# $^{18}O$ and Secondary $^{2}H$ Kinetic Isotope Effects Confirm the Existence of Two Pathways for Acid-catalysed Hydrolyses of α-Arabinofuranosides

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The $^{18}O$ kinetic isotope effect on the $HClO_4$-catalysed hydrolysis of 4-nitrophenyl [1-$^{18}O$]-α-arabinofuranoside ($k_{16}/k_{18}$) is 1.023 ± 0.003 at 80.0 °C; that for isopropyl [1-$^{18}O$]-α-arabinofuranoside is 0.988 at 30.2 °C and the secondary deuterium effect on the hydrolysis of [2-$^{2}H$]propan-2-yl α-arabinofuranoside ($k_H/k_D$) is 0.979. The nitrophenyl glycoside reacts with exocyclic C–O cleavage and the propan-2-yl glycoside by endocyclic C–O cleavage.

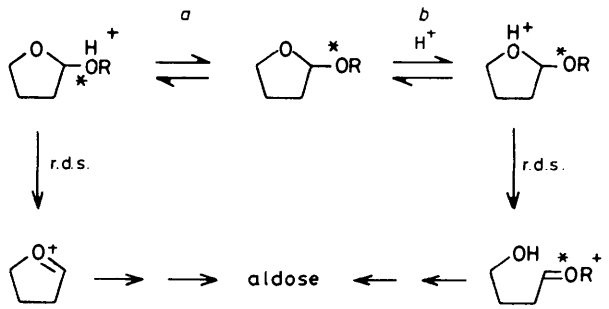
Since glycosides are unsymmetrical acetals, two pathways are available in principle for their acid-catalysed hydrolysis. Pyranosides invariably react *via* a glycopyranosyl cation (or cation-like transition state),[1] but the situation with aldofuranosides is more complex (Scheme). The increased reactivity of alkyl aldofuranosides, as compared with their pyranoside isomers, has been attributed to the predominance of the ring-opening mechanism[2] (pathway *b*), perhaps associated with nucleophilic assistance from water solvent.[3] However, subsequent work by Lönnberg and his co-workers[4] has indicated that both pathways *a* and *b* may obtain. Which pathway is taken depends on both the glycone and the electronegativity of the group R. If R is electron-withdrawing, both the pre-equilibrium and the ring-opening steps in pathway *b* will be disfavoured, but the overall effects on pathway *a* will be small since the effects on the two steps are in opposite senses; protonation will be disfavoured but, once protonated, ROH will be a better leaving group. (Thus, substituent effects on the acid-catalysed hydrolyses of aryl glycopyranosides, which take pathway *a*, are small.[1,5]) Therefore, in any given aldofuranosyl series, altering the electronegativity of R should in principle bring about a change in mechanism.

The Finnish workers showed that in a number of aldofuranosyl series[4,6,7] there was at some stage a sudden increase in the entropy of activation as the electronegativity of R was gradually increased—in accord with a change to the mechanism of pathway *a* in which two molecular fragements are generated—and that this was associated with a change in the composition-dependence of the decrease in rate brought about by added dimethyl sulphoxide.

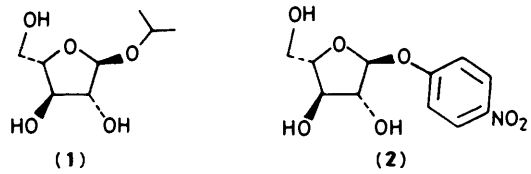
Convincing in aggregate though these indications of a change in mechanism are, it would be helpful to have, in a representative case, a more direct indication of a change in mechanism. The kinetic isotope effect consequent upon $^{18}O$ substitution at C-1 of the sugar (Scheme) would provide such direct demonstration of a change in mechanism. If pathway *a* is followed, then a normal effect ($k_{16}/k_{18}$ = 1.02)[8] should be observed; if pathway *b* is followed the effect should be inverse ($k_{16}/k_{18}$ < 1.00) since at the transition state bonding to the site of isotopic substitution is increased.

The experimental problems associated with the measurement of very small kinetic isotope effects have been greatly ameliorated by the advent of Robinson's quasi-racemate method,[9] in which the optical rotation of an equimolar mixture of a substrate and its isotopically labelled optical antipode is followed as the reaction is carried out. In the ideal case, when the concentrations of quasi-antipodal substrates are identical and there is no isotope effect on the optical rotation, the appearance and disappearance of optical activity is caused wholly by the kinetic isotope effect.

Reactions of those carbohydrates commercially available at modest cost in both antipodal forms are clearly well suited to investigation by this method. We therefore addressed the problem of the two mechanistic pathways for acid-catalysed hydrolysis of aldofuranosides in the α-*arabino*-series; labelled substrates could then be used in concurrent mechanistic investigations of α-L-arabinofuranosidase.[10]




**Scheme.** Pathways for hydrolysis of aldofuranosides; the kinetic effects of $^{18}O$ substitution at the starred oxygen atom will be in opposite senses




(1) (2)

Lönnberg and Kulonpää[6] showed that, by the criteria of entropy of activation and effect of added dimethyl sulphoxide, all α-D-arabinofuranosides with aglycones more electronegative than propan-2-yl were hydrolysed by pathway *a*, and that this pathway was probably taken by the propan-2-yl compound at high dimethyl sulphoxide concentration. We therefore examined $^{18}O$ kinetic isotope effects in the acid-catalysed hydrolyses of propan-2-yl α-arabinofuranoside [L-isomer (**1**)] and 4-nitrophenyl α-arabinofuranoside, [L-isomer (**2**)], the $^{18}O$-labelled L-enantiomer of the latter being also of use in enzymic work.

We also examined the effect of deuterium substitution at the 2-position of the propan-2-yl group of α-L-arabinofuranoside. Under many circumstances deuterium behaves as if it has a slight electron-donating effect with respect to protium;[11] this has been attributed to the anharmonicity of the C–H bond vibration resulting in a higher average electron density closer to the heavy atom in the ground-state vibration of the deuterium compound, which is of lower amplitude.[11] Therefore a small inverse effect should be observed if pathway *b* is taken, but effectively none if pathway *a* is taken.

## Results and Discussion

At 80.1 °C the rate of hydrolysis of 4-nitrophenyl α-D-arabinofuranoside was proportional to the stoicheiometric concentration of perchloric acid, a second-order rate constant of $1.60 \times 10^{-2}$ l mol$^{-1}$ s$^{-1}$ being calculated from first-order rate constants at five acid concentrations between 0.015 and 0.098M. First-order rate constants at five temperatures between 60 and 80 °C in aqueous 0.098M-perchloric acid were measured, and gave the activation parameters $\Delta H^{\ddagger} = 104 \pm 1.7$ kJ mol$^{-1}$, $\Delta S^{\ddagger} = +8 \pm 6$ J mol$^{-1}$ K$^{-1}$, calculated for a second-order reaction and a molar standard state. The entropy of activation is in the range considered typical of pathway *a*.[4,6,7,12]

Figure (i) illustrates the change in optical rotation with time of a 1 dm path-length polarimeter cell containing 7.5 mg each of 4-nitrophenyl α-D-arabinofuranoside and 4-nitrophenyl [1-$^{18}$O]-α-L-arabinofuranoside in aqueous 0.019M-perchloric acid at 80.1 ± 0.1 °C. The solid line is the least-squares fit of the data to equation (1), where $\alpha_t$ is the rotation at time $t$, $k_L$ the first-

$$\alpha_t = Ae^{-k_L t} + Be^{-k_L t/C} + \alpha' \qquad (1)$$

order rate constant for the light isotope, $\alpha'$ the end-point, $A$ the optical rotation change, under the same conditions, for the light isotopomer, $B$ that for the quasi-antipodal heavy isomer, and $C$ the kinetic isotope effect, $k_{light}/k_{heavy}$. The value of $A$ was measured in a separate experiment; $B$, $C$, $k_L$, and $\alpha'$ are the unknowns which were varied to obtain the best fit. In this way the effects of slightly unequal concentrations of the two compounds, and isotope effects on optical rotations, were accounted for. In a typical case an 8.4% error in $A$ results in a change in $C$ of only 0.00066. The mean and standard deviation of $C$ ($k_{16}/k_{18}$) from five such runs is 1.023 ± 0.003. 4-Nitrophenyl α-arabinofuranoside is therefore hydrolysed by aqueous acid by pathway *a*.

Figure (ii) shows a similar experiment with 12 mg each of propan-2-yl α-D-arabinofuranoside and propan-2-yl [1-$^{18}$O]-α-L-arabinofuranoside in aqueous 1.01M-perchloric acid at 30.2 °C. This run gave a value for $k_{16}/k_{18}$ of $0.987_7$; a duplicate run gave a value of $0.988_4$.

Figure (iii) shows a similar experiment with [2-$^2$H]propan-2-yl α-L-arabinofuranoside in place of the $^{18}$O-labelled material. This gave a value for $k_H/k_D$ of $0.979_4$; a duplicate run gave a value of $0.978_3$.

Deuterium and $^{18}$O kinetic isotope effects therefore both indicate that at 30.2 °C the propan-2-yl α-arabinofuranoside is hydrolysed by pathway *b*.

The lower temperature at which the kinetic isotope effects for the propan-2-yl glycoside were obtained of itself disfavours the dissociative pathway *a*. We made several attempts to measure the $^{18}$O kinetic isotope effect at 80.1 °C (in aqueous 0.020M-perchloric acid), but in the process of developing the technique for making measurements on this non-crystalline, hygroscopic material used up most of the $^{18}$O-labelled compound. The one quantitatively acceptable run gave $k_{16}/k_{18} = 0.989_4$, and this value, in conjunction with those from several less acceptable runs, gives us confidence that at 80.1 °C the $^{18}$O kinetic isotope effect on the hydrolysis of propan-2-yl [1-$^{18}$O]-α-arabinofuranoside is still inverse.

Our results thus provide direct evidence, in the α-arabinofuranosyl series, for the change in the mechanism of acid-catalysed hydrolysis of aldofuranosides with aglycone structure, proposed by Lönnberg *et al.*[4,6,7,12] on the basis of entropies of activation and solvent effects.

The magnitude of the measured kinetic isotope effects deserves comment. Rosenberg and Kirsch[8] calculated an equilibrium isotope effect, $K_{16}/K_{18}$, of 1.024 for the conversion of 4-nitrophenyl β-D-glucopyranoside into 4-nitrophenol and the glucosyl cation. They measured a value for $k_{16}/k_{18}$ of

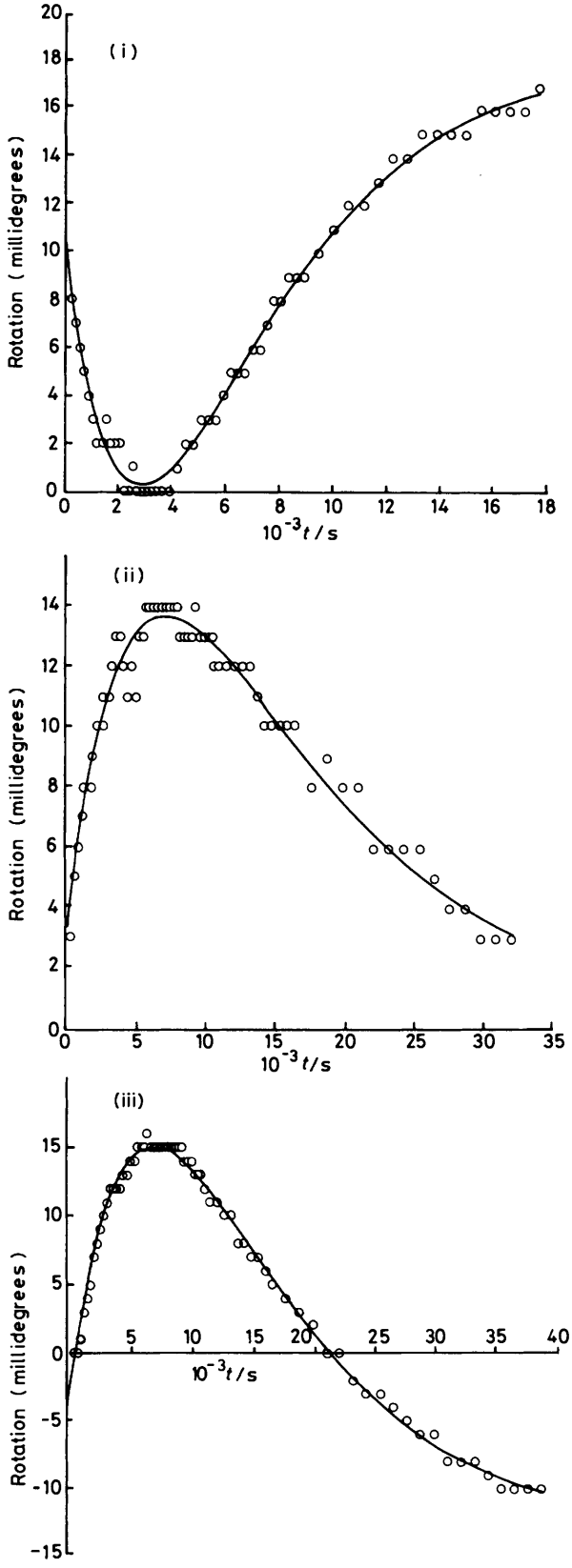



**Figure.** Optical rotations of isotopic quasi-racemates of (i) 4-nitrophenyl [1-$^{18}$O]-α-L-arabinofuranoside and 4-nitrophenyl α-D-arabinofuranoside, (ii) propan-2-yl [1-$^{18}$O]-α-arabinofuranoside and propan-2-yl α-D-arabinofuranoside, and (iii) [2-$^2$H]propan-2-yl α-L-arabinofuranoside and propan-2-yl α-D-arabinofuranoside, as functions of time during acid-catalysed hydrolysis; for details see text

$1.035_5 \pm 0.0015$ for the acid-catalysed hydrolysis of this glycoside,* and suggested that this effect required that proton donation be only partly complete at the transition state, and therefore that general acid catalysis should be observable in principle. Our measured effect for the α-arabinofuranosyl glycoside of the same aglycone is $\frac{2}{3}$ the size of the measured effect of Rosenberg and Kirsch, and is entirely consistent with the long held[13] view that glycoside hydrolysis proceeds *via* a specific, not a general, acid pathway and has a late transition state.†

The equilibrium isotope effect for reaction (i) ($K_{16}/K_{18}$) has

$$H^{18}OCH_2OH + H^+ \rightleftharpoons H^{18}O^+{=}CH_2 + H_2O \quad (i)$$

been calculated as 0.977.[14] The observed isotope effect for hydrolysis of propan-2-yl α-arabinofuranoside of 0.988 therefore indicates a transition state in which the charge on oxygen is about half developed.

The deuterium kinetic isotope effect ($k_H/k_D = 0.979$) is that expected from a simple inductive effect on the formation of the oxocarbocation. From the deuterium effect on the protonation of methylamine, $K_a(CH_3NH_3^+)/K_a(CD_3NH_3^+) = 0.86 \pm 0.03$,[15] one expects an effect of around 4—6% per deuterium atom for the introduction of a full positive charge β to the site of deuterium substitution. The observed inverse effect of 2.1% thus concurs with the $^{18}O$ effect and indicate the charge on oxygen is about half-developed in the transition state.

## Experimental

*4-Nitro*[$^{18}$O]*phenol.*—Lithium hydride (1.4 g) was added to dry dimethyl sulphoxide (50 ml) and the suspension was stirred at 70—75 °C under argon until reaction ceased. The resulting solution of methylsulphonylmethyl-lithium was allowed to cool to room temperature, [$^{18}$O]methanol (1.27 ml) was added, and the solution was stirred for 20 min. {The [$^{18}$O]methanol had been made from $H_2{}^{18}O$ [98% enriched, *ex* Amersham plc, batch No. B1(DD)] and tributyl orthoformate by the method of Sawyer.[16]}

To the resulting solution of lithium methoxide was added redistilled 1-fluoro-4-nitrobenzene (3.3 ml); the mixture was stirred at 50—55 °C for 16 h, cooled, poured into aqueous tetrahydrofuran (100 ml), and extracted with ether (3 × 100 ml). The ethereal extracts were washed with saturated brine (2 × 100 ml), and distilled water (100 ml), dried ($MgSO_4$), and evaporated, and the residue was recrystallised to afford 4-nitro[$^{18}$O]anisole, m.p. 53—54 °C, in 61% yield from methanol. The nitroanisole was demethylated by the method of Prey,[17] to give a 67% yield of 4-nitro[$^{18}$O]phenol, m.p. 113—114 °C, with an isotopic enrichment of 97%, as estimated from the relative intensities of *m/z* 139 and 141 ($M^+$) in the mass spectrum.

*4-Nitrophenyl α-Arabinofuranosides.*—These glycosides were made from the 4-nitrophenol and 2,3,5-tri-*O*-benzoyl α-arabinofuranosyl bromide as described elsewhere.[18] M.p.s, rotations ($[\alpha]_D^{25}$, *c* 1, $H_2O$), and yields (from 4-nitrophenol) were, for the [$^{18}$O]-α-L-compound, 155—157 °C, −207°, 19%; for the unlabelled α-L-compound, 155—157 °C, −202°, 8%; and the unlabelled α-D-compound, 156—157 °C, +205°, 22%.

When fairly concentrated, but not saturated, aqueous solutions of the antipodal 4-nitrophenyl α-arabinofuranosides were mixed, a sparingly soluble racemic compound, m.p. 182—185 °C, crystallised out.

[2-$^{18}$O]- *and* [2-$^2$H]-*Propan-2-ol.*—[$^{18}$O]Water (0.5 ml; same batch as for nitro[$^{18}$O]phenol) was added to dry 1,2-*O*-isopropylidene DL-glycerol (4 ml), stirred under nitrogen, and anhydrous hydrogen chloride gas (10 ml) was introduced with a syringe. The temperature was slowly increased and the [$^{18}$O]acetone was distilled off through a short Vigreux column and collected in a 5 ml flask containing dry 2,5,8-trioxanonane (2 ml). The resulting solution of [$^{18}$O]acetone was added to a stirred suspension of lithium aluminium hydride (0.4 g) in dry trioxanonane (8 ml) at 0 °C. The solution was allowed to warm to room temperature, stirred for 20 min, and re-cooled to 0 °C, then butan-1-ol (6 ml) was added dropwise to destroy the excess of lithium aluminium hydride. The [2-$^{18}$O]propan-2-ol (0.78 ml; 37% from water) was then distilled out of the reaction mixture through a short Vigreux column, and used directly for glycosidation.

[2-$^2$H]Propan-2-ol was made analogously (in 73% yield from acetone) by reduction of acetone with lithium aluminium deuteride in trioxanonane.

*Propan-2-yl α-Arabinofuranosides.*—To the 2,3,5-tri-*O*-benzoyl α-arabinofuranosyl bromide[19] (5 g) in dry dichloromethane (95 ml) under nitrogen at 0 °C was added redistilled tin(IV) chloride (1.14 ml) and the whole was stirred for 10 min. The propan-2-ol (0.7—0.8 ml) was added, then the mixture was stirred for 4 h at room temperature and poured into saturated $NaHCO_3$ solution (100 ml). The organic layer was extracted with saturated $NaHCO_3$ solution until the aqueous phase was clear; it was then washed with water, dried ($MgSO_4$), and evaporated. The resulting syrup (4.2 g) was purified by flash column chromatography[20] on a 4.5 × 25 cm column of Merck 230—400 mesh silica gel (light petroleum–ethyl acetate 15:1 v/v as eluant). The protected propan-2-yl arabinofuranosides appeared in fractions 23—33 (20 ml fractions).

The syrup obtained by evaporation of fractions 23—30 (0.85 g) was dissolved in dry methanol (6 ml) and methanolic 0.2M-sodium methoxide (1.2 ml) was added. The mixture was kept for 3 h at 0°C, then freshly regenerated Duolite 225 ion-exchange resin ($H^+$ form) was added to neutralise the sodium methoxide, filtered off, and washed with methanol. The combined solution and washings were evaporated; the residue was dissolved in water, and the aqueous solution was extracted with ether to remove methyl benzoate, before being reconcentrated to a syrup. Purification of this syrup by h.p.l.c. [10% aqueous methanol at 2.5 ml $min^{-1}$ as eluant through a 'Spherisorb' column (S5 ODS2, Phase Separations Ltd., Queensferry, Clwyd)] resulted in the elution of contaminating β-anomer (m.p. 68 °C; lit.,[7] 70—71 °C) before the desired α-anomer; the latter fractions were concentrated and lyophilised.

All propan-2-yl α-arabinofuranosides were non-crystalline. The α-D-*compound* $[\alpha]_D^{25}$ +99° (*c* 1, $H_2O$), was obtained in 9% yield (from propan-2-ol) (Found: C, 49.8; H, 8.5. $C_8H_{16}O_5$ requires C, 50.0; H, 8.4%). For the other propan-2-yl α-arabinofuranosides, yields and rotations ($[\alpha]_D^{25}$; *c* 1, $H_2O$) are as follows: α-L, 11%, −100°; [1-$^{18}$O]-α-L, 5%, −102°; [*propanyl*-2-$^2$H] α-L, 7%, −109°. The $^{13}$C n.m.r. spectrum showed δ ($D_2O$; referenced *via* acetonitrile) 105.7 (C-1), 83.3, 81.5, 76.3 (C-2, C-3 and C-4), 61.4 (C-5), 71.6 (prop. C-2), 22.8, 21.3 (prop. C-1 and C-3); the signal at 71.6 appears as a 1:1:1 triplet in the case of the [$^2$H]-compound. The $^1$H n.m.r. spectrum was as reported,[7] except that at 200 MHz the diastereoisotopic methyl groups are distinguishable.

Isotopic enrichments of 97% for the $^{18}$O-labelled glycoside and 94% for the deuteriated glycoside were estimated from relative intensities of the glycoside molecular ions.

* This value pertains to 50 °C, and ours, for the α-arabinofuranosyl compound, to 80 °C, but if the effects are classical, *i.e.* In $(k_{light}/k_{heavy})$ = constant, then the temperature corrections are of similar magnitude to estimated errors.

† If a fraction of the reaction went by pathway *b*, this would reduce the observed isotope effect, but pathway *b* is unlikely to be taken by the glycoside of such an electronegative aglycone.

*Kinetic Measurements.*—Reactions were followed by monitoring the change in optical rotation of the appropriate solution (1.0 ml) in a jacketted 1 dm path-length cell, in a Perkin-Elmer 241MC spectropolarimeter, equipped with the manufacturer's digital printer. Water, thermostatted to ±0.2 °C (Julabo Paratherm U4 water-bath) was passed through the cell jacket. Mercury spectral lines at 546 and 404.6 nm were used for monitoring the hydrolysis of 4-nitrophenyl and propan-2-yl glycosides, respectively.

AnalaR 60—62% aqueous perchloric acid was diluted with h.p.l.c.-grade water and standardised by titration against B.D.H. standard sodium hydroxide solutions.

First-order rate constants were calculated from linear least-squares treatment of $\log(\alpha_t - \alpha_\infty)$ *versus* time plots, $\alpha_\infty$ being calculated by the Kezdy-Swinbourne[21] method. Particular attention was paid to the kinetics of hydrolysis of propan-2-yl α-arabinofuranoside, since the acyclic oxocarbocation could in principle ring close to propan-2-yl arabinopyranosides, which would be hydrolysed more slowly than the furanoside. In fact the kinetics of hydrolysis were cleanly first order, the rate at 80.1 °C and in 0.020M-perchloric acid being within 12% of that calculated from the data of Lönnberg and Kulonpää,[6] on the assumption of a proportionality of rate and acid concentration. Since these authors followed the reaction by reducing sugar analysis, we can be confident that the observed rotation change refers to hydrolysis rather than isomerisation.

Kinetic isotope effects were calculated by fitting the time course of optical activity of a reacting quasi-racemate to equation (1), using the EO4 HFF non-linear least-squares routine from the NAG library on the Bristol University Multics computer system. The reaction was followed for 6—7 half-lives and about 100 data points were used. The rotation change ($A$) for the light isotopomer during the reaction was determined from the initial reading and the infinity reading, calculated by the Kezdy-Swinbourne method, of the hydrolysis with only the light isomer present. In all cases it was checked that the estimated value of $B$ was approximately $-A$, that $k_L$ corresponded, within experimental error, to that measured independently, and, visually, that experimental points were randomly distributed about the calculated line of best fit at all points in the progress curve. The highest r.m.s. deviation of points from the calculated curve was 0.78 millidegrees; because of the digital output of the polarimeter a perfect fit would give an r.m.s. deviation of 0.29 millidegrees.

The problem of the insolubility of racemic 4-nitrophenyl α-arabinofuranoside was surmounted by making up 500 μl of each enantiomer separately, and mixing them only in a preheated polarimeter cell. Hydrolysis of the unlabelled racemic compound gave no detectable change in optical rotation, other than a very slow, linear, electronic drift.

The concentrations of the non-crystalline, hygroscopic propan-2-yl α-arabinofuranosides were estimated by pipetting fixed volumes of an aqueous solution into a preweighed flask and lyophilising.

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