

Hydrogen Sulfates of Natural Estrogens

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The preparation and properties of alkali salts of sulfates of estrone, estradiol, and estriol are described. Partly sulfated estrogens were obtained by hydrolysis either of the corresponding acetates or of the completely sulfated forms. A single step method for the preparation of the aryl sulfates from their parent compounds is presented. The synthesis of various acetates is also reported.

The importance of steroid sulfates as biological intermediates is generally recognized.¹ This is also true of estrogen sulfates.

Several years ago we have prepared some hydrogen sulfates of estrone, estradiol and estriol, which were used for identification purposes in investigations of estrogen metabolism in man.^{2,3} The sulfation of hydroxy groups of steroids is usually carried out by interaction with a sulfur trioxide-pyridine complex.⁴ In many cases this complex was not isolated but the reaction product between chlorosulfonic acid and pyridine was used directly.⁵ Benzene,⁶ chloroform,⁷ chloroform-pyridine⁸ or pyridine^{9,10} were the solvents commonly used. Sulfamic acid in pyridine^{11,12} and pyridine sulfate in the presence of acetic anhydride in pyridine^{10,13} have also been used as sulfating agents for steroids. Usually the steroid hydrogen sulfates were obtained in the form of their pyridinium salts and these salts have then been transformed into the corresponding alkaline salts.

The first estrogen sulfate, the sodium salt of estrone sulfate, was prepared in 1939 by Butenandt and Hofstetter.⁵ The sodium salt of estradiol-3-sulfate was first prepared by hydrogenation of estrone sulfate.¹⁴ Later on it has been synthesized from estradiol-17-acetate *via* the sodium salt of estradiol-3-sulfate-17-acetate.¹⁵ The sodium salt of estradiol-17-sulfate was synthesized from estradiol-3-benzoate *via* the sodium salt of estradiol-3-benzoate-17-sulfate.⁸ The preparation of the quinidine and the potassium salts of estradiol-3,17-disulfate has been described; chlorosulfonic acid with pyridine in chloroform or carbon tetrachloride was used.^{14,16} Recently the preparation of the three sulfates of estradiol has again been described.¹⁰ The only previously reported sulfate of estriol is the sodium salt of estriol-3,16,17-trisulfate.¹⁴ In this paper we wish to report on the synthesis of several sulfates of estriol, which have not been described previously.

Table 1. Analytical data of the estrogen sulfates synthesized.

No.	Compound (as the alkaline salts, see Formula)	Method ^a	Formula	Calculated %				Found %					
				C	H	S	Na (K)	H ₂ O	C	H	S	Na (K)	H ₂ O ^c
1	Estrone-3-sulfate	A1	C ₁₈ H ₂₁ O ₆ SNa ₂ H ₂ O	55.37	5.94	8.21	5.89	4.61	54.5	6.39	7.90	—	5.3
2	Estradiol-3-sulfate ^b	A4, D	C ₁₈ H ₁₉ O ₆ SNa ₂ H ₂ O	55.09	6.42	8.17	5.86	4.59	54.5	6.74	8.23	6.0	4.2
3	Estradiol-3-sulfate- 17-acetate	B	C ₂₀ H ₂₅ O ₆ SNa ₂ H ₂ O ^d	—	—	—	—	—	—	—	—	—	—
4	Estradiol-17-sulfate	A4, C	C ₁₈ H ₁₉ O ₆ SNa ₂ 2H ₂ O	52.67	6.63	7.81	5.60	8.78	52.4	7.09	8.04	—	9.1
5	Estradiol-3,17- disulfate ^b	A1	C ₁₈ H ₁₇ O ₈ S ₂ Na ₂ 2H ₂ O	42.18	5.11	12.51	8.97	7.03	41.8	5.59	12.30	8.6	6.3
6	Estradiol-3-sulfate ^b	A4, D	C ₁₈ H ₁₉ O ₆ SNa ₂ H ₂ O	52.93	6.17	7.85	5.63	4.41	52.8	6.32	7.74	5.6	5.0
7	Estradiol-3-sulfate- 16,17-diacetate	A3, B	C ₂₂ H ₂₇ O ₈ SNa ₂ H ₂ O ^e	53.65	5.94	6.51	4.67	3.66	52.0	6.14	6.19	—	—
8	Estradiol-17-sulfate	A4, C	C ₁₈ H ₁₉ O ₆ SNa ₂ 3H ₂ O	48.63	6.58	7.21	5.01	12.16	47.8	6.62	7.24	—	—
9	Estradiol-3,16-diacetate- 17-sulfate	B	C ₂₂ H ₂₇ O ₈ SNa ₂ H ₂ O ^f	—	—	6.51	—	—	—	—	6.51	—	—
10	Estradiol-3,17-disulfate	A4	C ₁₈ H ₁₇ O ₈ S ₂ Na ₂ 2H ₂ O	40.91	4.96	12.13	8.70	6.82	41.5	5.51	12.10	—	—
11	Estradiol-16,17-di- sulfate	A5, C	C ₁₈ H ₁₇ O ₈ S ₂ Na ₂ 2H ₂ O	40.91	4.96	12.13	8.70	6.82	40.9	5.28	12.35	8.7	7.1
12	Estradiol-3,16,17- trisulfate	A2	C ₁₈ H ₁₇ O ₉ S ₃ K ₃	33.63	3.29	14.96	18.25	—	33.2	3.55	14.56	18.6	—

^a See Experimental part.^b The corresponding potassium salts were prepared according to Method E.No 2. Found: K 10.0. Calc. for C₁₈H₂₃O₆SK: K 9.99.No 5. Found: K 15.3; S 12.8. Calc. for C₁₈H₂₃O₆S₂K₂: K 15.37; S 12.61.No 6. Found: K 9.2; S 7.7. Calc. for C₁₈H₂₃O₆SK: K 9.62; S 7.89.^c According to Karl Fischer.^d Acetyl. Found: 10.0 Calc.: 9.91.^e Acetyl. Found: 17.5 Calc.: 17.48.^f Acetyl. Found: 16.2 Calc.: 17.48.

The different sulfates reported here (see Table 1) were all obtained in good yields by shaking a solution of the steroid in pyridine with an excess of sulfur trioxide-pyridine complex at room temperature overnight (Method A).

The steroid sulfates were transformed into their alkali salts. If protecting acetyl groups were present, these were removed by hydrolysis with sodium hydroxide in aqueous methanol. The sodium salts were then isolated from aqueous solutions of sodium acetate. The products prepared in this way were normally homogeneous when assayed by thin-layer chromatography (TLC). No unreacted starting material was detected. Under the experimental conditions used for the isolation of the sulfates, only the isolation of the sodium salt of estriol-3,16,17-trisulfate presented some difficulties. However, the potassium salt could be obtained in good yield. In most cases the salts were purified by precipitation with ether from their methanolic solutions.

Partly sulfated compounds were prepared from their corresponding acetates. The only one of these sulfo-acetates which was isolated as such was the sodium salt of estriol-3-sulfate-16,17-diacetate. The same diacetate was also obtained by acetylating the sodium salt of estriol-3-sulfate with acetic anhydride in pyridine solution (Method B).

The sodium salts of the steroid sulfates could easily be transformed to their corresponding potassium salts with potassium acetate in aqueous solutions (Method E). Aryl sulfates have also been prepared in alkaline aqueous solutions, usually by reaction with the sulfur trioxide-trimethylamine (or triethylamine) complex.⁴ Using this method we obtained estradiol-3-sulfate and estriol-3-sulfate as their alkaline salts in a single step, using the free steroids as starting material (Method D).

Hydrolysis of steroid sulfates by aqueous acids and solvolysis in organic solvents have been the subject of numerous studies.^{7,9,14} It could be expected that the phenolic sulfate group is easier split off than the alicyclic ones. Therefore, we have investigated the possibility to use hydrolysis or solvolysis in preparing estrogen sulfates with a free 3-hydroxy group from the corresponding completely sulfated estrogens. The reaction could easily be followed with TLC and from the change of absorption in UV. It was found that estradiol-17-sulfate and estriol-16,17-disulfate both could be prepared in this manner. Although 70 % aqueous dioxane at 60° gave a selective solvolysis, the method preferred in preparing these compounds was hydrolysis with hydrochloric acid in aqueous methanol (Method C). Further hydrolysis of the estriol-16,17-disulfate resulted in the formation of an estriol monosulfate, identified as estriol-17-sulfate.

It has been reported previously⁵ that the sodium salt of estrone sulfate is unstable during storage. A crude product of our preparation, containing a small amount of sodium acetate was found to be stable for more than a year at -20°. At 35° it remained unchanged for some 2 days, but was disintegrated within 10 days. All other sulfates described here were found to be stable when stored at room temperature as their alkali salts.

Estradiol-3-acetate and the previously not described estriol-3-acetate were both obtained by acetylation with acetic anhydride in alkaline aqueous solution.

Estriol-3,16,17-triacetate was obtained in good yield following acetylation with acetic anhydride, using *p*-toluenesulfonic acid as a catalyst. The 16,17-diacetate of estriol was prepared from the triacetate by careful alkaline hydrolysis. This compound has also been synthesized by Tsuneda *et al.*¹⁷ both *via* direct acetylation of estriol with acetic acid and from estriol-triacetate following treatment with sodium borohydride.

The 16-acetate of estriol was obtained according to Tsuneda *et al.*¹⁷ by boiling estriol in acetic acid, and estriol-3,16-diacetate in the same way using estriol-3-acetate as the starting material. The latter reaction yields a mixture of estriol-3,16-diacetate and estriol-3,16,17-triacetate. The diacetate was not isolated, but its identity was proved by methylation.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. UV spectra were recorded with a Beckman DB spectrophotometer, IR spectra in potassium bromide discs with a Perkin-Elmer model 21 instrument. TLC was carried out on activated silica gel (Merck, Kieselgel G) according to Stahl.¹⁸ The spots were made visible by spraying with 10 % sulfuric acid in ethanol followed by heating to 110°.

Estrogen sulfates

The physical constants and analytical data of the various sulfates prepared are compiled in Tables 1 and 2.

Table 2. Some physicochemical data of the estrogen sulfates synthesized.

No.	[α] _D ^a	Ultraviolet absorption ^c				R_F values in system		
		λ_{\max}	ϵ	λ_{\max}	ϵ	A ^d	B ^e	C ^f
1	+ 94	269	780	275	756	0.52	0.37	0.39
2	+ 46	269	783	275	768	0.44	0.36	0.36
3	+ 14 ^b	269	771	275	696	0.52	0.36	—
4	+ 35 ^b	279	1978	—	—	0.45	0.36	0.32
5	+ 33	269	825	275	762	0.16	0.10	0.08
6	+ 36	269	790	275	737	0.33	0.29	0.29
7	— 9	269	711	275	664	0.52	0.33	—
8	+ 6	279	2005	—	—	0.62	0.36	0.26
9	—	269	699	275	645	0.71	0.31	—
10	+ 11	269	775	275	717	0.35	0.11	0.07
11	— 11	279	1965	—	—	0.52	0.20	0.03
12	— 7	269	813	275	776	0.21	0.04	0.01

^a Unless otherwise indicated rotations were recorded in water ($c = 1$).

^b In 33 % ethanol ($c = 1$).

^c In 50 % ethanol.

^d Methanol-benzene (1:1).

^e Butanol saturated with water.

^f Butanol-5 N NH₄OH (9:1).

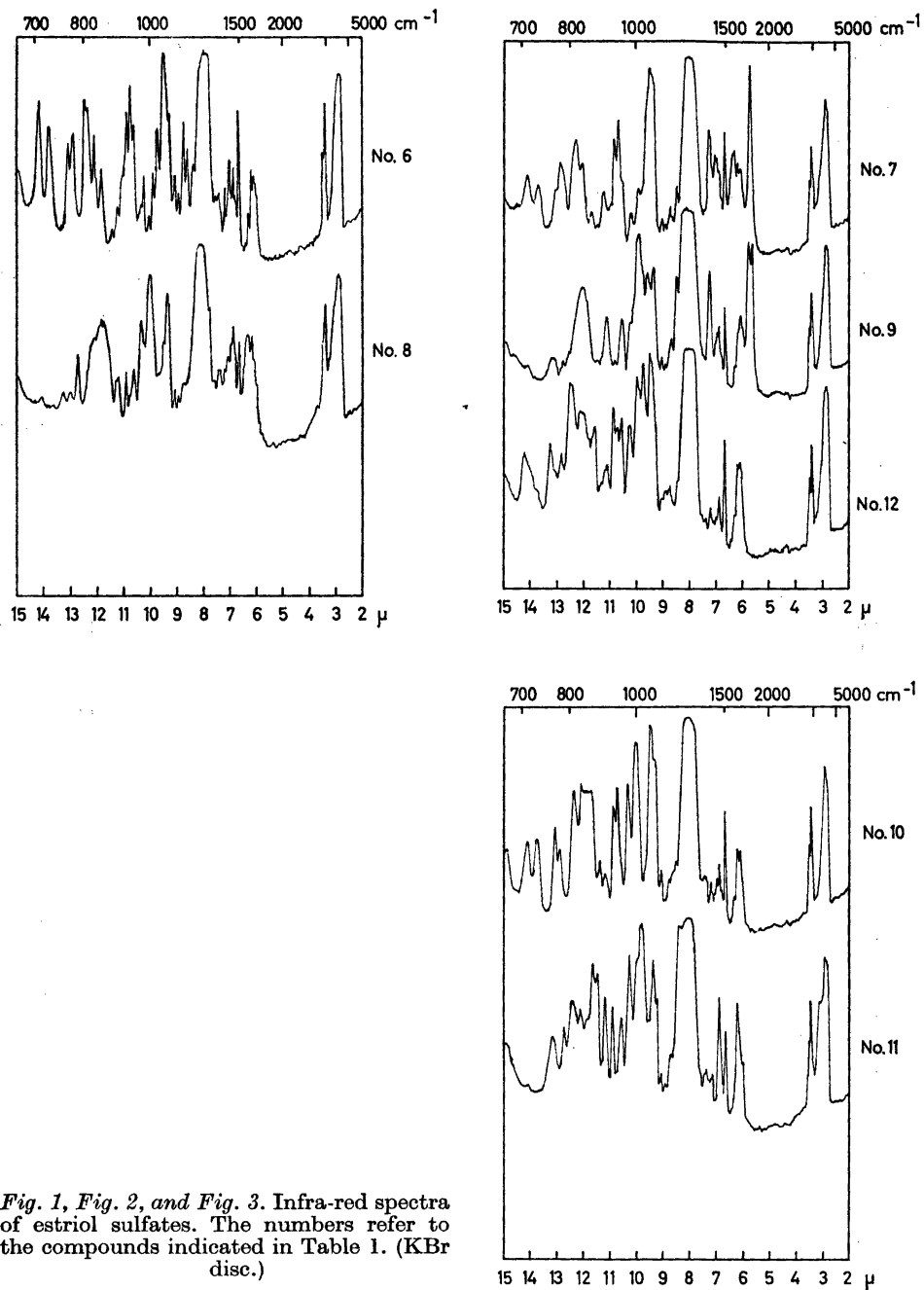


Fig. 1, Fig. 2, and Fig. 3. Infra-red spectra of estriol sulfates. The numbers refer to the compounds indicated in Table I. (KBr disc.)

Before analysis the compounds were dried at room temperature *in vacuo*. Melting points were found to be of little, if any, use for the characterization of the sulfates, since they were influenced by the way of heating. All compounds showed decomposition at temperatures higher than 150°.

The infra-red spectra of the sulfates of estradiol were found to be in good agreement with those recently published.¹⁰ The spectra of the various sulfates of estriol are shown in Figs. 1–3.

The sulfates prepared were synthesized principally by the same procedure (Method A), only the methods of isolation were different. Typical examples of these isolation methods are given below, indicated as A1, A2 etc. Some of the partly sulfated estrogens could also be prepared by selective hydrolysis of the completely sulfated steroid (Method C) or, in the case of the 3-monosulfates, directly from the parent steroid (Method D).

Method A. The free or partly acetylated steroid (1 g) was dissolved in dry pyridine (25 ml) and the sulfur trioxide-pyridine complex⁴ added. For compounds with only one free hydroxy group 1.5 g of the complex was used, if two or three hydroxy groups were present the amount was increased to 2.5 g. The reaction mixture was then shaken under anhydrous conditions overnight at room temperature. The clear, or nearly clear, solution was evaporated *in vacuo* until most of the pyridine was removed. The residue was treated with petroleum ether (2 × 50 ml) and the solid or semisolid residue was dissolved in methanol (50 ml). This methanol solution was then treated differently (see below) according to the compound used as starting material and the desired product.

The crude alkali salts obtained from the alkali acetate solutions (see below) were always homogeneous when subjected to thin layer chromatography but contained a small amount of alkali acetate as impurity (normally < 1 %). The yields of these crude products were about 70–90 %. They were purified by repeated precipitations from methanol with ether if not otherwise stated. The final product was controlled by TLC.

A1. Estradiol-3,17-disulfate (sodium salt). The methanol solution (see Method A) was neutralized with 1 N methanolic sodium hydroxide solution (19 ml). After the removal of the precipitated sodium sulfate by filtration most of the solvent was removed *in vacuo* and the residue was shaken with a saturated sodium acetate solution (20 ml). The precipitated solid was collected by filtration, washed with 10 % sodium acetate solution (5–10 ml) and with a small amount of ice-cold water (1–2 ml). The crude sodium salt of estradiol-3,17-disulfate (1.5 g) was precipitated twice from methanol (65 ml) and ether (65 ml). Yield 1.2 g.

A2. Estriol-3,16,17-trisulfate (potassium salt). This salt was prepared according to Method A1 from estriol but instead of a saturated sodium acetate solution a 70 % potassium acetate solution (35 ml) was used in the isolation. The crude product obtained was purified by precipitation with hot methanol (150 ml) from an aqueous solution (50 ml). Yield 80 %.

A3. Estriol-3-sulfate-16,17-diacetate (sodium salt). From estriol-16,17-diacetate according to Method A. The methanol solution was carefully neutralized (excess avoided) with 1 N methanolic sodium hydroxide solution at –5°. The precipitated sodium sulfate was immediately removed by filtration and the filtrate was allowed to run directly into cold ether. Then the precipitate was treated with saturated sodium acetate solution according to A1. The twice reprecipitated compound was identical with that obtained by Method B. Yield 31 %.

A4. Estriol-3-sulfate (sodium salt). From estriol-16,17-diacetate according to Method A. After the methanol solution had been neutralized and the sodium sulfate removed (see A1), 10 N aqueous sodium hydroxide solution (10 ml) was added and the reaction mixture was boiled 15 min. After cooling and neutralizing with acetic acid to pH 7.5–8, most of the solvent was removed *in vacuo*. The residue was treated with water (20 ml) and filtered. The solid was washed with 10 % sodium acetate solution (10 ml) and finally with ice-cold water (5 ml). The crude sodium salt of estriol-3-sulfate (0.9 g) was reprecipitated twice. Yield 0.8 g.

A5. Estriol-16,17-disulfate (sodium salt). From estriol-3-acetate according to Method A. To the methanol solution was added 10 N aqueous sodium hydroxide solution (10 ml) and the reaction mixture was boiled 15 min. After cooling and neutralizing with acetic acid the sodium salt of estriol-16,17-disulfate was isolated as described in A4. The crude salt (1.3 g) was purified by precipitation from an aqueous solution (40 ml) by adding hot ethanol (150 ml). Yield 1.0 g.

Method B. Acetates of sulfated estrogens. With the exception of the sodium salt of estriol-3-sulfate-16,17-diacetate, which also was prepared as described above (A3), the acetates of partly sulfated estrogens were obtained by acetylation of the sodium salt of the sulfates, using pyridine and acetic anhydride at room temperature. The reaction mixture was allowed to stand overnight, then most of the solvent was removed *in vacuo* and the semisolid residue was treated with petroleum ether. The sodium salt was obtained from the residue after dissolving in methanol and precipitating with ether. Yields about 60–90 %.

Method C. Partly sulfated estrogens formed by solvolysis and hydrolysis. The reactions below were followed by TLC (butanol saturated with water). In all experiments the potassium salt of estriol-3,16,17-trisulfate was used as the starting material.

Solvolysis in aqueous dioxane (3 mg/ml). In 50 % dioxane only unchanged starting material was found after 32 h at 50°. Changing to 70% dioxane the trisulfate was practically unchanged after 48 h at room temperature but at 50° only estriol-16,17-disulfate was found after 32 h. At 60° this result was obtained already after 4 h. After further 4 h under the same conditions a small amount of a compound was obtained with the same R_F -value as that of estriol-17-monosulfate. Free estriol was formed only after 20 h of solvolysis at 80°.

Acid hydrolysis. This was investigated at a concentration of 1–3 mg/ml in 90 % acetic acid and with 1 N hydrochloric acid in aqueous methanol.

In 90 % acetic acid at 50° the selectivity was not so good; after 20 h about the same amount of di- and monosulfate (but no free estriol) was formed. At 100° only free estriol was found after 4 h.

Some of the results with 1 N hydrochloric acid in aqueous methanol are given in Table 3. Practically only estriol-16,17-disulfate (no estriol-17-monosulfate) was formed in 80% methanol after 48 h at 0° and practically only free estriol after boiling for 2 h.

Estrogen sulfates prepared by hydrolysis. All sulfates prepared according to this method were compared with the same compounds obtained with Method A and were found to be identical in all respects (IR, UV, TLC, melting behaviour).

Estradiol-17-monosulfate (sodium salt). Sodium estradiol-3,17-disulfate (1 g) was dissolved in a mixture of 2 N hydrochloric acid (50 ml) and methanol (50 ml). The reaction mixture was warmed at 50° for 4 h. After cooling, 5 N sodium hydroxide (20 ml) was added to yield a pH of about 8.5 and most of the solvent removed *in vacuo*. The residue was treated with an amount of water (30 ml) sufficient for dissolving the sodium chloride formed. After filtration and washing with cold water the crude sodium salt of estradiol-17-monosulfate (0.8 g) showed only a trace contamination with free estradiol.

Table 3. Hydrolysis of estriol-3,16,17-trisulfate with 1 N hydrochloric acid in aqueous methanol (see Experimental part).

Compound	20°					50°					
	50 % methanol		80 % methanol			50 % methanol			80 % methanol		
	8 h	50 h	4 h	8 h	50 h	2 h	6 h	20 h	1.5 h	2 h	30 h
E ₃ -3,16,17-trisulfate	6	2	trace	0	0	0	0	0	0	0	0
E ₃ -16,17-disulfate	4	8	10	10	5	10	8	trace	trace	0	0
E ₃ -17-monosulfate	0	trace	0	trace	5	trace	2	8	10	9	0
Free E ₃					0		0	2	trace	1	10

The figures represent the relative amount formed estimated by TLC (butanol saturated with water).

The compound was precipitated from ethanol with ether to yield 0.56 g (70 %) of pure material.

Estriol-16,17-disulfate (sodium salt). From the potassium salt of estriol-3,16,17-trisulfate (1 g) as described above for estradiol-17-monosulfate. The crude sodium salt of estriol-16,17-disulfate (0.52 g) obtained was free from starting material and estriol but was contaminated with a small amount of estriol-17-sulfate, as indicated by TLC. Recrystallization from 80 % ethanol gave 0.3 g (37 %) of the pure compound.

Estriol-17-monosulfate (sodium salt). The potassium salt of estriol-3,16,17-trisulfate (1 g) was hydrolysed in a mixture of 5 N hydrochloric acid (70 ml) and methanol (280 ml) at 50° for 3.5 h. After isolation as described for estradiol-17-monosulfate, the crude sodium salt of an estriol-monosulfate (0.3 g) was obtained, which in TLC showed only a small impurity of estriol. Precipitation from ethanol with ether gave 0.25 g (36%) of the pure compound. This product was found to be identical with the sodium salt of estriol-17-monosulfate prepared according to Method A (IR, UV, R_F -values, melting behaviour and hydrolysis).

After acetylation according to Method B both monosulfates gave identical diacetates (IR, UV, R_F -values). In 90 % acetic acid at 100° for 4 h both acetates were hydrolysed to estriol diacetates with melting points (152–153°, no depression in mixed m.p.) in agreement with the value reported¹⁷ for estriol-3,16-diacetate.

Method D. The 3-sulfates prepared were found to be identical with the same compounds obtained according to Method A (IR, UV, R_F -values and melting behaviour).

Estradiol-3-sulfate (sodium salt). Estradiol (1.09 g) was dissolved in acetone (20 ml) and this solution was added slowly to 0.4 N aqueous sodium hydroxide (100 ml). Most of the acetone was then removed *in vacuo* and the clear solution warmed to about 55°. At once sulfur trioxide-triethylamine complex (3.6 g, prepared by adding chlorosulfonic acid to the amine in chlorobenzene at 10°⁴) was added during vigorous stirring. The clear solution was then kept at 55° for 7 h. At that time the colour had changed to yellow-reddish (if the acetone was not removed, the colour was dark). Hydrochloric acid was added to a pH of about 8 and the solution extracted with ether (from this ether-extract unchanged estradiol (0.36 g) was isolated).

From the aqueous solution the crude sodium salt (0.65 g) was precipitated and isolated after adding solid sodium acetate. After dissolving in methanol and decolorizing with Norit the pure sodium estradiol-3-sulfate was precipitated with ether in 60 % yield (corrected for recovered estradiol).

Estriol-3-sulfate (sodium salt). Estriol (0.46 g) was dissolved in acetone (75 ml) and this solution was added slowly to 0.3 N aqueous sodium hydroxide (50 ml). After removal of the acetone *in vacuo* the reaction with sulfur trioxide-trimethylamine complex (1.5 g) and the isolation and purification of the formed estriol-3-sulfate as its sodium salt was performed in the same manner as described for estradiol-3-sulfate above. Yield 31 % (corrected for recovered estriol).

Method E. Conversion of the sodium salts of the sulfates to the corresponding potassium salts. This was done by dissolving the sodium salt in a small amount of water and adding the same volume of saturated potassium acetate solution. The potassium salts precipitated almost immediately and after filtration and washing with a small amount of ice-cold water, they were reprecipitated from methanol with ether.

Estrogen acetates

The data for the estrogen acetates prepared are found in Table 4. The preparation of some of them is given below.

Estradiol-3-monoacetate. Estradiol (5 g) was dissolved in acetone (100 ml) and this solution poured into 0.5 N aqueous sodium hydroxide (2500 ml). The clear solution obtained was cooled to 0–5°, acetic anhydride (60 ml) was added at once and the reaction mixture vigorously shaken during 5 min. The precipitated 3-acetate (about 5 g) was collected by filtration and carefully washed with water. The product obtained melted at 136–138° and was pure in TLC.

Estriol-3-monoacetate. This product was prepared as the corresponding estradiol-3-acetate. The best yield was obtained by dissolving estriol (2 g) in dimethylformamide (25 ml) and pouring this solution into 0.25 N sodium hydroxide (2000 ml). At 0° acetic

Table 4. Some physicochemical data of the estrogen acetates synthesized.

Compound	Formula	Acetyl		M.p. °C	[α] _D ⁱ	Ultraviolet absorption ^m			R _F values in system		
		Calc.	Found			λ_{\max}	ϵ	λ_{\max}	ϵ	D ⁿ	E ^o
Estrone-3-acetate	C ₂₀ H ₂₄ O ₃	—	—	125–6 ^{a,b}	+ 137	269	796	275	744	0.79	0.13
Estradiol-3-acetate	C ₂₀ H ₂₆ O ₃	13.69	13.4	141–2 ^{a,c}	+ 58	269	768	275	762	0.67	0.05
Estradiol-17-acetate	C ₂₀ H ₂₆ O ₃	13.69	13.4	217–8 ^{a,d}	+ 39	279	2120	—	—	0.67	0.09
Estradiol-3,17-diacetate	C ₂₂ H ₂₈ O ₄	24.16	23.5	129–30 ^{a,e}	+ 30	269	731	275	721	0.80	0.26
Estriol-3-acetate	C ₁₈ H ₂₀ O ₄	13.03	12.8	186–7 ^f	+ 45	269	734	275	720	0.25	0.00
Estriol-16-acetate	C ₂₀ H ₂₆ O ₄	13.03	12.7	192–4 ^{g,h}	+ 26	279	2175	—	—	0.52	0.00
Estriol-3,16-diacetate	C ₂₂ H ₂₈ O ₅	23.12	21.3 ^p	152–3 ^{g,i}	—	269	771	275	721	0.77	—
Estriol-16,17-diacetate	C ₂₂ H ₂₈ O ₅	23.12	22.7	180–1 ^{g,j}	— 19	279	2070	—	—	0.64	0.02
Estriol-3,16,17-triacetate	C ₂₄ H ₃₀ O ₆	31.16	30.9	123–9 ^{a,k}	— 28	269	914	275	889	0.80	0.02

^a After recrystallization from aqueous methanol.^b Lit.²² m.p. 125.5°.^c Lit.²⁰ m.p. 136.5–7.5°.^d Lit.²⁰ m.p. 215–7.5°.^e Lit.²¹ m.p. 126°.^f After recrystallization from ethylacetate-hexane.^g After recrystallization from ether-hexane.^h Lit.¹⁷ m.p. 191–3°. Lit.²³ m.p. 198–200°.ⁱ Lit.¹⁷ m.p. 150–2°. Lit.²³ m.p. 158–60°.^j Lit.¹⁷ m.p. 171–3°.^k Lit.²² m.p. 126°.^l Rotations were recorded in dioxane (c = 1).^m In ethanol.ⁿ Chloroform-ethanol (9:1).^o Methylene chloride.^p Crystallization with solvent has been reported.¹⁹

anhydride (25 ml) was added at once. After 5 min of vigorous shaking, the reaction mixture was extracted with ethyl acetate. The extract was washed with water and dried over anhydrous sodium sulfate. After removal of the solvent 2.2 g estriol-3-acetate, m.p. 182–184° was obtained, which was pure in TLC.

Estriol-3,16,17-triacetate. Estriol (1 g), *p*-toluenesulfonic acid (1 g), and acetic anhydride (40 ml) were heated on a steam bath under anhydrous conditions until a clear solution was obtained (5–10 min), and then left at room temperature for 2 days. After that time the solution was poured into a mixture of pyridine (30 ml) and water (400 ml). The resulting precipitate was collected and washed with water. The crude triacetate was dissolved in methanol (75 ml) on the steam bath and water (40 ml) was added. After cooling pure estriol-3,16,17-triacetate (1.4 g) was obtained; m.p. 125–127°.

Estradiol-3,17-diacetate. This compound was prepared in the same manner as the estriol-3,16,17-triacetate above. From 7.5 g estradiol 9.2 g of crude estradiol-3,17-diacetate was obtained; m.p. 126–128°.

Estradiol-17-monoacetate. This compound was prepared from estradiol-3,17-diacetate (4.5 g) according to the method described by Miescher and Scholz.²⁰ Hydrolysis was carried out at room temperature during 1 h. The reaction mixture was then poured into a mixture of water (1000 ml) and 2 N hydrochloric acid (40 ml). 3.8 g of crude estradiol-17-monoacetate was obtained; m.p. 216–217°.

Estriol-16,17-diacetate. This diacetate was prepared by a slight modification of the method used in preparing estradiol-17-acetate. Estriol-3,16,17-triacetate (1 g) was dissolved in ethanol-acetone (1:1, 200 ml). The solution was cooled to +3° and a cold (+3°) solution of potassium carbonate (0.5 g) in 95% methanol (200 ml) was added. After 1/2 h at +3° the solution was acidified to pH 6–7 with hydrochloric acid. Most of the solvent was evaporated *in vacuo* and the residue was dissolved in ether and washed with water. The ether solution was dried over anhydrous sodium sulfate and then evaporated *in vacuo* to a volume of about 30 ml. This solution was brought to boiling on a steam bath and then hexane (200 ml) was added. After cooling crude estriol-16,17-diacetate (0.62 g) was obtained with m.p. 170–173°.

Estriol-3,16-diacetate. This compound has already been described above (see under Estriol-17-sulfate). For synthetic purposes estriol-3,16-diacetate was prepared as a mixture with estriol-3,16,17-triacetate using the method of Tsuneda *et al.*¹⁷ for the preparation of estriol-16-monoacetate.

Estriol-3-acetate (1 g) was boiled in acetic acid (50 ml) for 5 h. (TLC showed that after that time no more estriol-3-acetate was present in the reaction mixture.) Most of the acetic acid was removed *in vacuo* and the residue was dissolved in ether (150 ml), washed with cold sodium bicarbonate solution and finally water. After drying over anhydrous sodium sulfate the ether was evaporated *in vacuo*. 1.1 g of a solid product was obtained which in TLC gave only two spots of about the same size and with the same R_F -values as those found for estriol-3,16,17-triacetate and estriol-3,16-diacetate. The mixture was used without further purification.

Methylation of this product according to Neeman *et al.*¹⁹ followed by hydrolysis in boiling 1 N methanolic sodium hydroxide solution (15 min) gave a mixture of estriol and estriol-17-monomethyl ether (TLC). When the mixture was chromatographed on aluminium oxide the estriol-17-monomethyl ether could be eluted with ether:ethanol (10:1). After recrystallization from benzene it had a m.p. of 188–190° (lit.¹⁹ 192–192.5°), λ_{\max} 280 (2280). (Found: CH₃O 9.7. Calc. for C₁₅H₃₆O₃: CH₃O 10.26).

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