The Use of an Immobilized Aldolase in the First Synthesis of a Natural Deaminated Neuraminic Acid

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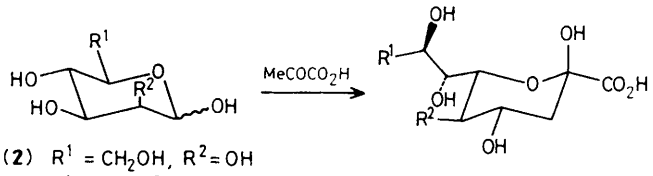
The first synthesis of 3-deoxy-D-*glycero*-D-*galacto*-nonulosonic acid (KDN) (**1**) was achieved by condensation of the non-nitrogeneous substrate D-mannose (**2**) with pyruvate, catalysed by immobilized acylneuraminate pyruvate lyase.

The natural occurrence of a deaminated sialic acid, 3-deoxy-D-*glycero*-D-*galacto*-nonulosonic acid (KDN) (**1**) has been reported recently.[1] KDN was isolated on the μg scale from rainbow trout egg polysialoglycoprotein; it is exclusively located at the non-reducing end of the sialyl chains, and therefore may be involved in egg activation of salmonid fishes by protecting these chains against sialidases. A mixture of KDN isomers has been prepared by the base-catalysed condensation of oxaloacetate with D-altrose;[2] these were studied by Unger as analogues of 3-deoxy-D-*manno*-octulosonic acid (KDO), an important component of bacterial cell walls. No synthesis of KDN itself has been described so far. We now report a



(2) $R^1 = CH_2OH$, $R^2 = OH$
(4) $R^1 = H$, $R^2 = OH$
(6) $R^1 = CH_2OH$, $R^2 = H$

(1) $R^1 = CH_2OH$, $R^2 = OH$
(3) $R^1 = H$, $R^2 = OH$
(5) $R^1 = CH_2OH$, $R^2 = H$

straightforward access to this compound, with the help of the immobilized aldolase which we have also used in the preparation of *N*-acylneuraminic acids and some of their naturally occurring esters.[3—5]

A 0.1 M solution of D-mannose (**2**) (1 mmol) was treated with 10 equiv. of sodium pyruvate in the presence of acylneuraminate pyruvate lyase (E.C.4.1.3.3; 15 U) covalently bound to 4% agarose,[4] in 0.05 M potassium phosphate buffer, pH 7.2, containing 0.01 M dithiothreitol and 0.02% sodium azide, at 37 °C under nitrogen, with gentle stirring for 1 day. After filtration of the gel, chromatography of the solution on Dowex-1 formate (elution with a 0—2 M formic acid gradient) afforded pure (**1**). KDN (**1**) was characterized as its ammonium salt, after treatment with Dowex-50 (H^+), neutralisation to pH 7 with dilute ammonia, and freeze-drying: 84% yield; $[\alpha]_D^{20}$ −41° (H_2O); ^{1}H n.m.r. (250 MHz, D_2O) δ 1.80 (t, 1H, $J_{3ax.,\ 3eq.} = J_{3ax.,\ 4}$ 12 Hz, 3ax.-H), 2.23 (dd, 1H, $J_{3eq.,\ 4}$ 5 Hz, 3 eq.-H), 3.56 (t, 1H, $J_{4,5} = J_{5,6}$ 9 Hz, 5-H), 3.63 (dd, 1H, $J_{9,9'}$, 11 Hz, $J_{9,8}$ 5.5 Hz, 9-H), 3.73 (m, 1H, $J_{8,7}$ 8.5 Hz, $J_{8,9'}$, 2 Hz, 8-H), 3.88 (dd, 2H, 9′-, 7-H), 4.01 (dd, 1H, $J_{7,6}$ 1 Hz, 6-H), and 4.02 (m, 1H, 4-H); ^{13}C n.m.r. (62.9 MHz, internal 1,4-dioxane reference, δ 67.40) δ 39.19 (C-3), 63.86 (C-9), 68.51 (C-4), 69.27 (C-7), 70.67 (C-5), 71.06 (C-6), 72.29 (C-8), 96.02 (C-2), 174.29 (C-1). The composition found (C, H, N, O) was satisfactory for the formula $C_9H_{19}O_9N$.

There are no problems in the scaling up of the preparation, as the recovered gel retained about 95% of its enzymatic activity, and could be used again at least seven times. With the same gel, we found that D-lyxose (**4**) and 2-deoxy-D-glucose (**6**) were excellent substrates, giving acids (**3**) and (**5**) respectively, which were isolated as described for (**1**). ^{1}H N.m.r. spectra were consistent with the presumed structures. While D-arabinose also reacted readily, to give presumably the 4-epimer of KDO, the complexity of the ^{1}H n.m.r. spectrum of the compound obtained precluded a definitive identification.

N-acylglucosamines are not substrates of acylneuraminate pyruvate lyase, a fact which in our hands allowed inexpensive syntheses of *N*-acetyl[4] and *N*-glycolylneuraminic acid.[5] However, it seems that the replacement of NHAc by OH, or even by hydrogen, makes no great difference to the enzyme. The specificity of this aldolase may prove as broad as that of fructose of 1,6-diphosphate aldolase, studied by Whitesides *et al.*[6]

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