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16α-Hydroxy Steroids. VII.[1] The Isomerization of Triamcinolone

BY LELAND L. SMITH, MICHAEL MARX, JOHN J. GARBARINI, THEODORE FOELL, VICTOR E. ORIGONI AND JOSEPH J. GOODMAN

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Triamcinolone isomerizes under a variety of conditions to give stereoselectively a D-homoannulated isomer, tentatively 9α-fluoro-11β,16α,17aα-trihydroxy-17aβ-hydroxymethyl-1,4-D-homoandrostadiene-3,17-dione. The conditions involved in the isomerization are described and some chemical and physical evidence for the structure assigned is presented.

Triamcinolone (9α-fluoro-11β,16α,17α,21-tetrahydroxy-1,4-pregnadiene-3,20-dione) (I) isomerizes stereoselectively under a variety of conditions to give biologically inactive triamcinolone isomer, II. The Δ^4-3-ketone analog of triamcinolone, 9α-fluoro-11β,16α,17α,21-tetrahydroxy-4-pregnene-3,20-dione (III), similarly rearranges to give its respective isomer, 1,2-dihydrotriamcinolone isomer IV. Some mention of these isomers has been made previously.[2]

Triamcinolone and its 1,2-dihydro analog III isomerize (a) during certain microbiological fermentations, (b) during alkaline hydrolysis of the 16α,21-diesters, (c) in certain solvents, (d) in the presence of certain metal cations, and (e) during chromatography on silica gel. The microbiological 16α-hydroxylation of 9α-fluorohydrocortisone[3] affords 9α-fluoro-16α-hydroxyhydrocortisone (III), together with varying amounts of the rearranged product 1,2-dihydrotriamcinolone isomer IV. Similar 16α-hydroxylation of several other related steroids yield analogous isomeric products. These results will be discussed elsewhere.[4]

Triamcinolone and its 1,2-dihydro analog III are unstable in alkaline solution. Rapid loss in reducing power results and the isomers II and IV are formed. Conditions commonly employed for hydrolysis of steroid acetates (sodium methoxide, aqueous potassium carbonate) cause varying degrees of isomerization. Extensive degradation occurs on exposure of triamcinolone to hot methanolic alkali or to the hot sodium sulfite conditions of Cooley, *et al.*[5] (for cleavage of 16α,17α-cyclic osmate esters).

Triamcinolone and 9α-fluoro-16α-hydroxyhydrocortisone are isomerized on standing or on heating in certain solvents. Some commerical reagent methanol contains unidentified non-volatile agent(s) which isomerize rapidly and completely both normal steroids (see Table I). Initial isomerization rates at room temperature (half-life one to three days) are greatly increased at reflux temperature (half-life about five minutes). Warm dimethylformamide also isomerizes triamcinolone completely. Isomerization has been observed when partition column fractions obtained using the dioxane–cyclohexane–water systems[2b] are concentrated.

TABLE I

ISOMERIZATION IN METHANOL SOLUTIONS

Condition	Time, hr.	Triamcinolone experiment I found, %[a]	1,2-Dihydrotriamcinolone experiment III found, %
Reagent methanol, "as is," room temperature	0	100(100)	..
	24	71(73)	..
	48	44(45)	..
	72	43(45)	..
Reagent methanol, "as is" reflux	0	92(90)	..
	0.25	23(23)	..
	0.50	1(3.5)	..
	1.0	0(4.4)	..
	2.0	6.6(4.3)	..
Still concentrate methanol, room temperature	0	97	87
	24	64	66
	48	57	58
Distilled methanol, room temperature	0	98	100
	24	99	98
	48	91	100
Reagent 2-propanol, "as is," reflux	0	98	..
	0.25	99	..
	0.50	97	..
	1.0	95	..

[a] Spectrophotometric assay on papergram eluates; colorimetric assays with tetrazolium blue are in parentheses.

Solutions of triamcinolone in redistilled methanol (free from isomerizing agents) are isomerized rapidly in the presence of traces of metal cations. The most effective cation found was ferric cation, which caused measurable isomerization of triamcinolone at cation levels as low as 1 μg./ml. Ferrous, aluminum and vanadyl cations are also effective at 10 μg./ml. levels. At 10–100 μg./ml. levels both ferric and aluminum cation isomerization is accompanied by formation of unrecognized polar non-reducing products. The isomerizing effects of ferric and ferrous iron on I and III have also been demonstrated in submerged aerated microbiological fermentations.[4] Triamcinolone isomer II is converted by ferric cation to polar non-reducing components at about the same rate as is triamcinolone (see Table II).

Acid hydrolysis conditions do not result in any detectable isomerization; thus no triamcinolone isomer is found on acid cleavage of triamcinolone 16α,17α-acetonide with a variety of organic acids. Acidity generated by partial hydrolysis of ferric chloride hexahydrate in the methanol system does not isomerize triamcinolone (Table III) although

(1) Paper VI, J. J. Goodman, M. May and L. L. Smith, *J. Biol. Chem.*, **235**, 965 (1960).

(2) (a) L. L. Smith and M. Halwer, *J. Am. Pharm. Assoc.*, **48**, 348 (1959); (b) L. L. Smith, T. Foell, R. De Maio and M. Halwer, *ibid.*, **48**, 528 (1959).

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

(4) J. J. Goodman and L. L. Smith, *Applied Microbiology*, in press.

(5) G. Cooley, B. Ellis, F. Hartley and V. Petrow, *J. Chem. Soc.*, 4377 (1955).

TABLE II

EFFECTS OF FERRIC CATION ON TRIAMCINOLONE

Ferric cation, μg./ml.	Time, hr.	Components analyzed, % I	II	"Origin"[a]
		Triamcinolone		
0	0	98	2	0
	24	99	1	0
0.1	0	100	0	0
	24	99	1	0
1.0	0	92	7	1
	24	70	30	0
10.0	0	17	81	2
	24	5	82	13
100	0	..	..	..
	24	4	67	29
		Triamcinolone isomer		
0	0	..	100	0
	24	..	100	0
0.1	0	..	100	0
	24	..	100	0
1.0	0	..	100	0
	24	..	99	1
10.0	0	..	100	0
	24	..	87	13

[a] Immobile components.

TABLE III

EFFECT OF ACIDITY ON TRIAMCINOLONE AND ITS ISOMER

Steroid	Time, hr.	Components analyzed,[a] % I	II	"Origin"[b]
Triamcinolone	0	99	1	0
	24	100(87)	0(0)	0(13)
	48	63(80)	0(4)	37(16)
Triamcinolone isomer	0	.....	100	0
	24	.....	87(92)	13(8)
	48	.....	80(89)	20(11)

[a] The results of a second, independent experiment are in parentheses. [b] Immobile components.

unrecognized non-reducing immobile components were formed from both triamcinolone and its isomer II.

The isomers II and IV are characterized as reducing[6] steroids having about half the mobility of their respective parent steroid in a variety of paper chromatographic systems.[7] While the isomers II and IV have infrared spectra similar to those of the parent steroid, prominent bands in the 10.0–10.1 μ region together with diminished absorption in the 9.3–9.7 μ region distinguish the isomers over the normal steroid.[8]

The isomers II and IV are tetrahydroxy diketones. On the basis of spectral data one ketone group was formulated as the unsaturated 3-ketone group present in the parent steroids I and III. The Δ^4-3-ketone IV was recognized as the 1,2-dihydro analog of the $\Delta^{1,4}$-3-ketone triamcinolone isomer II by their common modes of formation, spectral data, optical rotation similarities[9] and papergram mobility behavior.[11]

(6) Both isomers are significantly slower in their response to alkaline tetrazolium blue, whether used as a papergram spray or colorimetrically, in comparison with the parent 16α,17α,21-trihydroxy-20-ketone or with the related non-16α-hydroxylated 17α,21-dihydroxy-20-ketone.

(7) In Bush-type systems[2b] triamcinolone isomer II is *ca.* 46–57% as mobile as triamcinolone; 1,2-dihydrotriamcinolone isomer IV is *ca.* 50–59% as mobile as 9α-fluoro-16α-hydroxyhydrocortisone.

(8) We are indebted to Mr. W. Fulmor of these laboratories for pointing out these spectral characteristics.

Microbiological dehydrogenation of the Δ^4-3-ketone isomer IV with *Nocardia corallina* gave triamcinolone isomer II identical with II prepared by the several other methods, thus establishing the analog relationship between II and IV. The following structural arguments are all based on chemistry of the $\Delta^{1,4}$-3-ketone isomer II. Wherever examined, the 1,2-dihydro isomer IV gave identical chemistry.

Triamcinolone isomer readily forms a reducing diacetate IIa with acetic anhydride–pyridine and a reducing monoacetate VI on limitation of the acetic anhydride to 1.1 equivalents. These reactions imply the presence of a primary and an unhindered secondary hydroxyl group in the molecule.

Triamcinolone isomer forms a non-reducing cyclic acetonide V with acetone–hydrochloric acid,[12] which yields a non-reducing acetonide monoacetate VII on acetylation. Treatment of triamcinolone isomer monoacetate VI with acetone–hydrochloric acid gives the same isomer acetonide acetate VII.

By heating the isomer diacetate IIa in acetic anhydride–pyridine a more mobile reducing component is formed, interpreted as involving a triacetate XIVa formed on acetylation of the 11β-hydroxyl group.[13] The presence of the 11β-hydroxyl group is established by chromium trioxide–pyridine oxidation of the isomer acetonide V to an 11-ketone VIII. The 11-ketone acetonide VIII forms a monoacetate IX.

This behavior with chromium trioxide–pyridine implies in an independent fashion the presence in the triamcinolone isomer molecule of a primary hydroxyl group, resistant to such oxidation.[15]

(9) The molecular rotational increment attendant on isomerization of I to II is −84° (methanol)[10]; of III to IV, −86°. The isomer diacetates IIa and IVa show increments of −280° and −252° in comparison with their respective normal parent diacetates Ia and IIIa.[10] The increments associated with 1,2-dehydrogenation are: for isomers IV → II, −162°; IVa → IIa, −198°; for the normal parent steroids III → I, −156°; IIIa → Ia, −170°.

(10) Rotations in methanol used for this purpose were $[\alpha]^{22}D$ for I, +67.1°[2a]; for Ia, +63°; for III, +108°; for IIIa, +98.2°.

(11) Triamcinolone isomer II has 60–83% of the mobility of isomer IV, which retardation is typical of the mobility relationship between $\Delta^{1,4}$-3-ketone and Δ^4-3-ketone analogs in these systems; see also ref. 7.

(12) A second non-reducing, less polar ketal Va, $C_{27}H_{31}O_7F$, was also formed. Its relative amount depended on the level of acid used. The more mobile Va was converted to the acetonide V by heating in pyridine, and to the isomer acetonide monoacetate VII on acetylation. The structural integrity of Va, which corresponds to an acetone solvate of V by elemental analysis, through isolation procedures, drying in vacuum over refluxing butanol, refluxing in solution, and chromatographic irrigation on papergrams precludes its formulation as a solvate in the usual sense. The formation of the ketal Va as a by-product of the formation of V does not alter the arguments advanced herein for the structure of triamcinolone isomer.

(13) The indicated triacetate XIVa was not isolated, but was recognized by characteristic paper chromatographic behavior analogous to the behavior of the pair triamcinolone 16α,21-diacetate Ia and triamcinolone 11β,16α,21-triacetate[14] XIIIa. Preparation of a component with identical chromatographic and color test behavior by acetylation of triamcinolone isomer 11β-acetate XIV supports the recognized triacetate nature of XIVa.

(14) S. M. Fox, V. E. Origoni and L. L. Smith, THIS JOURNAL, **82**, 2580 (1960).

(15) There are several instances where primary hydroxyl not part of an intact α-ketol system is not oxidized by the chromium trioxide–pyridine reagent of Poos, *et al.*[16] See W. S. Allen, S. Bernstein and R. Littell, THIS JOURNAL, **76**, 6116 (1954); S. Bernstein and R. H. Lenhard, *ibid.*, **77**, 2331 (1955); R. Littell and S. Bernstein, *ibid.*, **78**, 984 (1956); S. Bernstein and R. Littell, *ibid.*, **82**, 1235 (1960); H. J. Lowenthal, *Tetrahedron*, **6**, 269 (1959).

(16) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, THIS JOURNAL, **75**, 422 (1953).

A fourth hydroxyl group is indicated by elemental analysis and infrared spectra, and is formulated as an unacylable tertiary hydroxyl group.

The primary hydroxyl group indicated is not involved in the cyclic acetonide feature in that triamcinolone isomer monoacetate (primary hydroxyl acetylated) forms the same acetonide acetate VII as is derived from the isomer acetonide V on mild acetylation. Furthermore the 11-ketone acetonide VIII is readily acetylated at the indicated primary hydroxyl group. Therefore, the tertiary and unhindered secondary hydroxyl groups are involved in acetonide formation, and are either *cis*-1,2- or 1,3-diaxial relative to one another.[17]

The primary hydroxyl group is also not involved in the α-ketol feature indicated by the reducing properties of the isomer II, as otherwise the isomer acetonide V should be cleaved by sodium bismuthate to an acid acetonide as is triamcinolone 16α,17α-acetonide, which forms the etianic acid acetonide X. The isomer acetonide V is recovered unaltered from the reaction. Also the acetonide V should retain its reducing properties were a primary α-ketol involved. Finally, no vicinal interactions in the infrared spectra of the isomer monoacetate VI are evident as would be anticipated of primary α-ketol acetates.[18] Thus the α-ketol involves either the secondary or tertiary hydroxyl group or both.

The isomerization of triamcinolone was early recognized as involving the D-ring and side-chain of the molecule. Diminished response to tetrazolium salts, loss of significant absorption in concentrated sulfuric acid in the 375–390 mμ region characteristic of triamcinolone and related 16α,17α,21-trihydroxy-20-ketones,[19] failure to give any selective light absorption in the 350–450 mμ region after treatment with phenylhydrazine–sulfuric acid (Porter–Silber reaction),[20] biological inactivity of the isomers II and IV and of their several derivatives, the non-reducing character of the isomer acetonides V, Va, VII, VIII and IX,[23] together with the failure of the triamcinolone isomer acetonide V to undergo oxidative cleavage with sodium bismuthate all strongly suggest that a five-membered D-ring and a primary α-ketol side-chain are not present in triamcinolone isomer II.

Were a five-membered D-ring still present in the isomer molecule, the only structure of concern would be that of a 16,17,20-trihydroxy-21-aldehyde (XI). However, triamcinolone isomer is not rearranged to triamcinolone by refluxing pyridine[24,25] (II is recovered unaltered), nor does it form bisulfite addition compounds typical of steroid 21-aldehydes.[24] Further, evidence for a primary hydroxyl group in the molecule and the selective chromic acid oxidation of the C_{11}-hydroxyl in the isomer acetonide V cannot be reconciled with the 21-aldehyde structure XI.

The alternate formulation possible for a D-ring/side-chain trihydroxyketone consistent with the chemical evidence presented involves D-homoannulated structures, a reasonable formulation in view of recent work on D-homo steroids arising from 16α,17α-dihydroxy-20-ketones.[5,26]

Direct evidence for the presence of a six-membered D-ring is found in the proton nuclear magnetic resonance spectra[27] of three triamcinolone isomer derivatives, the diacetate IIa, the acetonide V and the acetonide acetate VII. Compared with the respective normal parent steroid derivatives, each isomer derivative shows a characteristic shift to lower fields of proton resonance associated with the C_{18} and the downfield shift for each pair are: triamcinolone isomer diacetate IIa, 297 c.p.s., triamcinolone 16α,17α-diacetate Ia, 323 c.p.s., Δ −26 c.p.s.; triamcinolone isomer acetonide V, 308 c.p.s., triamcinolone 16α,17α-acetonide, 331 c.p.s., Δ −23 c.p.s.; triamcinolone isomer acetonide acetate VII, 308 c.p.s., triamcinolone 16α,17α-acetonide 21-acetate, 328 c.p.s., Δ −20 c.p.s. The C_{19}-methyl proton resonances are constant at 291–292 c.p.s. in all derivatives.[28]

The shift in frequency of *ca.* −20 to −26 c.p.s. (60 mc.) is of the same order and direction deduced for the separation between the protons of an angular methyl group situated at the juncture between a five-membered ring and a six-membered ring (C_{18}) and the protons of an angular methyl group located at the juncture of two six-membered rings (C_{19}) in a variety of normal steroids[29] (15–17 c.p.s. at 40 mc., 22–25 c.p.s. at 60 mc.). These spectral data established the presence of an expanded six-membered D-ring in the several triamcinolone isomer derivatives IIa, V and VII, and thus in triamcinolone isomer II. A shift of 18 c.p.s. (60 mc.) to lower fields for the C_{18}-angular methyl protons in the pair cortisone 21-acetate and D-homocortisone acetate[30] has been reported.[31]

(17) S. J. Angyal and C. G. Macdonald, *J. Chem. Soc.*, 686 (1952); P. A. Sneeden and R. B. Turner, THIS JOURNAL, **75**, 3510 (1953).

(18) R. N. Jones, P. Humphries, F. Herling and K. Dobriner, *ibid.*, 2820 (1952).

(19) L. L. Smith and W. H. Muller, *J. Org. Chem.*, **23**, 900 (1958).

(20) Both triamcinolone and its 1,2-dihydro analog III have a much diminished response in the Porter–Silber[21] reaction, but selective absorption still is observed in the spectral region normally associated with the Porter–Silber chromogen.[2a,22] Triamcinolone isomer II and its 1,2-dihydro analog IV show no selective absorption at all, the total absorbance of the treated solutions being but *ca.* 1% of that of steroids bearing the normal dihydroxyacetone side-chain.

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

(22) A. Walser and H. P. Schlunke, *Experientia*, **15**, 71 (1959).

(23) 16α,17α-Cyclic acetonides of normal 16α,17α,21-trihydroxy-20-ketones are reducing[2a]; J. Fried, A. Borman, W. B. Kessler, P. Grabowich and E. F. Sabo, THIS JOURNAL, **80**, 2338 (1958).

(24) D. Taub, R. H. Pettebone, N. L. Wendler and M. Tishler, *ibid.*, **76**, 4094 (1954).

(25) T. Reichstein and J. von Euw, *Helv. Chim. Acta*, **23**, 1258 (1940).

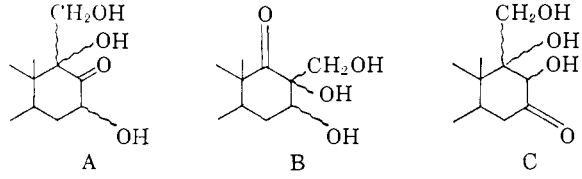
(26) (a) H. H. Inhoffen, F. Blomeyer and K. Bruckner, *Chem. Ber.*, **87**, 593 (1954); (b) K. Heusler and A. Wettstein, *ibid.*, **87**, 1301 (1954); (c) J. Romo and A. Romo de Vivar, *J. Org. Chem.*, **21**, 902 (1956); (d) N. L. Wendler and D. Taub, *Chemistry & Industry*, 1237 (1957); (e) N. L. Wendler, *ibid.*, 20 (1959).

(27) Proton nuclear magnetic resonance spectra were obtained on 99.5% deuteriochloroform solutions of the samples using a 60 mc instrument with an internal reference standard of tetramethylsilane incorporated into each sample. The tetramethylsilane signal was found to be at 385 ± 1 c.p.s. relative to an external reference of benzene. All chemical shifts have been converted to the benzene reference. The 60 mc. data were obtained from Varian Associates, Palo Alto, Calif.

(28) In order to compare the angular methyl proton resonances of triamcinolone 16α,21-diacetate with a previously published 9α-fluoro steroid, data were obtained using a 40 mc. applied field also. For triamcinolone diacetate Ia the C_{19}-methyl protons were found at 193 c.p.s., in agreement with 193 c.p.s. reported for the C_{19}-protons of 9α-fluorocorticosterone.[29] The C_{18}-protons of Ia were found at 215 c.p.s., somewhat less than the 218 c.p.s. reported for the non-17α-hydroxylated-9α-fluoro analog.

(29) J. N. Shoolery and M. T. Rogers, THIS JOURNAL, **80**, 5121 (1958).

Three possible partial structures A, B and C can be devised to conform with the chemical data presented for triamcinolone isomer. Partial structure C involving a 16-ketone is eliminated from further consideration in that more drastic conditions are



suggested for formation of such postulated structures[5,26e] than are involved in the formation of triamcinolone isomer. Also neither isomer II or IV gives a positive Zimmermann test[32] as expected of a 16-ketone of partial structure C.

Only those conformations of partial structures A and B which can form cyclic acetonides need be considered, thus only 16,17a-diaxial diol-17-ketones and 16,17-*cis*-diol-17a-ketones. Both "chair" and "boat" conformations need examination.[33]

In view of the diverse new chemistry being developed in the 16-substituted-17α-hydroxy-20-ketone series[34] and the unsettled literature dealing with 16-oxygenated D-homo steroids[5,26,33b], no choice between 17-ketone and 17a-ketone structure for II can be made by analogy. In the one case where sound chemical evidence supports a 17a-ketone structure, the parent 16-oxygenated steroid is in the 11-keto-21-deoxy series[26d,33b], and both 17- and 17a-ketones were actually obtained.[26e] The present instance of D-homoannulation does not involve formation of both 17- and 17a-ketones; indeed, the formation of but a single isomer under a wide variety of conditions suggests that the Lewis acid–base catalyzed D-homoannulation concepts of Turner[35] are not applicable in the present study.

A choice between 17-ketone and 17a-ketone can be made on infrared absorption data. The absorption bands of the non-conjugated carbonyl group in several derivatives of the isomers II and IV fall in a region (5.84–5.89 μ) normally associated with an unperturbed 20-ketone group in this series.[36] None of these derivatives is acetylated at the secondary hydroxyl group associated with the D-ring. In contrast those derivatives of isomers II and IV which are acetylated at the D-ring secondary hydroxyl group exhibit infrared absorption typical of the interactions anticipated of α-ketol acetates.[37] Thus triamcinolone isomer diacetate IIa absorbs at 5.70 (acetate), 5.78 (interactions) and 5.83 μ (non-conjugated carbonyl) in potassium bromide disk, at 5.68 (acetate) and 5.77 μ (non-conjugated carbonyl) in ethanol. 1,2-Dihydrotriamcinolone isomer diacetate IVa absorbs at 5.73 (acetate) and 5.79 μ (non-conjugated carbonyl) in potassium bromide disk, and its 3-*p*-nitrophenylhydrazone at 5.78 μ.

Such vicinal interactions require that the acetylated secondary hydroxyl group be adjacent to the non-conjugated ketone group of the D-ring. The α-ketol acetate feature thus induced in the isomer diacetates IIa and IVa cannot be reconciled with any of the four possible (chair and boat) conformations arising from the 17a-ketone partial structure B.[40]

A further consideration of the proton nuclear magnetic resonance spectra of triamcinolone isomer diacetate IIa (Fig. 1) shows an uncoupled proton resonance at 61 c.p.s. The methylene protons of the primary hydroxymethyl group (acetylated) being found at higher fields (125 c.p.s.), the 61 c.p.s. resonance is assigned to the C_{16}-proton at which point the acetylated secondary alcohol function is attached in either of partial structures A or B. The C_{16}-proton is equatorial in conformation, as axial protons are expected at higher fields and with more pronounced axial–axial coupling patterns.[41] On this basis both chair and boat conformations are acceptable as possible structures for triamcino-

(30) R. O. Clinton, H. C. Neumann, A. J. Manson, S. C. Laskowski and R. G. Christiansen, THIS JOURNAL, **80**, 3395 (1958).

(31) "NMR at Work," No. 51, Varian Associates Instrument Division, *Chem. and Eng. News*, Sept. 22, 1958, p. 59.

(32) D. K. Fukushima, S. Dobriner, M. F. Heffler, T. Kritchevsky and G. Roberts, *Federation Proc.*, **14**, 216 (1955); THIS JOURNAL, **77**, 6585 (1955); N. L. Wendler, D. Taub, S. Dobriner and D. K. Fukushima, *ibid.*, **78**, 5027 (1956); E. P. Oliveto, C. Gerold, R. Rausser and E. B. Hershberg, *ibid.*, **79**, 3594 (1957).

(33) (a) R. S. Rosenfeld, *ibid.*, **79**, 5540 (1957); (b) N. L. Wendler, *Chemistry & Industry*, 1662 (1958).

(34) S. Bernstein, M. Heller and S. M. Stolar, THIS JOURNAL, **81**, 1256 (1959); S. Bernstein, J. J. Brown, L. I. Feldman and N. E. Rigler, *ibid.*, **81**, 4956 (1959); G. R. Allen and M. J. Weiss, *ibid.*, **81**, 4968 (1959); R. D. Hoffsommer, H. L. Slates, D. Taub and N. L. Wendler, *J. Org. Chem.*, **24**, 1617 (1959).

(35) R. B. Turner, THIS JOURNAL, **75**, 3484 (1953).

(36) Compare triamcinolone isomer II, 5.89 μ; triamcinolone isomer monoacetate VI, 5.85 μ; triamcinolone isomer 11β-acetate XIV, 5.84 μ; triamcinolone isomer acetonide V, 5.85 μ; triamcinolone isomer ketal Va, 5.84 μ; triamcinolone isomer acetonide monoacetate VII, 5.85 μ; 1,2-dihydrotriamcinolone isomer IV, 5.87 μ; *versus* triamcinolone I, 5.85 μ; and 9α-fluoro-16α-hydrocortisone III, 5.84 μ.

(37) Vicinal infrared interactions for a variety of primary α-ketol acetates,[18] a series of 11-acetoxy-12-ketones and 12-acetoxy-11-ketones,[38] a series of 16-acetoxy-17-ketones and 17-acetoxy-16-ketones,[39] and D-homo-17aβ-acetoxy-17-ketones,[38a] etc., have been established. In addition the normal parent steroid diacetates exhibit vicinal interactions; thus, triamcinolone 16α,21-diacetate Ia, 5.74 μ, triamcinolone 11β,16α,21-triacetate XIII, 5.71, 5.76 μ; triamcinolone 16α,17α-acetonide 21-acetate XIIa, 5.71, 5.79 μ; 9α-fluoro-16α-hydroxyhydrocortisone 16α,21-diacetate IIIa, 5.71 μ.

(38) (a) R. N. Jones and F. Herling, *J. Org. Chem.*, **19**, 1252 (1954); (b) D. H. W. Dickson and J. E. Page, *J. Chem. Soc.*, 447 (1955).

(39) M. Okada, D. K. Fukushima and T. F. Gallagher, *J. Biol. Chem.*, **234**, 1688 (1959); L. J. Bellamy and R. L. Williams, *J. Chem. Soc.*, 861 (1957); R. N. Jones and G. Roberts, *Chemistry & Industry*, 1269 (1957).

(40) Were the indicated α-ketol acetate feature imposed on a 16,17 dihydroxy-17a-ketone of partial structure B, the tertiary 17-hydroxyl group would be acetylated. Acetylation of tertiary hydroxyl groups is generally unlikely under the conditions used; also a triacetate would thus be required whereas a diacetate is actually found. (The required triacetate is not to be confused with the triacetate XIVa which involves the 11β-hydroxyl group and not the tertiary hydroxyl group.)

(41) These interpretations were kindly made by Mr. L. Johnson and Dr. J. N. Shoolery of Varian Associates. From their unpublished studies with six-membered A-ring steroid acetates, equatorial protons (of axial acetates) have resonances near 80 c.p.s. while axial protons (of equatorial acetates) have resonances at 105 c.p.s. Some indication that equatorial protons of acetylated inositols, sugar derivatives, etc., are to be found at lower fields than are axial protons has been published.[42] A more full account of the use of proton nuclear magnetic resonance spectra with D-homoannulated steroids of another series, in which both C_{16}-axial and C_{16}-equatorial protons are involved with their appropriate 17a- and 17-ketone structures, will be submitted for publication by S. Bernstein, M. Heller and S. M. Stolar at a later date.

(42) R. U. Lemieux, R. K. Kullnig, H. J. Bernstein and W. G. Schneider, THIS JOURNAL, **79**, 1005 (1957); **80**, 6098 (1958). See also the general discussion in J. A. Pople, W. G. Schneider and H. J. Bernstein, "High-resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959. Chapter 14, pp. 387–399.

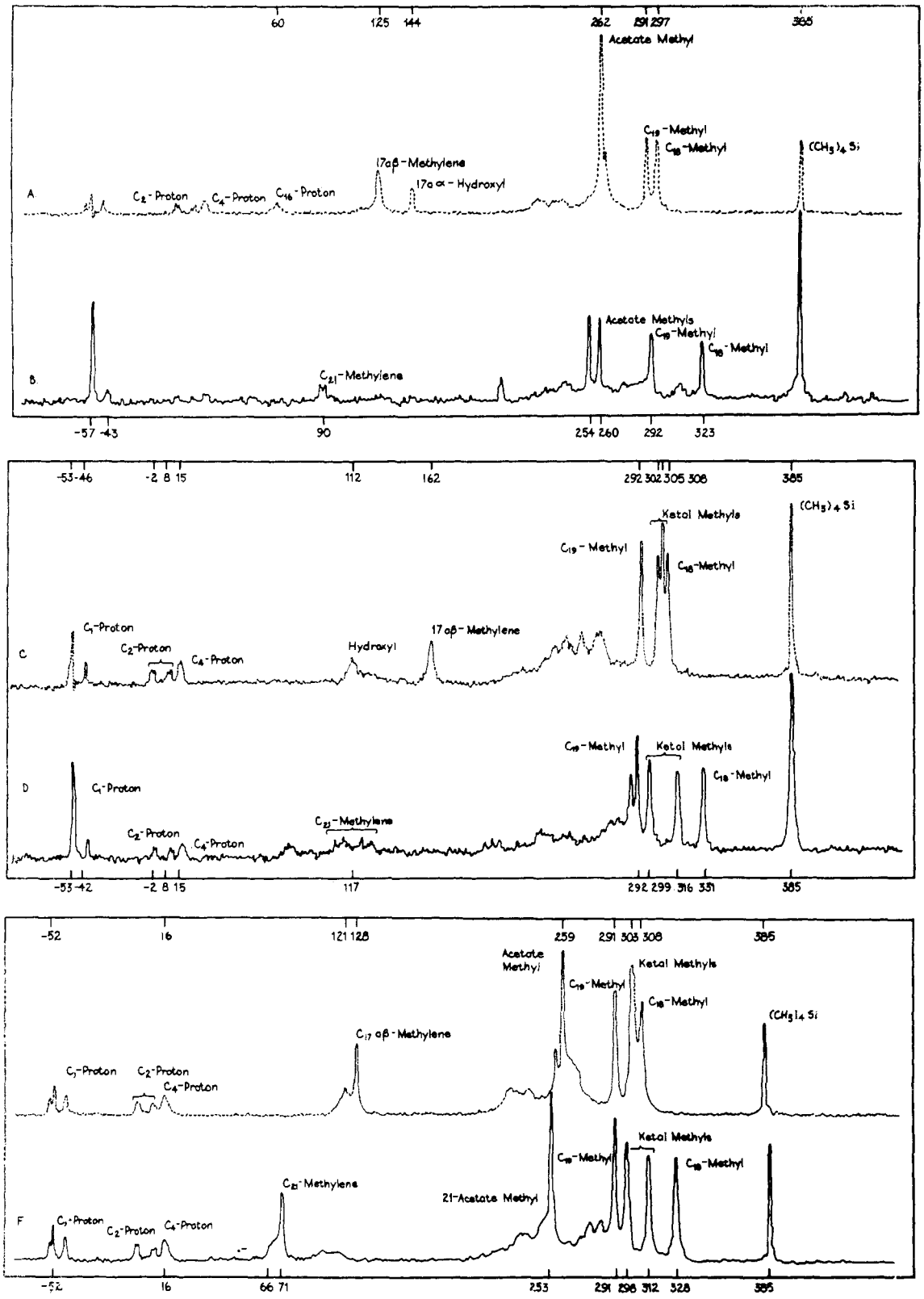
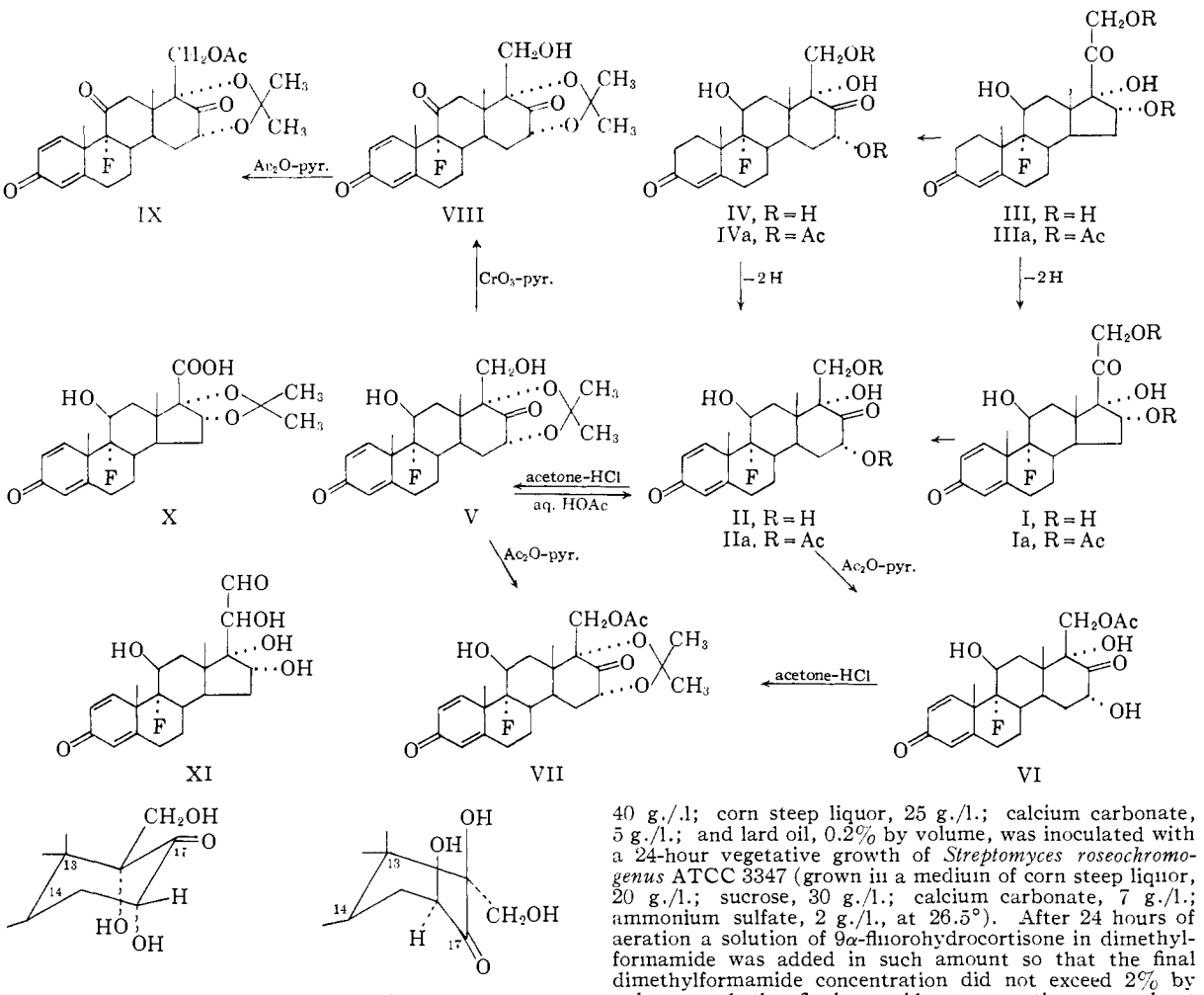


Fig. 1.—Proton n.m.r. spectra of triamcinolone and triamcinolone isomer derivatives: A, triamcinolone isomer diacetate; B, triamcinolone 16α,21-diacetate; C, triamcinolone isomer acetonide; D, triamcinolone 16α,17α-acetonide; E, triamcinolone isomer acetate acetonide; F, triamcinolone 21-acetate 16α,17α-acetonide.

lone isomer. However, the boat conformation represents a very hindered structure, for which it was not possible to construct a 16β,17$\alpha\beta$-cyclic acetonide with Stuart–Briegleb molecular models.



The structure of triamcinolone isomer II is thus tentatively assigned that of 9α-fluoro-11β,16α,17aα-trihydroxy-17aβ-hydroxymethyl-1,4-D-homoandrostadiene-3,17-dione; of 1,2-dihydrotriamcinolone IV, tentatively that of 9α-fluoro-11β,16α,17aα-trihydroxy-17aβ-hydroxymethyl-4-D-homoandrostene-3,17-dione.

Neither the isomers II and IV nor their several derivatives were biologically active as glucocorticoids or mineralocorticoids in adrenalectomized rats.

Acknowledgment.—The authors are grateful to W. Muller and I. Palestro for ultraviolet absorption measurements and for optical rotations, to W. Fulmor for infrared absorption spectra and interpretations thereof, to Dr. M. Halwer for polarographic and colorimetric determinations, to L. Brancone and staff for elemental analyses, and to Dr. I. Ringler for animal assays.

Experimental[43]

9α-Fluoro-11β,16α,17aα-trihydroxy-17aβ-hydroxymethyl-4-D-homoandrostene-3,17-dione (IV). A. Fermentation Source.—A fermentation medium consisting of starch, 40 g./l.; corn steep liquor, 25 g./l.; calcium carbonate, 5 g./l.; and lard oil, 0.2% by volume, was inoculated with a 24-hour vegetative growth of *Streptomyces roseochromogenus* ATCC 3347 (grown in a medium of corn steep liquor, 20 g./l.; sucrose, 30 g./l.; calcium carbonate, 7 g./l.; ammonium sulfate, 2 g./l., at 26.5°). After 24 hours of aeration a solution of 9α-fluorohydrocortisone in dimethylformamide was added in such amount so that the final dimethylformamide concentration did not exceed 2% by volume and the final steroid concentration was about 250 μg./ml. Fermentation was continued under aerated conditions for varying time intervals depending on instrumental and papergram analyses for each individual fermentor. In each case the unaltered substrate, the 16α-hydroxylated product III and the rearrangement product IV were detected on papergrams.

A typical broth was harvested after 48 hours of fermentation, the broth adjusted to *p*H 4 with dilute sulfuric acid, filtered from the mycelium, the filtrate readjusted to neutrality with sodium carbonate solution, and then extracted with an equal volume of methyl isobutyl ketone. The spent broth was re-extracted and the combined organic extracts were evaporated under diminished pressure to incipient crystallization. The solids were filtered, washed with methyl isobutyl ketone, and dried *in vacuo*. From the mother liquor more solids were obtained on further concentration, which solids had the same composition as the first crop by papergram. Still further concentration resulted in a viscous oil which was of a complex composition.

The crude solids from the fermentation conducted in iron tanks were mainly the isomer IV by papergram. The solids were dissolved in the lower phase of the solvent mixture dioxane–cyclohexane–water 5:2:1 and partitioned on Celite diatomaceous earth using the lower phase as stationary phase, the upper phase as the mobile phase. Each fraction was papergrammed and the appropriate fractions combined. The eluates, 3.16 l., were diluted with a half-volume of petroleum ether, precipitating 23.75 g. of crude IV. Further dilution with petroleum ether gave a second crop, 115.6 g., and a third crop, 17.9 g. The second crop was homogeneous

(43) All melting points were obtained in capillary tubes using Anschütz thermometers unless noted "Kofler," when the determinations were made under a microscope with a Kofler block. Optical rotations were made on 0.5–1.0% solutions in the solvent specified. Ultraviolet absorption spectra in absolute ethanol and in concentrated sulfuric acid were recorded on a Cary model 11S recording spectrophotometer. Infrared spectra were obtained on potassium bromide disks using a Perkin–Elmer model 21 double beam instrument. Paper chromatographic analyses were conducted using techniques already described.[2b]

as IV by papergram, and was dissolved in tetrahydrofuran, decolorized with charcoal, and the clarified filtrate was concentrated to about 80% of its original volume, cooled, and the crystals filtered, washed with ethyl acetate, and dried, yielding 32 g. of IV, m.p. 228–232°, resolidifying and remelting 256° dec., homogeneous on papergrams, and assaying polarographically 81% Δ^4-3-ketosteroid. Recrystallization from dimethylformamide–chloroform–water and then from pyridine–water gave the pure isomer IV hemihydrate, m.p. 252–254° dec.; $[\alpha]^{22}D$ + 84.4° (methanol); λ_{max} 238 mμ (ε 16,540), 303 mμ (ε 75),[44] λ_{max}^{KBr} 2.93, 3.41, 5.88, 6.05, 6.17, 8.06, 9.35, 10.10, 11.32 μ, etc.; $\lambda_{max}^{H_2SO_4}$ ($E_{1cm}^{1\%}$): at 15 min., 283 mμ (400); at 2 hr., 283 mμ (420), at 20 hr., 283 mμ (530); $\lambda_{max}^{0.066\ N\ NaOH}$ (60°), 241 mμ (ε 11,150), 310 mμ (ε 3,450); polarographic $E_{1/2}$ −1.19v., diffusion current identical with 9α-fluoro-16α-hydroxyhydrocortisone (III).

Anal. Calcd. for $C_{21}H_{29}O_6F \cdot 1/2\ H_2O$: C, 62.21; H, 7.46; F, 4.69. Found: C, 62.40; H, 7.63; F, 4.62.

Another isolation *via* chromatography afforded a crude IV which was recrystallized from tetrahydrofuran twice and from methanol–2-propanol several times, m.p. 244–256°, with slightly altered infrared spectra, λ_{max}^{KBr} 2.90, 3.03, 3.40, 5.87, 6.03, 6.15 μ, etc., which also analyzed as a hemihydrate; paper chromatographic mobility of IV[2b]: system I, R_f 0.12; system II, R_f 0.30; system III, R_f 0.13; system IV, R_f 0.10; yellow fluorescence with alkali,[45] and isonicotinic acid hydrazide,[46] positive tests with *p*-phenylenediamine phthalate,[47] tetrazolium blue, etc.

Anal. Found: C, 62.50; H, 7.47; F, 4.64.

The reducing properties of 1,2-dihydrotriamcinolone isomer IV toward alkaline tetrazolium blue[2a] are: with 9α-fluoro-16α-hydroxyhydrocortisone (III) as unit color intensity, after 0.5 hr., 28%; after 1 hr., 36%; after 1.5 hr., 43%; after 2 hr., 46%.

B. Solvent Isomerization.—A solution of 200 mg. of 9α-fluoro-16α-hydroxyhydrocortisone (III) in 2 ml. of N,N-dimethylformamide was heated for 30 minutes on a steam-bath, then diluted with 20 ml. of chloroform. Crystallization did not occur so the solution was concentrated *in vacuo* and diluted with water. After standing overnight at 4° the product was filtered and dried, yielding 170 mg. of crystals homogeneous on papergrams. Recrystallization from aqueous ethanol gave 135 mg. of IV, m.p. 249.5–251.5° dec., identical with IV isolated from fermentation sources as evidenced by infrared absorption spectra, papergram behavior and mixture melting point determination.

Repetition of this experiment failed to produce the expected isomerization of III.

16α-Acetoxy-17aβ-acetoxymethyl-9α-fluoro-11β,17aα-dihydroxy-4-D-homoandrostene-3,17-dione (IVa).—Five grams of IV was acetylated with 15 ml. of acetic anhydride and 25 ml. of pyridine at room temperature. After removal of the solvents *in vacuo*, crystallization and recrystallization from acetone–petroleum ether, pure IVa was obtained, m.p. 208–210°; $[\alpha]^{22}D$ +45.5° (methanol); λ_{max} 238 mμ (ε 14,560); λ_{max}^{KBr} 2.89, 3.00, 3.39, 5.73, 5.79, 6.02, 7.27, 8.00, 8.12, 9.55, 9.65, 9.95 μ, etc.; $\lambda_{max}^{H_2SO_4}$ ($E_{1cm}^{1\%}$): at 15 min., 224 mμ (162), 282 (287), at 2 hr., 224 mμ (161), 282 (285), 390 (83); at 20 hr., 224 mμ (155), 281 (281), 390 (83).

Despite several attempts to prepare an analytical sample of the diacetate IVa, a satisfactory elemental analysis could not be obtained.

Anal. Calcd. for $C_{25}H_{35}O_8F$: C, 62.49; H, 6.92; F, 3.95; O-acetyl, 17.93. Found: C, 64.43, 64.10; H, 7.73, 7.51; F, 3.25; O-acetyl, 17.74.

In order to establish the diacetate formulation IVa by elemental analysis, the isomer 3-*p*-nitrophenylhydrazone diacetate and the isomer 17-dihydro derivative triacetate XV were prepared and analyzed satisfactorily.

The 3-*p*-nitrophenylhydrazone of IVa was prepared by adding a drop of acetic acid to a suspension of 0.75 g. of the diacetate IVa and 0.75 g. of *p*-nitrophenylhydrazine in 25 ml. of warm methanol. The solids dissolved on warming to reflux. After 1 hour at reflux the cooled solution was diluted with water and the precipitated solids filtered and recrystallized from methanol–tetrahydrofuran, yielding 687 mg. of crystals, m.p. 261–261.5° dec.; λ_{max}^{KBr} 2.89, 3.00, 3.40, 5.78, 6.11 (shoulder), 6.27, 6.65, 7.05, 7.40, 9.00, 9.56, 10.00, 11.86 μ, etc.

Anal. Calcd. for $C_{31}H_{38}N_3FO_9$: C, 60.48; H, 6.21; N, 6.83; F, 3.09. Found: C, 60.22; H, 6.57; N, 6.23, 6.08; F, 3.01.

16α,17ξ-Diacetoxy-17aβ-acetoxymethyl-9α-fluoro-11β,-17aα-dihydroxy-4-D-homoandrosten-3-one (XV).—To a solution of 1.0 g. of pure diacetate IVa in 20 ml. of dry methanol at 0° was added 0.0695 g. of sodium borohydride (1.5 meq.). After standing 1 hour at 0°, 0.3 ml. of glacial acetic acid was added, and the solution was concentrated in vacuum. The residue was taken up in ethyl acetate, the extract washed with sodium bicarbonate and with water, then dried over anhydrous magnesium sulfate. After evaporation the residue was acetylated with 3 ml. of acetic anhydride and 5 ml. of pyridine at room temperature. After the usual isolation, 350 mg. of crystals was recovered, which were recrystallized from acetone–petroleum ether twice, yielding the analytical sample, m.p. 221–223.5°, $[\alpha]^{22}D$ −2.5° (methanol), λ_{max} 239 mμ (ε 17,050); λ_{max}^{KBr} 2.92, 3.42, 5.75, 5.81, 6.02, 8.00, 9.57 μ, etc.

Anal. Calcd. for $C_{27}H_{37}O_9F$: C, 61.82; H, 7.11; F, 3.62; O-acetyl, 24.61. Found: C, 61.98; H, 7.35; F, 3.32; O-acetyl, 23.09.

By heating the acetylation mixture containing XV, a more mobile component could be produced, which was formulated as a tetraacetate on the basis of infrared spectra: λ_{max}^{KBr} 2.75 (unbonded hydroxyl), 3.39, 5.72 (acetate), 6.00 (conjugated carbonyl), 6.11, 7.27, 8.05 (C–O–C), 8.15, 9.55 μ etc. No further characterization was made.

9α-Fluoro-11β,16α,17aα-trihydroxy-17aβ-hydroxymethyl-1,4-D-androstadiene-3,17-dione (II). A. From Triamcinolone 16α,21-Diacetate.—A solution of 20 g. of Ia in 3 l. of absolute methanol was purged of air with nitrogen and a solution of 1.93 g. of sodium in 400 ml. of absolute methanol similarly purged with nitrogen was added under a nitrogen atmosphere. After 15 minutes of stirring the solution was neutralized with 68 ml. of acetic acid, concentrated in vacuum, and the residue washed with water. After washing with acetone, the solids were dried in vacuum; 14.8 g., m. 251–258° dec.

Recrystallization of this typical hydrolysis product from methanol gave a first crop of pure triamcinolone, and second and third crops containing increasing amounts of the isomer. An enriched crop was partitioned on a Celite diatomaceous earth column with the dioxane–cyclohexane–water 5:2:1 system, from which triamcinolone was recovered in the 2.8–4.7 hold-back volume (H.B.V.) fractions, triamcinolone isomer from the 4.7–6.7 H.B.V. fractions. From 1.2 g. of mixed steroids was recovered 0.7 g. of isomer, which was recrystallized several times from methanol; m.p. 255–258° dec., $[\alpha]^{22}D$ +45.6° (methanol); λ_{max} 238 mμ (ε 15,300), 320 mμ (ε 47, infl.); $\lambda_{max}^{H_2SO_4}$ ($E_{1cm}^{1\%}$): at 15 min., 259 mμ (311), 310 (159); at 2 hr., 260 mμ (317), 310 (164); at 20 hr., 262 mμ (365), 310 (175); λ_{max}^{KBr} 2.93, 2.97, 3.40, 3.45, 5.89, 6.00, 6.21, 9.40, 10.08, 11.25 μ etc.; $\lambda_{max}^{0.066\ N\ NaOH}$ (60°),[48] 240 mμ (ε 12,900), 265 (6,570, infl.), 312 (3,640); polarographic response, $E_{1/2}$ −1.02 v., diffusion current identical with triamcinolone[2a]; paper chromatographic mobility of II: system I, R_f 0.10; system II, R_f 0.22; system III, R_f 0.08; system IV, R_f 0.06; no fluorescence with alkali[45]; negative test with *p*-phenylenediamine phthalate[47]; detected with isonicotinic acid hydrazide differentially[46] as a $\Delta^{1,4}$-3-ketone.

Anal. Calcd. for $C_{21}H_{27}O_6F$: C, 63.94; H, 6.90; F, 4.82. Found: C, 63.65; H, 6.93; F, 4.78.

The reducing properties of triamcinolone isomer II toward alkaline tetrazolium blue[2a] are: with triamcinolone as unit color intensity, immediately after solution, 0%; after 3 hr. 29%; after 6 hr., 46%; after 9.5 hr., 55%.

B. From Solvent Isomerization.—A solution of 200 mg. of triamcinolone in 2 ml. of dimethylformamide was heated on a steam-bath for 30 minutes, diluted with 30 ml. of chloroform, seeded with a crystal of the isomer, and scratched. After aging overnight at room temperature the crystalline precipitate was filtered, washed, dried at 40° in vacuum and

(44) The normal parent III has a weak band in ethanol at 296 mμ (ε 136) as well as the major 238 mμ (ε 16,300) band.

(45) I. E. Bush, *Biochem. J.*, **50**, 370 (1951).

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

(47) A. Bodánzsky and J. Kollonitsch, *Nature*, **175**, 729 (1955).

(48) A. S. Meyer, *J. Org. Chem.*, **20**, 1240 (1955); the data compare well with those of triamcinolone.[2a]

weighed 170 mg., identified as pure triamcinolone isomer by infrared spectra, melting point and papergram behavior.

C. From 1,2-Dihydrotriamcinolone Isomer.—Medium consisting of commercial dextrose, 10 g./1., yeast extract, 1 g./1., peptone, 4 g./1., beef extract, 4 g./1., sodium chloride, 2.5 g./1., was inoculated with 2% by volume of a 24-hour growth *Nocardia corallina* (grown in the same medium) and grown in aerated flasks for 24 hours. A solution of 1,2-dihydrotriamcinolone isomer (IV) in propylene glycol was added so that the final steroid concentration was 250 μg./ml. and the final propylene glycol concentration was 1% by volume (total of 500 mg. of IV added). After 24 hours of aeration a polarographic analysis of an extract of the broth indicated 133 μg./ml. of Δ^4-3-ketone, 86 μg./ml. of $\Delta^{1,4}$-3-ketone, identified as IV and II by paper chromatography.

The harvested broth was extracted with ethyl acetate, the extracts concentrated in vacuum, and the residue analyzed by papergram, indicating the presence of IV and II, together with more polar unknown materials. The oily residue was dissolved in ethyl acetate and precipitated by addition of petroleum ether. The crystalline product, 120 mg., contained 64% of triamcinolone isomer, 36% of unaltered substrate IV by quantitative papergram analysis. Column chromatography using the dioxane–cyclohexane–water 5:2:1 system on diatomaceous earth gave 1,2-dihydrotriamcinolone isomer in the 3.6 H.B.V. fraction, triamcinolone isomer in the 6 H.B.V. fraction, 40 mg., m. 248–250°. After recrystallization twice from pyridine–water and from methanol twice the sample melted 254–255° dec. with presoftening from 238°, not lowered on admixture with II prepared by alkaline hydrolysis of Ia. The product was shown to be identical with authentic II by infrared absorption spectra and by papergram behavior in a number of systems.

16α-Acetoxy-17aβ-acetoxymethyl-9α-fluoro-11β,17aα-dihydroxy-1,4-D-homoandrostadiene-3,17-dione (IIa).—Acetylation of triamcinolone isomer II with acetic anhydride–pyridine at room temperature in the usual manner afforded a diacetate, which when recrystallized from acetone–petroleum ether melted 227–229° dec., $[\alpha]^{22}D$ +5.4° (methanol), λ_{max} 238 mμ (ε 14,700); $\lambda_{max}^{H_2SO_4}$ ($E_{1cm}^{1\%}$): at 15 min. and 2 hr., 261 mμ (269), 310 (127); at 20 hr., 261 mμ (272), 310 (129); λ_{max}^{KBr} 2.92, 3.02, 3.39, 3.47, 5.71, 5.78, 5.82, 6.00, 6.17, 6.21, 9.62 μ, 10.02 μ, 11.20 μ, etc. In ethanol the carbonyl region lost the extra band, λ_{max}^{EtOH} 5.68, 5.77, 5.97, 6.11, 6.17 μ.

Anal. Calcd. for $C_{25}H_{31}O_8F$: C, 62.75; H, 6.53; F, 3.97. Found: C, 62.46; H, 6.80; F, 4.02.

9α-Fluoro-11β-hydroxy-17aβ-hydroxymethyl-16α,17aα-isopropylidenedioxy-1,4-D-homoandrostadiene-3,17-dione (V).—To a solution of 1.62 g. of triamcinolone isomer in 1 l. of acetone (prepared by heating) was added 2.5 ml. of concentrated hydrochloric acid. After standing overnight at room temperature the solution was neutralized with 20 ml. of saturated aqueous sodium bicarbonate solution and 150 ml. of water was added. The oil obtained on vacuum concentration was extracted into ethyl acetate, the extracts washed with brine and water, dried over anhydrous magnesium sulfate, and concentrated in vacuum to yield an amorphous glassy residue, which was crystallized from acetone–petroleum ether. Crystals, 1.06 g., m. 275.5–278.5° dec., were recovered, which were homogeneous on papergrams (negative reaction to tetrazolium blue, located *via* ultraviolet absorption properties on paper). An additional 160 mg. was recovered from the filtrates, m. 260–262° dec. Further recrystallization from acetone–petroleum ether gave the pure acetonide, m.p. 290–291° dec., $[\alpha]^{22}D$ +39.6° (chloroform), λ_{max} 237 mμ (ε 14,800); $\lambda_{max}^{H_2SO_4}$ ($E_{1cm}^{1\%}$): at 15 min. and 2 hr., 258 mμ (282), 309 (145), at 20 hr., 261 mμ (304), 309 (141); λ_{max}^{KBr} 2.91, 3.37, 3.42, 5.84, 6.00, 6.11, 6.21, 7.21, 7.27, 8.05, 8.17, 9.33, 10.11, 11.21, 11.76 μ, etc.

Anal. Calcd. for $C_{24}H_{31}O_6F$: C, 66.34; H, 7.19; F, 4.37. Found: C, 66.37; H, 7.21; F, 4.03.

Triamcinolone Isomer Ketal Va.—Limitation of the amount of hydrochloric acid used led to the formation of a mixture of triamcinolone isomer acetonide V and another more mobile component Va. A solution of 400 mg. of triamcinolone isomer in 400 ml. of acetone was treated with 4 drops of concentrated hydrochloric acid. After standing overnight at room temperature the solution was neutralized with 5 ml. of saturated sodium bicarbonate solution, and the precipitated inorganic salts were filtered. Paper chromatographic analysis of the filtrate indicated the presence of two non-reducing components (R_f 0.57 and 0.69 in system IV). Dilution of the filtrate with 150 ml. of water was followed by vacuum concentration to remove acetone. Crystallization occurred from the aqueous solution on storing at 4° for 3 hours. The crystals, 300 mg., were homogeneous on papergram (R_f 0.65), and were identified as the more mobile component initially in the reaction filtrate. Recrystallization from acetone–petroleum ether gave the analytical sample, m.p. 248–250° dec., λ_{max} 238 mμ ($E_{1cm}^{1\%}$ 324); λ_{max}^{KBr} 2.92, 3.42, 5.84, 6.00, 6.15, 6.21, 9.38, 10.08, 11.06, 11.20 μ, etc.

Anal. Calcd. for $C_{27}H_{37}O_7F$: C, 65.78; H, 7.57; F, 3.86. Found: C, 65.65; H, 7.98; F, 3.78.

The spent aqueous mother liquor from which Va crystallized was extracted with ethyl acetate, the extracts evaporated, and the residue crystallized from acetone–petroleum ether, yielding 200 mg. of crystalline V, m.p. 283–286° dec. Infrared spectra of the preparation indicated identity with V prepared previously, as did a comparison of papergram behavior.

Repetition of the low acid level experiment gave the same results.

Reaction of triamcinolone isomer on papergrams using the technique of Smith and Foell[49] (20 μg. of steroid, spotted with 5–20 μl. of the diluted perchloric acid reagent, 16–64 hr. exposure to acetone atmosphere) resulted in partial formation of the isomer acetonide V (R_f 0.88 in system II, R_f 0.09 in system V) with unreacted isomer II after the standard 16-hour exposure to acetone. After 64-hour exposure, unaltered triamcinolone isomer, triamcinolone isomer acetonide V (R_f 0.09, system V), and a substantial amount of the more mobile acetonide Va (R_f 0.14 system V) were detected.

The same behavior was evidenced by 1,2-dihydrotriamcinolone isomer IV, two non-reducing components at R_f 0.15 (major) and at R_f 0.22 (minor) (system V) being formed after 64 hours of exposure to acetone. Only the R_f 0.15 acetonide was present after 16-hour exposure. Unaltered isomer IV remained in either instance.

Conversion of Triamcinolone Isomer Ketal Va to Triamcinolone Isomer Acetonide V.—Unweighed small samples of Va were heated on the steam-bath for 2 hours as solutions in ethanol, chloroform, water and pyridine, and the treated solutions were chromatographed on paper. The ethanol, chloroform and water solutions contained Va and no other detectable component, while the pyridine solution contained unaltered Va and a minor amount of a component with the mobility of and color reactions of triamcinolone isomer acetonide V. After 2 days the amount of the minor component had increased in quantity, although unaltered Va was still the major component.

Hydrolysis of Triamcinolone Isomer Acetonide.—Ten milligrams of triamcinolone isomer acetonide V was dissolved in 1 ml. of 60% aqueous formic acid and heated on a steam-bath for 90 minutes. Partial evaporation of the reaction mixture, followed by dilution with water, yielded crystalline triamcinolone isomer II, identified by infrared spectra and papergram behavior.

Ten milligrams of the triamcinolone isomer ketal Va treated similarly yielded triamcinolone isomer II, also identified by papergram behavior and infrared spectra. In both cases no alteration product other than triamcinolone isomer was detected by paper chromatography.

17aβ-Acetoxymethyl-9α-fluoro-11β,16α,17aα-trihydroxy-1,4-D-homoandrostadiene-3,17-dione (VI).—To a solution of 1.41 g. of triamcinolone isomer in 17 ml. of dry pyridine was added 0.40 ml. (1.1 equivalents) of acetic anhydride and the solution was stored at −10° for 44 hours. The reaction mixture was quenched with large volumes of methanol and evaporated to dryness in vacuum. The evaporation procedure was repeated until the odor of pyridine, etc., was eliminated. The residue obtained was composed of a major component less mobile on papergrams than the isomer diacetate, together with some isomer diacetate and unacetylated isomer. The mixture was dissolved in a minimum volume of methanol, to which was added 1.6 g. of a similar mixture prepared the same way. The steroids were slurried with 40 g. of silica gel, the slurry

(49) L. L. Smith and T. Foell, *J. Chromatography*, **3**, 381 (1960).

digested with petroleum ether (Skellysolve C) at reflux. As the methanol was displaced more petroleum ether was added. After 2 hours the methanol was considered removed. The slurry was packed onto the top of a column of silica gel (160 g.) prepared in petroleum ether. Elution with diethyl ether–methylene chloride (90:10) was followed by diethyl ether–methylene chloride (80:20) and (75:25), which eluted the first component. From these fractions was recovered 1.705 g. of crystalline triamcinolone isomer diacetate. Further elution with methylene chloride and with ethyl acetate removed the monoacetate from the column; 1.00 g. of crystals homogeneous on papergrams. Recrystallization from acetone–petroleum ether yielded white needles, m.p. 244.5–246.0° dec., $[\alpha]^{22}D$ +62.5° (chloroform), λ_{max} 237 mμ (ε 14,720); $\lambda_{max}^{H_2SO_4}$ ($E_{1\,cm}^{1\%}$): at 15 min., 258 mμ (302), 307 (151); at 2 hr., 258 mμ (295), 307 (146); at 20 hr., 258 mμ (288), 307 (137); λ_{max}^{KBr} 2.91, 3.41, 3.46, 5.76, 5.85, 6.00, 6.15, 6.21, 7.92, 8.03, 9.40, 10.09, 11.18 μ, etc.

Anal. Calcd. for $C_{23}H_{29}O_7F$: C, 63.29; H, 6.70; F, 4.35. Found: C, 63.41; H, 6.85; F, 4.33.

17aβ-Acetoxymethyl-9α-fluoro-11β-hydroxy-16α,17aα-isopropylidenedioxy-1,4-D-homoandrostadiene-3,17-dione (VII). A. From Triamcinolone Isomer Acetonide.—To a solution of 160 mg. of triamcinolone isomer cyclic acetonide V in 10 ml. of pyridine was added 0.50 ml. of acetic anhydride. After 24 hours the solution was diluted with methanol and evaporated in vacuum. The residue was crystallized from acetone–petroleum ether, m.p. 240°, resolidifying and remelting 263–275°. After several recrystallizations from acetone–petroleum ether the sample melted 307.5–308.0° dec., $[\alpha]^{22}D$ +7.6° (chloroform), λ_{max} 237 mμ (ε 14,700); $\lambda_{max}^{H_2SO_4}$ ($E_{1\,cm}^{1\%}$): at 15 min. and 2 hr., 258 mμ (273), 309 (135); at 20 hr., 258 mμ (269), 309 (130); λ_{max}^{KBr} 2.87, 2.92, 3.35, 3.40, 5.71, 5.85, 6.00, 6.15, 6.21, 6.90, 7.25, 7.30, 10.18, 11.19 μ, etc.

Anal. Calcd. for $C_{26}H_{31}O_7F$: C, 65.53; H, 6.98; F, 3.99. Found: C, 65.21; H, 7.22; F, 4.41.

B. From Triamcinolone Isomer Monoacetate.—To a solution of 75 mg. of triamcinolone isomer monoacetate VI in 45 ml. of warm acetone was added 3 drops of concentrated hydrochloric acid. After standing overnight the mixture was neutralized and evaporated to dryness in vacuum. The residue was digested with hot acetone, the digest dried over anhydrous magnesium sulfate, filtered and crystallized by dilution with petroleum ether. The product, 50 mg., m. 260–262° dec., was recrystallized from acetone–petroleum ether yielding crystals, m. 272–274° dec. Paper chromatographic examination of the product obtained *via* routes A and B indicated identity (R_f 0.85 in system III); both products were further recognized as being identical by infrared absorption spectra. Both products are negative to tetrazolium blue.

The lower melting point of the product *via* route B was accepted in that the analytical sample from route A exhibited melting point behavior of this type, and only after repeated recrystallizations was the melting point of the analytical sample raised to 307.5–308° dec.

C. From Triamcinolone Isomer Ketal Va.—Sixty milligrams of Va was acetylated with 1 ml. of acetic anhydride and 0.4 ml. of pyridine. After isolation in the usual manner and crystallization from acetone–petroleum ether, 40 mg. of needles was recovered, and identified as triamcinolone isomer acetonide acetate VII by melting point, infrared spectral and papergram behavior.

9α-Fluoro-17aβ-hydroxymethyl-16α,17aα-isopropylidenedioxy-1,4-D-homoandrostadiene-3,11,17-trione (VIII).—To a suspension of 750 mg. of chromium trioxide in 7.5 ml. of pyridine was added 500 mg. of triamcinolone isomer acetonide V dissolved in 5 ml. of pyridine. After standing at room temperature overnight the mixture was diluted with 20 ml. of water, shaken with ethyl acetate and filtered. The aqueous phase was separated and extracted four times with ethyl acetate, and the combined extracts were washed with saturated salt solution, with 5% sodium hydroxide solution and water. The aqueous washes were re-extracted with ethyl acetate and these extracts combined with the original extracts which combined extracts were again washed with brine to neutral reaction. The extracts were dried over anhydrous magnesium sulfate, treated with charcoal and filtered through magnesium silicate (Magnesol). The clarified extract was evaporated in vacuum, yielding 275 mg. of solids.

From the spent aqueous extracts (acidified to *p*H 5 with HCl) was obtained a further quantity of product (45 mg.) by extraction with ethyl acetate, etc. Papergram examination of both fractions indicated a single major component at R_f 0.41–0.43 (system V), negative to tetrazolium blue.

Recrystallization of the two fractions separately from acetone–petroleum ether yielded material melting 246–249° dec. and 242–245° dec., respectively. From the first fraction an analytical sample, prepared by recrystallization from acetone–petroleum ether, was dried over refluxing toluene, m.p. 245–246° dec., $[\alpha]^{22}D$ +95.4° (chloroform), λ_{max} 233 mμ (ε 15,270); $\lambda_{max}^{H_2SO_4}$ ($E_{1\,cm}^{1\%}$): at 15 min. and 2 hr., 256 mμ (339), 307 (152), at 20 hr., 256 mμ (303), 307 (145); λ_{max}^{KBr} 2.87, 3.35, 3.39, 5.77, 5.97, 6.10, 6.17, 7.21, 8.00, 10.03, 11.12 μ, etc.

Anal. Calcd. for $C_{24}H_{29}O_6F$: C, 66.65; H, 6.76; F, 4.39. Found: C, 66.56; H, 6.97; F, 4.22.

17aβ-Acetoxymethyl-9α-fluoro-16α,17aα-isopropylidenedioxy-1,4-D-homoandrostadiene-3,11,17-trione (IX).—To a solution of 100 mg. of the trione VIII in 3 ml. of pyridine was added 0.2 ml. of acetic anhydride. After standing overnight at room temperature the reaction product was isolated in the usual manner. The residue was crystallized from acetone–petroleum ether; 95 mg., m. 240–242° dec. After several further recrystallizations from the same solvents the analytical sample showed m.p. 241.0–242.5° dec., $[\alpha]^{22}D$ +95.7° (chloroform), λ_{max} 233 mμ (ε 14,800); $\lambda_{max}^{H_2SO_4}$ ($E_{1\,cm}^{1\%}$): at 15 min. and 2 hr., 256 mμ (317), 305 (140); at 20 hr., 256 mμ (302), 305 (127); λ_{max}^{KBr} 3.31, 3.35, 5.71, 5.76, 5.97, 6.11, 6.18, 7.20, 10.02, 11.12, 11.25 μ, etc.

Anal. Calcd. for $C_{26}H_{31}O_7F$: C, 65.80; H, 6.59; F, 4.00. Found: C, 65.34; H, 6.74; F, 3.98.

9α-Fluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-3-oxo-1,4-androstadiene-17β-oic Acid (X).—To a solution of 1.2 g. of triamcinolone 16α,17α-acetonide in 360 ml. of warm 50% aqueous acetic acid was added 20.4 g. of sodium bismuthate. After shaking for 40 hours at room temperature 130 ml. of 18 *N* sodium hydroxide solution and ethyl acetate was added. The mixture was filtered, the filter cake washed with ethyl acetate, and the two-phase filtrate was separated. The aqueous portion was re-extracted with ethyl acetate three times, the combined ethyl acetate extracts being washed then with saturated aqueous sodium bicarbonate solution, with brine, and then dried over anhydrous magnesium sulfate. The dried extracts were concentrated in vacuum to a crystalline residue, which analyzed by papergram as a mixture of starting material and product.

The residue was chromatographed on silica gel and 230 mg. of triamcinolone 16α,17α-acetonide was recovered. The column was then stripped with methanol, from which was recovered a solid residue which was slurried in acetone. The insoluble portion was dissolved in water and precipitated with sulfuric acid. The acetone extract residue was extracted with water, acidified and a second crop of solids collected. A total of 150 mg. of crystalline material was recovered, which was recrystallized from dilute aqueous acid and from acetone–petroleum ether, and dried over refluxing toluene; m.p. 302.5–303.5°, $[\alpha]^{22}D$ + 82.1° (methanol), λ_{max} 238 mμ (ε 15,180); $\lambda_{max}^{H_2SO_4}$ ($E_{1\,cm}^{1\%}$): at 15 min. and 2 hr., 258 mμ, (267), 309 (133); at 20 hr., 220 mμ (489), 278 (277), 370 (132), 468 (106); λ_{max}^{KBr} 2.85, 2.93, 3.37, 3.80, 5.85, 6.01, 6.27, 7.22, 7.91, 8.27, 8.52, 9.05, 9.45, 10.28, 11.00, 11.15, 11.53 μ, etc.

Anal. Calcd. for $C_{23}H_{29}O_6F$: C, 65.70; H, 6.95; F, 4.52. Found: C, 65.01; H, 7.33; F, 4.72.

Treatment of triamcinolone isomer acetonide V under identical conditions resulted in the recovery of the isomer acetonide V unchanged.

11β-Acetoxy-9α-fluoro-16α,17aα-dihydroxy-17aβ-hydroxymethyl-1,4-D-homoandrostadiene-3,17-dione (XIV).—A solution of triamcinolone 11β-acetate in chloroform was allowed to stand at room temperature for 8 months, at which time the solution was evaporated, m.p. (Kofler) 212.0–215.0° dec. with softening from 205.0°; papergram mobility in system III 1.40 relative to triamcinolone as unity (triamcinolone 11β-acetate mobility 2.4); positive to tetrazolium blue; λ_{max}^{KBr} 2.85, 3.36, 5.71, 5.84, 5.98, 6.11, 6.18, 8.11, 9.44, 10.05, 11.15 μ, etc. Insufficient sample

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]

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