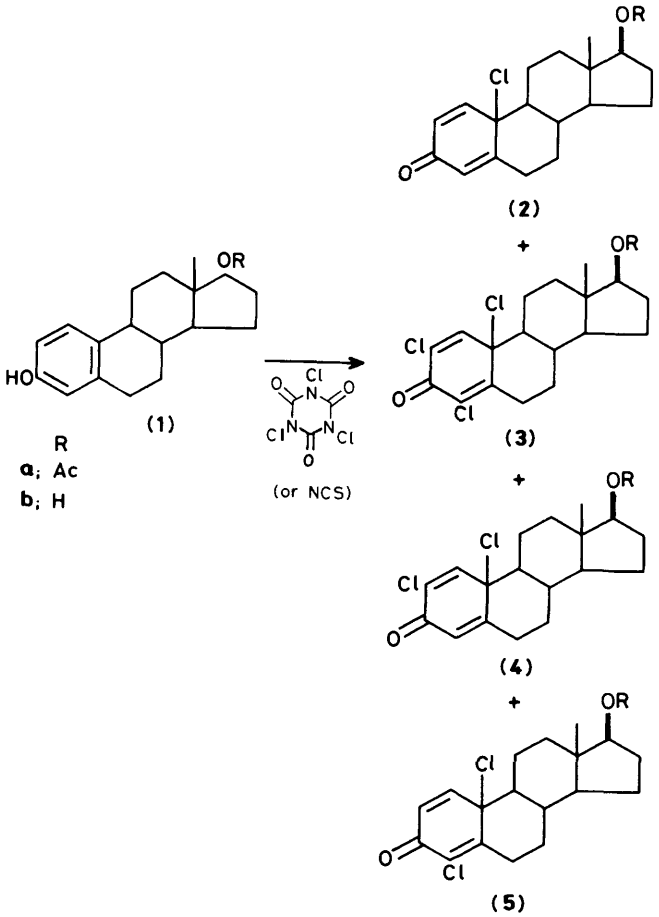
10β-Chloro-17β-hydroxyestra-1,4-dien-3-one and its Related Compounds

Fumikazu Mukawa
Shionogi Research Laboratories, Shionogi & Co., Ltd., Fukushima-ku, Osaka, 553 Japan

Chlorination of steroidal ring A phenols with *N*-chloro imide reagents (*e.g.* *N*-chlorosuccinimide and trichloroisocyanuric acid) afforded the 10β-chloroestra-1,4-dien-3-one (**2**) together with a smaller proportion of 2,4,10β-trichloroestra-1,4-dien-3-one (**3**), 2,10β-, and 4,10β-dichloroestra-1,4-dien-3-one [(**3**) and (**5**)]. In contrast with the results of bromination using *N*-bromo imide reagents, only substitution of aromatic ring A was observed. The structure of compound (**2b**) was established by an *X*-ray diffraction study. Compounds (**2a**) and (**2b**) were readily reduced to the original phenol, and compound (**2a**) underwent dienone–phenol rearrangement with acetic anhydride–sulphuric acid to yield 4-chloroestra-1,3,5(10)-triene-1,17-diyl diacetate (**7**).

Although ring A bromination of estrogens has been well described in the literature,[1] there are few reports of chlorination.[2] In an earlier study, the configuration of the 10β-chlorine substituent was assigned only on the basis of the similarity with the rotatory dispersion (r.d.) curve of cholesta-1,4-dien-3-one.[2a] Recent work has shown that the halogeno-labelled estrogen might be used to detect and/or determine the course of therapy of hormone-dependent tumours, and a high stability of fluoro-, chloro-, bromo-, and iodo-estradiol derivatives would then enable a correlation study between the size of the halogen and receptor-binding affinities.[3] Thus the chlorination of estrane derivatives was investigated.

Treatment of estradiol (**1b**) with trichloroisocyanuric acid



Scheme 1.

Table 1. 10β-Chloro-17β-hydroxyestra-1,4-dien-3-one derivatives (Scheme 1)

Compd.	Formula	M.p. (from acetone) (°C)	$[\alpha]_D$	^{1}H n.m.r. δ($CDCl_3$) (p.p.m.)
(**2b**)[a]	$C_{18}H_{23}ClO_2$	160	−15.4	0.83 (3 H, s, 18-Me), 6.03 (1 H, m, 4-H), 6.20 (1 H, m, 2-H), and 7.08 (1 H, d, *J* 4 Hz, 1-H)
(**3b**)	$C_{18}H_{21}Cl_3O_2$	196	−21.2	0.83 (3 H, s, 18-Me) and 6.78 (1 H, s, 1-H)
(**4b**)	$C_{18}H_{22}Cl_2O_2$	187	−24.2	0.83 (3 H, s, 18-Me), 6.08 (1 H, s, 4-H), and 7.23 (1 H, s, 1-H)
(**5b**)	$C_{18}H_{22}Cl_2O_2$	190	−18.1	0.83 (3 H, s, 18-Me), 6.18 (1 H, d, *J* 4 Hz, 2-H), and 7.09 (1 H, d, *J* 4 Hz, 1-H)

[a] Observed ^{13}C n.m.r. chemical shifts (p.p.m.): 185.0 (3), 161.1 (5), 147.8 (1), 126.7 (2), 123.8 (4), 81.4 (17), 67.7 (10), 53.6 (9), 50.0 (14), 43.1 (13), 36.0 (8), 35.8 (12), 32.3 (6), 32.3 (7), 30.4 (16), 24.0 (15), 22.9 (11), and 11.0 (18). Observed c.d.(MeOH) (nm) {[θ]}: 220 {17 500}, 2.45 {−19 900}, 276 {8 510}, and 352 {−1 770}.

(TCA) in t-butyl alcohol was initially carried out with or without a small amount of aqueous acetic acid. Better yields, however, were obtained in acetonitrile. The chlorinated products were separated and purified by medium-pressure column chromatography using a Merck 'Lobar' pre-packed column. They were subsequently identified as 10β-chloro-17β-hydroxyestra-1,4-dien-3-one (**2b**), 2,4,10β-trichloro-17β-hydroxyestra-1,4-dien-3-one (**3b**), 2-, and 4,10β-dichloro-17β-hydroxyestra-1,4-dien-3-one [(**4b**) and (**5b**)] (Table 1) (Scheme 1).

In contrast, when estradiol (**1b**) was treated with a slight excess of *N*-chlorosuccinimide (NCS), or with a large excess, the principal product was compound (**2b**) with low yields of compounds (**3b**), (**4b**), and (**5b**). Also, losses due to oxidation of the 17β-hydroxy entity were smaller than they were for TCA.

The structure of 10β-chloro-17β-hydroxyestra-1,4-dien-3-one (**2b**) is compared with that of 17β-hydroxyandrosta-1,4-dien-3-one[4] (**6**) in Figure (*a*) and its *X*-ray structure is given in Figure (*b*). Figure (*a*) shows that the chlorine atom of compound (**2b**) is oriented over ring A of the steroid nucleus in a manner virtually identical with that of the 10-methyl substituent in compound (**6**). A detailed study of the structure of the solution state of compound (**2b**) is now in progress.

In a bioassay study,[5] compound (**2b**) showed estrogenic activity with about 30% of the binding activity of estradiol for

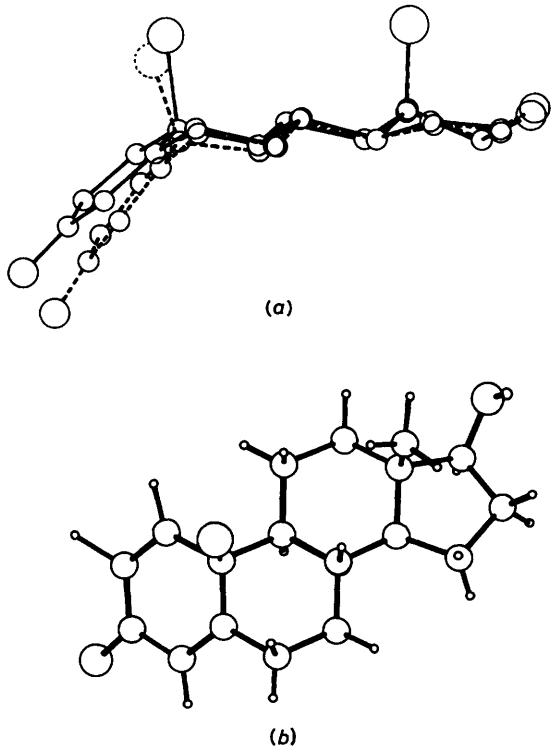
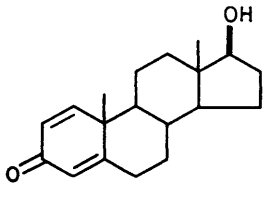


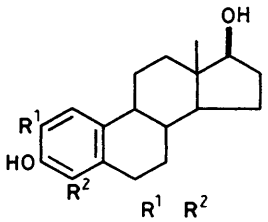
Figure. (*a*) The superposition of 17β-hydroxyandrosta-1,4-dien-3-one (**6**) and 10β-chloro-17β-hydroxyestra-1,4-dien-3-one (**2b**). The open circles and solid line represent compound (**6**) whereas open circles and broken line represent compound (**2b**). (*b*) *X*-Ray structure of compound (**2b**)

the estrogenic receptor (uterine cytosol). *In vivo* assay of compound (**2b**) showed long-lasting estrogenic action when tested with adult overectomized mice. A hormonal response of steroids is contingent to their binding to specific receptors in the target tissue and the common feature of compounds that compete for the estrogen receptor is a phenol ring.[6] These findings coupled with the observation that compound (**2b**) undergoes further *in vivo* pathways, particularly enzymatic or non-enzymatic reduction at ring A, prompted investigation of its chemical characteristics.

Reduction Dehalogenation of Compounds (**2**)—(**5**).—The preferential removal of the 10β-chloro substituent by ordinary chemical reduction was studied. Reduction of compound (**2b**) with sodium borohydride or zinc dust in ethanol regiospecifically give back estradiol. In a similar way, the 2- and 4,10β-dichloro compounds (**4b**) and (**5b**) were efficiently demonochlorinated to give the corresponding 2-chloroestradiol (**8a**) or 4-chloroestradiol (**8b**) as the major products, accompanied by estradiol (**1b**) as the minor product. These observations afford a convenient method for preparing 2- and 4-chloroestradiol.* Treatment of compound (**3b**) with an excess of sodium borohydride gave a mixture of 2,4-dichloroestradiol (**8c**), 4-chloroestradiol (**8b**), and estradiol,† which were isolated by silica gel chromatography.

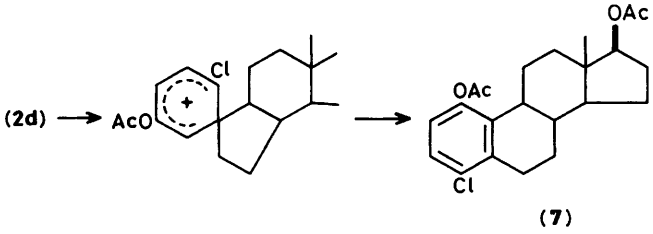


(**6**)



	R^1	R^2
(**8**) **a**;	Cl	H
b;	H	Cl
c;	Cl	Cl

Acid-catalyzed Rearrangement of Compound (**2a**).—Mills *et al.*[2b] have reported that 10β-fluoro-17β-hydroxyestra-1,4-dien-3-one readily undergoes dienone–phenol rearrangement with acetic anhydride–sulphuric acid to yield the 4-fluoroestratrienediyl diacetate. In a similar way, treatment of compound (**2a**) with acetic anhydride containing a little sulphuric acid gave a higher yield of a 4-chloroestratrienediyl diacetate (**7**) (Scheme 2).



(**7**)

Scheme 2.

Experimental

M.p.s were determined with a Yanagimoto melting point apparatus and were uncorrected. I.r. spectra were recorded on a Jasco-A-702 spectrophotometer. N.m.r. spectra were obtained with a Varian XL-200 spectrometer for ^1H and with a Varian XL-100-12 for ^{13}C. Chemical shifts are reported in δ (p.p.m.) from the internal standard Me_4Si in [^2H]chloroform. Low resolution mass spectra were recorded on a Hitachi M68 mass spectrometer. Optical rotation was measured on a Perkin-Elmer polarimeter 241. Medium-pressure column chromatography on Merck 'Lobar' pressure columns packed with Lichroprep Si60 [size C (440—37 mm, 63—125 μm), size B (310—25 mm, 43—63 μm), and size A (240—10 mm, 43—63 μm)] were carried out for separation. Elemental analyses were with 0.3% of the calculated values and are available as a supplementary publication [SUP. NO. 56715 (1 pp.)].‡

Chlorination of Estradiol with Trichloroisocyanuric Acid.—TCA (2.1 g) was added in one portion to an ice-cooled solution of the estradiol (**1b**) (5 g) in acetonitrile (200 ml). After 30 min, the reaction was complete (neg. starch–KI paper). The solution was poured into ice–water and the products were extracted with dichloromethane. The organic layer was successively washed with aqueous sodium hydrogen sulphite (5%), 5% aqueous sodium hydrogen carbonate and water, dried (Na_2SO_4), evaporated, and the resulting oil applied to a 'Lobar' prepacked column (size C). Elution with toluene–ethyl acetate (9:1) gave compound (**3b**) (1.3 g, 25%, non-polar fraction), a mixture of compounds (**4b**) and (**5b**) (0.8 g, 13%, next most polar fraction) which ^1H n.m.r. analysis showed to be about 1:1, and compound (**2b**) (1.1 g, 20%, most polar fraction). By rechromatography on silica gel, compounds (**4b**) and (**5b**) were separated and purified (Table 1).

For estradiol 17β-monoacetate, three chlorinated products were obtained, although separation of compounds (**4a**) and (**5b**) did not proceed smoothly on silica gel column chromatography.

* Chlorination of estradiol with chlorine or sulphuryl chloride failed to afford the desired 4-chloroestradiol. See ref. 2*c*.

† Reduction dechlorination was observed, although the reason for this behaviour is not yet clear. See ref. 7.

‡ For details of the supplementary publications scheme, see Instructions for Authors (1988), *J. Chem. Soc., Perkin Trans. 1*, 1988, Issue 1.

Table 2. Atomic co-ordinates with e.s.d.s in parentheses

	x	*y*	*z*
C(1)	0.861 6(2)	0.121 7(2)	0.895 7(5)
C(2)	0.755 4(2)	0.136 3(2)	0.860 1(6)
C(3)	0.707 5(2)	0.119 3(2)	0.679 8(6)
C(4)	0.771 1(2)	0.080 7(2)	0.537 0(5)
C(5)	0.877 4(2)	0.064 0(1)	0.572 1(5)
C(6)	0.945 2(2)	0.027 5(2)	0.424 9(5)
C(7)	1.042 7(2)	0.075 9(1)	0.371 8(4)
C(8)	1.106 7(2)	0.096 7(1)	0.547 9(4)
C(9)	1.032 1(2)	0.133 7(1)	0.695 9(4)
C(10)	0.932 5(2)	0.087 0(1)	0.749 4(4)
C(11)	1.095 9(2)	0.161 4(2)	0.874 6(4)
C(12)	1.194 1(2)	0.208 3(2)	0.816 0(5)
C(13)	1.266 9(2)	0.170 9(1)	0.672 7(4)
C(14)	1.199 7(2)	0.148 6(1)	0.497 9(5)
C(15)	1.283 8(2)	0.126 2(2)	0.349 0(4)
C(16)	1.380 6(2)	0.178 8(2)	0.388 0(5)
C(17)	1.351 8(2)	0.219 0(1)	0.570 8(5)
C(18)	1.327 1(2)	0.107 9(1)	0.772 0(5)
O(3)	0.612 7(2)	0.137 1(1)	0.639 0(6)
Cl(10)	0.980 9(1)	0.005 0(1)	0.869 9(1)
O(17)	0.442 7(2)	0.240 8(1)	0.683 1(4)

Reaction of Estradiol with N-*Chlorosuccinimide.*—A solution of estradiol (1.0 g) in acetonitrile (40 ml) was warmed to 80—85 °C and NCS (0.73 g) was added in one portion. After 2 h the solution was poured into ice–water. The product was isolated with dichloromethane and subjected to a work-up similar to that described above. The resulting oil was purified by silica gel column chromatography to give pure samples (Table 1).

X-*Ray Analysis** *of Compound* (**2b**).—*Crystal data.* $C_{18}H_{23}O_2Cl$, M = 306.5. Orthorhombic, space group $P2_12_12_1$, a = 12.293(20), b = 18.692(3), c = 6.986(1) Å, U = 1 605.2(4) Å^3, Z = 4, D_c = 1.270 g cm^{-3}. 1 287 Reflections of 1 588 unique ones were observed on a RIGAKU AFC-5R diffractometer using Cu-K_α radiation. The structure was solved by direct methods and refined by the block-diagonal least-squares technique to R = 0.038. All the hydrogen atoms were located on a difference electron density map, and their positional parameters were refined at the last stage of the least-squares refinement. Atomic co-ordinates for compound (**2b**) are given in Table 2. Tables of anisotropic thermal parameters, bond lengths, and bond angles are available on request from the Cambridge Crystallographic Data Centre.*

Reduction of 10β-*Chloroestra*-1,4-*dien*-3-*one* (**2**) *and its Chloro Derivatives* (**3**), (**4**), *and* (**5**).—*General procedure.* Each of the 10β-chloro dienones suspended in 99% ethanol was treated with a large excess of sodium borohydride whilst being stirred at room temperature for 2 h. Hydrogen was evolved and all of the material dissolved. A few drops of acetic acid were added to destroy the excess of sodium borohydride and after dilution with water, the product was extracted with dichloromethane. Pure samples were obtained by silica gel column chromatography using toluene–ethyl acetate (8:1) as eluant.

(*i*) *Reduction of compound* (**2b**). Reduction of compound (**2b**) (30 mg) with sodium borohydride (10 mg) gave estradiol (21 mg), m.p. 178—179 °C. The t.l.c. and spectral data were identical with those of the authentic sample.

(*ii*) *Reduction of compound* (**3b**). Reduction of compound (**3b**) (70 mg) with sodium borohydride (20 mg) gave a gum which was separated by silica gel column chromatography. From the non-polar fraction, 2,4-dichloroestradiol (**8c**) (21 mg) was obtained and recrystallized from MeOH, m.p. 208—210 °C; δ($CDCl_3$) 0.83 (3 H, s, 18-Me) and 7.26 (1 H, s, 1-H). From the next most polar fraction, estradiol (15 mg) was obtained, m.p. 175—179 °C. It was identical with the authentic sample according to i.r., t.l.c., and mixed m.p. From the most polar fraction, 4-chloroestradiol (**8b**) (25 mg) was obtained. It was crystallized from chloroform–MeOH, m.p. 255—261 °C. δ($CDCl_3$) 0.79 (3 H, s, 18-Me), 3.68 (1 H, t, *J* 2 Hz, 17α-H), 6.82 (1 H, d, *J* 9 Hz, 2-H), and 7.15 (1 H, d, *J* 9 Hz, 1-H).

(*iii*) *Reduction of compound* (**4b**). Reduction of compound (**4b**) (150 mg) with sodium borohydride (43 mg) gave a gum which was separated by silica gel chromatography. 2-Chloroestradiol (**8a**) (70 mg,), m.p. 271—273 °C; δ($CDCl_3$) 0.78 (3 H, s, 18-Me), 6.73 (1 H, s, 4-H), and 7.33 (1 H, s, 1-H).

(*iv*) *Reduction of compound* (**5b**). Reduction of compound (**5b**) (70 mg) with sodium borohydride (20 mg) gave a gum which was separated by silica gel chromatography. Initial elution afforded estradiol, m.p. 173—175 °C, and continued elution gave 4-chloroestradiol (**8b**) (35 mg), m.p. 253—255 °C. These compounds were identified by comparison of mixed m.p., t.l.c., and i.r. spectral data with those of the authentic samples.

Zinc–ethanol reduction. A solution of compound (**5b**) (0.1 g) in ethanol (10 ml) was refluxed for 15 min with zinc dust (washed with dilute hydrogen chloride) (200 mg). The clear solution was decanted into ice–water and the zinc residue washed with ethanol. The precipitate was filtered off and compound (**8b**) (73 mg) was separated by silica gel column chromatography. The t.l.c. and i.r. spectral data were identical with those of the authentic specimen.

Reduction with zinc–acetic acid[2a,c] gave a mixture of 17β-acetoxy- and 17β-hydroxy-4-chloroestradiol.

Dienone–phenol Rearrangement of Compound (**2a**).—Compound (**2a**) (0.3 g) in acetic anhydride (3 ml) was treated with concentrated sulphuric acid (2 drops). After 3 h at room temperature, the solution was poured into aqueous potassium hydrogen carbonate and recrystallized from cyclohexane–benzene to yield compound (**7**) (0.18 g), m.p. 120—121 °C; $[\alpha]_D^{23.5}$ +134.3 (*c*, 1.0, chloroform); *m/z* 390; δ($CDCl_3$) 0.80 (3 H, s, 18-Me), 2.05 (3 H, s, 17-OAc), 2.25 (3 H, s, 1-OAc), 4.70 (1 H, t, *J* 7.5 Hz, 17α-H), 6.75 (1 H, d, *J* 8.0 Hz, 2-H), and 7.17 (1 H, d, *J* 8.0 Hz, 3-H).

In a similar way, compound (**2b**) gave compound (**7**).

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