Studies on anabolic steroids. 9. Tertiary sulfates of anabolic 17α-methyl steroids: synthesis and rearrangement

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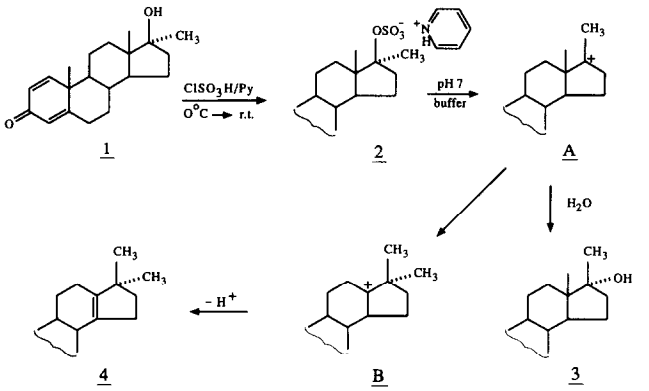
A simple and convenient method has been developed to prepare sulfates of anabolic 17β-hydroxy-17α-methyl steroids. The sulfates of methandienone, 17α-methyltestosterone, mestanolone, oxandrolone, and stanozolol were prepared. Different A-ring functions were not affected under the sulfation condition. The buffered hydrolyses of these sulfates provided the 17-epimers of the original steroids and 17,17-dimethyl-18-nor-13(14)-ene steroids, presumably via the 17-carbocations. (*Steroids* **57**:306–312, 1992)

Keywords: steroids, tertiary sulfates; 17α-methyl steroids; 17β-methyl steroids; 17,17-dimethyl-18-norandrost-13(14)-enes; NMR

Introduction

Sulfates are important steroid metabolites in living organisms. Steroid sulfates are not merely end products of metabolism that are excreted in urine. They are also found in high concentrations in human plasma, and they can serve as intermediates in steroid metabolism and as precursor pools of biologically active androgens and estrogens.[1] In a previous paper of this series on the study of anabolic steroid metabolism in humans,[2] we reported preliminary data about the role of sulfate conjugates of 17β-hydroxy-17α-methyl steroids in the in vivo formation of their corresponding 17α-hydroxy-17β-methyl epimers. Rongone and Segaloff[3] were the first researchers to report the occurrence of 17-epimethandienone **3** (Scheme 1) as a probable urinary metabolite of methandienone **1.** It was synthesized and identified by MacDonald et al.[4] in 1971. More recently, Dürbeck and Büker[5] reported the detection of the 18-nor-13(14)-ene steroid **4** as an artifact resulting from the dehydration and rearrangement of **1** under the acidic conditions prevailing in the gut. A mechanism accounting for the formation of the epimer **3** was recently proposed by Edlund et al.[6] These investigators showed that 17β-methyl-17α-alcohol **3** was produced from the 17β-sulfate conjugate of **1** through the formation of a tertiary carbocation, which upon nucleophilic attack by one molecule of water afforded the corresponding 17-epimer **3** (Scheme 1). This reaction is apparently governed by the intrinsic lability of the tertiary 17β-sulfate moiety of the steroid in aqueous media. In addition, the presence of a methyl group at the vicinal C_{13} position leads to further rearrangement products through migration of the methyl group from C_{13} to C_{17}.



Scheme 1 Synthesis, epimerization, and rearrangement of methandienone 17β-sulfate.

Part of this paper will be included in the Ph.D. thesis of H. Bi. Address reprint requests to Dr. Massé at the Institut National de la Recherche Scientifique, INRS-Santé, Université du Québec, 245 Hymus Boulevard, Pointe-Claire, Québec H9R 1G6, Canada. Received October 8, 1991; accepted February 6, 1992.

Anabolic 17β-hydroxy-17α-methyl steroids are well known for their strong liver toxicities and carcinogenicities. This may perhaps be due to conjugation with sulfuric acid, leading to highly reactive alkylating agents (17-carbocation) and their probable ability to alkylate nucleic acids and/or other biological macromolecules. Therefore, the preparation of these sulfates is of great importance to further studies about their covalent binding characteristics, which may be crucial to initiation of events leading to hepatic side effects.

The chemical synthesis of steroid sulfates have been extensively reviewed.[7–9] The synthesis of tertiary alcohol sulfates usually requires higher temperatures and/or longer reaction times than that of secondary sulfates.[7,10,11] This may cause side reactions complicating the purification steps and lowering the yield of the desired sulfates.

In our previous metabolism studies of several 17β-hydroxy-17α-methyl anabolic steroids, 17-epimers were often encountered as important urinary metabolites.[2,12,13] 17-Epimerization is a common metabolic route for these anabolic steroids. For certain steroids, 17,17-dimethyl-18-nor-13(14)-enes, proposed as artifacts,[5] can be detected in the postadministration urine. The confirmation of these urinary metabolites and artifacts relies on the synthesis of authentic substances. However, the synthetic method used by MacDonald et al.[4] to prepare the 17-epimer **3** was lengthy and the overall yield was only 3%. It seemed to us that 17β-sulfates of these anabolic steroids could be very good precursors to these 17-epimers and rearrangement products. In order to investigate the transformation of 17β-hydroxy sulfates to the 17-epimers and other rearrangement products, we have developed a simple and convenient synthetic method to prepare 17β-hydroxy sulfates and their rearrangement products. Literature methods were modified to prepare the 17,17-dimethyl-18-nor-13(14)-ene steroids **4, 8, 12, 16,** and **20,** in better yields, directly from anabolic steroids **1, 5, 9, 13,** and **17** (the structure of all the steroids being discussed are presented in Figure 1).

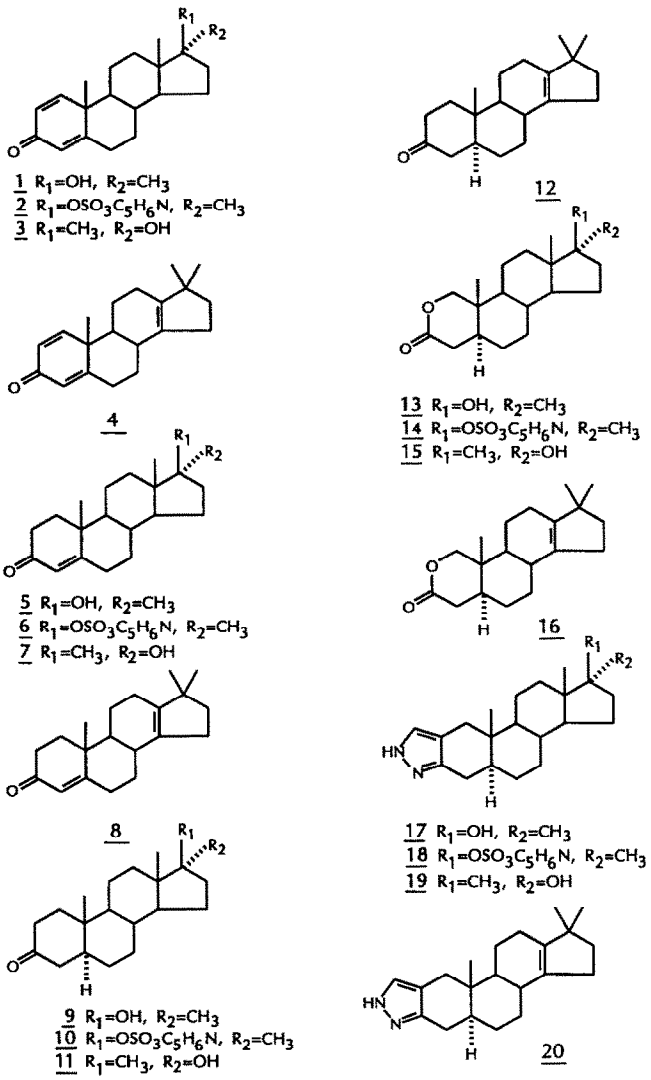


Figure 1 Chemical structures of steroids **1–20.**

Experimental

Steroids were purchased from Sigma Chemical Co. (St. Louis, MO, USA) or Steraloids Inc. (Wilton, NH, USA); chlorosulfonic acid ($ClSO_3H$, 99%) was supplied by Aldrich Chemical Co. (Milwaukee, WI, USA); pyridine (distilled in glass) and other organic solvents (HPLC grade) were from Caledon Laboratories Ltd. (Georgetown, Ontario, Canada); inorganic salts were purchased from Caledon or J. T. Baker (Philipsburg, NJ, USA); pyridine was distilled from KOH before use.

Melting points were measured on an electrothermal melting points apparatus and are reported without correction. IR spectra were taken in Nujol on a Perkin-Elmer 267 infrared spectrophotometer. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ (unless otherwise indicated) on a Varian XL-200, Varian XL-300, or Bruker WH-400 spectrometer with tetramethylsilane as internal standard. Low-resolution mass spectra (LRMS, electron-impact) were recorded on Hewlett Packard 5890-5970 GC-MSD instruments. Positive or negative high-resolution mass spectra [HRMS, chemical ionization (NH_3 as reagent gas) or FAB (glycerol as matrix)] were taken on a Kratos MS50TC mass spectrometer.

General method for the preparation of steroid 17β-tertiary sulfates 2, 6, 10, 14, and 18[14]

To 5 ml of cooled pyridine (−5 C) under N_2 was added slowly with stirring via a narrow-gauge syringe (No. 22) 160 μl of $ClSO_3H$ (2.4 mmol), leading to the formation of a white precipitate. **(Caution: Addition of $ClSO_3H$ must be very slow to prevent a violent explosion!)** A solution of 2 mmol of the steroid in 5 ml pyridine was added. After stirring for 5 minutes, the cold bath was removed and the reaction was allowed to continue at room temperature for 2–3 hours. At the end of the reaction, as established by the disappearance of the starting steroid (TLC condition was hexane: EtOAc, 1 : 1, v/v), 10–15 ml of hexane was added to the gelatinous mixture to completely precipitate the sulfate. After stirring for 10–15 minutes, the precipitate was filtered and washed with hexane. The resulting white solid was further dried in vacuo. The resulting pyridinium salt of the sulfate

was kept under dry and cold (−20 C) conditions to prevent decomposition.

General procedure for the preparation of the 17-epimers (3, 7, 11, 15, and 19) from 17β-sulfates

A solution of 0.1 mmol of sulfate in 2 ml of 0.2 M phosphate buffer (pH 7.0) was left at room temperature overnight. The precipitated steroids were extracted with 2 × 10 ml of Et_2O, and the Et_2O phase was washed with H_2O, dried with Na_2SO_4, and evaporated in vacuo. The products were separated on a Sep-Pak Silica cartridge (Millipore Co., Milford, MA, USA). The cartridge was first washed with 5 ml hexane, and then a solution of the above reaction product in 5 ml of hexane/$CHCl_3$ (10:1, v/v) was applied on the cartridge. The rearrangement products (**4, 8, 12, 16,** and other isomeric steroids) were eluted with a mixture of hexane/EtOAc (10:2, v/v), and then the 17-epimers (**3, 7, 11,** and **15**) were eluted with EtOAc. For stanozolol, the separation of the 17-epimer and rearrangement products was performed on a Sep-Pak NH_2 cartridge, rearrangement products (**20** and other isomeric steroids) were eluted with hexane/$CHCl_3$/EtOAc (10:2:2, v/v/v), and 17-epistanozolol (**19**) was eluted with EtOAc.

General method for preparation of the 17,17-dimethyl-18-nor-13(14)-ene steroids (4, 8, 12, 16, 20)

Method 1. For methandienone **1** and methyltestosterone **5,** 0.1 mmol of steroid was suspended in 3 ml of 2 M HCl and allowed to react for 30 minutes at 60 C. The reaction mixture was extracted with 2 × 10 ml of Et_2O. The ether layer was washed with aqueous K_2CO_3 and H_2O, and dried over Na_2SO_4. After evaporation of the solvent, the extract was purified by silica cartridges as described.

Method 2. For mestanolone **9,** oxandrolone **13,** and stanozolol **17,**[15] 0.1 mmol of steroid was treated with 2 ml of concentrated HCl at room temperature for 15 minutes. The resulting mixture was diluted with H_2O and extracted with Et_2O. The ethereal phase was washed with aqueous K_2CO_3 and H_2O, and dried over Na_2SO_4. After removing the solvent in vacuo, the residue was crystallized.

Methandienone 17β-sulfate pyridinium salt 2. After the general synthetic method, evaporation of the solvent yielded 960 mg of **2** (quantitative yield). MP, 140–145 C. IR, ν_{max} 1,220 (SO), 1,180 (SO), 1,150 (SO), 1,040 (SO) cm^{-1}. HRMS (FAB, negative ion), m/z 379.1563 ([M − H]$^-$, 100%) for $C_{20}H_{27}O_5S$ (theoretical value, 379.1579). ^1H NMR (200 MHz, CD_3OD), δ 0.92 (s, 3H, 18-H_3), 1.17 (s, 3H, 19-H_3), 1.23 (s, 3H, 17α-CH_3), 6.05 (s, 1H, 4-H), 6.21 (d, 1H, J 10 Hz, 2-H), 7.05 (d, 1H, J 10 Hz, 1-H). ^{13}C NMR, see Table 1.

17α-Hydroxy-17β-methylandrosta-1,4-dien-3-one 3 (17-epimethandienone). Separation on Si cartridge and crystallization from $CHCl_3$/hexane afforded 5.1 mg of **3** (yield 17%). MP, 224–225 C (reported[4] 221 C). IR, ν_{max} 3,430 (OH), 1,656 (C=C—C=O), 1,620 (C=C), 1,600 (C=C) cm^{-1}. HRMS (CI), m/z 301.2149 ([M + H]$^+$, 78%) for $C_{20}H_{29}O_2$ (theoretical value, 301.2167), 282 (28%), 161 (34%), 122 (78%). ^1H NMR (400 MHz), δ 0.76 (s, 3H, 18-H_3), 1.21 (s, 3H, 17β-CH_3), 1.25 (s, 3H, 19-H_3), 6.07 (s, 1H, 4-H), 6.23 (d-d, 1H, 2-H, J_1.9 Hz, J_2 10.2 Hz), 7.07 (d, 1H, 1-H, 1-H, J 10.2 Hz).^{13}C NMR, see Table 1.

17,17-Dimethyl-18-norandrosta-1,4,13(14)-trien-3-one 4. Treatment of methandienone **1** by the acidic rearrangement method (1) afforded 18 mg of **4** as a light yellow oil (yield 60%). Repeated attempts failed to give a crystalline product. The purity was assessed by gas chromatography/mass spectrometry (GC/MS) analysis (>99%). IR, ν_{max} 1,660 (C=C—C=O), 1,620 (C=C), 1,600 (C=C) cm^{-1}. HRMS (CI), m/z 283.2049 ([M + H]$^+$, 100%) for $C_{20}H_{27}O$ (theoretical value, 283.2062), 267 (30%), 161 (36%), 159 (24%), 122 (83%). ^1H NMR (300 MHz), δ 0.92 (s, 3H, 17-CH_3), 0.96 (s, 3H, 17-CH_3), 1.21 (s, 3H, 19-H_3), 6.08 (s, 1H, 4-H), 6.25 (d, 1H, J 10.1 Hz, 2-H), 7.14 (d, 1H, J 10.1 Hz, 1-H). ^{13}C NMR, see Table 1.

Methyltestosterone 17β-sulfate pyridinium salt 6. Evaporation of solvent afforded 966 mg of **6** (quantitative yield). MP, 125–130 C. IR, ν_{max} 1,250 (SO), 1,180 (SO), 1,150 (SO), 1,040 (SO) cm^{-1}. HRMS (FAB, negative ion), m/z 381.1736 ([M − H]$^-$, 100%) for $C_{20}H_{29}O_5S$ (theoretical value, 381.1746). ^1H NMR (200 MHz, pyridine-d_5), δ 0.93 (s, 3H, 18-H_3), 1.16 (s, 3H, 19-H_3), 1.89 (s, 3H, 17α-CH_3), 5.87 (s, 1H, 4-H). ^{13}C NMR, see Table 1.

17α-Hydroxy-17β-methylandrost-4-en-3-one 7 (17-epimethyltestosterone). Separation on Si cartridge and crystallization from $CHCl_3$/hexane gave 6.2 mg of **7** as needles (yield 21%). MP, 180–181 C (reported[16] 182 C). IR, ν_{max} 3,430 (OH), 1,665 (C=C—C=O), 1,600 (C=C) cm^{-1}. HRMS (CI), m/z 303.2328 ([M + H]$^+$, 100%) for $C_{20}H_{31}O_2$ (theoretical value, 303.2324), 284 (21%), 269 (18%), 161 (9%), 124 (23%). ^1H NMR (400 MHz), δ 0.73 (s, 3H, 18-H_3), 1.20 (s, 3H, 19-H_3), 1.21 (s, 3H, 17β-CH_3), 5.73 (s, 1H, 4-H). ^{13}C NMR, see Table 1.

17,17-Dimethyl-18-norandrosta-4,13(14)-dien-3-one 8. Treatment of 17α-methyltestosterone **5** by the acid rearrangement method (method 1) afforded 19.5 mg of **8** as light yellow oil (yield 65%). Its purity was assessed by GC/MS analysis (>98%). IR, ν_{max} 1,665 (C=C—C=O), 1620 (C=C) cm^{-1}. HRMS (CI), m/z 285.2235 ([M + H]$^+$, 87%) for 3H, 17-CH_3), 0.96 (s, 3H, 17-CH_3), 1.15 (s, 3H, 19-H_3), 5.73 (s, 1H, 4-H). ^{13}C NMR, see Table 1.

Mestanolone 17β-sulfate pyridinium salt 10. Evaporation of solvent afforded 970 mg of **10.** MP, 140–146 C. IR, ν_{max} 1,250 (SO), 1,180–1,150 (br, SO), 1,050–1,030 (br, SO) cm^{-1}. HRMS (FAB, negative ion), m/z 383.1934 ([M − H]$^-$, 100%) for $C_{20}H_{31}O_5S$ (theoretical value, 383.1892). ^1H NMR (400 MHz), δ 0.86 (s, 3H, 18-H_3), 0.95 (s, 3H, 19-H_3), 1.50 (s, 3H, 17α-CH_3). ^{13}C NMR, see Table 1.

17α-Hydroxy-17β-methyl-5α-androstan-3-one 11 (17-epimestanolone). Separation on Si cartridge and crystallization from $CHCl_3$/hexane afforded **11** as needles (7.0 mg, yield 23.3%). MP, 234–235 C. IR, ν_{max} 3,480 (OH), 1,705 (C=O) cm^{-1}. HRMS (CI): m/z 307.2485 ([M + H]$^+$, 18%) for $C_{20}H_{33}O_2$ (theoretical value, 305.2480), 289 (31%), 286 (52%), 271 (100%), 161 (9%). ^1H NMR (400 MHz), δ 0.70 (s, 3H, 18-H_3), 1.03 (s, 3H, 19-H_3), 1.20 (s, 3H, 17β-CH_3). ^{13}C NMR, see Table 1.

17,17-Dimethyl-18-nor-5α-androst-13(14)-en-3-one 12. Treatment of mestanolone **9** by the acidic rearrangement method (method 2) and crystallization from aqueous ethanol gave 20 mg of **12** (yield 65%). MP, 140–143 C (reported[15] 132–134 C). IR,

Table 1 ^{13}C resonances (δ_C in ppm) of steroids **1–20**

Steroid	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20
1($CDCl_3$)	155.5	127.3	186.1	123.7	168.8	32.7	33.2	36.3	52.4	43.5	22.5	38.7	45.6	49.7	23.3	31.3	81.2	13.9	18.7	25.7
1(CD_3OD)	159.5	127.6	188.6	124.0	173.5	33.9	34.7	37.6	54.3	45.5	23.8	39.1	47.0	51.2	24.4	32.7	82.0	14.7	19.1	26.1
2(CD_3OD)	159.5	127.5	188.6	124.0	173.5	33.9	34.7	37.4	54.2	45.4	23.8	36.1	48.9	49.6	24.5	32.7	93.9	15.2	19.1	23.3
3($CDCl_3$)	155.6	127.4	186.1	123.8	168.9	32.9	33.7	36.1	52.2	43.5	22.5	38.4	46.7	48.9	24.0	29.6	81.7	15.8	18.6	22.7
4($CDCl_3$)	155.4	127.5	186.0	124.2	168.6	32.5	33.2	36.6	49.5	43.3	24.0	22.0	(141.8)	(134.1)	29.9	39.4	45.3	(26.3)	18.5	(26.5)
5($CDCl_3$)	35.6	33.8	199.2	123.7	171.0	32.7	31.6	36.4	53.7	38.6	20.6	38.8	45.3	50.1	23.1	31.3	81.3	13.8	17.3	25.7
5(Py-d_5)	36.0	34.5	198.4	124.1	170.8	32.9	(32.1)	36.6	54.1	39.4	21.0	38.9	46.0	50.6	23.8	(32.1)	80.5	14.8	17.3	26.7
6(Py-d_5)	36.0	34.5	198.4	124.2	170.8	32.9	(32.2)	36.0	54.0	38.9	21.0	36.4	47.6	49.1	23.8	(32.1)	91.4	15.4	17.3	24.0
7($CDCl_3$)	35.8	33.9	199.1	123.8	171.0	32.9	32.1	36.1	53.7	38.6	20.6	38.4	46.5	49.3	23.8	29.6	81.8	15.7	17.4	22.7
8($CDCl_3$)	35.4	33.8	199.2	124.1	171.2	33.3	31.1	36.5	51.6	38.5	22.5	22.1	(141.9)	(134.6)	29.7	39.4	45.3	(26.3)	16.9	(26.6)
9($CDCl_3$)	38.4	38.0	212.0	44.6	46.6	28.7	31.3	36.1	53.7	35.6	20.9	38.8	45.4	50.3	23.1	31.4	81.5	13.9	11.4	25.7
10($CDCl_3$)	38.4	37.9	211.5	44.4	46.5	28.6	31.2	35.8	53.6	35.5	20.9	37.9	47.0	48.7	23.3	31.5	93.2	14.6	11.3	22.8
11($CDCl_3$)	38.4	38.1	211.4	44.6	46.8	29.0	31.9	35.9	53.7	35.7	21.0	38.6	46.7	49.8	23.9	29.9	82.0	15.9	11.4	22.6
12($CDCl_3$)	38.2	38.0	211.5	44.5	46.4	29.3	30.9	36.5	51.4	35.6	23.0	22.2	(141.4)	(135.3)	29.7	39.5	45.3	(26.3)	11.0	(26.6)
13($CDCl_3$)	81.0		170.5	33.7	40.4	27.1	30.7	35.5	49.6	34.6	20.9	38.6	45.3	50.2	23.2	31.2	81.5	13.9	10.1	25.8
14($CDCl_3$)	81.0		170.5	33.7	40.3	27.1	30.7	34.5	49.5	34.6	20.9	35.4	46.9	48.6	23.5	31.3	93.3	14.7	10.1	22.9
15($CDCl_3$)	81.0		170.3	33.8	40.5	27.2	31.1	35.2	49.6	34.7	20.9	38.2	46.4	49.4	23.8	29.5	81.9	15.7	10.1	22.6
16($CDCl_3$)	81.1		170.3	33.7	40.3	27.5	30.3	35.7	47.2	34.7	22.9	21.7	(141.4)	(134.9)	29.7	39.3	45.5	(26.3)	9.8	(26.6)
17($CDCl_3$)	34.7	113.2	131.2	26.4	42.4	29.2	31.6	36.5	53.8	36.4	20.7	38.9	45.3	50.5	23.2	31.4	81.6	13.8	11.4	25.9
17($DMSOd_6$)	34.6	113.2	131.2	26.3	42.2	29.0	31.5	36.2	53.4	36.1	20.5	38.4	45.0	50.2	23.2	31.3	79.8	14.1	11.4	26.2
18(CD_3OD)	35.0	116.2	133.1	26.0	42.9	29.9	32.5	36.1	54.8	37.3	21.9	37.7	48.2	50.1	23.3	32.9	94.3	15.2	11.7	24.5
19($CDCl_3$)	34.7	113.2	131.2	26.1	42.2	29.9	31.6	36.2	53.7	36.5	20.7	38.4	46.5	49.8	23.9	29.2	82.0	15.8	11.5	22.6
20($CDCl_3$)	34.3	113.2	131.2	25.7	42.0	29.8	30.8	36.7	51.5	36.3	22.8	22.2	(141.4)	(135.6)	29.5	39.5	45.4	(26.4)	11.2	(26.7)

Note. The resonances of 3′ carbons in the pyrazole rings of stanozolol derivatives are 145.1 ppm for **18**, and 141.0 for **17**, **19**, and **20**. The δ_C in parentheses may be interchangeable.

ν_{max} 1,705 (C=O), 1650 (C=C) cm^{-1}. HRMS (CI), m/z 287.2362 ([M + H]$^+$, 27%) for $C_{20}H_{31}O$ (theoretical value, 287.2375), 271 (100%), 161 (17%). ^1H NMR (400 MHz), δ 0.96 (s, 6H, 17-2CH_3), 0.98 (s, 3H, 19-H_3). ^{13}C NMR, see Table 1.

Oxandrolone 17β-sulfate pyridinium salt 14. Evaporation of solvent afforded 970 mg of **14.** MP, 150–155 C. IR, ν_{max} 1,250 (SO), 1,180 (SO), 1,040 (SO) cm^{-1}. HRMS (FAB, negative ion), m/z 385.1685 ([M − H]$^-$, 100%) for $C_{19}H_{29}O_6S$ (theoretical value, 385.1694). ^1H NMR (200 MHz), δ 0.90 (s, 3H, 18-H_3), 0.95 (s, 3H, 19-H_3), 1.53 (s, 3H, 17α-CH_3). 2.20 (d-d, 1H,4β-H, $J_{4\beta\text{-}5}$ 13 Hz, $J_{\alpha\text{-}\beta}$ 18.7 Hz), 2.50 (d-d, 1H, 4α-H, $J_{4\alpha\text{-}5}$ 6 Hz, $J_{\alpha\text{-}\beta}$ 18.7 Hz), 3.89 (d, 1H, 1β-H, $J_{\alpha\text{-}\beta}$ 10.7 Hz), 4.20 (d, 1H, 1α-H, $J_{\alpha\text{-}\beta}$ 10.7 Hz). ^{13}C NMR, see Table 1.

17α-Hydroxy-17β-methyl-2-oxa-5α-androstan-3-one 15 (17-epioxandrolone). Separation on Si cartridge and crystallization from $CHCl_3$/hexane afforded 7.3 mg of **15** (yield 24%). MP, 205–206 C. IR, ν_{max} 3,500 (OH), 1,735 (lactone) cm^{-1}. HRMS (CI), m/z 307.2263 ([M + H]$^+$, 10%) for $C_{19}H_{31}O_3$ (theoretical value, 307.2273), 291 (12%), 288 (28%), 273 (100%). ^1H NMR (400 MHz), δ 0.69 (s, 3H, 18-H_3), 1.00 (s, 3H, 19-H_3), 1.20 (s, 3H, 17β-CH_3), 2.22(d-d, 1H, 4β-H, $J_{4\beta\text{-}5}$ 13 Hz, $J_{\alpha\text{-}\beta}$ 18.5 Hz), 2.51 (d-d, 1H, 4α-H, $J_{4\alpha\text{-}5}$ 6 Hz, $J_{\alpha\text{-}\beta}$ 18.5 Hz), 3.94 (d, 1H, 1β-H, $J_{\alpha\text{-}\beta}$ 10.7 Hz), 4.25 (d, 1H,1α-H, $J_{\alpha\text{-}\beta}$ 10.7 Hz). ^{13}C NMR, see Table 1.

17,17-Dimethyl-18-nor-2-oxa-5α-androst-13(14)-en-3-one 16. Treatment of oxandrolone **13** by the acidic rearrangement method (method 2) and crystallization from $CHCl_3$/hexane yielded 22.5 mg (yield 73%) of **16.** MP, 106–107 C. IR, ν_{max} 1,740 (lactone), 1,650 (C=C) cm^{-1}. HRMS (CI), m/z 289.2155 ([M + H]$^+$, 23%) for $C_{19}H_{29}O_2$ (theoretical value, 289.2167), 273 (100%), 161 (3%). ^1H NMR (400 MHz), δ 0.96 (s, 6H, 17-2CH_3), 0.97 (s, 3H, 19-H_3), 2.23 (d-d, 1H, 4β-H, $J_{4\beta\text{-}5}$ 13.2 Hz, $J_{\alpha\text{-}\beta}$ 18.6 Hz), 2.54 (d-d, 1H, 4α-H, $J_{4\alpha\text{-}5}$. 8 Hz, $J_{\alpha\text{-}\beta}$ 18.6 Hz), 3.97 (d, 1H, 1β-H, $J_{\alpha\text{-}\beta}$ 10.7 Hz), 4.32 (d, 1H, 1α-H, $J_{\alpha\text{-}\beta}$ 10.7 Hz). ^{13}C NMR, see Table 1.

Stanozolol 17β-sulfate pyridinium salt 18. Stanozolol **17** was reacted with 2.4 equivalents of $ClSO_3H$ to complete the sulfation. Complete evaporation of solvent afforded 1,390 mg of **18**. The final sulfate salt contained about 30% of SO_3-Py as impurities (net weight of the sulfate should be 975 mg), which did not affect the preparation of the 17-epimer. MP, 205–215 C. IR, ν_{max} 1,260 (SO), 1,230 (SO), 1,180 (SO), 1,055 (SO) cm^{-1}. HRMS (FAB, negative ion), m/z 407.1925 ([M − H]$^-$, 100%) for $C_{21}H_{31}N_2O_4S$ (theoretical value, 407.2004). ^1H NMR (200 MHz, CD_3OD), δ 0.78 (s, 3H 18-H_3), 0.93 (s, 3H, 19-H_3), 1.51 (s, 3H, 17α-CH_3), 7.79 (br s, 1H, 3′-H). ^{13}C NMR, see Table 1.

17α-Hydroxy-17β-methyl-5α-androstano[3,2c]pyrazole 19 (17-epistanozolol). Separation on Sep-Pak NH_2 cartridges and crystallization from $CHCl_3$/hexane afforded 6.6 mg (yield 20%) of **19.** MP, 175–180 C. IR, ν_{max} 3,150 (br, s, H-bonded OH and NH), 1,600 (C=N) cm^{-1}. HRMS (CI), m/z 329.2571 ([M + H]$^+$, 15%) for $C_{21}H_{33}N_2O$ (theoretical value, 329.2593), 310 (46%), 295 (100%), 270 (40%), 258 (28%). ^1H NMR (400 MHz), δ 0.71 (s, 3H, 18-H_3), 0.76 (s, 3H, 19-H_3), 1.21 (s, 3H, 17β-CH_3), 7.36 (br s, 1H, 3′-H). ^{13}C NMR, see Table 1.

17,17-Dimethyl-18-nor-5α-androst-13(14)-eno[3,2c]pyrazole 20. Treatment of stanozolol **17** by the acidic rearrangement method (method 2) and crystallization from $CHCl_3$/hexane afforded 20.5 mg (yield 66%) of **20.** MP, 230–235 C. IR, ν_{max} 3,160 (br, s, H-bonded NH), 1,600 (C=N) cm^{-1}. HRMS (CI), m/z 311.2477 ([M + H]$^+$, 26%) for $C_{21}H_{31}N_2$ (theoretical value, 311.2487), 295 (100%), 161 (3%). ^1H NMR (400 MHz), δ 0.72 (s, 3H, 19-H_3), 0.98 (s, 6H, 17-2CH_3), 7.41 (br s, 1H, 3′-H). ^{13}C NMR, see Table 1.

Results and discussion

Synthesis of tertiary sulfates

Because the five anabolic steroids studied (methandienone **1,** methyltestosterone **5,** mestanolone **9,** oxandrolone **13,** and stanozolol **17**) bear different functions in their respective A ring, mild reagents such as commercial SO_3-pyridine (SO_3-Py) complex and SO_3-Et_3N complex were first used so as not to affect the A-ring functions. However, the sulfation yields were very low (<15%), even if elevated temperatures and prolonged reaction times were used. Under such conditions, reaction mixtures became yellow or brown, indicating decomposition of the starting material. It became obvious that the sulfation of these tertiary hydroxy groups requires stronger sulfation reagents. A modified sulfation method[14] by which SO_3-Py was prepared in situ by adding $ClSO_3H$ to pyridine was then used. The reaction was convenient and very simple to perform and allowed for the rapid transformation of the tertiary alcohol function into the corresponding sulfate pyridinium salt. The reaction temperature was kept below 0 C for the first 10 minutes to minimize side reactions, because with higher temperatures the reaction mixture darkened and yields were lower. The reaction was then allowed to proceed at room temperature for 2–3 hours. At the end of the reaction, hexane was added to precipitate the sulfate salt. The yield was almost quantitative. The tertiary sulfate is very labile in the presence of water. The pyridinium salts, which may contain some unreacted SO_3-Py, were stable for more than 14 months when kept as such in dry and cold (-20 C) conditions. In the case of stanozolol **17,** an additional equivalent of $ClSO_3H$ was needed for completion of the reaction. Although there was no evidence to indicate the formation of an N-sulfate, less $ClSO_3H$ led to incomplete sulfation.

Preparation of 17-epimers

Tertiary sulfates are very labile in aqueous systems. Edlund *et al.*[6] reported that the half-lives of **2** in different aqueous buffers and equine urine are on the order of minutes. Under our reaction conditions, the major decomposition products of sulfate **2** were the 17-epimer **3** and 17,17-dimethyl-18-norandrosta-1,4,13(14)-trien-3-one **4.** The formation of 17-epimers from the 17β-sulfates indicates that a carbocation at carbon 17 is the most likely intermediate (Scheme 1). However, no detailed kinetic studies were performed to assess the proposed mechanism. Nucleophilic attack of H_2O on the planar cation at C-17 occurs as expected[17] from the α side of the D ring because steric hindrance is expected from the angular methyl group at C-13, which appears to prevent any nucleophilic attack from the β side. Alternatively, the carbocation A (Scheme 1) rearranges to carbocation B, which eliminates a proton to give a 17,17-dimethyl-18-nor-13(14)-ene **4.** According to GC/MS analyses, the major rearrangement product of these reactions is accompanied by minor, isomeric products. The separation of the 17-epimers from the major rearrangement products was performed using Sep-Pak Si cartridges, with the exception of stanozolol derivatives. In that specific case, 17-epistanozolol could not be separated from the corresponding rearrangement products, probably because the polar pyrazole moiety determines their chromatographic retention on silica gel. To circumvent this problem, a bonded silica ion-exchange phase (NH_2) was used and the epimer **19** was isolated as a homogeneous compound.

Preparation of 17,17-dimethyl-18-nor-13(14)-ene steroids

The Wagner-Meerwein rearrangement of tertiary sulfates gave 17,17-dimethyl-18-nor-13(14)-enes and other isomeric by-products. Because of their similar chromatographic properties, these unsaturated steroids could not be efficiently separated by column chromatography. An alternative method, based on an acid catalyzed rearrangement, was therefore used to prepare pure 17,17-dimethyl-18-nor-13(14)-ene steroids. Methods from the literature in which strong acidic conditions (e.g. concentrated HCl or HOAc-HCl)[15,18] are used were found suitable to prepare steroids **12, 16,** and **20.** However, under these strong acidic conditions, steroids **1** and **5,** which bear one and two double bonds respectively in their A ring, afforded a brown oily residue containing several unidentified products. In order to prepare the steroids **4** and **8,** much milder reaction conditions (2 M HCl) were used. Besides 5–10% of the starting steroids and traces of other by-products, the latter reaction afforded **4** and **8** as the major products resulting from the parent steroids **1** and **5,** respectively.

General ^{13}C NMR spectral features of sulfates, 17-epimers, and 18-nor-13(14)-enes

The ^{13}C NMR spectral assignments presented in Table 1 are based on those of the parent steroids **1, 5, 9, 13,** and **17** and data from the literature.[19,20]

17β-Sulfates (**2, 6, 10, 14,** *and* **18**)

Examination of the ^{13}C NMR data reveals, as expected,[8] downfield shifts of 10.9–11.7 ppm for the tertiary 17-carbon atoms of the sulfate derivatives with respect to the corresponding signals from the parent steroids, due to the strong electronegativity and polarity of the sulfate group. The chemical shifts of neighboring carbons were much less affected. For example, the C-13s and the C-20s showed downfield shifts of 1.3–1.6 ppm and upfield shifts of 2.7–2.9 ppm, respectively. A small β effect from the 17β-sulfate group was noticed for signals associated with C-12 and C-14 and was reflected by upfield shifts of 0.9–3.2 ppm and 1.6 ppm, respectively, relative to parent steroids, except for stanozolol for which the corresponding signals were shielded by 0.7 and 0.4 ppm, respectively. No β effect was observed at the C-15 methylene group and no long range effect was noticed on the A- and B-ring carbons. These spectral data indicate that the carbon atom bear-

ing the β-sulfate group exhibit the largest deviation whereas α and β effects were negligible, indicating that the introduction of the latter group at C-17 did not induce any significant conformational change in any of the studied steroids with respect to those of the parent steroids.

17-Epimers (**3, 7, 11, 15,** *and* **19**)

Although epimerization at C-17 brings about new short-range interactions between the α and β substituents at this position and neighboring carbon atoms, shifts of ^{13}C resonances were relatively small. The steric interaction between C-18 and C-20 induced a shielding effect on C-20s, the resonance of which was shifted upfield by 3.0–3.6 ppm. Likewise, resonances of C-12s, C-14s and C-16s were shifted upfield by 0.2–0.5 ppm, 0.5–0.8 ppm and 1.5–2.2 ppm, respectively. Conversely, carbon nuclei at the 13, 15, 17, and 18 positions were slightly deshielded by 1.1–1.3 ppm, 0.6–0.8 ppm, 0.4–0.5 ppm, and 1.8–2.0 ppm, respectively. The upfield shifts of the C-16 and C-20 resonances combined with the downfield shift exhibited by C-18 constituted an adequate spectral probe to differentiate 17β-hydroxy-17α-methyl steroids from their 17-epimers.

17,17-dimethyl-18-nor-13(14)-enes (**4, 8, 12, 16,** *and* **20**)

The introduction of a 13(14) double bond in these steroids brings about the transformation of the chair conformation of the C ring to a half-chair structure. The two olefinic signals at 134.9 ± 0.7 ppm and 141.7 ± 0.2 ppm were associated to C-13s and C-14s. Because of the absence of olefinic proton at these positions, $^{13}C/^{1}H$ heteronuclear correlated experiments that separate the proton chemical shifts relative to the carbon resonances could not be performed for C-13 and C-14 and definitive assignment of their respective resonances could not be made. However, given that the more highly substituted carbons normally absorb at lower field than the less substituted carbons,[19] it seems reasonable to propose that the resonances at 134 and 141 ppm are characteristic of C-14 and C-13, respectively. The two methyl groups at C-17 showed similar resonances of 26.3 ± 0.1 ppm and 26.6 ± 0.1 ppm, whereas that of C-17 was shielded from 81.4 ± 0.2 ppm to 45.4 ± 0.1 ppm relative to their corresponding parent 17β-hydroxy-17α-methyl steroids. It is of interest to note that the 13(14)-ene function induced notable downfield shifts of the C-15s and C-16s resonances by 6.4 ± 0.2 ppm and 8.1 ppm, respectively. Because of the presence of a 13(14)-ene function, the C ring adopted a half-chair conformation that brings the C-12 methylene group into closer proximity with the 17β-methyl group.[20] As a result, the signals associated with C-12s are shielded by 16.6–16.9 ppm relative to the parent steroids. Resonances of other neighboring carbon atoms in the C ring were also shifted, but to a lesser extent. For example, there are small upfield shifts of 2.1–2.9 ppm and downfield shifts of 1.5–2.1 ppm of the signals associated with C-9s and C-11s, respectively, compared with their corresponding parent steroids. Finally, resonances of carbons remote from the 13(14)-ene function in the A and B rings were not noticeably affected. The specific resonances exhibited by the olefinic carbons in compounds **4, 8, 12, 16,** and **20** do not overlap and provide for the unambiguous identification of steroids bearing a 13(14)-ene group and their differentiation from other unsaturated analogs.[20]

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