was available for proper classical characterization of the compound.

Acetylation of a few crystals of XIV at room temperature gave a single additional mobile reducing component, R_f 0.39 (system V) (with triamcinolone 16α,21-diacetate R_f 0.29, triamcinolone 11β,16α,21-triacetate R_f 0.53, run at the same time), which was the same papergram mobility as observed on heating an acetic anhydride–pyridine solution of the isomer diacetate IIa. The indicated triacetate was not isolated.

Solvent Isomerization Rates.—Comparison of solvent isomerization rates were made with steroid solutions of 1 mg./ml. in methanol. Commercial reagent methanol was used "as is," after filtering through Darco G 60 charcoal, and after distillation. The still concentrate, representing 10–15% of the original volume of methanol taken, was also used for isomerization experiments. The steroids were analyzed by streaking the methanol solution (100 μl.) onto washed sheets of Whatman No. 1 filter paper, chromatographically resolving the sample components (system I) eluting from the paper, and determination by ultraviolet absorption measurements and by colorimetric measurements with tetrazolium blue. Pure triamcinolone and triamcinolone isomer were run as standards. Results are expressed in terms of percentage of steroid found. In all cases the only ultraviolet absorbing zones or tetrazolium reducing zones were those of the parent steroid and its isomer.

Metal Cation Isomerization.—Solutions of selected salts were made in redistilled methanol so that the cation concentration was 10 μg./ml. To these solutions sufficient triamcinolone was added so the final steroid concentration was 1 mg./ml. After standing 24 hours at room temperature the solutions were analyzed by paper chromatography (system II). The metal salts isomerizing triamcinolone were: ferric chloride hexahydrate, ferrous ammonium sulfate (under nitrogen), vanadyl sulfate, aluminum sulfate, aluminum chloride hexahydrate. Salts not isomerizing triamcinolone were: chromium nitrate, manganese sulfate, nickel chloride, zinc sulfate, cupric sulfate, cobalt chloride, magnesium sulfate, silver nitrate, calcium carbonate, sodium chloride.

Ferric cation levels were also studied at 0.1, 1, 10 and 100 μg./ml. using the same steroid concentration. The degree of isomerization was determined spectrophotometrically on eluates from the paper chromatograms.

The equivalent acidity produced by the hydrolysis of 10 μg./ml. of ferric chloride hexahydrate was used with the same steroids and the products analyzed after 24 hours.

[Contribution from the Chemical Process Improvement Department, Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.]

16α-Hydroxy Steroids. VIII.[1] 16α,17α-Cyclic Orthoesters of Triamcinolone

By Leland L. Smith and Michael Marx

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Triamcinolone reacts with a variety of aliphatic orthoesters to form steroidal 16α,17α-cyclic orthoester derivatives. With the higher aliphatic orthoester homologs both 16α,17α-cyclic orthoester and 16α- and 21-normal monoesters are obtained, the proportion of cyclic orthoester decreasing as the molecular size of the aliphatic orthoester used increases.

Steroidal 16α,17α-diols react with a variety of reagents known to form cyclic derivatives or complexes with acyclic vicinal diols or with simple cyclic *cis*-1,2-diols. In the pregnane series 16α,17α-cyclic osmate esters,[2b] 16α,17α-cyclic acetals and ketals,[2a,3] and 16α,17α-cyclic borates[4] have been described for several steroids.

Preparation of other 16α,17α-cyclic derivatives formally related to ketals and acetals became attractive in view of the interesting biological activity exhibited by triamcinolone (9α-fluoro-11β,16α,17α,21 - tetrahydroxy - 1,4 - pregnadiene-3,20-dione) (I) cyclic ketals and acetals.[3a] Formal replacement of an alkyl group of cyclic ketal derivatives by alkoxyl groups results in cyclic orthoester structures; 16α,17α-cyclic orthoesters of triamcinolone are the subject of this report.

Cyclic orthoesters have been formed with acyclic vicinal diols (ethylene glycol),[5] acyclic 1,3-diols (pentaerythritol),[5] and cyclic polyhydroxy compounds (ouabagenin,[6] cevin alkaloids[7]) by acid-catalyzed ester interchange or by direct reaction with acetic anhydride–pyridine, etc. Formation of a rich variety of cyclic orthoesters of several structural types is well known in the carbohydrate field.[8]

Treatment of a slurry of triamcinolone in an orthoester with a small amount of perchloric acid resulted in rapid solution accompanied by coloration. The major product isolated in most cases is the 16α,17α-cyclic orthoester II. Papergram analysis of the reaction mixture indicates that the reaction is complete almost immediately after solution of the steroid. Other reducing steroids are formed in varying amounts, depending on the orthoester used.

Triamcinolone forms the 16α,17α-ethoxymethylenedioxy derivative IIa with ethyl orthoformate, the 16α,17α-methoxymethylenedioxy derivative IIb with methyl orthoformate, etc. With methyl orthoacetate and orthopropionate, and with ethyl orthoacetate, the respective 16α,17α-alkyloxyalkylidenedioxy derivatives are formed.

Triamcinolone 16α,17α-cyclic orthoesters reduce tetrazolium blue, although the color intensity developed is less than that obtained with triamcinolone or its normal 16α,21-diacetate. The increased tetrazolium blue reducing power of triamcinolone[9]

(1) Paper VII, L. L. Smith, M. Marx, J. J. Garbarini, T. Foell, V. E. Origoni and J. J. Goodman, This Journal, **82**, 4616 (1960).

(2) (a) G. Cooley, B. Ellis, F. Hartley and V. Petrow, *J. Chem. Soc.*, 4373 (1955); (b) 4377 (1955).

(3) (a) J. Fried, A. Borman, W. B. Kessler, P. Grabowich and E. F. Sabo, This Journal, **80**, 2338 (1958); (b) S. Bernstein, *Recent Progress in Hormone Research*, **14**, 1 (1958); (c) S. Bernstein, R. H. Lenhard, W. S. Allen, M. Heller, R. Littell, S. M. Stolar, L. I. Feldman and R. H. Blank, This Journal, **81**, 1689 (1959).

(4) G. H. Thomas, U. S. Patent 2,831,003, April 15, 1958.

(5) V. G. Mkhitaryan, *Zhur. Obschchei Khim.*, **10**, 667 (1940).

(6) R. Tschesche and G. Snatzke, *Chem. Ber.*, **88**, 1558 (1955).

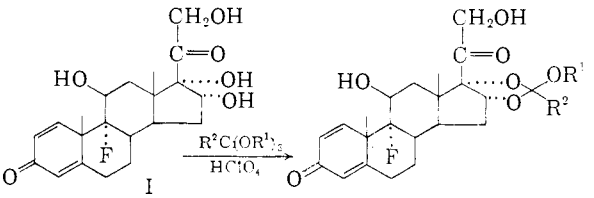
(7) A. Stoll and E. Seebeck, *Helv. Chim. Acta*, **37**, 824 (1954); D. H. R. Barton, C. J. W. Brooks and J. S. Fawcett, *J. Chem. Soc.*, 2137 (1954); S. M. Kupchan and D. Lavie, This Journal, **77**, 683 (1955); S. M. Kupchan, *ibid.*, **77**, 686 (1955); H. Auterhoff and H. Möhrle, *Arch. Pharm.*, **291**, 288 (1958).

(8) E. Pacsu, *Adv. Carbohydrate Chem.*, **1**, 77 (1945).

(9) L. L. Smith and M. Halwer, *J. Am. Pharm. Assoc.*, **48**, 348 (1959). Triamcinolone also has increased reducing power toward alkaline ferrocyanide.[10]

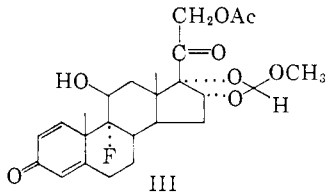
(10) N. R. Stephenson, *Can. J. Biochem. Physiol.*, **37**, 391 (1959).

compared to its non-16α-hydroxylated analog, 9α-fluoroprednisolone, is diminished in turn by 16α,17α-cyclic acetonide formation.[9] Orthoester formation diminishes tetrazolium blue reducing power still further. Thus with triamcinolone response to alkaline tetrazolium blue at 100%, the 16α,17α-cyclic acetonide has 48% response and the 16α,17α-cyclic orthoesters of triamcinolone have the following relative responses: ethyl orthoformate ester IIa, 9%; ethyl orthoacetate ester IIc, 23%; methyl orthoformate ester IIb, 8%; methyl orthoacetate ester IId, 13%; methyl orthopropionate ester IIe, 15%; methyl orthoformate 21-acetate derivative III, 11%. On papergrams the normal diformazan colors appear with indistinguishable intensity compared with triamcinolone.

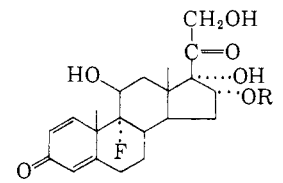


I

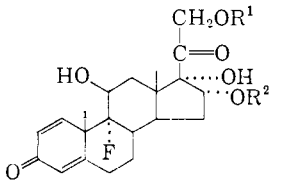
IIa, $R^1 = C_2H_5$; $R^2 = H$
IIb, $R^1 = CH_3$; $R^2 = H$
IIc, $R^1 = C_2H_5$; $R^2 = CH_3$
IId, $R^1 = CH_3$; $R^2 = CH_3$
IIe, $R^1 = CH_3$; $R^2 = C_2H_5$



III



IV and VI
IV, R = $COCH_3$
VI, R = $CO(CH_2)_3CH_3$



V
Va, $R^1 = H$; $R^2 = COC_2H_5$
Vb, $R^1 = COC_2H_5$; $R^2 = H$
Vc, $R^1 = R^2 = COC_2H_5$

The cyclic methyl orthoformate ester IIb forms a monoacetate III on treatment with acetic anhydride–pyridine, to which the 21-acetate structure is assigned on the basis of typical 21-acetoxy-20-ketone interaction in the infrared absorption spectra.

Acid hydrolysis (methanolic hydrochloric acid) of the cyclic orthoesters II yields triamcinolone, thus suggesting potential use of the cyclic orthoester as a protective group for the 16α,17α-diol feature. As anticipated of orthoesters, alkaline hydrolysis with several bases did not lead to any detectable triamcinolone (paper chromatographic analysis). No triamcinolone isomer[1] was detected either. Under conditions which lead readily and completely to triamcinolone from triamcinolone 16α,21-diesters (sodium in methanol, aqueous potassium carbonate) the orthoesters II are but incompletely hydrolyzed to reducing products having papergram mobilities on the order of triamcinolone monoesters IV and Va. The identity of these hydrolysis products was not investigated further. Substantial amounts of the parent orthoester II remained in the reaction mixture.

The triamcinolone orthoesters II are characterized by intense multiple-band infrared absorption in the 9–10 μ region. This absorption is associated with increased C–O–C content of the orthoester molecule, and is readily differentiated from C–O–C absorption of normal 16α,21-diesters and 16α,17α-cyclic ketals of triamcinolone. The 20-carbonyl absorption of the orthoesters II is in the 5.80–5.82 μ region, whereas triamcinolone absorbs at 5.85 μ.[3c,9] However, this displacement is less than that found in triamcinolone 16α,21-diacetate (5.74 μ[9]), triamcinolone 16α,17α-acetonide 21-acetate (5.71, 5.79 μ[3a,3c]) or triamcinolone methyl orthoformate 21-acetate (III) (5.70, 5.75 μ), and more nearly approximates the 16-acetoxy-20-ketone interactions described by Hirschmann and Daus.[11]

These several chemical and physical properties of the orthoester products II are sufficient to establish the 16α,17α-(1-alkyloxy)-alkylidenedioxy structures which have been assigned.

Minor reducing products can be detected in each reaction by papergram. In a few cases their proportion favored isolation and characterization. In the case of reaction with ethyl orthopropionate and methyl orthovalerate no steroidal orthoester was isolated.

In reaction with ethyl orthopropionate two reducing components Va and Vb were formed in the proportion of 2:1, both with less papergram mobility than that anticipated of the sought 16α,17α-orthopropionate. Both were recognized as monopropionates from papergram mobility, elemental analysis and infrared spectra (C–O–C absorption at 8.40–8.48 μ). Both Va and Vb were converted to triamcinolone 16α,21-dipropionate Vc by propionic anhydride–pyridine. The less mobile Va did not form a cyclic acetonide with acetone–perchloric acid, while the more mobile Vb did.[12]

Infrared spectra of Vb have bands at 5.73 and 5.78 μ, typical of the vicinal interactions characterisitic of 21-acyloxy-20-ketones,[13] while Va showed a single band at 5.80 μ, more in keeping with the lesser displacement of the 20-carbonyl absorption in the 16α,17α-cyclic orthoesters II. The carbonyl region of the 16α,21-dipropionate Vc includes bands at 5.72 and 5.77 μ, essentially identical with spectra of Vb. Thus Vb is assigned the structure of a 21-propionate and Va of a 16α-propionate.

In reaction with ethyl orthoacetate triamcinolone forms the 16α,17α-cyclic orthoacetate deriva-

(11) H. Hirschmann and M. A. Daus, *J. Org. Chem.*, **24**, 1114 (1959).

(12) The acetonide derivative was formed on filter paper and recognized by its mobility after chromatographic irrigation, but was not isolated; see L. L. Smith and T. Foell, *J. Chromatography*, **3**, 381 (1960).

(13) R. N. Jones, P. Humphries, F. Herling and K. Dobriner, THIS JOURNAL, **74**, 2820 (1952).

tive IIc and a second major reducing product IV, in approximately equal proportions. On the basis of elemental analysis, infrared spectra and papergram mobility, IV was recognized as a monoacetate. Acetylation of IV gave triamcinolone 16α,21-diacetate. The absence of a large vicinal interaction in the carbonyl region of the infrared spectra and the failure to form an acetonide with acetone–perchloric acid established the structure of IV as that of triamcinolone 16α-acetate. The papergram mobility of IV also favors the 16α-acetate structure in view of the relative mobilities of the 16α- and 21-propionates Va and Vb already described.

In reaction with methyl orthovalerate the major product isolated was a normal ester VI, to which the structure triamcinolone 16α-valerate was assigned on the basis of papergram mobility and infrared spectral analogies with the 16α-acetate IV and 16α-propionate Va. A minor reducing component (not isolated) was suggested as being an orthovalerate on the basis of papergram mobility.

Reaction with other methyl orthoesters (formate, acetate, propionate) gave only minor amounts of reducing steroids less mobile than the 16α,17α-cyclic orthoester, which traces were not isolated. Characterization of these traces as the 16α- and 21-normal esters is made strictly on the basis of analogy with the results obtained with the ethyl orthoester series. In all cases where both normal esters were detected the 16α-ester was detected in the greater amount.

As the size of the orthoacylate portion of the ethyl orthoester molecule increases from orthoformate through orthopropionate the proportion of 16α,17α-cyclic orthoester decreases. The ratio of orthoester product to normal ester product(s) is: for orthoformate, 1:0; for orthoacetate, 1:1; for orthopropionate, 0:1. Although this effect is not displayed in the methyl orthoester series, in the one instance of a large orthoacyl group—the orthovalerate—the normal 16α-valerate was the major product of the reaction.

Papergram mobilities of the normal 16α-esters are substantially less than those of the normal 21-esters, indicating the pronounced differential between the polarity influences of the 16α-hydroxyl and 21-hydroxyl of the triamcinolone molecule.

The papergram mobility of the 16α,17α-cyclic orthoesters (as well as 16α,17α-cyclic acetals and ketals) is related to their carbon content. For derivatives with the same orthoacyl portion of the molecule the ethyl esters are slightly more mobile than the methyl esters, and the orthoesters in turn are slightly more mobile than 16α,17α-cyclic acetals or ketals of the same carbon content.

Each of the orthoesters of triamcinolone was biologically active as a glucocorticoid when tested in adrenalectomized rats. Relative to hydrocortisone on a liver glycogen assay, IIa was 4× (3–7, 95% confidence limits), IIb was 10× (6.3–16) as active; on a thymus involution test, IIa was 3× (2–3), IIb was 8.9× (6.8–11.7) as active. None of the orthoester derivatives was as active as triamcinolone 16α,17α-acetonide; however, each of the orthoesters II did elicit increased sodium and fluid excretion in the adrenalectomized rat.

Acknowledgment—The authors are grateful for the assistance of Mr. Theodore Foell for papergram analyses, to Mr. William Fulmor for infrared spectra, to Mr. Walter H. Muller for ultraviolet spectra and rotations, and to Mr. L. Brancone for elemental analyses. The animal assay data were kindly supplied by Dr. Ira Ringler of these laboratories.

Experimental[14]

9α - Fluoro - 11β,21 - dihydroxy - 16α,17α - methoxymethylenedioxy - 1,4 - pregnadiene - 3,20 - dione (IIb).—To a slurry of 1.0 g. of triamcinolone in 30 ml. of methyl orthoformate was added 0.30 ml. of reagent 70% perchloric acid. A dark red coloration developed as the steroid dissolved. After shaking for 10 minutes the reaction mixture was neutralized with 10 ml. of saturated aqueous sodium bicarbonate solution, which treatment discharged the red color and precipitated inorganic solids. After filtration, the filtrate was diluted with 20 ml. of water and concentrated *in vacuo* to remove organic solvent. The resulting gum was extracted with three portions of methylene chloride, the combined extracts washed with water, dried over anhydrous magnesium sulfate, and evaporated *in vacuo*. The residue was crystallized from acetone–petroleum ether, yielding 600 mg. of crude orthoester, m.p. 208.5–215.5°. Two recrystallizations from acetone–petroleum ether gave the pure product, m.p. 218.5–222.0°, $[\alpha]^{22}D$ +127°, λ_{max} 238 mμ (ε 15,700); λ_{max}^{KBr} 2.92, 3.40 , 5.80, 6.00, 6.14, 6.21, 6.90, 8.86, 9.35, 9.51, 9.74, 10.14, 11.15 μ, etc.; $\lambda_{max}^{H_2SO_4}$ ($E_{1\ cm}^{1\ \%}$): 2 hr., 260 (303), 309 (134), 375 mμ (14); 20 hr., 260 (312), 309 (134), 375 mμ (111); papergram mobility, system V, R_f 0.30; system VI, R_f 0.10, positive to alkaline tetrazolium blue and to concentrated isonicotinic acid hydrazide reagent.

Anal. Calcd. for $C_{23}H_{29}O_7F$: C, 63.28; H, 6.70; F, 4.35. Found: C, 63.58; H, 6.89; F, 4.46.

Papergram examination of the crude residue indicated the methyl orthoformate derivative IIb to be the major component, with but traces of a reducing component at R_f *ca.* 0.01 (system V).

9α - Fluoro - 11β,21 - dihydroxy - 16α,17α - (1 - methoxy)-ethylidenedioxy - 1,4 - pregnadiene - 3,20 - dione (IId).—One gram of triamcinolone was shaken with 30 ml. of methyl orthoacetate and 0.30 ml. of perchloric acid for 40 minutes, the reaction mixture neutralized and worked up in the same manner as applied to the orthoformate IIb. Crystallization from acetone–petroleum ether gave 595 mg. of crude product, m.p. 197–200°, resolidifying and remelting 243–247° dec. After three recrystallizations from acetone–petroleum ether the pure orthoester was obtained as transparent hexagonal platelets, m.p. 210–214°, resolidifying and remelting 241.5–245° dec., $[\alpha]^{22}D$ + 123°, λ_{max} 238 mμ (ε 15,400); λ_{max}^{KBr} 2.94, 3.40, 5.82, 6.00, 6.16, 6.21, 7.18, 7.84, 8.53, 8.87, 9.34, 9.48, 9.71, 9.87, 10.55, 10.73, 11.45 μ, etc.; $\lambda_{max}^{H_2SO_4}$ ($E_{1\ cm}^{1\ \%}$): 2 hr., 260 (255), 309 (113), 375 mμ (27), 20 hr., 260 (276), 309 (119), 374 mμ (159); papergram mobility, system V, R_f 0.44; system VI, 0.17, positive to both tetrazolium blue and isonicotinic acid hydrazide.

Anal. Calcd. for $C_{24}H_{31}O_7F$: C, 63.99; H, 6.94; F, 4.22. Found: C, 64.07; H, 6.94; F, 4.23.

Papergram examination of the crude product revealed the presence of: the major orthoester component IId;

(14) All melting points were made in capillary tubes and are uncorrected. Optical rotations are all in methanol on a 0.5% solution. Ultraviolet absorption spectra in absolute ethanol and in concentrated sulfuric acid were recorded on a Cary recording spectrophotometer model 11S. Infrared absorption spectra were obtained on potassium bromide disks using a Perkin–Elmer model 21 double beam instrument. Paper chromatographic analyses were conducted using Bush-type systems previously described[15]: system V, toluene–petroleum ether–methanol–water 12:8:13:7; and system VI, benzene–petroleum ether–methanol–water 5:5:7:3. Tetrazolium blue and isonicotinic acid hydrazide color tests[16] were used.

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

(16) L. L. Smith and T. Foell, *Anal. Chem.*, **31**, 102 (1959).

a minor trace component (21-acetate?), R_f 0.21 (system V), R_f 0.10 (system VI), reactive toward tetrazolium blue and isonicotinic acid hydrazide; and a trace component (16α-acetate?), R_f 0.02 (system V), R_f 0.01 (system VI), reactive toward tetrazolium blue and isonicotinic acid hydrazide.

9α - Fluoro - 11β,21 - dihydroxy - 16α,17α - (1 - methoxy)-propylidenedioxy - 1,4 - pregnadiene - 3,20- dione (IIe).—The reaction between 750 mg. of triamcinolone and 15 ml. of methyl orthopropionate with 0.20 ml. of 70% perchloric acid was terminated after 20 minutes and worked up in the typical manner. The crude material was crystallized from acetone–petroleum ether, 420 mg., m.p. 160–164°, resolidifying and remelting 256–261° dec. After three recrystallizations from acetone–petroleum ether the pure orthoester was obtained as needles, m.p. 266–267° dec., $[\alpha]^{22}D$ + 111°, λ_{max} 238 mμ (ε 15,150); λ_{max}^{KBr} 2.92, 3.40, 5.82, 6.00, 6.17, 6.21, 7.97, 9.12, 9.36, 9.49, 9.68, 9.85, 10.22, 10.30, 11.16 μ, etc.; $\lambda_{max}^{H_2SO_4}$ ($E_{1\ cm}^{1\ \%}$): 2 hr., 260 (273), 309 (122), 375 mμ (28); 20 hr., 260 (296), 309 (124), 373 mμ (172); papergram mobility, system V, R_f 0.55; system VI, R_f 0.26.

Anal. Calcd. for $C_{25}H_{33}O_7F$: C, 64.64; H, 7.16; F, 4.09. Found: C, 64.87; H, 7.32; F, 4.25.

Papergram examination of the crude product indicated the presence of the orthopropionate as the major product, together with a trace component (16α-propionate?) at R_f 0.04 (system V), R_f 0.02 (system VI) and a minor trace component at R_f 0.19 (system VI) (21-propionate?). Both trace components were detected by tetrazolium blue and isonicotinic acid hydrazide.

9α - Fluoro - 11β,17α,21 - trihydroxy - 16α - *n* - valeryloxy-1,4-pregnadiene-3,20-dione (VI).—A slurry of 1.0 g. of triamcinolone in 15 ml. of methyl ortho-*n*-valerate and 0.20 ml. of 70% perchloric acid was shaken for one hour, neutralized, and the product isolated in the usual manner. The crude product failed to crystallize, and was thus adsorbed onto silica gel from chloroform. Elution with chloroform–ethyl acetate 3:2 gave a reducing fraction, 85 mg., which analyzed as a mixture of supposed orthovalerate derivative (R_f 0.55, system V) and 16α-valerate VI (R_f 0.17) and from which only the 16α-monovalerate could be isolated as crystalline material. Further elution with chloroform–ethyl acetate 1:1 gave 180 mg. of crystals, m.p. 175–180°, R_f 0.19, which after several recrystallizations from acetone–petroleum ether gave the pure monovalerate, m.p. 212–216°, $[\alpha]^{22}D$ +27°, λ_{max} 238 mμ (ε 14,900); λ_{max}^{KBr} 2.87, 3.39, 5.75, 5.81, 6.00, 6.17, 6.21, 8.50, 8.86, 9.40, 10.22 μ, etc.

Anal. Calcd. for $C_{26}H_{35}O_7F$: C, 65.25; H, 7.37; F, 3.97. Found: C, 65.43; H, 7.82; F, 4.05.

16α,17α - Ethoxymethylenedioxy - 9α - fluoro - 11β,21-dihydroxy - 1,4 - pregnadiene - 3,20 - dione (IIa).—One gram of triamcinolone, 50 ml. of ethyl orthoformate and 0.3 ml. of 70% perchloric acid were shaken for 30 minutes, neutralized, and worked up in the usual manner. The gummy solids obtained from the aqueous solution were extracted into chloroform, the chloroform extracts dried over anhydrous magnesium sulfate, and adsorbed onto silica gel. Elution of the orthoester fraction was accomplished with chloroform containing 15–20% ethyl acetate, v./v. Evaporation of the solvent from the appropriate fractions yielded a glass, which crystallized upon trituration with methylene chloride. Recrystallization from acetone–petroleum ether furnished 545 mg. of crude crystals, m.p. 206–209°. After three recrystallizations from acetone–petroleum ether the pure orthoformate derivative was obtained, m.p. below 150°, resolidifying and remelting 207.5–209°, $[\alpha]^{22}D$ + 127°, λ_{max} 238 mμ (ε 15,170); λ_{max}^{KBr} 2.93, 3.40, 5.82, 6.00, 6.16, 6.21, 8.86, 9.14, 9.36, 9.51, 9.83, 10.04, 11.16 μ, etc.; $\lambda_{max}^{H_2SO_4}$ ($E_{1cm}^{1\%}$): 2 hr., 260 (304), 309 (136), 380 mμ (19), 20 hr., 260 (314), 309 (135), 374 mμ (124); papergram mobility, system V, R_f 0.46; system VI, R_f 0.18.

Anal. Calcd. for $C_{24}H_{31}O_7F$: C, 63.99; H, 6.94; F, 4.22. Found: C, 63.81; H, 7.28; F, 4.30.

Papergram examination of the gummy solids obtained above revealed the presence of four reducing steroids: (1) the major orthoformate component, (2) a more mobile minor trace, (3) a minor component, R_f 0.21 (system V), R_f 0.10 (system VI) (21-formate ?), and a trace R_f 0.02 (system V), R_f 0.01 (system VI) (16α-formate ?).

16α,17α - (1-Ethoxy) - ethylidenedioxy - 9α - fluoro-11β,21 - dihydroxy - 1,4 - pregnadiene - 3,20 - dione (IIc).—One gram of triamcinolone, 30 ml. of ethyl orthoacetate and 0.30 ml. of 70% perchloric acid were mixed in the usual way, and after 30 minutes the reaction was neutralized and worked up. The crude reaction product was crystallized from acetone–petroleum ether, 640 mg., m.p. 164–167°, resolidifying and remelting 237–241° dec. Papergram examination indicated the crystals to be a mixture of two major components, at R_f 0.01 and R_f 0.62 (system V), both reducing toward tetrazolium blue, both reactive toward isonicotinic acid hydrazide. The composition of the mixture was not altered by three recrystallizations from acetone–petroleum ether. The crystals and mother liquor solids were combined, dissolved in chloroform and adsorbed onto silica gel. Elution with chloroform–ethyl acetate 1:1 yielded the crude orthoacetate IIc, 245 mg., which after three recrystallizations from acetone–petroleum ether gave an analytical sample, m.p. below 200°, resolidifying and remelting 239–242° dec., $[\alpha]^{22}D$ +87.5°, λ_{max} 238 mμ (ε 15,150); λ_{max}^{KBr} 2.92, 3.40, 5.81, 6.00, 6.15, 6.21, 7.16, 7.90, 8.50, 9.35, 9.50, 9.70, 9.88, 10.53, 11.21 μ, etc.; $\lambda_{max}^{H_2SO_4}$ ($E_{1\ cm}^{1\ \%}$): 2 hr., 260 (272), 309 (122), 377 mμ (34); 20 hr., 260 (293), 308 (124), 373 mμ (176); papergram mobility, system V, R_f 0.62; system VI, R_f 0.30.

Anal. Calcd. for $C_{25}H_{33}O_7F \cdot {}^1/_2H_2O$: C, 63.41; H, 7.24; F, 4.01. Found: C, 63.37; H, 6.76; F, 4.09.

16α - Acetoxy - 9α - fluoro - 11β,17α,21 - trihydroxy - 1,4-pregnadiene - 3,20 - dione (IV).—Further elution of the silica gel column above with chloroform–ethyl acetate 3:7 afforded the second reducing component of lower papergram mobility. Evaporation of the eluates gave a residue, 350 mg., which was crystallized from acetone–petroleum ether giving 270 mg. of needles, m.p. 228–231° dec. Three further recrystallizations gave the pure 16α-acetate IV (acetone solvate), m.p. 224–228° dec., $[\alpha]^{22}D$ +49.7° λ_{max} 238 mμ (ε 15,450); λ_{max}^{KBr} 2.93, 3.41, 5.80, 6.01, 6.16, 6.21, 7.98, 8.85, 9.42, 11.20μ, etc.; papergram mobility, system V, R_f 0.01; system VI, R_f 0.01.

Anal. Calcd. for $C_{23}H_{29}O_7F \cdot C_2H_6O$: C, 62.23; H, 7.31; F, 3.94. Found: C, 62.17; H, 7.34; F, 4.21.

Acetylation of IV with acetic anhydride–pyridine in the usual manner gave triamcinolone 16α,21-diacetate, identified with an authentic sample by comparison of melting point behavior, paper chromatographic behavior and infrared spectra.

9α - Fluoro - 11β,16α,17α - trihydroxy - 21 - propionoxy-1,4 - pregnadiene - 3,20 - dione (Vb).—To a slurry of 1.0 g. of triamcinolone in 30 ml. of ethyl orthopropionate was added 0.30 ml. of 70% perchloric acid. Over a period of 1 hour on the rotary shaker a portion of the solids failed to dissolve. The mixture was neutralized with 8 ml. of sodium bicarbonate solution, which discharged the coloration, etc., and produced a precipitate of inorganic salts. After the usual work-up the residue obtained was crystallized from acetone–petroleum ether. Paper chromatographic examination of the crystals indicated two major reducing zones at R_f 0.06 (predominant) and R_f 0.26 (secondary) (system V) with no reducing zone in the position to be expected of the anticipated orthoester. The mixture was dissolved in chloroform and adsorbed onto silica gel. Elution with chloroform–ethyl acetate 45:55 yielded 55 mg. of the 21-propionate Vb in the first fraction. A second fraction yielded 80 mg. of 21-propionate contaminated with small amounts of the 16α-propionate Va, as evidenced by paper chromatography. Recrystallization from acetone–petroleum ether gave the 21-propionate, m.p. 229–231° dec., $[\alpha]^{22}D$ +66.5°, λ_{max} 238 mμ (ε 15,480); λ_{max}^{KBr} 2.89, 2.95, 3.40, 5.73, 5.78, 6.00, 6.15, 6.21, 8.40, 8.48, 9.20, 9.33, 11.20 μ, etc.; papergram mobility, system V, R_f 0.25; system VI, R_f 0.09.

Anal. Calcd. for $C_{24}H_{31}O_7F$: C, 63.99; H, 6.94; F, 4.22. Found: C, 63.59; H, 7.31; F, 4.49.

Acylation of Vb with propionic anhydride–pyridine in the usual manner yielded triamcinolone 16α,21-dipropionate (Vc) identical with an authentic sample.

9α - Fluoro - 11β,17α,21 - trihydroxy - 16α - propionoxy-1,4 - pregnadiene - 3,20 - dione (Va).—Further elution of the above silica gel column with chloroform–ethyl acetate 45:55 yielded an intermediate fraction of the 16α-propionate contaminated with small amounts of 21-propionate, and finally in the latter fractions, the 16α-propionate Va, crystal-

lized from acetone–petroleum ether, 280 mg., m.p. 210–215° dec. Recrystallization from acetone–petroleum ether gave the pure 16α-propionate, m.p. 219–222° dec., $[\alpha]^{22}D$ +56.4°, λ_{max} 239 mμ (ε 15,600); λ_{max}^{KBr} 2.93, 3.41, 5.75, 5.80, 6.00, 6.16, 6.21, 8.44, 8.86, 9.21, 9.37, 9.50, 11.20 μ, etc.; papergram mobility, system V, R_f 0.05; system VI, R_f 0.04.

Anal. Calcd. for $C_{24}H_{31}O_7F$: C, 63.99; H, 6.94; F, 4.22. Found: C, 64.27; H, 7.33; F, 4.22.

Acylation of Va with propionic anhydride–pyridine yielded triamcinolone 16α,21-dipropionate (Vc), m.p. 178–181°, identical with the authentic sample.

9α - Fluoro - 11β,17α - dihydroxy - 16α,21 - dipropionoxy-1,4-pregnadiene-3,20-dione (Vc).—Five hundred milligrams of triamcinolone was acylated with 5 ml. of propionic anhydride and 10 ml. of pyridine. After standing overnight the product was isolated in the usual manner and recrystallized twice from acetone–petroleum ether, 220 mg., m.p. 181–183°, $[\alpha]^{22}D$ +67.0°, λ_{max} 238 mμ (ε 15,300); λ_{max}^{KBr} 2.90, 3.40, 5.72, 5.77, 5.99, 6.15, 6.20, 8.43, 11.18 μ, etc.; papergram mobility, system V, R_f 0.67; system VI, R_f 0.45.

Anal. Calcd. for $C_{27}H_{35}O_8F$: C, 64.02; H, 6.96; F, 3.75. Found: C, 63.81; H, 7.16; F, 4.15.

21 - Acetoxy - 9α - fluoro - 11β - hydroxy - 16α,17α - methoxymethylenedioxy - 1,4 - pregnadiene - 3,20 - dione (III).—A solution of 300 mg. of triamcinolone methyl orthoformate derivative IIb in 4 ml. of dry pyridine was treated with 0.8 ml. of acetic anhydride. After 16 hours the reaction was terminated by addition of methanol and removal of solvents *in vacuo*. The residue was crystallized from acetone–petroleum ether to yield impure crystals which, on recrystallization from the same solvent pair, weighed 240 mg., m.p. 188–191°. After two recrystallizations more the orthoester acetate was obtained, m.p. 192–194°, $[\alpha]^{22}D$ +123°, λ_{max} 238 μ (ε 15,620); λ_{max}^{KBr} 2.92, 3.39, 5.70(shoulder), 5.75, 5.98, 6.15, 6.20, 8.17, 8.85, 9.32, 9.42, 9.70, 10.05, 11.16, etc.; $\lambda_{max}^{H_2SO_4}$ ($E_1^{1\%}_{cm}$): 2 hr., 260 (257), 309 (116), 380 mμ (17); 20 hr., 260 (274), 309 (116), 374 mμ (104); papergram mobility, system V, R_f 0.93; system VI, R_f 0.72; positive to tetrazolium blue and to isonicotinic acid hydrazide.

Anal. Calcd. for $C_{25}H_{31}O_8F$: C, 62.75; H, 6.53; F, 3.97. Found: C, 62.98; H, 6.91; F, 4.01.

[CONTRIBUTION FROM THE STERLING-WINTHROP RESEARCH INSTITUTE]

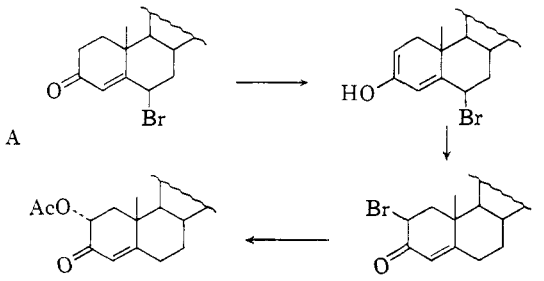
Some Reactions of 2-Hydroxytestosterone and its Diacetate. I

BY ROBERT L. CLARKE

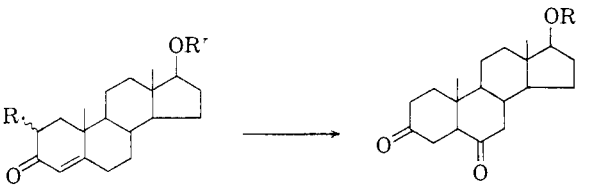
RECEIVED MARCH 1, 1960

Treatment of 2β-hydroxytestosterone diacetate (Ia) with *p*-toluenesulfonic acid in methanol produced 17β-hydroxy-5α-androstan-3,6-dione. Oxidation of Ia, its 2α-epimer Ib or 2α-hydroxytestosterone with molecular oxygen under alkaline conditions gave 2,17β-dihydroxy-1,4-androstadien-3-one (IIIa).

Rivett and Wallis[1] reported that 6β-bromo-4-cholesten-3-one reacted with potassium acetate in acetic acid to give "6α-acetoxy-4-cholesten-3-one." Fieser and Romero[2] later showed that this reaction product actually was 2α-acetoxy-4-cholesten-3-one and suggested a possible mechanism for the rearrangement as shown by equation A. This same reaction has been observed with 6β-bromotestosterone acetate.[3,4] The present paper reports a reaction which is essentially the reverse of this rearrangement, *i.e.*, a 2→6-migration.



In the course of continuing investigations of 2-oxygenated steroids in this Laboratory, 2β-hydroxytestosterone diacetate[3] (Ia) was subjected to the action of *p*-toluenesulfonic acid (tosyl acid) in boiling methanol. 17β-Hydroxy-5α-androstane-3,6-dione[3] (IIa) (51% yield) and its acetate[5] (IIb) (4% crude yield) were isolated from the reaction mixture as the only products identified. For characterization purposes the 3,6-dione IIa was converted to its acetate ester[5] and to 5α-androstane-3,6,17-trione.[6]



Ia, R = β-OAc, R' = Ac
b, R = α-OAc, R' = Ac
c, R = α-OH, R' = H

IIa, R = H
b, R = Ac

This 2→6-rearrangement is dependent upon substantially anhydrous conditions. The presence of 5% water prevents it. Lowering the reaction temperature to 26° also essentially stops the process. The rate of reaction is dependent upon the concentration of the tosyl acid. For example, using 200 mg. of Ia and 10 mg. of tosyl acid, the ultraviolet absorption of the solution due to the unsaturated ketone chromophore dropped by 56% in 48 hr. With a fivefold increase in tosyl acid concentration the absorption dropped by 92% in 8 hr. When 200 mg. of 2α-hydroxytestosterone diacetate (Ib) was refluxed with methanol containing 10 mg. of tosyl acid, a drop of only 10% in ultraviolet absorption occurred in 48 hr. The rearrangement propensity thus appears related to the β-configuration at C-2.

The mechanism of the rearrangement is perhaps that shown by equation B. The water required for

(1) D. E. A. Rivett and E. S. Wallis, *J. Org. Chem.*, **15**, 35 (1950).
(2) L. F. Fieser and M. A. Romero, THIS JOURNAL, **75**, 4716 (1953).
(3) F. Sondheimer, St. Kaufmann, J. Romo, H. Martinez and G. Rosenkranz, *ibid.*, **75**, 4712 (1953).
(4) R. L. Clarke, K. Dobriner, A. Mooradian and C. M. Martini, *ibid.*, **77**, 661 (1955).
(5) S. H. Eppstein, P. D. Meister, H. M. Leigh, D. H. Peterson, H. C. Murray, L. M. Reineke and A. Weintraub, *ibid.*, **76**, 3174 (1954).
(6) C. P. Balant and M. Ehrenstein, *J. Org. Chem.*, **17**, 1587 (1952).