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.

Acknowledgments.—The assistance of Mrs. Clara McGrew, who carried out the elementary microanalyses, and of H. A. Davis, who synthesized the methyl β-D-glucoside used, is gratefully acknowledged.

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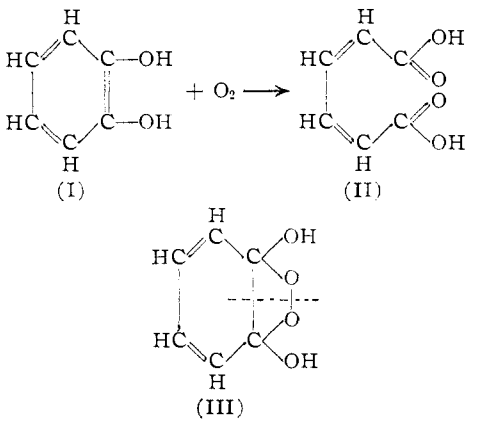
COMMUNICATIONS TO THE EDITOR

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).

TABLE I

ENZYMATIC INCORPORATION OF O_2^{18} INTO *cis–cis*-MUCONIC ACID

Experiment I. Catechol (0.6 mmole), 4.8 mg. of purified pyrocatechase (specific activity 112 units/mg. protein),[a] 0.6 millimole of glutathione and 4.5 millimoles of potassium phosphate buffer (*p*H 7.5) were incubated in a 500-ml. Erlenmeyer flask in a total volume of 60 ml. H_2O^{18}, 0.701 atom % excess. Experiment II. A 500-ml. Büchner suction flask was modified with a high vacuum stopcock at the top; the hose connection was sealed with a rubber vaccine cap. The same reaction components as in Expt. I were employed except that H_2O was used instead of H_2O^{18} and to the degassed container was added a O_2^{18}–N_2 mixture with an approximate ratio of O_2^{18}/N_2 of 18.2%.[b] In Expt. II the O^{18} content of the flask was determined before the addition of the enzyme and after the completion of the reaction. The atom % excess was 1.354 and 1.331 respectively. The reactions were run at 25° with gentle mechanical shaking and the reaction rate was followed by the increased absorption at 260 mμ. Aliquots were removed by syringe through the rubber vaccine cap in Expt. II. After two hours of incubation 2 ml. of 2 *N* H_2SO_4 was added to the reaction mixture. *cis–cis*-Muconic acid was isolated and was recrystallized from absolute ethanol. The recrystallized material was pyrolyzed by the method of W. E. Doering and E. Dorfman (THIS JOURNAL, **75**, 5595 (1953)[c]. The O^{18} content was determined by a Consolidated Nier Model 21-201 Mass Spectrometer, measuring $CO^{16}O^{18}/CO_2$ (46/44) ratio.

Expt.	Medium	Atom % O^{18} in Catechol	Atom % O^{18} in *cis–cis*-Muconic acid[d]	Atom % excess Experiment	Atom % excess Theory[e]
I	$O_2^{16} + H_2O^{18}$	0.207	0.207	0.000	0.701
			0.207	0.000	
II	$O_2^{18} + H_2O^{16}$	0.207	1.421	1.217	1.343
			1.433	1.229	

[a] M. Katagiri and O. Hayaishi, unpublished procedure. [b] O_2^{18} was prepared by electrolysis of H_2O^{18} obtained from the Stuart Oxygen Co. [c] We are indebted to Mr. S. Ishihara of the National Bureau of Standards for use of the pyrolysis equipment. [d] Calculated for the oxygen atoms incorporated. [e] Theoretical atom % excess when two oxygen atoms are derived from O^{18}.

oxygen transferase rather than a dehydrogenase and no hydration reaction is involved in the over-all process. *cis–cis*-Muconic acid semialdehyde is therefore excluded as an intermediate since any known mechanism of enzymatic aldehyde oxidation involves hydration. A compound such as (III) appears to be a more likely intermediate in the pyrocatechase reaction. Orthobenzoquinone appears unlikely as an intermediate since H_2O_2 was previously shown not to participate in the reaction.[1,4] This compound, however, cannot be completely ruled out as an intermediate because of the possibility of a tightly bound enzyme–H_2O_2 complex acting as a peroxidase.

The similarity of pyrocatechase to other enzymes which catalyze oxidative rupture of aromatic rings of certain phenolic compounds was recently reviewed by Crandall.[5] In addition to pyrocatechase, homogentisicase,[6] 3-hydroxyanthranilic acid oxidase[7,8] and protocatechuic acid oxidase[4] appear to belong to this new class of metallo-protein enzymes which introduce two oxygen atoms directly across the aromatic bond adjacent to the phenolic group with simultaneous rupture of the aromatic structure.

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(5) D. L. Crandall, "A Symposium on Amino Acid Metabolism," ed. by D. McElroy and B. Glass, Johns Hopkins Press, Baltimore, Md., 1955, p. 867.

(6) M. Suda and Y. Takeda, *J. Biochem. (Japan)*, **37**, 381 (1950).

(7) L. M. Henderson, Abstract of paper, Amer. Chem. Soc. 121st Meeting, Milwaukee, 1952, p. 23C.

(8) A. Miyake, A. H. Beckman and B. S. Schweigert, Abstract of Paper, Amer. Chem. Soc. 124th Meeting Chicago, 1953, p. 11C.

DECOMPOSITION OF PRIMARY HYDROPEROXIDES

Sir:

We should like to report that *n*-butyl hydroperoxide when decomposed by heating the neat liquid at 85° in absence of added catalyst gives, as the primary gaseous product, hydrogen. Furthermore this behavior is also general for the higher primary hydroperoxides; isobutyl, *n*-amyl, isoamyl, *n*-heptyl, *n*-octyl and *n*-decyl hydroperoxides all give hydrogen as the major gaseous component on heating to 100°. Oxygen was not produced in significant amounts in any case.

Milas[1] states that "at relatively low temperatures primary hydroperoxides decompose to give aldehydes and water, while secondary and tertiary hydroperoxides give the corresponding alcohols and oxygen." That hydrogen was the gas evolved in the decomposition of *n*-butyl hydroperoxide was therefore totally unexpected.

A preliminary study on *n*-butyl hydroperoxide reveals two major reactions: approximately 50% goes to hydrogen and butyric acid and 40% goes to *n*-butyl *n*-butyrate and water. The remaining minor products are carbon dioxide, carbon monoxide, propane, propyl butyrate, butyl propionate, propionic acid and an unknown compound, probably an hydroxy acid.

Redistilled *n*-butyl hydroperoxide,[2] 0.605 g. (0.0067 mole), which was shown by gas-liquid partition chromatography[3] to contain no appreciable amounts of impurities, was heated in the vapor of boiling trichloroethylene (b.p. 86°) for 47 hours until gas evolution had ceased and the peroxide titer was zero. The gas, 83.9 ml. at standard conditions, was collected over mercury; it showed the following analysis: hydrogen, 80.0% (0.0030 mole); carbon dioxide, 4.4% (0.00016 mole); carbon monoxide, 0.1%; propane, 6.8% (0.00025 mole); oxygen, 0.5%; residue 8.2%. The identity of the propane, as well as carbon monoxide and carbon dioxide, was confirmed by its infrared spectrum. The liquid products, 0.532 g., were separated by gas–liquid partition chromatography[3] on a silicone oil–Celite column and identified by infrared spectra. The composition of the liquid mixture was determined, by direct comparison of the partitionograms of the unknown with a synthetic mixture,[4] to be as follows: water, 9.9% (0.0029

(1) N. A. Milas, "Encyclopedia of Chemical Technology," Vol. 10, Interscience Encyclopedia Inc., New York, 1954, p. 63.

(2) H. R. Williams and H. S. Mosher, THIS JOURNAL, **76**, 2984 (1954).

(3) (a) A. T. James and A. J. P. Martin, *Analyst*, **77**, 915 (1952); (b) A. J. P. Martin and A. T. James, *Biochem. J.*, **50**, 679 (1952).

(4) G. Dijkstra, J. G. Keppler and J. A. Schols, *Rec. trav. chim.*, **74**, 805 (1955).