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 *p*H 10 or standing in solution for short periods at *p*H 4 caused alteration in the electrophoresis patterns. At *p*H 10, an asymmetry was noted without resolution into components; a more definite separation was apparent at *p*H 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 *p*H 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 *p*H 10 and a somewhat lower positive value at *p*H 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)

MERCK SHARP & DOHME RESEARCH LABORATORIES
MERCK & CO., INC. U. J. LEWIS
RAHWAY, NEW JERSEY NORMAN G. BRINK

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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 diphosphate (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 *p*H 7.0, and the particulate material which sedimented at 18,000 × *g* for 30 minutes, after removal of coarse debris, was suspended in 0.5 ml. of 0.1 *M* tris-(hydroxymethyl)-aminomethane chloride buffer, *p*H 7.5.

The electrophoretic separations were carried out in 0.2 *M* ammonium formate, *p*H 3.6, or in 0.2 *M* ammonium acetate, *p*H 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 μmoles (10^6 c.p.m.) of UDP glucuronic acid[11] in a total volume of 1.2 ml., and after one hour the components were separated by paper electrophoresis at *p*H 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 arabinose[10] at *p*H 3.6 ($M_{picrate}$, 1.20) and at *p*H 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 *p*H 3.6 ($M_{picrate}$, 1.26) and at *p*H 5.8 ($M_{picrate}$, 1.45), and by coelectrophoresis with authentic galacturonic acid at *p*H 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 C^{14}-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.

(1) This investigation was supported in part by a research grant (No. A-1418) from the United States Public Health Service, National Institutes of Health, and by a research contract with the United States Atomic Energy Commission.

(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.

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

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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.

DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY
UNIVERSITY OF CALIFORNIA
BERKELEY 4, CALIFORNIA
E. F. NEUFELD
D. S. FEINGOLD
W. Z. HASSID

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16-ALKYLATED CORTICOIDS. II. 9α-FLUORO-16α-METHYLPREDNISOLONE 21-ACETATE[1]

Sir:

A recent report[2] on preliminary clinical trials of 9α-fluoro-16α-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.

16α-Methylpregnenolone[3] was hydrogenated with palladium in acetic acid to 3β-hydroxy-16α-methylallopregnan-20-one, m.p. 203–205°, $[\alpha]_D$ +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 3β-17α-dihydroxy-16α-methylallopregnan-20-one, m.p. 257–259°, $[\alpha]_D$ +11.9°. *Anal.* Found: C, 75.91; H, 10.04. Bromination and acetoxylation at C-21 produced 21-acetoxy-3β,17α - dihydroxy - 16α - methylallopregnan-20-one, m.p. 181–185°, $[\alpha]_D$ +21.0. *Anal.* Found: C, 70.59; H, 8.51. Oxidation with chromium trioxide–acetone–sulfuric acid gave 21-acetoxy - 17α - hydroxy - 16α -methylallopregnane-3,20-dione, m.p. 205–207°, $[\alpha]_D$ +46°. *Anal.* Found: C, 71.15; H, 8.74. Dibromination at C-2 and C-4, then dehydrobromination with dimethylformamide produced 21-acetoxy-17α-hydroxy-16α-methyl-1,4-pregnadiene-3,20-dione (16α-methyl-1-dehydro-Compound S 21-acetate) which without purification was hydrolyzed with sodium hydroxide to the 21-alcohol, m.p. 209–212°, $[\alpha]_D$ +45.7°, λ_{max}^{MeOH} 244 mμ (ε 14,900). *Anal.* Found: C, 73.98; H, 8.38. 11α-Hydroxylation with *Pestalotia foedans*[4] gave 11α,17α,21-trihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione, m.p. 236–238°, $[\alpha]_D$ +23.9°, λ_{max}^{MeOH} 247 mμ (ε 18,200). *Anal.* Found: C, 70.56; H, 8.02. 21-Monoacetate: m.p. 188–190°, $[\alpha]_D$ +45.6, λ_{max}^{MeOH} 247 mμ (ε 19,000). *Anal.* Found: C, 66.96; H, 7.64 (1 mole ethyl acetate). 11α-Tosylate-21-acetate: m.p. 182–184° (dec.), $[\alpha]_D$ +87.7°, λ_{max}^{MeOH} 229.5 mμ (ε 22,200), shoulder at 241 mμ. *Anal.* Found: C, 65.53; H, 6.78. Dehydrotosylation with sodium acetate in acetic acid gave 21-acetoxy-17α-hydroxy - 16α - methyl - 1,4,9(11) - pregnatriene-3,20-dione, m.p. 210–213°, λ_{max}^{MeOH} 238 mμ (ε 15,500). *Anal.* Found: C, 72.68; H, 7.65. Addition of hypobromous acid (N-bromoacetamide and perchloric acid) gave a 9α,11β-bromohydrin, which was epoxidized by sodium acetate treatment to 21 - acetoxy - 17α - hydroxy - 16α - methyl - 9β,11β-epoxy-1,4-pregnadiene-3,20-dione, m.p. 198–200°, $[\alpha]_D$ +40.1°, λ_{max}^{MeOH} 249 mμ (ε 15,600). *Anal.* Found: C, 69.55; H, 7.18. Ring opening with hydrogen fluoride in chloroform–tetrahydrofuran produced the desired product, 9α-fluoro-16α-methylprednisolone 21-acetate (I), m.p. 229–231°, $[\alpha]_D$ +77.6, λ_{max}^{MeOH} 239 mμ (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 studies[6] 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.

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RESEARCH LABORATORIES
SCHERING CORP.
BLOOMFIELD, N. J.
EUGENE P. OLIVETO
RICHARD RAUSSER
LOIS WEBER
A. L. NUSSBAUM
WILLIAM GEBERT
C. THOMAS CONIGLIO
E. B. HERSHBERG
S. TOLKSDORF
MILTON EISLER
P. L. PERLMAN
M. M. PECHET
MASSACHUSETTS GENERAL HOSPITAL
BOSTON, MASS.

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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,

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