to enhance the natural quenching of these compounds under ultra-violet light.

The paper ionophoresis and chromatography techniques of Runeckles and Krotkov[1] are ideally suited for this dip procedure since volatile buffers and solvents are used. The presence of inorganic salts in the developing solvents for the papers causes rather severe interference with spot detection. Interference from compounds such as trichloracetic acid, however, can be minimized by washing the papers with absolute ethanol or other suitable solvents prior to the quinine sulphate dip.

The dip procedure has worked well with a variety of papers including Whatman No. 1 and Schleicher and Schuell No. 507. In most cases, prior washing of the paper has been found beneficial. With some papers, however, washing has not always been necessary if the solvent front is sufficiently removed from the spots under study.

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[1] Runeckles, V. C., and Krotkov, G., *Arch. Biochem. Biophys.*, **70**, 442 (1957).
[2] Wade, H. E., and Morgan, D. M., *Nature*, **171**, 529 (1953).
[3] Pfennig, N., *Naturwiss.*, **44**, 537 (1957).

Isolation of Kojibiose from Honey

Previously, we reported[1] that fifteen honey samples produced mainly in the Tohoku region of Japan were analysed, and sugar components of one kind of honey (*Lespedeza bicolor*) were fractionated by chromatography, using a carbon : 'Celite' column, and the sugars in each fraction were estimated. Glucose, fructose, sucrose, maltose, melezitose, eight di- or tri-saccharides and nine higher oligosaccharides were detected by paper chromatography, and fifteen spots of them contained ketose.

On the other hand, it has been reported that Goldschmidt *et al.*[2] detected *iso*maltose in honey by paper chromatography and White *et al.*[3] isolated nigerose from honey.

We now report the isolation of kojibiose. Honey (*Lespedeza bicolor*, 300 gm.) was fractionated with a carbon : 'Celite' column (550 mm. × 125 mm.) using water (22 l.), and ethanol, 2·5 per cent (22 l.), 5 per cent (14 l.), 10 per cent (18 l.), 15 per cent (18 l.), 20 per cent (14 l.), 25 per cent (12 l.) and 30 per cent (30 l.) as successive elution solvents. Kojibiose was detected in 2·5–5 per cent ethanol effluent by paper chromatography. The effluent portions containing kojibiose from three columns (900 gm. honey) were combined (16·5 gm. sugar mixture). These portions were contaminated with a considerable amount of *iso*maltose, two oligosaccharides containing ketose, glucose, fructose, sucrose and a trace of leucrose.

The sugar mixture was then rechromatographed on a carbon : 'Celite' column (530 mm. × 80 mm.) using the gradient elution method with 0–3·0 per cent aqueous ethanol containing borate buffer (*p*H 10·0)[4]. Substantially perfect separation of kojibiose from other sugars was achieved. The water fractions contained monosaccharides, and the 0·5–1·5 per cent ethanol fractions contained *iso*maltose and several sugars containing ketose. Kojibiose appeared in the 2·0–3·0 per cent ethanol fractions.

By the removal of borate from the sugar complex by repeated distillation with methanol, 1·3 gm. of chromatographically pure kojibiose was obtained. Since direct crystallization of the sugar acetylated in the usual way was not successful, the benzene solution of the crude acetate was poured on a column (300 mm. × 40 mm.) of 'Magnesol' : 'Celite' (5 : 1) and developed with 1,000 ml. of benzene/*t*-butanol (100 : 1 by vol.). A zone appeared at 165–215 mm. from the top of the column, which, by means of potassium permanganate streak indicator, was sectioned from the column and eluted with acetone. Removal of the solvent left 0·5 gm. of syrup, from which 208 mgm. of fine prisms were obtained upon crystallization from ethanol. It had m.p. 117–118° C., $[\alpha]_D$ + 113·8° (*c.* 1·2 in chloroform).

The upper portion (10–165 mm.) of the column was eluted with acetone, and removal of the solvent left 0·7 gm. of syrup. This syrup was rechromatographed on the 'Magnesol' : 'Celite' column. From the zone at the bottom of the column, 0·1 gm. of syrup was obtained which upon crystallization from ethanol gave 11 mgm. of fine prisms. It had an m.p. of 166° C.

Neither acetate showed depression of melting point on admixture with α- and β-kojibiose octa-acetates (166° and 117° C.) synthesized chemically by Matsuda[5].

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[1] Aso, K., Watanabe, T., and Yamao, K., *Hakko-kogaku Zasshi* (Japan), **36**, 39 (1958).
[2] Goldschmidt, S., and Burkert, H., *Hoppe-Seyler's Z.* (Physiol. Chem.), **300**, 188 (1955).
[3] White, jun., J. W., Eddy, C. R., Petty, J., and Hoban, N., *Anal. Chem.*, **30**, 506 (1958).
[4] Barker, S. A., Bourne, E. J., and Theander, O., *J. Chem. Soc.*, 4276 (1955).
[5] Matsuda, K., *Nature*, **180**, 985 (1957).

Purification of Bacterial Neuraminidase (Receptor-destroying Enzyme)

Neuraminidase is the name now given to an enzyme the action of which is the hydrolytic cleavage of the glycosidic bond joining the keto group of N-acetylneuraminic acid to D-galactose or D-galactosamine[1]. The enzyme was discovered in 1946 when Burnet, McCrea and Stone[2] found that filtrates of *V. cholerae* rendered human cells inagglutinable by influenza viruses. The factor responsible for this action was called the receptor-destroying enzyme by Burnet and Stone, who obtained some purification of the enzyme present in *V. cholerae* (strain 4 *Z*) filtrates by adsorption to and elution from red cells[3]. This technique was later incorporated into a three-step procedure for the partial purification of the enzyme[4]. Crude or semi-purified receptor-destroying enzyme has since been used as a standard reagent in influenza virus studies: further work in this field, together with increasing interest in the biological and chemical properties of compounds containing