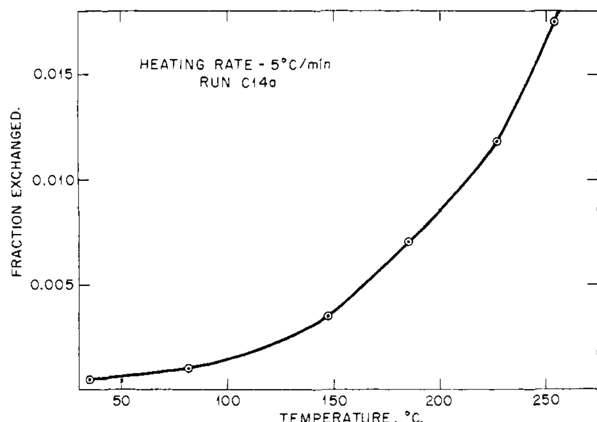

Fig. 1.—The Exchange of F^{18} between C_3F_6 and RbF as a Function of Temperature.

per square meter of surface was observed to be Cs > Rb > K, Na, Li.[3]

Perfluoropropene with any single salt exchanged more readily than CF_4, C_4F_{10} and $(C_2F_5)_2O$, although appreciable exchange has been observed in all cases. Sulfur hexafluoride failed to exchange with any of these salts below 300°. Above that temperature, complicated reactions set in. Silicon tetrafluoride exchanged rapidly at room temperature with the alkali fluorides but manometric measurements showed complex fluoride formation at higher temperatures. Currently, a survey of exchange reactions in other fluorocarbon-fluoride systems is under way.

(3) Surface areas were determined by the BET method on representative alkali fluorides using krypton. We are indebted to P. K. Melroy of the Special Analytical Services Department of the Oak Ridge Gaseous Diffusion Plant for these measurements.

(4) Oak Ridge Institute of Nuclear Studies Fellow, 1955–57.

(5) National Carbon Company, A Division of Union Carbide and Carbon Corporation, Cleveland, Ohio.

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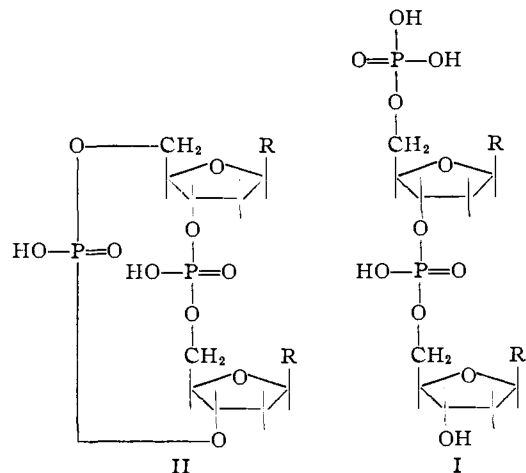
RECEIVED NOVEMBER 23, 1956

SYNTHESES OF DIDEOXYRIBONUCLEOTIDES

Sir:

A new method for the synthesis of diesters of phosphoric acid recently has been reported from this Laboratory.[1] This approach involved the phosphorylation of a hydroxylic compound by a *mono*ester of phosphoric acid in the presence of *p*-toluenesulfonyl chloride. We now wish to report that dicyclohexylcarbodiimide (DCC) can not only replace *p*-toluenesulfonyl chloride but is, in fact, the preferred reagent. The method has now been applied successfully to syntheses of dideoxyribonucleotides containing 5′ to 3′ phosphodiester linkages and bearing 5′-phosphate end-groups (I).

Thymidine 5′-phosphate was treated with acetic anhydride in pyridine to give the 3′-acetyl derivative in quantitative yield. The latter (1 mole) was brought into reaction in anhydrous pyridine with thymidine 5′-dibenzyl phosphate[2] (1.15 mole) in the presence of DCC (5 moles). Water was added after a reaction period of 24 hours at room temperature and the solvent removed by evaporation. The benzyl groups were then removed by hydrogenation in 10% acetic acid using palladium oxide on barium sulfate[3] as catalyst and the acetyl group by mild alkaline treatment. The dinucleotide (I, R = R′ = thymine) was purified by preparative paper chromatography using isopropyl alcohol–ammonia–water[4] (7–1–2) (Solvent A) and was finally obtained as the highly crystalline trisodium salt in 40% yield.[5] A sample was recrystallized from aqueous ethyl alcohol and dried at 110° in a high vacuum for 12 hours; $[\alpha]^{20}D + 10.9°$ (Michelson and Todd report $[\alpha]^{20}D + 7.0°$ for the hydrated calcium salt).[2] *Anal.* Calcd. for $C_{20}H_{25}O_{15}N_4P_2Na_3$; P, 8.95. Found.[6] P, 8.80. The ratio of total phosphorus to thymidine, using a figure of 9700 for E_{max} at 267 mμ for thymidine, was 1.054.



Hydrolysis with 1 *N* hydrochloric acid required 2 hours at 100°, the products being thymidine 3′,5′-diphosphate, thymidine and inorganic phosphate. An electrometric titration of the free acid, obtained by passing the sodium salt through Dowex-50(H^+) resin, showed that the ratio of primary (*p*H range 1–3.5) to secondary (*p*H range 5.5–8) phosphoryl dissociations was 2.

Incubation of the dinucleotide in tris-(hydroxymethyl)-aminomethane buffer (*p*H 8.8) with unfractionated snake venom[7] resulted in quantitative degradation to thymidine and inorganic phosphate as shown by paper chromatography in Solvent A, whereas a purified phosphodiesterase fraction[8] gave thymidine 5′-phosphate (chromatography in *n*-butyl alcohol–acetic acid–water (5–2–3) (Solvent

(1) H. G. Khorana, G. M. Tener, J. G. Moffatt and E. H. Pol, *Chemistry and Industry*, 1523 (1956).

(2) Prepared in quantitative yield from 3′-acetyl thymidine by a modification of the procedure of Michelson and Todd, (*J. Chem. Soc.*, 2632 (1955).

(3) R. Kuhn and H. J. Haas, *Angew. Chem.*, **67**, 785 (1955).

(4) D. M. Brown and A. R. Todd, *J. Chem. Soc.*, 2040 (1953).

(5) The yield can probably be greatly improved since most of the coproduct is thymidine 5′-phosphate.

(6) E. J. King, *Biochem. J.*, **26**, 292 (1932).

(7) *Crotalus adamenteus*, purchased from Ross Allen's Reptile Farm, Florida.

(8) The procedure used to purify the diesterase involves fractional acetone precipitation in the cold (unpublished work of Dr. R. L. Sinsheimer).

B)). Digestion with prostatic phosphomonoesterase[9] gave thymidine-5′ thymidine-3′ phosphate, identical on paper chromatograms (Solvent A) with a sample of this substance synthesized earlier.[1] The dithymidine phosphate produced above was degraded by the snake venom diesterase[8] fraction to give approximately equivalent amounts of thymidine and thymidine 5′-phosphate (Solvent A).

By the above synthetic procedure but using so far only *p*-toluenesulfonyl chloride as the reagent, we have prepared 3′-(2′-deoxyadenylyl)-thymidine 5′-phosphate (I, R = thymine; R′ = adenine).

Some progress has been made in the identification of the products which result when thymidine 5′-phosphate alone is treated with *p*-toluenesulfonyl chloride or DCC in anhydrous pyridine.[1] One product, present in the dialysable fraction,[10] travels faster than thymidine 5′-phosphate in Solvent A but slower in Solvent B. The substance which is more stable than thymidine dinucleotide, (I, R = R′ = thymine) to hydrochloric acid, gives thymidine 3′,5′-diphosphate on hydrolysis. Incubation with crude snake venom gives thymidine and inorganic phosphate whereas the diesterase[8] fraction slowly yields thymidine 5′-phosphate (Solvent B). An electrometric titration shows the absence of any groups titratable in the *p*H range 5–8. The total evidence indicates that this substance has the cyclic structure II (R = thymine). This finding demonstrates that in the thymidine 5′-phosphate "polymerization reaction" *inter*molecular and *intra*molecular phosphorylations are competing reactions.

It is clear that the above approach offers promise for the synthesis of the higher oligonucleotides.

Acknowledgments.—This work has been supported by grants from the National Research Council and National Cancer Institute of Canada.

(9) G. Schmidt, "Methods in Enzymology," Vol. II, Academic Press, Inc., New York, N. Y., 1955, p. 523–530.

(10) Dialysis against distilled water; a large proportion of the nucleotidic material is non-dialysable under these conditions.

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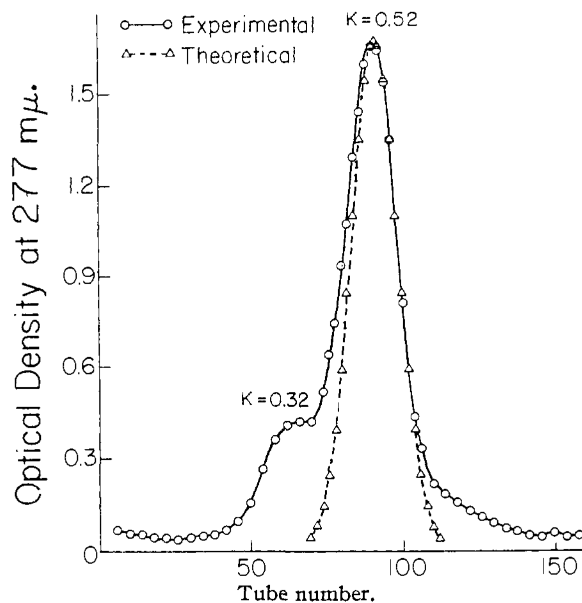
RECEIVED JANUARY 18, 1957

THE ISOLATION AND STRUCTURE OF A MELANOCYTE-STIMULATING HORMONE FROM BOVINE PITUITARY GLANDS

Sir:

The purification, isolation and structure of a porcine melanocyte-stimulating hormone (β-MSH) have recently been reported by various investigators.[1,2,3,4] We now wish to describe the isolation and structure of a melanocyte-stimulating peptide from the posterior-intermediate lobes of bovine pituitaries. In its countercurrent distribution behavior and amino acid composition, this peptide differs considerably from the bovine preparation.[2]

The isolation procedure was the same as that previously described for β-MSH,[4,5] with commercially available bovine posterior pituitary powder used as the starting material. Zone electrophoresis on starch[6] was carried out with pyridine–acetic acid buffers at *p*H 4.9 and at *p*H 6.4; under these conditions, it was observed that the MSH activity[7] possessed a cathodic mobility greater than that of β-MSH. When the active fraction obtained by zone electrophoresis was further purified by countercurrent distribution at 20° in the system 0.5% trichloroacetic acid *vs.* *sec*-BuOH (266 transfers), a main peak with a partition coefficient of 0.52 was obtained (Fig. 1); the MSH activity



Tube number.

Fig. 1.

was concentrated in this peak. The material falling within the theoretical distribution curve for a partition coefficient of 0.52 (tubes 80–104) was recovered and submitted to zone electrophoresis on starch (*vide infra*) at *p*H's 4.9, 5.7, 6.4, 6.9, 7.3 and 8.4; at each *p*H the material migrated as a single zone which contained all the MSH activity. Moreover, after reaction of the material with carboxypeptidase and fluorodinitrobenzene, the sole amino acid found at either terminus was aspartic acid. Finally, quantitative amino acid analysis of the 24-hour acid hydrolysates by the paper-fluorodinitrobenzene method[8,9] gave the following molar ratio for the constituent amino acids

$Asp_{2.0}, Glu_{1.0}, Ser_{1.7}, Gly_{2.0}, Pro_{3.0}, Met_{1.1}, Phe_{1.0}, Tyr_{1.0}, Lys_{1.8}, His_{1.0}, Arg_{0.9}, Try_{1}$

(1) J. Porath, P. Roos, F. W. Landgrebe and G. M. Mitchell, *Biochim. et Biophys. Acta*, **17**, 598 (1955).

(2) B. G. Benfey and J. L. Purvis, *Biochem. J.*, **62**, 588 (1956).

(3) J. I. Harris and P. Roos, *Nature*, **178**, 90 (1956). We wish to apologize for the omission in our earlier communication[4] of mention of the independent work of Harris and Roos, giving the complete structure of β-MSH (porcine). A discussion of the lack of purity of the β-MSH preparations of other workers[1,2] also was not included because of space limitations. Reference to all of the above work has been made in two detailed papers on this subject.[5,15]

(4) I. I. Geschwind, C. H. Li and L. Barnafi, THIS JOURNAL, **78**, 4494 (1956).

(5) I. I. Geschwind and C. H. Li, *ibid.*, **79**, 615 (1957).

(6) H. G. Kunkel and R. J. Slater, *Proc. Soc. Exper. Biol. Med.*, **80**, 42 (1952).

(7) The melanocyte-stimulating potency of each fraction was estimated by the method described by K. Shizume, A. B. Lerner and T. B. Fitzpatrick (*Endocrinology*, **54**, 553 (1954)).

(8) A. L. Levy, *Nature*, **174**, 126 (1954).

(9) Tryptophan was determined by the method of T. W. Goodwin and R. A. Morton (*Biochem. J.*, **40**, 628 (1946)).