The Crystal and Molecular Structure of 8-Azaestrone Hydrobromide

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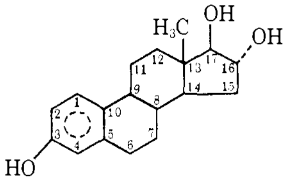
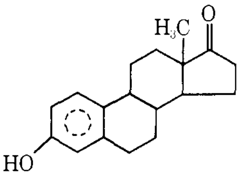
Abstract: The crystal and molecular structure of 8-azaestrone hydrobromide has been determined by a single crystal, X-ray diffraction study. The compound crystallizes in the monoclinic space group $P2_1/c$ with cell dimension $a = 8.65 \pm 0.01$, $b = 12.64$, and $c = 15.42$ Å and $\beta = 111° 55' \pm 5'$. The cation is shown to have the same conformation as natural estrone. Its molecular parameters are further shown to be essentially those observed in the natural estrogens. Hence its relative lack of estrogenic activity is not due to either conformational differences or variations in molecular parameters. The van der Waals packing of the crystal with the bromide ion between the oxygens of adjacent molecules disrupts the hydrogen-bonding scheme characterizing the natural estrogens. A mechanism is suggested to account for its lack of estrogenic activity. The 1422 independent reflections were refined to a final value of $R = 0.09$ with all estimated standard deviations being less than 0.02 Å in bond distances and 1.0° in bond angles.

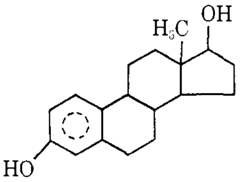
The estrogens are the essential hormones for the development of primary and secondary female sex characteristics. The ring structures of the three primary estrogens found in humans (I–III) have in common a characteristic β-OH group at the 3 position and an angular methyl at the 13-ring juncture position. Both estrone (with a keto group at the 17 position) and estradiol (with an alcohol at the 17β position) are metabolized to estriol (I). Although the mechanism is not clearly understood, it has been suggested that a fundamental action of estrogens is activation of a transhydrogenase that catalyzes transfer of hydrogen from NADPH + H^+ to NAD^+, forming $NADH^+$ + H^+ and connecting the two NAD systems to each other.[1]



I, estriol

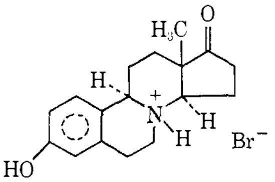


II, estrone



III, estradiol

The preparation of aza analogs of these steroids was predicated on the belief that molecular modifications of these basic structures could lead to new compounds of enhanced pharmaceutical value.[2] 8-Azaestrone was prepared[3,4] and of the various stereoconformers formed, the hydrobromide derivative (IV) of the compound presumed to be the exact analog of natural estrone was separated and recrystallized. Support for this conformation of the 8-azaestrone compound was ingeniously drawn from a variety of indirect evidence:[3] A, ir studies (the presence of prominent Bohlmann bands);[5] B, synthetic evidence for the *trans* configuration of the B/C rings; C, nmr chemical shifts of the C-9 proton; D, nmr solvent shifts of the methyl singlet.



IV

Indications are that the 8-azaestrone itself has limited estrogenic activity.[6] A three-dimensional X-ray diffraction study was thus undertaken to answer a variety of questions. (1) What is the structure of the 8-azaestrone hydrobromide? (2) Does it have the same conformation as estrone itself? A previous structure study[7] of the monobromo derivative, 4-bromoestrone, has already been published. (3) Are the indirect methods used in the conformational assignments in the 8-azaestrone valid? If so, this would presumably validate such methods of stereochemical assignments throughout the azasteroid series. (4) Does the insertion of a quaternary nitrogen within the ring structure cause small or large modifications in bond distances, bond angles, and other intimate molecular parameters? (5) To what can one ascribe the lack of estrogenic activity in the 8-azaestrone?

Crystal Data

Samples of the 8-azaestrone hydrobromide were made

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(1) B. Harrow and A. Mazur, "Textbook of Biochemistry," 9th ed, W. B. Saunders and Co., Philadelphia, Pa., 1966.

(2) M. Martin-Smith and M. G. Sugrue, *J. Pharm. Pharmacol.*, 16, 569 (1964).

(3) A. I. Meyers and J. C. Sircar, *Tetrahedron*, 23, 785 (1967).

(4) K. I. Meltzer, D. M. Lustgarten, R. J. Stanaback, and R. E. Brown, *Tetrahedron Letters*, 1581 (1963).

(5) F. Bohlmann, *Chem. Ber.*, 91, 2157 (1958).

(6) R. L. Kroc and T. Mischler, Werner-Lambert, private communication, 1968.

(7) D. A. Norton, G. Kartha, and C. T. Lu, *Acta Cryst.*, 16, 89 (1963).

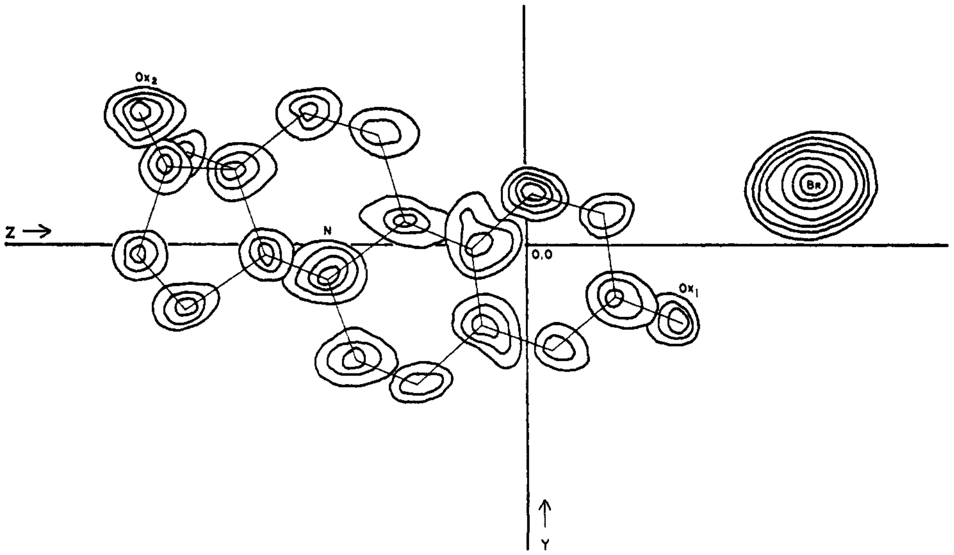

Figure 1.

available to us by Brown[8] and samples of the methoxy derivative by Meyers.[9] Attempts were made to recrystallize both, and finally single crystals of the 8-azaestrone hydrobromide were grown from absolute methanol. One such crystal, sufficiently small so that absorption errors would be minimized, was selected for this study.

Intensity data were collected using a precession camera and filtered Mo Kα radiation. Intensity data of the zero and first seven levels about the [001] axis, the zero and first five levels about the [010] axis, and an additional zone (*hkk*) were collected. Systematic absences for the *h*0*l* reflections when *l* was odd and the 0*k*0 reflections when *k* was odd led to the space group $P2_1/c$.

The cell dimensions of this crystal were $a = 8.655 \pm 0.003$, $b = 12.639$, and $c = 15.422$ Å with a monoclinic angle of $\beta = 111° 55' \pm 5'$. The experimentally measured density (using flotation techniques) of 1.491 ± 0.005 g/cc agreed with a density of 1.494 g/cc calculated by assuming four molecules per unit cell. A total of 1422 independent reflections were recorded, with all intensities estimated using a Nonius integrating densitometer.

Structure Determination

A three-dimensional Patterson synthesis based on the squares of the observed structure factors was calculated and led immediately to trial coordinates for the bromine. Least-squares refinement of these trial coordinates, using all data, led to *R*, *r* values[10] = 0.358, 0.455. A three-dimensional Fourier map was then calculated, based on the phases resulting from the least-squares refinement of the bromine position and the magnitudes of the observed structure factors. This map (Figure 1) yielded electron density peaks corresponding to all 20 atoms in addition to the bromine peak. The peak heights (in arbitrary units) were subsequently shown to correspond to the atoms as: bromine (260), oxygen (42 = carbonyl, 36 = phenolic), nitrogen (41), and carbons (32–40). Repeated cycles of isotropic least-squares refinements, using a full matrix and unit weights ultimately reduced the values of the reliability indices to *R*, *r* = 0.121, 0.131. The isotropic temperature factors were then converted to

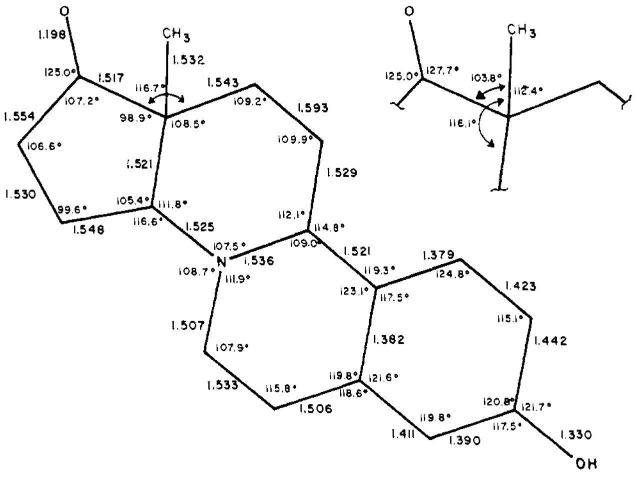

Figure 2.

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(8) R. E. Brown, private communication, 1967.

(9) A. I. Meyers, private communication, 1967.

(10) The least-squares program used in these calculations is a version of ORFLS by Busing, Martin, and Levy, modified for use on a 32k IBM 7040. The program uses the full matrix and fractional shifts and minimizes a weighted residual factor. The expression for the reliability factor (*R*) and the weighted reliability factor (*r*) are

$$R = \frac{\sum \big| |F_o| - |F_c| \big|}{\sum |F_o|}$$

$$r = \left[\frac{\sum (wF_o - kF_c)^2}{\sum wF_o^2}\right]^{1/2}$$

Unit weighting factors were used in refining this structure. Material supplementary to this article has been deposited as Document No. NAPS-00161 with the ASIS National Auxiliary Publication Service, c/o CCM Information Sciences, Inc., 22 West 34th St., New York, N. Y., 10001. A copy may be secured by citing the document number and by remitting $1.00 for microfiche or $3.00 for photocopies. Advance payment is required. Make checks or money orders payable to: ASIS–NAPS.

Table I. Coordinates and Temperature Factors[a]

Atom	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
Br	0.1434	0.0749	0.3112	0.0116	0.0082	0.0043	−0.0025	0.0017	0.0005
O_1(OH)	0.1618	0.8946	0.1689	0.0243	0.0095	0.0046	−0.0005	−0.0052	0.0032
O_2(C═O)	0.4258	0.1764	0.5751	0.0276	0.0072	0.0067	−0.0045	0.0075	−0.0006
N	0.2618	0.9554	0.7811	0.0130	0.0034	0.0031	0.0001	−0.0003	0.0001
C1	0.2525	0.0678	0.0038	0.0116	0.0046	0.0037	0.0014	0.0028	0.0002
C2	0.2129	0.0420	0.0830	0.0135	0.0060	0.0043	−0.0007	0.0025	−0.0012
C3	0.1885	0.9309	0.0947	0.0110	0.0064	0.0040	0.0015	0.0021	−0.0008
C4	0.1955	0.8573	0.0294	0.0097	0.0065	0.0035	−0.0003	0.0020	0.0013
C5	0.2277	0.8912	0.9505	0.0101	0.0037	0.0048	0.0014	0.0026	0.0016
C6	0.2211	0.8108	0.8771	0.0198	0.0029	0.0024	−0.0002	0.0045	0.0004
C7	0.3085	0.8426	0.8109	0.0207	0.0025	0.0040	0.0009	0.0047	0.0022
C9	0.3255	0.0323	0.8640	0.0137	0.0039	0.0021	−0.0004	0.0014	0.0000
C10	0.2645	0.9955	0.9396	0.0067	0.0039	0.0029	0.0002	0.0017	0.0004
C11	0.2848	0.1474	0.8336	0.0308	0.0029	0.0048	0.0025	0.0086	0.0012
C12	0.3596	0.1768	0.7567	0.0240	0.0022	0.0038	−0.0012	0.0052	0.0013
C13	0.2929	0.0980	0.6745	0.0115	0.0036	0.0040	−0.0003	0.0015	0.0008
C14	0.3419	0.9869	0.7121	0.0132	0.0033	0.0027	0.0003	0.0026	0.0003
C15	0.3026	0.9157	0.6246	0.0208	0.0045	0.0035	−0.0004	0.0050	−0.0007
C16	0.3722	0.9858	0.5669	0.0194	0.0033	0.0069	−0.0003	0.0066	0.0007
C17	0.3752	0.1006	0.6031	0.0144	0.0054	0.0032	−0.0021	0.0025	−0.0011
C18	0.1018	0.1158	0.6162	0.0085	0.0067	0.0067	−0.0004	0.0009	0.0042

[a] The temperature factors are given in the form: $T = \exp\{-(B_{11}h^2 + B_{22}k^2 + B_{33}l^2 + B_{12}2hk + B_{13}2hl + B_{23}2kl)\}$.

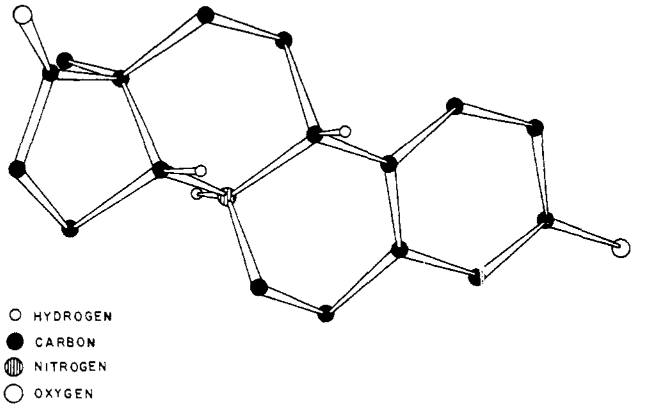


Figure 3.

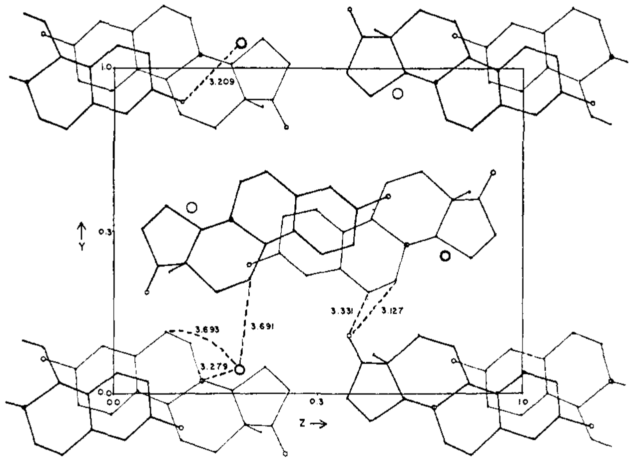


Figure 4.

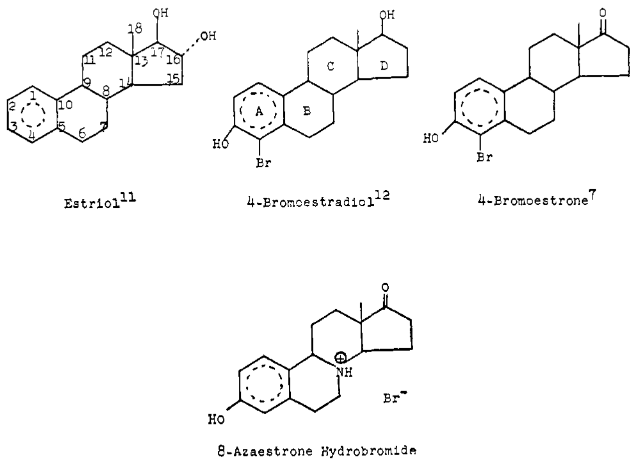
anisotropic temperature factors and six additional cycles of refinement reduced the values to R, r = 0.088, 0.097. At this point, the estimated standard deviations for all coordinates were below 0.02 Å, and the estimated standard deviations for all temperature factors were below 0.04 Å^2. Our least-squares program contains a subroutine which terminates refinements when the maximum calculated shift in both the coordinates and the temperature factors drop below 0.0001. At this point, the computer terminated the refinements.

To confirm our results, three-dimensional Fourier and difference Fourier maps were calculated based on the results of the last cycle of least-squares refinement. Both maps were checked for the existence of any extraneous peaks comparable in height to the atoms in our structure. No extraneous peaks of this magnitude were found, the highest extraneous positive region in the maps (except for small residual bromine ripples) having a peak height of 1.2 e/Å^3. Hydrogen peak positions for all but the phenyl hydrogens were then calculated based on geometric considerations (C–H = 1.10 Å, ∠HCH = 109° and all other angles equally split and near 109°). These calculated positions were compared to the final difference Fourier map and all of the calculated peaks fell in positive regions ranging in height from 0.4 to 1.2 e/Å^3. Consequently, we were satisfied that the structural parameters had been refined to the limit of our data.

Discussion

All bond distances and bond angles in this structure study have estimated standard deviations of less than 0.02 Å and 1.0°, respectively. Table I contains the final values of the coordinates and anisotropic temperature factors. A drawing of the molecule, Figure 2, oriented in the same fashion as the Fourier map, shows the actual bond distances and angles obtained.

The structure of the compound studied has the natural conformation with α-hydrogens at the C-9 and C-14 positions and a β-hydrogen at the C-8 position. This is illustrated in the perspective drawing, Figure 3, which shows the molecular framework including the



Estriol[11] 4-Bromoestradiol[12] 4-Bromoestrone[7]

8-Azaestrone Hydrobromide

Figure 5.

hydrogens in question. Its stereochemistry is thus identical with the previously determined 4-bromoestrone.[7] The stereoisomeric assignments based upon ir and nmr methods are correct and so these indirect methods would thus seem to be of high validity in future aza steroid work.

Bond scans were made of closest inter- and intramolecular contact distances for the carbon ring skeleton and for the structure including the calculated hydrogen positions. Figure 4 shows the contents of the unit cell, projected down the x axis (hydrogens excluded). All distances less than 3.3 Å for contacts not involving the bromine and all distances less than 3.7 Å for contacts involving the bromine are shown. The structure does not show the end-to-end O···H···O hydrogen bonding scheme exhibited in the other estrogen structures but rather indicates a van der Waals pattern characteristic of salts of organic moieties. Within this pattern, the bromide ion lies between the oxygens (3.21 Å from the phenolic oxygen and 4.09 Å from the carbonyl oxygen) of different molecules and thus would disrupt the hydrogen bonding which would have been anticipated.

Since the structures of all of the principal members of the estrogen family have been determined, it is of interest to compare those results with the parameters obtained in this study. All of the compounds in the form of the derivative studied, are illustrated in Figure 5 with the labeling to be used in this discussion.

One can check the internal reliability of the various determinations by focusing on the phenyl (A ring) parameters. The average values for the phenyl angles vary from 119.8 ± 0.1° in the estriol[11] to 121.3 ± 1.6° in the 4-bromoestrone with those in this study having values of 119.9 ± 2.4°. All of these values are well within one esd of the accepted value of 120° in benzene itself and all are internally consistent. The average value of the bond distances in the phenyl ring varies from 1.39_1 ± 0.014 Å in 4-bromoestradiol[12] to 1.40_4 ± 0.02_1 Å in this study. Again, within one esd all of these values are in agreement with each other and with the accepted C–C distance in benzene of 1.39_6 Å.

Table II. Comparison of Distances

Bond	8-Azaestrone hydrobromide	4-Bromoestrone	4-Bromoestradiol	Estriol (av)
1-2	1.42	1.39	1.39	1.388
2-3 ring A	1.44	1.34	1.40	1.373
3-4 (phenyl)	1.39	1.42	1.39	1.396
4-5	1.41	1.43	1.37	1.406
5-10 fusion A/B	1.38	1.40	1.42	1.400
5-6	1.51	1.46	1.52	1.510
6-7	1.53	1.56	1.56	1.505
7-8 ring B	1.51	1.53	1.54	1.540
9-10	1.52	1.55	1.54	1.530
8-9 fusion B/C	1.54	1.56	1.56	1.545
9-11	1.53	1.58	1.56	1.550
11-12	1.59	1.61	1.57	1.542
12-13 ring C	1.54	1.55	1.54	1.526
8-14	1.53	1.50	1.53	1.520
13-14 fusion C/D	1.52	1.49	1.53	1.560
14-15	1.55	1.57	1.57	1.536
15-16	1.53	1.56	1.58	1.536
16-17 ring D	1.55	1.57	1.57	1.534
17-13	1.52	1.53	1.54	1.538
13-CH_3	1.53	1.58	1.59	1.537
3-OH	1.33	1.37	1.36	1.380
17-O	1.20	1.20	...	...

Table III. Comparison of Angles

Angles	8-Azaestrone hydrobromide	4-Bromoestrone	4-Bromoestradiol	Estriol (av)
Internal				
10,1,2	125	121	123	122.9
1,2,3	115	121	119	118.4
2,3,4 ring A	121	119	119	121.0
3,4,5 (phenyl)	120	122	123	119.7
4,5,10	122	116	119	120.1
5,10,1	118	121	119	117.7
9,10,5	123	118	121	120.2
10,5,6	120	123	124	122.3
5,6,7	116	117	110	114.8
6,7,8 ring B	108	108	109	110.2
7,8,9	112	108	108	108.6
8,9,10	109	109	110	110.6
9,8,14	108	106	105	107.7
8,14,13	112	112	111	113.7
14,13,12	109	108	114	107.9
13,12,11 ring C	109	106	109	111.2
12,11,9	110	106	113	107.9
11,9,8	112	111	110	112.5
13,14,15	105	106	104	103.8
14,15,16	100	102	101	104.1
15,16,17 ring D	107	102	106	105.5
16,17,13	107	108	105	104.5
17,13,14	99	103	99	96.9
External				
16,17, oxygen C=O	125	124	...	...
13,17, oxygen	128	127	...	...
17,13, methyl	104	101	110	109.9
12,13 methyl	112	113	110	111.4
14,13, methyl	116	116	117	113.0
17,13,12	117	114	114	116.3
2,3, oxygen C—OH	122	122	123	119.0
4,3, oxygen	118	119	121	120.0

The C-3 phenolic hydroxyl group common to all of the structures has an average value of 1.37_1 ± 0.00_6 Å in the members of the estrogen family and 1.33_0 in the azaestrone. Thus it is shorter by 2σ in the azaestrone. Although this is not a statistically significant difference,

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(11) A. Cooper, D. A. Norton, and H. Hauptman, submitted for publication.

(12) D. A. Norton, G. Kartha, and C. T. Lu, *Acta Cryst.*, 17, 77 (1964).

it should be borne in mind that one of the principal differences in the azaestrone structure from all of the other estrogens determined is its lack of intermolecular hydrogen bonding. Furthermore, the estrogens themselves are removed from the body through the urine in the form of water-soluble compounds such as estrone sulfate and estriol glucuronidate which form compounds utilizing the phenolic–OH at the 3 position.

If any other differences in molecular parameters exist between the azaestrone and the other structures, these differences should be most pronounced in those bonds and angles about the 8 position. In actual fact, the distances and angles in this region agree within one esd for both the 8-azaestrone salt and the estrone compound.

The stereochemistry, bond distances, and bond angles for this compound and the three estrogens are thus strikingly similar. A detailed comparison of the four structures is given in Tables II and III. One rather obvious conclusion is that the lack of estrogenic activity in 8-azaestrone is *not* a function of any large conformational changes.

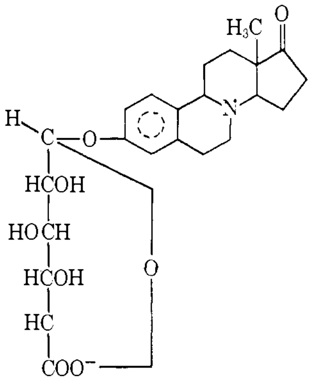
It has been suggested that the presence in the molecule of two groups capable of entering into hydrogen bonding and which are held in a certain steric relationship[11,13–15] affect the estrogenic activity. If one compares the separation of the oxygen on the 3 and the 17β positions for the three natural estrogens to the same distance in the azaestrone no appreciable differences are observable (Table IV). Thus, since the 8-azaestrone hydrobromide conforms again to the trends in the natural estrogens, its lack of estrogenic activity can not arise from any such differences.

Table IV. Intramolecular Oxygen Distances

Compound	Ref	O···O distance, Å
4-Bromoestrone	7	10.78 ± 0.04
8-Azaestrone	This study	10.83 ± 0.02
4-Bromo-17β-estradiol	12	10.95 ± 0.04
Estriol 3-17β (molecule 1)	11	10.952 ± 0.007
Estriol 3-17β (molecule 2)	11	11.085 ± 0.007

Studies on the conjugation of estrogens with glucuronic acid indicate that the preferential glucuronidation occurs at the 3 position.[16] The reactivity of estradiol far exceeds that of estrone. However, the conjugation of the 3-methyl ester of estradiol is much less than that of estrone.[17] Further evidence for the specificity was given by the results of a large-scale experiment in which 17β-estradiol-6,7-^{3}H was incubated with liver homogenate in the presence of both UDP-glucosiduronic acid and UDP-N-acetylglucosamine.[18] The product formed was the estradiol-3-glucuronoside-17α-N-acetylglucosaminide. Thus, there is speculation as to whether those results together with the preferential formation of 3-glucuronosides of estradiols can be solely for the purpose of excretion. Rather, . . . "this marked specificity in the attachment of the sugars to the two conjugating sites may be related to some effect of the estrogen on mucopolysaccharide. . . ."[18]

The lack of estrogenic activity for the 8-azaestrone thus may in some way be related to this glucuronidation at the 3 position. If, in the case of the normal estrogens, the product of this glucuronidation is similar to the acetal V, then this reaction would be inhibited by the preferential protonation of the tertiary nitrogen.



V

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(13) E. Macovski and J. Georescu, *Bull. Sci. Acad., Roumaine*, **28**, 645 (1946).

(14) A. L. Fisher, H. H. Keasling, and F. W. Shueler, *Proc. Soc. Exptl. Biol. Med.*, **81**, 439 (1952).

(15) M. Oki, *J. Chem. Soc. Japan*, **73**, 252 (1952).

(16) C. G. Beling, *Acta Endocrinol. Suppl.*, **67**, 79 (1962).

(17) E. R. Smith and H. Breuer, *Biochem. J.*, **88**, 168 (1963).

(18) H. Jirku and D. S. Layne, *Biochemistry*, **4**, 2126 (1965).