

The biological activities of the compounds herein described are compared with those of other anti-inflammatory steroids in an accompanying Communication.¹⁷

(17) G. E. Arth, J. Fried, D. B. R. Johnston, D. R. Hoff, L. H. Starett, R. H. Silber, H. C. Stoerk, C. A. Winter, *THIS JOURNAL*, **80**, 3161 (1958).

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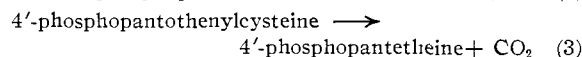
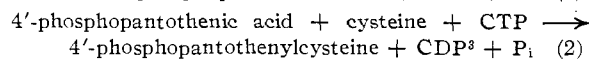
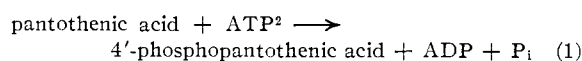
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REQUIREMENT OF CYTIDINE TRIPHOSPHATE FOR THE BIOSYNTHESIS OF PHOSPHOPANTETHEINE¹

Sir:

The enzymes, present in cell-free extracts of *Proteus morgani*, which catalyze reactions 1, 2 and 3



have been named, respectively: (1) pantothenic acid kinase^{4,5}; (2) phosphopantothenic acid-cysteine coupling enzyme⁵; and (3) phosphopantothencylcysteine decarboxylase. A crude extract, prepared from cells ruptured in a Hughes press, was treated with ammonium sulfate and calcium phosphate gel to yield a preparation of the coupling enzyme which was purified 20-fold and was free of the other two enzymes.

The substrates for the reaction catalyzed by the purified coupling enzyme were found to be 4'-phosphopantothenic acid and cysteine. The amount of product formed, 4'-phosphopantothencylcysteine, was determined by measurement of the disappearance of 4'-phosphopantothenic acid by determining the amount of pantothenic acid which could be regenerated by treatment with phosphatase. For this purpose, pantothenic acid assays were performed with *Saccharomyces carlsbergensis* 4228.⁶ Unexpectedly, it was found that the purified coupling enzyme did not function unless a small amount of crude extract was also included in the reaction mixture. The activating factor in the crude extract was heat labile and appeared to be an enzyme. Of a large number of compounds which were tested only one, CTP, was able to replace the requirement for this extra enzyme. The activating effects of the crude extract and CTP are shown in Table I. Other nucleoside di- and triphosphates were inactive. Additional experiments

(1) This investigation was supported by National Science Foundation Grant G4580.

(2) ATP, ADP, CTP and CDP are adenosine and cytidine tri- and diphosphates.

(3) Fragmentary evidence only indicates that CDP and inorganic phosphate (P_i) are products of this reaction.

(4) G. B. Ward, G. M. Brown and E. E. Snell, *J. Biol. Chem.*, **213**, 869 (1955).

(5) G. M. Brown, *Federation Proc.*, **17**, 197 (1958).

(6) L. Atkin, W. L. Williams, A. S. Shultz and C. N. Frey, *Ind. Eng. Chem., Anal. Ed.*, **16**, 67 (1944).

have shown that only 0.2 μM of CTP is required to give maximal activity. It seems probable that the extra enzyme required in the absence of added CTP was nucleoside diphosphate kinase, whose function was to replenish the small amount of CTP which was present in the enzyme preparations.

TABLE I

REQUIREMENT OF CTP FOR SYNTHESIS OF 4'-PHOSPHOPANTOTHENCYLCYSTEINE

The reaction mixture contained: 0.08 μM 4'-phosphopantothenic acid, 5 μM ATP, 10 μM MgCl₂, 80 μM tris-(hydroxymethyl)-aminomethane buffer at pH 7.4, 10 μM cysteine, 700 γ of purified coupling enzyme and additions as shown below in a total volume of 2 ml. Incubation was for 3 hr. at 37°, followed by heating for 5 min. at 100° and centrifugation to separate denatured protein. The supernatant solutions were analyzed as described in the text.

Addition	4'-Phosphopantothencylcysteine formed, $\mu\text{M} \times 10^2$
None	1.91
ATP, 5 μM	1.25
ATP, 5 μM + crude extract	4.38
ATP, 5 μM + boiled extract	1.91
CTP, 5 μM	6.92

Incubation of the purified enzyme with cysteine and CTP gave no detectable cytidine-containing, sulfur-containing compound. Thus it seems likely that the CTP requirement in the reaction is for the activation of the carboxyl group of 4'-phosphopantothenic acid in a manner similar to the way ATP functions in the synthesis of pantothenic acid from pantoic acid and β -alanine.⁷

(7) W. K. Maas, *Federation Proc.*, **15**, 305 (1956).

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16-METHYLATED STEROIDS. II. 16 α -METHYL ANALOGS OF CORTISONE, A NEW GROUP OF ANTI-INFLAMMATORY STEROIDS. 9 α -HALO DERIVATIVES

Sir:

Syntheses of the acetates of 16 α -methylated analogs of cortisone, hydrocortisone and their 1,2-unsaturated derivatives, I and II, respectively, are reported in an accompanying communication.¹ The enhanced activity and freedom from salt retention characteristic of this group of compounds prompted extension to 9-halogenated analogs.

A dimethylformamide-pyridine solution of 16 α -methylhydrocortisone acetate¹ (III) was treated with methanesulfonyl chloride,² affording 16 α -methyl-4,9(11)-pregnadiene-17 α , 21-diol-3,20-dione 21-acetate (IV), m.p. 205–208° λ_{max} 239 m μ , ϵ 17,300, $[\alpha] + 93^\circ$.³ (*Anal.* Found: C, 71.96; H, 8.30) which was converted to 16 α -methyl-9 α -

(1) G. E. Arth, D. B. R. Johnston, J. Fried, W. W. Spooner, D. R. Hoff and L. H. Sarett, *THIS JOURNAL*, **80**, 3160 (1958).

(2) Modification of unpublished procedure of E. M. Chamberlain and J. M. Chemerda; cf. J. Fried, K. Florey, E. Sabo, J. Herz, A. Restivo, A. Borman and F. Singer, *ibid.*, **77**, 4181 (1955).

(3) Ultraviolet spectra are of methanolic solutions of the compounds. Rotations were determined in chloroform at 25°, concn. = 100 mg./10 ml. using the sodium-D line, unless otherwise noted.