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## Preliminary communication

# A novel method for specific labelling of carbohydrates with deuterium by catalytic exchange*

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It has been observed in this laboratory that deuterated Raney nickel in refluxing deuterium oxide causes deuterium exchange with carbon-bound hydrogen geminal to the hydroxyl group in primary and secondary alcohols.

$$RCH_2O^2H \xrightarrow[^2H_2O]{Ni} RC^2H_2O^2H$$

Harness and Hughes[1] reported the isomerization of some deoxyalditols under conditions of reductive desulfurization with Raney nickel. This isomerization is believed to involve an initial dehydrogenation of the alcohol to a ketone, followed by re-hydrogenation to an alcohol with loss of configuration[1,2]. As the hydrogen adsorbed on Raney nickel exchanges rapidly with the deuterium of heavy water, the deuterium exchange of an alcohol at the geminal position may proceed by this simplified scheme:

$$R_2CHO^2H + Ni \rightleftarrows R_2C{=}O + Ni(H^2H)$$

$$Ni(H^2H) \xrightarrow{^2H_2O} Ni(^2H^2H)$$

$$R_2C{=}O + Ni(^2H^2H) \rightleftarrows R_2C^2HO^2H + Ni$$

The use of Raney nickel as a hydrogen-transfer catalyst between alcohols and ketones or aldehydes is well known[3–5].

For a number of years, this exchange reaction has been applied successfully to many different alcohols and particularly to carbohydrate derivatives; only when large neighboring groups are present has it failed. The experimental conditions are simple. The alcohol (3 g) is pre-exchanged (when possible) by evaporating deuterium oxide (~20 mL) from it. Then, deuterium oxide (80 mL) and deuterated Raney nickel (20 mL, settled

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volume) are added. This exchange mixture is boiled under reflux for ~10 h, the catalyst is filtered off, and the filtrate is passed through a little Amberlite IR-120 ($H^+$) cation-exchange resin and evaporated to dryness. The residue is generally purified by recrystallization.

Deuterated Raney nickel was prepared by washing Raney nickel prepared in the usual way[6], or commercial Raney nickel (W. R. Grace and Co., No. 28), with deuterium oxide in a chromatography column.

Simple alcohols exchange rapidly at the geminal position and slowly at the vicinal position; thus, in these cases, this method is not useful for specific deuterium labelling. The deuterium exchange, with loss of configuration, at C-1 of the various *cis*- and *trans*-methylcyclohexanols is rapid; however, some vicinal deuteration occurs. For chiral, polyhydric alcohols, the rate of isomerization is lower than the rate of exchange. This is consistent with the hypothesis that attachment of more than one conformationally acceptable hydroxyl group to the catalyst causes retention of chirality by making desorption and rotation of the molecule (leading to isomerization) more difficult; this is particularly noticeable for cyclitols and glyco-furanosides and -pyranosides.

Analogous to the work of Karabinos and Ballun[5], free aldoses are converted into the corresponding deuterated alditols if a deuterated alcohol such as ethanol-$^2H_6$ is added to the exchange mixture to act as a deuterium donor; otherwise, the nickel is deactivated rapidly. Alditols exchange at all positions; however, considerable isomerization occurs.

As was indicated previously, this exchange reaction is most useful for the deuteration of cyclic, polyhydric alcohols. The exchange of *myo*-inositol at all positions occurs readily during a reaction time of ~10 h. The product was identified by its melting point, and mixture melting point with the light* compound. It has neither a p.m.r. nor a $^{13}$C-n.m.r. spectrum. It was converted into its hexaacetate, and this was compared to an authentic, light sample, by melting point, mixture melting point, chromatographic behavior, and n.m.r. spectrum. In both the p.m.r. and $^{13}$C-n.m.r. spectra, the signals due to the *O*-acetyl groups were identical to those of the light compound; however, the signals due to the cyclitol moiety were missing, as the $^{13}$C-n.m.r. signals of carbon atoms bonded to deuterium are extremely weak[7]. This is due to coupling with the deuterium, loss of nuclear Overhauser effect, and quadrupole broadening caused by the deuterium nucleus. The deuterium content was measured mass-spectrometrically by using the m – 59 peak, and was found to be 97 atom-percent.

The exchange reaction is particularly useful for the deuteration of methyl glycopyranosides. The reaction should be monitored by n.m.r. spectroscopy, as excessive reaction-times may lead to (usually undesirable) isomerizations. The exchange of methyl α-D-mannopyranoside gives the corresponding deuterated product, namely, methyl α-D-mannopyranoside-2,3,4,6,6′-$^2H_5$, which was purified by recrystallization, and also identified as its tetraacetate. No loss of absolute configuration was observed. $^{13}$C-N.m.r. spectroscopy is most useful for the identification of partially *C*-deuterated carbohydrates.

*The term "light" is used to denote an unlabelled compound.

Interesting isomerizations may be observed when the exchange mixture is heated for extended periods of time. Methyl β-D-galactopyranoside shows complete deuterium exchange at C-2, 3, 4, 6, and 6′ after heating for ~10 h under reflux. There is little isomerization, and the deuterated product can be readily isolated. However, after 2 days, considerable conversion into the correspondingly deuterated D-*gluco* isomer has occurred. Similarly, exchange of methyl β-D-glucopyranoside gives traces of methyl β-D-galactopyranoside-2,3,4,6,6′-$^2H_5$ and at least one other glycoside, presumably, methyl β-D-mannopyranoside-2,3,4,6,6′-$^2H_5$. It is also noteworthy that, after two days of exchange, no deuterium has been introduced at C-1 and C-5, as determined from the p.m.r. and $^{13}$C-n.m.r. spectra. However, mass-spectrometric examination of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose, obtained from methyl α-D-glucopyranoside which had been subjected to the exchange conditions for 3 days, showed the presence of a small proportion of a $^2H_6$ species.

Both methyl α-D-galactopyranoside and methyl α-D-mannopyranoside exhibit isomerization to the *gluco* isomer during extended periods of contact with nickel under the exchange conditions. Again, deuteration is much faster than isomerization. Methyl α-D-glucopyranoside isomerizes only slightly to the deuterated α-D-*galacto* and α-D-*manno* isomers. It appears that, for both α- and β-hexopyranosides, an equilibrium mixture consisting of all possible isomers may eventually form, with the *gluco* isomer, the most thermodynamically stable, greatly preponderating.

The proximity of isopropylidene groups seems to inhibit the exchange. Presumably their size prevents effective interaction of the alcohol with the catalytic surface. Thus, neither 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose nor 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose showed any exchange. 2,3:4,5-Di-*O*-isopropylidene-β-D-fructopyranose also failed to exchange at C-1 with tritiated water and Raney nickel[8]. However, 1,2-*O*-isopropylidene-α-D-glucofuranose exchanges readily at C-5 and C-6, but not at C-3. Some isomerization (~30%) occurs at C-5, leading to the L-*ido* derivative. Similarly, 1,2-*O*-isopropylidene-α-D-xylofuranose exchanges at C-5, but not at C-3, giving 1,2-*O*-isopropylidene-α-D-xylofuranose-5,5′-$^2H_2$. This compound was converted into its diacetate, in order to have substituents not present during the exchange and thus allow the detection of any unsuspected introduction of deuterium. However, integration of the p.m.r. spectrum showed no deuterium exchange other than at C-5. The signals of H-1, H-2, and H-3 are unaltered in the p.m.r. spectrum of the deuterated compound, compared to that of authentic, light material, and the signals due to the protons at C-5 are no longer present; H-4 is now coupled only with H-3, giving rise to a doublet. This signal is broadened, because of the coupling of H-4 with the two deuterium atoms on C-5. The fine structure of the H-1 signal is due to coupling with one of the isopropylidene methyl groups[9]. In the $^{13}$C-n.m.r. spectrum, the signal due to C-5 is missing, as expected (see Fig. 1).

Subjecting a combination of various derivatives to the exchange reaction allows the preparation of a considerable number of deuterated carbohydrates. For instance, D-glucose-2,3,4,5,6,6′-$^2H_6$ has been prepared from 1,2-*O*-isopropylidene-α-D-glucofuranose. This compound was first converted into 1,2-*O*-isopropylidene-α-D-glucofuranose-5,6,6′-$^2H_3$;

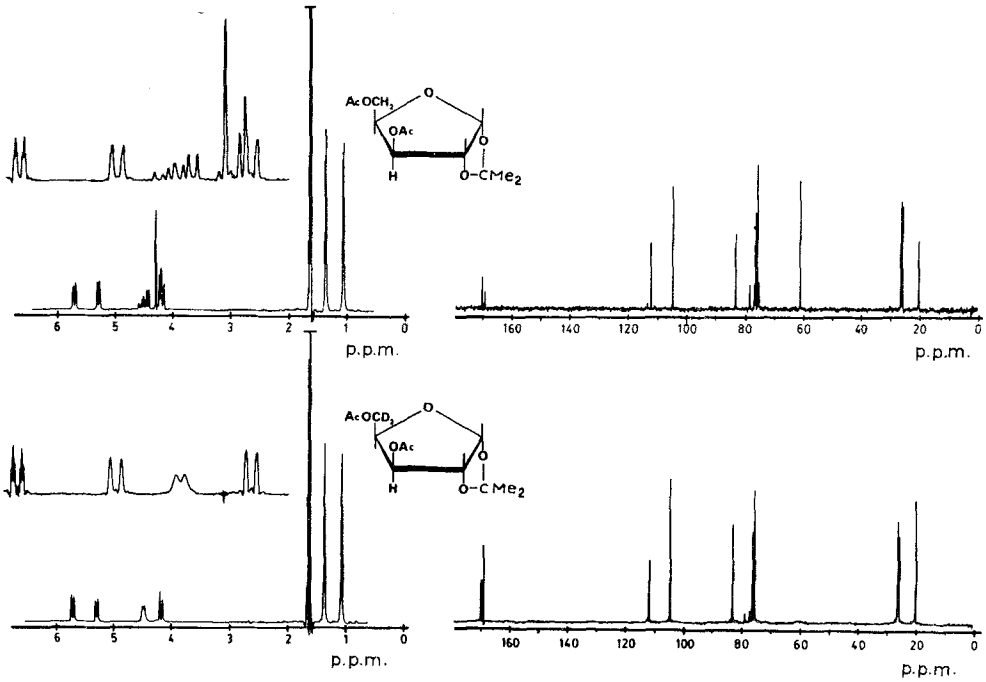


Fig. 1. The proton (80 MHz), and proton-decoupled (20 MHz), $^{13}$C-n.m.r. spectra of 3,5-di-*O*-acetyl-1,2-*O*-isopropylidene-α-D-xylofuranose and 3,5-di-*O*-acetyl-1,2-*O*-isopropylidene-α-D-xylofuranose-5,5′-$^{2}H_2$. The proton spectra were measured for a solution in benzene-$^{2}H_6$, and the $^{13}$C-spectra for a solution in chloroform-$^{2}H_1$, with $Me_4Si$ as the internal standard.

treatment with methanolic hydrogen chloride gave methyl α-D-glucopyranoside-5,6,6′-$^{2}H_3$, which, in turn, was converted into methyl α-D-glucopyranoside-2,3,4,5,6,6′-$^{2}H_6$ by nickel-catalyzed deuterium exchange. Similarly, perdeuterated D-glucose was prepared by starting with D-glucose-1-$^{2}$H, synthesized by reduction of D-glucono-1,5-lactone with deuterated borane[10], followed by conversion into 1,2-*O*-isopropylidene-α-D-glucofuranose-1-$^{2}$H.

Nonreducing di- and tri-saccharides undergo deuterium exchange in the expected way. Reducing oligosaccharides have first to be converted into glycosides to prevent reduction at the anomeric carbon atom. α,α-Trehalose exchanges at C-2, 3, 4, 6, 6′ of both α-D-glucose moieties, and the $^{13}$C-n.m.r. spectrum shows the retention of two peaks upon deuteration, namely, those of C-1 and C-5. The assignment of the C-3 signal is based on the sluggish exchange at this position, which is also observed for the methyl α-D-hexopyranosides, and which may be due to an interaction of the axial H-3 with the axial methoxyl group at C-1, making attachment to the catalyst at C-3 more difficult (see Fig. 2). Sucrose undergoes exchange at all carbon atoms except C-1 and C-5 of the D-glucose moiety and C-5 of the D-fructose moiety (see Fig. 2). Similarly, the trisaccharides melezitose and raffinose give correspondingly deuterated analogs. However, it appears that the D-galacto pyranosyl group of raffinose is slowly converted into a D-glucopyranosyl group, which is

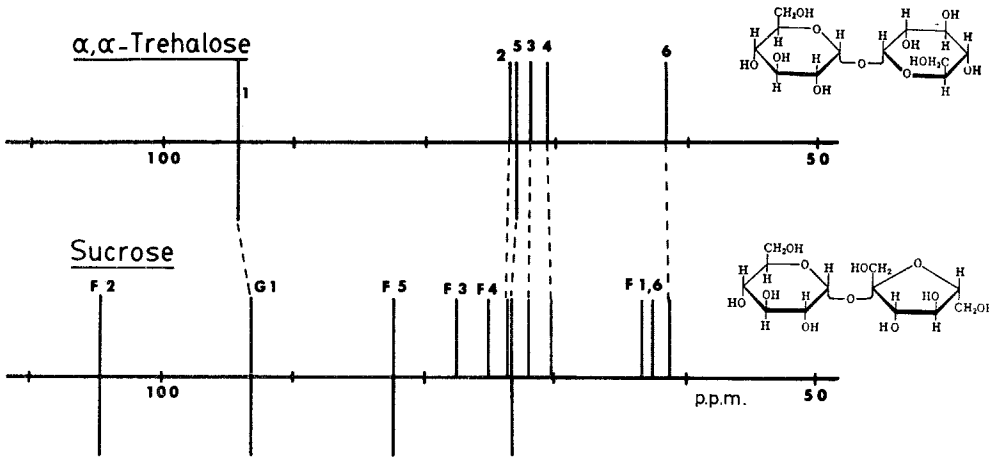


Fig. 2. The proton de-coupled (20 MHz), $^{13}$C-n.m.r. spectra of α,α-trehalose and sucrose measured in deuterium oxide before (above the scale) and after (below the scale) deuterium exchange, with $Me_4Si$ as the external reference-standard.

analogous to the conversion of the D-galactopyranosides into the corresponding D-glucopyranosides, as already described.

During the catalytic exchange of cyclohexaamylose, the deuteration at C-6 of the D-glucopyranosyl residues was rapid; however, deuteration at C-2 and C-3 was very slow (5 days). This observation is not surprising, as the macrocyclic structure of cyclohexaamylose would make interaction with the catalyst difficult at these atoms. Inulin, a water-soluble polysaccharide, was deuterated readily under these conditions (10 h). The $^{13}$C-n.m.r. spectrum of the deuterated product showed that deuteration had taken place at C-3, 4, and 6 of the D-fructosyl residues.

A number of nucleosides (adenosine, guanosine, thymidine, and uridine) were subjected to the exchange conditions. No incorporation of deuterium into the D-glycosyl group was observed for any of them. However, the base groups were fully deuterated. As the methyl D-ribofuranosides exchange readily at C-2, 3, and 5, the failure of exchange of the D-glycosyl groups of the nucleosides may be due to the favored interaction of the base group of the nucleoside with the catalyst. Similarly, dihydrostreptomycin does not appear to exchange in the streptidine residue, probably because of the strongly basic guanidino groups. Both the dihydrostreptosyl residue and the 2-deoxy-2-(methylamino)-L-glucosyl group, including the *N*-methyl group, exchange.

This exchange process is not only useful for the preparation of deuterated carbohydrates but, as it allows the rapid and facile introduction of deuterium into existing molecular structures at specific positions, may also constitute a useful aid in spectroscopic studies. The introduction of tritium by exchange of carbon-bound hydrogen atoms of carbohydrates with water containing tritium oxide and nickel should proceed in an analogous way, and its usefulness in $^{3}$H-n.m.r. studies has been suggested[11].

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