EXPERIMENTAL[10]

3-Ethylenedioxy-5-androsten-17-one (I). To a chilled solution of 1.64 g. (4.94 mmoles) of 3-ethylenedioxy-17β-hydroxy-5-androstene[4d] in 15 cc. of dry pyridine was added the chromic oxide–pyridine reagent[5] prepared from 2.06 g. of chromic oxide and 15 cc. of pyridine. The stirred mixture was kept at room temperature over night and was then added to 100 cc. of ice water. Chloroform was added and the mixture was filtered through diatomaceous earth. The precipitate was washed with more chloroform and the layers of the filtrate were separated. The chloroform phase was washed with water, and was dried and partially decolorized over magnesium sulfate and activated charcoal. The solution was filtered and freed from solvents by evaporation. The residue was crystallized from methanol to afford 1.18 g. (73%), m.p. 189–197° (lit. m.p. 185–192°,[4c] 197–198°[4d]).

16-Ethoxalyl-3-ethylenedioxy-5-androsten-17-one (II). Sodium (75 mg.) was dissolved with heating in a mixture of 30 cc. of benzene and 2 cc. of methanol. Solvent was distilled from the stirred mixture until the distillation temperature reached 80°. To the resulting stirred suspension, cooled to room temperature, was added 1 g. (3 mmoles) of 3-ethylenedioxy-5-androsten-17-one (I) and 1 cc. of ethyl oxalate. The mixture was stirred at room temperature for 16 hr. and was then extracted with several portions of 1% aqueous potassium hydroxide solution. The extracts were neutralized with 30% aqueous sodium dihydrogen phosphate solution and the mixture was extracted several times with chloroform. The combined chloroform extracts were washed with water, dried, and evaporated. The residue was crystallized from ether to afford 0.795 g. (72%) of white solid with m.p. 161–164°. A sample recrystallized from ether showed m.p. 161–163; $[\alpha]_D^{25}$ −103° (*c*, 0.793 in chloroform); λ_{max} 5.76 μ (ester), 5.94 μ, and 6.17 μ (enolized β-diketone system); positive enol test.

Anal. Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found: C, 69.98; H, 8.20.

3-Ethylenedioxy-16ζ-fluoro-5-androsten-17-one (III). To a suspension of 860 mg. (2 mmoles) of 16-ethoxalyl-3-ethylenedioxy-5-androsten-17-one (II) in 20 cc. of methanol, cooled to −15°, was added 3 cc. of a 1*N* methanolic sodium methoxide solution and the stirred, cooled solution was saturated with a rapid stream of perchloryl fluoride gas.[11] Nitrogen was then blown through the solution to remove excess perchloryl fluoride and the reaction mixture (neutral, negative enol test) was evaporated *in vacuo* at room temperature. The residue was dissolved in chloroform and water, and the layers were separated. The organic phase was dried and evaporated and the residue was redissolved in 20 cc. of methanol. Potassium acetate (1.5 g.) was added and the stirred mixture was heated under reflux for 1 hr. The solvent was evaporated and the residue was dissolved in chloroform and water. The chloroform layer was washed with a little water and was dried and evaporated to afford 913 mg. of a glass. This was crystallized from 10 cc. of ether and the solid was collected and washed twice with 5-cc. portions of ether. There was obtained 170 mg. (24%) with m.p. 227–230°. The analytical sample was recrystallized twice from methylene chloride–ether; m.p. 240–243°; $[\alpha]_D^{25}$ + 26.8° (*c*, 1.64 in chloroform); λ_{max} 5.70 μ (17-one), 9.08 μ (3-ketal).

Anal. Calcd. for $C_{21}H_{29}FO_3$: C, 72.37; H, 8.39; F, 5.45. Found: C, 72.01; H, 8.73; F, 5.82.

Total evaporation of the ether mother liquors and trituration with a small amount of fresh ether afford 360 mg. of a solid with m.p. 100–105°. This was recrystallized from acetone-hexane and from ether; m.p. 109–111°; $[\alpha]_D^{25}$ −63.5° (*c*, 0.38 in chloroform); λ_{max} 2.83 μ (OH), 5.70 μ (shoulder, α-fluoroketone), 5.78 μ (ester?), 7.90–8.02 μ (ester?), 9.08 μ (3-ketal).

Anal. Calcd. for $C_{21}H_{29}FO_5 \cdot H_2O$: C, 63.29; H, 7.84; F, 4.77; H_2O, 4.52. Found: C, 62.86; H, 7.87; F, 4.53; H_2O, 4.84.

16ζ-Fluoro-4-androstene-3,17-dione (*16ζ-Fluorotestosterone*, IV). A solution of 348 mg. (1 mmole) of 3-ethylenedioxy-16ζ-fluoro-5-androsten-17-one (III), in 10 cc. of methanol and 0.5 cc. of water was reduced with 175 mg. of sodium borohydride at the reflux point for 3 hr. The mixture was cooled and poured into 25 cc. of water to give a suspension which was extracted with four 10-cc. portions of chloroform. The combined extracts were washed with water, dried and evaporated. The residue was crystallized once from methanol to afford 0.3 g. (86%) with m.p. 189–195°; λ_{max} 2.80 μ (OH region), no absorption in the C=O region. The solid was redissolved in 20 cc. of methanol containing 1 cc. of 8% aqueous sulfuric acid and the mixture was allowed to reflux for 2 hr. and was then diluted with an additional 30 cc. of methanol. Duolite A-4 anion exchange resin (OH form)[12] was added with stirring until the solution was neutral. The resin was removed by filtration and was washed well with methanol. The combined filtrate and washings were evaporated and the residue was dissolved in chloroform and water. The chloroform phase was washed with a little water and was dried and evaporated to give a crystalline residue which was recrystallized from ether to afford 0.17 g. (55% over-all from III), m.p. 154–156°; $[\alpha]_D^{25}$ +117° (*c*, 0.5 in chloroform) (lit. values[3] for 16α-fluorotestosterone: m.p. 153–158°, $[\alpha]_D$ +113° in chloroform); λ_{max} 240 mμ (ε, 15,800); λ_{max} 2.94 μ (OH), 6.01 μ (Δ⁴-3-one).

Anal. Calcd. for $C_{19}H_{27}O_2F$: C, 74.47; H, 8.88; F, 6.20. Found: C, 74.46; H, 9.03; F, 6.00.

Acknowledgment. We would like to thank Mr. W. Fulmor and staff for spectrophotometric and polarimetric data and Mr. L. Brancone and staff for microanalyses.

ORGANIC CHEMICAL RESEARCH SECTION
LEDERLE LABORATORIES DIVISION
AMERICAN CYANAMID CO.
PEARL RIVER, N. Y.

(10) Melting points were taken on a Kofler micro hot-stage and are corrected. Ultraviolet spectra were determined in methanol on a Cary recording spectrophotometer and infrared spectra (potassium bromide disks) on a Perkin Elmer spectrophotometer (Model 21). An ethanolic solution of ferric chloride was used for the enol test. Solutions were dried over magnesium sulfate and evaporated under reduced pressure.

(11) V. Papesh, *Chem. Eng. News*, **37**, (No. 28), 60 (1959) has reported an explosion resulting from the addition of sodium methoxide to a vessel containing the mixed vapors of methanol and perchloryl fluoride.

(12) Duolite A-4 is the trademark of the Chemical Process Co., Redwood City, Calif., for a weakly basic anion exchange resin.

6β-Hydroxylation of 9α-Fluorohydrocortisone

LELAND L. SMITH,[1] JOSEPH J. GOODMAN, HAROLD MENDELSOHN, JOHN P. DUSZA, AND SEYMOUR BERNSTEIN

Received June 15, 1960

In a study of the hydroxylation of 9α-fluorohydrocortisone (I) by a variety of *Streptomyces* species we have found that a strain of *S. rimosus* (Lederle Laboratories Collection No. T1686B) affords as a major product a very polar reducing monohydroxylated 9α-fluorohydrocortisone II different from the previously described 1ξ- and 16α-hy-

(1) Present address: Wyeth Laboratories, Inc., Philadelphia, Pa.

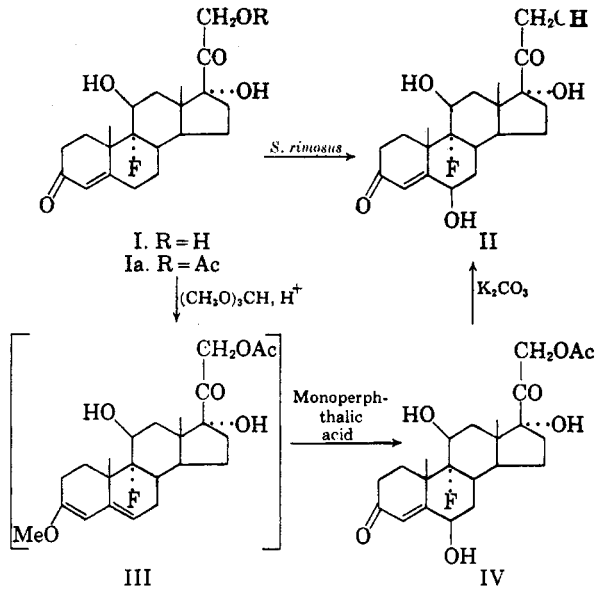
droxy-9α-fluorohydrocortisones.[2a,2b] Isolation of the polar steroid II was accomplished *via* partition chromatography. In addition a more polar reducing steroid was recovered from later column fractions.

The polar steroid II was recognized as retaining the dihydroxyacetone sidechain by comparison of its reducing capacity towards alkaline Tetrazolium Blue over a period of several hours versus that of 9α-fluorohydrocortisone (essentially identical time curves) and by consideration that II gave a typical Porter-Silber chromogen. A hypsochromic shift of 7 mμ in the ultraviolet absorption spectra of II indicated the point of attachment of the additional hydroxyl group was involved in the A-ring chromophore, the 6β-position being suggested. Confirmation of the 6β-hydroxy formulation was made by a study of ultraviolet light absorption spectra in alkaline ethanol,[3] wherein spectra characteristic of 6β-hydroxy-Δ⁴-3-ketosteroids were obtained.

The polarity of the monohydroxy-9α-fluorohydrocortisone II in several paper partition chromatographic systems was strikingly more polar than the known 1ξ-hydroxy- and 16α-hydroxy-9α-fluorohydrocortisones, which is consistent with the suggested 6β-hydroxy structure for II. The structure of II is thus assigned that of 9α-fluoro-6β-hydroxyhydrocortisone (9α-fluoro-6β,11β,17α,-21-tetrahydroxy-4-pregnene-3,20-dione).

An opportunity for a complete proof of structure of the 6β-hydroxy derivative II, at the same time offering a nonfermentative preparative method for the compound from 9α-fluorohydrocortisone 21-acetate (Ia) followed from our finding that the 3-methyl enol ether III (not isolated) of Ia was oxidized by monoperphthalic acid directly to the 6β-hydroxy-Δ⁴-3-ketosteroid IV. This appears to be the first report of the reaction of an enol ether with a peracid to yield the respective 6β-hydroxy-Δ⁴-3-ketone. Similar reaction of a Δ³,⁵-3-enol acetate to yield the 6β-hydroxy-Δ⁴-3-ketone system has been recorded previously.[4]

Saponification of the 6β-hydroxy-21-acetate IV afforded 9α-fluoro-6β-hydroxyhydrocortisone (II) identical with the product II isolated from *S. rimosus* hydroxylation of 9α-fluorohydrocortisone.[5]



EXPERIMENTAL

Fermentation of 9α-fluorohydrocortisone. An inoculum medium consisting of corn steep liquor (20 g./l.), sucrose (30 g./l.), calcium carbonate (7 g./l.), and ammonium sulfate (2 g./l.) was prepared and inoculated with spores of *S. rimosus* (Lederle Laboratories Collection No. T1686B). After 24 hr. of growth the inoculum was used at 4% levels to inoculate medium consisting of corn steep liquor (25 g./l.), starch (40 g./l.), calcium carbonate (5 g./l.), and lard oil (0.2% by volume). After a further 24 hr. of growth a solution of 9α-fluorohydrocortisone dissolved in propylene glycol was added in such an amount that the final steroid concentration was 500 μg./ml. After 48 hr. of aerated fermentation, analyses indicated that *ca.* 430 μg./ml. of steroid product had been formed, and after 72 hr., 375 μg./ml. Paper chromatographic analyses indicated that the major product was the polar 6β-hydroxy-9α-fluorohydrocortisone II (R_f 0.25 in System II[6]) together with a lesser amount of a still more polar reducing steroid (R_f 0.06). No unaltered I was detected.

The harvested broth (fermentation time 48 hr., 1330 ml.) was filtered through diatomaceous earth, the filter cake washed with water, methanol, and chloroform, and the combined filtrates extracted with equal volumes of ethyl acetate three times. The combined extracts were evaporated under vacuum to a viscous residue which analyzed on paper chromatograms as a mixture of about ten different steroidal components, of which the major component was the 6β-hydroxy derivative II. The total steroid sample was partitioned on Celite diatomaceous earth using the system dioxane–cyclohexane–water (5:2:1).[6] Two major reducing products were eluted, the desired 6β-hydroxy derivative II at hold-back volume 2.1, and a more polar reducing steroid present in lesser amounts at hold-back volume 3.2. The appropriate fractions were evaporated under vacuum to yield 47 mg. of crystalline 6β-hydroxy-9α-fluorohydrocortisone (II), m.p. 206–209°. After several recrystallizations from methanol/acetone the product melted at 222–224° dec. (Kofler block), 224–227° dec. (capillary); $[\alpha]_D$ + 56° (methanol); λ_{max} 232 mμ (in ethanol); $\lambda_{max}^{H_2SO_4}$ ($E_{1cm}^{1\%}$) at 2 hrs., 259 mμ (239), 337 mμ (317), 405 mμ (203), 442 mμ (200, inflection), 470 mμ (210), 500 mμ (177, inflection); λ_{max}^{KBr} 2.90 μ, 5.84 μ, 6.00 μ, 6.12 μ, 9.47 μ, 10.04 μ, 10.62 μ, 11.30 μ, etc.

(2a) W. J. McAleer, M. A. Kozlowski, T. H. Stoudt, and J. M. Chemerda, *J. Org. Chem.*, **23**, 508 (1958).

(2b) S. Bernstein, R. H. Lenhard, W. S. Allen, M. Heller, R. Littell, S. M. Stolar, L. I. Feldman, and R. H. Blank, *J. Am. Chem. Soc.*, **78**, 5693 (1956); **81**, 1689 (1959).

(3) A. S. Meyer, *J. Org. Chem.*, **20**, 1240 (1955).

(4) J. Romo, G. Rosenkrantz, C. Djerassi, and F. Sondheimer, *J. Org. Chem.*, **19**, 1509 (1954).

(5) 6β-Hydroxy-9α-fluorohydrocortisone has been isolated (without characterization or proof of structure) by McAleer *et al.*,[2a] from *Streptomyces sp.* fermentations with 9α-fluorohydrocortisone and by N. E. Rigler, A. J. Shay, L. I. Feldman, and B. Nielsen of these laboratories from *Mortierella sp.* fermentations with the same substrate.

(6) L. L. Smith, T. Foell, R. De Maio, and M. Halwer, *J. Am. Pharm. Assoc.*, **48**, 528 (1959).

Papergram mobilities[6] relative to 16α-hydroxy-9α-fluorohydrocortisone include: System I, 0.35; System II, 0.48; System III, 0.42. The steroid reduces alkaline Tetrazolium Blue reagent and gives a yellow fluorescent reaction with isonicotinic acid hydrazide[7] on papergrams. A normal Porter-Silber chromogen[8] with λ_{max} 413 mμ ($E^{1\%}_{1cm}$. 395) *vs.* λ_{max} 414 mμ. ($E^{1\%}_{1cm}$. 374) *for 9α-fluorohydrocortisone is found. Maximum* color development with alkaline Tetrazolium Blue[9] occurred at 30 min., with λ_{max} 520 mμ ($E^{1\%}_{1cm}$. 580) *vs.* λ_{max} 525 mμ ($E^{1\%}_{1cm}$. 654) for 9α-fluorohydrocortisone, also maximum at 30 min.

Spectra in 0.066*N* ethanolic potassium hydroxide at room temperature (22°) showed a slow shift from λ_{max} 233 mμ ($E^{1\%}_{1cm}$. 312) at 3 min. to λ_{max} 235 mμ ($E^{1\%}_{1cm}$. 226), 380 mμ (28) at 24 hr. At 60° the spectra changed from λ_{max} 232 mμ ($E^{1\%}_{1cm}$. 308) at 3 min., λ_{max} 236 mμ (172) at 1 hr., λ_{max} 239 mμ (132), 378 mμ (62) at 2 hr., to λ_{max} 248 mμ (142), 378 mμ (91) at 3 hr., typical of spectral changes associated with the 6β-hydroxy-Δ⁴-3-ketone chromophore.[8]

The fractions eluted about hold-back volume 3.2 were reduced in volume under vacuum, analyzing by paper chromatography as a single major reducing component of R_f 0.06 in System II. Crystallization of the material from acetone–chloroform–ethyl acetate (1:1:2) yielded 7.1 mg. of crystals, whose infrared absorption spectra indicated the same general structural features to be present as were present in the spectra of II. No further characterization was made of this component.

21-Acetoxy-9α-fluoro-6β,11β,17α,21-tetrahydroxy-4-pregnene-3,20-dione IV. Ten grams of 9α-fluorohydrocortisone 21-acetate (Ia) was added to a solution of 75 ml. of dioxane, 10 ml. of trimethyl orthoformate, and 0.5 ml. of absolute methanol. To this suspension was added 10 ml. of dioxane containing 0.5 ml. of concd. sulfuric acid. After 10 min. a clear solution resulted. After 20 min. at room temperature pyridine was added dropwise until the deep red color of the solution was discharged (total pyridine used, about 1 ml.). The reaction mixture was poured into water, giving an oily material which was extracted into ether. the ether extract washed with saline, and then dried. The ether volume was increased to 200 ml. and 120 ml. of 0.31*N* monoperphthalic acid in ether was added. The reaction mixture was then stored in the dark for 15 hr., at which time the precipitated crystalline product was filtered and washed with ether (4.4 g. weight). Recrystallization from ethyl acetate yielded 1.635 g. of pure IV, m.p. 248–252° dec. (capillary); $[\alpha]^{25}_D$ + 70° (pyridine); λ_{max} 232 mμ (ε 14,000); λ^{KBr}_{max} 2.87, 5.72 (Sh), 5.87, 5.92 (Sh), 5.97, 8.06, 10.04, and 10.61 μ.

Anal. Calcd. for $C_{23}H_{31}O_7F$: C, 63.00; H, 7.13; F, 4.33. Found: C, 62.46; H, 7.26; F. 4.44.

The mother liquors on standing deposited an additional 0.60 g. of crude product, which was recrystallized from ethyl acetate/heptane, 0.24 g., m.p. 234–239° (capillary).

9α-Fluoro-6β,11β,17α,21-tetrahydroxy-4-pregnene-3,20-dione (II). A solution of 600 mg. of the 21-acetate IV in 100 ml. of absolute methanol was prepared under a nitrogen atmosphere. To this solution was added 3.0 ml. of a 10% aqueous potassium carbonate solution. After 1 hr. (under nitrogen) the reaction mixture was neutralized with acetic acid, most of the methanol removed under vacuum, and the reaction concentrate was poured into water. The solids recovered by filtration were recrystallized several times from ethyl acetate–heptane, yielding 145 mg. of plates, m.p. 214–244° (capillary), which after drying in vacuum melted at 235–239° dec. (capillary), 223–225° dec. (Kofler block); $[\alpha]^{25}_D$ + 57.7° (pyridine); λ_{max} 232 mμ (ε 15,000).

Anal. Calcd. for $C_{21}H_{29}O_6F$: C, 63.62; H, 7.37; F, 4.79. Found: C, 63.54; H, 7.60; F, 4.65.

The identity of the microbiologically derived II with II derived chemically was established by comparison of infrared spectra, sulfuric acid spectra, melting points and papergram mobility, and color test behavior.

CHEMICAL PROCESS IMPROVEMENT DEPARTMENT AND THE ORGANIC CHEMICAL RESEARCH SECTION
LEDERLE LABORATORIES
AMERICAN CYANAMID CO.
PEARL RIVER, N. Y.

(7) L. L. Smith and T. Foell, *Anal. Chem.*, **31**, 109 (1959).

(8) C. C. Porter and R. H. Silber, *J. Biol. Chem.*, **185**, 201 (1950).

(9) L. L. Smith and M. Halwer, *J. Am. Pharm. Assoc.*, **48**, 348 (1959).

Chloromethylation. A Novel Route to 4-Methylsteroids

JOHN H. FRIED, ANTHONY N. NUTILE, AND GLEN E. ARTH

Received June 27, 1960

Enol acetates of Δ⁴-3-ketosteroids have been utilized to introduce halogen,[1,2] nitro and hydroxyl substituents at C_6 of steroids by an electrophilic process. An extension of this reaction to include carbonium ions could be a useful path to the biologically important C_6 alkylated steroids.

The solvolytic reaction of chloromethyl methyl ether in acetic acid appeared to be a suitable source of an electrophilic fragment, the chloromethyl carbonium ion.[3] A solution of this reagent and 17α,-20,20,21-bismethylenedioxy-3-acetoxy-3,5-pregnadiene-11-one, II, afforded a crystalline product after percolation through alumina. This product was shown to be a mixture by NMR[4] analysis but could not be resolved by recrystallization or chromatography. However, reduction of the crude crystalline product with zinc in acetic acid afforded 4-methylcortisone BMD,[5] V, in *ca.* 11% overall yield. It follows that 4-chloromethylcortisone BMD, III, was one of the components of the chloromethylation mixture. All attempts to isolate this product failed. However, repeated crystallizations from methanol did afford a second component of the mixture IV in addition to hydrogen chloride. A simpler procedure for isolating IV was to reflux the mixture with dilute hydrochloric acid in methanol. This treatment destroyed the 4-chloromethyl-

(1) A. Bowers, L. C. Ibanez, and H. J. Ringold, *J. Am. Chem. Soc.*, 81, 3707 (1959) and references cited.

(2) B. M. Bloom, V. V. Bogert, and R. Pinson, *Chem. Ind.*, 1317 (1959).

(3) The attacking species is represented as $^+CH_2Cl$ as a matter of convenience but could equally well be $CH_3O^+CH_2Cl$ (H) or one of several ion pairs.

(4) NMR spectra were run on a Varian 60MC Spectrometer at a concentration of *ca.* 20 mg. in 0.3 ml. deuterochloroform. $\tau = \gamma/60 + 3.5$ where γ is the observed band position in c.p.s. relative to benzene as external standard. *Cf.* G. V. D. Tiers, *J. Phys. Chem.*, 62, 1151 (1958). We wish to thank B. Arison and Dr. N. R. Trenner for the determination and interpretation of the NMR spectra.

(5) N. G. Steinberg, R. Hirschmann, and J. M. Chemerda, *Chem. Ind.*, 975 (1958).