

LETTERS TO THE EDITOR

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A SELF-STABILISED HIGH VOLTAGE SOURCE FOR GEIGER COUNTERS

SEVERAL methods* of obtaining stabilised high voltages have been described. In all of them, a separate rectifier and a separate stabiliser, bias batteries, etc., have been used. A self-stabilised high voltage source is being developed and its circuit is shown in Fig. 1.

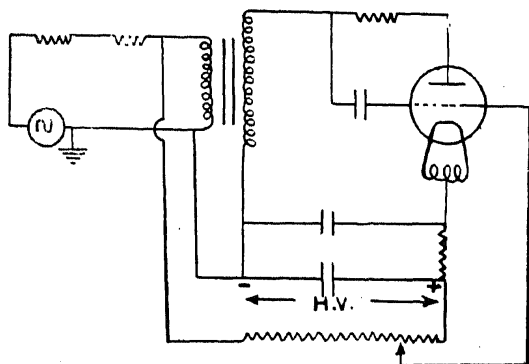


FIG. 1

The circuit is self-explanatory. By introducing an air gap in the central leg of the high voltage transformer, some degree of stability is obtained. By adjusting the bias to the grid of the rectifier, the stability is increased. Feeding back the D.C. output current to the primary further improves the stability of the output voltage. The coupling between the plate and grid by a condenser reduces the ripple to an unnoticeable extent and also improves the stability. The primary circuit contains lamp resistances which reduce surges and regulate the output voltage.

In such a circuit, it is found that a mains voltage variation of 60 volts in a 230 volt supply produces a variation in the output voltage of less than 10 volts. An output current change of ten micro-amperes produces no perceptible change in the output voltage. In this circuit, a power tube of the receiving type is used and as such cannot be operated with a high current drain at voltages above 1,000 volts. Consequently the polarisation of the core by D.C. flow has to be reduced considerably at such voltages. Even if this is done, the worst instability obtained corresponds to one volt in the output per one volt change of the mains

voltage. A complete description of its operation will be published in due course.

Cosmic Ray Research Unit,
Indian Institute of Science,
Bangalore, S. V. CHANDRASHEKHAR AIYA.
August 16, 1943.

* Webster, *Proc. Camb. Phil. Soc.*, 1931, 28, 121. Street and Johnson, *J. Frankl. Inst.*, 1932, 214, 155. Richards, *Rev. Sc. Inst.*, 1933, 4, 479. Evans, *Ibid.*, 1934, 5, 371. Ginrich, *Ibid.*, 1936, 7, 207. Ashworth and Muzon, *Ibid.*, 1937, 8, 127. Neher and Pickering, *Ibid.*, 1939, 10, 53. Hartman, *Electronics*, 1932, 6, 43.

THE TEA POLYPHENOL OXIDASE—ITS MATERIAL NATURE

IN previous studies¹ the tea oxidising enzyme was characterised as a polyphenol oxidase, mainly by reason of its substrate specificity. The question then arose whether tea oxidase was also analogous in its material and chemical nature to such other polyphenol oxidases^{2,3} which are known to be copper-protein compounds.

Copper has been detected in all preparations of tea enzyme. But the crude preparations were found also to contain manganese and iron, both associated with other types of oxidising enzymes.^{4,5} On purification, however, iron and manganese were completely eliminated from the enzyme while the preparations got enriched in their copper content.

The purification of enzyme was effected as follows:—The acetone preparation of the enzyme was extracted with Sorenson's glycine buffer at pH 10.1, and after adjusting the pH of extract to 6.0 the enzyme was precipitated by a fractional saturation with Am_2SO_4 . The precipitate obtained between half and full saturation was collected, dispersed in water and dialysed. Further purification consisted in an adsorption on freshly prepared calcium orthophosphate gel and subsequent elution. By this method tea oxidase has been purified to a concentration of at least 800 times that present in fresh leaf.

Some typical results for the activities and the copper contents of the preparations during the different stages of purification are given

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