.

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