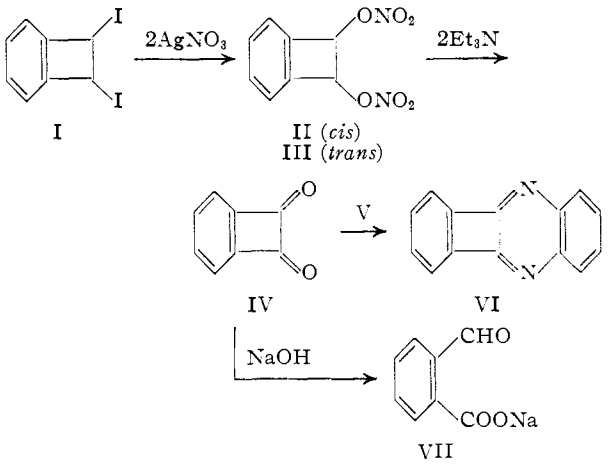

The quinone IV was oxidized rapidly and quantitatively by peracetic acid to phthalic acid.

A dilute solution of IV in 5% aqueous methanolic sodium hydroxide was decolorized completely after five hours at 25°, the sodium salt of phthalaldehydic acid (VII) being produced in 94% yield.

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THE FORMATION OF A CYCLIC DIANHYDRODIADENYLIC ACID (I) BY THE ALKALINE DEGRADATION OF ADENOSINE-5′-TRIPHOSPHORIC ACID (II)[1]

Sir:

It has been shown that barium hydroxide hydrolysis of (II) yields adenosine-5′-phosphoric acid (III) and inorganic pyrophosphate.[2] Hock and Huber[3] recently demonstrated that at least one other product is formed in this reaction.

Experiments in this laboratory show that the degradation of (II) by barium hydroxide is complex and that a number of previously unreported products are formed. One of these (I) exhibits sufficiently unexpected properties to merit description, particularly since it appears to be identical with a recently isolated, naturally-occurring compound.[4] In the preparation of (I), 250 mg. of (II)[5] and 3 ml. of 0.4 *N* barium hydroxide were heated for 30 min. at 100° and then the barium ion was precipitated. The solution was chromatographed on paper using 70% 2-propanol-ammonia as the developing solvent.[6] The fastest-moving substance is adenosine and this is closely followed by (I) (yield *ca.* 5–10%[7]).

(1) This research was supported in part by the U. S. Atomic Energy Commission.

(2) K. Lohmann, *Biochem. Z.*, **233**, 460 (1931); S. E. Kerr, *J. Biol. Chem.*, **139**, 131 (1941).

(3) A. Hock and G. Huber, *Biochem. Z.*, **328**, 44 (1956).

(4) E. W. Sutherland and T. W. Rall, This Journal, **79**, 3608 (1957).

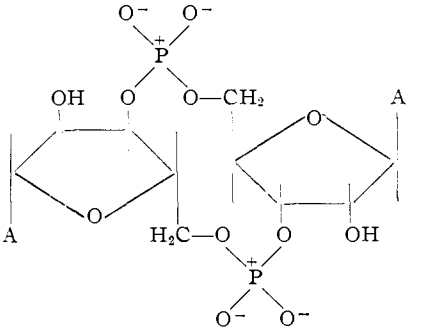
(5) We wish to thank Pabst Laboratories for the gift of a generous sample of (II).

(6) R. Markham and J. D. Smith, *Biochem. J.*, **52**, 552 (1952).

(7) Low yields of (I) also are obtained by the action of barium hydroxide on adenosine-5′-pyrophosphoric acid or by trifluoroacetic anhydride on (III).

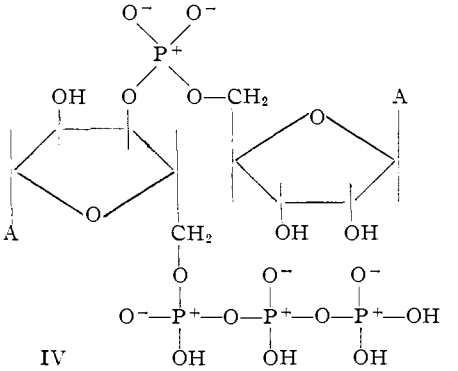
The new compound (I) has the following properties: (1) the molar ratio of adenine:phosphorus is unity; (2) adenosine is slowly formed from it by the action of *Crotalus adamanteus* venom; (3) it is not attacked by prostate phosphomonoesterase; (4) it is not oxidized by periodate; (5) it is deaminated by nitrite to the inosine analog, which also has been prepared from inosine-5′-triphosphoric acid; (6) its electrophoretic mobility on paper[8] relative to (III) is 1.2 at *p*H 3.5 (formate), 0.57 at *p*H 7.2 (phosphate) and 0.49 at *p*H 9.2 (borate); and (7) it is eluted from a Dowex-2 formate column by ammonium formate (*p*H 5.0) after all four known adenine-containing monoribonucleotides and just prior to adenosine-5′-pyrophosphoric acid.

A structural formula for (I) which is in accord with the above data is (A = adenine-9 residue)



There is no evidence at present to indicate that the phosphate ester bonds involve only the 3′, in addition to the 5′, carbon atoms. A study of models indicates, however, that this is the most likely phosphate diester structure and explains the surprising stability of (I) toward alkali. The positions of the phosphorus atoms in (I) are such that the formation of a 2,′ 3′ cyclic phosphate ester is hindered. The compound also is exceptionally stable toward acid hydrolysis.

The formation of (I) may take place by one of two paths. Two molecules of (II) suitably oriented may react simultaneously at two sites to give (I) directly, or (IV)



and inorganic pyrophosphate may form followed by intramolecular cyclization to give (I). Alternatively, (IV) could yield equivalent amounts of adenosine and 3′(or 2′)-phosphoadenosine-5′-phosphoric acids. The latter, which we have tenta-

(8) R. Markham in K. Paech and M. V. Tracey, "Modern Methods of Plant Analysis," Springer-Verlag, Berlin, 1955, Vol. IV, p. 278.

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.[9,10]

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

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(9) M. Grunberg-Manago and S. Ochoa, THIS JOURNAL, **77**, 3165 (1955).

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

(11) H. G. Khorana, *et al.*, THIS JOURNAL, **79**, 1002 (1957).

(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^{++}, 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^{++}, and epinephrine or glucagon) stimulated the formation of phosphorylase in supernatant fractions of homogenates.[1] This factor was isolated by ion-exchange chromatography and proved to be an adenine ribonucleotide (I).[1] Similar or identical compounds have been isolated from heart, skeletal muscle and brain. Crystals formed when 2×10^{-2} *M* aqueous solutions of (I) were chilled at acid *p*H. (I) was not attacked by several monoesterases[1] and upon titration with alkali exhibited no buffering capacity between *p*H 5 and *p*H 8. Descending paper chromatography, using an ethanol–ammonium citrate (*p*H 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^+). 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.[2] 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 quantitatively 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.[3] 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 $Ba(OH)_2$ digestion of ATP.[4] This knowledge prompted an exchange of information and samples were kindly provided for comparison.[5] 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,[1] moderate amounts of Russell's viper venom did not attack (I)).[6]

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

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

(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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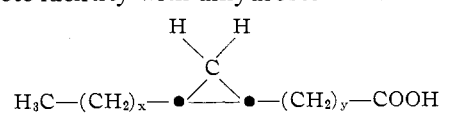
UNEQUIVOCAL SYNTHESES OF DL-*cis*-9,10-METHYLENEOCTADECANOIC ACID (DIHYDROSTERCULIC ACID) AND DL-*cis*-11,12-METHYLENEOCTADECANOIC ACID[1]

Sir:

The recent interest regarding the structure of sterculic acid[2-7] 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.[2,8]



(I) x and $y = 7$; (VI) $x = 5$; $y = 9$

(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).