TABLE I

DISTRIBUTION OF C^{14} IN PHENYLALANINE AND TYROSINE FROM YEAST UTILIZING CH_3–$C^{14}O$–COOH OR CH_3–$C^{14}OOH$

	Phenylalanine						Tyrosine					
	Aerobic pyruvate		Anaerobic pyruvate		Aerobic acetate		Aerobic pyruvate		Anaerobic pyruvate		Aerobic acetate	
Conditions →	C.p.m./ mmole × 10^4	% of total	C.p.m./ mmole × 10^4	% of total	C.p.m./ mmole × 10^4	% of total	C.p.m./ mmole × 10^4	% of total	C.p.m./ mmole × 10^4	% of total	C.p.m./ mmole × 10^4	% of total
Whole molecule	38.8	100	19.0	100	4.58	100	49.1	100	19.0	100	4.83	100
Side chain	18.4	47.3	10.0	52.7	1.33	29	22.3	48	7.7	41	2.43	50
Ring	20.4	52.7	9.0	47.3	3.25	71	25.9	53	10.5	55	2.59	53
Distribution of C^{14} within the side chain												
CH_2CHNH_2COOH	18.4	100	10.0	100	1.33	100	22.3	100	7.7	100	2.43	100
COOH	2.7	14.7	2.7	27	1.33	100	4.3	19	2.8	36	2.43	100
$CHNH_2$	14.8	80.3	6.5	65	0	0	18.0	81	4.9	64	0	0
CH_2	0.9	5	0.8	8	0	0	0	0	0	0	0	0
Distribution of C^{14} within the ring												
Ring	20.4	100	9.0	100	3.25	100	25.9	100	10.5	100	2.59	100
C_{1+3+5}	16.5	81	6.0	66.6	2.50	77	20.1	78	5.4	52	0.74	28
C_{2+4+6}	3.9	19	3.0	33.3	0.75	23	5.7	22	5.0	48	1.74	72
C_1							8.3	32	1.6	15	0	0
C_{3+5}							11.8	46	3.0	36	0.74	29
C_4	1.7	8.3	1.4	15.6	0.58	17.6						
C_{2+6}	2.2	10.8	1.6	17.8	0.17	5.1						

acid and a C_3 unit, is an intermediate in the biosynthesis of phenylalanine.

Although the precise mechanism of formation of phenylalanine in yeast is conjectural, the present data permit some pertinent conclusions regarding the process. First, tyrosine is not directly interconvertible with phenylalanine, at least in the acetate samples. Second, whereas from pyruvate or glucose a single route of synthesis of phenylalanine and tyrosine may function,[4,6] with acetate as substrate there are two important differences: the orientation of the side chain in terms of isotopic distribution in the ring, and the unequal distributions of isotope between the rings and the side chains (Table I). The different orientations of the side chains might be either the result of a change in the site of attachment, or a migration of the side chains after attachment.

The picture is further complicated by the differing isotope contributions of the ring and side chain. With *pyruvate* as the carbon source, the labeling in the two amino acids is similar in amount and almost identical in pattern (Table I), but with acetate as substrate, phenylalanine is preferentially labeled in the ring. It would appear from these observations that the routes of synthesis of the *aromatic ring* may also differ in one or more important respects when derived from acetate or pyruvate.

Acetate, although not a "normal" substrate for yeast, has been widely used to suggest pathways of amino acid biosynthesis. In view of the present results, a note of caution seems appropriate when observations on acetate metabolism are interpreted in terms of pyruvate, glucose, or other carbohydrate intermediates.

Efforts are continuing to outline the routes of biosynthesis of these amino acids.

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Methyl D-Glucoside Tetra-(chlorocarbanilates)

BY I. A. WOLFF AND R. L. MELLIES

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In previous studies at this Laboratory[1] it was shown that tri-*o*-chlorocarbanilates of corn starch and its fractions had solubility properties, optical rotations and melting ranges considerably different from the corresponding *meta* isomers. This was attributed to the existence of a chelate structure in the *ortho* compounds. It also was pointed out that the observed properties might result from the additive effect of the small individual contribution of each chelated chlorine atom caused by the large total number of such groupings in the polymer molecules. To investigate this point further, it was considered desirable to compare the properties of isomeric chlorocarbanilates of non-polymeric materials. The anomeric methyl D-glucosides were selected for derivatization and study.

Experimental

Materials.—Eastman Kodak Co. white label isocyanates were used as received without further purification. They were characterized by conversion in high yield to the corresponding ureas which had melting points corresponding to literature values. Anomeric methyl D-glucosides were prepared by conventional procedures and dried before use.

Preparations.—Only minor variation of the following general preparative procedure was employed for the individual compounds. To 1.94 g. (10 millimoles) of the glucoside in 15 ml. of dry pyridine was added 1.1 to 1.2 times the theoretical amount of isocyanate required for complete substitution. All reactions were exothermic. After allowing the reaction mixtures to stand overnight at room temperature 10–15 ml. of dry methanol was added, followed, after about an hour, by 25–50 ml. of water. Total yields of crude carbanilates were usually slightly above theoretical due to contamination with by-products from the excess reagent used. Preferred solvent systems for purification of the products, together with properties of the compounds, are listed in Table I.

Similar to the glycoside carbanilates described by Wol-

(1) I. A. Wolff, P. R. Watson and C. E. Rist, THIS JOURNAL, **74**, 3061 (1952).

TABLE I

PROPERTIES OF THE TETRA-(CHLOROCARBANILATES)

Anomeric methyl D-glucoside	Isomeric chlorocarbanilate	Purification solvents	M.p., °C.	$[\alpha]^{25}D$ ($C \cong 1$) Pyridine	Morpholine	Analyses, found, %[a] C	H	Cl
α	*ortho*	Abs. EtOH	139.5–140.5	+83.6°	+50.4°	52.1	3.75	17.4
α	*meta*	Me_2CO–heptane; MeOH–H_2O	208.5–210.5	+79.1	+52.2	51.9	3.78	17.7
β	*ortho*	Me_2CO–H_2O; *n*-BuOH	202–205	+3.5	−1.3	52.0	3.99	17.5
β	*meta*	EtOH–H_2O	223–226	+6.3	−3.0	52.4	3.82	17.8

[a] Calculated: C, 52.0; H, 3.74; Cl, 17.5.

from and Pletcher,[2] these compounds showed considerable tendency to precipitate as partially crystalline gels which were slow filtering and difficult to handle. The melting points were difficult to determine exactly since the melt became clear slowly, and the temperature of melting varied with the rate of heating. The melting point values reported were determined at a rate of temperature rise of about a degree per minute.

Discussion

Unlike the isomeric chlorocarbanilates of the amylaceous materials previously studied,[1] there was no significant difference in optical rotation between the *o*- and *m*-chlorocarbanilates of the methyl glucosides reported here. The data enable calculation of 2A and 2B values according to Hudson's Isorotation Rules[3] as follows:

Solvent	Isomer	2A	2B
Pyridine	*Ortho*	64,800	70,400
Pyridine	*Meta*	58,900	69,100
Morpholine	*Ortho*	41,800	39,700
Morpholine	*Meta*	44,600	39,800

(2) M. L. Wolfrom and D. E. Pletcher, THIS JOURNAL, **62**, 1151 (1940).

(3) W. W. Pigman and R. M. Goepp, Jr., "Chemistry of the Carbohydrates," Academic Press, Inc., New York, N. Y., 1948, pp. 80-88.

This also shows the absence of any significant rotational effects attributable to position isomerism of the chlorine substituent on the benzene ring.

In contrast to the lack of rotational differences, the *ortho* isomers did have lower melting ranges, and were considerably more soluble in chloroform and benzene than the *meta* isomers. All of the compounds were readily soluble in acetone, ethyl acetate and morpholine and insoluble in heptane. We believe on the basis of these solubility and melting point differences that there is probably a discernible chelation effect in these *o*-chlorocarbanilates and that our previous observations were not entirely dependent on the polymeric character of the materials.

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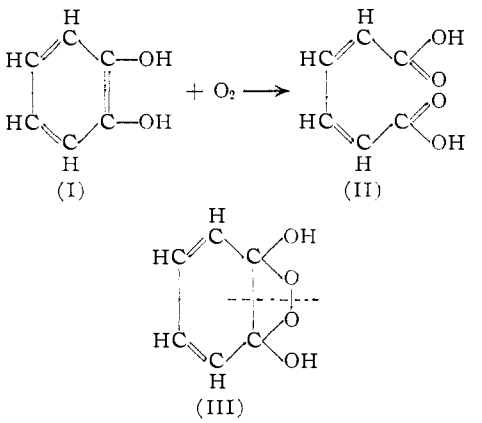
COMMUNICATIONS TO THE EDITOR

MECHANISM OF THE PYROCATECHASE REACTION

Sir:

Pyrocatechase[1,2] of *Pseudomonas* sp. catalyzes the oxidative cleavage of the aromatic ring of catechol (I) to *cis–cis*-muconic acid (II). Subsequent work has shown that pyrocatechase requires ferrous ion[3] and sulfhydryl containing compounds[4] for maximum activity, although the mechanism of electron transport as well as the nature of intermediate steps has remained unknown.

We wish to report some experimental results using O_2^{18} and H_2O^{18} which may aid in elucidating the mechanism of this unique enzymatic reaction. When the reaction was conducted in the presence of H_2O^{18}, O^{18} was not detected in the product, *cis–cis*-muconic acid. In the presence of O_2^{18}, however, essentially all the oxygen enzymatically introduced into *cis–cis*-muconic acid was shown to be derived from molecular oxygen (Table I). The results clearly demonstrate that pyrocatechase is an



(I) (II) (III)

(1) O. Hayaishi and K. Hashimoto, *J. Biochem. (Japan)*, **37**, 371 (1950).

(2) O. Hayaishi and R. Y. Stanier, *J. Bact.*, **62**, 691 (1951).

(3) M. Suda, K. Hashimoto, H. Matsuoka and T. Kamahora, *J. Biochem. (Japan)*, **38**, 289 (1951).

(4) R. Y. Stanier and J. L. Ingraham, *J. Biol. Chem.*, **210**, 799 (1954).