Hz, 2 H, CH_2), 5.31 (d, J = 9.6 Hz, 1 H, CH).

Preparation of 2-Acetyl-2,3,3a,4,6,7-hexahydro-3-[3-(trifluoromethyl)phenyl]-7-[[3-(trifluoromethyl)phenyl]methylene]thiopyrano[4,3-*c*]pyrazole 5,5-Dioxide (28). A mixture of 1.9 g (4.23 mmol) of ketone **74** and 170 mg (5.3 mmol) of anhydrous hydrazine in 100 mL of MeOH was heated at reflux temperature for 0.5 h. Water (20 mL) was added, and the mixture was allowed to cool to 5 °C. Product was collected and dried in vacuo over P_2O_5 at 50 °C to give 1.6 g, mp 183–185 °C.

This hydrazine-cyclized product in 25 mL of HOAc and 2 mL of Ac_2O was heated on a steam bath for 15 min. Water (10 mL) was added, and the mixture was allowed to cool to room temperature. The product was collected, washed with HOAc/H_2O (3:2) and H_2O, and dried in vacuo over P_2O_5 at 50 °C to give 1.56 g: mp 200–202.5 °C; IR (KBr) 1675 (C=O), 1110, 1310 (SO_2) cm^{-1}; NMR ($CDCl_3$) δ 2.39 (s, 3 H, CH_3), 3.53 (s, 2 H, CH_2), 4.14 (s, 2 H, CH_2), 5.12 (d, J = 7.8 Hz, 1 H, CH).

Preparation of 2-Acetyl-2,3,3a,4,6,7-hexahydro-3-(3-pyridinyl)-7-[(3-pyridinyl)methylene]thiopyrano[4,3-*c*]pyrazole (37). A solution 2.94 g (10 mmol) of ketone **69** and 480 mg (15 mmol) of anhydrous hydrazine in 100 mL of $CHCl_3$/MeOH (1:3) was concentrated on a steam bath for 1 h while MeOH was added periodically to maintain volume. After the solution was cooled, the solids were collected and washed with MeOH to give 2.8 g, mp 146–151 °C.

The above hydrazine-cyclized product in 20 mL of HOAc and 2 mL of Ac_2O was heated on a steam bath for 20 min. Water was added, and solvent was removed in vacuo. The residue was crystallized from $CHCl_3$/hexane to give 1.8 g of product: mp 202–203 °C; IR (KBr), 1565, 1580, 1595 (C=CC=N), 1675 (C=O) cm^{-1}; NMR ($CDCl_3$), δ 2.34 (s, 3 H, CH_3), 4.94 (d, J = 9 Hz, 1 H, CH).

Preparation of 2-Acetyl-2,3,3a,4,6,7-hexahydro-3-[3-(trifluoromethyl)phenyl]-7-[[3-(trifluoromethyl)phenyl]methyl]thiopyrano[4,3-*c*]pyrazole 5,5-Dioxide (38). A solution of 1.0 g (1.94 mmol) of compound **28** in 50 mL of EtOH containing 0.5 g of 10% Pd/C was hydrogenated at atmospheric pressure until uptake ceased (52 mL). The catalyst was filtered, and the filtrate was concentrated in vacuo. Chromatography on Baker silica gel and elution with hexane/$CHCl_3$ (100:0 to 0:100) and then MeOH/$CHCl_3$ (5:95) afforded the product, which was recrystallized twice from $CHCl_3$/hexane to give 685 mg of product: mp 196–200 °C; IR (KBr), 1680 (C=O), 1125, 1335, (SO_2) cm^{-1}; NMR ($CDCl_3$) δ 2.35 (s, 3 H, CH_3), 5.0–5.25 (m, 1 H, CH).

General Procedure for Preparation of [3-(*N*-Methylpiperazino)propyl]hexahydrothiopyrano[4,3-*c*]pyrazoles (39-51, Table II). A mixture of 1.0 equiv of ketone IV and 1.1 equiv of 3-(*N*-methylpiperazino)propylhydrazine in chloroform/methanol (1:4) is heated at reflux temperature for 3–6 h. Solvent is removed in vacuo, and the oily residue, dissolved in acetonitrile, is treated with 2.0 equiv of oxalic acid in acetonitrile. After the solution is stirred at room temperature for 0.5 h, the dioxalate salt is collected and, if necessary, recrystallized from dimethylformamide/acetonitrile.

The dioxalate salt is suspended in a mixture of water and chloroform and made basic with solid potassium carbonate. The organic phase is separated, washed with water, dried over anhydrous magnesium sulfate, and concentrated in vacuo. In three cases (**40**, **47**, and **50**), the free base was obtained in crystalline form. The free base, dissolved in acetonitrile, is treated with 2 equiv of maleic acid in acetonitrile to obtain the dimaleate salt (additional data in Table II): NMR (maleate salts, Me_2SO-d_6) δ 7.0–7.2 (s, =CH), 2.60–2.65 (s, NCH_3); aromatic substituent 2.32 (**41**, s, CH_3), 3.75 (**42**, s, OCH_3), 2.28, 2.35 (**43**, s, CH_3), 2.95 (**44**, s, S-CH_3), 3.76 (**46**, s, OCH_3), 2.28, 2.33 (**48**, s, CH_3), 2.74 (**51**, s, S-CH_3); other absorption are complex and not resolved.

Acknowledgment. The authors thank Dr. A. I. Cohen, Dr. M. Puar, and Ms. Mary Young and their associates for the NMR; IR, and microanalytical data. We also thank Dr. M. B. Goldlust and his associates for the mouse active Arthus data and Dr. R. J. Wojnar and his associates for the delayed hypersensitivity skin reaction data. Dr. B. R. Vogt provided helpful guidance and discussion throughout this project.

Selenium Labeling in Nuclear Medicine. 2.[1] D Ring Substituted Estrogens

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A series of seven D ring substituted selenium derivatives of estrone/estradiol has been prepared and characterized. Competitive binding studies using the rat uterine cytosol assay are reported for each compound. The most promising agent currently appears to be 17α-[(methylseleno)ethynyl]-17β-estradiol, which possesses 19% of the binding affinity of natural 17β-estradiol.

A radiopharmaceutical for imaging breast tumors would be of immense value in diagnostic nuclear medicine;[3] to date no satisfactory agent is available, though extensive efforts are being expended to this end.[4-7] In considering the rational development of a γ-emitting estrogen, one must examine the biological and chemical consequences of introducing a label. While radioactive halogens (^{123}I or ^{77}Br) offer certain advantages (ease of preparation and desirable radionuclide characteristics), these nuclides also exhibit disadvantages, such as biological instability and short half-lives, which allow little time for synthetic manipulations.

(1) A previous publication has been designated Part 1 in this series: Sadek, S. A.; Shaw, S. M.; Kessler, W. V.; Wolf, G. C. *J. Org. Chem.* **1981**, *46*, 3259.

(2) (a) Department of Bionucleonics and School of Health Sciences. (b) The current work is taken in part from the Ph.D. Thesis of S. A. S., Purdue University, 1982. (c) Department of Biological Sciences. (d) Department of Medicinal Chemistry and Pharmacognosy.

(3) Krohn, K. A. *J. Nucl. Med.* **1980**, *21*, 593.

(4) Longcope, C.; Arunachalam, T.; Rafkin, I.; Caspi, E. *J. Steroid Biochem.* **1981**, *14*, 261.

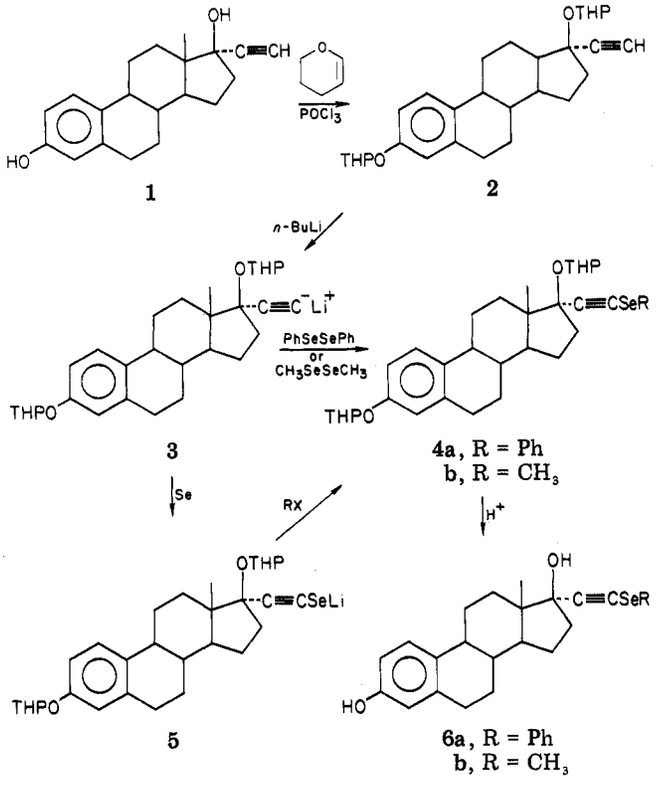
(5) Gatley, S. J.; Shaughnessy, W. J.; Inhorn, L.; Lieberman, L. M. *J. Nucl. Med.* **1981**, *22*, 459.

(6) Katzenellenbogen, J. A.; Senderoff, S. G.; McElvany, K. D.; O'Brien, H. A., Jr.; Welch, M. J. *J. Nucl. Med.* **1981**, *22*, 42.

(7) Heiman, D. F.; Senderoff, S. G.; Katzenellenbogen, J. A.; Neeley, R. J. *J. Med. Chem.* **1980**, *23*, 994.

0022-2623/82/1825-1488$01.25/0

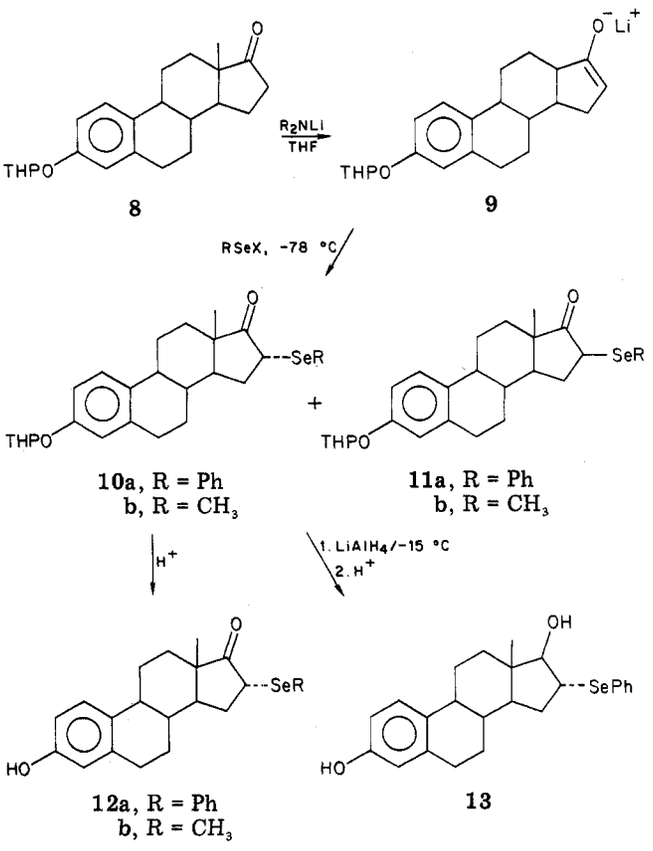
Scheme I



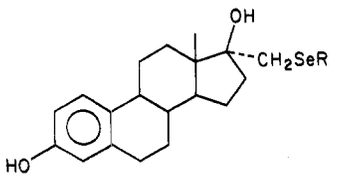
Although ^{75}Se may not be the ideal radionuclide, it does possess four attractive features to be considered in developing a breast tumor imaging agent: (a) its long half-life allows adequate time for synthesis and handling; (b) introduction in a covalently bound manner into the desired organic molecule can be performed with minimal difficulty by a variety of techniques; (c) once incorporated, it is less easily removed by in vivo biochemical reactions than halogens; and (d) preliminary scanning studies with ^{75}Se could pave the way for the potentially more useful ^{73}Se-labeled compounds. ^{73}Se is not now readily available. However, it can be produced by the $^{70}Ge(\alpha,n)^{73}Se$ reaction with a specific activity high enough to synthesize ^{73}Se-labeled estrogens with specific activities that would be in the range (ca. 1000 Ci/mmol) for use as imaging agents. The specific activity of pure ^{73}Se is 4.4×10^5 Ci/mg-atom. Our past efforts[1] revealed the potential value of C ring substituted selenoestrogens, and we now report expanded synthetic studies along with assay of receptor binding affinities of these current derivatives.

17α-[(Phenylseleno)ethynyl]-17β-estradiol (**6a**) and 17α-[(methylseleno)ethynyl]-17β-estradiol (**6b**) were prepared as outlined in Scheme I. Protection of the 3- and 17β-hydroxy groups of ethynylestradiol (1) was accomplished by treating with dihydropyran in tetrahydrofuran, with catalytic amounts of phosphorus oxychloride. The 20-lithio derivative, **3**, was generated without difficulty employing *n*-butyllithium and anhydrous ether. Subsequent introduction of selenium was achieved by two alternative pathways. Treatment of **3** with diphenyl diselenide (or dimethyl diselenide) provided immediate access to **4a** (and **4b**), whereas initial reaction with metallic selenium afforded **5** as an intermediate, which could then be treated with iodomethane to give **4b**. Subsequent mild acid hydrolysis led to the appropriate ethynylestradiol derivative **6**. In fact, the pathway involving metallic selenium (**3** → **5** → **4** → **6**) gave the desired product in an overall yield of less than 10%, while the more direct route provided **6** in approximately 40% yield. Considering the attractiveness of the metallic selenium pathway for introducing ^{75}Se, this is somewhat disappointing.

Scheme II



The preparation of **7a**, 17α-[(phenylseleno)methyl]-



17β-estradiol, has been described in our previous publication;[1] in a like manner, **7b**, 17α-[(methylseleno)-methyl]-17β-estradiol, has been synthesized in 65% yield from estrone, via the 17-epoxy derivative.[8]

Scheme II outlines the preparation of 16α- and 16β-substituted steroids. The reaction of ketone enolates with phenylselenyl bromide or chloride is a method generally successful for the preparation of α-phenylseleno ketones; this approach has previously been used for nonestrogenic steroids.[9]

Accordingly, the lithium enolate of estrone 3-tetrahydropyranyl ether (**9**) was combined with phenylselenyl chloride to provide 16α- and 16β-(phenylseleno)estrone 3-tetrahydropyranyl ether (**10a** and **11a**) in an approximate

(8) During the preparation of this manuscript, a paper (Arunachalam, T.; Caspi, E. *J. Org. Chem.* **1981**, *46*, 3415) appeared that provides an alternate synthesis of our previous compound **2a** and also an alternate synthesis of our current products **7b**, **12a**, and **12b**.

(9) Konopelski, J. P.; Djerassi, C.; Raynaud, J. P. *J. Med. Chem.* **1980**, *23*, 722.

(10) Leonard-Coppens, A. M.; Krief, A. *Tetrahedron Lett.* **1976**, *36*, 3227.

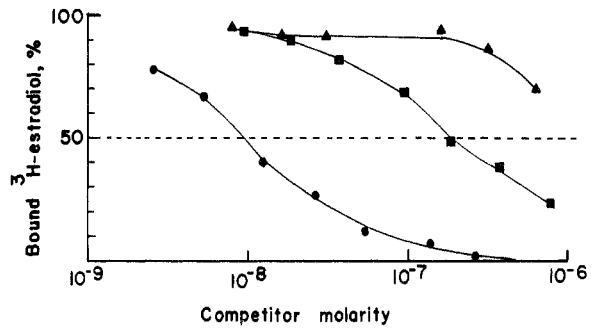


Figure 1. Effect of estradiol (●–●), 17α[(phenylseleno)-methyl]-17β-estradiol (▲–▲), and 17α-[(methylseleno)methyl]-17β-estradiol (■–■) on [^{3}H]estradiol binding to estrogen receptors.

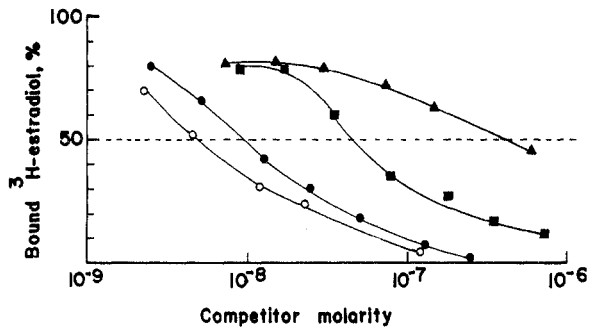


Figure 2. Effect of estradiol (●–●), ethynylestradiol (○–○), 17α-[(phenylseleno)ethynyl]-17β-estradiol (▲–▲), and 17α-[(methylseleno)ethynyl]-17β-estradiol (■–■) on [^{3}H]estradiol binding to estrogen receptors.

ratio of 4:1. The major component, **10a**, could be isolated by fractional crystallization from ethanol in 44% yield. The presumed 16β derivative was not purified. Similarly, methylselenyl bromide was used to provide **10b**, which was obtained by chromatographic separation. Mild acid hydrolysis of **10a** and **10b** provided **12a** and **12b**, respectively. It should be noted that the absolute stereochemistry of carbon-16 has not been rigidly established; however, our physical data for **12a** [mp 184–185 °C; NMR δ 4.05 (m, C_{16} H)] agree with those recently published for this compound [mp 185–188 °C; NMR δ 4.1 (m, C_{16} H)].[8] Also, similar D ring substitution in 3β-hydroxy-16α-(phenylseleno)-androst-5-en-17-one provides virtually identical data [δ 4.1 (m, C_{16} H)].[9]

Finally, **13** was obtained from **10a** via lithium aluminum hydride reduction and subsequent mild acid hydrolysis. Though this reduction is not stereospecific, it has been shown to be selective for preferential formation of the 17β-ol substitution.[8,9] Note is made, however, that when applied to **12b**, this reduction resulted in the concurrent cleavage of the 16α-methylseleno group to produce mainly 17β-estradiol.

Binding Affinity Studies. The influence of increasing the concentration of nonradioactive estrogen derivatives on the binding of [^{3}H]estradiol by the rat uterine cytosol is shown graphically in Figures 1–3. That our assay procedure is valid was confirmed by the close agreement of our values for the relative binding affinity (RBA × 100) for ethynylestradiol (173%; lit.[11] 191%) and estrone (20%; lit.[12] 24%).

Among the seleno steroids that have been studied (Table I), 17α-[(methylseleno)ethynyl]-17β-estradiol (**6b**) has the highest relative affinity (18.7%). This is probably due, in part, to the high affinity of the parent ligand (ethynylestradiol). It was observed in all cases that replacing the methyl group by a phenyl group decreased the binding affinity five- to tenfold. According to the results obtained, the affinity decreases as the steric size of the substituent increases: $SeCH_3$ > CH_2SeCH_3 > SePh > CH_2SePh.

From Table I, 16α-(methylseleno)estrone (11.2%) has an affinity that is more than 50% of that of estrone (19.6%), while the 17α-[(methylseleno)ethynyl]-17β-estradiol has roughly one-tenth the affinity of ethynylestradiol (18.7 vs. 173%). It seems that the 16α position of estrone tolerates a methylseleno group more readily than position 20 of ethynylestradiol.

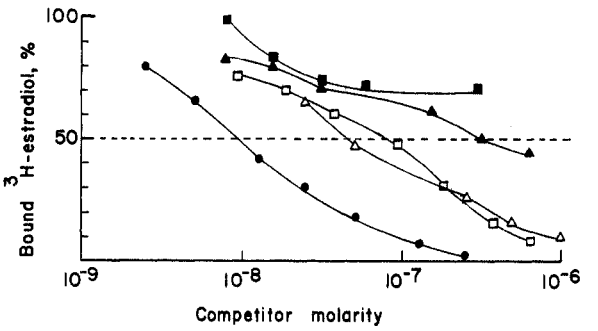
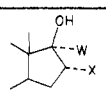
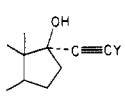
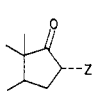


Figure 3. Effect of estradiol (●–●), estrone (△–△), 16α-(methylseleno)estrone (□–□), 16α-(phenylseleno)estrone (▲–▲), and 16α-(phenylseleno)-17β-estradiol (■–■) on [^{3}H]estradiol binding to estrogen receptors.

Table I. Binding Affinity of Seleno Steroids for Estrogen Receptors

compd	structure	substituent W	substituent X	RBA × 100[a]
estradiol		H	H	100
		CH_2SePh	H	<1.0
		CH_2SeCH_3	H	5.1
		H	SePh	1.3
		Y		
ethynyl-estradiol		H		173
		SePh		2.0
		$SeCH_3$		18.7
		Z		
estrone		H		19.6
		SePh		2.8
		$SeCH_3$		11.2

[a] RBA × 100 = the ratio of the concentration of nonradioactive estradiol required to inhibit 50% of [^{3}H]estradiol binding to that of the competitor multiplied by 100.

Discussion

The synthesis of the various seleno steroids bearing a phenylseleno or methylseleno group has been described. We have measured the estrogen binding affinities of these compounds and have compared these binding affinities with the affinities of the parent compounds.

Among the seleno steroids studied, the methylseleno derivatives were found to have higher affinity for receptors than the corresponding phenylseleno derivatives. 17α-[(Methylseleno)ethynyl]-17β-estradiol and 16α-(methylseleno)estrone had the highest receptor binding affinity.

(11) Korenman, S. G. *Steroids* **1969**, *13*, 163.
(12) Chernayaev, G. A.; Barkova, T. I.; Egorova, V. V.; Sorokina, I. B.; Anachenko, S. N.; Mataradze, G. D.; Sokolova, N. A.; Rozen, V. B. *J. Steroid Biochem.* **1975**, *6*, 1483.

The 16α position seems to be able to tolerate the methylseleno group; 16α-(methylseleno)estrone had 60% of the binding affinity of estrone. Consequently, it is anticipated that the 16α-(methylseleno)-17β-estradiol will have even greater binding, perhaps 50–60% of that of estradiol. The compound appears to be a good candidate as an estrogen receptor imaging agent.

The presence of the ethynyl decreases the metabolism of estrogens.[13] Thus, 17α-[(methylseleno)ethynyl]-17β-estradiol may have a lowered metabolic destruction rate and may accumulate in the uterus, in vivo, in spite of its low relative binding affinity (RBA % = 19%). We are currently testing this compound more extensively. Additionally, we are expanding the above series to include other seleno-substituted estrogens. The preparation of ^{75}Se-labeled compounds is underway.

Experimental Section

All starting materials and reactants were obtained from commercial suppliers, except for dimethyl diselenide which was prepared in our laboratory according to the method described by Günther.[14] All reactions involving organometallic reagents were carried out under a nitrogen atmosphere. Melting points were determined with a Büchi capillary melting point apparatus and are uncorrected. ^1H NMR spectra were obtained with a Varian FT-80 or a Varian EM-360. Chemical shifts are reported in parts per million (δ) relative to $(CH_3)_4Si$ as an internal standard, and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). NMR spectra of all compounds and intermediates were consistent with the assigned structures. Mass spectra were obtained with a DuPont 21-492B or a CEC 110 double-focusing mass spectrometer. Elemental analyses were performed by the Microanalytical Laboratory, Department of Chemistry, Purdue University, and all were within 0.4% of theoretical values.

17α-[(Methylseleno)methyl]-17β-estradiol (7b). To dimethyl diselenide (0.46 g, 2.44 mmol) in absolute ethanol (20 mL) was added $NaBH_4$ (0.19 g, 4.94 mmol) in small portions, while stirring under nitrogen, until the bright yellow solution turned colorless. The 17-spiro steroid[1] derived from estrone (1.62 g, 4.27 mmol) was added in THF (10 mL) to the reaction mixture, 0.2 g of *p*-toluenesulfonic acid was added, and the solution was stirred at 55 °C for 1 h. To the reaction mixture was added 200 mL of water; this was extracted with ether (2 × 100 mL). The organic layer was washed with water, dried over anhydrous magnesium sulfate, and evaporated to dryness. Crystallization from benzene/hexane provided white crystals of **7b** (1.34 g, 81% yield): mp 62–65 °C; NMR δ 0.92 (s, 3 H, $C_{18}H$), 2.2 (s, 3 H, $SeCH_3$), 3.27 (AB quartet, 2 H, C_{19} H), 6.55–7.25 (m, 3 H, Ar H); MS, *m/e* 381 (M^{+1} for ^{80}Se), 379 (M^{+1} for ^{78}Se), 363 ($M^{+1} - H_2O$), 285 (M – SePh). Anal. ($C_{20}H_{28}O_2Se$) C, H.

17α-[(Phenylseleno)ethynyl]-17β-estradiol (6a). Ethynylestradiol (1) (5 g, 16.9 mmol) in THF (20 mL) and dihydropyran (17 mL, 18.4 g, 219.2 mmol) were treated with $POCl_3$ (0.15 mL). After 1 h the mixture was poured into water and extracted with chloroform. Standard workup and crystallization from absolute ethanol, containing 2 drops of pyridine, provided compound **2** (4.1 g, 66% yield), mp 165–166 °C.

To *n*-BuLi (2.5 mL of 1.6 M in hexane, 4 mmol) in dry ether at −15 °C (under nitrogen), the bis(ether) **2** (1.02 g, 2.2 mmol) in dry ether was added dropwise in 15 min. The reaction flask was placed for 1 h at 25 °C and again cooled to −10 °C. Diphenyl diselenide (Aldrich Chemical Co., 1.31 g, 4.2 mmol) in dry ether was added in 30 min, and the stirring was continued for 2 h at room temperature. The mixture was poured into water, the organic layer was separated, washed with water, and evaporated in vacuo, and the residue was dissolved in 95% ethanol (50 mL) and water (10 mL). To this solution was added 0.15 g of *p*-toluenesulfonic acid. After 4 h at 40 °C, 200 mL of water was added, and the solution was extracted with ether. The organic layer was washed with water, dried over anhydrous magnesium sulfate, and evaporated in vacuo, and the residue was crystallized from benzene to give compound **6a** (0.51 g, 57% yield): mp 201–202 °C; NMR δ 0.90 (s, 3 H, C_{18} H), 6.56–7.55 (m, 8 H, Ar H); MS, *m/e* 453 (M^{+1} for ^{80}Se), 451 (M^{+1} – for ^{78}Se), 435 ($M^{+1} - H_2O$), 296 (M^{+1} – SePh), 271 (M – C≡CSePh). Anal. ($C_{26}H_{28}O_2Se$) C, H.

17α-[(Methylseleno)ethynyl]-17β-estradiol (6b). Method A. This was prepared similarly from compound **2** utilizing dimethyl diselenide instead of diphenyl diselenide. Crystallization from benzene/hexane furnished yellow crystals of the desired compound (0.51 g, 60% yield): mp 80–82 °C; NMR δ 0.87 (s, 3 H, C_{18} H), 2.3 (s, 3 H, $SeCH_3$), 6.56–7.25 (m, 3 H, Ar H); MS, *m/e* 391 (M^{+1} for ^{80}Se), 389 (M^{+1} for ^{78}Se), 373 ($M^{+1} - H_2O$), 297 ($M^{+2} - SeCH_3$), 279 ($M^{+2} - H_2O - SeCH_3$), 271 (M – C≡CSeCH$_3$). Anal. ($C_{21}H_{26}O_2Se$) C, H.

Method B. To *n*-BuLi (2.5 mL of 1.6 M solution in hexane, 4.00 mmol) in dry ether (20 mL) at −15 °C, under nitrogen, the bis(ether) **2** (1.022 g, 2.2 mmol) in dry ether was added dropwise in 15 min. The temperature was maintained below 0 °C. After the addition, the reaction flask was placed at room temperature for 1 h, then cooled to −10 °C. Selenium powder (0.31 g, 0.004 g-atom) was quickly added to the white suspension of the lithium salt. The reaction mixture was allowed to warm to 15–20 °C, and stirring was continued for 2 h. Methyl iodide (0.564 g, 4.00 mmol) in dry ether was then added at −20 °C to −30 °C. Again the mixture was allowed to warm to room temperature. Thin-layer chromatography of the residual oil, after working with water, separating, drying, and evaporating, provided evidence of starting material (75%) and **4b** (10%). Preparative TLC ultimately gave 0.055 g of **4b**. Mild acid hydrolysis and standard workup provided 0.035 g of **6b** (mp 81–83 °C).

16α-(Phenylseleno)estrone (12a). To THF (20 mL), cooled to −78 °C (under nitrogen), was added diisopropylamine (2.4 mL, 16 mmol), followed by *n*-BuLi (10 mL of 1.6 M solution in hexane, 16 mmol). A solution of compound **8** (4.25 g, 12 mmol) in THF was added dropwise, and the resulting mixture was stirred for 15 min. PhSeCl (3.06 g, 16 mmol) in THF was added rapidly. The solution was poured into 50 mL of H_2O and 50 mL of ether. The organic layer was washed with water, dried over anhydrous magnesium sulfate, and evaporated in vacuo. Crystallization from absolute ethanol gave 3 g of white prisms of **10a** (44% yield), mp 151–152 °C. One gram of **10a** was dissolved in 95% ethanol (50 mL) and water (10 mL). To this solution was added 0.2 g of *p*-toluenesulfonic acid. After 3 h at 40 °C, 200 mL of water was added, and the solution was extracted with ether. The organic layer was washed with water, dried over anhydrous magnesium sulfate, and evaporated in vacuo. Crystallization from benzene provided 0.7 g of **12a** (84% yield): mp 184–185 °C; NMR δ 0.77 (s, 3 H, C_{18} H), 4.05 (m, 1 H, C_{16} H), 6.78–7.73 (m, 8 H, Ar H); MS, *m/e* 427 (M^{+1} for ^{80}Se), 425 (M^{+1} for ^{78}Se), 409 ($M^{+1} - H_2O$), 271 (M^{+2} – SePH). Anal. ($C_{24}H_{26}O_2Se$) C, H.

16α-(Methylseleno)estrone (12b). *n*-BuLi (7 mL of 1.6 M solution in hexane, 11.2 mmol) was added to a cold solution of diisopropylamine (1.7 mL, 11.2 mmol) in THF (20 mL), at −78 °C, under nitrogen. A solution of compound **8** (2.96 g, 8.39 mmol) in THF was added dropwise, and the solution was stirred for 15 min. Methylselenyl bromide was prepared by adding bromine (0.31 mL, 0.92 g, 5.75 mmol) in THF dropwise with agitation to a solution of dimethyl diselenide (1.05 g, 5.75 mmol) in THF. The CH_3SeBr solution was added rapidly through a syringe to the enolate solution. Immediate decolorization was noticed. The cold reaction mixture was poured into 50 mL of water and 40 mL of ether. The organic layer was washed with water and then with saturated NaCl solution and was dried over anhydrous magnesium sulfate. The solvent was evaporated under vacuum. The residue was dissolved in a minimum amount of benzene and chromatographed on silica gel (60–200 mesh) with ethanol/benzene (1:100, v/v) as an eluent. Fractions containing 20 mL were collected. A pale yellow crystalline solid was obtained from fractions 7–15, and was dissolved in 95% ethanol (30 mL) and water (5 mL). To this solution was added 0.1 g of *p*-toluenesulfonic acid. After 1 h at 50 °C, 200 mL of water was added, and the solution was extracted with ether. The organic layer was evaporated, and crystallization from benzene provided an analytical sample of the

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desired **12b**: mp 168–170 °C; NMR δ 0.89 (s, 3 H, C_{18} H), 2.3 (s, 3 H, $SeCH_3$), 6.78–7.25 (m, 3 H, Ar H); MS, *m/e* 365 (M^{+1} for ^{80}Se), 363 (M^{+1} for ^{78}Se), 347 ($M^{+1} - H_2O$), 271 ($M^{+1} - SeCH_3$). Anal. ($C_{19}H_{24}O_2Se$) C, H.

16α-(Phenylseleno)-17β-estradiol (13). Compound **10a** (509 mg, 1 mmol) was dissolved in a minimum amount of anhydrous ether (15 mL). This solution was slowly added to a precooled (−15 °C) suspension of $LiAlH_4$ (75 mg, 2 mmol) in ether (20 mL) under nitrogen. The reaction mixture was stirred at −15 °C for 2 h and then for another hour at room temperature. Three drops of 50% NaOH was added. The suspension was diluted with ether and filtered. Ether was evaporated, and the residue was dissolved in 95% ethanol (50 mL) and water (10 mL). To this solution was added 0.15 g of *p*-toluenesulfonic acid. After 1 h at 50 °C, 200 mL of water was added. The solution was allowed to cool to room temperature and extracted with ether. The organic layer was washed with water, dried over anhydrous magnesium sulfate, and filtered, and the filtrate was evaporated to dryness. Crystallization from benzene/hexane provided **13** as a fluffy mass of fine crystals (50% overall yield): mp 233–234 °C; NMR δ 0.77 (s, 3 H, C_{18} H), 1.55 (s, 1 H, C_{17} OH), 4.05 (m, 1 H, C_{16} H), 4.6 (s, 1 H, C_3 OH), 6.55–7.61 (m, 8 H, Ar H); MS, *m/e* 429 (M^{+1} for ^{80}Se), 427 (M^{+1} for ^{78}Se), 411 ($M^{+1} - H_2O$), 273 (M^{+2} – SePh), 255 ($M^{+2} - H_2O$ – SePh). Anal. ($C_{24}H_{28}O_2Se$) C, H.

Competitive Binding Assay Using Immature Rat Uterus Cytosol. Immature female Sprague–Dawley derived rats (21–25 days old) were killed by cervical dislocation. The uteri were removed, cleaned from adhering fat and mesentery, and placed in cold 0.9% NaCl. The uteri (2 uteri/mL) were homogenized at 4 °C in TEE buffer (10 mM Tris, 1.5 mM Na_2EDTA, and 1 mM dithiothreitol; pH 7.4 at 4 °C) in a motor-driven all-glass conical tissue homogenizer. The homogenizing vessel was held in an ice bath during the homogenization. The homogenate was centrifuged at 4 °C for 10 min. The fat-free supernatant was mixed with TEE buffer to provide a concentration equivalent to 1 uterus/mL.

An accurately weighed sample of nonradioactive competitor (25 mg) was dissolved in 25 mL of absolute ethanol. A 10-μL aliquot of this solution was diluted to 10 mL with TEE buffer to give a 1-μg/mL stock solution. Serial dilutions with TEE buffer were prepared to give concentrations ranging from 2.5 to 200 ng/0.2 mL. For cold estradiol and ethynylestradiol, the range in concentration was 0.5 to 200 ng/0.2 mL.

Microfuge tubes (1.5-mL capacity) were cooled on ice. To each tube was added 25 μL of a 2×10^{-7} M [^{3}H]estradiol solution in TEE buffer, followed by 200-μL aliquots of the competitor solutions, and the tubes were vortexed. After addition of 0.5 μL of cytosol to each tube, the tubes were again vortexed and placed on ice in a refrigerator. After 20 h, the incubation was terminated by the addition of 150 μL of a well-mixed cold dextran–charcoal suspension to each tube. These tubes were again vortexed and placed on ice in a refrigerator. After 10 min, the charcoal was spun down for 5 min. The last step was repeated with all the supernatant. The 250-μL aliquots of the supernatant were pipetted into scintillation vials containing 10 mL of Aquasol-2 (New England Nuclear). The radioactivity was measured in a liquid scintillation counter for a time that would give less than 2% counting error at the 95% confidence level.

17β-[6,7-^{3}H(N)]Estradiol, specific activity 53.0 Ci/mmol, was obtained from New England Nuclear. The radiochemical purity was greater than 98% when determined by TLC on silica gel G with the solvent system benzene/ethanol (9:1, v/v). Spots were located by autoradiography on X-Omat TL film (Eastman Kodak).

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Synthesis and Topical Antiinflammatory Activity of Some Steroidal [16α,17α-*d*]Isoxazolidines

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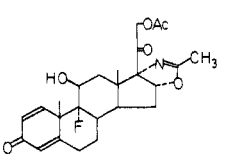
1,3-Dipolar cycloaddition of *N*-methylnitrone, *N*-benzylnitrone, and pyrroline *N*-oxide to 1,4,16-pregnatriene-3,20-diones is described. In each case only [16α,17α-*d*]isoxazolidines were isolated. The pentacyclic adducts **16–19** were active topical antiinflammatory agents in mice, with **18** being more potent than any of the standard compounds tested. The hexacyclic adduct **20** was inactive in this assay.

The fusion of heterocyclic rings onto steroid nuclei in order to alter the biological activity of the parent molecule has been a very productive endeavor for medicinal chemists. This is particularly true for the antiinflammatory steroids where several such analogues have found clinical use. Examples of corticosteroids with ring D fused heterocycles include the [17α,16α-*d*]-2′-methyloxazoline **1**[2] and the 16α-hydroxy-16,17-acetonides **2** and **3**. These compounds are potent, topical antiinflammatory agents in man,[3] and the acetonides in particular are widely used clinically for a variety of skin diseases.

In a program aimed at finding novel compounds with high topical antiinflammatory activity, we have sought to synthesize steroids with other heterocyclic rings fused to the 16,17-positions of corticosteroids. In this report we



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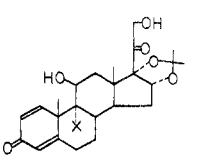
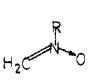


X = H, **2**
X = F, **3**



R = CH_3, **4**
R = CH_2Ph, **5**

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describe the preparation and topical antiinflammatory activity of a series of [16α,17α-*d*]isoxazolidines. We chose

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