STEROID CONJUGATES IV. THE PREPARATION OF STEROID SULFATES WITH TRIETHYLAMINE-SULFUR TRIOXIDE

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A lengthy series of steroid sulfate triethylammonium salts has been prepared using triethylamine-sulfur trioxide in pyridine as the sulfating agent. In addition to the expected sulfating capacity of the reagent, selective sulfation was observed in those cases where rate factors greatly differentiated between the reaction of various hydroxyl groups. Modifications of the general reaction conditions have allowed the sulfation of hindered hydroxyl groups, and the preparation of several disulfate salts. A simple procedure for the conversion of triethylammonium salts to the corresponding ammonium salts has been outlined.

The sulfation of alcohols with tertiary amine-sulfur trioxide complexes presents a process which has found ever increasing utility in synthetic organic chemistry.[2] Thus in the preparation of steroid sulfate conjugates, pyridine-sulfur trioxide[3] has been widely employed for this purpose. In this report we wish to present our experiences with triethylamine-sulfur trioxide.[2,4] We feel that this reagent offers some distinct advantages for the preparation of a wide variety of steroidal mono- and di-sulfates.

In the Table are outlined a number of representative steroid sulfates which have been prepared in this study. The triethylammonium sulfates[4,5] are generally highly crystalline derivatives which can be readily recrystallized. Analytical data indicate the salts are

non-hydrated, appear to be non-hygroscopic and stable under normal storage conditions.[6] An advantage of the triethylammonium salts is their solubility in methylene chloride which conveniently allows their recrystallization from methylene chloride-anhydrous ether. This solubility also aids in their purification since passage of a methylene chloride solution of the salt through a pad of an adsorbent such as Magnesol® removes colored impurities and, in some cases, unreacted starting material. The adsorbent also removes traces of water which would hamper recrystallization. The triethylammonium salts can easily be converted to their corresponding ammonium salts by passage of a stream of gaseous ammonia into a methylene chloride or methylene chloride-anhydrous ether (2:1) solution of the former. The precipitated ammonium salt can be recovered by filtration.

The preparation of the sulfates I through VIII listed in the Table was easily accomplished at room temperature (2 hr) in dry pyridine employing 1.1 equivalents of the sulfating agent. No difficulties arose in sulfation of the 17β-hydroxy group in III; in fact, 17β-sulfooxyandrost-4-en-3-one triethylammonium salt (III) was isolated in at least 80% yield (recrystallized-analytical quality). A base-labile hydroxyl group was successfully sulfated in the preparation of 6β-sulfooxypregn-4-ene-3,20-dione triethylammonium salt (XI). The latter was recrystallized without noticeable decomposition.

The use of the reagent for selective hydroxyl group sulfation was next investigated. Employing 1.1 equivalents of the reagent and normal reaction conditions (room temperature 2 hr) allowed the preparation of 17α-hydroxy-3β-sulfooxypregn-5-en-20-one triethylammonium salt (IX). When the quantity of the reagent was

increased to 5.0 equivalents and the reaction time extended to 17 hr, thin layer chromatography indicated the presence of a mixture of IX and the 3,17-disulfate. An increase in the reaction temperature to 90-95° (3 hr) brought about complete conversion to the disulfate. The ditriethylammonium salt appeared to be a low melting compound, and therefore, the disulfate in methylene chloride-ether solution was treated with gaseous ammonia, being converted to and characterized as the diammonium salt X. 17α-Sulfooxypregn-4-ene-3,20-dione triethylammonium salt (XII) was prepared, albeit in low yield (13%), using the reaction conditions triethylamine-sulfur trioxide (1.1 eq.) - room temperature - overnight reaction time. When the reaction conditions were made more vigorous, (70-95° for 3 hr), a 70% yield of XII was obtained.

Concerning the sulfation of the 21-hydroxyl group in the corticoid series, selective reaction has yielded the following triethylammonium salts, 21-sulfooxypregn-4-ene-3,20-dione (XIII), 17α-hydroxy-21-sulfooxypregn-4-ene-3,20-dione (XIV), 17α-hydroxy-21-sulfooxypregn-4-ene-3,11,20-trione (XVI) and 11β,17α-dihydroxy-21-sulfooxypregn-4-ene-3,20-dione[7] (XVIII). In addition, XVI was converted to its ammonium salt XVII with gaseous ammonia to illustrate the salt exchange in this series. The preparation of 17α,21-disulfooxypregn-4-ene-3,20-dione - reaction conditions: sulfating agent (5.0 eq.) - 70-90° - 3 hr - was successful with the ditriethylammonium salt XV being conveniently characterized in this instance.

The aromatic sulfates XIX through XXI have been included to illustrate examples in the estrogen series. The conversions are extremely facile and actually represent nearly ideal reaction

conditions for the preparation of estrogen sulfates. We have investigated the synthesis of estrogen sulfate conjugates in greater depth, which will be the subject of a future publication.

Experimental

Melting Points. - All melting points are uncorrected.

Optical Rotations. - The rotations are for chloroform solution or as otherwise stated, and were carried out at 25°.

Absorption Spectra. - The ultraviolet spectra were determined in methanol. The infrared spectra are for a pressed potassium bromide disc or as otherwise stated. NMR data were obtained on a Varian A-60 spectrometer using TMS as internal standard.

Petroleum Ether. - The fraction used had bp 30-75°.

Celite®. - (Johns-Manville Company), a diatomaceous silica product.

Florisil®. - (Floridin Corporation), a synthetic magnesium silicate adsorbent.

Magnesol®. - (Food Machinery Chemical Corp.), a hydrous magnesium silicate.

Thin Layer Chromatography Solvent System. - Benzene:acetone:water (2:1:2) upper phase 70%, methanol 30%.

Thin Layer Chromatography. - The thin layer chromatograms were carried out at room temperature on glass plates coated with approximately 0.25 mm of Silica Gel G (Merck, Darmstadt) prepared according to E. Stahl, CHEM. ZTG. 82, 323 (1958) with approximately 0.3% Radelin Phosphor GS-115 (United States Radium Corporation, Morristown, N.J.), added. After developing the chromatogram, the plates were viewed under a Mineralight® UVS.11 (Ultra Violet Products Inc., San Gabriel, California) ultraviolet source and then sprayed with a 10% phosphomolybdic acid-methanol solution (slight heating of plates to develop spots).

Purification of Triethylamine-Sulfur Trioxide.

Commercial grade triethylamine-sulfur trioxide (30 g) was dissolved in methylene chloride (50 ml) and this solution was passed through a 35 mm column of Magnesol® (packed in a 35 mm sintered glass funnel) followed by additional methylene chloride (250 ml). The clear effluent was heated to reflux on a steam bath, and anhydrous diethyl ether was added slowly with continued reflux until a heavy crystalline precipitate was evident. This suspension

was cooled and the reagent was recovered by filtration, 25.5 g, mp 92-93°, reported[4] mp 91.5°.

General Procedure for the Monosulfation of a Steroid.

The steroid was dissolved in the minimum volume of dry pyridine required to effect complete solution. To this solution was added solid triethylamine-sulfur trioxide (1.1 eq.).[8] After standing approximately 2 hr at room temperature the reaction mixture was poured into anhydrous ether (approximately 10 to 20 times the volume of the reaction mixture). The precipitated salt was filtered, washed several times with anhydrous ether, and then dissolved in a minimum volume of methylene chloride. This solution (usually colored) was added to the top of a sintered glass funnel which had previously been packed with Magnesol® (approximately 10 g/1 g of steroid). Additional methylene chloride was passed through the column under reduced pressure and the effluent stream was monitored for the termination of the steroid flow. The methylene chloride effluent was refluxed on a steam bath and anhydrous ether was continuously added until the precipitation of the salt was noted. The solution was then cooled and the crystals were collected by filtration. Additional compound could be obtained by the addition of more anhydrous ether to the initial mother liquor. The purity of the salt was established by thin layer chromatography using the system outlined in the introduction to the Experimental. Recrystallization of salts was accomplished from the same solvent pair, methylene chloride-anhydrous ether.

For the monosulfation of steroids having a hindered hydroxyl group (e.g. XII) the most favorable reaction conditions were 1.1 equivalents of reagent at 70-95° for 3 hr.

General Procedure for the Disulfation of Steroids.

The experimental conditions for the disulfation of steroids were essentially those outlined for monosulfation with the exception that the molar quantity of triethylamine-sulfur trioxide was increased from 1.1 to 5.0 equivalents, and the temperature of the reaction mixture was increased from room temperature to 90-95°. Product isolation has been detailed in the monosulfate section.

General Procedure for the Conversion of Triethylammonium Salts to the Corresponding Ammonium Salts.

A solution of the triethylammonium salt in methylene chloride or methylene chloride-anhydrous ether (2:1) was treated with a stream of gaseous ammonia with the immediate appearance of a precipitate. This process was continued for 2 min and then the product was recovered by filtration. Recrystallization of the ammonium salt was accomplished from methanol-anhydrous ether.

Table

Physical Constants of Steroid Sulfate Salts

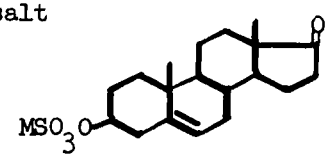
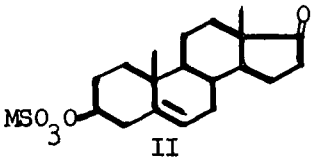
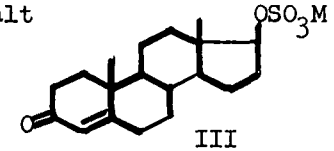
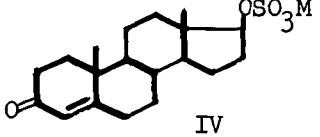
Compound	M^+	Mp	$[\alpha]_D^{25}$ $CHCl_3$	λ_{max}^{MeOH} mμ (ε)	Nmr Signals Hz [a]
3β-Sulfooxyandrost-5-en-17-one triethylammonium salt  I	$NH(C_2H_5)_3$	220-222°	+1°	-	18-CH_3 = 52 19-CH_3 = 61 H-3 = 255 (broad) H-6 = 324 (m) HN = 580 (broad)
Anal. Calcd for $C_{25}H_{43}NO_5S$ (469.66): C, 63.92; H, 9.22; N, 2.98; S, 6.82. Found: C, 63.67; H, 9.46; N, 2.94; S, 6.81					
3β-Sulfooxyandrost-5-en-17-one ammonium salt  II	NH_4	201-202° dec	-	-	-
Reported[b] 206-207° mp					
17β-Sulfooxyandrost-4-en-3-one triethylammonium salt  III	$NH(C_2H_5)_3$	158-163°	+64°	241 (16,400)	18-CH_3 = 52 19-CH_3 = 72 H-4 = 345 H-17 = 262 (t)
Anal. Calcd for $C_{25}H_{43}NO_5S$ (469.66): C, 63.92; H, 9.22; N, 2.98; S, 6.82. Found: C, 64.22; H, 9.14; N, 2.67; S, 6.78.					
17β-Sulfooxyandrost-4-en-3-one ammonium salt  IV	NH_4	202-204°	-	-	-
Reported[b] 206-207° mp					

Table (continued)

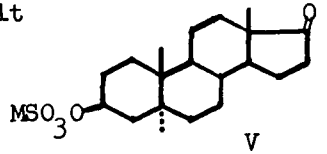
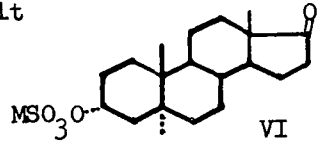
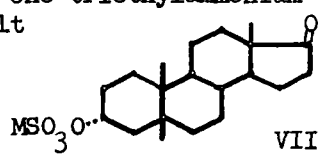
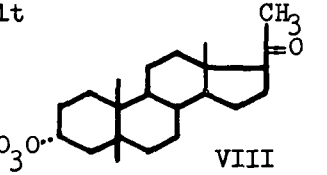
Compound	M^+	Mp	$[\alpha]_D^{25}$ $CHCl_3$	λ_{max}^{MeOH} mμ(ε)	Nmr Signals Hz [a]
3β-Sulfooxy-5α-androstan-17-one triethylammonium salt  V	$NH(C_2H_5)_3$	210-212°	+52°	-	18-CH_3 = 50, 51 19-CH_3 = H-3 = 257 (broad) HN = 588 (broad)
Anal. Calcd for $C_{25}H_{45}NO_5S$ (471.68): C, 63.65; H, 9.62; N, 2.97; S, 6.80. Found: C, 63.85; H, 9.70; N, 2.96; S, 6.71.					
3α-Sulfooxy-5α-androstan-17-one triethylammonium salt  VI	$NH(C_2H_5)_3$	206-207°	+53°	-	18-CH_3 = 49, 51 19-CH_3 = H-3 = 279 (m) HN = 585 (broad)
Anal. Calcd for $C_{25}H_{45}NO_5S$ (471.68): C, 63.65; H, 9.62; N, 2.97; S, 6.80. Found: C, 63.40; H, 9.41; N, 2.96; S, 6.60.					
3α-Sulfooxy-5β-androstan-17-one triethylammonium salt  VII	$NH(C_2H_5)_3$	171-173°	+62°	-	18-CH_3 = 51 19-CH_3 = 58 H-3 = 282 (m)
Anal. Calcd for $C_{25}H_{45}NO_5S$ (471.68): C, 63.65; H, 9.62; N, 2.97; S, 6.80. Found: C, 63.22; H, 9.69; N, 2.87; S, 6.73.					
3α-Sulfooxy-5β-pregnan-20-one triethylammonium salt  VIII	$NH(C_2H_5)_3$	224-226°	+83°		18-CH_3 = 35 19-CH_3 = 54 21-CH_3 = 126 H-3 = 260 (m)
Anal. Calcd for $C_{27}H_{49}NO_5S$ (499.77): C, 64.85; H, 9.88; N, 2.80; S, 6.41. Found: C, 64.50; H, 9.43; N, 2.97; S, 6.53.					

Table (continued)

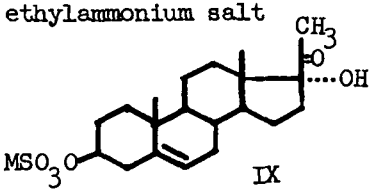
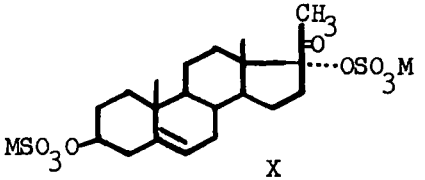
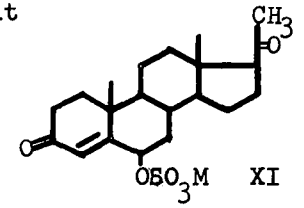
Compound	M^+	Mp	$[\alpha]_D^{25}$ $CHCl_3$	λ_{max}^{MeOH} mμ (ε)	Nmr Signals Hz
17α-Hydroxy-3β-sulfooxy-pregn-5-en-20-one triethylammonium salt  IX	$NH(C_2H_5)_3$	235-238°	-17° (MeOH)		18-CH_3 = 30 19-CH_3 = 56 H-3 = 220 (m) H-6 = 317 (m) OH-17 = 307 (s) d_6-DMSO
Anal. Calcd for $C_{27}H_{47}NO_6S$ (513.76): C, 63.12; H, 9.23; N, 2.93; S, 6.24. Found: C, 62.95; H, 9.09; N, 2.72; S, 6.31.					
3β,17α-Disulfooxypregn-5-en-20-one diammonium salt  X	NH_4	202-204° dec	+8° (MeOH)		18-CH_3 = 29 19-CH_3 = 57 21-CH_3 = 126 H-3 = 210 (m) H-6 = 318 (m) NH_4 = 425 d_6-DMSO
Anal. Calcd for $C_{21}H_{38}N_2O_9S_2$ (526.65): C, 47.89; H, 7.27; N, 5.32; S, 12.17. Found: C, 47.23, 47.67; H, 7.38, 6.94; N, 5.47; S, 11.78.					
6β-Sulfooxypregn-4-ene-3,20-dione triethylammonium salt  XI	$NH(C_2H_5)_3$	180-181°	+59°	236 (13,300)	18-CH_3 = 41 19-CH_3 = 80 21-CH_3 = 127 H-6 = 299 H-4 = 357
Anal. Calcd for $C_{27}H_{45}NO_6S$ (511.74): C, 63.35; H, 8.86; N, 2.74; S, 6.26. Found: C, 63.09; H, 8.89; N, 2.58; S, 6.24.					

Table (continued)

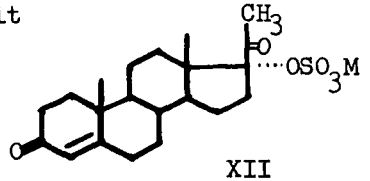
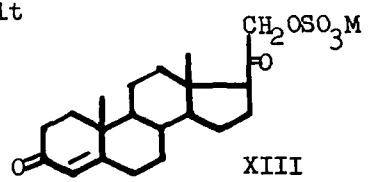
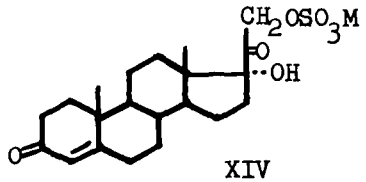
Compound	M^+	Mp	$[\alpha]_D^{25}$ $CHCl_3$	λ_{max}^{MeOH} mμ (ϵ)	Nmr Signals Hz
17α-Sulfooxypregn-4-ene-3,20-dione triethylammonium salt  XII	$NH(C_2H_5)_3$	209-212°	+104°	241 (17,600)	18-CH_3 = 39 19-CH_3 = 70 21-CH_3 = 137 H-4 = 342 (s)
Anal. Calcd for $C_{27}H_{45}NO_6S$ (511.74): C, 63.35; H, 8.86; N, 2.74; S, 6.26. Found: C, 63.78; H, 9.21; N, 3.23; S, 6.22.					
21-Sulfooxypregn-4-ene-3,20-dione triethylammonium salt  XIII	$NH(C_2H_5)_3$	141-142°	+120°	238 (19,400)	18-CH_3 = 40 19-CH_3 = 70 CH_2-21 = 273 (d) H-4 = 341 (s)
Anal. Calcd for $C_{27}H_{45}NO_6S$ (511.74): C, 63.35; H, 8.86; N, 2.74; S, 6.26. Found: C, 63.46; H, 8.68; N, 2.70; S, 6.16.					
17α-Hydroxy-21-sulfooxy-pregn-4-ene-3,20-dione triethylammonium salt  XIV	$NH(C_2H_5)_3$	224-225°	+88°	241 (16,600)	18-CH_3 = 39 19-CH_3 = 72 CH_2-21 = 296 (q) OH-17 = 287 (s) H-4 = 340 (s)
Anal. Calcd for $C_{27}H_{45}NO_7S$ (527.74): C, 61.32; H, 8.59; N, 2.66; S, 6.08. Found: C, 61.82; H, 8.48; N, 2.75; S, 5.86.					

Table (continued)

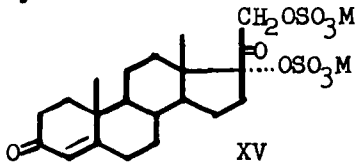
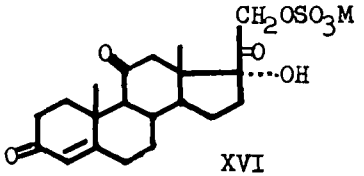
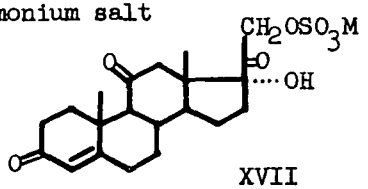
Compound	M^+	Mp	$[\alpha]_D^{25}$ $CHCl_3$	λ_{max}^{MeOH} mμ (ε)	Nmr Signals Hz
17α,21-Disulfooxypregn-4-ene-3,20-dione ditriethylammonium salt  XV	$NH(C_2H_5)_3$	201-202°	+90°	242 (16,600)	18-CH_3 = 39 19-CH_3 = 70 CH_2-21 = 302 (q) H-4 = 341 (s)
	Anal. Calcd for $C_{33}H_{60}N_2O_{10}S_2$ (708.95): C, 55.90; H, 8.53; N,3.96; S, 9.03. Found: C, 55.98, 55.85; H, 7.92, 8.30; N, 3.98, 3.99; S, 9.03.				
17α-Hydroxy-21-sulfooxy-pregn-4-ene-3,11,20-trione triethylammonium salt  XVI	$NH(C_2H_5)_3$	226-227°	+127° (MeOH)	238 (15,400)	18-CH_3 = 36 19-CH_3 = 85 CH_2-21 = 291 (d) H-4 = 341
	Anal. Calcd for $C_{27}H_{43}NO_8S$ (541.69): C, 59.86; H, 8.00; N, 2.58; S, 5.92. Found: C, 60.03; H, 8.28; N, 2.59; S, 5.83.				
17α-Hydroxy-21-sulfooxy-pregn-4-ene-3,11,20-trione ammonium salt  XVII	NH_4	275°	+153° (MeOH)	238 (15,600)	18-CH_3 = 29 19-CH_3 = 79 CH_2-21 = 278 (q) H-4 = 338 NH_4 = 427 (s) DMSO-d_6
	Anal. Calcd for $C_{21}H_{31}NO_8S$ (457.56): C, 55.13; H, 6.83; N, 3.07; S, 7.01. Found: C, 54.96; H, 6.64; N, 3.13; S, 6.80.				

Table (continued)

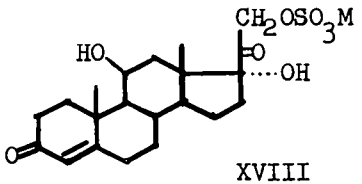
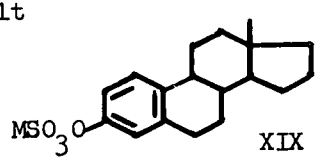
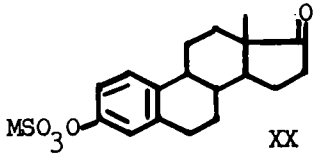
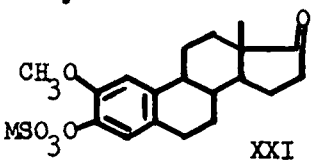
Compound	M^+	Mp	$[\alpha]_D^{25}$ $CHCl_3$	λ_{max}^{MeOH} mμ (ε)	Nmr Signals Hz
11β,17α-Dihydroxy-21-sulfooxypregn-4-ene-3,20-dione triethylammonium salt  XVIII	$NH(C_2H_5)_3$	189-191°	+110°	241 (16,850)	18-CH_3 = 53 19-CH_3 = 86 H-4 = 338 ($CDCl_3$ + d6-DMSO)
	Reported[c]	191-193°	+117.8° (H_2O)		
	Anal. Calcd for $C_{27}H_{45}NO_8S$ (543.70): C, 59.64; H, 8.34; N, 2.58; S, 5.92. Found: C, 59.37; H, 7.92; N, 2.34; S, 5.72.				
3-Sulfooxyestra-1,3,5(10)-triene triethylammonium salt  XIX	$NH(C_2H_5)_3$	135-136°	+47°	270 (987) 277 (940)	18-CH_3 = 43
	Anal. Calcd for $C_{24}H_{39}NO_4S$ (437.65): C, 65.87; H, 8.98; N, 3.20; S, 7.32. Found: C, 66.26; H, 9.03; N, 3.10; S, 7.29.				
3-Sulfooxyestra-1,3,5(10)-trien-17-one triethyl-  XX	$NH(C_2H_5)_3$	136-138°	+101°	268 (1100) 275 (1100)	18-CH_3 = 53 H-N = 523
	Anal. Calcd for $C_{24}H_{37}NO_5S$ (451.61): C, 63.83; H, 8.26; N, 3.10; S, 7.10. Found: C, 63.69; H, 8.31; N, 3.27; S, 7.06.				

Table (continued)

Compound	M^+	Mp	$[\alpha]_D^{25}$ $CHCl_3$	λ_{max}^{MeOH} mμ (ε)	Nmr Signals Hz
2-Methoxy-3-sulfooxyestra-1,3,5(10)-trien-17-one triethylammonium salt  XXI	$NH(C_2H_5)_3$	150-160°	+101°	278 (3390)	18-CH_3 = 55 OCH_3 = 229 H-1 = 439 H-4 = 410
	Anal. Calcd for $C_{25}H_{39}NO_6S$ (481.63): C, 62.34; H, 8.16; N, 2.91; S, 6.66. Found: C, 62.58; H, 8.22; N, 2.86; S, 6.77.				

[a] Spectra were determined in $CDCl_3$ unless otherwise stated. The signals ascribed to the ethyl groups attached to nitrogen are not recorded in the Table due to their consistent appearance in all samples as a triplet centered at 80 ± 5 and as a quartet centered at 185 ± 5 Hz.

[b] Joseph, J. P., Dusza, J. P., and Bernstein, S., STEROIDS, 7, 577 (1966).

[c] See Ref. 7.

Acknowledgment

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We wish to thank Dr. J. S. Webb, also of this Laboratory, for calling our attention to the possible utility of triethylamine-sulfur trioxide as a sulfating agent for steroids.

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1. Paper III, Joseph, J. P., Dusza, J. P., and Bernstein, S., J. AM. CHEM. SOC., 89, 5078 (1967).

2. Gilbert, E. E., CHEM. REVIEWS, 62, 549 (1962).

3. Sobel, A. E., Drekter, I. J., and Natelson, S., J. BIOL. CHEM. 115, 381 (1936); Sobel, A. E., and Spoerri, P. E., J. AM. CHEM. SOC., 63, 1259 (1941).

4. Beilstein, F., and Wiegand, E., BER., 16, 1264 (1883); Fex, H., Lundrall, K. E., and Olsson, A., have also employed triethylamine (or trimethylamine)-sulfur trioxide for the preparation of estrogen sulfates which were isolated as sodium or potassium salts. We wish to thank Dr. Fex (Research Dept., AB Leo, Halsingborg, Sweden) for this information.

   Hydorn, A. E., Lerner, L. J., and Schwartz, J., STEROIDS, 6, 247 (1965) have reported on the first use of trimethylamine-sulfur trioxide as a sulfating agent in the steroid field. The 21-sulfates of 9α-fluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxypregna-1,4-diene-3,20-dione and the Ring A saturated analog of the latter were prepared.

5. Griebsch, E., and Garn, W., Ger. 1,090,208 (Oct. 6, 1960), mention several triethylammonium 21-steroid sulfates. These, however, were prepared by exchange of the pyridinium salts.

6. Periodic examination of samples stored at room temperature in capped vials showed no signs of decompostion when examined by thin layer chromatography.

7. Griebsch, E., and Garn, W., U.S. Pat. 3,152,044 (Oct. 6, 1964).

8. Triethylamine-sulfur trioxide has a greater solubility at room temperature in pyridine than does its trimethyl analog. This solubility difference may offer a distinct advantage to the former as a sulfating agent for steroids.