That the mercapto groups are really bound to protein, and not merely the result of inefficient separation, was shown by ultracentrifugation with a colored azomercurial. At 60,000 r.p.m., the schlieren and color boundaries moved together.

The acetyl-S linkage of (II) is stable for days in aqueous solution at *p*H's as high as 9.5. Conversion of (II) into (III), if desired, can be accomplished in a few minutes in dilute sodium hydroxide, *p*H 11.5. Preliminary experiments indicate that some nitrogenous bases also work at *p*H's much nearer 7 (*e.g.*, imidazole).

DEPARTMENT OF CHEMISTRY
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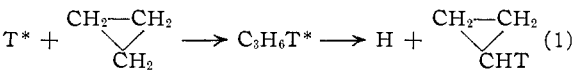
RECEIVED MAY 25, 1959

HIGH ENERGY EXCHANGE REACTION OF TRITIUM ATOMS WITH CYCLOPROPANE

Sir:

Our recent experiments with tritium atoms slowing down from very high energies in the presence of cyclopropane show a substantial incorporation into the organic molecule by reaction (1), in which the asterisk designates an energetic species



(1)

Previous experiments with thermal deuterium atoms have failed to show any exchange of D for H in the cyclopropane molecule.[1]

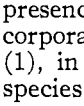
Gaseous mixtures of He^3 and cyclopropane, with oxygen or He^4 sometimes added, have been irradiated with thermal neutrons to produce tritium by the reaction $He^3(n,p)H^3$. The resulting radioactive products have been separated and measured with a proportional counter on the outlet end of a gas chromatographic column.[2] The percentage of radioactivity incorporated in each radioactive product is shown in Table I for several runs, both with and without added gases.

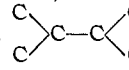
In these systems, O_2 serves as a very effective radical scavenger.[3] The essentially unchanged yield of cyclopropane in its presence indicates that free radicals are not involved, and that the reaction goes through an intermediate as indicated in (1). Presumably the absence of observable exchange with thermal deuterium atoms is the result of a high activation energy for this reaction; the recoil tritium atoms react as "hot" atoms before reaching thermal energies. Moderating collisions with He^3 or He^4 serve to reduce the average energy of the tritium atom at the time of reaction,[4] and hence reduce the possibility of exchange during collision. This is reflected in the lower yield of cyclopropane in the He^4 experiments.

Such irradiations cause degradation of the parent molecules by ordinary radiation effects. In the 70.4 cm. Hg Δ run of Table I. the final gaseous mixture contained about 1% other hydrocarbons than the parent, principally ethane and propane. Runs for higher *nvt* irradiations showed a higher percentage of radiation damage. Quantitative explanations of the distribution of radioactivity will require separation of the energetic tritium atom reactions from the accompanying macroscopic radiation damage.

TABLE I

RADIOACTIVE PRODUCTS OF THE GASEOUS REACTION OF ENERGETIC TRITIUM ATOMS WITH CYCLOPROPANE

Gas Pressure, cm.	Per cent. total observed tritium[a] 70.4Δ 2.0 He^3	31.4Δ 1.9 He^3	21.5Δ 1.9 He^3 8.6 O_2	10.1Δ 1.5 He^3 66.7 He^4	8.4Δ 1.9 He^3 24.3 He^4
Irradiation conditions n./cm.²/sec.	6 days at 3×10^9	12 hr. at 2×10^{12}	12 hr. at 2×10^{12}	6 days at 3×10^9	12 hr. at 2×10^{12}
Product					
Δ	22.1	16.4	15.3	10.9	7.4
HT	31.2	47.4	58.3	30.1	54.9
CH_3T	2.5	6.8	6.0	1.8	6.8
C–C	6.2	4.7	4.0	7.8	6.4
C=C	1.5	1.8	1.8	2.0	2.0
C–C–C	12.0	6.5	4.1	13.7	6.8
C–C=C	2.1	2.5	2.2	1.8	1.7
	3.0	1.5	1.0	3.1	1.8
C–C–C–C	8.6	4.9	1.8	13.8	5.0
	Low			Low	
	5.4	4.0	1.5	7.8	3.7
C–C–C–C–C	1.4	1.7	0.7	1.8	0.9
	0.7	0.5	0.1	0.5	<0.1

[a] Smaller amounts (<1% each) have been observed for C≡C, C=C=C, C–C≡C,  , *i*-C_6, *n*-C_6, and others.

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OXAMYCIN, A COMPETITIVE ANTAGONIST OF THE INCORPORATION OF D-ALANINE INTO A URIDINE NUCLEOTIDE IN *STAPHYLOCOCCUS AUREUS*

Sir:

Oxamycin (D-4-amino-3-isoxazolidone, D-cycloserine), like penicillin, bacitracin, novobiocin and gentian violet, induces uridine nucleotide accumulation in *S. aureus*.[1] The nucleotides which accumulate are bacterial cell wall precursors.[2] Their accumulation, as well as protoplast formation,[3] is the consequence of inhibition of cell wall synthesis by these antibacterial substances.

The major compound isolated from oxamycin-treated cells had a slower mobility in several solvents than UDP-GNAc-lactyl-(L)ala-(D)glu-(L)-lys-(D)ala-(D)ala,[4,5] the principal compound which

(1) H. I. Schiff and E. W. R. Steacie, *Canad. J. Chem.*, **29**, 1 (1951).
(2) R. Wolfgang and F. S. Rowland, *Anal. Chem.*, **30**, 903 (1958).
(3) J. K. Lee, B. Musgrave and F. S. Rowland, 134th A. C. S. Meeting, Chicago, Sept., 1958.
(4) See, for example, M. El-Sayed, P. Estrup and R. Wolfgang, *J. Phys. Chem.*, **62**, 1356 (1958).

(1) J. Ciak and F. E. Hahn, *Antibiotics and Chemo.*, **9**, 47 (1959).
(2) J. T. Park and J. L. Strominger, *Science*, **125**, 99 (1957).
(3) J. Lederberg, *J. Bacteriol.*, **73**, 144 (1957).
(4) In this abbreviation UDP refers to uridine diphosphate and GNAc-lactyl to an ether of acetylglucosamine and lactic acid (acetylmuramic acid). The peptide, for which the usual abbreviations are employed, is linked to the carboxyl group of the lactic acid. Its sequence recently has been determined.[5]
(5) (a) J. L. Strominger, *Compt. Rend. Trav. Lab. Carlsberg*, **31**, 181

accumulates in penicillin-treated cells.[6] It contained, in μmoles per μmole of uridine: phosphate, 2.0; GNAc-lactic, 0.98; alanine 0.96; glutamic acid, 1.00; and lysine, 1.04. Determination of the configuration of isolated alanine[5] gave 0.98 μmole of L-alanine and no D-alanine. These and other data[5] allow formulation of the structure of this previously unknown intermediate as UDP-GNAc-lactyl-(L)ala-(D)glu-(L)lys.

When D-alanine was added to a culture along with oxamycin, accumulation of nucleotides was greatly reduced. Similarly, D-alanine could *reverse* nucleotide accumulation previously induced by oxamycin (Table I). L-Alanine, D-serine or DL-alanyl-DL-alanine were ineffective antagonists of oxamycin.[8] Kinetic measurements of nucleotide accumulation indicated that the relationship between oxamycin and D-alanine is a true competitive one.[9] This is only the second example of competitive antagonism of an antibacterial substance by a natural substrate, the classical example being reversal of sulfonamide bacteriostasis by *p*-aminobenzoic acid.[10]

TABLE I

ANTAGONISM BY D-ALANINE OF URIDINE NUCLEOTIDE ACCUMULATION INDUCED BY OXAMYCIN

Measurements of nucleotide accumulation were made as described previously.[7] In experiment 1, oxamycin (75 μg./ml.) and possible antagonists were added together at 0 time. In experiment 2, oxamycin (75 μg./ml.) was added at 0 time. At 45 min., 20.4 μmoles of nucleotide had accumulated. At this time possible antagonists were added and incubation was continued for 45 minutes longer. Data are expressed as μmoles of uridine nucleotide per liter of culture at half-maximal growth.

Antagonist added	Expt. 1	Expt. 2
None	41.4	30.0
D-Alanine (500 μg./ml.)	17.0	12.1
D-Alanine (5000 μg./ml.)	4.5	6.9
L-Alanine (5000 μg./ml.)	41.5	32.2
DL-Alanyl-DL-alanine (5000 μg./ml.)	..	33.5
D-Serine (5000 μg./ml.)	..	34.2

The molecular basis for this phenomenon is undoubtedly the structural similarity between oxamycin[11] and D-alanine. It is noteworthy that oxamycin (D-cycloserine) does not inhibit incorporation of the L-alanine residue into the uridine nucleotide and that L-cycloserine does not induce nucleotide accumulation.[1] The enzymatic reactions which lead to synthesis of the peptide bonds in the nucleotide are under investigation.[12] It may also be possible to define the mechanism by which oxamycin inhibits bacterial growth at an enzymatic level. In any case, these observations should stimulate a search for D-amino acid analogs as possible chemotherapeutic agents.

J. L. Strominger, unpublished). The chromatographic position of the enzymatically synthesized compound was the first clue to the nature of the compound which accumulates with oxamycin.

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RECEIVED MAY 20, 1959

(1959); (b) J. L. Strominger and R. H. Threnn, *Biochim. Biophys. Acta*, **33**, 280 (1959), and *J. Pharm. Exper. Ther.*, **122**, 73A (1958).

(6) J. T. Park, *J. Biol. Chem.*, **194**, 877 (1952).

(7) J. L. Strominger, *ibid.*, **224**, 509 (1957).

(8) A. Bondi, J. Kornblum and C. Forte have reported that DL-alanine will permit growth of *S. aureus* in the presence of oxamycin (*Proc. Soc. Exper. Biol. Med.*, **96**, 270 (1957)).

(9) The reciprocal of the rate of nucleotide accumulation *vs.* the reciprocal of oxamycin concentration at four different concentrations of D-alanine gave four straigbt lines which intercepted the ordinate at the same point (*cf.* H. Lineweaver and D. Burke, THIS JOURNAL, **56**, 658 (1934)).

(10) (a) D. D. Woods, *Brit. J. Exper. Path.*, **21**, 74 (1940); (b) P. A. Fildes, *Lancet*, **1**, 955 (1940).

(11) (a) F. A. Kuehl, *et al.*, THIS JOURNAL, **77**, 2344 (1955); (b) P. H. Hidy, *et al.*, *ibid.*, **77**, 2345 (1955).

(12) The enzyme which catalyzes the synthesis of UDP-GNAc-lactyl-ala-glu-lys from UDP-GNAc-lactyl-ala-glu,[5b] lysine and ATP bas been purified about 500-fold from an extract of *S. aureus* (EI to and

A REQUIREMENT FOR VITAMIN B_{12} IN THE CONVERSION OF RIBOSE TO DEOXYRIBOSE BY LACTOBACILLUS LEICHMANNII

Sir:

The B_{12} requirement for *Lactobacillus leichmannii* may be replaced by a number of deoxynucleosides.[1] Subsequent reports have indicated that B_{12} functions in the biosynthesis of deoxyribose by this organism.[2] Two pathways for deoxyribose biosynthesis have been suggested. Acetaldehyde may condense with glyceraldehyde-3-phosphate to form deoxyribose[3]; however, a considerable body of data suggests that many organisms may convert ribose to deoxyribose. The present experiments were designed to determine which pathway is catalyzed by B_{12} in *L. leichmannii*.

The organism was grown in the basal medium previously described containing 2 mg. of deoxycytidine per liter.[4] The B_{12} concentration was varied from 0 to 20 mμg. per ml. The cells were grown for 24 hours in the presence of the C^{14} labeled substrates and then were fractionated as previously described.[4]

It was found that when cells were grown in the presence of acetaldehyde-1-C^{14} the addition of B_{12} slightly reduced the incorporation of the C^{14} into DNA, suggesting that B_{12} was not required for this pathway of deoxyribose biosynthesis.

Typical results obtained in experiments with ribose-1-C^{14} are given in Table I.

TABLE I

THE INFLUENCE OF VITAMIN B^{12} ON THE INCORPORATION OF RIBOSE-1-C^{14} INTO RIBONUCLEIC ACID (RNA) AND DEOXYRIBONUCLEIC ACID (DNA) BY *L. leichmannii*

Each flask contained 100,000 c.p.m. of ribose-1-C^{14}, specific activity 1 mc./mmole. The final volume of the incubation mixture was 30 ml.

B_{12} added, mμg./ml.	Specific activity (c.p.m./mg.) RNA	DNA
0	470	0
0.002	540	0
.02	420	0
.2	370	320
2	360	370
20	350	340

(1) E. E. Snell, E. Kitay and W. S. McNutt, *J. Biol. Chem.*, **175**, 473 (1948).

(2) M. Downing and B. S. Schweigert, *ibid.*, **220**, 521 (1956).

(3) E. Racker, *ibid.*, **196**, 347 (1952).

(4) J. S. Dinning, B. K. Allen, R. S. Young, and C. L. Day, *ibid.*, **233**, 647 (1958).