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organism have demonstrated the synthesis of C_4-dicarboxylic acids from ethanol by the $2C_2$ condensation reaction (unpublished data).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.

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RECEIVED JANUARY 19, 1950

(3) Foster and Carson, in press.

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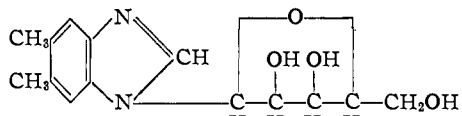
VITAMIN B_{12}. IX. 1-α-D-RIBOFURANOSIDO-5,6-DIMETHYLBENZIMIDAZOLE, A DEGRADATION PRODUCT OF VITAMIN B_{12}

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.

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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.

that the only significant products of low molecular weight (*i. e.*, in the water-soluble oligosaccharide range) are maltose, amylotriose and amylotetraose. These findings suggest that the substrate molecules, having once diffused close enough to the enzyme molecule that enzyme–substrate complex formation can take place, are attacked by the enzyme with sufficient speed that the fragments initially produced are further attacked before they have time to diffuse away from the enzyme molecule. This hypothesis has now been tested by conducting enzyme digests under unfavorable conditions of *p*H or temperature and examining the products by means of paper chromatography. At intermediate stages of salivary amylase–amylodextrin digests we find a complete array of amyloöligosaccharides, covering the range from maltose (even small amounts of glucose) to amylooctaose and higher unresolved saccharides. Similarly with beta amylase–amyloheptaose digests, maltose and amylopentaose are the initial products of the reaction at 26° and *p*H 10, the amylopentaose accumulating and finally disappearing as amylotriose and maltose are produced in increasing amount. Similar effects were obtained at *p*H 4.7 and 70°.

We interpret these findings to mean that either the enzyme–substrate affinity constants are seriously decreased at higher temperatures or unfavorable *p*H, or the rate at which the enzyme–substrate complex disintegrates into products is diminished, or possibly a combination of these two effects. Further studies, in progress, may show the quantitative roles of affinity, disintegration and diffusion under conditions favorable and unfavorable for rapid enzyme action.

It is generally recognized that the optimal conditions of *p*H and temperature for a given enzyme may vary with different substrates or with variation in the kinds and amounts of salts, etc., present. However, in the present case, it has been demonstrated that not only the *rate* but also the *relative amounts of the intermediate products* of an enzymatic reaction are dependent on the reaction conditions.

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RECEIVED FEBRUARY 11, 1950

CHROMATOGRAPHY ON TREATED FILTER PAPER[1]

Sir:

In purifying the 2,4-dinitrophenylhydrazones of aldehydes and ketones by the chromatographic separation method of Roberts and Green,[2] combinations of derivatives were found which could not be separated. Because of the time involved in column chromatography, we used the test-tube paper chromatographic method of Rockland and Dunn[3a] as applied to 2,4-dinitrophenylhydrazones by Keller, *et al.*,[3b] and found that it can be adapted as a rapid checking system for the evaluation of numerous solvents in order to select one that can be used to separate these derivatives on corresponding chromatographic columns. The modification consisted in impregnating the paper with silicic acid so that the paper strip acts as a micro-silicic acid column.

Flood[4] has used impregnated papers for inorganic ion analysis in which the paper was first treated with aluminum hydroxide or a synthetic zeolite and then with a suitable reagent for developing the spot test. Hopf[5] has used Flood's method as a spot test for ketones and aldehydes by treating the filter paper with alumina and 2,4-dinitrophenylhydrazine. In the present instance the 2,4-dinitrophenylhydrazones were applied directly to the paper as explained by Keller, *et al.*[3]

Before cutting the filter paper in strips it was treated as follows: A heavy grade of filter paper was soaked in sodium silicate solution (Bé 42° diluted with two volumes of water), drained of surplus liquid, and then immersed for five minutes in 6 *N* hydrochloric acid solution, followed by a brief washing to remove excess acid. Care should be taken to avoid excess washing, which will leach out some of the silicic acid. The paper was then dried at 110° and finally pressed between weights to remove most of the curl in the treated paper.

Treatment of the paper in this manner broadens the scope of paper chromatography for application to more fields and compounds, and has been tried on other separations with excellent results. In some cases separations could be achieved which could not be obtained on any of the filter papers available on the market. For example as shown in Table I, the separation of three compounds can be achieved using treated paper where untreated paper fails. The method can be applied to any adsorbent such as starch, sugar or calcium hydroxide, which can be impregnated into filter paper.

TABLE I

COMPARISON OF R_f VALUES FOR 2,4-DINITROPHENYLHYDRAZONES ON TREATED AND UNTREATED FILTER PAPER
Solvent = 5% ethyl ether in petroleum ether (b. p. 110°)

Ketone	R_f Untreated paper	R_f Treated paper
Methyl ethyl	0.90	0.38
Methyl propyl	.90	.54
Methyl isopropyl	.91	.48

Where separation of compounds on packed chromatographic columns can be achieved in the time that it takes the solvent to travel the length of the column, duplicate chromatograms on

(1) Report of a study made under the Research and Marketing Act of 1946.

(2) J. D. Roberts and Charlotte Green, *Anal. Chem.*, **18**, 335 (1946).

(3) (a) L. B. Rockland and M. S. Dunn, *Science*, **109**, 539 (1949); (b) G. J. Keller, J. G. Kirchner and R. G. Rice (unpublished).

(4) H. Flood, *Anal. Chem.*, **120**, 327 (1940).

(5) P. P. Hopf, *J. Chem. Soc.*, 785 (1946).