[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN CO.]

The Adrenal Hormones and Related Compounds. VI. Fluorinated Derivatives of Testosterone and Progesterone

BY ALAN H. NATHAN, JOHN C. BABCOCK AND JOHN A. HOGG

RECEIVED AUGUST 27, 1959

Preparation of 2-fluorotestosterone and several of its derivatives, and of 2-fluoroprogesterone is described. The physiological activity of 2-fluorotestosterone is briefly discussed.

The technique of fluorinating carbanions with perchloryl fluoride[1] has made accessible a number of α-fluoro ketosteroids.[2] The present paper reports some further results of our experiences with this technique.

Testosterone, 17α-methyltestosterone, 11β-hydroxy-17-methyltestosterone,[3] 9(11)-dehydro-17-methyltestosterone[3] and 9α-fluoro-11β-hydroxy-17-methyltestosterone[3] were fluorinated successfully in the 2-position by the reaction of perchloryl fluoride with the sodium salts of their 2-ethoxyoxalyl derivatives followed by base-catalyzed cleavage of the ethoxyoxalyl groups. The yields were good in all cases except in that of the 2,9α-difluoro compound which was prepared in better yield from 2-fluoro-9(11)-dehydro-17-methyltestosterone by reaction of hydrofluoric acid with the corresponding 9β,11β-oxide.[3]

2-Fluoroprogesterone was prepared by application of the perchloryl fluoride–glyoxalate procedure to 20(α + β)-hydroxy-4-pregnen-3-one[4] (obtained from lithium aluminum hydride reduction[5a] of the 3-pyrrolidinyl enamine of progesterone[5b]) and then by oxidation of the 20-hydroxyl group.

The configuration of the 2-fluorine atom, by analogy, would seem to be alpha. The infrared absorptions of the 3-keto groups in all cases are displaced by about 25 cm.$^{-1}$ toward higher wave numbers which is in the direction predicted for equatorial α-bromoketosteroids[6] and as was indeed observed for the 2-fluorocholestan-3-one reported by Gabbard and Jensen,[2a] to which they assigned the α-configuration. However, since the axial α-fluoro isomer may exert an effect upon the infrared absorption of a carbonyl group similar to that of the equatorial isomer, this evidence does not preclude the possibility that the configuration of the 2-fluorine atom is beta.

Bioassays performed in these laboratories[7] indicate that 2-fluorotestosterone, in the female rat, produced a marked increase in body weight, yet affected nearly 100% inhibition of the mammary fibroadenoma[8a] which had grown resistant to the action of testosterone propionate.[8b] In the male rat, 2-fluorotestosterone exhibited very little, if any, androgenic activity.

Experimental[9]

2-Fluorotestosterone (I).—A solution of 5.76 g. (0.02 mole) of testosterone in 100 ml. of t-butyl alcohol was stirred while heating to 65° with exclusion of air and moisture by maintaining a gentle flow of dry nitrogen above the surface. Heating was stopped and 5.45 ml. (0.04 mole) of ethyl oxalate was added all at once, and then sufficient commercial 25% sodium methoxide in methanol[10] to contain 0.03 mole of sodium methoxide (approximately 6.7 ml.). After stirring for one-half hour under nitrogen (without further heating), the solution, which was deep green, was cooled to 25–30°, and 300 ml. of dry ether was added. The sodium salt of the 2-ethoxyoxalyl derivative which precipitated was collected on a büchner funnel in as dry an atmosphere as possible (it was exceedingly hygroscopic) and dried in a vacuum desiccator over calcium chloride for three hours.

The dried salt then was dissolved in 170 ml. of methanol, the solution was cooled in an ice–salt-bath to −10°, and an ice-cold solution of 3.2 g. of perchloryl fluoride in 100 ml. of methanol was added at such a rate as to raise the temperature no higher than −5°; about 10 minutes was required for the addition. Then a volume of 25% methanolic sodium methoxide was added equal to 2.19 times the number of grams of perchloryl fluoride added (7.0 ml. in this case) and stirring was continued for 0.5 hour while the flask remained in the cooling bath.

The solution was evaporated under reduced pressure at 50° to about 50 ml. and diluted with one liter of cold water. The precipitated product was collected, washed thoroughly with water, dried and recrystallized from 95% ethanol. The yield was 4.3 g. (70%), m.p. 158–160°. The compound retained solvent of crystallization, which was driven off at 105° in a vacuum oven. The analytical sample, obtained by one further recrystallization, melted at 159.5–161°.

The acetate II and the propionate III were prepared from 2-fluorotestosterone (I) by reaction with acetic anhydride and propionic anhydride, respectively, in pyridine for 16 hours at room temperature (see Table I).

The other compounds listed in Table I were prepared from the corresponding steroids by the same procedure given above for 2-fluorotestosterone (I). In addition, 2,9α-difluoro-11β-hydroxy-17-methyltestosterone (VII) was prepared from 3.0 g. of 2-fluoro-9(11)-dehydro-17-methyltestosterone *via* the bromohydrin and 9β,11β-oxide by making use of the procedures of Fried and Sabo.[11] These two intermediates were not purified or characterized except for the m.p. of the oxide (177–178° dec.), but the oxide was opened with anhydrous hydrogen fluoride in a mixture of tetrahydro-

(1) C. E. Inman, E. A. Tyczkowski, R. E. Oesterling and F. L. Scott, *Experientia*, **14**, 355 (1958); C. E. Inman, R. E. Oesterling and E. A. Tyczkowski, THIS JOURNAL, **80**, 6533 (1958).

(2) (a) R. B. Gabbard and E. V. Jensen, *J. Org. Chem.*, **23**, 1406 (1958); (b) H. M. Kissman, A. M. Small and M. J. Weiss, THIS JOURNAL, **81**, 1262 (1959); (c) A. H. Nathan, B. J. Magerlein and J. A. Hogg, *J. Org. Chem.*, **24**, 1517 (1959).

(3) M. E. Herr, J. A. Hogg and R. H. Levin, THIS JOURNAL, **78**, 500 (1956).

(4) A. Butenandt and J. Schmidt, *Ber.*, **67**, 2092 (1934).

(5) (a) F. L. Weisenborn and H. E. Applegate, THIS JOURNAL, **81**, 1960 (1959); (b) F. W. Heyl and M. E. Herr, *ibid.*, **75**, 1918 (1953).

(6) E. J. Corey, *ibid.*, **76**, 175 (1954).

(7) We are grateful to E. M. Glenn, S. L. Richardson, S. C. Lyster, B. J. Bowman and W. E. Dulin of our Department of Endocrinology for biological data.

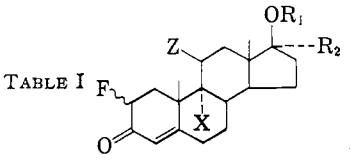
(8) (a) C. Huggins, Y. Torralba and K. Mainzer, *J. Exp. Med.*, **104**, 525 (1956); E. M. Glenn, S. L. Richardson and B. J. Bowman, *Endocrinol.*, **64**, 379 (1959); E. M. Glenn, S. L. Richardson, S. C. Lyster and B. J. Bowman, *ibid.*, **64**, 390 (1959). (b) Private communication from Dr. E. M. Glenn.

(9) Analyses unless otherwise indicated and spectral data were furnished by the members of our Department of Physical and Analytical Chemistry, to whom we are indebted for their unremitting coöperation. Melting points are given as observed on a Fisher–Johns block.

(10) Obtained from Olin Mathieson Chemical Co. and assayed titrimetrically for total alkali, which for the present purpose is expressed as sodium methoxide.

(11) J. Fried and E. F. Sabo, THIS JOURNAL, **75**, 2273 (1953).

TABLE I



	R_1	R_2	X	Z	M.p., °C.	Crystn. solvent	U.v. λ_{max}, (in EtOH)	I.r., cm.$^{-1}$ 3-C=O (ν)	Analyses, % Calcd. C	Calcd. H	Calcd. F	Found C	Found H	Found F	Yield, %
I	H	H	H	H	159.5–161	EtOH[a]	242	1690	74.47	8.88	6.20	74.54	9.12	5.91	70
II	CH_3CO–	H	H	H	182–183	EtOH–H_2O	..	1687	72.38	8.39	5.45	72.12	8.40	5.42	..
III	CH_3CH_2CO–	H	H	H	146.5–147	EtOH–H_2O	..	1688	72.90	8.62	5.24	72.49	8.94	5.32	..
IV	H	CH_3	H	H	174–174.5	EtOH[a]	242	1683	74.96	9.12	5.93	75.17	9.53	6.10	42
V	H	CH_3	H	β-OH	217–220	Acetone–petr. ether	243	1690	71.39	8.69	5.65	71.79	8.51	5.6[b]	60
VI	H	CH_3	Δ 9:11		182–182.5	EtOAc	240	1698	75.44	8.55	5.97	75.27	8.95	5.96	53
VII	H	CH_3	F	β-OH	288 d.[c]	EtOH–EtOAc	..	1680	67.77	7.96	10.72	68.08	8.29	9.67	8[d]

[a] Compound crystallized with bound solvent, driven off at 105° *in vacuo*. [b] Analysis by Huffman Micro-analytical Laboratories, Wheatridge, Colo. [c] Variable decomposition point, not indicative of purity. [d] Yield from VI *via* epoxide: 63.5%.

furan and methylene chloride[12] to give 2.0 g. of the 2,9α-difluoro compound VII melting at *ca.* 210–217° with decomposition, and having an infrared spectrum identical with that of the product prepared by the direct route. Recrystallization from a mixture of ethanol and ethyl acetate, in which it dissolved sparingly, brought the sample to a comparable degree of purity—m.p. 238–240° dec.

2-Fluoroprogesterone.—The mixture of isomeric alcohols (0.02 mole) obtained by reduction with lithium aluminum hydride of the 3-pyrrolidinyl enamine of progesterone,[5] and then removal of the pyrrolidine group,[13] was fluorinated with perchloryl fluoride *via* its glyoxalate, and the crude product was oxidized in 67 ml. of methylene chloride solution by stirring overnight at room temperature with a solution of 4 g. of sodium dichromate dihydrate and 5.4 ml. of sulfuric acid in 33 ml. of water. The oxidation products were chromatographed on Florisil; 2-fluoroprogesterone (2.05 g.) was eluted by 10% acetone in Skellysolve B. The substance melted at 187–191° and was analyzed; λ_{max} 242 mμ (*E* 15,475), $[\alpha]D$ + 220° ($CHCl_3$). Carbonyl bands appeared in the infrared spectrum at 1700 (C-20) and 1690 cm.$^{-1}$ (C-3).

Anal. Calcd. for $C_{21}H_{29}FO_3$: C, 75.87; H, 8.79; F, 5.71. Found: C, 75.98; H, 9.25; F, 5.24.

Acknowledgment.—We are indebted to Mr. John D. Highstrete for technical assistance in many of the preparations described.

KALAMAZOO, MICH.

(12) R. F. Hirschmann, R. Miller, J. Wood and R. E. Jones, THIS JOURNAL, **78**, 4956 (1956).

(13) M. E. Herr and F. W. Heyl, *ibid.*, **75**, 5928 (1953).

[CONTRIBUTION FROM THE CHEMICAL PROCESS IMPROVEMENT DEPARTMENT, LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID CO.]

16α-Hydroxy Steroids. IV.[1] Microbiological Reduction of Triamcinolone

BY LELAND L. SMITH, JOHN J. GARBARINI, JOSEPH J. GOODMAN, MICHAEL MARX AND HAROLD MENDELSOHN

RECEIVED AUGUST 20, 1959

Microbiological reduction of triamcinolone and of 9α-fluoro-16α-hydroxyhydrocortisone with *Corynebacterium simplex* or with *Bacterium cyclo-oxydans* yields the respective 20β-dihydro derivative. Further reduction of triamcinolone by these microorganisms results in the loss of the C-1,2 double bond as well as reduction of the C-20 ketone. 20β-Dihydrotriamcinolone forms a 16α,20β,21-triacetate, a 20β,21-acetonide and a 16α,17α;20β,21-bis-acetonide.

The several reported syntheses of triamcinolone (9α-fluoro-11β,16α,17α,21-tetrahydroxy-1,4-pregnadiene-3,20-dione) (II) involve microbiological dehydrogenation of an appropriate Δ^4-3-ketosteroid[2,3] with the organisms *Corynebacterium simplex*,[4] *Bacterium cyclo-oxydans*,[5] *Mycobacterium rhodochorus*,[3] *Nocardia corallina*,[2] etc. As a competing reaction we find that the 20-carbonyl group of triamcinolone and its Δ^4-3-ketone precursors is reduced by *C. simplex* and *B. cyclo-oxydans*, yielding the 20β-dihydro derivative III. The combination of C-1,2 dehydrogenation and 20-carbonyl reduction has been noted in other cases.[4-6]

Reduction of triamcinolone by either organism was manifested by the presence of a single very polar, non-reducing component (III) on papergrams of fermentation broth extracts and of crude steroid preparations isolated from fermentation broths. While possessing the common character-

(1) Paper III, L. L. Smith and T. Foell, *J. Chromatography*, in press.

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

(3) R. W. Thoma, J. Fried, S. Bonanno and P. Grabowich, *ibid.*, **79**, 4818 (1957).

(4) A. Nobile, W. Charney, P. L. Perlman, H. L. Herzog, C. C. Payne, M. E. Tully, M. A. Jevnik and E. B. Hershberg, *ibid.*, **77**, 4184 (1955); A. Nobile, U. S. Patent 2,837,464 (June 3, 1958).

(5) H. A. Kroll, J. F. Pagano and R. W. Thoma, U. S. Patent 2,822,318 (Feb. 4, 1958).

(6) (a) J. Fried and R. W. Thoma, U. S. Patent 2,756,179 (July 24, 1956); U. S. Patent 2,799,690 (July 16, 1957); (b) S. A. Szpilfogel, P. A. Van Hemert and M. S. De Winter, *Rec. trav. chim.*, **75**, 1227 (1956); (c) D. Sutter, W. Charney, P. L. O'Neill, F. Carvajal, H. L. Herzog and E. B. Hershberg, *J. Org. Chem.*, **22**, 578 (1957); (d) H. Nawa, M. Uchibayashi, R. Takeda, I. Nakanishi, T. Kusaka, J. Terumichi, M. Uchida, M. Katsumata, K. Yoshimo and H. Fujitami, *Tetrahedron*, **4**, 201 (1958).