# PREPARATION OF SULFATE ESTERS. THE SYNTHESIS OF STEROID SULFATES BY A DICYCLOHEXYLCARBODIIMIDE-MEDIATED SULFATION

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ABSTRACT

Steroid sulfates were synthesized in good yield under mild conditions by a dicyclohexylcarbodiimide-mediated sulfation. The reaction is especially suitable for the synthesis of $^{35}S$-labeled steroid sulfates owing to the readily available and reasonably priced $^{35}SO_4^{=}$. Alkyl hydroxyl groups can be sulfated under dilute reaction conditions while phenolic hydroxyl groups are not sulfated, as illustrated in the direct synthesis of 17β-estradiol-17-sulfate from 17β-estradiol. Phenolic hydroxyl groups, however, can be sulfated under concentrated reaction conditions. There is no noticeable alteration in the position of the double bonds or sulfonation of the aromatic rings. The yields of steroid sulfate seem to be partially dependent upon the type and position of the hydroxyl group being sulfated. The more sterically hindered the alkyl hydroxyl group the lower the yield.

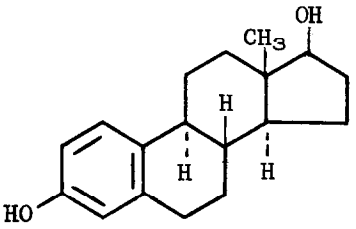
The recent biochemical interest in steroid sulfates makes it desirable to synthesize these conjugates. Steroid sulfates labeled selectively in the steroid nucleus ($^{14}C$ or $^{3}H$) or in the sulfate group ($^{35}S$) are useful for metabolic studies, and a number of sulfating agents have been described (1-5). Our laboratory has recently reported the selective synthesis of sulfate esters under mild conditions, in good yields, by a dicyclohexylcarbodiimide (DCC)-mediated reaction (6-8). Reaction conditions were found under which alkyl hydroxyl groups were sulfated and phenolic hydroxyl groups were not. Therefore, this new method of preparing

$$DCC + H_2SO_4 + ROH \xrightarrow{DMF} ROS(=O)_2-OH + \text{dicyclohexylurea}$$

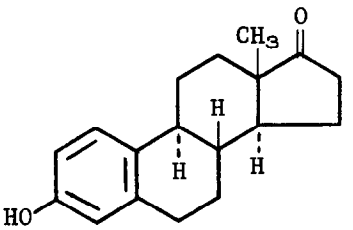
sulfate esters has great potential for the synthesis of steroid sulfates, owing to the mild conditions of the reaction, the readily available starting materials, and its selectivity. This publication describes the synthesis of a variety of steroid sulfates which demonstrate the usefulness and selectivity of the DCC-mediated sulfation. The steroid conjugates synthesized were the sodium or potassium salts of 17β-estradiol-17-sulfate (3-hydroxyestra-1,3,5[10]-trien-17β-yl sulfate), estrone sulfate (17-oxoestra-1,3,5[10]-trien-3-yl sulfate), androsterone sulfate (17-oxo-5α-androstan-3α-yl sulfate), dehydroepiandrosterone sulfate (17-oxoandrost-5-en-3β-yl sulfate), and deoxycorticosterone sulfate (3,20-dioxopregnene-21-yl sulfate). These steroids possess functional groups typical of many steroids.

The general procedure for the synthesis and isolation of the steroid sulfates is shown in Figure 1. A predetermined quantity of sulfuric acid (may be $^{35}S$-labeled) dissolved in dimethylformamide (DMF), was added to a solution containing DCC and a selected steroid dissolved in DMF at 0°. Reaction occurred immediately, as evidenced by the formation of the insoluble dicyclohexylurea. The molar ratio of the reactants used was steroid:$H_2SO_4$:DCC; 1:1.5:5. The concentration of the reactants was varied with the type of hydroxyl group being sulfated. One mmole of steroid per 40 ml of DMF was used for the sulfation of alkyl hydroxyl groups, and 1 mmole of steroid per 4 ml of DMF for the sulfation of phenolic hydroxyl groups.

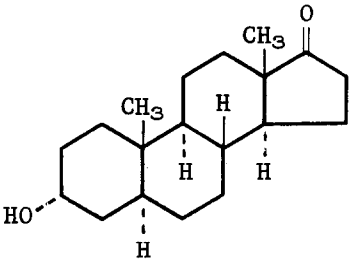
After 15 minutes the entire mixture was transferred to a diethylaminoethyl (DEAE) cellulose column (chloride form) and eluted with methanol. The slightly soluble dicyclohexylurea eluted slowly, requiring a



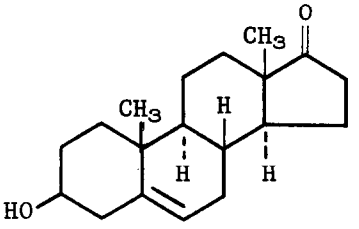

17β-Estradiol



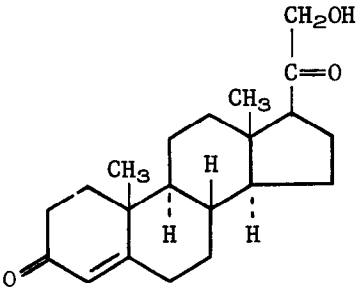

Estrone




Androsterone




Dehydroepiandrosterone




Deoxycorticosterone

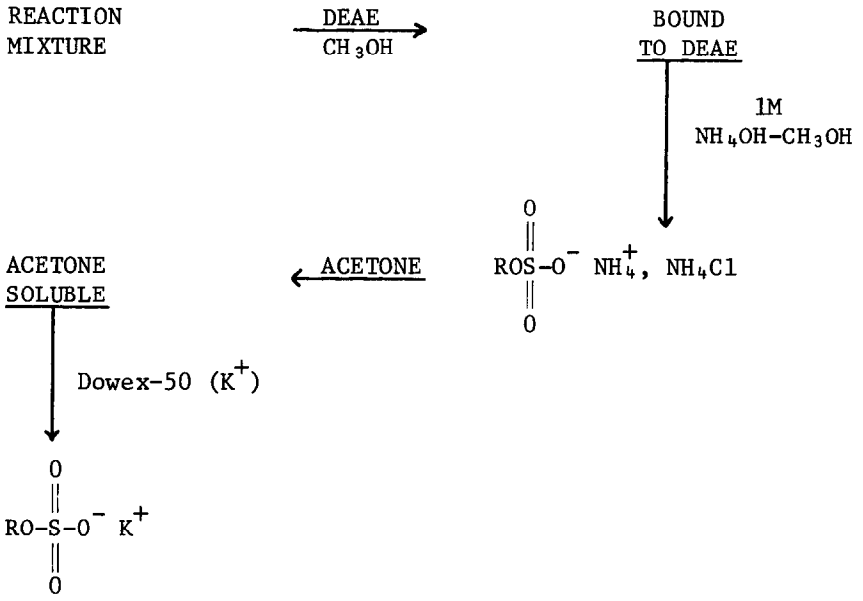



Figure 1

large volume of methanol. The DEAE-bound steroid sulfate was removed from the column with methanolic ammonium hydroxide. The eluate was then flash-evaporated. The residue, consisting of steroid sulfate and some ammonium chloride, was washed with a large volume of acetone. A partial purification was achieved because the steroid sulfate dissolved in the acetone, while the ammonium chloride essentially did not dissolve. The acetone solution was then passed through a Dowex-50 column ($Na^+$ or $K^+$ form). The resulting acetone solution was immediately flash-evaporated, the residue being relatively pure steroid sulfate. The sodium or potassium chloride impurity obtained from the ion exchange columns was insoluble in acetone.

The overall reaction time and the isolation of the product ($NH_4^+$ form) was approximately three hours. Thin-layer chromatography (TLC) ($CHCl_3$:$CH_3OH$:$H_2O$; 65:25:4,v/v) was used to check the completeness of the reaction and the purification procedures. TLC can also be used as a

method of isolation of steroid sulfates, especially when only radiochemical quantities are desired. All products were analyzed by infrared, ultraviolet, and mass spectroscopy, and their spectra are consistent with proposed structures and with published spectra.

The direct synthesis of 17β-estradiol-17-sulfate from 17β-estradiol illustrates the selective sulfation ability of the reaction. The secondary 17β-alkyl hydroxyl group was selectively sulfated while the phenolic hydroxyl group was not. The 17β-estradiol-17-sulfate was formed in 63% yield. The potassium salt gave good elemental analysis, while the sodium salt did not. Kirdani (1) reported the same problem with the sodium salt and attributed the difficulty to solvent of crystallization.

By choosing the right conditions, the DCC-mediated reaction can be employed in the sulfation of phenolic groups. An example of this sulfation is in the synthesis of estrone sulfate, where concentrated reaction conditions are required. Under these concentrated reaction conditions, the sulfation is not selective; a product in addition to the monosulfates is produced, and this product is presumed to be a sulfate ester anhydride (8) (pyrosulfate diester). The formation of a side product may explain the relatively poor yield of estrone sulfate (45%).

Two steroids, androsterone and dehydroepiandrosterone, were chosen to illustrate the ability of the reaction to sulfate the 3α- and 3β-hydroxyl groups (70-73% yields). The double bond in dehydroepiandrosterone remains unaltered. Deoxycorticosterone possesses a primary 21-hydroxyl group and was converted to the sulfate ester in good yield, 93%. Table I shows the percentage yields of steroid sulfates, relative to the position of the hydroxyl group. The more sterically hindered the hydroxyl group the lower the yield.

Table I. Yield of Steroid Sulfate

| Position and Type of Hydroxyl Group | % Yield |
|---|---|
| Secondary | |
| 17β | 56 |
| 3α | 70 |
| 3β | 73 |
| Primary | |
| 21 | 93 |

## EXPERIMENTAL

Materials and Methods. All solvents were distilled before use. The steroids were purchased from Supelco, Inc., Bellefonte, Pa., and their purity was checked with TLC (visualized with $I_2$ and with p-toluene sulfonic acid) (9). The DCC was purchased from Eastman Organic Chemicals, Rochester, N. Y., and the DMF (certified) was purchased from Fisher Scientific Co., Pittsburgh, Pa. Infrared spectra were obtained with a Perkin Elmer Model 521 spectrophotometer (KBr). A Cary Model 14 spectrophotometer was used to record ultraviolet spectra (ethanol). A Bendix Time of Flight Mass spectrometer was used for the laser ionization mass spectra (10). Elemental analyses were performed by Midwest Microlab, Inc., Indianapolis, Indiana. Whatman diethylaminoethyl cellulose (DE-11) and Dowex 50W-X8 columns were used as ion exchangers.

17β-estradiol-17-sulfate. One mmole of 17β-estradiol, dissolved in 12 ml of DMF, was added to 5.0 mmole of DCC dissolved in 16 ml of DMF in an ice bath. To this mixture was added 1.5 mmole of $H_2SO_4$, dissolved in 12 ml of DMF. After the last of the three components had been added, the mixture was occasionally shaken for 15 minutes and then transferred to a DEAE-cellulose column (4 gm, 2 cm x 9 cm, chloride form), and washed with one liter of methanol to elute the unreacted DCC, steroid, DMF, and dicyclohexylurea. The steroid sulfate was then eluted from the column with 200 ml of 1M methanolic $NH_4OH$ (65 ml of conc. $NH_4OH$ diluted to one liter with methanol). This fraction was immediately flash-evaporated, the residue suspended in 200 ml of acetone and filtered. The acetone solution was then passed through a Dowex-50 column (1 cm x 30 cm, potassium form) and after flash-evaporation 250 mg (56%) of potassium 17β-estradiol-17-sulfate was obtained, m.p. 175°-185°, $\lambda$ max = 281 mμ($\varepsilon$ = 2010).

Anal. calcd. for $C_{18}H_{23}O_5SK \cdot H_2O$: C, 52.92; H, 6.17; S, 7.85; K, 9.57
found: C, 52.47; H, 6.21; S, 7.81; K, 9.39

Estrone Sulfate. Estrone (1.5 mmole) was suspended in 1.8 ml of DMF and added to an ice-cooled flask containing DCC (7.5 mmole) in 2.4 ml of DMF. To this mixture was added $H_2SO_4$ (2.25 mmole) dissolved in 0.8 ml of DMF. The method of isolation was the same as with 17β-estradiol-17-sulfate except that the sodium salt was prepared. The yield of sodium estrone sulfate was 200 mg (45%), m.p. 225-230°, λ max = 276 mμ(ε = 742) and 270 mμ(ε = 761).

Anal. calcd. for $C_{18}H_{21}O_5SNa \cdot H_2O$: C, 55.37; H, 5.94; S, 8.21; Na, 5.89
found: C, 55.51; H, 5.85; S, 8.73; Na, 5.65

Androsterone Sulfate. Androsterone (1.0 mmole), DCC (5.0 mmole), and $H_2SO_4$ (1.5 mmole) were combined and the reaction product isolated in the same manner as in the synthesis of 17β-estradiol-17-sulfate. The yield of potassium androsterone sulfate was 325 mg (70%), m.p. 180°-181°.

Anal. calcd. for $C_{19}H_{29}O_5SK \cdot H_2O$: C, 53.50; H, 7.32; S, 7.52; K, 9.17
found: C, 53.43; H, 7.37; S, 7.56; K, 9.07

Dehydroepiandrosterone Sulfate. Dehydroepiandrosterone (1.0 mmole), DCC (5.0 mmole), and $H_2SO_4$ (1.5 mmole) were combined and the reaction product isolated in the same manner as in the synthesis of 17β-estradiol-17-sulfate. The yield of potassium dehydroepiandrosterone sulfate was 310 mg (73%), m.p. 225°-229°.

Anal. calcd. for $C_{19}H_{27}O_5SK \cdot H_2O$: C, 53.75; H, 6.88; S, 7.55; K, 9.21
found: C, 53.87; H, 6.84; S, 7.53; K, 9.08

Deoxycorticosterone Sulfate. Deoxycorticosterone (1.0 mmole), DCC (5.0 mmole), and $H_2SO_4$ (1.5 mmole) were combined and the reaction product isolated in the same manner as in the synthesis of 17β-estradiol-17-sulfate. The yield of potassium deoxycorticosterone sulfate was 410 mg (93%), m.p. 155°-158°.

Anal calcd. for $C_{21}H_{29}O_6SK \cdot H_2O$: C, 54.05; H, 6.70; S, 6.87; K, 8.38
found: C, 54.43; H, 6.68; S, 6.82; K, 8.51

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