reaction flask was taken off the boiling water bath, placed on a hot plate, and brought to a slow boil for 30 min. The reaction mixture was then cooled and hydrolyzed on 50 g of ice. The product was filtered, washed with water, and recrystallized from methanol/acetone to give 980 mg (1.28 mmol, 71%) of white, microcrystalline **5a**: mp 281–284 °C; IR 2230 (nitrile) cm^{-1}; NMR 1.52 (d, 12 H, J = 7 Hz), 2.08 (s, 6 H), 2.28 (s, 6 H), 2.48 (s, 12 H), 3.85 (sept, 2 H), 8.13 (s, 2 H). Anal. ($C_{42}H_{40}N_2O_{12}$) C, H, N.

Gossylic Nitrile 1,1′-Diacetate (6a; 6,6′,7,7′-Tetrahydroxy-3,3′-dimethyl-5,5′-bis(1-methylethyl)-1,1′-bis(ethanoyloxy)[2,2′-binaphthalene]-8,8′-dinitrile). Compound **5a** (500 mg, 0.65 mmol) was added to 5 mL of methanol. One milliliter of water and 500 mg of potassium carbonate were added, and the mixture was refluxed for 30 min. The mixture was allowed to cool and was acidified by dropwise addition of acetic acid. Ten milliliters of water was added, and the reaction mixture was chilled. The off-white product was filtered, washed with water, and dried. It was recrystallized once from methanol/water and once from toluene/acetone to give 275 mg (0.46 mmol, 71%) of microcrystalline needles: mp 300–302 °C dec; IR 2220 (nitrile), 1765 (acetyl) cm^{-1}; NMR 1.58 (d, 12 H, J = 7 Hz), 1.98 (s, 6 H), 2.24 (s, 6 H), 3.96 (sept, 2 H, J = 7 Hz), 6.4 (br s, 4 H), 7.98 (s, 2 H). Anal. ($C_{34}H_{32}N_2O_8$) C, H, N.

Compounds **6b–d** were synthesized by using the same procedures and molar ratios of the corresponding acid anhydrides and acid salts that were used for **6a**. Intermediates were not isolated, and yields are based on **2** as starting material.

Gossylic Nitrile 1,1′-Dipropionate (6b; 6,6′,7,7′-Tetrahydroxy-3,3′-dimethyl-5,5′-bis(1-methylethyl)-1,1′-bis(propanoyloxy)[2,2′-binaphthalene]-8,8′-dinitrile). Compound **6b** was recrystallized from toluene to give a tan powder in 62% yield: darkened at 145 °C, dec without melting; IR 2220 (nitrile), 1770 (propionyl) cm^{-1}; NMR 0.69 (t, 6 H, J = 7 Hz), 1.52 (d, 12 H, J = 7 Hz) 2.21 (s, 6 H), 2.24 (q, 4 H, overlaps 2.21s), 3.96 (sept, 2 H, J = 7 Hz), 6.05 (br s, 4 H), 7.93 (s, 2 H). Anal. ($C_{36}H_{36}N_2O_8$) C, H, N.

Gossylic Nitrile 1,1′-Dibutyrate (6c; 6,6′,7,7′-Tetrahydroxy-3,3′-dimethyl-5,5′-bis(1-methylethyl)-1,1′-bis(butanoyloxy)[2,2′-binaphthalene]-8,8′-dinitrile). Compound **6c** was recrystallized from toluene to give tan microcrystalline plates in 58% yield: darkened at 150 °C, dec without melting; IR 2240 (nitrile), 1775 (butyryl) cm^{-1}; NMR 0.43 (t, 6 H, J = 7 Hz), 1.10 (m, 4 H), 1.51 (d, 12 H, J = 7 Hz), 2.20 (s, 6 H), 2.27 (t, 4 H, overlaps 2.20s), 3.96 (sept, 2 H, J = 7 Hz), 6.05 (br s, 4 H), 7.91 (s, 2 H). Anal. ($C_{38}H_{40}N_2O_8$) C, H, N.

Gossylic Nitrile 1,1′-Divalerate (6d; 6,6′,7,7′-Tetrahydroxy-3,3′-dimethyl-5,5′-bis(1-methylethyl)-1,1′-bis(pentanoyloxy)[2,2′-binaphthalene]-8,8′-dinitrile). Compound **6d** was recrystallized from toluene to give a tan powder in 58% yield: softened at 135 °C, dec without melting; IR 2230 (nitrile), 1765 (valeryl) cm^{-1}; NMR 0.47 (t, 6 H, J = 7 Hz), 0.92 (m, 8 H), 1.53 (d, 12 H, J = 7 Hz), 2.22 (s, 6 H), 2.29 (t, 4 H, overlaps 2.22s), 3.96 (sept, 2 H, J = 7 Hz), 6.05 (br s, 4 H), 7.93 (s, 2 H). Anal. ($C_{40}H_{44}N_2O_8$) C, H, N.

Strains of *P. falciparum*. The chloroquine-resistant strain FCB, a Colombian strain of *P. falciparum*, was cloned by limiting dilution. The clone used in the present study is denoted as clone NC-1. The chloroquine-sensitive strain of *P. falciparum*, strain CDC/I/HB-3, is a Honduras strain that was cloned by Prof. W. Trager, Rockefeller University. This clone was obtained from the Malaria Branch, Center for Disease Control, Altanta, GA, with permission from Prof. Trager.

Parasite Growth. *P. falciparum* was grown in vitro in human erythrocytes. Culture dishes contained 2% erythrocytes in RPMI 1640 medium supplemented with 5 mM glutamine, 35 mM Hepes, 24 mM sodium bicarbonate, and gentamicin, 33 mg/L. The medium contained 10% (v/v) horse serum, pH 7.2. Parasite growth was monitored by measuring the uptake of [^{3}H]hypoxanthine and its incorporation into nucleic acid. Aliquots of media, 250 μL, containing parasitized erythrocytes, 0.5% parasetemia, were added to microtiter wells. Drug at the appropriate concentration was added to the wells in 1 μL of Me_2SO. Control wells received 1 μL of Me_2SO. Samples were run in triplicate at each concentration of drug. After addition of the drug, [^{3}H]hypoxanthine (New England Nuclear), 0.5 μCi, was added to each well. Microtiter plates were incubated for 3 days, 37 °C, in 5:95 CO_2/air, after which the contents were collected on glass fiber filters, using a cell harvester. The filters were washed with distilled water and then were counted by liquid scintillation. In separate experiments, the [^{3}H]hypoxanthine was omitted, and parasite growth was monitored by differential cell counting of Giemsa-stained slides. This confirmed that the uptake of [^{3}H]hypoxanthine provided a good indication of parasite growth.

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Studies in Antifertility Agents. 50. Stereoselective Binding of *d*- and *l*-Centchromans to Estrogen Receptors and Their Antifertility Activity[1]

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Centchroman [*dl*-3,4-*trans*-2,2-dimethyl-3-phenyl-4-[*p*-(β-pyrrolidinoethoxy)phenyl]-7-methoxychroman hydrochloride], an antifertility agent under clinical evaluation, has been resolved into its optical enantiomers. The cytosol estrogen receptor binding affinity and estrogenic, antiestrogenic and antiimplantation activities of the two enantiomers have been determined. The enantiomers display a 7-fold difference in receptor affinity, and a corresponding difference in stimulation of the uterine growth and antiimplantation activity was observed in rats.

As a result of studies carried out in this laboratory on estrogen receptor (ER) binding to various types of di- and triarylethylenes, -ethanes[4] and -alkanones,[5] some generalizations have emerged for the contribution of different substituents of the prototypes of ligand–receptor binding

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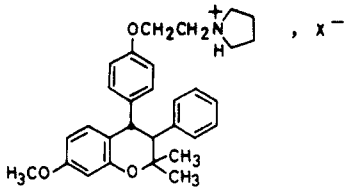
(1) CDRI Communication No. 3735.
(2) Ray, S.; Grover, P. K.; Kamboj, V. P.; Setty, B. S.; Kar, A. B.; Anand, N. *J. Med. Chem.* **1976**, *19*, 276.
(3) Anand, N.; Ray, S. *Indian J. Exp. Biol.* **1977**, *15*, 1142.
(4) Durani, S.; Agarwal, A. K.; Saxena, R.; Setty, B. S.; Gupta, R. C.; Kole, P. L.; Ray, S.; Anand, N. *J. Steroid Biochem.* **1979**, *11*, 67.
(5) Gopalachari, R.; Iyer, R. N.; Kamboj, V. P.; Kar, A. B. *Contraception* **1970**, *22*, 962.

0022-2623/86/1829-1801$01.50/0

Table I. Relative Binding Affinity (RBA) and Uterotrophic and Antiimplantation Activities of 17β-Estradiol, *dl*-Centchroman, and Its Optical Isomers

compd	dose, μg (×3 days)	uterine wet wt, mean ± SEM (*n*)	% of control	rel uterotrophic act. (E_2 = 100%)	RBA, %: mean ± SEM	antiimplantation act. (MED, mg/kg)
17β-estradiol	0	17.0 ± 1.0 (18)[a]	100			
	0.05	33.2 ± 3.6 (18)[a]	195			
	0.10	43.3 ± 3.4 (16)[a]	255	100	100	
	0.20	50.1 ± 2.9 (17)[a]	195			
	0.50	55.8 ± 3.3 (18)[a]	328			
	1.00	55.6 ± 2.9 (18)[a]	327			
dl-centchroman	0.05	16.6 ± 1.0 (5)	98			
	0.50	16.3 ± 0.7 (4)	96			
	5.00	29.5 ± 1.0 (16)	174	0.56	5.24 ± 1.45	0.25
	50.00	47.7 ± 1.8 (6)	281			
	100.00	49.0 ± 2.6 (6)	288			
l-centchroman	0.50	15.8 ± 1.6 (5)	93			
	2.50	25.8 ± 2.3 (6)	152			
	5.00	37.8 ± 0.9 (11)[b]	222	1.41	15.70 ± 3.10	0.15
	50.0	48.6 ± 1.8 (18)[a]	286			
	100.00	51.4 ± 3.2 (16)[a]	303			
d-centchroman	0.50	16.2 ± 1.1 (5)	95			
	5.00	16.8 ± 1.0 (5)	99			
	25.00	31.8 ± 2.9 (4)	187	0.24	2.10 ± 0.90	5.0
	50.0	40.5 ± 1.7 (10)[b]	238			
	100.00	40.4 ± 3.7 (5)	238			
	500.00	45.4 ± 196 (5)	267			
nafoxidine[c]				5.5	9.29 ± 1.80	0.10

[a] Mean of three different experiments; *n* number of animals. [b] Mean of two different experiments. [c] 3 See ref 4.



Compound no	Isomer	X
1	dl	Cl⁻
2	d	di-p-toluoyl-d-tartrate
3	d	Cl⁻
4	l	di-p-toluoyl-l-tartrate
5	l	Cl⁻

Figure 1.

and to biological activity.[4,6,7] These studies have provided useful information for the design of molecules, termed atypical estrogens, with modified estrogenic responses. Some have clinical promise as pregnancy inhibiting agents. One such agent in an advanced stage of clinical development (as a weekly administered contraceptive) is *dl*-3,4-*trans*-2,2-dimethyl-3-phenyl-4-[*p*-(β-pyrrolidinoethoxy)-phenyl]-7-methoxychroman hydrochloride (centchroman[3] 1). This compound has weak estrogenic activity, strong antiestrogenic activity, and significant antiimplantation actvity as compared to 17β-estradiol.[8] In an attempt to separate undesirable estrogenicity of the molecule and to provide further insight into the stereoselectivity of the receptor sites for different biological activities, the relative binding affinity (RBA) and estrogenic, antiestrogenic and antiimplantation activities of the two enantiomers of centchroman were studied.

Chemistry. *dl*-Centchroman (1) was resolved into its *d* and *l* enantiomer **3** and **5** by fractional crystallization of its salts with *d*- and *l*-di-*p*-toluoyltartaric acid to constant rotation and subsequent liberation of the free bases by alkali treatment.[9]

Results

Results on RBA presented in Table I are in agreement with the stereoselective requirement of ER.[10] The racemic mixture of centchroman (*dl*) has nearly 20-fold lower receptor affinity than 17β-estradiol (E_2). The *l* isomer with an affinity of 15% is nearly 3-fold more potent than the *dl* mixture. On the other hand, *d*-centchroman is only half as potent in binding as *dl*-centchroman.

The binding of these isomers to ER is reflected in their uterotrophic activity as well (Table I). The *l* isomer, in accordance with its RBA, is a more potent uterotrophic agent than the *dl* mixture. The *d* isomer has decreased uterotrophic activity, nearly half of that of *dl*-centchroman. At nearly 200 times the dose of E_2, *dl*- and *l*-centchroman stimulate the growth of the uterus to almost the same extent as E_2. However, even at 1000 times the dose of E_2, *d*-centchroman fails to induce a similar growth of the uterus. The fact that uterotrophic activity is related to the affinity is clearly evident at 5-μg dose, where the *l* isomer is most active followed by the *dl* mixture. The *d* isomer, however, at this dose does not produce any response.

All three test compounds at 100-μg dose inhibit the growth of uterus stimulated by 0.5 μg of E_2 (Table II). At lower doses (5 and 50 μg), varying degrees of antagonism were evident with *l*- and *dl*-centchroman. The *d* isomer, however, has no inhibitory effect at these lower doses.

Discussion

2,2-Dimethyl-3-phenyl-4-[*p*-(β-pyrrolidinoethoxy)-phenyl]-7-methoxychroman has two chiral centers and thus exists as two diastereomeric racemate pairs, 3,4-trans and 3,4-cis. In earlier studies it was reported[4,11] that the 3,4-

(6) Agarwal, A. K. Ph.D. Thesis, Kanpur University, 1980.
(7) Durani, S.; Anand, N. *Int. J. Quantum Chem.* **1981**, *20*, 71.
(8) Anand, N. *Proc. Indian Natl. Sci. Acad.* **1983**, *49A*, 233.
(9) Salman, M.; Ray, S.; Kamboj, V. P.; Anand, N. Indian Patent No. 476/DEL/79, June 30, 1979.
(10) Chernayaev, G. A.; Barkova, T. I.; Egorova, V. V.; Sorokina, I. B.; Ananchenko, S. N.; Mataradze, G. D.; Sokalova, N. A.; Rozen, V. B. *J. Steroid Biochem.* **1975**, *6*, 1483.
(11) Kamboj, V. P.; Setty, B. S.; Chandra, H.; Roy, S. K.; Kar, A. B. *Indian J. Exp. Biol.* **1977**, *15*, 1144.

Table II. Antiuterotrophic Activity of *dl*-Centchroman and Its Optical Isomers in Immature Rats

compd	dose, μg	uterine wet wt, mean ± SEM (*n*)[a]	% stimuln of E_2[b]	% inhibn[c]
dl-centchroman	control	14.7 ± 0.9 (6)	23	
	17β-E_2 (0.5)	64.3 ± 3.7 (6)	100	
	5 ± 0.5	52.5 ± 2.4 (4)	82	23.8
	50 ± 0.5	60.3 ± 3.5 (6)	94	8.1
	100 ± 0.5	56.0 ± 1.4 (4)	87	16.7
l-centchroman	control	14.0 ± 1.5 (6)	21	
	17β-E_2 (0.5)	68.3 ± 2.8 (6)	100	
	5 ± 0.5	52.7 ± 3.2 (6)	77	28.7
	50 ± 0.5	52.6 ± 1.6 (5)	77	28.7
	100 ± 0.5	46.4 ± 3.3 (5)	68	40.3
d-centchroman	control	15.3 ± 0.8 (6)	29	
	17β-E_2 (0.5)	53.0 ± 2.0 (6)	100	
	5 ± 0.5	52.3 ± 3.8 (6)	99	1.9
	50 ± 0.5	55.7 ± 3.6 (6)	105	
	100 ± 0.5	42.3 ± 2.6 (6)	80	28.4

[a] *n* = number of animals. [b] 0.5 μg of E_2 = 100%. [c] $[1 - (E_{S,T} - E_V)/(E_S - E_V)] \times 100$, where E_V = effect of vehicle, E_S = effect of 17β-estradiol standard, and $E_{S,T}$ = effect of standard under simultaneous application of test substance.

trans isomer, named centchroman, had a higher order of antifertility activity, weak estrogenic and rather strong antiestrogenic activities, and significant RBA, while the corresponding cis isomer was practically devoid of any of these activities.[4] The *l* enantiomer of centchroman has now been shown to have an almost 7-fold higher affinity for the cytosol estrogen receptor than the *d* isomer, which emphasizes the stereodynamic nature of ligand–receptor interactions in this group of molecules and a causal relationship with the biological responses such as estrogenic effects and antifertility activity. In principle there could be a high RBA (high affinity) but weak in vivo estrogenic activity (weak intrinsic activity) and possibly a dissociation of antifertility and estrogenic activities, but in the present case the two seem to run parallel to each other. The *l* isomer is about 33 times more active than the *d* isomer in the antiimplantation activity assay and is also considerably more active in uterotrophic responses.

The quantitation of agonistic and antagonistic activities in these compounds is difficult as they have both estrogenic and antiestrogenic activities and any situation observed is a net balance of different responses. The *l* enantiomer may thus have advantage over the racemic compound in clinical usage by preventing unnecessary loading of the system with an inactive constituent.

Experimental Section

Resolution of *dl*-Centchroman. The melting points were determined in sulfuric acid bath and are uncorrected. The ^1H NMR spectra were recorded on a Perkin-Elmer R-32 (90-MHz) spectrophotometer using Me_4Si as internal standard. Mass spectra were recorded on Jeol JMS-D 300 instrument. Optical rotations were taken on Jasco DIP-180 polarimeter. The purity of the compounds was checked by silica gel G or basic/neutral alumina thin-layer chromatographic (TLC) technique.

***d*-3,4-*trans*-2,2-Dimethyl-3-phenyl-4-[*p*-(β-pyrrolidinioethoxy)phenyl]-7-methoxychroman Di-*p*-toluoyl-*d*-tartrate (2).** A solution of *dl*-3,4-*trans*-2,2-dimethyl-3-phenyl-4-[*p*-(β-pyrrolidinoethoxy)phenyl]-7-methoxychroman (1; 457 mg, 1.0 mmol) and di-*p*-toluoyl-*d*-tartaric acid (193 mg, 0.5 mmol) in 30 mL of dry methanol was stirred for 2 h at room temperature. The solvent was evaporated, and the residue was repeatedly crystallized from methanol to afford **2** as a colorless crystalline solid: yield 150 mg (46%); mp 138 °C; $[\alpha]^{20}_D$ + 117.2° (*c* 1.0, $CHCl_3$); ^1H NMR δ 1.15, 1.35 (2 s, 6 H, *gem*-$(CH_3)_2$), 1.8 (m, 4 H, $\overline{NCH_2(CH_2)_2CH_2}$), 2.25 (s, 6 H, Ar CH_3), 3.2 (m, 7 H, 3 NCH_2, monobenzylic acid H), 3.7 (s, 3 H, OCH_3), 4.0 (m, 2 H, OCH_2), 4.2 (d, 1 H, dibenzylic H, *J* = 12 Hz), 5.7 (s, 2 H, 2 CH, tartaric acid), 6.3–7.9 (m, 20 H), Ar H); mass, *m/e* 457 (M^+ – 386, di-*p*-toluoyl-*d*-tartaric acid). Anal. C, H.

***d*-3,4-*trans*-2,2-Dimethyl-3-phenyl-4-[*p*-(β-pyrrolidinoethoxy)phenyl]-7-methoxychroman Hydrochloride (3).** The *d* salt **2** was dissolved in chloroform and shaken with dilute aqueous NaOH (10%). The organic layer was washed with water to neutral, dried (Na_2SO_4), and concentrated to give **3**, which was converted to its hydrochloride: mp (as hydrochloride salt) 197 °C; $[\alpha]^{20}_D$ + 192.9° (*c*, 1.0, $CHCl_3$); ^1H NMR δ 1.12, 1.25 (2 s, 6 H, *gem*-$(CH_3)_2$), 1.75 (m, 4 H, $\overline{NCH_2(CH_2)_2CH_2}$), 2.65 (m, 6 H, 3 NCH_2), 3.85 (d, 1 H), monobenzylic H, *J* = 12 Hz), 3.65 (s, 3 H, OCH_3), 3.9 (m, 2 H, OCH_2), 4.18 (d, 1 H, dibenzylic H), 6.15–7.1 (m, 12 H), Ar H); mass; *m/e* 457 (M^+ – 37, HCl). Anal. C, H.

***l*-3,4-*trans*-2,2-Dimethyl-3-phenyl-4-[*p*-(β-pyrrolidinioethoxy)benzyl]-7-methoxychroman Di-*p*-toluoyl-*l*-tartrate (4).** This was obtained in 40% yield when **1** was treated with di-*p*-toluoyl-*l*-tartaric acid monohydrate under similar conditions as described for **2**: mp 120 °C $[\alpha]^{20}_D$ –117.2° (*c*].0, $CHCl_3$); ^1H NMR and mass spectra were similar to that of **2**. Anal. C, H.

***l*-3,4-*trans*-2,2-Dimethyl-3-phenyl-4-[*p*-(β-pyrrolidinoethoxy)phenyl]-7-methoxychroman Hydrochloride (5).** The *l* salt on alkali treatment as described for **3** afforded **5**: mp (as hydrochloride salt) 197 °C; $[\alpha]^{20}_D$ –192.9° (*c* 1.0, $CHCl_3$); ^1H NMR and mass spectra were similar to that of **3**. Anal. C, H.

Biochemical and Biological Methods. Receptor Affinity. Relative binding affinities (RBA) for uterine cytosol estrogen receptors, obtained from immature Charles Foster rats, 21–25 days old, were determined by a competitive inhibition assay, employing dextran-coated charcoal (DCC) for separation of unbound steroids as reported earlier.[12]

Estrogenic Activity. The estrogenic activity of the compounds was evaluated in immature rats (25–30 g) as assayed by uterine weight gain. The compounds were administered subcutaneously once daily, over a 3-day period in 0.5 mL of saline/propylene glycol (1:1, v/v). Relative activities were determined as reported earlier.[12]

Antiestrogenic Activity. The antiestrogenic activity of the compounds was assayed by uterine weight gain in immature rats (25–30 g). The compounds were administered subcutaneously in 0.5 mL of propylene glycol/saline (1:1 v/v) along with 0.5 μg of 17β-E_2 (in 0.2 mL of olive oil) at two different sites for 3 consecutive days. Inhibition is expressed as percent inhibition from the formula of Hartman et al.[13]

Antiimplantation Activity. This was studied in sperm-positive female albino rats mated to coeval males of proven fertility. The compounds were administered orally as a suspension in gum acacia to colony-bred adult female rats (150–170 g) on days 1–7 postcoitum, using five to seven animals in each group. The animals were examined by laparotomy on day 10 of pregnancy for the number of implants. The results were scored as positive only if implants were totally absent in both the uterine horns.[12]

Registry No. **1**, 103150-29-4; **2**, 103150-30-7; **3**, 92083-15-3; **4**, 103150-31-8; **5**, 79386-05-3.

(12) Salman, M.; Ray, S.; Agarwal, A. K.; Durani, S.; Setty, B. S.; Kamboj, V. P.; Anand, N. *J. Med. Chem.* **1983**, *26*, 592.
(13) Hartman, R. W.; Kranzfelder, G.; Angerer, E. V.; Schonenberger, H. *J. Med. Chem.* **1980**, *23*, 841.