quired the ordinate scale expansion were 0.96, 0.91, 0.62, and 0.58% for trihexiphenidyl hydrochloride, benztropine mesylate, methadone hydrochloride, and oxyphenonium bromide, respectively.

Assay Results—Table I records the results obtained for 18 solid dosage formulations of benzenoid drugs which, with the exception of four, are official in the British Pharmacopoeia 1980 (17). The analytical concentration for each drug is given, and the measured amplitude is specified by the wavelength of the minimum in the derivative spectrum, after correction for scan speed effects (14, 15), measured either to its shorter or longer wavelength satellite. The specific amplitude is included to indicate the relative derivative responses of the drugs on a weight basis.

The presence of irrelevant absorption in all the sample extracts was confirmed by the observation of nonspecific absorption above 285 nm (where the drugs, except methadone, have zero absorptivity) and of the increasing distortion of the spectra toward lower wavelengths. The extent of the interference in the sample extracts is evident from the results (in the last column of Table I) showing the assay results calculated from the absorbance of the extracts at the λ_{max} in the region of 255–270 nm, uncorrected for irrelevant absorption. The background absorption at the λ_{max} of one drug formulation, that of phenelzine sulfate, exceeded the absorbance of the drug itself and many formulations showed interference >10% of that of the drug. The derivative spectra of the sample extracts, however, showed no apparent distortion and were identical in shape to the corresponding standard solutions, indicating the elimination of the broad-band irrelevant absorption of the formulation excipients from the derivative spectra of the sample solutions. In the case of two benzenoid drugs, atropine sulfate and scopolamine hydrobromide, formulated at very low doses (0.3 and 0.6 mg/tablet), there was unacceptable distortion of the second- and even of the fourth-derivative spectra by the tablet excipients. The derivative spectrophotometric assay of these very low-dose formulations by an alternative procedure is the subject of another report (18).

The assay results for the formulations, obtained by the derivative procedure, are in excellent agreement with the declared amount and, where appropriate, with the results given by official procedures of the BP 1980 (17), confirming that second-derivative spectrophotometry is a simple, rapid, and selective technique which has general application in the assay of tablet and capsule formulations containing a single benzenoid drug. The specificity of the procedures for drugs in the presence of degradation products has not been investigated in the present study. It is likely, however, that degradation products will contribute to the derivative spectrum of the sample solution and therefore interfere in the assay, if their zero-order UV spectra, like that of the parent drug, show fine structure in the 250–270-nm region.

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Estrogenic and Antiestrogenic Activity of Novel Selenosteroids

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Received November 22, 1982, from the **Faculty of Medicine, Kuwait University, Kuwait*, and the ‡ *College of Pharmacy, The University of Oklahoma, Oklahoma City Campus Health Science Center, Oklahoma City, OK 73190.* Accepted for publication February 8, 1983.

Abstract □ An assay for estrogenic and antiestrogenic activity of seven selenoestrogens has been carried out in immature female rats. The estrogenic activity was compared to the *in vitro* binding affinity data. The study reveals that introduction of selenium substituents on C-16 or C-17 of the steroid nucleus produced a marked reduction in the estrogenic activity. The selenium analogues of ethynylestradiol produced the highest estrogenic activity. None of the compounds produced antiestrogenic activity.

Keyphrases □ Selenoestrogens—estrogenic and antiestrogenic activity in immature rats, comparison with *in vitro* binding affinity □ Estrogens—selenium substituted, estrogenic and antiestrogenic activity in immature rats, comparison with *in vitro* binding affinity □ Binding affinity, *in vitro*—selenium-substituted estrogens, correlation with *in vivo* estrogenic and antiestrogenic activity, rats

The differentiation between estrogen receptor-negative and receptor-positive breast tumors and their metastases is important in the management and treatment of such tumors (1, 2). Present techniques are based on the *in vitro* measurement of estrogen receptors in freshly obtained biopsies of breast tissue. Therefore, a radiopharmaceutical which could be used to image estrogen-dependent breast tumors and metastatic foci by external detection could be a useful diagnostic tool. A number of investigators have attempted to develop such a radiopharmaceutical using radiohalogenated (*e.g.*, iodine-125, bromine-77) estrogens (3, 4). However, most of these studies have met with limited success. Although selenium-75 may not be an ideal radionuclide for diagnostic use, it offers the following advantages (5, 6): (*a*) its long half-life (120 d) allows enough time for synthesis and handling; (*b*) it can be incorporated into organic molecules with minimal difficulty; (*c*) the organoselenium compounds are more stable *in vivo* than the corresponding halogenated derivatives; and (*d*) preliminary studies with selenium-75 could determine the feasibility of using the potentially more useful ^{73}Se-labeled compounds ($t_{1/2}$ = 7 h).

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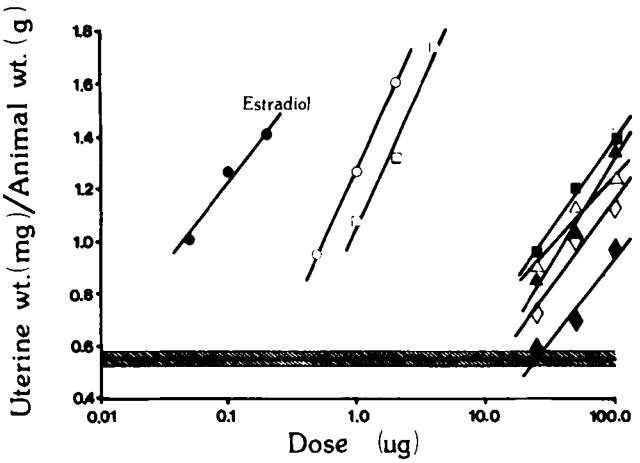


Figure 1—*Dose–uterotropic response curves of the selenosteroids. The horizontal bar represents the mean ± SEM of the control group. Key: (◆) I; (▲) II; (○) III; (□) IV; (△) V; (◇) VI; (■) VII.*

In previous studies (7, 8) we reported the synthesis and the relative binding affinity of various selenosteroids to the cytoplasmic estrogen receptor of rat uteri *in vitro*. In the present study, the uterotropic activity of these selenosteroids was examined and a correlation made between the *in vivo* uterotropic activity and *in vitro* receptor affinity.

EXPERIMENTAL

The uterotropic assay for estrogenic activity was a modification (9) of the original method of Rubin *et al.* (10). Immature female Sprague–Dawley rats weighing 40–50 g (~21 d old) were randomly distributed into groups containing five rats each. Estradiol and the test compounds were dissolved separately in corn oil and administered subcutaneously in a volume of 0.1 mL. Control animals were treated with the same volume of corn oil alone.

All animals were treated daily for three consecutive days with the same test compounds. On the fourth day the animals were sacrificed, and the uteri were carefully dissected, blotted lightly, and weighed to the nearest 0.1 mg; body weights were also recorded. Estradiol was used in a dosage range of 0.5–2 μg (total dose) as the assay standard. Each compound was examined over a dosage range of 5–100 μg (total dose).

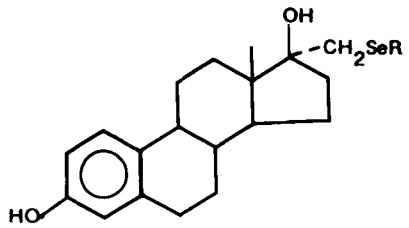
The uterotropic assay was also used to evaluate the antiestrogenic activity of the test compounds. The antiestrogenic assay was conducted as described above for estrogenic activity except that each animal in the selenosteroid treatment groups received a standard stimulating dose of estradiol (2 μg). A separate control group which was treated with estradiol alone (2 μg) was also included in this assay. The test compounds and estradiol were administered separately at different injection sites to minimize possible physical interaction or reduce absorption of either compound. The antiestrogenic activity was measured as a decrease in estradiol-stimulated uterotropic response in groups which received both the test compound and estradiol as compared with the control group, which was treated with estradiol alone.

A line of best fit was plotted for each compound that produced an estrogenic response. Regression analysis was used to determine the dose–response line for each test compound. The slope of the response to each

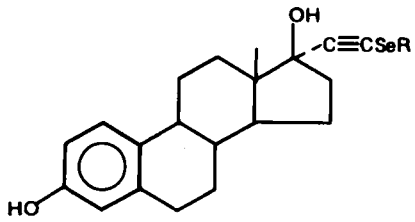
Table I—Relative Uterotropic Activity of the Selenosteroids

Compound	Relative Uterotropic Activity[a]	RBA × 100[b]
Estradiol	100.00	100.00
I	0.06	1.0
II	0.14	5.1
III	10.46	2.0
IV	6.44	18.7
V	0.14	2.8
VI	0.11	0.1
VII	0.17	1.3

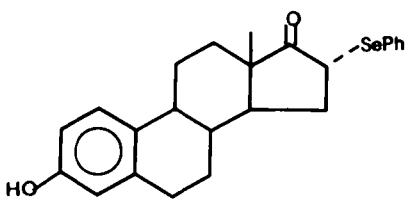
[a] Expressed as percentage of estradiol activity according to the method of Bliss (Ref. 11). [b] Ratio of the concentration of nonradioactive estradiol required to inhibit 50% of [^{3}H]estradiol binding to that of the competitor multiplied by 100 (from Ref. 8).



I : R = Ph
II : R = CH_3



III : R = Ph
IV : R = CH_3



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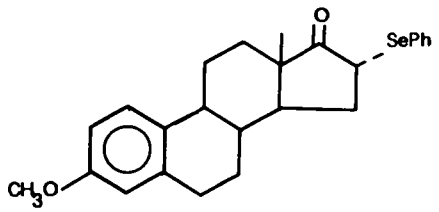

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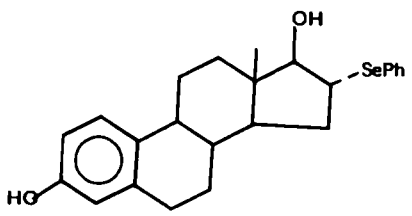

VII

analogue was compared with the slope of the estradiol response to determine parallelism in this assay system. The relative uterotropic activity of each compound was expressed as a percentage of estradiol activity according to the method of Bliss (11).

RESULTS

The dose–response curve produced by each of the selenium analogues is presented in Fig. 1. The potency of each compound relative to estradiol is summarized in Table I.

Among the selenosteroids that were examined in this study, III produced the greatest uterotropic activity while I produced the least activity. The selenoanalogues of ethynylestradiol (III and IV) were more active than those of estradiol or estrone. This may be due to the differences in the activity of the parent compounds (12). None of the test compounds produced a significant degree of antiestrogenic activity in this study.

DISCUSSION

In Table I the relative binding affinity of the test compounds was compared with the uterotropic activity. The potency estimates were in good agreement with the relative binding affinities with few exceptions. To correlate *in vitro* and *in vivo* results, metabolism has to be taken into consideration. Even though VI demonstrated a low *in vitro* binding affinity, its *in vivo* potency is almost equal to that of V. This could be as a result of demethylation *in vivo*. Sulfur-containing organic compounds are metabolized to sulfoxides (13); analogously, selenides are expected to oxidize to selenoxides *in vivo*. Reports indicate that aromatic selenoxides are less stable than the aliphatic derivatives (14–16). Therefore, we assume that the greater activity of III as compared with IV, despite a lower affinity for estrogen receptors, is due to the degradation of III to the more potent ethynylestradiol.

It is reasonable to assume that VII has a greater *in vivo* uterotropic activity than V because estradiol (the parent compound of analogue VII) is more potent than estrone (the parent compound of analogue V) (12).

This study indicates that increasing the size of the substituent on C-17 decreases the *in vitro* binding and the *in vivo* potency. These data confirm the importance of the β-hydroxyl group on C-17 to retain the estrogenic activity (17). Since these selenosteroids retain a high degree of estrogenic activity (especially III and IV) *in vivo*, as shown in the present study, and display potent receptor binding *in vitro* (8), it is possible that one of these derivatives might be a suitable imaging agent for estrogen-dependent tumors and metastatic foci. Selenium-75 labeling of II has been accomplished utilizing newly developed methodology (18). Biodistribution studies of this ^{75}Se-labeled compound in tumor-bearing animals are underway.

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Diaspirins of Methylenecitric Acid

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Abstract □ A series of bissalicylic esters of methylenecitric acid have been prepared and, as a probe of their potential as antisickling agents, tested for their ability to modify hemoglobin. Substantial acylation of hemoglobin was obtained with these dicarboxylate esters at 1–5 mM concentrations.

Keyphrases □ Methylenecitric acid—bissalicylic esters, synthesis, hemoglobin-modifying potential □ Antisickling agents—potential, bissalicylic esters of methylenecitric acid, synthesis, hemoglobin-modifying ability

Diaspirins have been found (1) to modify hemoglobin S and to change its solubility markedly. Consequently, this class of compounds may provide promising antisickling agents. Just as aspirin is an acetate of salicylic acid, so are diaspirins the alkanedioates of salicylic acid. Alkanedioic acids of 4–5 carbon chain length seem to be optimal in modification of hemoglobins, probably because they can readily span the β-cleft of the hemoglobin molecule and form a covalent bridge between the two Lys 82 residues. Such a bridge evidently locks the protein into a conformation out of register for the aggregation that occurs in sickling.

The modified pentanedioic acid, 4-oxo-1,3-dioxolane-5,5′-diacetic acid, also known as methylenecitric acid (A), is a five-carbon span dicarboxylic acid whose monoesters have been examined previously for pharmacological ac-

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