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Review

The physicochemical properties of oestradiol

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Abstract: The spectral, solubility and related physicochemical characteristics of the ovarian hormone are presented. A review of its crystal properties indicates that the common crystalline form of the steroid is the hemihydrate.

Keywords: *Oestradiol; physicochemical properties; crystal characteristics.*

Introduction

Oestradiol (oestra-1,3,5(10)-triene-3,17β-diol; 17β-oestradiol; CAS 50-28-2; Fig. 1) is the most potent oestrogenic hormone secreted by the ovaries of pre-menopausal women. Although subcutaneous implants of the steroid have been used for post-climacteric replacement therapy since shortly after the steroid was isolated in the mid-1930s, there has recently been a resurgence of interest in the clinical use of the natural hormone in view both of the hepatic, metabolic and vascular side-effects associated with long-term administration of synthetic analogues and conjugated equine oestrogens [1] and developments in methods of drug delivery, notably by the transdermal route [2, 3]. In this paper the physicochemical characteristics of oestradiol pertinent to its formulation are reviewed: some data of pharmaceutical interest are collated and, in particular, its crystal properties are reassessed. A more comprehensive monograph on the drug has been prepared [4].

Figure 1
17β-Oestradiol; $C_{18}H_{24}O_2$.

Absorption, Resonance and Mass Spectra

Infrared spectrum

The IR spectrum of oestradiol hemihydrate is illustrated in Fig. 2; detailed assignments have been proposed by Smakula *et al.* [5].

The spectrum in Fig. 2 is identical to those reported by Hayden *et al.* [6], presented in the Atlas of Steroid Spectra [7] and supplied by the MRC Steroid Reference Collection, London [8], but differs from the spectrum in the Sadtler Standard Spectra File [9] and in the compilation of Sammul *et al.* [10], which also differ from each other.

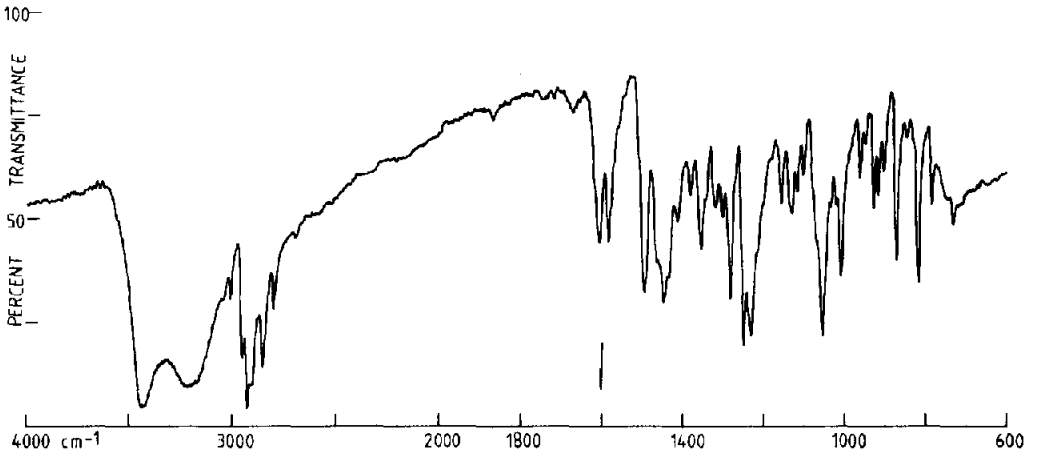


Figure 2
IR spectrum of oestradiol hemihydrate (KCl disc, polystyrene marker at 1602 cm $^{-1}$).

Nuclear magnetic resonance spectra

^{1}H- and ^{13}C-NMR spectra of oestradiol hemihydrate in acetone-d_6 were obtained with a Brucker WM250 spectrometer at 250.13 and 62.9 MHz, respectively (TMS internal standard). Assignments for the proton spectrum (Fig. 3; unpublished results) are:

δ(ppm)	
7.09	(1H, *d*, *J* = 8.3 Hz, H-1)
6.59	(1H, *dd*, *J* = 8.3, 2.8 Hz, H-2)
6.52	(1H, *d*, *J* = 2.8 Hz, H-4)
4.10	(3H, *br.s*, 2 × OH and ½ H_2O)
3.67	(1H, *dd*, *J* = 8.8, 8.1 Hz, H-17α)
2.76	(2H, *m*)
2.28	(1H, *m*)
2.20–1.10	(12H, *m*)
0.77	(3H, *s*, Me-18).

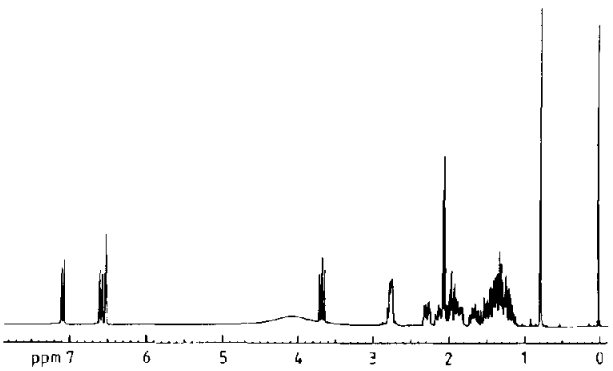


Figure 3
^{1}H-NMR spectrum of oestradiol hemihydrate.

The following assignments for the ^{13}C-NMR spectrum (Fig. 4; unpublished results) concur with the literature [11]:

δ(ppm)	δ(ppm)
155.89 (*s*, C-3)	44.04 (*s*, C-13)
138.38 (*s*, C-5)	40.03 (*d*, C-8)
132.09 (*s*, C-10)	37.76 (*t*, C-12)
126.94 (*d*, C-1)	31.02 (*t*, C-16)
115.96 (*d*, C-4)	30.30 (*t*, C-6)
113.58 (*d*, C-2)	28.13 (*t*, C-7)
81.86 (*d*, C-17)	27.26 (*t*, C-11)
50.96 (*d*, C-14)	23.80 (*t*, C-15)
44.90 (*d*, C-9)	11.63 (*q*, C-18).

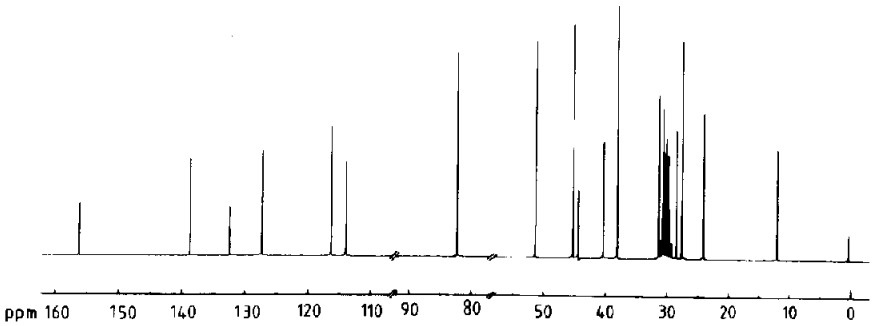


Figure 4
^{13}C-NMR spectrum of oestradiol hemihydrate.

Ultraviolet spectrum

In 2% v/v methanol, oestradiol hemihydrate exhibits maxima at 221 nm ($A1\%$ 1 cm 289) and 280 nm ($A1\%$ 1 cm 75), with a shoulder at 287 nm. Absorbance values quoted for oestradiol in other media are:

	λ_{max}(nm)	$A1\%$ 1 cm
0.1 M hydrochloric acid [12]	278	76
80% v/v methanol, alkalinified [7]	285.5	69
0.1 M sodium hydroxide [12]	238	341
	296	102

The absorption spectrum of oestradiol undergoes bathochromic shifts with increase in pH (isosbestic point at 285.5 nm) [7]. Spectra in concentrated sulphuric and other mineral acids are illustrated in the monograph by Engel [13].

Mass spectrum

The following assignments of a high-resolution mass spectrum, obtained using an AEI (Kratos) MS9 spectrometer (electron impact ionization, direct insertion), agree to six

significant figures with the experimental mass data for oestradiol hemihydrate (unpublished results):

m/z	Composition	Relative intensity
272	$C_{18}H_{24}O_2$ [M^+]	100
213	$C_{15}H_{17}O$	46
186	$C_{13}H_{14}O$	14
185	$C_{13}H_{13}O$	13
172	$C_{12}H_{12}O$	27
160	$C_{11}H_{12}O$	43
159	$C_{11}H_{11}O$	29
158	$C_{11}H_{10}O$	13
146	$C_{10}H_{10}O$	28
145	$C_{10}H_9O$	18
133	C_9H_9O	23

A simulated low-resolution plot of the spectrum is illustrated in Fig. 5. The chemical ionization mass spectrum has been described [14].

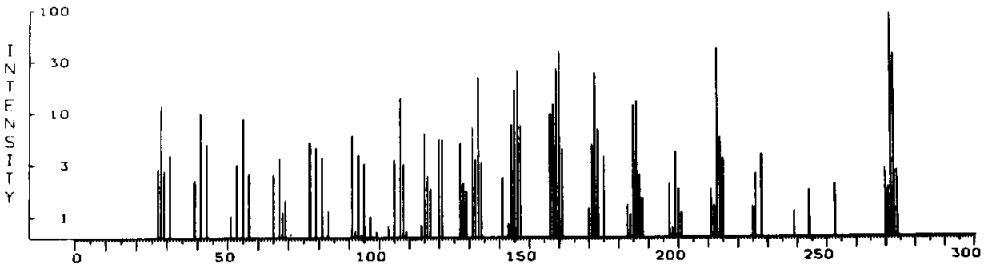


Figure 5
Simulated mass spectrum (low resolution) of oestradiol hemihydrate.

Dipole Moment, Optical Rotation and pKa

The *dipole moment* of "anhydrous" oestradiol in dioxane was determined to be 2.33 D (7.772×10^{-30} Cm) [7].

Specific optical rotation $[\alpha]_D$ values reported are: (22–24°C, dioxane) +76° [7]; (18°C, ethanol) +78° [15]. Values at different wavelengths and temperatures, and in other solvents, are quoted in Beilstein [16].

Apparent pKa values for the phenolic OH, determined spectrophotometrically, are: 10.12 ± 0.025 s.d. [17]; 10.30 ± 0.10 m.s.e. [18]; 10.71 ± 0.02 s.d. [19].

Solubility, Complexation and Distribution Ratios

The solubilities of oestradiol in some aqueous and organic media are listed in Tables 1–3. However these values, particularly for aqueous solutions, should be accepted cautiously because (as is often the case with poorly soluble drugs) the apparent solubility of oestradiol depends to a degree upon the procedure used. For instance, the equilibrium concentrations achieved by initially unsaturated solutions were found to differ from

Table 1
Solubility (mg dl^{-1}) of oestradiol in aqueous solvents

	Temperature (°C)						
	20	25	35	37	42.5	50	Ref.
Water			0.399				[20]
	0.17	0.30	0.56		0.77	0.97	[21]
0.02 M sodium chloride				0.38			[22]
0.20 M sodium chloride				0.34			
0.40 M sodium chloride				0.28			
Phosphate buffer,							
pH 7.2, 0.05 I				0.38 ± 0.046			
pH 7.2, 0.10 I				0.36 ± 0.016			
pH 7.2, 0.20 I				0.34 ± 0.024			
0.005 M L-tyrosine in phosphate buffer,							
pH 7.2, 0.10 I				0.66 ± 0.049			
Phosphate buffer,							
pH 7.4, 0.15 I				0.512			[23]
0.02 M sodium deoxycholate in phosphate buffer,							
pH 7.4, 0.15 I				3.92			

Table 2
Solubility (mg dl^{-1}) of oestradiol in organic solvents [24]

	Temperature (°C)				
	0	15	25	30	40
Acetone	2594.1	4291.2	7068.8	8914.8	13715.2
Benzene	—	21.7	46.7	80.1	198.3
Chloroform	140.5	251.2	411.4	642.7	762.0
Cyclohexane	—	0.5	1.3	2.2	7.6
Dichloromethane	45.1	108.4	192.6	267.1	—
Dioxane	—	7075.2	12085.6	19868.4	30968.0
Ethanol	1541.8	2387.2	3134.4	3727.4	4890.7
95% v/v Ethanol	1060.4	1606.9	2908.8	4186.3	4805.3
Diethyl ether	533.2	700.8	754.5	836.7	—
Hexane	<0.5	<0.5	<0.5	0.9	—
Methanol	862.1	1811.3	2548.8	3525.6	5342.4
Tetrahydrofuran	25447.2	28006.4	29222.4	33788.8	43313.6
Toluene	4.3	14.8	33.1	51.5	95.0

Table 3
Solubility (mg g^{-1}) of oestradiol in organic solvents at 22°C [25–27]

1-Decanol	28
Dimethyl sulfoxide	>500
Ethyl oleate	16
Ethylene glycol	16
Ethylene glycol: polyethylene glycol 400, 1:1 w/w	78
Glycerol	1.5
Polyethylene glycol 400	105*
Polysorbate 80 (Tween 80)	36
Propylene glycol	75
80% w/w Propylene glycol	2.8
60% w/w Propylene glycol	0.52
40% w/w Propylene glycol	0.10
20% w/w Propylene glycol	0.023
Propylene glycol: glycerol, 1:1 w/w	25

*167×10^2 mg dl^{-1} in dry solvent at 35°C [20].

those attained by supersaturated solutions [24], and whereas shaking an aqueous suspension for 48 h resulted in a solubility of 0.319 mg dl $^{-1}$ at 25°C, ultra-sonication for 0.5 h instead produced a concentration of 0.613 mg dl $^{-1}$ [28]. Also, although oestradiol does not (unlike its metabolite oestrone) appreciably adsorb to glass or plastic containers [29], it may be taken up by filters used to clarify supernatants [30].

Although the high (25 mg dl^{-1}) concentration rapidly (and transiently) achieved by a 1:9 solid co-precipitate of oestradiol in polyvinylpyrrolidone 40 000 was attributed to the steroid being molecularly dispersed within the polymer [23], the aqueous solubility of oestradiol may be modified by association with a variety of compounds. The amino acids tyrosine (Table 1), arginine and lysine promote solubility [22], as do progesterone [30] and human serum albumin [31, 32] (which admixed with propylene glycol has been used to prepare intravenous injections of 100 mg dl^{-1} concentration [33]). Polysorbate 20 (at 20°C) and sodium dodecyl sulphate (at 40°C) maximally solubilize oestradiol at 13 and 25 mmol per mole surfactant, respectively [34], the solubilizing capacity approximately doubling over the range 20–50°C [35]. During the incorporation of oestradiol into egg lecithin vesicles the ratio was found to change from 0.0176 to 0.0422 mmol per mole lipid depending on the method of preparation [28]. Polyethylene glycol (PEG) 400 is an excellent co-solvent for oestradiol, the solubility of which rises exponentially with increase in PEG concentration [20, 26, 36]. β-Cyclodextrin complexes with oestradiol in aqueous solution [37]; phase solubility analysis at 25°C indicated a Type-B_S curve (Fig. 6), with a formation constant of $3.2 \times 10^4 M^{-1}$ (unpublished results), comparable with those for other steroids [38]. The cyclodextrin complex, isolated as a white amorphous powder by interfacial co-precipitation [39], exhibited a rapid rate of dissolution and a solubility of 12 mg dl^{-1} at 25°C (Fig. 7). In contrast, both urea and digitonin form only sparingly soluble complexes with oestradiol; columnar orthorhombic crystals of the 1:1 complex precipitate from a solution in benzene of oestradiol and urea in 1:10 mole ratio [40], whereas needles of the digitonide complex (m.p. 265°C) may be obtained by mixing

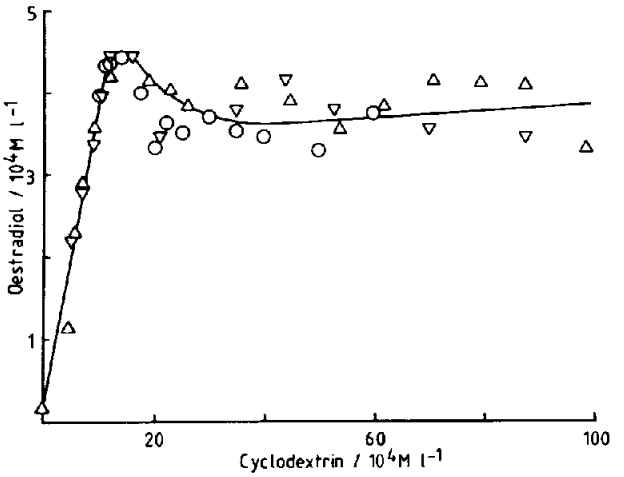


Figure 6
Phase solubility analysis curve for oestradiol and β-cyclodextrin in water at 25°C: symbols represent replicate analyses.

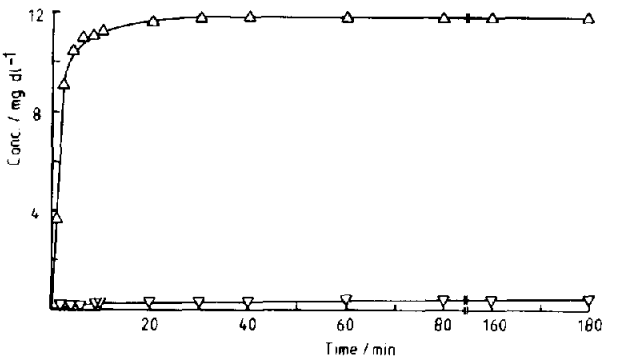


Figure 7
Dissolution curves for oestradiol in water at 25°C: △, oestradiol–cyclodextrin complex; ▽, commercial micronised oestradiol hemihydrate.

solutions of the steroid with 1–4% w/v digitonin in 80% v/v ethanol [41, 42]. Apparently the urea complex is not of the usual clathrate type: crystallographic analysis indicates a $P2_12_12_1$ space group, the molecular arrangement consisting of alternate layers of oestradiol and urea hydrogen bonded in planes roughly perpendicular to each other [40]. Both urea and digitonide complexes are readily cleaved by warming in water and dry pyridine, respectively.

Oestradiol is sufficiently soluble in peanut and sesame oils for them to be used as vehicles for intramuscular injections [43]; its solubility in soybean oil at 37°C is 160 mg dl^{-1} [44].

The distribution ratios of oestradiol in several dozen systems have been compiled by Engel [13]; a selection of values at room temperature are listed in Table 4. Lundberg [21] has determined the thermodynamic parameters associated with the partitioning of oestradiol between octanol and water.

Table 4
Distribution ratios of oestradiol [13]

Solvent system	D_c
Benzene–water	∞
Benzene–1.54 M hydrochloric acid	∞
Benzene–0.10 M sodium hydroxide	0.23
Benzene–1.0 M sodium hydroxide	0.04
Benzene:petroleum ether, 1:1–water	24
50% v/v Methanol–carbon tetrachloride	2.10
Water–carbon tetrachloride	0.08
Diethyl ether–water	55
Diethyl ether–1.6 M hydrochloric acid	50
Diethyl ether–0.10 M sodium hydroxide	2.0
Diethyl ether–1.0 M sodium hydroxide	0.7
Ethyl acetate–water	28
Hexane–water	1.07
Petroleum ether (35–60°)–water	0.79

Crystal Properties

Oestradiol, a white, odourless, tasteless powder, exhibits a variety of phases and transformations: this has led to a degree of confusion in the somewhat fragmented literature, e.g. different IR spectra in reference collections. Here the crystal properties of oestradiol are put into perspective by presenting a resumé of published work, aspects of which are reinterpreted in the light of a close comparison of data and some unpublished results.

The most noteworthy property of crystalline oestradiol is its tendency to adopt the hemihydrated form, as which phase it precipitates from not only partially aqueous solutions but also from ethyl acetate [5], chloroform [6], absolute ethanol [23, 45] and other apparently anhydrous solvents [46]. In ignorance of this characteristic some IR and crystallographic data have been erroneously ascribed: for instance, the X-ray powder diffraction photograph and data of Parsons and Beher [47] and the data cited in the JCPDS File [48] properly refer to oestradiol hemihydrate (Table 5); similarly the 'anhydrous' single crystal analysis reported by Norton *et al.* [49], and the 'monohydrate' data cited in *Structure Reports* [50] also probably refer to the hemihydrate. The powder diffractometer curve for oestradiol hemihydrate is illustrated in Fig. 8 and the interplanar spacings calculated from the most prominent peak positions are listed in Table 5 (unpublished results). Its crystallographic parameters are [45]: $a = 12.055 \pm 0.003$ Å, $b = 19.280 \pm 0.003$ Å, $c = 6.632 \pm 0.002$ Å, $Z = 4$, $D_x = 1.21$ g cm^{-3}, $D_m = 1.20$ g cm^{-3}, orthorhombic system, space group $P2_12_12$. The water molecules are located on a binary axis in association with the A- and D-rings of steroidal molecules packed 'head-to-tail', and participate in the hydrogen bonding which supports the lattice (Fig. 9).

The differential thermal analysis (DTA) curve of oestradiol hemihydrate exhibits endothermic peaks beginning at 112 and 174°C prior to the melting endotherm at 179°C [51] (Fig. 10a; similar curves have been obtained using differential scanning calorimetry (DSC) [23, 46, 52]). Simultaneous effluent gas analysis of DSC specimens indicated that

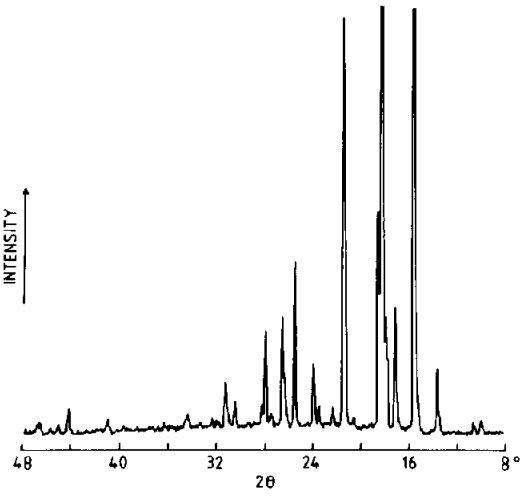


Figure 8
X-Ray powder diffractometer curve for oestradiol hemihydrate (2° 2θ min^{-1} scanning rate).

Table 5
X-Ray powder diffraction data for oestradiol

Hemihydrate*			Parsons and Beher [47]†		JCPDS [48]‡	
2θ	d(Å)	I/I_0	d(Å)	I/I_0	d(Å)	I/I_0
10.06	10.21	2	7.50	4	10.18	1
10.72	9.57	2	6.71	9	9.65	<1
13.66	7.53	11	6.03	4	7.51	7
15.60	6.60	100	5.64	10	6.63	100
17.16	6.00	20	5.00	4	6.01	9
18.20	5.66	100	4.78	9	5.75	6
18.60	5.54	36	4.63	4	5.67	50
20.60	5.01	2	4.32	6	5.56	95
21.43	4.81	68	4.08	4	4.99	20
22.40	4.61	3	3.92	6	4.82	80
23.40	4.41	3	3.72	5	4.61	20
23.94	4.31	11	3.35	6	4.39	2
25.50	4.06	28	3.24	3	4.31	45
26.60	3.89	18	3.13	3	4.04	18
27.92	3.71	15	3.02	3	3.92	6
28.20	3.67	3	2.90	2	3.90	50
30.44	3.410	4	2.80	4	3.76	1
31.26	3.323	7	2.65	1	3.70	20
			2.56	2	3.67	3
			2.49	4	3.40	3
			2.40	2	3.33	40
			2.32	1	3.25	2
			2.27	2	3.21	5
			2.21	2	3.16	1
			2.14	4	3.10	3
			2.08	2	3.03	4
			2.00	2	3.02	1
			1.93	3	2.917	3
			1.86	2	2.876	3
			1.79	2	2.784	5
					2.716	1
					2.665	2
					2.638	1
					2.562	3
					2.535	2
					2.489	8
					2.460	5
					2.381	3
					2.338	2
					2.308	3
					2.266	3
					2.225	3
					2.207	3

*Phillips PW 1050/35 scanning goniometer. Co K_α λ = 1.79026 Å, Cr_2O_3 internal standard (Fig. 8).
†Debye–Scherrer camera, Cu K_α λ = 1.5418 Å.
‡Guinier camera, Cu $K_{\alpha 1}$ λ = 1.5405 Å; unit-cell data: a = 12.04 Å, b = 19.26 Å, c = 6.642 Å, orthorhombic system.

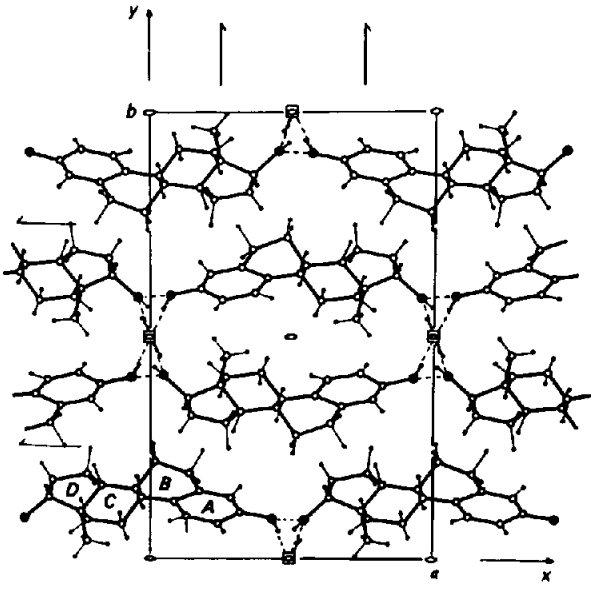


Figure 9
Packing diagram (projection along z-axis) for oestradiol hemihydrate [45]. The location of water molecules on a two-fold axis is illustrated; broken lines indicate hydrogen bonds.

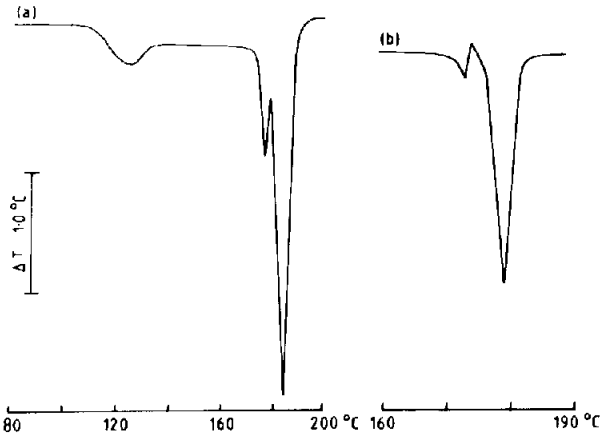
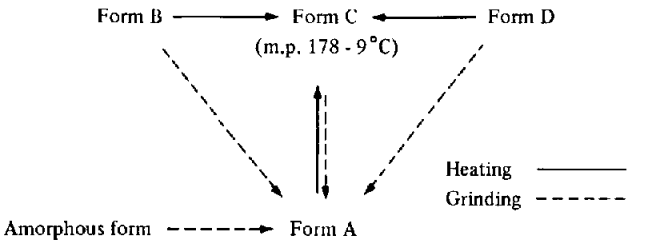


Figure 10
DTA curves for oestradiol hemihydrate: (a) 10°C min^{-1} and (b) 2°C min^{-1} heating rate (Stanton Redcroft 671B analyser, 8 mg specimens, open cups in static ambient atmosphere, alumina reference).

the two pre-melting endotherms were associated with solvent loss [46, 52]. Thermogravimetric analysis was inconclusive (due to the small mass changes involved), but Karl Fischer titration of a sample which had been maintained for 1 h at 148°C (i.e. above the temperature of the first DTA peak, which was abolished) indicated 1.8% w/w residual moisture, cf. 3.5% w/w initially (unpublished results). Thermomicroscopic examination of hemihydrated crystals had suggested that some structural rearrangement occurred prior to melting [46]; the pre-melting endotherm–exotherm doublet on DTA (Fig. 10b)

and DSC [23, 52] curves obtained at low heating rates would support this view. It appears then that oestradiol hemihydrate desolvates in two stages, first at about 112°C and again at 174°C, the complete loss of lattice water resulting in simultaneous transformation to an anhydrous phase.

Smakula *et al.* [5], examining the effects of sample preparation on IR spectra, first reported the apparent polymorphism of oestradiol. On the basis of both IR spectra and X-ray powder diffraction photographs they identified four crystalline modifications (Forms A–D) and an amorphous phase obtained from solvents and melts. Interestingly, all five forms transformed when subjected to heat and/or grinding, as summarised below:



Independently, Kuhnert–Brandstatter concluded (primarily from the thermomicroscopic examination of cooling melts [53]) that oestradiol was dimorphic, Form II (m.p. 169°C) being the unstable monotrope of Form I (m.p. 178°C), the two modifications differing also in habit, birefringence [53] and IR spectrum [54]. Close examination of the reports and data showed that Form A of Smakula *et al.* (which had a diffraction pattern identical to the one reported by Parsons and Beher [47]) was oestradiol hemihydrate (Table 5) and that Forms C and D (which exhibited different diffraction patterns and IR spectra) corresponded to Kuhnert–Brandstatter and colleagues' Forms I and II, respectively (Form B, recrystallised from methanol, was probably a solvate — see below). It appears then that *anhydrous* oestradiol is dimorphic and that in the process of desolvation the hemihydrate usually transforms to anhydrous Form I; the interplanar spaces and corresponding peak intensities of this stable monotrope, estimated from the diffractometric curve presented by Resetarits *et al.* [23], are approximately as follows:

d(Å)	I/I_o	d(Å)	I/I_o
7.6	3	4.2	3
6.4	5	4.1	3
5.8	10	4.0	3
5.6	4	3.9	1
5.3	3	3.7	2
4.9	4	3.6	1
4.6	2	3.3	2.

As noted above, grinding apparently transforms the anhydrous modifications of oestradiol to the hydrated form, an unusual solid-state phenomenon. However, crystalline oestradiol hemihydrate is itself affected by comminution: grinding resulted in no change in IR spectrum, but the DTA curve exhibited only a single, enlarged, pre-melting peak at about 120°C [51] — similar changes in DSC curves were reported for

samples which had been milled (these also exhibited diffuse X-ray powder diffraction patterns) [52] or obtained by rapid precipitation from ethanol [23]. These changes suggest that comminution can structurally deform the crystalline hemihydrate to the extent that desolvation and simultaneous transformation to anhydrous Form I are facilitated, occurring at a temperature about 60°C lower than usual. (It has been noted by this author that commercial 'micronized' oestradiol may apparently vary in degree of crystallinity, with different batches, even from the same supplier, exhibiting altered DTA curves.)

Oestradiol also forms solvates with organic solvents: a hemisolvate with methanol [46] and a monosolvate with ethanol [23], both of which desolvate (at 155 and 119°C, respectively) to Form I [46]. The crystallographic parameters of the monosolvate with propanol are [55]: a = 12.215 Å, b = 24.251 Å, c = 6.671 Å, Z = 4 and space group $P2_12_12_1$. The presence of propanol in the lattice was found to only very slightly perturb the conformation of oestradiol molecules [55], which may explain the apparent facility with which some polar solvents can substitute for the water of crystallisation.

Oestradiol recrystallises from organic solvents in a variety of habits (Table 6), photomicrographs of some of which have been presented by Kuhnert–Brandstatter [56];

Table 6
Oestradiol crystal habits [24, 56]

Habit	Crystallisation solvent
Amorphous	Acetone, benzene, dioxane, ether
Bladed	Dioxane, ethanol, aq. ethanol, ether, tetrahydrofuran
Platy	Chloroform, dichloromethane, hexanol, isopropanol, methanol
Prismatic	Benzene, chlorobenzene, methanol

in view of its tendency to incorporate solvent of crystallisation, some of these probably represent solvated forms, the hemihydrate in particular.

To summarise, the outstanding solid-state characteristic of oestradiol is the tenacity with which it adopts the hemihydrate form. In the experience of this author (and others [46, 52]) commercial samples are invariably composed of this phase. As described above, it has been demonstrated that water is selectively incorporated into the lattice when oestradiol crystallises from solution, that dehydration is difficult (usually occurring completely only at temperatures close to melting) and that other solvates are difficult to obtain and may be unstable [46, 55]. In addition, both crystalline and amorphous, anhydrous phases transform to the hemihydrate (this author has noted that the DTA curves of shavings from the surfaces of aged proprietary fusion-moulded implants were often typical of the hemihydrate). Clearly the presence of water molecules is a crucial requirement for the crystallographic stability of oestradiol under normal conditions; for this reason, and considering the effect of hydrogen bonding on molecular conformation, it has been suggested that water may even play a significant part in the hormone's interactions with its receptors [57]. However, proper cognisance of this solid-state characteristic has not been taken by the major pharmaceutical compendia, although the NF XIV [58] stated that oestradiol is 'hygroscopic' and the USP XXI [59] imposes a 3.5% limit on weight loss on drying at 105°C (at which temperature there may not be any discernible loss [46] or consequent alteration in DTA curve). To conclude, the evidence

summarised above presents a substantial case for oestradiol hemihydrate to be properly acknowledged as the common solid form of the steriod, with the molecular formula and relative molecular mass:

$$C_{18}H_{24}O_2 \cdot \tfrac{1}{2}H_2O,\ M_r\ 281.39_5.$$

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