B. Blood Pressure of Spontaneously Hypertensive Rats. Male spontaneously hypertensive rats (SHR) of the Wistar/Kyoto strain were supplied by Laboratory Supply Co., Indianapolis, IN. They were kept four per cage and fed Purina Laboratory Chow and water ad libitum. Lights were on 12 h a day. At the time of experiments, rats were about 6 months of age and weighed between 300 and 400 g.

Two methods were employed in measuring the systolic blood pressure of SHR indirectly. In the beginning of this study, systolic blood pressure was measured by a modified method of Friedman and Freed.[7] Using this method, rats were warmed for 30 to 45 s with a microwave oven set at 32 °C. The details of that procedure have been published elsewhere.[7]

Most compounds in this study were, however, tested by the method using photoelectric sensors at 25 °C.[7] A detailed description of that procedure was published previously.[7]

All compounds reported here were synthesized at our Laboratories, except Prazosin which was a gift of Pfizer, Inc. Saline containing 2% Emulphor EL-620 (General Aniline and Film), a polyoxyethylated vegetable oil, was used as the vehicle to facilitate the suspension of all compounds.

Acknowledgment. The authors are indebted to Donovan V. Pearson for his technical assistance in testing compounds in SHR and to Tommy Smith for his technical assistance in testing compounds in rabbits.

Synthesis and Biochemical Screening of Phenylselenium-Substituted Steroid Hormones

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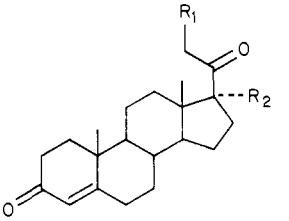
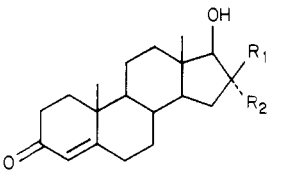
The syntheses of phenylselenium-substituted progesterone [21- (**1**) and 17α-(phenylseleno)progesterone (**2**)] and testosterone [16β- (**3**) and 16α-(phenylseleno)testosterone (**4**)] derivatives are described, along with data which help to establish the stereochemistry of the substituents in the testosterone molecules. Except for 21-(phenylseleno)-progesterone, the molecules exhibit greatly reduced receptor-binding capabilities.

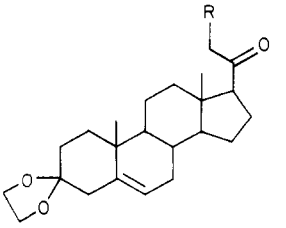
Interest in the biological effects of organoselenium compounds stems from the dual nature of the element: selenium is both a toxic and essential substance for many living organisms, including humans.[1] Recent advances made in the introduction of organoselenium substituents into organic molecules[2] have also helped to stimulate research into the effects of selenium on natural systems. However, although steroids are among the most potent biological molecules, regulating many important biological functions, few organoselenium-substituted steroids have been prepared,[1e] and there is no report of organoselenium steroids bearing the Δ^4-3-ketone functionality common to many important steroid hormones. Recently, in connection with an unrelated problem, we had occasion to prepare a number of steroid hormones containing organoselenium substituents, and we report herein the synthesis of certain analogues of progesterone [21- and 17α-(phenylseleno)-progesterone (**1** and **2**, respectively)] and testosterone [16β- and 16α-(phenylseleno)testosterone (**3** and **4**, respectively)], together with the results of the biochemical screening of these molecules.



1, R_1 = SePh; R_2 = H
2, R_1 = H; R_2 = SePh



3, R_1 = SePh; R_2 = H
4, R_1 = H; R_2 = SePh
5, R_1 = R_2 = H



6, R = H
7, R = SePh

Chemistry. The syntheses of the desired phenylselenium-substituted steroids proved to be straightforward. 3,3-(Ethylenedioxy)pregn-5-en-20-one (**6**)[3] was treated with lithium diisopropylamide and PhSeCl[4] to give the ketoselenide **7**, identified by the singlet signal in the NMR spectrum at δ 3.6 for the two protons on C-21. Hydrolysis of the ketal functionality gave the progesterone derivative **1**.

The synthesis of the related compound 17α-(phenylseleno)progesterone (**2**) started with pregnenolone (**8**),

(1) For reviews of this area, see (a) K. Schwarz and K. D. Pathak, *Chem. Scr.*, **8A**, 85 (1975); (b) D. V. Frost and D. Ingvoldstad, *ibid.*, **8A**, 96 (1975); (c) M. L. Scott, in "Organic Selenium Compounds: Their Chemistry and Biology", D. L. Klayman and W. H. H. Günther, Eds., Wiley, New York, 1973, p 629; (d) J. R. Shapiro, *ibid.*, p 693; (e) D. L. Klayman, *ibid.*, p 727.

(2) For a comprehensive review, see D. L. J. Clive, *Tetrahedron*, **34**, 1049 (1978).

(3) (a) W. S. Allen, S. Bernstein, and R. Littell, *J. Am. Chem. Soc.*, **76**, 6116 (1954); (b) A. Bowers, L. Cuéllar Ibañez, and H. J. Ringold, *Tetrahedron*, **7**, 138 (1959).

(4) H. J. Reich, J. M. Renga, and I. L. Reich, *J. Am. Chem. Soc.*, **97**, 5434 (1975).

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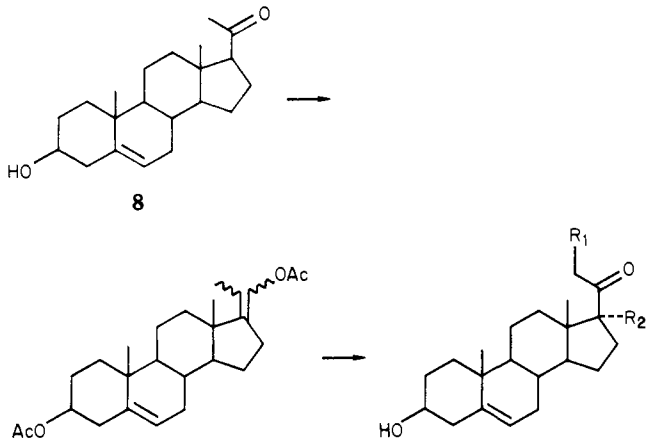
Scheme I



8

9

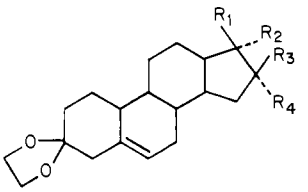
10, R_1 = SePh; R_2 = H
11, R_1 = H; R_2 = SePh

which was treated with perchloric acid and acetic anhydride[5] to give the enol acetate **9** (Scheme I).[6] Regiospecific enolate formation[7] (by treatment with 4 equiv of methyllithium), followed by quenching of the reaction with PhSeCl, led to 17α-(phenylseleno)pregnenolone (**11**) as the major product. Oppenauer oxidation[8] of **11** afforded the desired 17α-(phenylseleno)progesterone (**2**).

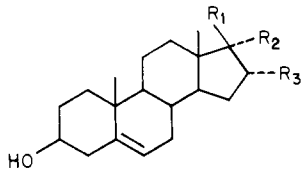
The mass spectra of compounds **1** and **2** are distinctly different and diagnostic. The most abundant ions in the mass spectrum of **1** (aside from the base peak molecular ion at m/z 470) originate from cleavages α to the C-20 ketone, affording the fragment ions m/z 271 (17–20 bond rupture, 84% of the base peak) and 299 (20–21 bond rupture, 62% of the base peak). On the other hand, the MS of compound **2** is dominated by the base peak at m/z 313, resulting from the loss of the PhSe group at C-17. These results indicate that the fragmentations in **1** and **2** are directed by the phenylselenium substituent, in contrast to the mass spectra of other progesterone analogues,[9] which show more diverse fragmentation patterns (e.g., cleavage reactions associated with the α,β-unsaturated ketone functionality).

Testosterone (**5**) was the starting material for the synthesis of the epimeric derivatives **3** and **4**. 3,3-(Ethylenedioxy)androst-5-en-17-one (**13**), prepared from testosterone by standard literature procedures,[3a,10] was treated with lithium diisopropylamide and PhSeCl to give two compounds (separable by column chromatography) which were identified (vide infra) as the ketoselenides **14** and **15**. Reduction of the ketone functionality in each compound by treatment with $LiAlH_4$ at −15 °C in ether[11] gave the respective hydroxy ketals **16** and **17**, which were hydrolyzed to the desired testosterone derivatives **3** and **4**.

The absolute stereochemistry at C-17 in these derivatives (**3** and **4**) was established by replacement of the PhSe substituent with hydrogen using triphenyltin hydride,[12] which resulted in the isolation of testosterone (rather than its 17α epimer) in both cases. The stereochemistry at C-16 in **3** and **4** was not as firmly established, although there was evidence to suggest the current assignments. When 3β-hydroxyandrost-5-en-17-one (**18**) was treated with PhSeCl in EtOAc,[13] only one product was isolated, which was converted (via reduction of the C-17 ketone to the alcohol followed by Oppenauer oxidation) to the testosterone derivative **4**, identical with the previously prepared sample in all respects. Since additions to C-16 of 17-ketoandrostanes usually result in formation of the 16α product,[14] it seems reasonable to assume that compound **4** is, indeed, 16α-(phenylseleno)testosterone.



12, R_1 = OH; R_2 = R_3 = R_4 = H
13, R_1, R_2 = O; R_3 = R_4 = H
14, R_1, R_2 = O; R_3 = SePh; R_4 = H
15, R_1, R_2 = O; R_3 = H; R_4 = SePh
16, R_1 = OH; R_3 = SePh; R_2 = R_4 = H
17, R_1 = OH; R_4 = SePh; R_2 = R_3 = H



18, R_1, R_2 = O; R_3 = H
19, R_1, R_2 = O; R_3 = SePh
20, R_1 = OH; R_2 = H; R_3 = SePh

Biochemical Results. The progesterone derivatives **1** and **2** and the testosterone derivatives **3** and **4** were assayed for binding affinity to various steroid hormone receptors in a routine screening system,[15] and the relative binding affinity[16] in each case was determined. Table I gives the source of the cytosols used in the screening, Table II shows the labeled steroids used to tag the steroid hormone receptors, and Table III gives the incubation conditions and the results of the present study. Apart from 21-(phenylseleno)progesterone (**1**), which competes quite

(5) D. H. R. Barton, R. M. Evans, J. C. Hamlet, P. G. Jones, and T. Walker, *J. Chem. Soc.*, 747 (1954).
(6) This compound was obtained as a mixture of geometrical isomers about the C-17 double bond and was used without isolation of the individual isomers in the synthesis of **10**. See L. F. Fieser and Huang-Minlon, *J. Am. Chem. Soc.*, 71, 1840 (1949).
(7) H. O. House and B. M. Trost, *J. Org. Chem.*, **30**, 2052 (1965).
(8) C. Djerassi, *Org. React.*, **6**, 207 (1951).
(9) S. Hammerum and C. Djerassi, *Tetrahedron*, **31**, 2391 (1975).
(10) R. Ratcliffe and R. Rodehorst, *J. Org. Chem.*, **35**, 4000 (1970).
(11) A. M. Léonard-Coppens and A. Krief, *Tetrahedron Lett.*, 3227 (1976).
(12) D. L. J. Clive, G. Chittattu, and C. K. Wong, *J. Chem. Soc., Chem. Commun.*, 41 (1978).
(13) K. B. Sharpless, R. F. Lauer, and A. Y. Teranishi, *J. Am. Chem. Soc.*, **95**, 6137 (1973).
(14) J. Fajkos, *Collect. Czech. Chem. Commun.*, **20**, 312 (1955).
(15) (a) T. Ojasoo and J. P. Raynaud, *Cancer Res.*, **38**, 4186 (1978); (b) J. P. Raynaud, T. Ojasoo, M. M. Bouton, and D. Philibert, in "Drug Design", Vol. VIII, E. J. Ariëns, Ed., Academic Press, New York, 1979, p 169.
(16) The percentage of radioligand bound in the presence of competitor compared to that bound in its absence was plotted against the concentration of unlabeled competitor. From this plot, the molar concentrations of unlabeled radioligand or competitor that reduced radioligand binding by 50% was determined. The effectiveness of a competitor was established using the ratio of unlabeled radioligand concentration for 50% competition to competitor concentration for 50% competition. This ratio was multiplied by 100 and termed the relative binding affinity (RBA). The RBA's of the natural hormones and that of dexamethasone were taken to be equal to 100.

Table I. Preparation of Cytosols Used to Study Steroid Hormone Receptors

species[a] and strain	status	organ	buffer	homogenization tissue/buffer ratio, w/v	cytosol prepn
mouse (Swiss)	female, 18-days old	uterus	10 mM Tris-HCl (pH 7.4), 0.25 M sucrose (TS buffer)	1/50	centrifugation of homogenate at 105000g for 60 min at 4 °C
rabbit (New Zealand)	female (1 kg), 50-55 days old, estrogen-primed[b]	uterus		1/50	
rat (Sprague-Dawley)	male, 140-160 g, castrated for 24 h	prostate		1/5	
	male, 140-160 g, adrenalectomized for 4-7 days	perfused kidney	Krebs-Ringer phosphate buffer	1/3	
		perfused liver	TS buffer	1/10	105000g for 60 min at 4 °C

[a] All animals were supplied by Iffa-Credo, France, except for the rabbits which were obtained from Elevage Cunicole, Chatillon-Coligny, France. [b] The rabbits were primed with 25 μg of estradiol in ethanol applied to the dorsal skin and killed 4 days later.

effectively for binding to the progestin receptor, the compounds are inactive. Preparation of 21-(phenylseleno) analogues of more potent progestins (e.g., 19-norprogesterone) and more extensive biochemical evaluation is therefore indicated.

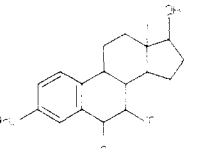
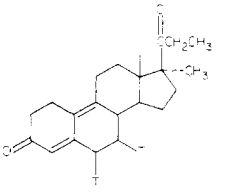
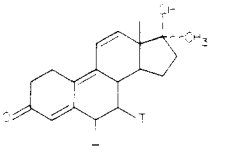
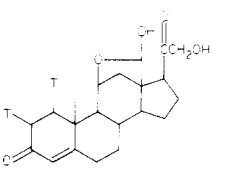
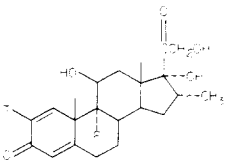
Experimental Section

Melting points were measured on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Optical rotations were determined on a Rudolph Autopol III polarimeter in thermostated 1.00-dm cells with fixed end plates, for solutions in chloroform. Infrared (IR) spectra were recorded for solutions in chloroform on a Perkin-Elmer Model 700A spectrometer. Nuclear magnetic resonance (NMR) spectra were obtained on either a Varian T-60 (60 MHz) spectrometer or a Varian XL-100 spectrometer by Dr. L. J. Durham using deuteriochloroform as solvent and tetramethylsilane as internal reference. Low- and high-resolution mass spectra (MS) were determined by A. Wegmann on a Varian MAT-711 spectrometer operated at 70 eV with a direct-inlet system. Elemental analyses were determined by E. Meier of the Stanford Microanalytical Laboratory. Column chromatography was done using E. Merck silica gel 60 (70–230 mesh ASTM).

3,3-(Ethylenedioxy)-21-(phenylseleno)-5-pregnen-20-one (7). Into a 25-mL two-neck round-bottom flask (nitrogen inlet, serum stopper, magnetic stirrer) was added THF (5 mL) under nitrogen. The flask was cooled to −78 °C and diisopropylamine (0.33 mmol, 46 μL) was added, followed by *n*-BuLi (0.33 mmol, 206 μL of a 1.6 M solution). A solution of 3,3-(ethylenedioxy)-pregn-5-en-20-one (**6**;[3] 0.25 mmol, 90 mg) in a small amount of THF was added dropwise. The resulting solution was stirred for 15 min at −78 °C, at which time PhSeCl (0.33 mmol, 64 mg) was added. The reaction mixture was poured into dilute HCl and extracted with ether. The combined ether extracts were washed with saturated $NaHCO_3$ solution, water, and brine, dried over anhydrous Na_2SO_4, and concentrated. The resulting solid was purified by preparative TLC (eluting with 10% acetone–benzene) to give 50 mg (39%) of the desired α-(phenylseleno) ketone (**7**): mp 138–139 °C; $[\alpha]^{20}_D$ +37.4° (*c* 0.53). M_r Calcd for $C_{29}H_{38}O_3Se$: 514.19860. Found: 514.20028.

21-(Phenylseleno)progesterone (1). A solution of the ketal **7** (0.43 mmol, 220 mg) in 10 mL of acetone was treated with *p*-toluenesulfonic acid (15 mg) and stirred for 8 h at room temperature. The reaction mixture was diluted with water and extracted with ether. The combined ether extracts were washed with saturated $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4, and concentrated to give 200 mg (99%) of the crude progesterone derivative **1**. Chromatography (eluting with 5% acetone–hexane), followed by recrystallization from ether–hexane, gave the pure α-(phenylseleno) ketone **1**: mp 114–115 °C; $[\alpha]^{20}_D$ +133° (*c* 0.15); IR 1660 (C=O), 1220 cm^{-1}; NMR δ 0.68 (s, 3 H, 18-CH_3), 1.17 (s, 3 H, 19-CH_3), 3.6 (s, 2 H, CH_2SePh), 5.7 (s, 1 H, 4-H), 7.29 and 7.45 (2 m, 5 H, aromatic); MS *m/z* 470 (100, M^+ for Se 80), 468 (55, M^+ for Se 78), 299 (62, M − CH_2SePh),

Table II. Radioligands Used to Label Steroid Hormone Receptors

radioligand[a,b]	structure	sp act., Ci/mmol
[6,7-^{3}H]-estradiol		50
[6,7-^{3}H]-R 5020		57
[6,7-^{3}H]-R 1881		56
[1,2-^{3}H]-aldosterone		53
[2-^{3}H]-dexamethasone		26

[a] All the radioligands were synthesized by the Roussel-Uclaf Research Center. [b] Prior to use, radiochemical purity was checked by TLC using Merck silica gel plates; all the radioligands were more than 98% pure.

Table III. Relative Binding Affinities for Steroid Hormone Receptors[a]

seleno steroids	ES,[b] 2 h, 0 °C	PG, 24 h, 0 °C	ANDR, 2 h, 0 °C	MIN, 30 min, 25 °C	GLU, 4 h, 0 °C
1	<0.1	59 ± 14 (4)	1.7 ± 0.2 (3)	2.1 ± 0.3 (3)	0.6 ± 0.3 (4)
2	<0.1	2.1 ± 0.5 (5)	<0.1	0.23 ± 0.09 (3)	<0.1
3	<0.1	<0.1 (2)	1.9 ± 0.6 (3)	<0.1	<0.1
4	<0.1	0.8 ± 0.4 (3)	0.33 ± 0.07 (3)	0.15 (2)	0.23 ± 0.07 (3)

[a] The RBA's of the natural hormones, estradiol, progesterone, testosterone, aldosterone, and of dexamethasone, are taken to be equal to 100. [b] ES, PG, ANDR, MIN, GLU: estrogen, progestin, androgen, mineralocorticoid and glucocorticoid receptors, respectively. The values are the means of either three or more experiments (mean ± SEM) or of two experiments giving results which differ by less than 15%. The number of determinations is indicated in parentheses.

271 (84, M – $COCH_2SePh$), 253 (48), 147 (42). M_r Calcd for $C_{27}H_{34}O_2Se$: 470.17239. Found: 470.17360.

17α-(Phenylseleno)pregnenolone (11). A solution of the enol acetate **9** [prepared from pregnenolone (**8**);[5,6] 0.5 mmol, 200 mg] in THF (3 mL) was added dropwise to a solution of MeLi (2.5 mmol, 1.4 mL of a 1.8 M solution) in THF (3 mL) which had been cooled to −20 °C under nitrogen. The resulting solution was warmed to 0 °C and stirred for 20 min, then cooled to −78 °C, and treated with PhSeCl (2.75 mmol, 0.53 g) in a small amount of THF. Dilute HCl was added and the mixture was extracted with hexane. The combined hexane extracts were washed with saturated $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4, concentrated, and chromatographed (eluting with 5% acetone–benzene) to give 21-(phenylseleno)pregnenolone (**10**; 35 mg) and the desired 17α-(phenylseleno)pregnenolone (**11**; 170 mg, 72%): mp 189–191 °C (recrystallized from MeOH); $[\alpha]^{20}_D$ −193.5° (*c* 0.97). Anal. ($C_{27}H_{36}O_2Se$) C, H.

17α-(Phenylseleno)progesterone (2). A solution of the alcohol **11** (0.39 mmol, 182 mg) in toluene (16 mL) and cyclohexanone (4 mL) was treated with aluminum *tert*-butoxide (200 mg) and refluxed for 10 min. The reaction mixture was cooled, diluted with 1 N H_2SO_4, and extracted with toluene. The combined toluene extracts were washed with water and brine, dried over anhydrous Na_2SO_4, concentrated, and chromatographed (eluting with 5% acetone–hexane) to give the enone **2** (135 mg, 74%): mp 206–208 °C (recrystallized from acetone–hexane); $[\alpha]^{20}_D$ −137.2° (*c* 0.5); IR 1660 (C=O) cm^{-1}; NMR δ 0.88 (s, 3 H, 18-CH_3), 1.2 (s, 3 H, 19-CH_3), 2.43 (s, 3 H, 21-CH_3), 5.7 (s, 1 H, 4-H), 7.5 (m, 5 H, aromatic); MS *m/z* 470 (19, M^+), 313 (100, M – SePh). M_r Calcd for $C_{27}H_{34}O_2Se$: 470.17238. Found: 470.16889.

3,3-(Ethylenedioxy)androst-5-en-17-one (13). A solution of CrO_3–(pyridine)$_2$ complex was prepared by adding anhydrous CrO_3 (5.0 mmol, 500 mg) to a solution of pyridine (10.0 mmol, 8.1 mL) in CH_2Cl_2 (12 mL). The solution was stirred for 15 min and 3,3-(ethylenedioxy)androst-5-en-17β-ol (**12**; 1.5 mmol, 500 mg) was added. After stirring for 12 h at room temperature, the reaction solution was decanted, concentrated, diluted with ether, and filtered through a column of Florisil. The resulting solution was washed with dilute HCl solution, saturated $NaHCO_3$ solution, and brine, dried over anhydrous $MgSO_4$, and concentrated to give 447 mg (90%) of the ketone **13**: mp 195–197 °C (recrystallized from MeOH), lit.[17] 197–198; $[\alpha]^{20}_D$ +13.9° (*c* 1.05), lit. +15.4°. M_r Calcd for $C_{21}H_{30}O_3$: 330.21337. Found: 330.21638.

3,3-(Ethylenedioxy)-16β-(phenylseleno)androst-5-en-17-one (14) and 3,3-(Ethylenedioxy)-16α-(phenylseleno)androst-5-en-17-one (15). Following the procedure for the synthesis of **7**, the ketone **13** (0.68 mmol, 226 mg) yielded two compounds identified[18] as the ketoselenides **14** (77 mg, 23%) and its epimer **15** (190 mg, 57%). Ketone **14**: mp 181–183 °C; $[\alpha]^{20}_D$ +40.6° (*c* 0.53); NMR δ 3.7 (t, 1 H, *J* = 9 Hz, 16-H). M_r Calcd for $C_{27}H_{34}O_3Se$: 486.16729. Found: 486.16619. Ketone **15**: mp 177–179 °C; $[\alpha]^{20}_D$ −88.4° (*c* 1.18); NMR δ 4.05 (m, 1 H, 16-H). M_r Calcd for $C_{27}H_{34}O_3Se$: 486.16729. Found: 486.16849.

3β-Hydroxy-16α-(phenylseleno)androst-5-en-17-one (19). This procedure follows that of Sharpless et al.[13] A solution of 3β-hydroxyandrost-5-en-17-one (**18**; 3.47 mmol, 1.0 g) in ethyl acetate (30 mL) was treated with PhSeCl (4.17 mmol, 800 mg) and stirred for 2 h at room temperature. The reaction mixture was diluted with water and extracted with ether. The combined ether extracts were washed with saturated $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4, concentrated, and chromatographed (eluting with 5% acetone–benzene) to give the ketoselenide **19** (859 mg, 56%): mp 181–182 °C (recrystallized from ether–hexane); $[\alpha]^{20}_D$ −86° (*c* 0.07); NMR δ 4.1 (m, 1 H, 16-H). M_r Calcd for $C_{25}H_{32}O_2Se$: 444.15672. Found: 444.15638.

3β-Hydroxy-16α-(phenylseleno)androst-5-en-17-ol (20). A solution of the ketone **19** (0.68 mmol, 300 mg) in a minimum amount of ether was added to a stirred solution of $LiAlH_4$ (1.4 mmol, 52 mg) in ether (20 mL) which had been cooled to −15 °C under nitrogen. The resulting mixture was stirred at −15 °C for 2 h, then allowed to warm to room temperature, and stirred for 1 h. Water (52 μL) was added dropwise, followed by the addition of 15% NaOH solution (52 μL) and water (156 μL). The resulting solution was filtered and concentrated to give 280 mg (93%) of the alcohol **20**: mp 135–136 °C (recrystallized from MeOH); $[\alpha]^{20}_D$ −80.2° (*c* 0.29); IR: 3600, 3450 (–OH), 1200, 1040 cm^{-1}. M_r Calcd for $C_{25}H_{34}O_2Se$: 446.17237. Found: 446.17284.

16β-(Phenylseleno)testosterone (3). Following the procedure given above for the synthesis of the seleno alcohol **20**, the ketone **14** (0.61 mmol, 300 mg) was reduced with $LiAlH_4$ to give the ketal **16** (271 mg), which was dissolved, without purification, in acetone (30 mL) and treated with *p*-toluenesulfonic acid (25 mg). The solution was refluxed for 2 h, cooled, diluted with water, and extracted with ether. The combined ether extracts were washed with saturated $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4, and concentrated to give the testosterone derivative **3** (241 mg, 88% from **14**): mp 160–161 °C (recrystallized from acetone–water); $[\alpha]^{20}_D$ +45.6° (*c* 0.8); IR 3450 (–OH), 1660 (C=O) cm^{-1}; NMR δ 0.80 (s, 3 H, 18-CH_3), 1.19 (s, 3 H, 19-CH_3), 3.65 and 3.95 (2 m, 2 H, 16-H and 17-H), 5.7 (s, 1 H, 4-H), 7.25 and 7.55 (2 m, 5 H, aromatic); MS *m/z* 444 (100, M^+ for Se 80), 442 (58, M^+ for Se 78), 287 (40, M – SePh), 269 (15, M – H_2O + SePh), 229 (32), 145 (24). M_r Calcd for $C_{25}H_{32}O_2Se$: 444.15672. Found: 444.15575.

16α-(Phenylseleno)testosterone (4). Method I. Following the procedure given above for the synthesis of the testosterone derivative **3** from the ketone **14**, the ketone **15** (0.361 mmol, 175 mg) was reduced with $LiAlH_4$ and deketalized to give the testosterone derivative **4** (160 mg, 99%): mp 155–156 °C (recrystallized from MeOH).

Method II. Following the procedure for the synthesis of the ketone **2**, the alcohol **20** (0.34 mmol, 150 mg) was oxidized to the ketone **4** (105 mg, 70%): mp 121–123 °C (recrystallized from acetone–hexane); $[\alpha]^{20}_D$ −7° (*c* 0.23); IR 3600, 3450 (–OH), 1660 (C=O), 1615, 1060 cm^{-1}; NMR δ 0.83 (s, 3 H, 18-CH_3), 1.17 (s, 3 H, 19-CH_3), 3.5 (2 m, 2 H, 16-H and 17-H), 5.7 (s, 1 H, 4-H), 7.25 and 7.55 (2 m, 5 H, aromatic); MS *m/z* 444 (100, M^+ for Se 80), 442 (61, M^+ for Se 78), 287 (34, M – SePh), 269 (10, M – H_2O + SePh), 229 (23). M_r Calcd for $C_{25}H_{32}O_2Se$: 444.15672. Found: 444.15661.

Reduction of Organoselenium Substituent. This procedure follows that of Clive et al.[12] A solution of 16α-(phenylseleno)-testosterone (**4**; 0.04 mmol, 20 mg) in 1 mL of toluene was treated with triphenyltin hydride (0.168 mmol, 59 mg) and heated to reflux for 2 h under nitrogen. The resulting mixture was diluted with ether and washed with dilute HCl and brine, dried over anhydrous Na_2SO_4, concentrated, and chromatographed (eluting with 20% ethyl acetate–toluene) to give testosterone (5 mg, 39%). This procedure, when used on 16β-(phenylseleno)testosterone (**3**), also provided testosterone, identical with an authentic sample by NMR spectroscopy.

(17) H. J. Dauben, Jr., B. Löken, and H. J. Ringold, *J. Am. Chem., Soc.*, **76**, 1359 (1954).
(18) See the text for a discussion of the stereochemistry at C-16.

Biochemical Screening. Cytosol Preparation and Incubation. Cytosols were prepared by centrifuging homogenates obtained from the organs of the species indicated in Table I and incubated with the corresponding radioligand (Table II); i.e., 5 nM [^{3}H]estradiol was incubated for 2 h at 0 °C with mouse uterus cytosol to label the estrogen receptor, 2.5 nM [^{3}H]R 5020 was incubated for 24 h at 0 °C with rabbit uterus cytosol to label the progestin receptor, 2.5 nM [^{3}H]R 1881 was incubated for 2 h at 0 °C with rat prostate cytosol to label the androgen receptor, 5 nM [^{3}H]dexamethasone was incubated for 4 h at 0 °C with rat liver cytosol to label the glucocorticoid receptor, and 2.5 nM [^{3}H]aldosterone was incubated for 30 min at 25 °C with rat kidney homogenates, which was then centrifuged at 800g for 10 min at 0 °C to label the mineralocorticoid receptor. All incubations were performed in the absence and in the presence of 0 to 2500 nM unlabeled competing steroids.

Bound Steroid Measurement. A 100-μL aliquot of incubated cytosol was stirred for 10 min at 0–4 °C with 100 μL of DCC (0.625% Dextran 80000–1.25% charcoal Norit A) in a microtiter plate and then centrifuged for 10 min at 800g. The radioactivity of a 100-μL supernatant sample was measured.

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Synthesis and Analgesic Activity of Some 5-(4-Hydroxyphenyl)-2-azabicyclo[3.2.1]octanes

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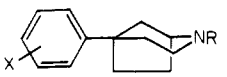
A representative series of 5-(4-hydroxyphenyl)-2-azabicyclo[3.2.1]octanes was synthesized and evaluated in vitro, as well as in vivo, as potential analgetic agents. In general, moderate to good activity (**19** twice as active as morphine) was observed in the phenylquinone writhing assay (PQW), while only marginal activity was detected by the tail-flick method. Compounds **19** and **18**, being the most active in the PQW model, also demonstrated weak binding affinity for the opiate receptors labeled by [^{3}H]naloxone in rat brain homogenates.

In a previous publication[1] from this laboratory, we described the synthesis and analgesic activity of a series of 5-(3-hydroxyphenyl)-2-azabicyclo[3.2.1]octane derivatives (I), some of which displayed a mixed agonist–antagonist



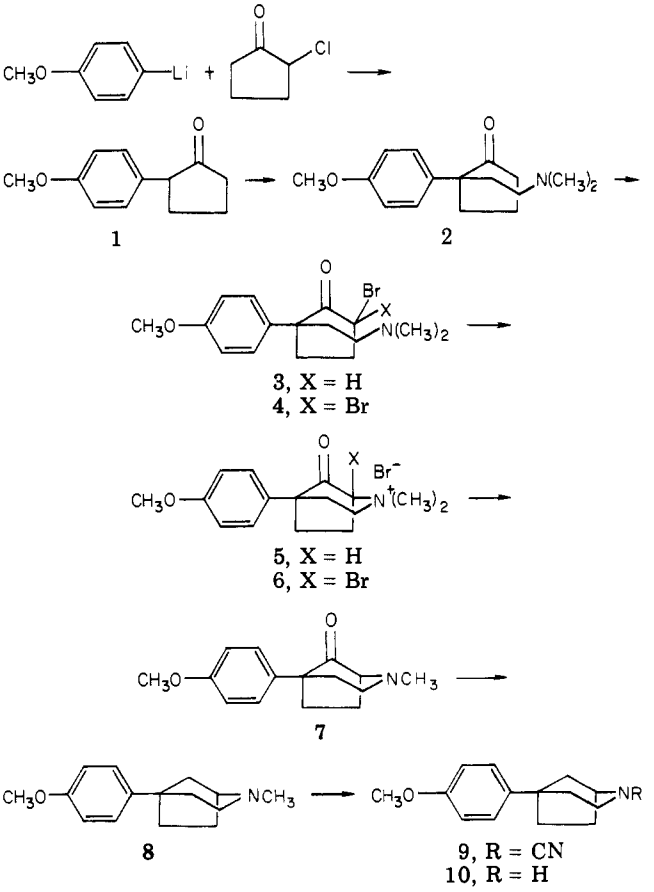
I, X = 3-OH
II, X = 4-OH

profile with a potency range comparable to that of morphine. Structure–activity studies of these compounds centered mostly upon the modification of N-substituents, and it was also demonstrated that analogues bearing a 3-hydroxy substituent on the aromatic nucleus were much more active than their 3-methoxy counterparts when other structural parameters were held at optimum.

Although the activity-enhancing effect of a free phenolic hydroxy group located meta to the quarternary carbon is widely recognized for many classes of centrally mediated (polycyclic) analgesics,[2a,b] evidence supporting a similar conclusion has yet to be documented for bicyclic strong analgetics, which possess not only a greater degree of conformational flexibility but also markedly different spatial relationships between the basic nitrogen and the aromatic ring. In this article we report the synthesis and biological activity of a number of 5-(4-hydroxyphenyl)-2-azabicyclo[3.2.1]octanes (II).

Chemistry. The title compounds **15–20** were synthesized by previously published routes.[1] 2-Chlorocyclopentanone reacted with 4-methoxyphenyllithium at −50 °C to give a mixture of chlorohydrins, which underwent a "one-pot" thermal rearrangement to afford **1** in high purity. Alkylation of **1** with β-(dimethylamino)ethyl

Scheme I



1 2

3, X = H
4, X = Br

5, X = H
6, X = Br

7

8

9, R = CN
10, R = H

(1) H. H. Ong, V. B. Anderson, and J. C. Wilker, *J. Med. Chem.*, **21**, 758 (1978).

(2) (a) J. Hellerbach, O. Schnider, H. Besendor, and B. Pellmount, "Synthetic Analgesics, Part IIA, Morphinans", Pergamon Press, Elmsford, N.Y., 1966, pp 75. (b) N. B. Eddy and E. L. May, "Synthetic Analgesics, Part IIB, 6,7-Benzamorphans", Pergamon Press, Elmsford, N.Y., 1966, pp 138.

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