STEROID STRUCTURE AND FUNCTION VII. REMARKABLE ESTROGENICITY OF 3-HYDROXY-9β-ESTRA-1,3,5(10)-TRIENE-11,17-DIONE

Albert Segaloff, R. Bruce Gabbard, Albert Flores
Alton Ochsner Medical Foundation
New Orleans, Louisiana 70121

Ronald F. Borne, John K. Baker
University of Mississippi School of Pharmacy
University, Mississippi 38677

William L. Duax, Phyllis D. Strong and Douglas C. Rohrer
Medical Foundation of Buffalo, Inc.
73 High Street, Buffalo, New York 14203

Received: 1-31-80

ABSTRACT

Remarkably high estrogenic activity was observed for 3-hydroxy-9β-estra-1,3,5(10)-triene-11,17-dione despite its unusual bent conformation. The 9α epimer of this compound has markedly less activity despite the fact that its overall shape is nearly identical to that of estrone. The potency of these compounds in enhancing uterine weight in Fischer rats and reducing ovarian weight in parabiosed rats was compared with that of estrone, and the structures were unambiguously identified by X-ray crystallographic study. The results underscore the importance of the phenolic ring A to estrogenic activity, and suggest a tolerance of the putative estrogenic receptor to flexibility in overall molecular shape.

INTRODUCTION

As an approach to discovering agents with a favorable distribution of biological activities, we have been interested in the effects of altering the structural planarity of biologically active steroids (1-9).

There is a growing knowledge that substitution in the 11-position of various estrogens leads to compounds of widely differing activities. For one example, Ojasso and Raynaud have reported that 11α-methoxyethynylestradiol is an anti-estrogen while 11β-methoxyethynylestradiol

is an extremely potent estrogen (10).

Since we were unaware of any biological studies on 11-ketoestrones, we decided to synthesize and study them.

EXPERIMENTAL

Melting points were observed in a Köfler micro hot stage, and are uncorrected. Infrared (ir) spectra were from KBr pellets, and were obtained on a Perkin-Elmer Infrared Spectrophotometer Model 710B.

For the nmr spectral determinations, the compounds were dissolved in pyridine-d_5. Proton nmr spectra were obtained on a Jeolco C-60 H 1 Spectrometer, and signals are reported relative to tetramethylsilane (TMS) as internal standard. ^{13}C-nmr spectra were obtained on a JEOL FX-60 Fourier transform spectrometer, and the ^{13}C-nmr assignments are summarized in Table 1.

Table 1. ^{13}C-nmr Spectra of 11-Ketoestrone and 11-Keto-9β-estrone[a]

Position No.	11-Ketoestrone	11-Keto-9β-estrone
1	132.5	126.6
2	114.1	115.1
3	157.5	158.1
4	116.3	117.3
5	139.1	137.6
6	27.1	24.9
7	24.9	23.5
8	47.0	41.7
9	56.4	54.4
10	(b)	(b)
11	208.5	210.4
12	30.2	33.3
13	49.9	46.9
14	52.1	50.5
15	21.3	21.6
16	40.0	36.2
17	216.8	217.3
18	14.8	14.8

(a) Reported in ppm from tetramethylsilane (TMS).
(b) Obscured by solvent (pyridine-d_5) peaks.

Crystal data and refinement statistics are given in Table 2. Cell dimensions were determined from diffraction angles measured on an Enraf-Nonius CAD-4 diffractometer, and intensities for reflections with $\theta<75°$ were measured on the same instrument. The structures were solved by direct methods (11,12) and refined by full matrix least squares procdures. The positions of all non-hydrogen atoms were refined anisotropically. The hydrogen atoms were refined isotropically. The atomic coordinates are given in Table 3.

Table 2. Crystal Data and Refinement Statistics

	11-Ketoestrone	11-Keto-9β-estrone
Molecular Formula	$C_{18}H_{20}O_3$	$C_{18}H_{20}O_3$
Molecular Weight	284.3	284.3
Crystal System	Monoclinic	Orthorhombic
Space Group	$P2_1$	$P2_12_12_1$
a (Å)	9.7508(4)	10.8358(8)
b (Å)	11.1359(5)	12.546(1)
c (Å)	7.1072(3)	10.6532(9)
β (°)	110.530(3)	90.0
Volume (Å^3)	722.7	1447.3
Z	2	4
Density g/cm^3	1.307	1.304
Reliability indices		
observed data $I>2\sigma_I$	4.4% (1530)	4.5% (1587)
all data	4.5% (1562)	4.8% (1711)

3-Hydroxyestra-1,3,5(10)-triene-11,17-dione (11-ketoestrone). 11β-Hydroxyestrone (13), 1.77 g, was dissolved in 400 ml acetone, placed in an ice bath, and Kiliani's reagent (13.25 g CrO_3, 20 g H_2SO_4, and enough H_2O to make 100 ml) was added dropwise until the solution became pale brown in color. Ethanol (1 ml) was added to decompose excess CrO_3, 400 ml H_2O was added, and the mixture was extracted with 200 ml dichloromethane. The dichloromethane extract was dried with anhydrous Na_2SO_4, and was evaporated to dryness under N_2 on a steam bath. The residue was taken up in a minimal amount of dichloromethane, filtered through 20 g silica gel to remove colored impurities, and the elution was completed with approximately 200 ml dichloromethane. The eluate was evaporated to dryness under N_2 on a steam bath, and the residue was recrystallized from methanol containing a small amount of dichloromethane to afford 0.67 g (38%) of 11-ketoestrone, pale yellow cubes, mp 205-215°C. (Lit. (14) mp, 199-203°C).

nmr: δ 0.89 (s; 13β-CH_3); 3.70 (m, $W_{1/2}$ = 13 Hz; 9α-H)

ir: ν_{max} 3475 (phenolic OH), 1735 (17-ketone), 1700 (11-ketone), 1618, 1580, 1500 (benzene ring), and 1221 cm^{-1} (phenolic C-O)

3-Hydroxy-9β-estra-1,3,5(10)-triene-11,17-dione (11-keto-9β-estrone). 17-Ethylenedioxyestra-1,3,5(10)-triene-3,11β-diol (13), 3.5 g, was dissolved in 25 ml pyridine, 25 ml acetic anhydride was added, and the solution was allowed to stand for 30 min at room temperature. After dilution with H_2O, gummy material resulted, and this was separated from

Table 3a. Atomic coordinates for 3-hydroxy-1,3,5(10)-estratriene-11,17-dione (nonhydrogen $\times 10^4$; hydrogen $\times 10^3$; std. dev. in parentheses). The form of the anisotropic thermal parameters ($\times 10^4$) is $\exp[-2\pi^2(U_{11}h^2a^{*2} + 2U_{12}hka^*b^* + ,,,)]$. Isotropic thermal parameters are $\times 10$.

ATOM	X/A(σ)	Y/B(σ)	Z/C(σ)	U11(σ)	U22(σ)	U33(σ)	U12(σ)	U13(σ)	U23(σ)
C(1)	4120(2)	288(1)	4281(2)	41(1)	48(1)	42(1)	8(1)	0(1)	-5(1)
C(2)	3633(2)	609(2)	3137(2)	44(1)	51(1)	46(1)	9(1)	-5(1)	0(1)
C(3)	4205(2)	285(2)	2029(2)	41(1)	53(1)	44(1)	-4(1)	-4(1)	4(1)
C(4)	5267(2)	-333(2)	2080(2)	43(1)	52(1)	37(1)	-4(1)	3(1)	1(1)
C(5)	5756(1)	-657(1)	3233(1)	36(1)	41(1)	39(1)	-2(1)	4(1)	0(1)
C(6)	6917(2)	-1335(2)	3226(2)	44(1)	55(1)	40(1)	8(1)	9(1)	1(1)
C(7)	7586(2)	-1315(2)	4481(2)	36(1)	60(1)	43(1)	8(1)	7(1)	3(1)
C(8)	6684(1)	-1570(1)	5533(1)	35(1)	37(1)	37(1)	3(1)	3(1)	-1(1)
C(9)	5709(1)	-658(1)	5604(1)	35(1)	39(1)	33(1)	4(1)	1(1)	-3(1)
C(10)	5171(1)	-360(1)	4346(1)	36(1)	38(1)	36(1)	0(1)	-2(1)	-2(1)
C(11)	4778(2)	-985(2)	6611(2)	40(1)	50(1)	39(1)	11(1)	4(1)	-2(1)
C(12)	5510(2)	-1107(2)	7924(2)	48(1)	78(1)	36(1)	15(1)	5(1)	-1(1)
C(13)	6320(2)	-1976(1)	7846(2)	45(1)	51(1)	37(1)	5(1)	-1(1)	2(1)
C(14)	7266(1)	-1676(1)	6823(1)	36(1)	38(1)	44(1)	3(1)	-1(1)	-3(1)
C(15)	8343(2)	-2445(2)	7059(2)	47(1)	57(1)	49(1)	15(1)	-6(1)	-3(1)
C(16)	8417(2)	-2457(2)	8507(2)	60(1)	66(1)	55(1)	16(1)	-14(1)	-3(1)
C(17)	7176(2)	-2075(2)	8982(2)	65(1)	54(1)	42(1)	10(1)	-8(1)	-1(1)
C(18)	5723(2)	-3085(2)	7644(2)	67(1)	59(1)	56(1)	-15(1)	-5(1)	12(1)
O(3)	3767(1)	568(1)	871(1)	54(1)	93(1)	42(1)	11(1)	-4(1)	10(1)
O(11)	3709(1)	-1189(1)	6388(1)	36(1)	81(1)	53(1)	3(1)	5(1)	8(1)
O(17)	6921(2)	-1859(2)	10035(2)	100(1)	124(1)	40(1)	47(1)	-9(1)	-12(1)

ATOM	X/A(σ)	Y/B(σ)	Z/C(σ)	BISO(σ)	ATOM	X/A(σ)	Y/B(σ)	Z/C(σ)	BISO(σ)
H(1)	369(2)	52(1)	502(2)	39(4)	H(12B)	465(2)	-133(2)	837(2)	37(4)
H(2)	295(2)	112(2)	313(2)	49(5)	H(14A)	761(2)	-99(1)	705(2)	44(5)
H(4)	574(2)	-53(2)	134(2)	51(5)	H(15A)	924(2)	-218(2)	680(2)	43(5)
H(6A)	747(2)	-117(1)	267(2)	38(4)	H(15B)	815(2)	-312(2)	676(2)	64(7)
H(6B)	674(2)	-211(2)	304(2)	44(5)	H(16A)	916(2)	-202(2)	877(3)	61(6)
H(7A)	798(2)	-57(1)	467(2)	40(4)	H(16B)	856(2)	-323(2)	885(3)	65(7)
H(7B)	819(2)	-190(1)	447(2)	41(5)	H(18A)	522(2)	-315(2)	691(2)	57(6)
H(8B)	620(2)	-226(1)	533(2)	27(4)	H(18B)	634(2)	-367(2)	757(2)	61(6)
H(9A)	615(2)	4(2)	584(2)	36(4)	H(18C)	508(3)	-325(2)	837(2)	75(7)
H(12A)	573(2)	-41(2)	837(2)	61(6)	H(30)	309(2)	77(2)	88(2)	62(6)

Table 3b. Atomic coordinates for 3-hydroxy-9β-estra-1,3,5(10)-triene-11,17-dione (nonhydrogen $\times 10^4$; hydrogen $\times 10^3$; std. dev. in parentheses). The form of the anisotropic thermal parameters ($\times 10^4$) is $\exp[-2\pi^2(U_{11}h^2a^{*2} + 2U_{12}hka^*b^* + \ldots)]$. Isotropic thermal parameters are $\times 10$.

ATOM	X/A(σ)	Y/B(σ)	Z/C(σ)	U11(σ)	U22(σ)	U33(σ)	U12(σ)	U13(σ)	U23(σ)
C(1)	6816(2)	7302(2)	2021(2)	45(1)	50(1)	45(1)	-5(1)	20(1)	-10(1)
C(2)	5783(2)	7999(2)	606(3)	41(1)	46(1)	60(1)	-1(1)	22(1)	-12(1)
C(3)	5415(2)	7725(2)	-1412(3)	33(1)	42(1)	59(1)	-3(1)	12(1)	-7(1)
C(4)	6057(2)	6740(2)	-1992(2)	36(1)	44(1)	45(1)	-6(1)	10(1)	-10(1)
C(5)	7070(1)	6016(1)	-563(2)	36(1)	35(1)	45(1)	-7(1)	14(1)	-9(1)
C(6)	7660(2)	4925(2)	-1290(2)	47(1)	40(1)	43(1)	-3(1)	12(1)	-12(1)
C(7)	8418(2)	4051(2)	399(3)	48(1)	33(1)	51(1)	-7(1)	13(1)	-8(1)
C(8)	9486(2)	4710(-)	2219(2)	43(1)	50(1)	44(1)	-6(1)	13(1)	-1(1)
C(9)	8618(2)	5569(2)	3120(2)	44(1)	58(1)	45(1)	-8(1)	20(1)	-2(1)
C(10)	7491(1)	6315(2)	1482(2)	35(1)	38(1)	45(1)	-8(1)	17(1)	-5(1)
C(11)	9625(2)	6418(2)	4712(2)	46(1)	47(1)	39(1)	-6(1)	17(1)	-4(1)
C(12)	10876(2)	7027(2)	4272(2)	45(1)	40(1)	42(1)	-9(1)	17(1)	-7(1)
C(13)	11704(2)	6068(1)	3575(2)	39(1)	33(1)	36(1)	-3(1)	11(1)	2(1)
C(14)	10644(1)	5428(1)	1710(2)	39(1)	30(1)	36(1)	0(1)	12(1)	0(1)
C(15)	11659(2)	4787(2)	794(3)	47(1)	41(1)	58(1)	2(1)	20(1)	-8(1)
C(16)	12866(2)	5723(2)	1034(3)	51(1)	51(1)	61(1)	0(1)	28(1)	-5(1)
C(17)	12884(2)	6508(2)	2788(2)	36(1)	37(1)	36(1)	4(1)	9(1)	6(1)
C(18)	12517(2)	5214(2)	5324(3)	52(1)	51(1)	43(1)	-3(1)	8(1)	15(1)
O(3)	4417(1)	8444(2)	-2774(2)	52(1)	58(1)	63(1)	12(1)	6(1)	-9(1)
O(11)	9458(2)	6594(2)	6309(2)	84(1)	88(1)	50(1)	-28(1)	39(1)	-17(1)
O(17)	13707(1)	7350(1)	3456(2)	44(1)	43(1)	53(1)	-7(1)	14(1)	6(1)

ATOM	X/A(σ)	Y/B(σ)	Z/C(σ)	BISO(σ)	ATOM	X/A(σ)	Y/B(σ)	Z/C(σ)	BISO(σ)
H(1)	710(2)	745(3)	348(3)	45(5)	H(12A)	1053(2)	758(3)	307(3)	58(6)
H(2)	527(2)	858(3)	92(3)	57(6)	H(14A)	1021(2)	609(2)	76(3)	34(4)
H(4)	585(2)	660(3)	-332(3)	47(5)	H(15A)	1109(3)	461(3)	-71(4)	50(5)
H(6A)	836(2)	521(3)	-192(4)	45(5)	H(15B)	1210(2)	412(2)	140(3)	33(4)
H(6B)	681(2)	455(3)	-234(3)	52(6)	H(16A)	1257(3)	631(4)	-26(4)	72(8)
H(7A)	896(2)	346(3)	-1(3)	39(4)	H(16B)	1387(2)	541(2)	121(3)	41(5)
H(7B)	765(2)	372(2)	70(3)	42(5)	H(18A)	1189(2)	489(2)	593(3)	41(5)
H(8B)	999(3)	407(3)	329(4)	48(5)	H(18B)	1301(3)	456(2)	474(4)	47(5)
H(9A)	808(2)	514(2)	372(3)	29(4)	H(18C)	1316(2)	573(2)	645(4)	43(5)
H(12B)	1137(3)	744(3)	530(4)	58(6)	H(30)	422(2)	825(3)	-389(3)	39(4)

the aqueous layer by decantation. The gummy residue was extracted with dichloromethane, dried with anhydrous Na_2SO_4, and evaporated to dryness under N_2 on a steam bath. The residue, assumed to be 17-ethylenedioxy-3-acetoxyestra-1,3,5(10)-trien-11β-ol, was dissolved in 200 ml acetone, placed in an ice bath, and Jones' reagent (25 g CrO_3, 25 ml H_2SO_4, and enough H_2O to make 100 ml) was added dropwise until the solution became reddish brown in color. Ethanol (1 ml) was added to decompose excess CrO_3, 200 ml H_2O was added, and the mixture was extracted with 200 ml dichloromethane. The dichloromethane extract was dried with anhydrous Na_2SO_4, and evaporated to dryness under N_2 on a steam bath. The residue was crystallized from methanol (containing a small amount of pyridine) to afford 2.82 g (80%) of 17-ethylenedioxy-3-acetoxyestra-1,3,5(10)-trien-11-one, colorless plates, mp 145-7°C. (ir: ν_{max} 1752 (acetate C=O), 1710 (11-ketone), and 1200 cm^{-1} (C-O-C of phenyl acetate). The last named compound was refluxed in 50 ml methanol containing a 2 ml conc HCℓ for 15 min, and diluted with 100 ml H_2O. The precipitate was collected by filtration, and recrystallized from methanol to afford 1.5 g (43% overall yield) of 11-keto-9β-estrone, colorless plates, mp 206-214°C. (Lit. (14) mp, 204-207°C (decomp).)

nmr: δ 0.92 (s; 13β-CH_3); 3.83 (s, $W_{1/2}$ = 8 Hz; 9β-H).

ir: ν_{max} 3308 (phenolic OH), 1730 (17-ketone), 1698 (11-ketone), 1610, 1510 (benzene ring), and 1230 cm^{-1} (phenolic C-O).

Conversion of 11-ketoestrone to 11-keto-9β-estrone. Although no kinetic studies were undertaken, the following conditions were found to convert 11-ketoestrone to 11-keto-9β-estrone in nearly quantitative yields:

(1) Refluxing in methanol containing conc HCℓ for 15 min,

(2) refluxing in piperidine for 15 min and neutralizing with aqueous 20% HCℓ afterwards, or

(3) dissolving in ethanolic KOH and nuetralizing with aqueous 20% HCℓ afterwards.

Sodium borohydride reductions. General procedure. The ketonic compound (100 mg) was dissolved in 10 ml ethanol, 50 mg sodium borohydride was added, a few drops of H_2O was added until solution of the sodium borohydride was complete, and the solution was allowed to stand for 15 min. The solution was diluted with 50 ml aqueous 10% HCℓ, the precipitate was collected by filtration, and recrystallized from an appropriate solvent. Yields were from 75 to 90%.

After sodium borohydride reduction, either 11β-hydroxyestrone or 11-ketoestrone afforded the same product, 11β-hydroxyestradiol, as colorless small plates from ethyl acetate, mp 291-6°C. (Lit. (15) mp, 291-5°C.)

11-Keto-9β-estrone did not give 11β-hydroxyestradiol after sodium borohydride reduction, but gave instead 11-keto-9β-estradiol (3,17β-dihydroxy-9β-estra-1,3,5(10)-trien-11-one), colorless small prisms

from ethyl acetate, mp 238-242°C. (Lit. (16) mp, 255-257°C.) ir: ν_{max} 3375, 3225 (two OHs), 1685 (11-ketone), 1610, 1500 (benzene ring), and 1225 cm^{-1} (phenolic C-O).

Bioassays. We use the stimulation of uterine weight as our bioassay for estrogenicity. For this purpose, we use weanling female Fischer rats of our inbred line. The total dose of steroid to be administered is dissolved in 1 ml cottonseed oil, and in the late afternoon of the next two days, each animal is given 0.2 ml subcutaneously. Controls are given the cottonseed oil vehicle. On the morning of the fourth day, animals are sacrificed with ether anesthesia and the uteri dissected free, blotted, and weighed.

The bioassay to assess inhibition of gonadotropin secretion uses Fischer rats weighing from 70 to 80 grams. The males are castrated before parabiosis. After a skin incision is made from the neck to the hip, the pair (castrated male on the right and intact female on the left) are joined by sewing through the adjacent scapulae. After incisions through the lateral abdominal muscles and peritoneum, they are joined with a continuous suture. Then, the skin is finally closed throughout. Treatment is started on the day of parabiosis. The steroid, in 0.1 ml cottonseed oil, is administered subcutaneously to the castrated male daily for 10 days. The pairs are sacrificed 24 hrs after the last injection. The male accessory organs are weighed to assess possible side effects, and the ovarian weight in the female is used as the index of gonadotropin secretion by the male.

Because the estrogens under study do not have parallel dose-response curves, for comparative purposes we have adopted a quantal response where the amount of estrogen which would be required to double the uterine weight of the controls is extrapolated from the dose-response curve obtained by means of a computer program. For the same reason, the results from the gonadotropin-secretion inhibiting assay in parabiotic rats are also reduced to quantal assay in which the amount of steroid required to halve the ovarian weight of the controls is calculated.

RESULTS

Bioassays. Figure 1 shows the striking difference in biologic activities between the 9α and 9β forms of 11-ketoestrone. It required about 10 times as much of the 9α as the 9β form to double the uterine weight of the immature female rat. Estrone itself was included for comparison; it can be seen that the activities of 11-keto-9β-estrone approximated those of estrone. 11-Ketoestrone, in contrast to its 9β isomer, was inactive in the antigonadotropin assay.

Table 4 shows the quantal numerical values that were derived to

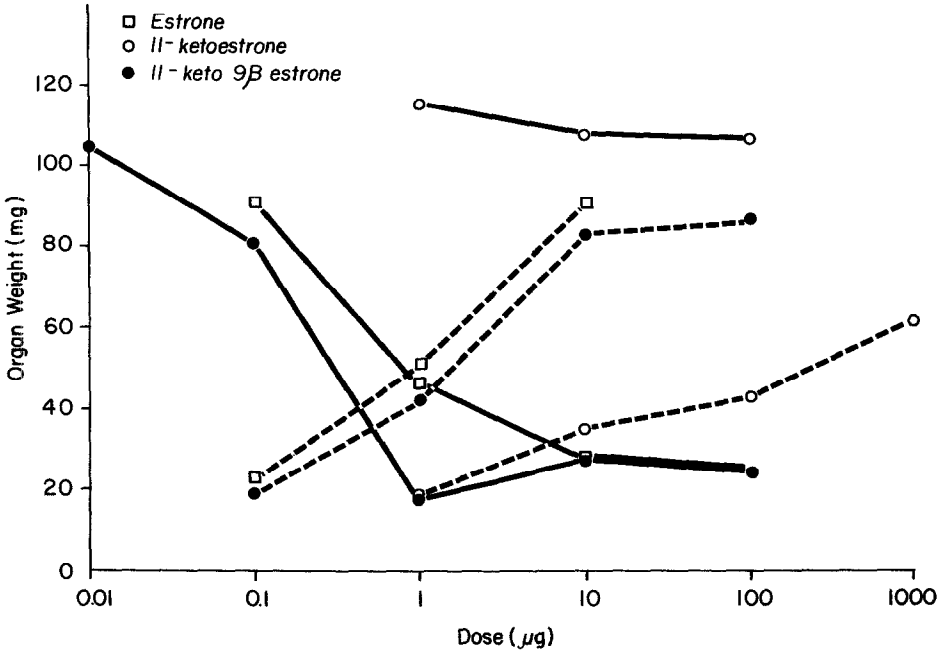

Figure 1. Biologic activities of estrone, 11-ketoestrone and 11-keto-9β-estrone. Solid lines: Ovarian weights in the parabiotic assays. Dashed lines: Uterine weights of immature female rats in the estrogenic assays.

Table 4. Relative quantal activities.

Steroid	Uterine weight assay; total dose in ug/rat	Ovarian weight assay; total dose in ug/rat
Estrone	0.53	0.42
11-Ketoestrone	8.13	<100
11-Keto-9β-estrone	0.67	0.47

compensate for the lack of parallelism in the dose-response curves.

Chemistry. The procedure that we described for the preparation of 11-keto-9β-estrone was originally intended for the preparation of 11-ketoestrone. We suspected that our product was 11-keto-9β-estrone when it failed to give the expected, and previously reported (15), 11β-hydroxyestradiol, but gave instead the unexpected, and previously reported (16), 11-keto-9β-estradiol after sodium borohydride reduction. The ir

spectrum of our product (11-keto-9β-estrone) was not superimposable with that of an authentic sample of 11-ketoestrone (17) obtained by the method of Hasegawa *et al* (14). Hasegawa *et al* reported that basic conditions (KOH in MeOH) isomerized 11-ketoestrone to its 9β isomer. We have found that acidic conditions will do as well.

Proton nmr spectra were helpful in identifying 11-ketoestrone and 11-keto-9β-estrone. In 11-ketoestrone, the proton at C-9 is axial, while in the 9β isomer the proton is equatorial with respect to ring C. In 11-ketoestrone, a multiplet for this proton is centered at δ 3.70 and has a peak half-width ($W_{1/2}$) of 13 Hz. In 11-keto-9β-estrone, the signal is centered at δ 3.83 and has a $W_{1/2}$ of 8 Hz. Since axial protons have the larger coupling constants and appear further downfield than equatorial protons (18), the nmr data were consistent with the structures of 11-ketoestrone and 11-keto-9β-estrone. Also, the proton nmr data were similar to those reported by Hasegawa *et al* (14).

X-Ray crystallography. The X-ray crystal structure determinations confirmed the spectral assignments (Figure 2).

The bond lengths and valence and torsion angles of the two steroids are compared in Figure 3. The bond lengths are in excellent agreement despite the difference in C(9) configuration. The largest differences in valence angles are around C(9) and the signs of the torsion angles in the B-ring are reversed as a result of the configurational change. The observed conformations of the two structures agree with prediction based upon force field calculations (16).

In 11-ketoestrone, the B-ring has a nearly symmetric 7α,8β-half chair conformation (19), the C-ring has a very symmetric chair conformation and the D-ring has a conformation midway between a 14α-envelope

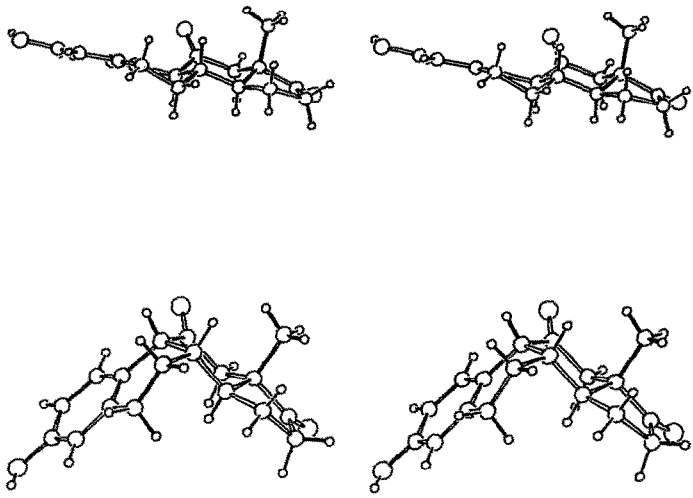

Figure 2. Stereo views of the conformation of the 9α and 9β epimers of 3β-hydroxy-1,3,5(10)-estratriene-11,17-dione.

and a 13β,14α-half chair. As predicted (16), repulsive interactions between the 11-oxo substituent and the hydrogen atom on C(1) open the C(10)-C(9)-C(11) valence angle to 115.8° and the C(1)-C(10)-C(19)-C(11) torsion angle to 43.5°. The latter differs by more than 10° from the average of four crystallographically independent observations of estrone, 28.1°, 33.1°, 32.9° and 35.0° (20).

In 11-keto-9β-estrone, the B ring also has a nearly perfect half chair conformation with atoms C(7) and C(8) displaced equally on opposite sides of the plane of atoms C(9), C(10), C(5) and C(6). The C ring has a distorted chair conformation which is flattened at the C(9) position as indicated by the 15° decrease in the magnitude of the torsion angles C(14)-C(8)-C(9)-C(11)-C(12) when compared to those in the 9α epimer. This flattening is probably due to 1,3-diaxial interaction between C(10) and the hydrogen on C(14). The D ring has a nearly perfect 14α-envelope conformation. The D ring conformational

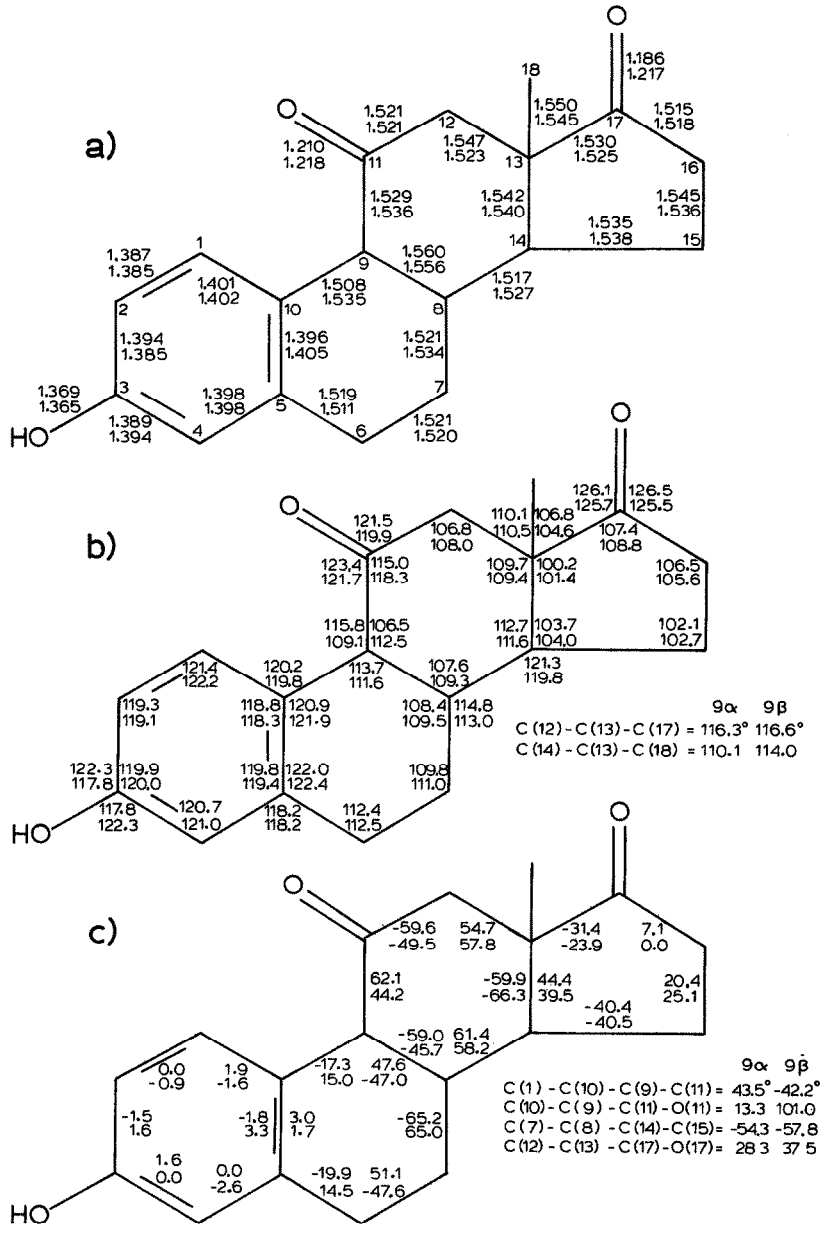

Figure 3. Bond distances (a) (Å, σ range = 0.002-0.003Å), valence angles (b) (°, σ ≈ 0.1°) and torsion angles (c) (°, σ range = 0.2-0.3°) in 3-hydroxy-1,3,5(10)-estratriene-11,17-dione (above) and 3-hydroxy-9β-estra-1,3,5(10)-triene-11,17-dione (below) A torsion angle α-β-γ-δ is positive if, when viewed down the β-γ bond, the α-β bond will eclipse the γ-δ bond when rotated less than 180° in a clockwise direction.

difference is not necessarily linked to the change in C(9) configuration. The D-rings of the four crystallographically independent estrone structures include two having conformations midway between the 14α-envelope and the 13β,14α-half chair forms and one of each of these symmetric forms. (23)

DISCUSSION

The 9α-to-9β isomerization of 11-ketoestratrienes under acidic or basic conditions was first described by Bailey *et al* (21) for 1-methyl-11-ketoestratrienes. Liang *et al* (16) reported that the 9β isomer of 11-ketoestradiol was more stable than the 9α conformer, and postulated that it was due to the hydrogen atom at C-1 being closer to the 11-keto group in the 9α conformer than in the 9β isomer ("peri-planar effect"). However, we feel that dipole-dipole repulsions may also account for the unexpected stability of the 9β isomer. In the 11-ketoestratrienes, the C-O bond of the 11-ketone eclipses the C(9)/C(10) bond, so that the electronegative O atom of the 11-ketone is on the same plane as the electronegative C(10) position of the benzene ring A. Therefore, it would be expected that these electronegative charges would repel each other to favor the 9β conformation in which the C(10) position and the oxygen atom of the 11-ketone would be farther apart from each other.

If we assume that binding to the estrogen receptor in the cytosol is indeed a prerequisite for estrogenic activity, and if we further assume that the striking uterotropic effects of 11-keto-9β-estrone are indeed evidence of estrogenic activity, then we are faced with a dilemma. One would really expect that the 9α conformer should have a greater abilty to approach the estrogen receptor than the 9β isomer.

However, since it has recently been reported that simple alkyl phenols are capable of displacing estradiol-17β from the estrogen receptor (22), this together with our data suggests a key role for the phenolic group in receptor binding.

We could rationalize that the 11-keto substitution in 11-keto-estrone might reduce estrogenicity as a consequence of loss of affinity for the receptor sites or increased competition for metabolizing enzymes. But it is still difficult to understand how the combination of the 11-keto substitution and 9β configuration results in such striking biologic activity. It is doubtful that equilibration in some solvents of the 9α and 9β structures (16) could play a significant role in determining the observed activity *in vivo*, since if they came to equilibrium, the biologic activity should be independent of which form is administered.

Although the O(3) to O(17) distance in natural estrogens (23) differs substantially from distance between terminal hydroxyls observed in synthetic estrogens (24,25), it is still thought to be a relevant factor in determining activity. This distance in the 9β-epimer is only 8.66Å as compared to the more typical value of 10.78Å found in the 9α-epimer. It would appear that 11-keto-9β-estrone has the phenolic A-ring and O(3)-O(17) parameters required for binding to receptors and activity; its overall shape probably protects it from other *in vivo* interactions such as metabolism that may prevent 11-ketoestrone (the 9α isomer) from achieving biological activity similar to that of its 9β isomer.

At any rate, the results suggest that the putative estrogenic receptor is tolerant to flexibility in the overall molecular shape of

the ligand, but other factors operate to determine biological activity.

ACKNOWLEDGEMENTS

This work was supported in part by Grant No. AM-26546 from the National Institute of Arthritis, Metabolism and Digestive Diseases, DHEW, and in part by the annual gift support program of the Alton Ochsner Medical Foundation through donors who have designated their gifts to this project. We thank Miss F. E. DeJarnette, Miss Gloria Del Bel, Mrs. Brenda Giacchi, Miss Deanna Hefner and Miss Melda Tugac of the Medical Foundation of Buffalo, Inc., and Mrs. Debbie L. Braud, Mr. Roy H. Coleman, Mrs. Leslie Hamer and Miss Cynthia A. Prehmus of the Alton Ochsner Medical Foudnation for valuable technical assistance.

APPENDIX

IUPAC nomenclature and trivial names:

11α-Methoxyethynylestradiol:	11α-methoxy-17-ethynyl-1,3,5(10)-estratriene-3,17β-diol
11β-Methoxyethynylestradiol:	11β-methoxy-17-ethynyl-1,3,5(10)-estratriene-3,17β-diol
11-Ketoestrone:	3-hydroxy-1,3,5(10)-estratriene-11,17-dione
11-Keto-9β-estrone:	3-hydroxy-9β-estra-1,3,5(10)-triene-11,17-dione
11β-Hydroxyestrone:	3,11β-dihydroxy-1,3,5(10)-estratrien-17-one
11β-Hydroxyestradiol:	1,3,5(10)-estratriene-3,11β,17β-triol
11-Keto-9β-estradiol:	3,17β-dihydroxy-9β-estra-1,3,5(10)-trien-11-one

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