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Note

Preparation of deuterium-labeled methyl glycosides of *N*-acetylneuraminic acid

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Sialic acids are involved in numerous biological processes, including cell–cell recognition, cell differentiation, and viral infection[1]. They are principally found in plasma membranes of animal cells at the non-reducing terminus of the carbohydrate moieties of glycoproteins and glycolipids, where they appear to function as receptors for the biomolecules which initiate the aforementioned processes[2]. Of the thirty or so naturally occurring sialic acids, the most prevalent in animal cells is *N*-acetylneuraminic acid[3] (NeuAc, **1**), a nine-carbon amino sugar acetylated at the 5 position. The solution structure of **1** and other sialic acids have been extensively studied. However, to comprehend fully the biological function of these molecules we must also determine their conformational properties as they exist in nature, that is at water–membrane interfaces. Deuterium n.m.r. has been of tremendous value in the study of conformational properties of molecules at membrane surfaces[4]. Applications of deuterium n.m.r. include the use of simple deuterium-labeled carbohydrates and glycolipids in media that mimic a biological membrane environment[5]. Because of the low natural abundance of deuterium, enrichment is a prerequisite for application of these methods. The development of a mild and efficient deuteration procedure for sialic acids, along the lines of those for other carbohydrates, is therefore important. Here we extend the applicability of a Raney nickel-catalyzed hydrogen–deuterium exchange method to include the carboxylate-containing sialic acids, a class of compounds not heretofore been shown to undergo exchange by this method.

Friebolin *et al.*[6] have reported the deuteration of sialic acid at C-3 upon treatment with NaOH in D_2O, and Julina *et al.*[7] have reported the synthesis of 6-deuteriosialic acid. Both of these methods have their merits. However, neither of them are suitable for the direct deuteration of sialic acid-containing glycosides and hence are not useful for labeling natural products. Also, conformational analysis

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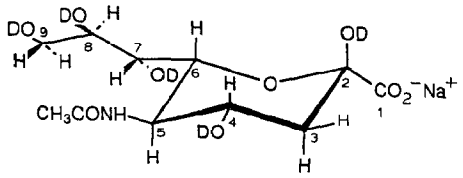

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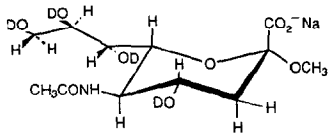
by deuterium n.m.r. requires a deuterium label at several sites within the molecule to completely define a conformation. A procedure that would accomplish this in one step would be ideal. A method, which we have found to be both mild and efficient for the deuteration of neutral glycosides, is the use of Raney nickel catalyst in deuterium oxide coupled with sonication of the reaction mixture[8]. This reaction results in the substitution of deuterium for hydrogen directly bonded to hydroxylated carbon atoms. Sonication greatly improves the efficiency of the reaction when conducted at lower temperatures in mixed solvents. The mechanistic effects of sonication are as yet undetermined, although evidence does indicate that the con-



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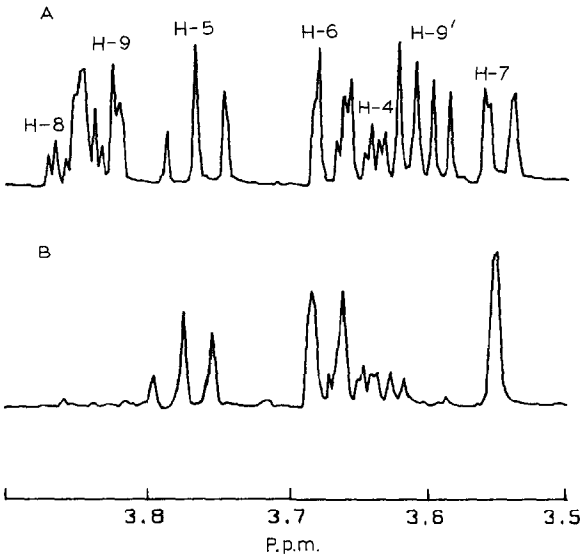


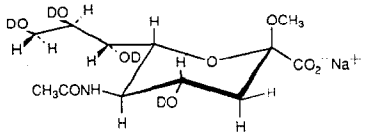
Fig. 1. The 490-MHz ^{1}H-n.m.r. spectrum of *N*-acetylneuraminic acid methyl α-glycoside (**2**), A; before exchange, B; after exchange.

tinual sonication of the catalyst results in removal of inhibitory deposits from the catalyst surface[8].

To demonstrate the applicability of this method to the deuteration of sialic acid-containing glycolipids and hence to carboxylate-containing sugars in general we employed, as a simple model, the methyl α-glycoside[9] (**2**) of NeuAc (**1**). For comparison we also investigated the deuteration of the methyl β-glycoside[9] (**3**).

The extent and position of deuteration may be monitored by the disappearance of signals in the ^{1}H-n.m.r. spectrum. The spectral assignments[10] are given in Figs. 1 and 2. Initial attempts at deuteration employing previously reported conditions[8] (0.5 h, 40°) resulted in little chemical degradation but also produces little deuterated material, as judged by ^{1}H-n.m.r. spectroscopy. Raising the temperature had little or no effect. However a combination of elevated temperature, increased reaction time, and replenishment of the catalyst midway through the reaction furnished deuterium-enriched products.

As may be seen from the n.m.r. spectrum of the methyl α-glycoside (Fig. 1) the signals of H-8, -9, and -9′ (at δ 3.86, 3.84 and 3.61, respectively) have almost completely disappeared, indicating essentially quantitative exchange. The upfield



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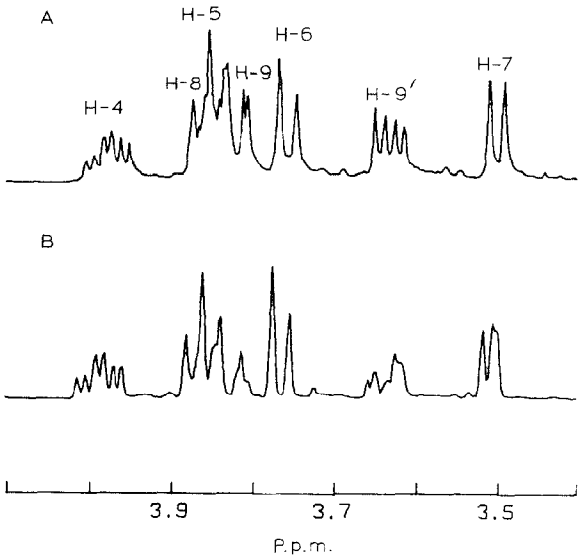

Fig. 2. The 490-MHz ^{1}H-n.m.r. spectrum of *N*-acetylneuraminic acid methyl β-glycoside (**3**), A; before exchange, B; after exchange.

signals (not shown) for H-3 and H-3′ are unchanged and, except for some broadening, the remaining lines show only the expected changes in multiplicity at H-7. Despite filtration of the reaction product through an ion-exchange resin, we believe traces of nickel impurities may still be present and may be responsible for the observed line broadening in the spectrum of deuterated **2**.

Examination of the corresponding spectra for the methyl β-glycoside reveals an identical substitution pattern, although the extent of substitution is considerably less. Little degradation was observed in either case. Complete exchange of H-8, -9, and -9′ in the β-glycoside could be achieved by increasing the reaction time; however this resulted in the formation of a small proportion of byproducts. One of these was the methyl glycoside of neuraminic acid, a product readily formed by base-catalyzed hydrolysis of the acetamido group. The appearance of this product suggests that care should be taken when the catalyst is being prepared, as an insufficiently washed catalyst will produce a basic solution.

The observed positions of deuteration may be explained by the qualitative rules for Raney nickel-catalyzed hydrogen–deuterium exchange originally reported by Balza and Perlin[11] for methyl glucopyranoside and later elaborated by Angyal and Stevens[12] to include methyl glycosides in general. That is, exchange occurs only at hydroxylated carbon atoms and occurs very slowly at these positions when either there is no hydroxyl group on the neighboring carbon atom or the hydrogen atom is axial. Therefore, we would except that, for both the methyl α- and β-glycosides of *N*-acetylneuraminic acid, the rate of exchange would be C-8 > C-9 > C-7 ⩾ C-4 and this is the result obtained. The lower rates observed for the methyl β-glycoside may be the result of less-effective binding to the catalyst surface when the sialic acid carboxylate group is equatorially disposed.

We briefly investigated the applicability of this method for the deuteration of free NeuAc (**1**). However, initial attempts at deuteration of **1** under the aforementioned conditions resulted in severe decomposition of the starting material. Lowering the reaction temperature or decreasing the reaction time gave back **1**. This observation is not unexpected in view of previous results on free reducing sugars[12].

In this report we have introduced a mild, efficient method for the deuteration of *N*-acetylneuraminic glycosides at the 8 and 9 positions, thereby extending Raney nickel-catalyzed deuteration procedures to include sugars that contain a carboxylate group. This methodology, with adequate safety precautions, should also be applicable for the introduction of tritium.

EXPERIMENTAL

Materials and methods. — Raney nickel (50% slurry in water, Aldrich) was thoroughly washed with distilled water and exchanged twice with deuterium oxide prior to use. Deuterium oxide (99.9%) was purchased from MSD Isotopes (St Louis, MO) and *N*-acetylneuraminic acid (synthetic, 95%) from Sigma. A

Bransonic Model W-200 P sonicator apparatus equipped with a titanium tip was employed in all reactions, and ^{1}H-n.m.r. spectra were obtained on a home-built 490-MHz instrument operating in the pulse f.t. mode.

*Methyl α- and β-glycosides (**2** and **3**) of 8,9,9'-trideuterio-*N*-acetylneuraminic acid.* — The Raney-nickel catalyst (0.5 mL settled volume in D_2O, 1 mL in an oven-dried flask), was placed under an argon atmosphere, and D_2O (5 mL) was added. To this mixture was added *N*-acetylneuraminic acid (0.025 g) in D_2O (5 mL). The mixture was placed in a water bath at 65° and allowed to equilibrate for 20 min. The sonicator probe tip was immersed just below the surface of the liquid and sonication was begun. After 1 h the sonication was halted, and the mixture was cooled to room temperature and centrifuged. The clear solution obtained was filtered through a short bed of Chelex-100, a second portion of catalyst was added, and the procedure was repeated. After a further 1 h the reaction was halted, worked up as previously described, and lyophilized to give the desired product in near-quantitative yield.

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