ESTRADIOL VALERATE

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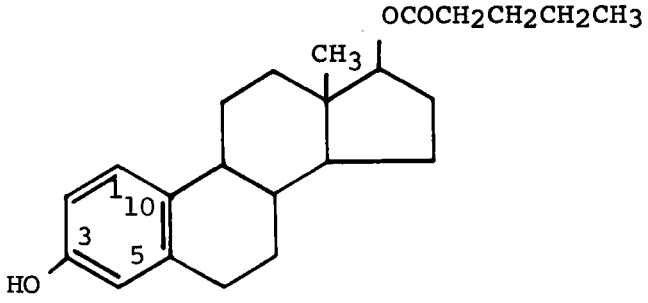
CONTENTS

1. Description

1.1 Name, Formula, Molecular Weight

Estradiol Valerate is estra-1,3,5 (10) triene-3,17β-diol-17-valerate (pentanoate).



$C_{23}H_{32}O_3$ Mol. Wt. 356.51

1.2 Appearance, Color, Odor

White, odorless, crystalline powder.

2. Physical Properties

2.1 Infrared Spectrum

The infrared spectrum of estradiol valerate[1] is presented in Figure 1.

2.2 Nuclear Magnetic Resonance Spectrum

The NMR spectrum is presented in Figure 2. It was obtained on a 60 MHz spectrometer in deuterochloroform containing tetramethylsilane as an internal reference. The following proton assignments were made[2]:

Protons at	Chemical Shift τ	Coupling Constants T (in Hz)
C-1H	2.86 (doublet)	1H,2H = 9
C-2H	3.38 (quartet)	1H,2H = 9;2H,4H =2.5
C-4H	3.43 (multiplet)	2H,4H = 2.5;4H,6H =1
C-18H	9.19 (singlet)	
C-17αH	5.25 (triplet)	16H,17H = 7.5
ester-CH_3	9.09 (triplet)	6.5
phenolic-OH	4.94 (singlet)	Concentration dependent

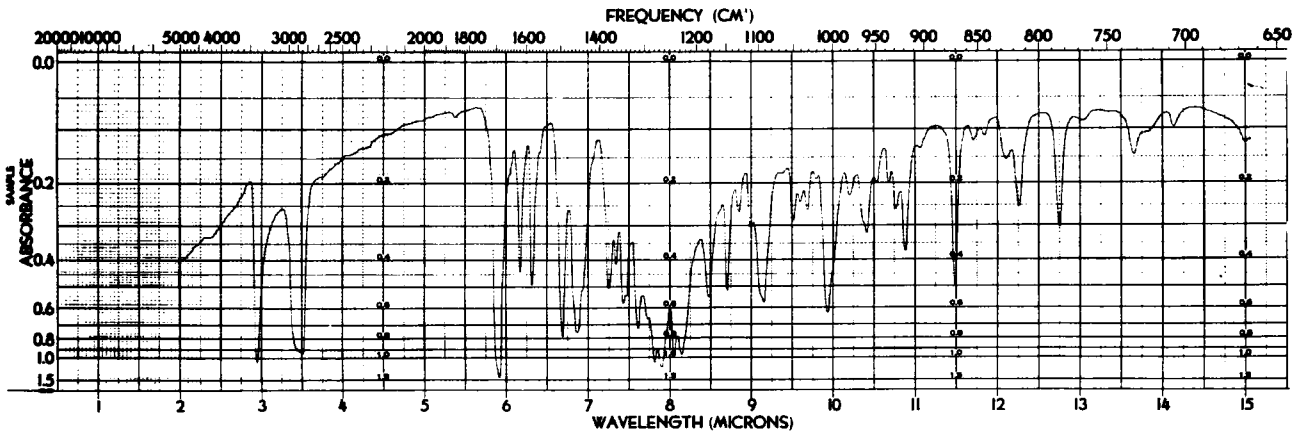


Figure 1. Infrared Spectrum of Estradiol Valerate in Mineral Mull. Instrument: Perkin-Elmer 621.

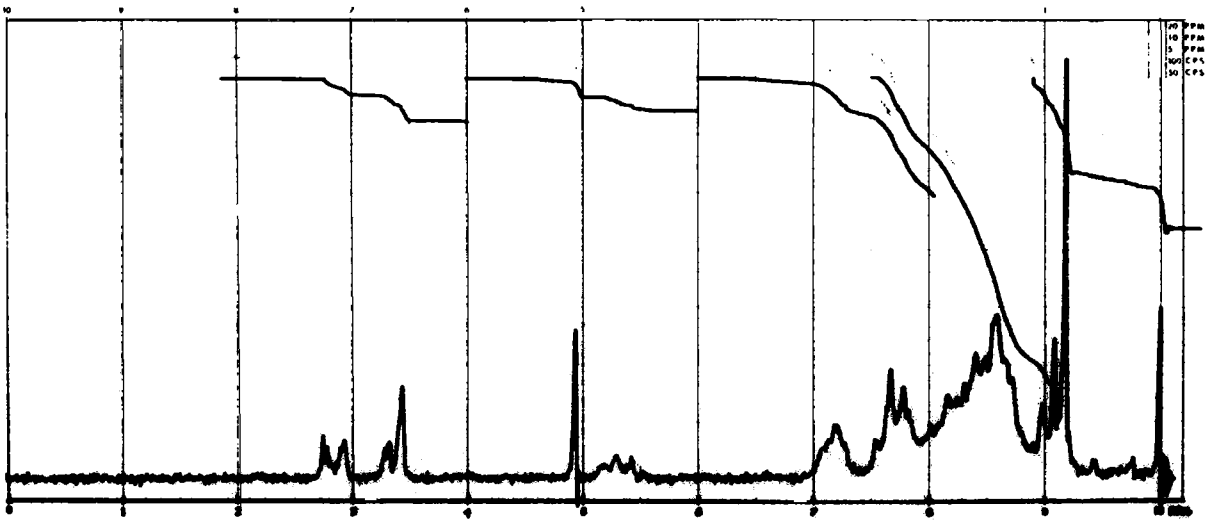


Figure 2. NMR Spectrum of Estradiol Valerate in Deuterated Chloroform. Perkin-Elmer R-12B.

2.3 Ultraviolet Spectrum

λ_{max}^{EtOH} 281 nm $E_{1cm}^{1\%}$ 61[3]

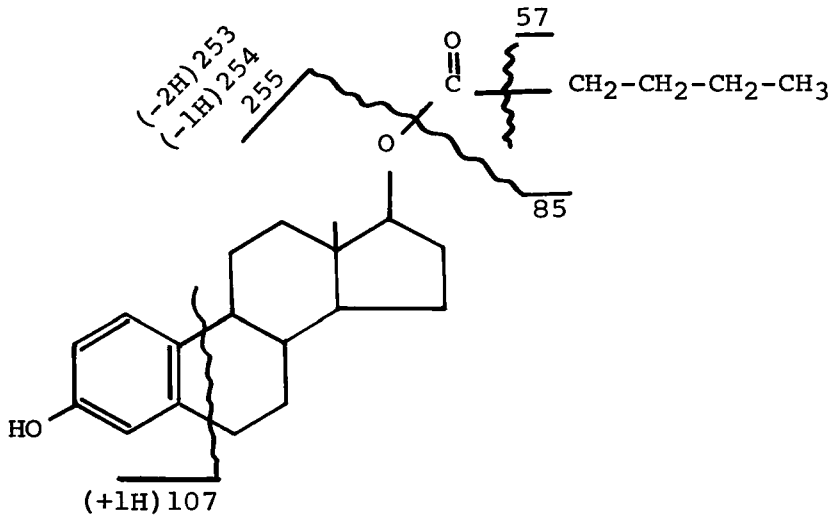
λ max 281 nm; = 2090[6]

λ max 287 nm; = 1800 (small peak)[6]

2.4 Mass Spectrum

The low resolution mass spectrum, shown in Figure 3, was obtained on an AEI MS-902 spectrometer equipped with a frequency modulated analog recorder[2].

It demonstrates the expected M^+ of m/e 356. There is also the M^+ of an acid homolog at m/e 370 present as a minor component. The high mass fragment ion at m/e 271 occurs by the cleavage of the acyl portion of the ester at the C-17 position. There is complement fragment ion at m/e 85 that represents the other portion of the molecule. The ions at m/e 253-255 represent the cleavage of the entire C-17 moiety. Because it is an aromatic steroid, there are a series of strong fragment ions with the progressive loss of first D-ring carbons, then C-ring carbons and finally B-ring carbons. The m/e 107 ion contains the A-ring and the C-6 carbon. Phenols can also eliminate the oxygen by explusion of CO or HCO. Thus a second series of progressive loss of carbons can occur but does not produce as many ions. The assignment of some of the diagnostic ions is depicted below.


(-2H)253
(-1H)254
255
O
C
57
CH2-CH2-CH2-CH3
O
85
HO
(+1H)107

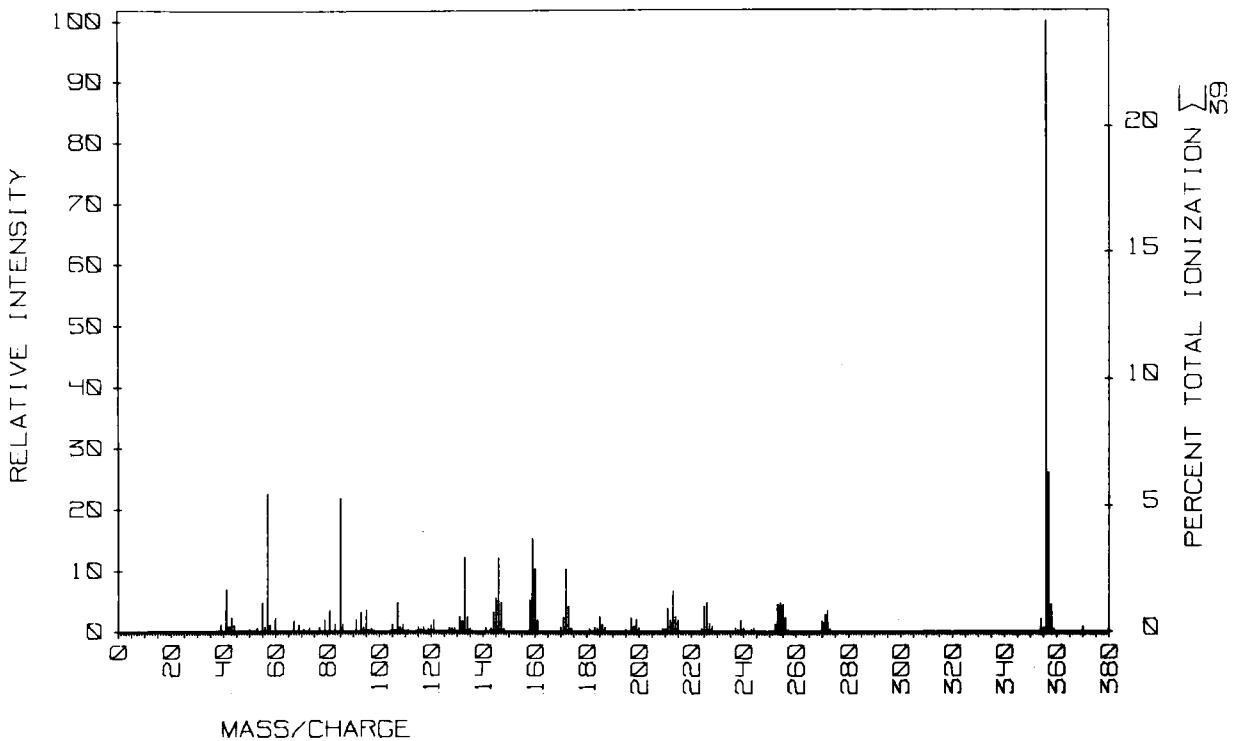


Figure 3. Low Resolution Mass Spectra of Estradiol Valerate. Instrument: AE1-902.

2.5 Rotation

$[\alpha]_D$ + 44° (dioxane)[3]

2.6 Melting Range

Like many steroids, Estradiol Valerate does not melt sharply. The following melting range temperatures (°C) were reported:

144-145[4] (from methanol-water)
150[5]
146-148[6]
147[7]
142-144[27]

The thermomicroscopic[6] and fusion properties[5] of estradiol valerate have also been described.

2.7 Solubility

Practically insoluble in water; soluble in caster oil, in methanol, in benzyl-benzoate and in dioxane; sparingly soluble in sesame oil and in peanut oil[8].

2.8 Crystal Properties

No polymorphism was found[7]. Isomorphic mixed crystals were formed with estradiol propionate[5].

The powder X-ray diffraction pattern is presented in Table I[9].

Table I
Powder X-ray Diffraction Pattern
of Estradiol Valerate

d(Å)*	I/I$_1$**		
11.5	0.06	3.50	0.19
10.0	0.60	3.31	0.34
6.95	0.21	3.22	0.08
6.21	0.24	3.14	0.07
5.97	0.51	3.02	0.09
4.98	1.00	2.96	0.08
4.68	0.46	2.32	0.05
4.12	0.34		
3.96	0.32		
3.83	0.13		

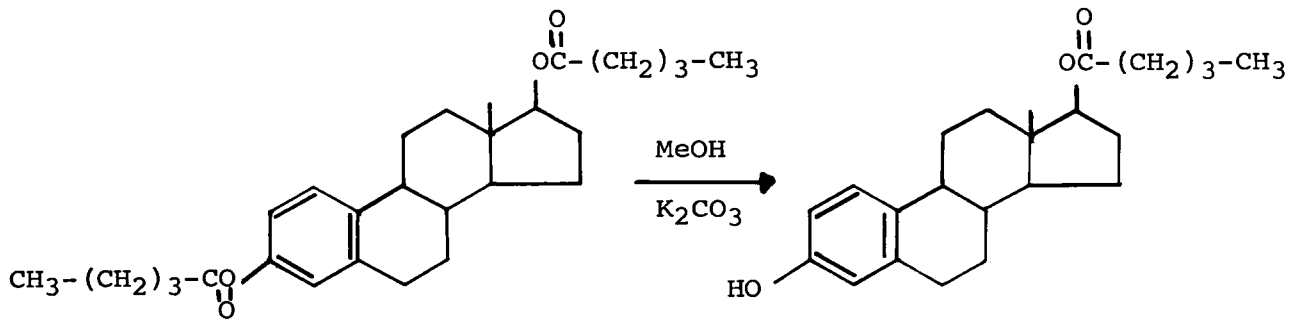
*d = interplanar distance $\frac{n\lambda}{2 \sin\Theta}$

λ = 1.539Å; Radiation: Kα_1 and Kα_1 Copper

**Relative intensity based on highest intensity of 1.00

3. Synthesis

The synthesis of estradiol valerate was first described by Miescher and Scholz[4] by methanlysis of estradiol 3,17-divalerate.



Alternatively the 3-benzoate has been removed selectively with sodium borohydride[27].

4. Stability - Degradation

Estradiol Valerate is very stable as a solid. In solution under certain conditions, particularily alkaline, saponification to valeric acid and estradiol can occur.

5. Drug Metabolic Products

An increase in blood and urine estrogen levels was noted after administration of estradiol valerate to ovarectomized [10] and postmenopausal [11] women. No metabolites have been identified so far, although estradiol valerate most likely follows the pathway of estradiol metabolism.

6. Methods of Analysis

6.1 Elemental Analysis

	% Calc.	% Found[4]
C	77.49	77.54;77.42
H	9.05	9.00;9.20
O	13.46	--

6.2 Spectrophotometric Analysis

The U.V. absorption at 282 nm can be used to determine estradiol valerate in sesame oil after extraction with 80% methanol, however, with an accuracy of not better than ± 10% because of interference from the oil[15]. In the compendial assay the absorbance of an alkaline assay solution at 300 nm is determined against an acidic assay preparation[8].

6.3 Spectroflurometric Analysis

A quantitative fluorometric method for determination of estradiol valerate in oil has been described[16]. After column clean up the steroid concentration in ethanol is determined in a spectrofluorometer (Excitation wave length: 285 nm; Fluorescence maximum ca. 328 nm).

6.4 Colorimetric Analysis

Estradiol valerate responds to colorimetric tests used for estrogens such as the phenol reagent (Folin-Ciocalteau reagent, phosphomolybdic phosphotungstic acid)[8], iron-phenol solution[17] and the Ittrich modification of the Kober

reaction (see section 7).

6.5 Chromatographic Analysis

6.51 Paper

The quantitative determination of estradiol valerate by paper chromatography has been described[18]. After spotting the paper strip strips were impregnated with 20% diethylene glycol monoethylether(Carbitol) in chloroform and developed for 3 hours with methylcyclohexane saturated with diethylene glycol monoethyl ether. The steroid is located on a guide-strip by spraying with Folin-Ciocalteau reagent, eluted with ethanol and quantitated fluorometrically. (Activation wave length at 280 nm, fluorescence at 310 nm). In this system estradiol stays at the origin.

6.52 Thin-Layer

Thin-layer chromatographic systems have been tabulated in Table II.

Table II

Absorbant	Solvent System	Ref.	Ref.
Magnesium silicate	Benzene-ethanol(9:1)	0.52	19
Magnesium silicate	Chloroform	0.62	19
Silica gel G	Stationery phase:mineral oil Mobile phase:50% acetic acid	0.64	20
Silica gel G	Stationery phase:propylene glycol Mobile phase:cyclohexane-pet.ether(1:1)	0.60	22
Silica gel G	Stationery phase:tetraethylene glycol Mobile phase:xylene	--	24

Detection systems. Phosphoric acid (1:5)spray(green color);conc. sulfuric acid, p-toluenesulfonic acid.

Quantitative determination in pharmaceutical preparation has been described[23]. The tetra-ethylene glycol-xylene system[24] separates estradiol valerate from estradiol and is the basis for a compendial limit test for free estradiol.

6.53 Gas-Liquid

Estradiol valerate can be determined quantitatively by gas chromatography using a 3% OV-17 on 80-100 mesh Varaport 30 column at a column temperature of 260°[25]. Alternately a 3% JXR (methylsilicone polymer)on silanized 100-200 mesh Gas Chrom P column at a column temperature of 230° can be used for the tetramethylsilyl derivate[26].

7. Identification and Determination in Body Fluids and Tissues.

A method[12] has been developed to determine extremely low concentration of total estrogens in bovine tissues, making use of a shortened version of the extraction procedure of Goldzieher[13], followed by fluorometric read out using the Ittrich[14] modification of the Kober reaction.

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