This final fraction was converted to the barium salt, reconverted to the cyclohexylammonium salt, and then subjected to preparative paper chromatography to remove R_f 0 material, as described for the α-anomer. The **cyclohexylammonium β-D-ribofuranose 1-phosphate** thus purified showed $[\alpha]^{23}_D$ −15.8° in water (*c* 1.2) and was chromatographically pure although it could not be distinguished from its α-anomer using isopropyl alcohol–ammonia–water.

Anal. Calcd. for $C_{17}H_{37}N_2O_7P$ (412.47): C, 49.50; H, 9.04; N, 6.79; P, 7.51. Found: C, 49.13; H, 9.30; N, 6.79; P, 7.11.

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

16α-Hydroxy Steroids. XII.[1a] 21-Amino Derivatives of 9α-Fluorohydrocortisone

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Fermentations of 9α-fluorohydrocortisone with *Streptomyces roseochromogenus* afford as a minor product a steroidal amide, 21-acetylamino-9α-fluoro-11β,17α-dihydroxy-4-pregnene-3,20-dione, whose isolation, characterization, structural elucidation, and synthesis from 9α-fluorohydrocortisone are described. A synthesis of the 21-amino analog of 9α-fluorohydrocortisone is also described.

The complex alteration of 9α-fluorohydrocortisone (I) by *Streptomyces roseochromogenus* includes 16α-hydroxylation,[2] 2β-hydroxylation,[3] 20-carbonyl reduction,[4] together with D-homoannulation of the 16α-hydroxylated products[5] and conversion to other non-reducing products. In a continuing study of the bioconversions of this microörganism on 9α-fluorohydrocortisone, we have noted the regular occurrence in a variety of fermentation samples of one non-reducing component II, located midway between the substrate I and the major fermentation product 16α-hydroxy-9α-fluorohydrocortisone (III) on standard paper chromatograms. Instrumental evaluation of the fluorescence of the isonicotinic acid hydrazones on paper chromatograms indicated that about 4–5% of the substrate could be accounted for as the non-reducing component II.

The steroidal nature of II was supported by infrared spectra of papergram eluates. However, major concern for the structure of the compound was provoked by strong bands at 6.5 μ, implying that the steroid contained nitrogen.

Isolation of II from very complex extract concentrates from which 16α-hydroxy-9α-fluorohydrocortisone had been removed by crystallization and from which other 16α,17α-diols were removed as their water-soluble 16α,17α-cyclo borates has been described.[3] The pure II was recognized as a neutral polyhydroxy-Δ⁴-3-ketosteroidal amide, $C_{23}H_{32}O_5NF$, from which a Δ¹·⁴-3-ketone analog IV, $C_{23}H_{30}O_5NF$, was prepared microbiologically. Physical properties of the amides II and IV differentiated them from naturally occurring steroidal amides such as the toad poisons, bile acid conjugates, etc., and from the nitrogenous steroid preparations of Voigt and Schroeder,[6] steroid-nucleotide/purine complexes, steroid-polypeptide/protein complexes, and from various synthetic and derived steroid amine and amide compounds.[7]

From the characteristic infrared absorption near 3, 6 and 6.5 μ exhibited by both amides II and IV, a non-cyclic secondary amide was suggested. Absorption in these regions is characteristic for non-cyclic secondary amides in general[8] and for such steroidal amides in particular.[9] Whereas some non-cyclic steroidal secondary amides have been reported without the amide II bands near 6.5 μ, no steroidal secondary amide (cyclic) or tertiary amide is known to us with absorption near 6.5 μ.

Neither amide II nor IV was acetylated by acetic anhydride and pyridine at room temperature,[10] nor was a cyclic acetonide derivative formed between II or IV with acetone–perchloric acid. Mild acid hydrolysis of IV afforded a 16-dehydro amide V, recognized as such by loss of absorption at 5.8 μ and increased absorption at 6.0 μ and at 238 mμ. No hydrolysis occurred with base (as strong as 10 *N*) at room temperature, and no steroids could be isolated when hot alkali was used. Further experiments aimed at amide hydrolysis were not fruitful.[11]

(1) (a) Paper XI, J. J. Goodman and L. L. Smith, *App. Microbiol.*, **9**, 372 (1961); (b) Wyeth Laboratories, Philadelphia, Pa.

(2) R. W. Thoma, J. Fried, S. Bonanno and P. Grabowich, *J. Am. Chem. Soc.*, **79**, 4818 (1957).

(3) L. L. Smith, H. Mendelsohn, T. Foell and J. J. Goodman, *J. Org. Chem.*, **26**, 2859 (1961).

(4) L. L. Smith, T. Foell and J. J. Goodman, *Biochemistry*, in press.

(5) L. L. Smith, M. Marx, J. J. Garbarini, T. Foell, V. E. Origoni and J. J. Goodman, *J. Am. Chem. Soc.*, **82**, 4616 (1960).

(6) K. D. Voigt and W. Schroeder, *Nature*, **176**, 599 (1955); W. Schroeder and K. D. Voigt, *Acta Endocrinol.*, **21**, 343 (1956); **27**, 110 (1958).

(7) K. D. Voigt and G. Kallistratos, *Endokrinol.*, **35**, 56 (1958).

(8) L. J. Bellamy, "The Infra-red Spectra of Complex Molecules," 1st Edition, Methuen and Co., Ltd., London, 1954, pp. 175–196.

(9) G. Rosenkrantz, O. Mancera, F. Sondheimer and C. Djerassi, *J. Org. Chem.*, **21**, 520 (1956); K. Heusler, P. Wieland and A. Wettstein, *Helv. Chim. Acta*, **41**, 997 (1958); Y. Sato, *et al.*, *J. Org. Chem.*, **22**, 1496 (1957); **24**, 893 (1959); **25**, 783, 786, 789 (1960); V. Černý and F. Šorm, *Coll. Czech. Chem. Commun.*, **24**, 4015 (1959); **25**, 2841 (1960); B. G. Ketchum and A. Taurins, *Can. J. Chem.*, **38**, 981 (1960); M. M. Janot, Q. Khuang-Huu and R. Goutarel, *Bull. soc. chim. France*, 1640 (1960).

(10) Forced acetylation with acid catalysis gave complex products which did not absorb at 6.5 μ, but exhibited O-acetate bands.

(11) 6 *N* hydrochloric acid–acetic acid gave complex products without 6.5 μ absorption, which on reacetylation absorbed in the region of O-acetates rather than near 6.5 μ. Hydrolysis of steroidal amides has generally been a problem; *cf.* L. Lábler and F. Šorm, *Coll. Czech. Chem. Commun.*, **25**, 265 (1960); F. Ramirez and S. Stafiej, *J. Am. Chem. Soc.*, **77**, 134 (1955); R. H. Mazur, *ibid.*, **81**, 1454 (1959); J. Schmidt-Thomé, *Chem. Ber.*, **88**, 895 (1955); ref. 12.

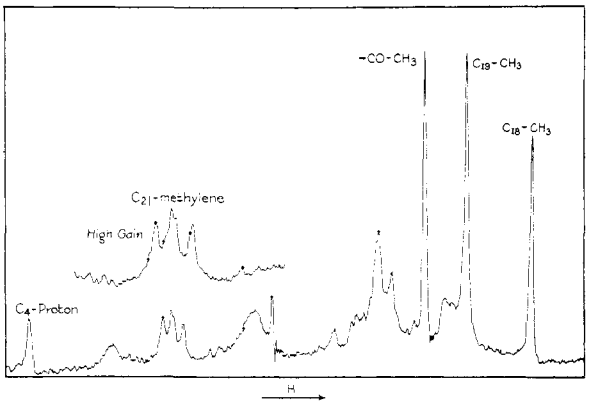


Fig. 1.—Proton nuclear magnetic resonance spectra (60 mc.) of 21-acetylamino-9α-fluoro-11β,17α-dihydroxy-4-pregnene-3,20-dione (II) in pyridine–deuteriochloroform (1:7).

The $\Delta^{1,4}$-3-ketone IV was degraded by sodium borohydride reduction, followed by sodium bismuthate oxidation to a known 17-ketone 9α-fluoro - 11β - hydroxy - 1,4 - androstadiene - 3,17-dione (VII).[12] Preparation of VII from 9α-fluoroprednisolone (VIII) by bismuthate oxidation and from 9α-fluoro-11β-hydroxy-4-androstene-3,17-dione by selenium dioxide oxidation established its structure.

A structure thus suggested for IV is that of 21-acetylamino - 9α - fluoro - 11β,17α - dihydroxy-1,4-pregnadiene-3,20-dione, and for II that of 21-acetylamino - 9α - fluoro - 11β,17α - dihydroxy-4-pregnene-3,20-dione.

Proton nuclear magnetic resonance spectra support in detail the suggested structures. Three non-equivalent methyl group resonances are present in the spectra of both amides II and IV in pyridine. The two high-field resonances are associated with the angular C_{18}- and C_{19}-methyl groups. The third resonance is associated with a methyl group adjacent to a carbonyl group, thus an acetyl group.[13] Spectra in pyridine diluted with deuteriochloroform gave additional information. The three high-field methyl resonances, vinyl proton resonances (A-ring) and a characteristic multiplet in the methylene region (for II, 266 c.p.s. from an internal standard of tetramethylsilane) were present. The multiplet was recognized as the AB-portion of the spectra of an ABX-grouping, tentatively associated with the $-CH_2-NH-$ feature of the molecule.[15] Spectra of the Δ^4-3-ketoamide II are presented in Fig. 1.

The structures of II and IV thus induced were confirmed by synthesis of the indicated compounds. Copper acetate oxidation of the α-ketol I led to the 21-aldehyde IX, which was characterized as the aldehyde hydrate, so established by elemental analyses and by paper chromatographic mobility typical of a tetrahydroxy-diketone. A 21-oxime X was obtained selectively from reaction of IX with 1.1 equivalents of hydroxylamine. The oxime was more mobile on paper chromatograms than anticipated, and thus appeared to be associated under the chromatographic conditions.

Reduction of the 21-oxime X was accomplished using the zinc dust–acetic acid–acetic anhydride conditions of Treibs and Sutter.[16] Catalytic and sodium–alcohol reduction methods were rejected in view of the other reducible functional groups in the molecule. The 21-acetylamino compound isolated in 60% yield was identical in every respect with the amide II isolated from *S. roseochromogenus* fermentations on 9α-fluorohydrocortisone.

Later work showed that the inclusion of the inorganic salts as catalysts in the reduction was unnecessary, and that the same reduction product was formed when zinc–acetic acid–acetic anhydride was used alone. No unaltered oxime X was detected chromatographically nor were other reduction products found.

Elimination of the acetic anhydride from the reduction system resulted in the formation of an amine, 21-amino-9α-fluoro-11β,17α-dihydroxy-4-pregnene-3,20-dione(XI), isolated as a hydrochloride. This synthesis marks the first reported successful attempt at preparation of 21-amino-21-deoxy analogs in the corticosteroid series.

Acetylation of the 21-amine XI with acetic anhydride and pyridine gave the 21-acetylamino derivative II identical with the amide samples previously prepared. Acylation with phthalic anhydride and pyridine gave the 21-phthalimido derivative 9α-fluoro-11β,17α-dihydroxy-21-phthalimido-4-pregnene-3,20-dione (XII) previously prepared in these laboratories by another route.[17]

Fermentation of the 21-amine XI hydrochloride with *S. roseochromogenus* gave a single component, the amide II, with no detectable amine remaining. This result establishes yet another bioconversion of *S. roseochromogenus*, that of acetylation of primary amines (microbiological acetylation of amines is a known process[18,19]). Although this finding

(12) The intermediate 20β-dihydro amide VI was not isolated. A similar 20-ketone reduction of 21-nitrogenous 20-ketones has been reported, R. A. Micheli and C. K. Bradsher, *J. Am. Chem. Soc.*, **77**, 4788 (1955).

(13) Spectra run on pyridine solutions of the amides were recorded with a 40 mc. applied field, all frequencies being measured relative to benzene as zero. Spectra of the amide II are: 243 (C_{18}-methyl), 229 (C_{19}-methyl), 212 c.p.s. (acetyl methyl); for amide IV: 235 (C_{18}-methyl), 223 (C_{19}-methyl), 209 c.p.s. (acetyl methyl). For 9α-fluorohydrocortisone 21-acetate: 243 (C_{18}-methyl), 229 (C_{19}-methyl), 215 c.p.s. (21-O-acetate); for 9α-fluoroprednisolone 21-acetate: 237 (C_{18}-methyl), 223 (C_{19}-methyl), 209 c.p.s. (21-O-acetate).

Although pyridine has been used as a solvent in nuclear magnetic resonance spectral studies,[14] its use in this instance obscured the vinyl proton region and made interpretation of the methylene region unreliable. Dilution of pyridine solutions of the amides II and IV with deuteriochloroform gave solutions whose spectra did show reliable features in the regions of interest. These spectra were recorded using a 60 mc. applied field, on pyridine solutions of the amides diluted with 95% deuteriochloroform until satisfactory spectra in the methylene region were obtained. An internal standard of tetramethylsilane was used in this case.

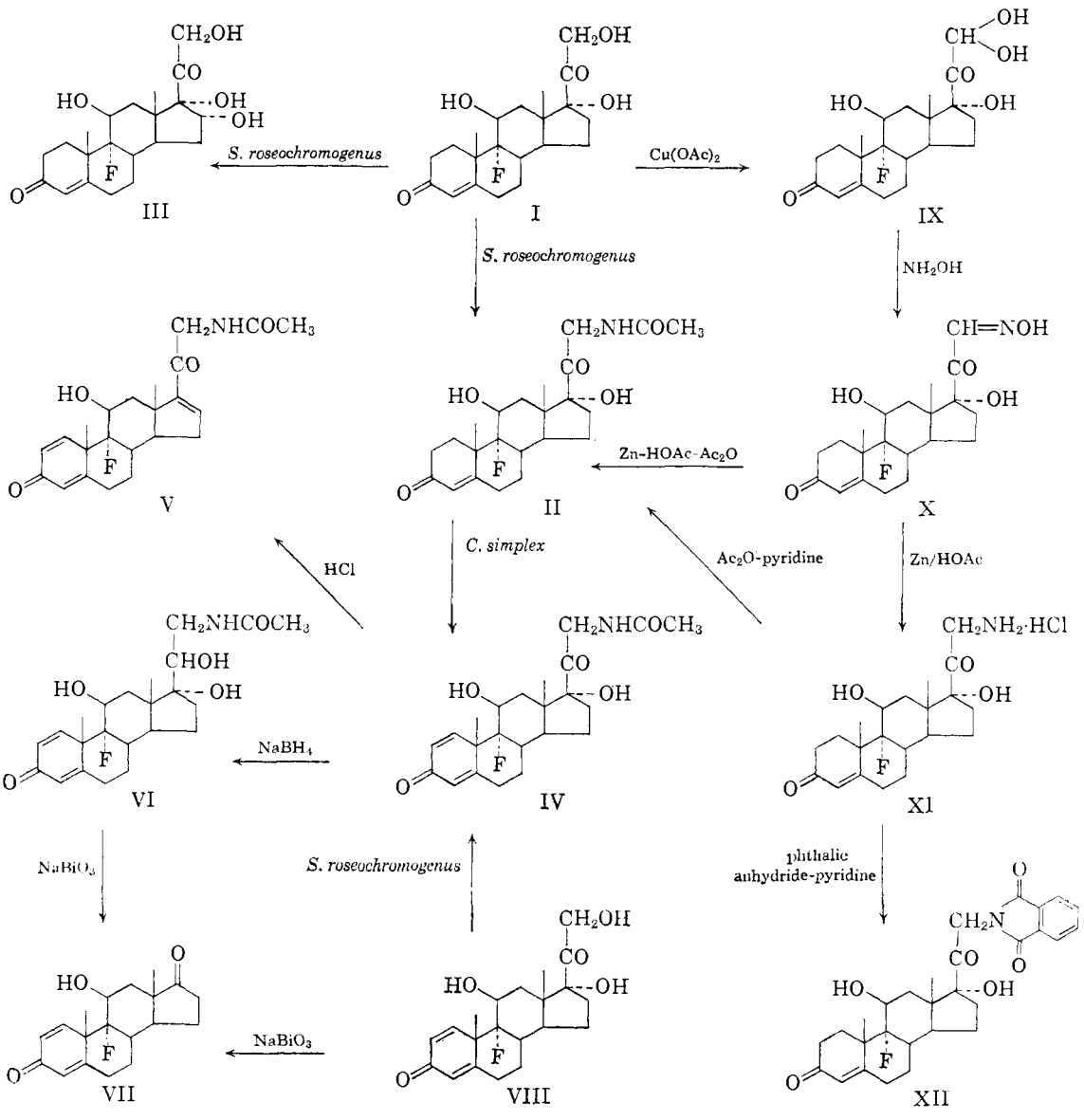
(14) G. Slomp and F. MacKellar, *J. Am. Chem. Soc.*, **82**, 999 (1960); see also S. M. Kupchan, W. S. Johnson and S. Rajagopalan, *Tetrahedron*, **7**, 47 (1959).

(15) No bands could be associated with the X-portion of the spectra with certainty even though the spectra were scanned below the vinyl proton region. The NH-proton of simple secondary amides has been reported to split the protons of the adjacent N-methyl group, with no NH-proton resonances being found, see H. S. Gutowsky and C. H. Holm, *J. Chem. Phys.*, **25**, 1228 (1957).

(16) A. Treibs and W. Sutter, *Chem. Ber.*, **84**, 96 (1951).

(17) R. E. Schaub and M. J. Weiss, *J. Org. Chem.*, **26**, 1223 (1961).

(18) F. H. Stodola, "Chemical Transformations by Microörganisms," John Wiley and Sons, Inc., New York, N. Y., 1958; L. L. Walen, F. H. Stodola and R. W. Jackson, "Type Reactions in Fermentation Chemistry," ARS-71-13, May, 1959, Agricultural Research Service, U. S. Dept. of Agriculture.



suggests that the amine XI may be a biological intermediate between 9α-fluorohydrocortisone and the 21-acetylamino derivative II, no other evidence has been developed yet which supports this possibility.

Neither amide II nor IV was 16α-hydroxylated by *S. roseochromogenus*, but selected strains[1a] did apparently hydroxylate the Δ^4-3-ketoamide II at the 2β-position.[20]

Steroids of chromatographic properties entirely analogous to those of II and IV have been observed in fermentations with various strains of *S. roseochromogenus* on several other corticoids. Thus hydrocortisone, 9α-fluoroprednisolone, 2β-hydroxy-9α-fluorohydrocortisone, 6α,9α-difluorohydrocortisone, etc., all yield non-reducing components suspected of being the 21-acetylamino-21-deoxy derivatives. Where sufficient material has been available the components were isolated from papergram and their infrared spectra shown to contain characteristic amide I and II bands[8] near 6.1 and 6.5 μ. The component derived from 9α-fluoroprednisolone is indistinguishable from the amide IV obtained by dehydrogenation of II.

Biological conversion of the 21-hydroxyl group of the active corticoid molecule to the 21-acetylamino group has not been previously described.[18] The present bioconversion to the 21-acetylamino-21-deoxy steroid appears to be general, and it is tempting to speculate on the possibilities of still other new microbiological reactions wherein elements other than oxygen and hydrogen are introduced into the steroid molecule.

Acknowledgment.—The authors acknowledge the capable assistance of Roberta Zimmerman and Barbara Laird in paper chromatographic analyses

(19) Microbiological acetylation of 21-hydroxy steroids has also been recently discovered; C. E. Holmlund, L. I. Feldman, N. E. Rigler, B. E. Nielsen and R. H. Evans, *J. Am. Chem. Soc.*, **83**, 2586 (1961).

(20) The major alteration product from II, located at R_f 0.30 in system II,[22] was eluted and gave spectra typical of 2β-hydroxy-Δ^4-3-ketones: $\lambda_{max}^{0.056\ N\ NaOH}$ ($E_{1\ cm.}^{1\%}$, 3 hr. at 60°) 228 mμ (260), 249 (mμ 120 infl.), 350 mμ (30).

PAPER CHROMATOGRAPHIC BEHAVIOR

Steroid	Solvent system[22] I[a]	II[b]	III[a]	IV[a]	V[b]	INH[d] dil.	INH[d] str.	BT[e]	$SbCl_3$[f]	H_3PO_4[g]
21-Acetylamino-9α-fluoro-11β,17α-dihydroxy-4-pregnene-3,20 dione (II)	1.76	0.69	2.02	2.88	0.06	+	+	−	Pe	Y
21-Acetylamino-9α-fluoro-11β,17α-dihydroxy-1,4-pregnadiene-3,20-dione (IV)	1.56	.65	1.53	2.35	.04	−	+	−	−	−
9α-Fluorohydrocortisone (I)	1.65	.80	1.73	2.72	.06	+	+	+	Pe	Y
9α-Fluoroprednisolone (VIII)	..	.76	1.53	2.04	..	−	+	+	−	Y
16α-Hydroxy-9α-fluorohydrocortisone (III)	1.00	.50	1.00	1.00[c]	.01	+	+	+	Pe	Y

[a] Mobility relative to 16α-hydroxy-9α-fluorohydrocortisone (III) as unity. [b] Conventional R_f value. [c] R_f of unit marker was measured, R_f 0.18. [d] Isonicotinic acid hydrazide, dilute and strong reagents; see L. L. Smith and T. Foell, *Anal. Chem.*, **31**, 102 (1959). [e] Alkaline tetrazolium blue reagent. [f] Saturated solution of antimony trichloride in chloroform, fluorescences under 365 mμ light; Pe = peach color, Y = yellow. [g] A 15% aqueous solution of phosphoric acid, viewed under 365 mμ light; see R. Neher and A. Wettstein, *Helv. Chim. Acta*, **34**, 2278 (1951).

and of W. H. Muller and Irene Palestro for optical rotations and spectrophotometric analyses. Elemental analyses were performed by L. Brancone and staff, infrared absorption measurements by W. Fulmor and staff.

The authors are especially indebted to Dr. J. Lancaster and to Dr. J. N. Shoolery and Mr. LeRoy Johnson, Varian Associates, Palo Alto, Calif., for nuclear magnetic resonance spectra and interpretations.

Experimental[21]

21-Acetylamino-9α-fluoro-11β,17α-dihydroxy-4-pregnene-3,20-dione (II). Microbiological Source.—Isolation of II was accomplished from a concentrated mother liquor fraction rich in steroidal by-products from typical *S. roseochromogenus* fermentations. The initial isolation is described elsewhere.[3] The appropriate column fractions therein described (fractions 155–180) were evaporated in vacuum, yielding a residue which was crystallized from methanol. Two crops weighing 5.32 and 3.52 g. were taken. Recrystallization of 5.20 g. of the first crop from methanol gave 3.38 g. of crystals, m.p. 263–271°, whereas the second crop similarly recrystallized gave 2.45 g., m.p. 258–265°, both preparations being identical and homogeneous on paper chromatographic examination. Another recrystallization from methanol with charcoal treatment gave crystals, m.p. 275–276°; however, a third recrystallization from methanol gave material melting 254–258° (not raised by further recrystallization from methanol) with λ_{max} 238 mμ ($E^{1\%}_{1\,cm.}$ 407). The sample was chromatographically homogeneous and had characteristic infrared spectra; however, satisfactory elemental analyses could not be obtained on this sample, possibly because of solvation.

Anal. Found: C, 64.29, 64.58; H, 7.89,7.93; N, 3.29; F, 3.84; O, 20.28.

Recrystallizations from aqueous pyridine gave an unsolvated analytical sample, m.p. 263–264° dec., $[\alpha]^{22}D$ +132°, λ_{max} 238 mμ (ε 16,200); λ^{KBr}_{max} 2.93 (OH), 3.00 (NH), 5.80 (CO), 6.02 (conjugated CO and amide I band), 6.13, 6.54 μ (-CONH-), etc.

Anal. Calcd. for $C_{23}H_{32}O_5NF$: C, 65.54; H, 7.65; N, 3.32; F, 4.51. Found: C, 65.45; H, 7.53; N, 4.88 (Dumas); F, 4.44.

The amide II is characterized by absorption spectra in concentrated sulfuric acid: at 2 hr., $\lambda^{H_2SO_4}_{max}$ ($E^{1\%}_{1\,cm.}$) 241 (310), 283 (326), 335 mμ (21, infl.); $\lambda^{H_2SO_4}_{min}$ ($E^{1\%}_{1\,cm}$) 254 mμ (140). No selective absorption in the 415 mμ region was exhibited in the Porter–Silber reaction, but non-specific absorption at 415 mμ ($E^{1\%}_{1\,cm.}$ 8) was 2.5% of the response of 9α-fluorohydrocortisone run at the same time. Reaction with alkaline tetrazolium blue at room temperature in solution gave the typical diformazan color with λ_{max} 520 mμ. Color maximum was attained within an hour with a response of 83.0% of that of 9α-fluorohydrocortisone run at the same time (1-hour reactions).

21-Acetylamino-9α-fluoro-11β,17α-dihydroxy-1,4-pregnadiene-3,20 dione (IV).—21-Acetylamino-9α-fluoro-11β,17α-dihydroxy-4-pregnene-3,20-dione was dehydrogenated as follows: the substrate II was dissolved in dimethylformamide and added to a 24-hour vegetative growth of *Corynebacterium simplex* grown in a medium consisting of commercial glucose 20 g./l., yeast extract 5 g./l., tryptone 5 g./l., peptone 5 g./l., calcium carbonate 2.5 g./l. The steroid was added so as to have a 250 μg./ml. concentration in the fermenting growth. Aerated fermentation was continued until polarographic analyses indicated that dehydrogenation was complete. The broth was extracted three times with equal portions of ethyl acetate, and the pooled extracts were evaporated under vacuum. The residue obtained was chromatographed using Celite diatomaceous earth and the solvent system dioxane–cyclohexane–water (8:5:1) and the amide IV was eluted in fractions centered about 6.5 hold-back volumes. Evaporation of these fractions yielded 300 mg. of IV, homogeneous on paper chromatograms (R_f 0.63 in system II, non-reactive with dilute isonicotinic acid hydrazide, detected with strong isonicotinic acid hydrazide reagent, negative to alkaline tetrazolium blue).

The crude amide IV was recrystallized from hot benzene–ethanol, yielding 210 mg. of crystals, m.p. 231–235°, and then from ethanol, yielding the ethanol solvate, 172 mg., m.p. 251–255° dec. (softening from 238°), λ_{max} 238 mμ (ε 14,900). Further recrystallization from acetone–ethanol gave crystals, m.p. 252–255° dec. (softening from 236°), $[\alpha]^{22}D$ + 106.9°, λ_{max} 239 mμ (ε 14,900); spectra in concd. sulfuric acid at 2 hr.: $\lambda^{H_2SO_4}_{max}$ ($E^{1\%}_{1\,cm.}$), 231 (367, infl.), 267 (350), 276, (326, infl.), 310 mμ (214); $\lambda^{H_2SO_4}_{min.}$ ($E^{1\%}_{1\,cm.}$) 251 (314), 295 mμ (201); infrared absorption, λ^{KBr}_{max} 2.95 (OH), 3.03 (NH), 5.82 (CO), 6.04 (conjugated CO and amide I band), 6.19, 6.26, 6.50 (CONH-), 6.55 μ, etc.

Anal. Calcd. for $C_{23}H_{30}O_5NF \cdot C_2H_6O$: C, 64.50; H, 7.80; N, 3.01; F, 4.08. Found: C, 64.69; H, 7.83; N, 4.52; F, 4.09.

Recrystallization of the amide ethanol solvate from aqueous pyridine or from acetone gave the non-solvated amide.

Anal. Calcd. for $C_{23}H_{30}O_5NF$: C, 65.85; H, 7.21; N, 3.34; F, 4.53. Found: C, 65.57; H, 7.48; N, 4.01 (Dumas); F, 4.49.

The amide IV reduces alkaline tetrazolium blue in solution, λ_{max} 520 mμ, with maximum color development at 1 hour, at which time the response was 67.6% of that of 9α-fluorohydrocortisone run at the same time. Very weak selective absorption was present in the Porter–Silber reaction, λ_{max} 415 mμ ($E^{1\%}_{1\,cm}$ 2.5, *i.e.*, 0.8% of that of 9α-fluorohydrocortisone).

No specific color test for amides could be used for the detection of either II or IV on paper chromatograms. Exposure of the amides II and IV to chlorine gas and to various

(21) All melting points were taken on a Kofler block under a microscope and are corrected. Spectrophotometric measurements in absolute ethanol and in concentrated sulfuric acid and colorimetric determinations (tetrazolium blue, Porter–Silber reaction, etc.) were made using a Cary model 11S recording spectrophotometer. Infrared spectra were recorded on Perkin–Elmer model 21 and Infracord double beam spectrometers using pressed potassium bromide disks. Paper chromatographic procedures have been described previously.[22] All rotations were made on 0.5% solutions in methanol.

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

treatments with sodium hypochlorite solutions, followed by application of a variety of detection methods involving iodine,[23] either failed to give a sufficiently sensitive reaction to 10–20 μg. of amide or else gave the same colorations with 9α-fluorohydrocortisone and other non-nitrogenous steroids. Application of other color tests for N-acetylamino sugars,[24] etc., also failed to give satisfactory results with the amides II and IV.

9α-Fluoro-11β,17α,21,21-tetrahydroxy-4-pregnene-3,20-dione (IX).—To a hot solution of 1.0 g. of 9α-fluorohydrocortisone in 100 ml. of methanol was added a hot solution of 1.5 g. of cupric acetate monohydrate in 50 ml. of methanol containing 1 ml. of glacial acetic acid. After refluxing 30 minutes, 50 ml. of water was added and heating continued for 30 minutes. The reaction mixture was filtered through diatomaceous earth, the filter cake washed with methanol, and the combined filtrate and washes diluted with 25 ml. of water and concentrated in vacuum to about 20% of the original volume. The crystals formed were aged at 4° for 1 hour, filtered, and washed with water, then dried overnight in vacuum at 40°. The yellow crystalline product, 610 mg., gave positive tests with Tollens and Schiff reagents. Recrystallization of the aldehyde from aqueous methanol, from aqueous dimethylformamide, and twice again from aqueous methanol gave the pure hydrated aldehyde, decomposing 240–242° without melting (sintering beginning at 130°), $[\alpha]^{22}D$ + 152°, λ_{max} 239 mμ (ε 17,150); λ_{max}^{KBr} 2.90, 5.88, 6.10, 9.65μ, etc.[25]; paper chromatogram mobility in system II: R_f 0.43 (*vs.* R_f 0.52 for III); negative reaction to alkaline tetrazolium blue, positive to isonicotinic acid hydrazide, immediate yellow color with the Porter–Silber reagent on papergrams.[26]

Anal. Calcd. for $C_{21}H_{29}O_6F$: C, 63.62; H, 7.37; F, 4.79. Found: C, 63.51, 63.73; H, 7.79, 7.53; F, 5.06.

9α-Fluoro-11β,17α-dihydroxy-21-oximino-4-pregnene-3,20-dione (X).—A solution of 1.0 g. of the hydrated aldehyde IX and 0.20 g. (1.1 equiv.) of reagent hydroxylamine hydrochloride in 50% ethanolic pyridine was warmed on a steam-bath for 90 minutes. The reaction mixture was concentrated to a small volume in vacuum and water was added to precipitate a gum. The gum was washed several times by decantation with water, and was then crystallized from aqueous methanol. The crude oxime, 625 mg., m.p. 195–197° dec., was recrystallized several times from aqueous methanol to give the analytical sample as a methanol solvate, m.p. 210–211° dec., $[\alpha]^{22}D$ + 165°, λ_{max} 235 mμ. (ε 25,600); λ_{max}^{KBr} 2.85, 3.05, 5.90, 6.16, 6.97, 9.60 μ, etc.

Anal. Calcd. for $C_{21}H_{28}O_6NF \cdot CH_3OH$: C, 62.10; H, 7.55; N, 3.29; F, 4.47. Found: C, 62.10; H, 7.49; N, 5.31 (Dumas), 2.23 (Kjeldahl); F, 4.49.

21-Acetylamino-9α-fluoro-11β,17α-dihydroxy-4-pregnene-3,20-dione (II). Chemical Source.—To a stirred solution of 100 mg. of the 21-aldehyde oxime X, 20 mg. of sodium acetate and 5 mg. of mercuric chloride in a mixture of 6 ml. of glacial acetic acid and 5 ml. of acetic anhydride was added 300 mg. of zinc dust. The addition of zinc was made in small portions over 30 minutes. Stirring was continued for 90 minutes after addition was complete. The reaction mixture was filtered from zinc, the cake washed with glacial acetic acid, and the combined filtrates and washes were concentrated to near dryness in vacuum. A small quantity of solids precipitated on addition of water to the residue, and these were filtered off. The filtrate was extracted several times with methylene chloride and the combined extracts washed well with water, dried over anhydrous magnesium sulfate, and evaporated in vacuum to yield 100 mg. of a residue. Crystallization from acetone–petroleum ether gave the crude amide II, m.p. 243–248°. A second experiment using 600 mg. of the oxime was conducted, and the crude amide therefrom recrystallized several times from ethyl acetate–petroleum ether and from aqueous pyridine to give the analytical sample, m.p. 262.5–264° dec., identical in physical properties with the amide isolated from the biological source.

Anal. Calcd. for $C_{23}H_{32}O_5NF$: C, 65.54; H, 7.65; N, 3.32; F, 4.51. Found: C, 65.18; H, 7.63; N, 3.19 (Kjeldahl); F, 4.64.

21-Amino-9α-fluoro-11β,17α-dihydroxy-4-pregnene-3,20-dione (XI) Hydrochloride.—A solution of 500 mg. of the oxime X in 50 ml. of glacial acetic acid was treated with 1.0 g. of zinc dust added over a period of 1 hour. The reaction mixture was then filtered from zinc and the filtrate was evaporated to near dryness under reduced pressure. Dilution of the concentrate with 50 ml. of water produced a yellow precipitate which was filtered off. The filtrate was further diluted with an equal volume of methanol and passed through a column of analytical grade Dowex 1-X2 anion exchange resin in the chloride form. The eluates giving a positive tetrazolium blue test were combined and evaporated under reduced pressure. The residue was dissolved in a mixture of 1-butanol and water and the phases were separated. The aqueous phase was extracted twice with butanol, and the combined butanol extracts were washed three times with saturated salt solution and then dried over anhydrous magnesium sulfate. To the dried extracts was added 50 ml. of concentrated hydrochloric acid; however, a precipitate did not occur on dilution with diethyl ether and aging. The acidified mixture was evaporated under vacuum and the glassy residue solidified on trituration with diethyl ether, yielding 550 mg. of solids, λ_{max} 239 mμ ($E^{1\%}_{1cm}$ 261). The salt was recrystallized three times from methanol–diethyl ether, giving white needles, browning but not melting below 300°, λ_{max} 238 mμ ($E^{1\%}_{1cm}$ 370); λ_{max}^{KBr} 2.95–4.0 (multiple bands), 5.80, 6.03–6.30 (multiple bands), 8.04, 8.80, 8.94, 9.14, 9.56, 11.20, 11.53 μ, etc.

Anal. Calcd. for $C_{21}H_{30}O_4NF \cdot HCl \cdot 2H_2O$: C, 55.81; H, 7.81; N, 3.10; F, 4.20; Cl, 7.85. Found: C, 56.32; H, 8.39; N, 3.26 (Dumas); F, 4.23; Cl, 7.71.

Attempted isolation of other salts of the amine XI generally led to poor, non-crystalline preparations with low ultraviolet extinctions, difficult to purify. Reduction of 100 mg. of oxime using the exact directions employed in the synthesis of the amide II but without the acetic anhydride being added, filtration of the zinc, diluting with ether and filtering off insolubles, and addition of 50 μl. of 70% perchloric acid led to an eventual crystallization of the amine hydroperchlorate, 65 mg., m.p. 206–210°. Two recrystallizations from acetone–petroleum ether raised the melting point to 211–213°, λ_{max} 238 mμ ($E^{1\%}_{1cm}$ 296).

Acetylation of either hydrochloride or hydroperchlorate salt with acetic anhydride and pyridine gave the 21-acetylamino compound II, with no other reaction product detected on paper chromatograms. The homogeneity of each preparation was conveniently checked by acetylation and paper chromatography, and in all cases examined only one component was found identified as the amide II.

The amine XI was essentially immobile in most of the Bush-type paper chromatographic solvent systems used; however, suitable mobilities were obtained using Whatman No. 1 filter paper impregnated with *p*H 2 phosphate buffer developed with chloroform–butanol–0.3 *M* monobasic sodium phosphate (*p*H 2) (3:1:2), a system of use in the separation of some tetracycline antibiotic derivatives.[27] The hydrochloride and hydroperchlorate salts of XI moved *ca.* 10 cm./6 hr.

The amine XI salts give typical violet colors with alkaline tetrazolium blue and intense yellow colors with 0.2% ninhydrin (the amides II and IV are negative to tetrazolium blue and ninhydrin on papergrams).

9α-Fluoro-11β,17α-dihydroxy-21-phthalimido-4-pregnene-3,20-dione (XII).—A solution of 50 mg. of the amine XI hydrochloride, 50 mg. of phthalic anhydride and 50 mg. of sodium acetate in 5 ml. of glacial acetic acid was heated for 30 minutes at reflux. The reaction solution was cooled, diluted with 20 ml. of water, and the resulting precipitate filtered. The solids were washed with water and dried, yielding 30 mg., λ_{max}^{KBr} 2.84, 3.41, 5.68, 5.78, 6.04, 7.10, 13.88 μ, etc., identical with an authentic sample of XII previously prepared by another route.[17]

21-Acetylamino-9α-fluoro-11β-hydroxy-1,4,16-pregnatriene-3,20-dione (V).—To a solution of 100 mg. of IV in

(23) H. N. Rydon and P. W. G. Smith, *Nature*, **169**, 922 (1952); S. C. Pan and J. D. Dutcher, *Anal. Chem.*, **28**, 836 (1956); J. J. Wren and H. K. Mitchell, *J. Biol. Chem.*, **234**, 2823 (1959).

(24) M. R. J. Salton, *Biochim. Biophys. Acta*, **34**, 308 (1959).

(25) The aldehyde IX has been mentioned in the patent literature without adequate physical description; see Belgian Patent 574,645, British Patent 791,065, February 19, 1958; M. Tishler, U. S. Patent 2,982,774, May 2, 1961.

(26) M. K. Birmingham, *Nature*, **184**, 67 (1959).

(27) J. R. D. McCormick, E. R. Jensen, P. A. Miller and A. P. Doerschuk, *J. Am. Chem. Soc.*, **82**, 3381 (1960).

2 ml. of absolute ethanol was added 10 ml. of concentrated hydrochloric acid. After 24 hours of agitation at room temperature the mixture was extracted several times with methylene chloride, the combined extracts were washed with aqueous sodium bicarbonate solution, dried over anhydrous magnesium sulfate, and evaporated in vacuum. The residue weighed 10 mg. The aqueous reaction mixture was neutralized with sodium hydroxide solution and extracted three times with ethyl acetate, the combined extracts were washed with sodium bicarbonate solution, with brine, and then dried and evaporated in vacuum. The residue, weighing 125 mg., was combined with the previous residue isolated and chromatographed on Celite diatomaceous earth using a solvent system benzene–acetone–water (2.5:1:2). The appropriate fractions were combined and evaporated, yielding 30 mg. of crystals analyzing by paper chromatography as a mixture of a major component (product V) and a minor component (starting material IV). An additional 30 mg. of material was eluted, which analyzed as mainly unaltered starting material. The first fraction was recrystallized from aqueous alcohol and from alcohol, yielding the 16-dehydro amide, m.p. 228–232° dec., λ_{max} 238 mμ (ε 22,900); λ_{max}^{KBr} 2.87, 2.97, 6.03, 6.22, 6.32, 6.51 μ, etc.

Anal. Calcd. for $C_{23}H_{28}O_4NF$: C, 68.80; H, 7.03. Found: C, 68.25; H, 7.32.

21-Acetylamino-9α-fluoro-11β,17α,20β-trihydroxy-1,4-pregnadien-3-one (VI).—A solution of 100 mg. of 21-acetylamino-9α-fluoro-11β,17α-dihydroxy-1,4-pregnadiene-3,20-dione in 5 ml. of methanol and 5 ml. of dimethylformamide was cooled to 0°, and 13 mg. of sodium borohydride was added. After 90 minutes (occasional shaking, 0°) 0.5 ml. of glacial acetic acid was added. The reaction mixture was analyzed by paper chromatogram and shown to contain one non-reducing product at R_f 0.16 (system II), reactive toward isonicotinic acid hydrazide. No unaltered amide II (R_f 0.65) was detected.

9α-Fluoro-11β-hydroxy-1,4-androstadiene-3,17-dione (VII). A. From 21-Acetylamino-9α-fluoro-11β,17α,20β-trihydroxy-1,4-pregnadien-3-one.—The reaction mixture from the previous experiment, containing VI as the only detectable steroid, was mixed with 50 ml. of a solution containing 4% by weight of trichloroacetic acid in 75% aqueous ethanol. To this was added 1.7 g. of sodium bismuthate and the mixture was agitated for 75 minutes at room temperature. The reaction mixture was adjusted to near neutrality by careful addition of strong sodium hydroxide solution. The solids which precipitated were filtered and washed with three portions of hot acetone, and the combined filtrates and washes were concentrated in vacuum until the organic solvent had evaporated. Two drops of concentrated hydrochloric acid was added to ensure an acid reaction and the solution was held at 4° overnight. Paper-gram examination of the solution indicated that no reaction had occurred and that the starting material VI was the major component in the preparation. The solution was extracted with methylene chloride, the extracts washed with saturated aqueous sodium bicarbonate solution, dried over magnesium sulfate, and evaporated in vacuum, and the residue again treated with sodium bismuthate in the same proportions as used before, except the reaction ran for 5 hours. Work-up of the reaction mixture in the same way as before yielded a residue which analyzed by paper chromatography as containing starting material VI and a more mobile component. The residue was chromatographed on Florisil; elution with methylene chloride–5% acetone gave 28 mg. of a material which, on recrystallization from acetone–petroleum ether, gave 16 mg. of 9α-fluoro-11β-hydroxy-1,4-androstadiene-3,17-dione, m.p. 244–246°, identified by comparison with the authentic sample prepared under B below.

B. **From 9α-Fluoro-11β,17α,21-trihydroxy-1,4-pregnadiene-3,20-dione.**—To a solution of 600 mg. of 9α-fluoro-11β,17α,21-trihydroxy-1,4-pregnadiene-3,20-dione in 350 ml. of a 4% solution of trichloroacetic acid in 75% aqueous ethanol was added 17 g. of sodium bismuthate. The resulting slurry was shaken for 90 minutes, at which time a sample withdrawn from the mixture failed to give a positive test with alkaline tetrazolium blue. The reaction mixture was adjusted to near neutrality with strong sodium hydroxide solution, the solids filtered, the filter cake extracted thoroughly with hot acetone, and the combined filtrates and acetone washes concentrated in vacuum to remove the organic solvent. The aqueous solution was acidified with 4 drops of concentrated hydrochloric acid and cooled at 4°. The product crystals were collected in two crops, 90 mg., m.p. 246–249.5°, and 135 mg., m.p. 244.5–249°. Extraction of the mother liquor with methylene chloride after making alkaline gave an additional 148 mg., m.p. 248–250°. Recrystallization from acetone–petroleum ether several times gave the pure 9α-fluoro-11β-hydroxy-1,4-androstadiene-3,17-dione, λ_{max} 236 mμ (ε 14,720); λ_{max}^{KBr} 2.88, 5.75, 6.01, 6.20, 9.73, 11.17 μ, etc.

Anal. Calcd. for $C_{19}H_{23}O_3F$: C, 71.68; H, 7.28; F, 5.97. Found: C, 71.41; H, 7.49; F, 6.10.

Both samples were identical with another sample, m.p. 247–251° (capillary), prepared by R. H. Lenhard of these laboratories by selenium dioxide dehydrogenation of 9α-fluoro-11β-hydroxy-4-androstene-3,17-dione. Identity of all three samples was established by comparisons of infrared spectra, melting point behavior, paper chromatographic mobility and color test behavior (positive Zimmermann test).[28]

The mobility of the 17-ketone VII was: system IV, R_f 0.86; system V, R_f 0.56; system VI, R_f 0.29.

(28) A. Nobile, U. S. Patent 2,955,118, October 14, 1960, describes 9α-fluoro-11β-hydroxy-1,4-androstadiene-3,17-dione, m.p. 252–253°, $[\alpha]_D$ +113.6° (dioxane).

[CONTRIBUTION FROM THE MERCK SHARP & DOHME RESEARCH LABORATORIES, DIVISION OF MERCK & CO., INC., RAHWAY N. J.]

A General Synthesis of 4,5-Unsaturated 2-Oxasteroids. A Synthesis of 2-Oxacortisol[1]

BY RALPH HIRSCHMANN, N. G. STEINBERG AND ROBERT WALKER

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The synthesis of 2-oxacortisol and its 9α-chloro-16α-methyl analog is described. Osmylation of 17α,20;20,21-bismethylenedioxy-1,4-pregnadiene-3,11-dione (prednisone BMD) followed by lead tetraacetate cleavage gave the required tricyclic steroid derivative III. Saponification led to the sodium salt of the aldehydic acid which was reduced with hydride under carefully controlled conditions to afford the BMD of 2-oxacortisol. In the 9α-fluoro series osmylation took place primarily at the 4,5-double bond.

The decade after the discovery of the therapeutic effects of cortisone in the treatment of rheumatoid arthritis has witnessed a great effort to prepare analogs which are superior to the hormone, cortisol. The syntheses of the very potent 16-epimeric 9α-fluoro-11β,17,21-trihydroxy-16-methyl-pregna-1,4-dien-3,20-diones[2] are examples of recent advances

(1) Presented in part at the 140th Meeting of the American Chemical Society, Chicago, Ill., September, 1961.

(2) G. E. Arth, J. Fried, D. B. R. Johnston, D. R. Hoff, L. H. Sarett, R. H. Silber, H. C. Stoerk and C. A. Winter, *J. Am. Chem. Soc.*, **80**, 3161 (1958); D. Taub, R. D. Hoffsommer, H. L. Slates and N. L.