

have revealed the rôle of elasticity of organogels on hysteresis in sorption.

KITTUR SUBBA RAO.

Department of Chemistry,  
Central College,  
Bangalore,  
December 27, 1939.

<sup>1</sup> Bernhart, Earle Arnow and Bratton, *J. Ind. Eng. Chem., Anal. Edn.*, 1937, 9, 387.

<sup>2</sup> Briggs, D. R., *J. Phys. Chem.*, 1934, 38, 867.

<sup>3</sup> McBain, *J. Amer. Chem. Soc.*, 1935, 57, 699.

<sup>4</sup> —, *The Sorption of Gases and Vapours by Solids*, George Routledge and Sons, Ltd., London, 1932, 433.

<sup>5</sup> —and Bakr, *J. Amer. Chem. Soc.*, 1926, 48, 690.

<sup>6</sup> Rao, B. S., *Curr. Sci.*, 1938, 6, 446.

<sup>7</sup> Rao, K. S., *Ibid.*, 1939, 8, 256.

<sup>8</sup> —, *Ibid.*, 1939, 8, 468; 1939, 8, 546.

<sup>9</sup> —, Unpublished.

<sup>10</sup> Thomson, *Phil. Mag.*, 1871, (4), 42, 448.

### The 'Tyrosinase' from *Dolichos lablab*

IN view of the recent work<sup>1-5</sup> on phenolases which has revealed conflicting views regarding the existence of distinctly separate enzymes for the oxidation of the mono- and dihydroxy phenols, it was of interest to purify and elucidate the nature of the "tyrosinase" present in *Dolichos lablab*. The enzyme extract was obtained by an extraction of the dried and powdered seeds with saline (5 per cent. NaCl). On removal of the salt by dialysis, the globulins of the extract were thrown down, which were subsequently filtered off. The resulting clear light brown filtrate containing most of the enzyme was saturated with ammonium sulphate, when the enzyme was completely precipitated. A further purification of this precipitate was effected by fractional precipitations with alcohol and acetone and by adsorption on calcium phosphate gel.

The enzyme preparations oxidise catechol with great ease, while the oxidation of phenol, *p*-cresol, tyrosine, pyrogallol and "dopa" does not proceed with the same vigour. The course of oxidation of these substrates has been followed manometrically by measuring the oxygen uptake in a Warburg. Through a series of

preliminary trials, the conditions for obtaining a measure of the activity of the enzyme preparations, were standardised. The oxygen uptake with phenol and *p*-cresol was found to be linear (after a short initial induction period) and proportional to the concentration of the enzyme. With catechol, as reported by Wagreich and Nelson,<sup>5,6</sup> the oxygen uptake falls off rapidly, presumably due to inactivation of the enzyme. But the secondary oxidation of hydroquinone or ascorbic acid through the aid of the catechol as "carrier" proceeds at a rate which is a measure of the enzyme. The rate of oxygen uptake is proportional to the concentration of carrier within narrow limits (0.01 to 0.03 mgm. in 2 c.c.).

For a comparative study of the activity of the enzyme preparations towards mono- and dihydroxy phenols, the oxygen uptake with ascorbic acid/catechol and with phenol were measured. The oxygen uptake with hydroquinone/catechol and *p*-cresol also follow closely the above. The measurements were made at pH 6.2 on 1 mgm. quantities of substrate in a final volume of 2 c.c.

The following table indicates the effect of a preliminary purification on the activity of the enzyme towards the two different sets of substrates.

It will be observed that the activity towards phenol decreases as compared to catechol, with progressive purification. The induction period with phenol becomes prolonged while the oxidation of catechol begins instantaneously in every case. The addition of minute amounts of catechol (0.02 mgm.) to the phenol practically abolishes the induction period, increases the oxygen uptake, which, however, tends to fall off instead of remaining steady. In the fractional precipitations with alcohol and acetone, no other fractions of the enzyme with a comparatively greater activity towards phenol than catechol, could be obtained. The centrifugate after adsorption was also not more active towards phenol. These results suggest that the activity towards phenol (or *p*-cresol or tyrosine) is a secondary reaction depending on the

No.	Purification stage	Total solids mgm. per c.c.	Activity in mm. min./mgm. enzyme		Ratio (A)/(B)
			Ascorbic acid/ cate- chol (A)	Phenol (B)	
1	Initial extract precipitated with $\text{Am}_2\text{SO}_4$ and precipitate suspended in water and dialysed .. .. .	10	0.25	0.2	1.25
2	(1) Precipitated with alcohol in the cold 30-60 per cent. and precipitate taken up in water .. .. .	6	6.0	5.0	1.20
3	(2) Precipitated with acetone in the cold 33-50 per cent. and precipitate taken up in water .. .. .	2	3.7	2.1	1.8
4	(3) Adsorbed on $\text{Ca}_3(\text{PO}_4)_2$ gel at pH 5.0 and eluted with M/20 $\text{Na}_2\text{HPO}_4$ .. .. .	1.0	11.0	2.5	5.6
5	Further precipitation of (4) with acetone in the cold 33-60 per cent. .. .. .	0.5	20.0	1.6	12.5

presence of a subsidiary factor, in addition to the main portion of the catechol (or diphenol) oxidase. This factor gets eliminated during the purification and is partly replaceable by catechol. Work on the further purification of the enzyme is being continued.

S. L. VENKATISWARAN.

M. SREENIVASAYA.

Department of Biochemistry,

Indian Institute of Science,

Bangalore,

December 20, 1939.

<sup>1</sup> Graubard and Nelson, *Jour. Biol. Chem.*, **111**, 757.

<sup>2</sup> Kubowitz, *Biochem. Zeits.*, **292**, 221.

<sup>3</sup> Califano and Stefani, *Nature*, **142**, 1036.

<sup>4</sup> Graubard, *Enzymologia*, **5**, 332.

<sup>5</sup> Wagreich and Nelson, *J. Amer. Chem. Soc.*, **60**, 1544.

<sup>6</sup> Adams and Nelson, *Ibid.*, **60**, 2474.

### Vascular Anatomy of the Flower of *Macadamia ternifolia* F. Muell. (Proteaceæ)

SINCE the publication of an account of floral anatomy in *Macadamia ternifolia* F. Muell. some time back<sup>1</sup> the writer had occasion to make a more detailed examination of the floral structure in the same plant. Some of the previous interpretations concerning the nature of the perianth traces and the morphology of the nectar-secreting disc at the base of the ovary

are now found to be incorrect in certain respects. The writer is grateful to Prof. Arthur J. Eames, of Cornell University, U.S.A., who, being requested to give an opinion in the matter, very kindly pointed out the discrepancy in the earlier account.

It is stated in the paper cited above that the perianth in the modern Proteaceæ represents the whorl of calyx and that the marginal strands of the perianth segments which arise by forking of four large strands separating from the receptacular stole represent traces to a lost corolla. Such an interpretation was offered on the strength of the remarks by Joshi and Rao<sup>2</sup> in their work on the floral anatomy of some Nyctaginaceæ; these authors state with regard to the two sets of traces to the perianth that one method of interpreting is "that each set of traces belongs to a separate whorl of leaves and formerly in this family there were two whorls of perianth leaves, the traces of the lower set belonging to the sepals and those of the upper to the petals. At present these two sets of traces are running in the same whorl owing to the disappearance of one whorl." This interpretation is inconsistent with the detailed observations now made in *Macadamia ternifolia*. The perianth segments are strictly sepals in nature and their vascular connections are quite normal as in many other angiosperms