

below. Activities represent mg. ascorbic acid oxidised in a catechol-ascorbic acid substrate in 1 hour at room temperature.

Stage of Purification	Activity per g. dry enzyme	Cu content μ g. per g.	Cu per unit activity
1. Acetone prepared enzyme	151	32	0.21
2. Crude extract	330	60	0.18
3. Am_2SO_4 full saturation precipitate	3546	335	0.09
4. After one adsorption on Ca_3PO_4 gel and elution	5000	357	0.07
5. After a second adsorption and elution	10100	800	0.08

Thus in the more active preparations there exists a fair proportionality between activity and copper content, showing thereby that Cu forms the active group of the enzyme.

Further proof of this is furnished by the fact that on dialysis against KCN solution tea oxidase becomes completely inactivated due to removal of the bound Cu.

It is, therefore, concluded that tea oxidase is a metallo-protein with Cu as its prosthetic group. As such it takes its place along with the other polyphenol oxidases whose constitution and mechanism have been fully worked out.

Tea Research Institute of Ceylon,
Talawakelle,
Ceylon,

H. B. SREERANGACHAR.

June 10, 1943.

1. Lamb and Sreerangachar, *Biochem. J.*, 1940, **34**, 1472.
2. Krbowitz, *Biochem. Z.*, 1938, **299**, 32. 3. Keilin and Mann, *Proc. Roy. Soc.*, 1938, **B125**, 187. 4. Bertrand, *Compt. Rend.*, 1895, **121**, 726. 5. Green, *Mechanism of Biological Oxidations*, 1940, p. 11, Cambridge.

PREPARATION OF DIAZOMETHANE

A FAIRLY quick and economical method of preparing diazomethane from acetamide is described.

The preparation of diazomethane by Arndt's method¹ is expensive and the starting substance, methyl urea, is not readily obtainable. An alternative method has been suggested by Adamson and Kenner² which is economical but which involves long and troublesome preparative work.

During work on the synthesis of the ketonic terpene, umbellulone from isovalerianic acid, and of caryophyllanic acid, supplies of diazomethane in quantity were required and a method of preparation from acetamide, based on the work of Brüning³ was found economical and quick.

EXPERIMENTAL

Aqueous sodium hydroxide (10 per cent.) was added slowly to acetamide (100 gms.) and bromine (45 c.c.) with shaking until the solution was permanently pale yellow, first with ice-cooling and then after heating on the water-bath. On cooling, the acetyl methyl urea

(m.p. 179-180° C.) was collected, a further quantity being obtained by concentration of the filtrate (total yield 75 gms.). The acetate was hydrolysed by heating for 3 hours with 8 per cent. hydrochloric acid (200 c.c.), the solution cooled in ice and a saturated solution of sodium nitrite (37 gms.) added with the stem of the tap funnel below the level of the liquid. The nitrosomethylurea (52 gms.) was collected, washed with a small quantity of cold water, and dried in a vacuum. The nitrosomethylurea may be stored in quantity provided it is kept at 0° C., as at ordinary temperature it decomposes slowly.

Diazomethane was then prepared in ether solution by adding aqueous potash and ether to the nitrosomethylurea and distilling from a water-bath at about 60° C.

This work was carried out in the University College of North Wales, Bngor, under the direction of Dr. G. R. Ramage and Prof. J. L. Simonsen.

Inspectorate of Military Explosives,
Kirkee,

M. D. OWEN.

August 14, 1943.

1. *Organic Syntheses*, **15**, p. 48. 2. *J.C.S.*, 1937, p. 1551. 3. *Ber.*, 1888, **21**, 1809; and *Annalen*, 1889, **253**, 6.

A NOTE ON THE ALKALOIDS OF *COSCINIUM FENESTRATUM* (COLEBR.)

THOUGH previous workers^{1,2} had suggested the presence of the alkaloid berberine in the stems, Katti and Shintre,³ in the course of a complete investigation of the stems, noted the probable presence of two alkaloids. They reported that the alcoholic extract of the plant stems contained ceryl alcohol, hentriacontane, sitosterol, palmitic and oleic acids, sitosterol glucoside, saponins, glucose and a large amount of a mixture of alkaloids, together with some resinous material. The melting points of the two alkaloids obtained by them in a pure condition did not correspond with that of berberine recorded in the literature (145° C.).

The present work was undertaken to verify the presence or absence of berberine and of any other alkaloid.

An alcoholic extract of the roots was thoroughly extracted, first with water and then with dilute acetic acid. The insoluble residue did not give any test with alkaloidal reagents. From the aqueous and acetic acid extracts the alkaloids were completely precipitated as nitrate (1.6 per cent.) by adding a solution of potassium nitrate. The filtrates did not show the presence of any other alkaloid in solution. The yellow alkaloidal nitrate was found to contain only berberine from a study of the nitrate, the hydrochloride, the platinichloride and the acetone compound as well as the free base regenerated from the acetone compound. These compounds were compared with the corresponding compounds prepared from pure berberine and found to be identical. The free base also gave the usual colour reactions for berberine.

As a result of the investigation it was proved beyond any doubt that the alkaloidal content of *Cosciniium fenestratum* consists of berberine