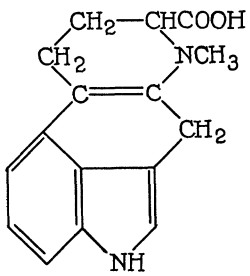
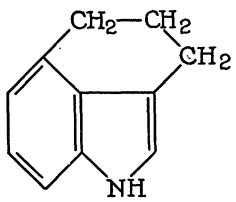


I



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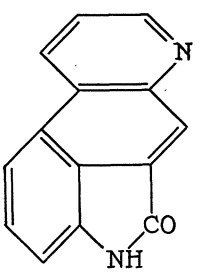
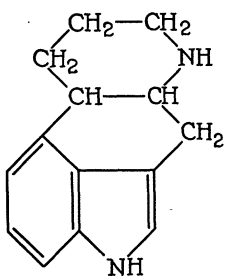


III



IV

itself has more recently been achieved as follows. 3-amino-1-naphthoic acid[2] by the Skraup reaction has given the corresponding β-naphthoquinoline carbonic acid which on nitration yielded a nitro-β-naphthoquinoline carbonic acid. The position occupied by the nitro group became evident after its reduction to the amino group, since lactamization then readily occurred with production of the substance given in formula III. In preliminary experiments, reduction of the latter with sodium and butyl alcohol yielded a mixture containing a substance apparently with the structure given in formula IV, since this mixture gave color reactions closely approaching those which are so characteristic of lysergic acid and its derivatives.

There is now in progress in this laboratory a logical extension of this work to include other substances related to lysergic acid and eventually to lysergic acid itself.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

AN AUTOMATIC DEHYDRATING DEVICE

A SIMPLE and satisfactory apparatus whereby histological and cytological tissues may be dehydrated gradually has been devised, thus preventing any shrinkage which may be due to improper upgrading in the alcohol series. By this method tissues for paraffin impregnation may be run up in twelve hours to three days, the length of time depending upon the rate of flow from the siphon.

Apparatus used: A 500 cc flask (A) filled with absolute alcohol is fitted with a one-holed rubber stopper to which is connected a piece of glass tubing about eight or nine inches long, the unattached end having a 15° bevel. The flask is placed upside down and held in place by a clamp on an iron stand (B).

Some distance below the flask, a stender (C) filled with water and containing the tissues is placed. The flask is lowered until the bottom of the bevel of the glass tube extends 1/16 inch below the water level in the stender. Leading from the stender, a piece of capillary tubing, bent at a 50° angle 1½ inches from one end and at the other end drawn to half the original bore, is held in place by a piece of plastic clay moulded over the edges of the stender (D). If the long arm of the siphon is placed in almost a vertical position, the liquid will drain off the tissues at a very rapid rate, but the greater the tendency toward a horizontal position, the more noticeable will be the decrease in the number of drops siphoned per minute. However, the siphon will not work unless the overflow end is placed on a lower level than the suction end, and the suction end must be placed near the bottom of the stender.

As the siphon drains the liquid off the tissues, the level of the mixture of alcohol and water falls below

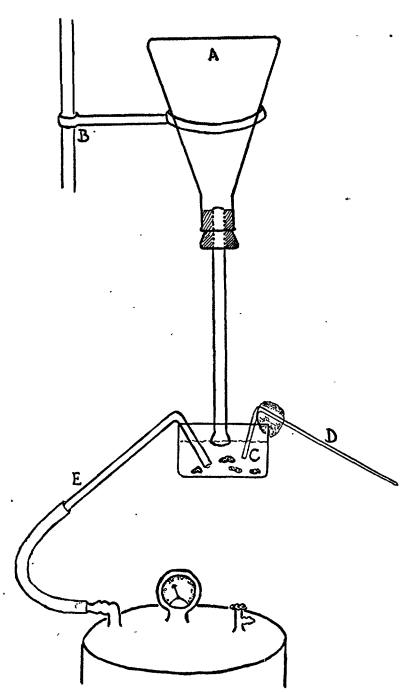

FIG. 1

[2] G. J. Leuck, R. P. Perkins and F. C. Whitmore, *Jour. Am. Chem. Soc.*, 51: 1834, 1929.

the lower edge of the bevel, which causes air to be drawn back into the flask and more alcohol is then introduced into the stender from the flask; when the level of the liquid in the stender reaches the level of the bevel, the flask of alcohol will no longer empty itself.

A provision has been made to insure the proper mixing of the alcohol and water in the stender. An L-shaped piece of glass tubing is connected to a rubber hose which runs to any source of compressed air (E). In the apparatus shown above, an old gas tank, provided with a pressure gauge, inlet and outlet stopcocks, was filled with air by an ordinary automobile air pump, so as to yield a pressure of 20 to 25 pounds. The rubber tube was attached to the outlet and the stopcock was regulated so that there would be 50 to 75 bubbles of air per minute when placed in water. Care must be taken to place the outlet of the L-shaped tube near the bottom of the stender; otherwise the alcohol and water will not mix properly.

If the siphon is regulated at eight drops per minute, the tissue (if placed in 20 cc of water to begin with) will be in approximately 97 per cent. alcohol after twenty-four hours, and is then ready to be transferred into absolute alcohol for 3 to 6 hours, before being placed in the clearing agent.

The apparatus is effective and easy to construct. One should be available on each table of the technique laboratory.

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THE ISOLATION OF MUSCLE NUCLEI

As a matter of cytological interest and as a means of freeing the nuclei from the cytoplasm for chemical analysis of their constituents, a method has been devised for the isolation of the nuclei of smooth, striated and cardiac muscle. The following procedure yields a permanently stained preparation suitable for cytological study. Cardiac muscle is suggested as an initial preparation.

(1) Smear a slide with Mayer's egg albumen according to the usual method.

(2) Place a drop of 5 per cent. citric acid in the center of the slide.

(3) Place a small piece of fresh muscle in the drop. Gradually the tissue is infiltrated and assumes a translucent state. The citric acid becomes cloudy, due to the released nuclei. Gentle teasing will hasten the reaction. If the preparation is examined microscopically at this time, one will note large numbers of nuclei streaming from the muscle substance.

(4) Remove the muscle from the drop with forceps. The resulting preparation will contain nuclei free of cytoplasm.

(5) Allow the preparation to dry almost completely. The slide is then placed in 95 per cent. ethyl alcohol, which coagulates the albumen, thus holding the nuclei in place.

(6) Rinse in several changes of tap water, followed by distilled water.

(7) Transfer to Mayer's hemalum until the nuclei are stained.

(8) Wash in tap water until blue.

(9) Counterstain in eosin.

(10) Dehydrate, clear and mount.

The technique is comparatively simple. The only possible cause of failure is the washing off of the preparation in step five. Perhaps a repeated attempt may be necessary for determination of the optimum time for drying the slide before placing in the 95 per cent. alcohol.

The resulting preparation shows an abundance of nuclei stained blue on a quite homogeneous eosin-stained background. The latter is presumed to consist of egg albumen and any muscle fraction soluble in the citric acid. Although the technique is applicable to a number of other body tissues, somewhat inferior results are obtained.

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