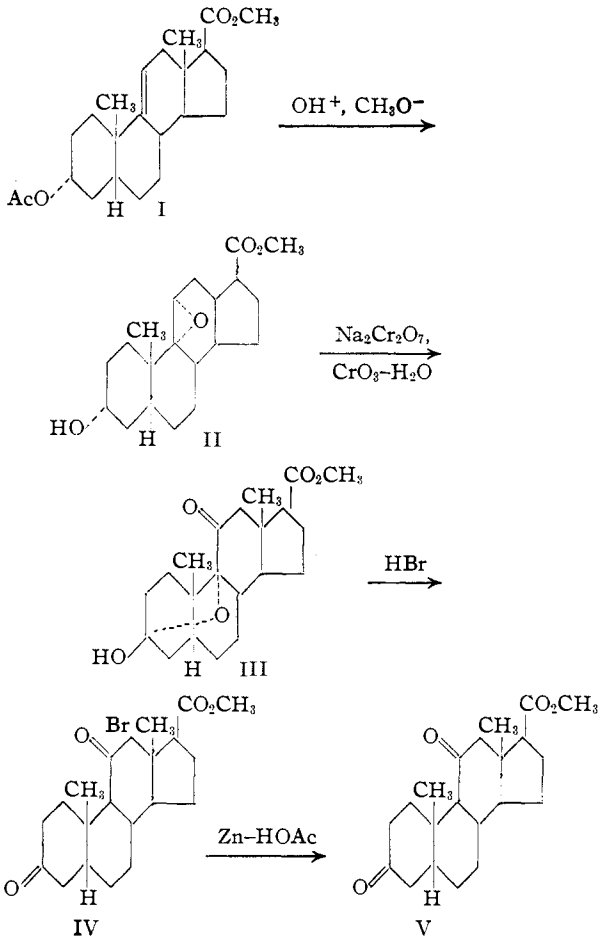

After completion of this work, Dr. Woodward kindly informed us of the two-stage reduction and acetylation of synthetic methyl *d*-3-keto-$\Delta^{4,9(11),16}$ etiocholatrienate[5] to our starting material I, as reported in a parallel Communication. Our product V has been transformed by reactions reported by Reichstein[4,6] into an intermediate of Sarett's synthesis of cortisone.[7]

(5) R. B. Woodward, F. Sondheimer and D. Taub, THIS JOURNAL, **73**, 4057 (1951).

(6) J. v. Euw, A. Lardon and T. Reichstein, *Helv. Chim. Acta.*, **27**, 1287 (1944).

(7) L. H. Sarett, THIS JOURNAL, **70**, 1454 (1948); **21**, 2443 (1949).

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STEROIDS. XXV.[1] SYNTHESIS OF CORTISONE

Sir:

There was recorded recently the partial synthesis of allopregnane-11,20-dione-3β-ol from a number of ring C unsubstituted plant steroids such as diosgenin,[1,2] ergosterol,[2] and two degradation products (Δ^5-3β-hydroxybisnorcholenic acid[2] and Δ^5-pregnen-3β-ol-20-one[3]) of stigmasterol. Since allopregnane-3β,17α,21-triol-11,20-dione 21-monoacetate (monoacetate of Reichstein's compound D[4]) has already been transformed[5] into cortisone, there remains only the interconversion of allopregnane-11,-20-dione-3β-ol into Reichstein's compound D monoacetate in order to complete the partial synthesis of cortisone from readily available plant steroids. The present report is concerned with the successful completion of these missing steps.

Allopregnane-11,20-dione-3β-ol was converted (acetic anhydride–*p*-toluenesulfonic acid) into its 11,20-dienol acetate,[6] which without isolation was treated with excess perbenzoic acid in chloroform solution.[7] Brief saponification with 2 *N* sodium hydroxide solution afforded in good yield allopregnane-3β,17α-diol-11,20-dione (m.p. 270–272° (uncor.), $[\alpha]^{20}D$ + 76° (dioxane); found: C, 72.44; H, 9.46). Bromination in chloroform solution smoothly led to 21-bromoallopregnane-3β,17α-diol-11,20-dione (m.p. 243–245° (dec.), $[\alpha]^{20}D$ + 73° (dioxane)) which was treated with sodium iodide in acetone solution followed by refluxing with potassium acetate exactly as described recently[8] for the preparation of Reichstein's compound P. The resulting allopregnane-3β,17α,21-triol-11,20-dione 21-acetate (m.p. 235–237°, $[\alpha]^{20}D$ + 66° (acetone); found: C, 68.22; H, 8.73) was identified with Reichstein's compound D[4] by direct comparison of the 3,21-diacetates (m.p. 220°). We are grateful to Prof. T. Reichstein of the University of Basle for carrying out the mixed melting point determination.

Formally speaking the above sequence of reactions completes the total synthesis of cortisone. Androstan-3β-ol-17-one has been synthesized totally[9] and is convertible[10–13] into methyl 3-ketoetioallocholanate, which has also been synthesized totally.[14] The latter substance has already been transformed[15] into methyl Δ^4-3-ketoetiocholenate and thence *via* methyl Δ^5-3β-acetoxyetiocholenate[16] and Δ^5-3β-acetoxyetiocholenic acid[17] into Δ^5-preg-

(1) Paper XXIV, G. Stork, J. Romo, G. Rosenkranz and C. Djerassi, THIS JOURNAL, **73**, 3546 (1951).

(2) E. M. Chamberlin, W. V. Ruyle, A. E. Erickson, J. M. Chemerda, L. M. Aliminosa, R. L. Erickson, G. E. Sita and M. Tishler, *ibid.*, **73**, 2396 (1951).

(3) C. Djerassi, J. Romo and G. Rosenkranz, *J. Org. Chem.*, **16**, 754 (1951); J. Romo, G. Rosenkranz and C. Djerassi, THIS JOURNAL, **73**, in press (1951).

(4) J. v. Euw and T. Reichstein, *Helv. Chim. Acta*, **25**, 1009 (1942).

(5) G. Rosenkranz, C. Djerassi, R. Yashin and J. Pataki, *Nature*, **168**, 28 (1951). The reactions involve N-bromoacetamide oxidation to allopregnane-3,11,20-trione-17α,21-diol 21-acetate, dibromination and treatment with sodium iodide (*cf.* ref. 15).

(6) *Cf.* B. A. Koechlin, D. L. Garmaise, T. H. Kritchevsky and T. F. Gallagher, THIS JOURNAL, **71**, 3262 (1949), for the analogous reaction with pregnane-11,20-dione-3α-ol.

(7) General method of T. H. Kritchevsky and T. F. Gallagher (*J. Biol. Chem.*, **179**, 507 (1949); THIS JOURNAL, **73**, 184 (1951)) for the introduction of the 17α-hydroxy group.

(8) G. Rosenkranz, J. Pataki, St. Kaufmann, J. Berlin and C. Djerassi, *ibid.*, **72**, 4081 (1950).

(9) H. M. E. Cartwell, J. W. Cornforth, S. R. Duff, H. Holtermann and R. Robinson, *Chemistry and Industry*, 389 (1951).

(10) L. Ruzicka, P. A. Plattner, H. Heusser and J. Pataki, *Helv. Chim. Acta*, **29**, 936 (1946).

(11) P. A. Plattner, L. Ruzicka, H. Heusser, J. Pataki and K. Meier, *ibid.*, **29**, 943 (1946).

(12) M. Sorkin and T. Reichstein, *ibid.*, **29**, 1209 (1946).

(13) M. Steiger and T. Reichstein, *ibid.*, **20**, 1040 (1938); C. Djerassi and C. R. Scholz, THIS JOURNAL, **69**, 2409 (1947).

(14) R. B. Woodward, F. Sondheimer and D. Taub, *ibid.*, **73**, 3547 (1951).

(15) C. Djerassi and C. R. Scholz, *ibid.*, **69**, 2410 (1947); G. Rosenkranz, C. Djerassi and co-workers, *ibid.*, **72**, 1046, 4077 (1950).

(16) H. Reich and A. Lardon, *Helv. Chim. Acta*, **29**, 671 (1946).

(17) This step is carried out industrially (*cf.* F.I.A.T. Final Report No. 996, London, H. M. Stationery Office 1949, pp. 87–89).

nen-3β-ol-20-one,[18] which represents one of the intermediates[3] in our synthesis of cortisone.

(18) H. B. MacPhillamy and C. R. Scholz, *J. Biol. Chem.*, **178**, 37 (1949).

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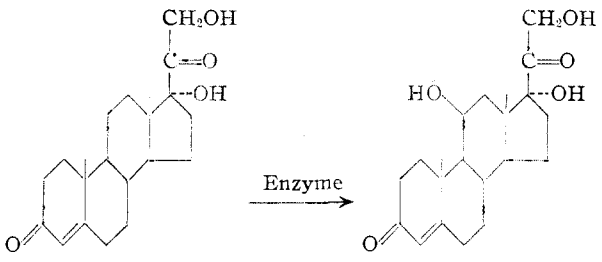
ENZYMATIC SYNTHESIS OF 17-HYDROXYCORTICOSTERONE

Sir:

The biosynthesis of 17-hydroxycorticosterone has been demonstrated in intact adrenal glands,[1] extirpated adrenal glands perfused with a medium containing 17-hydroxy-11-desoxycorticosterone acetate[2] and adrenal gland homogenates to which 17-hydroxy-11-desoxycorticosterone had been added as the substrate.[3] We wish to report the oxidation of 17-hydroxy-11-desoxycorticosterone to 17-hydroxycorticosterone by an enzyme system associated with the insoluble cellular constituents of the adrenal cells.



17-Hydroxy-11-desoxycorticosterone — 17-Hydroxycorticosterone

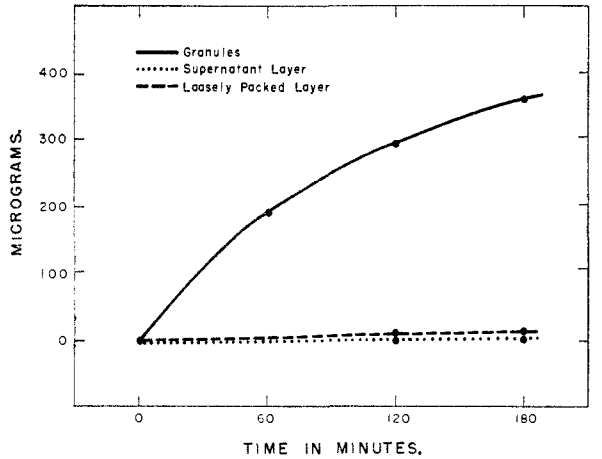


Fig. 1.—17-Hydroxycorticosterone formed in differentially centrifuged fractions of a beef adrenal homogenate incubated with 400 micrograms of 17-hydroxy-11-desoxycorticosterone in a buffer composed of 0.01 *M* glucose, 0.062 *M* NaCl, 0.04 *M* ($NaHPO_4$–NaH_2PO_4, *p*H 7.0), 0.025 *M* KCl, 0.01 *M* sodium fumarate and 0.004 *M* $MgSO_4$.

(1) D. H. Nelson, H. Reich and L. T. Samuels, *Science*, **111**, 578 (1950).

(2) O. Hechter, R. P. Jacobsen, R. Jeanloz, H. Levy, C. W. Marshall, G. Pincus and V. Schenker, *Arch. Biochem.*, **25**, 457 (1950).

(3) D. A. McGinty, G. N. Smith, M. L. Wilson and C. S. Worrel, *Science*, **112**, 506 (1950).

Frozen beef adrenals were thawed at 2°, minced, suspended in buffer solution or 0.88 *M* sucrose solution and homogenized with a Potter–Elvehjem type homogenizer. The resulting homogenates were differentially centrifuged according to the methods of Schneider and Hogeboom[4] and the various supernatant and sedimented layers were tested for enzyme activity.

To test for enzyme activity, portions of the sediments or supernatants in amounts equivalent to 1 g. of original tissue were incubated from one to five hours in 5 ml. of buffer containing 400 micrograms of 17-hydroxy-11-desoxycorticosterone. After incubation, the mixture was extracted with chloroform, the chloroform evaporated to dryness and the residue partitioned between petroleum ether and 70% ethanol. The 70% ethanol phase was evaporated to dryness and the 17-hydroxycorticosterone formed was determined by the fluorescence and by chromogens produced with sulfuric acid. For the identification of the products, the residues of several incubations were combined, dissolved in chloroform and chromatographed on silica gel with 7–10% ethanol in chloroform.

The figure shows the amount of 17-hydroxycorticosterone formed by fractions separated by differential centrifugation between 2000 *g* for ten minutes and 19000 *g* for 30 minutes. It will be seen that essentially all of the activity is in the granules while only traces are present in the supernatant layer or the loosely packed layer above the granules. The reaction proceeds best at a *p*H near the neutral point and at temperatures between 35 and 40°. Above 40° there is a rapid decline in activity. It is inhibited by 0.01 *M* diethyl dithiocarbamate, partially inhibited with 0.01 *M* HCN and disodium versonate and slightly inhibited with 0.01 *M* sodium azide. With the buffer used, fumarate ion is necessary for the reaction. Adenosinetriphosphate does not enhance the rate of conversion.

The product of the reaction has been isolated in crystalline form. The crystals separated in small striated cylinders and cruciform aggregates and melted at 199–205°. A second crystallization yielded crystals which melted at 204–208° and gave no depression of melting point with an authentic sample of 17-hydroxycorticosterone obtained by saponification of 17-hydroxycorticosterone acetate. The product gave a distinct green fluorescence when treated with sulfuric acid and when chromatographed according to the methods of Zaffaroni[5] positioned itself in the regions characteristic of 17-hydroxycorticosterone. Both the specific rotation and infrared spectrum were shown to conform to those of 17-hydroxycorticosterone.

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(4) W. C. Schneider and G. H. Hogeboom, *J. Biol. Chem.*, **183**, 123 (1950).

(5) R. B. Burton, A. Zaffaroni and E. H. Keutmann, *ibid.*, **188**, 763 (1951).