Synthesis and Estrogenic Properties of 7α,8α-Epoxy- and 7α,8α-Methyleneestradiols

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A series of 7α,8α-epoxyestradiol derivatives with ethynyl, 2- or 3-furyl, or 2-thienyl substituents in the 17α position was prepared. The products were highly active orally in the Allen–Doisy test in rats, but most of them were only weakly active in the uterotrophic assay in the mouse. A 7α,8α-methylene analog and a 7α,8α-difluoromethylene analog were less active than the corresponding epoxides.

As part of a study of the chemistry and pharmacology of steroids derived from equilin, a number of 7α,8α-epoxy- and 7α,8α-methylene estrogens have been prepared and screened for estrogenic activity.

Chemistry. Reaction of appropriate 1,3,5(10),7-estratetraenes (equilin derivatives) with *m*-chloroperbenzoic acid afforded in each case a single epoxide.† The 7α,8α configuration was asigned on the basis of the expected preference for α-side attack. Thus from 3-ethers of 17α-ethynyl-1,3,5(10),7-estratetraene-3,17-diol, the 7α,8α-epoxides **8** and **9** were obtained (Table II). In order to prepare 7α,8α-epoxides corresponding to 17α-furyl[2] and 2-thienyl[3] estrogens, and because of the sensitivity of furans[4] and thiophenes[5] to peracids, 7α,8α-epoxy 17-ketones were prepared (Table I) and then allowed to react with the appropriate organolithium compounds.

In view of the high degree of estrogenic activity shown by the epoxyestrogens so obtained (see below), the synthesis of some 7α,8α-methylene analogs was undertaken. Under the conditions of the Simmons-Smith reaction[6] equilin did not afford detectable amounts of cyclopropane derivatives. Reaction of 17β-dihydroequilin bis(tetrahydropyranyl) ether with diethylzinc and methylene iodide[7] afforded a crude product containing some cyclopropane ring (nmr). However, no crystalline product could be isolated due, apparently, to attack of the reagent on the ether linkages.[8] It was eventually found that reaction of triethylaluminum and methylene iodide with equilin in refluxing benzene gave up to 70% yields of 7α,8α-methyleneestra-1,3,5(10)-triene-3,17β-diol.‡ The stereochemistry, initially assigned on the assumption of "rear attack" by the reagent, was later confirmed by means of an X-ray crystallographic analysis[10] of the derived 3-methyl ether 17-bromoacetate. The X-ray analysis showed that, in the latter compound, ring B has a conformation very close to that of an ideal boat.

Oxidation of the 7α,8α-methylenediol to the corresponding 17-ketone with sulfur trioxide–triethylamine complex in dimethyl sulfoxide,[11] followed by reaction with sodium acetylide, afforded 17α-ethynyl-7α,8α-methyleneestra-1,3,5(10)-triene-3,17-diol (**19**).

A difluoromethylene analog **20** was obtained by reaction of equilin methyl ether with difluorocarbene (derived from sodium chlorodifluoroacetate)[12] followed by conventional introduction of the 17α-ethynyl group. The physical properties of the 7α,8α-substituted estradiols are given in Table II.

Pharmacology. The oral estrogenic activity of the compounds was determined by means of two standard assays.

(a) Allen–Doisy test[13a] with a slight modification.[13b] The ED_{50} in this experiment is the dose which induces cornification of the vaginal epithelial cells in 50% of the ovariectomized rats. The ED_{50} was calculated by using an average of 40 (20–100) animals per compound. Dose–response curves were used to determine the ED_{50} graphically.[14] At least four dose levels were used for the determination of the ED_{50} for each compound.

(b) Uterotrophic assay in mice.[15] This test was done with intact immature mice with a minimum of five doses for each compound. Five to ten animals were used at each dose level. The results are expressed as the minimum effective dose which increased the uterine weight threefold over that of the controls.

Results are given in Table II together wih data for 17α-ethynyl-3-methoxyestra-1,3,5(10)-trien-17-ol (**6**) and 17α-ethynyl-3-methoxyestra-1,3,5(10),7-estratetraen-17-ol (**7**). In the Allen–Doisy assay, the 17α-substituted epoxides were approximately as active as **7** which is more active than **6**.§ The 17α-furyl-substituted epoxides **10–16** had about the same degree of activity in this assay as the corresponding 17α-furyl-1,3,5(10),7-tetraen-17-ols.[2] It may be relevant to mention that models indicate that the distance between the C-3 and C-17 oxygen atoms in the epoxides is very nearly the same as in the corresponding steroids with an unsubstituted ring B and having the normal 8β configuration.

All the epoxides, with the exception of **9**, were much less active than **6** or **7** in the uterotrophic assay. Other 17α-furyl-substituted estrogens also show weak uterotrophic activity.[2]

The 7α,8α-methylene compound **19** was somewhat more active than **6** in the Allen–Doisy assay but was less active than **7**. The 7α,8α-difluoromethylene compound **20** had only marginal activity.

Experimental Section

The compounds gave satisfactory analyses for C and H and, where applicable, for S and F. The uv, ir, and nmr spectra were in agreement with the proposed structures. The melting points are uncorrected.

7α,8α-Epoxides Derived from Equilin Derivatives. The general procedure may be illustrated by the following example. *m*-Chloroperbenzoic acid (4.05 g) was added in portions over a period of 30 min to an ice-cold solution of equilin methyl ether (5.0 g) in $CHCl_3$ (125 ml). After stirring the mixture for 2.5 hr at 0° and 30 min at room temperature, the solution was washed to neutrality ($NaHCO_3$, H_2O), dried ($MgSO_4$), and evaporated. The residue was chromatographed on Al_2O_3. The fractions eluted with C_6H_6–petroleum ether were combined and crystallized from methanol to afford pure **1** (4.1 g, 77%). An analytical sample was obtained from CH_2Cl_2–MeOH.

Similarly, epoxidation of equilin THP and cyclopentyl ethers, respectively, prepared according to the procedures of Cross[17] and Ercoli[18] gave **2** and **3**.

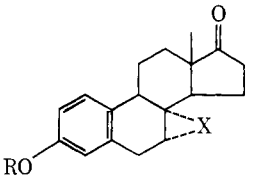
7α,8α-Methyleneestra-1,3,5(10)-triene-3,17β-diol. A 20% solution of triethylaluminum in hexane (494 ml) was drained directly from a cylinder into a three-necked flask. After dilution with C_6H_6 (300 ml), equilin (36 g) was added in portions, followed by a solution of redistilled CH_2I_2 (52 ml, 169 g) in C_6H_6 (70 ml). The mixture was refluxed for 24 hr. After cooling to 0°, MeOH (110 ml) was added dropwise, followed by H_2O (100 ml). The mixture was acidified (dilute HCl) and extracted with EtOAc. The extract was successively washed with $Na_2S_2O_3$ and H_2O, dried, and evaporated. The residue was crystallized from acetone–hexane to

†Some 7,8-epoxides derived from equilin are mentioned in patents.[1]

‡The formation in low yield of bicyclo[4.1.0]heptane in the reaction of cyclohexene with triethylaluminum and methylene iodide has been reported.[9]

§Other estrogens with the unnatural 8α configuration have been found to be as active as the corresponding 8β compounds.[16]

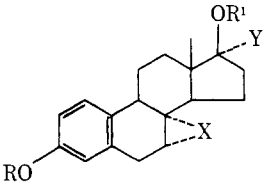
Table I. Physical Properties of the 7α,8α-Substituted Estrone Derivatives



No.	R	X	Mol formula[e]	$[\alpha]_D$[a]	Mp, °C	Solvent of crystn[b]	Yield, %
1	CH_3	O	$C_{19}H_{22}O_3$	+238	176–178	Mc–M	77
2	c-C_5H_{10}	O	$C_{23}H_{28}O_3$	+202	143–145	Mc–*i*E	73
3	2-THP[f]	O	$C_{23}H_{28}O_4$	+110.3	167–168	M	
4	H	CH_2	$C_{19}H_{22}O_2$	+249[c]	218–219	Mc–M	28[d]
5	CH_3	CF_2	$C_{20}H_{22}F_2O_2$	+209.6	135–136	Mc–H	73

[a]Rotations were determined in 1% $CHCl_3$ solutions at 24–25°. [b]The solvents of crystallization are *i*E, $(i\text{-Pr})_2O$; H, *n*-hexane; M, MeOH; Mc, CH_2Cl_2. [c]Rotation determined in dioxane. [d]Overall yield from equilin (two steps). [e]All compounds were analyzed for C and H and **5** also for F. The results were all within 0.4% of the calculated values. [f]2-Tetrahydropyranyl.

Table II. Physical and Biological Properties of 7α,8α-Substituted Estradiol Derivatives



No.	R	R^1	Y	X	Mol formula[f]	$[\alpha]_D$[a]	Mp, °C	Solvents of crystn[b]	Yield, %	Activity po: Allen–Doisy in rats, ED_{50}, μg	Activity po: Utero-trophic in mice, μg
6	17α-Ethynyl-3-methoxyestra-1,3,5(10)-trien-17-ol (mestranol)									46	0.5
7	17α-Ethynyl-3-methoxyestra-1,3,5(10),7-tetraen-17-ol									3.5	0.06
8	CH_3	H	C≡CH	O	$C_{21}H_{24}O_3$	+99.5	138–140 150–151	E–H	64.5	6	128
9	c-C_5H_{10}	H	C≡CH	O	$C_{25}H_{30}O_3$	+86.8	187–188	B–H	32	3.5	0.03
10	H	H	3-Furyl	O	$C_{22}H_{24}O_4$	+148.2[c]	154–156	N	49[d]	2.1	128
11	CH_3	H	3-Furyl	O	$C_{23}H_{26}O_4$	+120.9	223–225	Mc–E	64	4	4
12	CH_3	$COCH_3$	3-Furyl	O	$C_{25}H_{28}O_5$	+147.1	183–185	M	41	13	>512
13	$COCH_3$	$COCH_3$	3-Furyl	O	$C_{26}H_{28}O_6$	+137.9	209–210	Mc–M	27	16.5	>512
14	c-C_5H_{10}	H	3-Furyl	O	$C_{27}H_{32}O_4$	+108	162–163	A–H	57	4.6	128
15	CH_3	H	2-Furyl	O	$C_{23}H_{26}O_4$	+135.7	188–190	Mc–E	75	4.5	512
16	c-C_5H_{10}	H	2-Furyl	O	$C_{27}H_{32}O_4$	+115	152–153	A–H	67	14	256
17	CH_3	H	2-Thienyl	O	$C_{23}H_{26}O_3S$	+139.5	206–207	A–M	39	1.5	256
18	c-C_5H_{10}	H	2-Thienyl	O	$C_{27}H_{32}O_3S$	+115.1	Amorphous		25	4.2	64
19	H	H	C≡CH	CH_2	$C_{21}H_{24}O_2$	+137[e]	218–219	A–H	53.5	24	64
20	CH_3	H	C≡CH	CF_2	$C_{22}H_{24}F_2O_2$	+65.8	133–135	Mc–H	66	340	>512

[a]Rotations were determined in 1% $CHCl_3$ solutions at 24–25° unless otherwise indicated. [b]Solvents of crystallization: A, Me_2CO; B, C_6H_6; E, Et_2O; H, *n*-hexane; M, MeOH; Mc, CH_2Cl_2; N, CH_3NO_2. [c]Rotations in MeOH. [d]Overall yield from **3**. [e]Rotation in dioxane. [f]All compounds were analyzed for C and H. **17** and **18** were also analyzed for S. The results obtained were within 0.4% of the calculated values.

afford pure **19** (20 g, 52.5%): mp 182–184°; $[\alpha]_D$ +165° (dioxane). *Anal.* ($C_{19}H_{24}O_2$) C, H.

3-Hydroxy-7α,8α-methyleneestra-1,3,5(10)-trien-17-one (4). A solution of $N(Et)_3$-SO_3 complex[19] (7.8 g) in DMSO (25 ml) was added dropwise to a solution of **19** (5 g) in DMSO (50 ml) and $N(Et)_3$ (11 ml) cooled to 15°. The mixture was stirred for 30 min. The mixture was cooled, acidified (35 ml of 10% HCl), and diluted with H_2O (150 ml). The resulting gummy precipitate was dissolved in Et_2O and the solution was washed to neutrality. Crystallization of the residue from CH_2Cl_2–MeOH afforded pure **4** (2.6 g, 52.5%).

7α,8α-Difluoromethylene-3-methoxyestra-1,3,5(10)-trien-17-one (5). A warm (60°) solution of sodium chlorodifluoroacetate (86 g) in anhydrous diglyme (210 ml) was added over a period of 6.5 hr to a refluxing solution of equilin methyl ether (10.0 g) in diglyme (165 ml). The mixture was refluxed for an additional 90 min and left overnight at room temperature. The solid was filtered and the filtrate evaporated *in vacuo* at a temperature not exceeding 40°. The residue was chromatographed on silica gel. Elution with EtOAc–hexane (2:8) afforded pure **5** (8.6 g, 73%).

17α-Ethynyl-3-methoxyestra-1,3,5(10),7-tetraen-17-ol (7). A 29% suspension of sodium acetylide in xylene (18 ml) was centrifuged and the solid was washed three times by centrifugation with THF. A suspension of this solid in dry DMSO (60 ml) was added to an ice-cold suspension of equilin methyl ether (6 g) in THF (36 ml) and DMSO (48 ml). After stirring for 1 hr at room temperature, the mixture was again cooled and H_2O (240 ml) was added. The resulting solid was filtered, thoroughly washed (H_2O), and dried. Filtration through a column of silica gel (90 g, deactivated with 6% H_2O) and crystallization of the eluted product from MeOH–H_2O gave the title product (5 g, 76%). An analytical sample was obtained from Et_2O–C_6H_6: mp 178–180°; $[\alpha]_D$ +117.5° ($CHCl_3$). *Anal.* ($C_{21}H_{24}O_2$) C, H.

By the same procedure, 3-cyclopentyloxy-17α-ethynylestra-1,3,5(10),7-tetraen-17-ol was obtained from equilin cyclopentyl ether as an amorphous compound: $[\alpha]_D$ +75.6° ($CHCl_3$). *Anal.* ($C_{25}H_{30}O_2$) C, H. Similarly the ketones **4** and **5** were converted to **19** and **20**, respectively.

17α-Substituted 7α,8α-Epoxyestra-1,3,5(10)-triene-3,17-diol Derivatives (10–18). Reaction of **3** with 3-furyllithium,[2] followed

by removal of the THP group, afforded 10. Treatment of the ketones 1 and 2 with 2- and 3-furyllithium gave 11, 14, 15, and 16. The acetates 12 and 13 were obtained by heating a solution of 10 or 11 in Ac_2O and pyridine at 100° for 24 hr.[2]

Compounds 17 and 18 were prepared by treating the ketones 1 and 2 with 2-thienyllithium,[20] prepared *in situ* by reacting thiophene with *n*-BuLi. A solution of freshly distilled thiophene (4.2 g), Et_2O (84 ml), and *n*-BuLi in Et_2O (1.4 *N*, 34 ml) was stirred at −10° for 1 hr. A solution of 1 (4.2 g) in toluene (168 ml) was added and the mixture was stirred overnight at room temperature. After work-up, the crude product was chromatographed on basic Al_2O_3. The fractions eluted with C_6H_6–hexane (2:1) were combined and crystallized from Me_2CO–MeOH to give pure 17 (2.0 g). The cyclopentyl ether 18 was prepared by the same method.

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Quinazolines and 1,4-Benzodiazepines. 67.[1] 5-Ferrocenyl-1,4-benzodiazepin-2-ones

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In a continuation of our search for medicinally useful benzodiazepines, we have prepared 1,4-benzodiazepin-2-ones containing a ferrocenyl group at the 5 position. These compounds are, to our knowledge, the first stable benzodiazepines containing an organometallic substituent. The preparation and the evaluation of the CNS activity of these 5-ferrocenylbenzodiazepinones were of particular interest because of the reported similarity of many of the physical and chemical properties of the phenyl and ferrocenyl groups.[2]

Chemistry. The key step in the synthesis of these benzodiazepinones (Scheme I) was the preparation of the alcohol 1 (83% yield) *via* the condensation of 2-lithionitrobenzene[3] with ferrocenecarboxaldehyde at −90 to −95°. Despite the known sensitivity of ferrocenes to oxidation, the conversion of 1 to 2 was effected in high yield with activated MnO_2 at 25°. Surprisingly, the reduction of 2 to 3 was more difficult to achieve. Metal reducing agents such as $SnCl_2$ and Fe–HCl were without effect. The conversion of compound 2 to 3 by catalytic hydrogenation (Pd or Pt) was slow (48–72 hr) but did give 60–70% yields of the desired amino ketone. However, the use of the Pd-catalyzed hydrogen-transfer reaction of Braude, *et al.*,[4] gave 3 in almost quantitative yield in a clean, relatively rapid (*ca.* 18 hr) reaction. The conversion of 3 to the benzodiazepinones 5 and 6 was accomplished in the standard fashion *via* bromoacetylation and then ammonolysis of the resulting α-bromoacetanilide with liquid ammonia to give the (uncharacterized) α-aminoacetanilide which was cyclized with acid to the parent benzodiazepinone 5. Finally, N-methylation of 5 gave the desired benzodiazepinone 6.

Because of the known beneficial effect of 7-halogen substituents on the activity of benzodiazepines,[5] the 7-iodobenzodiazepinones 9 and 10 were also prepared. Iodination of the amino ketone 3 with ICl was erratic but gave the desired iodoamino ketone 7 in *ca.* 19% yield. The position of the introduced iodine was established by spectral data (see Experimental Section). In particular, there was no evidence of any product resulting from either iodination ortho to the amino group or in the "less-deactivated" bottom, unsubstituted π-cyclopentadienyl ring of the ferrocenyl substituent. Conversion of 7 to the benzodiazepinones 9 and 10 was accomplished in the usual fashion.

Pharmacology. Benzodiazepines 6 and 10 are both relatively nontoxic: compound 6, LD_{50} (mice) 775 mg/kg ip and 900 mg/kg po; compound 10, LD_{50} (mice) 900 mg/kg ip and 450 mg/kg po. Both compounds were inactive at the highest doses used when screened for muscle relaxant, anticonvulsant, and taming activity in mice: muscle relaxant activity (inclined screen, po), compounds 6 and 10 ED_{50} >400 mg/kg; anticonvulsant activity (antimetrazole po), compounds 6 and 10, ED_{50} >800 mg/kg; taming activity (foot shock, po), compounds 6 and 10, ED_{50} >100 mg/kg. The methods used in these tests have been previously described.[6]

Experimental Section

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. All new compounds possess ir (Perkin-Elmer Model 621 and Beckman IR-9 spectrophotomers), nmr (Varian Associates A-60 and HA-100 spectrometers, TMS internal standard), uv (Cary 14 and 15 spectrophotomers), and mass spectral data (CEC 110-21B and Jeolco 01SG double-focusing spectrometers, Hitachi RMU 6L single-focusing spectrometer) in agreement with their assigned structures. Where analyses are indicated only by the symbols of the elements, the analytical results obtained for those elements were within ±0.4% of the theoretical values.