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MICROBIOLOGICAL TRANSFORMATION OF STEROIDS—X

1-DEHYDRO ANALOGS OF CORTICAL STEROIDS

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Abstract—The transformation of the principal adrenocortical hormones into the corresponding 1-dehydro analogs by the action of *Corynebacterium simplex* (A.T.C.C. 6946) is described.

THE most important advance in the treatment of dermatologic, allergic and collagen diseases came with the introduction of adrenocorticosteroids as therapeutic and palliative agents in 1949 (cortisone)[1,2] and 1952 (hydrocortisone).[2] It was soon apparent however that a significant portion of the population could not tolerate the chronic systemic use of either of these hormones because of their excessively high salt-retaining component of action at the required dose, or did not respond to the anti-inflammatory action of these drugs after prolonged administration. Accordingly we directed substantial research efforts in the period 1951–1954 toward the solution of these problems, and in 1954 we elucidated a principle which was employed effectively to eliminate salt retention as a consideration in adrenocorticoid therapy and to decrease the incidence of drug "fastness."[3-9]

The embodiment of this principle was that the insertion of a double bond between C-1 and C-2 in cortisone and hydrocortisone resulted in the formation of products [respectively, prednisone (Id) and prednisolone (IId)] which were 3–5 times as active

[1] P. S. Hench, E. C. Kendall, C. H. Slocumb and H. F. Polley, *Arch. Int. Med.* **85**, 545 (1950).
[2] E. W. Boland, *Annals N.Y. Acad. Sci.* **61**, 349 (1955) and references cited therein.
[3] A. Nobile, U.S. 2,837,464.
[4] H. L. Herzog, A. Nobile, S. Tolksdorf, W. Charney, E. B. Hershberg, P. L. Perlman and M. M. Pechet, *Science* **121**, 176 (1955).
[5] J. J. Bunim, M. M. Pechet and A. J. Bollet, *J. Amer. Med. Assoc.* **157**, 311 (1955).
[6] H. L. Herzog, C. C. Payne, M. A. Jevnik, D. Gould, E. L. Shapiro, E. P. Oliveto and E. B. Hershberg, *J. Amer. Chem. Soc.* **77**, 4781 (1955).
[7] A. Nobile, W. Charney, P. L. Perlman, H. L. Herzog, C. C. Payne, M. E. Tully, M. A. Jevnik and E. B. Hershberg, *J. Amer. Chem. Soc.* **77**, 4184 (1955).
[8] H. L. Herzog, *Gordon Conference on Steroids and Related Natural Products*. New Hampton, August 3 (1955).
[9] S. Tolksdorf, M. L. Batten, J. W. Cassidy, R. M. McLeod, F. H. Warren and P. L. Perlman, *Proc. Soc. Exper. Biol. and Med.* **92**, 207 (1956).

as the parent compounds by a variety of biological and therapeutic criteria, and which were effectively free of a salt-retaining component of activity in the therapeutic dose range. We recognized the universal utility of 1-dehydrogenation,[3] which was later confirmed by us insofar as its application to the preparation of other corticosteroid derivatives was concerned,[10–14] and also by workers in many other laboratories.[15–23a] Since 1954 every new adrenocorticoid which has found clinical application has contained the 1,2-double bond.[12–23a]

The history of the application of microbial transformations of steroids to synthesis began in 1937 with Mamoli's school, which employed bacteria and yeasts to effect a variety of oxidations, hydrogenations and isomerizations.[24] While the period 1940–1950 was on the whole a quiet one insofar as the volume of new work in the microbial transformations was concerned, two papers did appear which foreshadowed the extensive developements of the 1950's. These papers, by Kramli and Horvath, illustrated the two major classes of economically important microbial transformations of steroids, namely oxygenation and dehydrogenation, as exemplified by the

[10] D. Gould, E. L. Shapiro, H. L. Herzog, M. Jevnik Gentles, E. B. Hershberg, W. Charney, M. Gilmore, S. Tolksdorf, M. Eisler, P. L. Perlman and M. M. Pechet, *J. Amer. Chem. Soc.* **79**, 502 (1957).

[11] E. P. Oliveto, R. Rausser, A. Nussbaum, W. Gebert, C. Coniglio, E. B. Hershberg, S. Tolksdorf, M. Eisler, P. L. Perlman and M. M. Pechet, *J. Amer. Chem. Soc.* **80**, 4428 (1958).

[12] E. P. Oliveto, R. Rausser, L. Weber, A. Nussbaum, W. Gebert, C. Coniglio, E. B. Hershberg, S. Tolksdorf, M. Eisler, P. L. Perlman and M. M. Pechet, *J. Amer. Chem. Soc.* **80**, 4431 (1958)—dexamethasone.

[13] E. P. Oliveto, R. Rausser, H. L. Herzog, E. B. Hershberg, S. Tolksdorf, M. Eisler, P. L. Perlman and M. M. Pechet, *J. Amer. Chem. Soc.* **80**, 6687 (1958). —betamethasone

[14] C. H. Robinson, L. Finckenor, E. P. Oliveto and D. Gould, *J. Amer. Chem. Soc.* **81**, 2191 (1959). —dichlorisone

[15] G. B. Spero, J. L. Thompson, B. J. Magerlein, A. R. Hanze, H. C. Murray, O. Sebek, J. A. Hogg, *J. Chem. Amer. Soc.* **78**, 6213 (1956). —6α-methylprednisolone.

[16] S. Bernstein, R. H. Lenhard, W. S. Allen, M. Heller, R. Littell, S. M. Stolar, L. I. Feldman and R. H. Blank, *J. Amer. Chem. Soc.* **78**, 5693 (1956). —triamcinolone (9α-fluoro-16α-hydroxyprednisolone)

[17] H. J. Ringold, O. Mancera, C. Djerassi, H. Bowers, E. Batres, H. Martinez, E. Necoecha, J. Edwards, M. Velasco, C. Casas Campillo and R. J. Dorfman, *J. Amer. Chem. Soc.* **80**, 6464 (1958). —chloroprednisone (6α-chloroprednisone).

[18] J. A. Hogg, G. B. Spero, J. L. Thompson, B. J. Magerlein, W. P. Schneider, D. H. Peterson, O. K. Sebek, H. C. Murray, J. C. Babcock, R. L. Pederson and J. A. Campbell, *Chem. & Ind.* 1002 (1958); A. Bowers, E. Denot, M. Blanca Sanchez and H. J. Ringold, *Tetrahedron* **7**, 153 (1959).

[19] G. Arth, D. Johnston, J. Fried, W. Spooncer, D. Hoff and L. Sarett. *J. Amer. Chem. Soc.* **80**, 3160 (1958).

[20] D. Taub, R. Hoffsommer, H. Slates, and N. Wendler, *J. Amer. Chem. Soc.* **80**, 4435 (1958).

[21] G. Arth, J. Fried, D. Johnston, D. Hoff, L. Sarett, R. Silber, H. Stoerk and C. Winter, *J. Amer. Chem. Soc.* **80**, 3161 (1958). —dexamethasone

[22] J. A. Edwards, H. J. Ringold and C. Djerassi, *J. Amer. Chem. Soc.* 82, 2318 (1960); W. P. Schneider, F. H. Lincoln, G. B. Spero, H. C. Murray and J. L. Thompson, *Ibid*, **81**, 3167 (1959). —paramethasone (6α-fluoro-16α-methylprednisolone)

[23] H. J. Mannhardt, F. V. Werder, K. H. Bork, H. Metz and K. Brückner, *Tetrahedron Letters* No. 16, 21 (1960).

[23a] J. S. Mills, A. Bowers, C. Djerassi and H. J. Ringold, *J. Amer. Chem. Soc.* **82**, 3399 (1960). —6α-fluorotriamcinolone 16,17-acetonide

[24] This work has been reviewed by C. Arnaudi, *Zentr. Parasitenk*, **105**, 352 (1952) and by F. G. Fischer, *Newer Methods of Preparative Organic Chemistry* p. 182. Interscience, New York (1948).

7-hydroxylation[25] and 6-dehydrogenation[26] of cholesterol by *Proactinomyces roseus* and *Azotobacter sp.* respectively.

In 1949 Hechter *et al.*[27] showed that enzymatic 11β-hydroxylation of steroid substrates could be accomplished by perfusion through isolated adrenal glands.

Following the introduction of cortisone into the pharmaceutical marketplace by Merck in 1949 the medically and economically important uses of this product provided an incentive to develop improved methods of synthesis. A rationale for a microbiological approach to direct 11-oxygenation of steroids was provided by the work of Krámli and Horváth and of Hechter *et al.* A successful microbiological solution to this problem was announced by Peterson and Murray[28] in 1952, when they reported 11α-hydroxylation of progesterone by the fungus *Rhizopus arrhizus* and more generally by fungi of the order Mucorales. This hydroxylation technique provided a powerful tool for both steroid research and production and has been applied widely with dramatic results. While the fall in the price of cortisone cannot be attributed directly to introduction of microbiological methods, there is no question that a note of healthy competition was provided by the development of a synthesis from a microbiological base which undoubtedly contributed to the further efforts being made on the improvement of the "classical," bile acid-derived synthesis. Today, the bile acid syntheses of cortisone and hydrocortisone are still competing successfully with the microbiological route.

The economic significance of microbial 11-hydroxylation was also appreciated and a solution sought in other laboratories. While the breakthrough clearly belongs to the Upjohn workers, other important, independent efforts were reported by the Squibb group.[29,30] The latter investigators incidentally discovered the 16α-hydroxylation of progesterone by an unknown actinomycete,[29] a class of organisms which had heretofore been investigated principally for antibiotic production. It developed later that this fermentation, too, was to have great economic significance in the production of an important anti-inflammatory agent, 16α-hydroxy-9α-fluoroprednisolone (triamcinolone).[16,31]

Since Schering had been a producer of cortisone from bile acids since 1951, we had a strong interest in these new developments in microbial transformations and decided to reinvestigate the use of bacteria in the transformation of steroids, with the aid of the powerful new technique of paper chromatographic analysis. Early in the course of this work it was observed that *Corynebacterium simplex* (A.T.C.C. 6946) afforded clean dehydrogenation of a variety of 3-keto-Δ^4-steroidal substrates to the corresponding 1-dehydro species.[3,7,8] In this article and that which follows[32] we describe some of our studies with this organism.

[25] A. Krámli and J. Horváth, *Nature, Lond.* **162,** 619 (1948); **163,** 219 (1949).
[26] J. Horváth and A. Krámli, *Nature,* **160,** 639 (1947).
[27] O. Hechter, R. P. Jacobsen, R. Jeanloz, H. Levy, C. W. Marshall, G. Pincus and V. Schenker, *J. Amer. Chem. Soc.* **71,** 3261 (1949).
[28] D. H. Peterson and H. C. Murray, *J. Amer. Chem. Soc.* **74,** 1871 (1952).
[29] D. Perlman, E. Titus and J. Fried, *J. Amer. Chem. Soc.* **74,** 2126 (1952).
[30] J. Fried, R. W. Thoma, J. R. Gerke, J. E. Herz, M. N. Donin and D. Perlman, *J. Amer. Chem. Soc.* **74,** 3962 (1952).
[31] R. W. Thoma, J. Fried, S. Bonanno and P. Grabowich, *J. Amer. Chem. Soc.* **79,** 4818 (1957).
[32] W. Charney, A. Nobile, C. Federbush, D. Sutter, P. L. Perlman, H. L. Herzog, C. C. Payne, M. E. Tully, M. J. Gentles and E. B. Hershberg, *Tetrahedron.*

It has been observed that *C. simplex* transforms the following adrenocortical hormones and related compounds into the corresponding 1-dehydro derivatives:

4-pregnene-17α,21-diol-3,20-dione (Reichstein's Compound S, III), 4-pregnene-17α,21-diol-3,11,20-trione (cortisone, I), 4-pregnene-11β,17α,21-triol-3,20-dione (cortisol, II), 4-pregnene-11β,21-diol-3,20-dione (corticosterone, IV), 4-pregnene-11α,17α,21-triol-3,20-dione (V), 4-pregnene-21-ol-3,20-dione 21-actetate (desoxycorticosterone acetate, VI), 4-pregnene-11β,17α,21-triol-3,20-dione-11,21-diacetate (VII), and 9α-fluoro-4-pregnene-11β,17α,21-triol-3,20-dione (VIII).

In most cases the yields of diene produced were excellent. The progress of the reaction could be followed with either the polarograph[33] or paper chromatogram. The latter method was usually more convenient, and the reaction was terminated when the chromatogram indicated that the starting material was completely transformed. Isolation of the product was accomplished by extraction with chloroform, concentration of the extract to a residue and subsequent crystallization from a suitable solvent. When Compound S (III) was incubated, with shaking at 28°, in the presence of growing bacteria, either (or both) of two identifiable products were produced, depending on the medium employed. When the nutrient source was 0·1 per cent yeast extract the only product isolated after 48 hours was $\Delta^{1,4}$-pregnadiene-17α,21-diol-3,20-dione (IIId). On the other hand, when the medium contained 1 per cent "condensed fish solubles" and 0·1 per cent yeast extract both IIId and $\Delta^{1,4}$-pregnadiene-17α,20β,21-triol-3-one (IX)[33a] were produced. It is likely that IIId is an intermediate in the formation of IX, the reduction of the 20-carbonyl being a slow reaction. Experiments were made in which the reaction was permitted to proceed for as long as 48 hours with the "fish-soluble medium." While some of IIId had been formed after 6 hours, and was the predominant, identifiable, transformed product after 12 hours, it was not possible to isolate IIId after 24 hours. Compound IX first appeared after 12 hours and could be isolated in substantial yield after 48 hours.

The structures of IIId and IX were proved in the following manner. The presence of the Δ^1-unsaturation was inferred from the appearance of the characteristic triad in the infra-red spectra[34] at approximately 6·0, 6·16 and 6·20 μ, from the shift of the ultra-violet absorption maxima to higher wave lengths and from the decrease in the molecular rotations relative to Compound S. Both IIId and IX were degraded by the action of sodium bismuthate in aqueous acetic acid[35] to $\Delta^{1,4}$-androstadiene-3, 17-dione, which established the presence of the added unsaturation at C-1, and indicated the absence of any other changes in the nuclei of the steroids. The structures of the side-chains in IIId and IX were established by the preparation of the respective 21-acetate[36] and 20, 21-diacetate[37] with acetic anhydride in pyridine. The 21-acetate

[33] P. Kabàsakalian and J. McGlotten, *J. Amer. Chem. Soc.* **78,** 5032 (1956); P. Kabàsakalian, S. DeLorenzo and J. McGlotten, *Anal. Chem.* **28,** 1669 (1956).

[33a] Similar reduction has been described in the triamcinolone series by L. L. Smith, J. J. Garbarini, J. J. Goodman, M. Marx and H. Mendelsohn, *J. Amer. Chem. Soc.* **82,** 1437 (1960).

[34] J. Fried, R. W. Thoma and A. Klingsberg, *J. Amer. Chem. Soc.* **75,** 5764 (1953). These authors noted also the reduction of the 20-carbonyl in progesterone by *Streptomyces lavendulae* to give 4-pregnene-20β-ol-3-one.

[35] C. J. W. Brooks and J. K. Normyberski, *Biochem. J.* **55,** 371 (1953).

[36] G. Rosenkranz, J. Pataki, St. Kaufmann, J. Berlin and C. Djerassi, *J. Amer. Chem. Soc.* **72,** 4081 (1950).

[37a] Cf. D. Sutter, W. Charney, P. L. O'Neill, F. Carvajal, H. I. Herzog and E. B. Hershberg, *J. Org. Chem.* **22,** 578 (1957); [b] M. Uchibayashi, *Chem. and. Pharm. Bull. Japan.* **8,** 112 and 117 (1960).

I, X = =O , Y = OH, Z = H, R = H,
II, X = OH / H , Y = OH, Z = H, R = H,
III, X = H / H , Y = OH, Z = H, R = H,
IV, X = OH / H , Y = H, Z = H, R = H,
V, X = H / OH , Y = OH, Z = H, R = H,
VIII, X = OH / H , Y = OH, Z = F, R = H,
VI, X = H / H , Y = H, Z = H, R = CCH3, =O
VII, X = OCCH3, =O / H , Y = OH, Z = H, R = CCH3, =O
Id
IId
IIId
IVd
Vd
VIIId
VId
VIId
IX, X = H / H , Y = OH, Z = H, R = H
X, X = =O, Y = OH, Z = H, R = H

group of IIId showed the characteristic interaction with the 20-carbonyl group in the infra-red.[38] The configuration of the 20-hydroxyl group in IX was shown to be β by the characteristic, large positive shift in the molecular rotation which resulted upon acetylation.[39]

By the use of procedures similar to that employed with Compound S (yeast extract medium), cortisone (I) and cortisol (II) were transformed into their respective 1-dehydro derivatives Id and IId. These were shown to be identical with synthetic samples of established structure.[6] Acetylation of the mother liquors from the preparation of Id, followed by careful separation through chromatography made possible the isolation of a compound whose empirical formula corresponded to the 20,21-diacetate of X. It is reasonable to suppose that reduction of the 20-carbonyl group of Id (or I) occurs to some extent in the yeast extract medium (as well as the "fish solubles" medium) even though the analogous product was not isolated from the reaction of Compound S in this medium. The structure of X follows from that of IX.

The conversion of IV, V, VI, VII and VIII into the corresponding 1-dehydro derivatives was accomplished in a similar fashion. It is interesting to note that the acetate groups of VI and VII were not hydrolysed during the transformations. Related bacteria are known to possess a deacetylase capable of splitting the 21-acetate group.[24] It was observed also that while the infra-red triad at ca. 6·00, 6·16 and 6·20 μ is positive evidence for the presence of a $\Delta^{1,4}$-diene-3-keto steroid, its absence does not exclude the diene structure. For example, Vd and VIId showed absorption bands at 6·07 and 6·30 μ, and 6·04 and 6·23 μ respectively, which would usually be interpreted as indicating Δ^4-3-keto-steroids. However, in each case the polarographic reduction potential, paper chromatographic behaviour, and rotation were clearly consistent with the assigned $\Delta^{1,4}$-diene-3-one structure.

Dehydrogenation reactions had been observed earlier by Fried, *et al.*[34] and by Vischer,[40a] with several species at filamentous fungi and actinomycetes. In all of the work reported prior to our announcement[4] of the structure and activity of compounds Id and IId, the identified products containing the $\Delta^{1,4}$-diene-3-one system, from the action of *Cylindrocarpon radicicola*,[34] *Streptomyces lavendulae*[34] and *Fusarium Solani*[40a] on a variety of C-21 steroid substrates, were all C-19 steroids. In these studies[34,40a] degradation of the side-chain had occurred concomitantly with dehydrogenation in ring A.

Since the discovery of prednisone and predisolone great interest has been manifested in microbial, dehydrogenation processes and many reports have appeared describing fungal and bacterial species capable of causing this reaction with a host of steroidal substrates.[40b]

The biological effects of Id and IId have been described. Studies in animal and human subjects using IIId, IVd, VId and VIIId have appeared elsewhere.[41]

[38] R. N. Jones, P. Humphries, F. Herling and K. Dobriner, *J. Amer. Chem. Soc.* **74**, 2820 (1952).

[39] L. F. Fieser and M. Fieser, *Natural Products Related to Phenanthrene* 3rd Ed., p. 434. Reinhold, New York (1949).

[40a] E. Vischer and A. Wettstein, *Experientia* **9**, 371 (1953).

[40b] A recent summary of microbial 1-dehydrogenation is given by E. Vischer and A. Wettstein, *Adv. in Enzym.* **XX**, 262 (1958).

[41] M. M. Pechet, B. Bowers and F. C. Bartter, *J. Clin. Invest.* **38**, 681, 691 (1959).

EXPERIMENTAL[42]

General procedure for the introduction of Δ^1 *unsaturation in a 3-keto-*Δ^4*-steroid.* To a mixture containing 3·0 g yeast extract (Difco), 4·49 g potassium dihydrogen phosphate, 8·83 g disodium hydrogen phosphate heptahydrate and 1 l. of tap water is added one loopful of cell material from an agar slant, prepared by growing a culture of *Corynebacterium simplex* (A.T.C.C. 6946) on 1% yeast extract-dextrose agar, and the resulting mixture is incubated for 18–24 hr. One ml of the resulting culture is used as a standard inoculum for the medium for steroid transformation.

A mixture of 1 g yeast extract (Difco) in 1 l. of tap water, the pH of which is adjusted to 6·8–7·0, is distributed among ten 300 ml erlenmeyer flasks and to each flask is added the standard inoculum. The resulting suspensions are incubated at 30° on a shaking machine and growth is followed turbidimetrically. The appropriate steroid (0·5 g) is dissolved in a minimum amount of methanol or acetone and the resulting solution is distributed equally among the 10 flasks when the peak of the log phase of growth of the organism is reached. Progress of the reaction is then followed by withdrawing flasks from the shaking machine at periodic intervals, extracting the mixture with chloroform and chromatographing the extracted steroids according to the method of Schull[43] or in another appropriate system. When the desired transformation is complete (3–48 hr), as evidenced by the disappearance of the starting material or the absence of further change in the composition of the reaction mixture, the contents of the remaining flasks are combined and extracted with chloroform. The residue remaining after the removal of the chloroform is then crystallized or chromatographed over Florisil as the case requires to complete the purification of the desired $\Delta^{1,4}$-diene-3-keto-steroid.

1,4-*Pregnadiene*-17α,21-*diol*-3,20-*dione* (IIId). The general procedure described previously was employed. From 1·5 g III, there was obtained by crystallization from acetone–hexane of the reaction products extracted by chloroform, 0·8 g IIId, m.p. 246–250° dec. Further crystallization from the same solvent mixture did not change the m.p. significantly; $[\alpha]_D^{25}$ +76° (chloroform), $\lambda_{max}^{methanol}$ 244 mμ (ε = 15,900), λ_{max}^{Nujol} 3·05 μ (OH), 5·80 μ (20-carbonyl), 6·0, 6·16 and 6·22 μ ($\Delta^{1,4}$-diene-3-one).[43a]

(Found: C, 73·56; H, 8·40. Calc. for $C_{21}H_{28}O_4$: C, 73·22; H, 8·19%).

1,4-*Pregnadiene*-17α,21-*diol*-3,20-*dione* 21-*acetate*. A solution of 1·1 g IIId in 5 ml pyridine was treated with 2 ml acetic anhydride and allowed to stand overnight at room temp. Water was then added and the resulting precipitate was removed by filtration. There was recovered 1·2 g, m.p. 218–220°. Recrystallization from acetone–hexane did not change the m.p.; $[\alpha]_D^{25}$ +97° (dioxane); $\lambda_{max}^{ethanol}$ 244 mμ (15,900); λ_{max}^{Nujol} 2·96 μ (OH), 5·72 and 5·80 μ (20-carbonyl, 21-acetate interaction), 6·02, 6·18 and 6·23 μ ($\Delta^{1,4}$-diene-3-one) and 8·08 μ (C—O—C of acetate). Lit. m.p. 218–220°; $[\alpha]_D^{30}$ +88° ($CHCl_3$); $\lambda_{max}^{ethanol}$ 244 mμ (ε = 18,200)[36]; m.p. 218–222; $[\alpha]_D^{23}$ +86° (dioxane); $\lambda_{max}^{ethanol}$ 244 mμ (ε = 15,400).[43a]

1,4-*Pregnadiene*-17α,20β,21-*triol*-3-*one* (IX). The general procedure described previously was employed with the exception that 1% of concentrated fish solubles[44] was added to the medium in which the reaction was carried out. After a 48 hr incubation period employing 6·0 g III, extraction with chloroform and chromatography of the extracts over Florisil afforded 0·57 g III, (off in 0·5% methanol in methylene chloride), 0·97 g of a mixture of III and IX (off in 0·75% methanol in methylene chloride), and 2·72 g IX (off in 1–4% methanol in methylene chloride). Recrystallization of IX from acetone–hexane yielded 1·48 g purified product, m.p. 194–195°. Further recrystallization from the same solvents gave IX, m.p. 195–196°, $[\alpha]_D^{25}$ + 33° (methanol), λ_{max}^{Nujol} 3·02 μ (OH), 6·02, 6·19 and 6·24 μ ($\Delta^{1,4}$-diene-3-one). Lit[37b] m.p. 194–195, $[\alpha]_D^{20}$ +33° ($CHCl_3$).

(Found: C, 72·79; H, 9·08. Calc for $C_{21}H_{30}O_4$: C, 72·80; 8·73%).

After 6 hr of reaction in the aforedescribed experiment chromatography gave principally III. After 12 hr the proportions of III:IIId:IX were approximately 7:2:1.

Transformation after 24 hr afforded only III and IX in isolable amount with IX being preponderant.

[42] All melting points are corrected. Analyses and optical data were obtained by the Microanalytical and Physical Chemistry Departments of these laboratories and by the Galbraith Laboratories, Knoxville, Tennessee.

[43] G. M. Shull, *Abstracts of Papers, 126th Meeting* of the *Amer. Chem. Soc.* p. 9A. Sept. 12–17, New York (1954).

[43a] E. Vischer, Ch. Meystre and A. Wettstein, *Helv. Chim. Acta*, **38**, 835 (1955).

[44] Condensed fish solubles containing 50% solids obtained from the Mead Corp., N.Y., see also W. Charney, Ph.D. Thesis, Rutgers Univ. (1953).

1,4-*Pregnadiene*-17α,20β,21-*triol*-3-*one* 20,21-*diacetate*. A solution of 250 mg IX in 2 ml pyridine and 1 ml acetic anhydride was allowed to stand at room temp overnight. The reaction mixture was poured into ice-water and the resulting precipitate was removed by filtration. There resulted 280 mg 20,21-diacetate of IX, which upon recrystallization from methylene chloride–hexane gave 240 mg m.p. 181–182°, $[\alpha]_D^{25}$ +123° (methanol), $\lambda_{max}^{methanol}$ 245 mμ (ε = 15,250), λ_{max}^{Nujol} 2.86 μ (OH), 5·75 μ (acetate carbonyl), 6·01, 6·14 and 6·22 μ ($\Delta^{1,4}$-diene-3-one) and 8·1 μ (C—O—C of acetate). Lit[37b] m.p. 178–179°, $[\alpha]_D^{20}$ +100° ($CHCl_3$). (Found: C, 70·03; H, 8·30; Calc. for $C_{25}H_{34}O_6$: C, 69·74; H, 7·96%).

Degradation of IIId *with sodium bismuthate*. A mixture of 0·1 g IIId dissolved in 10 ml acetic acid with 10 ml water and 2·0 g sodium bismuthate was shaken intermittently for 3 hr, and then was allowed to stand overnight. The sodium bismuthate was then removed by filtration and washed with 20 ml 1:1 acetic acid-water. The combined filtrates were diluted with 100 ml water and extracted with methylene chloride. The methylene chloride extracts were washed free of acetic acid with water and were dried ($MgSO_4$). Concentration of the dry solution followed by the addition of hexane afforded 30 mg $\Delta^{1,4}$-androstadiene-3,17-dione, m.p. 128–132°. Recrystallization from ether–hexane raised the m.p. to 139–140°. The sample so obtained had an infrared spectrum which was identical with that of authentic material. No depression of m.p. resulted from admixture of the recrystallized sample with authentic material.

Degradation of IX *with sodium bismuthate*. By the aforedescribed procedure 100 mg IX was degraded to 30 mg $\Delta^{1,4}$-androstadiene-3,17-dione, m.p. 139–140°. The sample so obtained had an infrared spectrum which was identical with that of authentic material.

1,4-*Pregnadiene*-17α,21-*diol*-3,11,20-*trione* (Id). By the general procedure described previously 4·0 g I was transformed. Recrystallization of the chloroform-extracted product from acetone–hexane gave 2·3 g Id, m.p. 222–225° dec. By repeated crystallization from acetone the m.p. was raised to 233–235° dec, $[\alpha]_D^{25}$ +172° (dioxane), $\lambda_{max}^{methanol}$ 238 mμ (ε = 15,500), λ_{max}^{Nujol} 3·04 μ (OH), 5·84 μ (11- and 20-carbonyls), 5·98, 6·16 and 6·21 μ ($\Delta^{1,4}$-diene-3-one).

(Found: C, 70·35; H, 7·45; Calc. for $C_{21}H_{26}O_5$: C, 70·37; H, 7·31%).

1,4-*Pregnadiene*-17α,20β,21-*triol*-3,11-*dione* 20,21-*diacetate* (*diacetate of* X). The mother liquors from the crystallization of a series of preparations of Id were pooled and evaporated to dryness. Five grams of the residue was taken up in 10 ml acetic anhydride and 10 ml pyridine. The reaction was permitted to stand overnight and was then poured into ice-water. The products were extracted with methylene chloride and the extracts were washed successively with water, dil sulfuric acid and water. The methylene chloride solution was dried and concentrated and the residue was crystallized from acetone–hexane. There resulted 2·18 g of the 21-acetate of IIId, m.p. 230–236°.[4] Further concentration of the mother liquor followed by crystallization afforded 1·28 g of a mixture, m.p. 205–229°. Chromatography of 1·0 g of this mixture over 15 g Florisil afforded a series of fractions (eluted with 75% ether in hexane) which were combined according to the appearance of their infrared spectra. A group (fractions #27–#46) which had the most intense acetate absorption and the weakest C_{11}—C_{20} carbonyl band was collected and crystallized from acetone–hexane. There resulted 0·14 g of the 20,21-diacetate of X, m.p. 239–242°, $[\alpha]_D^{25}$ +160° (dioxane), $\lambda_{max}^{ethanol}$ 239 mμ (ε = 16,100), λ_{max}^{Nujol} 2·92 μ (OH), 5·74 and 5·76 μ (20,21-diacetate), 5·90 μ (11-carbonyl), 6·01, 6·14 and 6·22 μ ($\Delta^{1,4}$-diene-3-one) and 8·06 μ (acetate C—O—C).[45]

(Found: C, 67·71; H, 7·04; Calc. for $C_{25}H_{32}O_7$: C, 67·55; H, 7·26%).

1,4-*Pregnadiene*-11β,17α,21-*triol*-3,20-*dione* (IId). By the general procedure described previously 4·0 g II was transformed. There was isolated from the chloroform extract 3·75 g crude IId, m.p. 227–232° dec. Repeated crystallization from acetone raised the m.p. to 240–241° dec $[\alpha]_D^{25}$ +102° (dioxane), $\lambda_{max}^{methanol}$ 242 mμ (ε = 15,000). λ_{max}^{Nujol} 2·96 μ (OH), 5·82 μ (20-carbonyl), 6·04, 6·19 and 6·25 μ ($\Delta^{1,4}$-diene-3-one).

(Found: C, 70·24; H, 8·13; Calc. for $C_{21}H_{28}O_5$: C, 69·97; H, 7·83%).

1,4-*Pregnadiene*-11β,21-*diol*-3,20-*dione* (IVd). By the general procedure described previously 0·9 g IV was transformed. Crystallization of the crude product, isolated by chloroform extraction, from acetone–hexane afforded 0·3 g IVd, m.p. 215–220° dec. Several recrystallizations from acetone–hexane raised the m.p. to 227·5–230·5°, $[\alpha]_D^{25}$ +173° (methanol), $\lambda_{max}^{methanol}$ 243 mμ (ε = 14, 300), λ_{max}^{Nujol}

[45] Cf. F. Carvajal, O. F. Vitale, M. J. Gentles, H. L. Herzog and E. B. Hershberg, *J. Org. Chem.* **24**, 695 (1959).

2·88 and 2·97 μ (OH), 5·88 μ (20-carbonyl), 6·07, 6·20 and 6·25 μ ($\Delta^{1,4}$-diene-3-one). Lit[43a] m.p. 216–220°, $[\alpha]_D^{28}$ +158° (ethanol).

(Found: C, 73·49; H, 8·12; Calc. for $C_{21}H_{28}O_4$: C, 73·22; H, 8·19%).

1,4-*Pregnadiene*-11α,17α,21-*triol*-3,20-*dione* (Vd). By the general procedure described previously 0·5 g V was transformed. Crystallization of the crude product from acetone yielded 250 mg Vd, m.p. 244–245° dec. Recrystallization from acetone raised the m.p. to 246–247° dec, $[\alpha]_D^{25}$ +73° (methanol), $\lambda_{max}^{methanol}$ 248 mμ (ε = 17,300). λ_{max}^{nujol} 3·00 μ (OH), 5·86 μ (20-carbonyl), 6·07 and 6·30 μ ($\Delta^{1,4}$-diene-3-one).

(Found: C, 70·09; H, 7·90; Calc. for $C_{21}H_{28}O_5$: C, 69·97; H, 7·83%).

1,4-*Pregnadiene*-21-*ol*-3,20-*dione* 21-*acetate* (VId). By the general procedure described previously 3·0 g VI was transformed. Crystallization of the crude product from methylene chloride–hexane afforded 2·0 g VId, m.p. 195–198°. Recrystallization from methylene chloride–hexane raised the m.p. to 202–204°, $[\alpha]_D^{25}$ +143° (chloroform), +152° (ethanol), $\lambda_{max}^{ethanol}$ 243 mμ (ε = 15,800), λ_{max}^{Nujol} 2·93 μ (OH), 5·72 and 5·80 μ (20 carbonyl, 21-acetate interaction), 6·01, 6·16 and 6·23 μ ($\Delta^{1,4}$-diene-3-one) and 8·06 μ (C—O—C of acetate). Lit m.p. 202·6–204°, $[\alpha]_D^{25}$ +125·6° (ethanol);[48] m.p. 203–206, $[\alpha_D^{23}]$ +134° ($CHCl_3$).[43a]

(Found: C, 74·46; H, 8·24; Calc. for $C_{23}H_{30}O_4$: C 74·56; H, 8·16%).

1,4-*Pregnadiene*-11β,17α,21-*triol*-3,20-*dione* 11,21-*diacetate* (VIId). By the general procedure described previously 3·0 g VII was transformed. Crystallization of the crude product from acetone–hexane afforded 1·76 g m.p. 216–218°. Recrystallization from the same solvents raised the m.p. to 219–221°, $[\alpha]_D^{25}$ +152° (chloroform), $\lambda_{max}^{ethanol}$ 240 mμ (ε = 15,200), λ_{max}^{Nujol} 2·97 μ (OH), 5·72 and 5·79 μ (20-carbonyl, 21-acetate interaction), 6·04 and 6·23 μ ($\Delta^{1,4}$-diene-3-one) and 8·1 μ (C—O—C of acetate).

(Found: C, 67·46; H, 7·64; Calc. for $C_{25}H_{32}O_7$: C,67·55; H, 7·26%).

9α-*Fluoro*-1,4-*pregnadiene*-11β,17α,21-*triol*-3,20-*dione* (VIIId). By the general procedure described previously 3·0 g VIII was transformed. The crude product from chloroform extraction was chromatographed on Florisil to separate some unreacted starting material from VIIId. Starting material was eluted with 1% methanol in methylene chloride while VIIId came off in 2–4% methanol in methylene chloride. Crystallization of the combined VIIId fractions from methanol–water afforded 0·65 g VIIId as a methanol solvate, m.p. 265–269° dec $[\alpha]_D^{25}$ +111° (ethanol), $\lambda_{max}^{methanol}$ 239 mμ (ε = 16,100). λ_{max}^{Nujol} 3·00 μ (OH), 5·82 μ (20-carbonyl), 6·02, 6·18 and 6·23 μ ($\Delta^{1,4}$-diene-3-one). Lit m.p. 263–266°, $[\alpha]_D^{28}$ +108° (ethanol);[43a] m.p. 274–275°; $[\alpha]_D^{23}$ +94° (ethanol).[47]

(Found: C, 64·22; H, 7·51; Calc. for $C_{21}H_{27}O_5F.CH_4O$: C, 64·37; H, 7·61%).

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[46] R. L. Clarke, K. Dobriner, A. Mooradian and C. M. Martini, *J. Amer. Chem. Soc.* **77**, 661 (1955).
[47] J. Fried, K. Florey, E. F. Sabo, J. E. Herz, A. R. Restivo, A. Borman and F. M. Singer, *J. Amer Chem. Soc.*, **77**, 4181 (1955).