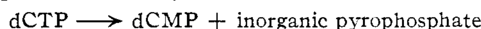
the host, *Escherichia coli*.[2a,b,c] A brief description[3] has been made of some enzymatic events pertaining to the synthesis of DNA in T2 infected *E. coli*. When C^{14} labeled dTTP was incubated with primer DNA and an enzyme system isolated from uninfected cells, radioactive DNA was formed only in the presence of deoxyadenosine triphosphate, deoxyguanosine triphosphate and dCTP[4] as has been demonstrated previously by Bessman, *et al.*[5] We have reported that dHTP will replace dCTP in the system containing the enzyme prepared from uninfected *E. coli* cells. However, with extracts from *E. coli* cells infected with T2 bacteriophage, synthesis of radioactive DNA is observed in the presence of dHTP but *not* when dCTP replaces dHTP. This led to the finding that dCTP is specifically destroyed by an enzyme produced in the infected cell.[3]

We wish to report further details of this system. The dCTP degrading enzyme and the DNA polymerase have been separated by chromatography on a column of hydroxylapatite. The dCTP degrading enzyme fraction, free from interfering phosphatases, catalyzes the reaction



$$\text{dCTP} \longrightarrow \text{dCMP} + \text{inorganic pyrophosphate}$$

Evidence for this reaction is based on the demonstration that one mole of inorganic pyrophosphate is produced for each mole of dCMP formed. This enzyme does not act upon dTTP, deoxycytidine diphosphate, or the ribosyl analog of dCTP, cytidine triphosphate. The appearance of this new enzymatic activity takes place shortly after infection of the cells with T2 bacteriophage, and approximately 50% of the maximum level observed is attained seven minutes after infection of a culture at 37°.

The purified DNA polymerase of infected cells (freed from the dCTP splitting enzyme by the chromatographic procedure and of 30-fold purity compared to the crude extract) utilizes dCTP or dHTP to an equal extent at similar concentrations. At this level of purification it is indistinguishable from the polymerase of uninfected cells by the criterion of substrate specificity.

When dCTP or dHTP was replaced with deoxy-5-glucosylhydroxymethylcytidine triphosphate, there was either little or no synthesis of labeled DNA from C^{14}-dTTP and the other substrates in the presence of either the crude or purified polymerase.[6] It is therefore probable that this glucosyl compound is not an intermediate in the formation of the glucosylated DNA of T2 bacteriophage.

This dCTP degrading enzyme may have a dual function in the promotion of the synthesis of phage DNA. First it degrades dCTP to a form which is not utilized as a substrate for the DNA polymerase and, secondly, it provides dCMP which is known to be the substrate for the synthesis of deoxy-5-hydroxymethylcytidine monophosphate by the reaction of Flaks and Cohen.[7] Furthermore, the production of the dCTP degrading enzyme may be one of the mechanisms responsible for the exclusion of phages other than the T-even variety when *E. coli* cells undergo mixed infection with T2 phage and a phage which contains cytosine in its DNA.[8]

(2) (a) G. R. Wyatt and S. S. Cohen, *Biochem. J.*, **55**, 774 (1953); (b) R. L. Sinsheimer, *Science*, **120**, 551 (1954); (c) E. Volkin, THIS JOURNAL, **76**, 5892 (1954).

(3) J. F. Koerner and M. S. Smith, *Federation Proc.*, in press.

(4) dCTP and deoxyadenosine triphosphate were prepared from the corresponding nucleotides by the method of L. I. Hecht, V. R. Potter, and E. Herbert, *Biochim. et Biophys. Acta*, **15**, 134 (1954). dHTP, deoxy-5-glucosylhydroxymethylcytidine triphosphate, dTTP, and deoxyguanosine triphosphate were prepared by the procedure of V. M. Clark, G. W. Kirby and A. Todd, *J. Chem. Soc.*, 1497 (1957).

(5) M. J. Bessman, I. R. Lehman, E. S. Simms and A. Kornberg, *J. Biol. Chem.*, **233**, 171 (1958).

(6) Experiments carried out in collaboration with Dr. John S. Wiberg.

(7) J. G. Flaks and S. S. Cohen, *Biochim. et Biophys. Acta*, **25**, 667 (1957).

(8) M. Delbrück and S. E. Luria, *Arch. of Biochem.*, **1**, 111 (1942).

(9) Postdoctoral Fellow of the National Cancer Institute, United States Public Health Service, 1956–58.

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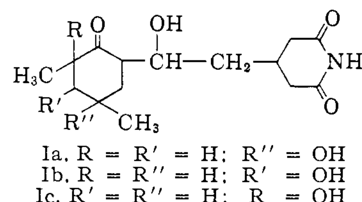
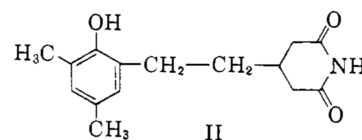
STRUCTURES OF THE STREPTOVITACINS

Sir:

The streptovitacins, a group of closely related compounds isolated from a *Streptomyces griseus* fermentation, have been found to have broad-spectrum antitumor activity.[1–6] The chemical evidence described below, coupled with preliminary studies reported elsewhere,[3,7] permit structural assignments (Ia-c) to several components of the complex.



That streptovitacins A and B possess the cycloheximide[8] (I, R = R′ = R″ = H) type structure was shown[7] by (i) spectral comparisons, (ii) the present of an imide system (NMR), (iii) acid catalyzed dehydration to II, identical with material derived



from cycloheximide, and (iv) facile alkali-catalyzed retroaldol degradation to six-membered ring ketones. Although certain of the early experiments

(1) J. B. Field, F. Costa and A. Boryczka, "Antibiotics Annual 1958–1959," Medical Encyclopedia, Inc., New York, N. Y., 1959, in press.

(2) W. T. Sokolski, N. J. Eilers and G. M. Savage, *ibid.*, in press.

(3) T. E. Eble, M. E. Bergy, C. M. Large, R. R. Herr and W. G. Jackson, *ibid.*, in press.

(4) J. S. Evans, G. D. Mengel, J. Ceru and R. L. Johnston, *ibid.*, in press.

(5) J. B. Field, A. Mireles, H. R. Pachl, L. Bascoy, L. Cano and W. K. Bullock, *ibid.*, in press.

(6) J. S. Evans, G. D. Mengel and J. Ceru, *Proc. Soc. Exper. Biol. Med.*, in press.

(7) R. R. Herr, "Antibiotics Annual 1958–1959," Medical Encyclopedia, Inc., New York, New York, 1959, in press.

(8) The trade name of The Upjohn Company for cycloheximide is Acti-dione.

on the hydroxydimethylcyclohexanone obtained by alkaline degradation of streptovitacin A ($C_{15}H_{23}NO_5$) suggested the 3-position for the hydroxyl group,[7] (i) Wolff–Kishner reduction of the hydroxyketone to 1,3-dimethylcyclohexanol, and (ii) resistance of streptovitacin A to oxidation by periodate, demonstrated that the hydroxyketone is 4-hydroxy-2,4-dimethylcyclohexanone. Streptovitacin A therefore is 3-[2-(5-hydroxy-3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-glutarimide (Ia).[9]

Alkaline degradation of streptovitacin B, an isomeric ring-hydroxylated cycloheximide, gave predominantly 2,4-dimethyl-2-cyclohexenone,[7] thereby suggesting the 3-hydroxy-2,4-dimethylcyclohexanone structure for the hydroxyketone moiety of streptovitacin B. Also, while acetylation of streptovitacin A under mild conditions gave a monoacetate, m.p. 165–168° (calcd. for one acetyl, 12.7; found, acetyl, 11.5) similar treatment of streptovitacin B gave a diacetate, m.p. 155–158° (calcd. for two acetyls, 22.6; found, acetyl, 21.6). We therefore propose the structure of streptovitacin B to be 3-[2-(4-hydroxy-3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-glutarimide (Ib).

Elemental analyses of streptovitacins C_2, m.p. 91–96° (C, 60.53; H, 7.98; N, 4.67; O, 27.71; C-CH_3, 8.2) and D, m.p. 67–69° (C, 60.42; H, 7.89; N, 4.83; O, 27.00; C-CH_3, 9.0) showed these compounds also to have the empirical formula $C_{15}H_{23}NO_5$ (calcd. C, 60.58; H, 7.69; N, 4.71; O, 26.91; C-CH_3, 10.1).

Acid-catalyzed dehydration of streptovitacin C_2 gave the phenolic derivative (II), while alkaline degradation provided a hydroxyketone which appeared from infrared spectra to be similar but not identical to that obtained from streptovitacin A. Periodate oxidation indicated one mole of oxidant consumed per mole of C_2. From these facts, we propose the structure of streptovitacin C_2 to be 3-[2-(3-hydroxy-3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-glutarimide (Ic).

Acid degradation of streptovitacin D gave the phenolic derivative (II) showing this compound also to be a ring-hydroxylated cycloheximide. At the present time the position of the hydroxyl group in streptovitacin D is not known.

The author wishes to thank Mr. W. A. Struck and associates for microanalyses, Mr. M. F. Grostic for infrared spectra, Dr. G. Slomp for nuclear magnetic resonance measurements, and Dr. E. E. van Tamelen for helpful discussions and suggestions.

(9) This structure is the same as that shown by K. V. Rao for a degradation product "desacetyl E-73" (Abstract, 134th Am. Chem. Soc. Meeting, Chicago, Sept., 1958). The stereochemical relationship of the two compounds is not known.

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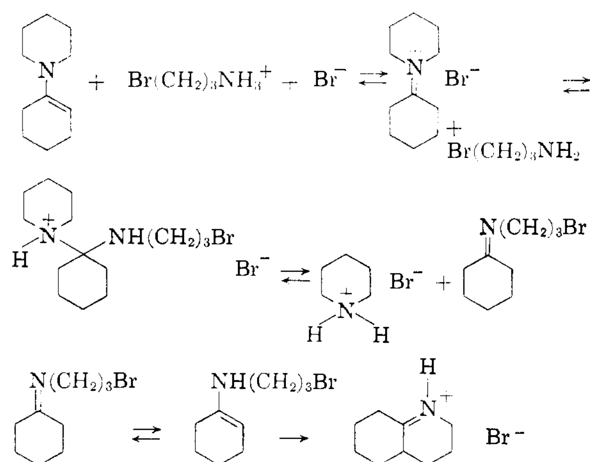
RECEIVED MARCH 27, 1959

A NEW REACTION OF ENAMINES

Sir:

It has been found that $\Delta^{1(9)}$-octahydroquinoline (2,3,4,4a,5,6,7,8-octahydroquinoline) may be prepared in 80–84% yields in one step by the exothermic reaction of 3-bromopropylamine hydrobromide with 1-(1-cyclohexenyl)-piperidine in dimethylformamide. After dilution with water and basification, the product is extracted with ether, dried and distilled. The infrared absorption and the melting point of its picrate salt are identical with those of a sample prepared by an earlier route[1] involving three steps with an over-all yield of 44%.

The alkylation of enamines with alkyl halides is well known.[2] However, N,N-diethyl-3-bromopropylamine hydrobromide does not react with 1-(1-cyclohexenyl)-piperidine under these conditions to produce isolable amounts of 2-(3-diethylaminopropyl)-cyclohexanone, indicating that the reaction of 3-bromopropylamine hydrobromide is probably not a simple alkylation of the enamine followed by ring closure. It is postulated that the reaction occurs through loss of a proton from the 3-bromopropylammonium ion to the more strongly basic enamine, addition of 3-bromopropylamine to the resulting enamine salt, and elimination of piperidinium ion to form N-cyclohexylidene-3-bromopropylamine. This imine is in equilibrium with its enamine form, and, as the enamine, it cyclizes to $\Delta^{1(9)}$-octahydroquinoline hydrobromide.



The conversion of an enamine to an imine under these conditions is illustrated by warming equivalent amounts of 1-(1-cyclohexenyl)-piperidine and butylamine hydrobromide in dimethylformamide; dilution with ether precipitates an 84% yield of piperidine hydrobromide. Evaporation of the filtrate, and then distillation, produces a moderate amount of N-cyclohexylidenebutylamine. That these imines exist partially in the enamine form is suggested by the fact that N-cyclohexylidene-ethylamine and -isopropylamine react similarly with 3-bromopropylamine hydrobromide to produce $\Delta^{1(9)}$-octahydroquinoline in 75–82% yields. This equilibrium also is suggested by the failure of 3-bromopropylamine hydrobromide to react with 1-(1-cyclopentenyl)-piperidine to produce 2,3,4,-4a,6,7-hexahydro-5H-1-pyrindine, due to a lesser tendency of the imine double bond to migrate into the five-membered ring. However, N-methyl-3-bromopropylamine hydrobromide reacts with 1-

(1) L. A. Cohen and B. Witkop, THIS JOURNAL, **77**, 6595 (1955).

(2) J. N. Collie, *Ann.*, **226**, 316 (1884); R. Robinson, *J. Chem. Soc.*, **109**, 1038 (1916); G. Stork, R. Terrell and J. Szmuszkovicz, THIS JOURNAL, **76**, 2029 (1954).