Isomeric Aryl Monosulfates of Estrogen Catechols

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The syntheses of sodium estra-1,3,5(10)-triene-2,3,17β-triol 2-sulfate (Va) and sodium estra-1,3,5(10)-triene-2,3,17β-triol 3-sulfate (Vb) by procedures leading uniquely to each are described. The isomeric monosulfates Va and Vb were differentiated and their structures confirmed by means of the nmr spectra of their aromatic protons.

Steroid conjugates have been shown repeatedly[3] to be active intermediates in steroid hormone transformations. These results have raised new questions about the physiological role of the conjugates and have renewed interest in these derivatives, particularly sulfate esters. Our interest in the chemistry and biochemistry of the female sex hormone has prompted us to initiate work on a potentially important aspect of estrogen conjugation. The catechols, 2-hydroxy estrogens, are among the most significant derivatives of the female hormone.[4] The synthesis of the two isomeric aryl monosulfate esters of 2-hydroxyestradiol was undertaken in order to provide reference material as well as information about the properties of these conjugates.

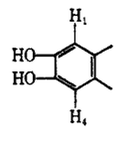
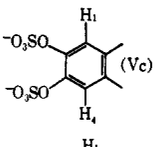
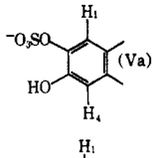
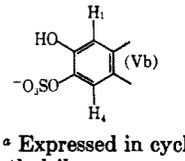
The expected difficulty in separation of mixtures of the two monosulfates required procedures leading uniquely to each compound. The chemical equivalence of the two phenolic groups precluded the selective sulfation or blocking of either of the phenolic hydroxyl groups. The route originally used for preparation of 2-hydroxy estrogens[5] provided an advantageous means for the preparation of the corresponding 2-sulfate ester.

Estradiol was converted into the 5-nitrobenzophenone ether (Ia) (Chart I), acetylated at C-17 to Ib, and then oxidized to the 2-hydroxy compound Ic. Reaction of Ic with sulfur trioxide–trimethylamine complex[6] in pyridine solution gave the 2-sulfate Id which was purified by preparative thin layer chromatography. Treatment of Id with 5% aqueous sodium hydroxide at room temperature for 18 hr removed both the benzophenone ether and acetate ester groups, leaving the sulfate ester intact. The sodium salt of 2-hydroxyestradiol 2-sulfate Va was isolated by butanol extraction and preparative thin layer chromatography as a semicrystalline homogenous compound which melted with decomposition at 210°.

The 3-monosulfate of 2-hydroxyestradiol was prepared by a parallel sequence starting with the estradiol isomer 1,3,5(10)-estratriene-2,17β-diol (IIa) previously prepared in these laboratories.[7] Diol IIa with 2-chloro-5-nitrobenzophenone gave the ether IIb, which was acetylated to monoacetate IIc. The latter was cyclized with sulfuric acid to the intermediate xanthylium salt III which was oxidized directly with H_2O_2 to give the catechol derivative IVa. No evidence for the alternative cyclization to C-1 was obtained. This is presumably due to the steric interference of the C-11 methylene with the planarity of the C-1 xanthylium ion and the resultant loss of resonance stabilization. Sulfation of the free phenol in IVa with the sulfur trioxide–trimethylamine complex led smoothly to the sulfate salt IVb, mp 190–194°. Treatment of IVb with aqueous NaOH gave the desired 2-hydroxyestradiol 3-sulfate as the sodium salt Vb.

The two isomeric sulfates Va and Vb exhibited identical polarities in several tlc systems and thus confirmed the expected difficulty in separation of their mixtures. On acid hydrolysis both Va and Vb yielded 2-hydroxyestradiol. The infrared spectra of the two sulfates showed only minor differences which were insufficient for ready differentiation. Confirmation of their structure was therefore achieved by means of nmr spectroscopy. From consideration of the two separate aromatic proton resonances of various derivatives of 2-hydroxy estrogens, it was possible to assign chemical shift values to the C-1 and C-4 protons which are 12 cps apart.[8] Further, it was shown that an electron-withdrawing substituent on the 2-hydroxy group will increase the distance between the aromatic resonances to more than 12 cps, while a similar substitution at C-3 will have the opposite effect.[8] The pertinent regions of the nmr spectra in dimethyl sulfoxide of the two monosulfates Va and Vb (Table I) clearly show that

TABLE I

	δ_{H_1}[a]	$\Delta\delta_{H_1}$	δ_{H_4}	$\Delta\delta_{H_4}$	$\Delta(\delta_{H_1} - \delta_{H_4})$
	400	...	388	...	12
 (Vc)	450	+50	438	+50	12
 (Va)	424	+24	395	+7	29
 (Vb)	407	+7	411	+23	−4

[a] Expressed in cycles per second downfield from internal tetramethylsilane.

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(5) J. Fishman, *J. Am. Chem. Soc.*, **80**, 1213 (1958).

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(7) J. Fishman and M. Tomasz, *J. Org. Chem.*, **27**, 365 (1962).

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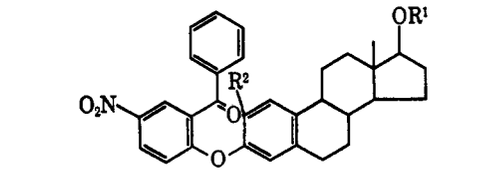
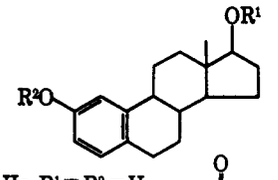
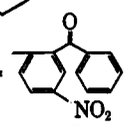
CHART I



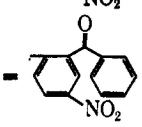
Ia, $R^1 = R^2 = H$
b, $R^1 = Ac; R^2 = H$
c, $R^1 = Ac; R^2 = OH$
d, $R^1 = Ac; R^2 = OSO_3^-$

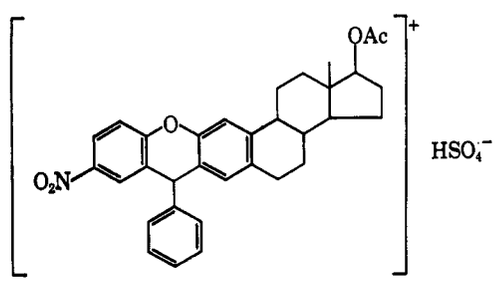


IIa, $R^1 = R^2 = H$
b, $R^1 = H; R^2 =$

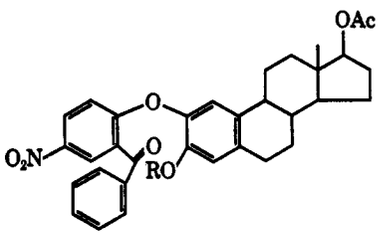


c, $R^1 = Ac, R^2 =$

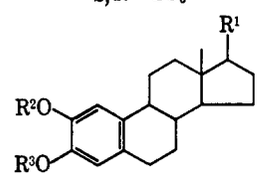




III



IVa, R = H
b, $R = SO_3^-$



Va, $R^1 = OH; R^2 = SO_3^-; R^3 = H$
b, $R^1 = OH; R^2 = H; R^3 = SO_3^-$
c, $R^1 = O; R^2 = R^3 = SO_3^-$

the two compounds are different and correspond to the structure anticipated from the synthetic sequences employed, as well as to the electron-withdrawing nature of the sulfate group. The H_1 and H_4 resonances of Va are 29 cps apart at 424 and 395 cps, respectively. In contrast, those observed for Vb are 4 cps apart at 411 and 407 cps with the H_4 resonance being the more downfield one. For comparison purposes 2-hydroxy estrone disulfate Vc was prepared by sulfation with excess reagent. The nmr spectrum of this compound shows that both the H_1 and H_4 resonance has been equally shifted downfield by 50 cps to 450 and 438 cps, leaving the separation between them constant at 12 cps. It is of interest that the nmr spectrum of the product obtained from sulfation of 2-hydroxyestrone with 1 equiv of reagent exhibited aromatic proton absorptions of equal intensity at 395, 407, 411, and 424 cps, indicating that it consists of a 1:1 mixture of two monosulfates and that there is no preferential sulfation of either of the two phenolic groups. From the lack of any bands further downfield, it appears that no disulfate was formed. This is expected since sulfation of one of the *o*-hydroxyl groups deactivates the other and it ceases to be an effective competitor for the available reagents.

The *o*-catechol monosulfates Va and Vb are at least as stable as phenol sulfates but are hygroscopic and retain moisture with tenacity. The hydrolysis of these sulfates and that of other estrogen sulfates will be the subject of a subsequent report.

The procedures described provide methods for the syntheses of potential naturally occurring conjugates of the catechol estrogens and permit the study of their involvement in estrogen metabolism and particularly their participation in the subsequent O-methylation. With modifications, the syntheses given can be applied to the preparation of monoderivatives of other unsymmetrical catechols and may be useful in the preparation of the particularly interesting isomeric catechol amine monosulfates.

Experimental Section[9]

Trimethylammonium 17β-Acetoxy-$\Delta^{1,3,5(10)}$-estratrien-2-ol 3-(2-Benzoyl-4-nitro)phenyl Ether 2-Sulfate (Id).—To a solution of 83.9 mg (0.150 mmole) of 2-hydroxy-17β-acetoxy-$\Delta^{1,3,5(10)}$-estratriene 3-(2-benzoyl-4-nitro)phenyl ether (Ic)[5] in 1 ml of pyridine, 105.6 mg (0.757 mmole) of sulfur trioxide–trimethylamine was added. The mixture was stirred at room temperature for 18 hr and then the solvent was removed at reduced pressure at 45°. The oily residue was dissolved in 1 ml of methanol and, after preparative thin layer chromatography on Merck silica gel GF in the system methanol–ethyl acetate (1:3), gave 54.5 mg of orange-yellow powder which on trituration with methanol–ether gave a crystalline solid: mp 191–194°; uv, λ_{max} 257 mμ, λ_{min} 237 mμ.

Sodium $\Delta^{1,3,5(10)}$-Estratriene-2,3,17β-triol 2-Sulfate (Va).—Compound Id (56.9 mg) was suspended in 5 ml of 5% sodium hydroxide solution and stirred at room temperature for 18 hr. To this suspension, 20 ml of water was added and the mixture was extracted three times with 30 ml of 1-butanol. The butanol extract was evaporated below 45° to give a dark red oil. Trituration with ether gave a yellowish brown precipitate, which was filtered, washed with ether, and dried over sodium hydroxide *in vacuo* to give 57.7 mg of material. The crude product was decolorized with charcoal and was submitted to preparative thin layer chromatography on silica gel, in the system methanol–ethyl acetate (1:3). Trituration with methanol–ether gave 21.1 mg of semicrystalline solid, which on heating became pink at 185°, red at 206°, and melted at 209°. The nmr spectrum showed aromatic proton resonances at 395 and 424 cps downfield from tetramethylsilane: λ_{max} 281 mμ (ε 2700); λ_{min} 245.5 mμ (ε 450).

Anal. Calcd for $C_{18}H_{23}O_6SNa \cdot 2.5H_2O$: C, 49.64; H, 6.48. Found: C, 49.27; H, 6.78.

17β-Hydroxy-$\Delta^{1,3,5(10)}$-estratriene 2-(2-Benzoyl-4-nitro)phenyl Ether (IIb).—To a solution of 700.2 mg (2.57 mmoles) of $\Delta^{1,3,5(10)}$-estratriene-2,17β-diol (IIa) and 107 mg (1.91 mmoles) of potas-

(9) Melting points were determined on a hot-stage apparatus and are corrected. Ultraviolet spectra were measured in ethanol using a Cary recording spectrophotometer. Nmr spectra were obtained on a Varian A-60 instrument in dimethyl sulfoxide using tetramethylsilane as an internal standard. Chemical shift values are given in cycles per second. Analyses were performed by Spang Microanalytical Laboratory.

sium hydroxide in 30 ml of 95% ethanol, 344.2 mg (1.32 mmoles) of 2-chloro-5-nitrobenzophenone was added. After refluxing for 48 hr the dark orange solution was poured into 100 ml of cold 1 *N* sodium hydroxide solution. The white suspension was extracted three times with 50 ml of chloroform and after drying over anhydrous sodium sulfate and evaporation a viscous brown-red oil was obtained; this was dissolved in 10 ml of 1:1 petroleum ether (bp 60°)–benzene and chromatographed on 15 g of alumina. Elution with 1:1 to 1:2 petroleum ether–benzene and crystallization from ether gave 412 mg of IIb: mp 125–127°; ir carbonyl absorption at 1672 cm^{-1} in chloroform; λ_{max} 255.5 mμ (ε 18,800), 297 (12,000); λ_{min} 237 mμ (ε 14,300), 287 (11,900).

Anal. Calcd for $C_{31}H_{31}O_5N \cdot 0.5H_2O$: C, 73.49; H, 6.37. Found: C, 73.27; H, 6.31.

17β-Acetoxy-$\Delta^{1,3,5(10)}$-estratriene 2-(2-Benzoyl-4-nitro)phenyl Ether (IIc).—To a solution of 250 mg (0.494 mmole) of IIb in 3 ml of pyridine 1.5 ml of acetic anhydride was added and the mixture was kept at room temperature overnight. The reaction mixture was poured into ice–water and extracted four times with 50 ml of chloroform. The chloroform extracts were washed with 5% sulfuric acid and 5% sodium carbonate solutions and with water. After drying over sodium sulfate, the extract was evaporated to dryness to give an oily residue which was triturated with petroleum ether to give a white yellow powder (255 mg, 96%). Crystallization from petroleum ether–ether gave 207 mg of colorless needles, mp 171.5–173.5°. The analytical sample melted at 172–174°.

Anal. Calcd for $C_{33}H_{33}O_6N$: C, 73.45; H, 6.16. Found: C, 73.03; H, 6.23.

3-Hydroxy-17β-acetoxy-$\Delta^{1,3,5(10)}$-estratriene 2-(2-Benzoyl-4-nitro)phenyl Ether (IVa).—To a solution of 160.8 mg (0.298 mmole) of IIc in 0.23 ml of glacial acetic acid, 0.8 ml of cold, concentrated sulfuric acid was added slowly with cooling and shaking. The dark red mixture was kept at room temperature for 30 min. It was then diluted with 3.2 ml of glacial acetic acid and 0.8 ml of a 1:1 mixture of acetic acid and 30% hydrogen peroxide was added dropwise with shaking. The color of the solution began immediately to lighten and a tan precipitate appeared. The mixture was allowed to stand 30 min more, poured into ice–water, and the yellowish white precipitate was filtered. The precipitate, after washing well with 5% sodium bicarbonate solution and water, was air dried. The material was dissolved in a small amount of 1:1 petroleum ether–benzene and chromatographed on 2 g of alumina (Merck, acid washed). Elution with 1:1 petroleum ether–benzene and crystallization from methanol gave 143 mg (83%) of IVa: mp 123–126° (the analytical sample melted at 126–129°); λ_{max} 256 mμ (ε 18,000), 289 (15,000); λ_{min} 237 mμ (13,000), 276 (14,000); ir C=O absorption at 1655 cm^{-1} in chloroform, indicating strong hydrogen bonding.

Anal. Calcd for $C_{33}H_{33}O_7N \cdot H_2O$: C, 69.09; H, 6.15. Found: C, 69.09; H, 5.83.

Trimethylammonium 17β-Acetoxy-$\Delta^{1,3,5(10)}$-estratrien-3-ol 2-(2-Benzoyl-4-nitro)phenyl Ether 3-Sulfate (IVb).—To a solution of 80.7 mg (0.141 mmole) of IVa in 1 ml of pyridine, 105.0 mg (0.754 mmole) of sulfur trioxide–trimethylamine complex was added and the mixture was stirred at room temperature for 18 hr. The reaction was worked up as previously described for the preparation of Id. A light yellow crystalline solid, 67.1 mg, mp 189–195°, was obtained which on trituration with methanol–ether gave a crystalline material melting at 190–194°.

Sodium $\Delta^{1,3,5(10)}$-Estratriene-2,3-17β-triol 3-Sulfate (Vb).—Compound IVb (40.0 mg) was treated with 5 ml of 5% sodium hydroxide solution in the same way as described for the preparation of Va. The crude product, 44.2 mg, was decolorized with charcoal and further purified by preparative thin layer chromatography on silica in the system methanol–ethyl acetate (1:3). Crystallization of the purified product from a small amount of methanol gave 7.0 mg of crystals which melted with decomposition at 192–193°. The nmr spectrum exhibited aromatic proton bands at 407 and 410 cps; uv showed λ_{max} 282 mμ (ε 2730), λ_{min} 247 mμ (ε 580).

Anal. Calcd for $C_{18}H_{23}O_6SNa \cdot 1.5H_2O$: C, 51.79; H, 6.28. Found: C, 51.89; H, 5.98.

Disodium 17-Oxo-$\Delta^{1,3,5(10)}$-estratriene-2,3-diol 2,3-Disulfate (Vc).—To 3.2 ml of pyridine, 250 mg (0.98 mmole) of pyridine sulfate and 0.1 ml (1.06 mmoles) of acetic anhydride were added. The mixture was stirred for 30 min and 62.6 mg (0.22 mmole) of 2-hydroxyestrone dissolved in 0.6 ml of pyridine was added and the stirring was continued for 18 hr. The reaction mixture was evaporated to dryness at 40° *in vacuo* and the residue was dissolved in 20 ml of water. After the pH was adjusted to 8.0 with 5% sodium hydroxide solution, the solution was extracted with ether to remove unreacted materials. The aqueous solution was then further adjusted to pH 12 and extracted five times with 10-ml portions of 1-butanol. The butanol extracts were combined and evaporated to dryness to give 67.7 mg of crude product. Examination of the crude product by thin layer chromatography showed that it was a mixture of disulfate and monosulfates of 2-hydroxyestrone. The disulfate was obtained by dissolving the crude product in 1 ml of methanol and adding ether to the solution until the disulfate precipitated. Crystallization from methanol–ether gave 10.2 mg (9%) of slightly brownish white product which turned red without melting at 233° and then decomposed at about 260°. The aromatic proton resonances in dimethyl sulfoxide were at 438 and 451 cps; uv showed λ_{max} 247 mμ (ε 1740), λ_{min} 249.5 mμ (ε 260).

Anal. Calcd for $C_{18}H_{20}O_9S_2Na_2 \cdot 2H_2O$: C, 41.00; H, 4.58. Found: C, 41.09; H, 4.45.

Partial Sulfation of 2-Hydroxyestrone.—To a solution of 50.6 mg (0.176 mmole) of 2-hydroxyestrone in 1 ml of pyridine, 30.1 mg (0.216 mmole) of sulfur trioxide–trimethylamine was added. The mixture was stirred at room temperature for 18 hr. After the reaction ended, the mixture was poured into 50 ml of ether and the white precipitate which soon turned slightly brownish was filtered, washed with 50 ml of ether, and finally dried over sodium hydroxide *in vacuo* to give 56.0 mg of hygroscopic product. The nmr spectrum of the crude product in dimethyl sulfoxide showed aromatic resonances at 395, 407, 410, and 424 cps. The bands were of equal intensity, indicating a 1:1 mixture of 2- and 3-monosulfates. No bands were visible at 438 or 450 cps, indicating the absence of the disulfate.

Hydrolysis of Monosulfates Va and Vb.—A small sample (1 mg) of each compound was dissolved in 1 ml of 0.1 *N* HCl and warmed at 50° for 4 hr. The aqueous solution was then extracted with chloroform which was dried and evaporated. The residue was run on thin layer chromatography in the system 50% cyclohexane–48% ethyl acetate–2% acetic acid to produce material which was identical with the authentic 2-hydroxyestradiol standard.

Registry No.—Id, 15448-42-7; IIb, 15448-43-8; IIc, 15448-44-9; IVa, 15448-45-0; IVb, 15448-46-1; Va, 15448-41-6; Vb, 15448-39-2; Vc, 15448-40-5.