filtrate with MnO_2 yields an apparent retention of 80 per cent instead of the value of 8 per cent which is observed if the first MnO_2 treatment is omitted. The same result is obtained following the addition of Mn_2O_3 or $Mn(ClO_4)_2$. In the absence of water these substances thus cause the conversion of most of the active species to a form which is not made removable by subsequent addition of water and shaking with MnO_2. The high apparent retention of 80 per cent is not due to conversion of the active species to permanganate since the retention as permanganate, determined after dilution and precipitation of silver permanganate, is 8 per cent. The increase in permanganate retention which was observed when the diluted solution was treated with barium hydroxide suggests that the remaining 72 per cent of the activity was present as colloidal MnO_2, not easily removable by shaking with solid MnO_2.

In conclusion, it has not been demonstrated that the active species in dry pyridine solution is similar in its reactions to the active species in the solid. After addition of water to the pyridine solution, the active species appears to be present as manganese dioxide which, under some conditions, forms a colloidal dispersion in the pyridine-water solution.

We are grateful to the National Research Council of Canada for generous financial assistance.

J. R. Bolton
K. J. McCallum

Department of Chemistry,
University of Saskatchewan,
Saskatoon, Saskatchewan.
Aug. 27.

[1] McCallum, K. J., and Maddock, A. G., *Trans. Faraday Soc.*, **49**, 1150 (1953).
[2] Libby, W. F., *J. Amer. Chem. Soc.*, **62**, 1930 (1940).

N-Methylation of Methyl 2-Acetamido-2-Deoxy-α-D-Glucopyranoside

Type XIV *Pneumococcus* polysaccharide, which contains *N*-acetyl glucosamine, glucose and galactose residues[1], has been methylated using dimethyl sulphate and sodium hydroxide (Haworth method[2]) to yield a methyl ether (I) which on hydrolysis gave 3-*O*-methyl D-glucosamine as the only methylated hexosamine.

By contrast, hydrolysis of a methyl ether (II) obtained from Type XIV *Pneumococcus* polysaccharide using sodium and methyl iodide in liquid ammonia (Freudenberg method[3]) gave as the only methylated amino-sugar a compound having the properties expected of 3-*O*-methyl-2-methylamino-2-deoxy-D-glucose. Moreover, the infra-red spectrum of II differed from that of I in that only the latter showed absorption at 1540 cm.$^{-1}$ attributable to the N-H deformation of an acetamido group[4].

These findings, together with the lack of mobility of II in acetate buffer *p*H 5 and its failure to give a colour with ninhydrin indicated that II contained *N*-methyl acetamido groups. This novel feature of the Freudenberg methylation procedure[3] has now been confirmed using methyl 2-acetamido-2-deoxy-α-D-glucopyranoside as a model compound.

D-Glucosamine hydrochloride (5 gm.) in 10 per cent aqueous methanol (110 ml.) was stirred for 90 min. at 0–5° with 'Amberlite *IRA*-400' (CO_3^{--}, 140 ml.) and acetic anhydride (3 ml.)[5]. The filtrate was passed through a column of 'Amberlite *IR*-120' (H^+, 25 ml.) and concentrated. The product (4·47 gm.) was refluxed with dry methanol (150 ml.) and 'Amberlite *IR*-120' (H^+ form dried over phosphorus pentoxide at 80°, 4·47 gm.) for 3 hr.[6]. Concentration of the filtrate yielded methyl-2-acetamido-2-deoxy-α-D-glucoside (3·82 gm.; m.p. 184°). This model compound was submitted to one methylation[3] involving four alternate additions of sodium (total, 12 gm.) and methyl iodide (total, 36 ml.) in liquid ammonia (300 ml.). After removal of ammonia, an aqueous solution of the product was extracted with water-washed chloroform and the extracts concentrated to a yellow syrup. Most of the syrup (2·2 gm.) distilled at 128°/0·11 mm., had $n^{18\cdot5}$ 1·4660 and showed $[\alpha]_D^{21}$ +93·5 (*c.*, 3·3 in chloroform). Its infra-red spectrum included strong absorption at 1640 cm.$^{-1}$ (C=O stretching vibration) but none at 1540 cm.$^{-1}$ (absence of N-H deformation). Analysis indicated that it was a crude specimen of methyl-3:4:6-tri-*O*-methyl-2-*N*-methylacetamido-2-deoxy-α-D-glucoside. Found: OMe, 41·2; CH_3CO, 16·2 per cent. $C_{13}H_{25}O_6N$ requires OMe, 42·5; CH_3CO, 14·8 per cent. To confirm this a portion (500 mgm.) was hydrolysed with 3N-hydrochloric acid (3 ml.) at 100° for 6 hr. and the product crystallized. After recrystallization from dry ethanol it decomposed in the range 170–200°, and showed $[\alpha]_D^{20}$ +105° equil. (*c.*, 0·12 in water) as expected for 3:4:6-tri-*O*-methyl-2-methylamino-2-deoxy-D-glucose hydrochloride.

Fried and Stavely[7] quote a decomposition range of 160–210°, $[\alpha]_D$ -108° for the L-isomer. On paper chromatograms this hydrochloride and 3:4:6-tri:*O*-methyl-2-amino-2-deoxy-D-glucose hydrochloride had respectively R_F values of 0·65 and 0·56 in butanol-ethanol-water (4:1:5), 0·51 and 0·46 in butanol-acetic acid-water (4:1:5) and of 0·60 and 0·53 in butanol-ethanol-water-ammonia (4:1:4·9:0·1). Part of the hydrochloride (284 mgm.) was dissolved in water (2 ml.) and passed through a pad of charcoal and silver oxide onto freshly precipitated yellow mercuric oxide (5 gm.). The mixture was heated on a boiling water bath for 15 min. and then refluxed for 15 min. On concentration and addition of alcohol, crystals of 3:4:6-tri-*O*-methyl-2-methylamino-2-deoxy-D-gluconic acid were obtained, m.p. 204° (Fried and Walz[8] quote m.p. 206–207°).

One of us (D.J.T.) thanks Courtaulds Scientific and Educational Trust Fund for the award of a Scholarship.

S. A. Barker
M. Stacey
D. J. Tipper

Chemistry Department,
The University,
Birmingham, 15.
Sept. 1.

[1] Barker, Heidelberger, Stacey and Tipper, *J. Chem. Soc.*, 3468 (1958).
[2] Haworth, J. *Chem. Soc.*, **107**, 8 (1915).
[3] Freudenberg and Boppel, *Ber.*, **71**, 2505 (1938).
[4] Orr and Harris, *Nature*, **169**, 544 (1952).
[5] Roseman and Ludowieg, *J. Amer. Chem. Soc.*, **76**, 301 (1954).
[6] Zilliken, Brown, Rose and György, *Arch. Biochem. Biophys.*, **54**, 392 (1955).
[7] Fried and Stavely, *J. Amer. Chem. Soc.*, **74**, 5461 (1952).
[8] Fried and Walz, *J. Amer. Chem. Soc.*, **74**, 5468 (1952).

The Unit Cell of Mercuripapain Crystals

Mercuripapain was prepared and crystallized for the first time by Kimmel and Smith[1]. It contains one atom of mercury for two molecules of papain, but the nature of the mercury-protein complex is not exactly known. Papain is a proteolytic enzyme and has a molecular weight of 20,500 (ref. 2).