Synthesis and Evaluation of Potential Radioligands for the Progesterone Receptor

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Several steroidal analogues were synthesized as potential γ-emitting radioligands for the progesterone receptor. Each of these compounds was tested as an inhibitor of the specific binding of [^{3}H]-17α,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione (R5020) to the progesterone receptor in rabbit uterine cytosol. R5020 is a well-known progestin with high affinity for the receptor. Of the compounds synthesized, aromatic N-substituted C-17 steroidal carboxamides inhibited the binding only poorly. Three compounds, 16α-iodo-4-estren-17β-ol-3-one, 17α-[2(*E*)-iodovinyl]-4-estren-17β-ol-3-one, and 17α-[2(*Z*)-iodovinyl]-4-estren-17β-ol-3-one were excellent competitors, each having a K_i less than or equal to that of the natural progestin, progesterone. Since similar iodinated analogues of estrogens have been shown to be extremely stable both in vivo and in vitro, these compounds are potentially useful ligands for the progesterone receptor.

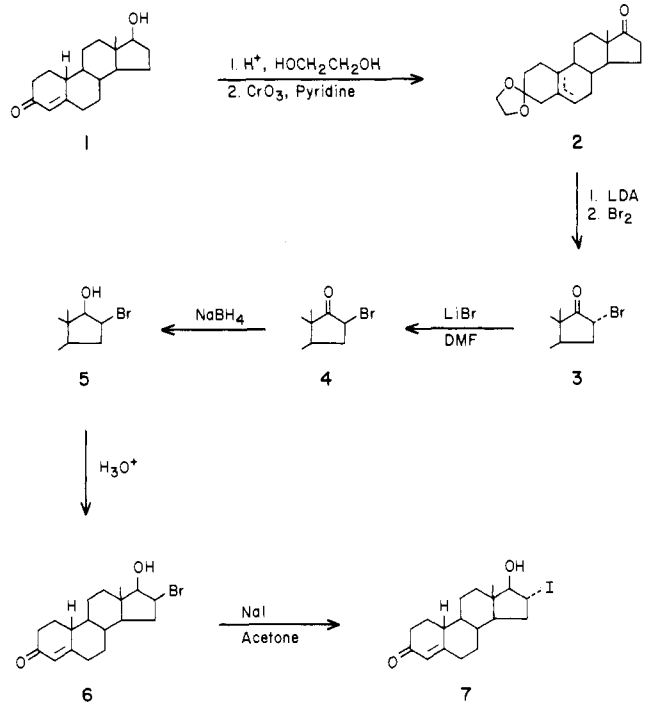
While the synthesis of γ-emitting halogenated steroids is not unusually difficult, the synthesis of such compounds which have biological activity has, for the most part, resisted intensive efforts. The complex information encoded within the steroid hormone molecule is contained within such a small structure that substituents often interfere with the exquisitely sensitive interaction of the hormone with its receptor. Thus, analogues labeled, for example, with the useful isotopes of iodine are usually biologically inactive. Such difficulties have limited the development of these potentially useful biological and clinical probes of hormone action. γ-emitting halogens have many advantages over the β-emitting radionuclide, ^{3}H, which is the usual radioactive label in ligands for steroid receptors. For example, ^{125}I has a very high specific activity and its γ-emission is easily detected, making it an attractive analytical tool. ^{123}I, as well as many other radioactive halogens, has energetic emissions that are suitable for clinical imaging. To date, the only radiodinated, biologically active, steroids that have been synthesized are estrogens. We synthesized[1-3] the 16α-iodo analogue of estradiol, which because of its high affinity has proved to be an extremely useful probe for the estrogen receptor.[4-7] Subsequently, the 17α-[2(*E*)-iodovinyl] analogues of estradiol were prepared; they also bind to the estrogen receptor and concentrate in estrogen target tissues.[8,9] Quantification of the estrogen receptor is an important adjunct in determining therapy in patients with carcinoma of the breast;[10] 16α-[^{125}I]iodoestradiol is frequently used for the accurate detection of this trace protein.[4-7]

More recently it has become clear that measurement of the progesterone receptor (an estrogen-induced protein[11]) in addition to the estrogen receptor yields more accurate guidelines for hormonal therapy of breast cancer.[12] To obtain a ligand for the precise and sensitive monitoring of the progesterone receptor, we have synthesized several iodinated analogues of 19-nortestosterone and some aromatic steroidal analogues that can be easily iodinated (Figure 1). We tested the affinity of all of these compounds for the progesterone receptor by measuring their ability to inhibit the binding of the synthetic progestin, [^{3}H]-R5020.

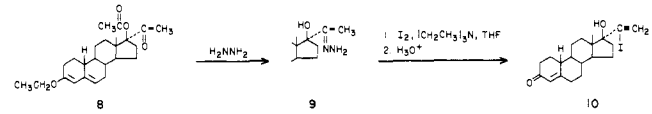
Chemistry

16α-Iodo-19-nortestosterone (16α-iodo-4-estren-17β-ol-3-one (**7**)) was synthesized by the route outlined in Scheme I: A mixture of Δ^5- and $\Delta^{5,10}$-3-ethylene ketals was prepared from 4-extren-17β-ol-3-one (**1**) and oxidized to the C-17 ketone, **2**, with CrO_3. After purification on a silica

Scheme I



Scheme II



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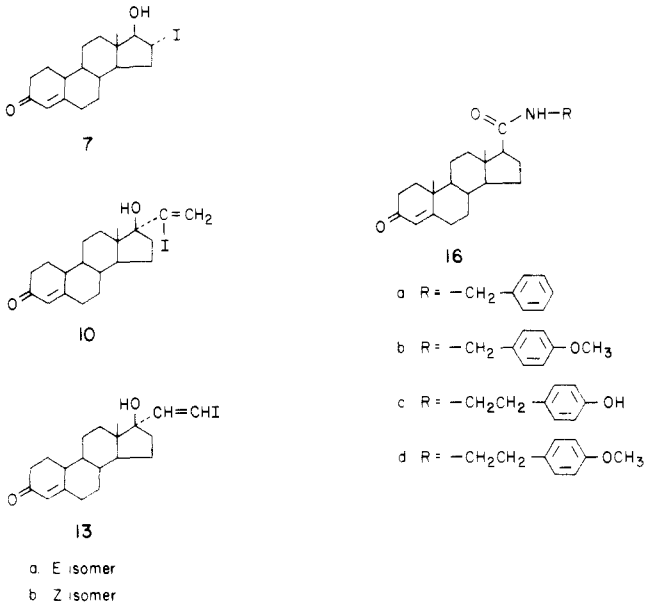
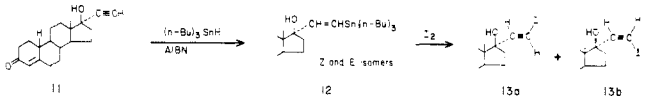
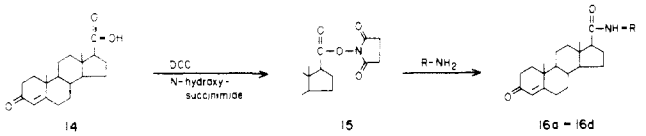


Figure 1. Potential ligands for the progesterone receptor.

Scheme III



Scheme IV[a]



[a] The structures of **16a–d** are given in Figure 1.

gel column, the product was converted to the 16α-bromo analogue **3**, by reaction with lithium diisopropylamide and Br_2. The 16β-bromo 17-ketone was formed by epimerization of **3** with LiBr, following which the C-17 ketone was reduced with $NaBH_4$ in ethanol to yield **5**. 16β-Bromo-4-estren-17β-ol-3-one (**6**) was prepared by hydrolysis of the C-3 ketal with HCl in aqueous dioxane. Reaction of **6** with NaI in acetone gave the final product, **7**.

The 17α-(1-iodovinyl) derivative of 19-nortestosterone (**10**) was prepared as in Scheme II: The 3-ethoxy 17β-acetoxy derivative of 3,5-pregnadien-20-one, **8**, was converted into the 20-hydrazone, **9**, by reaction with hydrazine. Hydrazone **9** was then treated with I_2 and triethylamine in tetrahydrofuran, which converted the C-17 side chain into the 1-iodovinyl group. The enol ether protecting group was hydrolyzed with HCl in aqueous methanol. The product, **10**, was purified by HPLC.

The 17α-(2-iodovinyl) derivatives of 19-nortestosterone, **13a** and **13b**, were synthesized from 17α-ethinyl-19-nortestosterone, **11**, as shown in Scheme III: Reaction of **11** with tri-*n*-butyltin hydride and AIBN gave 17α-[2-(tri-*n*-butylstannyl)vinyl]-4-estren-17β-ol-3-one (**12**) as a mixture of *E* and *Z* isomers. This intermediate product was iodinated directly with I_2, and the final products, **13a** and **13b**, were isolated from the reaction mixture by preparative HPLC.

The amides **16a-d**, were prepared as shown in Scheme IV: 4-Androsten-3-one-17β-carboxylic acid (**14**) was converted into its *N*-succinimidyl ester, **15**, by treatment with *N*-hydroxysuccinimide and dicyclohexylcarbodiimide. The appropriate amines were reacted with **15** producing compounds **16a-d** which were crystallized directly.

Table I. Binding of Ligands to the Progesterone Receptor[a]

compd	$10^{-9}K_i$, M
17α,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione, R5020	1.053 ± 0.19 (5)[b]
17α-[2(*Z*)-iodovinyl]-4-estren-17β-ol-3-one (**13b**)	1.81 (2)
17α-[2(*E*)-iodovinyl]-4-estren-17β-ol-3-one (**13a**)	4.26 ± 0.94 (4)
progesterone	10.4 (2)
16α-iodo-4-estren-17β-ol-3-one (**7**)	39.8 (2)
17α-(1-iodovinyl)-4-estren-17β-ol-3-one (**10**)	80.1 (2)

[a] The inhibition constants were determined with progesterone receptor derived from rabbit uterine cytosol by incubating varying amounts of the indicated compounds with a fixed concentration of [^{3}H]-R5020 as described in the text. The values in parentheses are the number of complete experiments that were conducted. The binding constants are means ± SEM. [b] K_d.

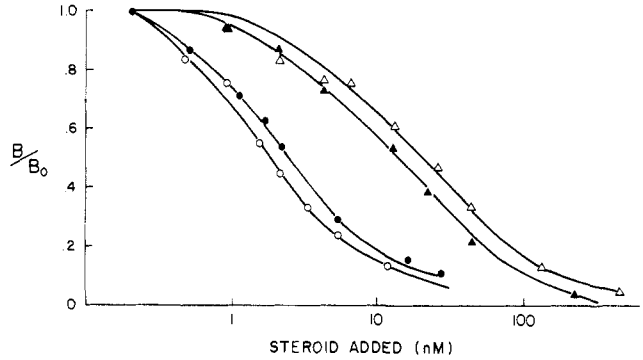


Figure 2. Inhibition of the binding of [^{3}H]-R5020 to the progesterone receptor: ○, R5020, 17α,21-dimethyl-19-nor-4,9-pregnadiene-3-20-dione; ●, **13a**, 17α-[2(*E*)-iodovinyl]-4-estren-17β-ol-3-one; ▲, progesterone; △, 1, 16α-iodo-4-estren-17β-ol-3-one. B_0 = specifically bound [^{3}H]-R5020 alone; B = specifically bound [^{3}H]-R5020 in the presence of designated concentration of competitor. The curve for **13b**, 17α-[2(*Z*)-iodovinyl]-4-estren-17β-ol-3-one, overlaps portions of the curves for both R5020 and **13a**. It has been ommitted for clarity.

Results and Discussion

We have synthesized eight steroids as potential ligands for the progesterone receptor and tested their ability to compete with the binding of [^{3}H]-R5020, the standard ligand for this receptor.[13] Four of these compounds, **7**, **10**, **13a**, and **13b**, are analogues of 19-nortestosterone and were synthesized because other derivatives of 19-nortestosterone are known to be potent progestins.[16] In addition, it has been shown that iodination at the 16α and 17α-[2(*E*)-vinyl] positions do not interfere with binding of estrogens to the estrogen receptor.[1,8] It was anticipated that this might also hold for progestins. Three of these compounds, **7**, **13a**, and **13b**, has high affinity for the progesterone receptor (Table I; Figure 2). It is apparent that all three of these iodinated steroids inhibit the specific binding of [^{3}H]-R5020 in a substantive manner; **13a** and **13b** are both impressively better ligands than progesterone, whereas **7** is approximately equal to progesterone in its binding affinity (Table I). The K_d for R5020 and the K_i's

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for the competing radioinert compounds were calculated with computerized programs.[14,15] As can be seen in Table I, R5020 had a K_d of 1×10^{-9} M, compound **7** had a K_i of 4.0×10^{-8} M, compound **13a** had a K_i of 4.3×10^{-9} M, and compound **13b** had a K_i of 1.8×10^{-9} M, while progesterone had a K_i of 1.0×10^{-8} M. Judging from their inhibition of the binding of R5020, all three of these steroids are potentially excellent radioligands for the progesterone receptor.

Compound **10** had a K_i of 8×10^{-8} M, almost 10-fold weaker than progesterone and about 20–40-fold less than the 17α-(2-iodovinyl) compounds, **13a** and **13b**. Small changes in steroid structure can have remarkable effects on biological activity, and so it is not necessarily surprising that movement of the bulky iodine from C-2 to C-1 on the vinyl group results in a compound, **10**, that binds more weakly to the receptor than compounds **13a** and **13b**. While this might not have been surprising, it would have been difficult to predict.

The results of the inhibition in the progesterone receptor assay with compounds **16a–d** showed that although all of these compounds compete for binding to the progesterone receptor, none of them caused any displacement of the tracer until their added concentration exceeded the K_d of R5020 by a factor of 10000 (data not shown). We synthesized them because a similar steroidal amide has been used as the ligand portion of an affinity column for the purification of the progesterone receptor.[17] In addition, similar analogues of 17α,11β-dihydroxy steroids are antiglucocorticoids that bind to the glucocorticoid receptor.[18] Because many progestins also bind to the glucocorticoid receptor,[19] compounds **16a–d** were reasonable candidates as ligands for the progesterone receptor. If the outcome was favorable, then compounds like **16c** could be iodinated easily. However, none of them were good inhibitors of binding.

While there are many compounds that can serve to insert halogens into organic molecules, few are available as radioactive reagents. All of the chemicals necessary to produce the radioactive form of the potential ligands, **7**, **13a**, and **13b** are commercially available. Therefore, the use of radioisotopes of iodide or iodine (generated in situ from iodide) can be utilized in syntheses essentially the same as those described above. Furthermore, because it has been shown that the analogous compounds, 16α-iodo and 17α-[2(*E*)-iodovinyl] estrogens, are stable and active in vitro and in vivo,[1-3,8,9] we are optimistic that these iodinated analogues of 19-nortestosterone will also have the necessary stability and specificity to make them excellent radiopharmaceuticals.

Experimental Section

Melting points were obtained in a Koffler hot stage or in a Mel-temp apparatus and are uncorrected. Infrared spectra were recorded in potassium bromide disks on a Beckman Acculab 4 spectrophotometer. NMR spectra, unless otherwise indicated, were obtained at 60 MHz with a Varian EM360A spectrometer equipped with EM3630 lock decoupler and V2048 signal averager. Mass spectra were recorded on Hewlett-Packard Models 5985A and 5890A spectrometers at 20 or 70 eV with a direct-insertion probe. High-performance liquid chromatography was performed on a Waters modular system consisting of a U6K injector, M-45 pump, and Model 440 detector or a Beckman Model 334 gradient system equipped with Model 421 controller, Altex CR-IA integrator–recorder, and Hitachi Model 100-10 variable-wavelength detector.

Progesterone Receptor Assay.[20] A 2.5-kg female rabbit was injected intramuscularly for four successive days with 0.1 mg of estradiol cypionate in 0.1 mL of sesame oil. On the fifth day the uterus was excised, weighed, and homogenized at 0 °C in 1.5 volumes of iced buffer containing 1 mM dithiothreitol, 0.15 mM Na_2EDTA, 30% glycerol, 20 mM sodium molybdate, and 10 mM Tris-HCl, pH 7.4 (4 °C). Homogenization was accomplished with three 10-s bursts of a Polytron homogenizer. The homogenate was centrifuged at 3000*g* for 10 min, and the resulting supernatant was centrifuged at 100000*g* for 1 h. The final supernatant (cytosol) was diluted with homogenization buffer to afford 20–40% binding of a trace of [^{3}H]-R5020. The protein concentration was generally 1–2 mg/mL. Binding assays were performed in duplicate by incubating 50 μL of cytosol, 50 μL of [^{3}H]-R5020 (90 Ci/mmol), 10000 dpm, and 50 μL of the appropriate ratioinert compound. Nonspecific binding was evaluated in tubes containing 2 μM R5020. After incubating at 4 °C overnight, free and bound steroids were separated by the addition of 100 μL of a stirred suspension of charcoal (5 mg/mL) in 10 mM Tris-HCl, pH 7.4 (4 °C), 0.15 mM Na_2EDTA. After mixing and standing on ice for 10 min, the tubes were centrifuged at 1500*g* for 10 min, and the "bound" radioactivity, in a 0.15-mL aliquot of the supernatant, was measured in a liquid scintillation counter. The effect of the competitors on the binding of [^{3}H]-R5020 is shown in Table I and Figure 2.

3,3-Ethylenedioxy-5-estren-17-one (2). 4-Estren-17β-ol-3-one (1, 19-nortestosterone 5 g), was converted in 92% yield to a 2:1 mixture of $\Delta^{5,10}$- and Δ^5-3-ethylene, ketals according to the method described by Djerassi and co-workers.[21] The ratio of isomers was computed from the ratio of NMR peaks at δ 5.4 (H-6), 3.97 (ketal), and 3.6 (H-17α). Oxidation of this material by chromium trioxide–pyridine complex according to standard methods[22] and chromatography on basic alumina (1% ethyl acetate in benzene) gave a 57% yield of the product, **2**, which was a mixture of the $\Delta^{5(10)}$ and Δ^5 isomers. This mixture of isomers was used for the synthesis of **7** as outlined in Scheme I.

16α-Bromo-3,3-ethylenedioxy-5-estren-17-one (3). A 1.07 M solution of lithium diisopropylamide in hexane (0.98 mL 1.05 mmol) was placed in a dry flask under nitrogen. A solution of 300 mg of 3,3-ethylenedioxy-5-estren-17-one (**2**, 0.948 mmol) in 3 mL of dry tetrahydrofuran was added with stirring over 5 min. After an additional 15 min, the mixture was cooled in a dry ice–acetone bath, and a solution of 167 mg (1.04 mmol) of bromine in 3.61 mL of methylene chloride was added rapidly. After 5 min, 6 mL of a saturated aqueous solution of $NaHCO_3$ was added and the mixture was slowly allowed to warm to room temperature. The mixture was transferred to a separatory funnel with the aid of 90 mL of ether. The organic phase was washed with three 30-mL portions of water and then dried over anhydrous sodium sulfate. Filtration and evaporation of the solvent gave an oil that was crystallized from methanol to give 180 mg (48%) of product **3**: mp 157–163 °C; IR (KBr) 1740 cm^{-1} (strong); NMR ($CDCl_3$) δ 5.50 (m, 1, H-6), 4.57 (m, 1, H-16β), 3.97 (s, 4, ketal), 0.95 (s, 3, H-18).

16β-Bromo-3,3-ethylenedioxy-5-estren-17-one (4). Epimerization was achieved by stirring 141 mg of 16α-bromo-3,3-ethylenedioxy-5-estren-17-one (**3**) with 360 mg of lithium bromide in 2.9 mL of *N,N*-dimethylformamide for 19 h at room temperature. The mixture was pipetted into 150 mL of hot water with stirring. After cooling slowly to 0 °C, the resulting precipitate was collected to give 129 mg (91.5%) of the 16β-bromo ketone **4**. NMR analysis indicated this material to be at least 90% 16β

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epimer: mp 190–200 °C; (KBr) 1752 cm^{-1} (strong); NMR ($CDCl_3$) δ 5.42 (m, 1, H-6), 4.17 (m, 1, H-16α), 3.98 (s, 4, ketal), 1.1 (s, 3, H-18).

16β-Bromo-3,3-ethylenedioxy-5-estren-17β-ol (5). A mixture of 128.5 mg (0.325 mmol) of the 16β-bromo ketone 4 was stirred with 51 mg (1.34 mmol) of sodium borohydride and 191 mL of absolute ethanol at 4 °C for 20 h. The mixture was then poured into 110 mL of water at 0 °C and stirred for 1 h. The resulting precipitate was collected by filtration and amounted to 110.2 mg (85%): IR (KBr) 3460 cm^{-1} (br, 17β-OH); NMR ($CDCl_3$) δ 5.47 (m, 1, H-6), 4.57 (m, 1, H-16α), 3.97 (s, 4, ketal), 3.37 (d, 1, H-17α), 0.91 (s, 3, H-18).

16β-Bromo-4-estren-17β-ol-3-one (6). The protected 16β-Br 17β-ol, **5** (110.2 mg, 0.277 mmol), 38 mL of distilled dioxane, and 9.75 mL of 0.2 N HCl were shaken at 37 °C for 22 h. The mixture was neutralized with 271 μL of triethylamine and evaporated to dryness, and the residue was stirred with 50 mL of water at 0 °C. This gave a filterable white solid that amounted to 77.4 mg (79%). It was recrystallized from ethanol: mp 148–151 °C; IR (KBr) 3440 (br, 17β-OH), 1660 (strong, C=O), 1610 (w, C=C) cm^{-1}; NMR ($CDCl_3$) δ 5.82 (s, 1, H-4), 4.63 (m, 1, H-16α), 3.40 (d, 1, H-17α), 0.96 (s, 3, H-18); mass spectrum *m/e* 352, 354 (15.9, 15.3 Parents, M), 273 (80.5, M – Br), 255 (13.9, M – Br – H_2O), 217 (29.2, M – D ring), 213 (4.8, A-ring cleavage – Br – H_2O), 110 (23.1 B-ring cleavage).

16α-Iodo-4-estren-17β-ol-3-one (16α-Iodo-19-nortestosterone, 7). Thirty milligrams (0.085 mmol) of 16β-bromo-4-estren-17β-ol-3-one (**6**) and 127 mg (0.85 mmol) of sodium iodide dissolved in 3 mL of acetone were heated at 60 °C in a sealed tube for 20 h. The mixture was then added to 50 mL of water at 0 °C with stirring. The resulting cloudy solution was extracted with four 20-mL portions of methylene chloride. The organic extracts were combined, washed with 20 mL of 10% sodium thiosulfate and then with three 20-mL portions of water, and dried over anhydrous sodium sulfate. Filtration and evaporation gave an oil. Crystallization from aqueous methanol gave 20 mg (59%) of a pale yellow, low-melting solid. Further recrystallization gave needlelike crystals, mp 120–140 °C. Analysis by high-performance liquid chromatography revealed one major component and several minor components (starting material and epimeric products). The major component was successfully separated by preparative HPLC in two systems: 43% THF–H_2O on 25 cm × 4.6 mm Ultrasphere ODS (C-18) at 0.75 mL/min, R_T = 12 min; 1% isopropyl alcohol–methylene chloride on 25 cm × 4.6 mm Partisil-10 (silica) at 1 mL/min, R_T = 14 min, detector 280 nm. The second HPLC system, necessary to remove minor impurities, gave material that crystallized from aqueous ethanol and was homogeneous by TLC and analytical HPLC (Partisil-10 column, system described above): mp 142–144 °C; IR (KBr) 3430 (br, 17β-OH), 1666 (strong, C=O), 1610 cm^{-1} (weak, C=C); NMR ($CDCl_3$) δ 5.87 (s, 1, H-4), 3.9–4.2 (m, 2, H-16β, H-17α), 0.8 (s, 3, H-18); mass spectrum *m/e* 400 (4.1, parent, M), 273 (base, M – I), 255 (19.6, M – I – H_2O), 217 (60.6, M – D-ring), 213, (2.8, A-ring cleavage – I – H_2O), 110 (5.6, B-ring cleavage); high-resolution M_r for $C_{18}H_{25}O_2I$, calcd 400.0899, found 400.0898.

3-Ethoxy-17β-acetoxy-19-nor-3,5-pregnadien-20-one Hydrazone (9). A mixture of 50 mg (0.131 mmol) of 3-ethoxy-17β-acetoxy-19-nor-3,5-pregnadien-20-one (**8**) (prepared as described by Djerassi and co-workers),[23] 0.23 mL of 85% hydrazine hydrate, 0.31 mL of water, and 1.7 mL of absolute ethanol were heated in a sealed tube at 95 °C for 72 h. The mixture was poured into 50 mL of water at 0 °C, and the resulting white precipitate was collected. This gave 33.7 mg (65% yield) of TLC-homogeneous material: mp 210 °C dec; IR (KBr pellet) 1645 and 1622 (diene, hydrazone), 1170 (enol ether CO), 3240, 3340 cm^{-1} (hydrazone N–H superimposed on 3600–3100 (br, 17β-OH).

17α-(1-Iodovinyl)-4-estren-17β-ol-3-one (10). This procedure is an adaptation of that described by Barton and co-workers:[24] A solution of iodine (0.214 mmol in 0.27 mL of THF) was added via syringe to a mixture of 33.7 mg (0.085 mmol) of hydrazone **9** in 1.4 mL of THF and 0.82 mL of triethylamine. The mixture was stirred for 90 min at room temperature and poured into 70 mL of ether. The ether solution was washed sequentially with water, 10% sodium thiosulfate, water, 0.1 N HCl, and water again. After filtration and evaporation of the solvent, the residue was hydrolyzed by stirring overnight in 1.25 mL of methanol, 0.5 mL of water, and 0.25 mL of concentrated HCl. The mixture was diluted with 50 mL of ether, washed with water and saturated sodium chloride, and dried over anhydrous sodium sulfate. After filtration and evaporation, the residue was submitted to preparative HPLC using a 25 cm × 4.6 mm μ-Bondapak column, 45% THF in water, flow rate 1 mL/min. The product eluted at 23 min. Evaporation of solvents gave an oil that was crystallized from aqueous acetone. This compound, **10**, was homogeneous by TLC and analytical HPLC in the above system: mp 159–161 °C IR 3425 (br, 17β-OH), 1649 (strong, C=O), 1600 (w, C=C), 917 cm^{-1} (m, C=CH_2); 270-MHz NMR ($CDCl_3$) δ 6.17 and 6.09 (AB pattern, 2, *J* = 2.6 Hz, H-21), 5.83 (s, 1, H-4), 0.99 (s, 3, H-18); mass spectrum *m/e* 426 (1.6, parent, M), 299 (base, M – 1), 281 (10.8, M – I – H_2O), 110 (59.5, B-ring cleavage); high-resolution M_r for $C_{20}H_{27}O_2I$, calcd 426.1056, found 426.1051.

17α-[2-(Tri-*n*-butylstannyl)vinyl]-4-estren-17β-ol-3-one. This procedure is an adaptation of that described by Easley and co-workers:[25] Ten milliliters of dry benzene and 100 mg (0.335 mmol) of 17β-hydroxy-17α-ethinyl-4-estren-3-one (**11**, 17α-ethinyl-19-nortestosterone) were heated at 80 °C with nitrogen slowly bubbling through the mixture for 10 min. The solution was then cooled to 50 °C. Tri-*n*-butyltin hydride (150 mg, 0.515 mmol) was added by syringe into the emerging stream of nitrogen, followed by 25 mg of AIBN. The reaction mixture was refluxed under nitrogen with stirring for 24 h. The solution was evaporated, and the residue was iodinated as described below.

17α-[2(*E*)- and 17α-[2(*Z*)-Iodovinyl]-4-estren-17β-ol-3-one (13a,b). Iodine (152 mg, 0.6 mmol), dissolved in 4 mL of methylene chloride, was added to the solution of 17α-[2-(tri-*n*-butylstannyl)vinyl]-4-estren-17β-ol-3-one (**12**) described above. After stirring was continued for 30 min, the mixture was diluted with 10 mL of a solution containing 10% sodium bisulfite and 1% potassium fluoride, then transferred to a separatory funnel, and shaken. After separation of layers, the organic solution was washed with two 10-mL portions of water and dried over anhydrous sodium sulfate. HPLC analysis (25 cm × 4.6 mm Partisil PXS column, Whatman, 0.8% 2-propanol in methylene chloride, 1 mL/min) of this solution showed the presence of 34% of the *E* isomer of the product **13a** (R_T = 19.1 min) 14% of the *Z* isomer **13b** (R_T = 15.6 min), and 46% of unreacted 17α-ethinyl-19-nortestosterone (**11**, R_T = 25.4 min). Preparative HPLC was conducted on the mixture using a 25 cm × 1 cm silica column, Rainin (0.8% 2-propanol in methylene chloride, 5 mL/min). After two additional repurifications under these conditions, **13a** (R_T = 18.1 min) and **13b** (R_T = 15.4 min) were obtained. These materials were each homogeneous by TLC and analytical HPLC (Partisil PXS system described above). 17α-[2(*E*)-iodovinyl]-4-estren-17β-ol-3-one (**13a**): mp 111–114 °C dec; IR (KBr) 3425 (br, 17β-OH), 1660 (C=O), 1620 (C=C), 965 cm^{-1} (*trans*-CH=CH out of plane); NMR ($CDCl_3$) δ 6.7 and 6.2 (AB pattern, 2, *trans*-CH=CH, *J* = 15.2 Hz), 5.84 (s, 1, H-4), 0.97 (s, 3, H-18); mass spectrum *m/e* 426 (10.2, parent, M), 299 (base, M – I), 281 (22.6, M – I – H_2O), 231 (34.4, A-ring cleavage 17α side chain); high-resolution M_r for $C_{20}H_{27}O_2I$, calcd for 426.1056, found 426.1054. 17α-[2(*Z*)-iodovinyl]-4-estren-17β-ol-3-one (**13b**): mp 117–118 °C dec; IR (KBr pellet) 3430 cm^{-1} (br, 17β-OH); NMR ($CDCl_3$) δ 6.77 and 6.33 (AB pattern, 2, *cis*-CH=CH, *J* = 8.6 Hz), 5.83 (s, 1, H-4), 1.01 (s, 3, H-18); mass spectrum *m/e* 426 (2.5, parent, M), 299 (97.2, M – I), 281 (38.7, M – I – H_2O), 231 (base, A-ring cleavage 17α side chain); high-resolution M_r for $C_{20}H_{27}O_2I$, calcd 426.1056, found 426.1045.

***N*-Hydroxysuccinimide Ester of 4-Androsten-3-one-17β-carboxylic Acid (15).** 4-Androsten-3-one-17β-carboxylic acid (**14**; 948 mg, 3 mmol) was added to a solution of *N*-hydroxysuccinimide (345 mg, 3 mmol) in 20 mL of tetrahydrofuran. Dicyclohexylcarbodimide (618 mg, 3 mmol) dissolved in 10 mL

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of tetrahydrofuran was then added, and the reaction was left overnight at room temperature. Dicyclohexylurea was removed by filtration, and the filtrate was evaporated under vacuum. The residue was purified on a silica gel column (15 cm × 2.5 cm) with chloroform. Those fractions containing the ester were evaporated, and the residue was crystallized from methanol–water: yield 1.05 g (85%); mp 236–238 °C; mass spectrum m/e 413 (6.0, parent, M), 55 (base).

Amides of 4-Androsten-3-one-17β-carboxylic Acid (16a–d). A solution of *N*-hydroxysuccinimide ester of 4-androsten-3-one-17β-carboxylic acid (**15**; (316 mg, 1 mmol) in 10 mL of tetrahydrofuran was added to a mixture of the appropriate amine (1 mmol) and $NaHCO_3$ (84 mg, 1 mmol) dissolved in 10 mL of tetrahydrofuran–water (1:1). The reaction was left at room temperature for 16 h and then acidified with 1 N HCl. The organic solvent was removed under vacuum, and the precipitate in the resulting aqueous media was collected by filtration, dried, and crystallized from aqueous ethanol. The yields ranged from 70 to 75%. These crystalline products were homogeneous by TLC and analytical HPLC on a 25 cm × 4.6 mm LiChrosorb-Diol column with methylene chloride for **16c** and methylene chloride–isooctane (7:3) for **16a,b,d**. All of these amides, **16a–d**, gave satisfactory (±0.4%) combustion analysis. The steroidal amides were readily characterized by their mass spectra. **16a**: mp 194–196 °C; mass spectrum m/e 405 (29, parent, M), 91 (base, $CH_2C_6H_5$). Anal. ($C_{27}H_{35}NO_2$) C, H, N. **16b**: mp 182–184 °C; mass spectrum, m/e 435 (7, parent, M), 121 (base, $CH_2C_6H_4OCH_3$). Anal. ($C_{28}H_{37}NO_3$) C, H, N. **16c**: mp 242–245 °C; mass spectrum m/e 435 (0.6, parent, M), 120 (base, $CH_2CHC_6H_4OH$). Anal. ($C_{28}H_{37}NO_3$) C, H, N. **16d**: mp 183–184 °C; mass spectrum m/e 449 (1.0, parent, M), 134 (base, $CH_2CHC_6H_4OCH_3$). Anal. ($C_{29}H_{39}NO_3$) C, H. N.

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Alkylating β-Blockers: Activity of Isomeric Bromoacetyl Alprenolol Menthanes

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An affinity label for β-adrenoceptors, *N*-(bromoacetyl)-*N'*-[3-(o-allylphenoxy)-2-hydroxypropyl]-1,8-diamino-*p*-menthane, has been extensively used in the form of a mixture of four isomers. In the present study, all four isomers were isolated, their structures elucidated, and their interactions with β-adrenoceptors characterized. The isomer with the aromatic (pharmacophore) group on carbon 1 of *p*-menthane and with the *Z* configuration (*Z*-1) predominates in the mixture and has the highest affinity for β-adrenoceptors of rat heart ($K_D = 3 \times 10^{-8}$ M) and lungs ($K_D = 2 \times 10^{-8}$ M). This isomer acts as a ligand that binds irreversibly at the drug binding site of the receptor (i.e., after treatment and extensive washing of the membrane preparation, the concentration of the receptors is decreased in a dose-dependent manner), while binding characteristics of the remaining receptors are not changed. The corresponding *E* diastereomer (*E*-1) also binds irreversibly to the drug binding site of the receptor. The isomer with the aromatic group on carbon 8 and the *Z* configuration (*Z*-8) modifies the receptor noticeably only at higher concentrations and then on a site apparently different from the drug-binding site, i.e., affinity of receptors after the treatment and washing is changed. The corresponding *E* diastereomer (*E*-8) modified both the drug-binding and alternative binding site. The results suggest that there is some flexibility in the conformation of the β-adrenoceptor that enables pairs of ligands, differing by axial or equatorial positions of critical groups, to alkylate the receptor in an analogous manner.

Affinity labeling of receptors is a useful technique for biochemical and physiological studies of these proteins. Photoaffinity probes have been extensively used for biochemical studies of β-adrenoceptors.[1-4] Chemically reactive probes were also prepared and used.[5-10] One of these probes, called bromoacetyl alprenolol menthane or BAAM, has proven to be very well suited for in vivo studies and its use has made possible the selective destruction of β-adrenoceptors in living cells or in animals. This procedure enabled us (a) to study the rates of de novo synthesis of β-adrenoceptors in rat heart and lungs and to measure the decrease in these rates in senescent animals,[11,12] (b) to study the effects of a decrease in β-adrenoceptor number on the adenylate cyclase activity in rat heart,[13] (c) to study the release of amylase from cells of rat salivary glands activated by catecholamines,[14] (d) to study de novo synthesis of β-adrenoceptors in guinea pig lungs and to show that regeneration of physiological functionality is slower than regeneration of antagonist binding capacity of re-

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