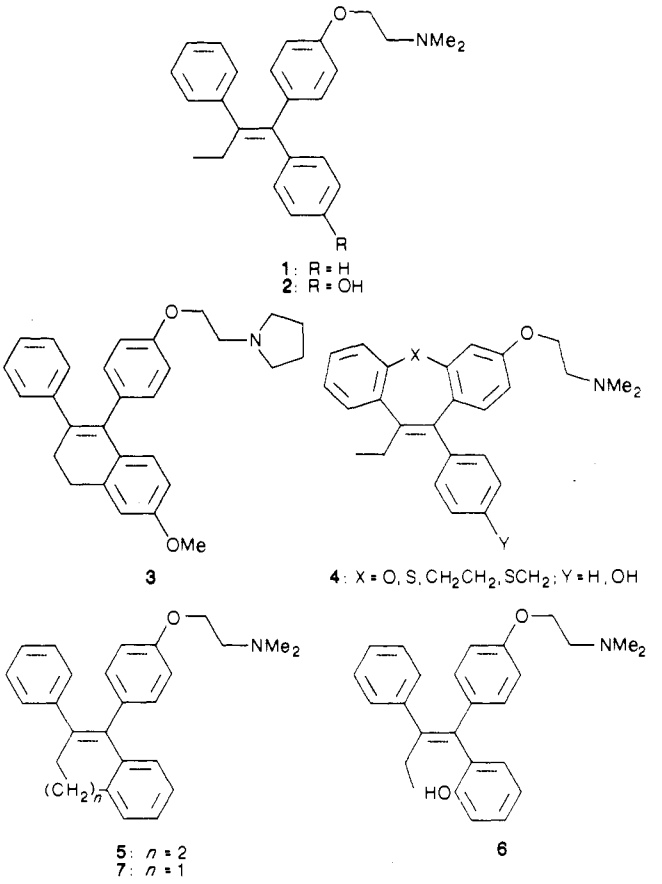
Synthesis and Estrogen Receptor Binding of 6,7-Dihydro-8-phenyl-9-[4-[2-(dimethylamino)ethoxy]phenyl]-5*H*-benzocycloheptene, a Nonisomerizable Analogue of Tamoxifen. X-ray Crystallographic Studies

Raymond McCague,*† Reiko Kuroda,‡ Guy Leclercq,§ and Susanna Stoessel§

Drug Development Section, Cancer Research Campaign Laboratory, and Cancer Research Campaign Biomolecular Structure Unit, Institute of Cancer Research, Sutton, Surrey, SM2 5PX, U.K., and Laboratoire de Cancérologie Mammaire, Service de Médecine, Institut Jules Bordet, 1000 Bruxelles, Belgium. *Received January 22, 1986*

Syntheses of the title compound (**5**), a novel nonisomerizable antiestrogen containing a seven-membered ring, are described. In one method, 6,7-dihydro-9-(4-methoxyphenyl)-5*H*-benzocycloheptene was brominated at the 8-position and the bromine displaced by phenylzinc chloride with palladium complex catalysis to introduce the 8-phenyl substituent. Alternatively, benzosuberone was α phenylated with tricarbonyl(η^6-fluorobenzene)chromium(0) and the product treated with the appropriate aryllithium reagent to introduce the 9-aryl group last. The relative binding affinities for estrogen receptors in cell cytosol and whole cells and growth inhibitory activity against the MCF-7 human breast tumor cell line in vitro were for **5** comparable to those of tamoxifen (**1**) and the corresponding six-membered ring analogue (**7**). X-ray crystallographic analyses of **10** and **15**, which are methoxy derivatives of **5** and **7**, show that in some respects **5** bears a closer structural relationship to tamoxifen than does nafoxidine (**3**) or **7**. Thus, the aromatic ring, which is fused in the cyclic analogues, was twisted 64, 45, 20, and 19° out of the plane of the double bond for **1**, **10**, **3**, and **15**, respectively. Low-temperature NMR studies indicate that **5** is more rigid than tamoxifen; interconversion between enantiomeric conformers is slow on the NMR time scale at −75 °C.

Suitably substituted triarylethylene derivatives have antiestrogenic activity.[1] Of these, tamoxifen [(*Z*)-1,2-diphenyl-1-[4-[2-(dimethylamino)ethoxy]phenyl]-1-butene, **1**] is in current clinical use for the treatment of hormone-dependent breast cancer[2-4] and is thought to act mainly by competing with estradiol for its receptor (ER).[1] Pharmaceutical preparations of tamoxifen should contain only the isomer of *Z* stereochemistry since the *E* isomer has unwanted opposing estrogenic properties.[5,6] Although pure (*Z*)-tamoxifen can be prepared,[7-9] there is evidence that its 4-hydroxy derivative **2**, which is more potent than tamoxifen in vitro and is a minor metabolite in patients,[10-12] isomerizes at least in vitro to a mixture of *Z* and *E* isomers.[13] The conjugation of the hydroxyl group with the central double bond is responsible for the facile interconversion.[9] One solution to this problem is the introduction of a methyl substituent into the 2-position of the hydroxylated ring, which appears to prevent isomerization.[14] Alternatively, the configuration of the double bond can be fixed by its incorporation into a ring such as in nafoxidine (**3**)[15] or as in the series of tricyclic analogues of type **4** recently reported by workers at Imperial Chemical Industries.[16] However, in these compounds there are significant differences from tamoxifen in their detailed stereochemistry. X-ray crystallographic studies have demonstrated that the phenyl rings in tamoxifen and a methoxy derivative are twisted out of the plane of the double bond by more than 50°.[17,18] In **3**, introduction of the six-membered ring alters the orientation of the ring, which becomes fused,[19] and in **4** two of the phenyl rings are inclined toward each other[16] rather than being in a propeller-like conformation as in tamoxifen. We investigated the benzocycloheptene derivative **5** in the belief that it would show greater stereochemical similarity to tamoxifen than do known fused-ring analogues. This followed the observation that, in the crystal structure of 2-hydroxytamoxifen (**6**),[20] the hydroxyl group lies close to the ethyl group such that it could be envisaged that forming a seven-membered ring by joining the oxygen atom to the methyl group would not appreciably affect the overall conformation of the molecule. The six-membered ring analogue **7**, which is closely related to nafoxidine (**3**), was also prepared for comparison with **5**.



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† Drug Development Section, Cancer Research Campaign Laboratory.
‡ Cancer Research Campaign Biomolecular Structure Unit.
§ Institut Jules Bordet.

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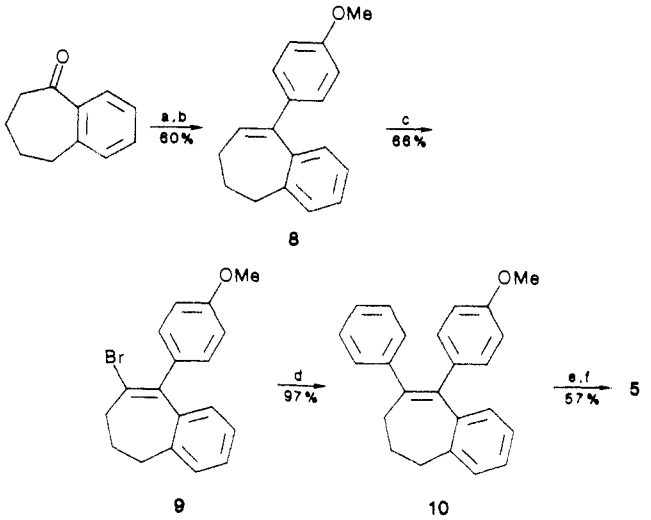
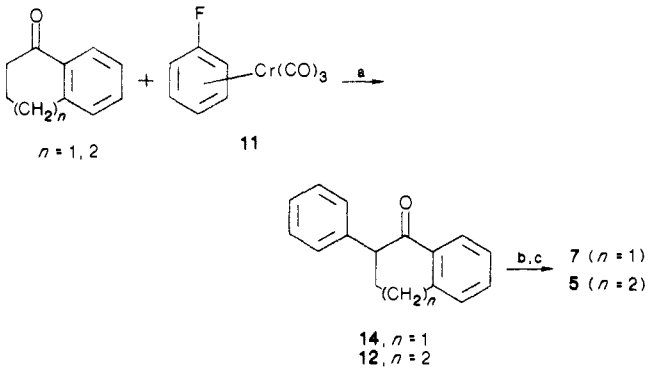
Scheme I[a]



[a] Reagents: (a) BrC_6H_4OMe, Mg, Et_2O, 34 °C; (b) HCl(aq), EtOH, 80 °C; (c) py·HBr_3, CH_2Cl_2, 20 °C; (d) PhZnCl, $Pd(PPh_3)_4$, THF, 60 °C; (e) py·HCl, 220 °C; (f) $Me_2NCH_2CH_2Cl$·HCl, NaH, DMF, 60 °C.

Scheme II[a]



[a] Reagents: (a) NaH, THF, 60 °C; (b) $BrC_6H_4OCH_2CH_2NMe_2$ (**13**), *n*-BuLi, THF, 20 °C; (c) HCl, EtOH, 80 °C.

Compounds **5** and **7** were assayed for relative binding affinity to estrogen receptors in both cytosol and whole cells and for growth inhibition of the MCF-7 human breast cancer cell line in vitro. In addition, derivatives of **5** and **7** were examined by X-ray crystallography in order to gain a fuller understanding of the stereochemical features of these compounds. It was thought that **5** could be more conformationally rigid than tamoxifen, and a low-temperature dynamic NMR study of **5** was made to determine the rate of interconversion of its enantiomeric conformers.

Table I. Relative Binding Affinity of Tamoxifen and Compounds **5** and **7** for Estrogen Receptor

	cytosol assay[a]	whole cell assay[a]
tamoxifen	1.0	0.06
5	1.0	0.02
7	0.5	0.02

[a] Estradiol = 100.

Table II. Effects of Compounds **5** and **7** on MCF-7 Cell Growth

		opt density (mean ± SD)[a]	
	concn, M	compd alone	compd + 10^{-8} M estradiol
control[c]		0.418 ± .084 (100)[b]	0.460 ± 0.033 (110)
5	10^{-7}	0.244 ± 0.022 (58)	0.379 ± 0.043 (91)
	10^{-6}	0.182 ± 0.014 (43)	0.438 ± 0.021 (105)
	10^{-5}	0.058 ± 0.001 (14)	0.098 ± 0.022 (23)
7	10^{-7}	0.390 ± 0.044 (93)	0.450 ± 0.087 (108)
	10^{-6}	0.288 ± 0.009 (69)	0.385 ± 0.019 (92)
	10^{-5}	0.049 ± 0.016 (11)	0.079 ± 0.020 (19)

[a] Each value corresponds to the mean of optical density measurements from four separate cultures. [b] Percentage of control value. [c] Effect of tamoxifen: control, 0.450 ± 0.037 (100); at 10^{-6} M, 0.246 ± 0.032 (55); at 10^{-7} M, 0.367 ± 0.050 (82).

Results and Discussion

Synthesis. The seven-membered ring tamoxifen analogue (**5**) was synthesized from benzosuberone as shown in Schemes I and II. In Scheme I the known methyl ether (**8**) was obtained by the reported procedure[21] and brominated to give **9**. Treatment of **9** with phenylzinc chloride in the presence of tetrakis(triphenylphosphine)palladium(0) catalyst, a method used in a stereospecific synthesis of tamoxifen,[22] gave a high yield of **10**. Replacement of the methyl group in **10** by the required basic side chain using the standard conditions[8] proceeded without problems. The resulting tamoxifen analogue (**5**) was crystalline.

In Scheme II, tricarbonyl(η^6-fluorobenzene)chromium(0) (**11**), which readily undergoes displacement of the fluorine atom by suitable nucleophiles,[23,24] was used to arylate the sodium enolate of benzosuberone. After oxidative decomplexation of the product, 2-phenylbenzosuberone (**12**) was obtained. 2-Phenylbenzosuberone had been previously prepared in moderate yield from benzosuberone by phenylation with benzyne.[25] Treatment of the ketone (**12**) with the anion derived by lithiation of 1-bromo-4-[2-(dimethylamino)ethoxy]benzene (**13**) gave, after acid-catalyzed dehydration of the resulting carbinol, the bicyclic tamoxifen analogue (**5**). The six-membered ring analogue (**7**) was prepared similarly from α-tetralone via the ketone **14**.

Both of the above routes represent novel syntheses of this type of compound, and either may be preferred in the preparation of specific further analogues. The second route is the shorter, but the first route is the more amenable to large-scale work.

Relative Estrogen Receptor Binding Affinities and Effect on MCF-7 Cell Growth. Table I gives the relative binding affinities (RBA) of **5**, **7**, and tamoxifen as measured for rat uterine cytosol (biochemical assay) and MCF-7 cells

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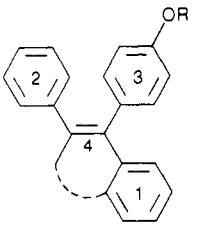
Table III. Dihedral Angles (deg) between Selected Planes in Various Triarylethylenes



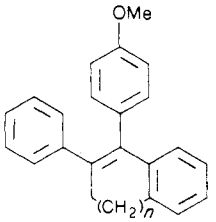
	tamoxifen (1)	2-hydroxy-tamoxifen (6)	3-hydroxy-tamoxifen	7-memb ring analogue (10)	6-memb ring analogue (15)	nafoxidine (3)
1–2[a]	59	57	60	90	119	112
1–3	87	92	94	103	97	110
2–3	57	58	57	55	62	55
1–4	64	65	59	45	19	20
2–4	57	59	61	45	44	50
3–4	50	52	60	53	69	56

[a] Planes calculated from the atomic coordinates of six atoms and defined as follows: 1 = aryl ring on C-1 trans to phenyl; 2 = phenyl ring on C-2; 3 = aryl ring on C-1 cis to phenyl; 4 = ethylene bond and adjoining atoms. Figures for compounds 1, 3, and 6 and 3-hydroxytamoxifen were calculated from reported atomic coordinates.[18-20]

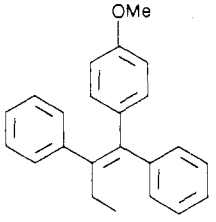
in monolayer culture (whole-cell assay). In each test, both compounds displayed RBA values of the same order of magnitude, which were weak in comparison with estradiol. Moreover, as for other antiestrogens of the triarylethylene category,[26] the whole-cell assay gave lower values than the biochemical assay.

Table II shows that compounds **5** and **7** both inhibited the growth of MCF-7 human breast cancer cells in vitro at concentrations between 10^{-5} and 10^{-6} M. Compound **5** appeared to be the more potent since it, but not **7**, produced a significant growth inhibition at 10^{-7} M. A routine control run in parallel showed that tamoxifen limited tumor growth to 55% at 10^{-6} M and was quite ineffective at 10^{-7} M, consistent with values recorded previously[14] (84% growth at 10^{-7} M and 63% at 10^{-6} M). In view of these data, compound **5** appears to be at least as potent as tamoxifen. It is noteworthy that the inhibitions observed were suppressed by 10^{-8} M estradiol at concentrations of compound of 10^{-7} and 10^{-6} M, but not at a concentration of 10^{-5} M. This behavior is identical with that previously described for tamoxifen[27] and is consistent with the binding affinity measurements.

X-ray Crystal Structure Determinations. For the purpose of structure determinations, compounds with methoxy rather than the basic side chain were chosen because of the greater ease of obtaining suitable crystals. Different substitution of the oxygen would not be expected to affect the stereochemistry of the triarylethylene framework. Crystals of the seven-membered ring compound **10** were obtained from ethanol. The corresponding



15, $n = 1$
10, $n = 2$



16

six-membered ring compound **15** was prepared from **14** by the reported procedure,[28] and crystals were obtained from light petroleum ether. Table III gives selected dihedral angles for **10** and **15** in comparison with those for tamoxifen, nafoxidine (**3**), and the 2- and 3-hydroxytamoxifen analogues described previously.[20] The angles between the planes of the aromatic rings (planes 1–3 in Table III) give an indication of the overall topology of the molecule and have been used previously in a discussion of the stereochemical features of nafoxidine.[19] The close correlation of the figures for the three non-ring-fused analogues is evidence that the structure of tamoxifen found in the crystal is a suitable representation of the favored conformation in solution, which is in accordance with conformational energy calculations.[20] Assuming that the seven-membered ring analogue **5** has the same stereochemistry as the methoxy derivative **10** studied, **5** has stereochemical features approximately midway between those of tamoxifen and nafoxidine. As expected, the dihedral angles for **15** were similar to those of nafoxidine. In order to consider the orientation of the individual rings independently of those of other rings, dihedral angles between the planes of the rings and the plane of the double bond (plane 4 in Table III) were calculated. From these figures, it is clear that the ring that is fused in nafoxidine (**3**) is much less twisted out of the plane of the double bond than in tamoxifen (**1**), and in **5** the orientation of the ring is between that in **1** and **3**, being closer to that of **1**. The lowered dihedral angles of the ring at C-2 in the bicyclic analogues are possibly the result of there being less steric repulsion between this ring and the protons of a cycloalkyl group compared with an ethyl group. Interestingly, the orientation of the ring cis to the 2-phenyl group (ring 3 in Table III) was fairly constant, being essentially unaffected by the varying orientations of the two adjacent rings. The slight variation nevertheless seen could be accounted for by different crystal-packing forces. Molecular structures of compounds **10** and **15** are given in Figure 1. Figure 2 illustrates superpositions of the structures of compounds **10** and **15**, with the methoxy analogue **16** corresponding to tamoxifen.[17] The overall conformations of the molecules are similar. However, it is clear from this figure that compound **10** with a seven-membered ring has the closer similarity to **16**.

Low-Temperature NMR Studies. The proton NMR spectra of both tamoxifen and the benzocycloheptene (**5**) at room temperature show no evidence for chiral forms; thus, the protons allylic to the olefinic bond were found to be equivalent, as were the pairs of protons on the 4-substituted ring that appear as an AB quartet. In the case

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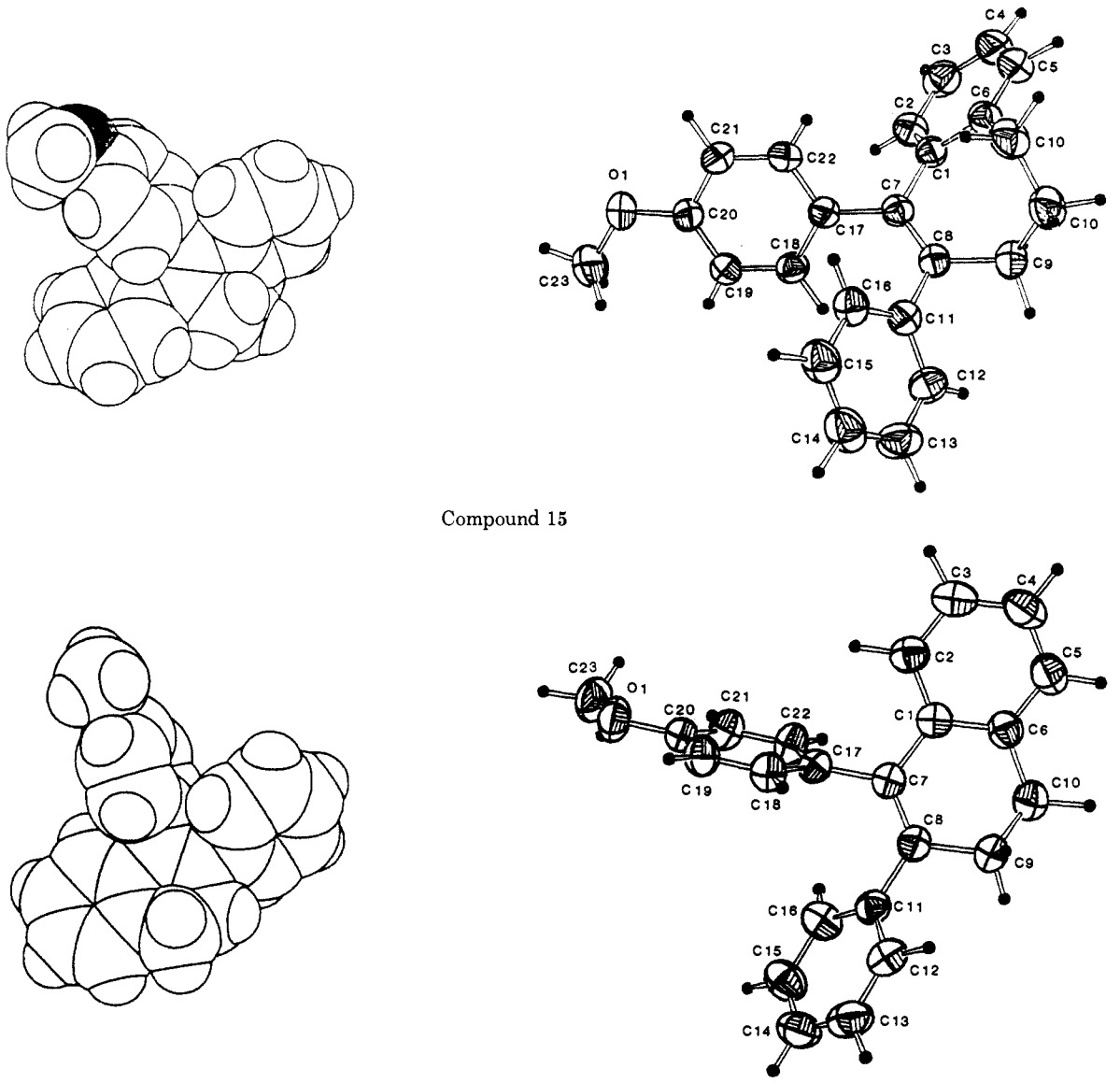
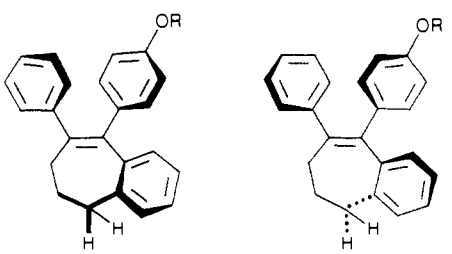


Figure 1. Computer-drawn representations of compounds **10** and **15**. Space-filling diagrams are with atoms drawn at their van der Waals radii. Framework representations illustrate the numbering system used in the supplementary data and 50% probability thermal ellipsoids.

of tamoxifen, cooling to −75 °C has no effect on the proton NMR spectrum apart from line broadening. However, in the case of **5**, the benzylic protons in the seven-membered ring appear as a broadened multiplet and at −85 °C as two separate multiplets (Figure 3), indicating the presence of distinct chiral forms (**5a** and **5b**), on the NMR time scale



5a **5b**

R = $CH_2CH_2NMe_2$

in which the benzylic protons shown are chemically nonequivalent. However, even at −85 °C, we saw no effect on the resonances of the alkoxy-bearing ring, indicating that this ring can rotate independently of the bicyclic ring system. Spectra below −85 °C were not attainable owing to solubility problems and excessive line broadening.

Conclusions. The novel analogue **5** of tamoxifen described herein has the advantage over tamoxifen that the ring fusion circumvents the requirement for a stereospecific synthesis or the separation of *Z* and *E* isomers. The X-ray crystallographic study shows that the orientation of the fused aromatic ring in **5** is closer to that of the corresponding ring in tamoxifen than is that in nafoxidine. On this evidence, compound **5** is probably closer in its stereochemistry to tamoxifen than are other known nonisomerizable analogues. The relative binding affinity of compound **5** to the estrogen receptor in cytosol was indistinguishable from that of tamoxifen, as might be expected from the good correlation between its stereochemistry and that of tamoxifen. It is noteworthy that the tricyclic analogues of type **4** all had lower binding affinity than the corresponding 4-hydroxytamoxifen, and the most active was 5 times less potent in a test for antiestrogenicity than tamoxifen.[16] These results indicate that, for good activity, the phenyl rings should not be inclined toward each other but that the propeller-like conformation of tamoxifen should be maintained as is the case for **5**. The results also

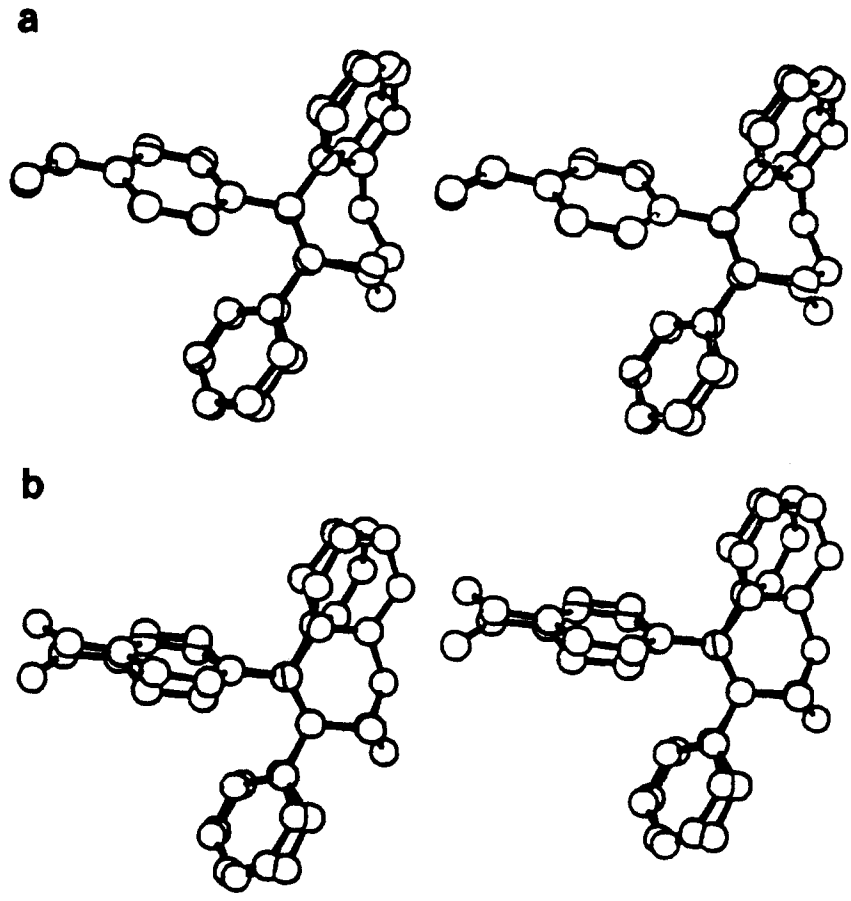


Figure 2. Superpositions of stereoviews of the molecular structures: (a) seven-membered ring compound **10** and the tamoxifen analogue **16**; (b) six-membered ring compound **15** and **16**.

indicate that the extra steric bulk in **5** caused by the added methylene group has little effect on the RBA. A similar situation was observed in the case of 2-methyl-4-hydroxytamoxifen, which had an RBA identical with that of 4-hydroxytamoxifen.[14] However, in the crystal structure at least, the methyl group in 2-methyl-4-hydroxytamoxifen protrudes into a region of space away from the ethyl group,[20] different from that of the benzylic methylene group in **5**.

In a whole-cell assay, the cyclic compounds **5** and **7**, like tamoxifen and other triarylethylene antiestrogens,[26] had RBA values somewhat lower than in cytosol. The origin of this reduced RBA is unknown. It has been suggested that it may reflect poor binding of these antiestrogens to the activated conformation of the receptor in the nucleus, the whole-cell assay therefore giving a measure of estrogenicity.[26] Alternatively, poor permeability of the cell membrane to these compounds would account for the whole-cell RBA values observed, but this explanation is unlikely in view of the marked MCF-7 cell growth inhibition effects seen. Experiments to ascertain the cause of the reduced RBA in whole cells should be valuable in the search for a strategy to obtain improved antiestrogens for the treatment of hormone-dependent breast cancer.

The analogue **5** inhibited the growth of the MCF-7 human breast cancer cell line in vitro, and it is therefore an antiestrogen. Its potency was at least as high as that of tamoxifen, and like tamoxifen, the growth inhibition was reversible by estradiol at concentrations of 10^{-7} and 10^{-6} M but not at 10^{-5} M, indicating a hormone-independent cytotoxicity at this higher concentration. The good correlation between the biological properties of **5** and those of tamoxifen indicates that **5** would be a good substitute for tamoxifen in studies where possible isomerism in the case of tamoxifen would cause complications. The slightly lower antitumor potency of the six-membered ring analogue **7** compared to **5** might be due to the different orientation of the fused phenyl ring.

The results of the low-temperature NMR studies show that somewhat more rigidity needs to be introduced into the molecule before it is likely that two chiral forms (atropisomers) can be isolated and do not interconvert at physiological temperature. Other workers have shown that alkenes suitably substituted with bulky groups can be separated into the constituent atropisomers.[29] Presumably sufficiently bulky groups could be incorporated into **5**, but it is questionable whether the resulting compound would retain any useful activity. However, if two atropisomeric derivatives of **5** could be isolated, these could be useful compounds with which to gain a fuller understanding of the three-dimensional nature of the ER binding site and the precise mode of action of antiestrogens.

Experimental Section

Chemical Methods. General Procedures. ^{1}H NMR spectra were recorded on solutions in $CDCl_3$ containing Me_4Si as internal standard. Routine 60-MHz spectra were recorded on a Perkin-Elmer R12B spectrometer. Spectra (250 MHz) were obtained by courtesy of the University of London Intercollegiate Research Service. Infrared spectra were recorded on a Perkin-Elmer 1310 spectrophotometer, and mass spectra (electron impact, 70 eV) were obtained with a VG 7070H spectrometer and VG 2235 data system using the direct-insertion method. Column chromatography was carried out with silica gel 60 (Merck 7736). Melting points were determined on a Kofler hot stage and are uncorrected.

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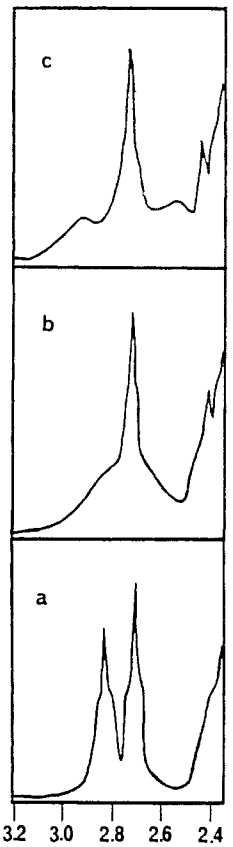

Figure 3. Part of the ^1H NMR spectrum of the benzocycloheptene (**5**) showing the temperature variance of the benzylic protons. Spectra were recorded on solutions in $CDCl_3$ containing 25% $CFCl_3$ at (a) −55 °C, (b) −75 °C, and (c) −85 °C. The invarient signal at δ 2.65 is for protons of the methylene adjacent to nitrogen in the side chain.

6,7-Dihydro-8-bromo-9-(4-methoxyphenyl)-5*H*-benzocycloheptene (9). 6,7-Dihydro-9-(4-methoxyphenyl)-5*H*-benzocycloheptene (**8**; 2.26 g, 9.0 mmol), prepared as reported[21] (60% yield) from 1-benzosuberone, was dissolved in CH_2Cl_2 (20 mL) and the solution stirred at 0 °C. Pyridine hydrobromide perbromide (2.88 g, 9.0 mmol) was added in portions over 15 min, and the solution was stirred for a further 30 min, then washed with aqueous $NaHSO_3$ (10% w/v; 20 mL), and concentrated. The residue was crystallized from light petroleum ether (bp 80–100 °C) to give **9**: 2.62 g (88%); mp 109.5–110 °C; ^1H NMR (60 MHz) δ 2.0–3.0 (m, 6, H-5, 6, 7), 3.77 (s, 3, OMe), 6.82 (d, 2, J = 9 Hz, ArH ortho to OMe), 6.9–7.3 (m, 6, remaining ArH); MS, m/z 330/328 (1:1, 47%, M^+•), 249 (26%, M^+ − Br), 121 (100%). Anal. ($C_{18}H_{17}BrO$) C, H, Br.

6,7-Dihydro-8-phenyl-9-(4-methoxyphenyl)-5*H*-benzocycloheptene (10). A solution of $ZnCl_2$ (1.86 g, 13.7 mmol) in dry THF (4 mL) was stirred under N_2 while phenyllithium (7.6 mL of a 1.8 M solution, 13.7 mmol) was added over 3 min. The mixture was then refluxed for 30 min. Separately, a stirred solution of $Pd(PPh_3)_4$ (263 mg, 0.23 mmol) and **9** (1.5 g, 4.56 mmol) was prepared under N_2 at 20 °C and the above solution of phenylzinc chloride added. The mixture was refluxed for 4 h and then poured into dilute HCl (1 N; 100 mL) and the product extracted with Et_2O (100 mL). The Et_2O solution was dried (Na_2SO_4) and concentrated and the residue chromatographed on silica gel (80 g). Elution with 3% CH_2Cl_2 in light petroleum ether (bp 40–60 °C) gave **10**: 1.44 g (97%); mp 136–137 °C (EtOH); ^1H NMR (60 MHz) δ 2.0–2.5 (m, 4, H-6, 7), 2.81 (t, 2 J = 6 Hz, H-5), 3.62 (s, 3, OMe), 6.58 (d, 2 J = 9 Hz, ArH ortho to OMe), 6.83 (d, 2, J = 9 Hz, ArH meta to OMe), 6.9–7.4 (m, 9, remaining ArH); MS, m/z 326 (M^+•, 100%), 325 (57%), 166 (7%), 91 (11%). Anal. ($C_{24}H_{22}O$) C, H.

6,7-Dihydro-8-phenyl-9-[4-[2-(dimethylamino)ethoxy]phenyl]-5*H*-benzocycloheptene (5). Method 1. A mixture of **10** (300 mg, 0.92 mmol) and pyridine hydrochloride (600 mg, 5.2 mmol) was heated to reflux for 4 h. The mixture was then cooled and partitioned between Et_2O (30 mL) and 10 N H_2SO_4 (30 mL). The Et_2O solution was washed with H_2O (30 mL) and dried (Na_2SO_4) and the solvent evaporated to give an oil that from light petroleum ether (bp 80–100 °C; 15 mL) crystallized **6,7-dihydro-8-phenyl-9-(4-hydroxyphenyl)-5*H*-benzocycloheptene**: 233 mg (81%); mp 184–185 °C. Anal. ($C_{23}H_{20}O$) C, H. A solution of this phenol (222 mg, 0.71 mmol) in dry DMF (4 mL) was stirred under N_2 at 20 °C while NaH (200 mg, 8.3 mmol) was added. The mixture was warmed to 60 °C, and then $Me_2NCH_2CH_2Cl$·HCl (250 mg, 1.78 mmol) was added in portions over 30 min. After 1 h, the solution was poured into ice–water (20 mL) and the product extracted with Et_2O (20 mL). The Et_2O solution was washed with H_2O (20 mL) and dried (Na_2SO_4) and the solvent evaporated. Crystallization of the residue from light petroleum ether (bp 80–100 °C) gave **5**: 204 mg (75%, 61% overall from **10**); mp 145–147 °C; ^1H NMR (250 MHz) δ 2.19 (quint, 2, J = 7 Hz, H-6), 2.30 (s, 6, NMe_2), 2.37 (t, 2, J = 6.8 Hz, H-7), 2.67 (t, 2, J = 5.8 Hz, CH_2NMe_2), 2.80 (t, 2, J = 7.0 Hz, H-5), 3.97 (t, 2, J = 5.8 Hz, OCH_2), 6.61 (d, 2, J = 8.7 Hz, ArH ortho to $OCH_2CH_2NMe_2$), 6.80 (d, 2, J = 8.7 Hz, ArH meta to $OCH_2CH_2NMe_2$), 6.91 (m, 1, H-1), 7.05–7.30 (m, 7, ArH); MS, m/z 383 (M^+•, 18%), 72 (Me_2N^+=CH_2, 49%), 58 (Me_2N^+=CH_2, 100%). Anal. ($C_{27}H_{29}NO$) C, H, N.

2-Phenyl-1-benzosuberone (12). To a stirred solution of 1-benzosuberone (0.865 g, 5.4 mmol) and tricarbonyl(η^6-fluorobenzene)chromium(0)[30] (1.25 g, 5.4 mmol) in dry THF (10 mL) under N_2 was added NaH (0.40 g, 17 mmol) and the mixture refluxed. After 5 h, the deep yellow mixture was poured carefully into ice–water (150 mL), the solution was acidified with 2 N H_2SO_4, and the products were extracted with Et_2O (2 × 125 mL). The combined Et_2O solutions were dried (Na_2SO_4) and the chromium complexes photolyzed by means of three 500-W tungsten lamps, while a slow stream of air was drawn through the solution. When the yellow color of the solution had discharged (ca. 3 H), precipitated Cr_2O_3 was removed by filtration through Celite, the filtrate concentrated, and the residue chromatographed on silica gel. Elution with 1:3 CH_2Cl_2–light petroleum ether (bp 40–60 °C) gave **12**[24] as an oil: 0.83 g (65%); ^1H NMR (60 MHz) δ 1.5–2.4 (m, 4), 2.7–3.1 (m, 2, $ArCH_2$), 4.02 (t, 1, J = 7 Hz, PhC*H*CO), 7.0–7.7 (m, 9, ArH).

2-Phenyl-1-tetralone (14). α-Tetralone was α phenylated as described above for 1-benzosuberone except that column chromatography was not necessary and instead the crude product was crystallized from light petroleum ether (bp 80–100 °C) to give **14** (70%), mp 78–79 °C (lit.[24] mp 79 °C).

6,7-Dihydro-8-phenyl-9-[4-[2-(dimethylamino)ethoxy]phenyl]-5*H*-benzocycloheptene (5). Method 2. To a stirred solution of 1-bromo-4-[2-(dimethylamino)ethoxy]benzene[31] (**13**; 598 mg, 2.45 mmol) in dry THF (2 mL) under N_2 at −78 °C was added a solution of *n*-BuLi in hexane (1.6 M; 1.53 mL, 2.45 mmol). After 5 min, a solution of 2-phenylbenzosuberone (**12**; 232 mg, 0.98 mmol) was added and the mixture allowed to warm to 20 °C over 1 h and then refluxed 3 h. The mixture was then poured into H_2O (50 mL) and extracted with Et_2O (50 mL). The Et_2O solution was concentrated and the residual oil dissolved in EtOH (15 mL). Concentrated HCl (10 mL) was added, and the mixture was refluxed for 3 h, then poured into NaOH (3 N; 100 mL), and extracted with Et_2O (2 × 50 mL). The Et_2O solution was washed with H_2O (50 mL), dried (Na_2SO_4), and concentrated. The residue was chromatographed on silica gel. Elution with 1:10:10 NEt_3–Et_2O–light petroleum ether (bp 40–60 °C) gave **5**, which was recrystallized from light petroleum ether (bp 80–100 °C); yield 204 mg (55%). This product was identical with the sample obtained previously in method 1.

1-[4-[2-(Dimethylamino)ethoxy]phenyl]-2-phenyl-3,4-dihydronaphthalene (7). The compound was prepared (55% yield) as described above for **5** but from 2-phenyltetralone (**14**) instead of **12**: mp 99–101 °C (from light petroleum ether, bp 80–100 °C); ^1H NMR (60 MHz) δ 2.29 (s, 6, NMe_2), 2.5–3.1 (m, 6, CH_2CH_2 and OCH_2CH_2N), 3.99 (t, 2, J = 6 Hz, OCH_2CH_2N), 6.80 (d, 2, J = 9 Hz, ArH ortho to $OCH_2CH_2NMe_2$), 6.93 (d, 2, J = 9 Hz, ArH meta to $OCH_2CH_2NMe_2$), 6.95–7.2 (m, 9, remaining

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ArH). Anal. ($C_{26}H_{27}NO$) C, H, N.

Binding Studies. Biochemical Assay.[32] Immature rat uterine cytosol was incubated at 18 °C for 30 min with 5×10^{-9} M [^{3}H]estradiol in the absence and presence of increasing amounts (10^{-9}–10^{-5} M) of the test compound or unlabeled estradiol (control). Unbound compounds were then removed with dextran-coated charcoal, and the amounts of estrogen receptor bound [^{3}H]estradiol were measured. The relative concentrations of estradiol and test compound required to achieve 50% inhibition of [^{3}H]-estradiol binding are the RBA: RBA = ($[I_{50}]$(estradiol)/$[I_{50}]$(test compound)) × 100.

Whole-Cell Assay.[26,33] MCF-7 cells were incubated at 37 °C for 50 min with 10^{-9} M [^{3}H]estradiol in the absence or presence of increasing amounts (10^{-10}–10^{-5} M) of the test compound or unlabeled estradiol (control). Bound compounds were then extracted with ethanol, and the amounts of estrogen receptor bound [^{3}H]estradiol were measured. The RBA values were calculated as for the biochemical assay.

Effect of Compounds on MCF-7 Cell Growth. MCF-7 cells were plated in 96-multiwell dishes (Falcon; plating density 10000 cells/mL). After 24 h of culture, compounds **5** or **7** were added to the culture dishes according to the protocol described previously.[34] Estradiol was also added to evaluate its potential antagonism of the growth inhibition of compounds **5** and **7**. Final concentrations were as follows: estradiol, 10^{-8} M; compounds **5** and **7**, 10^{-7}, 10^{-6}, and 10^{-5} M. After 5 days of culture, the monolayer was fixed with 90% ethanol and colored with hematoxylin (for experimental procedure see ref 34). The intensity of the coloration, which is a measurement of the number of cells, was determined with a multiscan spectrophotometer at 540 nm (Flow Laboratories Inc.). A control evaluation of the inhibitory potency of tamoxifen was run in parallel in another multiwell dish.

X-ray Crystal Structure Determinations. Accurate cell dimensions were obtained by measurement of 25 θ values on an Enraf-Nonius CAD4 diffractometer, after a preliminary examination by Weissenberg photography. The space groups were uniquely determined from systematic absences: compound **10**, ($h00$) $h = 2n + 1$, ($0k0$) $k = 2n + 1$, ($00l$) $l = 2n + 1$; compound **15**, ($h0l$) $h = 2n + 1$, ($0k0$) $k = 2n + 1$. Intensity data were collected on the diffractometer with Cu Kα radiation operated in an ω–2θ scan mode up to $\theta = 72°$ for **10** and 70° for **15**. Three strong reflections were monitored every 3600 s, and no decay was observed during the data collection in both cases.

Crystal Data. (i) Compound 10: $C_{24}H_{22}O$; MW = 326.44; orthorhombic; a = 8.949 (4), b = 11.426 (2), c = 18.104 (3) Å; V = 1851 (1) Å^3; d_{calcd} = 1.172 g cm^{-3}; $Z = 4$, $F(000) = 696$; space group $P2_12_12_1$; μ(Cu Kα) = 5.03 cm^{-1}; crystal dimensions 0.15 × 0.55 × 0.70 mm.

(ii) Compound 15: $C_{23}H_{20}O$; MW = 312.42; monoclinic; a = 9.957 (3), b = 16.164 (1), c = 10.608 (1) Å; β = 91.19 (1)°; V = 1706.9 (8) Å^3; d_{calcd} = 1.216 g cm^{-3}; $Z = 4$, $F(000) = 664$; space group $P2_1/a$; μ(Cu Kα) = 5.25 cm^{-1}; crystal dimensions 0.70 × 0.21 × 0.43 mm.

Both structures were solved by direct methods using the program MULTAN82[35] and refined by a full-matrix least-squares method. In the case of **10**, out of 2079 unique observed reflections, 1880 with $I > 1.5\sigma(I)$ were used for structure refinement. The positions of all the hydrogen atoms except H4 were revealed in a difference Fourier map at $R = 0.103$. The remaining hydrogen atom was located in a subsequent difference map. The positional and isotropic thermal parameters of hydrogen atoms were refined except for those of the methyl group. Non-hydrogen atoms were refined anisotropically. Empirical absorption[36] and extinction corrections ($g = 2.821 \times 10^{-6}$) were applied. The final R value, $R = \sum||F_o| - |F_c||/\sum|F_o|$, was 0.0386. Unit weight was assigned to each reflection. The maximum shift/error was 0.05 and 0.13 for non-hydrogen and hydrogen atoms, respectively. The highest residual electron density was 0.10 e/Å^3. In the case of **15**, 2472 out of 2669 observed reflections with $I > 1.5\sigma(I)$ were used for the refinements. All the hydrogen atoms were clearly revealed in a difference Fourier map following least-square refinements with anisotropic temperature factors for all the non-hydrogen atoms ($R = 0.094$). The positional and isotropic thermal parameters of hydrogen atoms were included in the refinement. An empirical absorption[36] was applied. The final R value was 0.048, and the maximum shift/error ratios were 0.00 and 0.01 for non-hydrogen and hydrogen atoms, respectively. The final difference Fourier map did not show any peaks > 0.12 e/Å^3. Unit weight was assigned to each reflection. Atomic scattering factors were taken from ref 37. All the calculations were carried out on a PDP 11/34A computer using the SDP program system.[38]

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Registry No. 1, 10540-29-1; **3**, 1845-11-0; **5**, 103304-52-5; **7**, 103304-53-6; **8**, 21855-86-7; **9**, 103304-54-7; **10**, 103304-55-8; 11, 12082-05-2; **12**, 51197-89-8; **13**, 2474-07-9; 14, 7498-87-5; **15**, 145-55-1; **16**, 6462-18-6; $Me_2NCH_2CH_2Cl$·HCl, 4584-46-7; 6,7-dihydro-8-phenyl-9-(4-hydroxyphenyl)-5*H*-benzocycloheptene, 103304-51-4; 1-benzosuberone, 826-73-3; α-tetralone, 529-34-0.

Supplementary Material Available: Tables of positional parameters, bond lengths, and bond angles for compounds **10** and **15** (7 pages). Ordering information is given on any current masthead page.

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