in addition to undergoing reactions (3b), $C_6H_5S\cdot$ enters into two termination processes:

$$C_6H_5S\cdot + (C_6H_5)_2CH\cdot \longrightarrow (C_6H_5)_2CHSC_6H_5 \text{ (V)} \quad (4a)$$
$$2\ C_6H_5S\cdot \longrightarrow C_6H_5SSC_6H_5 \text{ (VI)} \quad (4b)$$

The infrared spectrum of residues after isolation of III was identical with that of a solution prepared from an authentic sample of V[6] and VI.[7] Evidence for the occurrence of reaction (3b) was found in the formation of tetraphenylethane III in 72% yield by exposure of a dilute solution of diphenyl disulfide VI in diphenylmethane to a sun lamp. Studies with S^{35} have shown that disulfides dissociate into mercaptyl radicals on irradiation.[8] Blank experiments indicated that compounds IV, V and VI did not cause formation of III from II, nor did they adversely affect the isolation of III from II.[9]

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(7) T. Zinke and W. Frohneberg, *Ber.*, **43**, 840 (1910).
(8) E. N. Guryanova and V. N. Vasileva, *Zhur. Fiz. Khim.*, **28**, 60 (1954).
(9) This work was supported by a grant from the National Science Foundation.

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CONCERNING THE STEREOSPECIFICITY OF THE FUMARASE REACTION AND THE DEMONSTRATION OF A NEW INTERMEDIATE[1]

Sir:

When fumarase catalyzes the addition of water to fumarate only the L-isomer of malate is produced.[2] While the hydroxyl group is added stereospecifically, the question still arises as to the stereospecificity of the addition of the hydrogen atom. We have been able to demonstrate that this process is absolutely stereospecific.[3] Dipotassium fumarate was added to a reaction mixture containing crystalline fumarase,[4] 0.038 *M* K_2HPO_4, and 0.015 *M* KH_2PO_4 in a medium of 99.5% D_2O at 25°. After equilibration it was found that the resulting L-malate, isolated as diphenacyl-L-malate, containing 0.97 excess atom of deuterium per molecule after exhaustive washing with water. The fumaric acid isolated had incorporated less than 1×10^{-4} atom of non-exchangeable deuterium per molecule. This shows that the entering hydrogen atom is added in only one of the two possible positions and that a hydrogen atom from the identical position is removed in the dehydration reaction.

The stereospecificity with regard to the hydrogen atom having been established, the question as to whether the hydrogen and hydroxyl groups are added to fumarate in a *cis* or *trans* manner is currently being investigated in this laboratory.

The availability of the particular diamer of monodeutero-L-malate which is produced enzymatically made it possible to determine whether the breaking of the methylene carbon–hydrogen bond is the rate determining step in the enzymatic reaction. The breaking of the methylene carbon–deuterium bond in deutero-L-malate must proceed at approximately one-sixth the rate of that for the corresponding carbon–hydrogen bond.[5] If the breaking of a carbon–hydrogen bond were rate limiting, the over-all rate of reaction would be decreased by a factor of six in the dehydration of deutero-L-malate.

When monodeutero-L-malate was dehydrated enzymatically at *p*H 8.0 in 0.005 *M* tris-(hydroxymethyl)-aminomethane perchlorate buffer it was found that both the maximum initial velocity and Michaelis constant were unchanged after a correction for a small amount of fumarate in the malate preparation. Thus the breaking of the carbon–hydrogen bond is not involved in the rate-determining step. It seems likely from kinetic arguments[6,7] that the dissociation of fumarate from the enzyme is not the rate-determining step for the dehydration reaction at high L-malate concentrations.

The fact that the deuterium of monodeutero-L-malate was removed in a step which is not rate limiting suggested that there should be a sterically specific exchange of this hydrogen atom proceeding at a rate faster than the dehydration reaction. Such a rapid exchange was indeed found to occur in an experiment in which L-malate was dehydrated enzymatically in 99.5% D_2O to the extent of 0.04%. The amount of deutero-L-malate which could have been formed by the reverse reaction from the fumarate so produced and initially present in the sample would be immeasurably small. However, the dipotassium malate recovered had incorporated about 0.003 atom of deuterium per molecule. This relatively rapid exchange demonstrates the existence of an intermediate in which the hydrogen atom has been removed from the methylene carbon of L-malate and which may be converted either into the enzyme–fumarate or enzyme–L-malate complex.

(1) This work was supported by research grants from the Research Committee of the University of Wisconsin (Rockefeller Grant) and from the National Science Foundation.
(2) H. D. Dakin, *J. Biol. Chem.*, **52**, 183 (1922).
(3) S. Englard and S. P. Colowick (personal communication) have arrived at this same conclusion from indirect evidence using a particulate preparation from heart muscle.
(4) C. Frieden, R. M. Bock and R. A. Alberty, THIS JOURNAL, **76**, 2482 (1954).
(5) F. H. Westheimer and N. Nicolaides, *ibid.*, **71**, 25 (1949).
(6) C. Frieden and R. A. Alberty, *J. Biol. Chem.*, **212**, 859 (1955).
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THE ADRENAL HORMONES AND RELATED COMPOUNDS. I. A "DIRECT" SYNTHESIS OF HYDROCORTISONE ACETATE AND CORTISONE ACETATE FROM 11α-HYDROXYPROGESTERONE

Sir:

The microbiological oxidation of progesterone described by Peterson, Murray, *et al.*,[1] provides an elegant method for the synthesis of 11α-hydroxyprogesterone (I) in high yield. The synthesis of

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cortisone acetate[2] and hydrocortisone acetate[3] from I has been described. In both of these schemes the 3-keto-Δ^4-system was selectively reduced and reconstituted at the end of the synthesis. We now report the synthesis of these and other members of the cortical family from I *via* "direct" chemical procedures which avoid this undesirable expedient.

11-Ketoprogesterone (II), obtained by oxidation of I, was treated with ethyl oxalate (1.1 mole) and sodium methoxide (1 mole) in benzene (25–30°). The sodium salt of 21-ethoxyoxalyl-11-ketoprogesterone (III)[4] was precipitated in 85–90% yield by the addition of ether. Treatment of III with iodine in aqueous potassium iodide and subsequently with potassium acetate in methanol produced 11-dehydrocorticosterone acetate (IV) (m.p. 181–182.5°) in approximately 40% yield. In a similar way corticosterone acetate (VI) was prepared from 11β-hydroxyprogesterone (V).[4]

A solution of III in *t*-butyl alcohol and methanol, neutralized with acetic acid, was treated (0°) with bromine (2 moles) in the presence of sodium acetate. Subsequent addition of sodium methoxide in methanol gave methyl 3,11-diketo-4,17(20)-[*cis*]-pregnadien-21-oate (VII)[5] (60%), m.p. 212–216°, $[\alpha]^{23}D$ +189° (acetone), $\lambda_{max.}^{EtOH}$ 233 mμ, ϵ = 24,375. (*Anal.* Calcd. for $C_{22}H_{28}O_4$: C, 74.13; H, 7.92. Found: C, 74.40; H, 7.78). Methyl 3-keto-11α-hydroxy-4,17(20)-[*cis*]-pregnadien-21-oate (IX),[6] m.p. 205–210°; $[\alpha]^{23}D$ +133° (acetone), $\lambda_{max.}^{EtOH}$ 239 mμ, ϵ = 22,425. (*Anal.* Calcd. for $C_{22}H_{30}O_4$: C, 73.75; H, 8.48. Found: C, 73.77; H, 8.38), was prepared in a like manner from the salt of 21-ethoxyoxalyl-11α-hydroprogesterone (VIII).[4]

The hydrolysis of VII with potassium hydroxide in aqueous methanol gave the corresponding acid (X), m.p. 252–254° (dec.), and 3,11-diketo-4,16-pregnadien-21-oic acid (XI), m.p. 169–172° [methyl ester (XII), m.p. 114–115°, $[\alpha]^{23}D$ +179° (acetone), $\lambda_{max.}^{EtOH}$ 239 mμ, ϵ = 15,450. *Anal.* Calcd. for $C_{22}H_{28}O_4$: C, 74.13; H, 7.92. Found: C, 74.34; H, 7.69]. Esterification of X with diazomethane gave VII. However, sodium methoxide in anhydrous methanol converted VII to its 17,20-*trans* isomer (XIII), m.p. 155–159°, $[\alpha]^{23}D$ +121° (acetone), $\lambda_{max.}^{EtOH}$ 230 mμ, ϵ = 24,300. (*Anal.* Calcd. for $C_{22}H_{28}O_4$: C, 74.13; H, 7.92. Found: C, 73.93; H, 7.66).

Treatment of VII with pyrrolidine and *p*-toluenesulfonic acid according to procedures described by Heyl and Herr,[7] resulted in the quantitative formation of the corresponding 3-enamine (XIV). An analytical sample melted at 181–183°; $[\alpha]^{23}D$ −110° ($CHCl_3$); $\lambda_{max.}^{5\% HCl-MeOH}$ 225 mμ, ϵ = 14,550; 274 mμ, ϵ = 21,825. (*Anal.* Calcd. for $C_{26}H_{35}NO_3$: C, 76.24; H, 8.61; N, 3.42. Found: C, 76.34; H, 8.31; N, 3.64). The crude enamine (XIV) was reduced with lithium aluminum hydride in dibutyl ether, N-ethylmorpholine or ether–benzene to 3-pyrrolidyl-11β,21-dihydroxy-3,5,17(20)-[*cis*]-pregnatriene (XV), m.p. 228–232° (dec.), $[\alpha]^{23}D$ −88° (chloroform). (*Anal.* Calcd. for $C_{25}H_{37}NO_2$: C, 78.28; H, 9.72; N, 3.65. Found: C, 78.31; H, 9.82; N, 4.03). The 3-keto-Δ^4-system was regenerated by hydrolysis with alkali in methanol–water yielding 11β,21-dihydroxy-4,17(20)-[*cis*]-pregnadien-3-one (XVI),[5] m.p. 156–158°, $[\alpha]^{23}D$ +128° (acetone), $\lambda_{max.}^{EtOH}$ 242.5 mμ, ϵ = 16,100. (*Anal.* Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.04; H, 9.43). Acetylation of the total crude XVI yielded the 21-acetate (XVII)[5] (70–76% over-all from VII), m.p. 190–191°, $[\alpha]^{23}D$ +128° (acetone), $\lambda_{max.}^{EtOH}$ 243 mμ, ϵ = 15,750. (*Anal.* Calcd. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 74.18; H, 8.45). Chromic acid oxidation of XVII gave 21-hydroxy-4,17(20)-[*cis*]-pregnadien-3,11-dione 21-acetate (XVIII), m.p. 196–199°, $[\alpha]^{23}D$ +145° (acetone). (*Anal.* Calcd. for $C_{23}H_{30}O_4$: C, 74.50; H, 8.22. Found: C, 74.37; H, 8.43).

Alternatively, reaction of VII with ethylene glycol and *p*-toluenesulfonic acid[5] afforded the 3-ketal (XIX), m.p. 188–190°, $[\alpha]^{23}D$ +9°, $\lambda_{max.}^{EtOH}$ 225 mμ, ϵ = 13,525. (*Anal.* Calcd. for $C_{24}H_{32}O_5$: C, 71.94; H, 8.05. Found: C, 71.90; H, 7.95). Reduction of XIX with lithium aluminum hydride gave the 3-ethylene ketal (XX) of XVI, m.p. 185–188°, $[\alpha]^{23}D$ +4° (acetone). (*Anal.* Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.87; H, 9.22). Acid hydrolysis of XX yielded XVI. In a similar manner XIII was converted to 11β,21-dihydroxy-4,17-4,17(20)-[*trans*]-pregnadien-3-one (XXI), m.p. 181–185°, $[\alpha]^{23}D$ +120° (acetone). (*Anal.* Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.60; H, 9.33). The acetate (XXII) of XXI melted at 107–110°; $[\alpha]^{23}D$ +113° (acetone). (*Anal.* Calcd. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 74.16; H, 9.07).

Evidence for the stereochemical assignments at the 17,20-double bonds in XVII and XXII and their precursors was provided by oxidation studies at these positions. Hydroxylation of XVII with osmium tetroxide according to procedures similar to those described by Milas[8] or Reichstein[9] gave 11β,17α,20α,21-tetrahydroxy-4-pregnen-3-one 21-acetate (XXII), m.p. 228–229°, $[\alpha]^{23}D$ +89° (acetone). (*Anal.* Calcd. for $C_{23}H_{34}O_6$: C, 67.95; H, 8.43. Found: C, 68.33; H, 8.29). Upon saponification, XXIII yielded the corresponding tetrol (XXIV), m.p. 256–260°, $[\alpha]^{23}D$ + 65° (acetone). (*Anal.* Calcd. for $C_{21}H_{32}O_5 \cdot 2H_2O$: C, 63.00; H, 9.05. Found: C, 63.45; H, 9.32). Similarly, the *trans*-isomer[10] (XXII) was converted to 11β,17α,20β,21-tetrahydroxy-4-pregnen-3-one

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(3) R. H. Levin, B. J. Magerlein, A. V. McIntosh, A. R. Hanze, G. S. Fonken, J. L. Thompson, A. M. Searcy, M. A. Scheri and E. S. Gutsell, *ibid.*, **75**, 502 (1953).

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(5) J. A. Hogg, P. F. Beal and F. H. Lincoln, U. S. Patent 2,707,184 (1955).

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(7) F. W. Heyl and M. E. Herr, THIS JOURNAL, **75**, 1918 (1953).

(8) N. A. Milas and S. Sussman, *ibid.*, **58**, 1302 (1936).

(9) D. A. Prins and T. Reichstein, *Helv. Chim. Acta*, **25**, 300 (1942).

(10) It has been shown that *trans*-17,20-steroid olefins yield 17α,20β-glycols: see Fieser and Fieser, "Natural Products Related to Phenanthrene," Third Edition, Reinhold Publishing Corporation, New York, N. Y., 1949, pp. 410-419.

(XXV, Reichstein's Substance E[11]), m.p. 130–132°, identified as the 20,21-diacetate XXVI, m.p. 230–231.5°; $[\alpha]^{23}D$ +168° (acetone). [Reported[11] m.p. 229–230°; $[\alpha]^{23}D$ +163° (acetone)]. (*Anal.* Calcd. for $C_{25}H_{36}O_7$: C, 66.94; H, 8.09. Found: C, 66.69; H, 8.07). That XXIV is the 20α-epimer of Reichstein's Substance E was established by the oxidation of its 21-acetate (XXIII) with manganese dioxide to hydrocortisone acetate (XXVII), m.p. 217–220°, $[\alpha]^{23}D$ +159° (dioxane), $\lambda_{max.}^{EtOH}$ 243 mμ, ε = 15,900. Also, the oxidation of XXIII with chromium trioxide in pyridine yielded cortisone acetate (XXVIII), m.p. 244–248°, identified by its infrared spectrum and paper chromatogram.

The oxidative hydroxylation of XVII in *t*-butyl alcohol–pyridine with phenyl iodosoacetate in the presence of catalytic amounts of osmium tetroxide afforded hydrocortisone acetate (XXVII) in 65% yield.[12] In like manner XXII and XVIII were converted to hydrocortisone acetate (XXVII) and cortisone acetate (XXVIII), respectively.

The methods described have also been applied to progesterone and other 20-ketosteroids.

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(11) T. Reichstein and J. von Euw, *Helv. Chim. Acta*, **24**, 247E (1941).

(12) K. Miescher and J. Schmidlin, *ibid.*, **33**, 1840 (1950), have described the use of hydrogen peroxide and osmium tetroxide for the oxidative hydroxylation of the steroidal 17,20-double bond. This procedure in the present studies gave hydrocortisone acetate in 35–40% yield when applied to XVII.

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THE ADRENAL HORMONES AND RELATED COMPOUNDS. II. SYNTHESIS OF 1-DEHYDRO ANALOGS[1]

Sir:

In the previous paper of this series we described the preparation of 21-ethoxyoxalyl-11-ketoprogesterone and its conversion to certain of the adrenal hormones. We now report the preparation of 2,21-di-(ethoxyoxalyl)-11-ketoprogesterone (I) and its conversion to 9α-fluoro-1-dehydrohydrocortisone acetate *via* 1-dehydrohydrocortisone acetate.[2]

11-Ketoprogesterone, prepared by the method of Peterson, Murray, *et al.*,[3] was treated with two moles of diethyl oxalate and sodium methoxide in *t*-butyl alcohol. The yellow disodium salt of I precipitated at once. This slurry was neutralized with acetic acid–methanol and treated with three moles of bromine in the presence of sodium acetate. Subsequent treatment with sodium methoxide in methanol gave a 58.3% yield of methyl 2-bromo-3,11-diketo-4,17(20)-[*cis*]-pregnadien-21-oate (II), m.p. 160–162°; $[\alpha]^{23}D$ +209° (chloroform); (*Anal.* Calcd. for $C_{22}H_{27}BrO_4$: Br, 18.36. Found: Br, 18.46). Debromination of a sample of II with zinc and acetic acid yielded methyl 3,11-diketo-4,17(20)-[*cis*]-pregnadien-2-oate (III).[1] Dehydrohalogenation of III with collidine or with lithium chloride in dimethyl formamide[4] produced methyl 3,11-diketo-1,4,17(20)-[*cis*]-pregnatrien-21-oate (IV) (70% yield), m.p. 240–243°; $[\alpha]^{24}D$ +210° (chloroform); (*Anal.* Calcd. for $C_{22}H_{26}O_4$: C, 74.55; H, 7.39. Found: C, 74.50; H, 7.14). Conversion of IV to the pyrrolidyl enamine followed by reduction with lithium aluminum hydride and removal of the enamine, as previously described,[1] afforded 11β,21-dihydroxy-1,4,17(20)-[*cis*]-pregnatrien-3-one (V), m.p. 174–175°; $[\alpha]^{25}D$ +110° (chloroform); (*Anal.* Calcd. for $C_{21}H_{28}O_3$: C, 76.78; H, 8.59. Found: C, 76.98; H, 8.73). The conversion of IV to V also can be effected by blocking the 3-keto grouping as the ethylene ketal instead of the enamine. Acetylation of V gives quantitatively the 21-acetate (VI), m.p. 220–222.5°; $[\alpha]D$ +122° (chloroform). (*Anal.* Calcd. for $C_{23}H_{30}O_4$: C, 74.56; H, 8.16. Found: C, 74.58; H, 8.50). In 65% yield[5] VI is converted by phenyl iodosoacetate in the presence of osmium tetroxide to 11β,17α,21-trihydroxy-1,4-pregnadiene-3,20-dione-21-acetate (VII),[2] m.p. 240–242°; $[\alpha]^{24}D$ +116° (dioxane); $\lambda_{max.}^{EtOH}$ 242 mμ; ε 15,225; (*Anal.* Calcd. for $C_{23}H_{30}O_6$: C, 68.63; H, 7.51. Found: C, 68.89; H, 7.52). Saponification of VII produced 11β,17α,21-trihydroxy-1,4-pregnadiene-3,20-dione (VIII),[2] (90% yield), m.p. 232–236°; $[\alpha]D$ +102° (dioxane); (*Anal.* Calcd. for $C_{21}H_{28}O_5$: C, 69.97; H, 7.83. Found: C, 69.79; H, 7.88). Oxidation of VII with N-bromoacetamide in *t*-butyl alcohol–pyridine formed 17,21-dihydroxy-1,4-pregnadiene-3,11,20-trione-21-acetate (IX)[2] (85% yield), m.p. 230–232° (dec.); $[\alpha]D$ +185° (dioxane); (*Anal.* Calcd. for $C_{23}H_{28}O_5$: C, 68.98; H, 7.05. Found: C, 68.95; H, 6.65). Hydrolysis of IX with potassium bicarbonate in aqueous methanol gave 17α,21-dihydroxy-1,4-pregnadiene-3,11,20-trione (X)[2] (91% yield), m.p. 230.5–232.5°; $[\alpha]^{23}D$ +169° (dioxane); (*Anal.* Calcd. for $C_{21}H_{26}O_5$: C, 70.37; H, 7.31. Found: C, 70.64; H, 7.20). Dehydration of VII with thionyl chloride–pyridine formed 17α,21-dihydroxy-1,4,9(11)-pregnatriene-3,20-dione-21-acetate (XI) (48% yield), m.p. 223–226°; $[\alpha]^{24}D$ +75° (chloroform); (*Anal.* Calcd. for $C_{23}H_{28}O_5$: C, 71.85; H, 7.34. Found: C, 72.10; H, 7.66). Treatment of XI with N-bromo-

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(4) R. P. Holysz, *ibid.*, **75**, 4432 (1953).

(5) K. Miescher and J. Schmidlin, *Helv. chim. acta*, **33**, 1840 (1950), have described the oxidative hydroxylation of the steroidal 17,20 double bond with hydrogen peroxide and osmium tetroxide. In this work 1-dehydrohydrocortisone acetate was produced in 43% yield from VI by their procedure. Prolonged workup with sodium bisulfite resulted in appreciable hydrolysis to the free alcohol.