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.

DEPARTMENT OF CHEMISTRY
WASHINGTON UNIVERSITY
ST. LOUIS 5, MISSOURI

WILLIAM H. COOK
DAVID LIPKIN
ROY MARKHAM[12]

(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).

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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^{++}, 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.

DEPARTMENT OF PHARMACOLOGY
SCHOOL OF MEDICINE
WESTERN RESERVE UNIVERSITY
CLEVELAND 6, OHIO

EARL W. SUTHERLAND
T. W. RALL

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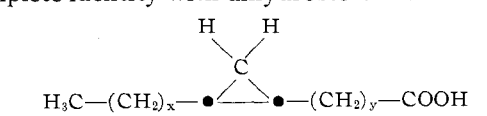
UNEQUIVOCAL SYNTHESES OF DL-*cis*-9,10-METHYLENEOCTADECANOIC ACID (DIHYDROSTERCULIC ACID) AND DL-*cis*-11,12-METHYLENEOCTADECANOIC ACID[1]

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.

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(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).

The addition of dibromocarbene to 1,4-cyclohexadiene[9] afforded 7,7-dibromonorcar-3-ene (II), m.p. 36.8–37.0°; *Anal.* Calcd. for $C_7H_8Br_2$: C, 33.5; H, 3.2; Br, 63.3. Found: C, 33.0; H, 3.5; Br, 63.0. Oxidation converted (II) into *cis*-3,3-dibromocyclopropane-1,2-diacetic acid (III), m.p. 179.4–181.2°; *Anal.* Calcd. for $C_7H_8O_4Br_2$: C, 26.8; H, 2.5; Br, 50.5; neut. equiv., 158. Found: C, 26.5; H, 2.2; Br, 50.5; neut. equiv., 159. Hydrogenolysis of (III) gave *cis*-cyclopropane-1,2-diacetic acid (IV), m.p. 131–133°; *Anal.* Calcd. for $C_7H_{10}O_4$: C, 53.3; H, 6.4; neut. equiv., 79.4. Found: C, 53.0; H, 6.2; neut. equiv., 79.6. The monomethyl ester chloride of (IV) on treatment with *n*-hexylcadmium followed by alkaline hydrolysis afforded DL-*cis*-6-keto-3,4-methylene-dodecanoic acid which was reduced[10] to DL-*cis*-3,4-methylenedodecanoic acid (V), b.p. 153–154° at 3 mm.; *Anal.* Calcd. for $C_{13}H_{24}O_2$: C, 73.7; H, 11.4; neut. equiv., 213. Found: C, 73.6; H, 11.3; neut. equiv., 208. Conversion of (V) into DL-*cis*-9,10-methyleneoctadecanoic acid (I) was effected by procedures previously described,[8,11] m.p. 38.6–39.6°; *Anal.* Calcd. for $C_{19}H_{36}O_2$: C, 77.0; H, 12.2; neut. equiv., 296. Found: C, 77.0; H, 12.2; neut. equiv., 298. Amide, m.p. 86.4–87.6°; *Anal.* Calcd. for $C_{19}H_{37}ON$: C, 77.2; H, 12.6; N, 4.7. Found: C, 77.0; H, 12.3; N, 5.0. Mixed melting point determinations and comparison of the infrared absorption spectra and X-ray diffraction patterns of (I) and dihydrosterculic acid demonstrated the complete identity of these compounds. The X-ray diffraction patterns of the amide of (I) and of dihydrosterculamide were identical also, and admixture of the amides did not result in a depression of the melting point. These findings establish the structure of dihydrosterculic acid.[12]

In connection with studies on the structure of lactobacillic acid, we have converted *cis*-cyclopropane-1,2-diacetic acid (IV) into DL-*cis*-11,12-methylene-octadecanoic acid (VI), m.p. 31.0–33.6°; Found: C, 76.5; H, 12.0; neut. equiv., 294. Amide, m.p. 84.0–85.2°; Found: C, 77.0; H, 12.6; N, 4.8. Although exhibiting an identical infrared absorption spectrum, the synthetic specimen differed from lactobacillic acid in melting point and X-ray diffraction pattern. The non-identity of lactobacillic acid (which has been shown to represent one of the stereoisomeric forms of 11,12-methyleneoctadecanoic acid[13]) with either *cis*- or *trans*[8]-DL-11,12-methyleneoctadecanoic acids demonstrates clearly that lactobacillic acid must be a distinct optical isomer. Synthetic DL-*cis*-11,12-methyleneoctadecanoic acid exhibits approximately one-half the growth promoting activity of natural lactobacillic acid for *Lactobacillus delbrueckii*. DL-*trans*-11,12-Methyleneoctadecanoic acid fails to support growth of this organism under identical experimental conditions.[14] These observations point to either D- or L-*cis*-11,12-methyleneoctadecanoic acid as the structure for lactobacillic acid.

(9) W. von E. Doering and A. K. Hoffmann, THIS JOURNAL, **76**, 6162 (1954).

(10) Huang-Minlon, *ibid.*, **68**, 2487 (1946).

(11) S. Stallberg-Stenhagen, *Archiv Kemi Mineral. Geol.*, **A22**, No. 19, 1 (1946).

(12) We wish to express our sincere appreciation to Professor G. A. Jeffrey, Department of Chemistry, University of Pittsburgh, for the X-ray studies. Details of this work will be presented in THIS JOURNAL.

(13) G. J. Marco and K. Hofmann, *Federation Proc.*, **15**, 308 (1956).

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BIOCHEMISTRY DEPARTMENT
UNIVERSITY OF PITTSBURGH
SCHOOL OF MEDICINE
PITTSBURGH, PENNSYLVANIA

KLAUS HOFMANN
SALVADOR F. OROCHENA
CLAYTON W. YOHO

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OXIDATIONS AT MERCURY HALIDE ANODES

Sir:

It is well known in polarography that the anodic potential range of a mercury electrode is severely limited by oxidation of the mercury itself. A mercury electrode which can be used at highly anodic potentials might well be considered a significant advance in polarographic practice. Such an electrode has been developed as a result of chronopotentiometric studies at a mercury pool in chloride media. This electrode operates through formation of an "inert" film of mercurous chloride prior to oxidation of electroactive species in the bulk of solution. This phenomena has not been observed previously since conventional polarography at the dropping mercury electrode does not allow sufficient time for the proper film formation.

The theory of chronopotentiometry has been thoroughly reviewed by Delahay.[1] The equipment used in the present study was conventional in all respects. The background electrolyte was a solution 0.3 M in potassium chloride and 2×10^{-3} M in hydrochloric acid (pH 2.7). The anodic chronopotentiogram of this solution (Hg pool area 1.47 cm.^{2}, current 350 microamperes) shows four small waves at *ca.* +0.07, 0.5, 0.8, and 1.2 v. *vs.* S.C.E. The first wave is due to formation of mercurous chloride. The variation of $E_{1/4}$ for this wave follows closely

$$E_{Hg} = E^0 - 0.059 \log [Cl^-]/2$$

where $E^0 = 0.022$ v., the standard potential vs. S.C.E. of the reaction

$$2Hg^0 + 2Cl^- = Hg_2Cl_2 + 2e^-$$

Further interpretation of these potential–time patterns will be reported in the near future.

The chronopotentiogram of a 2×10^{-3} M solution of N,N′-dimethyl-p-phenylenediamine (DPP) in the same background electrolyte shows an identical first wave for mercurous chloride formation. It is followed by a clearly defined wave for the oxidation of DPP. The $E_{1/4}$ of DPP (*ca.* +0.4 v.) wave is identical with that obtained at a platinum electrode under the same pH conditions. A plot of $\tau^{1/2}$ *vs.* concentration shows excellent linearity between 4×10^{-4} to 3×10^{-3} M DPP.

The anodic oxidations of aniline, o-phenylenediamine and hydroquinone, which take place at

(1) P. Delahay, "New Instrumental Methods in Electrochemistry," Interscience Publishers, Inc., New York, N. Y., 1954.