there is an equally good possibility that the C^{13} in citrate entered by simple metabolic exchange.[3]

The essentially equal specific activities of the non-carboxyl carbon chain is interpreted to mean that it arose by condensation of methyl groups of acetate, probably thusly, $2C_2 \rightarrow C_4$; $C_4 + C_2 \rightarrow C_6$. Isotope dilution experiments with this organism have demonstrated the synthesis of C_4-dicarboxylic acids from ethanol by the $2C_2$ condensation reaction (unpublished data). The observed distribution of C^{14} in citrate indicates a very active C_4-dicarboxylic acid respiratory cycle. Such a cycle moves methyl activity to carboxyl, and thus one finds C^{14} in all 3 citrate carboxyls; whereas C^{13} from $C^{13}O_2$ enters primary carboxyls only (CO_2 fixation and/or exchange). Detailed discussion will be presented elsewhere.

BIOLOGY DIVISION
OAK RIDGE NATIONAL LABORATORY J. W. FOSTER[4]
OAK RIDGE, TENNESSEE S. F. CARSON

RECEIVED JANUARY 19, 1950

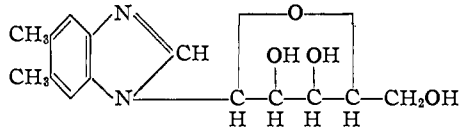
(3) Foster and Carson, in press.

(4) On leave of absence from the University of Texas.

VITAMIN B_{12}. IX. 1-α-D-RIBOFURANOSIDO-5,6-DIMETHYLBENZIMIDAZOLE, A DEGRADATION PRODUCT OF VITAMIN B_{12}

Sir:

1-α-D-Ribofuranosido-5,6-dimethylbenzimidazole (I) has been obtained by degradation of vitamin B_{12} and by synthesis.



I

The degradation of vitamin B_{12} to 5,6-dimethylbenzimidazole by acid hydrolysis has been reported.[1,2] Further investigation of the hydrolytic reaction yielded a basic product with an absorption spectrum of the benzimidazole type, and which gave a positive carbohydrate test.[3] A crystalline picrate, m. p. 213–214°, $[\alpha]^{23}D$ +9.9 ± 1.6° (*c*, 2.4 in pyridine), was prepared. *Anal.* Calcd. for $C_{14}H_{18}N_2O_4 \cdot C_6H_3N_3O_7$: C, 47.34; H, 4.17; N, 13.80; picric acid, 45.3. Found: C, 47.52; H, 3.92; N, 14.07; picric acid, 45.9 (spectrophotometric). In acidic ethanol solution, the absorption spectrum showed maxima at 2760 Å. (E_M 10,950), 2850 Å. (E_M 10,600), and 3590 Å. (E_M 13,000). The picrate consumed 0.92 mole of periodate per mole, demonstrating a 1-pentofuranosido-5,6-dimethylbenzimidazole structure. The oxidation gave a crystalline picrate of m. p. 180–185° and $[\alpha]^{23}D$ +24 ± 4° (*c*, 0.58 in pyridine). Conditions which cleaved the glycosidic linkage in the degradation product also caused extensive decomposition of the pentose.

Concomitant syntheses of 1-glycosidobenzimidazoles yielded one identical with the degradation product.

2-Nitro-4,5-dimethylaniline and 5-trityl-D-ribofuranose reacted to give 2-nitro-4,5-dimethyl-N-(5′-trityl-D-ribofuranosido)-aniline. Hydrogenation, condensation with ethyl formimino ether hydrochloride, and acid hydrolysis yielded crystalline 1-α-D-ribofuranosido-5,6-dimethylbenzimidazole picrate, m. p. and mixed m. p. 212–214°, $[\alpha]^{23}D$ +9.1 ± 1° (*c*, 4.0 in pyridine). *Anal.* Found: C, 47.55; H, 4.28; N, 13.74. Its absorption spectrum was identical with that of the degradation product. It consumed one mole of periodate per mole, and gave an α-(5,6-dimethylbenzimidazole-1)-α′-hydroxymethyldiglycolic aldehyde picrate of m. p. 183–185°, $[\alpha]^{23}D$ +20 ± 4° (*c*, 5.5 in pyridine), which did not depress the melting point of the corresponding derivative of the natural picrate. *Anal.* Calcd. for $C_{14}H_{16}N_2O_4 \cdot C_6H_3N_3O_7$: N, 13.86. Found: N, 13.08.

When 2-nitro-4,5-dimethyl-N-(5′-trityl-D-ribofuranosido)-aniline was acetylated and hydrogenated, the product after condensation with ethyl formimino ether hydrochloride and hydrolysis yielded 1-β-D-ribofuranosido-5,6-dimethylbenzimidazole picrate, m. p. 175–177°, $[\alpha]^{23}D$ −24 ± 2° (*c*, 2.1 in pyridine). *Anal.* Found: C, 47.55: H, 4.00; N, 13.92. This anomeric picrate consumed 1.1 moles of periodate per mole.

For convenience, the names α- and β-ribazole have been designated for the corresponding 1-D-ribofuranosido-5,6-dimethylbenzimidazoles.

NORMAN G. BRINK
FREDERICK W. HOLLY
CLIFFORD H. SHUNK
RESEARCH LABORATORIES ELIZABETH W. PEEL
MERCK & CO., INC. JOSEPH J. CAHILL
RAHWAY, N. J. KARL FOLKERS

RECEIVED FEBRUARY 27, 1950

(1) Brink and Folkers, THIS JOURNAL, **71**, 2951 (1949).

(2) Holliday and Petrow, *J. Pharm. Pharmacol.*, **1**, 734 (1949); Beavan, Holliday, Johnson, Ellis, Mamalis, Petrow and Sturgeon, *ibid.*, **1**, 957 (1949).

(3) Feigl, "Qualitative Analyses by Spot Tests," Third English Edition, Elsevier, New York, 1946, p. 410.

AMYLASE ACTION UNDER CONDITIONS OF UNFAVORABLE TEMPERATURE OR HYDROGEN ION CONCENTRATION[1]

Sir:

It was pointed out in a recent paper[2] that when acting under optimal conditions of *p*H and temperature soybean beta amylase characteristically degrades amyloheptaose and other amylaceous substrates without appreciable formation of saccharides intermediate between the original substrate and the final products. We have also observed[3] in the initial phase of salivary amylase acting under optimal conditions on amylodextrin

(1) Journal Paper No. J-1744 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1116. Supported in part by a grant from the Corn Industries Research Foundation.

(2) French, Levine, Pazur and Norberg, THIS JOURNAL, **72**, 1746 (1950).

(3) French, Pazur and Knapp, unpublished observations.