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DETERMINATION OF THE ABSOLUTE CONFIGURATION OF THE NONSTEROIDAL CONTRACEPTIVE AGENT CENTCHROMAN BY X-RAY CRYSTALLOGRAPHY ON ITS *N*-METHYL IODIDE SALT

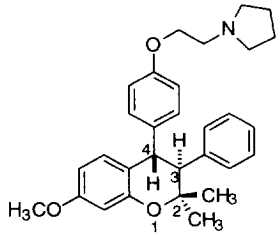
Neeta Srivastava,† Suprabhat Ray,*† Indra Dwivedy,†
Scott R. Wilson,‡ Roy K. Hom,‡ and John A. Katzenellenbogen‡

†*CDRI Communication No. 5539, from the Medicinal Chemistry Division, Central Drug Research Institute, Lucknow–226001, India,*
and ‡*Department of Chemistry, University of Illinois, 600 South Mathews Avenue, Urbana, IL 61801 USA*

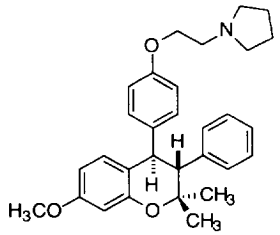
Abstract: X-ray crystallographic analysis of the *N*-methyl iodide salt of the *d*-enantiomer of the non-steroidal estrogen contraceptive agent centchroman (less active) indicates that the more active *l*-centchroman enantiomer has a *3R,4R* configuration. This confirms a prediction made by us earlier and suggests that this ligand is bound to the estrogen receptor in a chroman/AB ring orientation relative to *d*-estradiol.

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-*dl***) is a non-steroidal estrogen that is widely used as a contraceptive agent.[1] A delicate balance between its estrogen agonist and antagonist activities is thought to underlie its contraceptive effectiveness.[2] Centchroman is chiral and the relative configuration at its two stereogenic centers C-3 and C-4 is trans. Although the marketed drug is a racemate, the *d*- and *l*-enantiomers of centchroman (**1-*d*** and **1-*l***) are known to have very different estrogen receptor binding affinities, as well as estrogen agonist and antagonist potencies, with the *l*-enantiomer (**1-*l***) being the more potent of the two.[3]

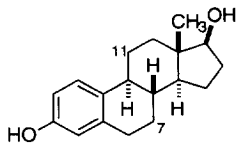
Recently, we reported the X-ray crystallographic structure of *dl*-centchroman as the *N*-methyl iodide salt.[4] The rectangular crystal used in that structural analysis was one of two crystalline forms obtained from a nearly fully resolved sample of *l*-centchroman; only upon X-ray analysis was this form found to be the racemate. Using the conformation for centchroman revealed by that X-ray analysis, we compared both centchroman enantiomers with *d*-estradiol (**2**), the natural, active enantiomer, and we concluded that the more active *l*-centchroman should have the 3*R*,4*R* configuration.



(3*S*, 4*S*)- or *d*-centchroman (**1-*d***)
[less active]



(3*R*, 4*R*)- or *l*-centchroman (**1-*l***)
[more active]



d-Estradiol (**2**)

In this report, we describe the X-ray analysis of a fully resolved sample of the *d*-enantiomer of centchroman as the *N*-methyl iodide salt, which shows that this less active enantiomer has the 3*S*,4*S* configuration (Figure 1), confirming that the active *l*-centchroman has the 3*R*,4*R* configuration, as we predicted.

Preparation of *d*-Centchroman *N*-Methyl Iodide

A mixture of *d*-centchroman[3] (0.1 g, 0.21 mmol), anhydrous K_2CO_3 (0.4 g, 2.8 mmol), methyl iodide (1.7 mL, 2.8 mmol), and dry acetone (25 mL) was heated under reflux for 10 h. The potassium carbonate was removed by filtration. Upon concentration, the filtrate gave a crystalline solid which, on slow crystallization from methanol, yielded colorless crystals of *d*-centchroman *N*-methyl iodide: mp 225-227 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16-7.26 (m, 5H), 6.92 (d, *J* = 8.6 Hz, 2H), 6.62 (d, *J* = 8.6 Hz, 2H), 6.55 (d, *J* = 8.5 Hz, 1H), 6.44 (d, *J* = 2.5 Hz, 1H), 6.35 (dd, *J* = 8.5, 2.5 Hz, 1H), 4.26-4.36 (m, OCH_2CH_2N, 4-C*H*, 5H), 3.82-3.85 (m, pyrrolidine NCH_2, 4H), 3.77 (s, OCH_3, 3H), 3.33 (s, NCH_3, 3H), 3.16 (d, *J* = 12 Hz, 3-C*H*, 1H), 2.25 (m, pyrrolidine NCH_2CH_2, 4H), 1.37 (s, CH_3, 3H), 1.25 (s, CH_3, 3H); FABMS (*m*/*z*) 472 (M-^{127}I).

X-Ray Crystallographic Analysis of *d*-Centchroman *N*-Methyl Iodide Salt

The colorless, tabular crystal was mounted using oil (Paratone-N) to a thin glass fiber, with the (1 0 1) scattering planes roughly normal to the spindle axis. Crystal and refinement details are given in Table 1. Systematic conditions suggested the ambiguous space group $P2_1$; optical activity supported this choice. Scattering factors and anomalous dispersion terms were taken from standard tables.[5]

Table 1. Crystal data and structure refinement for **1**-*d*.

Temperature	198(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1$
Unit cell dimensions	a = 9.5731(2) Å α = 90 deg
from 7880 reflections	b = 53.2618(9) Å β = 90.5100(10) deg
$4 \le \theta \le 28$	c = 11.8727(2) Å γ = 90 deg
Density (calculated)	1.386 Mg/m^3
Absorption coefficient	1.093 mm^{-1}
Crystal size	0.20 x 0.36 x 0.48 mm
θ range for data collection	1.15 - 22.50 deg
Index ranges	$-11 \le h \le 11$, $-63 \le k \le 63$, $-14 \le l \le 11$
Reflections collected	24175 [R(int) = 0.0417]
Independent reflections	12893 [11587 obs, $I > 2\sigma(I)$]
Absorption correction	Semi-empirical from φ-scans
Maximum and minimum transmission	0.6457 and 0.4865
Refinement (shift/err = 0.005)	Full-matrix least-squares on F^2
Data / restraints / parameters	12860 / 436 / 731
Goodness-of-fit on F^2	1.081
Final R indices (obs data)	R_1=0.281, R_{2w}=0.2902
R indices (all data)	R_1=0.1387, R_{2w}=0.2995
calc w=1/[$\sigma^2(F_o^2)$+(0.0789P)^2+148.7885P] where P=(F_o^2+2F_c^2)/3	
Absolute structure parameter	0.15(5)
Largest difference peak and hole	1.902 and -1.50 e.A^{-3}

The structure was solved by direct methods;[6] correct positions for the iodide ions were deduced from an E-map. One cycle of isotropic least-squares refinement followed by an unweighted difference Fourier synthesis revealed positions for the remaining ordered non-H atoms. The proposed model for the unit cells included four *N*-methyl centchroman cations, four iodide anions, and four methanol solvate molecules. Two of the cations had disordered ethylpyrrolidine moieties, and one anion was severely disordered. Ethylpyrrolidine moieties were restrained to equivalent, idealized geometries, with an effective standard deviation of 0.02 Å. Oxygen atoms bound to disordered ethylpyrrolidine groups were assigned equivalent thermal parameters. Remaining disordered non-hydrogen atoms separated by less than 1.3 Å were restrained to have similar displacement parameters with an esd of 0.01. Methanol C–O bond lengths were restrained (esd = 0.02). Ordered hydrogen atoms were included as fixed idealized contributors. Hydrogen atom displacement coefficients were assigned as 1.2 times the displacement coefficient of the adjacent C atom. Iodide ions were refined with anisotropic displacement parameters; the remaining non-H parameters were refined with isotropic parameters. Successful convergence of the full-matrix least-squares refinement on F^2 was indicated by the maximum shift/error for the last cycle.[7] The absolute configuration was supported by refinement.[8] The highest peaks in the final difference Fourier map were in the vicinity of iodide anions; the final map had no other significant features. A final analysis of variance between observed and calculated structure factors showed dependence on amplitude.

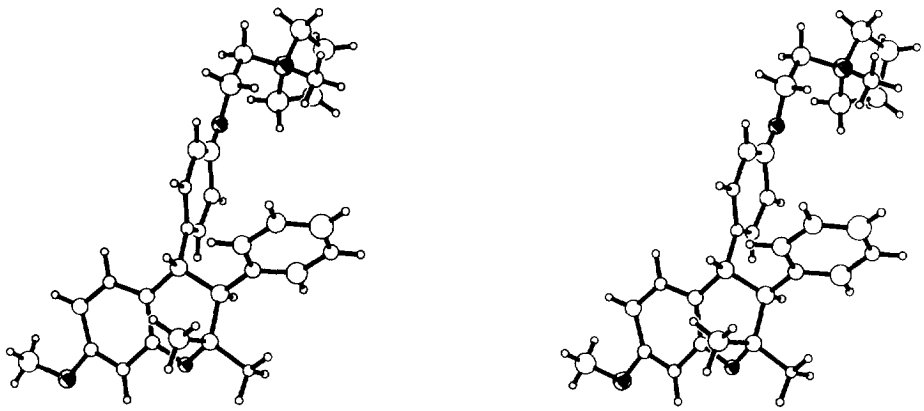


Figure 1. SHELXTL[9] relaxed stereo ORTEP plot for *d*-centchroman *N*-methyl iodide, showing 35% probability ellipsoids for non-H atoms and circles of arbitrary size for H atoms.

Configuration-Activity Correlations of *d*- and *l*-Centchroman and other Steroidal and Non-Steroidal Ligands for the Estrogen Receptor

The binding affinities of centchroman and 7-*O*-desmethylcentchroman derivatives as *d*- and *l*-enantiomers, and the estradiol enantiomers are given in Table 2. It is clear from the estrogen receptor binding affinities of centchroman, and especially from that of the 7-*O*-desmethyl derivatives (which are presumed to be the metabolites with the highest activity in vivo),[10] that the *l* enantiomers are the higher affinity ligands.

This is reflected in the higher uterotropic potency of the *l*-centchroman as well. (The uterotropic potencies of the desmethyl centchromans are not known.)

Table 2. Biological activity and estrogen receptor binding affinity of estradiol and centchroman enantiomers and related derivatives.

compound	estrogen receptor binding[a]	relative uterotrophic activity[b]
estradiol (**2**)		
d (8β,9α,13β,14α,17β)	100	100
l (8α,9β,13α,14β,17α)	1[c]	<0.1[d]
centchroman[e] (**1**)		
l (3*R*,4*R*)	15.7	1.41
d (3*S*,4*S*)	2.1	0.24
7-*O*-desmethyl centchroman[f]		
l (3*R*,4*R*)	78 ± 11	ND[g]
d (3*S*,4*S*)	1.7 ± 0.2	ND

[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 using rat uterus estrogen receptor.[11] [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 12. [c] Binding data are from unpublished work (K. E. Carlson and J. A. Katzenellenbogen) that was quoted in ref 13. [d] Biological data are from ref 14. 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 15. [f] Values represent the average of two determinations ± range. [g] ND = not determined

Our previous analysis of the configurational relationship between centchroman and estradiol was based on a superposition in which the two chroman rings of centchroman overlaid the AB-ring system of estradiol ("chroman/AB superposition") (Figure 2).[4] When centchroman is in the orientation revealed in the X-ray structures of both the *dl*- and the *l*-*N*-methyl iodide salts,[16] an excellent steric overlap between the chroman system with the estradiol AB ring systems is obtained with centchroman in the 3*R*,4*R* configuration (Figure 2). This configuration placed, as well, one of the C-2 methyl groups in a steroidal 7α-direction and disposed the C-4 aryl substituent in a somewhat 11β-orientation. Large substituents at both of these sites in estradiol are known to be well tolerated by the estrogen receptor.[17] By contrast, in this chroman/AB superposition, (3*S*,4*S*)-centchroman disposed these substituents in a 7β- and more 11α-like orientation (not

shown, see ref 4). With both enantiomers, the C-3 phenyl group occupied a region analogous to the D-ring of estradiol. As a result of this analysis, we suggested that the higher affinity, more active *l*-enantiomer of centchroman would have the 3*R*,4*R* configuration.[18]

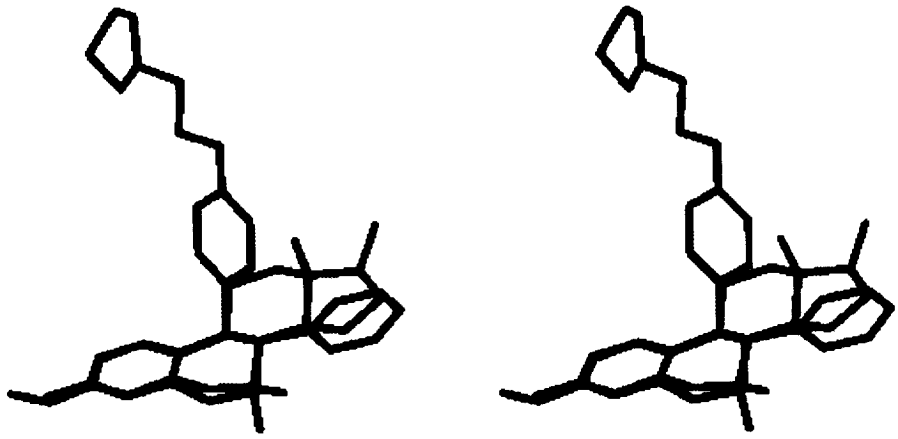


Figure 2. The "chroman/AB superposition" of *l*- or (3*R*,4*R*)-centchroman (blue) with *d*-estradiol (red) (relaxed stereoview).

In conclusion, the absolute configuration of *l*-centchroman, the more active enantiomer of the contraceptive non-steroidal estrogen centchroman, is now known to have a 3*R*,4*R* configuration. This confirms the configuration we predicted for -centchroman based on an earlier analysis,[4] and consequently it suggests that when bound to the estrogen receptor, centchroman may adopt a chroman/AB ring orientation relative to estradiol.

Acknowledgment

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18. One is inclined to favor the original chroman/AB ring superposition because it places the methoxy group of centchroman over the phenol of estradiol. One should certainly expect that in vivo this methyl group would be lost by oxidative metabolism. On the other hand, the alternate pendant ring/A-ring orientation (c.f. ref 4) might be favored if a *p*-hydroxyl group were introduced into the C-3 pendant ring. Such hydroxylations are well known in drug metabolism and specifically in the metabolic activation of the antiestrogen tamoxifen to 4-hydroxytamoxifen.[19] However, although hydroxylation at the para position in the C-3 phenyl ring in centchroman is known to increase binding affinity to the estrogen receptor, the effect is smaller than with the introduction of a hydroxy group at C-7.[15]
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