

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.

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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.

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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

only. The alkaloidal crystals obtained by Katti and Shintre, and also obtained by us by following their method, were not free berberine but salts of berberine and, therefore, naturally did not give the melting point of pure berberine. Presumably they were salts of two different acids, as they obtained crystals with two different melting points. It was not thought to be of sufficient importance to isolate and identify these acids.

EXPERIMENTAL

The drug was purchased from a local dealer and identified in the botanical department of the University College.

The powdered air-dried stem (180 gms.) was defatted with petrol and soxhleted with alcohol. From the alcoholic extract (9.2 gms.) warm water dissolved 6.2 gms. From the insoluble residue, the remaining alkaloids were dissolved out with warm 4 per cent. acetic acid and precipitated as nitrate with strong potassium nitrate solution (A).

The aqueous extract, on concentration and cooling, gave a crystalline alkaloidal material (.8 gm.; B), which was also converted to the nitrate. The aqueous filtrate, also gave a nitrate (2.6 gms.; C).

BERBERINE-ACETONE COMPOUND

0.1 gm. of the nitrate was dissolved in water (10 c.c.) and mixed with 2 c.c. of 10 per cent. aqueous sodium hydroxide, heated to 50° C. mixed with 5 c.c. of acetone and set aside. A lemon yellow powder separated. Melting point 167-169° C. (decomp.).

BERBERINE REGENERATED FROM ACETONE COMPOUND

The free base was liberated from the acetone compound by boiling 0.2 gm. of it with alcohol on a water-bath. The alcohol was driven off and the residue recrystallised from water. It melted at 145° C. both alone and after admixture with a sample of pure berberine.

The authors thank Dr. K. L. Moudgill, Director of Research, for his interest in this work.

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June 11, 1943.

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CATALYSIS OF VANADATE-HYDRIODIC ACID REACTION BY THE OXALATE ION

In a previous publication¹ we reported the catalysis of the reaction between dichromate and hydriodic acid by the oxalate ion. We have carried out a survey of numerous reactions involving the oxidation of hydriodic acid by such substances as hydrogen peroxide, potassium persulphate, sodium arsenate, potassium chlorate, potassium bromate, and potassium iodate to ascertain the possible catalytic effect of oxalate ion. No catalytic effect was observed in these cases. It was, however, found that the oxalate ion has a profound accelerating action on the reaction between vanadic acid and hydriodic acid.

The reaction was followed by titration of the iodine liberated with sodium thiosulphate. The

concentration of sodium vanadate was varied from 0.025 N to 0.00025 N and that of sodium oxalate from 0.225 N to 0.0005 N. The reaction was studied in the presence of air, in vacuum, and in an atmosphere of carbon dioxide.

In seeking an explanation for the mechanism of the catalytic action of oxalate ion on these reactions, we have to take into account the numerous resemblances between chromates and vanadates. Both chromic acid and vanadic acid form poly-acids, and, possibly, complexes with oxalic acid. It seems, therefore, that in the reaction between chromate and hydriodic acid, the oxalate catalysis is more concerned with the chromate than with the hydriodic acid. This idea received further support from our recent observation² that the reaction between dichromate and hydrobromic acid is also catalysed by oxalate.

Full details will be published elsewhere.

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July 6, 1943. G. GOPALA RAO.

1. Viswanadham, C. R., and Gopala Rao, G., *Curr. Sci.*, March 1942, **11**, No. 3, pp. 102-103. 2. *Ibid.*, June 1943, **12**, No. 6.

A NEW STEM-BASE DISEASE OF *ALTISSIMA* CAUSED BY A SPECIES OF *PHYTOPHTHORA*

For the first time during the year 1930 *Phytophthora* was reported¹ by this section to cause diseased lesions on the stem of *altissima* (*Hibiscus sabdariffa* Lin. var. *altissima*). *Altissima* supplies the Roselle Hemp of Commerce, and is noted for its good silky fibres, much stronger than jute and can be used in some proportion in the manufacture of ropes, cordage, etc.

The disease as observed since 1930 is characterised by the production of discoloured patches on the stem. If the stem is still green, the patch appears at first as a water-soaked, slightly yellow patch at the base of the stem; with time the lesion enlarges, darkens and becomes brown in colour and the infected tissues (the excambial layers) dry up resulting in shreds and cracks and thereby exposing the pith inside. Ultimately the leaves begin to wilt and the plants gradually dry up and die prematurely. In case they do not completely succumb, the fibres at the infected regions are damaged thereby depreciating greatly the quality of the yield.

The first infection usually takes place on the lower portion of the stem and more often near about the collar region; but the production of these lesions are confined within 2 to 3 feet from the ground level. The number of lesions in any single plant varies from a few to half a dozen and the size of the individual lesions from half an inch to many inches in length and may partially or completely girdle the stem. One or more lesions may coalesce together to form diseased surface of considerable length. If rain or very humid conditions prevail for a number of days gums are sometimes seen exuding from old and large lesions; such conditions also favour the growth of fungus mycelium from the margins of these spots.

The plant may be attacked at any stage of