Note

An improved preparation of methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside*

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The assay[1] of β-glucosiduronase activity by enzymic hydrolysis of methylumbelliferyl β-D-glucopyranosiduronic acid, followed by fluorimetric estimation of the liberated methylumbelliferone, has been widely applied[2]. In 1961, Leaback and Walker[2] introduced methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**1**) as a substrate for the assay of β-acetylglucosaminidase from ram testis. Compound **1** is now commonly used for the assay of glucosaminidases, but difficulties have been experienced by biochemists in obtaining a reliable preparation or commercial source. Consequently, we now report a minor modification of the method of preparation of **1** which is in current use in our Biochemistry Department and provides an improved synthesis.

A solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl bromide[3] in dichloromethane is allowed to react overnight with 4-methylumbelliferone, and subsequent concentration of the reaction mixture results in precipitation of the acetylated glycoside which, on *O*-deacetylation with sodium ethoxide, yields the required glycoside **1** in 25% overall yield.

EXPERIMENTAL

Methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**1**). — 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl bromide was prepared by the method of Inouye *et al.*[3], except that a commercial solution of hydrogen bromide in acetic acid (45% w/v; 15 ml for 5 g of 2-acetamido-2-deoxy-D-glucose tetra-acetate) was used. The reaction was complete after 6 h at room temperature, and the product was extracted with dichloromethane rather than chloroform. The dry dichloromethane extract was concentrated to ~20 ml, and then acetone (70 ml), M sodium hydroxide (17 ml), and 4-methylumbelliferone (1.9 g) were added together. The reaction mixture was stirred overnight at room temperature and then concentrated to half its original

*4-Methyl-2-oxo-1,2-benzopyran-7-yl 2-acetamido-2-deoxy-β-D-glucopyranoside.

volume, whereupon the acetylated glycoside was precipitated. Recrystallisation from ethanol gave **1** triacetate (2 g, 32%), m.p. 253–254°, $[\alpha]_D^{20}$ −18.7° (*c* 1.0, chloroform).

Subsequent *O*-deacetylation and further purification were carried out according to the method of Leaback and Walker[2].

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