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

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

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]

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.

(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 straight 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 has been purified about 500-fold from an extract of *S. aureus* (EI to and 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

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).

It should be pointed out that since all flasks contained deoxycytidine growth was the same regardless of the B_{12} concentration. Samples of DNA from cells grown with 2 mμg. of B_{12} per ml. and with ribose-1-C^{14} were degraded with sulfuric acid and the deoxyribose converted to levulinic acid which was isolated as the 2,4-dinitrophenylhydrazone.[5] All the C^{14} activity of the original DNA was recovered in this derivative.

The results of these experiments demonstrate that in *L. leichmannii* vitamin B_{12} is required for the conversion of ribose to deoxyribose.

Acknowledgment.—This investigation was supported by research grant A-721, National Institutes of Health, Public Health Service.

(5) M. C. Lanning and S. S. Cohen, *J. Biol. Chem.*, **216**, 413 (1955).

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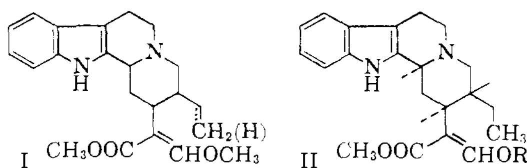
RECEIVED MAY 25, 1959

THE TOTAL SYNTHESIS OF *dl*-DIHYDROCORYNANTHEINE

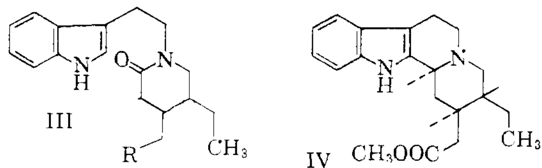
Sir:

Structural[1] and stereochemical[2] investigations have revealed the characteristic alkaloids (I) of *Pseudocinchona africana* A. Chev. as "missing



links" in the biogenetic sequences which involve among other substances, the venerable cinchona and yohimbine bases. We wish to record the first total synthesis of the racemic form of a naturally occurring representative of the corynantheine group, dihydrocorynantheine (II, R = CH_3).

Nickel-catalyzed reduction of diethyl β-(1-cyano-1-propyl)-glutarate[3] in the presence of excess tryptamine afforded *cis* and *trans* ethyl *dl*-N-(β-3′-indolyl)-5-ethylpiperidone-4-acetates (III, R



= C_2H_5OOC-), which were separated as the acids (III, R = COOH) by chromatography on silicic acid (*cis* acid, m.p. 221–223°, C = 69.35; H = 7.54; *trans* acid, m.p. 203–205°, C = 69.78; H = 7.63). These isomers were distinguished stereochemically by correlation of the lower-melting substance with *trans*-N-(β-3′-indolyl)-4,5-diethylpiperidone (III, R = CH_3),[4] achieved by means of this series of transformations: selective reduction with lithium borohydride to the piperidone alcohol (m.p. 145–146°; C, 72.55; H, 8.28); without deliberate purification of intermediates, formation of the O-tosylate, then conversion to the isothiouronium salt, and reductive desulfurization of the latter by means of Raney nickel. Bischler–Napieralski cyclization of *trans* piperidone (III, R = CH_3OOC-), and catalytic reduction over platinum of the resulting imine salt, resulted in formation of the *dl*-tetracyclic ester IV, m.p. 143.5–145.5° (HCl salt, m.p. 274.5–275°; C, 66.29; H 7.47). Treatment of IV with sodium triphenylmethyl then with methyl formate provided the α-hydroxymethylene ester (II, R = H), m.p. 185–186° (dec.) (infrared bands at 2.84 and 6.04μ) (C, 70.68; H, 7.47). Dimethyl sulfate and alkali, or, better, diazomethane in ethanol–ether, effected O-methylation, giving rise to *dl*-dihydrocorynantheine, which was characterized as the crystalline hydrochloride, m.p. 242–243° (C, 64.90; H, 7.17). The infrared spectrum (infrared bands at 2.85, 5.94 and 6.11μ) of the corresponding free base in chloroform solution was identical with the spectrum of *d*-dihydrocorynantheine in the same solvent.

Acknowledgment.—This work was supported by a grant from the Research Committee of the University of Wisconsin, with funds supplied by the Wisconsin Alumni Research Foundation.

(1) R. H. F. Manske and H. L. Holmes, "The Alkaloids," Vol. II, Academic Press, Inc., New York, N. Y., 1952, p. 420.

(2) (a) E. E. van Tamelen, P. E. Aldrich and T. J. Katz, *Chemistry and Industry*, 793 (1956); THIS JOURNAL, **79**, 6426 (1957); (b) M.-M. Janot, R. Goutarel, A. Le Hir, G. Tsatsas and V. Prelog, *Helv. Chim. Acta*, **38**, 1073 (1955).

(3) R. P. Evstigneeva, R. S. Livshits, L. I. Zakharkin, M. S. Bainova and N. A. Preobrazhensky, *Doklady Akad. Nauk.. U.S.S.R.*, **75**, 539 (1950); N. A. Preobrazhensky, R. P. Evstigneeva, T. S. Leuchenko and K. M. Fedyshkina, *ibid.*, **81**, 421 (1951).

(4) E. E. van Tamelen, P. E. Aldrich and J. B. Hester, Jr., THIS JOURNAL, **79**, 4817 (1957).

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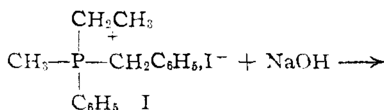
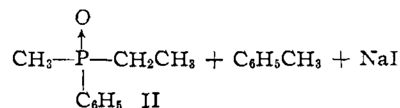
STEREOSPECIFIC CONVERSION OF METHYLETHYLPHENYLBENZYLPHOSPHONIUM IODIDE TO METHYLETHYLPHENYLPHOSPHINE OXIDE

Sir:

In the following communication[1] data were provided to show that the conversion by the action of sodium hydroxide solution of methylethylphenylbenzylphosphonium iodide (I) to methylethylphenylphosphine oxide (II), with elimination of toluene, is a third order reaction. We also wish to report that the reaction is completely stereospecific.





Treatment of optically pure levorotatory I[2]

(1) M. Zanger, C. A. VanderWerf and W. E. McEwen, THIS JOURNAL, **81**, 3806 (1959).

(2) K. F. Kumli, W. E. McEwen and C. A. VanderWerf, *ibid.*, **81**, 248 (1959).