Reagents for Photoaffinity Labeling of Estrogen Binding Proteins. Synthesis of Some Azide and Diazo Derivatives of Estradiol, Estrone, and Hexestrol

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Fourteen estrogen derivatives bearing a photosensitive functional group have been synthesized for use as photoaffinity labels for estrogen binding proteins: diazoacetate derivatives of 17β-estradiol and estrone, 16-diazoestrone, and 3-diazo-2-ketopropyl ether and ortho azide derivatives of estradiol, estrone, and hexestrol. All of these compounds are reasonably stable and easily purifiable. Attempts to introduce a diazo function at B-ring positions 6 and 7 of the estrogens have been unsuccessful: the 6-diazo derivative is thermally labile and the 6-keto-7-diazo derivative fails to form. This is presumably due to the hindrance at position 7, as the corresponding position (2) in 7-hydroxy-1-tetralone is easily functionalized.

There are a number of interesting interactions between hormonal steroids and binding sites on proteins. Enzymes involved in steroidogenesis and steroid metabolism display affinity for their steroid substrates,[1] and the activity of certain other enzymes is affected in an allosteric fashion by steroid ligands.[2] Circulatory transport of steroids is assisted by binding to serum albumin and to several globulins.[3] Perhaps the most interesting interactions involve the binding shown by certain proteins present in steroid hormone target tissues. This binding is stereospecific and of very high affinity, and it has been demonstrated for estrogens,[4] androgens,[5] progestins,[4] and corticosteroids[6] in a number of different target tissues. In some cases, early biochemical events resulting from hormone administration have been correlated with steroid binding and subcellular movement of the steroid–protein complex.

The characterization of these target tissue binding proteins, often termed "receptors," has been a challenging task. Their low concentrations and often their thermal lability make purification difficult, and the fact that they are labeled only by virtue of their ability to complex a labeled steroid in a noncovalent fashion places severe limits on the types of investigations that can be carried out. In most studies, the availability of a radiolabel attached in a covalent fashion to these proteins would be of great advantage.

We have undertaken a study of the covalent labeling of the estrogen binding protein found in immature rat uterus, using the photoaffinity labeling technique. In this report we describe the synthesis of several derivatives of estrone (**5**), estradiol (**1**), and hexestrol (**44**)[7] that bear photosensitive diazocarbonyl or azide groups and have the potential to act as photoaffinity labeling reagents. The binding affinity of these compounds for the uterine estrogen binding protein has been determined by competitive assay and will be reported elsewhere.[8] Studies on their effectiveness as selective labeling reagents for this protein are currently in progress.

Results and Discussion

A number of reports have described the synthesis of electrophilic steroid derivatives for affinity labeling of steroid binding sites.[9-12] Some labeling studies involving pure enzymes have been successful, but those investigations directed at the high-affinity binding proteins from target tissues have given unsatisfactory results.[13]

Considerations of the heterogeneity of readily available preparations of the rat uterine estrogen binding protein led us to choose the technique of photoaffinity labeling over the conventional affinity labeling based on alkylation or acylation.[14,15] Although the technique of photoaffinity labeling[15b] was introduced more than a decade ago, it has not been used to study steroid binding sites, and other applications have been rather sporadic.[16] Most investigations have utilized the diazocarbonyl function, originally introduced by Westheimer,[17] as the group through which covalent attachment is to take place under photolytic conditions.

(1) (a) U. Westphal, "Steroid Protein Interactions," Springer-Verlag, West Berlin, 1971, Chapter 16; (b) R. B. Clayton, "Methods in Enzymology," Vol. 15, Academic Press, New York, N. Y., 1969, Section 5.

(2) A. W. Douville and J. C. Warren, *Biochemistry*, **7**, 4052 (1968), and references cited therein.

(3) (a) Reference 1a, Chapters 6–8, 10–13; (b) E. Milgrom, P. Allouch, M. Atger, and E-E. Baulieu, *J. Biol. Chem.*, **248**, 1106 (1973); (c) P. L. Corvol, A. Chrambach, D. Rodbard, and C. W. Bardin, *ibid.*, **246**, 3435 (1971).

(4) E. V. Jensen and E. R. DeSombre, *Annu. Rev. Biochem.*, **41**, 203 (1972).

(5) S. Liao and S. Fang, *Vitamins Hormones*, **27**, 17 (1969).

(6) M. Beato and P. Fiegelson, *J. Biol. Chem.*, **247**, 7890 (1972).

(7) Common names used in this paper are estradiol = 1,3,5(10)-estratriene-3,17β-diol; estrone = 3-hydroxy-1,3,5(10)-estratriene-17-one; hexestrol = *meso*-3,4-bis(4′-hydroxyphenyl)hexane.

(8) J. A. Katzenellenbogen, H. J. Johnson, Jr., and H. N. Myers, *Biochemistry*, in press.

(9) C. Liarakos and M. May, *Endocrinology*, **84**, 1247 (1969).

(10) (a) A. J. Solo and J. O. Gardner, *Steroids*, **11**, 37 (1968); (b) A. J. Solo and J. O. Gardner, *J. Med. Chem.*, **14**, 222 (1971).

(11) (a) C-C. Chin and J. C. Warren, *J. Biol. Chem.*, **243**, 5056 (1968); (b) T. G. Muldoon and J. C. Warren, *ibid.*, **244**, 5430 (1969); (c) C-C. Chin and J. C. Warren, *Biochemistry*, **9**, 1917 (1970); (d) M. Ganguly and J. C. Warren, *J. Biol. Chem.*, **246**, 3646 (1971); (e) F. Sweet, F. Arias, and J. C. Warren, *ibid.*, **247**, 3424 (1972); (f) C-C. Chin and J. C. Warren, *Biochemistry*, **11**, 2720 (1972).

(12) J. Kallos and K. P. Shaw, *Proc. Nat. Acad. Sci. U. S.*, **68**, 916 (1971).

(13) 4-Mercuriestradiol appears to react with a sulfhydryl group in the binding site of the estradiol binding protein from rat uterus (ref 11b). However, the mercury–sulfur link is quite labile, so that detailed characterization of this protein derivative is difficult.

(14) (a) H. Kiefer, J. Lindstrom, E. S. Lennox, and S. J. Singer, *Proc. Nat. Acad. Sci. U. S.*, **67**, 1688 (1970); (b) a detailed discussion of this point is given in ref 8.

(15) (a) For recent reviews affinity labeling, see S. J. Singer, *Advan. Protein Chem.*, **22**, 1 (1967), and E. Shaw, *Physiol. Rev.*, **50**, 244 (1970); (b) for a recent review of photoaffinity labeling, see J. R. Knowles, *Accounts Chem. Res.*, **5**, 155 (1972).

(16) Recent examples include (a) D. T. Browne, S. S. Hixson, and F. H. Westheimer, *J. Biol. Chem.*, **246**, 4477 (1971); (b) C. Hexter and F. H. Westheimer, *ibid.*, **246**, 3928, 3934 (1971); (c) C. A. Converse and F. F. Richards, *Biochemistry*, **8**, 4431 (1969); (d) D. J. Brunswick and B. S. Cooperman, *Proc. Nat. Acad. Sci. U. S.*, **68**, 1801 (1971); (e) J. Frank and R. Schwyzer, *Experientia*, **26**, 1207 (1970); (f) P. G. Waser, A. Hofmann, and W. Hopff, *ibid.*, **26**, 1342 (1970).

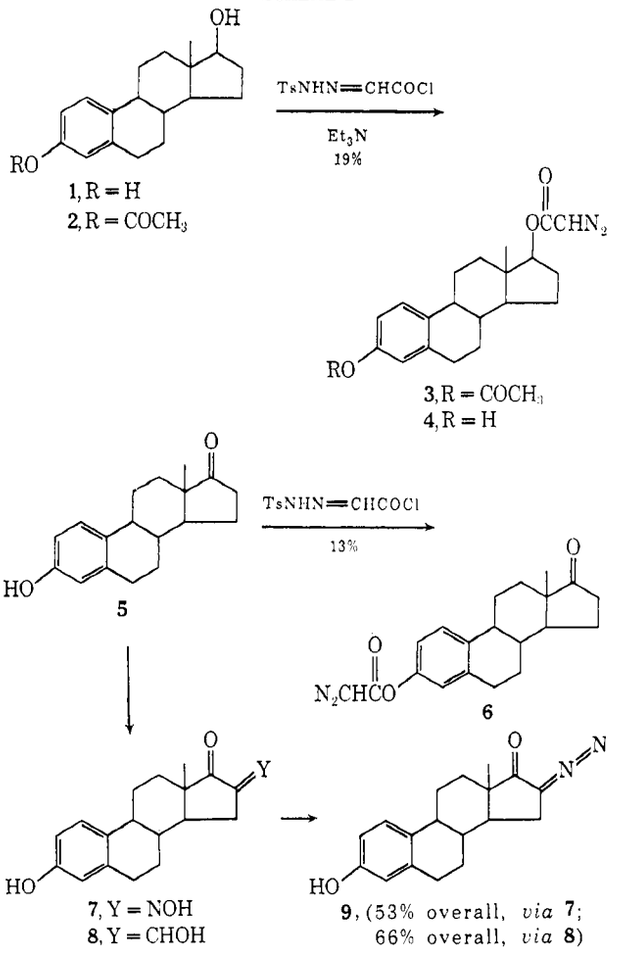
(17) A. Singh, E. R. Thornton, and F. H. Westheimer, *J. Biol. Chem.*, **237**, PC 3006 (1962).

More recently, the use of aryl azides has been described.[14a,15b,18]

Ideally, the inclusion of a photosensitive attaching function into the architecture of the steroidal ligand should result in a minimum disruption in the binding between steroid and protein. However, because so little is known about the nature of the binding site of the rat uterine protein we are interested in, we have chosen to restrict our initial efforts to relatively simple estrogen derivatives. The compounds we have prepared are functionalized at the chemically most accessible positions, the two hydroxyl groups, the positions ortho to the phenolic hydroxyl, and the positions α to the 17-keto group in estrone, and they bear one of the two known photoattaching functions. We have also investigated the synthesis of certain B-ring derivatives.

Diazo Compounds.—The preparation of estrone 3-diazoacetate (**6**) and estradiol 17-diazoacetate (**4**) is outlined in Scheme I. Treatment of estradiol (**1**) with

SCHEME I



1 equiv of the acid chloride of glyoxylic acid tosylhydrazone[19] allows the isolation of the 17-diazoacetate derivative **4** in 19% yield after purification.[20] None of the 3-diazoacetate or the 3,17-bis diazoacetate of estradiol was found. The apparent selectivity of this reagent for the 17 hydroxyl, rather than the phenolic hydroxyl, is curious, as most reported acylations occur preferentially at position 3.[21]

In order to establish the position of acylation rigorously, the 17-diazoacetate **4** was prepared *via* the well-characterized estradiol 3-acetate **2**.[22] The product obtained after potassium carbonate–methanol hydrolysis of the 3-acetate–17-diazoacetate **3** was identical in all respects with the product prepared by direct acylation of estradiol. The nmr spectrum of **4** (pyridine-d_5) further confirms the position of the diazoacetate: the 17α proton in **4** resonates as a triplet at δ 4.85, a position characteristic of 17β-acetates; the corresponding proton in 17β-estradiol resonates at δ 4.00.

Estrone 3-diazoacetate (**6**) was prepared directly from estrone by treatment with the tosylhydrazone of glyoxylic acid chloride. The diazo esters **4** and **6** are highly crystalline and are readily recrystallized from benzene or methanol. They are quite light sensitive since exposure to laboratory illumination at 25° results in some decomposition within 24 hr.

The diazo ketone D-ring derivative, 16-diazoestrone **9**, was prepared by two different routes (Scheme I). Nitrosation of estrone[23] gave 16-oximoestrone **7**, which, upon chloramine oxidation,[24] gave **9** in 53% overall yield from estrone. Alternatively 16-hydroxymethyleneestrone,[25] obtained by condensation of estrone with ethyl formate, was treated with tosyl azide and triethylamine[26] to give **9** in 66% yield overall from **5**. The diazo ketone **9** is a yellow, highly crystalline solid that is quite thermally stable. It can be repeatedly recrystallized from acetone and, in the crystalline state, can be stored for several months at room temperature, under normal laboratory illumination, without noticeable degradation.

In order to avoid problems that might arise from having a hydrolyzable ester function between the site of attachment and the position of radiolabel (generally, steroid positions 6 and 7), as in the diazoacetate derivatives **4** and **6**, three ether-linked diazo ketone derivatives (**12**, **17**, and **23**) were synthesized (Scheme II).

Treatment of estrone with excess ethyl bromoacetate in ethanolic sodium ethoxide gave the oxyacetic ester **10** in high yield. Hydrolysis with potassium hydroxide in ethanol was quantitative and gave the corresponding acid which was converted into the acid chloride with thionyl chloride and treated with excess diazomethane to give the diazo ketone **12** in 79% yield. This compound is a yellow crystalline solid that recrystallizes well from acetone and is quite thermally stable. Recrystallization from ethanol, however, causes some decomposition.

(18) (a) G. W. J. Fleet, J. R. Knowles, and R. R. Porter, *Biochem. J.*, **128**, 499 (1972); (b) R. Schwyzer and M. Coviezel, *Helv. Chim. Acta*, **54**, 1395 (1971); (c) M. B. Perry and L. L. W. Heung, *Can. J. Biochem.*, **50**, 510 (1972).

(19) (a) H. O. House and C. J. Blankley, *J. Org. Chem.*, **33**, 53 (1968); (b) H. O. House, F. J. Santer, and C. J. Blankley, *Org. Syn.*, **49**, 22 (1969).

(20) Although higher yields of diazoacetates have been obtained using this reagent in aliphatic systems (ref 19), alteration in reaction conditions and reagent stoichiometry failed to increase the yield of **4**.

(21) G. E. Abraham and P. K. Grover in "Principles of Competitive Protein Binding Assays," D. Odell and W. H. Daughaday, Ed., Lippincott, Philadelphia, Pa., 1971, p 140. We have also found that treatment of estradiol with 2 equiv of bromoacetyl bromide in tetrahydrofuran at 0° with no base present gives the 17β-bromoacetate (crude yield 95%; twice recrystallized, 66%).

(22) O. O. Orazi and R. A. Corral, *J. Amer. Chem. Soc.*, **91**, 2162 (1969).

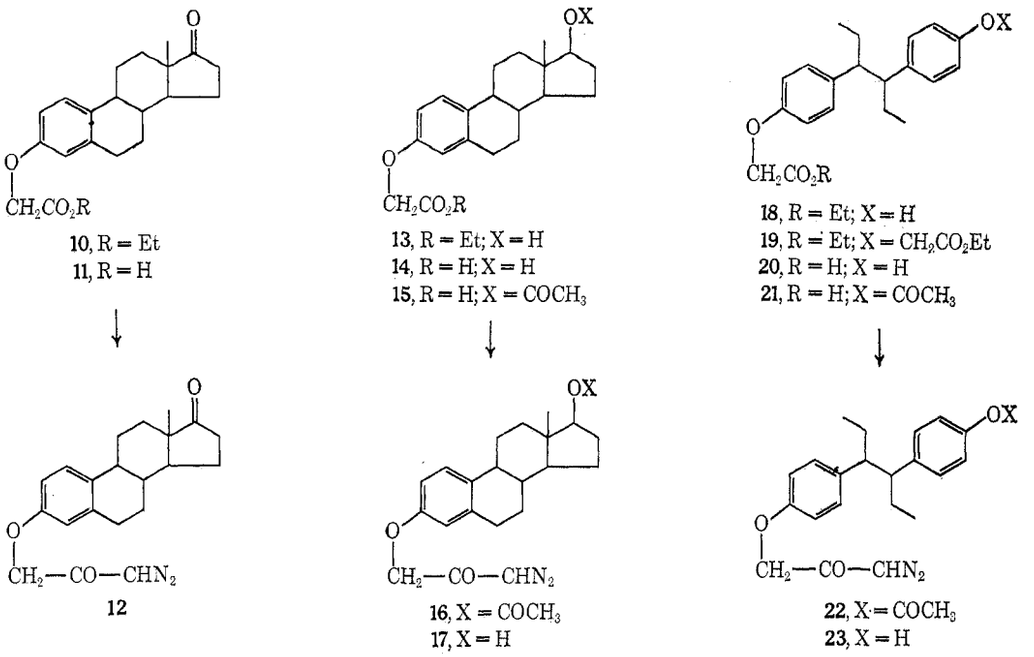
(23) M. N. Huffman and H. H. Darby, *J. Amer. Chem. Soc.*, **66**, 150 (1944).

(24) M. P. Cava and B. R. Vogt, *J. Org. Chem.*, **30**, 3775 (1965).

(25) R. O. Clinton, *Chem. Abstr.*, **70**, P4412*d* (1969).

(26) M. Regitz and J. Ruter, *Chem. Ber.*, **101**, 1263 (1968).

SCHEME II



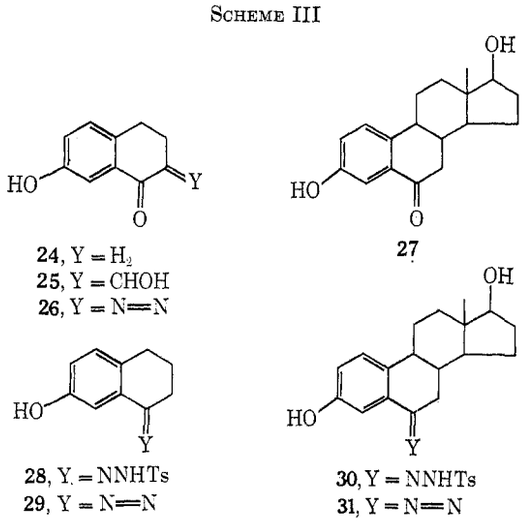
Synthesis of the corresponding diazo ketones in the estradiol and hexestrol series required selective reaction and protection of the two hydroxyl functions. Reaction of estradiol with excess ethyl bromoacetate was highly selective; only traces of the bisalkylated material could be detected by tlc after complete consumption of the starting estradiol. However, with hexestrol **44**, the equal reactivity of the two hydroxyl functions demanded more selective conditions. Reaction with a limited excess of ethyl bromoacetate and base for short time periods allowed the isolation of the monoalkylated hexestrol **18** in 44% yield, along with starting material (19%) and bis ester **19** (19%). The monoesters in both series were carried through an analogous series of steps: hydrolysis with potassium hydroxide in ethanol, protection of the free hydroxyl by acetylation with acetic anhydride–pyridine, conversion into the acid chloride with thionyl chloride, and reaction with excess diazomethane to give the protected diazo ketones **16** and **22**, respectively. With potassium carbonate, deacetylation of the hexestrol derivative was rapid, but the more hindered estradiol derivative required longer hydrolysis time. Hydrolysis with potassium hydroxide was less satisfactory.

The diazo ketones **17** and **23** are both yellow solids that can be readily recrystallized. They are somewhat less stable than the estrone derivative (**12**) but can be stored without decomposition at −20°.

All the diazo derivatives we have prepared show prominent molecular ions in their 70-eV mass spectra, a characteristic diazo stretching band in the infrared (2250–2050 cm^{-1}), and strong ultraviolet absorption at 250–280 nm (with a weak band or tail in the region 350–400 nm).

Several attempts were made to introduce photosensitive functional groups into positions 6 and 7 of the steroid (Scheme III). Due to the tedious and inefficient procedure involved in the preparation of 6-ketoestradiol (**27**),[27,28] 7-hydroxytetralone (**24**) was

SCHEME III



used as a model system. As done previously in the preparation of **9** (Scheme I), the diazo ketone **26** could be conveniently prepared *via* the hydroxymethylene derivative **25**. Nitrosation of this system also proceeded smoothly. However, despite numerous attempts, both nitrosation and condensation with ethyl formate failed completely in the steroid system (**27**). In each case, starting material could be recovered from the reaction mixtures in high yield. Construction of a space filling model attests to the greater steric hindrance of position 7 in **27** than the corresponding position in the tetralone system (**24**).

The possibility of introducing a diazo function directly into position 6 of the steroid was also investigated (Scheme III). The tosylhydrazone derivatives in both the steroid (**30**) and the model tetralin system (**28**) were decomposed with sodium methoxide in pyridine.[29] Direct assay of the reaction mixture during

(27) B. Longwell and O. Wintersteiner, *J. Biol. Chem.*, **133**, 219 (1940).

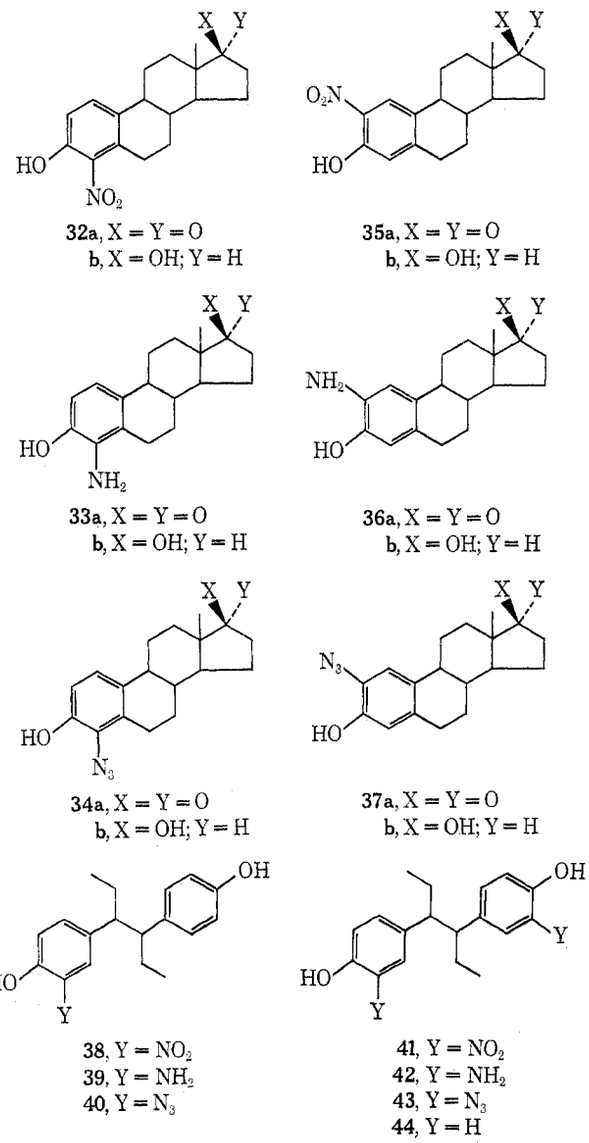
(28) P. D. G. Dean, D. Exley, and M. W. Johnson, *Steroids*, **18**, 593 (1971).

(29) D. G. Farnum, *J. Org. Chem.*, **28**, 870 (1963).

the initial phase of the reaction showed an ir band at 2240 cm^{-1}, characteristic of the desired diazo derivatives **29** and **31**. However, in both cases these derivatives proved to be thermally unstable; the deep red color of the reaction mixture (also characteristic of aryl-substituted diazomethanes[29,30]) soon faded, and no diazo products could be isolated.

Azides.—The azide derivatives **34a** and **b**, **37a** and **b**, **40**, and **43** were prepared by standard techniques (Scheme IV). Nitration of estrone **5** with 1 equiv of

SCHEME IV



nitric acid in glacial acetic acid gave a 1:1 mixture of the 2- and 4-nitroestrones **35a** and **32a**.[11c,31] These isomers could be separated readily by column chromatography and were isolated in 39 and 32% yield, respectively. Reduction with hydrogen over a palladium catalyst,[11c] or more conveniently using sodium dithionite,[32] furnished the amino compounds **33a** and **36a** in greater than 80% yield. As direct nitration of estradiol produced a complex mixture of products from which the desired nitroestradiols **32b** and **35b** could be obtained only with great difficulty, entry into the estradiol series was achieved through the nitro estrones. Sodium borohydride reduced the ketone in preference to the aryl nitro group, permitting the conversion of **32a** into **32b** and **35a** into **35b**. Alternatively, reduction of the amino estrones **33a** and **36a** with lithium aluminum hydride gave the amino estradiols **33b** and **36b** directly. The β orientation of the 17-hydroxyl in **32b** and **35b** was confirmed by 220-MHz nmr (in DMSO-d_6). Both compounds show a sharp singlet for the 18-methyl group at δ 0.68. The 18-methyl in 17β-estradiol also resonates at this position, but in the 17α epimer it is shifted to δ 0.62.[33,34]

Final transformation of the four amino estrogens **33a** and **33b** and **36a** and **36b** to the corresponding azides was achieved by diazotization with sodium nitrite in hydrochloric acid, followed by treatment with sodium azide.[35]

The same sequence of reactions was used to convert hexestrol into the 3-azide and the 3,3′-bisazide derivatives **40** and **43**. The initial nitration products **38** and **41** could be separated by careful column chromatography, and the identity of **41** as the symmetrical dinitration product was confirmed by its nmr and mass spectra.

All of the azides prepared are tan solids with ultraviolet absorption bands near 250 and 300 nm. They exhibit a characteristic azide stretch in the infrared (2120 cm^{-1}) and have weak, but detectable, molecular ions in their 70-eV mass spectra. Upon standing at room temperature, under normal laboratory light, all the azides show some decomposition; however, they may be stored for long periods of time at −20° without decomposition.

Conclusions

The photoaffinity labeling reagents that we have prepared should be widely applicable to the study of estrogen binding sites. The effect that structural modification of the steroidal ligand (resulting from embodiment of the photoattaching function) has on the binding affinity and the selectivity with which these compounds are capable of labeling binding sites are questions that must be answered individually in each system studied.[8]

Our particular interest is in labeling the estrogen binding protein of rat uterus. As pure preparations of this protein are not readily available, high binding affinity is crucial for high labeling selectivity. We have determined the binding affinity of these compounds for this protein by competitive assay. Certain ones display high affinity for the binding protein, and preliminary investigations indicate that they may indeed be capable of labeling the estrogen binding site.[8]

Experimental Section

The following chemicals were obtained from the sources indicated: estrone and 17β-estradiol (G. D. Searle; Steraloids) and

(30) B. Eistert, M. Regitz, G. Heck, and H. Schwall in "Houben-Weyl," Vol X/4, 4th ed., E. Muller, Ed., G. Thieme Verlag, Stuttgart, 1968, p 468.
(31) A. J. Tomson and J. P. Horwitz, *J. Org. Chem.*, **24**, 2056 (1959).
(32) S. Kraychy, *J. Amer. Chem. Soc.*, **81**, 1702 (1959).
(33) L. M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," 2nd ed, Pergamon Press, Elmsford, N. Y., 1969, p 242.
(34) In most of the nmr spectra run in DMSO-d_6, the resonance due to the 17α hydrogen, which generally falls at δ 3.7 (6, J = 7 Hz), was obscured by broad signals due to water absorbed by the samples.
(35) E. Noetling and O. Michael, *Chem. Ber.*, **26**, 86 (1893).

hexestrol (Mann). Ethanol-free chloroform was obtained by passing chloroform reagent over alumina. Diazomethane was freshly prepared as alcohol-free ethereal solution from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (Diazald, Aldrich) using preparation II as given. Spectroscopic data were obtained on the instruments listed: ir (Perkin-Elmer 237), nmr (Varian A-60, HA-100, or HR 220), mass spectra (Varian-MAT CH-5 and 731), uv (Cary-White 15), and molecular rotation (Bendix NPL polarimeter). Microanalysis was performed by the microanalytical service of the University of Illinois.

A standard procedure for product isolation was used in all reactions: exhaustive extraction with a solvent, drying over an anhydrous salt, filtration, and evaporation of solvent under reduced pressure on a rotary evaporator. In each procedure, the extraction solvent and drying agent used are given in parentheses.

Analytical thin layer chromatography (tlc) was performed either on Eastman Chromatosheets 6060 or Brinkmann F-254 glass-backed plates. The following solvent systems were often used: system A, chloroform–ether (97:3); system B, benzene–ethyl acetate–chloroform (5:1:5); system C, benzene–ethyl acetate–acetic acid (90:10:0.5); system D, ether–hexane (1:1). The R_f values for estrone, estradiol, and hexestrol on Eastman Chromatosheets in these system follow (respectively): A, 0.45, 0.33, 0.25; B, 0.35, 0.22, 0.23; C, 0.43, 0.32, 0.41; D, 0.14, 0.10, 0.23.

Estradiol 3-Acetate (2).—A solution of 400 mg (1.47 mmol) of estradiol (1) and 357 mg (1.9 mmol) of 3-acetyl-1,5,5-trimethylhydantoin[22] in 8 ml of THF was refluxed under nitrogen for 31 hr. After the solution was allowed to cool, the solvent was removed under reduced pressure, and water was added. Product isolation (EtOAc; $MgSO_4$) gave 360 mg of a pale yellow oil which was purified by preparative tlc (R_f 0.4, system A). Recrystallization from ethyl acetate–hexane furnished 280 mg (60%) of pure white product (2), mp 139–140° (lit.[22] mp 136–139°).

Estradiol 3-Acetate 17-Diazoacetate (3).—A solution of 246 mg (0.95 mmol) of glyoxylic acid chloride *p*-toluenesulfonylhydrazone and 100 mg (0.316 mmol) of estradiol 3-acetate (2) in 10 ml of ethyl acetate was treated dropwise at 0° with 95 mg (0.95 mmol) of triethylamine in 1 ml of ethyl acetate. An additional 190 mg of triethylamine was added, and the reaction mixture was stirred at 0° for another hour. After the solution was filtered to separate triethylamine hydrochloride, dilute sodium bicarbonate was added. Product isolation (EtOAc; $MgSO_4$) gave a pale yellow liquid which after preparative tlc (R_f 0.70, system A) yielded 40 mg (32%) of product **3** as an oil that failed to crystallize: ir ($CHCl_3$) 2120 (C=N=N), 1750 (C=O, acetyl), and 1680 cm^{-1} (C=O, diazo ester); nmr ($CDCl_3$) δ 0.8 (s, 3 H), 2.4 (s, 3 H), 4.7 (s, 1 H), 6.8–7.7 (m, 3 H).

Anal. Calcd for $C_{22}H_{26}N_2O_4$: mol wt, 382.1891. Found: mol wt (high-resolution mass spectrum), 382.1893.

Estradiol 17-Diazoacetate (4) [from Estradiol (1)].—A solution of 384 mg (1.47 mmol) of glyoxylic acid chloride *p*-toluenesulfonylhydrazone and 405 mg (1.48 mmol) of estradiol (1) in 10 ml of ethyl acetate was treated dropwise at 0° with 154 mg (1.52 mmol) of redistilled triethylamine in 2 ml of ethyl acetate. After 0.5 hr, an additional 225 mg (2.22 mmol) of triethylamine was added, and the resulting solution was stirred at room temperature for 1 hr. The reaction mixture was filtered to separate 180 mg of triethylamine hydrochloride, and the solvent was removed under reduced pressure. The crude diazoacetate was purified by preparative tlc (R_f 0.63, system A) to yield 95 mg (19%) of a yellow solid. Recrystallization from methanol gave pure estradiol 17-diazoacetate (4): mp 172–182° dec; ir (KBr) 2120 (C=N=N) and 1655 cm^{-1} (C=O, diazo ester); uv (EtOH) λ_{max} 246 (ϵ 1600), 283 (23,400); nmr ($CDCl_3$) δ 0.78 (s, 3 H, $-CH_3$), 1.0–3.1 (m, 15 H), 4.75 (t, 1 H), 4.80 (s, 1 H, N=CH), 6.5–7.2 (m, 3 H, aromatic); mass spectrum (70 eV) *m/e* (rel intensity) 340 (M^+, 89) 312 (1), 270 (3), 239 (2), 211 (4), 159 (100).

Anal. Calcd for $C_{20}H_{24}N_2O_3$: C, 70.57; H, 7.11; N, 8.23. Found: C, 70.29; H, 7.13; N, 8.21.

Estradiol 17-Diazoacetate (4) (from 3).—A solution of 40 mg (0.105 mmol) of estradiol 3-acetate 17-diazoacetate (3) in 1 ml of methanol was mixed with 2 ml of methanolic potassium carbonate and stirred at room temperature. After 24 hr, water was added, and the product was isolated (ether; $MgSO_4$). The crude product was purified by preparative tlc (R_f 0.63, system A) to yield 20 mg (57%) of pure ester (4), spectroscopically and chromatographically identical with the ester 4 prepared directly from estradiol.

Estrone 3-Diazoacetate (6).—Glyoxylic acid chloride *p*-toluenesulfonylhydrazone (400 mg, 1.54 mmol) in 5 ml of methylene chloride was treated dropwise at 0° with a solution of estrone (5) (400 mg, 1.48 mmol) and redistilled triethylamine (150 mg, 1.48 mmol) in 4 ml of methylene chloride–THF (1:1). After 0.5 hr, an additional 225 mg (2.22 mmol) of triethylamine was added, and the resulting solution was allowed to warm to room temperature and to stir for an additional hour. The reaction mixture was filtered to separate 100 mg of triethylamine hydrochloride, and the solvent was removed at 25° under reduced pressure. The crude product was purified by preparative tlc on alumina (chloroform, 2 developments) to yield 69 mg (13%) of a pale yellow solid. Recrystallization from benzene–petroleum ether gave estrone diazoacetate (6) as pale yellow prisms: mp 159–160°; uv (EtOH) λ_{max} 215 nm (ϵ 9200), 250 (17,350); ir (KBr) 2125 (C=N=N), 1637 (C=O, cyclopentyl), 1655 cm^{-1} (C=O, diazo ester); nmr ($CDCl_3$) δ 0.9 (s, 3 H, $-CH_3$), 1–3.0 (m, 15 H), 4.7 (s, 1 H, N=CH—), 6.8–7.5 (m, 3 H, aromatic); mass spectrum (70 eV) *m/e* (rel intensity) 338 (M^+, 12), 270 (100), 213 (7), 186 (6), 160 (7), 159 (6), 146 (14), 115 (8), 91 (7), 69 (9), 55 (6), 28 (25).

Anal. Calcd for $C_{20}H_{22}N_2O_3$: C, 70.99; H, 6.55; N, 8.28. Found: C, 70.80; H, 6.48; N, 8.35.

16-Oximoestrone (7).[23]—Estrone (22.2 mmol, 6.06 g) was stirred under nitrogen in 50 ml of freshly distilled *tert*-butyl alcohol for 10 min (dissolution incomplete). Potassium *tert*-butoxide (110 mmol, 12.0 g) was added, and stirring was continued for 30 min (dissolution complete). *n*-Butyl nitrite (35 mmol, 5 ml) was added *via* syringe, and the solution turned deep red, and then orange within a few minutes. A second portion of *n*-butyl nitrite (35 mmol, 5.0 ml) was added after 8 hr, and stirring was continued an additional 16 hr. The reaction mixture was quenched in 100 ml of ice and acidified with glacial acetic acid. The precipitate that formed was redissolved in THF–EtOAc (1:1), and product isolation ($MgSO_4$) gave a yellow solid that was triturated with three small portions of cold acetone to give 5.71 g (85%) of 16-oximo estrone as a off-white powder. This material was homogeneous by tlc (R_f 0.17, system B). Further purification by recrystallization from THF–hexane afforded white crystals: mp 238–239°; uv (EtOH) λ_{max} 280 nm (ϵ 2880), 232 (13,900); $[\alpha]^{24}D$ +87° (*c* 0.4416, THF); ir 3600–3000 (broad, OH), 1750 (C=O), 1650, 1510, 950 cm^{-1}; nmr (DMSO-d_6) δ 0.88 (s, 3 H), 6.3–7.1 (3 H, aromatic); mass spectrum (70 eV) *m/e* (rel intensity) 299 (M^+, 99), 254 (100), 213 (46), 159 (55), 146 (21), 145 (22), 133 (56), 62 (19).

Anal. Calcd for $C_{18}H_{21}NO_3$: C, 72.22; H, 7.07; N, 4.68. Found: C, 72.10; H, 7.06; N, 4.86.

16-Hydroxymethylenestrone (8).[25]—Estrone (5) (5 g, 18.5 mmol) was dissolved in 25 ml of THF with warming on the steam bath, and 125 ml of dry benzene was added. Sodium hydride (13.1 g of a 57% dispersion in mineral oil, 315 mmol) was washed with three portions of dry benzene to remove the mineral oil and transferred as a slurry to the steroid solution with vigorous stirring. Five or six drops of absolute ethanol were added, and then the flask was placed under a nitrogen atmosphere. After stirring for 25 min, ethyl formate (20.2 g, 22.0 ml, 272 mmol) was added dropwise over a 30-min period. The reaction mixture was stirred vigorously at 25°, and the progress of the reaction was monitored periodically by tlc of an acidified aliquot. After 24 hr, the reaction vessel was cooled in ice, and 2 *N* HCl added cautiously (frothing) until the mixture was acidic. Product isolation (EtOAc, $MgSO_4$) gave 6.5 g of a solid. Tlc analysis (R_f 0.27, system B) showed a small amount of contaminating estrone that could be removed by chromatography on silica gel, but the product became discolored by this procedure. Recrystallization from acetone was inefficient, but gave chromatographically homogeneous material, mp 229–231° (lit.[25] 229–232°). As the next product (16-diazo estrone 9) crystallized readily, the crude product was used without further purification: $[\alpha]^{24}D$ +144° (*c* 0.357, THF); ir (KBr) 3400 (broad, OH), 1720 (C=O), 1640 cm^{-1}; nmr (DMSO-d_6) δ 0.82 (s, 3 H), 2.73 (br t), 6.4–7.3 (3 H, aromatic), 7.43 (br s, 1 H); mass spectrum (70 eV) *m/e* (rel intensity) 298 (M^+, 100), 214 (17), 213 (59), 160 (24), 159 (38), 146 (33), 133 (26).

16-Diazoestrone (9) [(from 16-Hydroxymethyleneestrone (8)].—The crude 16-hydroxymethylenestrone (8) (5.9 g, 20.4 mmol) was dissolved in 50 ml of THF and 400 ml of methylene chloride and 12 ml (71.4 mmol) of triethylamine were added. After

flushing the flask with nitrogen, 10.5 ml (52.4 mmol) of tosyl azide dissolved in 30 ml of methylene chloride was added over a 10-min period. After stirring at 25° for 2–3 hr, product isolation (THF–EtOAc; $MgSO_4$) gave the yellow, crystalline solid which was freed from excess tosyl azide by trituration with benzene and ether. The yellow, crystalline product (3.9 g, 66%) was homogeneous by tlc analysis (R_f 0.44, system B). Recrystallization of 500 mg from acetone gave 386 mg of analytically pure material: mp 215–217° dec; uv (EtOH) λ_{max} 251 nm (ϵ 3200), 282 (1130), 288 (1140), 305 (sh), 381 (ϵ 4); $[\alpha]^{24}D$ −38° (*c* 0.5136, THF); ir (KBr) 3470 (br), 2040 (s), 1665, 1640, 1600, 1575, 1495, 1460, 1445, 1320, 1250 cm^{-1}; nmr (DMSO-d_6) δ 0.95 (s, 3 H), 2.75 (m), 6.3–7.1 (3 H, aromatic), 8.92 (s, 1 H, OH); mass spectrum (70 eV) *m/e* (rel intensity) 296 (M^+, 100), 211 (22), 172 (25), 160 (23), 159 (56), 158 (23), 157 (31), 146 (22), 145 (27), 133 (40), 79 (21).

Anal. Calcd for $C_{18}H_{20}N_2O_2$: C, 72.95; H, 6.80; N, 9.45. Found: C, 72.87; H, 6.92; N, 9.25.

16-Diazoestrone (9) [(from 16-Oximoestrone (7)].—16-Oximoestrone (7) (135 mg, 0.45 mmol) was dissolved in 4 ml of THF and 0.5 ml of a 5 *N* sodium hydroxide solution, and 0.8 ml of a 28% ammonium hydroxide solution was added. A solution of sodium hypochlorite (1 ml, 5.25%, 1 mmol; Clorox) was added, and the reaction mixture was allowed to stir under nitrogen for 6 hr at 25°. Product isolation (EtOAc; $MgSO_4$) gave 100 mg of crude product. Preparative tlc (chloroform–ethanol 9:1, 2 developments; 8:2, 2 developments) furnished 83 mg (62%) of the yellow, crystalline product 9 that was identical in all respects with the material prepared by the preceding method.

3-*O*-Carbethoxymethylestrone (10).—Estrone (2 g, 7.4 mmol) was dissolved in 35 ml of ethanol and 10 ml of THF. Sodium ethoxide (11 mmol; 5.5 ml of 2 *M* solution in ethanol) and 3.7 g (3 ml, 22.2 mmol) of ethyl bromoacetate were added, and the reaction mixture was refluxed for 24 hr. An additional portion of sodium ethoxide and ethyl bromoacetate was added, and refluxing continued for 2 hr. After the reaction mixture was quenched in saturated NaCl solution, product isolation (EtOAc–THF; $MgSO_4$) gave 2.41 g of crude product. A small amount of contaminating ethyl bromoacetate was removed by trituration with hexane to give 2.39 g (91%) of a white crystalline solid that was homogeneous by tlc (R_f 0.58, system A). An analytical sample was prepared by recrystallization from acetone–hexane: mp 98–100°; $[\alpha]^{24}D$ +138° (*c* 0.5253, THF); ir ($CHCl_3$) 1755–1720 (s), 1605, 1575, 1495 cm^{-1}; nmr (DMSO-d_6) δ 0.83 (s, 3 H), 1.22 (t, J = 7 Hz, 3 H), 2.8 (br t), 4.16 (q, J = 7 Hz, 2 H), 4.67 (s, 2 H), 6.5–7.3 (3 H, aromatic); mass spectrum (70 eV) *m/e* (rel intensity) 356 (M^+, 32), 136 (24), 121 (21), 109 (33), 107 (20), 95 (21), 93 (21), 81 (47), 69 (100).

Anal. Calcd for $C_{22}H_{28}O_4$: C, 74.13; H, 7.92. Found: C, 73.94; H, 7.90.

3-*O*-Carboxymethylestrone (11).—3-*O*-Carbethoxymethylestrone (10) (2.29 g, 7.1 mmol) was dissolved in 35 ml of ethanol and 8 ml of a 1 *M* potassium hydroxide solution (8 mmol) was added. The reaction mixture was stirred overnight at room temperature, the solvent evaporated, and the residual salt washed with ether. The washed residue was dissolved in water and acidified with 10% H_2SO_4. Product isolation (ether–THF; $MgSO_4$) gave 1.78 g of crude acid. Recrystallization from benzene–ethyl acetate gave 1.46 g (63%) of a white crystalline solid, mp 214–215°, (R_f 0.39, system C). An analytical sample was prepared by three recrystallizations from the same solvent system: $[\alpha]^{24}D$ +159° (*c* 0.4651, THF); ir (KBr) 3600–2200 (br OH), 1750 (C=O), 1620, 1510, 1250 cm^{-1}; nmr ($CDCl_3$) δ 0.92 (s, 3 H), 2.85 (br, t, 2 H), 4.63 (s, 2 H), 6.5–7.4 (3 H, aromatic), 7.8 (br s, 1 H, OH); mass spectrum (70 eV) *m/e* (rel intensity) 328 (M^+, 100), 243 (22), 204 (24), 115 (20), 97 (33), 92 (23), 91 (36).

Anal. Calcd for $C_{20}H_{24}O_4$: C, 73.15; H, 7.37. Found: C, 72.80; H, 7.27.

3-*O*-(3′-Diazo-2′-ketopropyl)estrone (12).—3-*O*-Carboxymethylestrone (11) (200 mg, 0.61 mmol) was dissolved in 12 ml of ethanol-free chloroform and 435 mg (0.262 ml, 3.66 mmol) of thionyl chloride and 0.003 ml of pyridine were added. After 18 hr at 25°, the solvent was evaporated at room temperature under vacuum, and excess thionyl chloride was removed by evaporation of two additional 5-ml portions of ethanol-free chloroform. The acid chloride in a small volume of chloroform was added to a large excess (*ca.* 6 mmol) of distilled diazomethane in ether at 0°. After 3 hr at 0° and 18 hr at 25°, half of the solvent was distilled off on the steam bath, and the solution remaining was filtered (Celite) and evaporated to give 258 mg of a yellow crystalline solid. Purification by preparative tlc (ether–hexane 1:3, 2 developments) gave 170 mg (79%) of a yellow crystalline solid (R_f 0.39, system D). An analytical sample was prepared by recrystallization from acetone: mp 156–158°; uvλ (EtOH) 272 nm (ϵ 7700); $[\alpha]^{24}D$ +125° (*c* 0.5058, THF); ir (KBr) 2120 (C=N=N), 1750 (C=O), 1650, 1510, 1380 cm^{-1}; nmr ($CDCl_3$) δ 0.92 (s, 3 H), 2.83 (2 H), 4.50 (s, 2 H), 5.75 (s, 1 H), 6.5–7.3 (3 H, aromatic); mass spectrum (70 eV) *m/e* (rel intensity) 352 (M^+, 19), 326 (17), 324 (54), 296 (6), 270 (100), 238 (12), 226 (16), 213 (26), 199 (13).

Anal. Calcd for $C_{21}H_{24}N_2O_3$: C, 71.57; H, 6.86; N, 7.95. Found: C, 71.21; H, 7.10; N, 7.82.

3-*O*-Carbethoxymethylestradiol (13).—Estradiol (2 g, 7.4 mmol) dissolved in 30 ml of absolute ethanol, 5.5 ml of a 2 *M* solution of sodium ethoxide in ethanol, and 2.45 ml (3.67 g, 22 mmol) of ethyl bromoacetate were allowed to react by the procedure used to prepare 3-*O*-carbethoxymethylestrone (10). Evaporation of excess ethyl bromoacetate (40°, 1 mm) gave 2.66 g of a yellow oil which was purified by column chromatography ($CHCl_3$ and $CHCl_3$–EtOH 97:3, as eluent). The chromatographically homogeneous fractions were pooled to give 2.43 g (85%) of a white solid, mp 88–90°. An analytical sample was prepared by recrystallization from hexane: $[\alpha]^{24}D$ +60° (*c* 0.6959, THF); ir (KBr) 3500 (OH), 1760 (C=O), 1500, 1220 cm^{-1}; nmr ($CDCl_3$) δ 0.75 (s, 3 H), 1.28 (t, J = 7 Hz, 3 H), 2.8 (br t, 2 H), 3.68 (t, J = 7 Hz, 1 H), 4.22 (q, J = 7 Hz, 2 H), 4.52 (s, 2 H), 6.5–7.3 (3 H, aromatic); mass spectrum (70 eV) *m/e* (rel intensity) 358 (M^+, 100), 299 (10), 285 (6), 271 (15), 258 (16), 253 (21), 246 (10), 232 (11), 219 (7), 159 (23).

Anal. Calcd for $C_{22}H_{30}O_4$: C, 73.71; H, 8.44. Found: C, 73.47; H, 8.22.

3-*O*-Carboxymethylestradiol (14).—3-*O*-Carbethoxymethylestradiol (13) (2.43 g, 5.5 mmol) was dissolved in 35 ml of ethanol, and 6 ml of 1 *M* aqueous potassium hydroxide solution was added. Reaction and product isolation as described for 3-*O*-carboxymethylestrone gave 1.93 g of crude product. After recrystallization from ethyl acetate, 1.64 g (91%) of a white crystalline solid was obtained. An analytical sample was prepared by three recrystallizations from toluene–ethyl acetate: mp 214–215°; $[\alpha]^{24}D$ +50° (*c* 0.5998, THF); ir (KBr) 3600–2200 (broad, OH), 1735 (C=O), 1610, 1500, 1220 cm^{-1}; nmr (DMSO-d_6) δ 0.67 (s, 3 H), 2.75 (br t), 3.54 (t, J = 6 Hz, 1 H), 4.57 (s, 2 H), 6.5–7.3 (3 H, aromatic); mass spectrum (70 eV) *m/e* (rel intensity) 330 (M^+, 100), 271 (30), 253 (13), 230 (21), 218 (23), 204 (18), 159 (17), 157 (15), 114 (17).

Anal. Calcd for $C_{20}H_{26}O_4$: C, 72.70; H, 7.93. Found: C, 72.39; H, 7.92.

3-*O*-Carboxymethylestradiol 17-Acetate (15).—3-*O*-Carboxymethylestradiol (14) (1.64 g, 5.0 mmol) was dissolved in 12 ml of pyridine and 4 ml (40 mmol) of acetic anhydride was added. After stirring at 25° for 18 hr, water was added and the product extracted with ethyl acetate. The extracts were washed with 10% sulfuric acid, dried ($MgSO_4$), and evaporated to give 1.75 g (94%) of crude acetate. Recrystallization from benzene gave 1.13 g (61%) of a white, crystalline solid that was chromatographically homogeneous (R_f 0.48, system C). An analytical sample was recrystallized three times from ethyl acetate–hexane: mp 195–197°; $[\alpha]^{24}D$ +56° (*c* 0.422, THF); ir (KBr) 3600–2200 (broad, OH), 1770 (ester C=O), 1750 (acid C=O), 1510, 1250 cm^{-1}; nmr (DMSO-d_6)^{34} δ 0.80 (s, 3 H), 2.00 (s, 3 H), 2.75 (br t), 4.57 (s, 2 H), 6.5–7.3 (3 H, aromatic); mass spectrum (70 eV) *m/e* (rel intensity) 372 (M^+, 100), 271 (16), 253 (20), 230 (22), 217 (18), 204 (16), 191 (13), 158 (17), 145 (13), 115 (13), 81 (17), 77 (28).

Anal. Calcd for $C_{22}H_{28}O_5$: C, 70.94; H, 7.58. Found: C, 71.14; H, 7.53.

3-*O*-(3′-Diazo-2′-ketopropyl)estradiol 17-Acetate (16).—3-*O*-Carboxymethylestradiol 17-acetate (15) (120 mg, 0.32 mmol) was dissolved in 10 ml of ethanol-free chloroform and treated with 0.15 ml (357 mg, 3.0 mmol) of thionyl chloride. Three drops of pyridine were added, and the reaction mixture was stirred at 50° for 5 hr. Reaction according to the procedure used in the preparation of the corresponding estrone derivative 12 gave 132 mg of a solid, which contained a small amount of 3-carboxymethylestradiol 17-acetate (tlc analysis). Purification by preparative tlc (ether–hexane, 1:3, 1 development; 1:1, 2 developments) gave 45 mg (35%) of a pale yellow solid that was chromatographically homogeneous (R_f 0.55, system D) and analytically pure: mp 97° dec; uv λ_{max} (EtOH) 2700 nm (ϵ

9480); $[\alpha]^{24}D$ +24° (*c* 0.6800, THF); ir (KBr) 2140 (C=N=N), 1750 (broad C=O), 1650, 1510, 1380, 1260 cm^{-1}; nmr ($CDCl_3$) δ 0.83 (s, 3 H), 2.04 (s, 3 H), 2.8 (br t, 2 H), 4.48 (s, 2 H), 4.70 (t, *J* = 8 Hz, 1 H), 5.73 (s, 1 H) 6.5–7.3 (3 H, aromatic); mass spectrum (70 eV) *m/e* (rel intensity) 396 (M^+, 3), 368 (3), 326 (3), 314 (5), 213 (2), 172 (3), 160 (5), 147 (4), 133 (4), 55 (12), 43 (21), 28 (100).

Anal. Calcd for $C_{23}H_{28}N_2O_4$: C, 69.68; H, 7.12; N, 7.07. Found: C, 69.68, H, 7.06; N, 7.17.

3-*O*-(3′-Diazo-2′-ketopropyl)estradiol (17).—Diazoketopropylestradiol acetate (16) (252 mg, 0.64 mmol) was dissolved in 75 ml of methanol–THF (2:1) and 440 mg (3.8 mmol) of potassium carbonate in 10 ml of methanol was added. After stirring 22 hr at 25°, the crude product was isolated (EtOAc, $MgSO_4$). Purification by preparative tlc (system A, 2 developments) gave 108 mg (48%) of a pale yellowc rystalline solid: mp 150° dec; uv λ_{max} (EtOH) 273 nm (ε 8000); $[\alpha]^{24}D$ −12° (*c* 0.3374, THF); ir (KBr) 3500 (OH), 2120 (C=N=N), 1650 (C=O), 1510, 1380, 1260 cm^{-1}; nmr ($CDCl_3$) δ 0.8 (s, 3 H), 3.7 (t, 1 H), 4.5 (s, 2 H), 5.7 (s, 1 H), 6.6–7.3 (3 H, aromatic); mass spectrum (70 eV) *m/e* (rel intensity) 354 (M^+, 2), 326 (3), 272 (11), 213 (4), 160 (5), 159 (5), 43 (100).

Anal. Calcd for $C_{21}H_{26}N_2O_3$: C, 71.16; H, 7.39; N, 7.90. Found: C, 70.90; H, 7.26; N, 7.78.

4-*O*-Carbethoxymethylhexestrol (18).—Hexestrol (6.5 g, 24.1 mmol) was dissolved in 200 ml of absolute ethanol, and 14.5 ml of a 2 *M* solution of sodium ethoxide in ethanol was added, followed by 8.1 ml (12.1 g, 72.3 mmol) of ethyl bromoacetate. After 0.5 hr at 25°, tlc analysis (product R_f 0.43, system A) indicated that dialkylated product (19) was beginning to form. Saturated sodium chloride solution (100 ml) was added, and product isolation (THF–ether; $MgSO_4$) gave a pale yellow oil which was chromatographed on a column of silica gel, eluting with chloroform–ethanol (99:1). Fractions containing 4-*O*-carbethoxymethylhexestrol (18) were pooled and the solvent was removed to yield 3.7 g (43.5%) of a white solid. Recrystallization from benzene gave plates: mp 134–136°; ir (KBr) 3440 (OH), 1740 (ester C=O), 1220 cm^{-1} (O–C); nmr (DMSO-d_6) δ 0.5 (t, 6 H), 1.2 (m, 7 H), 2.5 (m, 2 H), 4.2 (q, 2 H), 4.7 (s, 2 H), 6.6–7.1 (m, 8 H, aromatic); mass spectrum (70 eV) *m/e* (rel intensity) 263 (2), 223 (2), 222 (16), 221 (100), 219 (10), 193 (10), 147 (20), 135 (30).

Anal. Calcd for $C_{22}H_{28}O_4$: C, 74.13; H, 7.92. Found: C, 74.25; H, 7.92.

4,4′-*O*-Bis(carbethoxymethyl)hexestrol (19).—Fractions containing the product 19 were pooled and solvent was removed to yield 2.0 g (18.6%) of a white solid. Recrystallization from benzene gave plates: mp 110–112°; ir (KBr) 1760 (ester C=O), 1230 cm^{-1} (O–C); nmr (DMSO-d_6) δ 0.5 (t, 6 H), 1.3 (m, 10 H), 2.5 (m, 2 H), 4.1 (q, 4 H), 4.7 (s, 4 H), 6.8–7.2 (m, 8 H, aromatic); mass spectrum (70 eV) *m/e* (rel intensity) 442 (M^+, 8), 369 (14), 223 (39), 222 (100), 221 (84), 207 (10), 206 (14).

Anal. Calcd for $C_{26}H_{34}O_6$: C, 70.56; H, 7.74. Found: C, 70.82; H, 7.96.

4-*O*-Carboxymethylhexestrol (20).—4-*O*-Carbethoxymethylhexestrol (18) (3.0 g, 8.45 mmol) was dissolved in 60 ml of absolute ethanol, and 10 ml of 1 *M* aqueous potassium hydroxide solution was added. Reaction and product isolation according to the procedure for 3-*O*-carboxymethylestrone (11) gave 2.68 g (98%) of a white solid. Recrystallization from benzene gave white crystals: mp 137–140°; ir (KBr) 3400 (OH), 1750 (C=O), 1230 cm^{-1} (O–CH_2); nmr (DMSO-d_6) δ 0.5 (t, 6 H), 1.2 (m, 4 H), 2.5 (m, 2 H), 4.6 (s, 2 H), 6.8–7.2 (m, 8 H, aromatic), 7.35 (s, 1 H, phenolic OH); mass spectrum (70 eV) *m/e* (rel intensity) 328 (M^+, 1), 193 (100), 165 (42), 147 (48), 134 (42).

Anal. Calcd for $C_{20}H_{24}O_4$: C, 73.15; H, 7.37. Found: C, 72.93; H, 7.38.

4-*O*-Carboxymethylhexestrol 4′-Acetate (21).—4-*O*-Carboxymethylhexestrol (20) (1.4 g, 4.26 mmol) was dissolved in 16 ml of pyridine, and 3.94 ml (4.26 g, 42.6 mmol) of acetic anhydride was added. Reaction and product isolation according to the procedure for 3-*O*-carboxymethylestradiol 17-acetate (15) gave after recrystallization from benzene 1.5 g (95%) of a fine white powder: mp 125–127°; ir (KBr) 1770 (acid C=O), 1730 s (ester C=O), 1220 cm^{-1} (O–C); nmr (DMSO-d_6) δ 0.5 (t, 6 H), 1.2 (m, 4 H), 2.3 (s, 3 H), 2.5 (m, 2 H), 4.6 (s, 2 H), 6.8–7.4 (m, 8 H, aromatic); mass spectrum (70 eV) *m/e* (rel intensity) 370 (M^+, 1), 221 (2), 193 (100), 164 (10), 147 (13), 135 (26), 107 (20), 91 (60).

Anal. Calcd for $C_{22}H_{26}O_5$: C, 71.33; H, 7.07. Found: C, 71.44; H, 6.94.

4-*O*-(3′-Diazo-2′-ketopropyl)hexestrol 4′-Acetate (22).—Carboxymethylhexestrol acetate (21) (1.5 g, 4.05 mmol) was dissolved in 25 ml of ethanol-free chloroform and treated with 2.9 ml (4.8 g, 40.5 mmol) of thionyl chloride. One drop of pyridine was added. Reaction and product isolation according to the procedure used in the preparation of 3-*O*-(3′-diazo-2′-ketopropyl)-estradiol 17-acetate (16) gave 1.56 g of a brown solid. Purification by preparative tlc (system A, 2 developments) gave after recrystallization from benzene 320 mg (20%) of a pale yellow crystalline solid: mp 122° dec; uv λ_{max} (EtOH) 268 nm (ε 9350); ir (KBr) 2120 (C=N=N), 1760 (ester C=O), 1635 cm^{-1} (ketone C=O); nmr (DMSO-d_6) δ 0.5 (t, 6 H), 1.3 (m, 4 H), 2.2 (s, 3 H), 2.5 (m, 2 H), 4.6 (s, 2 H), 6.2 (s, 1 H), 6.8–7.2 (m, 8 H, aromatic); mass spectrum (70 eV) *m/e* (rel intensity) 394 (M^+, 4), 221 (22), 217 (100), 207 (59), 189 (45), 147 (44), 136 (52).

Anal. Calcd for $C_{23}H_{26}N_2O_4$: C, 70.03; H, 6.64; N, 7.10. Found: C, 69.90: H, 6.38; N, 7.19.

4-*O*-(3′-Diazo-2′-ketopropyl)hexestrol (23).—Diazoketopropylhexestrol acetate (160 mg, 0.405 mmol) was dissolved in 10 ml of methanol, and 2 ml of saturated potassium carbonate solution was added. Reaction and product isolation according to the procedure for 3-*O*-(3′-diazo-2′-ketopropyl)estradiol (17) gave 92 mg (64%) of a pale yellow solid, after purification by preparative tlc ($CHCl_3$, 2 developments): mp 92° dec; uv λ_{max} (EtOH) 274 nm (ε 9920); ir (KBr) 3400 (OH), 2120 (C=N=N), 1620 cm^{-1} (ketone C=O); nmr (DMSO-d_6) δ 0.5 (t, 6 H), 1.3 (m, 4 H), 2.5 (m, 2 H), 4.6 (s, 2 H), 6.2 (s, 1 H), 6.8–7.2 (m, 8 H, aromatic), 9.1 (s, 1 H, OH); mass spectrum (70 eV) *m/e* (rel intensity) 352 (M^+, 4), 271 (14), 270 (64), 216 (67), 191 (44), 165 (37), 137 (33), 136 (100).

Anal. Calcd for $C_{21}H_{24}N_2O_3$: C, 71.57; H, 6.86; N, 7.95. Found: C, 71.83; H, 6.99; N, 7.80.

4-Nitroesterone (32a) and 2-Nitroestrone (35a).[11c,31]—Estrone (5 g, 18.5 mmol) was dissolved in boiling glacial acetic acid. The solution was allowed to cool to 70°, and 1.17 ml of concentrated nitric acid was added in one portion (color change, yellow to deep orange). A precipitate formed as the reaction medium was cooled to room temperature, and after 24 hr it was collected by filtration, washed with cold glacial acetic acid, and dried *in vacuo*, to give 1.84 g (32%) 4-nitroestrone (32a), mp 250° dec. An analytical sample, obtained after one recrystallization from aqueous acetic acid, melted with decomposition above 270° (lit.[31] 270–280° dec): $[\alpha]^{24}D$ +286° (*c* 0.59, THF); ir (KBr) 3450 (OH), 1740 cm^{-1} (C=O); nmr (DMSO-d_6) δ 0.80 (s, 3 H), 1.20–2.40 (15 H), 7.00 (AB quartet, 2 H), 10.3 (s, 1 H); mass spectrum (70 eV) *m/e* (rel intensity) 315 (M^+, 35), 298 (14), 181 (15), 169 (15), 131 (24), 119 (25), 97 (21), 69 (100), 41 (17), 28 (12).

Anal. Calcd for $C_{18}H_{21}NO_4$: C, 68.55; H, 6.71; N, 4.44. Found: C, 68.23; H, 6.88; N, 4.28.

The filtrate was poured into 350 ml of distilled water to precipitate the remaining nitro derivatives and the yellow solid (3.67 g) was collected and dried *in vacuo*. It was dissolved in benzene and purified by column chromatography. Elution with benzene yielded, after recrystallization from 80% ethanol, 2.29 g (40%) of 2-nitroestrone (35a): mp 183–185° (lit.[31] 183.5–184°); $[\alpha]^{24}D$ +190° (*c* 0.6135, $CHCl_3$); ir (KBr), 3440 (OH), 1740 cm^{-1} (C=O); nmr (DMSO-d_6) δ 0.82 (s, 3 H), 1.20–2.40 (15 H), 6.80 (s, 1 H), 7.73 (s, 1 H); mass spectrum (70 eV) *m/e* (rel intensity) 315 (M^+, 100), 271 (51), 259 (29), 258 (49), 217 (33), 205 (25), 97 (23), 55 (23), 41 (30), 28 (33).

Anal. Calcd for $C_{18}H_{21}NO_4$: C, 68.55; H, 6.71; N, 4.44. Found: C, 68.40; H, 6.65; N, 4.55.

4-Nitroestradiol (32b).—A solution of 315 mg (1.0 mmol) of 4-nitroestrone (32a) in 25 ml of dry THF was added dropwise to a stirred solution of 608 mg (16.0 mmol) of sodium borohydride in 20 ml of dry THF, 4 ml of 1 *N* sodium hydroxide, and 3 ml of distilled water. The resulting mixture was refluxed for 6 hr, after which time the alkaline reaction mixture was acidified with 1 *N* hydrochloric acid. Product isolation (ether; Na_2SO_4) gave 135 mg (43%) 4-nitroestradiol (32b) after two crystallizations from ethanol: mp 210° dec; $[\alpha]^{24}D$ +210° (*c* 0.5515, THF); ir (KBr) 3600–3000 (broad OH), 1525, 1365 cm^{-1} (C–NO); nmr (DMSO-d_6, 220 MHz)[34] δ 0.68 (s, 3 H), 4.62 (s, 1 H, C-17 OH), 7.12 (AB quartet, 2 H), 10.55 (s, 1 H); mass spectrum (70 eV) *m/e* (rel intensity) 317 (M^+, 25), 300 (26), 258 (25), 128 (27),

115 (34), 107 (23), 95 (26), 93 (26), 91 (29), 81 (30), 79 (25), 77 (32), 67 (25).

Anal. Calcd for $C_{18}H_{23}NO_4$: C, 68.12; H, 7.30; N, 4.41. Found: C, 67.78; H, 7.18; N, 4.30.

4-Aminoestrone (33a).—To a refluxing solution of 4-nitroestrone (**32a**) (196 mg, 0.62 mmol) in 50 ml of acetone, 10 ml of water, and 5 ml of 1 *N* sodium hydroxide was added 1.0 g of sodium dithionite. The red reaction mixture was stirred under reflux for 30 min; then 700 mg of sodium dithionite was added. Small portions of 1 *N* sodium hydroxide were added periodically to maintain alkalinity. After another 30 min, a final 300-mg portion of sodium dithionite was added, the reaction mixture was cooled briefly, and acetone was removed under reduced pressure. The alkaline solution was neutralized with 10% acetic acid and allowed to stand at 0° for 2 hr. The white precipitate collected by filtration under nitrogen was washed with water and dried *in vacuo* to yield 171 mg (96%) of 4-aminoestrone (**33a**): mp 254° dec. Recrystallization from methanol–benzene gave 150 mg (88%) of **33a**: mp 260–261° dec (lit.[33] 260–262°); $[\alpha]^{24}D$ +171° (*c* 0.4974, THF); ir (KBr) 3600–3100 cm^{-1} (broad OH and NH); mass spectrum (70 eV) *m/e* (rel intensity) 285 (M^+, 100).

Anal. Calcd for $C_{18}H_{23}NO_2$: C, 75.76; H, 8.12; N, 4.91. Found: C, 75.66; H, 8.12; N, 4.99.

4-Aminoestradiol (33b).—To a round-bottom flask containing 175 mg (5 mmol) of lithium aluminum hydride in 25 ml of dry THF was added 285 mg (1 mmol) of 4-aminoestrone (**33a**) in 20 ml of dry THF. The reaction mixture was stirred at 25° for 2 hr, and excess $LiAlH_4$ was then destroyed by dropwise addition of water, followed by an equal volume of saturated ammonium chloride. Product isolation (ether; Na_2SO_4) gave 182 mg (63%) 4-aminoestradiol after recrystallization from aqueous methanol. An analytical sample was obtained by three recrystallizations from aqueous methanol: mp 260° dec; $[\alpha]^{24}D$ +37° (*c* 0.22, THF); ir (KBr) 3680–3000 (broad OH, NH), 1610 cm^{-1} (phenyl); mass spectrum (70 eV) *m/e* (rel intensity) 287 (M^+, 100), 122 (16).

Anal. Calcd for $C_{18}H_{25}NO_2$: C, 75.22; H, 8.77; N, 4.87. Found: C, 74.92; H, 8.67; N, 4.77.

4-Azidoestrone (34a).—A solution of 4-aminoestrone (**33a**) (200 mg, 0.52 mmol) in 10 ml of acetone and 5 ml of 2 *N* hydrochloric acid was cooled to 0° and diazotized with 49 mg of sodium nitrite in 2 ml of water. After 30 min the cold solution of diazonium salt was added to a tenfold excess (455 mg, 0.70 mmol) of sodium azide in 10 ml of water. The solution was overlayed with 100 ml of ether and stirred for about 30 min. Product isolation (ether; Na_2SO_4) gave crude material which was purified by preparative tlc (ether). The chromatogram was protected from light during development and subsequent product isolation. Recrystallization from methanol–benzene gave 124 mg (57%) 4-azidoestrone (**34a**) as straw-colored needles: mp 135° dec; uv λ_{max} (EtOH) 258 nm (ϵ 8350), 289 (4930); $[\alpha]^{24}D$ +167° (*c* 0.192, EtOH); ir (KBr) 3300 (broad OH), 2120 (N_3), 1710 cm^{-1} (C=O); mass spectrum (70 eV) *m/e* (rel intensity) 311 (M^+, 2), 285 (100), 283 (18), 187 (13), 161 (17), 131 (14), 122 (30), 115 (16), 97 (14), 91 (14), 77 (17), 41 (21).

Anal. Calcd for $C_{18}H_{21}N_3O_2$: C, 69.43; H, 6.80; N, 13.49. Found: C, 69.22; H, 6.84; N, 13.45.

4-Azidoestradiol (34b).—4-Aminoestradiol (**33b**) (110 mg, 0.39 mmol) in 5 ml of 2 N hydrochloric acid and 10 ml of acetone was diazotized with 27 mg of sodium nitrite and treated with tenfold excess sodium azide according to the procedure used in the preparation of 4-azidoestrone (**34a**). Preparative tlc of the crude azide (ether) gave 72 mg (59%) of 4-azidoestradiol (**34b**) after recrystallization from aqueous acetone: mp 140° dec; uv λ_{max} (EtOH) 257 nm (ϵ 6290), 292 (3130); $[\alpha]^{24}D$ +82° (*c* 0.7788, EtOH); ir (KBr) 3500–3100 (OH), 2120 cm^{-1} (N_3); mass spectrum (70 eV) *m/e* (rel intensity) 313 (M^+, 0.4), 288 (22), 287 (100), 286 (13), 285 (49), 187 (11), 175 (14), 174 (10), 162 (11), 161 (10), 160 (11), 122 (22).

Anal. Calcd for $C_{18}H_{23}N_3O_2$: C, 68.98; H, 7.40; N, 13.41. Found: C, 69.07; H, 7.28; N, 13.48.

2-Nitroestradiol (35b).—2-Nitroestrone (**35a**) (315 mg, 1 mmol) was reduced with sodium borohydride by the same procedure used in the preparation of 4-nitroestradiol (**32b**). Recrystallization of the crude product from aqueous ethanol gave 182 mg (58%) of **35b**, mp 168–172°. An analytical sample was recrystallized another two times from aqueous ethanol: mp 169–171°; $[\alpha]^{24}D$ +164° (*c* 0.5121, $CHCl_3$); ir (KBr) 3600–3280 (broad OH), 1540, 1310 (C–NO_2), but absence of C=O near 1700 cm^{-1}; nmr (DMSO-d_6, 220 MHz)[34] δ 0.68 (s, 3 H), 0.91–2.75 (16 H), 4.55 (s, C-17 OH), 6.82 (s, 1 H), 7.73 (s, 1 H), 10.46 (broad s, phenolic OH); mass spectrum (70 eV) *m/e* (rel intensity) 317 (M^+, 56), 259 (21), 258 (100), 205 (59), 71 (24), 57 (36), 43 (20), 28 (22).

Anal. Calcd for $C_{18}H_{23}NO_4$: C, 68.12; H, 7.30; N, 4.41. Found: C, 68.37; H, 7.31; N, 4.27.

2-Aminoestrone (36a).—2-Nitroestrone (**35a**) (500 mg, 1.59 mmol) in 150 ml of acetone, 30 ml of water, and 20 ml of 1 *N* sodium hydroxide was reduced with 1.38 g of sodium dithionite by the same procedure used in the preparation of 4-aminoestrone (**33a**); the additional portion of dithionite added was 276 mg. After recrystallization from aqueous methanol, 396 mg (87%) 2-aminoestrone (**36a**), mp 210° dec, was obtained. An analytical sample was recrystallized twice from aqueous methanol: mp 220° dec (lit.[35] 220° dec); $[\alpha]^{24}D$ 208° (*c* 0.558, THF); ir (KBr) 3100–2600 (broad, OH and NH_2 stretch), 1725 cm^{-1} (C=O); mass spectrum (70 eV) *m/e* (rel intensity) 286 (24), 285 (M^+, 100), 161 (11), 135 (14), 122 (23), 32 (15), 28 (63).

Anal. Calcd for $C_{18}H_{23}NO_2$: C, 75.76; H, 8.12, N, 4.91. Found: C, 75.52; H, 7.90; N, 4.93.

2-Aminoestradiol (36b).—Reduction of 300 mg (1.06 mmol) of 2-aminoestrone (**36a**) in 15 ml of THF with 163 mg (4.64 mmol) of $LiAlH_4$ in 40 ml of THF was accomplished by the same procedure used in the preparation of 4-aminoestradiol (**33b**). Recrystallization of the crude product from methanol–benzene gave 128 mg (42%) of 2-aminoestradiol (**36b**), mp 198–200°. An analytical sample was prepared by an additional recrystallization from methanol–benzene: mp 200–201°; $[\alpha]^{24}D$ +122° (*c* 0.1100, THF); ir (KBr) 3580–3000 (very broad, OH, NH), 1610 cm^{-1} (phenyl); mass spectrum (70 eV) *m/e* (rel intensity) 287 (M^+, 100), 135 (15), 122 (30), 78 (45), 77 (14), 28 (15).

Anal. Calcd for $C_{18}H_{25}NO_2$: C, 75.22; H, 8.77; N, 4.87. Found: C, 75.06; H, 8.56; N, 5.06.

2-Azidoestrone (37a).—A solution of 2-aminoestrone (260 mg, 0.92 mmol) in 5 ml of 2 *N* hydrochloric acid and 10 ml of acetone was diazotized at 0° with 68 mg of sodium nitrite in 2 ml of distilled water by the same procedure used to prepare 4-azidoestrone (**34a**). Recrystallization of the crude product from aqueous methanol gave 230 mg (81%) 2-azidoestrone (**37a**): mp 140–150° dec; uv λ_{max} (EtOH) 253 nm (ϵ 7200), 300 (5390); $[\alpha]^{24}D$ +191° (*c* 0.9062, EtOH); ir (KBr) 3420 (broad OH), 2130 (N_3), 1730 cm^{-1} (C=O); nmr (DMSO-d_6) δ 0.81 (s, 3 H), 1.20–2.40 (15 H), 6.56 (s, 1 H), 6.73 (s, 1 H); mass spectrum (70 eV) *m/e* (rel intensity) 311 (M^+, 2), 285 (100), 283 (21), 172 (17), 161 (13), 160 (10), 135 (10), 122 (25), 58 (13), 43 (46).

Anal. Calcd for $C_{18}H_{21}N_3O_2$: C, 69.43; H, 6.80; N, 13.49. Found: C, 69.29; H, 6.63; 13.34.

2-Azidoestradiol (37b).—A solution of 2-aminoestradiol (**36b**) (60 mg, 9.21 mmol) in 3 ml of 2 *N* hydrochloric acid and 6 ml of acetone was diazotized with 14.4 mg of sodium nitrite in 1 ml of water by the same procedure used in the preparation of 4-azidoestradiol (**34b**). The crude azide was purified by preparative tlc using ether–hexane (1:3) as developing solvent, to give 25 mg (38%) of 2-azidoestradiol (**37b**) as tan crystals from THF–hexane: mp 140° dec; uv λ_{max} (EtOH) 254 nm (ϵ 7650), 301 (5600); $[\alpha]^{24}D$ +135° (*c* 0.8118, EtOH), ir (KBr) 3560–3400 (broad OH), 2120 cm^{-1} (N_3); mass spectrum (70 eV) *m/e* (rel intensity) 313 (M^+, 0.6), 287 (3), 28 (100).

Anal. Calcd for $C_{18}H_{23}N_3O_2$: C, 68.98; H, 7.40; N, 13.41. Found: C, 68.82; H, 7.26; N, 13.29.

3-Nitrohexestrol (38) and 3,3′-Dinitrohexestrol (41).—Hexestrol (6.0 g, 19 mmol) was dissolved in 250 ml of glacial acetic acid at its boiling point. When the solution had cooled to 70°, 1.5 ml of concentrated nitric acid was added dropwise. The resulting orange reaction mixture was cooled to 25° and allowed to stand overnight. A yellow precipitate which formed in the reaction medium was collected, and the filtrate was neutralized with sodium bicarbonate. Product isolation (ether; Na_2SO_4) from the aqueous solution gave an orange residue. Purification of the products of nitration was accomplished by careful column chromatography. The residue from the filtrate was dissolved in THF and adsorbed onto a small amount of silica gel. The THF was removed under vacuum, and the coated silica gel was placed on top of a silica gel column (100:1 silica gel–compound). Elution with 30% benzene in hexane gave a yellow product (dinitrohexestrol **41**) which recrystallized from ethanol as bright yellow flakes (182 mg): mp 240° dec; ir (KBr) 3440 (OH), 1535, 1320 cm^{-1} (C–NO_2); nmr (220 MHz, DMSO-d_6) δ 0.52 (t, 6 H), 1.23–

1.46 (m, 4 H), 7.34 (q, 2 H), 7.78 (s, 1 H); mass spectrum (70 eV) *m/e* (rel intensity) 360 (M^+, 6), 285 (23), 181 (100), 180 (90), 153 (68), 152 (100), 106 (100), 58 (91), 79 (71), 78 (37), 77 (98), 65 (36), 55 (26), 51 (26), 41 (48), 39 (24), 28 (37), 27 (26).

Anal. Calcd for $C_{18}H_{20}N_2O_6$: C, 59.99; H, 5.59; N, 7.77. Found: C, 59.95; H, 5.56; N, 7.53.

Elution of the column with 70% benzene in hexane and finally benzene gave 1.85 g of another yellow solid (3-nitrohexestrol **38**) which could be recrystallized from aqueous ethanol only with difficulty: mp 139–142°; ir (KBr) 3520–3300 (OH), 1540, 1320 cm^{-1} (C–NO_2); nmr ($CDCl_3$) δ 0.58 (t, 6 H), 1.10–1.65 (m, 4 H), 2.40–2.70 (m, 2 H), 4.93 (broad s, 1 H), 6.68–7.91 (m, 7 H, aromatic), 10.40 (sharp s, 1 H); mass spectrum (70 eV) *m/e* (rel intensity) 315 (M^+, 1), 136 (12), 135 (100), 107 (37).

Anal. Calcd for $C_{18}H_{21}NO_4$: C, 68.55; H, 6.71; N, 4.44. Found: C, 68.75; H, 6.50; N, 4.27.

Chromatographic purification of the solid which had separated from the reaction medium gave an additional 765 mg 3,3′-dinitrohexestrol, mp 240° dec, after recrystallization from aqueous ethanol. The combined yields of the products were 3-nitrohexestrol (**38**), 1.85 g (30%), and 3,3′-dinitrohexestrol (**41**), 947 mg (14%).

3-Aminohexestrol (39).—3-Nitrohexestrol (**38**) (315 mg, 1 mmol) in 40 ml of acetone, 20 ml of water, and 10 ml of 1 *N* sodium hydroxide was reduced with 1 g of sodium dithionite by the same procedure used in the preparation of 4-aminoestrone (**33a**); a final portion of 174 mg of dithionite was added. Recrystallization of the crude precipitate from aqueous methanol gave 263 mg (92%) of 3-aminohexestrol: mp 200–202° dec; ir (KBr) 3600–3100 (OH, NH), 1610 cm^{-1} (C=C, aromatic); mass spectrum (70 eV) *m/e* (rel intensity) 285 (M^+, 18), 151 (18), 150 (100), 135 (24), 133 (14), 107 (14).

Anal. Calcd for $C_{18}H_{23}NO_2$: C, 75.76; H, 8.12; N, 4.91. Found: C, 75.80; H, 8.14; N, 4.87.

3-Azidohexestrol (40).—3-Aminohexestrol (**39**) (200 mg, 0.7 mmol) in 5 ml of 1 *N* hydrochloric acid and 15 ml of acetone was diazotized with 49 mg of sodium nitrite in 2 ml of water according to the procedure used in the preparation of 4-azidoestrone (**34a**). After purification of the crude product by preparative tlc (5% MeOH in $CHCl_3$, protect from light), recrystallization from aqueous methanol gave 150 mg (69%) 3-azidohexestrol (**40**): mp 145° dec; uv λ_{max} (EtOH) 250 nm (ε 6930), 288 (4850), 297 (4550); ir (KBr) 3440 (broad OH), 2130 (N_3), 1600, and 1610 cm^{-1} (phenyl); mass spectrum (70 eV) *m/e* (rel intensity) 311 (M^+, 6), 150 (27), 136 (12), 135 (99), 134 (14), 107 (39), 28 (100).

Anal. Calcd for $C_{18}H_{21}N_3O_2$: C, 69.43; H, 6.80; N, 13.49. Found: C, 69.30; H, 6.86; N, 13.56.

3,3′-Diaminohexestrol (42).—3,3′-Dinitrohexestrol (**41**) (360 mg, 1 mmol) in 40 ml of acetone, 20 ml of distilled water, and 10 ml of 1 *N* sodium hydroxide was reduced with 1.74 g of sodium dithionite by the same procedure used in the preparation of 4-aminoestrone (**33a**); a final portion of 174 mg of dithionite was added. The crude precipitate was recrystallized from aqueous methanol, giving 224 mg (75%) of 3,3′-diaminohexestrol (**42**): mp 240° dec; ir (KBr) 3600–3100 (broad OH, NH), 1600 cm^{-1} (phenyl); mass spectrum (70 eV), *m/e* (rel intensity) 300 (M^+, 18), 151 (33), 150 (100), 133 (17), 122 (13).

Anal. Calcd for $C_{18}H_{24}N_2O_2$: C, 71.97; H, 8.05; N, 9.33. Found: C, 71.71; H, 8.10; N, 9.39.

3,3′-Diazidohexestrol (43).—A solution of 3,3′-diaminohexestrol (**42**) in 10 ml of acetone and 5 ml of 2 *N* hydrochloric acid was cooled to 0°, diazotized with 34.5 mg of sodium nitrite in 2 ml of distilled water, and treated with a tenfold excess (325 mg, 5.0 mmol) of sodium azide in 10 ml of distilled water, according to the procedure used in preparing 4-azidoestrone (**34a**). The crude product was purified by preparative tlc (chloroform; protect from light during development) and recrystallization from aqueous ethanol, giving 55 mg (31%) of 3,3′-diazidohexestrol (**43**): mp 115° dec; uv λ_{max} (EtOH) 297 nm (ε 9430), 244 (14,400); ir (KBr) 3420 (OH), 2130 (N_3), 1600 cm^{-1}; mass spectrum (70 eV) *m/e* (rel intensity) 352 (M^+, 4), 176 (23), 28 (100).

Anal. Calcd for $C_{18}H_{20}N_6O_2$: C, 61.35; H, 5.72; N, 23.82. Found: C, 61.07; H, 5.71; N, 23.53.

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Registry No.—**1,** 50-28-2; **2,** 3434-88-6; **3,** 41164-27-6; **4,** 41164-28-7; **5,** 53-16-7; **6,** 41164-29-8; **7,** 6038-23-9; **8,** 3460-92-2; **9,** 41164-31-2; **10,** 41164-32-3; **11,** 1428-66-6; **12,** 41164-34-5; **13,** 41164-35-6; **14,** 41164-36-7; **15,** 41164-37-8; **16,** 41164-38-9; **17,** 41164-39-0; **18,** 41172-42-3; **19,** 6325-61-7; **20,** 6737-97-9; **21,** 41172-45-6; **22,** 41172-46-7; **23,** 41172-47-8; **32a,** 5976-74-9; **32b,** 6936-94-3; **33a,** 14984-42-0; **33b,** 6301-88-8; **34a,** 41164-44-7; **34b,** 41164-45-8; **35a,** 5976-73-8; **35b,** 6298-51-7; **36a,** 14984-43-1; **36b,** 6301-87-7; **37a,** 41164-50-5; **37b,** 41259-43-2; **38,** 41172-48-9; **39,** 41172-49-0; **40,** 41172-50-3; **41,** 41172-51-4; **42,** 41172-52-5; **43,** 41172-53-6; **44,** 5635-50-7; glyoxylic acid chloride *p*-toluenesulfonylhydrazone, 14661-69-9; potassium *tert*-butoxide, 865-47-4; thionyl chloride, 7719-09-7; piperidine, 110-86-1; ethyl bromoacetate, 105-36-2; acetic anhydride, 108-24-7; potassium carbonate, 584-08-7; sodium ethoxide, 141-52-6.