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Structural Studies of Thiophilic *N*-Chloroazasteroids

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Abstract

N-Chloroazasteroids form covalent S—N bonds with thiol groups and so are of interest as chemoselective irreversible binding agents for the active sites of steroid receptors and enzymes. The solid-state structures of *N*-chloro-3-methoxy-17-aza-*D*-homo-1,3,5(10)-estratrien-16-one (1), *N*-chloro-3-methoxy-17-aza-*D*-homo-1,3,5(10)-estratrien-17a-one (2) and *N*-chloro-3-methoxy-16-aza-1,3,5(10)-estratrien-17-one (3) were determined to obtain information about the spatial arrangement of the N—Cl groups. Crystal data: (1) $C_{19}H_{24}ClNO_2$, $M_r = 333{\cdot}84$, orthorhombic, $P2_12_12_1$, $a = 8{\cdot}0190\ (3)$, $b = 12{\cdot}7175\ (5)$, $c = 16{\cdot}5047\ (9)$ Å, $V = 1683{\cdot}2\ (1)$ Å^3, $Z = 4$, $D_x = 1{\cdot}317$ g cm^{-3}, λ(Cu $K\alpha$) = 1·54178 Å, $\mu = 20{\cdot}9$ cm^{-1}, $F(000) = 712$, $T = 295$ K, $R = 0{\cdot}045$ for 1786 observed reflections; (2) $C_{19}H_{24}ClNO_2$, $M_r = 333{\cdot}84$, orthorhombic, $P2_12_12_1$, $a = 10{\cdot}9853\ (7)$, $b = 11{\cdot}8216\ (5)$, $c = 12{\cdot}9851\ (9)$ Å, $V = 1686{\cdot}3\ (2)$ Å^3, $Z = 4$, $D_x = 1{\cdot}315$ g cm^{-3}, λ(Cu $K\alpha$) = 1·54178 Å, $\mu = 20{\cdot}9$ cm^{-1}, $F(000) = 712$, $T = 295$ K, $R = 0{\cdot}035$ for 1891 observed reflections; (3) $C_{18}H_{22}ClNO_2$, $M_r = 319{\cdot}81$, monoclinic, $P2_1$, $a = 13{\cdot}246\ (2)$, $b = 7{\cdot}972\ (2)$, $c = 7{\cdot}696\ (3)$ Å, $\beta = 90{\cdot}24\ (2)^\circ$, $V = 812{\cdot}7\ (4)$ Å^3, $Z = 2$, $D_x = 1{\cdot}307$ g cm^{-3}, λ(Cu $K\alpha$) = 1·54178 Å, $\mu = 21{\cdot}4$ cm^{-1}, $F(000) = 340$, $T = 295$ K, $R = 0{\cdot}045$ for 1636 observed reflections. Conformational analysis and comparisons of molecules of (1), (2) and (3) are presented. If the phenolic rings of these molecules are superimposed, the orientation of their reactive *N*-chloro groups is similar in (1) and (2) and different in (3), due to the different five-membered *D* ring in (3) and to the different *B*-ring conformation of (3). The distances in this superposition between the Cl atom of steroid (3) and the Cl atoms of steroids (1) and (2) are 1·87 and 2·27 Å, respectively.

Introduction

Modified steroids containing alkylating or other reactive functional groups that are capable of bonding covalently to the active sites of their receptor proteins, or to those of enzymes that use them as substrates, are of biological and medicinal interest. They can be used as affinity labels, as enzyme inhibitors, and in the treatment of breast and prostatic carcinomas (Dence, 1980).

Jensen, Hurst, DeSombre & Jungblut (1967) demonstrated that thiol (sulfhydryl) groups in the estrogen receptor play a crucial role in the binding of estradiol and, presumably, other estrogens as well. Reactive estrogen analogues that are capable of selectively forming covalent bonds with thiol groups are therefore of special interest (Simons, Pons & Johnson, 1980; Chin & Warren, 1968).

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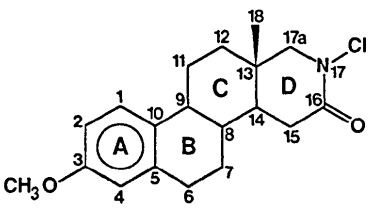
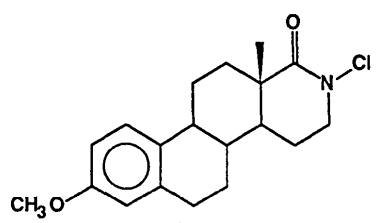
Recently, Back & Brunner (1989) reported the preparation of several novel *N*-chloroazasteroids that react chemoselectively with model thiols, resulting in the formation of covalent S—N (sulfenamide) bonds. If these compounds behave similarly with thiol residues in the active sites of enzymes or recognition sites of receptors, then potential medicinal applications can be envisaged. Since such behaviour will depend on the precise spatial disposition of the reactive functional group (*i.e.* the *N*-chloro group) relative to any available thiol groups in the active site, a precise knowledge of the conformations and spatial characteristics of the *N*-chloroazasteroids would be of interest.

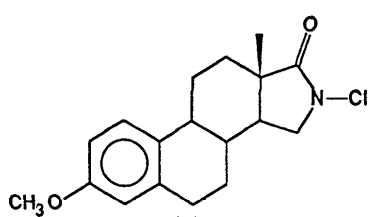
X-ray crystal structure analysis of three *N*-chloroazasteroids was undertaken to determine their conformations and the spatial arrangement of the reactive N—Cl groups relative to the common phenolic ring. Molecular structures of *N*-chloro-3-methoxy-17-aza-*D*-homo-1,3,5(10)-estratrien-16-one (1), *N*-chloro-3-methoxy-17-aza-*D*-homo-1,3,5(10)-estratrien-17a-one (2) and *N*-chloro-3-methoxy-16-aza-1,3,5(10)-estratrien-17-one (3) (see below for structural diagrams) are presented in this paper.



(1)



(2)



(3)

These compounds, because of their similarity to estradiol and because of a common phenolic *A* ring (Duax & Griffin, 1985, 1987; Hähnel, Twaddle & Ratajczak, 1973; Raynaud, Ojasoo, Bouton & Philibert, 1979), are expected to bind to the estrogen receptor. They, however, do not possess the 17β-hydroxy function important for high-affinity binding; when the 17β-hydroxy substituent is replaced with a 17-carbonyl group (*e.g.* in estrone), the affinity is decreased (Hähnel *et al.*, 1973; Raynaud *et al.*, 1979; Katzenellenbogen, Heiman, Carlson, Payne & Lloyd, 1980). The binding affinity of steroids (2) and (3) to the estrogen receptor may therefore be similar to that of estrone. It has also been suggested that absence of an oxygen substituent comparable to the estradiol 17-O accounts for the inactivity or antagonistic properties observed for some ligands (Duax, Griffin, Weeks & Korach, 1985). Thus, *N*-chloroazasteroids (1), (2) and (3) may be antagonists or weak agonists at the estrogen receptor. In either case, however, they would function as estrogen-receptor antagonists if, after binding to the estrogen receptor, covalent bonding between their *N*-chloro groups and a thiol residue in the receptor occurs.

Results of this study may help to interpret the results of future biological testing and, thereafter, provide guidance for the synthesis of more effective compounds. A sufficiently diverse array of *N*-chloroazasteroids could also be used to map the position(s) of the thiol group(s) in the binding site of the receptor or enzyme, relative to the binding position of the *A* ring, as only those compounds with the reactive group in close proximity to a thiol residue would bind covalently.

Experimental

All three *N*-chloroazasteroids were prepared from the corresponding parent lactams by treatment with potassium *tert*-butoxide in THF, followed by chlorination with *N*-chlorosuccinimide, as described previously (Back & Brunner, 1989). The products were homogeneous on TLC and had melting points and spectral properties in accordance with those reported earlier. IR spectra were recorded using KBr disks on a Nicolet 5DX spectrometer.

Transparent, colorless crystals of (1) and (2) were grown from methanol, and of (3) from ethyl acetate, by slow evaporation. Lattice cell parameters and intensities were measured at room temperature on an Enraf–Nonius CAD-4F diffractometer using Ni-filtered Cu $K\alpha$ radiation (λ = 1·54178 Å) and the ω–2θ scan mode. The intensities of three standard reflections were measured every 2000 s and showed a variation of less than 3% during the data collection. Corrections for Lorentz and polarization effects, but not for absorption, were applied. All structures were solved by direct methods with use of the program *GENTAN* (Hall, 1987) for (1) and (2) and the program *SHELXS*86 (Sheldrick, 1985) for (3); the positions of all of the non-H atoms were located in

Table 1. *Crystallographic data for steroids* (1), (2) *and* (3)

	(1)	(2)	(3)
Crystal dimensions (mm)	0·24 × 0·32 × 0·34	0·40 × 0·46 × 0·46	0·15 × 0·34 × 0·40
θ range of reflections used for cell parameters (°)	33–68	36–49	35–52
hkl range	0/10, 0/15, 0/20	0/13, 0/14, 0/16	0/16, 0/9, −9/9
Maximum $(\sin\theta/\lambda)$ (Å^{-1})	0·6258	0·6254	0·6254
No. of unique reflections measured	1979	1978	1791
No. of observed reflections $[I > 2{\cdot}5\sigma(I)]$	1786	1891	1636
No. of parameters refined	305	305	288
Weighting scheme	$w^{-1} = \sigma^2(F) + 0{\cdot}00015F^2$	$w^{-1} = \sigma^2(F) + 0{\cdot}0001F^2$	$w^{-1} = \sigma^2(F) + 0{\cdot}00015F^2$
R	0·045	0·035	0·045
wR	0·054	0·046	0·054
Goodness of fit, S	1·14	1·08	1·03
Maximum Δ/σ	0·003	0·015	0·018
Maximum $\Delta\rho$ (e Å^{-3})	0·17	0·18	0·36
Minimum $\Delta\rho$ (e Å^{-3})	−0·25	−0·20	−0·23
Empirical extinction parameter	0·09 (2) × 10^{-3}	0·28 (3) × 10^{-3}	0·35 (6) × 10^{-3}

each case. In structure (3), the cell origin was defined by holding the y coordinate of atom C(1) invariant. Blocked least-squares refinement on F was performed with the *XTAL*2.2 package (Hall & Stewart, 1987) for steroids (1) and (2) and with the *XRAY*76 package (Stewart, 1976) for steroid (3). Calculations were carried out on a VAX (VMS) computer (*XTAL*2.2) or a Honeywell (Multics) computer (*XRAY*76 and *SHELXS*86). All H atoms were found in difference Fourier syntheses and were included in the refinement with isotropic thermal parameters. In the final cycles of refinement all non-H atoms were refined with anisotropic thermal parameters. The final cycles of refinement varied the coordinates of all the atoms, the anisotropic thermal parameters of the non-H atoms, the isotropic thermal parameters of the H atoms, and the isotropic secondary-extinction parameter. The final difference Fourier syntheses were clean: the highest peak for steroid (3) was approximately in the middle of the N—Cl bond; no notable peaks were found for steroids (1) and (2). The feature in the difference Fourier synthesis of steroid (3) may be due to the sp^2 hybridization of the N atom and the consequent higher bond density in the N—Cl bond (see below). Neutral-atom scattering factors and anomalous-dispersion corrections for the non-H atoms were taken from Cromer & Mann (1968) and for the H atoms from Stewart, Davidson & Simpson (1965). Isotropic secondary-extinction corrections were based on the approach of Zachariasen (1967) and Larson (1967). Drawings of the molecules were prepared with the program *ORTEP*II (Johnson, 1976), molecular comparisons were made using the program *PROFIT* (Smith, 1983) and visualized with the program *MMS* (Dempsey, 1986). A summary of the crystal data and the structure refinements is given in Table 1.

Results

The final fractional coordinates and the equivalent isotropic thermal parameters for the non-H atoms of (1), (2) and (3) are reported in Table 2.* Selected torsion angles are listed in Table 3. Table 4 presents the conformations and selected asymmetry parameters (Duax & Norton, 1975) for rings *A*, *B*, *C* and *D* of each molecule. The molecular conformations and atomic labeling schemes for (1), (2) and (3) are shown in Figs. 1, 2 and 3, respectively.

The *N*-chlorolactam moiety results in a flattening of the *D* ring in all three steroids. The extent of this flattening and the specific conformation of the ring depends on the position of the lactam carbonyl group and the size of the ring. Amide resonance in the *N*-chlorolactam is evident from the lengths of the C(=O)—N bonds and from the planarity of the O=C—N—Cl group (torsion angle near 0°). The bond lengths are 1·372 (5), 1·354 (3) and 1·362 (6) Å, and the torsion angles are 7·5 (5), −4·7 (4) and 6·3 (8)°, for compounds (1), (2) and (3), respectively. However, as expected, the inductive effect of the *N*-chloro substituent suppresses the resonance interaction relative to that in the parent lactams. This is confirmed by IR measurements, which show higher carbonyl stretching frequencies, and therefore higher C=O bond orders, for the *N*-chloro derivatives compared to the unsubstituted lactams [(1) 1675 *versus* 1656 cm^{-1}; (2) 1685 *versus* 1649 cm^{-1}; (3) 1720 *versus* 1704 cm^{-1}]. Moreover, as indicated by the endocyclic torsion angle C—C(=O)—N—C, the *D* ring is most flattened in the five-membered ring of steroid (3), which has a torsion angle of only 6·1 (6)°, whereas in compounds (1) and (2) the angle is −18·1 (5) and 28·7 (4)°. The larger departure from planarity in the *D* rings of steroids (1) and (2) is associated with a non-planar hybridization of the N atoms: the sum of the bond angles around N are 356·8 and 354·5° for (1) and (2), respectively,

* Lists of anisotropic thermal parameters for non-H atoms, H-atom parameters, bond distances and angles for all atoms, and structure factors have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 53702 (48 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 2. *Atomic coordinates and equivalent isotropic thermal parameters* ($Å^2$) *for the non-*H *atoms of steroids* (1), (2) *and* (3)

$U_{eq} = (1/3)\sum_i \sum_j U_{ij} a_i^* a_j^* \mathbf{a}_i . \mathbf{a}_j .$

	x	y	z	U_{eq}
Steroid (1)				
C(1)	0·8527 (4)	0·3423 (3)	0·7969 (2)	0·061 (2)
C(2)	0·9152 (5)	0·4420 (3)	0·7930 (2)	0·064 (2)
C(3)	0·8231 (5)	0·5259 (3)	0·8216 (2)	0·056 (2)
C(4)	0·6657 (5)	0·5077 (3)	0·8520 (2)	0·055 (2)
C(5)	0·5997 (4)	0·4059 (3)	0·8551 (2)	0·051 (2)
C(6)	0·4255 (4)	0·3913 (3)	0·8885 (2)	0·057 (2)
C(7)	0·3499 (4)	0·2852 (3)	0·8687 (2)	0·057 (2)
C(8)	0·4742 (4)	0·1971 (3)	0·8863 (2)	0·048 (2)
C(9)	0·6274 (4)	0·2093 (3)	0·8302 (2)	0·049 (2)
C(10)	0·6938 (4)	0·3205 (3)	0·8284 (2)	0·049 (2)
C(11)	0·7569 (4)	0·1262 (3)	0·8507 (2)	0·060 (2)
C(12)	0·6865 (5)	0·0156 (3)	0·8467 (2)	0·064 (2)
C(13)	0·5330 (4)	0·0006 (3)	0·9008 (2)	0·051 (2)
C(14)	0·4028 (4)	0·0856 (3)	0·8792 (2)	0·051 (2)
C(15)	0·2440 (4)	0·0680 (3)	0·9280 (2)	0·061 (2)
C(16)	0·1783 (5)	−0·0436 (3)	0·9318 (2)	0·064 (2)
N(17)	0·2957 (4)	−0·1202 (2)	0·9188 (2)	0·066 (2)
C(17a)	0·4581 (5)	−0·1061 (3)	0·8811 (2)	0·063 (2)
C(18)	0·5823 (6)	0·0029 (4)	0·9905 (2)	0·063 (2)
O(3)	0·8954 (4)	0·6233 (2)	0·8158 (2)	0·073 (2)
C(31)	0·8136 (7)	0·7088 (4)	0·8522 (3)	0·073 (3)
O(16)	0·0353 (3)	−0·0639 (2)	0·9496 (2)	0·082 (2)
Cl(17)	0·22597 (14)	−0·24753 (8)	0·91699 (7)	0·085 (1)
Steroid (2)				
C(1)	0·3420 (2)	0·8369 (2)	0·6929 (2)	0·059 (1)
C(2)	0·3879 (2)	0·9408 (2)	0·6645 (2)	0·061 (1)
C(3)	0·3360 (2)	1·0384 (2)	0·7024 (2)	0·055 (1)
C(4)	0·2382 (2)	1·0310 (2)	0·7701 (2)	0·052 (1)
C(5)	0·1927 (2)	0·9257 (2)	0·8007 (2)	0·049 (1)
C(6)	0·0861 (3)	0·9211 (2)	0·8747 (2)	0·062 (1)
C(7)	0·0222 (2)	0·8082 (2)	0·8762 (2)	0·059 (1)
C(8)	0·1139 (2)	0·7117 (2)	0·8874 (2)	0·048 (1)
C(9)	0·1961 (2)	0·7097 (2)	0·7912 (2)	0·049 (1)
C(10)	0·2452 (2)	0·8259 (2)	0·7626 (2)	0·048 (1)
C(11)	0·2952 (2)	0·6202 (2)	0·8039 (2)	0·061 (1)
C(12)	0·2405 (3)	0·5031 (2)	0·8228 (2)	0·062 (1)
C(13)	0·1541 (2)	0·5012 (2)	0·9173 (2)	0·050 (1)
C(14)	0·0562 (2)	0·5944 (2)	0·9025 (2)	0·048 (1)
C(15)	−0·0375 (3)	0·5853 (2)	0·9888 (2)	0·063 (1)
C(16)	−0·0973 (3)	0·4706 (3)	0·9900 (2)	0·071 (2)
N(17)	−0·0094 (2)	0·3785 (2)	0·9773 (2)	0·064 (1)
C(17a)	0·0958 (2)	0·3833 (2)	0·9232 (2)	0·056 (1)
C(18)	0·2276 (3)	0·5145 (3)	1·0179 (3)	0·071 (2)
O(3)	0·3870 (2)	1·1378 (2)	0·6686 (2)	0·070 (1)
C(31)	0·3447 (4)	1·2396 (3)	0·7130 (3)	0·075 (2)
O(17a)	0·1443 (2)	0·2993 (2)	0·8890 (2)	0·084 (2)
Cl(17)	−0·07565 (7)	0·24775 (7)	0·98579 (6)	0·0789 (5)
Steroid (3)				
C(1)	0·8932 (3)	0·0614*	−0·3816 (5)	0·052 (2)
C(2)	0·9873 (3)	0·0622 (8)	−0·4580 (5)	0·054 (2)
C(3)	1·0661 (3)	0·1457 (7)	−0·3768 (5)	0·051 (2)
C(4)	1·0476 (3)	0·2240 (8)	−0·2215 (5)	0·054 (2)
C(5)	0·9541 (3)	0·2247 (7)	−0·1433 (4)	0·047 (2)
C(6)	0·9434 (3)	0·3053 (8)	0·0341 (6)	0·073 (3)
C(7)	0·8346 (3)	0·3285 (8)	0·0910 (6)	0·068 (3)
C(8)	0·7739 (3)	0·1697 (7)	0·0537 (4)	0·046 (2)
C(9)	0·7674 (2)	0·1444 (7)	−0·1464 (4)	0·045 (2)
C(10)	0·8728 (2)	0·1417 (7)	−0·2254 (4)	0·043 (2)
C(11)	0·7013 (3)	−0·0060 (8)	−0·1965 (5)	0·059 (2)
C(12)	0·5970 (3)	−0·0059 (8)	−0·1103 (5)	0·059 (2)
C(13)	0·6091 (2)	0·0116 (8)	0·0856 (5)	0·047 (2)
C(14)	0·6671 (3)	0·1750 (8)	0·1209 (4)	0·047 (2)
C(15)	0·6455 (3)	0·2117 (9)	0·3134 (5)	0·062 (2)
N(16)	0·5423 (2)	0·1462 (7)	0·3206 (4)	0·060 (2)
C(17)	0·5134 (3)	0·0406 (8)	0·1905 (5)	0·053 (2)
C(18)	0·6580 (3)	−0·1455 (8)	0·1642 (6)	0·065 (3)
O(3)	1·1624 (2)	0·1549 (7)	−0·4399 (4)	0·064 (2)
C(31)	1·1836 (3)	0·0692 (9)	−0·5975 (6)	0·075 (3)
O(17)	0·4295 (2)	−0·0201 (7)	0·1719 (4)	0·068 (2)
Cl(16)	0·4653 (1)	0·1913 (6)	0·4876 (1)	0·074 (1)

* The y coordinate of C(1) was held invariant to define the cell origin.

Table 3. *Selected torsion angles* (°) *for steroids* (1), (2) *and* (3)

Steroid (1)	
C(10)—C(1)—C(2)—C(3)	0·9 (6)
C(1)—C(2)—C(3)—C(4)	−1·7 (6)
C(2)—C(3)—C(4)—C(5)	0·8 (5)
C(3)—C(4)—C(5)—C(10)	0·9 (5)
C(4)—C(5)—C(10)—C(1)	−1·7 (5)
C(5)—C(10)—C(1)—C(2)	0·8 (5)
C(10)—C(5)—C(6)—C(7)	−15·9 (4)
C(5)—C(6)—C(7)—C(8)	47·2 (4)
C(6)—C(7)—C(8)—C(9)	−63·5 (3)
C(7)—C(8)—C(9)—C(10)	47·5 (3)
C(8)—C(9)—C(10)—C(5)	−17·2 (4)
C(9)—C(10)—C(5)—C(6)	0·8 (4)
C(14)—C(8)—C(9)—C(11)	−56·5 (3)
C(8)—C(9)—C(11)—C(12)	55·6 (4)
C(9)—C(11)—C(12)—C(13)	−55·7 (4)
C(11)—C(12)—C(13)—C(14)	54·1 (4)
C(12)—C(13)—C(14)—C(8)	−55·9 (3)
C(13)—C(14)—C(8)—C(9)	58·2 (3)
C(17a)—C(13)—C(14)—C(15)	59·5 (3)
C(13)—C(14)—C(15)—C(16)	−46·0 (4)
C(14)—C(15)—C(16)—N(17)	23·8 (5)
C(15)—C(16)—N(17)—C(17a)	−18·1 (5)
C(16)—N(17)—C(17a)—C(13)	34·2 (5)
N(17)—C(17a)—C(13)—C(14)	−52·7 (4)
O(16)—C(16)—N(17)—Cl(17)	7·5 (5)
C(2)—C(3)—O(3)—C(31)	−173·1 (4)
C(4)—C(3)—O(3)—C(31)	8·4 (5)
Steroid (2)	
C(10)—C(1)—C(2)—C(3)	−2·1 (4)
C(1)—C(2)—C(3)—C(4)	0·7 (4)
C(2)—C(3)—C(4)—C(5)	0·5 (4)
C(3)—C(4)—C(5)—C(10)	−0·4 (4)
C(4)—C(5)—C(10)—C(1)	−0·9 (3)
C(5)—C(10)—C(1)—C(2)	2·1 (4)
C(10)—C(5)—C(6)—C(7)	−17·3 (3)
C(5)—C(6)—C(7)—C(8)	48·8 (3)
C(6)—C(7)—C(8)—C(9)	−64·3 (3)
C(7)—C(8)—C(9)—C(10)	47·5 (3)
C(8)—C(9)—C(10)—C(5)	−17·5 (3)
C(9)—C(10)—C(5)—C(6)	1·6 (4)
C(14)—C(8)—C(9)—C(11)	−58·9 (2)
C(8) C(9)—C(11)—C(12)	57·5 (3)
C(9)—C(11)—C(12)—C(13)	−56·2 (3)
C(11)—C(12)—C(13)—C(14)	54·5 (3)
C(12)—C(13)—C(14)—C(8)	−56·8 (3)
C(13)—C(14)—C(8)—C(9)	60·0 (2)
C(17a)—C(13)—C(14)—C(15)	55·4 (3)
C(13)—C(14)—C(15)—C(16)	−58·7 (3)
C(14)—C(15)—C(16)—N(17)	44·5 (3)
C(15)—C(16)—N(17)—C(17a)	−31·0 (4)
C(16)—N(17)—C(17a)—C(13)	28·7 (4)
N(17)—C(17a)—C(13)—C(14)	−39·8 (3)
O(17a)—C(17a)—N(17)—Cl(17)	−4·7 (4)
O(17a)—C(17a)—C(13)—C(18)	−91·4 (3)
C(2)—C(3)—O(3)—C(31)	−174·0 (3)
C(4)—C(3)—O(3)—C(31)	6·1 (4)
Steroid (3)	
C(10)—C(1)—C(2)—C(3)	0·3 (7)
C(1)—C(2)—C(3)—C(4)	0·5 (8)
C(2)—C(3)—C(4)—C(5)	−0·5 (8)
C(3)—C(4)—C(5)—C(10)	−0·1 (6)
C(4)—C(5)—C(10)—C(1)	0·9 (7)
C(5)—C(10)—C(1)—C(2)	−1·0 (6)
C(10)—C(5)—C(6)—C(7)	−15·1 (8)
C(5)—C(6)—C(7)—C(8)	44·8 (6)
C(6)—C(7)—C(8)—C(9)	−65·9 (5)
C(7)—C(8)—C(9)—C(10)	55·1 (5)
C(8)—C(9)—C(10)—C(5)	−26·0 (7)
C(9)—C(10)—C(5)—C(6)	6·0 (7)
C(14)—C(8)—C(9)—C(11)	−52·4 (5)
C(8)—C(9)—C(11)—C(12)	50·7 (5)
C(9)—C(11)—C(12)—C(13)	−53·3 (6)
C(11)—C(12)—C(13)—C(14)	58·0 (6)
C(12)—C(13)—C(14)—C(8)	−65·6 (4)
C(13)—C(14)—C(8)—C(9)	61·2 (5)
C(17)—C(13)—C(14)—C(15)	38·6 (4)
C(13)—C(14)—C(15)—N(16)	−34·0 (5)
C(14)—C(15)—N(16)—C(17)	18·0 (6)
C(15)—N(16)—C(17)—C(13)	6·1 (6)
N(16)—C(17)—C(13)—C(14)	−27·5 (5)
Cl(16)—N(16)—C(17)—O(17)	6·3 (8)
O(17)—C(17)—C(13)—C(18)	−85·1 (6)
C(2)—C(3)—O(3)—C(31)	−1·7 (8)
C(4)—C(3)—O(3)—C(31)	177·8 (5)

Table 4. *Ring conformations and selected asymmetry parameters for steroids* (1), (2) *and* (3)

	(1)	(2)	(3)
Ring *A*	Planar	Planar	Planar
Ring *B*	7α,8β-Half-chair ΔC_2(5,10) = 0·9	7α,8β-Half-chair ΔC_2(5,10) = 0·9	Intermediate between 7α,8β-half-chair and 8β-sofa ΔC_2(5,10) = 10·6, ΔC_s(5) = 13·6
Ring *C*	Chair	Chair	Distorted chair
Ring *D*	Intermediate between 13β-sofa and 13β,14α-half-chair ΔC_s(13) = 8·5, ΔC_2(13,14) = 8·7	Intermediate between 14α-sofa and 14α,15β-half-chair ΔC_s(14) = 3·6, ΔC_2(14,15) = 9·9	Intermediate between 14α-envelope and 13β,14α-half-chair ΔC_s(14) = 7·5, ΔC_2(13,14) = 9·6

whereas the sum for steroid (3) is 359·9° suggesting nearly perfect sp^2 hybridization. This difference in hybridization is also evident in the N—Cl bond lengths; steroids (1) and (2) have longer bonds: 1·713 (3) and 1·712 (2) Å, respectively, while the bond for the sp^2 hybridized N atom in steroid (3) is shorter: 1·683 (3) Å.

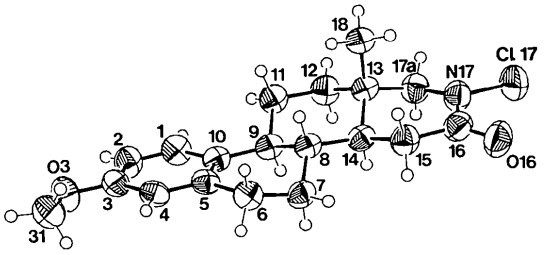


Fig. 1. Molecular conformation and atomic labeling scheme for steroid (1). The figure was drawn with *ORTEP*II (Johnson, 1976) and with thermal ellipsoids at the 50% probability level.

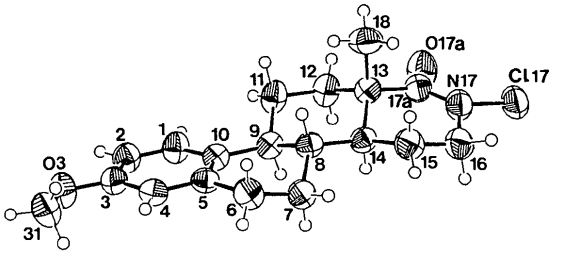


Fig. 2. Molecular conformation and atomic labeling scheme for steroid (2). The figure was drawn with *ORTEP*II (Johnson, 1976) and with thermal ellipsoids at the 50% probability level.

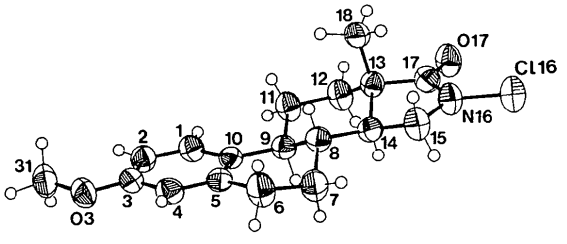


Fig. 3. Molecular conformation and atomic labeling scheme for steroid (3). The figure was drawn with *ORTEP*II (Johnson, 1976) and with thermal ellipsoids at the 50% probability level.

In steroids that have a five-membered *D* ring with a 17-keto substituent, the conformation of the *D* ring is a 14α-envelope or intermediate between a 14α-envelope and a 13β,14α-half-chair, and the pseudorotation parameter Δ (Altona, Geise & Romers, 1968) is lower than −12 (Griffin, Duax & Weeks, 1984). The pseudorotation parameter for ring *D* in (3) is −17·6 and the conformation is intermediate between a 14α-envelope and a 13β,14α-half-chair. Thus, the *D* ring of the *N*-chloro-γ-lactam (3) possesses a similar ring conformation to that of a 17-keto analogue.

The situation is more complicated in the *D*-homosteroids. Because of the relatively rigid, planar *N*-chloroamide group, a conformation of the *D* ring between that of a half-chair and of a sofa is expected; however, since the C—C(=O)—N—C torsion angle is far from zero, the conformation is somewhere between a strongly distorted chair and the forms mentioned above. When the carbonyl group of the *N*-chlorolactam is in the 16-position [compound (1)], the *D* ring has contributions from both a 13β-sofa and a 13β,14α-half-chair conformation. Preference for the 13β-sofa form over the 14α-sofa may be related to the fact that the carbonyl group is opposite to atom C(13). In steroid (2) the conformation of the *D* ring is closest to a 14α-sofa; the other significant contribution is from a 14α,15β-half-chair. A pure 14α,15β-half-chair conformation is disfavored because of the steric interactions between the axial H atom of C(15) and the H atoms of the C(18) methyl group. The symmetry of ring *D* in (3) is similar; a 14α-envelope is the major component observed.

The conformations of the *B* rings are restricted to half-chair or sofa forms as a result of the junction with the planar, aromatic *A* ring. A 7α,8β-half-chair conformation is most commonly observed in the 1,3,5(10)-estratrienes, while an 8β-sofa conformation, or both a 7α,8β-half-chair and an 8β-sofa in two molecules of the same compound, or the intermediate forms have also been observed (Duax & Norton, 1975). In steroids (1) and (2), ring *B* has a pure 7α,8β-half-chair form, while in steroid (3) a contribution from the 8β-sofa form is observed. This difference is probably the result of the difference in *C*-ring conformations. Ring *C* in (1) and (2) has a

highly symmetrical chair conformation; in (3), the chair conformation of ring *C* is distorted by strain at the junction with the five-membered *D* ring. That strain is reflected in an opening of the C(12)—C(13)—C(14)—C(8) torsion angle in (3) to −65·6 (4)°, which moves atom C(8) in the direction of the β-face more than in the half-chair conformation, and results in the 8β-sofa contribution in the conformation of ring *B*.

Ring-junction conformations are the same in all three compounds: *A/B* planar and *B/C* and *C/D* *trans*. The 3-methoxy group has the expected orientation (Hummel, Huml & Bürgi, 1988), approximately coplanar with the aromatic ring *A*. The methyl group of the 3-substituent is *cis* to atom C(2) in both *D*-homosteroids, while it is *trans* to C(2) in steroid (3). This difference probably results from the crystal packing.

Examination of the molecular packing in crystals of (1), (2) and (3) shows that only van der Waals interactions are involved; no unusually short intermolecular contacts are found.

Discussion

Assuming that the phenolic ring is necessary for binding of estrogens to the estrogen receptor (Duax & Griffin, 1985, 1987; Hähnel *et al.*, 1973; Raynaud *et al.*, 1979) and that irreversible covalent binding between the *N*-chlorolactam group and thiol groups on the receptor will occur, the O3···Cl distance can be used to assess the fit of the steroid in the receptor. The distance is about the same for both *D*-homosteroids, 12·420 (3) and 12·390 (2) Å in (1) and (2), respectively, and is 11·698 (4) Å in steroid (3). Because we assume the *A*-ring binding, *D*-ring acting model for steroid action (Duax & Griffin, 1985, 1987), the three molecules are compared by a superposition of the *A* rings and the O3 atoms (Fig. 4) obtained by a least-squares fit using the program *PROFIT* (Smith, 1983). In the superposition, the Cl atoms of (1) and (2) are 0·6 Å apart. However, because of the differences in *B*-ring conformation and in *D*-ring constitution, the distances between the Cl atom of (3) and the Cl atoms of (1) and (2) are 1·87 and 2·27 Å, respectively. Because of this difference, if there is a thiol group (or groups) in the receptor in the vicinity of the *D* ring, the ability of steroids (1) and (2) to form a covalent N—S bond will be different than in steroid (3). These structural data will help to interpret activity tests and to plan further syntheses to improve these estrogen receptor inhibitors. As an example, *N*-chloro-3-methoxy-16-aza-14β-1,3,5(10)-estratrien-15-one [(4), Back & Brunner, 1989], should show a different activity profile. The X-ray crystal structure of its parent lactam, 3-methoxy-16-aza-14β-1,3,5(10)-estratrien-15-one [(5), Back, Brunner, Codding & Roszak, 1989], shows that the 13β,14β-*cis* *C/D* ring junction orients the N—H group on the α side of the steroid perpendicular to the best plane through rings *A*, *B* and *C*. Assuming that the N—Cl group in (4) would be oriented similarly to the N—H group in (5), it is evident that the *C/D* ring junction would cause this steroid to map a different region of the receptor than that mapped by steroids (1)–(3).

If, however, the results of future activity tests show that *N*-chloroazasteroids (1), (2), (3) and (4) bind to the estrogen receptor reversibly, *i.e.*, compete with other estrogens for the same binding site but do not form the covalent N—S bond, the structural data should help to design other *N*-chloroazasteroids with the N—Cl group in locations other than the *D* ring. In this case, however, if the carbonyl O atoms of compounds (1)–(3) are involved in interactions with the estrogen receptor, then the comparison in Fig. 4 suggests that the affinities of compounds (2) and (3), and/or their biological profiles, should be different from the affinity and biological profile of compound (1).

Concluding remarks

The results of this study show that the position of the N—Cl groups in *N*-chloroazasteroids, relative to their phenolic *A* ring, varies with changes in the size of the *D* ring and in the position of the carbonyl group. This may lead to different reactivities for these compounds towards the formation of covalent N—S bonds with thiol groups in the estrogen receptor, and, in consequence, to differences in biological properties.

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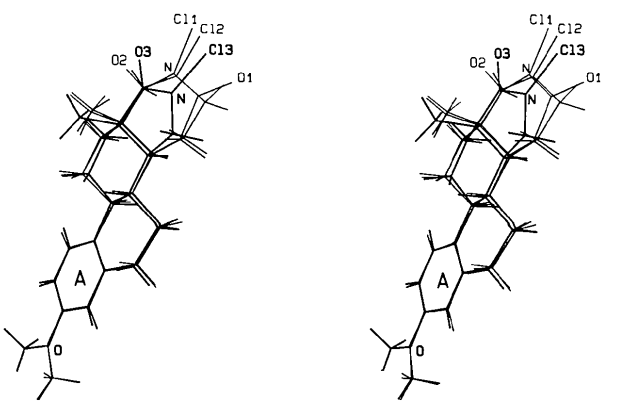

Fig. 4. Superposition of the structures of the three steroids which was obtained by a least-squares fit of atoms C(1) to C(6) plus atom O(3). Steroids (1) and (2) are drawn with thin lines and (3) with a thick line. The chlorine and carbonyl O atoms are labeled with the compound numbers.

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Structure of the Spherand* 6,12,18,24,30,36-Hexamethoxy-3,9,15,21,27,33-hexamethyl[0.$_6$]metacyclophane, $C_{48}H_{48}O_6$, and of Three Related Spherands and Spherand Complexes: $C_{48}H_{48}O_6$.LiCl, $C_{48}H_{48}O_6$.$NaSO_4CH_3$.$C_6H_5CH_3$ and $C_{42}H_{30}F_6$.$2CH_2Cl_2$

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Abstract

(I): $C_{48}H_{48}O_6$, $M_r = 720{\cdot}9$, rhombohedral, $R\bar{3}$, $a = 11{\cdot}697\ (3)$ Å, $\alpha = 114{\cdot}25\ (2)°$, $V = 954\ (2)$ Å^3, $Z = 1$, $D_m = 1{\cdot}267\ (5)$, $D_x = 1{\cdot}255$ g cm^{-3}, λ(Cu $K\alpha$) = 1·54184 Å, $\mu = 5{\cdot}7$ cm^{-1}, $F(000) = 384$, $T = 295$ K, $R = 0{\cdot}063$ for 1200 unique reflections with $I > \sigma(I)$. (II): $C_{48}H_{48}O_6$.LiCl, $M_r = 763{\cdot}3$, rhombohedral, $R\bar{3}$, $a = 11{\cdot}152\ (1)$ Å, $\alpha = 110{\cdot}60\ (1)°$, $V = 1020{\cdot}5\ (5)$ Å^3, $Z = 1$, $D_m = 1{\cdot}235\ (5)$, $D_x = 1{\cdot}242$ g cm^{-3}, λ(Cu $K\alpha$) = 1·54184 Å, $\mu = 11{\cdot}3$ cm^{-1}, $F(000) = 404$, $T = 295$ K, $R = 0{\cdot}050$ for 1150 unique reflections with $I > \sigma(I)$. (III): $C_{48}H_{48}O_6$.$NaSO_4CH_3$.C_7H_8, $M_r = 947{\cdot}1$, monoclinic, $P2/c$, $a = 11{\cdot}572\ (5)$, $b = 10{\cdot}467\ (5)$, $c = 22{\cdot}072\ (7)$ Å, $\beta = 108{\cdot}97\ (3)°$, $V = 2528\ (4)$ Å^3, $Z = 2$, $D_m = 1{\cdot}227\ (5)$, $D_x =$

* Nomenclature according to Vögtle & Neumann (1969, 1970). The three related compounds are: 6,12,18,24,30,36-hexamethoxy-3,9,15,21,27,33-hexamethyl[0.$_6$]metacyclophane–lithium chloride (1/1), $C_{48}H_{48}O_6$.LiCl; 6,12,18,24,30,36-hexamethoxy-3,9,15,21,27,33-hexamethyl[0.$_6$]metacyclophane–sodium methyl sulfate–toluene (1/1/1), $C_{48}H_{48}O_6$.$NaSO_4CH_3$.C_7H_8; 6,12,18,24,30,36-hexafluoro-3,9,15,21,27,33-hexamethyl[0.$_6$]metacyclophane–methylene chloride (1/2), $C_{42}H_{30}F_6$.$2CH_2Cl_2$.

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