

DEAE-cellulose chromatography of the crude acidic or alkaline potassium chloride extracts.

Approximately 100 mg. of growth hormone can be obtained from 1 g. of acetone powder by these procedures. This yield is very near the value reported by Li,¹ and is several times that obtained by Raben.³

Either column chromatography of human growth hormone at pH 10 or standing in solution for short periods at pH 4 caused alteration in the electrophoresis patterns. At pH 10, an asymmetry was noted without resolution into components; a more definite separation was apparent at pH 4. No significant change in biological activity was detected as a result of this evident alteration, nor was any asymmetry or separation into components observed during analytical ultracentrifugation of the altered material. A similar situation has been reported for bovine growth hormone.^{5,6}

The electrophoretic mobilities at pH 10 and 4 for the unaltered human hormone were -4.7×10^{-5} and $+3.4 \times 10^{-5}$ cm.²/volt/sec., respectively. The altered material had a slightly greater negative mobility at pH 10 and a somewhat lower positive value at pH 4. A sedimentation constant of 2.4 S was calculated. These values are in agreement with those already reported.^{1,2} Assayed in hypophysectomized rats, the human hormone had approximately the same growth activity as pure bovine growth hormone.

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(6) S. Ellis, G. Noda and M. E. Simpson, *J. Biol. Chem.*, **218**, 115 (1956)

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ENZYMATIC CONVERSION OF URIDINE DIPHOSPHATE D-GLUCURONIC ACID TO URIDINE DIPHOSPHATE GALACTURONIC ACID, URIDINE DIPHOSPHATE XYLOSE, AND URIDINE DIPHOSPHATE ARABINOSE^{1,2}

Sir:

Results of previous investigations indicate that D-glucuronic acid serves as a precursor of the D-galacturonic acid moiety of pectin,³ and that the D-xylose and L-arabinose constituent units of pentosans originate from uronic acid precursors by loss of C-6.⁴⁻⁹ It has been postulated that uridine di-

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(2) It has not been determined whether the carbohydrate moieties of these nucleotides are the D or L forms. However, since the galacturonic acid appears to arise from the glucuronic acid by epimerization of the 4-hydroxyl, it is assumed that the galacturonic acid is the D optical isomer. The xylose is assumed to be derived by decarboxylation of C-6 of D-glucuronic acid, and is therefore D-xylose. The arabinose is formed either by similar decarboxylation of D-galacturonic acid, or through epimerization of D-xylose, and is assumed to be L-arabinose.

(3) F. A. Loewus, R. Jang and C. G. Seegmiller, *J. Biol. Chem.*, in press.

(4) A. C. Neish, *Can. J. Biochem. Physiol.*, **33**, 658 (1955).

(5) C. G. Seegmiller, B. Axelrod and R. M. McCready, *J. Biol. Chem.*, **217**, 765 (1955).

phosphate (UDP) sugars are involved in these conversions.^{7,8}

In the present communication evidence is presented that particulate preparations from *Phaseolus aureus* (mung bean) seedlings are capable of catalyzing the formation of UDP galacturonic acid and UDP pentose from UDP glucuronic acid.

One hundred grams of mung bean seedlings was homogenized in 70 ml. of 0.01 M sodium-potassium phosphate buffer of pH 7.0, and the particulate material which sedimented at $18,000 \times g$ for 30 minutes, after removal of coarse debris, was suspended in 0.5 ml. of 0.1 M tris-(hydroxymethyl)-aminomethane chloride buffer, pH 7.5.

The electrophoretic separations were carried out in 0.2 M ammonium formate, pH 3.6, or in 0.2 M ammonium acetate, pH 5.8. Other methods used in this investigation have been described in a previous paper.¹⁰

The particulate suspension (0.2 ml.) was incubated at 23° with 0.27 μ moles (10^6 c.p.m.) of UDP glucuronic acid¹¹ in a total volume of 1.2 ml., and after one hour the components were separated by paper electrophoresis at pH 5.8. Residual UDP glucuronic acid and four new radioactive bands, representing 50% of the total radioactivity, were present in the mixture.

Band I, containing 34% of the total radioactivity, was shown to consist mainly of UDP pentose by co-electrophoresis with authentic UDP arabinose¹⁰ at pH 3.6 (M_{picrate} , 1.20) and at pH 5.8 (M_{picrate} , 1.08). The radioactive compounds liberated by hydrolysis with 1 N HCl for 15 minutes at 100° co-chromatographed with authentic D-xylose and L-arabinose. The ratio of radioactive xylose to arabinose was 6:1.

Band II, containing 6% of the total radioactivity, was identified as UDP galacturonic acid by co-electrophoresis with authentic UDP galacturonic acid^{12,13} at pH 3.6 (M_{picrate} , 1.26) and at pH 5.8 (M_{picrate} , 1.45), and by coelectrophoresis with authentic galacturonic acid at pH 3.6 (M_{picrate} , 0.54) of the radioactive compound liberated by hydrolysis in 1 N HCl for 15 minutes at 100°.

Band III, containing 1% of the total radioactivity, and Band IV, containing 9% of the total radioactivity, consisted of galacturonic acid 1-phosphate and glucuronic acid 1-phosphate, respectively.

Neither C¹⁴-labeled D-glucuronic acid 1-phosphate nor D-glucuronic acid was converted to galacturonic acid, pentose, or to their phosphorylated derivatives when incubated with the particulate suspension.

(6) C. G. Seegmiller, R. Jang and W. Mann, *Arch. Biochem. Biophys.*, **61**, 422 (1956).

(7) H. A. Altermatt and A. C. Neish, *Can. J. Biochem. Physiol.*, **34**, 405 (1956).

(8) W. G. Slater and H. Beevers, *Plant Physiol.*, **33**, 146 (1958).

(9) A. C. Neish, *Can. J. Biochem. Physiol.*, **36**, 187 (1958).

(10) E. F. Neufeld, V. Ginsburg, E. W. Putman, D. Fanshier and W. Z. Hassid, *Arch. Biochem. Biophys.*, **69**, 602 (1957).

(11) J. L. Strominger, E. S. Maxwell, J. Axelrod and H. M. Kalckar, *J. Biol. Chem.*, **224**, 79 (1957).

(12) D. S. Feingold and W. Z. Hassid, *Abstr. Proc. Am. Chem. Soc.*, Sept. 1957, p. 130.

(13) D. S. Feingold, E. F. Neufeld and W. Z. Hassid, *Arch. Biochem. Biophys.*, in press.

The mung bean particulate preparation thus contains a 4-epimerase capable of converting UDP glucuronic acid to UDP galacturonic acid, and a decarboxylase which decarboxylates the UDP uronic acid (or acids) to UDP pentose (or pentoses).

Particulate preparations from asparagus shoots, radish roots and leaves, and spinach leaves were also found to catalyze these reactions.

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16-ALKYLATED CORTICOIDS. II. 9 α -FLUORO-16 α -METHYLPREDNISOLONE 21-ACETATE¹

Sir:

A recent report² on preliminary clinical trials of 9 α -fluoro-16 α -methylprednisolone prompts us to describe our synthesis of its 21-acetate (I) from sapogenin intermediates. The biological activity of I in animal and human studies is similar to that reported for the corresponding alcohol. Additional pertinent animal and clinical data for the acetate are recorded below.

16 α -Methylpregnenolone³ was hydrogenated with palladium in acetic acid to 3 β -hydroxy-16 α -methylallopregnan-20-one, m.p. 203–205°, [α]_D +68.2° (all rotations in dioxane). *Anal.* Found: C, 79.42; H, 11.31. Enol acetylation at C-20 followed by treatment with peracetic acid, then alkaline hydrolysis, gave 3 β -17 α -dihydroxy-16 α -methylallopregnan-20-one, m.p. 257–259°, [α]_D +11.9°. *Anal.* Found: C, 75.91; H, 10.04. Bromination and acetoxylation at C-21 produced 21-acetoxy-3 β ,17 α -dihydroxy-16 α -methylallopregnan-20-one, m.p. 181–185°, [α]_D +21.0°. *Anal.* Found: C, 70.59; H, 8.51. Oxidation with chromium trioxide-acetone-sulfuric acid gave 21-acetoxy-17 α -hydroxy-16 α -methylallopregnane-3,20-dione, m.p. 205–207°, [α]_D +46°. *Anal.* Found: C, 71.15; H, 8.74. Dibromination at C-2 and C-4, then dehydrobromination with dimethylformamide produced 21-acetoxy-17 α -hydroxy-16 α -methyl-1,4-pregnadiene-3,20-dione (16 α -methyl-1-dehydro-Compound S 21-acetate) which without purification was hydrolyzed with sodium hydroxide to the 21-alcohol, m.p. 209–212°, [α]_D +45.7°, $\lambda_{\text{max}}^{\text{MeOH}}$ 244 m μ (ϵ 14,900). *Anal.* Found: C, 73.98; H, 8.38. 11 α -Hydroxylation with *Pestalotia foedans*⁴ gave 11 α ,17 α ,21-trihydroxy-16 α -methyl-1,4-pregnadiene-3,20-dione, m.p. 236–238°, [α]_D +23.9°, $\lambda_{\text{max}}^{\text{MeOH}}$ 247 m μ (ϵ 18,200). *Anal.* Found: C, 70.56; H, 8.02. 21-Monoacetate: m.p. 188–190°, [α]_D +45.6°, $\lambda_{\text{max}}^{\text{MeOH}}$ 247 m μ (ϵ 19,000). *Anal.* Found: C, 66.96; H, 7.64 (1 mole ethyl acetate). 11 α -Tosylate-21-acetate: m.p. 182–184° (dec.), [α]_D +87.7°, $\lambda_{\text{max}}^{\text{MeOH}}$ 229.5 m μ (ϵ 22,200), shoulder at 241 m μ . *Anal.* Found:

C, 65.53; H, 6.78. Dehydrotosylation with sodium acetate in acetic acid gave 21-acetoxy-17 α -hydroxy-16 α -methyl-1,4,9(11)-pregnatriene-3,20-dione, m.p. 210–213°, $\lambda_{\text{max}}^{\text{MeOH}}$ 238 m μ (ϵ 15,500). *Anal.* Found: C, 72.68; H, 7.65. Addition of hypobromous acid (N-bromoacetamide and perchloric acid) gave a 9 α ,11 β -bromohydrin, which was epoxidized by sodium acetate treatment to 21-acetoxy-17 α -hydroxy-16 α -methyl-9 β ,11 β -epoxy-1,4-pregnadiene-3,20-dione, m.p. 198–200°, [α]_D +40.1°, $\lambda_{\text{max}}^{\text{MeOH}}$ 249 m μ (ϵ 15,600). *Anal.* Found: C, 69.55; H, 7.18. Ring opening with hydrogen fluoride in chloroform-tetrahydrofuran produced the desired product, 9 α -fluoro-16 α -methylprednisolone 21-acetate (I), m.p. 229–231°, [α]_D +77.6°, $\lambda_{\text{max}}^{\text{MeOH}}$ 239 m μ (ϵ 14,500). *Anal.* Found: C, 66.27; H, 7.18.

Eosinopenic activity in the mouse, dog, and man, shows this compound to be at least four to six times as active as prednisone and prednisolone. In the granuloma pouch test,⁵ I is 6.5 times as active as prednisolone acetate, while thymus involution studies in rats and the nitrogen excretion in dogs reveals this compound to be about twenty-five times as active as prednisolone acetate and prednisone, respectively, in these tests.

Metabolic balance studies⁶ carried out with I in a human subject in doses of 15 mg. and 25 mg. per 24 hours caused an average increase over control values in urinary excretion per 24 hours of (1) phosphorus: 53 mg. at 15 mg. dose and 387 mg. at 25 mg. dose; (2) nitrogen: 3 g. at 15 mg. dose and 6.4 g. at 25 mg. dose; (3) sodium: 13.4 meq. at 15 mg. dose and 26.4 meq. at 25 mg. dose; (4) potassium: 8.9 meq. at 15 mg. dose and 17.9 meq. at 25 mg. dose.

Fasting blood sugar levels were consistently elevated above 120 mg. per cent. throughout the period of administration of I in doses of 15 mg. and 25 mg. per 24 hours. This is in marked contrast to the results obtained with prednisone at doses up to 70 mg. per 24 hours.

(5) A. Robert and J. E. Nezamis, *Acta Endocrinol.*, **25**, 105 (1957).

(6) E. C. Reifstein, F. Albright and S. Wells, *J. Clin. Endocrinol.*, **5**, 367 (1945).

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STUDIES OF THE PHOSPHOGLYCERIC ACID MUTASE REACTION WITH RADIOACTIVE SUBSTRATES

Sir:

In a previous communication it was confirmed that diphosphoglyceric acid (DPGA) activated phosphoglyceric acid mutase and that during the reaction one of the phosphate groups was transferred to a suitable acceptor.¹ There was observed,

(1) L. I. Pizer and C. E. Ballou, *THIS JOURNAL*, **79**, 3612 (1957).

(1) After submission of this manuscript, a Communication appeared [G. Arth, J. Fried, D. Johnston, D. Hoff, L. Sarett, R. Silber, H. Stoerk and C. Winter, *THIS JOURNAL*, **80**, 3161 (1958)] describing the preparation of 9 α -fluoro-16 α -methylprednisolone 21-acetate from bile acid intermediates.

(2) E. W. Boland, *Cal. Med.*, **88**, 417 (1958).

(3) R. E. Marker and H. Crooks, *THIS JOURNAL*, **64**, 1280 (1942).

(4) Canadian Patent 507,009.