A SIMPLE PHOTOMICROGRAPHIC APPARATUS FOR OBTAINING DEPTH OR FOCUS

THE generally prevailing use of the camera lucida as a means of illustrating Ostracoda, Foraminifera, insects and kindred small sub-spherical bodies evidences a difficulty commonly met with in achieving satisfactory photographic realization in combination with magnification. This is essentially due to the inability of the microscope's objective to provide depth of focus. The apparatus here described was constructed from the suggestions of Professor Albert Johannsen, of the University of Chicago. It furnished a magnification of from 12 to 28 diameters, depending upon the lens used, and was employed with excellent results in picturing the heads of flies and the wings and limbs of small insects. It proved satisfactory for the photographing of Ostracoda large enough to be retained by a forty-mesh screen, and offers the possibility of photographing even smaller ones.

The bellows of a good 5″ by 7″ view camera was extended by attaching to it an elongated wooden box, made by nailing together four boards, each eight inches wide by ten feet long. Cardboard diaphragms, properly sized, and containing circular openings four inches in diameter, were inserted into the box at distances of one third and two thirds of its length, respectively, to cut off any light reflected from its sides. The ground-glass plate and the plate-holder of the camera were then mounted in a cardboard support and fastened to one end of the tube, the seam being made light-proof by wrapping securely with black cloth. In a similar manner the bellows and lens of the camera were attached to the opposite end of the tube, and the whole apparatus firmly mounted upon a long table. Focusing was accomplished by means of the rack and pinion of the view camera. It required considerable care and precision, since very slight alterations of the distance between the lens and the object produce great changes in the focus on the ground-glass. Powerful illumination is essential, and two 250 candle-power bulbs, placed about eight inches from the object, were employed for this purpose. Under these conditions the time of exposure was between five and six minutes.

Since the size of the image obtained is a function of the length of the bellows extension, considerably higher magnification may be achieved by increasing the length of the wooden tube.

BRANDON H. GROVE

DEPARTMENT OF GEOLOGY,
THE UNIVERSITY OF CHICAGO

SPECIAL ARTICLES

CHEMICAL STUDIES ON TOAD POISONS, IV, BUFAGIN AND CINOBUFAGIN

DURING the past three years Dr. K. K. Chen and I have been studying toad poisons from both a chemical and pharmacological point of view. Due to the difficulty of obtaining somewhat large amounts of material the work has been rather slow. The isolation of the various principles from Ch'an Su, the dried venom of the Chinese toad, and from *Bufo marinus* has been reported[1]. A thorough pharmacological study of cinobufagin, isolated from Ch'an Su and of Abel's bufagin, isolated from *Bufo marinus*, undertaken by Dr. Chen, revealed that their physiological action is qualitatively about the same as that of the cardiac plant glucosides, but that there is a quantitative difference. The results of the pharmacological study of the principles isolated from Ch'an Su have been published[2]. These pharmacological findings make it appear very probable that the principles of toad poisons are chemically also very closely related to the cardiac aglucones of the plant kingdom. The reported formulae for cinobufagin $C_{29}H_{38}O_7$ (1c) and for Abel's bufagin $C_{28}H_{36}O_6$ (1d), however, do not indicate any close chemical relationship to the plant cardiac aglucones, which are C_{23} derivatives, as has been shown by Jacobs and coworkers[3] and by Windaus and coworkers[4]. Further investigation, and especially a careful analytical study of various derivatives of cinobufagin and bufagin, have led to the revision of the above formulae and the following ones are proposed, cinobufagin $C_{25}H_{32}O_6$ and bufagin $C_{24}H_{32}O_5$. The published analytical data check very well with these revised empirical formulae.

Cinobufagin, $C_{25}H_{32}O_6$, when treated with alkali, gives rise to a hydroxy-carboxylic acid with the opening of a lactone ring and the splitting off of acetic acid. On acetylation one acetyl group is introduced into the molecule. Under the influence of hydrochloric acid water and acetic acid are split off. On oxidation with chromic acid a mono-ketone is formed. From the foregoing one can conclude that the molecule of cinobufagin contains a lactone, an acetoxyl, a secondary hydroxy and a tertiary hydroxy group, thus accounting for the six oxygen atoms in the molecule. On catalytic reduction tetrahydrocinobufagin is

[1] (a) K. K. Chen and H. Jensen, *Proc. Soc. Exper. Biol. and Med.*, xxvi, 378, 1928–1929; (b) H. Jensen and K. K. Chen, *Jour. Biol. Chem.*, 82, 397, 1929; (c) *ibid.*, 87, 741, 1930; (d) *ibid.*, 87, 755, 1930.

[2] K. K. Chen, H. Jensen and A. Ling Chen, *J. Pharmacol. and Exper. Therap.*, 43, 13, 1931.

[3] W. A. Jacobs and E. L. Gustus, *Jour. Biol. Chem.*, 78, 573, 1928.

[4] A. Windaus, K. Westphal and Y. Stein, *Ber. d. deutsch. Chem. Gesell.*, 61, 1847, 1928; A. Windaus and Y. Stein, *ibid.*, 61, 2436, 1928.

formed, thus indicating the unsaturated nature of cinobufagin.

Bufagin, $C_{24}H_{32}O_5$, when treated with alkali, forms a hydroxy-carboxylic acid with the opening of a lactone ring and splitting off of formic acid. On acetylation one acetyl group is introduced. Under the influence of hydrochloric acid formic acid and one molecule of water are split off. Several attempts to secure a ketone by oxidation with chromic acid have failed. From these findings one may conclude that the molecule of bufagin contains a lactone, a formoyl and a tertiary hydroxy group, thus accounting for the five oxygen atoms in the molecule. On catalytic reduction tetrahydrobufagin is formed, indicating that bufagin is also of unsaturated nature. Catalytic reduction of cinobufagin and bufagin also gives by-products of acid character, probably formed by the opening of the lactone ring.

From the foregoing one can see that the chemical behavior of cinobufagin and of bufagin is quite similar to that of the plant cardiac aglucones. By splitting off the acid radical which is attached to a hydroxy group (acetic acid from cinobufagin and formic acid from bufagin), one obtains compounds which are C_{23} derivatives, as are the aglucones of the plant glucosides.

$$\underset{\text{Cinobufagin}}{C_{25}H_{32}O_6} \xrightarrow{+H_2O} C_{23}H_{30}O_5 + CH_3COOH$$

$$\underset{\text{Bufagin}}{C_{24}H_{32}O_5} \xrightarrow{+H_2O} C_{23}H_{32}O_4 + HCOOH$$

While the cardiac poisons of the plant kingdom are combined with carbohydrates these principles of toad poisons are coupled with acetic acid or formic acid, or, as in the case of the nitrogen containing principles, which also have a cardiac action, with suberylarginine and an acid radical.

It has already been mentioned that bufagin under the influence of hydrochloric acid will lose formic acid and one molecule of water.

$$\underset{\text{Bufagin}}{C_{24}H_{32}O_5} \xrightarrow{HCl} \underset{\text{Bufagien}}{C_{23}H_{30}O_3} + HCOOH + H_2O$$

Bufagin is a mono-hydroxy lactone containing three double bonds and should give on catalytic reduction a compound of the composition $C_{23}H_{36}O_3$. It is hoped that the latter substance may be identical with one of the known reduced anhydro compounds of the cardiac aglucones of the plant glucosides[5]. If this should be true it would be a direct proof of the chemical relationship between these two groups of natural compounds.

[5] See reference 4; W. A. Jacobs and A. M. Collins, *Jour. Biol. Chem.*, 63, 123, 1925; W. A. Jacobs, R. C. Elderfield, A. Hoffman and Th. B. Grave, *J. Biol. Chem.*, 93, 127, 1931.

This reaction is now being carried out and attempts are also being made to convert cinobufagin by similar steps into the corresponding compound. The results so far obtained and those which are being accumulated will be published later in greater detail.

Another method of approach to show the possible chemical relationship between the cardiac principles of toad poisons and those of the plants may be as follows: Bufagin and cinobufagin are converted with alkali into the corresponding hydroxy-acids and these are then catalytically reduced, giving C_{23} derivatives, which may be identical with compounds obtained from the plant aglucones in a similar manner. This method is being investigated.

The author wishes to express his thanks to Dr. K. K. Chen of the Lilly Research Laboratories, Indianapolis, for the supply of Ch'an Su and the secretion of *Bufo marinus*. The author is also indebted to Mr. Hutchison of the Antivenin Institute, Glenolden, Pennsylvania, for an additional supply of the secretion of *Bufo marinus*. The research has also been aided in part by a grant from the Committee on Scientific Research of the American Medical Association.

H. JENSEN

THE JOHNS HOPKINS
MEDICAL SCHOOL

CRYOLYSIS OF LYOPHILIC COLLOIDS, AND ITS BEARING ON THE MECHANISM OF ENZYME ACTION

IN a recent publication[1] evidence has been furnished that the surface tension of a solution containing lyophilic biocolloids is increased by ethylene as well as by acetylene. At the same time the observation was made that the viscosity of the same solutions was decreased. These observations were interpreted on the basis that ethylene or acetylene are in a state of physical adsorption on the surface of the lyophilic colloids. The gases exert accordingly a protector action on the combined carrier-enzyme surface, the latter being increased when submitted for shorter or longer time to the influence of freezing.[2] In order to obtain a detailed information of the latter observation solutions of different concentration of egg albumin, gelatin, gum arabic and sodium oleate were investigated by means of cryolysis. The measurements of the surface tension of all the solutions in all concentrations, having been frozen once or repeatedly at different temperatures, furnished a decided increase. The viscosity, however, was decidedly increased solely in the case of egg albumin, decreased when using solutions of gelatin or gum arabic and immaterially influenced in the case of sodium oleate solutions. The electrical conductivity

[1] F. F. Nord, *Trans. Faraday Soc.*, 26: 760. 1930.
[2] F. F. Nord, *Nature*, 120: 82. 1927.