aqueous ethanol and dried in the dark, m.p. 113.4–119° with previous sintering, λ_{max}^{EtOH} 278 mμ (ε 2270) with shoulders at 225 (ε 8070) and 284 mμ (ε 2060).

Anal. Calcd. for $C_{11}H_{13}NO_2$: C, 69.09; H, 6.85; N, 7.32. Found: C, 69.33; H, 6.56; N, 7.19.

1,2,3,4-Tetrahydro-6-methoxy-2-naphthylamine (IVb).—A solution of 9.0 g. of 6-methoxy-2-tetralone oxime in 200 ml. of methanol (saturated with ammonia at 0–5°) was hydrogenated in the presence of 1.5 teaspoons of W2-Raney nickel and an initial pressure of 1470 p.s.i. for 4 hours at 55°. The oil obtained by removal of the catalyst and concentration of the filtrate was dissolved in dilute hydrochloric acid. The resulting solution was washed with ether, made strongly basic with sodium hydroxide and the regenerated amine was extracted with ether. The dried ether extract was distilled giving 4.72 g. (57%) of 1,2,3,4-tetrahydro-6-methoxy-2-naphthylamine, b.p. 108–110° (0.2 mm.) which was characterized as the hydrochloride.

1,2,3,4-Tetrahydro-6-methoxy-2-naphthylamine hydrochloride was prepared in 94% yield by saturating an ethereal solution of the amine with hydrogen chloride, m.p. 234–236° with previous softening; λ_{max}^{EtOH} 221 (ε 7960), 279 (ε 2180) and 287 mμ (ε 2020).

Anal. Calcd. for $C_{11}H_{16}ClNO$: C, 61.82; H, 7.55; N, 6.55. Found: C, 61.74; H, 7.54; N, 6.41.

N-2-(1,2,3,4-Tetrahydro-6-methoxynaphthyl)-α-aminoisobutyric Acid (Vd).—To a mixture of 4.72 g. of 1,2,3,4-tetrahydro-6-methoxy-2-naphthylamine and 2.27 g. of acetone cyanohydrin, which had been allowed to stand overnight, was added 300 ml. of concentrated hydrochloric acid (saturated with hydrogen chloride at 0–5°). The resulting mixture was allowed to stand at room temperature for 15 hours and then was heated under reflux on the steam-bath for 3 hours before being concentrated to dryness. The residue was dissolved in 50 ml. each of water and methanol, the solution was clarified with Norit and adjusted to *p*H 5.5–6 with potassium carbonate. The precipitate was collected on a filter, washed and dried, giving 4.97 g. (70%) of N-2-(1,2,3,4-tetrahydro-6-methoxynaphthyl)-α-aminoisobutyric acid; the product sublimes without melting. The analytical sample was sublimed at 225° (10^{-4} mm.), ν_{max}^{KBr} 1604 (s, CO_2^-), λ_{max}^{EtOH} 220 (ε 8180), 279 (ε 2150) and 287 mμ (ε 2010).

Anal. Calcd. for $C_{15}H_{21}NO_3$: C, 68.41; H, 8.04; N, 5.32. Found: C, 68.49; H, 8.02; N, 5.24.

Methyl N-2-(1,2,3,4-Tetrahydro-6-methoxynaphthyl)-α-aminoisobutyrate (Ve).—In this preparation, the same procedure and apparatus was employed as in the synthesis of methyl (1,2,3,4-tetrahydronaphthyl)-α-aminoisobutyrate by procedure B above.

A mixture of 3.5 g. of N-2-(1,2,3,4-tetrahydro-6-methoxynaphthyl)-α-aminoisobutyric acid and 23.0 ml. of 0.589 *N* tetramethylammonium hydroxide was concentrated to a thick paste which was transferred to a distilling flask. Water was removed, finally at 120° (0.2 mm.) for 4 hours. The resulting solid was melted using a micro burner. Gas evolution was vigorous and on continued heating 2.29 g. (62%) of the crude product distilled, b.p. 160° (0.2–0.3 mm.). The analytical sample of Ve had b.p. 138–140° (0.06 mm.). $n^{27}D$ 1.5233, $\nu_{max}^{CCl_4}$ 1726 (s, CO), λ_{max}^{MeOH} 279.5 (ε 2180) and 288 mμ (ε 2060) with a plateau at 216–226 mμ (ε 7700).

Anal. Calcd. for $C_{16}H_{23}NO_3$: C, 69.28; H, 8.36; N, 5.05. Found: C, 69.38; H, 8.42; N, 5.00.

Acknowledgment.—We wish to thank Dr. V. A. Drill and his associates of the Division of Biological Research of G. D. Searle and Company for bioassays of some of the compounds. Compounds Vb–Ve showed essentially no lipodiatic, estrogenic, androgenic or anti-inflammatory activity. However, the hydrochloride salt of Vb exhibited a positive anti-inflammatory activity[23] at a level similar to that of Butazolidine.

(23) J. J. Selitto and L. O. Randall, *Federation Proc.*, Abstract No. 1323 (1954).

CAMBRIDGE 39, MASS.

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

16α-Hydroxy Steroids. V.[1] 11β-Esters of Triamcinolone

BY SIDNEY M. FOX, VICTOR E. ORIGONI AND LELAND L. SMITH

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Acetylation of the 11β- and 17α-hydroxyl groups of triamcinolone and of 16α-hydroxyhydrocortisone is readily accomplished with warm acetic anhydride and pyridine. Both a 11β,16α,21-triacetate and a 11β,16α,17α,21-tetraacetate are formed from triamcinolone. Hydrocortisone and 9α-fluorohydrocortisone were acetylated at the 11β-hydroxyl also. Some chemical proof for the assigned structures is presented.

It is commonly held that the 11β- and the 17α-hydroxyl groups of the active corticosteroids are not readily acetylated with acetic anhydride and pyridine, neither at room temperature nor at slightly elevated temperatures. Occasional exceptions in other steroid series have been noted,[2] but such treatment is generally regarded as a poor means of acetylation of these groups. We have found that acetic anhydride–pyridine smoothly acetylates the 11β- and 17α-hydroxyl groups of triamcinolone[3] (9α-fluoro-11β,16α,17α,21-tetrahydroxy-1,4-pregnadiene-3,20-dione) (IIa) yielding the 11β,16α,21-triacetate III and 11β,16α,17α,21-tetraacetate IV derivatives.

Triamcinolone triacetate (III) and tetraacetate (IV) were encountered unexpectedly in preparations of triamcinolone 16α,21-diacetate (IIb) obtained *via* microbiological dehydrogenation of 16α-hydroxy-9α-fluorohydrocortisone 16α,21-diacetate (I). *Nocardia corallina* dehydrogenates I[3] but also hydrolyzes partially the diesters involved, yielding a mixture of diacetates, monoacetates and free alcohols. Reacetylation of the fermentation extract residues without isolation of the purified steroids, using large excesses of acetic anhydride and pyridine and inadvertently warming on a steam-bath, gave the diacetate IIb which was contaminated with a major proportion of a new, more mobile component (paper chromatographic analyses) together with traces of a still more mobile component and unaltered substrate I.

(1) Paper IV, L. L. Smith, J. J. Garbarini, J. J. Goodman, M. Marx and H. Mendelsohn, THIS JOURNAL, **82**, 1437 (1960).

(2) M. Steiger and T. Reichstein, *Helv. Chim. Acta*, **20**, 817 (1937); A. D. Kemp, A. Kappas, I. I. Salamon, F. Herling and T. F. Gallagher, *J. Biol. Chem.*, **210**, 123 (1954).

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

Resolution of the mixture by partition chromatography yielded the more mobile components III and IV in the first fraction, mixed with non-steroidal fermentation impurities. From this first fraction was isolated in 27% weight yield (based on the weight of I charged) a triacetate III, contaminated with traces of IV. Unaltered substrate I was recovered in the middle fractions (8% by weight), and triamcinolone diacetate IIb in the final fractions in 24% weight yield.

Purified III was recognized as a triacetate, $C_{27}H_{33}O_9F$, on the basis of elemental analysis, papergram mobility and infrared absorption spectra. Alkaline hydrolysis of III gave a monoacetate V, $C_{23}H_{29}O_7F$, recognized as an 11β-monoacetate on the basis of mode of formation,[4] optical rotational increments (see Table I), and a hypsochromic shift of 2 mμ in the ultraviolet spectra previously associated with acetylation of the 11α- or 11β-hydroxyl group of Δ^4-3-ketones and $\Delta^{4,6}$-3-ketones.[4a,5]

The present instance is the first reported case of the hypsochromic effect on the ultraviolet absorption of 9α-fluoro-$\Delta^{1,4}$-3-ketones. Recently the ultraviolet spectra of a series of 11β-esters of 9α-chloro- and 9α-bromo-$\Delta^{1,4}$-3-ketones were reported, which spectra show the typical hypsochromic shift of *ca.* 1–2 mμ.[6]

Rearrangement of the steroid during alkaline hydrolysis was ruled out by acetylation of the monoacetate V, which yielded the original triacetate III. These evidences, coupled with spectral data, rotational data and papergram behavior, together with the complete loss of glucocorticoid activity associated with triamcinolone 16α,21-diacetate, lead to the assignment of the structure 11β,16α,21-triacetoxy-9α-fluoro-17α-hydroxy-1,4-pregnadiene-3,20-dione for the triacetate III and of the structure 11β-acetoxy-9α-fluoro-16α,17α,21-trihydroxy-1,4-pregnadiene-3,20-dione for the monoacetate V.

Since the initial observations of a relatively facile acetylation of the 11β-hydroxyl group were made on impure materials isolated directly from fermentation sources, further chemical confirmation of the suggested structures was made using pure triamcinolone and better controlled acetylation conditions. Using excessive amounts of acetic anhydride and pyridine and heating to about 80° gave products III and IV, as identified by paper chromatographic examination. Partition chromatography of the mixture gave IV as the major product, with the triacetate III as a minor product. Analysis of IV indicated it to be a tetraacetate, *i.e.*, 11β,16α,17α,21-tetraacetoxy-9α-fluoro-1,4-pregnadiene-3,20-dione. Infrared and ultraviolet absorption spectra and papergram mobility support this structural assignment. Hydrolysis of the tetraacetate IV with base also affords the monoacetate V, previously obtained from the triacetate III.

The facile acetylation of both the 11β- and 17α-hydroxyl groups of triamcinolone without evidences of dehydration, isomerization or other artifact formation, while not anticipated, was accomplished in good yield. Extension of the experiments to another 11β,16α,17α,21-tetrahydroxy-20-ketone, 16α-hydroxyhydrocortisone, afforded a tetraacetate VI, assigned the structure 11β,16α,17α,21-tetraacetoxy-4-pregnene, 3,20-dione.

Acetylation of both hydrocortisone 21-acetate (VIIa) and 9α-fluorohydrocortisone 21-acetate (VIIIa) afforded the respective 11β,21-diacetates VIIb and VIIIb. In each instance a hypsochromic shift in the ultraviolet spectra of *ca.* 2 mμ, together with increased papergram mobility and arguments of molecular rotation and biological inactivity, were used to support the assigned 11β-acetate ester structure.

The molecular rotational increments associated with 11β-acylation of 11β-hydroxy-3-ketosteroids are given in Table I. For several 11β-acetates and 11β-formates the $\Delta[M]_D$ ranges from +3° to +165°, with the one exception of −73° for the 11β-acetate of 11β,17α-dihydroxy-5β-pregnane-3,20-dione. The recently reported 11β-esters of 9α-chloroprednisolone 21-acetate[6] (21-acetoxy-9α-chloro-11β,17α-dihydroxy-1,4-pregnadiene-3,20-dione) conform to this range (+118° to +146°), but the 11β-esters of the 9α-bromo analogs[6] have significantly higher $\Delta[M]_D$ in the range of +202° to +255°. The 11β-acetates of triamcinolone range within these groups, being +157° to +279° (see Table I).

Some further chemical work was done to establish the structure of III, particularly to exclude the alternate possibility of a 16α,17α,21-triacetate. This alternate possibility is ruled against by molecular rotational arguments[7] by the formation of an alkali-stable monoacetate[8] and by the ultraviolet absorption spectra which show the typical hypsochromic shift associated with an 11β-acetate group; however, the following reactions establish the 11β,16α,21-triacetate structure firmly.

The monoacetate V formed from the triacetate III (or from the tetraacetate IV) forms an acetonide derivative IX with acetone–hydrochloric acid,[3,9]

(4) 11β- and 11α-acetates are resistant to alkaline hydrolysis; *cf.* (a) E. P. Oliveto, C. Gerold, L. Weber, H. E. Jorgensen, R. Rausser and E. B. Hershberg, THIS JOURNAL, **75**, 5486 (1953); (b) E. P. Oliveto, C. Gerold and E. B. Hershberg, *Arch. Biochem. Biophys.*, **43**, 234 (1953); (c) J. Romo, G. Rosenkranz, C. Djerassi and F. Sondheimer, THIS JOURNAL, **75**, 1277 (1953).

(5) (a) J. Romo, A. Zaffaroni, J. Hendrichs, G. Rosenkranz, C. Djerassi and F. Sondheimer, *Chemistry & Industry*, 783 (1952); (b) D. H. Peterson, S. H. Eppstein, P. D. Meister, B. J. Magerlein, H. C. Murray, H. M. Leigh, A. Weintaub and L. M. Reineke, THIS JOURNAL, **75**, 412 (1953); (c) P. D. Meister, D. H. Peterson, H. C. Murray, G. B. Spero, S. H. Eppstein, A. Weintaub, L. M. Reineke and H. M. Leigh, *ibid.*, **75**, 416 (1953); (d) D. H. Peterson, A. H. Nathan, P. D. Meister, S. H. Eppstein, H. C. Murray, A. Weinberg, L. M. Reineke and H. M. Leigh, *ibid.*, **75**, 419 (1953); (e) R. Antonucci, S. Bernstein, M. Heller, R. Lenhard, R. Littell and J. Williams, *J. Org. Chem.*, **18**, 70 (1953); (f) E. P. Oliveto, C. Gerold and E. B. Hershberg, *Arch. Biochem. Biophys.*, **49**, 244 (1954); (g) A. L. Nussbaum, G. Brabazon, E. P. Oliveto and E. B. Hershberg, *J. Org. Chem.*, **22**, 977 (1957); (h) E. P. Oliveto, R. Rausser, C. Gerold, E. B. Hershberg, M. Eisler, R. Neri and P. L. Perlman, *ibid.*, **23**, 121 (1958).

(6) C. H. Robinson, L. Finckenor, M. Kirtley, D. Gould and E. P. Oliveto, THIS JOURNAL, **81**, 2195 (1959). See also S. G. Levine and M. Wall, *ibid.*, **81**, 2826 (1959), for further examples of synthesis of 9α-bromo-11β-acetoxy steroids.

(7) Molecular rotational contribution for acetylation of the 17α-hydroxyl group is of the order of −209 to −299°; *cf.* R. B. Turner, *ibid.*, **75**, 3604 (1953).

(8) Alkaline hydrolysis of 17α-acetates proceeds as easily as the hydrolysis of 21-acetates; *cf.* Huang-Minlon, E. Wilson, N. L. Wendler and M. Tishler, *ibid.*, **74**, 5394 (1952).

(9) (a) J. Fried, A. Borman, W. B. Kessler, P. Grabowich and E. F. Sabo, *ibid.*, **80**, 2338 (1958); (b) S. Bernstein, *Recent Progress in Hormone Research*, **14**, 1 (1958).

TABLE I

MOLECULAR ROTATIONAL DATA FOR 11β-ESTERS

Steroid	[α]	Solvent	[M]D	Δ[M]D 11β-acylation	Reference
Hydrocortisone	+163°	Methanol	+591°		d
11β-acetate	+163.2	Chloroform	+687	+ 96°	a
11β,21-diacetate	+167.1	Chloroform	+746	+136	a,b
11β-formate 21-acetate	+176.1	Chloroform	+761	+124	c
21-acetate	+157.5	Dioxane	+637		d
5β-Dihydrohydrocortisone					
11β,21-diacetate	+ 92.8	Chloroform	+416	+ 64	a,b
11β-formate 21-acetate	+ 99	Chloroform	+430	+ 78	c
21-acetate	+ 86.6	Acetone	+352		d
11β,17α-Dihydroxyprogesterone	+112.3	Chloroform	+408		a
	+136	Acetone	+472		e
11β-acetate	+141.2	Chloroform	+573	+165	b
11β,17α-Dihydroxy-5β-pregnane-3,20-dione	+ 69.6	Acetone	+243		f
11β-acetate	+ 43.6	Chloroform	+170	(−73)	b
4ξ-Bromo-5β-dihydrohydrocortisone					
11β,21-diacetate	+ 95.5	Chloroform	+504	+ 24	
21-acetate	+ 99	Acetone	+480		d
11β-Hydroxy-4-androstene-3,17-dione	+203	Alcohol	+612		h
11β-acetate	+179	Dioxane	+615	+ 3	g
11β-Hydroxytestosterone 11β,17β-diacetate	+117.8	.....	+456	+ 31	g
17β-acetate	+121 to +125	Chloroform	+419 to +432		h,i,j
Triamcinolone	+ 67.1	Methanol	+264		l
11β-acetate	+ 97.5	Methanol	+426	+162	k
11β,16α,21-triacetate	+ 55.4	Chloroform	+289	+184	k
	+ 96.1	Methanol	+500	+200	
16α,21-diacetate	+ 22	Chloroform	+105		m
	+ 63	Methanol	+300		k
11β-acetate 16α,17α-acetonide	+128	Methanol	+610	+157	k
11β,21-diacetate 16α,17α-acetonide	+138	Methanol	+716	+279	k
16α,17α-acetonide	+109	Chloroform	+474		n
	+104	Methanol	+453		k
21-acetate 16α,17α-acetonide	+ 92	Chloroform	+437		n
9α-Fluorohydrocortisone					
11β,21-diacetate	+134	Methanol	+622	+ 82	k
21-acetate	+127	Acetone	+540		o
9α-Bromoprednisolone					
11β,21-diacetate	+159	Dioxane	+832	+240	p
11β-formate 21-acetate	+156	Dioxane	+794	+202	p
11β-trifluoroacetate 21-acetate	+141	Dioxane	+814	+222	p
11β-diethylacetate 21-acetate	+130	Dioxane	+847	+255	p
21-acetate	+123	Dioxane	+592		q
9α-Chloroprednisolone					
11β,21-diacetate	+163	Dioxane	+781	+146	p
11β-formate, 21-acetate	+162	Dioxane	+753	+118	p
21-acetate	+145	Alcohol	+635		q

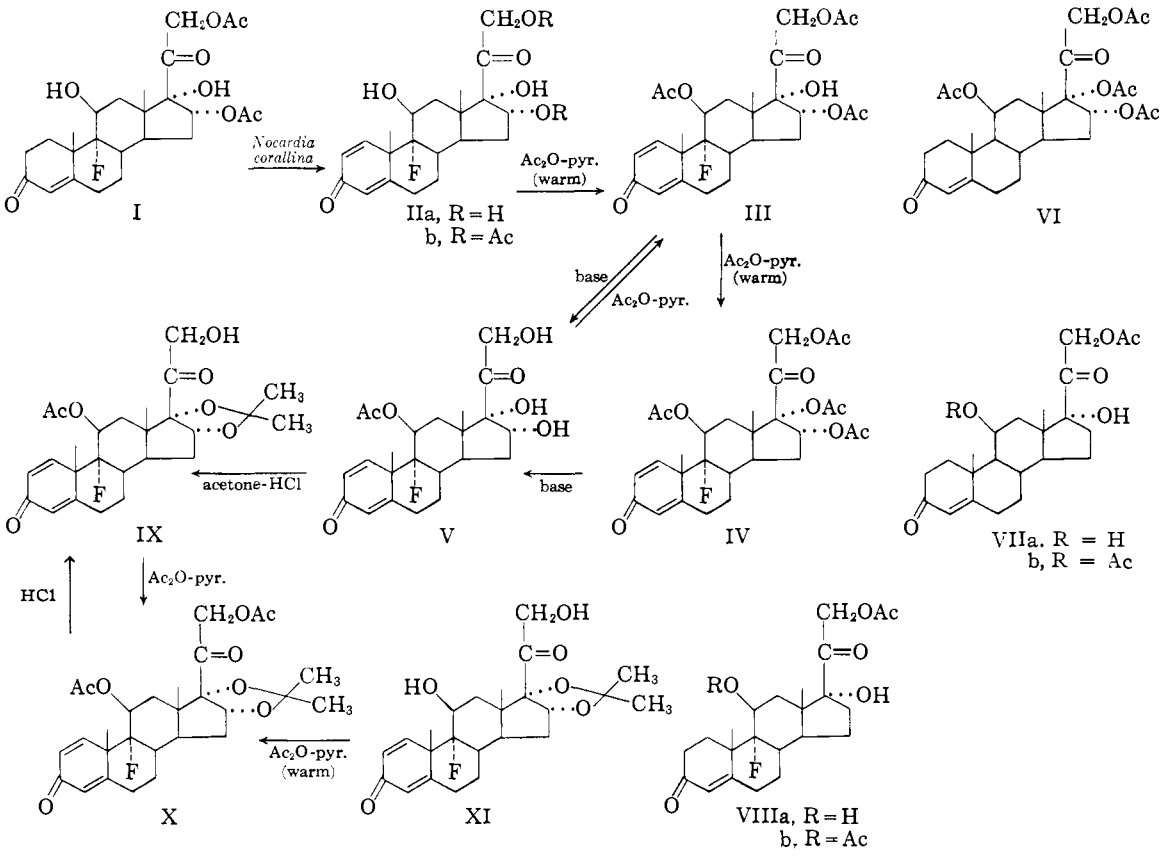
[a] See ref. 4b. [b] See ref. 4a. [c] See ref. 5f. [d] N. L. Wendler, R. P. Graber, R. E. Jones and M. Tishler, THIS JOURNAL, **74**, 3630 (1952). [e] R. H. Levin, *et al.*, *ibid.*, **75**, 546 (1953). [f] E. P. Oliveto, T. Clayton and E. B. Hershberg, *ibid.*, **75**, 486 (1953). [g] See ref. 5g. [h] S. Bernstein, R. H. Lenhard and J. H. Williams, *J. Org. Chem.*, 18, 1166 (1953). [i] M. E. Herr and F. W. Heyl, THIS JOURNAL, **75**, 5927 (1953). [j] O. Mancera, G. Rosenkranz and F. Sondheimer, *J. Chem. Soc.*, 2189 (1953). [k] This report. [l] See ref. 16. [m] See ref. 3. [n] See ref. 8a. [o] J. Fried and E. F. Sabo, THIS JOURNAL, **79**, 1130 (1957). [p] See ref. 6. [q] J. Fried, K. Florey, E. F. Sabo, J. E. Herz, A. R. Restivo, A. Borman and F. M. Singer, THIS JOURNAL, **77**, 4181 (1955).

which reaction is characteristic of *cis*-1,2-diols or 1,3-diaxial diols.[10] From these possibilities the 16α,17α-cyclic acetonide structure is assigned to IX, as acetylation affords a reducing diacetate acetonide derivative X, to which is assigned the structure 11β,21-diacetoxy-9α-fluoro-16α,17α-isopropylidenedioxy-1,4-pregnadiene-3,20-dione. Acetylation of triamcinolone 16α,17α-acetonide (XI)[3,9] with acetic anhydride and pyridine using an excess of reagent and warming, afforded the same diacetate acetonide X, which establishes the assigned structures of X and IX, and thus of V and III as 11β-acetates of triamcinolone.

In each instance ultraviolet absorption spectra, infrared absorption spectra, papergram mobilities and optical rotational data support the assigned structures.

The 11β,21-diacetate acetonide X could be hydrolyzed with hydrochloric acid to the 11β-acetate

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



acetonide IX, leaving both the 11β-ester group and the cyclic ketal group intact. Mineral acid is known to be ineffective in hydrolyzing 16α,17α-ketals of this series.[9a]

Previous understanding of the acetylation of the 11β-hydroxyl group of the corticosteroid molecule has been that strong acid-condensing agents be used for proper acetylation,[11] and that acetic anhydride–pyridine is not suitable for such acetylations. A recent report on the novel preparation of 9α-bromo- and chloro-11β-acylates of prednisolone by addition of the elements of the acyl hypohalite to the appropriate 9(11)-dehydro steroid points out that direct acetylation of the 11β-hydroxyl in the presence of the 17α-hydroxyl is not possible, and that use of *p*-toluenesulfonic acid–acetic anhydride–acetic acid produces the 11β,17α,21-triacetates.[6]

The reaction of triamcinolone and 9α-fluorohydrocortisone with warm acetic anhydride and pyridine at the 11β-hydroxyl group clearly permits one to prepare the 11β-acetates of these 9α-fluoro steroids without complications from the 17α-hydroxyl group. The facile acetylation of the 11β-hydroxyl might be attributed to the 9α-fluoro atom; however, similar acetylation experiments with hydrocortisone and 16α-hydroxyhydrocortisone afford 11β-acetylated products and thus require an alternate concept.

Acetylation of the 17α-hydroxyl group of triamcinolone and of 16α-hydroxyhydrocortisone is influenced by the presence of the 16α-hydroxyl group in the molecule, as the 17α-hydroxyl groups of the non-16α-hydroxylated steroids VII and VIII are not acetylated under the same conditions.[8]

The order of decreasing ease of acetylation of the four hydroxyl groups of triamcinolone becomes: 21 > 16α > 11β > 17α. With the recently reported high yields (90%) obtained for the microbiological deacetylation of hydrocortisone 11β,21-diacetate to yield hydrocortisone,[12] the combination of 11β-acetylation with warm acetic anhydride–pyridine and microbiological hydrolysis becomes more attractive as a protective measure in partial synthesis schemes for such 11β-hydroxy steroids.

Whereas previously described corticosteroids acetylated at the 11β-hydroxyl group are not biologically active,[3e,12] some of the triamcinolone 11β-acetates possess weak corticoid activities. Triamcinolone 11β-acetate 16α,17α-acetonide (IX) possesses glucocorticoid activity; liver glycogen, 2 × hydrocortisone (1–4, 95% confidence limits); thymus involution, 3 × hydrocortisone (1–5); pellet test, 1 × hydrocortisone (0.2–5). Triamcinolone 11β-acetate, while possessing no glucocorticoid activity in adrenalectomized rats, does exhibit a weak fluid diuretic activity in adrenalectomized and in normal rats. None of the other 11β-acetate derivatives showed any corticoid activity.

Acknowledgments.—The authors are grateful to Drs. I. Ringler and J. Cummings of these laboratories for biological data, to W. Fulmor for infrared

(11) (a) R. B. Turner, THIS JOURNAL, **74**, 4220 (1952); (b) R. B. Moffett and H. V. Anderson, *ibid.*, **76**, 747 (1954).

(12) W. Charney, L. Weber and E. P. Oliveto, *Arch. Biochem. Biophys.*, **79**, 402 (1959).

absorption spectra, to L. Brancone for microanalytical data, to W. Muller for ultraviolet absorption spectra, and T. Foell for papergram examinations.

Experimental[13]

11β,16α,21-Triacetoxy-9α-fluoro-17α-hydroxy-1,4-pregnadiene-3,20-dione (III). (A) Fermentation Source.—Thirty grams of 16α,21-diacetoxy-9α-fluoro-11β,17α-dihydroxy-4-pregnene-3,20-dione was dehydrogenated with *Nocardia corallina* under aerated submerged fermentation conditions in the manner described by Bernstein, *et al.*,[3] the steroids extracted with ethyl acetate, and the solvent removed *in vacuo*. The semi-solid residue so obtained was a mixture of triamcinolone diacetate, mixed monoacetates and triamcinolone alcohol, together with unaltered substrate, as evidenced by paper chromatography. Reacetylation of the residue was accomplished using 1000 ml. of acetic anhydride and 900 ml. of pyridine at room temperature for 16 hours. The reaction mixture was diluted with 1500 ml. of methanol and 1000 ml. of toluene and the solvents then removed under vacuum over a 4.5-hour period. During this period the temperature of the reaction mixture may have reached as high as 80° inadvertently. The solids were partitioned on a 6-inch diameter column packed with Celite diatomaceous earth using the solvent system ethylene glycol–petroleum ether–methylene chloride, 1:4:5. A forerun of 81. (0–1.0 hold-back volumes, HBV) contained the triacetate III and traces of IV and other tetrazolium blue-reducing impurities, together with non-steroidal lipid fermentation impurities. Further development of the column afforded fractions containing triamcinolone 16α,21-diacetate and unaltered substrate. Isolation of steroids from the fractions II and III required concentration in vacuum and crystallization from acetone–petroleum ether.

Fraction	HBV	Wt. isolated, g.	Identity	Papergram examination
I	0–1.0	8.5	Triacetate III	III + traces of IV
II	1.0–1.5	2.45	Substrate I	I only
III	1.5–3.0	7.28	Diacetate IIb	IIb only

Isolation of the steroids from fraction I followed concentration to a mobile oil and dilution with petroleum ether. The precipitated solids were filtered, washed with petroleum ether, and dried, yielding 8.5 g. of tan colored solids which analyzed on papergrams as a mixture of III (major) and IV (trace) (R_f values in system V[14] 0.60 and 0.90–0.96; system B_1 of Bush[15] 0.41–0.50 and 0.90–0.97; system B_3 of Bush[15] 0.47–0.56 and 0.84–0.90).

The impure triacetate of fraction I was partitioned on Celite diatomaceous earth (2.0 g. of crude III on 200 g. of Celite diatomaceous earth) with the ethylene glycol–methylene chloride–petroleum ether, 1:5:10 system, and the fraction containing III (fractions 4–10, 50-ml. fractions collected) was concentrated *in vacuo*, the residue dissolved in methanol and treated with charcoal and evaporated, yielding 650 mg. of III, m.p. 218–220°, homogeneous on papergrams, $[\alpha]^{22}D$ +55°. After two recrystallizations from acetone–petroleum ether the pure triacetate had m.p. 190.0–191.5°, resolidifying by 195° with remelting 221.0–223.0° dec. (Kofler), $[\alpha]^{22}D$ +55.4 (chloroform), λ_{max} 236 mμ (ε 14,900); $\lambda_{max}^{H_2SO_4}$ ($E_{1\,cm}^{1\%}$), at 15 min., 261 mμ (260), 308 mμ (114); at 2 hr., 260 mμ (266), 308 mμ (117); at 20 hr., 260 mμ (275), 308 mμ (156) 375 mμ (135); λ_{max}^{KBr} 3.05, 3.38, 5.71, 5.76 (shoulder), 5.98, 6.11, 6.17, 7.26, 8.07, 9.58, 11.16 μ, etc.; R_f system V[14] 0.57; propylene glycol–toluene system, 0.83 cm./hr.; positive to tetrazolium blue.

Anal. Calcd. for $C_{27}H_{33}O_9F$: C, 62.30; H, 6.39; F, 3.65. Found: C, 62.12; H, 6.84; F, 3.51.

(13) All melting points were taken in capillary except where noted (Kofler), which melting points were taken on a calibrated Kofler block under a microscope. Paper chromatographic examinations were conducted using systems already described.[14] Optical rotations were made on 0.5–1% solutions in methanol unless noted otherwise. Infrared spectra were obtained on potassium bromide disks using the Perkin–Elmer model 21 double beam instrument. Ultraviolet absorption spectra were obtained on absolute ethanol solutions using the Cary model 11S recording spectrophotometer.

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

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

(B) **Controlled Acetylation Source.**—Two grams of triamcinolone 16α, 21-diacetate was dissolved in a mixture of 50 ml. of pyridine and 20 ml. of acetic anhydride and the solution heated at 80° for 20 hours. After quenching with 40 ml. of methanol the solution was evaporated *in vacuo*, the residue dissolved in ethyl acetate, filtered through Florisil adsorbant to remove colored impurities, washed with bicarbonate and brine solutions, and dried over anhydrous magnesium sulfate. The solution was evaporated in vacuum to yield a crude product which analyzed as a mixture of III (minor component) and IV (major component) by papergram. Partition of the mixed acetates on Celite diatomaceous earth using the toluene–petroleum ether (b.p. 30–60°)–methanol–water, 12:8:13:7, system yielded 0.21 g. of triamcinolone 11β,16α, 21-triacetate (III), identical with III prepared under (A) or (C). The tetraacetate IV was recovered, and is described later in this section.

(C) **From Triamcinolone 11β-Acetate.**—A solution of 4.75 g. of V in 25 ml. of pyridine and 3 ml. of acetic anhydride was kept at room temperature for 6 hr. and then quenched with 10 ml. of methanol. After evaporation, etc., the residue was crystallized from ethyl acetate–petroleum ether, yielding 3.1 g. of triacetate III, m.p. 221–223° dec., identical with III prepared by A or B above, as evidenced by infrared spectra and paper chromatography.

11β,16α,17α,21-Tetraacetoxy-9α-fluoro-1,4-pregnadiene-3,20-dione (IV).—The reaction described under B above yielded 0.21 g. of the triacetate III on partition chromatography and 0.97 g. of the tetraacetate IV; after recrystallization from methanol, m.p. 217–219° dec., $[\alpha]^{22}D$ +99.8°, λ_{max} 235 mμ (ε 15,760); λ_{max}^{KBr} 3.40, 5.72, 5.99, 6.13, 6.20, 7.30, 8.15, 9.11 μ etc.; papergram mobility, system V[14] R_f 0.71.

Anal. Calcd. for $C_{29}H_{35}O_{10}F$: C, 61.91; H, 6.27; F, 3.38; acetyl, 29.88. Found: C, 62.01; H, 6.62; F, 3.16; acetyl, 29.02.

11β-Acetoxy-9α-fluoro-16α,17α,21-trihydroxy-1,4-pregnadiene-3,20-dione (V).—Ten grams of triamcinolone was acetylated by the means described for preparation of the triacetate and tetraacetate (method B above). The product isolated, 14.1 g., which was mainly the tetraacetate but with some triacetate, was dissolved in 150 ml. of methanol, purged of air with nitrogen, and 42.0 ml. of a 10% aqueous solution of potassium carbonate added dropwise over 20 minutes. After a further 15 minutes of stirring 4.2 ml. of glacial acetic acid was added, then 300 ml. of 12.5% sodium chloride solution. The crystals were filtered, 8.7 g., and recrystallized from 2-propanol; m.p. 228–230°, $[\alpha]^{22}D$ +156.6°, λ_{max} 236 mμ (ε 15,250); λ_{max}^{KBr} 2.93, 3.40, 5.73, 5.81 (shoulder), 5.99, 6.15, 6.20, 8.05, 8.12, 9.50, 11.26 μ, etc.; papergram mobility in system II, R_f 0.75 (*vs.* triamcinolone R_f 0.42).

Anal. Calcd. for $C_{23}H_{29}O_7F$: C, 63.27; H, 6.70; F, 4.35; acetyl, 9.63. Found: C, 63.10; H, 6.90; F, 4.10; acetyl, 10.7.

Hydrolysis of pure triamcinolone 11β,16α,21-triacetate (III) with sodium methoxide in methanol gave the same product, triamcinolone 11β-acetate, although papergram analyses of the products formed indicated some complete hydrolysis to triamcinolone, and some partial hydrolysis to unidentified diacetates, together with some isomerization to form triamcinolone isomer.[16] The product isolated from the reaction was the 11β-acetate V.

11β,16α,17α,21-Tetraacetoxy-4-pregnene-3,20-dione (VI).—A solution of 0.5 g. of 16α-hydroxyhydrocortisone in 5.0 ml. of pyridine and 2.5 ml. of acetic anhydride was heated at 80–85° for 26 hr., quenched with 10 ml. of methanol and worked up as usual. The product, 0.28 g., crystallized from acetone–petroleum ether, was recrystallized from aqueous acetone, m.p. 210–211°, $[\alpha]^{22}D$ +57.4°, λ_{max} 239 mμ (ε 15,900); λ_{max}^{KBr} 3.40, 5.70, 5.75, 5.97, 6.16, 7.28, 8.10, 9.42, 9.73, 10.62 μ, etc.

Anal. Calcd. for $C_{29}H_{38}O_{10}$: C, 63.72; H, 7.01; acetyl, 31.5. Found: C, 63.48; H, 7.19; acetyl, 27.64.

11β,21-Diacetoxy-9α-fluoro-17α-hydroxy-4-pregnene-3,-20-dione (VIIIb).—One gram of 9α-fluorohydrocortisone 21-acetate was dissolved in 40 ml. of pyridine and 10 ml. of acetic anhydride, warmed at 80° for 20 hr. and worked up as usual. The mixture of 21-acetate and 11β,21-diacetate was

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

partitioned on Celite diatomaceous earth using solvent system V[14]. A major fraction of 224 mg. of 11β,21-diacetate and a fraction, 421 mg. of diacetate–monoacetate mixture were obtained. Recrystallization of the pure diacetate fraction from acetone–petroleum ether gave crystals, m.p. 215–216, $[\alpha]^{22}D$ +134°, λ_{max} 236 mμ (ε 15,800); λ_{max}^{KBr} 2.90, 3.40, 5.73, 5.77(shoulder), 6.01, 6.15(shoulder), 7.29, 8.10, 9.07 μ, etc.; papergram mobility in system V[14] R_f 0.71.

Anal. Calcd. for $C_{25}H_{33}O_7F$: C, 64.64; H, 7.16; F, 4.09; acetyl, 18.53. Found: C, 64.10; H, 7.39; F, 4.04; acetyl, 19.23.

11β,21-Diacetoxy-17α-hydroxy-4-pregnene-3,20-dione (VIIb).—One gram of hydrocortisone 21-acetate was dissolved in 10 ml. of pyridine and 5.0 ml. of acetic anhydride, warmed at 80–85° for 24 hr., and worked up in the usual manner. The residue obtained was crystallized from ethyl acetate–petroleum ether, yielding 0.96 g. of the 11β,21-diacetate, m.p. 185.0–187.5° (Kofler), $[\alpha]^{22}D$ +168°, λ_{max} 238 mμ (ε 15,880).[17] Infrared spectra and papergram mobilities were consistent with the assigned 11β,21-diacetate structure.

11β,21-Diacetoxy-9α-fluoro-16α,17α-isopropyldienedioxy-1,4-pregnadiene-3,20-dione (X).—Acetylation of 0.6 g. of triamcinolone 16α,17α-acetonide[3,9] with 10 ml. of pyridine and 2.5 ml. of acetic anhydride by heating at 90° for 15 hr. afforded 0.7 g. of crystalline 11β,21-diacetate when worked up in the usual manner. Recrystallization from acetone–petroleum ether gave crystals, m.p. 230–232°, $[\alpha]^{22}D$ +138°, λ_{max} 236 mμ (ε 15,300); λ_{max}^{KBr} 3.40, 5.70, 5.75(shoulder), 5.98, 6.10, 6.19, 7.26, 8.13, 8.57, 9.25, 9.53, 10.98, 11.20, 11.68 μ, etc.; papergram mobiliy in system V[14] R_f 0.92.

(17) Hydrocortisone 11β,21-diacetate has been characterized[4a,4b] as follows: (a) m.p. 188–189, $[\alpha]^{25}D$ +167.1° (chloroform), λ_{max} 238 mμ (ε 17,200); (b) m. p. 191.0–191.8°, $[\alpha]D$ +167.1° (chloroform), $\lambda_{max}^{95\%EtOH}$ 240 mμ (17,200).

Anal. Calcd. for $C_{28}H_{35}O_8F$: C, 64.85; H, 6.80; F, 3.66; acetyl, 16.21. Found: C, 64.43; H, 7.33; F, 3.97; acetyl, 15.58.

Acetylation of triamcinolone 11β-acetate 16α,17α-acetonide with acetic anhydride and pyridine afforded the same 11β,21-diacetate 16α,17α-acetonide, as evidenced by infrared spectra, papergram mobility and melting point criteria.

11β-Acetoxy-9α-fluoro-21-hydroxy-16α,17α-isopropylidenedioxy-1,4-pregnadiene-3,20-dione (IX). (A). From Triamcinolone 11β-Acetate.—A solution of 1.5 g. of triamcinolone 11β-acetate in 500 ml. of acetone containing 2.25 ml. of concentrated hydrochloric acid was allowed to stand at room temperature for 24 hr., at which time the solution was neutralized with sodium bicarbonate solution, diluted with water, and concentrated in vacuum to a volume of 200 ml. The crystals formed were filtered, washed with water, and dried. The crystals, 1.4 g., were recrystallized from 50% aqueous methanol, m.p. 215–217°, $[\alpha]^{22}D$ +126.1°, λ_{max} 234 mμ (ε 15,490); λ_{max}^{KBr} 2.90, 3.40, 5.72, 5.80, 5.99, 6.10, 6.19, 6.91, 7.27, 8.13, 9.25, 9.55, 11.20, 11.67 μ, etc. papergram mobility in system V[14] R_f 0.69.

Anal. Calcd. for $C_{26}H_{33}O_7F$: C, 65.53; H, 6.98; F, 3.98; acetyl, 9.03. Found: C, 65.25; H, 7.13; F, 3.80; acetyl, 12.00.

(B) **From Triamcinolone 16α,17α-Acetonide 11β,21-Diacetate.**—A solution of 170 mg. of triamcinolone 16α,17α-acetonide 11β,21-diacetate in 16 ml. of methanol was diluted with 6.1 ml. of water and 2.0 ml. of concentrated hydrochloric acid, and the mixture was refluxed for 3 hours. After dilution with 15 ml. of water, the solution was concentrated in vacuum to about 15 ml. The crystals formed, 0.12 g., were recrystallized from aqueous methanol. Identity of the acetonide 11β-acetates formed by methods A and B was established by melting point, infrared spectral and paper chromatographic evidences.

PEARL RIVER, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY, CALIF.]

Reaction of D-Erythrose and 2,4-O-Ethylidene-D-erythrose with Methanolic Hydrogen Chloride[1]

BY CLINTON E. BALLOU

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The reaction of 2,4-O-ethylidene-D-erythrose with methanol containing hydrogen chloride gives, as the main product, methyl 2,3-O-ethylidene-β-D-erythroside. The migration of the ethylidene group and the formation of two fused five-membered rings apparently accounts for the stability of this product. Free D-erythrose reacts under the same conditions to give mostly methyl β-D-erythroside, contaminated with a little of the α-anomer. Pure methyl β-D-erythroside has a specific rotation of −149°. 2,4-O-Ethylidene-D-erythrose hydrolyzes completely in aqueous acid only if the acetaldehyde is allowed to escape. There is a strong tendency for the formation of 2,3-O-ethylidene-D-erythrose by the migration of the ethylidene group, but the same product is formed from free D-erythrose in aqueous acid containing 5% acetaldehyde. The reduction of the 2,3-O-ethylidene-D-erythrose gives 2,3-O-ethylidene-erythritol.

Several past investigations, particularly by Hockett and Maynard,[2] have been concerned with the reaction of the tetrose, erythrose, with methanol in the presence of an acid catalyst to give acetal derivatives. The only well defined product obtained has been a methyl D-erythroside; although the possible formation of acyclic acetals, as well as acetals of dimers of a dioxane type structure, has been the basis of conjecture.

During an attempt to prepare methyl D-erythroside by treating 2,4-O-ethylidene-D-erythrose with methanol containing 1% hydrogen chloride, we were surprised to find the rotation of the solution become strongly levorotatory. On the assumption that the ethylidene group was coming off by alcoholysis and the resulting free D-erythrose was being converted to methyl D-erythroside, the rotation indicated that the product must be primarily a β-anomer. This would be at variance with the observations of Hockett and Maynard that a solution of D-erythrose in methanolic hydrogen chloride became only slightly levorotatory. When the products from the above reaction of 2,4-O-ethylidene-D-erythrose were isolated, the major component was found to be methyl 2,3-O-ethylidene-D-erythroside, indicating that the ethylidene group had migrated, but had remained, for the most part, attached to the sugar molecule. A lesser amount of a methyl D-erythroside also was obtained. The rotations of both of these products were strongly levorotatory and suggestive of β-anomeric forms.

(1) Presented in part at the Meeting of the American Chemical Society in Chicago, Ill., September, 1958.

(2) R. C. Hockett and C. W. Maynard, Jr., THIS JOURNAL, **61**, 2111 (1939); G. F. Felton and W. Freudenberg, *ibid.*, **57**, 1637 (1935).