STRUCTURE-ACTIVITY RELATIONSHIPS OF 9β-ESTROGENS

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ABSTRACT

The 9β isomers of estradiol-17β, estradiol-17α, estrone and 17-ethinylestradiol-17β were synthesized and compared with their 9α-counterparts in the rat uterine cytosol estrogen receptor, uterotropic, and gonadotropin release inhibition assays. Except for 17-ethinyl-9β-estradiol-17β which was as active as its 9α isomer in the uterotropic assay, none of the 9β estrogens exhibited any biological activity which was equal to or greater than their 9α counterparts. For examples, 9β-estradiol-17β was 1/10 as active as estradiol-17β, and 9β-estrone was 1/4 as active as estrone in the uterotropic assay.

INTRODUCTION

Our discovery of the remarkable estrogenicity of 11-keto-9β-estrone (3) led us to investigate further the effects of the 9β configuration on the biological activities of other ring-A phenolic steroids. We have already reported that 11-hydroxy-9β-estrones are 1/10 (11β-hydroxy) to 1/2 (11α-hydroxy) as uterotropic as their 9α counterparts in the rat (4). Here we report on the syntheses and biological activities of the 9β isomers of familiar estrogens (estradiol-17β, estradiol-17α, estrone, and 17-ethinylestradiol-17β) which do not have functional groups at C-11.

EXPERIMENTAL

Melting points were determined by a Kofler micro-hotstage and are uncorrected. Infrared spectra (ir) were from KBr pellets and obtained from a Perkin Elmer infrared spectrophotometer model 710B. Proton nuclear magnetic resonance data (^{1}H-NMR) were obtained from a Varian EM-390 90 MHz NMR spectrometer and are reported in ppm relative to tetramethylsilane as internal standard (δ=0); deuteriochloroform was used as the NMR solvent. C,H analyses were done by Galbraith Laboratories, Knoxville, TN.

Estrone was a gift from Syntex; estradiol-17β and 17-ethinyl-estradiol-17β were purchased from Searle; estradiol-17α was a gift from Organon.

9β-Estrone.--- One gram of 9,11-dehydroestrone 17-ethyleneketal (4) was dissolved in 100 mL tetrahydrofuran (freshly distilled over KOH pellets), 100 mg 10% palladium on charcoal (Matheson Coleman and Bell) added, and hydrogenated at 40 lb/in^2 H_2 for 1 h in a Parr pressure reaction apparatus. The catalyst was deactivated with 1 g kaolin, filtered under reduced pressure, and the filtrate evaporated to dryness under N_2 on the steam bath. The oily residue was treated with 20 mL methanol containing 1 mL conc HCl and 2 mL water. After 15 min, the solution was diluted with water to precipitate a solid which was collected by filtration under reduced pressure. Recrystallization from 25 mL ethanol and dilution of the mother liquor with 5 mL water gave altogether 241 mg (27% yield) estrone, colorless cubes, identified by its ir spectrum, mp (255-257°C), and undepressed mixed mp with an authentic sample of estrone. Dilution of the leftover mother liquor with water precipitated a solid which was collected by filtration and recrystallized twice from aqueous ethanol (25 mL ethanol diluted with 30 mL water) to afford 427 mg (48% yield) 9β-estrone, colorless plates, mp 186-189°C (reported (2) for dl-9β-estrone: 184-186°C).

ir: 3275 (O-H), 2850, 2920 (C-H), 1718 (17-ketone), 1610, 1500 (benzene ring), 1445 (C-H), 1238 (phenolic C-O), 820 and 860 (benzene ring) cm^{-1}.

^{1}H-NMR: δ0.98 (s; 13 -CH_3), 3.02 (q, $W_{1/2}$=8.5 Hz; 9β-H).

Analyses: Calc for $C_{18}H_{22}O_2$: C, 79.96; H, 8.20%. Found: C, 79.97; H, 8.32%.

9β-Estradiol-17β.--- 9β-Estrone (300 mg) was dissolved in 10 mL ethanol, 150 mg sodium borohydride in 1 mL water added, and the mixture allowed to stand at room temperature for 45 min. The mixture was acidified with 10% aqueous HCl and diluted with water to precipitate a solid which was collected by filtration. Recrystallization from aqueous methanol afforded 149 mg (50% yield) of 9β-estradiol-17β, colorless flat needles, mp 217-221°C.

ir: 3380, 3275 (O-H), 2860, 2920 (C-H), 1608, 1500 (benzene ring), 1450 (C-H), 1230 (phenolic C-O), 1042 (C-O of 17 -OH), 870 and 810 (benzene ring) cm^{-1}.

Analyses: Calc for $C_{18}H_{24}O_2$ C, 79.37; H, 8.88%. Found: C, 79.13; H, 9.06%.

9β-Estradiol-17α.--- 9β-Estradiol-17β (910 mg) was dissolved in 4.5 mL t-butanol with the aid of approximately 1 mL of a saturated solution of KOH. Benzoyl chloride (1.8 mL) was added to the stirred solution, and after 15 min, water was added slowly until precipitation was complete. The solid was collected by filtration, and recrystallized from ethanol to afford 658 mg (58% yield) 9β-estradiol-17β 3-benzoate, colorless fine needles, mp 209-211°C.

ir: 3534 (strong and sharp, O-H of 17 -OH), 2910, 2850 (C-H), 1718 (benzoate carbonyl), 1600, 1583 (benzene ring), 1270 (C-O-C of a phenyl benzoate), and 700 (benzene ring of benzoate) cm^{-1}.

9β-Estradiol-17β 3-benzoate (658 mg) was dissolved in 10 mL tetrahydrofuran (freshly distilled over KOH pellets). To this was added 1.18 g triphenylphosphine, 0.7 mL diethyl azodicarboxylate (Aldrich), and 276 mg benzoic acid. The solution was allowed to stand overnight (18 h), and then heated to 60°C on a water bath for 2 h. Methanol (40 mL) was added, and the mixture was extracted three times with 40 mL portions of a 1:1 (v/v) mixture of diethyl ether and petroleum ether (bp 30-75°C). The combined diethyl ether-petroleum ether extract was washed with 60 mL methanol, the ether layer separated, and evaporated to dryness under N_2 on the steam bath. The residue was chromatographed through 30 g silica gel (60-200 mesh, Baker), using diethyl ether-petroleum ether (1:1, v/v) as the eluant. The composition of the collected fractions (after evaporation to dryness) was monitored by ir spectroscopy; those fractions that showed no -OH bands in the ir spectra were combined (these fractions were presumed to contain 9β-estradiol-17α 3,17-dibenzoate) and saponified by 25 mL ethanol containing 1 g KOH and 5 mL water on the steam bath for 30 min. After acidification with 10% HCl, precipitating with water, collecting the precipitate by filtration, and recrystallization from acetone-petroleum ether (bp 60-110°C), 139 mg (15% overall yield from 9β-estradiol-17β) of 9β-estradiol-17α, colorless needles, mp 232-235°C, was obtained.

ir: 3350 (broad, O-H), 2880, 2855 (C-H), 1622 (weak), 1590 (very strong), 1495 (benzene ring), 1460 (C-H), 1270 (phenolic C-O), 1038, 1085 (weak doublet, typical of 17α-OH), 860 and 810 (benzene ring) cm^{-1}.

Analyses: Calc for $C_{18}H_{24}O_2$: C, 79.37; H, 8.88%. Found: C, 79.18; H, 9.10%.

17-Ethinyl-9β-estradiol-17β.--- 9β-Estrone (590 mg) was dissolved in 50 mL dimethylsulfoxide, stirred under N_2, 1.2 g lithium acetylide-ethylenediamine complex (Aldrich) added all at once, and stirring continued for 6 h. Afterwards, the solution was allowed to stand overnight, poured onto 200 g crushed ice, and neutralized with 4N HCl. The solid was collected by filtration, taken up in dichloromethane, and chromatographed through 2 g silica gel. The eluants were dicholoromethane-petroleum ether (1:1, v/v), dichloromethane, and dichloromethane-ethyl acetate (3:1, v/v). The dichloromethane-ethyl acetate eluate was evaporated to dryness under N_2 on the steam bath, taken up in methanol, decolorized by activated charcoal (Norit A), and filtered. The filtrate was diluted with water, but no solid could be obtained. After extraction with dichloromethane, evaporating to dryness, and triturating the residue with petroleum ether, 217 mg (32% yield) of 17-ethinyl-9β-estradiol-17β (as the hydrate) was obtained as an amorphous solid, mp 75-80°C.

ir: 3375 (broad, O-H), 3275 (narrow, strong; ethinyl C-H), 2855, 2925 (C-H), 1610 (strong), 1580, 1495 (benzene ring), 1440 (C-H), 1225 (phenolic C-O), 815, 860 (benzene ring) cm^{-1}.

Analyses: Calc for $C_{20}H_{24}O_2.H_2O$: C, 76.40; H, 8.34%. Found: C, 76.29; H, 8.26%.

Pharmacology.--- The methodologies of the quantal bioassays for the determination of uterotropic and gonadotropin release inhibition activities have been previously described (3,7). Briefly, uterotropic activity (UT) is represented by the calculated total dose in μg that will double the weight of the ovaries of an immature female Fischer rat in 5 days. The gonadotropin release inhibition activity (OV) is represented by the calculated total dose in μg that will, in 10 days, halve the weight of the ovaries of a female Fischer rat joined in parabiosis to a castrated male Fischer rat that is treated with the test compound. The test compounds were administered in cottonseed oil by subcutaneous injections.

Estradiol-17β receptor assay.--- The methodology of determining the ability of a test compound to displace (^{3}H) estradiol-17β from an "estrogen receptor" has been previously described (7). Briefly, the relative displacing activity (RDA) is calculated from the concentration of the test compound that will displace 50% of (^{3}H) estradiol-17β from the cytosol prepared from the uteri of mature female AXC rats, with estradiol-17β being given the value of 100 for comparision.

Data. The data of the bioassays are presented in Table 1. The protocols of presenting the data are the same as those reported previously (7).

Table 1. Compilation of Data

Compound	RDA[a]	UT[b] (dose)	OV[c] (dose)	OV/UT[d]
1. Estradiol-17β	100	100 (0.03)	100 (0.04)	13
2. 9β-Estradiol-17β	13	10 (0.3)	6.7 (6.0)	20
3. 17-Ethinylestradiol-17β	91	60 (0.05)	13 (3.0)	60
4. 17-Ethinyl-9β-estradiol-17β	36	60 (0.05)	4 (10)	200
5. Estradiol-17α	25	0.8 (4.0)	0.8 (500)	125
6. 9β-Estradiol-17α	0	inactive[e]	inactive[f]	- -
7. Estrone	46	6 (0.5)	10 (4.0)	8
8. 9β-Estrone	26	1.5 (2.0)	0.4 (9.0)	45

[a]RDA: relative displacing activity; the ability of a compound to displace 50% of (^{3}H) estradiol-17β from rat uterus cytoplasmic preparations as compared to estradiol-17β which is given the value of 100.

[b]UT: relative uterotropic activity; the ability of a compound to double the weight of rat uterus as compared to estradiol-17β which is given the value of 100. (Total dose in μg is given in parentheses).

[c]OV: relative gonadotropin release inhibiting activity; the ability of a compound to halve the weight of parabiotic rat ovary as compared to estradiol-17β which is given the value of 100. (Total dose in μg is given in parentheses).

[d]OV/UT: The total dose to inhibit gonadotropin release divided by the total uterotropic dose.

[e]No dose-response curve could be obtained with 100 μg total dose.

[f]No dose-response curve could be obtained with 10,000 μg total dose.

RESULTS AND DISCUSSION

Chemistry: Smith et al (1) reported that the hydrogenation of dl-9,11-dehydroestrone 3-methyl ether 17-ethyleneketal over palladium-charcoal in a 1:1 mixture of acetic anhydride-acetic acid afforded an 1:1 mixture dl-estrone 3-methyl ether and dl-9β-estrone 3-methyl ether after deketalization. We modified the procedure of Smith et al by using tetrahydrofuran instead of the acetic anhydride-acetic acid mixture to avoid solubility problems and difficulties in the work-up. With this modification, 9,11-dehydroestrone 17-ethyleneketal was smoothly hydrogenated to an approximately 1:2 mixture of estrone and 9β-estrone after deketalization with methanolic HCl. These two compounds were easily separated from each other by recrystallization inasmuch as estrone is much less soluble in aqueous ethanol than 9β-estrone.

Apart from its mp that agreed with that reported by Anner and Miescher (2) for dl-9β-estrone, our 9β-estrone was conclusively identified by its chromatographic mobility, ir and ^{1}H-NMR data which considerably differed from those of estrone. In TLC (silica gel: chloroform-ethyl acetate 9:1 v/v), 9β -estrone had an Rf of 0.30 while estrone had an Rf of 0.43. In the ir spectra, 9β-estrone exhibited the characteristic benzene ring bands (1610 (very strong) and 1500 (moderate) cm^{-1}) that we have found to be typical of 9β-estrogens which we have synthesized so far (3,4). In the ^{1}H-NMR spectra, 9β-estrone exhibited a quartet centered at δ 3.02 ($W_{1/2}$=8.5 Hz). The 9α counterpart exhibited a quartet centered at δ 2.83 ($W_{1/2}$=13.5 Hz). Axial protons (such

as 9α-H) have larger coupling constants and appear further upfield than equatorial protons (such as 9β-H) (5). The ^{1}H-NMR data, therefore, were consistent with the structure of 9β-estrone.

The Mitsunobu reaction (6) was used for converting 9β-estradiol-17β to 9β-estradiol-17α. The sluggishly reactive 17β-hydroxyl group required longer time and heating (18 h at room temperature, then 2 h at 60°C) than usual (2 h at room temperature is sufficient to convert 3β-hydroxy-5α-androstan-17-one to 3α-hydroxy-5α-androstan-17-one 3-benzoate; unpublished results from this laboratory).

9β-Estrone was ethinylated with some difficulty by the lithium acetylide-ethylenediamine complex in dimethylsulfoxide to afford 17-ethinyl-9β-estradiol-17β hydrate in 32% yield.

Bioassays: In three of the four pairs studied, the 9β compound was considerably less effective as an uterotropic agent than its 9α isomer. For examples, 9β-estrone was 1/4 as active as estrone, and 9β-estradiol-17β was 1/10 as active as estradiol-17β. The exception was the ethinylestradiol pair where, in multiple assays done simultaneously, we found essentially identical uterotropic values for both 9α and 9β isomers.

On the other hand, in the evaluation of the inhibition of gonadotropin release, the 9α compounds were consistently more active than their 9β isomers. All 9α compounds had lower OV/UT ratios than their 9β isomers, which indicates that the 9β configuration does not benefit inhibition of gonadotropin release in comparison to uterotropic activity.

When the competitive displacement of (^{3}H) estradiol-17β in our RDA assay was evaluated, in every instance the 9β compound was considerably less effective than its 9α isomer. 9β-Estradiol-17α had an RDA of 0; this compound was also inactive in the uterotropic and gonadotropin release inhibition assays.

Other than the particular instance of 11-keto-9β-estrone (3), but confirming what was found for 11-hydroxyestrone isomers (4), the 9β configuration does not result in estrogens with advantageous biological activities. The 17α-ethinyl modification did not afford potent inhibitors of gonadotropin release, although it did result in potent uterotropic agents; as a consequence, the OV/UT ratios of 17α-ethinyl compounds are rather high (60 to 200). In the case of 17-ethinyl-9β-estradiol-17β, the 17α-ethinyl group apparently overrides the unfavorable influence of the 9β configuration on uterotropic activity. Thus, not only the spatial aspect of the phenolic ring A, but also the nature of the functional groups at ring D determine estrogenic activity. The 17α-hydroxyl group results in compounds having weak or no biological activities; estradiol-17α is a weak uterotropic agent (0.8% as active as estradiol-17β) and almost inactive as an inhibitor of gonadotropin release, while its 9β isomer is inactive in either parameter.

11-Keto-9β-estrone remains as the only example of an estrogenic steroid where the 9β configuration was more favorable than the "normal" 9α configuration in the uterotropic and gonadotropin secretion inhibition assays (3). When the biological activities of 9β-estrone and 11-keto-9β-estrone are compared, 11-keto-9β-estrone is 3 or 19 times as active as 9β-estrone in the uterotropic or gonadotropin release

inhibition assay, respectively. We have demonstrated that the estrogenic activity of 11-keto-9β-estrone cannot be due to metabolism to 11α- or 11β-hydroxyl compounds (4). The 11-oxo group, therefore, is important in determining the biological activities of 11-keto-9β-estrone. In regards to the possibility of metabolism at C-17, 11-keto-9β-estradiol-17β (the synthesis of which has been previously described (3)) is only 1/16 or 1/19 as active as 11-keto-9β-estrone in the uterotropic or gonadotropin release inhibition assay, respectively (unpublished data). This is a remarkable exception to the generalization that 17β-hydroxy estrogens are more potent than their corresponding 17-keto analogues (7). These facts strongly suggest the possibility of "estrogen receptors" specifically interacting with 11-keto-9β-estrone, but the actual existence of such "structurally specific receptors" remains to be verified (7).

The results of this work confirm what has been previously found (7): 1.) RDA cannot be quantitatively correlated with uterotropic activity or inhibition of gonadotropin release; 2.) uterotropic activity does not correlate with inhibition of gonadotropin release; and 3.) consistently successful predictions of the effects of a structural modification on RDA, uterotropic activity, or inhibition of gonadotropin release cannot be made a priori.

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APPENDIX: NOMENCLATURE

The trivial name is given first, then its IUPAC systematic nomenclature follows:

1. Estradiol-17β: 1,3,5(10)-estratriene-3,17β-diol
2. 9β-Estradiol-17β: 9β-estra-1,3,5(10)-triene-3,17β-diol
3. 17-Ethinylestradiol-17β: 17α-ethinyl-1,3,5(10)-estratriene-3,17-diol
4. 17-Ethinyl-9β-estradiol-17β: 17α-ethinyl-9β-estra-1,3,5(10)-triene-3,17-diol
5. Estradiol-17α: 1,3,5(10)-estratriene-3,17α-diol
6. 9β-Estradiol-17α: 9β-estra-1,3,5(10)-estratriene-3,17α-diol
7. Estrone: 3-hydroxy-1,3,5(10)-estratrien-17-one
8. 9β-Estrone: 3-hydroxy-9β-estra-1,3,5(10)-trien-17-one
9. 9,11-Dehydroestrone 17-ethyleneketal: 17,17-ethylenedioxy-1,3,5(10),9(11)-estratetraen-3-ol
10. 11-Keto-9β-estrone: 3-hydroxy-9β-estra-1,3,5(10)-triene-11,17-dione
11. 11-Keto-9β-estradiol-17β: 3,17β-dihydroxy-9β-estra-1,3,5(10)-trien-11-one

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