ELSEVIER

Papers

A comparative structural study of the steroid epimers: 17β-amino-1,3,5(10)-estratrien-3-ol, 17α-amino-1,3,5(10)-estratrien-3-ol, and some derivatives by ^{1}H NMR, and x-ray diffraction analysis

Cristina Lemini,* Elia Cruz–Ramos,* Rubén Alfredo Toscano,† and Raymundo Cruz–Almanza†

**Departamento de Farmacología, Facultad de Medicina, Universidad Nacional Autónoma de México (UNAM), CP 04510. Apdo. Postal 70–297 and †Instituto de Química, UNAM Circuito exterior CU, México D.F., CP 04510, México*

The epimers 17β-amino-1,3,5(10)-estratrien-3-ol and 17α-amino-1,3,5(10)-estratrien-3-ol were synthesized. 17β-Amino-1,3,5(10)-estratrien-3-ol was prepared by catalytic hydrogenation of the estrone–oxime. The 17α-amino epimer was obtained from estradiol, via tosylate, followed by nucleophilic displacement by sodium azide and subsequent reduction with $LiAlH_4$. They were characterized by spectroscopic methods. Determination of the crystal structures of 3-(toluene-4-sulfonyloxy)-17α-azido-1,3,5(10)-estratriene, 3-(toluene-4-sulfonyloxy)-17-α-amino-1,3,5(10)-estratriene hydrochloride, 17β-acetylamino-1,3,5(10)-estratriene-3-ol, and 3-acetoxy-17β-acetylamino-1,3,5(10)-estratriene enabled us to characterize the structure of the 17α and 17β amino epimers for the first time. (*Steroids* **63**:556–564, 1998) © *1998 by Elsevier Science Inc.*

Keywords: 17-aminosteroids; 17α-aminoestrogens; 17β-aminoestrogens; ^{1}H NMR; X-ray; epimers

Introduction

17β-aminosteroids have been used as precursors for the preparation of peptide derivatives for steroid-protein binding, and have been evaluated as anticancer drugs against hormone-dependent tumors, as well as immunoregulatory and antiarrhythmic agents.[1–4]

17β-aminoestrogens produce dose-dependent and prolonged anticoagulant effects in rodents,[5–10] contrasting with the procoagulant effects produced by estradiol and other synthetic estrogens when administered at high doses.[11] The anticoagulant effect of the 17β-aminoestrogens is related to the nitrogen function attached at the C17 position of the steroid. Compounds which have *N*-alkyl, *N*-alkyloxy and *N*-alkyl-*NN′*dialkyl C17 substitutions produce anticoagulant effects,[5–10] however, it is unknown whether substitution in the amino group is required or not to produce such effects. The effects on blood coagulation of 17α-aminoestrogens, which are epimers of 17β-aminoestrogens have not been studied yet.

To obtain this information, 17β-amino-1,3,5(10)-estratrien-3-ol (**1**) and 17α-amino-1,3,5(10)-estratrien-3-ol (**7**) were synthesized and characterized by spectroscopic methods. X-ray studies of 17β-acetylamino-1,3,5(10)-estratriene-3-ol (**2**), 3-acetoxy-17β-acetylamino-1,3,5(10)-estratriene (**3**), 3-(toluene-4-sulfonyloxy)-17α-azido-1,3,5(10)-estratriene (**6**), and 3-(toluene-4-sulfonyloxy)-17-α-amino-1,3,5(10)-estratriene hydrochloride (**8**) enabled us to characterize the structure of the 17β and 17α amino epimers **1** and **7**.

Experimental

All solvents and reagents used were of analytical reagent grade, and were dried and distilled under usual procedures.

Address reprint requests to Cristina Lemini, Departamento de Farmacología, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad Universitaria, CP 04510, Apdo. Postal 70–297, México D.F. Mexico.
Received September 11, 1997; accepted July 1, 1998.

655 Avenue of the Americas, New York, NY 10010

0039-128X/98/$19.00
PII S0039-128X(98)00063-4

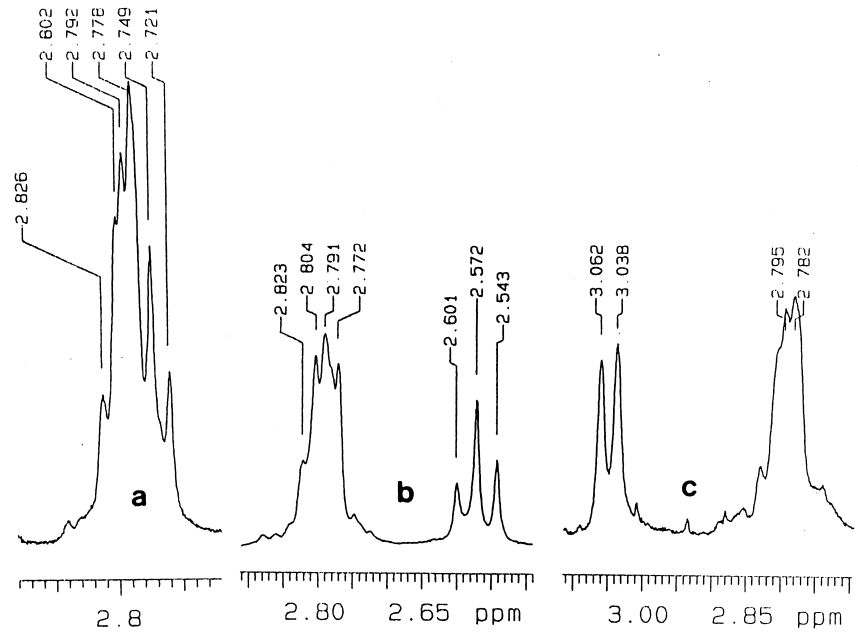


Figure 1 300 MHz^{1}H NMR of 17-H and benzylic protons; (**a**) epimer 17β-amino in $CDCl_3$-DMSO-d_6; (**b**) epimer 17β-amino in C_6D_6-DMSO-d_6; (**c**) epimer 17α-amino in $CDCl_3$-DMSO-d_6.

Estrone (3-hydroxy-1,3,5(10)-estratrien-17-one) and estradiol (3-hydroxy-1,3,5(10)-estratrien-17β-ol) were obtained from Syntex (Mexico City, Mexico). Reagents were purchased from Aldrich (Milwaukee, WI, USA). Reactions were monitored by thin-layer chromatography (TLC) with Merck precoated silica gel F254 plates, (0.25 mm thick) using hexane/ethyl acetate/methanol (5:4:1 v/v) and hexane-chloroform-methanol (5:4:1 v/v) as eluents. The spots were detected under ultraviolet light (254 nm and 366 nm) and revealed with iodine. Products were purified by flash chromatography (Merck, silica gel 60, 230–400 mesh ASTM or aluminum oxide). Proof of chemical purity was established by spectroscopy (IR, NMR, MS) and analytical techniques (TLC and chemical analysis). Elemental analyses were performed by Galbraith Laboratories Inc. Knoxville, TN, USA. Melting points were assessed on an Electrothermal 9100 capillary melting point apparatus, and are uncorrected. Optical rotation data were measured in methanol, ethanol or acetonitrile solution with a JASCO J-720 spectropolarimeter. IR spectra were obtained using a Nicolet FT-5SX or Perkin Elmer 283B spectrophotometer. ^{1}H NMR spectra were recorded on a Varian–Gemini 200 MHz and a Varian–Unity 300 MHz spectrometers, and are reported in ppm downfield from the internal standard tetramethylsilane. MS were obtained with a JEOL JMS-AX505HA mass spectrometer at 70 eV. The x-ray study was performed with a Siemens P4 diffractometer.

17β-Amino-1,3,5(10)-estratrien-3-ol (1)

Compound **1** was prepared by the reduction of estrone–oxime with sodium-propanol.[12–14] The resulting yellow pale solid was purified by TLC (silica-gel) using hexane-chloroform-methanol (85:4:1 v/v) and crystallized several

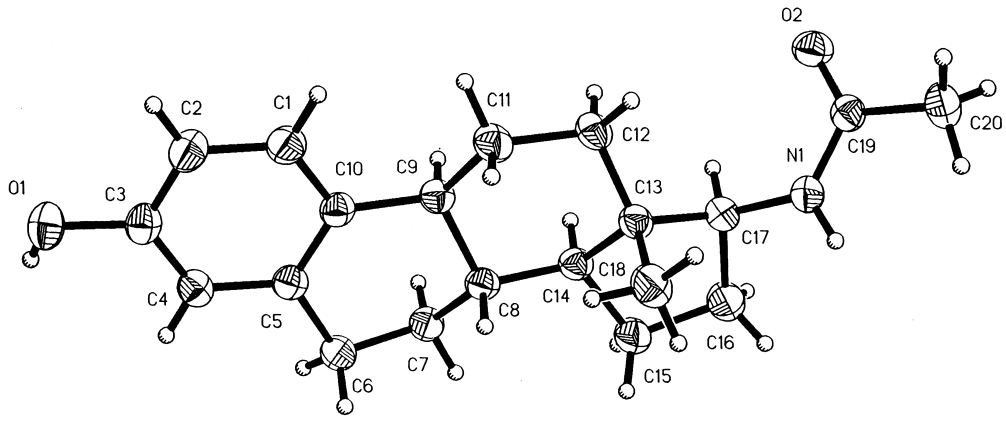


Figure 2 ORTEP-type plot of the **2** molecule. Ellipsoids are drawn at 30% probability level.

Table 1 Unit Cell, Data Collection, and Structure Solution Parameters

Compound	**2**	**3**	**6**	**8**
Molecular formula	$C_{20}H_{27}NO_2$	$C_{22}H_{29}NO_3$	$C_{25}H_{29}N_3O_3$ S	C_{25} H_{34} Cl N O_4 S
Mass	313.43	355.46	451.57	480.04
Space group	$P2_12_12_1$	$P2_1$	$P2_12_12_1$	$P2_12_12_1$
a (Å)	6.817(1)	7.0586(2)	6.372(1)	7.4871(3)
b (Å)	14.292(1)	30.830(1)	16.624(3)	7.610(1)
c (Å)	17.771(2)	9.3990(4)	21.770(2)	44.260(2)
β (°)	—	97.700(4)	—	—
V (Å^3)	1731.4(3)	2026.9(1)	2306.1(6)	2521.8(4)
Z	4	4	4	4
F(000)	680	768	960	1024
Radiation	CuKα	CuKα	CuKα	CuKα
μ (mm^{-1})	0.600	0.608	1.503	2.358
D (calc) (g cm^{-3})	1.202	1.165	1.301	1.264
Crystal dimensions (mm)	0.80, 0.24, 0.20	0.60, 0.46, 0.26	0.40, 0.14, 0.12	0.34, 0.26, 0.20
θ limits (°)	1.50–56.75	1.50–56.75	1.50–55.00	1.50–56.75
max. *h, k, l*	7, 15, 19 plus Friedel pairs	7, 33, 10 plus Friedel pairs	6, 17, 23	7, 8, 48 plus Friedel pairs
N measured	2319	5421	2888	3312
N observed [I $\geq 2\sigma$(I)]	2199	5052	2451	2945
Number of parameters	215	485	290	305
Max./min. residual Electron density (e/Å^3)	+0.12/−0.15	+0.50/−0.19	+0.21/−0.18	+0.22/−0.16
R	0.056	0.058	0.048	0.0458
wR	0.152	0.155	0.126	0.115
w[a]	w = 1/[σ^2(Fo^2) + (0.1026P)^2 + 0.3035P]	w = 1/[σ^2(Fo^2) + (0.0970P)^2 + 0.6211P]	w = 1/[σ^2(Fo^2) + (0.0721P)^2 + 0.6115P]	w = 1/[σ^2(Fo^2) + (0.0521P)^2 + 0.7501P]

[a] P = (Fo^2 + 2Fc^2)/3.

times from methanol to yield 0.302 g (1.10 mmol, 37%) of product **1** with m.p. 225–226° C, (lit. m.p. 200° C[12] 221–222.5° C[15] 222–224° C[16] 223–226° C[13] 231–233° C[1]). $[\alpha]_D^{25}$ = + 57.7° (MeOH), $[\alpha]_D^{30}$ = + 75.3° (EtOH), (lit. $[\alpha]_D^{22}$ = + 73.4° (EtOH)[1]. IR (KBr): 3426 and 3286 (N-H, O-H), 1611 (aromatic ring), cm^{-1}. ^{1}H NMR 300 MHz ($CDCl_3$-DMSO-d_6): δ 0.68 (s, 3H, 18-CH_3), 1.12–2.32 (m, 12H), 2.78 (m, 4H, 17-CH, benzylic protons), 6.56 (d, *J* = 2.4 Hz, 1H, H-4), 6.62 (dd, *J* = 8.1 Hz and 2.4 Hz, 1H, H-2), 7.14 (d, *J* = 8.1 Hz, 1H, H-1). ^{1}H NMR 300 MHz (C_6D_6-DMSO-d_6): δ 0.54 (s, 3H, 18-CH_3), 2.57 (t, *J* = 8.7 Hz 1H, 17-H), 2.79 (m, 3H, benzylic protons). MS *m/z* 271 (M$^+$, 95%), 254 (57.8%), 213 (50%), 56 (100%). Analysis calculated for: $C_{18}H_{25}NO$ (271.40) C, 79.66; H, 9.28; N, 5.16. Found: C, 79.68; H, 9.27; N, 5.27. Compound **1** was dissolved in methanol at room temperature and concentrated hydrochloric acid was added. The solution was evaporated to dryness and the residue was recrystallized from methanol/acetone. The hydrochloride decompound 286°C. $[\alpha]_D^{25}$ = + 80.2° (MeOH). IR (KBr): 3426–3062 (N-H, O-H), cm^{-1}. ^{1}H NMR 200 MHz ($CDCl_3$-DMSO-d_6): δ 0.83 (s, 3H, 18-CH_3), 1.18–2.38 (m, 12H), 2.75 (m, 3H, benzylic protons), 3.00 (br, 1H, 17α-H), 3.45 (br, 3H, $^+NH_3$), 6.49 (d, *J* = 2.5 Hz, 1H, H-4), 6.54 (dd, *J* = 8.3 Hz and 2.5 Hz, 1H, H-2), 7.00 (d, *J* = 8.3 Hz, 1H, H-1), 8.2 (br, 1H, OH).

17β-Acetylamino-1,3,5(10)-estratrien-3-ol (2)

Estrone–oxime (1.08 g, 3.78 mmol) was dissolved in 30 mL of acetic anhydride and shaken in the presence of 10% Pd/C and hydrogen at room temperature for 3 days. The catalyst was filtered off, washed with methanol and the solvent evaporated under reduced pressure. The solid residue was crystallized from methanol to give 0.452 g (1.44 mmol, 38% yield) of product **2**, with m.p. 266–268°C (lit. m.p. 264–265° C[17]). $[\alpha]_D^{25}$ = −37.2° (MeOH) (lit. $[\alpha]_D^{20}$ = + 6.5° (DMF)[17]. IR (KBr): 3399 and 3309 (N—H, O—H), 1625 (CO), cm^{-1}. ^{1}H NMR 200 MHz ($CDCl_3$): δ 0.74 (s, 3H, 18-CH_3), 1.24–2.62 (m, 10H), 1.95 (s, 3H, OC-CH_3), 2.75 (m, 5H, 16-H and benzylic protons), 3.87 (t, *J* = 9.4 Hz, 1H, 17α-H), 5.93 (d, *J* = 8.6 Hz, NH), 6.46 (d, *J* = 2.4 Hz, 1H, H-4), 6.52 (dd, *J* = 8.4 Hz and 2.4 Hz, 1H, H-2), 7.06 (d, *J* = 8.4 Hz, 1H, H-1), 8.2 (br, 1H, OH exchangeable with D_2O, -OH). MS *m/z* 313 (M$^+$, 100%), 254 (19%), 213 (75%), 160 (22%). Analysis calculated for: $C_{20}H_{27}NO_2$ (313.43) C, 76.64; H, 8.68; N, 4.47. Found: C, 76.60; H, 8.67; N, 4.37.

3-Acetoxy-17β-acetylamino-1,3,5(10)-estratriene (3)

A mixture of 1.5 g (5.26 mmol) of estrone–oxime was hydrogenated under the conditions described previously, maintaining the reaction for 5 days. The crude product **3** was crystallized from methanol/acetone to give 0.510 g (1.44 mmol, 27% yield), m.p. 225–227°C (lit. m.p. 220–222° C[17]). $[\alpha]_D^{25}$ = −26.4° (MeOH), $[\alpha]_D^{30}$ = −12.3° ($CHCl_3$), (lit: $[\alpha]_D^{21}$ = −10° ($CHCl_3$), $[\alpha]_D^{19}$ = + 4.7° (DMF)[17]. IR (KBr): 3393–3259 (N-H), 1760 (O-CO), 1643 (HN-CO) cm^{-1}. ^{1}H NMR 200 MHz ($CDCl_3$-DMSO-d_6): δ 0.71 (s, 3H, 18-CH_3), 1.28–2.40 (m, 12H), 2.0 (s, 3H, $NCOCH_3$), 2.28 (s, 3H, $OCOCH_3$), 2.85 (m, 3H, benzylic protons), 4.0 (dd, *J* = 9.2 Hz and 7.2 Hz, 1H, 17α-H), 5.38

Table 2 Atomic Coordinates ($\times 10^3$) and Equivalent Isotropic Thermal Parameters ($\times 10^2$ Å^2)

Atom	x/a	y/b	z/c	Ueq
Compound **2**				
O1	22123(4)	3289(2)	3650(1)	63(1)
O2	8452(4)	6532(2)	838(1)	60(1)
N1	9919(5)	5602(2)	−1(2)	60(1)
C1	18216(5)	4654(2)	2796(2)	56(1)
C2	19900(6)	4427(2)	3190(2)	59(1)
C3	20496(5)	3502(2)	3223(2)	51(1)
C4	19469(5)	2835(2)	2825(2)	49(1)
C5	17786(5)	3065(2)	2415(2)	46(1)
C6	16766(5)	2312(2)	1971(2)	53(1)
C7	14645(5)	2552(2)	1779(2)	51(1)
C8	14545(5)	3535(2)	1441(2)	45(1)
C9	15147(5)	4241(2)	2051(2)	45(1)
C10	17100(5)	3992(2)	2417(2)	46(1)
C11	15040(6)	5255(2)	1769(2)	55(1)
C12	13057(5)	5491(2)	1408(2)	52(1)
C13	12544(5)	4800(2)	792(2)	47(1)
C14	12550(5)	3805(2)	1137(2)	47(1)
C15	11544(6)	3207(2)	529(2)	67(1)
C16	10045(7)	3870(3)	161(3)	75(1)
C17	10394(5)	4840(2)	516(2)	54(1)
C18	13953(6)	4901(3)	125(2)	67(1)
C19	8977(4)	6380(2)	186(2)	47(1)
C20	8562(6)	7054(3)	−437(2)	64(1)
Compound **3**				
O1A	1531(4)	9554(1)	7841(3)	77(1)
O2A	4641(6)	9538(1)	8674(6)	123(2)
O3A	−216(5)	5943(1)	9453(3)	86(1)
N1A	−590(5)	6210(1)	11621(4)	59(1)
C1A	1678(6)	8366(1)	8221(4)	69(1)
C2A	2192(6)	8777(2)	7840(5)	72(1)
C3A	1184(6)	9125(1)	8287(4)	67(1)
C4A	−282(6)	9062(1)	9083(4)	63(1)
C5A	−800(5)	8647(1)	9457(4)	60(1)
C6A	−2451(6)	8592(1)	10334(5)	72(1)
C7A	−3196(6)	8130(1)	10336(5)	72(1)
C8A	−1560(5)	7805(1)	10616(4)	56(1)
C9A	−355(5)	7827(1)	9381(4)	57(1)
C10A	218(5)	8289(1)	9036(4)	58(1)
C11A	1355(6)	7515(1)	9634(6)	79(1)
C12A	704(7)	7046(1)	9836(5)	72(1)
C13A	−559(5)	7013(1)	11025(4)	54(1)
C14A	−2194(5)	7338(1)	10735(4)	58(1)
C15A	−3521(7)	7206(2)	11842(6)	84(1)
C16A	−3313(7)	6709(2)	11957(6)	81(1)
C17A	−1699(5)	6590(1)	11073(4)	58(1)
C18A	675(7)	7084(2)	12483(5)	78(1)
C19A	3288(7)	9725(2)	8046(5)	74(1)
C20A	3310(8)	10166(2)	7429(6)	93(1)
C21A	117(6)	5927(1)	10781(4)	64(1)
C22A	1352(8)	5575(2)	11519(5)	85(1)
O1B	5010(9)	2401(2)	14920(5)	130(2)
O2B	5509(25)	2470(3)	12792(12)	188(6)
O2B′	7062(41)	2513(4)	14068(18)	255(13)
O3B	804(5)	6002(1)	14561(3)	91(1)
N1B	1422(5)	5817(1)	16891(3)	58(1)
C1B	2981(10)	3499(2)	14535(6)	123(3)
C2B	3259(12)	3063(2)	14366(8)	144(3)
C3B	4873(10)	2872(2)	15024(6)	110(2)
C4B	6183(8)	3115(2)	15887(6)	85(1)
C5B	5927(6)	3553(1)	16098(5)	72(1)
C6B	7373(6)	3798(2)	17132(7)	97(2)
C7B	7232(6)	4286(2)	17013(7)	88(2)
C8B	5154(5)	4432(1)	16877(4)	56(1)
C9B	4038(6)	4246(1)	15496(4)	63(1)
C10B	4302(6)	3758(1)	15385(4)	69(1)
C11B	1943(6)	4388(2)	15269(5)	77(1)
C12B	1729(6)	4882(1)	15349(4)	72(1)
C13B	2820(5)	5067(1)	16710(3)	49(1)
C14B	4917(5)	4923(1)	16842(4)	59(1)
C15B	5925(6)	5200(2)	18081(6)	82(1)
C16B	4734(6)	5626(1)	18003(5)	72(1)
C17B	3092(5)	5561(1)	16759(4)	57(1)
C18B	1895(5)	4922(1)	18019(4)	61(1)
C19B	5629(15)	2235(2)	13921(7)	134(3)
C20B	6073(10)	1793(2)	13817(8)	113(2)
C21B	379(6)	6011(1)	15788(4)	62(1)
C22B	−1346(8)	6252(2)	16114(5)	83(1)
Compound **6**				
S1	1304(2)	5463(1)	5562(1)	54(1)
O1	2074(6)	4732(2)	5152(1)	60(1)
O2	−35(8)	5171(3)	6033(2)	83(1)
O3	3086(8)	5922(2)	5733(2)	87(1)
N1	7220(7)	−426(3)	7360(2)	64(1)
N2	7923(8)	−1039(3)	7583(2)	66(1)
N3	8731(11)	−1617(3)	7749(3)	107(2)
C1	5482(8)	3349(3)	6026(2)	47(1)
C2	4762(10)	4042(3)	5736(2)	51(1)
C3	2866(8)	4042(3)	5466(2)	46(1)
C4	1599(8)	3371(3)	5463(2)	46(1)
C5	2322(8)	2665(3)	5745(2)	43(1)
C6	848(8)	1943(3)	5718(2)	51(1)
C7	1880(8)	1175(3)	5947(2)	47(1)
C8	3097(7)	1348(2)	6534(2)	37(1)
C9	4983(7)	1899(3)	6379(2)	39(1)
C10	4220(7)	2655(2)	6040(2)	37(1)
C11	6308(8)	2081(3)	6951(2)	44(1)
C12	6973(8)	1310(3)	7293(2)	48(1)
C13	5086(8)	803(3)	7458(2)	42(1)
C14	3898(7)	603(2)	6863(2)	39(1)
C15	2287(9)	−43(3)	7057(3)	57(1)
C16	3399(8)	−497(3)	7593(2)	56(1)
C17	5516(8)	−45(3)	7711(2)	52(1)
C18	3720(9)	1245(3)	7936(2)	57(1)
C21	−160(8)	5976(3)	5009(2)	46(1)
C22	−2141(10)	6237(3)	5162(2)	63(2)
C23	−3326(10)	6676(3)	4731(3)	67(2)
C24	−2527(11)	6839(3)	4157(3)	62(2)
C25	−487(11)	6581(4)	4020(3)	72(2)
C26	708(9)	6153(3)	4436(2)	63(2)
C27	−3830(12)	7281(4)	3693(3)	84(2)
Compound **8**				
C11	7530(1)	4065(1)	7735(1)	60(1)
S1	−3471(2)	1059(2)	5707(1)	77(1)
O1	−1406(5)	539(4)	5733(1)	75(1)
O2	−4061(5)	1789(6)	5984(1)	89(1)
O3	−4268(7)	−496(6)	5589(1)	118(2)
O4	8613(6)	10077(6)	7539(1)	99(1)
N1	6490(5)	7372(5)	7309(1)	51(1)
C1	1303(5)	1599(5)	6395(1)	52(1)
C2	193(5)	721(6)	6199(1)	58(1)
C3	−300(6)	1518(6)	5934(1)	58(1)
C4	305(6)	3155(6)	5860(1)	60(1)
C5	1403(5)	4070(5)	6058(1)	49(1)
C6	1985(6)	5911(6)	5972(1)	64(1)
C7	2907(6)	6910(6)	6223(1)	68(1)
C8	4168(5)	5733(5)	6398(1)	45(1)
C9	3079(5)	4295(5)	6556(1)	44(1)
C10	1921(5)	3289(5)	6331(1)	46(1)
C11	4244(6)	3107(5)	6754(1)	56(1)
C12	5350(6)	4170(5)	6984(1)	55(1)
C13	6471(5)	5565(5)	6825(1)	47(1)
C14	5212(5)	6728(5)	6638(1)	48(1)
C15	6423(8)	8256(7)	6541(1)	79(2)
C16	7562(8)	8606(7)	6821(1)	81(2)
C17	7445(6)	6947(5)	7021(1)	54(1)
C18	7943(6)	4674(8)	6635(1)	79(2)
C19	−3465(6)	2663(7)	5421(1)	66(1)

Table 2 (Continued)

Atom	x/a	y/b	z/c	Ueq
C20	−3795(8)	4371(8)	5498(1)	90(2)
C21	−3791(9)	5647(9)	5278(1)	100(2)
C22	−3444(8)	5239(9)	4984(1)	91(2)
C23	−3090(8)	3542(10)	4913(1)	98(2)
C24	−3091(8)	2223(8)	5129(1)	85(2)
C25	−3463(11)	6645(11)	4743(2)	135(3)

(d, J = 9.2 Hz, 1H, H-N), 6.78 (d, J = 2.5 Hz 1H, H-4,), 6.83 (dd, J = 2.5 y 8.2 Hz 1H, H-2), 7.28 (d, J = 8.2 Hz 1H, H-1). MS *m/z* 355 (M^+, 16%), 313 (100%), 254 (20%), 213 (60%), 160 (16%). Analysis calculated for: $C_{22}H_{29}NO_3$ (355.46) C, 74.33; H, 8.22; N, 3.94. Found: C, 74.36; H, 8.17; N, 3.90.

3,17β-bis(toluene-4-sulfonyloxy)-1,3,5(10)-estratriene(5)

This compound was prepared from estradiol (**4**) using reported procedures[19–21] and was obtained with 87% yield, as pale pinkish white needles with m.p. 155–157°C. $[\alpha]_D^{25}$ = + 26.9° (CH_3CN). IR (KBr): 1372 and 1178 (SO_3), cm^{-1}. ^1H NMR 200 MHz ($CDCl_3$): δ 0.80 (s, 3H, 18-CH_3), 1.01–2.23 (m, 12 H), 2.43 (s, 6H, 2 CH_3-OTs), 2.74 (m, 3H, benzylic protons), 4.33 (d,d J = 9.0 Hz and J = 7.7 Hz, 1H, 17α-H), 6.63 (dd, J = 8.8 y 2.5 Hz, 1H, H-2), 6.72 (d, J = 2.5 Hz, 1H, H-4), 7.14 (d, J = 8.8 Hz, 1H, H-1), 7.29 (d, J = 8.1 Hz, 2H, 17-OTs), 7.31 (d, J = 8.1 Hz, 2H, 17-OTs), 7.71 (d, J = 8.5 Hz, 2H, 3-OTs), 7.78 (d, J = 8.5 Hz, 2H, 3-OTs,). MS *m/z* 580 (M^+ 100%), 408 (99%), 253 (100%), 91 (95%). Analysis calculated for: $C_{32}H_{36}O_6S_2$ (580.75): C, 66.18; H, 6.25. Found: C, 66.15; H, 6.27.

3-(Toluene-4-sulfonyloxy)-17α-azido-1,3,5(10)-estratriene(6)

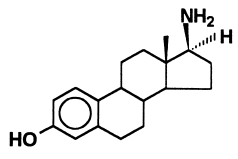
To a solution of 5 g (8.61 mmol) of compound **5** in 45 mL of hexamethylphosphoramide (HMPA), 6 g (92.29 mmol) of sodium azide was added and the mixture heated between 60–70 C for 5 days. The reaction mixture was poured on ice, the precipitate filtered and dissolved in ethyl acetate, washed with water, dried and the solvent removed under reduced pressure below 70 C. The brown oil obtained was purified by column chromatography (silica gel) using a mixture 95:5 (v/v) of hexane/ethyl acetate as eluent, obtaining 2.721 g (6.03 mmol, 70% yield) of compound **6**. Crystallization from ethyl acetate-methanol produced colorless hexagonal prisms, m.p. 94–96°C. $[\alpha]_D^{25}$ = + 22.2° (MeOH). IR (film $CHCl_3$): 2098 (N_3), 1187 and 1374 (-SO_3) cm^{-1}. ^1H NMR 200 MHz ($CDCl_3$): δ 0.76 (s, 3H, 18-CH_3), 1.20–2.35 (m, 12H), 2.44 (s, 3H, C3-OTs), 2.77 (m, 3H, benzylic protons), 3.56 (d, J = 6.2 Hz, 1H, 17β-H), 6.64 (dd, J = 8.4 y 2.5 Hz, 1H, H-2), 6.73 (d, J = 2.5 Hz, 1H, H-4), 7.14 (d, J = 8.4 Hz, 1H, H-1), 7.30 (d, J = 8.0 Hz, 2H, 3-OTs), 7.72 (d, J = 8.0 Hz 2H, 3-OTs). MS *m/z* 451 (M^+, 86.6%), 423 (23%), 268 (100%), 91 (34%). Analysis calculated for: $C_{25}H_{29}N_3O_3S$ (451.57): C, 66.49; H, 6.47 N, 9.31. Found: C, 66.51; H, 6.46; N, 9.32.

17α-Amino-1,3,5(10)-estratrien-3-ol (7)

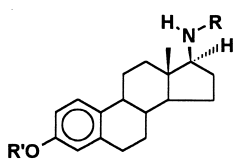
To a solution of 0.500 g (1.11 mmol) of compound **6** in 10 mL of anhydrous tetrahydrofuran, 1 g of lithium aluminum hydride was added and the reaction mixture stirred for 24 h at room temperature. After this time, water was added carefully, as well as a10% solution of hydrochloric acid to reach a pH of 1. The reaction mixture was extracted with ethyl ether, and the aqueous phase was made alkaline by adding a 10% solution of sodium hydroxide. After extraction with dichloromethane, the organic layer was dried and evaporated under reduced pressure. The oily residue was crystallized from methanol to yield 0.057 g (0.21 mmol, 17%) of product **7**, 205 C (decomp) m.p. 226–227°C. $[\alpha]_D^{25}$ = + 50.9° (MeOH). IR (KBr): 3348 and 3283 (O-H, N-H) cm^{-1}.[1] H NMR, 300 MHz ($CDCl_3$-DMSO-d_6): δ 0.75 (s, 3H, 18-CH_3), 1.25–2.39 (m, 12H), 2.79 (m, 3H, benzylic protons), 3.05 (d, J = 7.2 Hz, 1H, 17β-H), 6.57 (d, J = 2.4 Hz, 1H, H-4), 6.64 (dd, J = 8.1, J = 2.4 Hz, 1H, H-2), 7.10 (d, J = 8.1 Hz, 1H, H-1). MS *m/z* 271 (M^+52.5%), 254 (35%), 213 (33%), 56 (100%). Analysis calculated for: $C_{18}H_{25}NO$ (271.40): C, 79.66; H, 9.28, N, 5.16. Found: C, 79.68; H, 9.31; N, 5.20.

3-(Toluene-4-sulfonyloxy)-17α-amino-1,3,5(10)-estratriene hydrochloride (8)

A solution of 0.740 g (1.64 mmol) of compound **6** in methanol was added to a mixture of 0.24 g of 10% Pd/C in 30 mL of methanol. The mixture was hydrogenated for 2 h, filtered over celite, washed with ethyl acetate and methanol. The solvents were evaporated under reduced pressure, and the solid obtained was crystallized from methanol to give 0.290 g (0.63 mmol yield 39%) of crystals 170–174°C (decomp). $[\alpha]_D^{25}$ = + 35.4° (MeOH). IR (film): 3393 (N-H), 1372 and 1183 (SO_3) cm^{-1}. ^1H NMR 200 MHz ($CDCl_3$): δ 0.81 (s, 3H, 18-CH_3), 1.2–2.38 (m, 12H), 2.42 (s, 3H, 3-OTs), 2.70 (m, 3H, benzylic protons), 3.33 (br, when exchanged with D_2O d, J = 6.2 Hz, 1H, 17β-H), 6.58 (dd, J = 8.5 Hz and J = 2.5 Hz, 1H, H-2), 6.72 (d, J = 2.5 Hz, 1H, H-4), 7.0 (d, J = 8.5 Hz, 1H, H-1), 7.30 (d, J = 8.2 Hz, 2H, 3-OTs), 7.71 (d, J = 8.2 Hz, 2H, 3-OTs), 8.32 (br, 3H, NH_2.HCl). MS *m/z* 425 (M^+ 65%), 408 (15%), 270 (38.6%), 253 (43.3%), 56 (100%). Analysis calculated for: $C_{25}H_{32}O_3NSCl$ (462.0): C, 64.98; H, 6.98; N, 3.03. Found: C, 65.16; H, 6.77; N, 3.06.



1



2 R = $COCH_3$, R' = H

3 R = R' = $COCH_3$

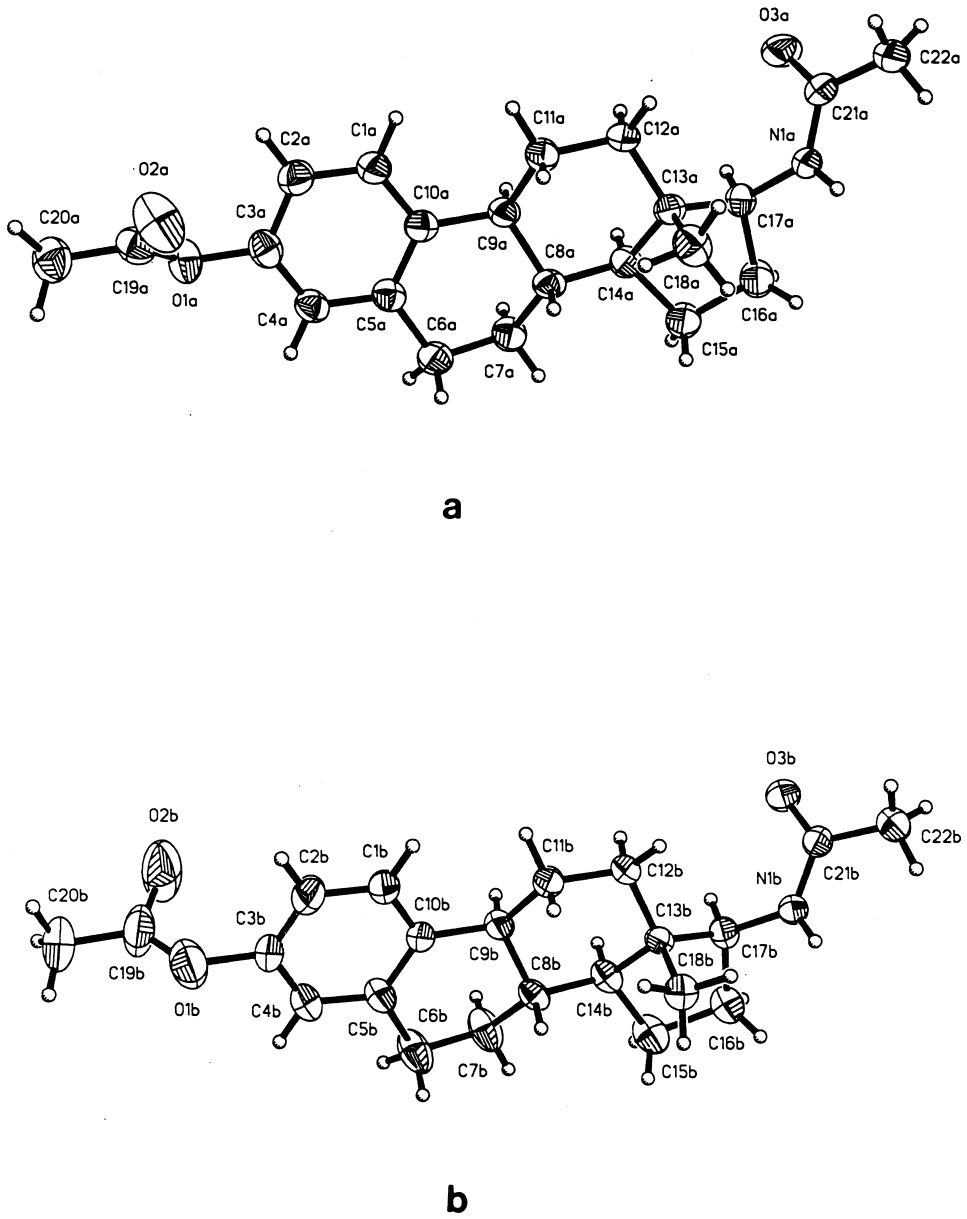


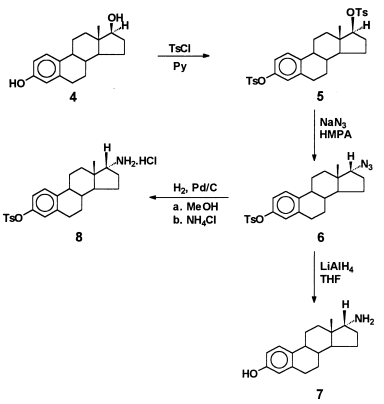
Figure 3 ORTEP-type plot of the **3** molecule. Ellipsoids are drawn at 30% probability level (top molecule **a**, bottom molecule **b**).

Results and discussion

17β-Amino-1,3,5(10)-estratrien-3-ol (**1**, Scheme 1) had been prepared previously by reduction of estrone–hydrazone in the presence of Ni/Ra[17] and by sodium reduction of estrone–oxime in n-propanol.[1,2,13,16] In our hands, sodium reduction of estrone–oxime gave, after recrystallization of the crude product, an 8:2 mixture (calculated from its ^{1}H NMR spectrum) of epimers **1** and **7**. From this mixture, two signals for the C-18 methyl group were observed: one at 0.68 ppm assigned to the 17β-amino epimer and the second at 0.75 ppm assigned to the 17α-amino epimer. The proton attached to C-17 in the 17β-amino appears as a multiplet centered at 2.78 ppm, whereas the corresponding proton in the 17α-amino is shifted to 3.05 ppm as a doublet signal. The mixture was purified by TLC and, after several recrystallizations from methanol, we were able to obtain compound **1** free from the epimer **7**.

Estrone–oxime, under catalytic hydrogenation over Pd/C in methanol-acetic anhydride medium, produced the 17β acetylamine **2**. Under prolonged reaction time 3-acetoxy-17β-acetylamine **3** was obtained. The ^{1}H NMR spectra of compound **2** showed the C-18 methyl group at 0.74 ppm and a triplet signal at 3.87 ppm assigned to the 17α-H. Compound **3** gave the C-18 methyl signal at 0.71 and a quartet (d,d) centered at 4.00 ppm corresponding to the 17α-H. These compounds were purified by recrystallization, obtaining the pure compounds **2**, **3** suitable for x-ray analysis.

Synthesis of 17α-amino-1,3,5(10)-estratrien-3-ol (**7**) was performed as outlined in Scheme 2. The di-tosylate **5**, prepared from estradiol in the usual fashion,[19–21] was



treated with sodium azide in hexamethylphosphoramide (HMPA) under reflux. Since the nucleophilic displacement of tosylate should follow an SN_2 mechanism, the azide ion attacks from the opposite side of the leaving tosylate group affording 17α-azide **6**. The stereochemistry of **6** was confirmed by x-ray analysis. The 17α-azide **6** was reduced to the corresponding amine **7** by treatment with $LiAlH_4$ in THF yielding 17%. When the reduction reaction was carried out by catalytic hydrogenation (Pd/C in methanol) the amine **8** was obtained with 39% yield and identified as its hydrochloride. The 17β-H of this compound appears as a doublet signal at 3.33 ppm. Crystals suitable for x-ray diffraction analysis of compound **6** and **8** were obtained by slow crystallization in the appropriate solvent (see Experimental).

Comparison of the pure epimers **1** and **7** by their infrared spectra, TLC behavior (ethyl acetate-chloroform-methanol 8:9:1 v/v), and HPLC gave nearly identical results. Proton magnetic resonance spectrum displacements of the individual 17β-amino and 17α-amino epimers exhibited C-18 angular methyl group signals as sharp singlets at 0.68 and 0.75 ppm, respectively. It has been reported that, in general, for the 17-substituted steroids, the 17α-H (quasi-axial) is found

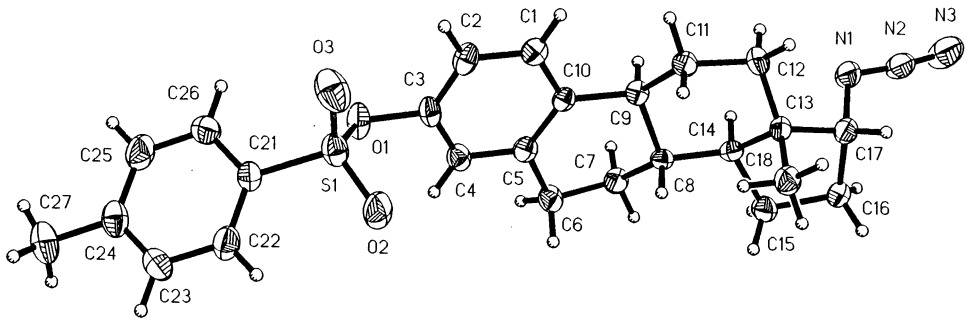


Figure 4 ORTEP-type plot of the **6** molecule. Ellipsoids are drawn at 30% probability level.

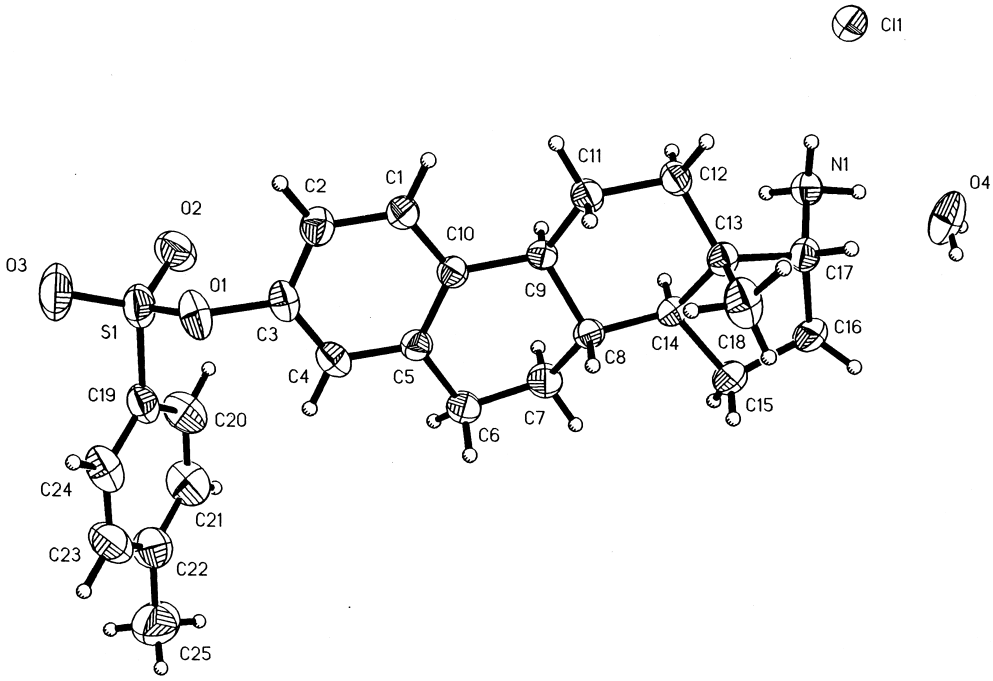


Figure 5 ORTEP-type plot of the **8** molecule. Ellipsoids are drawn at 30% probability level.

Table 3 Ring Conformations and the Puckering Parameters

		Molecule A	Molecule B		
	2	3		**6**	8
B-Ring	7α, 8β-half-chair (ϕ = 154.1°, Q = 0.374, θ = 44.3°)	7α, 8β-half-chair (ϕ = 155.3°, Q = 0.337, θ = 48.0°)	7α 8β-half-chair (ϕ = 161.2°, Q = 0.345, θ = 45.3°)	7α, 8β-half-chair (ϕ = 160.4°, Q = 0.341, θ = 51.1°)	intermediate between 7α, 8β-half-chair and 8β-envelope (ϕ = 166.0°, Q = 0.329, θ = 50.5°)
C-Ring	chair (ϕ = 276.4°, Q = 0.577, θ = 6.3°)	chair (ϕ = 5.1°, Q = 0.578, θ = 3.2°)	chair (ϕ = 305.2°, Q = 0.565, θ = 3.2°)	chair (ϕ = 268.9°, Q = 0.582, θ = 4.8°)	chair (ϕ = 267.4°, Q = 0.579, θ = 3.2°)
D-Ring	13β-envelope (ϕ = 186.6°, Q = 0.470)	13β-envelope (ϕ = 188.9°, Q = 0.471)	13β-envelope (ϕ = 181.6°, Q = 0.471)	13β-envelope (ϕ = 185.3°, Q = 0.453)	intermediate between 13β-envelope and 13β, 14α-half chair (ϕ = 204.6°, Q = 0.453)

at a higher field as a triplet signal, whereas the one corresponding to 17β-H (quasi-equatorial) appeared as a doublet.[22] In our spectrum the 17α-H of the 17β-amino epimer **1** could not be observed, since it appeared together with the benzylic protons as a multiplet centered at 2.78 ppm (Figure 1**a**). Other authors have described this proton as a triplet centered at 2.60 ppm[16] without mentioning the corresponding signals for the benzylic protons. However, in the ^{1}H NMR spectrum of the hydrochloride of **1,** the 17α-H could be observed as a broad signal shifted at 3.00 ppm separated from the benzylic protons. When the spectrum of **1** was determined in C_6D_6, part of the signal was shifted to a higher field; and the 17α-H could be observed as a triplet at 2.57 ppm J = 8.7 Hz (Figure 1**b**). For the 17α-amino epimer **7,** 17β-H appeared as a well-defined doublet centered a 3.05 ppm with a coupling constant of 7.2 Hz due to the interaction with a proton in C-16 (Figure 1**c**). To confirm the stereochemistry of the 17α-amino epimer **7,** a NOE DIF experiment was performed. The doublet signal centered at 3.05 ppm was irradiated and the signal corresponding to the methyl group at 0.75 ppm was observed.

Crystallographic studies

X-ray experiments were performed using a Siemens P4/PC four-circle diffractometer equipped with graphite monochromatized CuKα radiation; details are summarized in Table 1. All the structures were solved by direct methods (SIR92)[23] and refined with all non-hydrogen atoms having anisotropic temperature factors (SHELXL93).[24] Hydrogen atoms attached to carbon atoms were put at geometrical positions and forced to ride on their carbons, hydrogen atoms attached to a heteroatom were located on a difference Fourier map and their positional parameters refined; in both conditions an isotropic temperature factor 1.2 times the Ueq of the parent atoms was assumed.

The final coordinates and equivalent isotropic parameters for the non-H atoms of **2**, **3**, **6**, and **8** are reported on Table 2.[25] The molecular conformations and atomic labeling schemes for **2**, **3**, **6**, and **8** are shown in Figures 2–5, respectively, which also depict the spatial arrangement of the substituents at the chiral center C-17.

Compound **3** crystallized with two chemically similar but crystallographically different molecules (labeled **a** and **b**) forming "dimers" connected by hydrogen bonds between the amide groups. Compound **8** crystallized as the amine hydrochloride monohydrate, with nitrogen in the ammonium form. All bond lengths and bond angles of the four compounds correlate well with the average values observed for 1,3,5(10)-estratriene.[26] Amide resonance in compounds **2** and **3** (**a** and **b**) is evident from the values (1.326(4), 1.321(5), and 1.333(5) Å) of the lengths of N—C(═O) bonds and a planar hybridization of nitrogen atom (the sums

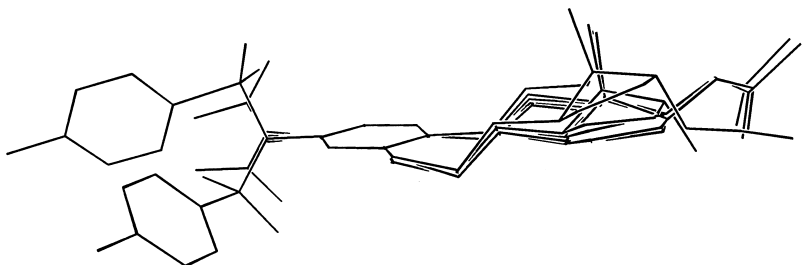


Figure 6 Least-square fit of the A rings of crystal structures determined in this work.

of the bond angles around N are 359.9, 359.9 and 359.3° for **2** and **3** (**a** and **b**), respectively. The *p*-tosyl group in **6** adopts a fully extended conformation (dihedral angle C3-O1-S1-C21: −163.3(4)°), whereas the same group in compound **8** is twisted toward the C4 carbon atom (dihedral angle C3-O1-S1-C21: 88.8(4)°). In both conditions, the plane of the phenyl group in this moiety bisects the angle subtended by O1-S1-O3.

Table 3 presents the conformations and the puckering parameters[27] for rings B, C, and D of each molecule, they are similar but not identical as can be seen in Figure 6. An 8β, 9α-half-chair conformation has been found for ring B of all molecules studied, although an increasing flattening at C7 is observed going from compound **2** to compound **8**, so the latter can be described as intermediate between half-chair and sofa conformations. The distorted chair conformation of ring C found in all four compounds presents a different direction of the distortion (phase angle ϕ, 5.1 and 305.2°) for both molecules of compound **3**. Four of the five molecules, determined in this work, show the 13β-envelope conformation of ring D, whereas the steroid molecule in compound **8** is intermediate between the 13β-envelope and the 13β, 14α-half-chair conformations.

The data presented for ^{1}H NMR spectra of compounds **1** and **7** and the determination of the crystal structure of compounds **2, 3, 6,** and **8** enabled us to characterize the structure of the 17α and 17β aminoepimers **1** and **7** for the first time. Pharmacological evaluation of the compounds synthesized here is in progress.

Acknowledgments

This work was supported by grants IN205396 from DGAPA-UNAM and 2400PM from CONACYT, Mexico. We are indebted to R. Patiño, L. Velasco, J. Pérez-Flores, R. Gaviño, S. Hernández and B. Quiroz for obtaining the IR, RMN, MS spectra, and x-ray analysis.

References

1. Ye Y, Huang Y, Wang Z, Chen S, Tian Y (1993). Synthesis of new amino acid and peptide derivatives of estradiol and their binding affinities for the estrogen receptor. *Steroids* **58**:35–39.
2. Kaspar P, Witzel H (1985). Steroid binding to the cytosolic estrogen receptor from rat uterus. Influence of the orientation of substituents in the 17-position of the 8β- and 8α-series. *J Steroid Biochem* **23**:259–265.
3. Blickenstaff RT, Foster E, Gerzon K, Young P (1985). Synthesis of some analogs of estradiol. *Steroids* **46**:889–902.
4. Vincze I, Hackler L, Szendi Z, Schneider G (1996). Steroids 54. Amino acylamidosteroids. *Steroids* **61**:697–702.
5. Rubio–Póo C, Mandoki JJ, Jayme V, Mendoza–Patiño N, Alvarado C, Silva G, Zavala E, Fernández–González JM, Rubio–Arroyo M (1983). Prodiame: a new estrogen with sustained anticoagulant effect. *Proc West Pharmacol Soc* **26**:111–113.
6. Rubio-Póo C, Mandoki JJ, Mendoza–Patiño N, Lemini C, de la Peña A, Cruz F, Zavala E, Silva G, García–Mondragón J, Fernández-G JM, Soriano–García M, Toscano AR (1985). The anticoagulant effect of prolame, *N*-(3-hydroxy-1,3,5(10)estratrien-17β-yl)-3-hydroxypropyl amine, a novel amino-estrogen. *Steroids* **45**:159–170.
7. Rubio–Póo C, Lemini C, García–Mondragón J, de la Peña A, Jayme V, Mendoza–Patiño N, Zavala E, Silva G, Blickenstaff RT, Fernandez–G JM, Mandoki JJ (1990). The anticoagulant effect of hexolame, *N*-(3-hydroxy-1,3,5(10)-estratrien-17β-yl)-6-hydroxyhexylamine, another amino-estrogen with prolonged anticoagulant effect. *Steroids* **55**:83–86.
8. Mandoki JJ, Rubio–Póo C, Lemini C, De la Peña A, Fernández-G JM, García–Mondragón J, Zavala E, Silva G, Mendoza–Patiño N (1991). The effects of five new 17β-amino-estrogens, buame, endiame, etolame, picae, and proacame on blood clotting time. *Proc West Pharmacol Soc* **34**:99–106.
9. Lemini C, Rubio–Póo C, Silva G, García–Mondragón J, Zavala E, Mendoza–Patiño N, Castro D, Cruz–Almanza R, Mandoki JJ (1993). Anticoagulant and estrogenic effects of two new 17β-aminoestrogens, butolame [17β-(4-hydroxy-1-butylamino)-1,3,5(10)-estratrien-3-ol] and pentolame [17β-(5-hydroxy-1-pentylamino)-1,3,5(10)-estratrien-3-ol]. *Steroids* **58**:457–461.
10. Rubio–Póo C, Lemini C, Silva G, Chávez–Lara B, Mendoza–Patiño N, Ortíz R, Mandoki JJ (1997). Effects of 17β-(*N*,*N*-diethylaminoethyl)amino-1,3,5(10)-estratrien-3-ol, and its androstane analog on blood clotting time. *Med Chem Res* **7**:67–75.
11. Mandoki JJ, Zavala E, Silva G, Mendoza–Patiño N, Rubio–Póo C, Medina–Martínez S, Domínguez–Escoto P (1983). The dual effects of estrogens on blood clotting time. *Proc West Pharmacol Soc* **26**:45–48.
12. Goodfriend L, Sehon AH, (1958). Preparation of an estrone-protein conjugate. *Can J Biochem Physiol* **35**:1177–1184.
13. Weeler OH, Reyes–Zamora C (1969). Steroid derivatives of cysteamine and cysteine. *Can J Chem* **47**:160–163.
14. Regan BM, Hayes N (1956). 17- And 17a-Aza-D-homosteroids *J Am Chem Soc* **78**:639–643.
15. Hecker E, Farthofer G (1963). Östrogene und antiöstrogene Wirkung einiger Östranabkömmlinge in vitro und in vivo. *Biochim Biophys Acta* **71**:196–198.
16. Chavis C, de Gourcy Ch, Borgna JL Imbach JL (1982). New steroidal nitrosoureas. *Steroids* **39**:129–147.
17. Hecker E, Walk E (1960). Zur Chemie der p-Chinole, III. o-Chinondiacetate und weitere Aminoanaloge der natürlichen Östrogene. *Chem Ber* **93**:2928–2937.
18. Robinson CH, Ermann C, Hollis DP (1965). The synthesis of 17α-amino-androsten-3β-ol, NMR spectra of 17-substituted androstanes. *Steroids* **6**:509–518.
19. Allais A, Hoffmann C (1958). US Patent 2,835,681. CA **52**:P 18531b.
20. Wang F, Chiba K, Tada M (1992). Facile deoxygenation of phenols and enols using sodium borohydride-nickel chloride. *J Chem Soc Perkin Trans* **1**: 897-1900.
21. Brunner H, Sperl G (1993). Synthese und antitumoraktivität von cis-dichloroplatin(II)-komplexen mit östradiolderivaten. *Monatsh Chem* **124**:83–102.
22. Szendi Z, Dombi G, Vincze I (1996). Steroids, LIII: New routes to aminosteroids. *Monatsh Chem* **127**:1189–1196.
23. Altomare A, Cascarano G, Giacovazzo C, Guagliardi A, Burla MC, Polidori G, Camalli M, (1994). SIR92 a program for automatic solution of crystal structures by direct methods. *J Appl Cryst* **27**:435.
24. Sheldrick GM (1993). SHELXL93 Program for Refinement of Crystal Structures. University of Göttingen, Germany.
25. Lists of anisotropic thermal parameters for non-H atoms, H-atoms parameters, bond distances and angles for all atoms have been deposited with the Editor.
26. Duax WL, Weeks CM, Rohrer DC (1976). Crystal structures of steroids. *Top Stereochem* **9**:271–383.
27. Cremer D, Pople JA (1975). A general definition of ring puckering coordinates. *J Am Chem Soc* **97**:1354–1358.