

were found in *Phytophthora cactorum* and its presence was noted in *Thamnidium elegans*. Tyrosine was present in small amount in several, but tryptophan was not detected in any of the fungi. Glutamine appeared to be present in larger amounts than asparagine. Four unidentified compounds were found in some of the fungi. These corresponded on chromatograms to the positions of  $\alpha$ -amino adipic acid, 3:4-dihydroxyphenylalanine, ethanolamine and taurine, but identification must await further work.

Under these experimental conditions, there do not appear to be any substantial differences between fungi belonging to different taxonomic and physiological groups. The number and relative amounts of free amino-acids present in fungi show an overall similarity to those in higher plants.

The assistance of Dr. E. G. Bollard is gratefully acknowledged.

R. CLOSE

Crop Research Division,  
Department of Scientific and Industrial Research,  
Christchurch, New Zealand.

<sup>1</sup> Fowden, L., *Nature*, **167**, 1030 (1951).

<sup>2</sup> Mansford, K., and Raper, R., *Nature*, **174**, 314 (1954).

<sup>3</sup> Fluck, V., and Richie, K. H., *Phytopath. Z.*, **24**, 455 (1955).

<sup>4</sup> Foster, J. W., "Chemical Activities of Fungi" (1940).

<sup>5</sup> Robbins, W. J., *Amer. J. Bot.*, **24**, 243 (1937).

### Specificity in vitro of a Phenoloxidase System from *Periplaneta americana* (L.)

ENZYMES capable of oxidizing mono- and diphenols are widely distributed in insects; some of these compounds are involved in the biosynthesis of exoskeletal substances, notably sclerotin<sup>1</sup>. While several *ortho*- and *para*-diphenols have been extracted from cuticle, relatively little is known about the corresponding synthetic enzymes. Recent work in connexion with the reaction of phenolic derivatives with proteins ('tanning reaction') has been reviewed by Hackman<sup>2</sup>, Mason<sup>3</sup> and Dennell<sup>4</sup>. The present results form part of an investigation of the intermediary metabolism of phenolic substrates involved in sclerotin formation in *Periplaneta*.

It has been already shown that the sclerotin walls of the egg capsule (ootheca) are formed by the intermingling of the secretions of the left and right colleterial glands<sup>1</sup>. As a result of this, protocatechuic acid<sup>5</sup> (3:4-dihydroxybenzoic acid) is liberated from its 4-O- $\beta$ -D-glucoside (secreted by the left gland) by the action of a  $\beta$ -glucosidase (from the right gland)<sup>6</sup>. Some evidence<sup>1</sup> suggests that the protocatechuic acid is oxidized enzymically by a secretion of the left gland<sup>7</sup>, afterwards to link with structural proteins to form sclerotin.

It has now been confirmed that the oxidative enzymes are present in the lumen of the left gland and are released in the form of a highly turbid suspension when the dissected left gland is allowed to stand for a few minutes in distilled water. The preparation can be freed from cellular debris by centrifugation at 0° C. and from endogenous substrates by dialysis ('Cellophane') against 0.01 M sodium chloride. Subsequent salt precipitation gave fractions of very high enzymic activity between 0.1 M and 0.2 M sodium chloride (final concentration).

The activity of the dialysed enzyme preparations was investigated in 0.1 M sodium phosphate buffer (pH 6.8) with a variety of phenolic compounds (each in a final concentration of 0.002 M) using Warburg

Table 1. SUBSTRATES FOR THE PHENOLOXIDASE SYSTEM FROM LEFT COLLETERIAL GLANDS OF *Periplaneta*

Active	Inactive
Protocatechuic acid	DL-3:4-Dihydroxyphenylalanine
3:4-Dihydroxybenzoic acid	L-Tyrosine ( <i>para</i> )
3:4-Dihydroxypropionic acid	L-Tyrosine ( <i>ortho</i> )
3:4-Dihydroxybenzaldehyde	<i>p</i> -Hydroxybenzoic acid
Catechol	DL-3:4-Dihydroxyphenylethylamine (dopamine)
Hydroquinone	Resorcinol
<i>p</i> -Phenylenediamine	

manometers (5-6 ml.) at 37° in an atmosphere of oxygen. The results are shown in Table 1.

The enzyme promoted rapid oxidation of a number of *o*- and *p*-diphenols with the notable exception of 3:4-dihydroxyphenylalanine and the corresponding amine. L-Tyrosine (either *ortho* or *para*) or other monophenols were not oxidized.

Thus, in the sense of Keilin and Mann<sup>8</sup> and Dawson and Tarpley<sup>9</sup>, this enzyme can be regarded as a laccase and in no sense a tyrosinase.

The enzyme activity is abolished by being heated above 70° and by 0.002 M potassium cyanide. It is unaffected by 4-chlororesorcinol and diethyldithiocarbamate. Preliminary investigations indicate that the enzyme is a protein-copper complex.

It is emphasized that the enzyme preparation has not yet been obtained in a soluble form but that it occurs in the gland in a finely divided state exhibiting a strong Tyndall effect. This is supported by electron microscopy<sup>10</sup>.

It has not yet been found possible to separate the enzyme from the protein-precursor of sclerotin, of which indeed the enzyme may be a part.

D. L. WHITEHEAD  
P. C. J. BRUNET  
P. W. KENT

Departments of Biochemistry and Zoology,  
University of Oxford.

<sup>1</sup> Pryor, M. G. M., *Proc. Roy. Soc.*, B, **128**, 378 (1940).

<sup>2</sup> Hackman, R. H., *Proc. Fourth Internat. Cong. Biochem.*, Vienna, **12**, 48 (1958).

<sup>3</sup> Mason, H. S., "Adv. in Enzymol.", **16**, 105 (1955).

<sup>4</sup> Dennell, R., *Biol. Rev.*, **33**, 178 (1958).

<sup>5</sup> Pryor, M. G. M., Russell, P. B., and Todd, A. R., *Biochem. J.*, **40**, 627 (1946).

<sup>6</sup> Brunet, P. C. J., and Kent, P. W., *Proc. Roy. Soc.*, B, **144**, 259 (1955).

<sup>7</sup> Brunet, P. C. J., *Quart. J. Micro. Sci.*, **93**, 47 (1952).

<sup>8</sup> Keilin, D., and Mann, T., *Nature*, **143**, 23 (1939).

<sup>9</sup> Dawson, C. R., and Tarpley, W. B., in "The Enzymes", ed. by Sumner, J. B., and Myrback, K., **2**, Pt. 1, 454 (1951).

<sup>10</sup> Mercer, E. H., and Brunet, P. C. J., *J. Biophys. Biochem. Cytol.*, **5**, 257 (1959).

### Infra-red Spectra of Capsular Polysaccharides from Thirteen Strains of *Cryptococcus neoformans*

STUDIES of infra-red absorption spectra of immunopolysaccharides have received little attention until recently. Thus, Levine, Stevenson and Kabler<sup>1</sup> were able to show that the serologically reactive and type specific pneumococcal polysaccharides have distinctive spectra by which they can be identified. Later, Watson, Marinetti and Scherp<sup>2</sup> working with the specific hapten of group C meningococcus were successful in assigning a provisional structure for the hapten by the aid of infra-red spectral analysis. Inasmuch as purified cryptococcal polysaccharides have shown serological reactivity by precipitation