p-Anisylmagnesium bromide was treated with ethyl pivalate to give 1,1-bis-(*p*-methoxyphenyl)-2,2-dimethylpropanol-1 (m. p. 81–83°. *Anal.* Calcd. for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05. Found: C, 75.51; H, 7.84), which was then reduced over copper chromite to 1,1-bis-(*p*-methoxyphenyl)-2,2-dimethylpropane (II) m. p. 59–61°. *Anal.* Calcd. for $C_{19}H_{24}O_2$: C, 80.24; H, 8.51. Found: C, 80.42; H, 8.55). This compound, more conveniently called 1,1-dianisylneopentane, is related to "methoxychlor"(III), the *p,p'*-dimethoxy analog of DDT, in the sense that the trichloromethyl group of "methoxychlor" has been replaced by a *t*-butyl group. The neopentane has insecticidal activity of the same order, although lower, as "methoxychlor." Some approximate LD 50 dosage ratios (1,1-dianisylneopentane: "methoxychlor") are as follows: German cockroaches (contact), 1:2; milkweed bugs (contact), 4:1; webbing clothes moth and carpet beetle larvae (wool impregnation), each 2:1; mosquito larvae (*A. aegypti*), 4:1; houseflies (spray), 4:1.[1] The tremors and paralysis characteristic of DDT and "methoxychlor" are produced by the neopentane. It has also been observed that the Ellenville strain of DDT-resistant houseflies is markedly more resistant to this compound than are ordinary strains of flies.[2,3]

The hypothesis of Martin and Wain,[4] that DDT toxicity is caused by hydrogen chloride release, obviously fails to explain the effectiveness of the chlorine-free product. Lauger's lipoid-solubility hypothesis[5] and a possible relationship between steroids and DDT-type compounds[6] will be discussed in a later publication.

(1) Tests by the Wisconsin Alumni Research Foundation.

(2) Barber and Schmitt, *N. J. Agr. Exp. Sta. Bull.*, 742 (1948); Barber, Starnes and Starnes, *Soap and San. Chem.*, **24** [11] 120 (1948).

(3) We are greatly obliged to the staff of our Entomological Laboratory for certain of the biological tests reported above and to Mr. Ordway Starnes and the late Dr. George W. Barber of the Department of Entomology, Rutgers University, and N. J. Agr. Exp. Station for tests with resistant strains of flies.

(4) Martin and Wain, *Nature*, **154**, 512 (1944).

(5) Lauger, Martin and Mueller, *Helv. Chim. Acta*, **27**, 892 (1944).

(6) Lauger, Pulver, Montigel, Weismann and Wild, "Mechanism of Intoxication of DDT Insecticides in Insects and Warm-Blooded Animals," Lecture, Washington, D. C., July 31, 1945, Geigy Company Inc., New York, N. Y., 1946.

RESEARCH LABORATORIES
MERCK & CO., INC. H. D. BROWN
RAHWAY, NEW JERSEY E. F. ROGERS

RECEIVED FEBRUARY 20, 1950

CITRIC ACID FORMATION BY *ASPERGILLUS NIGER* THROUGH CONDENSATION OF $3C_2$ MOIETIES

Sir:

Biogenesis of citric acid *via* condensation of oxalacetate and acetate (C_4 dicarboxylic acid + C_2) is a reasonably well-accepted hypothesis. The C_4 dicarboxylic acid is generally presumed to originate *via* the Wood–Werkman reaction (pyruvate and carbon dioxide), though now the C_2 condensation must also be considered.[1] The following experiments were intended to demonstrate the relative participation of the two modes of genesis of the C_4 moiety.

Radioactive citrate was produced from sucrose by washed *Aspergillus niger* submerged mycelium (200 mg. dry wt.) in the presence of 2 mg. of high specific activity methyl-C^{14}-labeled acetate and carbon dioxide containing 19.2 atom % $C^{13}O_2$. To the 38 mg. of citric acid produced in forty hours (at which time considerable unconsumed sucrose remained) carrier citric acid (350 mg.) was added; calcium citrate was isolated and purified by precipitation and twofold reprecipitation from hot solution.

The radioactive citric acid was converted to pentabromoacetone, which represents the non-carboxyl carbons of the citric acid. The non-carboxyl carbons were also obtained in the form of acetone, by dilute acid–dichromate oxidation of another portion of citric acid. The acetone was further degraded to iodoform and acetic acid; the acetic acid was then degraded[2] to methylamine and carbon dioxide. Specific activity measurements were made on barium carbonate obtained by wet combustion.

TABLE I

C^{13} AND C^{14} VALUES

	Fraction	Specific activity[a]	Atom % C^{13}
1	Total citric acid	0.16	1.107 ± 0.005[c]
2	Non-carboxyl carbons		
	Pentabromoacetone	.15	1.084 ± 0.002[c]
	Acetone	.15	
	Iodoform	.17	
	Acetic acid	.16	
	Methylamine	.15	
	Carbon dioxide	.17	
3	Carboxyl carbons		
	Primary carboxyls	.12	1.132 ± 0.009[c]
	Secondary carboxyl	.16	1.090 ± 0.010[c]
4	CO_2 in atmosphere		
	Initial	.00	19.2 ± 0.1
	Final	.43[b]	10.5 ± 0.1

[a] Counts/sec./mg. $BaC^{14}O_3$ (measured on citrate diluted with carrier). [b] Measured as 4.3 counts/sec./mg. $BaC^{14}O_3$, but calculated as if diluted same amount as the citrated. [c] Measurements made on citrate diluted with carrier, and its degradation products.

The mean C^{13} content of the atmospheric carbon dioxide (19.2 + 10.5/2 = 14.9 atom %) enables one to calculate that CO_2-carbon from the atmosphere entered citrate to the extent of 1.3% of the total citrate carbon; if the Wood–Werkman reaction were entirely responsible for net citrate synthesis, the figure should be 16.7%. Unlabeled intracellular carbon dioxide from sucrose theoretically could also account for some net synthesis; we have been unable to conceive a definitive experiment on this point. On the other hand,

(1) Foster, *et al.*, *Proc. Natl. Acad. Sci., U. S.*, **35**, 663–672 (1949).

(2) Phares, to be published.

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

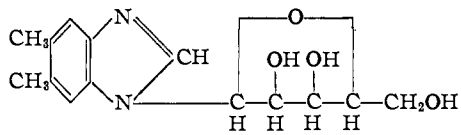
(3) Foster and Carson, in press.

(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.