

tively identified in our hydrolyzates, may be the product NF observed by Hock and Huber.

It is interesting to note that the reactions leading to the formation of (I) are similar in character to two of the enzymatic reactions involved in polynucleotide synthesis.<sup>9,10</sup>

Recently Khorana, *et al.*,<sup>11</sup> described a thymidine derivative analogous to (I) which they prepared by an entirely different procedure.

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(10) A. Kornberg, I. R. Lehman, M. J. Bessman and E. S. Simms, *Biochim. Biophys. Acta*, **21**, 197 (1956).

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(12) On leave from the Agricultural Research Council Virus Research Unit, Cambridge, and wishes to thank the Wellcome Foundation for a travel grant.

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#### THE PROPERTIES OF AN ADENINE RIBONUCLEOTIDE PRODUCED WITH CELLULAR PARTICLES, ATP, Mg<sup>++</sup>, AND EPINEPHRINE OR GLUCAGON

Sir:

Previous studies in this laboratory have shown that a heat-stable factor (formed by particulate fractions of liver homogenates in the presence of ATP, Mg<sup>++</sup>, and epinephrine or glucagon) stimulated the formation of phosphorylase in supernatant fractions of homogenates.<sup>1</sup> This factor was isolated by ion-exchange chromatography and proved to be an adenine ribonucleotide (I).<sup>1</sup> Similar or identical compounds have been isolated from heart, skeletal muscle and brain. Crystals formed when  $2 \times 10^{-2}$  M aqueous solutions of (I) were chilled at acid pH. (I) was not attacked by several monoesterases<sup>1</sup> and upon titration with alkali exhibited no buffering capacity between pH 5 and pH 8. Descending paper chromatography, using an ethanol-ammonium citrate (pH 4.4) solvent system, revealed that (I) moved more rapidly than 5'-AMP, but more slowly than adenosine. Heating at 98° for more than 60 minutes in 1 N HCl, or more than 40 minutes in 1 N NaOH, was required to destroy completely the biological activity of (I).

Biological activity of (I) was lost only after relatively prolonged heating at 98° in 0.05 N HCl in the presence of Dowex-50 (H<sup>+</sup>). The major products of such treatment, amounting to 85% of the total, were (1) adenine, identified by ion-exchange chromatography and ultraviolet spectrum, and (2) a mixture of ribose-3-phosphate (60%) and ribose-2-phosphate (40%), identified by ion-exchange chromatography in the presence of borate.<sup>2</sup> However, the biological activity of (I) was lost rapidly on incubation with crude or fractionated extracts of heart or brain. In the presence of a partially purified enzyme from heart, (I) was converted quanti-

(1) T. W. Rall, E. W. Sutherland and J. Berthet, *J. Biol. Chem.*, **224**, 463 (1957).

(2) J. X. Khyam and W. E. Cohn, *THIS JOURNAL*, **75**, 1153 (1953).

tatively to 5'-AMP, identified by paper chromatography and dephosphorylation by low concentrations of snake venom.

The biological activity of (I) was not destroyed by incubation with pancreatic ribonuclease or spleen phosphodiesterase.<sup>3</sup> Through private communication with Dr. Leon Heppel, we learned that the structure we had tentatively proposed for (I) was identical with that proposed by Cook, Lipkin and Markham for a product isolated from the Ba(OH)<sub>2</sub> digestion of ATP.<sup>4</sup> This knowledge prompted an exchange of information and samples were kindly provided for comparison.<sup>5</sup> These samples were identical with (I) by the following criteria: (1) ultraviolet spectrum, (2) biological activity, (3) paper chromatography, (4) loss of biological activity, when incubated with enzyme fractions from heart or brain, (5) quantitative conversion to 5'-AMP on incubation with a partially purified enzyme from heart, and (6) conversion to adenosine on prolonged incubation with large amounts of *Crotalus adamanteus* venom (although, as reported,<sup>1</sup> moderate amounts of Russell's viper venom did not attack (I)).<sup>6</sup>

(3) We thank Dr. Leon Heppel of the National Institutes of Health, Bethesda, Maryland, for supplying a sample of purified spleen phosphodiesterase, and for the information regarding the similarity of (I) with the compound reported in the accompanying paper by W. H. Cook, D. Lipkin and R. Markham.

(4) W. H. Cook, D. Lipkin and R. Markham, *THIS JOURNAL*, **79**, 3607 (1957).

(5) We thank Dr. Roy Markham for sending these samples and for furnishing a sample of *Crotalus adamanteus* venom.

(6) This investigation was supported (in part) by a research grant No. H-2745 from the National Heart Institute of the Public Health Service.

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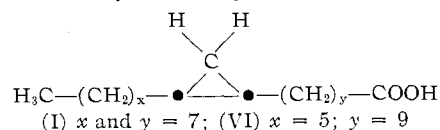
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#### UNEQUIVOCAL SYNTHESSES OF DL-*cis*-9,10-METHYLENEOCTADECANOIC ACID (DIHYDROSTERCULIC ACID) AND DL-*cis*-11,12-METHYLENEOCTADECANOIC ACID<sup>1</sup>

Sir:

The recent interest regarding the structure of sterculic acid<sup>2-7</sup> prompts us to record at this time an unequivocal synthesis of DL-*cis*-9,10-methyleneoctadecanoic acid (I) and the demonstration of its complete identity with dihydrosterculic acid.<sup>2,8</sup>



(1) Supported by grants from the American Cancer Society, recommended by the Committee on Growth of the National Research Council, and by the U. S. Public Health Service.

(2) J. R. Nunn, *J. Chem. Soc.*, 313 (1952).

(3) J. P. Verma, B. Nath and J. S. Aggarwal, *Nature*, **175**, 84 (1955).

(4) P. K. Faure and J. C. Smith, *J. Chem. Soc.*, 1818 (1956).

(5) D. G. Brooke and J. C. Smith, *Chem. and Ind.*, 49 (1957).

(6) B. A. Lewis and R. A. Raphael, *ibid.*, 50 (1957).

(7) V. V. Narayanan and B. C. L. Weedon, *ibid.*, 394 (1957).

(8) K. Hofmann, O. Jucker, W. R. Miller, A. C. Young, Jr., and F. Taussig, *THIS JOURNAL*, **76**, 1799 (1954).