

normal transmitting area, and the more distant portions of the clear field being of an intermediate shade. The substage 'field' portion must always be less bright visually than the transmitting area, and a valuable method of varying the intensity of the image contrast is to mount portions of 'Polaroid' over the field areas of this plate, and decrease or increase the intensity by rotating a 'Polaroid' screen in an understage filter carrier. In this case, as the crossed position was approached, the image assumed the characteristics of normal monochromatic phase-contrast, excepting that the field of view in the microscope took the colour of the transmitting portion. Further useful contrast effects may be obtained by placing an appropriate filter over the ocular of the microscope.

This method has been used with success on difficult sections of neurological material prepared by the Feulgen Light-Green technique<sup>2</sup> mounted in fluid or 'Euparal', and it greatly facilitates the examination of all faintly stained or coloured slides when suitably mounted. Contrast is obtained by virtue of the slight colour differences of fine detail not revealed by normal phase-contrast or ordinary light filters. The examination of insect parts is facilitated, and many applications will no doubt arise in the normal course of research.

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### Preparation of Acetobrome-Sugars

THE preparation of acetobrome-sugars involves generally the use of hydrogen bromide dissolved in glacial acetic acid or acetic anhydride. These solutions are very disagreeable to handle and to prepare; we therefore tried to circumvent their use, and after some preliminary experiments found a really satisfactory way of doing so.

We found that perchloric acid is a very efficient catalyst in acetylation<sup>1-4</sup>, as described by several authors. We did not find it necessary, however, to use a mixture of acetic anhydride and glacial acetic acid; the method works perfectly with acetic anhydride alone. The essential feature of our procedure is to generate hydrogen bromide within the reaction mixture. To do this we either added phosphorus tribromide and water to the mixture, or simply added phosphorus, bromine and water one after the other. The minimal amounts and reaction times necessary to accomplish the reaction were determined in a series of experiments.

We prepare acetobrome-glucose as follows: 400 ml. acetic anhydride is mixed with 2.4 ml. perchloric acid, and 100 gm. glucose is added in the course of half an hour, taking care that the temperature should not rise unnecessarily above 40° C. (to avoid caramelization) or fall below 30° C. (to keep up a steady rate of reaction). 30 gm. amorphous phosphorus is added and the vessel is cooled in ice or an ice-salt mixture. We then add 180 gm. bromine gradually

so as to keep the temperature below 20° C., and proceed directly to add 90 per cent of the stoichiometric amount of water, that is, 36 ml., carefully avoiding local rises of the internal temperature. This latter process takes about half an hour. The vessel is closed by a stopper and remains at room temperature for 1½–2 hr. Then 300 ml. chloroform is added and the mixture is poured into about 800 ml. ice-water and separated in a funnel. The chloroform layer occludes the rest of the phosphorus, and it is convenient to separate it at this stage by filtration. The filtered chloroform is extracted twice with an equal volume of ice water, taking care to extract the separated water twice with 30 ml. chloroform before discarding it. A final extraction with sodium bicarbonate solution in the separating funnel binds the last traces of acid and brings the  $\gamma_{\text{H}}$  to about 6. The yellow solution is dried with calcium chloride, adding a small amount of sodium bicarbonate, calcium carbonate or magnesium carbonate and about 5 gm. active charcoal with slight agitation. After half an hour the solution is filtered, evaporated *in vacuo* from a water-bath at 60° C. and dissolved in its own volume of dry ether, from which it crystallizes on cooling. Addition of double the volume of petrol-ether assures an improved yield. The product melts at 84° C. and is practically pure acetobrome-glucose. It may be used directly for further synthesis. Yield: 85 per cent; recrystallization from ether gives a melting point of 87° C.

We prepared the acetobrome derivatives of galactose, arabinose, lactose, cellobiose and maltose by the same method. It was necessary to use some glacial acetic acid for the acetylation of cellobiose, due to its poor solubility; and we only succeeded in obtaining a crystalline product from galactose when we dissolved the syrup in absolute ether. The syrup of brom-acetomaltose turned into a white powder on addition of ligroin (b.p. 80–100° C.) with the somewhat uncertain melting point of 78° C. instead of 84° C., but contained the theoretical percentage of bromine. Rhamnose, a sugar that is not easily acetobrominated except with titanium tetrabromide, yielded a syrup which we were not able to crystallize. Yields before recrystallizing were: lactose 85 per cent, arabinose 50 per cent, maltose 60 per cent, cellobiose 72 per cent, galactose 75 per cent.

The whole procedure is easily finished in a working day of eight hours, exclusive of recrystallization. The products are very pure and may be kept in a vacuum desiccator for weeks without decomposition. We think that the above method may be extended to similar compounds and represents the simplest way of obtaining them.

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