An X-ray Crystallographic Study of the Nonsteroidal Contraceptive Agent Centchroman

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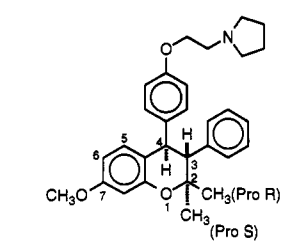
We have determined an X-ray crystal structure for the *N*-methyl iodide derivative of the nonsteroidal contraceptive centchroman. The pendant aromatic substituents on C-3 and C-4 of the chroman system are nearly perpendicular to the plane of the chroman system, an orientation expected in such a chroman, but perturbed to some degree by the *gem* dimethyl substituents at C-2. Structural superposition with other nonsteroidal antiestrogens, tamoxifen and nafoxidine, shows a similar disposition of the tertiary amine side chains responsible for antagonist activity. The aryl rings also show good superposition, but in contrast to tamoxifen and nafoxidine, which have the potential for ring double bond conjugation, the centchroman aryl rings show a larger dihedral twist. While different superpositions between the enantiomers of centchroman and the bioactive enantiomer of estradiol (*d*-estradiol, 8β,9α,13β,14α,17β) are possible, when the chroman ring system is positioned over the AB rings of estradiol, then (3*R*,4*R*)-centchroman makes the best fit. The aryl substituents in both enantiomers make comparable overlays with the steroidal skeleton, but the axial methyl group at C-2 in (3*R*,4*R*)-centchroman is directed downward along the C-7α axis of estradiol, a site where many substituents are known to be well tolerated by the estrogen receptor, while in the 3*S*,4*S*-enantiomer, this methyl group is projected upward. Thus, we suggest that the bioactive *l*-enantiomer of centchroman will have the 3*R*,4*R* absolute configuration.

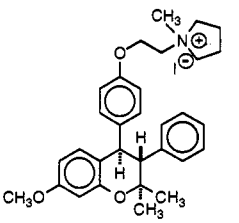
Introduction

Centchroman (*trans*-1-[2-[4-(7-methoxy-2,2-dimethyl-3-phenyl-3,4-dihydro-2*H*-1-benzopyran-4-yl)phenoxy]-ethyl]pyrrolidine hydrochloride (1)) has been introduced recently in the market as the first nonsteroidal oral contraceptive.[1] Its contraceptive efficiency has been attributed to its weak estrogenic, together with anitestrogenic, properties. Centchroman and other antiestrogens of the triarylethylene class (such as tamoxifen (3) and nafoxidine (4)) show this dual behavior, presumably by virtue of two molecular components present in their structural framework: (1) a *trans*-stilbene-like core which simulates diethylstilbestrol (5), hexestrol (6), or estradiol (7), accounting for their estrogenic nature, and (2) an additional binding unit, generally consisting of a (ω-*tert*-aminoalkoxy)phenyl residue,[2] that somehow interferes with the initiation of estrogenic activity and is thus responsible for their antiestrogenic property.

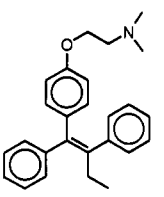
While centchroman is marketed as a racemate, its enantiomers are known to have different estrogen receptor binding affinities as well as estrogenic and antiestrogenic potencies, the *l*-enantiomer being more potent than the *d*.[3] The absolute configuration of the *l*-enantiomer, however, is not known. In this communication, we report the X-ray crystal structure determination of the *N*-methyl iodide derivative of *dl*-centchroman (2) and a molecular graphics comparison of centchroman with both estradiol and other nonsteroidal antiestrogens. On the basis of its comparison with estradiol, we suggest that *l*-centchroman will have the 3*R*,4*R* configuration.



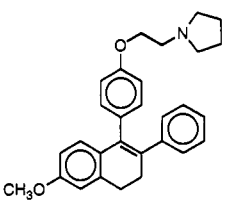
Centchroman (1)
(3R,4R)-enantiomer



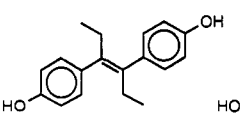
Centchroman N-Methyl Iodide Salt (2)



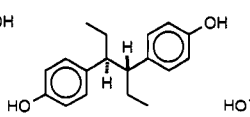
Tamoxifen (3)



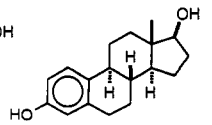
Nafoxidine (4)



Diethylstilbestrol (5)



Hexestrol (6)
(meso-diastereomer)



d-Estradiol (7)

Results and Discussion

Preparation of the *N*-Methyl Iodide Salt of Centchroman (2) and Crystallization. Neither *dl*-centchroman (1) itself nor either of its diastereomeric salts with di-*p*-toluoyltartaric acid used in its resolution[3] forms crystals suitable for X-ray analysis. Therefore, we prepared the *N*-methyl iodide derivative, as we expected that it would be more crystalline. Furthermore, if crystals of the *N*-methyl iodide salt could be obtained from a single enantiomer of centchroman, then it might be possible to

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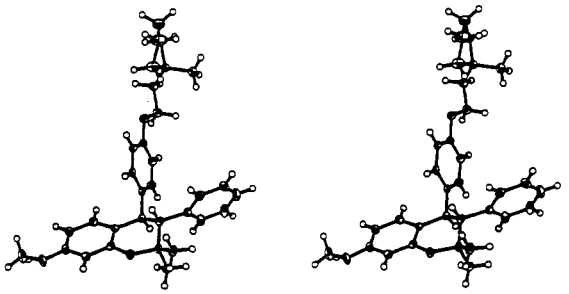


Figure 1. Relaxed stereostructure of centchroman, *N*-methyl iodide salt (**2**) as an ORTEP plot.

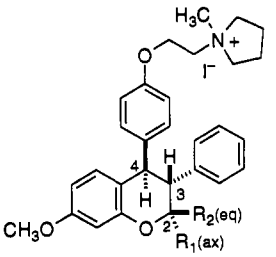
determine the absolute configuration of this enantiomer, using the method of Bijvoet.[4] A sample of centchroman (**1**) that had been resolved by selective crystallization of a *p*-toluolyltartrate salt[3] was used for preparation of the *N*-methyl iodide salt. This sample gave a substantial negative specific rotation (hydrochloride salt, $[\alpha]_D$ = −192.9°, c = 1, $CHCl_3$); however, its enantiomeric purity was not known, as a fully resolved standard for *l*-centchroman is not yet available.

Solid material obtained by the slow evaporation of a methanolic solution of the *N*-methyl iodide salt of centchroman consisted of two crystalline forms. The major form, as white crystalline needles, redissolved readily upon the addition of more methanol and was found to be unsuitable for X-ray analysis. The minor form, rectangular crystals with a yellow tint, estimated to account for only 5–10% of the material, was rather resistant to redissolution and proved to be suitable for X-ray analysis. This material turned out to be the *N*-methyl iodide salt of *dl*-centchroman.

Structure and Conformation of Centchroman, *N*-Methyl Iodide Salt (2). A stereostructure of the *N*-methyl iodide derivative of centchroman is shown in Figure 1. There are two notable conformational features in this structure. First, the two pendant phenyl substituents at C-3 and C-4 are twisted out of the plane of the chroman system, and second, the C-2, -3, and -4 atoms of the chroman system have a distinct chair cyclohexane-like pucker.

The pendant aryl ring twist is typical for triarylethylene- or -ethane-type nonsteroidal estrogens (cf. below), but in the case of centchroman, this twist, which is 71.5° for the C-3 ring and 98.7° for the C-4 ring, is somewhat larger than in other nonsteroidal estrogens, such as tamoxifen (**3**)[5] and nafoxidine (**4**),[6] which have twists of 58.9° and 93.0 or 67.6° and 70.9° for the corresponding rings, respectively. The factors that govern the dihedral twists in these antiestrogens are interesting: In contrast to the triarylethylene antiestrogens tamoxifen and nafoxidine, which have the potential for arene double bond conjugation that would tend to enforce ring double bond planarity, the two aryl substituents in centchroman are attached to tetrahedral centers. The preferred inherent conformation for such a linkage, inferred from studies of allylic systems[7] and exemplified in other crystal structures,[7c] is one in which the plane of the phenyl group is aligned with the benzylic C–H bond (i.e., dihedral angle of 0° or 180°), the orientation where nonbonding orbital repulsion would be at a minimum.[7b] The slight deviation of the rings in centchroman from this ideal orientation, which would have placed them essentially perpendicular to the best plane of the chroman system, appears to be due to the interaction of the C-3 phenyl group with the *gem* dimethyl substituents at C-2. The nature of this aryl ring–methyl group interaction was investigated further by a simple molecular mechanics study.

Table 1. Dihedral Angles in Energy-Minimized Structures of Centchroman and Centchroman Analogs



structures[a]		dihedral angle (degrees)[b]	
R_1 (ax)	R_2 (eq)	C-3 phenyl	C-4 aryl
CH_3	CH_3	64.1	109.3
H	CH_3	84.6	112.2
CH_3	H	63.9	109.4
H	H	85.0	112.9

[a] The structures are generated starting from the crystal structure of compound **2** (R_1 = CH_3, R_2 = CH_3) by progressive replacement of C-2 methyl substituents with hydrogen. All structures were energy minimized using the Tripos force field within SYBYL.[8] While, as noted in the text, the dihedral angles calculated for centchroman (first entry) deviate only slightly from those shown in the crystal structure, the relationship between the conformation of centchroman in the crystal and in solution is not certain. There are also obvious uncertainties in the absolute magnitude of the calculated values. The calculated *changes* in dihedral angles resulting from the alternate patterns of methylation (entries 2–4 vs entry 1) should be quite reliable, however. [b] The dihedral angle is measured between the plane of the pendant aromatic substituent at C-3 or C-4 and the best plane of the chroman ring.

When the crystal structure of centchroman was minimized with the Tripos force field within SYBYL,[18] the resulting structure deviated minimally from the X-ray structure; the aryl ring–chroman best plane dihedral angles changed by less than 7.5° (64.1° minimized vs 71.5° X-ray) and 11° (109.3° minimized vs 98.7° X-ray) for the C-3 and C-4 substituents, respectively. The structure was then further relaxed after removal of one or both of the methyl groups; the results are summarized in Table 1. While removal of the equatorial methyl group (the *pro-S* methyl group in (3*R*,4*R*)-centchroman) has essentially no effect on the ring torsion angles, removal of the axial methyl (or both methyl groups) allows the C-3 phenyl ring to turn to a more perpendicular orientation by more than 20°. Thus, the axial methyl group in centchroman appears to be responsible for twisting the C-3 phenyl group away from its preferred perpendicular disposition.

The second interesting conformational feature evident from the crystal structure of **2** is the chair cyclohexane-like puckering of carbon atoms C-2 to C-4 (cf. Figure 1). The axial methyl substituent at C-2 and the two hydrogen atoms at C-3 and C-4 have nearly perfectly antiperiplanar relationships: the dihedral angles are 172.1° and 171.5° between C-2 and C-3, and C-3 and C-4, respectively. The equatorial disposition of the two aryl groups on C-3 and C-4 places them in nearly contiguous volumes in space; this has an interesting consequence in terms of anticipated enantioselectivity of binding and bioactivity (see below).

Structure Comparison of Centchroman with Other Nonsteroidal Antiestrogens. An overlay of centchroman with the antiestrogens tamoxifen (**3**)[5] and nafoxidine (**4**),[6] based on the superposition of the aromatic ring centroids, is shown in Figure 2. In all three of these

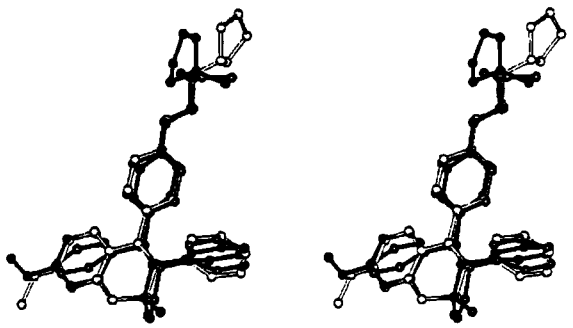


Figure 2. Relaxed stereostructure of the superposition of (3*R*,4*R*)-centchroman, *N*-methyl iodide salt (2) (black) with tamoxifen (3) (dark gray) and nafoxidine (4) (light gray). Superposition is done by the centroids of the three aromatic rings in each molecule.

Table 2. Enantioselectivity in Estrogen Receptor Binding and Bioactivity of Estradiol and Centchroman

compound (enantiomer)	estrogen receptor binding[a]	relative uterotrophic activity[b]
estradiol		
d (8β,9α,13β,14α,17β)	100	100
l (8α,9β,13α,14β,17α)	1[c]	<0.1[d]
centchroman[e]		
l	15.7	1.41
d	2.1	0.24

[a] Estrogen receptor binding is expressed as a percent of the affinity of *d*-estradiol. Binding assays are performed according to our previously described procedure.[12] [b] Bioactivity for estradiol enantiomers is relative potency in a standard 3-day uterine growth assay in immature female Sprague–Dawley rats. For a typical protocol, see ref 13. [c] Binding data are from unpublished work (K. E. Carlson and J. A. Katzenellenbogen) that was quoted in ref 9. [d] Biological data are from ref 10. The weak, impeded, and largely antagonistic activity of *l*-estradiol makes estimation of its uterotrophic potency difficult. [e] Binding and biological data are from ref 11.

antiestrogenic molecules, the tertiary aminoethoxy moiety, which is known to bind to the antiestrogen-binding site, is similarly disposed. As the data for these overlays were unaltered from the crystal structure, the different conformations of the side-chain ether, which is synclinal in centchroman (68.5°) and tamoxifen (78.7°)[5] and anti in nafoxidine (173.8°),[6] are most likely a result of crystal-packing-force stabilization rather than alterations in the core-ring structure; the different conformers are likely to be in equilibrium in solution and of comparable energy. The phenyl groups, as was discussed in the section above, have somewhat different dihedral conformations in centchroman than in the two other nonsteroidal antiestrogens.

Enantioselectivity of Binding and Bioactivity: Centchroman vs Estradiol. Both estradiol[9,10] and centchroman[11] show enantioselectivity in estrogen receptor binding and in bioactivity. These results are summarized in Table 2. The more potent isomer of estradiol is the *d*-enantiomer, with a 8β,9α,13β,14α,17β absolute configuration. The more potent isomer of centchroman is the *l*-enantiomer, but its absolute configuration is not known. While resolution of *dl*-centchroman has been achieved by crystallization of a tartrate salt derivative,[3] the enantiomeric purity of this material is not known. The fact that we have isolated a small amount of racemic material in our *N*-methyl iodide derivative of a "resolved" sample of *l*-centchroman indicates that the resolution was not complete.

Structural Comparison of Centchroman and Estradiol. In our previous work on the binding orientation of nonsteroidal estrogens vs estradiol, we considered four

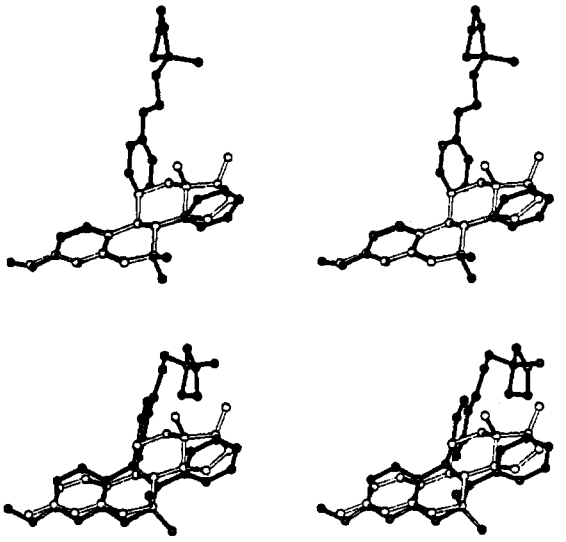


Figure 3. Relaxed stereostructure of the superposition of centchroman and *d*-estradiol. Top: (3*R*,4*R*)-centchroman (1) with *d*-estradiol. Bottom: (3*S*,4*S*)-centchroman (enantiomer of 1) with *d*-estradiol (7). Superpositions are based on matching carbons 3, 10, 11, and 7 with the equivalent atoms in centchroman.[16]

possible arrangements, two in which the fused ring system of the nonsteroidal compound (i.e., the chroman ring) overlaid the AB rings of estradiol and two in which the pendant phenyl group at C-3 overlaid the A ring of estradiol.[14] The choice between these two basic orientations was determined primarily by the placement of hydrogen-bonding substituents on these rings. Thus, in the case of centchroman, we would choose to superimpose the chroman ring system upon the AB rings of estradiol, which would place the hydrogen-bonding chroman methoxy group upon the phenolic hydroxyl of estradiol. This is substantiated by the observation that the centchroman analog with a free hydroxyl group has a 20-fold higher affinity than centchroman for the estrogen receptor, while introduction of a hydroxyl group at the *para* position of the C-3 phenyl group increases binding to a much lesser degree.[11]

Within each of these two basic orientations, the C-4 substituent (with the basic side chain) can be directed upward or downward. The latter orientation with the chroman AB ring overlap (not shown), however, places bulk from the nonaromatic ring of centchroman around C-1 of estradiol, a position known to be rather intolerant of substitution.[15] As a consequence, we favor the orientation shown in Figure 3 for the enantiomers of centchroman with estradiol. These structural overlays were obtained by the superposition of carbon atoms 3, 10, 11, and 7 in estradiol with the corresponding carbon atoms in centchroman. This pairing of atoms results in an optimum volume overlap of both enantiomers with estradiol; the result is quite instructive.[16]

In the overlay of (3*R*,4*R*)-centchroman with *d*-estradiol[17] (Figure 3, top), the configuration of the carbon atoms C-3 and C-4 in centchroman matches the configuration of carbons 8 and 9 in estradiol. Thus, the 7-methoxy group in centchroman overlays the 3-hydroxy group of estradiol, the C-3 phenyl group in centchroman projects into the D-ring region of estradiol, and the substituted C-4 phenyl group projects along the C-9,11 axis of estradiol, beyond the periphery of the C ring. A notable structural match is at C-2 in centchroman, where the axially disposed *pro-S* methyl group in (3*R*,4*R*)-centchroman is directed down-

ward, along the C-7α axis of estradiol. Many estrogens with a variety of 7α-substituents are known to have high affinity for the estrogen receptor.[15a,22,23]

The significance of the conformational disposition of these methyl groups is further evident from the superposition of (3*S*,4*S*)-centchroman with *d*-estradiol shown in Figure 3, bottom. While the configurations at carbons C-3 and C-4 of centchroman are now mismatched with the corresponding centers in estradiol, the equatorial disposition of the two aryl substituents in centchroman results in their spacial disposition in a way that is not greatly different from the 3*R*,4*R* superposition shown in Figure 3, top. A more marked deviation in this latter superposition, however, is with the methyl groups at C-2, where the equatorial methyl group lies slightly below the plane of the estradiol ring system and the axial methyl group (which is the *pro-R* methyl in (3*S*,4*S*)-centchroman) projects upward above C-7 in estradiol, a region not known to tolerate substitution. Also, the C-4 aryl substituent projects somewhat downward below the C ring of estradiol; this may also cause unfavorable interactions with the receptor.

It is on the basis of these comparisons that we suggest that the higher affinity, more potent *l*-centchroman enantiomer will have the 3*R*,4*R* configuration. Further work on determining the absolute configuration of *l*-centchroman is underway.[24]

Experimental Section

Centchroman, *N*-Methyl Iodide Salt (2). A mixture of *l*-centchroman (0.12 g, 0.3 mmol), anhydrous K_2CO_3 (0.2 g, 1.4 mmol), methyl iodide (0.2 g, 1.4 mmol), and dry acetone (5 mL) was heated under reflux for 8 h. The solid was removed by filtration and washed with dry acetone. Upon concentration, the filtrate gave a white crystalline solid: yield, 0.11 g (70%). Slow crystallization from methanol gave two types of crystals. Only a minor crop (estimated to be 5–10% of the material), which did not easily redissolve in methanol, was suitable for X-ray studies. These crystals turned out to be the *N*-methyl iodide salt of *dl*-centchroman (2) (mp 216 °C), indicating that the sample had not been completely resolved. ^{1}H NMR (400 MHz, CD_3OD): δ 7.22–7.09 (m, 5H), 6.98 (d, J = 8.5 Hz, 2H), 6.75 (d, J = 8.8 Hz, 2H), 6.50 (d, J = 8.5 Hz, 1H), 6.37 (d, J = 2.7 Hz, 1H), 6.30 (dd, J = 8.5, 2.7 Hz, 1H), 4.44 (d, J = 12.2 Hz, 1H, 3-CH_2), 4.34 (b t, J = 3.7 Hz, 2H, $ArOCH_2$), 3.79 (b t, J = 4.6 Hz, 2H, CH_2N), 3.72 (s, 3H, CH_3), 3.67–3.57 (m, 4H, pyrrolidine, CH_2N), 3.20 (d, J = 12.2 Hz, 1H, 4-CH), 3.13 (s, 3H, NCH_3), 2.22 (b m, 4H, pyrrolidine alkyl CH_2), 1.34 (s, 3H, 2-CH_3), 1.18 (s, 3H, 2-CH_3). ^{13}C NMR (100 MHz, CD_3OD): δ 160.74, 157.21, 155.48, 140.74, 138.93, 131.79, 131.59, 128.98, 127.79, 125.00, 119.91, 115.16, 108.12, 102.55, 79.15, 66.50, 64.13, 63.31, 58.63, 55.65, 44.88, 29.28, 22.39, 20.00. MS-FAB: m/z 472 (M – ^{127}I).

X-ray Crystallography. A colorless, transparent, prismatic crystal was mounted using oil (Paratone-N, Exxon) to a thin glass fiber with the (–1 –5 –2) scattering planes roughly normal to the spindle axis. Data were collected at 198 K on an Enraf-Nonius CAD4 diffractometer. Crystal and refinement details are listed in Table 3. The systematic absences and laue symmetry are consistent with two possible space groups: $P\bar{1}$ and $P1$. Refinement confirmed the space group as $P\bar{1}$. Periodically monitored standard intensities showed no significant decay. Step-scanned intensity data were reduced by profile analysis[25] and corrected for Lorentz-polarization effects and absorption.[26] Scattering factors and anomalous dispersion terms were taken from ref 27.

The structure was solved by direct methods (SHELXS-86);[28] correct positions for anion and cation non-H atoms were deduced from an E-map. One cycle of isotropic least-squares refinement followed by an unweighted difference Fourier synthesis revealed positions for a methanol solvate molecule and all H atoms. Methyl H atom positions, R–C-H_3, were optimized by rotation about R–C bonds with idealized C–H, R–H, and H–H distances. The hydroxyl H atom position was also optimized by rotation with idealized O–H and C–H distances. Remaining H atoms were included as fixed idealized contributors. H atom U's were assigned as $1.2U_{eq}$ of adjacent non-H atoms. In the final cycle of full-matrix least-squares refinement on F^2 (SHELXL-93; G. M. Sheldrick, in preparation), all non-H atoms were refined with anisotropic thermal coefficients. Successful convergence was indicated by the maximum shift/error for the last cycle. The highest peaks in the final difference Fourier map were in the vicinity of the anion; the final map had no other significant features. A final analysis of variance between observed and calculated structure factors showed no dependence on amplitude or resolution.

Table 3. Summary of Crystallographic Data for Compound 2

empirical formula	$C_{32}H_{42}INO_4$
formula weight	631.57
temperature	198(2) K
wavelength	0.71073 Å
crystal system	triclinic
space group	$P\bar{1}$
a, Å	11.769(3)
b, Å	11.830(7)
c, Å	12.770(3)
α, deg	66.20(4)
β, deg	68.09(2)
γ, deg	87.29(4)
volume, Å^3	1498.0(10)
Z	2
density calcd, mg/mm^3	1.400
absorption coefficient	1.105 mm^{-1}
F(000)	652
crystal dimensions, mm	0.20 × 0.16 × 0.14
θ range for data collection, deg	1.88–23.17
index ranges	$-13 \le h \le 13, -13 \le k \le 0, -14 \le l \le 12$
reflections collected	4590
independent reflections	4251 (R(int) = 0.0232)
absorption correction	integration
max and min transmission	0.885 and 0.856
refinement method	full-matrix least-squares on F^2
data/restraints/parameters	4251/0/349
goodness-of-fit on F^2	1.092
final R indices ($I > 2\sigma(I)$)	R_1 = 0.0335, R_{2w} = 0.0691
R indices (all data)	R_1 = 0.0513, R_{2w} = 0.0758
largest diff, peak and hole	0.464 and –0.325 e Å^{-3}

Acknowledgment. We are grateful to Dr. W. H. Pirkle for his advice on derivatization and to T. Wieckowska for assistance in X-ray crystallography. Support of this research was through grants from the National Institutes of Health (PHS R5R37 DK15556) and the Eli Lilly Co. J. P. O'Neil was supported in part by a National Institutes of Health training grant (PHS 5T32 CA09067), awarded by DHHS.

Supplementary Material Available: Number-labeled ORTEP drawing and listings of atomic and thermal parameters and bond lengths and angles (6 pages). Ordering information is given on any current masthead page.

References

(1) Kamboj, V. P.; Ray, S.; Dhawan, B. N. Centchroman. *Drugs Today* **1992**, *28*, 227–232.
(2) (a) Jordan, V. C.; Murphy, C. S. Endocrine Pharmacology of Antiestrogens as Antitumor Agents. *Endocr. Rev.* **1990**, *11*, 578–610. (b) Furr, B. J.; Jordan, V. C. Pharmacology and clinical uses of tamoxifen. *Pharmacol. Ther.* **1984**, *25*, 127–205.
(3) Salman, M.; Ray, S.; Anand, N.; Agarwal, A. K.; Singh, M. M.; Selty, B. S.; Kamboj, V. P. Studies in Antifertility Agents. 50. Stereoselective binding of *d*- and *l*-centromans to estrogen receptors and their antifertility activity. *J. Med. Chem.* **1986**, *29*, 1801–1803.
(4) Bijvoet, J. M.; Peedeman, A. F.; Van Bommel, A. J. Determination of the Absolute Configuration of Optically Active Compounds by Means of X-rays. *Nature* **1951**, *168*, 271–272.
(5) Precigoux, G.; Cowseille, C.; Geoffre, S.; Hôspital, M. [p-(Diméthyl-amino-2 éthoxy)phényl]-1 trans-Diphényl-1,2-Buténe-1 (Tamoxifène) (ICI-46474). *Acta Crystallogr., Sect. B* **1979**, *35*, 3070–2072.

(6) Camerman, N.; Chan, L. Y. Y.; Camerman, A. Crystal and Molecular Structure of Nafoxidine and Stereochemical Features of Anticancer Antiestrogens. *J. Med. Chem.* **1980**, *23*, 941–945.
(7) (a) Karabatsos, G. J.; Fenoglio, D. J. Rotational Isomerism about sp^2-sp^3 Carbon-Carbon Single Bonds. In *Topics in Stereochemistry*; Eliel, E. L., Allinger, N. L., Eds.; 1967; Vol. 5, pp 167–203. (b) Hehre, W. J.; Pople, J. A.; Devaquet, A. J. P. Torsional Potentials of Methyl Rotors Attached to Polar Linkages. *J. Am. Chem. Soc.* **1976**, *98*, 664–668. (c) A number of crystal structures in the Cambridge Structural Database (Allen, F. H.; Davies, J. E.; Galloy, J. J.; Johnson, O.; Kennard, O.; Macrae, C. F.; Mitchell, E. M.; Mitchell, G. F.; Smith, J. M.; Watson, D. G. The development of versions 3 and 4 of the Cambridge Structural Database System. *J. Chem. Inf. Comput. Sci.* **1993**, *31*, 187–204.) exemplify the fact that the dihedral angle between a phenyl group and the benzylic hydrogen at a secondary center is 0° ± 15°. See, for example: CIKFAD (9.8°, 0.8°), HEXTOL (17.5°, 15.4°), MEPHXB (–0.2°, –6.0°), SALROM (–2.0°, –9.3°), and MTPSOA (11.2°, 16.7°) (reference is made to database reference code and critical dihedral angles therein exemplified).
(8) SYBYL Molecular Modeling Package (Version 6.0), Tripos Associates, St. Louis, MO. Molecular modeling calculations were perfomed on a Silicon Graphics Indigo Elan computer. X-ray data for centchroman was energy minimized to a gradient of <0.05 kcal/(mol Å) with the standard Tripos force field applying the Powell gradient method and Gasteiger–Hückel charges.
(9) Landvatter, S. W.; Katzenellenbogen, J. A. Stereochemical Considerations in the Binding of Nonsteroidal Estrogens to the Estrogen Receptor. *Mol. Pharmacol.* **1981**, *20*, 43–51.
(10) Edgren, R. A.; Jones, R. C. Antiestradiol Effect of ent-17β-estradiol (*l*-estradiol). *Steroids* **1969**, *14*, 335–341.
(11) Salman, M. Potential Antifertility Agents. Ph.D. Thesis, University of Aligarh, India, 1982.
(12) Katzenellenbogen, J. A.; Johnson, H. J., Jr.; Myers, H. N. Photoaffinity Labels for Estrogen Binding Proteins of Rat Uterus. *Biochemistry* **1973**, *12*, 4085–4092.
(13) Lan, N. C.; Katzenellenbogen, B. S. Temporal Relationships Between Hormone Receptor Binding and Biological Responses in the Uterus: Studies with Short- and Long-Acting Derivatives of Estriol. *Endocrinology* **1975**, *98*, 220–227.
(14) Anstead, G. M.; Wilson, S. R.; Katzenellenbogen, J. A. 2-Arylindenes and 2-Arylindenones: Molecular Structures and Considerations in the Binding Orientation of Unsymmetrical Nonsteroidal Ligands to the Estrogen Receptor. *J. Med. Chem.* **1989**, *32*, 2163–2171.
(15) (a) Zeelen, F. J.; Bergink, E. W. Structure-Activity Relationships of Steroid Estrogens. In *Cytotoxic Estrogens in Hormone Receptor Tumors*; Raus, J., Martens H., LeClercq, G., Eds.; Academic Press: London, 1980; pp 39–48. (b) The RBA of 1-hydroxyestradiol was 19% using rat uterine cytosol: Carlson, K. E. Unpublished results. (c) Hylarides, M. D.; Duesler, E. N.; Mettler, F. A.; Leon, A. A. Structural Comparison of 1-Bromo and 1-Fluoroestradiol with D-Estradiol. *Acta Crystallogr.* **1988**, *C44*, 709–712.
(16) We have selected the atoms 3, 10, 11, and 7 in estradiol and the corresponding atoms in centchroman for the structural overlay with the following justifications: (a) atoms 3 and 10 align the fused aromatic rings without regard to twisting about the C-3 to C-10 axis. Such twisting appears to be of little consequence in binding to the estrogen receptor, as shown by a comparison of systems in which the phenolic substituent is fused to the B ring (e.g., estradiol,[17] nafoxidine,[6] centchroman, and a recently described tetrahydrochrysene[18]) vs those in which this ring is not fused (diethylstilbestrol,[19] hexestrol,[20] and tamoxifen[5]). (b) The atoms 11 and 7 provide appropriate points to align the midcore of the two ligand systems.
(17) Busetta, B.; Courseille, C.; Geoffre, S.; Hôspital, M. Structure Cristalline et Moléculaire du Complexe Oestradiol-Propanol. (Crystal and Molecular Structure of the Estradiol-Propanol Complex.) *Acta Crystallogr., Sect. B* **1972**, *28*, 1349–1351.
(18) Hwang, K. J.; O'Neil, J. P.; Katzenellenbogen, J. A. 5,6,11,12-Tetrahydrochrysenes: Synthesis of Rigid Stilbene Systems Designed to be Fluorescent Ligands for the Estrogen Receptor. *J. Org. Chem.* **1992**, *57*, 222–232.
(19) Busetta, B.; Courseille, C.; Hospital, M. Crystallographic study of different solvated forms of diethylstilbestrol. *Acta Crystallogr., Sect. B* **1973**, *29*, 2456–2463.
(20) Weeks, C. M.; Pokrywiecki, S.; Duax, W. L. (±)-Hexestrol, analog of a synthetic estrogen. *Acta Crystallogr., Sect. B* **1973**, *29*, 1729–1731.
(21) The estrogen receptor is tolerant of estrogens bearing sizable substituents at the 11β position: Pomper, M. G.; VanBrocklin, H.; Thieme, A. M.; Thomas, R. D.; Kiesewetter, D. O.; Carlson, K. E.; Mathias, C. J.; Welch, M. J.; Katzenellenbogen, J. A. 11β-Methoxy-, 11β-ethyl-, and 17α-Ethynyl-substituted 16α-Fluoroestradiols: Receptor-Based Imaging Agents with Enhanced Uptake Efficiency and Selectivity. *J. Med. Chem.* **1990**, *33*, 3143–3155 and references cited therein.
(22) French, A. N.; Wilson, S. R.; Welch, M. J.; Katzenellenbogen, J. A. A Synthesis of 7α-Substituted Estradiols: Synthesis and Biological Evaluation of a 7α-Pentyl-Substituted Bodipy Fluorescent Conjugate and a Fluorine-18 Substituted 7α-Pentyl Estradiol Analog. *Steroids* **1993**, *58*, 157–169 and references cited therein.
(23) An extensive study of receptor binding and uterotrophic activity has shown that the affinity and potency of various centchroman analogs are quite sensitive to the nature of C-2 substituents: Durani, S.; Agarwal, A. K.; Saxena, R.; Setty, B. S.; Gupta, R. C.; Kole, P. I.; Ray, S.; Anand, N. *Seco*-Oestradiol and Some Nonsteroidal Oestrogens: Structural Correlates of Oestrogenic Action. *J. Steroid Biochem.* **1979**, *11*, 67–77.
(24) As discussed earlier in this section, the prediction that the bioactive *R*-enantiomer of centchroman has the 3*R*,4*R* absolute configuration is based on the relative binding orientation that places the chroman ring system over the AB rings of estradiol. Other models would place the C-3 pendant ring in the A-ring region (see ref 14 and references cited therein), although this seems unlikely when this ring does not have a *para* hydroxyl group. A comparable analysis of the C-3-pendant-ring/A-ring orientation suggests that more favorable structural overlap occurs between *d*-estradiol and (3*S*,4*S*)-centchroman; in this overlay, the axial methyl group would be directed along the 11β axis of estradiol, a region in which the estrogen receptor is known to tolerate bulk (cf. ref 21).
(25) Coppens, P.; Blessing, R. H.; Becker, P. Computer Analysis of Step-Scanned X-ray Data. *J. Appl. Crystallogr.* **1972**, *7*, 488–492.
(26) Sheldrick, G. M. Program for crystal structure determination. SHELX-76; Cambridge University: England, 1976.
(27) Wilson, A. J. C., Ed. *International Tables for X-ray Crystallography*; Kluwer Academic Publishers: Dordrecht, 1992; Vol. C. (a) Scattering factors, pp 500–502. (b) Anomalous dispersion terms, pp 219–222.
(28) Sheldrick, G. M. Phase Annealing in SHELX-90: Direct Methods for Larger Structures. *Acta Crystallogr., Sect. A* **1990**, *46*, 467–473.