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E. S. D.

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Asunto : Solicitud de patente para **COMPUESTOS
ESTEROIDES CONDENSADOS 16,17-
CARBOXÍLICOS QUE TIENEN ACTIVIDAD
ESTROGENICA SELECTIVA**

Solicitante : N.V. ORGANON

Fecha de solicitud : 5 de diciembre de 2002

1. Yo, Emilio FERRERO, obrando como apoderado del solicitante en el asunto antes identificado, me refiero al Oficio No. 2615 notificado por fijación en lista del 23 de febrero de 2007, y atentamente presento junto con este escrito los siguientes documentos:

- 1.1 J.R. BULL ET AL: "Synthesis of 14,17-propane analogues of estradiol"
Journal of the chemical society, perkin transactions 1. 1997, pp 1179-1192.
- 1.2 EL GARROUJ DRISS ET AL: "Steroidal affinity labels of the estrogen receptor: 1.17 alpha-(bromoacetoxyl) alkyl/alkynylestradiols" Journal of medicinal chemistry, vol. 36, no. 20, 1993, pp 2973-2983.

3. Puesto que se han atendido a cabalidad las peticiones hechas por su Despacho sobre la presente solicitud de patente, atentamente solicito que se continúe su tramitación.

Bogotá,
Señora Jefe,



EMILIO FERRERO

T.P.A. No. 31.929

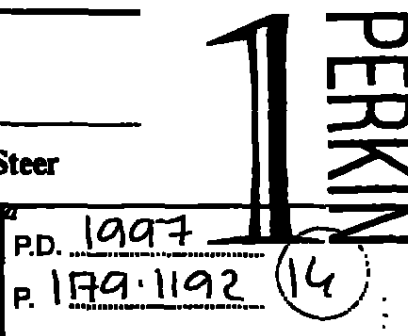
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Synthesis of 14,17-propano analogues of estradiol

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XP-002153748



Stereocontrolled syntheses of the 14 α ,17 α -propano and 14 β ,17 β -propano analogues of estradiol are described, together with those of numerous derivatives in which additional functionality is incorporated into the bridged system. Intramolecular aldol condensation of 17 β -acetoxy-3-methoxy-20-oxo-19-nor-17 α -pregna-1,3,5(10)-triene-14-carbaldehyde **1** furnishes 3-methoxy-17 α -oxo-14,17 α -prop-17 α -enoestra-1,3,5(10)-trien-17 β -yl acetate **2**, which is transformed into 14,17 α -propanoestra-1,3,5(10)-triene-3,17 β -diol **17**. In the first of two synthetic approaches to the 14 β ,17 β -propano series, cycloaddition of methyl propiolate to 3-methoxyestra-1,3,5(10),14,16-pentaen-17-yl acetate **25** gives a 14,17-bridged intermediate **26**, in which the latent propyne equivalency of the dienophile is elaborated through selective functional group transformations to give 17-acetoxy-3-methoxy-20-oxo-19-nor-14 β -pregna-1,3,5(10)-triene-14-carbaldehyde **50** and the derived product **52** of intramolecular aldol condensation. The second approach entails regioselective functionalisation of 14-allyl-3-methoxy-14 β -estra-1,3,5(10)-trien-17-one **54** at C-2' or C-3' to give intermediates for intramolecular closure between the chain terminus and C-17, leading to 14,17 β -propano-14 β -estra-1,3,5(10)-triene-3,17 α -diol **64**. The results of competitive binding assays of the hormone analogues **17** and **64** toward the estradiol receptor are reported, and compared with those of bridge-functionalised derivatives.

The quest for new predictive design principles in steroid hormone research gained impetus with the discovery that 14 α ,17 α -ethano analogues of estradiol^{1,2} and estriol^{3,4} bind efficiently to the estrogen receptor and display potent oral estrogenicity. This invites speculation upon the possible role of the 14,17-ethano bridge in bestowing resistance to metabolic degradation, whilst enabling receptor binding to proceed without evident steric impediment. An attendant question is whether alteration of the bridge size might influence this property. The 14 α ,17 α - and 14 β ,17 β -propano analogues of estradiol appeared to be synthetic candidates of particular interest, since modelling studies reveal subtle changes in the spatial disposition of polar functionality at C-3 and C-17 and in the steric environment of the 17-hydroxy group, associated with introduction of epimerically differentiated 14,17-propano bridges. A comparison of the receptor binding affinities of these targets was therefore expected to provide further insight into structure-activity relationships in ring D bridged analogues of estradiol, and possible pointers to predictive design of new targets in which binding affinity is optimised.

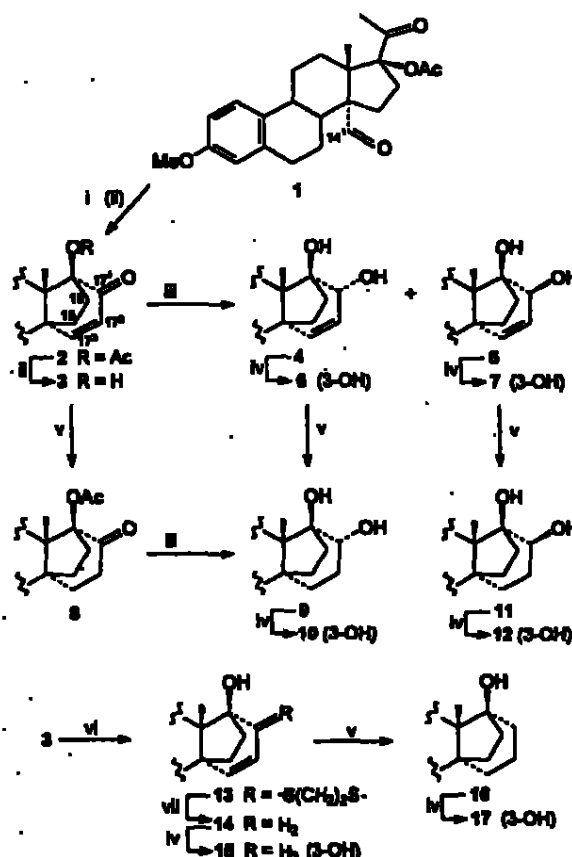
In this paper we describe stereocontrolled syntheses of 14,17 α -propanoestra-1,3,5(10)-triene-3,17 β -diol **17** and 14,17 β -propano-14 β -estra-1,3,5(10)-triene-3,17 α -diol **64**,^{5,6} as well as a number of bridge-functionalised derivatives of these parent systems. These products have been subjected to binding affinity and detailed structural studies.

Results and discussion

Synthesis of the 14 α ,17 α -propano series

A simple synthetic strategy for assembly of a 14 α ,17 α -propano bridge was suggested by the availability of 17 β -acetoxy-3-methoxy-20-oxo-19-nor-17 α -pregna-1,3,5(10)-triene-14-carbaldehyde **1**,⁷ in which the propitious array of ring D functionality was expected to lend itself to intramolecular aldol reaction, and conversion of the resultant 14 α ,17 α -bridged intermediates into the desired hormone analogues.

Indeed, treatment of compound **1** with hydrochloric acid in tetrahydrofuran (THF) at 50 °C resulted in near-quantitative conversion into the bridged enone **2**, the structure of which was evident from diagnostic spectroscopic data and rigorous struc-



Scheme 1 Reagents and conditions: i, HCl, THF, 50 °C (to **2**); ii, KOH, MeOH, 25 °C; iii, LiAlH₄, THF, 25 °C; iv, DIBAL, C₆H₅Me, reflux; v, Pd-C (10%), H₂; vi, (CH₃SH)₂, BF₃·Et₂O, HOAc, 30 °C; vii, Na, liquid NH₃.

tural elucidation of derived products. Intramolecular aldol reaction of compound **1** also proceeded in the presence of alkali, to give the product **3** arising from concomitant bridge-head hydrolysis, but accompanied by isomeric material which

was also detected during alkaline hydrolysis of compound 2. Although this product proved difficult to separate chromatographically, its presence was inferred from NMR spectroscopy of the reaction products.¹ The further implications of this interfering reaction will be reported elsewhere.

Reduction of the enone 2 with lithium aluminium hydride (LAH) in THF at 25 °C gave a separable mixture (~3:2) of the isomeric diols 4 and 5 arising from exclusive 1,2-addition of hydride. The configurational assignments were deduced from NMR data, which revealed significant coupling ($J_{17,17'}$ 4.1 Hz) in the 17 β ,17'-diol 4, consistent with a pseudo-axial (*exo*) orientation of 17'-OH, whereas the 17 β ,17'-diol 5 displayed the vicinal ($J_{17,17'}$ 2 Hz) and allylic ($J_{17,17'}$ 1.6 Hz) couplings expected for a pseudo-equatorial (*endo*) substituent at C-17'. The surprisingly slight preference for *endo*-entry of hydride in 2 suggests very little steric differentiation in reagent approach. The diols 4 and 5 were deprotected [diisobutylaluminium hydride (DIBALH) in refluxing toluene⁹] at C-3, to give the respective triols 6 and 7.

Further simplification of bridge functionality in the enone 2 was achieved through catalytic hydrogenation (Pd-C, H₂) to give the saturated 17'-ketone 8, LAH reduction of which was highly stereoselective giving only the 17 β ,17'-diol 9; the NMR signal for 17'-H appeared at δ 4.2 (*t*, J 2 $\times$ 3 Hz) consistent with axial (*exo*) orientation of 17'-OH and hence, exclusive *endo*-directed hydride addition. Correlation of 9 with the unsaturated 17 β ,17'-diol 4 was established through comparison with the product of catalytic hydrogenation of the latter, albeit in poor yield owing to unexplained rearrangements. By contrast, catalytic hydrogenation of the unsaturated 17 β ,17'-diol 5 proceeded smoothly, to give the saturated product 11. The parent hormone analogues 10 and 12 were prepared *via* conventional 3-deprotection of 9 and 11, respectively.

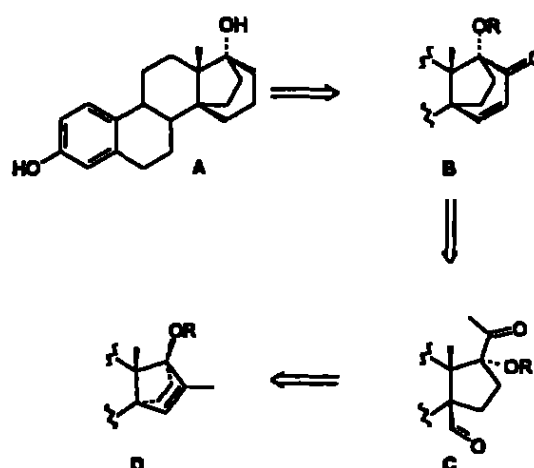
The remaining objective in this phase of the investigation was to prepare the parent 14 α ,17 α -propano analogue of estradiol. The most direct approach appeared to be *via* 17'-deoxygenation of the enone 2. Inexplicably, attempted dithioketalisation of 2 (ethanedithiol-boron trifluoride-diethyl ether complex, 30 °C) failed. Apparently this is associated with the presence of the bridgehead acetoxy group, since the saturated 17 β -acetoxy 17'-ketone 8 behaved similarly, but the 17 β -hydroxy enone 3 underwent slow but clean conversion into the corresponding 17',17'-dithioketal 13. Desulfurisation of 13 proceeded smoothly in the presence of sodium-liquid ammonia to give the bridged olefin 14, which underwent catalytic hydrogenation to give the 14 α ,17 α -propano compound 16. Deprotection of 14 and 16 at C-3 furnished the corresponding bridged estradiol analogues 15 and 17, respectively.

The foregoing synthetic sequence commended itself as a straightforward route to the target hormone analogues for evaluation purposes, but a necessary caveat is that the synthetic route to the pivotal intermediate 1 is flawed by imperfect stereoselectivity in the cycloaddition step.⁷ More direct and efficient access to 14 α ,17 α -propano systems can be envisaged through the synthesis of 14 α -alkyl 17-ketones in which a suitably functionalised three-carbon chain at C-14 can be primed for intramolecular 3',17-closure. Our preliminary studies toward these objectives will be reported elsewhere.

Synthesis of the 14 β ,17 β -propano series

The successful completion of the foregoing synthetic route invited consideration of the scope for implementing an analogous synthetic strategy for the 14 β ,17 β -propano series. The prerequisites would be ready access to the key intermediate C (a 14,17-epimer of 1) *via* oxidative cleavage of the bridged precursor D, and the reasonable presumption of comparable functional group reactivity implicit in the retrosynthetic step (A $\Rightarrow$ B, Scheme 2).

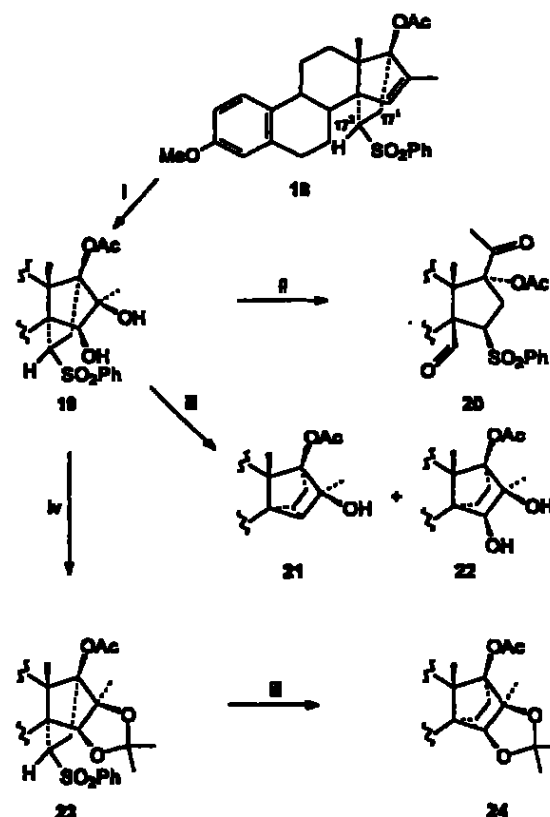
The fortuitous availability of the phenylsulfonyl compound 18, as the 'undesired' cycloadduct in the reaction sequence lead-



Scheme 2 Oxidative cleavage-intramolecular aldol condensation pathway to 14 β ,17 β -propanoestradiol

ing to compound 1,⁷ offered scope for testing the feasibility of the outlined approach. Earlier experiments⁹ had demonstrated the impracticality of converting 18 into a 16-methyl- Δ^{14} -intermediate D *via* direct reductive desulfonylation, owing to unacceptably high levels of attendant olefinic bond participation. Accordingly, it was decided to postpone this step until this source of intramolecular interference had been eliminated through necessary functionalisation of the olefinic bond.

cis-Hydroxylation of compound 18 (OsO₄-pyridine, 25 °C) proceeded slowly (168 h) but highly stereoselectively to give a single diol 19 (Scheme 3), confidently assigned a 15 β ,16 β -



Scheme 3 Reagents and conditions: i, OsO₄, C₂H₅N, 25 °C; ii, NaIO₄, EtOH-H₂O, 20 °C; iii, SmI₂, HMPA, THF, -20 °C; iv, Me₂CO, HClO₄ (70%), 20 °C

configuration owing to the severe steric congestion on the *endo*-face of the olefinic bond. The feasibility of oxidative cleavage at this stage was demonstrated by treatment of the diol 19 with sodium periodate in aqueous ethanol to give the dioxo com-

pound 20, further elaboration of which was deemed impractical owing to the plethora of ring D functionality. Instead, reductive desulfonylation of the diol 19 was attempted; reaction with samarium(II) iodide-HMPA¹⁰ at -20°C proceeded slowly (4 h) to give a mixture (~1:1) of products 21 and 22. The less polar product was formulated as the 15 α ,17 α -cyclo-14 α ,17 α -ethano compound 21, based upon NMR evidence for the presence of a cyclopropyl moiety and the absence of a secondary hydroxy group, whereas the properties of the more polar product were clearly consistent with the desulfonylated 15 β ,16 β -diol 22. The unexpected intrusion of an apparent participation process here, was circumvented by prior conversion of the diol 19 into the acetonide 23, which underwent efficient reductive desulfonylation to the expected product 24, deprotection of which to the diol 22 is described below.

Although these experiments demonstrated the feasibility of the planned approach, it was evident that a practical synthetic strategy would require a more direct method for preparation of the diol 22 or the precursor olefin D (Scheme 2). This, in turn, necessitated development of a plan for cycloaddition of a latent propyne equivalent to the diynyl acetate 25, which could lead *via* selective functional group modification to such an intermediate.

The potential of methyl propiolate to serve this purpose is implied in an earlier study upon a steroidal ring D diene,¹¹ in which the crucial steps demonstrated appropriate regioselectivity during cycloaddition, and chemoselective hydrogenation of the isolated olefinic bond in the primary cycloadduct.

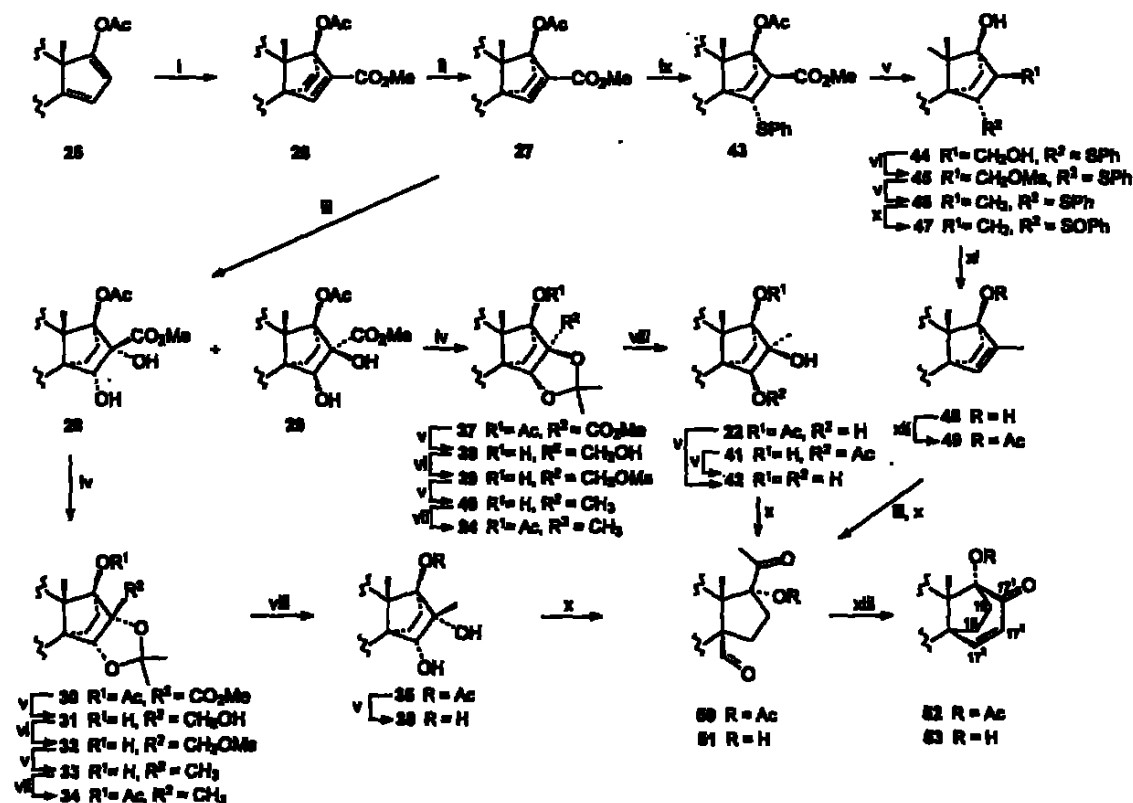
Treatment of the diynyl acetate 25 with methyl propiolate in benzene at 100°C (sealed tube, 23 h) resulted in efficient formation of a single cycloadduct 26 (85%) (Scheme 4), the structure of which was assigned by well-established analogy.¹¹ Catalytic hydrogenation (Pd-C, H_2) proceeded chemoselectively to give the 14 α ,17 α -ethano compound 27 (83%). Initial attempts to convert 27 into the 16-hydroxymethyl- Δ ¹²

compound for subsequent 16 β -deoxygenation were frustrated by negligible 1,2-regioselectivity during hydride reduction of the α,β -unsaturated ester grouping, under a variety of reaction conditions. Consequently, it was decided to postpone the necessary conversion of the 16-methoxycarbonyl group into a methyl group, and 27 was first osmylated to give a readily separable mixture (~7:3) of 15,16-diols 28 and 29, distinguished by the diagnostic four-bond coupling between 15 β -H and 17 β -H_{ax} (J 1.5 Hz) in the 15 α ,16 α -isomer 28. The conversion of these 16-methoxycarbonyl 15,16-diols into the corresponding 16-methyl 15,16-diols necessitated conventional multi-step reaction sequences.

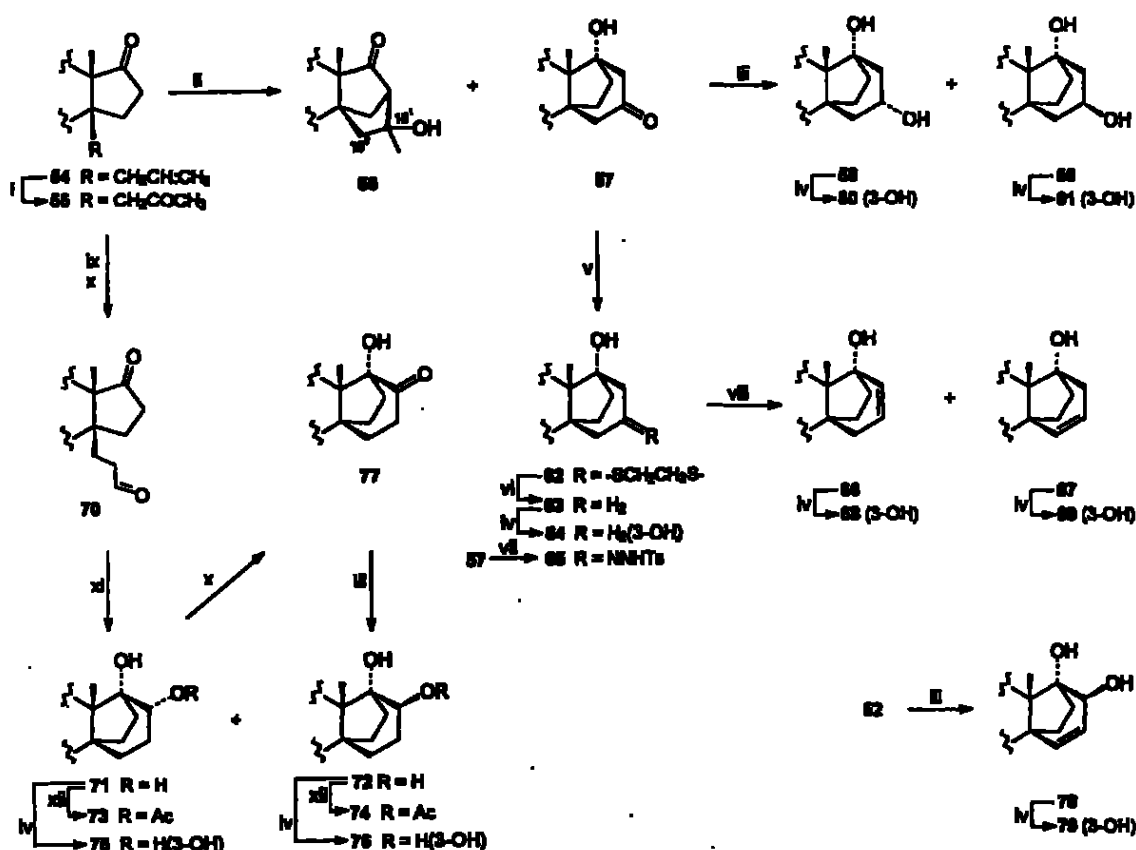
Thus, the major isomer 28 was first protected as the acetonide 30, whereupon sequential LAH reduction (to 31), selective 16 β -methanesulfonylation (to 32), LAH reduction (to 33), 17-acetylation (to 34) and finally, acetonide deprotection gave the desired 16 β -methyl 15 α ,16 α -diol 35 in an overall yield of ca. 25% from 28.

An analogous reaction sequence upon the minor isomer 29 ($\rightarrow$ 37 $\rightarrow$ 38 $\rightarrow$ 39 $\rightarrow$ 40 $\rightarrow$ 24) proceeded unexceptionally and as efficiently, to give the intermediate acetonide 24, identical with that obtained during reductive desulfonylation of 19. However, deprotection of 24 with iodine in methanol-THF¹² at 80°C furnished an inseparable mixture (~1.8:1 by NMR) of the 17 β -acetoxy 15 β ,16 β -diol 22 and the 15 β -acetoxy 16 β ,17 β -diol 41; the latter compound was isolated and characterised as the unreactive component in a subsequent reaction upon the mixture, and the structure was further inferred from treatment of the mixture 22 + 41 with LAH to give the triol 42 as a common product of hydrolysis.

It is evident that this two-fold *trans*-acetylation of 22 to give 41 requires a bridgehead-*exo* relationship of interacting functionality during the first step, since an analogous process was not observed during similarly mediated deprotection of the isomeric acetonide 34.



Scheme 4 Reagents and conditions: i, HO-COO-Me , C_6H_6 , 100°C ; ii, Pd-C (10%), H_2 ; iii, OsO_4 , $\text{C}_6\text{H}_5\text{N}$, 25°C ; iv, Me_2CO , HClO_4 (70%), 20°C ; v, LiAlH_4 , THF, 25°C ; vi, MeSO_2Cl , $\text{C}_6\text{H}_5\text{N}$, 0°C ; vii, Ac_2O , *p*- TsOH , THF, 20°C ; viii, I_2 , MeOH , reflux; ix, $\text{C}_6\text{H}_5\text{SH}$, $\text{P}_2\text{S}_5\text{NEt}_3$, 20°C ; x, NaIO_4 , THF-EtOH- H_2O , 20°C ; xi, Et_3N , C_6H_6 , 115°C ; xii, Ac_2O , DMAP, $\text{C}_6\text{H}_5\text{N}$, 20°C ; xiii, HCl , THF, 57°C or *p*- TsOH , C_6H_6 , reflux



Scheme 5 Reagents and conditions: i, PdCl_2 , CuCl , O_2 , $\text{DMF-H}_2\text{O}$, 65°C ; ii, KOH , MeOH , 20°C ; iii, LiAlH_4 , THF , 0°C ; iv, DIBAL , $\text{C}_6\text{H}_5\text{Me}$, reflux; v, $(\text{CH}_2\text{SH})_2$, $\text{Zn}(\text{OTf})_2$, CH_2Cl_2 , 20°C ; vi, Raney-Ni, EtOH , reflux; vii, $p\text{-TlNHNH}_2$, TFA , THF , 20°C ; viii, BuLi , THF , 0°C ; ix, $\text{BH}_3\text{-Me}_2\text{S}$, THF , reflux, then H_2O_2 , NaOH , 40°C ; x, $(\text{COCl})_2$, DMSO , THF , -78°C , then Et_3N , -78°C to 20°C ; xi, $\text{TiCl}_4\text{-(DME)}_{1.5}$, Zn-Cu , 20°C ; xii, Ac_2O , $\text{C}_6\text{H}_5\text{N}$, 20°C

Although the foregoing reaction sequences succeeded in furnishing the targeted intermediates 22 and 35 for oxidative cleavage (to C, Scheme 2), the need to process the isomeric diols 28 and 29 separately, and the complications attending functional group manipulation of the latter isomer 29, detracted from the overall efficiency of the method, and an alternative approach to elaborating the propyne equivalency implicit in the key intermediate 27 was sought.

This was readily achieved *via* conjugate phenylthiolation¹³ of 27, which proceeded efficiently and stereoselectively¹⁴ in the presence of thiophenol-diisopropylethylamine at 20°C to give the 15 α -phenylthio 16 β -carboxylate 43. The 15,16-*trans* relationship of functionality, crucial for the success of subsequent steps, was evident from NMR signals for 15 β -H and 16 α -H which revealed $J_{15\beta,16\alpha}$ 5.6 Hz, and the presence of a four-bond coupling, $J_{15\beta,17\alpha}$ 2.5 Hz confirmed the configuration at C-15. A three-step reduction-mesylation-reductive deoxygenation sequence (*via* 44 and 45) proceeded smoothly to give the corresponding 16 β -methyl compound 46 in high yield (78% from 43). The derived sulfoxide 47 underwent smooth *syn*-elimination at 115°C in the presence of triethylamine to give the olefin 48, thus fulfilling the objective of conferring effective propyne equivalency on methyl propiolate cycloaddition to the dienyl acetate 25.

cis-Hydroxylation of 48 (to 36 + 42) and of the derived 17-acetate 49 (to 22 + 35) completed the preferred synthetic routes to the key intermediates for oxidative cleavage. Treatment of 22 (or mixtures of 22 + 35) with sodium periodate in aqueous ethanol gave 17-acetoxy-3-methoxy-20-oxo-19-nor-14 β -pregna-1,3,5(10)-triene-14-carbaldehyde 50¹⁵ in high yield, whereas similar oxidative cleavage of the 15,16,17-triols 36 and 42 proceeded with high 15,16-regioselectivity to give the corresponding 17-hydroxy 14 β -carbaldehyde 51.

Intramolecular aldol reaction of 50 was readily achieved in

the presence of hydrochloric acid in THF at 57°C to give the expected bridged enone 52, whereas treatment with *p*-TsOH in refluxing benzene resulted in concomitant bridgehead hydrolysis to give 53.

The foregoing reaction sequence thus succeeded in demonstrating preparative and structural analogy with the corresponding 14,17-isomeric series, but preliminary experiments revealed poor regioselectivity during attempted functional group modification of 52. The need to explore this problem in more depth was overtaken by the development of an alternative and more amenable strategy for the synthesis of the 14 β ,17 β -propano analogues of estradiol, based upon regioselective modification of the readily available 14 β -allyl 17-ketone 54.¹

Thus, Wacker oxidation¹⁶ of 54 led readily to the 14 β -acetoxy 17-ketone 55 (70%), which underwent an intramolecular aldol reaction in the presence of alkali, to give the 14 β ,17 β -(17 α -oxopropano) compound 57 (70%) accompanied by minor amounts (6%) of the alternative aldol closure product 56 as a mixture (~2:1 by NMR) of 16 α -epimers. LAH reduction of 57 proceeded in accordance with expectations for a bicyclo[3.2.1]octan-2-one system, to give a separable mixture (~2:3) of axial and equatorial 17 α -alcohols 58 and 59, which were converted into the respective hormone analogues 60 and 61.

The primary purpose of this phase of the investigation was served through 17 α -deoxygenation of the bridged intermediate 57. The method of choice entailed dithioketalisation of 57 in the presence of zinc trifluoromethanesulfonate as catalyst,¹⁷ which ensured conditions sufficiently mild for preparation of the ethylenedithio derivative 62 without interference from retro aldol cleavage of the bridge. Desulfurisation of 62 in the presence of Raney nickel furnished the 14 β ,17 β -propano compound 63, which was deprotected to give the estradiol analogue 64.

A complementary 17²-deoxygenation routine provided access to 14 β ,17 β -propano analogues. Thus, the ketone 57 was converted into a separable *syn/anti* mixture (~2:1) of the 17²-tosylhydrazones 65, treatment of which with butyllithium gave the separable unsaturated products 66 and 67. Deprotection of these products at C-3 furnished the respective estradiol analogues 68 and 69. The spectroscopic properties of 66 and 67 were remarkably similar, and failed to furnish irrefutable evidence of their respective structures. However, an unambiguous synthesis of the 17¹-olefin 66⁶ was decisive for this purpose.

The scope for regioselective 3'-functionalisation of the 14 β -allyl 17-ketone 54 was also explored in order to gain access, *via* intramolecular 3',17-closure, to 14 β ,17 β -propano 17,17¹-diols. This was readily achieved through hydroboration-oxidation of compound 54, followed by Swern oxidation of the resultant 3',17 ϵ -diol mixture, to give the 14 β -formylethyl 17-ketone 70 (70%). The dioxo compound 70 underwent intramolecular reductive cyclisation in the presence of McMurry reagent¹⁸ [prepared by Zn-Cu couple reduction of TiCl₃·(DME)₃] in DME at 20 °C, to give a separable mixture of 14 β ,17 β -propano 17,17¹-diols 71 (53%) and 72 (4%). The configurational assignments followed from distinctive NMR data for the diols 71 and 72, and their derived 17¹-acetates 73 and 74, and the product distribution is consistent with the expectation of stereoselectivity mediated by favoured co-linear alignment of the 17- and 3'-oxo groups of 70 in a chair-like transition state. The consequent presentation of the *si*-face of the 3'-oxo group to C-17 thus leads to preferential formation of the 17¹*R*-isomer 71.

An attempt to prepare sufficient of the minor 17¹*S*-isomer 72 for further study, *via* Mitsunobu inversion of compound 71, was unsuccessful. However, Swern oxidation of 71 gave the 17¹-ketone 77, which underwent highly stereoselective LAH reduction to give 72. Deprotection of the diols 71 and 72 furnished the corresponding bridged analogues 75 and 76 of estriol.

In further experiments conducted for comparison, LAH reduction, or preferably for high-yield recovery, NaBH₄-CeCl₃ reduction of the bridged enone 52 gave only the corresponding 17,17¹*S*-diol 78, the structure of which was confirmed by catalytic hydrogenation to give compound 72. The stereoselectivity of hydride reduction in this case contrasts with that of the epimeric bridged enone 2, suggesting that the 13 β -methyl group in 52 is a more decisive sterically-inhibiting factor to *exo*-approach of hydride, than are the ring C elements in 2. The 17,17¹*S*-diol 78 was also converted into the corresponding 3,17,17¹-triol 79 for comparative biological evaluation.

Receptor affinity studies

Competitive binding affinities for the estradiol receptor have been determined for the hormone analogues described here,¹⁹ using the competition factor²⁰ (CF) as determinant. 14,17 α -Propanoestra-1,3,5(10)-triene-3,17 β -diol 17 displays highly competitive binding (CF 1.5), which is amplified in 14,17 α -prop-17²-enoestra-1,3,5(10)-triene-3,17 β -diol 15 (CF 0.4), but strongly attenuated by introduction of a 17¹-hydroxy group both in the 14 α ,17 α -propano and the 14 α ,17 α -prop-17²-eno series, as exemplified by 6 (CF 60), 7 (CF 44), 10 (CF 98) and 12 (CF >500). In the 14 β ,17 β -propano series, the weak competitive binding of the parent estradiol analogue, 14,17 β -propano-14 β -estra-1,3,5(10)-triene-3,17 α -diol 64 (CF 69), is even more pronounced in all of the derived variants bearing β -bridge functionality or unsaturation: 60 (CF ∞), 61 (CF ∞), 68 (CF 318), 69 (CF 470), 75 (CF 360), 76 (CF >500) and 79 (CF 180). It is therefore evident that the binding affinity associated with the presence of a 14 α ,17 α -ethano bridge in estradiol² is retained in the presence of a 14 α ,17 α -propano bridge, which suggests that the corresponding binding domain in the estradiol receptor is indifferent to the steric demand of these structural features on the α -face of ring D. By contrast, the poor binding affinity of

the epimeric 14 β ,17 β -propano analogue reveals a receptor domain associated with the β -surface near ring D which is exceptionally sensitive to skeletal change. This influence may be largely steric, since superimpositional modelling studies display very small differences in the spatial disposition of polar binding regions in these analogues. A further conclusion is that polar functionality on the bridges, exemplified by the analogues in this study, is inimical to receptor binding, even in the example (*viz.* 75) manifesting structural resemblance to estriol and its 14 α ,17 α -ethano analogue.

An interpretation of these findings will be presented in the context of more detailed structural and molecular modelling studies, to be published elsewhere, but the trends described here suffice to reveal a pattern of structure-activity relationships which has been instrumental in identifying additional synthetic targets based upon ring D bridged analogues of estradiol.

Experimental

Mps were determined on a Reichert-Jung Thermovar apparatus and are uncorrected. Unless otherwise stated, spectra were recorded as follows: IR, Perkin-Elmer 983, chloroform solutions; ¹H NMR, Varian VXR (200 MHz) and Varian Unity (400 MHz), deuteriochloroform solutions (*J* values are given in Hz); ¹³C NMR, Varian VXR (50 MHz) or Varian Unity (100 MHz), deuteriochloroform solutions; mass spectra (electron-impact), VG Micromass 16F. Optical rotations were measured on a Perkin-Elmer 141 polarimeter for chloroform solutions at 20 °C, and [α]_D-values are given in 10⁻¹ deg cm² g⁻¹. Microanalyses were performed on a Carlo Erba EA 1108 instrument. Silica gel for chromatography refers to Merck Kieselgel 60, 63–200 μ m (gravity) or 40–63 μ m (flash).

3-Methoxy-17¹-oxo-14,17 α -prop-17²-enoestra-1,3,5(10)-triene-17 β -yl acetate 2

17 β -Acetoxy-3-methoxy-20-oxo-19-nor-17 α -pregna-1,3,5(10)-triene-14-carbaldehyde 1 (250 mg, 0.63 mmol) was treated with hydrochloric acid (12 mol dm⁻³; 1 cm³) in tetrahydrofuran (THF; 10 cm³) at 50 °C for 3 h. The cooled reaction mixture was neutralised with saturated aq. NaHCO₃, and the product was isolated by extraction with toluene. Filtration of the residue (260 mg) through silica gel (20 g) with EtOAc-toluene (1:19) gave the product 2 (234 mg, 98%), mp 200–204 °C (from CHCl₃-MeOH); [α]_D +174 (c 1.0 in CHCl₃) (Found: C, 75.5; H, 7.1%; M⁺, 380; C₂₆H₃₆O₄ requires C, 75.8; H, 7.4%; M, 380); ν_{max} /cm⁻¹ 1739 and 1693; δ_{H} (400 MHz) 1.13 (3 H, d, *J* 0.8, 13 β -Me), 1.45 (1 H, td, *J* 2 $\times$ 13.1 and 4.5, 12 α -H), 1.68 (1 H, ddd, *J* 12.5, 9.0 and 2.8), 1.92 (1 H, ddd, *J* 12.5, 9.8 and 5.8), 2.15 (3 H, s, 17 β -OAc), 2.22–2.29 (1 H, m, 12 β -H), 2.34 (1 H, ddd, *J* 14.9, 9.8 and 2.8, 16 β -H), 2.47 (1 H, td, *J* 2 $\times$ 11.7 and 4.3, 9 α -H), 2.53 (1 H, br ddd, *J* 14.9, 9.0 and 5.1, 16 α -H), 2.86–2.93 (2 H, m, 6-H₂), 3.77 (3 H, s, 3-OMe), 6.11 (1 H, d, *J* 10.0, 17²-H), 6.62 (1 H, d, *J* 2.7, 4-H), 6.71 (1 H, dd, *J* 8.6 and 2.7, 2-H), 7.0 (1 H, d, *J* 10.0, 17¹-H) and 7.17 (1 H, d, *J* 8.6, 1-H); δ_{C} (100 MHz) 196.1 (s, C-17¹), 169.9 (s, OCOMe), 157.7 (s, C-3), 153.5 (d, C-17²), 137.6 (s, C-5), 131.7 (s, C-10), 127.0 (d, C-17²), 127.3 (d, C-1), 113.8 (d, C-4), 112.0 (d, C-2), 93.8 (s, C-17), 55.2 (q, 3-OMe), 54.3 (s, C-14^{*}), 53.5 (s, C-13^{*}), 41.1 (d, C-8), 39.3 (d, C-9), 31.0 (t, C-16), 30.4 (t, C-6), 29.5 (t, C-15), 27.9 (t, C-11), 26.9 (t, C-12), 23.5 (t, C-7), 21.2 (q, OCOMe) and 15.9 (q, C-18). Assignments marked * may be the other way round.

17 β -Hydroxy-3-methoxy-14,17 α -prop-17¹-enoestra-1,3,5(10)-triene-17¹-one 3

(a) Compound 1 (430 mg, 1.08 mmol) was dissolved in methanolic potassium hydroxide (1 mol dm⁻³; 20 cm³). After 3 h at 25 °C, the mixture was diluted with water and the product was isolated by extraction with toluene. Crystallisation of the residue (402 mg) from CHCl₃-MeOH gave the product 3 (114 mg), mp 177–180 °C; [α]_D +195 (c 0.8 in CHCl₃) (Found: C,

78.1; H, 7.8%; M⁺, 338. C₂₂H₂₈O₂ requires C, 78.1; H, 7.7; M, 338; $\nu_{\text{max}}/\text{cm}^{-1}$ 3460 and 1670; $\delta_{\text{H}}(200 \text{ MHz})$ 1.17 (3 H, s, 13 β -Me), 2.46 (1 H, td, J 2 $\times$ 10.3 and 3.9, 9 α -H), 2.86–2.96 (2 H, m, 6-H₂), 3.78 (3 H, s, 3-OMe), 6.21 (1 H, d, J 9.8, 17²-H), 6.64 (1 H, d, J 2.7, 4-H), 6.73 (1 H, dd, J 8.6 and 2.7, 2-H), 7.17 (2 H, superimposed d, J 9.8, 17¹-H and d, J 8.6, 1-H). Chromatography of the mother-liquor residue (280 mg) on silica gel (30 g) with EtOAc–toluene (1:9) as eluent gave further product 3 (197 mg) followed by mixed fractions (17 mg).

(b) Similar alkaline treatment of the 17 β -acetate 2 (50 mg, 0.13 mmol) for 5 h, followed by crystallisation of the product (42 mg) from CHCl₃–MeOH gave the 17 β -alcohol 3 (25 mg). The mother-liquor residue comprised an inseparable mixture (TLC) of 3 and an isomeric product (~25% by NMR).

Hydride reduction of the enone 2

Lithium aluminium hydride (LAH) (319 mg, 8.4 mmol) was added to a stirred solution of the enone 2 (958 mg, 2.8 mmol) in dry THF (100 cm³) at 25 °C. After 2 h at 25 °C, the mixture was treated with ethyl acetate, added slowly to destroy excess reagent, and then acidified with aq. acetic acid. The resultant mixture was extracted with EtOAc–toluene (3:7), and the extract, on work-up, gave a residue (1.05 g) which was adsorbed on silica gel (35 g). Elution with MeOH–CHCl₃ (3:97) gave (17¹R)-3-methoxy-14,17 α -prop-17²-enoestra-1,3,5(10)-triene-17 β ,17¹-diol 4 (483 mg, 56%), mp 139–143 °C (from CHCl₃–MeOH); $[\alpha]_{\text{D}}^{25} +63$ (c 0.9 in THF) (Found: C, 77.2; H, 8.2%; M⁺, 340. C₂₂H₂₈O₂ requires C, 77.6; H, 8.3%; M, 340); $\delta_{\text{H}}(200 \text{ MHz}, \text{MeOD}-\text{CDCl}_3, (7:93))$ 0.97 (3 H, s, 13 β -Me), 2.39 (1 H, m, 9 α -H), 2.70–2.82 (2 H, m, 6-H₂), 3.68 (3 H, s, 3-OMe), 3.8 (1 H, d, J 4.1, 17¹-H), 5.68 (1 H, dd, J 9.8 and 4.1, 17²-H), 5.87 (1 H, d, J 9.8, 17²-H), 6.52 (1 H, d, J 2.8, 4-H), 6.61 (1 H, dd, J 8.6 and 2.8, 2-H) and 7.09 (1 H, d, J 8.6, 1-H), followed by (17¹S)-3-methoxy-14,17 α -prop-17²-enoestra-1,3,5(10)-triene-17 β ,17¹-diol 5 (320 mg, 37%), mp 170–173 °C (from CHCl₃–MeOH); $[\alpha]_{\text{D}}^{25} +180$ (c 0.9 in THF) (Found: C, 77.4; H, 8.2; M⁺, 340); $\delta_{\text{H}}(200 \text{ MHz}, \text{MeOD}-\text{CDCl}_3, (7:93))$ 1.0 (3 H, s, 13 β -Me), 2.66–2.82 (2 H, m, 6-H₂), 3.69 (3 H, s, 3-OMe), 4.3 (1 H, br s, H_{in} 3, 17¹-H), 5.43 (1 H, dd, J 9.8 and 2.0, 17²-H), 5.55 (1 H, dd, J 9.8 and 1.6, 17²-H), 6.52 (1 H, d, J 2.8, 4-H), 6.63 (1 H, dd, J 8.6 and 2.8, 2-H) and 7.1 (1 H, d, J 8.6, 1-H).

3-Demethylation of 4 (120 mg, 0.35 mmol) [diisobutyl-aluminium hydride (DIBALH; 10 equiv.) in dry toluene (5 cm³); reflux, 72 h] gave (17¹R)-14,17 α -prop-17²-enoestra-1,3,5(10)-triene-3,17 β ,17¹-triol 6 (70 mg, 61%), mp 215–218 °C (from MeOH); $[\alpha]_{\text{D}}^{25} +67$ (c 0.5 in THF) (Found: C, 76.9; H, 7.8%; M⁺, 326. C₂₁H₂₆O₂ requires C, 77.3; H, 8.0%; M, 326).

Similar treatment of 5 (100 mg, 0.29 mmol) gave (17¹S)-14,17 α -prop-17²-enoestra-1,3,5(10)-triene-3,17 β ,17¹-triol 7 (64 mg, 67%), mp 177–181 °C (from CHCl₃–MeOH); $[\alpha]_{\text{D}}^{25} +170$ (c 0.9 in THF) (Found: C, 76.8; H, 7.8%; M⁺, 326).

3-Methoxy-17¹-oxo-14,17 α -propanoestra-1,3,5(10)-trien-17 β -yl acetate 8

A solution of the enone 2 (230 mg, 0.61 mmol) in ethanol (40 cm³) at 25 °C was hydrogenated for 8 h at 200 kPa in the presence of palladium–carbon (10%; 60 mg). The mixture was filtered, and the filtrate was evaporated under reduced pressure. Chromatography of the residue (225 mg) on silica gel (20 g) with EtOAc–toluene (1:9) as eluent gave the 17¹-ketone 8 (192 mg, 83%), mp 205–208 °C (from CHCl₃–MeOH); $[\alpha]_{\text{D}}^{25} +44$ (c 1.0 in CHCl₃) (Found: C, 75.4; H, 7.9%; M⁺, 382. C₂₄H₂₈O₄ requires C, 75.4; H, 7.9%; M, 382); $\nu_{\text{max}}/\text{cm}^{-1}$ 1743 and 1722; $\delta_{\text{H}}(400 \text{ MHz})$ 1.1 (3 H, s, 13 β -Me), 1.69 (1 H, dd, J 13.5 and 9.6), 2.05 (1 H, obac. td), 2.11 (3 H, s, 17 β -OAc), 2.37 (1 H, dd, J 14.8 and 7.3), 2.74 (2 H, m), 2.81 (1 H, ddd, J 14.6, 9.4 and 5.2), 2.86–2.93 (2 H, m, 6-H₂), 3.77 (3 H, s, 3-OMe), 6.62 (1 H, d, J 2.7, 4-H), 6.7 (1 H, dd, J 8.6 and 2.7, 2-H) and 7.14 (1 H, d,

J 8.6, 1-H); $\delta_{\text{C}}(100 \text{ MHz})$ 206.4 (s, C-17¹), 170.1 (s, OCOMe), 157.5 (s, C-3), 137.4 (s, C-5), 133.2 (s, C-10), 126.6 (d, C-1), 113.8 (d, C-4), 111.7 (d, C-2), 92.6 (s, C-17), 55.1 (q, 3-OMe), 55.1 (s, C-14), 46.2 (s, C-13), 42.4 (d, C-8), 36.9 (d, C-9), 34.7, 33.6, 32.1, 27.9 and 26.1 (each t, C-11, C-15, C-16, C-17² and C-17³), 30.7 (t, C-6), 27.2 (t, C-12), 24.1 (t, C-7), 21.0 (q, OCOMe) and 15.9 (q, C-18).

(17¹R)-3-Methoxy-14,17 α -propanoestra-1,3,5(10)-triene-17 β ,17¹-diol 9

The 17¹-ketone 8 (110 mg, 0.29 mmol) was reduced with LAH, as described for compound 2. Crystallisation of the product (105 mg) from Me₂CO furnished the (17¹R)-17 β ,17¹-diol 9 (76 mg, 77%), mp 180–183 °C; $[\alpha]_{\text{D}}^{25} +61$ (c 0.9 in THF) (Found: C, 77.0; H, 8.6%; M⁺, 342. C₂₂H₂₈O₂ requires C, 77.2; H, 8.8%; M, 342); $\nu_{\text{max}}/\text{cm}^{-1}$ 3553 and 3391; $\delta_{\text{H}}(200 \text{ MHz}, \text{C}_2\text{D}_2\text{N})$ 1.26 (3 H, s, 13 β -Me), 2.75–2.88 (2 H, m, 6-H₂), 3.15 (1 H, td, J 2 $\times$ 13.3 and 4.7, 9 α -H), 3.71 (3 H, s, 3-OMe), 4.2 (1 H, t, J 2 $\times$ 3.0, 17¹-H), 6.79 (1 H, d, J 2.7, 4-H), 6.91 (1 H, dd, J 8.6 and 2.7, 2-H) and 7.31 (1 H, d, J 8.6, 1-H).

The derived (17¹R)-3,17 β ,17¹-triol 10 had mp 262–265 °C (from CHCl₃–MeOH); $[\alpha]_{\text{D}}^{25} +81$ (c 1.0 in THF) (Found: C, 76.5; H, 8.5%; M⁺, 328. C₂₁H₂₆O₂ requires C, 76.8; H, 8.6%; M, 328).

(17¹S)-3-Methoxy-14,17 α -propanoestra-1,3,5(10)-triene-17 β ,17¹-diol 11

A solution of the olefinic diol 5 (50 mg, 0.15 mmol) in ethanol (5 cm³) at 25 °C was hydrogenated for 6 h at 100 kPa in the presence of palladium–carbon (10%; 10 mg). The mixture was filtered, and the residue (53 mg) obtained from concentration of the filtrate was chromatographed on silica gel (6 g) with MeOH–CHCl₃ (3:97) as eluent to give the (17¹S)-17 β ,17¹-diol 11 (37 mg, 74%), mp 185–187 °C (from CHCl₃–MeOH); $[\alpha]_{\text{D}}^{25} +76$ (c 0.8 in THF) (Found: C, 76.8; H, 8.5%; M⁺, 342. C₂₂H₂₈O₂ requires C, 77.2; H, 8.8%; M, 342); $\delta_{\text{H}}(200 \text{ MHz}, \text{MeOD}-\text{CDCl}_3, (7:93))$ 0.98 (3 H, s, 13 β -Me), 2.25 (1 H, m, 11 α -H), 2.62 (1 H, td, J 2 $\times$ 12.0 and 5.4, 9 α -H), 2.71–2.83 (2 H, m, 6-H₂), 3.7 (3 H, s, 3-OMe), 3.86 (1 H, dd, J 9.9 and 6.1, 17¹-H), 6.53 (1 H, d, J 2.7, 4-H), 6.62 (1 H, dd, J 8.5 and 2.7, 2-H) and 7.1 (1 H, d, J 8.5, 1-H).

The derived (17¹S)-3,17 β ,17¹-triol 12 had mp 286–290 °C (from Me₂CO); $[\alpha]_{\text{D}}^{25} +70$ (c 1.0 in THF) (Found: C, 76.3; H, 8.3%; M⁺, 328. C₂₁H₂₆O₂ requires C, 76.8; H, 8.6; M, 328).

3-Methoxy-14,17 α -prop-17²-enoestra-1,3,5(10)-trien-17 β -ol 14

Boron trifluoride–diethyl ether (0.12 cm³) was added in three equal aliquots during 30 h to a solution of compound 3 (466 mg, 1.4 mmol) in ethanedithiol (1 cm³) and glacial acetic acid (3 cm³) at 30 °C. The reaction mixture was then poured into saturated aq. NaHCO₃, and the product (618 mg) was isolated by extraction with toluene and adsorbed on silica gel (50 g). Elution with EtOAc–toluene (1:9) gave the crude dithioketal 13 (560 mg, 98%), $\nu_{\text{max}}/\text{cm}^{-1}$ 3481; $\delta_{\text{H}}(200 \text{ MHz})$ 1.09 (3 H, s, 13 β -Me), 3.10–3.45 (4 H, m, SCH₂CH₂S), 3.77 (3 H, s, 3-OMe), 5.69 and 5.8 (each 1 H, d, J 9.6, 17²- and 17³-H), 6.62 (1 H, d, J 2.7, 4-H), 6.71 (1 H, dd, J 8.6 and 2.7, 2-H) and 7.19 (1 H, d, J 8.6, 1-H); m/z 414 (M⁺), which was used directly in the next step.

Compound 13 (700 mg, 1.7 mmol) in dry THF (20 cm³) was added dropwise to a stirred solution of sodium (390 mg) in liquid ammonia (120 cm³). After 2 h, solid NH₄Cl was added to the mixture to disperse the blue colour, and the ammonia was allowed to evaporate. Extraction of the residue with toluene gave material (560 mg) which was chromatographed on silica gel (30 g) with EtOAc–toluene (1:19) as eluent, to give the product 14 (459 mg, 84%), mp 141–142 °C (from CHCl₃–MeOH); $[\alpha]_{\text{D}}^{25} +137$ (c 1.0 in CHCl₃) (Found: C, 81.3; H, 8.5%; M⁺, 324. C₂₂H₂₈O₂ requires C, 81.4; H, 8.7%;

M , 324); δ_{H} (200 MHz) 1.06 (3 H, s, 13 β -Me), 2.8–2.9 (2 H, m, 6-H₂), 3.78 (3 H, s, 3-OMe), 5.57 (1 H, ddd, J 9.7, 4.4 and 2.2, 17^a-H), 5.75 (1 H, br d, J 9.7, 17^a-H), 6.63 (1 H, d, J 2.7, 4-H), 6.72 (1 H, dd, J 8.6 and 2.7, 2-H) and 7.21 (1 H, d, J 8.6, 1-H).

The derived 3,17 β -diol 15 had mp 156–159 °C (from EtOAc); $[\alpha]_{\text{D}}^{25} +150$ (c 0.9 in THF) (Found: C, 81.0; H, 8.1%; M^+ , 310. $\text{C}_{21}\text{H}_{28}\text{O}_2$ requires C, 81.25; H, 8.4%; M , 310).

3-Methoxy-14,17 α -propanoestra-1,3,5(10)-trien-17 β -ol 16

A solution of the olefin 14 (175 mg, 0.54 mmol) in ethyl acetate (10 cm³) at 25 °C was hydrogenated in the presence of palladium–carbon (10%, 50 mg) for 30 h at 200 kPa. The mixture was filtered, and the filtrate was evaporated to give the 17 β -alcohol 16 (170 mg, 97%), mp 155–157 °C (from CHCl_3 –MeOH); $[\alpha]_{\text{D}}^{25} +86$ (c 0.9 in CHCl_3) (Found: C, 80.7; H, 9.0%; M^+ , 326. $\text{C}_{22}\text{H}_{30}\text{O}_2$ requires C, 80.9; H, 9.3%; M , 326); $\nu_{\text{max}}/\text{cm}^{-1}$ 3602; δ_{H} (200 MHz) 1.03 (3 H, s, 13 β -Me), 2.07 (1 H, td, J 2 $\times$ 12.5 and 4.9, 12 α -H), 2.38 (1 H, dddd, J 13.1, 5.9, 4.9 and 2.0, 11 α -H), 2.74 (1 H, td, J 2 $\times$ 11.9 and 5.9, 9 α -H), 2.8–2.9 (2 H, m, 6-H₂), 3.77 (3 H, s, 3-OMe), 6.61 (1 H, d, J 2.8, 4-H), 6.71 (1 H, dd, J 8.6 and 2.8, 2-H) and 7.21 (1 H, d, J 8.6, 1-H).

The derived 3,17 β -diol 17 had mp 228–231 °C (from EtOAc); $[\alpha]_{\text{D}}^{25} +87$ (c 0.6 in THF) (Found: C, 80.4; H, 8.8%; M^+ , 312. $\text{C}_{21}\text{H}_{28}\text{O}_2$ requires C, 80.7; H, 9.0%; M , 312).

(17^aR)-3-Methoxy-16 α -methyl-17^a-phenylsulfonyl-14,17 α -ethanoestra-1,3,5(10)-triene-15 β ,16 β ,17 β -triol 17-acetate 19

Compound 18 (1.18 g, 2.33 mmol) was treated with osmium tetroxide (600 mg, 2.36 mmol) in pyridine (45 cm³) at 25 °C for 168 h. Aq. sodium dithionite (10%, 90 cm³) was added to the mixture which was then stirred at 25 °C for 45 min; after this it was diluted with water and extracted with CHCl_3 . The extract was washed successively with dilute hydrochloric acid, saturated aq. NaHCO_3 and brine, dried (MgSO_4) and concentrated under reduced pressure. Chromatography of the residue (1.42 g) on silica gel (50 g) with EtOAc–toluene (1:4) as eluent gave starting material 18 (157 mg) followed by the 15 β ,16 β -diol 19 (923 mg, 73%), double mp 174–176 and 232–234 °C (from CHCl_3 –hexane), $[\alpha]_{\text{D}}^{25} +115$ (c 1.0 in CHCl_3) (Found: C, 66.4; H, 6.7%; M^+ , 540. $\text{C}_{28}\text{H}_{36}\text{O}_5\text{S}$ requires C, 66.6; H, 6.7%; M , 540); $\nu_{\text{max}}/\text{cm}^{-1}$ 3481 and 1708; δ_{H} (400 MHz) 1.32 (3 H, s, 13 β -Me), 1.78 (3 H, s, 16 α -Me), 2.06 (3 H, s, 17 β -OAc), 2.14 (2 H, m, 7 β - and 8 β -H), 2.24 (1 H, dd, J 14.1 and 12.2, 17^a-H_{ax}), 2.42 (1 H, m, 7 α -H), 2.58 (1 H, dd, J 14.1 and 5.5, 17^a-H_{eq}), 2.84 (1 H, s, exch. by D_2O , 16 β -OH), 2.8–3.1 (3 H, m, 6-H₂ and 9 α -H), 3.06 (1 H, d, J 5.8, exch. by D_2O , 15 β -OH), 3.79 (3 H, s, 3-OMe), 3.99 (1 H, dd, J 12.2 and 5.5, 17^a-H), 4.95 (1 H, d, J 5.8 $\rightarrow$ on D_2O exch., 15 α -H), 6.63 (1 H, d, J 2.7, 4-H), 6.72 (1 H, dd, J 8.4 and 2.7, 2-H), 7.24 (1 H, d, J 8.4, 1-H) and 7.5–7.9 (5 H, m, SO_2Ph); δ_{C} (100 MHz) 170.4 (s, 17 β -OCOMe), 157.5 (s, C-3), 140.9 (s, C-1'), 138.9 (s, C-5), 133.6 (d, C-4'), 132.5 (s, C-10), 129.5 (2 C, d, C-3' and C-5'), 127.8 (2 C, d, C-2' and C-6'), 127.1 (d, C-1), 113.6 (d, C-4), 111.9 (d, C-2), 89.2 (s, C-17), 79.5 (s, C-16), 74.7 (d, C-15), 61.6 (d, C-17'), 58.1 (s, C-14), 55.3 (q, 3-OMe), 54.4 (s, C-13), 36.3 (d, C-9), 35.6 (d, C-8), 31.2 (t, C-6), 30.5 (t, C-12), 28.9 (t, C-17'), 26.7 (t, C-11), 25.3 (q, 16-Me), 24.9 (t, C-7), 21.6 (q, 17 β -OCOMe) and 18.0 (q, C-18).

17-Acetoxy-3-methoxy-20-oxo-15 β -phenylsulfonyl-19-nor-14 β -pregna-1,3,5(10)-triene-14-carbaldehyde 20

Aq. sodium periodate (6%, 29 cm³) was added to a solution of the diol 19 (923 mg, 1.7 mmol) in ethanol (50 cm³). After 3.5 h at 20 °C, the mixture was diluted with water and the product (927 mg) was isolated by extraction with CHCl_3 and adsorbed on silica gel (36 g). Elution with EtOAc–toluene (1:4) gave the 14-carbaldehyde 20 (778 mg, 85%) as an oil, $\nu_{\text{max}}/\text{cm}^{-1}$ 1737 and 1714; δ_{H} (200 MHz) 1.01 (3 H, s, 13 β -Me), 1.81 (1 H, dd, J 15.9

and 9.8, 16 α -H), 2.03 (3 H, s, 20-Me), 2.07 (3 H, s, 17 α -OAc), 2.65–2.9 (3 H, m, 6-H₂ and 9 α -H), 3.65 (1 H, dd, J 15.9 and 11.3, 16 β -H), 3.7 (3 H, s, 3-OMe), 4.28 (1 H, dd, J 11.3 and 9.8, 15 α -H), 6.63 (1 H, d, J 2.7, 4-H), 6.72 (1 H, dd, J 8.4 and 2.7, 2-H), 7.24 (1 H, d, J 8.4, 1-H), 7.5–8.0 (5 H, m, SO_2Ph) and 10.29 (1 H, s, 14 β -CHO); m/z 538 (M^+).

Reductive desulfonylation of compound 19

A solution of the phenylsulfonyl compound 19 (360 mg, 0.6 mmol) in THF (12 cm³) was added to samarium(II) iodide [generated by reaction of samarium (1.14 g) with diiodoethane (1.93 g, 6.8 mmol)] in THF (68 cm³) at –20 °C with stirring, followed by hexamethylphosphoric triamide (HMPA) (5.5 cm³). After 4 h at –20 °C, the mixture was poured into saturated aq. NH_4Cl . The mixture was extracted with EtOAc, and the extract was washed successively with aq. sodium dithionite and brine, dried (MgSO_4) and evaporated under reduced pressure. The residue (303 mg) was chromatographed on silica gel (25 g) with EtOAc–hexane (3:2) as eluent to give (17^aS)-3-methoxy-16 α -methyl-15 α ,17^a-cyclo-14,17 α -ethanoestra-1,3,5(10)-triene-16 β ,17 β -diol 17-acetate 21 (78 mg, 31%), double mp 112–114 and 146–147 °C (from CHCl_3 –MeOH); $[\alpha]_{\text{D}}^{25} +41$ (c 1.0 in CHCl_3) (Found: M^+ , 382.214. $\text{C}_{28}\text{H}_{36}\text{O}_5$ requires M , 382.214); $\nu_{\text{max}}/\text{cm}^{-1}$ 3594 and 1738; δ_{H} (400 MHz) 1.09 (1 H, dd, J 5.2 and 1.1, 17^a-H), 1.29 (3 H, s, 13 β -Me), 1.33 (3 H, s, 16 α -Me), 1.55 (1 H, d, J 5.2, 15-H), 1.83 (1 H, d, J 11.4, 17^a-H_{ax}), 2.09 (3 H, s, 17 β -OAc), 2.34 (1 H, obsc. m, 9 α -H), 2.58 (1 H, dd, J 11.4 and 1.1, 17^a-H_{eq}), 2.8 (2 H, m, 6-H₂), 3.76 (3 H, s, 3-OMe), 6.67 (1 H, d, J 2.8, 4-H), 6.73 (1 H, dd, J 8.6 and 2.8, 2-H) and 7.21 (1 H, d, J 8.6, 1-H); δ_{C} (50 MHz) 171.5 (s, 17 β -OCOMe), 158.1 (s, C-3), 138.5 (s, C-5), 133.0 (s, C-10), 127.4 (d, C-1), 114.4 (d, C-4), 112.4 (d, C-2), 90.5 (s, C-17), 83.1 (s, C-16), 55.9 (q, 3-OMe), 47.8 (s, C-13), 43.6 (d, C-9), 36.4 (s, C-14), 35.6 (d, C-8), 33.3 (t, C-12), 31.7 (d, C-17'), 30.5 (t, C-6), 27.5 (t, C-11), 25.7 (t, C-15), 24.1 (t, C-7), 23.9 (q, 16 α -Me), 22.3 (q, 17 β -OCOMe), 17.9 (q, C-18) and 12.2 (d, C-17'); followed by 3-methoxy-16 α -methyl-14,17 α -ethanoestra-1,3,5(10)-triene-15 β ,16 β ,17 β -triol 17-acetate 22 (78 mg, 29%), mp 182–185 °C (from Me_2CO –MeOH); $[\alpha]_{\text{D}}^{25} +4$ (c 1.0 in CHCl_3) (Found: C, 72.0; H, 8.0%; M^+ , 400. $\text{C}_{28}\text{H}_{36}\text{O}_5$ requires C, 72.0; H, 8.05%; M , 400); $\nu_{\text{max}}/\text{cm}^{-1}$ 3600, 3575 and 1738; δ_{H} (400 MHz) 1.24 (3 H, s, 13 β -Me), 1.49 (3 H, s, 16 α -Me), 2.11 (3 H, s, 17 β -OAc), 2.64 (1 H, td, J 2 $\times$ 11.5 and 3.4, 9 α -H), 2.78–3.0 (2 H, m, 6-H₂), 3.61 (1 H, d, J 4.7 $\rightarrow$ on D_2O exch., 15 α -H), 3.76 (3 H, s, 3-OMe), 6.67 (1 H, d, J 2.8, 4-H), 6.73 (1 H, dd, J 8.6 and 2.8, 2-H) and 7.21 (1 H, d, J 8.6, 1-H); δ_{C} (50 MHz) 170.2 (s, 17 β -OCOMe), 157.4 (s, C-3), 137.9 (s, C-5), 133.1 (s, C-10), 126.3 (d, C-1), 113.7 (d, C-4), 111.5 (d, C-2), 91.6 (s, C-17), 82.3 (d, C-16), 78.7 (s, C-15), 55.2 (q, 3-OMe), 49.9 and 48.9 (each s, C-13 and C-14), 36.6 (d, C-9), 34.6 (d, C-8), 32.1 (t, C-6), 29.9 (t, C-17'), 27.1 (q, 16 α -Me), 25.8 (t, C-11), 23.7 (t, C-12), 23.4 (t, C-17'), 23.2 (t, C-7), 21.8 (q, 17 β -OCOMe) and 16.3 (q, C-18). Further elution gave starting material 19 (102 mg, 28%).

Acetonide formation–reductive desulfonylation of compound 19

The diol 19 (740 mg, 1.37 mmol) was treated with aq. perchloric acid (70%, 0.05 cm³) in acetone (20 cm³) at 20 °C for 5 h. NaHCO_3 was added to the mixture which was then concentrated under reduced pressure, treated with water and extracted with EtOAc. Work-up of the extract gave a brown oily residue (750 mg) which was adsorbed on silica gel (38 g). Elution with EtOAc–hexane (7:13) gave (17^aR)-15 β ,16 β -isopropylidene-dioxy-3-methoxy-16 α -methyl-17^a-phenylsulfonyl-14,17 α -ethanoestra-1,3,5(10)-trien-17 β -yl acetate 23 (625 mg, 79%), mp 185–188 °C (from Me_2CO –MeOH); $[\alpha]_{\text{D}}^{25} +102$ (c 1.0 in CHCl_3) (Found: C, 67.9; H, 6.9%; M^+ , 580. $\text{C}_{33}\text{H}_{40}\text{O}_5\text{S}$ requires C, 68.25; H, 6.9%; M , 580); $\nu_{\text{max}}/\text{cm}^{-1}$ 1735; δ_{H} (400 MHz) 1.43, 1.48 and 1.55 (each 3 H, s, 13 β -Me and CMe_2), 1.93 (3 H, s, 16 α -Me), 2.07 (3 H, s, 17 β -OAc), 3.79 (3 H, s, 3-OMe), 4.05 (1 H, dd, J

12.4 and 5.5, 17¹-H), 5.17 (1 H, s, 15 α -H), 6.67 (1 H, d, *J* 2.8, 4-H), 6.73 (1 H, dd, *J* 8.6 and 2.8, 2-H), 7.21 (1 H, d, *J* 8.6, 1-H) and 7.5–7.9 (5 H, m, SO₂Ph).

The acetonide 23 (200 mg, 0.34 mmol) was treated with samarium(II) iodide and HMPA in THF (18 cm³) at –20 °C for 1.5 h, as described for compound 19. Chromatography of the product (240 mg) on silica gel (15 g) with EtOAc–hexane (1:9→3:7) as eluent gave 15 β ,16 β -isopropylidenedioxy-3-methoxy-16 α -methyl-14,17 α -ethanoestra-1,3,5(10)-trien-17 β -yl acetate 24 (105 mg, 70%), mp 213–217 °C (from CHCl₃–MeOH); [α]_D –24 (c 1.0 in CHCl₃) (Found: C, 73.4; H, 8.0%; M⁺, 440. C₂₇H₃₄O₅ requires C, 73.6; H, 8.2%; M, 440); δ_H(400 MHz) 0.92 (1 H, ddd, *J* 13.4, 9.0 and 6.2, 12 α -H), 1.33 (3 H, s, 13 β -Me), 1.43 and 1.52 (each 3 H, s, CMe₂), 1.64 (3 H, s, 16 α -Me), 2.11 (3 H, s, 17 β -OAc), 2.24 (1 H, dq, *J* 13.2 and 3 × 3.9, 7 β -H), 2.62 (1 H, td, *J* 2 × 11.8 and 4.1, 9 α -H), 2.8–3.02 (2 H, m, 6-H₂), 3.76 (3 H, s, 3-OMe), 3.89 (1 H, s, 15 α -H), 6.67 (1 H, d, *J* 2.8, 4-H), 6.73 (1 H, dd, *J* 8.6 and 2.8, 2-H) and 7.21 (1 H, d, *J* 8.6, 1-H).

Methyl 17 β -acetoxy-3-methoxy-14,17 α -ethanoestra-1,3,5(10),15-tetraene-16-carboxylate 26

3-Methoxyestra-1,3,5(10),14,16-pentaen-17-yl acetate 25 (162 mg, 0.5 mmol) and methyl propiolate (0.09 cm³, 1 mmol) in dry benzene (2 cm³) was kept at 100 °C (sealed tube) for 23 h. The reaction mixture was then adsorbed directly on silica gel (16 g) and eluted with EtOAc–toluene (1:49) to give starting material (8 mg) followed by the cycloadduct 26 (174 mg, 85%), mp 141–144 °C (from CHCl₃–MeOH); [α]_D +1 (c 1.0 in CHCl₃) (Found: C, 73.5; H, 7.0%; M⁺, 408. C₂₅H₃₂O₅ requires C, 73.5; H, 6.9%; M, 408); δ_H(200 MHz) 1.19 (3 H, s, 13 β -Me), 2.16 (3 H, s, 17 β -OAc), 2.5 (1 H, td, *J* 2 × 11.4 and 3.4, 9 α -H), 2.86–2.98 (2 H, m, 6-H₂), 3.69 (3 H, s, 16-CO₂Me), 3.77 (3 H, s, 3-OMe), 6.58 (1 H, d, *J* 5.6, 17¹-H), 6.64 (1 H, d, *J* 2.4, 4-H), 6.73 (1 H, dd, *J* 8.6 and 2.4, 2-H), 7.05 (1 H, d, *J* 5.6, 17¹-H), 7.2 (1 H, d, *J* 8.6, 1-H) and 7.44 (1 H, s, 15-H); δ_C(50 MHz) 170.8 (s, 17 β -OCOMe), 164.8 (s, 16-CO₂Me), 157.6 (s, C-3), 152.4 (d, C-15), 147.1 (d, C-16), 140.6 and 139.1 (each d, C-17¹ and C-17²), 137.8 (s, C-5), 131.9 (s, C-10), 126.7 (d, C-1), 113.8 (d, C-4), 111.8 (d, C-2), 98.7 (s, C-17), 88.3 (s, C-13), 65.4 (s, C-14), 55.2 (q, 3-OMe), 51.3 (q, 16-CO₂Me), 39.8 (d, C-9), 35.9 (d, C-8), 30.2 (t, C-6), 29.9 (t, C-12), 26.3 (t, C-7), 25.2 (t, C-11), 21.4 (q, 17 β -OCOMe) and 17.0 (q, C-18).

Methyl 17 β -acetoxy-3-methoxy-14,17 α -ethanoestra-1,3,5(10),15-tetraene-16-carboxylate 27

A solution of the cycloadduct 26 (3.05 g, 7.5 mmol) in ethyl acetate (100 cm³) at 25 °C was hydrogenated for 3 h in the presence of palladium–carbon (10%; 1.02 g) and then filtered through Celite. The filtrate was evaporated and the residue (2.98 g) was chromatographed on silica gel (300 g) with EtOAc–hexane (3:17) as eluent, to give the dihydro compound 27 (2.55 g, 83%), mp 104–108 °C (from MeOH); [α]_D +9 (c 1.0 in CHCl₃) (Found: C, 73.1; H, 7.5%; M⁺, 410. C₂₉H₃₆O₅ requires C, 73.1; H, 7.4%; M, 410); ν_{max}/cm^{–1} 1737 and 1713; δ_H(200 MHz) 0.95 (3 H, s, 13 β -Me), 2.09 (3 H, s, 17 β -OAc), 2.74 (1 H, td, *J* 2 × 11.3 and 4, 9 α -H), 2.85–2.98 (2 H, m, 6-H₂), 3.71 (3 H, s, 16-CO₂Me), 3.77 (3 H, s, 3-OMe), 6.62 (1 H, d, *J* 2.7, 4-H), 6.7 (1 H, dd, *J* 8.6 and 2.7, 2-H), 6.91 (1 H, s, 15-H) and 7.18 (1 H, d, *J* 8.6, 1-H); δ_C(50 MHz) 170.4 (s, 17 β -OCOMe), 164.6 (s, 16-CO₂Me), 157.6 (s, C-3), 144.4 (d, C-15), 137.9 (s, C-5), 137.5 (s, C-16), 132.6 (s, C-10), 126.2 (d, C-1), 113.9 (d, C-4), 111.6 (d, C-2), 93.8 (s, C-17), 59.6 (s, C-14), 55.2 (q, 3-OMe), 54.1 (s, C-13), 51.3 (q, 16-CO₂Me), 37.1 (d, C-9), 36.0 (d, C-8), 29.9 (t, C-6), 29.7 (t, C-12), 28.1 (t, C-17¹), 25.9 (t, C-7), 25.4 (t, C-11), 24.7 (t, C-17²), 21.7 (q, 17 β -OCOMe) and 15.4 (q, C-18).

Hydroxylation of the unsaturated ester 27

Compound 27 (403 mg, 0.98 mmol) was treated with osmium tetroxide (250 mg, 0.9 mmol) in pyridine (5 cm³) at 25 °C for

48 h. Aq. sodium dithionite (10%, 40 cm³) was added to the mixture which was then stirred at 25 °C for 45 min. The product was isolated by extraction with CHCl₃ and chromatographed on silica gel (26 g) with EtOAc–toluene (1:9) as eluent to give starting material 27 (23 mg) followed by methyl 17 β -acetoxy-15 α ,16 α -dihydroxy-3-methoxy-14,17 α -ethanoestra-1,3,5(10)-triene-16 β -carboxylate 28 (281 mg, 65%), mp 169–170 °C (from Me₂CO); [α]_D +49 (c 1.0 in CHCl₃) (Found: C, 67.9; H, 7.2%; M⁺, 444. C₂₈H₃₂O₇ requires C, 67.5; H, 7.25%; M, 444); ν_{max}/cm^{–1} 3509br, 1733 and 1714; δ_H(200 MHz) 0.82 (3 H, s, 13 β -Me), 2.14 (3 H, s, 17 β -OAc), 2.7 (1 H, obsc. m, 9 α -H), 2.8–2.9 (2 H, m, 6-H₂), 3.73 (3 H, s, 16 β -CO₂Me), 3.76 (3 H, s, 3-OMe), 4.42 (1 H, d, *J* 1.5, 15 β -H), 5.9 (1 H, s, exch. by D₂O, 16 α -OH), 6.67 (1 H, d, *J* 2.8, 4-H), 6.73 (1 H, dd, *J* 8.6 and 2.8, 2-H) and 7.21 (1 H, d, *J* 8.6, 1-H); δ_C(50 MHz) 174.9 (s, 16-CO₂Me), 171.4 (s, 17 β -OCOMe), 157.5 (s, C-3), 137.9 (s, C-5), 132.4 (s, C-10), 126.3 (d, C-1), 113.6 (d, C-4), 111.6 (d, C-2), 93.7 (s, C-17), 75.9 (d, C-15), 75.6 (s, C-16), 55.1 (q, 3-OMe), 52.5 (q, 16-CO₂Me), 48.3 and 46.5 (each s, C-13 and C-14), 40.0 (d, C-8), 37.1 (d, C-9), 29.9 (t, C-6), 28.4 (t, C-12), 25.4 (t, C-7), 24.4 (t, C-11), 24.0 (t, C-17¹), 21.2 (q, 17 β -OCOMe), 17.8 (t, C-17²) and 13.4 (q, C-18) and methyl 17 β -acetoxy-15 β ,16 β -dihydroxy-3-methoxy-14,17 α -ethanoestra-1,3,5(10)-triene-16 α -carboxylate 29 (121 mg, 27%), mp 182–185 °C (from Me₂CO); [α]_D +45 (c 1.0 in CHCl₃) (Found: C, 67.7; H, 7.1%; M⁺, 444); ν_{max}/cm^{–1} 3410, 1730 and 1710; δ_H(200 MHz) 1.23 (3 H, s, 13 β -Me), 2.07 (3 H, s, 17 β -OAc), 2.7 (1 H, obsc. m, 9 α -H), 2.82–3.0 (2 H, m, 6-H₂), 3.76 (3 H, s, 3-OMe), 3.91 (3 H, s, 16 α -CO₂Me), 4.11 (1 H, s, 15 α -H), 4.45 (1 H, s, exch. by D₂O, 16 β -OH), 6.67 (1 H, d, *J* 2.8, 4-H), 6.73 (1 H, dd, *J* 8.6 and 2.8, 2-H) and 7.21 (1 H, d, *J* 8.6, 1-H); δ_C(50 MHz) 175.2 (s, 16 α -CO₂Me), 170.7 (s, 17 β -OCOMe), 157.4 (s, C-3), 137.8 (s, C-5), 132.9 (s, C-10), 126.2 (d, C-1), 113.7 (d, C-4), 111.4 (d, C-2), 90.3 (s, C-17), 80.8 (s, C-16), 79.9 (d, C-15), 55.1 (q, 3-OMe), 53.6 (q, 16 α -CO₂Me), 50.8 and 48.8 (each s, C-13 and C-14), 36.8 (d, C-9), 34.5 (d, C-8), 30.7 (t, C-6), 25.6 (t, C-7), 23.8 (t, C-11), 23.6 (t, C-17¹), 22.9 (t, C-17²), 21.0 (q, 17 β -OCOMe) and 15.8 (q, C-18).

Methyl 17 β -acetoxy-15 α ,16 α -isopropylidenedioxy-3-methoxy-14,17 α -ethanoestra-1,3,5(10)-triene-16 β -carboxylate 30

The diol 28 (4.37 g, 9.84 mmol) was treated with aq. perchloric acid (70%; 5.5 ml) in acetone (440 cm³) at 20 °C for 2 h. NaHCO₃ (5 g) was added to the mixture which was then concentrated under reduced pressure and diluted with water; the product was isolated by extraction with CHCl₃. Work-up follow by chromatography of the residue (5.9 g) on silica gel (150 g) with EtOAc–toluene (1:9) as eluent gave the acetonide 30 (4.04 g, 85%), mp 130–134 °C (from Me₂CO–MeOH); [α]_D +57 (c 1.0 in CHCl₃) (Found: C, 69.1; H, 7.2%; M⁺, 484. C₂₉H₃₄O₇ requires C, 69.4; H, 7.5%; M, 484); ν_{max}/cm^{–1} 1740; δ_H(200 MHz) 0.95 (3 H, s, 13 β -Me), 1.35 and 1.61 (each 3 H, s, CMe₂), 2.03 (3 H, s, 17 β -OAc), 2.68 (1 H, td, *J* 2 × 11.4 and 4.8, 9 α -H), 2.8–2.91 (2 H, m, 6-H₂), 3.14 (1 H, m), 3.76 (3 H, s, 16 β -CO₂Me), 3.78 (3 H, s, 3-OMe), 5.0 (1 H, d, *J* 1.1, 15 β -H), 6.67 (1 H, d, *J* 2.8, 4-H), 6.73 (1 H, dd, *J* 8.6 and 2.8, 2-H) and 7.21 (1 H, d, *J* 8.6, 1-H).

16 β -Hydroxymethyl-15 α ,16 α -isopropylidenedioxy-3-methoxy-14,17 α -ethanoestra-1,3,5(10)-trien-17 β -ol 31

LAH (918 mg, 80 mmol) was added in small portions to a stirred solution of the ester 30 (4.04 g, 8.4 mmol) in THF (50 cm³) at 25 °C under nitrogen. After 3 h at 25 °C, saturated aq. NH₄Cl was added to the mixture and the product was isolated by extraction with CHCl₃. Filtration of the product (4.05 g) through silica gel (90 g) with EtOAc–toluene (2:3) gave the diol 31 (3.47 g, 100%) as an oil, [α]_D +97 (c 1.0 in CHCl₃) (Found: C, 72.2; H, 8.2%; M⁺, 414. C₂₉H₃₆O₅ requires C, 72.4; H, 8.3%; M, 414); ν_{max}/cm^{–1} 3544br; δ_H(400 MHz) 0.92 (3 H, s, 13 β -Me), 1.48 and 1.62 (each 3 H, s, CMe₂), 2.68 (1 H, td, *J* 2 × 11.3 and 4.3, 9 α -H), 2.74–2.9 (2 H, m, 6-H₂), 3.76 (3 H, s, 3-OMe), 3.79

and 4.32 (each 1 H, d, J 11.9, 16 β -CH₂OH), 4.22 (1 H, d, J 1.2, 15 β -H), 6.67 (1 H, d, J 2.8, 4-H), 6.73 (1 H, dd, J 8.6 and 2.8, 2-H) and 7.21 (1 H, d, J 8.6, 1-H).

15 α ,16 α -Isopropylidenedioxy-3-methoxy-16 β -methyl-14,17 α -ethanoestra-1,3,5(10)-trien-17 β -ol 33

Methanesulfonyl chloride (2.1 cm³, 24 mmol) was added to a solution of the diol 31 (3.4 g, 8.2 mmol) in dry pyridine (20 cm³) at 0 °C under nitrogen. After 1.5 h at 0 °C, the mixture was diluted with water and the product was isolated by extraction with toluene. Work-up followed by chromatography of the residue (4.4 g) on silica gel (100 g) with EtOAc-toluene (3:7) as eluent gave the non-crystalline 16'-methanesulfonate 32 (4.04 g, 100%), δ_{H} (200 MHz) 0.99 (3 H, s, 13 β -Me), 1.5 and 1.6 (each 3 H, s, CMe₂), 3.05 (3 H, s, 16'-OMe), 3.75 (3 H, s, 3-OMe), 4.45 and 4.75 (each 1 H, d, J 11.1, 16-CH₂OMe), 4.6 (1 H, d, J 1.4, 15 β -H), 6.6 (1 H, d, J 2.8, 4-H), 6.7 (1 H, dd, J 8.6 and 2.8, 2-H) and 7.21 (1 H, d, J 8.6, 1-H).

The methanesulfonate 32 (4.04 g, 8.2 mmol) was treated with LAH (909 mg, 24 mmol) in dry THF (20 cm³) at 25 °C under nitrogen for 24 h. Saturated aq. NH₄Cl was added to the reaction mixture and the product (2.98 g) was isolated by extraction with toluene. Work-up followed by absorption of the residue on silica gel (200 g) and elution with EtOAc-toluene (3:17) gave the 16 β -methyl compound 33 (2.92 g, 89%), mp 98–102 °C (from Me₂CO-MeOH); $[\alpha]_{\text{D}}^{25} +111$ (c 1.0 in CHCl₃) (Found: C, 75.3; H, 8.6%; M⁺, 398. C₂₈H₃₄O₅ requires C, 75.3; H, 8.6%; M, 398); $\nu_{\text{max}}/\text{cm}^{-1}$ 3598; δ_{H} (400 MHz) 0.94 (3 H, s, 13 β -OMe), 1.45 and 1.63 (each 3 H, s, CMe₂), 1.54 (3 H, s, 16 β -Me), 2.7 (1 H, td, J 2 $\times$ 11.8 and 4.8, 9 α -H), 2.78–2.88 (2 H, m, 6-H₂), 3.73 (3 H, s, 3-OMe), 4.22 (1 H, d, J 1.6, 15 β -H), 6.6 (1 H, d, J 2.8, 4-H), 6.7 (1 H, dd, J 8.6 and 2.8, 2-H) and 7.21 (1 H, d, J 8.6, 1-H).

The derived (Ac₂O-*p*-TsOH-THF, 20 °C, 1 h) 17 β -acetate 34 had mp 191–193 °C (from CHCl₃-MeOH); $[\alpha]_{\text{D}}^{25} +96$ (c 1.0 in CHCl₃) (Found: C, 73.3; H, 8.2%; M⁺, 440. C₂₉H₃₄O₅ requires C, 73.6; H, 8.2%; M, 440); δ_{H} (200 MHz) 0.99 (3 H, s, 13 β -Me), 1.46 and 1.59 (each 3 H, s, CMe₂), 1.56 (3 H, s, 16 β -Me), 2.05 (3 H, s, 17 β -OAc), 3.76 (3 H, s, 3-OMe), 4.3 (1 H, d, J 1.3, 15 β -H), 6.6 (1 H, d, J 2.8, 4-H), 6.7 (1 H, dd, J 8.6 and 2.8, 2-H) and 7.21 (1 H, d, J 8.6, 1-H).

3-Methoxy-16 β -methyl-14,17 α -ethanoestra-1,3,5(10)-trien-15 α ,16 α ,17 β -triol 17-acetate 35

A solution of the acetone 34 (93 mg, 0.21 mmol) in methanolic iodine (1%; 13 cm³) was refluxed for 5.5 h, cooled and treated with aq. sodium thiosulfate. Extraction of the reaction mixture with EtOAc and work-up gave a product (80 mg) which was adsorbed on silica gel (10 g). Elution with EtOAc-hexane (1:9) gave mixed fractions (9 mg) followed by the 15 α ,16 α -diol 35 (63 mg, 79%), mp 145–149 °C (from Me₂CO-MeOH); $[\alpha]_{\text{D}}^{25} +91$ (c 1.0 in CHCl₃) (Found: C, 72.0; H, 8.1%; M⁺, 400. C₂₈H₃₂O₅ requires C, 72.0; H, 8.05%; M, 400); $\nu_{\text{max}}/\text{cm}^{-1}$ 3482br and 1710; δ_{H} (200 MHz) 0.99 (3 H, s, 13 β -Me), 1.35 (3 H, s, 16 β -Me), 2.12 (3 H, s, 17 β -OAc), 2.62 (1 H, obs. m, 9 α -H), 2.76–2.9 (2 H, m, 6-H₂), 3.75 (1 H, d, J 1.1, 15 β -H), 3.77 (3 H, s, 3-OMe), 6.6 (1 H, d, J 2.8, 4-H), 6.7 (1 H, dd, J 8.6 and 2.8, 2-H) and 7.21 (1 H, d, J 8.6, 1-H).

The derived (LAH-THF, 20 °C) 15 α ,16 α ,17 β -triol 36 had mp 183–185 °C (from CHCl₃-hexane); $[\alpha]_{\text{D}}^{25} +77$ (c 0.6 in CHCl₃) (Found: M⁺, 358.214. C₂₇H₃₀O₄ requires M, 358.214); δ_{H} (200 MHz) 0.93 (3 H, s, 13 β -Me), 1.44 (3 H, s, 16 β -Me), 2.78–2.9 (2 H, m, 6-H₂), 3.72 (1 H, dd, J 7.2 and 1.3 $\rightarrow$ d, J 1.3 on D₂O exch., 15 β -H), 3.77 (3 H, s, 3-OMe), 6.64 (1 H, d, J 2.4, 4-H), 6.73 (1 H, dd, J 8.6 and 2.4, 2-H) and 7.18 (1 H, d, J 8.6, 1-H).

15 β ,16 β -Isopropylidenedioxy-3-methoxy-16 α -methyl-14,17 α -ethanoestra-1,3,5(10)-trien-17 β -ol 40

A reaction sequence similar to that described in the foregoing experiments was used to convert the 16 α -carboxylate 29 into

the 16 α -methyl compound 40. The intermediates and product were characterised as follows.

Methyl 17 β -acetoxy-15 β ,16 β -isopropylidenedioxy-3-methoxy-14,17 α -ethanoestra-1,3,5(10)-trien-16 α -carboxylate 37, double mp 178–182 and 187–191 °C (from CHCl₃-hexane); $[\alpha]_{\text{D}}^{25} +4$ (c 1.0 in CHCl₃) (Found: C, 69.1; H, 7.2%; M⁺, 484. C₂₈H₃₄O₇ requires C, 69.4; H, 7.5%; M, 484); $\nu_{\text{max}}/\text{cm}^{-1}$ 1732; δ_{H} 1.22 (3 H, s, 13 β -Me), 1.35 and 1.55 (each 3 H, s, CMe₂), 2.09 (3 H, s, 17 β -OAc), 2.66 (1 H, td, J 2 $\times$ 11.3 and 4.0, 9 α -H), 2.8–3.02 (2 H, m, 6-H₂), 3.76 (3 H, s, 16 α -CO₂Me), 3.78 (3 H, s, 3-OMe), 4.64 (1 H, s, 15 α -H), 6.67 (1 H, d, J 2.8, 4-H), 6.73 (1 H, dd, J 8.6 and 2.8, 2-H) and 7.21 (1 H, d, J 8.6, 1-H).

16 α -Hydroxymethyl-15 β ,16 β -isopropylidenedioxy-3-methoxy-14,17 α -ethanoestra-1,3,5(10)-trien-17 β -ol 38, gum; $[\alpha]_{\text{D}}^{25} +5$ (c 1.0 in CHCl₃) (Found: C, 72.6; H, 8.2%; M⁺, 414. C₂₈H₃₄O₅ requires C, 72.4; H, 8.3%; M, 414); $\nu_{\text{max}}/\text{cm}^{-1}$ 3544br; δ_{H} (200 MHz) 1.31 (3 H, s, 13 β -Me), 1.41 and 1.56 (each 3 H, s, CMe₂), 2.64 (1 H, td, J 2 $\times$ 11.2 and 4.2, 9 α -H), 2.76–3.0 (2 H, m, 6-H₂), 3.77 (3 H, s, 3-OMe), 3.84 (1 H, s, 15 α -H), 4.02 and 4.1 (each 1 H, d, after D₂O exch., J 11.8, 16-H₂), 6.67 (1 H, d, J 2.8, 4-H), 6.73 (1 H, dd, J 8.6 and 2.8, 2-H) and 7.21 (1 H, d, J 8.6, 1-H).

15 β ,16 β -Isopropylidenedioxy-3-methoxy-16 α -methyl-14,17 α -ethanoestra-1,3,5(10)-trien-17 β -ol 40, gum; $[\alpha]_{\text{D}}^{25} +62$ (c 1.0 in CHCl₃) (Found: C, 75.4; H, 8.7%; M⁺, 398. C₂₈H₃₄O₅ requires C, 75.3; H, 8.6%; M, 398); $\nu_{\text{max}}/\text{cm}^{-1}$ 3572; δ_{H} (200 MHz) 1.26 (3 H, s, 13 β -Me), 1.43 and 1.54 (each 3 H, s, CMe₂), 1.5 (3 H, s, 16 α -Me), 2.65 (1 H, td, J 2 $\times$ 11.3 and 4.3, 9 α -H), 2.8–3.0 (2 H, m, 6-H₂), 3.78 (3 H, s, 3-OMe), 3.92 (1 H, s, 15 α -H), 6.6 (1 H, d, J 2.8, 4-H), 6.7 (1 H, dd, J 8.6 and 2.8, 2-H) and 7.21 (1 H, d, J 8.6, 1-H).

Acetylation (Ac₂O-*p*-TsOH-THF, 25 °C, 24 h) of 40 gave compound 24, identical with that prepared in a previous experiment.

Deprotection of the acetone 24

Treatment of compound 24 (970 mg, 2.2 mmol) with methanolic iodine in THF as described in a previous experiment, followed by flash chromatography of the product (914 mg) on silica gel (70 g) with EtOAc-hexane (7:13), gave starting material (17 mg) followed by an inseparable mixture (794 mg, 91%) of the isomers 22 and 41. A subsequent experiment (see later) resulted in isolation of pure 3-methoxy-16 α -methyl-14,17 α -ethanoestra-1,3,5(10)-trien-15 β ,16 β ,17 β -triol 15-acetate 41 (258 mg, 32%), mp 183–186 °C (from CHCl₃-MeOH); $[\alpha]_{\text{D}}^{25} -17$ (c 1.0 in CHCl₃) (Found: C, 72.1; H, 7.7%; M⁺, 400. C₂₈H₃₂O₅ requires C, 72.0; H, 8.05%; M, 400); $\nu_{\text{max}}/\text{cm}^{-1}$ 3597 and 1721; δ_{H} (200 MHz) 1.14 (3 H, s, 13 α -Me), 1.48 (3 H, s, 16 α -Me), 2.05 (3 H, s, 15 β -OAc), 2.64 (1 H, td, J 2 $\times$ 11.2 and 4.8, 9 α -H), 2.78–2.86 (2 H, m, 6-H₂), 3.77 (3 H, s, 3-OMe), 4.45 (1 H, s, 15 α -H), 6.67 (1 H, d, J 2.8, 4-H), 6.73 (1 H, dd, J 8.6 and 2.8, 2-H) and 7.21 (1 H, d, J 8.6, 1-H).

Hydrolysis (LAH-THF, 25 °C) of the mixture (65 mg, 0.16 mmol) of 22 and 41 gave the corresponding 15 β ,16 β ,17 β -triol 42 (57 mg, 98%), mp 190–193 °C (from CHCl₃-hexane); $[\alpha]_{\text{D}}^{25} +22$ (c 1.0 in CHCl₃) (Found: C, 74.0; H, 8.3%; M⁺, 358. C₂₇H₃₀O₄ requires C, 73.7; H, 8.4%; M, 358); $\nu_{\text{max}}/\text{cm}^{-1}$ 3552; δ_{H} (200 MHz) 1.11 (3 H, d, J 0.9, 13 β -Me), 1.35 (3 H, s, 16 α -Me), 2.8–3.0 (2 H, m, 6-H₂), 3.6 (1 H, s, 15 α -Me), 3.75 (3 H, s, 3-OMe), 6.67 (1 H, d, J 2.8, 4-H), 6.73 (1 H, dd, J 8.6 and 2.8, 2-H) and 7.21 (1 H, d, J 8.6, 1-H).

Methyl 17 β -acetoxy-3-methoxy-15 α -phenylthio-14,17 α -ethanoestra-1,3,5(10)-trien-16 β -carboxylate 43

Diisopropylethylamine (1.6 cm³) was added to a solution of the unsaturated ester 27 (3.06 g, 7.46 mmol) in thiophenol (10 cm³) at 20 °C. After 48 h at 20 °C, flash chromatography of the reaction mixture on silica gel (200 g) with EtOAc-hexane (1:9) as eluent gave the 15 α -phenylthio compound 43 (3.84 g, 98%), mp 153–155 °C (from CHCl₃-MeOH); $[\alpha]_{\text{D}}^{25} +42$ (c 0.8 in CHCl₃) (Found: C, 71.35; H, 7.0%; M⁺, 520. C₃₁H₃₈O₅S requires C, 71.5; H, 7.0%; M, 520); $\nu_{\text{max}}/\text{cm}^{-1}$ 1734, 1607, 1599, 1580 and

1571; δ_{H} (400 MHz) 1.23 (3 H, s, 13 β -Me), 1.96 (1 H, td, J 2 $\times$ 11.3 and 2.3, 8 β -H), 2.02 (3 H, s, 17 β -OAc), 2.16 (1 H, td, J 2 $\times$ 13.1 and 3.6), 2.72 (1 H, obsc. m, 9 α -H), 2.82–2.92 (2 H, m, 6-H₂), 3.36 (1 H, dd, J 5.6 and 2.5, 15 β -H), 3.53 (3 H, s, 16 β -CO₂Me), 3.78 (3 H, s, 3-OMe), 3.82 (1 H, d, J 5.6, 16 α -H), 6.64 (1 H, d, J 2.4, 4-H), 6.73 (1 H, dd, J 8.6 and 2.4, 2-H) and 7.15–7.45 (6 H, m, 1-H and 15 α -SPh); δ_{C} (50 MHz) 173.9 (s, 16 β -CO₂Me), 170.2 (s, 17 β -OCOMe), 157.5 (s, C-3), 137.9 (s, C-5), 136.2 (s, C-1'), 131.9 (s, C-10), 132.0 (2 $\times$ d, C-2' and C-6'), 128.9 (2 $\times$ d, C-3' and C-5'), 127.2 (d, C-4'), 126.2 (d, C-1), 113.8 (d, C-4), 111.5 (d, C-2), 90.8 (s, C-17), 58.9 (2 $\times$ d, C-15 and C-16), 55.2 (q, 3-OMe), 51.7 (q, 16-CO₂Me), 51.6 and 49.8 (each s, C-13 and C-14), 37.6 (d, C-8), 29.7 (t, C-6), 28.8 (t, C-12), 28.6 (t, C-17'), 25.7 (t, C-7), 24.1 (t, C-11), 23.8 (t, C-17'), 2.14 (q, 17 β -OCOMe) and 15.6 (q, C-18).

3-Methoxy-16 β -methyl-15 α -phenylthio-14,17 α -ethanoestra-1,3,5(10)-trien-17 β -ol 46

The ester 43 (3.84 g, 7.38 mmol) was treated with LAH (1.6 g, 44.2 mmol) in THF (50 cm³) at 20 °C for 45 min. The mixture was treated with aq. NH₄Cl, added slowly to destroy excess reagent, and then extracted with EtOAc. Work-up gave 16 β -hydroxymethyl-3-methoxy-15 α -phenylthio-14,17 α -ethanoestra-1,3,5(10)-trien-17 β -ol 44 (3.32 g, 100%), mp 207–210 °C (from CHCl₃); $[\alpha]_{\text{D}}^{25} +41$ (c 0.8 in CHCl₃) (Found: C, 74.8; H, 7.5; S, 6.6%; M^+ , 450. C₂₈H₃₄O₃S requires C, 74.6; H, 7.6; S, 7.1%; M , 450); $\nu_{\text{max}}/\text{cm}^{-1}$ 3593; δ_{H} (200 MHz) 1.2 (3 H, s, 13 β -Me), 2.74 (1 H, br d, J 6.1, 15 β -H), 3.77 (3 H, s, 3-OMe), 3.8 (2 H, obsc. m, 16 β -CH₂OH), 6.64 (1 H, d, J 2.4, 4-H), 6.73 (1 H, dd, J 8.6 and 2.4, 2-H) and 7.15–7.45 (6 H, m, 1-H and 15 α -SPh).

Methanesulfonylation (MsCl–pyridine, 0 °C, 1 h) of the diol 44 (3.32 g, 7.38 mmol), followed by dilution of the mixture with water and isolation of the product by extraction with EtOAc gave material (3.88 g) which was flash chromatographed on silica gel (100 g) with EtOAc–toluene (2:3) as eluent to give the non-crystalline 16 β -(methanesulfonyloxy)methyl derivative 45 (3.12 g, 81%).

The methanesulfonate 45 (3.12 g, 5.9 mmol) was treated with LAH (876 mg, 29 mmol) in THF (50 cm³) at 20 °C under nitrogen for 2.4 h. Excess reagent was destroyed by cautious addition of aq. NH₄Cl to the mixture, and the product (2.6 g) was isolated by extraction with EtOAc. Flash chromatography on silica gel (100 g) with EtOAc–toluene (1:9) as eluent gave the 16 β -methyl compound 46 (2.46 g, 96%), mp 135–136 °C (from MeOH); $[\alpha]_{\text{D}}^{25} +56$ (c 1.0 in CHCl₃) (Found: M^+ , 434.229. C₂₈H₃₄O₃S requires M , 434.228); $\nu_{\text{max}}/\text{cm}^{-1}$ 3594; δ_{H} (400 MHz) 0.9 (3 H, d, J 7.2, 16 β -Me), 1.1 (3 H, d, J 0.9, 13 β -Me), 1.42 (1 H, dq, J 12.4 and 3 $\times$ 2.6), 1.62 (1 H, dq, J 12.4 and 3 $\times$ 2.5), 1.85 (1 H, ddd, J 12.4, 9.5 and 3.1), 1.98 (1 H, td, J 2 $\times$ 11.5 and 2.1), 2.05 (1 H, td, J 2 $\times$ 12.7 and 3.4), 2.41 (1 H, ddd, J 7.0, 5.7 and 2.5), 2.66 (1 H, obsc., 8 β -H), 2.6–2.86 (2 H, m, 6-H₂), 2.84 (1 H, d, J 5.6, 15 β -H), 3.77 (3 H, s, 3-OMe), 6.64 (1 H, d, J 2.4, 4-H), 6.73 (1 H, dd, J 8.6 and 2.4, 2-H) and 7.15–7.45 (6 H, m, 1-H and 15 α -SPh).

Oxidation–elimination of the phenylthio compound 46

Aq. sodium periodate (0.5 mol dm³, 50 cm³) was added to a stirred solution of the phenylthio compound 46 (2.46 g, 5.66 mmol) in ethanol–THF (3:1, 80 cm³) at 20 °C. After 24 h at 20 °C, the reaction mixture was concentrated under reduced pressure and diluted with water. The product was isolated by extraction with chloroform. Flash chromatography of the residue (2.7 g) on silica gel (200 g) with EtOAc–toluene (2:5) as eluent gave the 15 α -phenylsulfinyl compound 47 (1.9 g, 75%), which was dissolved in triethylamine (0.2 cm³) and benzene (30 cm³), and heated at 115 °C (sealed tube) for 16 h. The reaction mixture was concentrated under reduced pressure and the residue (1.4 g) was flash chromatographed on silica gel (60 g) with EtOAc–toluene (1:9) as eluent, to give 3-methoxy-16-methyl-

14,17 α -ethanoestra-1,3,5(10),15-tetraen-17 β -ol 48 (1.4 g, 99%), mp 113–114 °C (from EtOAc–MeOH); $[\alpha]_{\text{D}}^{25} -22$ (c 1.0 in CHCl₃) (Found: C, 81.8; H, 8.7%; M^+ , 324. C₂₂H₂₈O₂ requires C, 81.4; H, 8.7%; M , 324); $\nu_{\text{max}}/\text{cm}^{-1}$ 3598; δ_{H} (200 MHz) 0.84 (3 H, s, 13 β -Me), 1.73 (3 H, d, J 1.6, 16-Me), 3.77 (3 H, s, 3-OMe), 5.62 (1 H, q, J 3 $\times$ 1.6, 15-H), 6.62 (1 H, d, J 2.7, 4-H), 6.7 (1 H, dd, J 8.6 and 2.7, 2-H) and 7.18 (1 H, d, J 8.6, 1-H); δ_{C} (100 MHz) 157.4 (s, C-3), 144.7 (s, C-16), 137.9 (s, C-5), 133.4 (s, C-10), 127.5 (d, C-15), 126.2 (d, C-1), 113.9 (d, C-4), 111.4 (d, C-2), 91.0 (s, C-17), 56.8 (s, C-14), 55.2 (q, 3-OMe), 54.1 (s, C-13), 37.4 (d, C-9), 37.2 (d, C-8), 30.1 (t, C-6), 27.8 (t, C-12), 27.5 (t, C-17'), 26.6 (t, C-17'), 25.6 (t, C-7), 24.6 (t, C-11), 14.7 (q, C-18) and 12.0 (q, 16-Me).

The derived (Ac₂O–DMAP–pyridine, 20 °C, 72 h) 17 β -acetate 49 had mp 108–110 °C (from CHCl₃–MeOH); $[\alpha]_{\text{D}}^{25} -40$ (c 1.0 in CHCl₃) (Found: C, 78.4; H, 8.4%; M^+ , 366. C₂₄H₂₈O₃ requires C, 78.65; H, 8.25%; M , 366); $\nu_{\text{max}}/\text{cm}^{-1}$ 1729; δ_{H} (200 MHz) 0.89 (3 H, s, 13 β -Me), 1.74 (3 H, d, J 1.5, 16-Me), 2.1 (3 H, s, 17 β -OAc), 3.78 (3 H, s, 3-OMe), 5.67 (1 H, q, J 3 $\times$ 1.5, 15-H), 6.64 (1 H, d, J 2.4, 4-H), 6.73 (1 H, dd, J 8.6 and 2.4, 2-H) and 7.18 (1 H, d, J 8.6, 1-H).

Hydroxylation of the 16-methyl Δ^5 -compounds 48 and 49

(a) Treatment of the 17 β -alcohol 48 (340 mg, 1.05 mmol) with osmium tetroxide (320 mg, 1.26 mmol) in pyridine (10 cm³) at 20 °C for 72 h, followed by standard work-up and chromatography of the product (360 mg) on silica gel (40 g) with EtOAc–toluene (1:1) as eluent, gave the 15 β ,16 β ,17 β -triol 42 (203 mg, 50%) followed by the 15 α ,16 α ,17 β -triol 36 (149 mg, 40%).

(b) Similar treatment of the 17 β -acetate 49 (1.4 g, 3.8 mmol), and chromatography of the product (1.8 g) on silica gel (150 g) with EtOAc–toluene (3:7) as eluent, gave the 17 β -acetoxyl 15 α ,16 α -diol 35 (958 mg, 63%), followed by the 17 β -acetoxyl 15 β ,16 β -diol 22 (441 mg, 29%).

Oxidative cleavage of the 15,16-diols

(a) The following procedure is representative. Aq. sodium periodate (0.5 mol dm³; 23 cm³) was added to a solution of the diols 22 + 35 (1.38 g, 3.5 mmol) in ethanol (100 cm³) at 20 °C. After 4 h at 20 °C, the mixture was diluted with water and the product (1.4 g) was isolated by extraction with chloroform. Flash chromatography of the residue on silica gel (90 g) with EtOAc–hexane (1:4) as eluent gave 17-acetoxyl-3-methoxy-20-oxo-19-nor-14 β -pregna-1,3,5(10)-triene-14-carbaldehyde 50 (1.24 g, 90%), mp 174–177 °C (from CHCl₃–MeOH); $[\alpha]_{\text{D}}^{25} +$ (c 1.0 in CHCl₃) (Found: C, 72.3; H, 7.6%; M^+ , 398. C₂₄H₃₀O₃ requires C, 72.3; H, 7.6; M , 398); $\nu_{\text{max}}/\text{cm}^{-1}$ 1731 and 1711; δ_{H} (400 MHz) 1.18 (3 H, s, 13 β -Me), 1.34 (1 H, qd, J 3 $\times$ 11.4 and 7, 7 α -H), 1.52 (1 H, obsc. m, 7 β -H), 1.74 (1 H, td, J 2 $\times$ 12 and 3.3, 8 β -H), 2.04 (3 H, s, 17 α -OAc), 2.1 (1 H, m), 2.15 (3 H, s, 20-Me), 2.6 (1 H, td, J 2 $\times$ 12 and 3.3, 9 α -H), 2.7–2.8 (2 H, m, 6-H₂), 2.92 (1 H, ddd, J 16.9, 10.0 and 6.9, 15 β -H), 3.77 (3 H, s, 3-OMe), 6.6 (1 H, d, J 2.8, 4-H), 6.7 (1 H, dd, J 8.6 and 2.8, 2-H), 7.21 (1 H, d, J 8.6, 1-H) and 9.67 (1 H, s, 14 β -CHO); δ_{C} (50 MHz) 209.2 (s, C-20), 207.2 (d, 14 β -CHO), 170.9 (s, 17 α -OCOMe), 157.7 (s, C-3), 137.5 (s, C-5), 131.4 (s, C-10), 126.4 (d, C-1), 113.4 (d, C-4), 111.9 (d, C-2), 96.4 (s, C-17), 61.0 (s, C-14), 55.2 (q, 3-OMe), 51.4 (s, C-13), 40.1 (d, C-8), 36.9 (d, C-9), 33.6 (t, C-12), 32.3 (t, C-15), 30.2 (t, C-6), 26.9 (q, 20-Me), 25.9 (t, C-16), 25.1 (t, C-11), 24.1 (t, C-7), 21.1 (q, 17 α -OCOMe) and 16.5 (q, C-18).

(b) Similar treatment of the 15,16,17-triols 36 or 42 for 2 h at 20 °C gave the 17 α -alcohol 51 (100%), mp 161–163 °C (from CHCl₃–hexane); $[\alpha]_{\text{D}}^{25} +134$ (c 1.0 in CHCl₃) (Found: C, 73.9; H, 7.9%; M^+ , 356. C₂₂H₂₈O₄ requires C, 74.1; H, 7.9%; M , 356); $\nu_{\text{max}}/\text{cm}^{-1}$ 3605 and 1708.

Intramolecular aldol condensation of the 14-carbaldehyde 50

(a) Compound 50 (100 mg, 0.25 mmol) was treated with hydrochloric acid (12 mol dm^{−3}; 1 cm³) in THF (9 cm³) at 57 °C for

2 h. The cooled reaction mixture was neutralised with saturated aq. NaHCO_3 , and the product was isolated by extraction with CHCl_3 . Work-up and chromatography of the residue (90 mg) on silica gel (9 g) with EtOAc-hexane (3:7) as eluent gave 3-methoxy-17 α -oxo-14,17 β -prop-17 α -eno-14 β -estra-1,3,5(10)-trien-17 α -yl acetate **52** (72 mg, 76%), mp 170–172 °C (from CHCl_3 -MeOH); $[\alpha]_D^{25}$ –1 (c 1.0 in CHCl_3) (Found: C, 75.9; H, 7.3%; M^+ , 380. $\text{C}_{27}\text{H}_{40}\text{O}_4$ requires C, 75.8; H, 7.4%; M , 380); $\nu_{\text{max}}/\text{cm}^{-1}$ 1739 and 1694; δ_{H} (200 MHz) 0.95 (3 H, s, 13 β -Me), 2.17 (3 H, s, 17 α -OAc), 2.8 (1 H, td, J 2 $\times$ 10.9 and 3.9, 9 α -H), 2.9–3.0 (2 H, m, 6-H $_2$), 3.79 (3 H, s, 3-OMe), 6.07 (1 H, d, J 9.9, 17 β -H), 6.67 (1 H, d, J 2.8, 4-H), 6.73 (1 H, dd, J 8.6 and 2.8, 2-H), 7.17 (1 H, d, J 9.9, 17 β -H) and 7.21 (1 H, d, J 8.6, 1-H).

(b) Treatment of compound **50** (436 mg, 1.1 mmol) with toluene-*p*-sulfonic acid (623 mg, 3.1 mmol) in benzene (10 cm 3) under reflux for 1.75 h, followed by work-up as described above gave 17 α -hydroxy-3-methoxy-14,17 β -prop-17 α -eno-14 β -estra-1,3,5(10)-trien-17 α -one **53** (370 mg, 100%), mp 158–161 °C (from CHCl_3 -MeOH); $[\alpha]_D^{25}$ +117 (c 1.0 in CHCl_3) (Found: C, 78.3; H, 7.6%; M^+ , 338. $\text{C}_{27}\text{H}_{40}\text{O}_3$ requires C, 78.1; H, 7.7%; M , 338); $\nu_{\text{max}}/\text{cm}^{-1}$ 3480 and 1677; $\lambda_{\text{max}}(\text{EtOH})/\text{nm}$ 243 (s/dm 2 mol $^{-1}$ 16 426); δ_{H} (200 MHz) 0.73 (3 H, s, 13 β -Me), 2.72 (1 H, td, J 2 $\times$ 11.3 and 4.3, 9 α -H), 2.81–2.9 (2 H, m, 6-H $_2$), 3.71 (3 H, s, 3-OMe), 6.07 (1 H, d, J 9.8, 17 β -H), 6.67 (1 H, d, J 2.8, 4-H), 6.73 (1 H, dd, J 8.6 and 2.8, 2-H), 7.17 (1 H, d, J 8.6, 1-H) and 7.26 (1 H, d, J 9.8, 17 β -H); δ_{C} (50 MHz) 203.0 (s, C-17 α), 158.9 (d, C-17 β), 157.6 (s, C-3), 137.4 (s, C-5), 132.3 (s, C-10), 126.2 (d, C-1), 125.0 (d, C-17 β), 113.3 (d, C-4), 111.6 (d, C-2), 88.7 (s, C-17), 55.2 (q, 3-OMe), 52.5 (2 C, s, C-13 and C-14), 38.7 (d, C-8), 37.4 (d, C-9), 30.2 (t, C-6), 28.5 (2 t, C-11 and C-12), 25.6 (t, C-15), 25.0 (t, C-16), 24.0 (t, C-7) and 13.8 (q, C-18).

14-Acetyl-3-methoxy-14 β -estra-1,3,5(10)-trien-17-one **55** Palladium(II) chloride (142 mg, 0.8 mmol) and copper(I) chloride (395 mg, 3.99 mmol) were added to water-dimethylformamide (1:10; 41 cm 3), and the mixture was stirred vigorously at 20 °C for 2.5 h under an oxygen atmosphere. 14-Allyl-3-methoxy-14 β -estra-1,3,5(10)-trien-17-one **54** (500 mg, 1.54 mmol) was added to the mixture which was then kept at 65 °C for 5 h, before being poured into water. The product (627 mg) was isolated by extraction with toluene and adsorbed on silica gel (55 g). Elution with EtOAc-toluene (1:9) gave starting material (22 mg) and uncharacterized material (191 mg), followed by the diketone **55** as an oil (363 mg, 69%), $[\alpha]_D^{25}$ +8 (c 0.8 in CHCl_3) (Found: M^+ , 340.203. $\text{C}_{27}\text{H}_{40}\text{O}_3$ requires M , 340.204); $\nu_{\text{max}}/\text{cm}^{-1}$ 1726br; δ_{H} (200 MHz) 1.01 (3 H, s, 13 β -Me), 2.15 (3 H, s, COMe), 2.39 and 2.62 (each 1 H, d, J 17.2, 14 β -H $_2$), 2.83–2.91 (2 H, m, 6-H $_2$), 3.78 (3 H, s, 3-OMe), 6.63 (1 H, d, J 2.7, 4-H), 6.74 (1 H, dd, J 8.7 and 2.7, 2-H) and 7.21 (1 H, d, J 8.7, 1-H).

Intramolecular aldol condensation of the diketone **55** Methanolic potassium hydroxide (1 mol dm $^{-3}$; 5.5 cm 3) was added to a stirred solution of the diketone **55** (470 mg, 1.38 mmol) in THF (17 cm 3) at 20 °C under nitrogen. After 30 min at 20 °C, the reaction was complete (TLC), and water was added followed by aqueous hydrochloric acid (2 mol dm $^{-3}$; 3 cm 3). Extraction with toluene and work-up gave material (420 mg) which was adsorbed on silica gel (21 g). Elution with EtOAc-toluene (2:3) gave an isomeric mixture (~2:1 by NMR, 27 mg) formulated as (16 β *R/S*)-16 β -hydroxy-3-methoxy-16 β -methyl-14,16 β -ethano-14 β -estra-1,3,5(10)-trien-17-ones **56**, $\nu_{\text{max}}/\text{cm}^{-1}$ 3584 and 1733; δ_{H} (200 MHz) 1.04 (3 H, s, 13 β -Me) and 1.35 (3 H, s, 16 β -Me) (minor component); 1.16 (3 H, s, 13 β -Me) and 1.45 (3 H, s, 16 β -Me) (major component); and 2.84–2.93 (2 H, m, 6-H $_2$), 3.78 (3 H, s, 3-OMe), 6.64 (1 H, d, J 2.9, 4-H), 6.73 (1 H, dd, 8.6 and 2.9, 2-H) and 7.21 (1 H, d, J 8.6, 1-H); m/z 340.203 (M^+), followed by mixed fractions (49 mg) and 17 α -hydroxy-3-methoxy-14,17 β -propano-14 β -estra-1,3,5(10)-trien-

17 α -one **57** (327 mg, 70%), mp 215–217 °C (from CH_2Cl_2 -MeOH); $[\alpha]_D^{25}$ +11 (c 1.0 in CHCl_3) (Found: C, 77.4; H, 8.4%; M^+ , 340. $\text{C}_{27}\text{H}_{40}\text{O}_3$ requires C, 77.6; H, 8.3%; M , 340); δ_{H} (400 MHz) 1.06 (3 H, s, 13 β -Me), 1.5 (1 H, qd, J 3 $\times$ 12.3 and 4.7), 2.04 (1 H, qt, J 3 $\times$ 13.1 and 2 $\times$ 3.6), 2.2 (1 H, dd, J 17.4 and 2.6, 17 β -H), 2.4 (1 H, dq, J 13.4 and 3 $\times$ 3.8, 11 α -H), 2.49 (1 H, dd, J 17.4 and 2, 17 β -H), 2.51 (1 H, dd, J 17.4 and 2, 17 β -H), 2.63 (1 H, td, J 2 $\times$ 14.6 and 3.8, 9 α -H), 2.8 (1 H, obsc. dd, J 17.4 and 2.8, 17 β -H), 2.8–2.84 (2 H, m, 6-H $_2$), 3.77 (3 H, s, 3-OMe), 6.64 (1 H, d, J 2.7, 4-H), 6.74 (1 H, dd, J 8.6 and 2.7, 2-H) and 7.24 (1 H, d, J 8.6, 1-H); δ_{C} (50 MHz) 209.7 (s, C-17 α), 157.6 (s, C-3), 137.6 (s, C-5), 132.4 (s, C-10), 126.5 (d, C-1), 113.5 (d, C-4), 111.8 (d, C-2), 81.2 (s, C-17 β), 55.2 (q, 3-OMe), 53.4 (t, C-17 β), 50.6 (t, C-17 β), 45.9 and 45.2 (each s, C-13 and C-14), 42.0 (d, C-8), 37.5 (d, C-9), 34.9 (t, C-16), 30.3 (t, C-6), 28.9 (t, C-12), 26.0 (t, C-15), 25.8 (t, C-11), 23.3 (t, C-7) and 13.5 (q, C-18).

Hydride reduction of the 17 α -ketone **57**

The ketone **57** (200 mg, 0.59 mmol) was treated with LAH (111 mg, 2.9 mmol) in THF (14 cm 3) at 0 °C for 1 h. Excess of reagent was destroyed by addition of saturated aq. NH_4Cl to the mixture which was then concentrated under reduced pressure. Extraction with EtOAc and work-up gave the product as a colourless oil (200 mg), which was adsorbed on silica gel (20 g). Elution with EtOAc-toluene (7:13) gave (17 β *R*)-3-methoxy-14,17 β -propano-14 β -estra-1,3,5(10)-trien-17 α ,17 β -diol **58** (70 mg, 35%), mp 179–182 °C (from CHCl_3 -hexane); $[\alpha]_D^{25}$ +9 (c 1.0 in CHCl_3) (Found: C, 77.3; H, 9.0%; M^+ , 342. $\text{C}_{27}\text{H}_{42}\text{O}_4$ requires C, 77.2; H, 8.8%; M , 342); $\nu_{\text{max}}/\text{cm}^{-1}$ 3602; δ_{H} (200 MHz) 0.82 (3 H, s, 13 β -Me), 2.55–2.69 (1 H, m, 9 α -H), 2.76–2.85 (2 H, m, 6-H $_2$), 3.77 (3 H, s, 3-OMe), 4.27 (1 H, t, J 2 $\times$ 6.2, 17 β -H), 6.62 (1 H, d, J 2.8, 4-H), 6.72 (1 H, dd, J 8.6 and 2.8, 2-H) and 7.23 (1 H, d, J 8.6, 1-H); δ_{C} (50 MHz) 157.5 (s, C-3), 137.9 (s, C-5), 133.4 (s, C-10), 126.4 (d, C-1), 113.5 (d, C-4), 111.6 (d, C-2), 80.8 (s, C-17), 66.3 (d, C-17 β), 55.2 (q, 3-OMe), 48.4 and 46.2 (each s, C-13 and C-14), 44.4 (t, C-17 β), 42.2 (d, C-8), 41.0 (t, C-17 β), 36.9 (d, C-9), 34.0 (t, C-16), 30.5 (t, C-6), 28.6 (t, C-12), 25.9 (t, C-15), 24.6 (t, C-11), 23.5 (t, C-7) and 13.5 (q, C-18); followed by (17 β *S*)-3-methoxy-14,17 β -propano-14 β -estra-1,3,5(10)-trien-17 α ,17 β -diol **59** (113 mg, 56%), mp 185–188 °C (from EtOAc); $[\alpha]_D^{25}$ +5 (c 1.0 in CHCl_3) (Found: C, 77.0; H, 8.95%; M^+ , 342); $\nu_{\text{max}}/\text{cm}^{-1}$ 3601; δ_{H} (200 MHz) 0.97 (3 H, s, 13 β -Me), 2.32 (1 H, dq, J 13.2 and 3 $\times$ 4.0, 11 α -H), 2.53–2.66 (1 H, m, 9 α -H), 2.77–2.85 (2 H, m, 6-H $_2$), 3.77 (3 H, s, 3-OMe), 4.04 (1 H, tt, J 2 $\times$ 10.5 and 2 $\times$ 7.4, 17 β -H), 6.62 (1 H, d, J 2.8, 4-H), 6.72 (1 H, dd, J 8.6 and 2.8, 2-H) and 7.22 (1 H, d, J 8.6, 1-H); δ_{C} (50 MHz) 157.5 (s, C-3), 137.9 (s, C-5), 133.3 (s, C-10), 126.3 (d, C-1), 113.5 (d, C-4), 111.6 (d, C-2), 81.0 (s, C-17), 66.0 (d, C-17 β), 55.2 (q, 3-OMe), 45.9 and 45.1 (each s, C-13 and C-14), 44.1 (t, C-17 β), 41.6 (d, C-8), 40.0 (t, C-17 β), 37.3 (d, C-9), 34.3 (t, C-16), 30.5 (t, C-6), 28.8 (t, C-12), 25.8 (t, C-15), 25.2 (t, C-11), 23.8 (t, C-7) and 13.7 (q, C-18).

3-Deacetylation (DIBALH-toluene, heat, 41 h) of compound **58** gave the corresponding (17 β *R*)-3,17 α ,17 β -triol **60**, mp 277–280 °C (from EtOAc); $[\alpha]_D^{25}$ –21 (c 1.0 in pyridine) (Found: C, 76.6; H, 8.6%; M^+ , 328. $\text{C}_{27}\text{H}_{42}\text{O}_3$ requires C, 76.8; H, 8.6%; M , 328).

Similarly, compound **59** was converted into the (17 β *S*)-3,17 α ,17 β -triol **61**, mp 251–253 °C (from Me_2CO -hexane); $[\alpha]_D^{25}$ –2 (c 0.5 in pyridine) (Found: C, 77.1; H, 8.5%; M^+ , 328).

3-Methoxy-14,17 β -propano-14 β -estra-1,3,5(10)-trien-17 α -ol **63** The 17 α -ketone **57** (100 mg, 0.29 mmol) in dichloromethane (4 cm 3) was added to vigorously stirred ethane-1,2-dithiol (0.1 cm 3 , 1.2 mmol) and zinc trifluoromethanesulfonate (210 mg, 0.58 mmol) in dichloromethane (4 cm 3) at 20 °C under nitrogen. After 3.5 h at 20 °C, the mixture was treated with saturated aq. NaHCO_3 and the product was isolated by extraction with CHCl_3 . Work-up gave a foam (132 mg) which was

adsorbed on silica gel (13 g). Elution with EtOAc-toluene (1:9) gave the 17²,17²-ethylenedithio compound 62 (102 mg, 85%), mp 86–90 °C (from CHCl₃-MeOH); [α]_D +8 (c 1.0 in CHCl₃) (Found: M⁺, 416.184. C₂₀H₂₂O₂S₂ requires M, 416.184); ν_{max}/cm⁻¹ 3598; δ_H(200 MHz) 0.94 (3 H, s, 13β-Me), 2.16 and 2.46 (each 1 H, d, J 14.9, 17²-H₂), 2.34 and 2.7 (each 1 H, d, J 13.9, 17¹-H₂), 2.78–2.83 (2 H, m, 6-H₂), 3.24–3.46 (4 H, m, SCH₂-CH₂S), 3.77 (3 H, s, 3-OMe), 6.62 (1 H, d, J 2.8, 4-H), 6.74 (1 H, dd, J 8.6 and 2.8, 2-H) and 7.24 (1 H, d, J 8.6, 1-H).

A mixture of the thioketal 62 (268 mg, 0.64 mmol) and Raney nickel (~1 g) in ethanol (10 cm³) was refluxed with vigorous stirring under nitrogen. After 3 h, the mixture was filtered, and the filtrate was evaporated under reduced pressure to give the 14β,17β-propeno compound 63 (199 mg, 95%), mp 155–156 °C (from CHCl₃-MeOH); [α]_D +8 (c 1.0 in CHCl₃) (Found: C, 81.1; H, 9.4%; M⁺, 326. C₂₂H₂₆O₂ requires C, 80.9; H, 9.3%; M, 326); ν_{max}/cm⁻¹ 3596; δ_H(200 MHz) 0.91 (3 H, s, 13β-Me), 2.31 (1 H, dq, J 12.6 and 3 × 3.6, 11α-H), 2.6 (1 H, br td, J 2 × 11.3 and 3.6, 9α-H), 2.76–2.83 (2 H, m, 6-H₂), 3.77 (3 H, s, 3-OMe), 6.62 (1 H, d, J 2.7, 4-H), 6.72 (1 H, dd, J 8.6 and 2.7, 2-H) and 7.24 (1 H, d, J 8.6, 1-H); δ_C(50 MHz) 157.4 (s, C-3), 138.0 (s, C-5), 133.6 (s, C-10), 126.3 (d, C-1), 113.4 (d, C-4), 111.4 (d, C-2), 82.0 (s, C-17), 55.2 (q, 3-OMe), 46.9 and 45.2 (each s, C-13 and C-14), 41.9 (d, C-8), 37.5 (d, C-9), 34.5 (t, C-16), 34.2 (t, C-17¹), 30.6 (t, C-12), 30.2 (t, C-6), 29.2 (t, C-17²), 25.9 (t, C-15), 25.2 (t, C-11), 23.7 (t, C-7), 18.8 (t, C-17²) and 13.4 (q, C-18).

3-Deoxygenation (DIBALH-toluene, heat, 24 h) of compound 63 gave the corresponding 3,17α-diol 64, mp 270–271 °C (from EtOAc); [α]_D +9 (c 1.0 in THF) (Found: C, 80.5; H, 9.0%; M⁺, 312. C₂₁H₂₆O₂ requires C, 80.7; H, 9.0%; M, 312).

Preparation of the 14β,17β-propeno compounds 66 and 67

A stirred solution of the 17²-ketone 57 (50 mg, 0.15 mmol) in THF (5 cm³) at 20 °C under nitrogen was treated with toluene-*p*-sulfonohydrazide (81 mg, 0.45 mmol) and trifluoroacetic acid (0.016 cm³). After 16 h at 20 °C, saturated aq. NaHCO₃ was added to the mixture and the product (149 mg) was isolated by extraction with chloroform. Work-up and chromatography of the residue on silica gel (15 g) with EtOAc-toluene (3:7) as eluent gave the 17²-toluene-*p*-sulfonylhydrazide 65 as separable *syn*- and *anti*-isomers: isomer *a* (49 mg, 64%), mp 154–158 °C (from CHCl₃-MeOH); [α]_D –3 (c 1.0 in CHCl₃) (Found: M⁺, 508.238. C₂₉H₃₄N₂O₆S requires M, 508.239); ν_{max}/cm⁻¹ 3599, 3290, 3216, 1336 and 1162; δ_H(200 MHz) 0.85 (3 H, s, 13β-Me), 2.41 (3 H, s, 4'-Me), 2.72–2.82 (2 H, m, 6-H₂), 3.75 (3 H, s, 3-OMe), 6.59 (1 H, d, J 2.0, 4-H), 6.69 (1 H, dd, J 8.6 and 2.0, 2-H), 7.18 (1 H, d, J 8.6, 1-H), 7.3 (2 H, d, J 8.2; 3'- and 5'-H) and 7.79 (2 H, d, J 8.2, 2'- and 6'-H); isomer *b* (27 mg, 36%), mp 177–180 °C (from Me₂CO-hexane); [α]_D –24 (c 1.0 in CHCl₃) (Found: M⁺, 508.239); ν_{max}/cm⁻¹ 3599, 3292, 3201, 1336 and 1163; δ_H(200 MHz) 0.89 (3 H, s, 13β-Me), 2.42 (3 H, s, 4'-Me), 2.75–2.85 (2 H, m, 6-H₂), 3.76 (3 H, s, 3-OMe), 6.61 (1 H, d, J 2.9, 4-H), 6.71 (1 H, dd, J 8.6 and 2.9, 2-H), 7.21 (1 H, d, J 8.6, 1-H), 7.32 (2 H, d, J 8.2, 3'- and 5'-H) and 7.84 (2 H, d, J 8.2, 2'- and 6'-H).

The *syn/anti* mixture of compound 65 (76 mg, 0.15 mmol) was treated with butyllithium (1.5 mol dm⁻³ in hexane; 0.5 cm³) in THF (5 cm³) at 0 °C under nitrogen. After 1.5 h at 0 °C, the mixture was treated with further reagent (0.5 cm³) and then kept at 0 °C for a further 1.5 h, whereupon the reaction was complete (TLC). After dilution with water to destroy excess reagent, the mixture was acidified and extracted with toluene. Work-up of the extract gave an oil (90 mg) which was chromatographed on silica gel (13.5 g) with EtOAc-toluene (1:9) to give 3-methoxy-14,17β-prop-17¹-eno-14β-estra-1,3,5(10)-trien-17α-ol 66 (15 mg, 31%), mp 112–114 °C (from CHCl₃-MeOH); [α]_D +2 (c 1.1 in CHCl₃) (Found: M⁺, 324.209. C₂₉H₃₈O₂ requires M, 324.209); ν_{max}/cm⁻¹ 3600; δ_H(200 MHz) 0.94 (3 H, d, J 0.6, 13β-Me), 2.18 (1 H, ddd, J 17.8, 3.9 and 1.5, 17²-H),

2.34 (1 H, dq, J 12.9 and 3 × 3.5, 11α-H), 2.46–2.61 (1 H, br td, 9α-H), 2.76–2.85 (1 H, m, 6-H₂), 3.77 (3 H, s, 3-OMe), 5.5 (1 H, ddd, J 9.8, 3.9 and 2.6, 17²-H), 5.75 (1 H, ddd, J 9.8, 2.3 and 1.5, 17¹-H), 6.62 (1 H, d, J 2.8, 4-H), 6.72 (1 H, dd, J 8.5 and 2.8, 2-H) and 7.24 (1 H, d, J 8.5, 1-H); δ_C(50 MHz) 157.5 (s, C-3), 137.9 (s, C-5), 136.9 (d, C-17¹), 133.0 (s, C-10), 126.6 (d, C-1), 123.8 (d, C-17²), 113.5 (d, C-4), 111.7 (d, C-2), 82.3 (s, C-17), 55.2 (q, 3-OMe), 46.0 and 44.5 (each s, C-13 and C-14), 42.8 (d, C-8), 40.0 (t, C-16), 38.6 (t, C-17²), 37.8 (d, C-9), 30.6 (t, C-6), 29.2 (t, C-12), 27.1 (t, C-15), 26.4 (t, C-11), 22.8 (t, C-7) and 14.0 (q, C-18).

Further elution gave 3-methoxy-14,17β-prop-17²-eno-14β-estra-1,3,5(10)-trien-17α-ol 67 (12 mg, 25%), mp 127–130 °C (from CHCl₃-MeOH); [α]_D –46 (c 0.8 in CHCl₃) (Found: M⁺, 324.209); ν_{max}/cm⁻¹ 3600; δ_H(200 MHz) 0.91 (3 H, s, 13β-Me), 2.59–2.72 (1 H, m, 9α-H), 2.82–2.88 (2 H, m, 6-H₂), 3.77 (3 H, s, 3-OMe), 5.51 (1 H, ddd, J 9.6, 3.8 and 2.7, 17²-H), 5.88 (1 H, ddd, J 9.6, 2.2 and 2.0, 17¹-H), 6.63 (1 H, d, J 2.7, 4-H), 6.72 (1 H, dd, J 8.6 and 2.7, 2-H) and 7.24 (1 H, d, J 8.6, 1-H); δ_C(50 MHz) 157.5 (s, C-3), 137.9 (s, C-5), 135.6 (d, C-17¹), 133.3 (s, C-10), 126.2 (d, C-1), 123.6 (d, C-17²), 113.6 (d, C-4), 111.5 (d, C-2), 81.7 (s, C-17), 55.2 (q, 3-OMe), 49.6 (s, C-14), 44.7 (s, C-13), 42.5 (t, C-17¹), 38.9 (d, C-8), 37.4 (d, C-9), 36.3 (t, C-16), 30.5 (t, C-6), 30.3 (t, C-15), 27.8 (t, C-12), 25.6 (t, C-11), 23.8 (t, C-7) and 13.2 (q, C-18).

3-Deoxygenation (DIBALH-toluene, heat, 25 h) of compound 66 gave the corresponding 3,17α-diol 68, mp 263–264 °C (from EtOAc); [α]_D +5 (c 0.4 in pyridine) (Found: C, 81.0; H, 8.5%; M⁺, 310. C₂₁H₂₆O₂ requires C, 81.25; H, 8.4%; M, 310).

Similarly, compound 67 was converted into the 3,17α-diol 69, mp 273–274 °C (from EtOAc); [α]_D –57 (c 0.5 in pyridine) (Found: C, 81.0; H, 8.5%; M⁺, 310).

14-Formylethyl-3-methoxy-14β-estra-1,3,5(10)-trien-17-one 70

Borane-dimethyl sulfide (2.5 cm³, 26 mmol) was added to a stirred solution of the 14β-allyl 17-ketone 54 (1.2 g, 3.7 mmol) in THF (90 cm³) at 20 °C under nitrogen. The solution was refluxed for 2 h, cooled to 0 °C and treated successively with aq. sodium hydroxide (6 mol dm⁻³, 8 cm³) and hydrogen peroxide (30%; 6 cm³) and then kept at 40 °C for 12 h. The mixture was concentrated under reduced pressure, diluted with water and the product was isolated as a crystalline solid (1.217 g) by extraction with ethyl acetate.

Dimethyl sulfoxide (1.5 cm³, 18 mmol) in THF (5 cm³) was added to a stirred solution of oxalyl-chloride (0.9 cm³, 9 mmol) in THF (21 cm³) at –78 °C under nitrogen. After 2 min, hydroboration product (317 mg, 0.9 mmol) in THF (21 cm³) was added over 5 min to the mixture which was then stirred at –78 °C for 45 min. Triethylamine (5.1 cm³, 36 mmol) was added to the mixture which was then stirred at –78 °C for 5 min before being allowed to warm to 20 °C. The mixture was concentrated under reduced pressure, diluted with water, and the product (310 mg) was isolated by extraction with CH₂Cl₂. Work-up followed by flash chromatography on silica gel (31 g) with EtOAc-toluene (3:17) as eluent gave the 14β-formylethyl compound 70 as a colourless oil (220 mg, 70%) (Found: M⁺, 340.203. C₂₉H₃₆O₂ requires M, 340.204); ν_{max}/cm⁻¹ 1726br; δ_H(200 MHz) 1.04 (3 H, s, 13β-Me), 2.82–2.9 (2 H, m, 6-H₂), 3.78 (3 H, s, 3-OMe), 6.64 (H, d, J 2.7, 4-H), 6.73 (1 H, dd, J 8.6 and 2.7, 2-H), 7.2 (1 H, d, J 8.6, 1-H) and 9.74 (1 H, t, J 2 × 1.2, CHO).

Intramolecular reductive coupling of the 14β-formylethyl 17-ketone 70

A mixture of TiCl₄·(DME)_{1.5} (4.57 g, 13.7 mmol) and zinc-copper couple (2.66 g, 41 mmol) in freshly distilled dimethoxyethane (DME) (100 cm³) was refluxed with vigorous stirring for 1.5 h. The resultant black suspension was cooled to 0 °C and compound 70 (310 mg, 0.91 mmol) in DME (150 cm³) was added to it over 10 min with stirring. The mixture was then allowed to warm to 20 °C. After 4 h at 20 °C, the reaction was

complete (TLC), and aq. K_2CO_3 (20%, 100 cm^3) was added to the mixture which was then stirred for 12 h. After concentration under reduced pressure, the mixture was diluted with water and the product was isolated by extraction with EtOAc. Work-up and crystallisation of the solid residue (295 mg) from EtOAc gave (17^R)-3-methoxy-14,17 β -propano-14 β -estra-1,3,5(10)-triene-17 α ,17¹-diol 71 (146 mg, 50%), mp 177–181 °C; $[\alpha]_D^{25} +18$ (c 1.0 in THF) (Found: M^+ , 342.217. $C_{22}H_{36}O_3$ requires M , 342.219); ν_{max}/cm^{-1} 3600 and 3450br; δ_H (200 MHz) 0.88 (3 H, s, 13 β -Me), 2.31 (1 H, br dq, J 12.6 and 3 $\times$ 3.3, 11 α -H), 2.6 (1 H, br td, J 2 $\times$ 10.8 and 3.1, 9 α -H), 2.75–2.85 (2 H, m, 6-H₂), 3.77 (3 H, s, 3-OMe), 3.99 (1 H, t, J 2 $\times$ 8.0, 17¹-H), 6.61 (1 H, d, J 2.6, 4-H), 6.71 (1 H, dd, J 8.6 and 2.6, 2-H) and 7.23 (1 H, d, J 8.6, 1-H).

Chromatography of the mother-liquor residue on silica gel (16 g) with EtOAc–toluene (3:7) gave starting material 70 (23 mg) followed by (17^S)-3-methoxy-14,17 β -propano-14 β -estra-1,3,5(10)-triene-17 α ,17¹-diol 72 (14 mg, 4%), mp 184–187 °C (from EtOAc); $[\alpha]_D^{25} +21$ (c 1.0 in THF) (Found: M^+ , 342.217); δ_H (200 MHz) 1.13 (3 H, s, 13 β -Me), 2.53–2.67 (1 H, m, 9 α -H), 2.77–2.85 (2 H, m, 6-H₂), 3.77 (3 H, s, 3-OMe), 3.84 (1 H, obsc. J 6.2, 17¹-H), 6.62 (1 H, d, J 2.8, 4-H), 6.71 (1 H, dd, J 8.5 and 2.8, 2-H) and 7.23 (1 H, d, J 8.5, 1-H); and the (17^R)-17 α ,17¹-diol 71 (10 mg, 3%).

3-Methoxy-14,17 β -propano-14 β -estra-1,3,5(10)-triene-17 α ,17¹-diol 17¹-acetates 73 and 74

(a) Treatment of the (17^R)-17 α ,17¹-diol 71 with acetic anhydride in pyridine at 20 °C gave the corresponding 17¹-acetate 73, mp 175–178 °C (from $CHCl_3$ –MeOH); $[\alpha]_D^{25} +27$ (c 1.0 in $CHCl_3$) (Found: C, 74.8; H, 8.3%; M^+ , 384. $C_{24}H_{38}O_4$ requires C, 75.0; H, 8.4%; M , 384); ν_{max}/cm^{-1} 3591 and 1709; δ_H (200 MHz) 0.94 (3 H, s, 13 β -Me), 2.09 (3 H, s, 17¹-OAc), 2.3 (1 H, dq, J 12.9 and 3 $\times$ 3.6, 11 α -H), 2.59 (1 H, td, J 2 $\times$ 11.2 and 4.5, 9 α -H), 2.76–2.84 (2 H, m, 6-H₂), 3.77 (3 H, s, 3-OMe), 5.32 (1 H, ddd, J 9.8, 6.8 and 1.8, 17¹-H), 6.61 (1 H, d, J 2.8, 4-H), 6.71 (1 H, dd, J 8.7 and 2.8, 2-H) and 7.23 (1 H, d, J 8.7, 1-H); δ_C (50 MHz) 172.0 (s, OCOMe), 157.5 (s, C-3), 137.8 (s, C-5), 133.3 (s, C-10), 126.4 (d, C-1), 113.5 (d, C-4), 111.5 (d, C-2), 83.2 (s, C-17), 75.4 (d, C-17¹), 55.2 (q, 3-OMe), 46.2 and 46.1 (each s, C-13 and C-14), 41.6 (d, C-8), 37.4 (d, C-9), 30.5 (t, C-6), 30.5 (t, C-16), 29.4 (t, C-12), 28.0 (t, C-17²), 25.7 (t, C-17³), 25.6 (t, C-15), 25.2 (t, C-11), 23.7 (t, C-7), 21.4 (q, 17¹-COMe) and 13.0 (q, C-18).

(b) Similarly prepared was the 17¹-acetate 74, mp 181–184 °C (from $CHCl_3$ –MeOH); $[\alpha]_D^{25} +36$ (c 1.0 in $CHCl_3$) (Found: C, 75.2; H, 8.2%; M^+ , 384); ν_{max}/cm^{-1} 3585 and 1727; δ_H (200 MHz) 1.08 (3 H, s, 13 β -Me), 2.09 (3 H, s, 17¹-OAc), 2.28 (1 H, br dq, J 13.3 and 3 $\times$ 3.4, 11 α -H), 2.62 (1 H, br td, J 2 $\times$ 10.7 and 3.8, 9 α -H), 2.78–2.86 (2 H, m, 6-H₂), 3.78 (3 H, s, 3-OMe), 4.9 (1 H, d, J 5.5, 17¹-H), 6.62 (1 H, d, J 2.7, 4-H), 6.72 (1 H, dd, J 8.6 and 2.7, 2-H) and 7.24 (1 H, d, J 8.6, 1-H); δ_C (50 MHz) 171.7 (s, 17¹-OCOMe), 157.4 (s, C-3), 137.8 (s, C-5), 133.4 (s, C-10), 126.3 (d, C-1), 113.5 (d, C-4), 111.5 (d, C-2), 82.7 (s, C-17), 78.2 (d, C-17¹), 55.2 (q, 3-OMe), 46.7 (s, C-14), 45.2 (s, C-13), 41.2 (d, C-8), 37.3 (d, C-9), 33.4 (t, C-16), 30.5 (t, C-6), 29.5 (t, C-12), 27.8 (t, C-17²), 25.1 (t, C-15), 24.8 (t, C-11), 24.0 (t, C-17³), 23.7 (t, C-7), 21.5 (q, 17¹-OCOMe) and 14.5 (q, C-18).

14,17 β -Propano-14 β -estra-1,3,5(10)-triene-3,17 β ,17¹-triols 75 and 76

(a) 3-Deoxygenation (DIBALH–toluene, heat, 24 h) of the (17^R)-17 α ,17¹-diol 71 gave the corresponding (17^R)-3,17 α ,17¹-triol 75, mp 269–270 °C (from EtOAc) (Found: C, 76.6; H, 8.8%; M^+ , 328. $C_{21}H_{34}O_3$ requires C, 76.8; H, 8.6%; M , 328).

(b) Similarly prepared was the (17^S)-3,17 α ,17¹-triol 76, mp 251–254 °C (from EtOAc) (Found: C, 77.0; H, 8.5%; M^+ , 328).

17 α -Hydroxy-3-methoxy-14,17 β -propano-14 β -estra-1,3,5(10)-triene-17¹-one 77

Dimethyl sulfoxide (0.5 cm^3 , 6 mmol) in THF (1.4 cm^3) was added to a stirred solution of oxalyl chloride (0.3 cm^3 , 3 mmol) in THF (7 cm^3) at –78 °C under nitrogen. After 2 min, the 17 α ,17¹-diol 71 (100 mg, 0.3 mmol) was added over 5 min to the mixture which was then stirred at –78 °C for 30 min. After this triethylamine (1.7 cm^3 , 12 mmol) was added to the mixture which was then stirred at –78 °C for 5 min before being allowed to warm to 20 °C. The mixture was then diluted with water and extracted with EtOAc. Work-up of the extract gave a residue (360 mg) which was chromatographed on silica gel (15 g) with EtOAc–toluene (3:17) as eluent, to give the 17¹-ketone 77 (62 mg, 63%), mp 153–156 °C (from $CHCl_3$ –MeOH); $[\alpha]_D^{25} +6$ (c 1.0 in $CHCl_3$) (Found: C, 77.4; H, 8.4%; M^+ , 340. $C_{22}H_{36}O_3$ requires C, 77.6; H, 8.3%; M , 340); ν_{max}/cm^{-1} 3480 and 1706; δ_H (400 MHz) 0.74 (3 H, s, 13 β -Me), 1.8 (1 H, qd, J 3 $\times$ 10.3 and 4.1), 2.26 (1 H, tdd, J 2 $\times$ 13.3, 4.5 and 2.5), 2.36 (1 H, dq, J 13.4 and 3 $\times$ 3.8, 11 α -H), 2.48 (1 H, ddd, J 17.1, 7.7 and 1.4, 17¹-H_{ax}), 2.56 (1 H, m, W 38, 17¹-H_{eq}), 2.65 (1 H, td, J 2 $\times$ 11 and 4.1, 9 α -H), 2.82–2.88 (2 H, m, 6-H₂), 3.69 (1 H, s, exch. by D_2O , 17 α -OH), 3.77 (3 H, s, 3-OMe), 6.62 (1 H, d, J 2.7, 4-H), 6.73 (1 H, dd, J 8.5 and 2.7, 2-H) and 7.23 (1 H, d, J 8.5, 1-H).

Hydride reduction of the 17¹-ketone 77

Treatment of compound 77 (113 mg, 0.33 mmol) in THF (8 cm^3) at 20 °C with LAH (63 mg, 1.66 mmol) for 45 min, followed by destruction of the excess of reagent with saturated aq. NH_4Cl , and extraction with EtOAc gave material (100 mg, 88%) which was crystallised from EtOAc to give the (17^S)-17 α ,17¹-diol 72.

(17^S)-3-Methoxy-14,17 β -prop-17¹-eno-14 β -estra-1,3,5(10)-triene-17 α ,17¹-diol 78

Sodium borohydride (74 mg, 1.95 mmol) was added to a stirred mixture of the enone 53 (219 mg, 0.65 mmol) and cerium(III) chloride heptahydrate (480 mg, 1.3 mmol) in methanol (10 cm^3) at 0 °C. After 45 min at 0 °C, the mixture was treated with saturated aq. $NaHCO_3$, and the product (208 mg) was isolated by extraction with EtOAc. Work-up of the extract and chromatography of the residue on silica gel (35 g) with EtOAc–hexane (7:13) as eluent gave the diol 78 (209 mg, 95%), mp 170–172 °C (from $CHCl_3$ –hexane); $[\alpha]_D^{25} +19$ (c 1.0 in $CHCl_3$) (Found: C, 77.6; H, 8.4%; M^+ , 340. $C_{22}H_{36}O_3$ requires C, 77.6; H, 8.3%; M , 340); ν_{max}/cm^{-1} 3607 and 3542; δ_H (200 MHz) 0.94 (3 H, s, 13 β -Me), 2.82–2.94 (2 H, m, 6-H₂), 3.76 (3 H, s, 3-OMe), 3.92 (1 H, dd, J 4.0 and 1.0, 17¹-H), 5.77 (1 H, dd, J 9.7 and 4.0, 17¹-H), 6.14 (1 H, dd, J 9.7 and 1.0, 17¹-H), 6.67 (1 H, d, J 2.8, 4-H), 6.73 (1 H, dd, J 8.6 and 2.8, 2-H) and 7.17 (1 H, d, J 8.6, 1-H).

The derived (17^S)-3,17 α ,17¹-triol 79 had mp 249–253 °C (from Me_2CO –hexane); $[\alpha]_D^{25} -28$ (c 1.0 in pyridine) (Found: C, 76.9; H, 7.8%; M^+ , 326. $C_{21}H_{34}O_3$ requires C, 77.3; H, 8.0%; M , 326).

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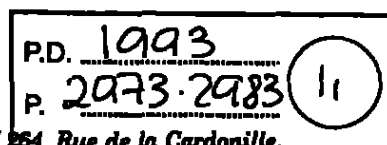
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Steroidal Affinity Labels of the Estrogen Receptor. 1. 17 α -(Bromoacetoxy)alkyl/alkynylestradiolsDriss El Garrouj,[†] André Aumelas,[‡] and Jean-Louis Borgna^{*,†}

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To develop steroidal affinity labels for the estrogen receptor, we prepared five electrophilic estradiol derivatives bearing the 17 α -propyl, 17 α -(1'-butynyl), or 17 α -(1'-octynyl) chain, with either a terminal epoxy function (for the 17 α -propyl substituent) or a terminal bromoacetoxy function (for all three 17 α -substituent types). These compounds displayed low affinity for the lamb uterine estrogen receptor; with apparent relative affinity constants ranging from 0.02% to 0.24% that of estradiol. They were also rapidly transformed in cytosol, probably to the corresponding vicinal diols (epoxy compounds) or primary alcohols (bromoacetoxy compounds). Nevertheless, bromoacetates induced irreversible inactivation of the hormone-binding site but only with ligand-free binding sites. The effect of bromoacetates was prevented by treatment of the cytosol with the thiol-specific reagent methyl methanethiosulfonate. Inactivation of the receptor at 0 °C was rapid (<1 h) and strongly dependent on both compound concentration and pH, with significant effects obtained at either >150 nM (at pH 9) or pH > 7.5 (at 5 μ M). Regardless of the conditions used, the order of efficiency for bromoacetates was always: 17 α -propyl derivative < 17 α -butynyl derivative < 17 α -octynyl derivative, with maximal inactivation of ~30% and ~70% of the hormone-binding sites obtained for the less active and more active compounds, respectively. Characteristics of the receptor inactivation suggest that (i) prepared bromoacetates are highly reactive affinity labels for the estrogen receptor, (ii) they react with similar (or even a single) nucleophilic amino acid residues located within or near the hormone-binding site of the receptor; these residues are probably the -SH of cysteines, and (iii) position 17 α of steroidal ligands is suitable for introducing electrophilic substituents to develop efficient affinity labels for the receptor.

Introduction

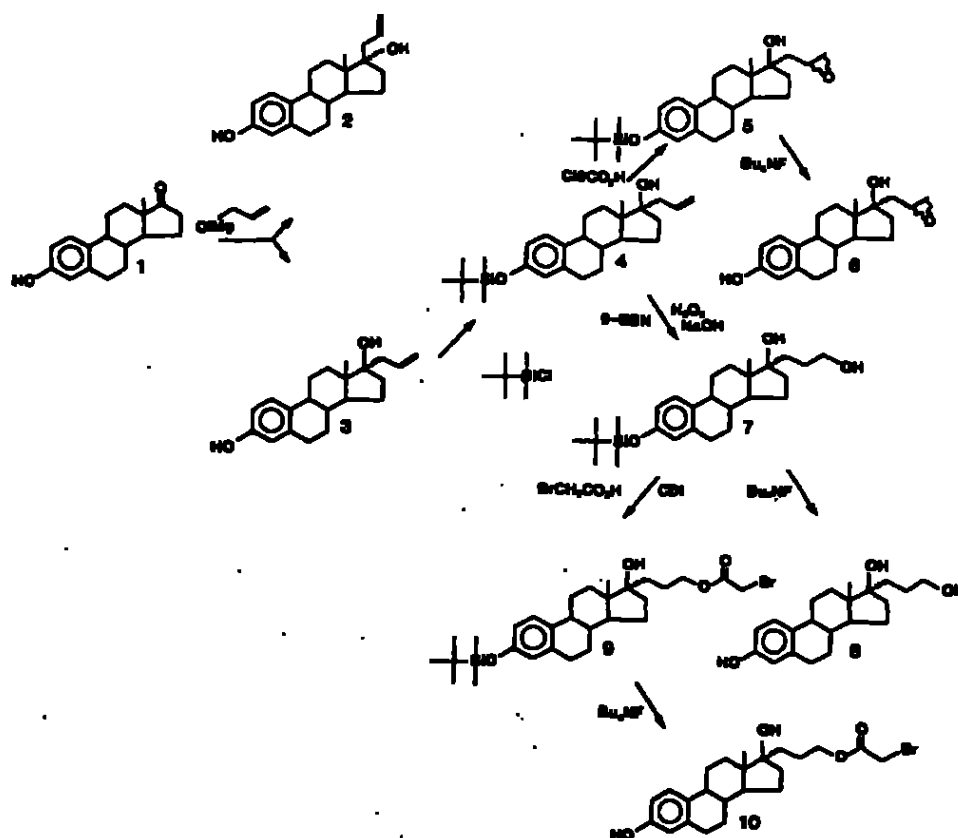
The hormone-binding domain of estrogen receptors¹ includes ~250 amino acids^{2,3} which are highly conserved.^{2,4} Mutation analyses have indicated that the whole domain is important for hormone binding,⁵ and hormone-binding properties are frequently altered due to substitution of single amino acids.⁶ Presently, very little is known about the molecular interaction between the hormone and the receptor. Because of the small size of estrogens, probably only a very small number of the 250 amino acids directly binds to the steroid. However, amino acids of the hormone-binding site that directly bind the hormone should be defined since this interaction results in receptor activation, the key process in triggering hormone action.

Affinity labels of the receptor, that can specifically recognize the hormone-binding site then covalently react with a suitable neighboring amino acid of this binding site, are useful for identifying amino acids in contact with the hormone. A battery of affinity labels of the estrogen receptor could be used to map the estrogen-binding site of the receptor and probably enhances our knowledge on molecular mechanisms of receptor activation. This could permit new potent and highly selective estrogens or antiestrogens to be defined for instance. Several steroids, including 4-mercuriestradiol,⁷ 16 α -hydroxyestrone,⁸ 17 β -(4'-nitrophenyl)dithio-17 β -deoxyestradiol,⁹ and organometallic derivatives of estradiol,^{10,11} were postulated as being able to alkylate the estrogen receptor. One or more of the four cysteines of the receptor hormone-binding

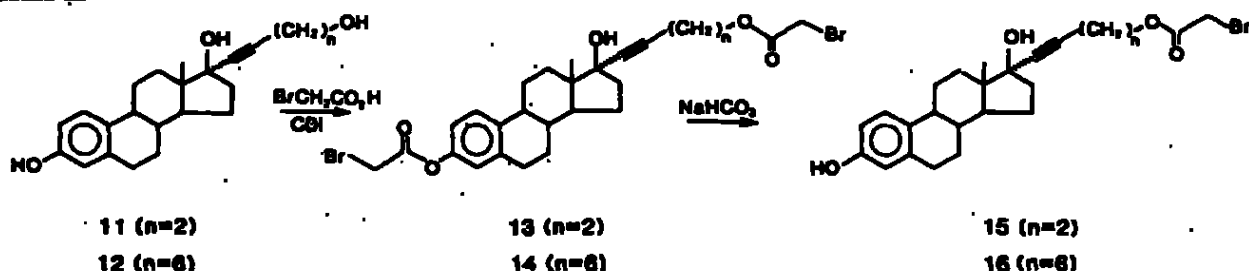
domain are probably involved in covalent attachment of 4-mercuriestradiol, 17 β -(4'-nitrophenyl)dithio-17 β -deoxyestradiol, or organometallic derivatives of estradiol to the receptor. Katzenellenbogen and co-workers developed two series of nonsteroidal affinity labels of the estrogen receptor bearing an aziridine function. One series was related to the triphenylethylene antiestrogen tamoxifen^{12,13} the other to the diphenylethane estrogen ketonolone.¹⁴ A single cysteine (cysteine 530 of the human estrogen receptor) was identified as the covalent attachment site of both estrogen and antiestrogen affinity labels.¹⁵ This does not reflect differential activation of the estrogen receptor by estrogen and antiestrogen but might be explained by the structural characteristics of these nonsteroidal affinity labels. Binding to the receptor of these flexible and relatively symmetrical ligands could be less constrained and less specific than that of the highly rigid and asymmetric steroidal ligands. We thus undertook to develop three series of steroidal affinity labels of the receptor, including the estradiol molecule bearing either a 17 α , 11 β , or 7 α aliphatic or aromatic chain with a terminal reactive function. The 17 α , 11 β , and 7 α substitution were chosen in light of (i) reports that mentioned high affinity for estradiol bearing such substitutions,¹⁶⁻¹⁹ and (ii) the hypothesis that each series of compounds labels a different part of the hormone-binding site. In this paper, we report the preparation of 17 α -alkyl or -alkynyl derivatives of estradiol bearing a terminal epoxy or bromoacetoxy function. We then demonstrate that the three synthesized bromoacetoxy derivatives irreversibly inactivated the hormone-binding site of the estrogen receptor. The characteristics of receptor inactivation strongly suggest that these compounds are efficient affinity labels

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Scheme I



Scheme II



of the receptor and that their targets are basic nucleophiles of the hormone-binding site, probably the -SH of cysteines.

Results

I. Synthesis of Electrophilic 17 α -Derivatives of Estradiol. Estrone was used as starting material for preparation of electrophilic derivatives of 17 α -propyl-estradiol including bromoacetate 10 and epoxides 6a and 6b (Scheme I). Syntheses of 17 α -allyl-estradiol 3,³⁰ epoxides 6a and 6b and triol 8¹⁶ were already reported. We used a similar approach to obtain 6a, 6b, and bromoacetate 10. Briefly, addition of the Grignard of allyl chloride to the ketone function of estrone afforded mixture of diastereoisomers: 17 α -hydroxy-17 β -(2'-propenyl)estradiol 2 (<5%) and 17 α -(2'-propenyl)-17 β -hydroxyestradiol 3 (>95%), which were separated by chromatography. The phenolic function of 3 was protected as TBDMS ether to give 4; then epoxidation of the terminal alkene function by *m*-CPBA gave a mixture of the two diastereoisomers 5a and 5b (75/25), which were separated by chromatography. Removal of the TBDMS group from 5a and 5b by tetrabutylammonium fluoride afforded 17 α -(2',3'-epoxypropyl)estradiol 6a and 6b, respectively. The terminal

alkene function of 4 was submitted to hydroboration-oxidation conditions (9-BBN and basic hydrogen peroxide) to afford the primary alcohol 7. Esterification of the dihydroxy compound 7 by bromoacetic acid in the presence of 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (CDI) afforded monoester 9. The sterically hindered tertiary 17 β -hydroxy function was not esterified, even with excess of bromoacetic acid and CDI. Phenol deprotection from 9 gave 17 α -(3'-(bromoacetoxymethyl)propyl)estradiol 10. For comparative purposes, triol 8 was prepared from 7 by phenol deprotection.

17 α -(4'-(bromoacetoxymethyl)alkynyl) derivatives of estradiol 15 and 16 were prepared from the corresponding triols 11 and 12 in two steps (Scheme II). Action of bromoacetic acid on 11 and 12 in the presence of CDI gave diesters 13 and 14, respectively. Selective saponification of the phenolic esters with NaHCO₃ afforded 17 α -(4'-(bromoacetoxymethyl)butynyl)estradiol 15 and 17 α -(8'-(bromoacetoxymethyl)octynyl)estradiol 16, respectively.

II. Estrogen Receptor Binding Affinity. Competition experiments using the cytosolic estrogen receptor were performed at pH 7 to determine the apparent relative affinity constant (RAC) of epoxides 6a and 6b, primary

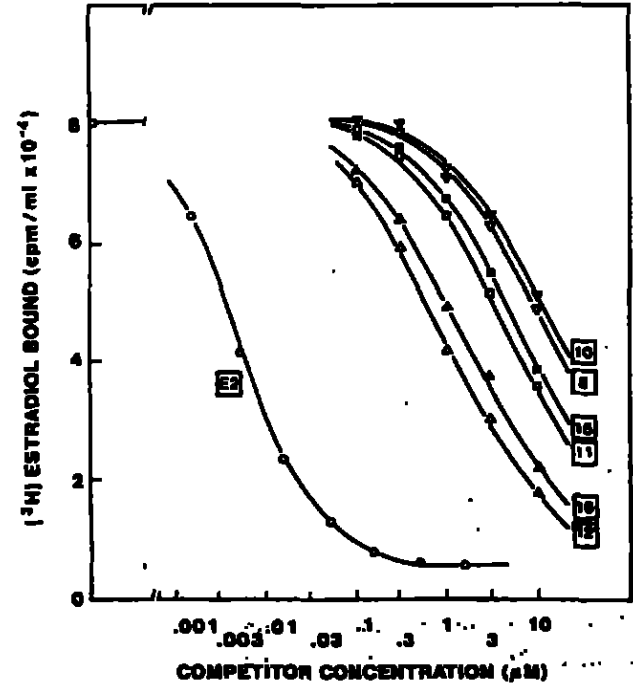


Figure 1. Inhibition of estradiol binding to the estrogen receptor by estradiol 17 α -derivatives. Cytosol (2 mg protein/mL, pH 7.0) was incubated with 5 nM [3 H]estradiol and increasing concentrations of either nonradioactive estradiol (E $_2$, O), 17 α -(bromoacetoxy)alkyl/alkynylestradiols (10v, 15w, and 16a) or corresponding primary alcohols (8v, 11z, and 12a) for 20 h at 20 $^{\circ}$ C. Binding of [3 H]estradiol in cytosol measured by charcoal assay is plotted against the competitor concentration. Experimental variation between duplicate determinations was less than 10%.

alcohols 8, 11, and 12, and bromoacetates 10, 15, and 16. At equilibrium, competitive binding assays allow determination of the RAC of reversibly binding ligands of the receptor. An apparent RAC can be calculated for (i) reversibly binding ligands under nonequilibrium conditions or (ii) reactive ligands able either to irreversibly bind to the receptor and cytosolic proteins or to be modified during incubation. At 0 $^{\circ}$ C, the displacement curves of [3 H]estradiol by the competitors moved to the right over the time course. They were not stabilized even after a 72-h incubation (not shown), indicating that equilibrium was considerably delayed at 0 $^{\circ}$ C. At 20 $^{\circ}$ C, this process was much more rapid since the competition curves were stabilized after a 20-h incubation (Figure 1). All compounds displayed low apparent RAC for the receptor, ranging from <0.02 to ~0.4 (with the RAC of estradiol = 100). The apparent RAC for epoxides dropped considerably from 4 h-0 $^{\circ}$ C competition to 20 h-20 $^{\circ}$ C competition; whereas variation in the RAC of the other compounds was much less pronounced (Table 1). For each of the three bromoacetate/related alcohol combinations, we observed a progressive time- and temperature-dependent decrease in the distance between the competition curves. In fact, these effects were due to instability of epoxides and bromoacetates in the cytosol. TLC analyses performed after the compound incubations showed that epoxides were stable when incubated at 0 or 20 $^{\circ}$ C in buffer. In cytosol they were transformed into more polar compounds displaying the same R_f as that of the corresponding low affinity vicinal diols (~80-100% conversion for 16 h-0 $^{\circ}$ C or 4 h-20 $^{\circ}$ C incubation). Bromoacetates were also fairly stable when incubated in buffer alone; in cytosol they were rapidly

Table 1. Apparent Relative Affinity Constants of Estradiol 17 α -Derivatives^a

compound	apparent RAC			RAC ratio (4 h, 0 °C/20 h, 20 °C)
	4 h, 0 °C	28 h, 0 °C	20 h, 20 °C	
estradiol	100	100	100	1
epoxide	6a	12	1.3	0.044
	8b	3.9	0.20	<0.017
bromoacetate	10	0.94	0.21	0.021
	15	2.0	0.79	0.066
	16	2.4	1.5	0.24
alcohol	8	1.1	0.28	0.024
	11	3.9	1.0	0.070
	12	6.5	3.4	0.40

^a Binding curves similar to those shown in Figure 1, corresponding to competition experiments, were used to determine the concentration of competing ligands which inhibited 50% of the specific binding of [3 H]estradiol in cytosol (2 mg protein/mL, pH 7.0) after 4 h-0 $^{\circ}$ C, 28 h-0 $^{\circ}$ C, or 20 h-20 $^{\circ}$ C competition. From these values, the apparent relative affinity constants (RACs) of competitors (relative to that of estradiol) were calculated for the various competition conditions as described in the Experimental Section, with the RAC of estradiol = 100. The ratio of the RAC determined for 4 h-0 $^{\circ}$ C incubation to the RAC determined for 20 h-20 $^{\circ}$ C incubation was then calculated. Data given for the apparent RACs correspond to means of duplicate determinations. Experimental variation was less than 10%.

transformed (even at 0 $^{\circ}$ C) to form compounds displaying the same R_f as that of the corresponding alcohols (>90% conversion for 1 h-0 $^{\circ}$ C incubation). We were unable to protect bromoacetates from hydrolysis using various esterase inhibitors (diisopropyl fluoro phosphate, diethyl *p*-nitrophenyl phosphate, and phenylmethylsulfonyl fluoride).

III. Inhibition of Ligand Binding to the Estrogen Receptor by Bromoacetates. Specific alkylation of a receptor by an affinity label normally hinders the receptor from further binding common ligands due to permanent occupancy of the ligand-binding site by the covalently bound affinity label. An irreversible decrease in the specific estradiol-binding capacity of the uterine cytosol following its incubation with a potential affinity label of the receptor is thus a strong indication that part of the estrogen receptor molecules were specifically alkylated by the reactive ligand. We applied this principle to evaluate the estrogen receptor-alkylating ability of synthesized estradiol electrophilic derivatives. Samples of uterine cytosol were first exposed to electrophilic derivatives, at various derivative concentrations, temperatures, times, and pH. Control samples were incubated under identical conditions, without steroid or with chemically inert steroids such as primary alcohols 8, 11, and 12. Thereafter, all samples were treated with charcoal to remove unbound or loosely bound steroids. The concentration of remaining specific binding sites for estradiol (corresponding to unfilled and/or reversibly steroid-filled estrogen receptor) was then determined by incubating, under exchange conditions, aliquots with excess [3 H]estradiol either alone, to measure total [3 H]estradiol binding, or with a large amount of nonradioactive estradiol, to measure nonspecific [3 H]estradiol binding.

Figure 2 shows the total (specific plus nonspecific) and nonspecific binding of [3 H]estradiol measured in uterine cytosol following a 1-h incubation at 0 $^{\circ}$ C, pH 8, without steroid or with 5 μ M of the various electrophiles or the primary alcohol 12 (the highest affinity compound of the three primary alcohols). Only bromoacetates induced significant decreases in specific binding of [3 H]estradiol in cytosol, with ~20, 30, and 50% decreases for compounds

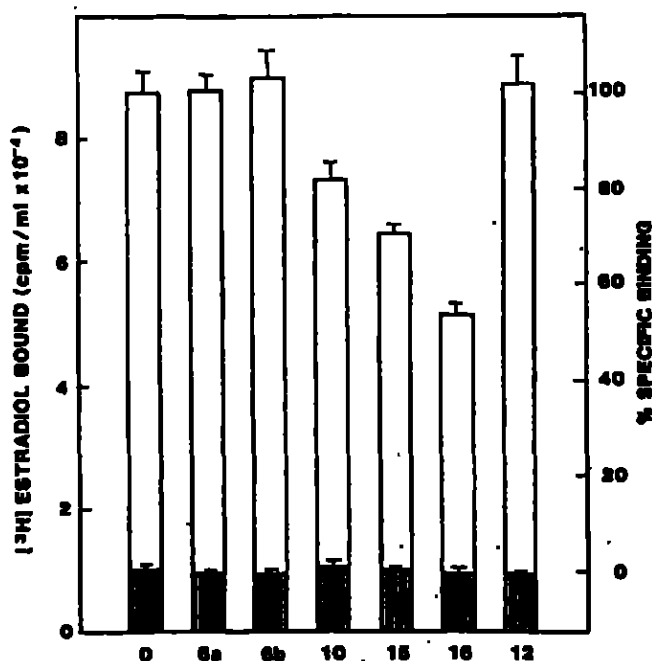


Figure 2. Irreversible inhibition of estradiol binding after exposure of uterine cytosol to estradiol 17 α -derivatives. Cytosol (pH 8.0) was incubated for 1 h at 0 °C either without steroid or with 5 μ M epoxide 6a or 6b, bromoacetate 10, 15, or 16 or primary alcohol 12 which is the homologue of 16. After treatment with an equal volume of charcoal suspension and centrifugation, concentrations of total (unfilled bars) and nonspecific (filled bars) bound [3 H]estradiol in the supernatant were determined, after 16 h - 20 °C incubation with 20 nM [3 H]estradiol, by charcoal assay. Values are means of triplicate determinations; error bars indicate standard deviations.

10, 15, and 16, respectively. Similar quantitative results were obtained when cytosol incubation with steroids was performed at 20 °C instead 0 °C or when the duration of incubation at 0 °C was changed from 1 to 0.25 or 25 h (not shown). However, the effect varied greatly depending on the steroid concentration or the pH in the cytosol (cf. parts VI and VII). Identical results were obtained when 4-hydroxy[3 H]tamoxifen, a high affinity triphenylethylene antiestrogen whose characteristics of interaction with the estrogen receptor differ from those of estrogens,²¹⁻²³ was used instead of [3 H]estradiol to measure the hormone-binding site concentration after incubation of the cytosol with or without steroids (not shown). Finally, the effect was specific to the prepared bromoacetates and was not observed with cytosol exposed to 5 μ M of acetates homologous to 10, 15, and 16, respectively, to 5 μ M of 11 β -(bromoacetoxy)-17 α -ethynylestradiol or 17 β -(O-bromoacetyl)testosterone. Bromoacetic acid and ethyl bromoacetate did not alter [3 H]estradiol specific binding in cytosol even at concentrations as high as 100 μ M (not shown).

IV. Evidence of Irreversible Inactivation of the Estrogen Receptor Hormone-Binding Site by Bromoacetates. Two kinds of experiments were undertaken to examine whether the observed decrease in [3 H]estradiol specific binding of cytosol induced by bromoacetates actually resulted from irreversible inactivation of the hormone-binding site of the receptor.

(A) The Saturable Binding of [3 H]Estradiol in Cytosol Measured After Exchange, Reflects Formation of the Estrogen Receptor: [3 H]Estradiol Complex. Following prolonged incubation of cytosol with [3 H]estradiol under exchange conditions and treatment

by charcoal, we analyzed the [3 H]estradiol-binding species of cytosol in high salt sucrose gradients. Figure 3 shows the total and nonspecific binding of [3 H]estradiol in gradients, from cytosol first exposed or not to 5 μ M steroids for 1 h at 0 °C, pH 9. For cytosol not exposed to steroid (Figure 3A), saturable binding of estradiol appeared to mainly involve a 4.5 S species, with minor species in the 8–10 S region. This saturable 4.5 S peak corresponded to the classical salt-dissociated estrogen receptor:[3 H]estradiol complex since it shifted to the right of the gradient when serum from mouse immunized with purified preparations of lamb uterine estrogen receptor²⁵ was added to cytosol before gradient analysis (not shown). The 8–10 S species probably represented aggregated forms of the receptor, resulting from 20 °C-20 h incubation of cytosol with [3 H]estradiol. Exposition of cytosol to the primary alcohol 12 (Figure 3C) did not significantly change the results observed with the control cytosol. However, exposition of cytosol to bromoacetate 16 resulted in a quantitative change which similarly affected the 4.5 S and the 8–10 S species. Finally, for all samples there was very close agreement between the specific binding of [3 H]estradiol measured by the charcoal assay and obtained by integration of specific binding in the sucrose gradient fractions. We conclude that saturable binding of [3 H]estradiol in uterine cytosol, measured by charcoal assay after incubation under exchange conditions, actually corresponded to the estrogen receptor:[3 H]estradiol complex.

(B) Bromoacetates Irreversibly Inactivate the Estrogen Receptor Hormone-Binding Site. The decrease in [3 H]estradiol specific binding in cytosol observed following its exposure to bromoacetates could have resulted from permanent occupancy of the hormone-binding site of a corresponding portion of receptor molecules by covalently linked bromoacetate. However, another possibility would be that this decrease resulted from affinity of [3 H]estradiol for the receptor, lower for bromoacetate-treated cytosol than for control cytosol and with a similar specific [3 H]estradiol-binding capacity for both types of cytosol, since we used a relatively high but single concentration of [3 H]estradiol to measure the concentration of accessible hormone-binding sites. To assess these two possibilities, we performed saturation experiments of control cytosol and bromoacetate-treated cytosol with increasing concentrations of [3 H]estradiol. Binding data obtained from cytosol incubated for 1 h at 0 °C, pH 9, either without steroid or with 5 μ M bromoacetate 16 or alcohol 12, are shown in Figure 4A. From the Scatchard plots of saturable binding (Figure 4B), calculated binding site concentration and the apparent equilibrium affinity constant of estradiol were $N = 1.03$ nM and $K_A = 4.83 \times 10^9$ M⁻¹ respectively, for the control cytosol. Binding data obtained for cytosol incubated with 12 reflected competitive inhibition of [3 H]estradiol binding, since a similar binding site concentration ($N = 0.977$ nM) but an ~ 2.5 -fold lower affinity constant ($K_A = 1.94 \times 10^9$ M⁻¹) than for control cytosol were determined. The presence of a low residual concentration of compound 12 in the cytosol after charcoal treatment could account for such competitive inhibition, since the efficiency of charcoal in removing

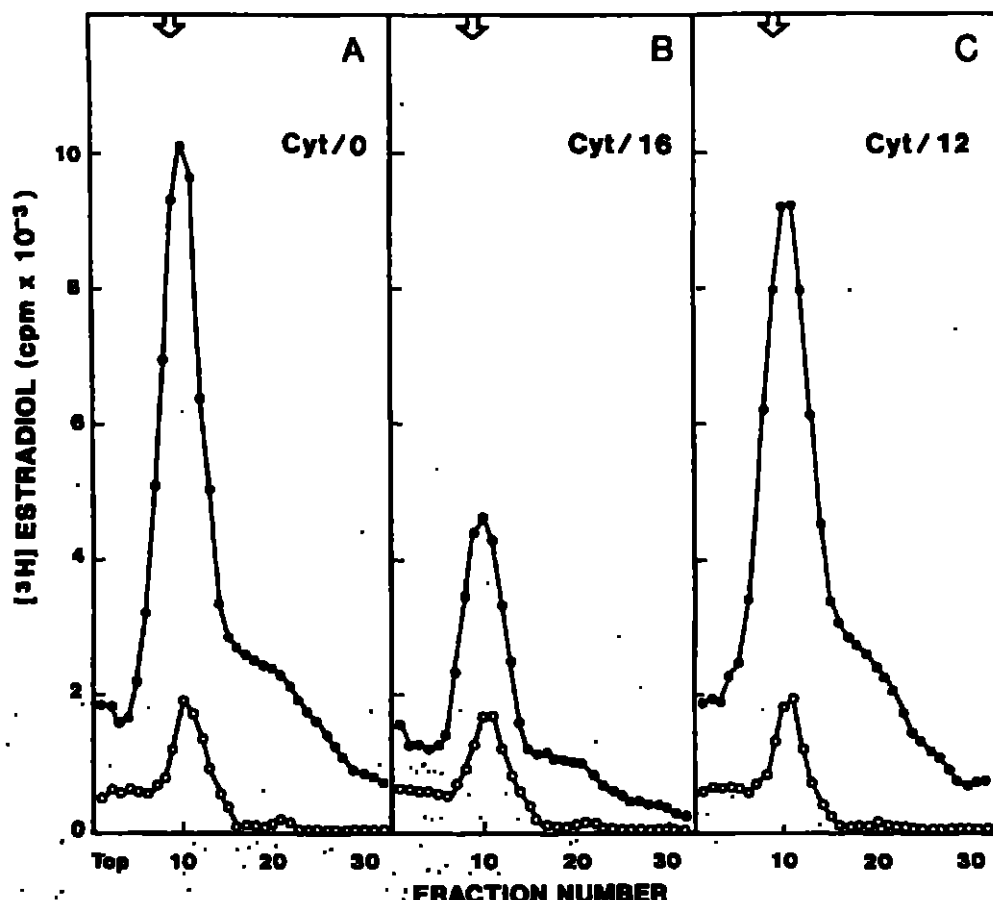


Figure 3. Sedimentation profile in high salt sucrose gradients of estradiol binding in cytosol following incubation under exchange conditions. Cytosol (pH 9.0) was incubated for 1 h at 0 °C either without steroid (A) with 5 μ M bromoacetate 16 (B) or corresponding alcohol 12 (C). After treatment with an equal volume of charcoal suspension and centrifugation, aliquots of the supernatant were incubated under exchange conditions with [3 H]estradiol in the absence or presence of nonradioactive estradiol to measure total and nonspecific [3 H]estradiol binding, respectively. Following a second charcoal treatment, residual [3 H]estradiol binding in aliquots was analysed by centrifugation on the sucrose gradients. Total (●) and nonspecific (○) bound [3 H]estradiol in gradient fractions are represented. The arrow indicates the sedimentation position of ovalbumin (3.7 S).

steroids from cytosol was never total (in these conditions, the efficiency of charcoal in removing [3 H]estradiol was ~95–97%). For cytosol incubated with bromoacetate 16, there was a strong decrease in the capacity of cytosol to specifically bind estradiol ($N = 0.408$ nM), whereas the apparent affinity constant ($K_A = 2.57 \times 10^9$ M $^{-1}$) was ~2-fold lower than that measured with control cytosol. In this case, inhibition of [3 H]estradiol binding resulting from incubation of cytosol with 16 was not overcome by increasing the [3 H]estradiol concentration. This sharply contrasted with the situation concerning the related compound 12, in spite of (i) an affinity of 12 for the receptor higher than that of 16 and (ii) the fact that much of 16 was rapidly converted to 12 in cytosol. We conclude that the decreased binding site concentration induced by 16 was due to irreversible inactivation of the hormone-binding site of a corresponding portion of the receptor molecules. Compared to the control cytosol, the ~2-fold decrease in the apparent affinity constant probably resulted, as stated for cytosol incubated with 12, from the presence of residual steroids (mainly 12, due to the instability of 16 in cytosol) in charcoal-treated cytosol.

V. Bromoacetate Inactivation of the Hormone-Binding Site Requires Unfilled Binding Site. To examine the specificity of the bromoacetate effect on inactivation of the estrogen-binding site, we determined the effect of bromoacetates when the hormone-binding site of the estrogen receptor was occupied with either

estradiol or 4-hydroxytamoxifen. As shown in Figure 5, the concentration of hormone-binding sites from cytosol not preexposed to estradiol or 4-hydroxytamoxifen, decreased by ~70% after a 1-h incubation of cytosol at 0 °C, pH 9, with 5 μ M 16. This effect was totally abolished for cytosol preexposed to either 50 nM estradiol or 4-hydroxytamoxifen before the addition of 16. In these latter two cases, the concentration of hormone-binding sites titrated with [3 H]estradiol was identical to that obtained for the control cytosol (incubated without steroid or with alcohol 12). Concentrations of hormone-binding sites measured with [3 H]estradiol in all samples preexposed to either estradiol or 4-hydroxytamoxifen were, respectively ~30% and ~40% lower than that measured in control cytosol not preexposed to the ligands, due to incomplete removal of these high affinity ligands by charcoal (mainly because of estrogen receptor-bound ligands which dissociated very slowly from the receptor at 0 °C). Hence, there was incomplete labeling of hormone-binding sites by [3 H]estradiol.

VI. Bromoacetate Inactivation of the Hormone-Binding Site Is Concentration-Dependent. Since bromoacetates 10, 15, and 16 are low affinity ligands of the estrogen receptor with low stability in cytosol, their efficiency for inactivating the hormone-binding site might be highly concentration-dependent. We therefore compared the abilities of increasing concentrations of the three bromoacetates to inactivate the binding site, with control

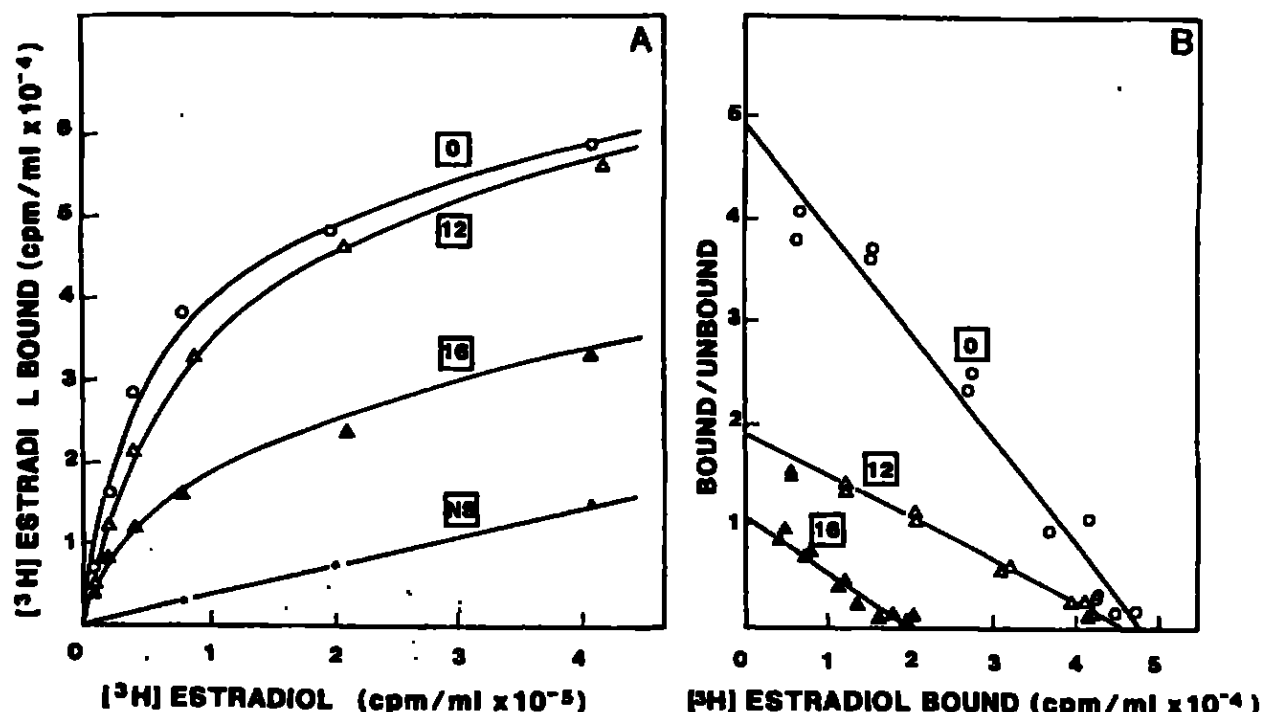


Figure 4. Saturation analysis of estradiol binding after cytosol exposure to alcohol 12 or bromoacetate 16. Cytosol (pH 9.0) was incubated for 1 h at 0 °C either without steroid (●), with 5 μM primary alcohol 12 (▲), or corresponding bromoacetate 16 (■). After charcoal treatment, and centrifugation, supernatant aliquots were incubated for 16 h at 20 °C with increasing concentrations (0.2 to 10 nM) of $[^3\text{H}]$ estradiol in the absence or presence of 5 μM nonradioactive estradiol. Binding of $[^3\text{H}]$ estradiol was measured by charcoal assay. (A) Mean values of duplicate determinations for concentrations of total (○, ▲, ■) and nonspecific (×) bound $[^3\text{H}]$ estradiol are plotted against the total concentration of $[^3\text{H}]$ estradiol; experimental variation was less than 10%. (B) Scatchard plots of individual specific $[^3\text{H}]$ estradiol binding data, considering specifically bound $[^3\text{H}]$ estradiol, as being the difference between the total bound and the nonspecific bound $[^3\text{H}]$ estradiol and unbound $[^3\text{H}]$ estradiol, as being the difference between the incubated and the total bound $[^3\text{H}]$ estradiol. Regression lines were determined respectively from plots relative to control cytosol ($K_A = 4.83 \times 10^6 \text{ M}^{-1}$, $N = 1.03 \text{ nM}$, correlation coefficient 0.981), cytosol exposed to alcohol 12 ($K_A = 1.94 \times 10^6 \text{ M}^{-1}$, $N = 0.977 \text{ nM}$, correlation coefficient 0.997), and cytosol exposed to bromoacetate 16 ($K_A = 2.57 \times 10^6 \text{ M}^{-1}$, $N = 0.408 \text{ nM}$, correlation coefficient 0.967).

incubations performed using increasing concentrations of alcohol 12. The results in Figure 6 illustrate the decreased concentration of accessible estradiol-binding sites, resulting from incubation of cytosol for 1 h at 0 °C, pH 9, with 15 nM to 5 μM of bromoacetates. Inactivation of the binding site by bromoacetates was practically undetectable until 50 nM, slight but significant receptor inactivation occurred at 150 nM; with an ~ 5 –10% decrease in the concentration of accessible binding sites, depending on the bromoacetate used. These effects were progressively more pronounced as the bromoacetate concentration increased. The strongest effects, observed at the highest bromoacetate concentration, corresponded to inactivation of 25, 45, and 60% of binding sites by 10, 15, and 16, respectively. In the 150 nM to 5 μM concentration range, the same order of efficiency ($10 < 15 < 16$) was observed for the three bromoacetates. Control compound 12 (up to 5 μM) did not significantly inhibit $[^3\text{H}]$ estradiol binding to the estrogen receptor. However, at higher concentrations ($> 15 \mu\text{M}$) there was a slight inhibition of $[^3\text{H}]$ estradiol binding, probably resulting from competition of the residual concentration of 12 in cytosol, which was not adsorbed by charcoal. We did not test concentrations of compounds higher than 5 μM so as to avoid such competition interference with bromoacetates.

VII. Bromoacetate Inactivation of the Hormone-Binding Site Is pH-Dependent. Since reactivity of most nucleophilic amino acids is strongly influenced by pH, we studied the extent of inactivation of hormone-binding sites by bromoacetates, relative to pH. We used the 7–9 pH-range, to preserve both the hormone-binding property of

the receptor and the chemical integrity of bromoacetates. Figure 7 shows that the hormone-binding capacity of the control cytosol, incubated for 1 h at 0 °C without or with 5 μM 12, decreased slightly above pH 8. Inactivation of the hormone-binding sites by 5 μM bromoacetates, was either slight ($\sim 15\%$ for bromoacetate 16) or practically undetectable ($\leq 5\%$ for bromoacetates 10 and 15) at pH 7. The proportion of inactivated binding sites greatly increased with increasing pH, reaching ~ 20 , 50, and 60% for 10, 15, and 16, respectively, at pH 9. As already observed when investigating the effect of the bromoacetate concentration on hormone-binding site inactivation, we observed the same order of efficiency ($10 < 15 < 16$) at any pH from 7 to 9.

VIII. Treatment of Cytosol with Methyl Methanethiosulfonate Prevents Inactivation of the Hormone-Binding Site by Bromoacetates. To examine the possible involvement of cysteine residues in the inactivation of the hormone-binding site, we studied the effect of the sterically small thiol-specific reagent methyl methanethiosulfonate (MMTS) on bromoacetate-induced inactivation of the hormone-binding site. This reagent, which converts thiol groups to $-\text{SSCH}_3$ groups, was previously used to study the involvement of thiols in binding of glucocorticoids to their receptors²⁴ and to avince the reaction of cysteine residues of wild-type¹⁵ and mutant²⁵ estrogen receptors with nonsteroidal affinity labels. In agreement with previous reports,^{14,25} we observed that preexposure of cytosol to MMTS did not impair the ability of the receptor to bind estradiol (not shown). However, the reagent progressively prevented inactivation

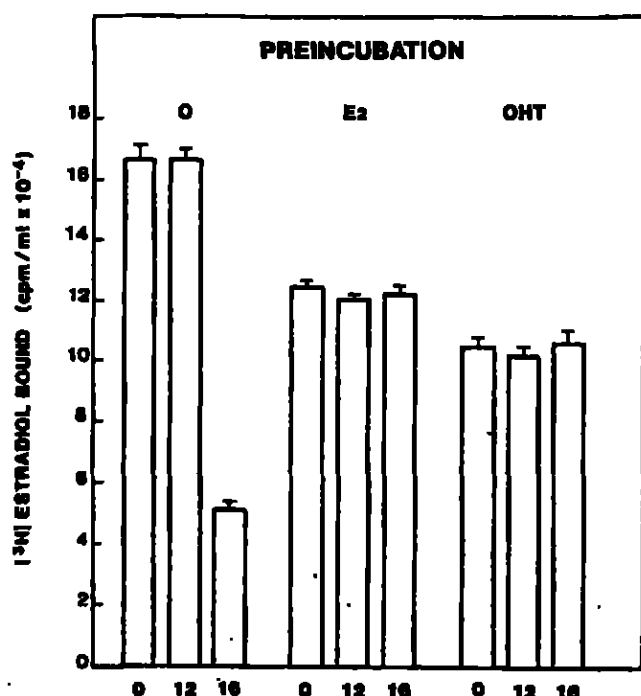


Figure 5. Inability of bromoacetate 16 to inactivate estrogen- or antiestrogen-filled hormone-binding site of the estrogen receptor. Cytosol (pH 8.0) was incubated for 4 h at 0 °C without ligand (O) with 50 nM estradiol (E₂) or 4-hydroxytamoxifen (OHT). Aliquots of the various samples were then incubated for 1 h at 0 °C, either without steroid (O) with 5 μM alcohol 12 or bromoacetate 16. After charcoal treatment and centrifugation, the supernatant was incubated with [³H]estradiol under exchange conditions to determine total and nonspecific [³H]estradiol binding. Mean specific [³H]estradiol binding from triplicate determinations are represented; error bars indicate standard deviations.

of the hormone-binding site by bromoacetates (Figure 5). The effect was detectable from 0.03 mM and practically complete at a reagent concentration equal to that of the total -SH group in cytosol. Finally, the IC₅₀s of MMTS were very similar (0.1–0.2 mM) for all three bromoacetates.

Discussion

I. 17 α -(Bromoacetoxy)alkyl/alkynylestradiols Are Estrogen Receptor Affinity Labels. In this paper, we describe the preparation and characterization of five 17 α -derivatives (two epoxides and three bromoacetates) of estradiol bearing an electrophilic group at the extremity of the 17 α -chain. Evaluation of these electrophiles revealed that all three bromoacetates irreversibly inactivated the hormone-binding site of the lamb uterine estrogen receptor in a concentration- and pH-dependent manner. The inactivation was due to the alkylating properties of these compounds since it did not occur with closely related but unreactive molecules, such as alcohol and acetate homologues of the three bromoacetates. Moreover this inactivation was a specific property of these compounds since other bromoacetoxy derivatives including nonsteroidal compounds (ethyl bromoacetate and bromoacetic acid) and steroidal compounds (17 β -(O-bromoacetyl) testosterone and 11 β -(bromoacetoxy)-17 α -ethynylestradiol) did not induce such effects. We cannot exclude that the observed inactivation of the hormone-binding site could have been due to alkylation of the estrogen receptor remote from the hormone-binding site, which would indirectly affect the hormone-binding properties of the receptor. However, this is less probable than receptor alkylation at

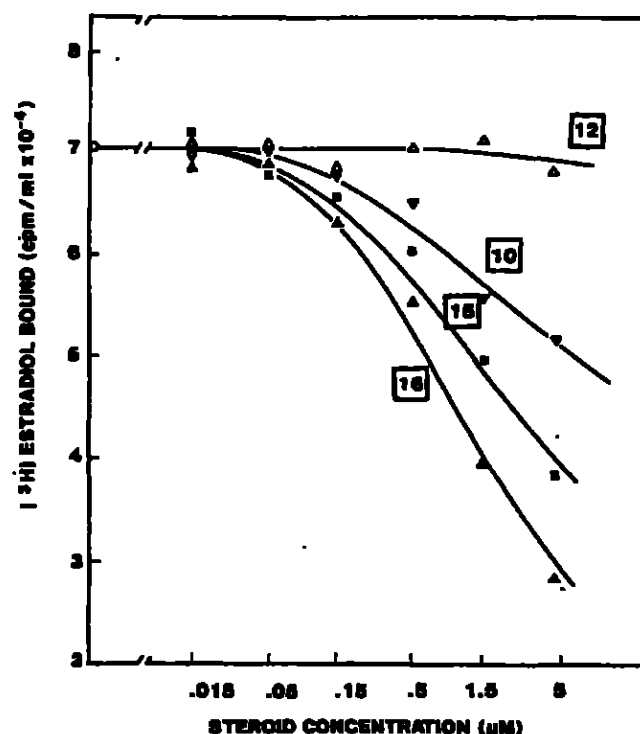


Figure 6. Concentration-dependent effects of 17 α -(bromoacetoxy)alkyl/alkynylestradiols on the inactivation of specific estradiol-binding sites. Cytosol (pH 8.0) was incubated for 1 h at 0 °C with increasing concentrations (0 to 5 μM) of bromoacetate 10 (v), 15 (m), or 16 (A) or alcohol 12 (Δ). After charcoal treatment and centrifugation, supernatant aliquots were incubated with [³H]estradiol under exchange conditions. Binding of [³H]estradiol was measured by charcoal assay. The specific binding of [³H]estradiol is plotted against the steroid concentration. Experimental variation between duplicate determinations was less than 10%.

or near the hormone-binding site since occupancy of the binding site by estrogen or antiestrogen totally prevented the bromoacetate effects. We therefore conclude that the three prepared bromoacetates are probably affinity labels of the estrogen receptor. Specific alkylation of a receptor by a reactive ligand normally occurs in two steps. As a conventional ligand the reactive molecule first reversibly associates with the receptor, and then a reaction takes place between the reactive function of the ligand and an appropriate amino acid of the ligand-binding site. Stability of this reversible receptor:reactive ligand complex is thus a crucial parameter which directly influences both the efficiency and specificity of the alkylation process, the higher the stability of the complex, the higher the probability that alkylation of the hormone-binding site occurs. Considering the ability of bromoacetates 10, 15 and 16, to specifically alkylate the receptor, two characteristics appear to be unfavorable.

(1) The low stability of compounds in cytosol: These bromoacetates are rapidly hydrolyzed to the corresponding primary alcohols. This process not only decreases the concentration of the compound able to alkylate the receptor but also generates a non-alkylating competitor whose affinity for the receptor is higher than that of the parent compound and which inhibits interaction of available receptors with intact bromoacetate. High concentrations of bromoacetates were thus required to permit alkylation of the hormone-binding site of receptors, since (1) to complex a given proportion of receptors, the higher the steroid concentration, the lower the duration of

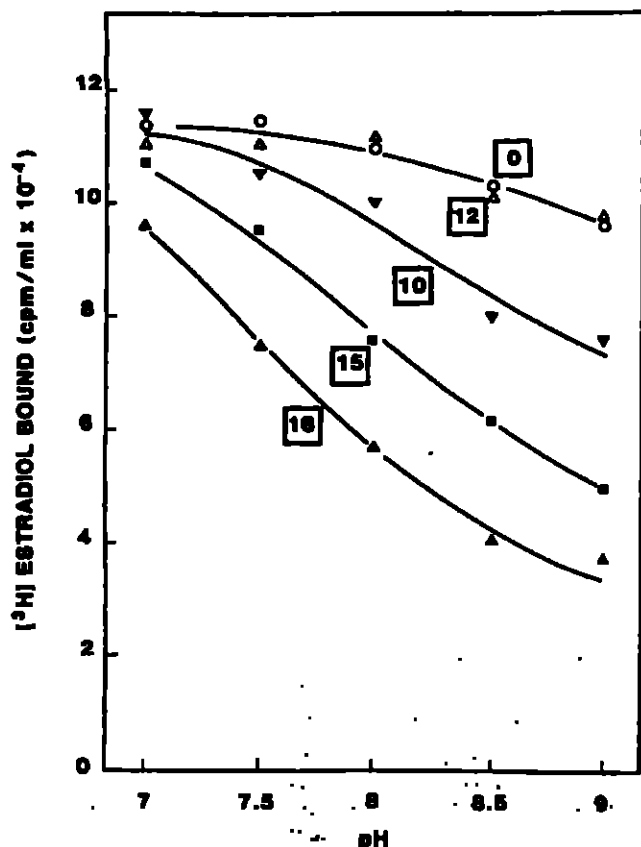


Figure 7. pH-dependent inactivation of specific estradiol binding sites by 17 α -(bromoacetoxy)alkyl/alkynylestradiols. Cytosol (pH 7.0) was progressively alkalinized by the addition of NaOH 0.5 M. Samples were removed at the following pH levels: 7.0, 7.5, 8.0, 8.5 and 9.0. Samples were incubated for 1 h at 0 °C either without steroid ($\bullet$), with 5 μ M bromoacetate 10 (∇), 15 ($\square$), or 16 (Δ) or alcohol 12 (Δ). After charcoal treatment and centrifugation, supernatant aliquots were incubated with [3 H]estradiol under exchange conditions. The specific binding of [3 H]estradiol is plotted against pH. Experimental variation between duplicate determinations was less than 15%.

exposure of receptors to steroid (relative to the steroid concentration, formation of the complex is a kinetic first-order process) and (2) the lower the duration of exposure, the higher the ratio of the bromoacetate concentration to that of the formed alcohol (at the beginning this ratio is roughly inversely proportional to time) and then the higher the ratio of the receptor:bromoacetate complex concentration to that of the receptor:alcohol complex.

(ii) The low stability of the receptor/bromoacetate complexes: The apparent RAC calculated for bromoacetates ranged from $\sim 0.02\%$ (compound 10) to $\sim 0.24\%$ (compound 16) to that of estradiol. Assuming, as usually observed, that affinity mainly reflects the dissociation rate of the complex, then from the half-dissociation times reported for ligands such as estradiol²⁸ and from the apparent affinity constants determined for bromoacetates, one can obtain a rough estimate of the half-lives of the receptor:bromoacetate complexes at 0 °C: <5 min with 10, <15 min with 15, and <60 min with 16.

When comparing the ability of bromoacetates to alkylate receptor molecules, according to either the compound concentration or pH, in both cases we observed strong differences between the three compounds, with the same efficiency order: $10 < 15 < 16$. These differences might partly reflect different intrinsic properties of the common electrophilic group in the three compounds, such as

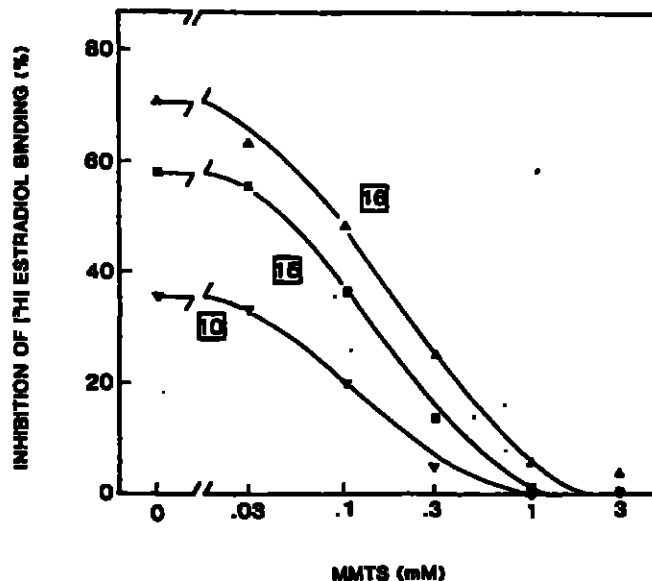


Figure 8. Effect of methyl methanethiosulfonate on the inactivation of specific estradiol-binding sites by bromoacetates. Cytosol (pH 9.0) was incubated for 2 h at 0 °C with increasing concentrations (0–3 mM) of methyl methanethiosulfonate (MMTS). Aliquots of the various samples were then incubated for 1 h at 0 °C with 5 μ M bromoacetate 10 (∇), 15 ($\square$), or 16 (Δ) or alcohol 12 as a control. After charcoal treatment and centrifugation, supernatant aliquots were incubated with [3 H]estradiol under exchange conditions. The specific binding of [3 H]estradiol in cytosol exposed to each of the bromoacetates, expressed as a percent of the specific binding of [3 H]estradiol in cytosol exposed to alcohol 12, is plotted against the MMTS concentration. Experimental variation between duplicate determinations was less than 10%. The 100% specific binding of [3 H]estradiol in the absence or presence of MMTS was 62 300 cpm/mL; the total -SH group concentration was ~ 1 mM.

chemical reactivity and/or location relative to those of nucleophiles in the hormone-binding site. For instance, the degree of freedom of the reactive terminal carbon is considerably higher in compound 16 than in compound 15 or 10. However, the more trivial previously described properties, i.e., the relative affinity and relative stability of compounds in cytosol, certainly played a major role in the compound efficiencies. It should be noted that there was a good correlation between the apparent relative affinity of bromoacetates and their ability to alkylate the hormone-binding site of the receptor.

II. A Single Cysteine Residue Is Probably the Target of All Three Bromoacetates. The marked pH-dependence of the alkylation processes; with very weak effects at pH 7 and then increasing effects up to pH 9, observed with all three bromoacetates, suggests that similar (or even a single) basic nucleophiles of the receptor hormone-binding site react with the three bromoacetates. It is unlikely that methionines or histidines are bromoacetate targets since these amino acids are usually reactive at neutral pH. Among the amino acids which contain a reactive side chain with a basic pK_a , cysteine ($pK_a \sim 8.3$) and lysine ($pK_a \sim 9.2$) are more probable candidates than tyrosine ($pK_a \sim 10.1$) and arginine ($pK_a \sim 12.5$).

Alkylation of the receptor by the three bromoacetates required intact cysteine residues since the cysteine-specific reagent MMTS totally prevented the effect of the compounds. One of the following two possibilities could therefore be considered: (i) cysteines are the actual targets of bromoacetates, and (ii) MMTS-modification of cysteine residues by steric hindrance impairs the reaction of

neighboring nucleophiles with bromoacetates. The second hypothesis appears unlikely since (1) the size of the functional group which is transferred by MMTS to cysteine residues is relatively small and (2) the fact that each bromoacetate displayed, for MMTS-modified receptor, a relative affinity which was equal to that of untreated receptor suggests that modification of the cysteine residues of the receptor does not significantly impair bromoacetate/receptor interactions. Therefore, cysteine residues of the receptor are probably bromoacetate targets. Moreover, the fact that the concentration-dependent blockade of the bromoacetate effects by MMTS was very similar for the three compounds with practically identical IC_{50} values strongly suggests that the same cysteine residue is the target of the three bromoacetates. pH-dependent alkylation of the receptor by bromoacetates was in sharp contrast to that displayed by the two nonsteroidal affinity labels: the antiestrogen tamoxifen aziridine and the estrogen ketononestrol aziridine which, at neutral pH, both efficiently react with the same cysteine residue of the hormone-binding site.¹⁵ This contrast could result from the low affinity and low stability of bromoacetates which, for efficient alkylation of the receptor, would require a highly reactive negatively charged cysteine residue. Finally, the suspected cysteine target of bromoacetates might differ from the lamb homologue of cysteine 530 of the human estrogen receptor since construction of steroidal analogues of tamoxifen aziridine and ketononestrol aziridines led to mean positions of the aziridine function nearer to the C11–C12 region of the C ring than to the C17 region of the D ring of the steroid. Another possibility could involve the difference in the chemical nature of the two types of electrophiles, since aziridines groups, which show very little reactivity in the uncharged form, become highly reactive after protonation.¹³ This suggests that aziridine compounds alkylate the receptor at neutral pH on cysteine 530, which could act as a proton donor.

In conclusion, estradiol derivatives bearing a 17 α , C3–C8-alkyl or alkynyl chain, with a terminal bromoacetate function, are highly reactive affinity labels of the estrogen receptor. At least one basic nucleophile, probably the -SH of a cysteine, appears to react with the electrophilic carbon of bromoacetates. Preparation of homologous compounds bearing smaller electrophilic groups linked to the estradiol molecule by means of a nonhydrolyzable 17 α -chain is in progress in our laboratory. Such compounds will probably be reactive affinity labels useful for identifying target amino acids in the hormone-binding site of the estrogen receptor.

Experimental Section

Synthesis and Characterization of Estradiol Derivatives. Estrone was purchased from Fluka, and 17 α -(4'-hydroxy-1'-butynyl)estradiol and 17 α -(8'-hydroxy-1'-octynyl)estradiol were donated by Dr. Poirier (Centre Hospitalier de l'Université Laval, Québec, Canada). Solvents and reagents were purchased from the following commercial sources: Aldrich, Carlo Erba, Fluka, Merck, and Sigma. Analytical TLC was performed with Merck aluminium sheet-baked silica gel (F-254, 0.2 mm). Column chromatography was performed with silica gel (0.063–0.2 mm). Nuclear magnetic resonance spectra were recorded on a WM 360 WB spectrometer at 360 MHz. Chemical shifts are reported downfield of tetramethylsilane internal standard (δ scale). High-resolution, electron-impact mass spectra were obtained on a LKB 2091 spectrometer. Elemental analyses were performed by the microanalytical service of CNRS (Versailles, France).

Except when otherwise stated, a standard procedure was used for product isolation. This involved quenching of the reaction

mixture in water or aqueous solution, followed by exhaustive extraction with ether or ethyl acetate, washing of extracts with aqueous solutions when necessary, drying of organic extracts over Na_2SO_4 , filtration, and evaporation of the solvent under reduced pressure. The solvents and the aqueous solutions used for quenching the reaction and washing are mentioned in parentheses after "product isolation".

17 α -(2'-Propen-1'-yl)estra-1,3,5(10)-trien-3,17 β -diol (3). Ten milliliters (120 mmol) of allyl chloride was added to 2.43 g (100 mmol) of magnesium turning under nitrogen. After starting of the reaction, dry THF (20 mL) was added, and the mixture was agitated until complete reaction of magnesium. A solution of 4.05 g of estrone (15 mmol) in 150 mL of dry THF and 10 mL of allyl chloride was added dropwise to the Grignard solution. After the addition of estrone was completed, the reaction mixture was heated to reflux for 2 h, and stirring was maintained for 15 h at 20 °C. The mixture was then poured into a saturated NH_4Cl solution. Product isolation (Et_2O , $NaHCO_3$, Na_2SO_4) afforded mixture of diastereoisomers 2 and 3 (3/3 < 1/20): chromatography (Et_2O /heptane 1/4) gave 4.1 g of 3 (88%); 1H NMR ($CDCl_3$) δ 0.92 (s, 3H, 18- CH_3), 5.19 (m, 2H, 3'- CH_2), 6.00 (m, 1H, 2'-CH), 6.55 (d, 1H, J = 2.7 Hz, 4-CH), 6.61 (q, 1H, J_1 = 2.7 Hz, J_2 = 8.3 Hz, 2-CH), 7.13 (d, 1H, J = 8.3 Hz, 1-CH); MS (70 eV), m/z (rel intensity) 312 (M^+ , 8), 294 (9), 272 (11), 43 (100). Anal. ($C_{27}H_{40}O_2$) C, H.

3-(*tert*-Butyldimethylsiloxy)-17 α -(2'-propen-1'-yl)estra-1,3,5(10)-trien-17 β -ol (4). A mixture of 1.68 g (11.1 mmol) of TBDMS chloride and 1.58 g (23.2 mmol) of imidazole in 7 mL of DMF was added to 2.9 g (9.3 mmol) of 3. After the mixture was stirred for 1 h at 35 °C, product isolation (Et_2O , $NaHCO_3$, Na_2SO_4) and then chromatography (Et_2O /heptane 1/9) gave 3.48 g of 4 (88%); 1H NMR ($CDCl_3$) δ 0.17 (s, 6H, $Si(CH_3)_2$), 0.92 (s, 3H, 18- CH_3), 0.98 (s, 9H, $Si(CH_3)_3$), 5.19 (m, 2H, 3'- CH_2), 6.00 (m, 1H, 2'-CH), 6.55 (d, 1H, J = 2.7 Hz, 4-CH), 6.61 (q, 1H, J_1 = 2.7 Hz, J_2 = 8.3 Hz, 2-CH), 7.13 (d, 1H, J = 8.3 Hz, 1-CH); MS (70 eV) m/z (rel intensity) 428 (M^+ , 43), 370 (21), 327 (27), 163 (12), 97 (100).

3-(*tert*-Butyldimethylsiloxy)-17 α -(2',3'-epoxyprop-1'-yl)estra-1,3,5(10)-trien-17 β -ol (5a) and (5b). *m*-CPBA (549 mg, 3.18 mmol) was added to a solution of alkene 4 (880 mg, 1.59 mmol) in 15 mL of CH_2Cl_2 . The mixture was stirred for 17 h at 20 °C and then poured into 80 mL of CH_2Cl_2 . Product isolation (KI 2%, $Na_2S_2O_5$ 10%, H_2O , Na_2SO_4) and then chromatography (Et_2O /heptane 4/6) afforded to 423 mg (80%) and 125 mg (18%) of less polar (5a) and more polar (5b) diastereoisomers, respectively.

Diastereoisomer 5a: 1H NMR ($CDCl_3$) δ 0.17 (s, 6H, $Si(CH_3)_2$), 0.91 (s, 3H, 18- CH_3), 0.98 (s, 9H, $Si(CH_3)_3$), 2.50 (q, 1H, J_1 = 2.7 Hz, J_2 = 5.0 Hz, 3'- CH_2), 2.78 (q, 1H, J_1 = 4.1 Hz, J_2 = 5.0 Hz, 3'- CH_2), 3.27 (m, 1H, 2'-CH), 6.53 (d, 1H, J = 2.7 Hz, 4-CH), 6.58 (q, 1H, J_1 = 2.7 Hz, J_2 = 8.3 Hz, 2-CH), 7.08 (d, 1H, J = 8.3 Hz, 1-CH); MS (70 eV), m/z (rel intensity) 442 (M^+ , 63), 385 (86), 327 (33), 272 (13), 270 (12), 41 (100). Anal. ($C_{37}H_{50}O_4Si$) C, H.

Diastereoisomer 5b: 1H NMR ($CDCl_3$) δ 0.17 (s, 6H, $Si(CH_3)_2$), 0.91 (s, 3H, 18- CH_3), 0.98 (s, 9H, $Si(CH_3)_3$), 2.53 (q, 1H, J_1 = 2.7 Hz, J_2 = 5.0 Hz, 3'- CH_2), 2.85 (q, 1H, J_1 = 4.1 Hz, J_2 = 5.0 Hz, 3'- CH_2), 3.28 (m, 1H, 2'-CH), 6.53 (d, 1H, J = 2.7 Hz, 4-CH), 6.60 (q, 1H, J_1 = 2.7 Hz, J_2 = 8.3 Hz, 2-CH), 7.09 (d, 1H, J = 8.3 Hz, 1-CH); MS (70 eV), m/z (rel intensity) 442 (M^+ , 35), 385 (28), 41 (100). Anal. ($C_{37}H_{50}O_4Si$) C, H.

17 α -(2',3'-Epoxyprop-1'-yl)estra-1,3,5(10)-trien-3,17 β -diol (6a). Tetrabutylammonium fluoride (90 mg, 0.34 mmol) was added to a solution of 100 mg (0.23 mmol) of diastereoisomer 5a in 1 mL of THF at 0 °C. After stirring for 5 min at 0 °C and then 15 min at 20 °C, product isolation ($NaHCO_3$, $AcOEt$, Na_2SO_4) then chromatography (Et_2O) afforded to 54 mg (73%) of (6a); 1H NMR ($CDCl_3$) δ 0.91 (s, 3H, 18- CH_3), 2.50 (q, 1H, J_1 = 2.7 Hz, J_2 = 5.0 Hz, 3'- CH_2), 2.80 (q, 1H, J_1 = 4.1 Hz, J_2 = 5.0 Hz, 3'- CH_2), 3.24 (m, 1H, 2'-CH), 6.54 (d, 1H, J = 2.7 Hz, 4-CH), 6.60 (q, 1H, J_1 = 2.7 Hz, J_2 = 8.3 Hz, 2-CH), 7.10 (d, 1H, J = 8.3 Hz, 1-CH); MS (70 eV), m/z (rel intensity) 328 (M^+ , 8), 310 (7), 270 (4), 43 (100). Anal. ($C_{27}H_{40}O_3$) C, H.

17 α -(2',3'-Epoxyprop-1'-yl)estra-1,3,5(10)-trien-3,17 β -diol (6b). Diastereoisomer 5b was prepared from the corresponding protected compound (diastereoisomer 5b) in 82% yield by the procedure described for compound 5a: 1H NMR ($CDCl_3$)

δ 0.91 (s, 3H, 18-CH₃), 2.52 (q, 1H, $J_1 = 2.7$ Hz, $J_2 = 5.0$ Hz, 3'-CH₂), 2.85 (q, 1H, $J_1 = 4.1$ Hz, $J_2 = 5.0$ Hz, 3'-CH₂), 3.24 (m, 1H, 2'-CH), 6.54 (d, 1H, $J = 2.7$ Hz, 4-CH), 6.80 (q, 1H, $J_1 = 2.7$ Hz, $J_2 = 8.3$ Hz, 2-CH), 7.10 (d, 1H, $J = 8.3$ Hz, 1-CH); MS (70 eV), m/z (rel intensity) 328 (M^+ , 39), 310 (13), 270 (10), 213 (100). Anal. (C₂₀H₂₈O₂) C, H.

3-(*tert*-Butyldimethylsiloxy)-17 α -(3'-hydroxyprop-1'-yl)estra-1,3,5(10)-trien-17 β -ol (7). 9-BBN (28.14 mmol, 0.5 M solution in THF) under nitrogen was added dropwise to a solution of 2 g (4.69 mmol) of alkene 4 in 15 mL of THF at 60 °C. After 40 min, the solution was cooled to 0 °C, 9 mL of water was added and stirring was continued for 5 min. Sodium hydroxide (9 mL, 3M) was added, and after an additional 5 min, 9 mL of hydrogen peroxide (30%) was added dropwise. After 30 min product isolation (NaHCO₃, Et₂O, Na₂SO₄) and then chromatography (CHCl₃) afforded to 1.77 g (86%) of the primary alcohol 7: ¹H NMR (CDCl₃) δ 0.16 (s, 6H, Si(CH₃)₂), 0.91 (s, 3H, 18-CH₃), 0.96 (s, 9H, SiC(CH₃)₃), 3.70 (m, 2H, 3'-CH₂), 6.53 (d, 1H, $J = 2.7$ Hz, 4-CH), 6.60 (q, 1H, $J_1 = 2.7$ Hz, $J_2 = 8.3$ Hz, 2-CH), 7.10 (d, 1H, $J = 8.3$ Hz, 1-CH); MS (70 eV), m/z (rel intensity) 444 (M^+ , 56), 428 (50), 41 (100). Anal. (C₂₇H₄₄O₃Si) C, H.

17 α -(3'-Hydroxyprop-1'-yl)estra-1,3,5(10)-trien-3,17 β -diol (8). Triol 8 was prepared from 7 in 86% yield, by the procedure described for compound 6a. It was purified by chromatography (Et₂O/CHCl₃ 1/4): ¹H NMR (DMSO) δ 0.79 (s, 1H, 18-CH₃), 3.41 (m, 2H, 3'-CH₂), 6.42 (d, 1H, $J = 2.7$ Hz, 4-CH), 6.50 (q, 1H, $J_1 = 2.7$ Hz, $J_2 = 8.3$ Hz, 2-CH), 7.03 (d, 1H, $J = 8.3$ Hz, 1-CH); MS (70 eV) m/z (rel intensity) 330 (M^+ , 12), 312 (81), 271 (9), 270 (7), 28 (100).

3-(*tert*-Butyldimethylsiloxy)-17 α -(3'-bromoacetoxyprop-1'-yl)estra-1,3,5(10)-trien-17 β -ol (9). Bromoacetic acid (561 mg, 4.04 mmol), 775 mg (4.04 mmol) of 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride, and 0.345 mL (3.03 mmol) of pyridine were added successively to a solution of 900 mg (2.02 mmol) of primary alcohol 7 in 30 mL of THF. The mixture was stirred for 2 h at 25 °C and then poured into Et₂O. Insoluble material was removed by filtration, product isolation (NaHCO₃, H₂O, and Na₂SO₄) and then chromatography (Et₂O/heptane 1/1) afforded to 917 mg (80%) of bromoacetate 9: ¹H NMR (CDCl₃) δ 0.16 (s, 6H, Si(CH₃)₂), 0.89 (s, 3H, 18-CH₃), 0.96 (s, 9H, SiC(CH₃)₃), 4.07 (s, 2H, CH₂Br), 4.23 (t, 2H, $J_1 = J_2 = 6.6$ Hz, 3'-CH₂), 6.53 (d, 1H, $J = 2.7$ Hz, 4-CH), 6.59 (q, 1H, $J_1 = 2.7$ Hz, $J_2 = 8.3$ Hz, 2-CH), 7.10 (d, 1H, $J = 8.3$ Hz, 1-CH); MS (70 eV) m/z (rel intensity) 557 (M^+ , 5), 555 (M^+ , 8), 428 (8), 408 (3), 272 (17), 97 (94), 73 (100).

17 α -(3'-(Bromoacetoxy)prop-1'-yl)estra-1,3,5(10)-trien-3,17 β -diol (10). Deprotected bromoacetate 10 was prepared from 9 in 67% yield by the procedure described for compound 6a. It was purified by chromatography (Et₂O/heptane 3/7): ¹H NMR (CDCl₃) δ 0.90 (s, 3H, 18-CH₃), 4.03 (s, 2H, CH₂Br), 4.25 (t, 2H, $J_1 = J_2 = 6.6$ Hz, 3'-CH₂), 6.54 (d, 1H, $J = 2.7$ Hz, 4-CH), 6.61 (q, 1H, $J_1 = 2.7$ Hz, $J_2 = 8.3$ Hz, 2-CH), 7.12 (d, 1H, $J = 8.3$ Hz, 1-CH); MS (70 eV), m/z (rel intensity) 452 (M^+ , 0.5), 450 (M^+ , 0.4), 408 (17), 406 (23), 390 (8), 388 (14), 312 (35), 294 (4), 213 (100).

17 α -(4'-(Bromoacetoxy)butyn-1'-yl)estra-1,3,5(10)-trien-3,17 β -diol (15). Bromoacetic acid (55.6 mg, 0.4 mmol), 76.6 mg (0.4 mmol) of 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride, and 24 μ L (0.3 mmol) of pyridine were added successively to a solution of 34 mg (0.10 mmol) of triol 11 in 2 mL of THF. The mixture was stirred for 2 h at 25 °C and then poured into Et₂O. Insoluble material was removed by filtration, product isolation (NaHCO₃, H₂O, and Na₂SO₄) gave ~80 mg of crude dibromoacetate 13. The compound was solubilized in 2 mL of THF after cooling to 0 °C, a solution of 12.6 mg NaHCO₃ (0.15 mmol) in 0.5 mL CH₃OH/H₂O (1/1) was added and the mixture was stirred for 15 h at 0 °C. Product isolation (Et₂O, H₂O, and Na₂SO₄) and then chromatography (Et₂O/heptane 3/7) afforded to 14 mg (30%) of monobromoacetate 15: ¹H NMR (CDCl₃) δ 0.85 (s, 3H, 18-CH₃), 2.63 (t, 2H, $J_1 = J_2 = 6.7$ Hz, 3'-CH₂), 4.05 (s, 2H, CH₂Br), 4.30 (t, 2H, $J_1 = J_2 = 6.7$ Hz, 4'-CH₂), 6.54 (d, 1H, $J = 2.7$ Hz, 4-CH), 6.60 (q, 1H, $J_1 = 2.7$ Hz, $J_2 = 8.3$ Hz, 2-CH), 7.14 (d, 1H, $J = 8.3$ Hz, 1-CH); MS (20 eV), m/z (rel intensity) 462 (M^+ , 0.6), 460 (M^+ , 0.5), 307 (15), 160 (100).

17 α -(8'-(Bromoacetoxy)octyn-1'-yl)estra-1,3,5(10)-trien-3,17 β -diol (16). The monobromoacetate 16 was prepared from triol 12 by the procedure described for compound 15. Compound 16 (47%) was purified by chromatography (Et₂O/heptane 1/3): ¹H NMR (CDCl₃) δ 0.85 (s, 3H, 18-CH₃), 2.24 (t, 2H, $J_1 = J_2 = 6.7$ Hz, 3'-CH₂), 3.99 (s, 2H, CH₂Br), 4.17 (t, 2H, $J_1 = J_2 = 6.77$ Hz, 8'-CH₂), 6.54 (d, 1H, $J = 8.3$ Hz, 4-CH), 6.60 (q, 1H, $J_1 = 2.7$ Hz, $J_2 = 8.3$ Hz, 2-CH), 7.14 (d, 1H, $J = 8.3$ Hz, 1-CH); MS (20 eV), m/z (rel intensity) 518 (M^+ , 1), 516 (M^+ , 1), 474 (10), 472 (10), 160 (100).

Estrogen Receptor Ligands. [6,7-³H]Estradiol (specific activity 70 Ci/mmol, radiochemical purity > 97%) was purchased from the Commissariat à l'Energie Atomique (Gif-Sur-Yvette, France). 4-Hydroxy[*N*-methyl-³H]tamoxifen (specific activity 83 Ci/mmol, radiochemical purity > 99%) was from Amersham International (Amersham, England). Estradiol, 4-hydroxytamoxifen, and all the 17 α -derivatives of estradiol used for binding studies were solubilized in absolute ethanol. Solutions were stored at -20 °C in the dark. Purity of solubilized compounds was checked before use by TLC.

Preparation of Cytosol. Immature lamb uteri were stripped from connective tissue, placed in liquid nitrogen, and then stored at -80 °C until use. The uteri were defrosted and then immediately homogenized in five volumes of chilled 20 mM Tris-HCl buffer, pH 7.0 (T₁₀), using an ultraturax. The homogenate was centrifuged at 10⁵ × g for 45 min. The protein concentration determined according to Layne²⁷ was then adjusted to 4 mg/mL. Depending on the experiments, the cytosol was used either at pH 7.0 or after alkalization with NaOH 0.5 M to reach pH values between 7.0 and 9.0.

Competitive Binding Assay: Apparent Relative Affinity Constants. Cytosol (pH 7.0) was diluted to 2 mg of protein/mL with T₁₀. It was then incubated with 5 nM [³H]estradiol and increasing concentrations of either nonradioactive estradiol (1.67 nM to 1.5 μ M) or estradiol 17 α -derivatives (0.1 to 10 μ M) either at 0 or 20 °C. Binding of [³H]estradiol in samples was determined after a 4-, 20-, or 72-h incubation. Aliquots were treated with an equal volume of charcoal suspension (0.5% charcoal, 0.06% dextran T₁₀ in T₁₀) for 30 min at 0 °C; charcoal was pelleted by 5-min centrifugation at 10⁵ × g and then radioactivity in the supernatant was measured. Apparent relative affinity constants (RACs) of competitors relative to that of estradiol were calculated from the concentration of unlabeled estradiol (E) and competitor (C) which inhibited 50% of the specific binding of [³H]estradiol, according to Korenman:²⁸

$$\text{RAC} = \frac{\frac{E_1 - E_b}{E_b} \cdot \frac{E}{C}}{\frac{E_1 - E_b}{E_b} + 1 - \frac{E}{C}}$$

where E_1^* is the total concentration of [³H]estradiol, E_b^* and E_{50}^* are the respective concentrations of bound and specifically bound [³H]estradiol at 50% inhibition; $E_1^* - E_b^*$ was assumed to be the unbound [³H]estradiol concentration at 50% inhibition.

Standard Irreversible Binding Assay. Cytosol was first incubated for 1 h at 0 °C with estradiol 17 α -derivatives or without steroid. Samples were then treated with an equal volume of charcoal suspension for 30 min at 0 °C. Thereafter charcoal was removed by centrifugation, and supernatant aliquots were incubated under "exchange conditions" (18 h at 20 °C) with 20 nM [³H]estradiol either in the absence (to measure total [³H]estradiol binding) or presence (to measure nonspecific [³H]estradiol binding) of 5 μ M nonradioactive estradiol. Binding of [³H]estradiol was determined by a 30 min-0 °C charcoal assay (of "competitive binding assay").

Sucrose Gradient Analyses. Cytosol aliquots (pH 9.0), incubated with or without steroids, treated with charcoal, then incubated with [³H]estradiol for 18 h at 20 °C, and finally submitted to a second charcoal treatment (cf "irreversible binding assay") were added with ¹⁴C-labeled ovalbumin. Then 200 μ L of the mixture was layered onto 10–30% sucrose gradient (4.8 mL) prepared in T₁₀ and containing 400 mM KCl. Gradients were centrifuged in a Beckman SW 50.1 rotor for 14 h at 216 000 g_{av} (48 000 rpm). Thirty-two successive fractions were collected from

the bottom of the tube, and their radioactivity measured. In some experiments, before centrifugation aliquots were incubated for 8 h at 0°C with 1% control serum from nonimmunized mouse or with serum from mouse immunized with purified preparations of lamb uterine estrogen receptor.²³

Radioactivity Determinations. The radioactivity of gradients fractions (~180 µL), and of the various cytosol samples (100 or 200 µL) was counted in 4 mL of Emulphor Safe (Packard).

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