to the limit of the solvent migration. Consequently, an elution chromatogram of a portion of Solution I was made by diluting with acetone and running on a silica gel column (0.9 × 30 cm.) using acetone–water mixtures. The radioactivity of the fractions was measured by counting evaporated drops of the eluate on aluminum discs with a G.M. counter. One fraction (Solution IX) behaving in the column in a manner similar to authentic B$_{12}$, was then subjected to further study. This solution exhibited absorption maxima at 2780 and 3610 Å. No radioactivity could be detected in the organic phase when IX was extracted with dithizone in chloroform. A paper chromatogram of IX revealed a concentrated spot with little tailing, coincident for both the radio- and bioactivity. The R_f of this spot (0.79–0.80) was in agreement with a B$_{12}$ standard run simultaneously.

The radioactivity of the original and purified samples was shown to be Co^{60} (5.3 y) by measurement of the gamma ray spectrum in a scintillation spectrometer. On a G.M. counter (geometry $\sim$9%), Solution I had 689 $\pm$ 16 cpm./γ, solution IX 567 $\pm$ 30 cpm./γ, giving a retention value of $\sim$80%. By employing serial dilutions of I and IX against standard B$_{12}$, it was determined that the *bio*-activity in Solution I was 80 $\pm$ 20% and in Solution IX was 100 $\pm$ 15% of the standard by weight.

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RECEIVED MAY 14, 1951

ENZYMATIC DISINTEGRATION OF WHEAT GERM DESOXYPENTOSE NUCLEIC ACID

Sir:

Previous work in this Laboratory[1] has shown that the degradation of calf thymus desoxypentose nucleic acid (DNA) by crystalline pancreatic desoxyribonuclease proceeded according to a complex pattern, resulting in the formation of dialyzable fragments and of a non-diffusible core which was characterized by greatly increased ratios of adenine to guanine, thymine to cytosine, and purines to pyrimidines.

A study of the generality of this phenomenon appeared important, since it offers an additional tool for the differentiation between DNA preparations of different origin and for the understanding of the relationship between nucleotide sequence and enzyme action. Wheat germ DNA, highly polymerized and entirely free of pentose nucleic acid, was employed. This DNA contains an appreciable quantity of a third pyrimidine, 5-methylcytosine,[2] and provides thereby one more marker, useful for the study of the enzymatic attack. The results summarized in Table I indicate the trend of degradation; "19% core" and "8% core" refer to the dialysis residues recovered after 81 and 92% of the DNA, respectively, had been converted to dialyzable products.

TABLE I

Wheat Germ DNA: Intact Preparation and Enzymatically Produced Cores (as moles per 100 moles P)

Constituent	Intact DNA	19% Core	8% Core
Adenine	26.3	33.2	35.4
Guanine	21.8	20.0	19.8
Cytosine	16.2	11.8	10.3
5-Methylcytosine	5.8	4.3	3.6
Thymine	26.1	26.2	23.4
Total	96.2	95.5	92.5
Purine to pyrimidine ratio	1.00	1.26	1.48

TABLE II

Liberation of Adenine (as moles per 100 moles P)

Agent	Intact DNA	19% Core	8% Core
1 *N* H$_2$SO$_4$, 100°, 1 hr.	26.5	31.2	31.6
98% HCOOH, 175°, 2 hr.	26.3	33.3	35.4
7.5 *N* HClO$_4$, 100°, 1 hr.		33.2	

Several points appear of interest. The ratio of cytosine to 5-methylcytosine remained constant in all stages, *viz.*, 2.8. In the intact DNA, the sum of these two pyrimidines equalled the molar concentration of guanine, a relationship observed with respect to the ratio of guanine to cytosine in almost all DNA specimens studied.[3] As judged from the extent of its liberation by various hydrolyzing agents, adenine seems to occur in two types of linkage, one of which is enriched in the cores (Table II). The procedures employed for the isolation and purification of the DNA will soon be discussed in detail. The enzyme used was supplied by the Worthington Biochemical Laboratory, Freehold, N. J. The analytical methods have been described before.[4]

This work was supported by research grants from the U. S. Public Health Service and the Rockefeller Foundation.

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(1) S. Zamenhof and E. Chargaff, *J. Biol. Chem.*, **178**, 531 (1949); **187**, 1 (1950).

(2) G. R. Wyatt, *Biochem. J.*, **48**, 584 (1951).

(3) E. Chargaff, *Experientia*, **6**, 201 (1950); *J. Cell. Comp. Physiol.*, in press; *Federation Proc.*, **10**, in press.

(4) E. Vischer and E. Chargaff, *J. Biol. Chem.*, **176**, 703, 715 (1948); E. Chargaff, E. Vischer, R. Doniger, C. Green and F. Misani, *ibid.*, **177**, 405 (1949); E. Chargaff, R. Lipshitz, C. Green and M. E. Hodes, *ibid.*, **192**, in press.

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RECEIVED JUNE 18, 1951

ON THE SYNTHESIS OF CORTISONE ACETATE

Sir:

We wish to report the synthesis of cortisone acetate from *allo*pregnane-3β-ol-11,20-dione acetate, I, made available recently from $\Delta^{5,6}$ steroids, devoid of functional groups in ring C.[1,2]

Hydroxylation of the *allo*pregnane I at the 17-position by conversion into its enol acetate and treatment with perbenzoic acid followed by caustic saponification[3] yielded *allo*pregnane-3β,17α-diol-

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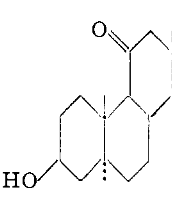
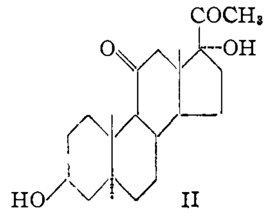
(1) Chamberlain, Ruyle, A. E. Erickson, Chemerda, Aliminosa, R. L. Erickson, Sita and Tishler, THIS JOURNAL, **73**, 2396 (1951).

(2) Stork, Romo, Rosenkranz and Djerassi, *ibid.*, **73**, 3546 (1951).

(3) Kritchevsky and Gallagher, *J. Biol. Chem.*, **179**, 507 (1949); Marshall, Kritchevsky, Lieberman and Gallagher, THIS JOURNAL, **70**, 1837 (1948).

11,20-dione(II); m.p. 290–292° (all m.p.s. are uncorrected); found: C, 72.98; H, 9.74; $(\alpha)^{24}_{\mathrm{D}}$ +43 (dioxane). Bromination of II in chloroform and subsequent treatment of the 21-bromo derivative (m.p. 238–240°; found: Br, 18.39) with sodium acetate afforded *allo*pregnane-3β,17α,21-triol-11,20-dione-21-acetate, III; m.p. 233–235°. The latter on oxidation with N-bromoacetamide in methanol was transformed into *allo*pregnane-17α,21-diol-3,11,20-trione (IV); m.p. 229–233°; $(\alpha)^{24}_{\mathrm{D}}$ +100° ($CHCl_3$); found: C, 68.46; H, 7.68 which proved to be identical with a sample prepared by the hydrogenation of cortisone acetate with palladium in methanol containing potassium hydroxide. Bromination and dehydrobromination of the *allo*-dihydrocortisone acetate by a procedure to be described in detail later produced cortisone acetate characterized by its melting point, optical rotation, ultraviolet and infra-red absorption spectra.


I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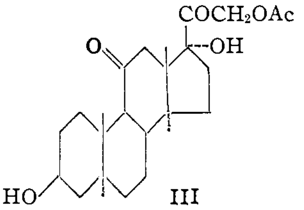
III

Complete details for the conversion of ergosterol, diosgenin, stigmasterol and cholesterol to cortisone acetate will be published in this Journal.

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MAX TISHLER

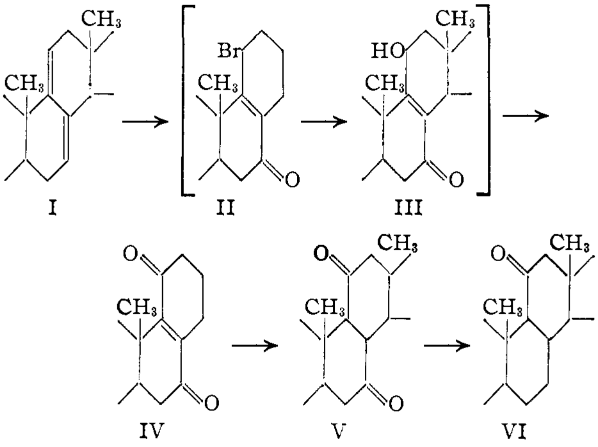
RECEIVED JULY 13, 1951

A FURTHER METHOD FOR PRODUCTION OF 11-KETOSTEROIDS FROM $\Delta^{7,9(11)}$-DIENES

Sir:

Three methods have been recorded for conversion of $\Delta^{7,9(11)}$-dienes into the corresponding 11-ketosteroids.[1,2,3] The first method[1] was shown to be applicable in both the cholestane and coprostane series; the third, demonstrated with sterol derivatives, is not applicable in the bile acid series, since we have found that methyl $\Delta^{7,9(11)}$-lithocholadienate reacts with performic acid to give methyl 3α-formoxy-7-keto-Δ^{8}-cholenate, m.p. 164–166°, $[\alpha]_{\mathrm{D}}$ −1.6° Di, λ^{EtOH} 255 mμ, log ε 3.85 (*Anal.* Calcd. for $C_{26}H_{38}O_5$: C, 72.52; H, 8.90. Found: C, 72.22; H, 9.12) in 63% yield; the hydroxy acid melts at 209–210°, $[\alpha]_{\mathrm{D}}$ −38° Di, (*Anal.* Calcd. for $C_{24}H_{36}O_4$: C, 74.19; H, 9.34. Found: C, 74.57; H, 9.33).

A fourth method, applicable in both series, consists in reaction of a diene (I) with excess N-bromosuccinimide in aqueous *t*-butanol and addition of silver nitrate followed by chromic acid. The resulting Δ^{8}-ene-7,11-dione IV is convertible through V to VI as previously described[1]; alternately, at least with methyl 3α-acetoxy-7,11-diketocholanate, V is convertible to VI by Raney nickel reduction of the 7-cycloethylenemercaptol derivative, m.p. 162–163.5°, $[\alpha]_{\mathrm{D}}$ +27.6° Di (*Anal.* Calcd. for $C_{29}H_{44}O_5S_2$: C, 64.89; H, 8.26; S, 11.96. Found: C, 65.20; H, 8.49; S, 11.86).



Methyl 3α-acetoxy-$\Delta^{7,9(11)}$-lithocholadienate (430 mg.), processed as indicated, gave 152 mg. of crude IV, λ^{EtOH} 272 mμ (3.77), which on reduction gave 90 mg. of pure V, m.p. 161–162° (no depression with previous sample; identical IR spectra), $[\alpha]_{\mathrm{D}}$ + 26.7° Di (*Anal.* Calcd. for $C_{27}H_{40}O_6$: C, 70.40; H, 8.75. Found: C, 70.71; H, 8.96). The reaction with NBS results in development of strong absorption at about 255 mμ; when the excess reagent was destroyed and the crude bromo derivative extracted and debrominated, the product, m.p. 179–180°, λ^{EtOH} 254, 310 mμ (3.88, 2.84) corresponded closely to methyl 3α-acetoxy-7-keto-Δ^{8}-cholenate[1] (no depression) and not at all to the isomeric 11-ketone[4]; hence II is a probable intermediate.

As applied to $\Delta^{7,9(11),22}$-ergostatrienyl acetate, the reaction sequence afforded a crude enedione that on reduction with zinc and acetic acid gave 7,11-diketo-Δ^{22}-erogostenyl acetate, m.p. 197–199°, $[\alpha]_{\mathrm{D}}$ −29.5° Chf (*Anal.* Calcd. for $C_{30}H_{46}O_4$: C, 76.55; H, 9.85. Found: C, 76.47; H, 9.77), in agreement with the properties reported.[2] $\Delta^{7,9(11)}$-Cholestadiene-3β-ol benzoate was converted similarly to the Δ^{8}-ene-7,11-dione benzoate,[1] m.p. 155–158°, $[\alpha]_{\mathrm{D}}$ +46° Di, λ^{EtOH} 269 mμ, log ε 3.80

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(1) L. F. Fieser, J. E. Herz and W. Y. Huang, THIS JOURNAL, **73**, 2397 (1951). The by-product of the oxidation (no ultraviolet absorption) described as methyl 3α-acetoxy-7,8-oxido-$\Delta^{9(11)}$-cholenate, actually is methyl 3α-acetoxy-7-keto-$\Delta^{9(11)}$-cholenate, since on reduction with sodium borohydride it yields an alcohol, m.p. 111–115°, $[\alpha]_{\mathrm{D}}$ +57.5° Di, λ^{Chf} 2.91, 5.80μ (*Anal.* Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48. Found: C, 72.56; H, 9.45), that on dichromate oxidation is reconverted to the starting material. The by-product can be isomerized to the conjugated isomer and the latter converted into the starting diene for recycling, by reduction of the free acid with, sodium and amyl alcohol followed by acid dehydration.

(2) E. M. Chamberlain, 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) G. Stork, J. Romo, G. Rosenkranz and C. Djerassi, *ibid.*, **73**, 3546 (1951).

(4) H. Heymann and L. F. Fieser, THIS JOURNAL, **73**, 4054 (1951).