described[12] using a *Neurospora sp.* (M 714). The methylene chloride extracts were concentrated to dryness, dissolved in 10% ethyl acetate in benzene and chromatographed on 1400 g. of silica gel. The chromatographic column was eluted successively with 10%, 12%, 15%, 25%, 35%, 40%, and 50% ethyl acetate in benzene. The 50% eluates were concentrated to dryness and the residue was crystallized from acetone-cyclohexane, then from methanol, and finally from acetone-cyclohexane again to yield 394 mg. of 7α-hydroxy-4-androstene-3,17-dione, m.p. 255–256.5°, $\lambda_{max}^{CH_3OH}$ 241 mμ, ε 16,000. The maximum in the ultraviolet spectrum in 0.10*N* methanolic potassium hydroxide shifted on standing from 241 mμ to 283 mμ.

Anal. Calcd. for: $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.36; H, 8.63.

7α-Acetoxy-4-androstene-3,17-dione. 7α-Hydroxy-4-androstene-3,17-dione, 0.10 g., was dissolved in 1.5 ml. of acetic anhydride and 3 ml. of pyridine and allowed to stand overnight at room temperature. Then the solution was concentrated under vacuum at 40–50°. Toluene was added and distilled twice followed by ether–petroleum ether (b.p. 35–40°). The residue solidified and was crystallized from acetone–petroleum ether (b.p. 65–70°) to yield 0.07 g. of 7α-acetoxy-4-androstene-3,17-dione, m.p. 177–179°, $\lambda_{max}^{CH_3OH}$ 238 mμ, ε 15,700.

Anal. Calcd. for: $C_{21}H_{28}O_4$: C, 73.23; H, 8.19. Found: C, 73.21; H, 8.18.

Sources of previously reported compounds. 7β-Hydroxy-4-androstene-3,17-dione was one of the products from a fermentation of 4-androstene-3,17-dione using *Rhizopus stolonifer*, ATCC No. 6227-B. The 7-position was proved by the shift of the ultraviolet maximum in base from 241 to 283 mμ. 7β,21-Diacetoxy-17α-hydroxy-4-pregnene-3,20-dione was isolated after acetylation of a portion of the product obtained by the action of a *Penicillium sp.*, ATCC No. 12556 on 17β,21-dihydroxy-4-pregnene-3,20-dione. We were unable to separate the free trihydroxy compound from a mixture with other products. The action of the same organism on progesterone produced 7β,15β-dihydroxy-4-pregene-3,20-dione which was selectively acetylated to give the 7β-monoacetate.[2d]

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

16α-Hydroxysteroids. X.[1] 2β-Hydroxylation of 9α-Fluorohydrocortisone by *Streptomyces roseochromogenus*

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9α-Fluorohydrocortisone is hydroxylated by *Streptomyces roseochromogenus* in the 16α- position, the 2β- position, and in both the 2β- and 16α- positions.

The fermentative 16α-hydroxylation of 9α-fluorohydrocortisone I by *Streptomyces roseochromogenus*[3] is accompanied by a complex spate of steroidal by-products. Isomerization of the major product 9α-fluoro-16α-hydroxyhydrocortisone II, has already been described.[1,4] The present paper deals with other reducing steroids formed in the fermentation. A later communication will deal with nonreducing steroids elaborated.

Paper chromatographic examination of broth extracts revealed the presence of several reducing steroids including 9α-fluoro-16α-hydroxyhydrocortisone, contaminated with a reducing steroid III of slightly less mobility, the D-homoannulation product 9α-fluoro-11β,16α,17aα-trihydroxy-17aβ-hydroxymethyl-4-D-homoandrostene-3,17-dione IV, and a still more polar component V.

Isolation of the steroid III was accomplished from enriched mother liquors from which remaining 16α,17α-diol II and other 16α,17α-diols were removed by extraction with aqueous sodium borate solution. The new monohydroxylated 9α-fluorohydrocortisone III was distinguished from other known monohydroxylated (1ξ-,[5] 6β-,[6] 16α-[7]) 9α-fluorohydrocortisones. The diacetate of III is further distinguished from the known diacetate of 9α-fluoro-16β-hydroxyhydrocortisone.[8]

The more polar steroid V was extracted into aqueous borate and was thus recognized as being 16α-hydroxylated. Satisfactory isolation from the borate extract was not possible as boron was present in the preparation after acidification and chromatography. A cyclic 16α,17α-acetonide VI was formed both by conventional means[7,9] using crystalline mixtures containing V and by micro reaction on

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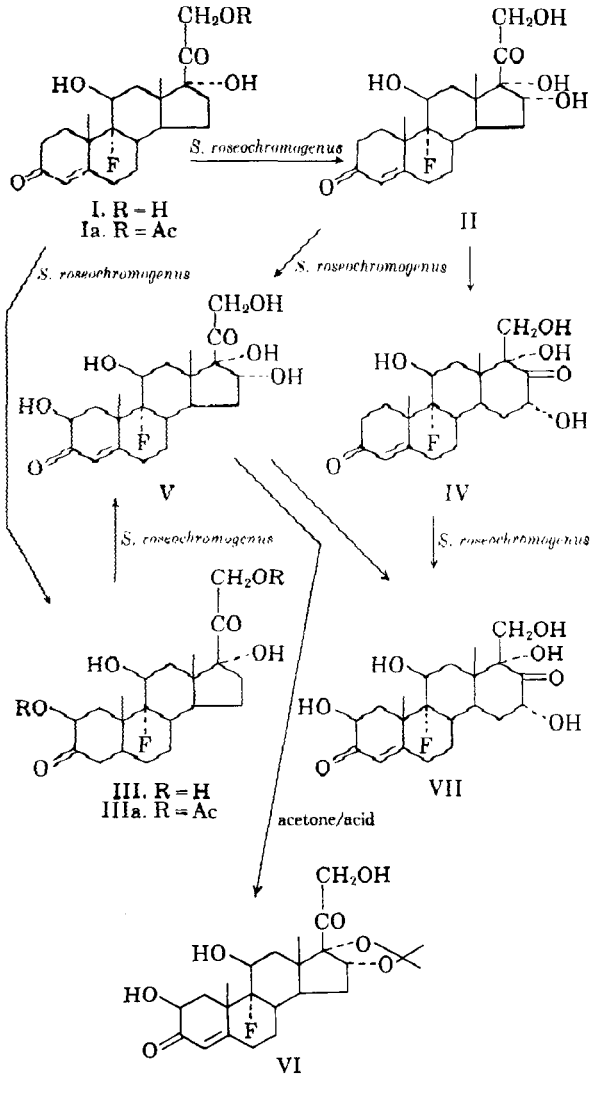
filter paper.[10] Both the free pentaol V and its acetonide VI were isolated from papergrams and centigram amounts of V were prepared by extended fermentation with a selected strain of *S. roseochromogenus*. Fermentation of the monohydroxy 9α-fluorohydrocortisone III under the same conditions also resulted in the formation of the pentaol V, as did fermentation of 9α-fluoro-16α-hydroxyhydrocortisone.

The compound III is thus an *x*-hydroxy-9α-fluorohydrocortisone, V is the *x*,16α-dihydroxy-9α-fluorohydrocortisone, and VI is the *x*, 16α-dihydroxy-9α-fluorohydrocortisone 16α,17α-acetonide.



The position of attachment of the *x*-hydroxyl group was determined by studies in alkaline ethanol at 60° followed by acidification of the solutions.[11] Spectral behavior typical of 2-hydroxy-Δ⁴-3-ketones was obtained in each case. The 2β-hydroxy orientation was assigned on the basis of the strong levorotatory contributions to the molecular rotation exhibited by the several steroids in comparison with the non-2β-hydroxylated analogs; thus the $\Delta[M]_D$ calculated for III, V, and VI are −519, −537, and −540 respectively. Other 2β-hydroxy-Δ⁴-3-ketones on record have $\Delta[M]_D$ of −535 to −768.[12]

The alkaline spectra of 9α-fluoro-2β,16α-dihydroxyhydrocortisone V is of interest in that the anticipated shift of the 238 mμ band to shorter wave length occurs, with an inflection in the 250 mμ region, but the anticipated band near 355 mμ (characteristic of 2β-hydroxy-Δ⁴-3-ketones in general[11] and of the other 2β-hydroxy steroids III, IIIa, and VI) is present as an inflection at 360 mμ. The 310 mμ band displayed by other 16α,17α,21-trihydroxy-20-ketosteroids[13,14] and their D-homoannulated isomers[4] in alkaline ethanol is also present in the spectra of the pentaol V, superimposed on the spectral detail arising from the 2β-hydroxy-Δ⁴-3-ketone functional group. The 16α,17α-acetonide VI lacks selective absorption in the 310–320 mμ region.

Examination of the infrared spectra of the 2β-hydroxylated steroids III, IIIa, V, and VI indicates a slight decrease in wave length (increased frequency) in the Δ⁴-3-ketone band position in comparison with the non-2β-oxygenated analog.[15] Vicinal interactions characteristic of α-ketol acetates have been reported for both 2α- and 2β-acetoxy-Δ⁴-3-ketones[12,16] and also for certain 2α- and 2β-hydroxy-Δ⁴-3-ketones.[12a,b,d,16c]

Among the poorly resolved, very polar steroids is one (VII) whose mobility suggests it to be the rearrangement isomer of 9α-fluoro-2β,16α-dihy-

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(15) The Δ⁴-3-ketone bands for 2β-hydroxylated and non-2β-hydroxylated analogs are: III, 5.97 μ, I, 6.00 μ, IIIa, 5.95 μ, Ia, 6.07 μ, V, 5.93 μ, II, 5.98 μ, VI, 5.93 μ, 9α-fluoro-16α-hydroxyhydrocortisone 16α,17α-acetonide, 6.02 μ.

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droxyhydrocortisone. The same component VII was also observed to form during isolation of 9α-fluoro-2β,16α-dihydroxyhydrocortisone *via* elution from paper chromatograms and by silica gel chromatography. This component, while not isolated and characterized, is formulated as 9α-fluoro-2β,-11β,16α,17aα-tetrahydroxy-17aβ-hydroxymethyl-4-D-homoandrostene-3,17-dione VII.

Some confirmation of the assigned structure is had in that fermentation of 9α-fluoro-11β,16α,-17aα-trihydroxy-17aβ-hydroxymethyl-4-D-homoandrostene-3,17-dione IV[4] with *S. roseochromogenus* affords a component with chromatographic and color test behavior identical with that of the component derived from the pentaol V. Also, fermentations of 9α-fluorohydrocortisone with 2β-hydroxylating strains of *S. roseochromogenus* conducted in the presence of iron wire[1] led to the final product isomer VII.

2β-Hydroxylation is thus as general a transformation in these strains of *S. roseochromogenus* as is 16α-hydroxylation. 2β-Hydroxylation of Δ[4]-3-ketosteroids by other *Streptomyces* species and by other microorganisms has been reported.[12,17] Early work involving 16α-hydroxylation of progesterone with actinomycete MD-2428[18] and with *S. argenteolus* ATCC 11,009[19] led to a progesterone diol which appears to be the 2β,16α-diol on the basis of the published optical rotation.

Dehydration of the 2β-hydroxylated steroids III, IIIa, V, and VI by a variety of acidic conditions could not be accomplished despite implications in the literature.[20] To date no authentic report of the preparation of a Δ[1,4]-3-ketosteroid from a 2β-hydroxy-Δ[4]-3-ketone has been found.

EXPERIMENTAL[21]

Papergram examination of a variety of *S. roseochromogenus* fermentations with 9α-fluorohydrocortisone as substrate indicated the regular presence of six reducing components, catalogued by their papergram mobility (R value) in System II relative to 9α-fluoro-16α-hydroxycortisone as unit mobility. The components are: R 1.6, R 1.00 (major product), R 0.9, R 0.58, R 0.33, and R 0.15. The R 1.6 component was recognized as unaltered substrate I, the R 1.00 component was 9α-fluoro-16α-hydroxyhydrocortisone II.

Isolation studies were made on solvent extract concentrates of *S. roseochromogenus* fermentations run according to the conditions of Fried *et al.*[22] The harvest broth was extracted several times with methyl isobutyl ketone, the extracts pooled and concentrated to incipient crystallization, and the product II filtered. Further concentration, crystallization, and filtration removed most of the major product II. The mother liquor (*ca.* 15% steroid by polarography) contained the R 0.9, R 0.58, R 0.33, R 0.15 components together with nonreducing steroids. A 150-ml. portion of the mother liquor was diluted with 300 ml. of methyl isobutyl ketone and extracted with six 600-ml. portions of 5% aqueous sodium tetraborate solution. The organic extracts were concentrated in vacuum and the residue slurried with 80 g. of silica gel and benzene was added. The residual methyl isobutyl ketone was removed by further evaporation, and the total mass was added to a column of 950 g. of silica gel prepared in benzene. Elution with 25–50% ether in benzene afforded 421 mg. of an unidentified phenol VIII, recrystallized from acetone/benzene, m.p. 290–293° dec.; $[\alpha]_D^{22}$ +2.5°; λ_{max} 262 mμ ($E_{1\,cm}^{1\%}$ 1268), 290 mμ ($E_{1\,cm}^{1\%}$ 422, shoulder), 330 mμ ($E_{1\,cm}^{1\%}$ 127, shoulder); λ_{min} 231 mμ; λ_{max}^{KBr} 2.95 μ, 3.18 μ, 3.40 μ, 3.53 μ, 3.85 μ, 4.00 μ, 6.03 μ, 6.19 μ, etc.

Anal. Found: C, 66.46; H, 4.27. Calcd. for $(C_8H_7O_3)_x$: C, 66.25; H, 4.32.

Elution with 50–75% ether in benzene afforded 449 mg. of another unidentified phenol IX, crystallized from methanol, subliming *ca.* 270°; optically inactive; λ_{max} 237–238 mμ. ($E_{1\,cm}^{1\%}$ 938, inflection), 248 mμ ($E_{1\,cm}^{1\%}$ 985), 292–301 mμ ($E_{1\,cm}^{1\%}$ 415, plateau); $\lambda_{max}^{0.01\ N\ NaOH}$ 258 mμ. ($E_{1\,cm}^{1\%}$ 1154), 290 mμ ($E_{1\,cm}^{1\%}$ 726), 331 mμ ($E_{1\,cm}^{1\%}$ 600); λ_{max}^{KBr} 3.10 μ, 3.35 μ, 3.43 μ, 3.50 μ, 3.70 μ, 3.80 μ, 4.00 μ, 6.12 μ, 6.25 μ, etc.

Anal. Found: C, 70.18; H, 4.35. Calcd. for $(C_{11}H_8O_3)_x$: C, 70.21; H, 4.29.

Both phenols VIII and IX were detected on papergrams (R_f 0.86 and 0.60 respectively in System II) by their ultraviolet light absorption properties. Neither responded to the steroidal detection reagents routinely employed.[23]

9α-Fluoro-2β,11β,17α,21-tetrahydroxy-4-pregnene-3,20-dione III. Elution with 5–75% chloroform in ether afforded a reducing steroid with about 90% of the mobility of the unit marker 9α-fluoro-16α-hydroxyhydrocortisone. After concentration of the fractions in vacuum the residue was crystallized from methanol/ether (1:5), yielding 198 mg. of crystals, homogeneous on papergrams. A second crop of 30 mg. was recovered from the filtrate, also homogeneous on papergrams. Recrystallizations from methanol and from acetone (with charcoal treatment) gave the analytical samples; m.p. 245–249°; $[\alpha]_D^{22}$ +1.5°; λ_{max} 239 mμ (ε 13,950); $\lambda_{max}^{H_2SO_4}$ ($E_{1\,cm}^{1\%}$) 2 hr., 255 mμ (224), 338 mμ (425), 411 mμ (173), 470 mμ (179); λ_{max}^{KB} 3.00μ , 3.40 μ, 5.85 μ, 5.97 μ, 6.14 μ, 8.10 μ, 8.80 μ, 9.10 μ, 9.16 μ, 9.57 μ, 11.25 μ, 11.45 μ, etc.

Anal. Calcd. for $C_{21}H_{29}O_6F$: C, 63.62; H, 7.37; F, 4.79. Found: C, 63.48; H, 7.50; F, 4.84.

Spectra in alkaline ethanol according to Meyer were recorded: $\lambda_{max}^{0.06\ N\ NaOH}$ ($E_{1\,cm}^{1\%}$) at 60°: 1 hr., 229 mμ (410), 250 mμ (175, inflection), 355 mμ (45), 4 hr., 229 mμ (410), 250 mμ (146, plateau), 360 mμ (60), not changed up to 6 hr. On acidification, λ_{max} 251 mμ and 300 mμ.

Paper chromatographic mobility of the 2β-hydroxylated 9α-fluorohydrocortisone in several solvent systems[22] was

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(20) H. L. Herzog, U. S. Patent 2,857,406, October 21, 1958; M. E. Wolff and C. B. Karash, *J. Org. Chem.*, 24, 1612 (1959).

(21) Melting points were taken on a Kofler block unless noted otherwise. Optical rotations were made on *ca.* 0.5% solutions in methanol or other specified solvent. Ultraviolet absorption spectra were recorded in absolute ethanol (other solvents specifically mentioned) with a Cary Model 11S Recording Spectrophotometer. Infrared spectra in macro (1 mg. sample in 200 mg. potassium bromide) and in micro (100 μg. sample in 20 mg. potassium bromide) disks were obtained with the Perkin-Elmer Model 21 double beam instrument. Paper chromatographic procedures employed have been previously described.[22]

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(23) J. Fried, D. Perlman, A. F. Langlykke, and E. O. Titus, U. S. Patents 2,855,343 and 2,855,410, October 7, 1958; see also references 1, 3, and 19.

compared with other monohydroxylated 9α-fluorohydrocortisones.

x-Hydroxy-9α-fluorohydrocortisone	System I	System II	System III	System IV
1ξ-Hydroxy	1.04	1.03	1.10	1.28
2β-Hydroxy	0.94	0.93	1.1	1.00
6β-Hydroxy	0.35	0.48	0.42	—
16α-Hydroxy	1.00	1.00	1.00	1.00

The rate of development of the diformazan color with alkaline tetrazolium blue for 9α-fluoro-2β-hydroxyhydrocortisone was identical with the rate for 9α-fluorohydrocortisone, with maximum color developed by 30 min. Porter-Silber assay[24] of 9α-fluoro-2β-hydroxyhydrocortisone gave λ_{max} 415 mμ ($E^{1\%}_{1cm.}$ 338), of 9α-fluorohydrocortisone run at the same time, λ_{max} 412–415 mμ ($E^{1\%}_{1cm.}$ 288).

2β,21-Diacetoxy-9α-fluoro-11β,17α-dihydroxy-4-pregnene-3,20-dione IIIa. Fifty milligrams of III was acetylated in the usual manner with acetic anhydride/pyridine, yielding 40 mg. of the crystalline diacetate IIIa, homogeneous on papergrams, m.p. 132–140°. Recrystallization from acetone and from methanol gave the pure diacetate methanol solvate m.p. 132–140.5°; $[\alpha]_D$ + 48.7°; λ_{max} 239 mμ (ε 14,520); $\lambda^{H_2SO_4}_{max}$ ($E^{1\%}_{1cm.}$) 2 hr., 259 mμ (211), 338 mμ (304), 410 mμ (179), 445 mμ (176, inflection), 467 mμ (179); λ^{KBr}_{max} 2.90 μ, 3.40 μ, 5.73 μ, 5.78 μ, 5.95 μ, 6.15 μ, 7.28 μ, 7.28 μ, 7.95 μ, 8.13 μ, 9.53 μ, 11.31 μ, etc.

Anal. Calcd. for $C_{25}H_{33}O_8F \cdot CH_4O$: C, 60.92; H, 7.08; F, 3.71. Found: C, 60.61, 60.74; H, 7.17, 7.26; F, 3.66.

Papergram mobility of the 2β,21-diacetate IIIa, of 9α-fluorohydrocortisone 21-acetate Ia and of triamcinolone 16α,21-diacetate are: System III, R_f 0.92, 0.86, 0.84; System V, R_f 0.51, 0.38, 0.26.

For isolation of the R 0.58 component, suspected of being the rearrangement product of 9α-fluoro-16α-hydroxyhydrocortisone, preparative paper chromatography of the extract concentrate was used.

9α-Fluoro-11β,16α,17aα-trihydroxy-17aβ-hydroxymethyl-4-D-homoandrostene-3,17-dione IV. The extract concentrate dissolved in ethyl acetate was adsorbed onto silica gel, washed with ethyl acetate, and eluted (no fractionation) with methanol. The concentrated methanol eluates were streaked onto 18 sheets of Whatman No. 1 filter paper (18 cm. wide, previously washed chromatographically with methanol) which were then developed chromatographically in System IV[22] (4 hr.). Four major bands were present (as detected by ultraviolet light absorption on paper) with R_f values at their centers of R_f 0.4, 0.36, 0.29, and 0.15. Elution of the polar regions (R_f less than 0.2) with hot methanol, concentration of the eluates and rechromatography in System II (4–6 hr.) resolved four zones with R_f 0.50, 0.36, 0.29, and 0.12. The R_f 0.29 zone was eluted with hot methanol, rechromatographed in System II (5 hr.), and the single zone eluted and evaporated. The residue was dissolved in acetone and cautiously evaporated. At a volume less than 200 μl. ether was added and the precipitated crystals centrifuged, decanted, dried, etc. Infrared spectra (100 μg. sample) indicated identity of the component with the D-homo isomer IV,[3] as did paper chromatographic behavior.

9α-Fluoro-2β,11β,16α,21-pentahydroxy-4-pregnene-3,20-dione V. (A) *From 9α-fluorohydrocortisone.* To a 24-hr. vegetative growth of *S. roseochromogenus ATCC* 3347 grown in 30 l. of medium containing 750 g. of corn steep liquor, 1200 g. of starch, 150 g. of calcium carbonate, 30 g. of dipotassium hydrogen phosphate, and 60 ml. of lard oil was added a solution of 7.5 g. of 9α-fluorohydrocortisone dissolved in 100 ml. of dimethylformamide. Fermentation was continued for 88 hr. at which time the total steroid content of the broth was 132 μg./ml. as assayed colorimetrically with tetrazolium blue. Quantitative papergram analysis indicated that the 9α-fluoro-2β,16α-dihydroxyhydrocortisone content was *ca.* 100 μg./ml.

The harvested fermentation broth was adjusted to *p*H 4.3 with 50% sulfuric acid, slurried with 2360 g. of diatomaceous earth filter aid, and filtered. The filter cake was reslurried with 30 l. of water (*p*H 3.6) and refiltered. The combined filtrates were readjusted to *p*H 6.4 with 20% sodium carbonate solution, then extracted three times with 25-l. portions of methyl isobutyl ketone. From the pooled extracts 4.1 g. of brown solids was isolated. The crude solids were dissolved in 45 ml. of pyridine, charcoal added, filtered, diluted with water, and concentrated *in vacuo*. The solids recovered were extracted with three portions of ethyl acetate, the ethyl acetate extracts evaporated, and the residue crystallized from acetone by addition of water, yielding 135 mg. of crystals assaying by paper chromatography as a mixture of 9α-fluoro-16α-hydroxyhydrocortisone II and 9α-fluoro-2β,16α-dihydroxyhydrocortisone V. From the filtrate a gummy residue was recovered which was crystallized from acetone, yielding 265 mg. of crystalline V contaminated with traces of II. After several further recrystallizations from hot acetone the last traces of contaminating II were removed and the analytical sample obtained, m.p. 220–223°; $[\alpha]^{22}_D$ −26.5°; λ_{max} 238 mμ (ε 14,700); $\lambda^{H_2SO_4}_{max}$ ($E^{1\%}_{1cm.}$) 2 hr., 280 mμ (103, inflection), 339 mμ (544); λ^{KBr}_{max} 2.90 μ, 2.95 μ, 3.40 μ, 5.80 μ, 5.93 μ, 6.11 μ, 8.22 μ, 8.85 μ, 9.21 μ, 9.40 μ, 9.55 μ, 11.30 μ, etc.

Anal. Calcd. for $C_{21}H_{29}O_7F$: C, 61.15; H, 7.08; F, 4.61. Found: C, 61.32; H, 7.14; F, 4.68.

Spectra in alkaline ethanol: $\lambda^{0.066\ N\ NaOH}_{max}$ ($E^{1\%}_{1cm.}$) (60°): at 30 min., 229 mμ (386), 250 mμ (172, inflection), 310 mμ (85), 360 mμ (38, inflection), at 3 hr., 228 mμ (367), 250 mμ (123, plateau), 320 mμ (73), 360 mμ (61, inflection).

Isolation of the R 0.33 component V from a selected crude crystalline product containing both II and V was also accomplished by preparative paper chromatography. The eluted, crystalline preparation was identical with the fully characterized V.

(b) *From 9α-fluoro-2β-hydroxyhydrocortisone.* To a 24-hr. vegetative growth of a strain of *S. roseochromogenus* ATCC 3347 in a medium consisting of corn steep liquor, 25 g./l., starch, 40 g./l., calcium carbonate, 2 g./l., lard oil, 0.2%, was added 25 mg. of 9α-fluoro-2β-hydroxyhydrocortisone in 0.5 ml. of dimethylformamide. After 48 hr. of aeration the fermentation broth was filtered from mycelium and the filtrate extracted six times with equal portions of ethyl acetate. The combined extracts were evaporated in vacuum and the oily residue analyzed on paper chromatograms. The R 0.33 component was the major product, with some unaltered substrate III and several other components. The steroid mixture was resolved preparatively on paper chromatograms in System II (16 hr.), the R 0.33 component eluted with hot alcohol, and the evaporated residue crystallized from ethanol. Comparison of papergram mobilities in several systems and of infrared spectra (micro disk) indicated identity of the sample with 9α-fluoro-2β,16α-dihydroxyhydrocortisone isolated under (a) above.

9α-Fluoro-2β,11β,21-trihydroxy-16α,17α-isopropylidenedioxy-4-pregnene-3,20-dione VI. Two hundred milligrams of the same selected sample of crystalline 9α-fluoro-16α-hydroxyhydrocortisone used for the papergram isolation of V above was suspended in 10 ml. of acetone and 0.06 ml. of 70% perchloric acid was added. After 45 min. of shaking the solution was neutralized with 1.6 ml. of saturated sodium bicarbonate solution, inorganic salts filtered, 7 ml. of water added, and the filtrates concentrated in vacuum. Crystals of 9α-fluoro-16α-hydroxyhydrocortisone 16α,17α-acetonide were filtered, and shown to be homogeneous by papergram with no by-product contamination present. The mother liquor evaporated to about 3 ml., was analyzed in System II, showing very mobile acetonide components VI at R_f 0.90 and 9α-fluoro-16α-hydrocortisone 16α,17α-acetonide at

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

R_f 0.96, and heretofore undetected 9α-fluoro-2β-hydroxyhydrocortisone III at R_f 0.49, together with two minor components.

The mother liquor was subjected to preparative paper chromatography using System V, the desired acetonide VI zone eluted with hot acetone, and the eluate evaporated to incipient crystallization. The product was recognized by comparison of infrared spectra (100 μg.) and papergram behavior as being identical with fully characterized 9α-fluoro-2β,16α-dihydroxhydrocortisone 16α,17α-acetonide VI prepared by another method in these Laboratories.

The acetonide VI is characterized by the spectral data: $\lambda_{max}^{H_2SO_4}$ ($E_{1\ cm.}^{1\%}$) 2 hr., 285 mμ (104, inflection), 340 mμ (497), 380 mμ (61, inflection), 550 mμ (11); λ_{max}^{KBr} 2.90 μ, 3.39 μ, 5.80 μ, 5.93 μ, 6.10 μ, 8.15 μ, 9.20 μ, 9.40 μ, 11.20 μ, 11.60 μ, etc.; $\lambda_{max}^{0.066\ N\ NaOH}$ ($E_{1\ cm.}^{1\%}$) at 60°: 1 hr. 230 mμ (354), 250 mμ (160, inflection), 355 mμ (54), 4 hr., 229 mμ (383), 250 mμ (159, plateau), 360 mμ (89).

The acetonide is further characterized by the following data kindly supplied by Dr. N. Rigler of these Laboratories: m.p. 260–261°; $[\alpha]_D$ + 12.9°; $\lambda_{max}^{CH_3OH}$ 239 mμ (ε 13,600).

Anal. Calcd. for $C_{24}H_{33}O_7F$: C, 63.75; H, 7.32; F, 4.22. Found: C, 62.88; H, 7.57; F, 4.53.

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[CONTRIBUTION FROM THE NATURAL PRODUCTS RESEARCH DEPARTMENT OF THE SCHERING CORP.]

17,21-Acetonide Derivatives of 9,11-Disubstituted Cortical Hormones

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A series of 9α,11β-dichloro- and 9α-chloro-11β-formoxycorticosteroids has been converted to the corresponding series of cyclic 17α,21-acetonides. The 17,21-acetonide system, useful as a means of side-chain protection, has also been found to enhance anti-inflammatory activity in the series studied.

The discovery of the ready reaction of the cortical dihydroxyacetone side chain with 2,2-dimethoxypropane, to give a cyclic 17α,21-acetonide,[1] has furnished not only a route to novel hormone analogs, but also a new means of side chain protection.

The variety of readily available 9α,11β-dihalo-[2] and 9α-halo-11β-acyloxycorticosteroids[3] led us to undertake the preparation of the corresponding 17α,21-acetonides, for possible further chemical transformations. The acetonides themselves, however, proved to be biologically interesting and this paper is concerned solely with the preparation, properties, and some reactions of this interesting group of compounds.

The 17α,21-acetonide (IVa) of 9α,11β-dichloro-1,4-pregnadiene-17α,21-diol-3,20-dione (IIIa) was first secured, in 50% yield, by the action of a 2,2-dimethoxypropane-dimethylformamide mixture, containing *p*-toluenesulfonic acid, for three hours under reflux. The course of the reaction was followed by periodically testing samples with triphenyltetrazolium chloride (TPTZ) reagent.

The acetonide (IVa) gave no color with TPTZ reagent, showed $\lambda_{max}^{CH_3OH}$ 236 mμ (16,400) and gave the correct analysis. The infrared spectrum showed that no hydroxyl was present, but absorption maxima were observed at 5.82 μ (20-ketone) and at 6.0, 6.12, and 6.20 μ (1,4-dien-3-one). When IVa was dissolved in 60% aqueous acetic acid and kept at steam bath temperature for ten minutes, cleavage of the acetonide grouping occurred, and 9α,11β-dichloro-1,4-pregnadiene-17α,21-diol-3,20-dione (IIIa), identical in all respects with authentic material, was obtained in excellent yield.

The 16α-methyl and 16β-methyl analogs (IVb and IVc, respectively) of IVa were next prepared by the following reaction sequences. Addition of chlorine, in carbon tetrachloride-pyridine, to 16α-methyl-1,4,9(11)-pregnatriene-17α,21-diol-3,20-dione 21-acetate[4] (Ib) and the 16β-methyl analog[5] gave the 9α,11β-dichloro derivatives (IIb and IIc). Although we had previously[2] used acetic acid as the solvent for the preparation of 9α,11β-dichloro compounds, we have since observed that both shorter reaction times and higher yields accompany the use of a carbon tetrachloride-pyridine system. (Thus, for example, 1,4,9(11)-pregnatriene-17α,21-diol-3,20-dione 21-acetate (Ia) gave the 9α,11β-dichloro compund (IIa) in about 75% yield by the carbon tetrachloride procedure, compared

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