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. 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.

(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 9α-fluoro-16α-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.

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

(6) E. C. Reifenstein, 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).

however, a small but definite amount of enzymatic activity even when special precautions were taken to eliminate DPGA from the reaction. This activity was ascribed to an activated form of the enzyme, probably a phosphorylated form. Another possibility was that cofactor DPGA was tightly bound to the enzyme and was therefore always present. In order to differentiate between these two alternatives, use has been made of C^{14}- and P^{32}-labeled substrates.

Carboxyl-labeled 3-phosphoglyceric acid (3PGA) was obtained by treating ribulose 1,5-diphosphate with C^{14}-sodium bicarbonate in the presence of purified carboxydismutase as described by Calvin, *et al.*[2,3] To a solution containing 0.17 μmole of C^{14}-3PGA, 0.15 μmole DPGA and 50 μmoles of imidazole-HCl buffer *p*H 7.0 was added 0.2 μmole of crystalline enzyme (final volume 1.5 ml.). After two hours of incubation at 30° the solution was dialyzed extensively against several changes of distilled water and sodium chloride solution. The final dialysate possessed no radioactivity but the protein solution was radioactive, and the amount of radioactivity in this solution indicated that there was about one mole of phosphoglyceric acid per 100 moles of protein. It appears, therefore, that although substrate is tightly bound to the enzyme, not enough is present to account for all the activity in the reaction mixtures where DPGA is excluded.

Phosphate-labeled DPGA was isolated from fluoride-poisoned fermentation mixtures by barium precipitation,[4] and was purified by paper chromatography with a 2-propanol/NH_4OH/water (70:10:20) solvent system. To a solution containing 50 μmoles of DPGA and 200 μmoles of imidazole–HCl buffer *p*H 7.0 was added 0.2 μmole of crystalline enzyme (final volume 5.6 ml.). After incubation and dialysis similar to that described previously, the protein solution was evaporated to dryness. It contained bound P^{32}, which was shown to be linked to the protein: Partial hydrolysis of the protein was carried out by dissolving it in 11 *N* hydrochloric acid and incubating at 40° for 72 hours. The hydrolysate, after removal of the hydrochloric acid, was passed through a Dowex 50 (H^+) resin column as described by Flavin.[5] The eluate was rechromatographed on paper with the 2-propanol solvent previously described and the region containing the P^{32}-labeled peptide material was eluted. This material after hydrolysis at 110° in a sealed tube with 5.7 *N* hydrochloric acid for 20 hours was chromatographed in the phenol–water system of Block.[6] Development of the chromatogram with ninhydrin indicated that the amino acids serine, alanine, and glutamic acid were present along with P^{32}-inorganic phosphate. Presumably in the peptide material resulting from partial hydrolysis the phosphate is esterified to the serine hydroxyl.

Determination of the P^{32} in the phosphorylated peptide fraction purified by chromatography indicated that 94% of the protein molecules had been phosphorylated. It appears that sufficient phosphate is present on the enzyme to account for the observed activity in the absence of DPGA, and that phosphoglyceric acid mutase has a mechanism of action similar to that of phosphoglucomutase.[7]

(2) J. Mayaudon, A. A. Benson and M. Calvin, *Biochim. Biophys. Acta*, **23**, 342 (1957).

(3) The C^{14}-3PGA was prepared by Dr. N. Pon and the author gratefully acknowledges the gift of this compound.

(4) C. Neuberg and H. Lustig, *Arch. Biochem.*, **1**, 311 (1942).

(5) M. Flavin, *J. Biol. Chem.*, **210**, 771 (1954).

(6) R. J. Block, *Anal. Chem.*, **22**, 1327 (1950).

(7) V. A. Najjar and M. E. Pullman, *Science*, **119**, 631 (1954). E. P. Kennedy and D. E. Koshland, *J. Biol. Chem.*, **228**, 419 (1957).

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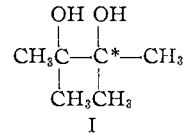
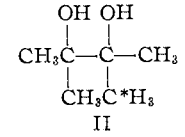
THE CARBON ISOTOPE EFFECT IN THE PINACOL-PINACOLONE REARRANGEMENT: A REINVESTIGATION

Sir:

Intermolecular carbon-isotope effects in the acid-catalyzed rearrangements, above 80°, of 2,3-dimethyl-2,3-butanediol-2-C^{14} [I] and 2,3-dimethyl-



I



II

2,3-butanediol-2-*methyl*-C^{14} [II] have been reported recently.[1] The magnitude of these reported isotope effects [k^*/k for I, 0.74; k^*/k for II, 0.48] is astonishing, particularly in the case of II, in which the isotopic bond should never be *directly* involved in the initial bond-breaking process. In addition the data of Duncan and Lynn, in two instances,[1b] fail to give a material balance. We have therefore reinvestigated the intermolecular isotope effects in the acid-catalyzed rearrangements of I and II.[1c] We find that in the presence of 0.295 *N* hydrochloric acid at 100°, within the limits of our experimental error, I exhibits an intermolecular isotope effect no greater than 3% [$k^*/k = 0.97$] and II exhibits no isotope effect at all [$k^*/k = 1.00$].

Acetic-1-C^{14} acid was converted to acetone-*carbonyl*-C^{14} by passage over manganous carbonate at 420–450°[2] [acetone-2,4-dinitrophenylhydrazone, m.p. 125–126°, radioactivity assay, 2.239 ± 0.001 mc./mole]. Isotope position isomer I (hexahydrate, m.p. 45.5–47°) was prepared from the foregoing acetone.[3] Periodate[1] cleavage of I produced acetone-*carbonyl*-C^{14} whose 2,4-dinitrophenylhydrazone had a m.p. of 125–126° and a radioactivity assay of 2.215 ± 0.005 mc./mole.

(1) a) J. F. Duncan and K. R. Lynn, *Australian Journal of Chemistry*, **10**, 7–25 (1957); (b) **10**, 18 (1957), Table II, line 3; (c) We are pleased to acknowledge a discussion with Professor J. F. Duncan in May of 1957, during which he brought his results to our attention. During this discussion (as in ref. 1a), Professor Duncan indicated that the isotope effect values calculated from his data may "be apparent, rather than real." Recently (personal communication), Professor Duncan has stated that some of his original results with K. R. Lynn may possibly be explainable as a consequence of a keto-enol equilibrium which prevents complete precipitation, at low temperature, of the 2,4-dinitrophenylhydrazone of pinacolone. If the pinacolone added as carrier were all in the keto-form, then an apparent but unreal isotope effect would be observed.

(2) W. M. Cumming, I. V. Hopper and T. S. Wheeler, "Systematic Organic Chemistry," 4th Ed., Constable and Co., Ltd., London, 1950, p. 101.

(3) "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 459.