Interactions of sterols with antiestrogen-binding sites: structural requirements for high-affinity binding

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Abstract Animal and human tissues contain a microsomal protein that binds nonsteroidal antiestrogens with high affinity and specificity. The functions of these binding sites and the identity of their natural ligands are unknown. Following a report that certain sterols inhibit [^{3}H]tamoxifen binding to this site, we attempted to define the structural requirements for maximal inhibition of [^{3}H]tamoxifen binding to rat liver antiestrogen-binding sites. Our studies identified 5α-cholestan-3β-ol-7-one (7-ketocholestanol) as the most potent sterol, having an inhibitory activity that was 12% that of unlabeled tamoxifen and an equilibrium dissociation constant of 6.3 nM. Structural features that appeared important for the inhibitory activity of this sterol include the presence of i) a hydrocarbon side chain at C17; ii) an oxygen function at C7; iii) a hydroxyl group at C3; and iv) the absence of a double-bond between C5 and C6. Saturation analysis and kinetic studies of [^{3}H]tamoxifen binding in the presence of varying concentrations of 7-ketocholestanol clearly indicated that this sterol competed directly with tamoxifen for the antiestrogen-binding site. Unlike tamoxifen, this sterol did not bind to the estrogen receptor. These features make 7-ketocholestanol a potentially valuable tool for studying the properties and functions of this site.—**Hwang, P. L. H., and A. Matin.** Interactions of sterols with antiestrogen-binding sites: structural requirements for high-affinity binding. *J. Lipid Res.* 1989. **30:** 239–245.

Supplementary key words tamoxifen • oxygenated sterols • rat liver • structural requirements • 7-ketocholestanol

It is now generally accepted that many animal and human tissues contain an intracellular protein, found predominantly in the microsomal fraction, that binds nonsteroidal antiestrogens such as tamoxifen with high affinity and specificity (1–5). The biological significance of this antiestrogen-binding site is currently unknown; the bulk of available evidence indicates that this site probably does not play a direct role in mediating the antagonism of estrogen action exhibited by the nonsteroidal antiestrogens (6–8). The nature and identity of endogenous ligands for these sites, it these exist, are also unknown. Several laboratories have attempted to identify endogenous ligands for the antiestrogen-binding site by looking for endogenous substances that inhibit [^{3}H]tamoxifen binding to these sites. Extracts of rat serum (9), rat liver (10), chicken serum and liver (11), and human serum (12) have all been shown to contain one or more substances that inhibited [^{3}H]tamoxifen binding. Murphy, Breckenridge, and Lazier (12) showed that two compounds present in human serum, namely 5-cholesten-3β-ol-7-one and 4-cholesten-3-one, inhibited [^{3}H]tamoxifen binding to antiestrogen-binding sites from cockerel liver. These compounds, however, had less than 0.5% of the potency of unlabeled tamoxifen in inhibiting [^{3}H]tamoxifen binding. Furthermore, the mechanism of their inhibitory activity was not examined and it is possible that they do not compete directly for the antiestrogen-binding site but act through noncompetitive mechanisms. Brandes, Macdonald and Bogdanovic (13) demonstrated that histamine antagonists also bound to antiestrogen-binding sites, raising the possibility that histamine or histamine-like substances might be endogenous ligands. There is no direct evidence to support this hypothesis at present. Our laboratory (9) recently showed that an extract of rat serum inhibited [^{3}H]tamoxifen binding to rat liver antiestrogen-binding sites and that the inhibitory activity in the extract was largely due to unsaturated fatty acids. The inhibitory effect of fatty acids, however, was clearly not competitive (14), making it unlikely that these compounds represent endogenous ligands.

We noted in our previous studies that rat serum extracts, in addition to unsaturated fatty acids, also contained a separate inhibitory activity which co-migrated with 5-cholesten-3β-ol-7-one (7-ketocholesterol) on TLC (9).

Abbreviations: TLC, thin-layer chromatography. The trivial names used are: tamoxifen, 1-[4-(2-dimethylaminoethyoxy)phenyl]-1,2-diphenylbut-1(Z)-ene; clomiphene, 1-[4-2-diethylaminoethoxy)phenyl]-1,2-diphenyl-2-chloroethylene; nafoxidine, 1-{2-[4(3,4-dihydro-6-methoxy-2-phenyl-1-naphthyl)phenoxy]ethyl} pyrrolidine hydrochloride; CI 628, α-(4-pyrrolidinoethoxy)phenyl-4-methoxy-α'-nitrostilbene; oleic acid, *cis*-9-octadecenoic acid.

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This observation, together with the earlier demonstration by Murphy et al. (12) that 7-ketocholesterol inhibited [^{3}H]tamoxifen binding, suggested that endogenous ligands of the antiestrogen-binding site might belong to a similar class of compounds. We felt it worthwhile to examine the structural requirements for the inhibition of [^{3}H]tamoxifen binding by studying a series of structurally related compounds in a ligand-binding assay using rat liver microsomes as the source of antiestrogen-binding sites. Such information may provide clues about the structure of putative endogenous ligands for the antiestrogen-binding site and may also lead to the identification of compounds which could be used to probe the function of these sites. We summarize here the results of these studies and further report that, among the sterols examined, 5α-cholestan-3β-ol-7-one (7-ketocholestanol) appears to bind specifically to the antiestrogen-binding site with the highest affinity (about 12% that of tamoxifen).

MATERIALS AND METHODS

Chemicals

[N-methyl-^{3}H]Tamoxifen (77 Ci or 2.85 TBq/mmol) was obtained from Amersham International and stored in ethanol at − 20°C protected from light. [^{3}H]Estradiol (90.4 Ci or 3.34 TBq/mmol) was purchased from New England Nuclear. Nafoxidine hydrochloride and CI 628 were graciously provided by the Upjohn Company. Tamoxifen citrate, clomiphene citrate, diethylstilbestrol, oleic acid, 5-cholesten-3-one, and 4-cholesten-3-one were obtained from Sigma Chemical Co. All other sterols and related compounds were purchased from Steraloids. Other chemicals were of analytical grade and were obtained from conventional sources.

Tissue preparation

For the preparation of antiestrogen-binding sites, rat livers from female Sprague-Dawley rats were processed and stored as previously described (9). A 20,000-*g* supernatant was used for ligand-binding studies. For the estrogen receptor binding assay, uteri from 6-week-old Sprague-Dawley rats were homogenized with 20 volumes of ice-cold buffer [10 mMTris-HCl, 1.5 mM EDTA, 20 mM sodium molybdate, 10% (v/v) glycerol, pH 7.5, at 4°C]. The homogenate was centrifuged at 100,000 *g* for 1 hr and the supernatant was stored at − 70°C. The cytosol was diluted, as required, to a protein concentration of 0.5–1.0 mg/ml for the binding assay.

Ligand-binding assays

Ligand-binding assays with [^{3}H]tamoxifen and rat liver antiestrogen-binding sites were carried out as described (9). In brief, the 20,000-*g* rat liver supernatant was incubated with 1–2 nM [^{3}H]tamoxifen in the absence or presence of various competing ligands. Except where indicated, all assay tubes contained 10^{-6} M diethylstilbestrol to eliminate binding to estrogen receptors. After incubation for 16 hr at 4°C, the assay was terminated by the addition of dextran-coated charcoal. Total bound counts were determined by counting the supernatants after pelleting the charcoal. Nonspecific binding was determined by parallel incubations in the presence of a 500-fold molar excess of nonradioactive tamoxifen. Specific binding was the difference between the total and nonspecifically bound counts. Assays using [^{3}H]estradiol as the labeled ligand were performed in a similar fashion except that diethylstilbestrol was omitted from the incubation mixture.

Saturation analysis

Saturation analysis was carried out by incubating the rat liver 20,000-*g* supernatant with various concentrations of [^{3}H]tamoxifen (0.25–8.0 nM) for 16 hr at 4°C in the absence or presence of various concentrations of 7-ketocholestanol. Details of the procedure have been described previously (14).

Dissociation kinetics

For the dissociation studies, the 20,000-*g* rat liver supernatant (diluted to a protein concentration of 1.1 mg/ml) was incubated for 16 hr at 4°C with 2 nM [^{3}H]tamoxifen in the presence of 0, 10, 20, and 40 nM 7-ketocholestanol. At zero time a 500-fold molar excess of nonradioactive tamoxifen was added. Aliquots (0.55 ml) of the incubation mixture were taken at 2, 4, 6, 8, 10, 12.5, and 15 min and treated with 0.25 ml of dextran-coated charcoal as described previously (14). Total and specific binding were determined for each time point as described above. The dissociation rate constant was determined from the plot of the specifically bound [^{3}H]tamoxifen against time as described (14).

Other procedures

Thin-layer chromatography was carried out as described (9) with the solvent systems specified in the text. Protein was determined by the method of Lowry et al. (15) using bovine serum albumin as standard.

RESULTS

Table 1 shows the relative binding affinities (defined in the legend of Table 1) of four nonsteroidal antiestrogens and 25 sterols and related compounds. The relative binding affinities of the four antiestrogens were similar and ranged from 100 for tamoxifen to 152 for clomiphene. Among the sterols studied, 5α-cholestan-3β-ol-7-one or 7-ketocholestanol (compound 5) had the highest relative

TABLE 1. Relative binding affinities of nonsteroidal antiestrogens, sterols, and related compounds for the rat liver antiestrogen-binding site

Compound Number	Compound Name	Relative Binding Affinity
		%
Nonsteroidal antiestrogens		
1	Tamoxifen	100.0
2	CI 628	116.7
3	Nafoxidine	140.0
4	Clomiphene	152.2
Sterols and related compounds		
5	5α-Cholestan-3β-ol-7-one (7-ketocholestanol)	11.8
6	5α-Cholestan-3β-ol-7-one acetate (7-ketocholestanol acetate)	5.9
7	5α-Cholestan-3β-ol-6-one (6-ketocholestanol)	4.0
8	5α-Cholestan-3β-ol-6-one acetate (6-ketocholestanol acetate)	2.8
9	5α-Cholesten-3β,7β-diol (7β-hydroxycholesterol)	0.37
10	5-Cholesten-3β-ol-7-one (7-ketocholesterol)	0.17
11	5-Cholesten-3β-ol-7-one acetate (7-ketocholesterol acetate)	0.13
12	Cholestan-3β,5α,6β-triol	0.06
13	5α-Cholesten-3β,7α-diol (7α-hydroxycholesterol)	0.04
14	4-Cholesten-3-one (cholestenone)	0.004
15	5-Cholesten-3β-ol (cholesterol)	< 0.004
16	5-Cholesten-3-one	< 0.004
17	5-Androsten-3β-ol-7,17-dione	< 0.004
18	5-Androsten-3β-ol	< 0.004
19	5-Pregnen-3β-ol-7,20-dione	< 0.004
20	5-Cholesten-3β,25-diol (25-hydroxycholesterol)	< 0.004
21	5α-Cholestane	< 0.001
22	5α-Cholestan-3α-ol (α-cholestanol)	< 0.001
23	5β-Cholestan-3-one	< 0.001
24	5α-Cholestan-3β-ol (β-cholestanol)	< 0.001
25	5β-Cholestan-3β-ol (coprostanol)	< 0.001
26	5α-Cholestan-3-one (cholestanone)	< 0.001
27	5α-Cholestan-3β-chloro-6-one	< 0.001
28	5α-Cholestan-3β,6α-diol	< 0.001
29	5α-Cholestan-3β,6β-diol	< 0.001

[^{3}H]Tamoxifen binding assays were set up (9) in the presence of various concentrations of competing ligand. The relative binding affinity was calculated as (IC_{50} of tamoxifen/IC_{50} of test compound) × 100% where IC_{50} is the concentration of the ligand required to reduce the specific binding of [^{3}H]tamoxifen by 50%. Trivial names of sterol derivatives are given in parentheses.

binding affinity, which amounted to 11.8% that of tamoxifen (mean of five experiments). It may be noted that in our hands the two sterol derivatives reported earlier by Murphy et al. (12) to inhibit [^{3}H]tamoxifen binding, namely 7-ketocholesterol (compound 10) and 4-cholesten-3-one (compound 14), had relative binding affinities of only 0.17% and 0.004%, respectively.

Our present study also identified several additional compounds with relative binding affinities that were only slightly lower than that of 7-ketocholestanol. The acetate of 7-ketocholestanol (compound 6), 6-ketocholestanol (compound 7), and its acetate (compound 8) had relative binding affinities of 5.9%, 4.0%, and 2.8%, respectively, relative to tamoxifen. Compounds 9–14 had lower but clearly detectable binding affinities. All the other compounds tested had negligible activity.

To facilitate an examination of the structural requirements for the inhibition of [^{3}H]tamoxifen binding to the antiestrogen-binding site, the structures of several selected compounds from Table I are depicted in **Fig. 1** together with their relative binding affinities (in parentheses). First, if one compares 7-ketocholestanol (Fig. 1, A) with 7-ketocholesterol (B) it is clear that the introduction of a double bond between C5 and C6 is associated with a dramatic 70-fold decrease in relative binding affinity. Secondly, if the oxygen function at C7 of either 7-ketocholestanol or 7-ketocholesterol is removed, there is almost complete loss of activity (A vs. C; B vs. D). It appears, however, that the requirement for a keto group at C7 can be partly satisfied by a keto group at C6 since 6-ketocholestanol (E) has a relative binding affinity of 4.0%, which is approximately one-third that of 7-ketocholestanol (A). Reduction of the keto group at C7 of 7-ketocholesterol (B) to a hydroxyl group is associated with either a modest increase in binding affinity in the case of 7β-hydroxycholesterol (Fig. 1, B vs. F) or a modest decrease in activity in the case of 7α-hydroxycholestanol (Table 1, compound 10 vs. 13). Thus for the oxygen function at C7, binding affinity was in the

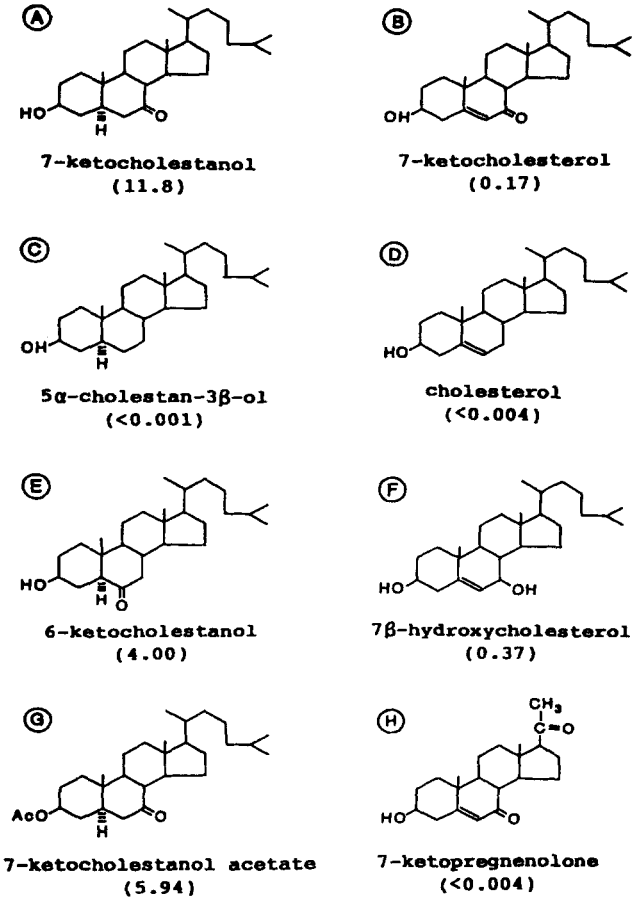

Fig. 1. Structures of selected compound from Table 1. Numbers in parentheses are the relative binding affinities.

order β-hydroxy > keto > α-hydroxy. Thirdly, comparing 7-ketocholestanol with 7-ketocholestanol acetate (A vs. G), it would appear that a free hydroxyl group at C3 may not be an absolute requirement for activity since acetylation is associated with only a twofold reduction in activity. This also appears to be the case for 6-ketocholestanol. Esterification of the hydroxyl group at C3 caused only a slight fall in the relative binding affinity of 6-ketocholestanol (Table 1, compound 7 vs. 8). These findings do not exclude the possibility of endogenous esterase activity. However, replacement of the hydroxyl group at C3 in 6-ketocholestanol by chlorine was associated with a total loss of activity (Table 1, compound 7 vs. 27). Finally, the hydrocarbon side chain appears to be essential for interaction with the antiestrogen-binding site. Neither the C21 compound 7-ketopregnenolone (Fig. 1, H) nor the C19 compound 5-androsten-3β-ol-7,17-dione (Table 1, compound 17) had significant activity when compared with 7-ketocholesterol (Fig. 1, B). These observations indicate that the following structural features are important, to varying degrees, for optimal interaction with the antiestrogen-binding site: *a*) the presence of a hydrocarbon side chain at C17; *b*) the presence of an oxygen function at C7 or C6; *c*) the presence of a nonesterified hydroxyl group at C3; and *d*) the absence of a double-bond between C5 and C6.

Since 7-ketocholestanol showed the highest relative binding affinity among the sterols studied, further studies were performed to examine the nature of the interaction between this particular compound and the antiestrogen-binding site. **Fig. 2** shows the inhibition of [^{3}H]tamoxifen binding by increasing concentrations of unlabeled tamoxifen, 7-ketocholestanol, 6-ketocholestanol, 7-ketocholesterol, and oleic acid. Of note is that the dose-response curves of the three sterols are parallel to that of tamoxifen, suggesting that the sterols may be competitive inhibitors of [^{3}H]tamoxifen binding. In contrast, the inhibition curve of oleic acid is clearly steeper, an observation compatible with our recent finding that fatty acids inhibit [^{3}H]tamoxifen binding through a noncompetitive mechanism (14).

Fig. 3 shows the effect of 7-ketocholestanol on [^{3}H]estradiol binding to the rat uterine estrogen receptor. While diethylstilbestrol and tamoxifen inhibited [^{3}H]estradiol binding as expected, 7-ketocholestanol had no effect on [^{3}H]estradiol binding.

Additional studies were carried out to determine whether 7-ketocholestanol competes directly with tamoxifen for the antiestrogen-binding site. **Fig. 4** shows the Scatchard (16) analysis of [^{3}H]tamoxifen binding to the antiestrogen-binding site in the presence of varying concentrations of 7-ketocholestanol. In the absence of 7-ketocholestanol, the plot indicated a single class of binding sites with an apparent equilibrium dissociation constant of 0.8 nM. The presence of 7-ketocholestanol did not significantly alter the number of binding sites (horizontal intercept), but clearly decreased the binding affinity (slope) in a dose-dependent

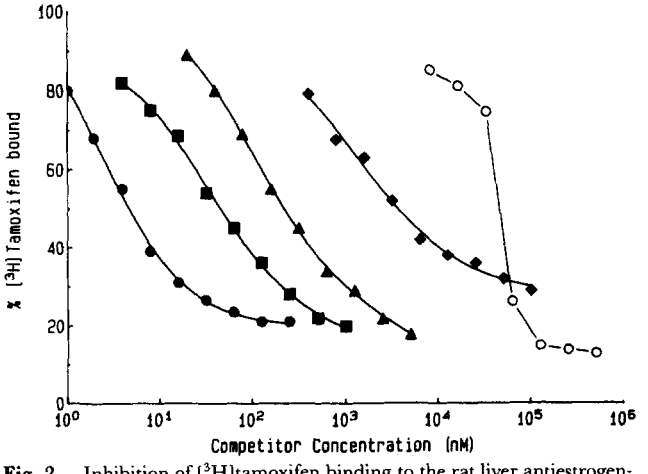

Fig. 2. Inhibition of [^{3}H]tamoxifen binding to the rat liver antiestrogen-binding site by various compounds. [^{3}H]Tamoxifen binding assays were set up as described in the Materials and Methods section in the presence of increasing concentrations of tamoxifen (●), 7-ketocholestanol (■), 6-ketocholestanol (▲), 7-ketocholesterol (♦), and oleic acid (○).

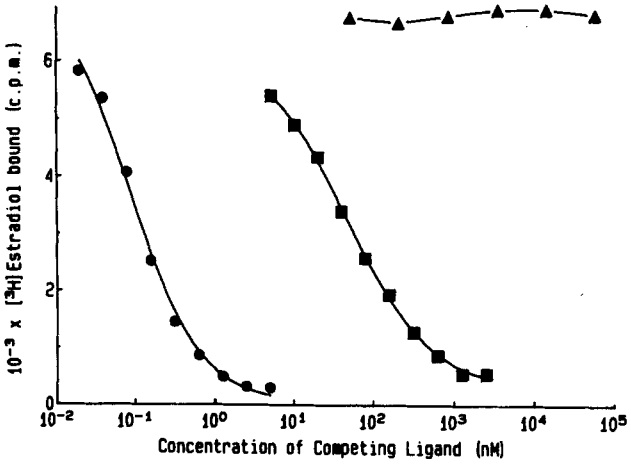

Fig. 3. Effect of 7-ketocholestanol on [^{3}H]estradiol binding to the estrogen receptor from rat uterine cytosol. [^{3}H]Estradiol binding assays were set up as described in the Materials and Methods section in the presence of varying concentrations of diethylstilbestrol (●), tamoxifen (■), and 7-ketocholestanol (▲).

manner. In the presence of 5, 10, 20, and 30 nM 7-ketocholestanol, the apparent equilibrium dissociation constants increased from 0.8 nM to 1.10, 1.66, 2.69, and 3.58 nM, respectively. If 7-ketocholestanol competes with [^{3}H]tamoxifen for the same binding site, then the change in the apparent dissociation constant should fit the expression

$$K'_d = K_d(1 + I/K_i)$$

where K_d is the dissociation constant for [^{3}H]tamoxifen in the absence of 7-ketocholestanol, K'_d the apparent dissociation constant for [^{3}H]tamoxifen in the presence of 7-ketocholestanol, I the concentration of 7-ketocholestanol, and K_i the dissociation constant for 7-ketocholestanol. This relationship predicts that, if 7-ketocholestanol competes directly with [^{3}H]tamoxifen for the antiestrogen-binding site, K'_d should be a linear function of the prevailing 7-ketocholestanol concentration (I). This is clearly the case (Fig. 4, inset), suggesting that 7-ketocholestanol indeed inhibits [^{3}H]tamoxifen binding by a competitive mechanism.

To obtain further support for this, kinetic studies were carried out to determine the dissociation rates of [^{3}H]tamoxifen from the antiestrogen-binding site in the presence of varying concentrations of 7-ketocholestanol. **Fig. 5** shows the dissociation of bound [^{3}H]tamoxifen from the antiestrogen-binding site as a function of time. In the presence of 0, 10, 20, and 40 nM 7-ketocholestanol, the times required for 50% of the specifically bound [^{3}H]tamoxifen to dissociate ($t_{½}$) were 31.3, 31.3, 29.5, and 30.8 min, respectively. The corresponding dissociation rate constants (which equal $\ln^2/t_{½}$) were 3.69, 3.69, 3.92, and 3.75×10^{-4} sec^{-1}, respectively. Therefore it would appear that 7-ketocholestanol had no effect on the dissociation rate of [^{3}H]tamoxifen from the antiestrogen-binding site. This provides additional evidence that 7-ketocholestanol is a competitive inhibitor of [^{3}H]tamoxifen binding.

The possibility that 7-ketocholestanol was metabolized during incubation and that its inhibitory effect on [^{3}H]tamoxifen binding was due to a metabolite was explored. 7-Ketocholestanol was incubated with the 20,000-*g* rat liver supernatant, the incubation mixture was extracted with diethylether, the extract was dried under nitrogen and then fractionated by TLC using three different solvent systems: chloroform, chloroform–methanol 19:1 (v/v), and hexane–diethylether–acetic acid 70:30:1 (v/v/v). The TLC segments were eluted and assayed for their ability to inhibit [^{3}H]tamoxifen binding. For each solvent system, it was observed that the major peak of inhibitory activity had the same mobility as that of authentic 7-ketocholestanol. These observations suggest that 7-ketocholestanol, and not a metabolite, is responsible for the inhibition of [^{3}H]tamoxifen binding. A minor degree of metabolic conversion of 7-ketocholestanol to compounds with inhibitory activity cannot, however, be totally excluded. Indeed, with the solvent system chloroform–methanol 19:1 (v/v), some inhibitory activity with a slower mobility appeared, but this constituted less than 1% of the total inhibitory activity recovered.

DISCUSSION

We have examined a series of sterols and related compounds in an attempt to define the structural requirements

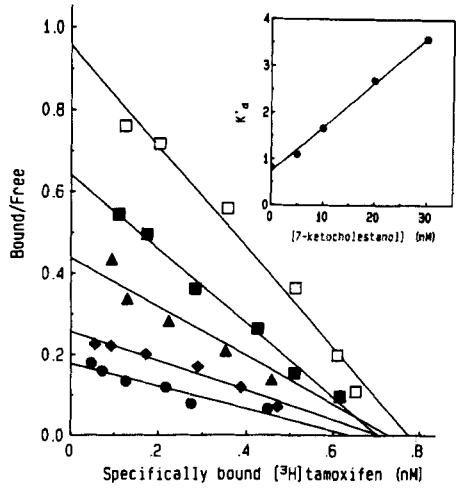

Fig. 4. Scatchard analysis of [^{3}H]tamoxifen binding to rat liver antiestrogen-binding sites in the presence of varying concentrations of 7-ketocholestanol. Saturation analysis was set up in duplicate as described in the Materials and Methods section in the presence of 0 (□), 5 (■), 10 (▲), 20 (♦), and 30 (●) nM 7-ketocholestanol. Inset: the prevailing 7-ketocholestanol concentration was plotted against the apparent equilibrium dissociation constant K'_d (nM). All lines were obtained by linear regression analysis.

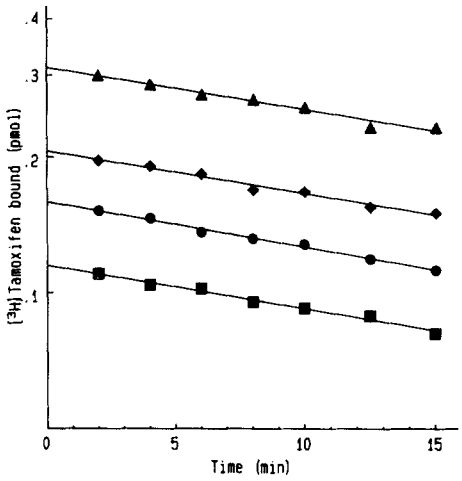

Fig. 5. Dissociation of bound [^{3}H]tamoxifen from rat liver antiestrogen-binding sites. The experimental procedure for the dissociation kinetics experiments was described in the Material and Methods section. The amount of specifically bound [^{3}H]tamoxifen in the absence (▲) or presence of 10 nM (♦), 20 nM (●), or 40 nM (■) 7-ketocholestanol is plotted against time. The results represent the mean of duplicate determinations. The straight lines and half-times of dissociation were obtained by linear regression analysis.

for the inhibition of [^{3}H]tamoxifen binding to the antiestrogen-binding site. The selection of compounds for inclusion in this study was limited to some extent by their availability. The information obtained is thus necessarily incomplete and it is likely that the structural requirements for optimal interaction with the antiestrogen-binding site would be better defined had a wider spectrum of sterols been available. Nevertheless, it is clear from a comparison of the relative binding affinities of the 25 compounds studied (Table 1 and Fig. 1) that the structural requirements for activity are rather stringent. Apparently minor modifications of the ring structure of 7-ketocholestanol, such as removal of the hydroxyl group at C3, removal of the oxygen function at C7, or the introduction of a C5-6 double bond, will cause a complete loss or a marked reduction of activity. The requirement for a side chain at C17 deserves additional comment. The ability of an uncharged hydrocarbon side chain to confer binding affinity to certain sterols stands in sharp contrast to what has been observed for all other compounds known to interact with the antiestrogen-binding site. In an extensive study that examined triphenylethylene antiestrogens, cyclofenil analogues, bibenzyl and stilbene derivatives, cytochrome P-450 inhibitors, and phenothiazine compounds, Watts and Sutherland (17) noted that all these nonsteroidal compounds had an absolute requirement for a basic aminoether side chain in order to interact with the antiestrogen-binding site. The remarkable difference between these compounds and sterols with respect to the side chain structure required for binding raises the possibility that nonsteroidal antiestrogens and sterols perhaps bind to distinct but overlapping sites.

We have studied the interaction of 7-ketocholestanol with the antiestrogen-binding site in some detail. Several points deserve emphasis. First, on a molar basis, it is 70 and 3,000 times, respectively, more potent than 7-ketocholesterol and 4-cholesten-3-one, which are the only two sterol derivatives hitherto reported to inhibit [^{3}H]tamoxifen binding. Using the method described by Horovitz and Levitzki (18), we have estimated the apparent equilibrium dissociation constant (K_d) of 7-ketocholestanol to be 6.3 nM (mean of six determinations), which is closer to the K_d of tamoxifen (0.8 nM) than that of any other sterol capable of inhibiting [^{3}H]tamoxifen binding. Secondly, 7-ketocholestanol differs from tamoxifen in that it appears to interact only with the antiestrogen-binding site but not with the estrogen receptor whereas tamoxifen binds to both sites (Fig. 3). Thirdly, data from Scatchard (16) analysis (Fig. 4) and dissociation kinetics (Fig. 5) clearly indicate that 7-ketocholestanol competes with tamoxifen for the antiestrogen-binding site. Taken together, these observations suggest that 7-ketocholestanol binds directly to the antiestrogen-binding site with high affinity and specificity although, as mentioned earlier, the possibility that it may bind to an adjacent overlapping site exists.

The ability of nanomolar concentrations of 7-ketocholestanol to specifically and competitively inhibit [^{3}H]tamoxifen binding makes it a potentially useful tool in addressing a number of questions in relation to the antiestrogen-binding site. Firstly, 7-ketocholestanol could be used as a specific probe to study the properties and possibly the functions of the antiestrogen-binding site. In this regard it would be more specific than tamoxifen because, unlike tamoxifen, it does not bind to the estrogen receptor. Secondly, the high affinity interaction of 7-ketocholestanol with the antiestrogen-binding sites suggests, for the first time, that sterols might be considered good candidates as putative endogenous ligands for these sites. There is, of course, no evidence at present that 7-ketocholestanol itself is the endogenous ligand. We have failed, in preliminary experiments, to identify this sterol in extracts of rat serum, rat liver, or bovine serum (results not shown). Indeed, to our knowledge, 7-ketocholestanol has not been clearly identified as a compound which is enzymatically produced in vivo, although it could represent a sterol autoxidation product (19). It would not be surprising, however, that should the endogenous ligand turn out to be a sterol, its structure might be similar to that of 7-ketocholestanol.

Finally, one may speculate on the possible biological significance of the specific and high affinity binding of 7-ketocholestanol to the antiestrogen-binding site. In recent years, considerable evidence has been presented to show that oxygenated sterols may have important biological functions such as the regulation of cholesterol biosynthesis and of cell proliferation (20). Two questions can be raised in this regard. *1*) Does 7-ketocholestanol play a role in the regulation of these processes? *2*) Is the antiestrogen-binding

site involved in mediating any of the multiple biological effects exhibited by the oxysterols (19)? The availability of 7-ketocholestanol as a specific high affinity ligand for the antiestrogen-binding site should facilitate further studies to answer these and related questions.

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