Tetrahedron Vol 24, pp. 2199 to 2204. Pergamon Press 1968. Printed in Great Britain

NMR SPECTRA OF ESTROGEN CATECHOLS*

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(*Received in USA* 30 *June* 1967; *accepted for publication* 28 *August* 1967)

Abstract—The NMR of various estrogen catechol derivatives were observed in $CDCl_3$ and DMSO. The chemical shifts of the aromatic protons permit the ready assignment of structure to isomeric mono-derivatives. The reasons for the different chemical shifts of the two aromatic protons in 2-hydroxyestrogens are discussed.

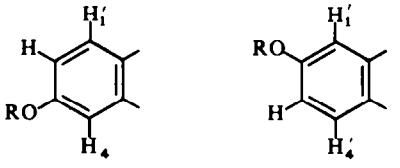
A MAJOR transformation of the female sex hormone in man is orthohydroxylation of the aromatic ring leading to 2-hydroxyestrone Ia,[1] which in turn is further metabolized to the monomethyl derivative, 2-methoxyestrone Ib.[2] The latter reaction appears specific for the C-2 OH group in that only the 2-OMe product has been demonstrated in man. The potential for alternative O-methylation,†[3] implicit in the presence of two essentially equivalent phenolic OH groups has stimulated interest in isomeric mono derivatives of the estrogen catechols and several of these have been synthesized.[4] The similarity of these isomers presents problems in structural assignment particularly in view of the limited quantities likely to be available from biological sources. It was, therefore, important to be able to differentiate and define the isomeric compounds by a simple and unequivocal procedure. The two aromatic protons of 2-hydroxy-estrone exhibit separate and distinct resonances in the downfield region of the NMR spectrum. Since an alteration of a phenolic group may be expected to have a greater effect on the chemical shift of the *ortho*-hydrogen compared to that situated *meta*, the structure of the isomeric monoderivatives of the catechol estrogens might be arrived at from the chemical shifts of their aromatic protons. In order for this criterion to be useful, however, it was essential to assign the two aromatic resonances in the parent compound. An assignment was possible from a first order analysis of the aromatic region of the NMR spectrum of the estradiol isomer estra-1,3,5(10)-trien-2,17β-diol IIa and its derivatives.[5] The chemical shifts of the aromatic protons listed in Table 1 show that the C-1 proton of the 2-hydroxy-3-desoxy compound resonates 12–14 c/s further downfield than the C-4 proton in a comparable estradiol IIb derivative. This value corresponds closely to the 13 c/s difference between the two aromatic hydrogens in 2-hydroxyestrone and suggests that the downfield resonance in that compound may be assigned to the C-1 hydrogen and the upfield one to the C-4 hydrogen. These assignments are supported by the NMR spectra of the various 2-hydroxyestrone derivatives listed in Table 2. As may be expected the 2,3-bis derivatives result in identical changes in the chemical shifts of the H-1 and H-4

* This investigation was supported by a grant from the American Cancer Society and by grant CA-07304 from the National Cancer Institute, National Institutes of Health, U.S.P.H.S.

† This potential has been realized only in *in vitro* studies with rat liver preparations where both possible monomethyl esters have been obtained.

protons so that their relative positions are unchanged. The monoderivatives, however, due to the unequal effect of the alteration on *ortho* and *meta* situated protons show distinct changes in the separation of the aromatic proton resonances. The formation of a methyl ether of one phenolic group affects the *ortho* and *meta* protons in opposite directions shifting the *ortho* proton 3–4 c/s upfield and deshielding the meta proton 1–2 c/s. This divergent effect where one would expect shifts in the same direction may be due to changes in intramolecular hydrogen bonding in going from the catechol to the guiacol structure.

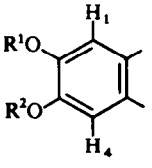
TABLE 1



R	δH_1	δH_4	$\delta H_1'$	$\delta H_4'$	$\Delta\delta(H_1'-H_4)$	$\Delta\delta(H_1-H_4')$
H*	428	394	406	415	12	13
Me	434	400	413	422	13	12
Ac	440	410	424	436	14	14

* The two isomeric free phenols were run in DMSO, the spectra of the derivatives were obtained in $CDCl_3$.

TABLE 2*



R^1	R^2	H_1	H_4	$\Delta(H_1-H_4)$
H	H	413	400	13
Me	Me	411	398	13
Ac	Ac	426	413	13
Me	H	410	402	8
H	Me	414	396	18
Ac	H	421	405	16
H	Ac	418	408	10

* All the spectra in this table were obtained in $CDCl_3$.

Acetylation of a phenolic group in the catechol produces deshielding of both *ortho* and *meta* protons, the former by 8 c/s and the latter by 5 c/s. The effect appears to be additive since in the diacetate both protons are shifted downfield equally by by 13 c/s. From the Table it is apparent that although the individual shifts produced

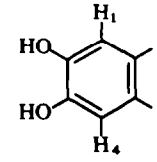
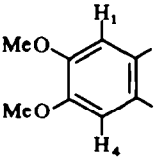
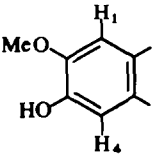
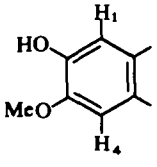
by mono ether or ester formation are small, a ready distinction between the C-2 or C-3 monoderivative of estrogen catechols is possible. An electron withdrawing substituent at C-2 will increase the distance between the aromatic resonances to more than 12 c/s, while a similar substitution on C-3 will decrease the separation to less than 12 c/s. A reverse situation will exist with electron donating substituents.

In order to establish more firmly the original assignments, and to ensure that the aromatic resonances of the derivatives in Table 2 did not "leapfrog" and reverse positions, the NMR spectra of the OME compounds were also measured in DMSO in the absence and presence of alkali. The effect of the hydrogen bonding of the free phenolic OH in DMSO is clearly apparent in the resonance shifts of the *ortho* and *meta* hydrogens which are affected to a different degree. The *ortho* hydrogen resonance is shifted by 10–11 c/s upfield while that of the *meta* hydrogen is virtually unchanged. These differences are considerably more emphatic than those reported in the Papaveracea alkaloids[6] which contain a similar guiacol structure. In that instance the adjacent nitrogen containing ring very likely affects the nature of bonded complexes, particularly since steric effects on DMSO hydrogen bonding have been reported.[7] The NMR shifts of the aromatic protons induced by a phenolic anion formation have been reported as useful in assigning structures to complex catechols,[8] and its application to the present problem was investigated. The chemical shift values for the aromatic protons for the various estrogen catechol derivatives in DMSO in the presence of base are listed in Table 3. It is apparent that while there are differences in the shifts of the protons *ortho* and *meta* to the phenolate anion, these differences are less diagnostic than those induced by hydrogen bonding in pure DMSO, and that the latter is preferable for structural assignments in this series. The separation between the aromatic resonances in $CDCl_3$ and DMSO readily distinguish which of the two *ortho* phenolic groups are free.

The reason for the greater deshielding of the C-1 proton in 2-hydroxyestrone is of considerable interest. Several possible explanations are available for this difference between the C-1 and C-4 protons. One is a possible difference in electron density between the two positions, another is additional steric compression of the C-1 hydrogen and finally the magnetic anisotropy of the substituents at C-5 and C-10 may be the contributing factor. With respect to the electron density difference numerous attempts have been made to relate electron distribution in aromatic systems with the chemical shift of the aromatic protons.[9–15] No general agreement has as yet been found but substantial partial correlations suggest a relationship.[11–15] If the deshielding of the C-1 proton is due to a lesser electron density at C-1 relative to C-4, it follows that electron density differences must exist between C-2 and C-3. This would imply a difference in the acidity of the two phenolic groups, a situation of considerable biochemical consequence. On the basis of evidence presented in a recent publication by Nagata *et al.*,[16] the steric compression between the C-1 hydrogen and the 11α-hydrogen would account satisfactorily for the greater deshielding of the proton at C-1. Indeed, a 12 c/s deshielding calculated from their data, is in excellent agreement with the value found in 2-hydroxyestrone. The contribution of the different magnetic anistropies of the B and C rings to the observed deshielding is considered by these authors, but is rejected on the basis of calculations, presumably by the McConnell[17] equation, which indicate this effect to be minimal. The use of the McConnell equation at distances of less than 3 Å, as is the case here, is however

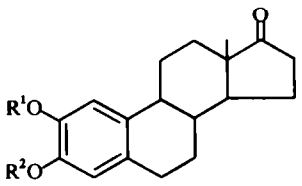
subject to substantial error,[17,18] and the contribution of magnetic anisotropy effects to the deshielding of the C-1 hydrogen may be significant. The recently published NMR data on the isomeric 6α and 6β methylestradiol derivatives IIIa and IIIb,[19] is pertinent to this question. The NMR spectra of these isomeric compounds show

TABLE 3

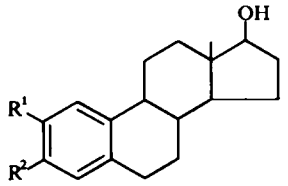
Compound	δCDCl	δDMSO	δDMSO + NaOD	Δδ(CDCl$_3$ −DMSO)	Δδ(DMSO− DMSO + NaOD)
	H_1 413 H_4 400	401 388	382 369	12 12	19 20
	H_1 411 H_4 398	417 404	— —	−6 −6	— —
	H_1 410 H_4 402	410 391	391 367	0 11	19 24
	H_1 414 H_4 396	404 396	381 378	10 0	23 18

that the 6β Me group has no effect on the chemical shift of the C-4 aromatic proton while the 6α Me group produces a 10 c/s downfield shift. The freely rotating 6α Me group involves a substantially lesser steric interaction with the C-4 hydrogen than that which exist between the 11α and C-1 hydrogens. Furthermore, the maximum interaction will occur in the half-chair form of ring B, and any conformational change in this flexible ring will only serve to increase the distance between the 6α Me hydrogens and the C-4 proton. The fact that despite these considerations the deshielding of the C-4 proton is comparable to that exhibited by the C-1 proton, suggests that the magnetic anistropy of the equatorial 6α Me group contributes significantly to the deshielding of the C-4 proton, and that similarly the anisotropy of the C-11 methylene group is responsible for some of the deshielding of the C-1 proton. The available evidence therefore, suggests that the deshielding of the C-1 proton in

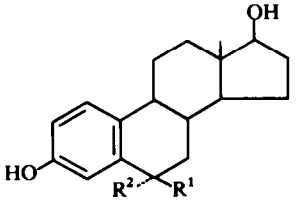
estrogen catechols is due to a combination of steric interaction and local magnetic anisotropy effects and is not due to a deficiency in electron density. Such a deficiency may exist and may result in different acidities of the two phenolic groups, but if present it is of a nature which is not apparent in deshielding at this carbon.



Ia: $R^1 = R^2 = H$
b: $R^1 = Me, R^2 = H$



IIa: $R^1 = OH, R^2 = H$
b: $R^1 = H, \quad R^2 = OH$



IIIa: $R^1 = H, \quad R^2 = Me$
b: $R^1 = Me, R^2 = H$

EXPERIMENTAL

The NMR spectra were determined on a Varian A60 instrument. The chemical shift values are given in c/s downfield from TMS as the internal standard and are accurate to ± 1 c/s. The solvents used were $CDCl_3$, DMSO and DMSO + p drop of 1N NaDO prepared from Na° and D_2O.

Acknowledgement—The authors are grateful to Dr. T. F. Gallagher for his interest and assistance in this work.

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