COUNTERCURRENT DISTRIBUTION OF LABELED ANOMERIC METHYL ARABINOSIDES AND METHYL α-D-MANNOSIDE*

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It was previously reported that lyxose-1-^{14}C [1] and arabinose 1-^{14}C showed an isotope effect during countercurrent distribution in cyclohexane–ethanol (2:1). For arabinose, at least, the effect was a positional one; for example arabinose-5-^{14}C showed the same mobility as its unlabeled counterpart. With the possibility that the isotope effect could be related to the anomerization of the sugar or to the distribution of the anomers at equilibrium, it was thought that the examination of the mobility of the glycosides, where the anomerization under the conditions could not prevail, would aid the understanding of the earlier observations. If the isotope effect were the same for the glycosides and the sugars from which they were synthesized, a role of the isotope effect in the anomerization would be excluded. On the other hand, a difference between the isotope effect for the sugar and this derivative would allow the possibility, if not prove, that the isotope could either influence the equilibrium mixture of the anomers or the rate at which mutarotation occurs.

METHODS

Preparation of methyl β-D-arabinoside-1-^{14}C

Seven grams of D-arabinose-1-^{14}C, specific activity, 1.3 μC per mmole, 2 g Dowex 50 (H^+) and 20 ml methanol were placed in a 50 ml double necked round bottom flask and heated under reflux with stirring. After 24 h, the Dowex was removed from the reaction mixture by filtration and washed with several 15 ml portions of methanol. The filtrate and washings were concentrated under vacuum. The residue was dissolved by suspending it in 20 ml of ethanol and boiling under reflux. On cooling the product from crystallization was methyl β-D-arabinoside-1-^{14}C, m.p. 167–170°, $[\alpha]_D^{22}$ + 248.

When the product was mixed with commercial methyl β-D-arabinoside there was no depression of melting point. When the product was chromatographed on paper with ethyl acetate–*n*-propanol–water (5:3:2), the mobility of the radioactive material was identical with that of the spot obtained after first spraying with 0.5 % NaIO and then after 5 min with benzidine dihydrochloride. The triacetate of the methyl D-arabinoside was prepared from 125 mg of the product, 15 mg of anhydrous sodium acetate and 0.3 ml acetic anhydride. The mixture was refluxed at 50° for 4 h.

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After adding 3.5 ml water, the mixture was stirred for 2 h and filtered. The filtrate contained methyl β-D-arabinoside triacetate, m.p. 69°.

Preparation of methyl α-D-arabinoside-1-^{14}C.

Eight grams of D-arabinose-1-^{14}C, specific activity, 1.13 μC per mmole, and 4 g Dowex 50 (H^+) were added to 40 ml of anhydrous methanol. The mixture was refluxed at 40°—instead of at boiling as in the above preparation—for 30 h, after which the Dowex was filtered off and discarded. Methyl β-D-arabinoside-1-^{14}C was precipitated from the filtrate on standing for less than 5 min. The filtrate contained methyl α-D-arabinoside-1-^{14}C, m.p. 128–131°.

Preparation of methyl α-D-mannopyranoside-1-^{14}C

The procedure closely followed that of MOWERY[2]. The chromatography of the products in the filtrate from the reaction mixture with Dowex appears in Fig. 1. The zones designated by Roman numerals represent the chromatographic positions

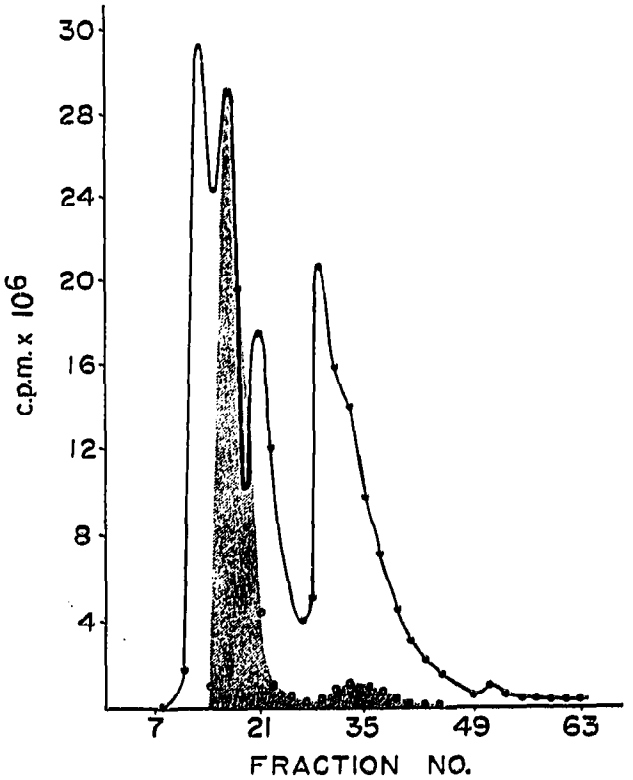


Fig. 1. Graphical representation of the chromatography of the filtrate of the reaction mixture with mannose, methanol and Dowex 50. Collected fractions were 2.5 ml. Columns were 1 × 17.5 cm. The shaded area represents the chromatography of α-D-mannopyranoside after a single crystallization.

for the methyl α-D-furanoside, α-D-pyranoside, β-D-furanoside and β-D-pyranoside of mannose. In this study no effort was made to identify solutes other than the solute from zone II. The shaded area of the plot, Fig. 1, represents the distribution on the cellulose columns of the product of a single crystallization from the reaction mixture. The twice crystallized product, methyl α-D-mannopyranoside-1-^{14}C, m.p. 185–187°, was a solute for the countercurrent distribution studies below.

Countercurrent distribution

The details for the distributions, employing the all glass machine of CRAIG AND POST, have been previously described[3]. All distributions were carried out in cyclohexane–ethanol (2:1). The assays for radioactivity were carried out on aliquots using a dioxane–naphthalene solvent, 2,5-diphenyloxazole (PPO) and 2,2′-*p*-phenylenebis(5-phenyloxazole) (POPOP) in the Nuclear Liquid Scintillation Counter, Model No. 8264. Glycosides after hydrolysis, as well as the pure sugars, were measured by the *o*-aminobiphenyl reaction through the absorption of the glycosylamine at 370 mμ[4].

RESULTS AND DISCUSSION

The data in Fig. 2 indicate that the isotope effect during the countercurrent distribution of methyl α-D-arabinoside-1-^{14}C is less than that of the arabinose resulting after the hydrolysis of the glycoside. Similar data were obtained for the methyl β-derivative (Fig. 2B). Plots of the type shown in Fig. 2 would ideally graphically represent an absence of an isotope effect as a straight line parallel to the abscissa;

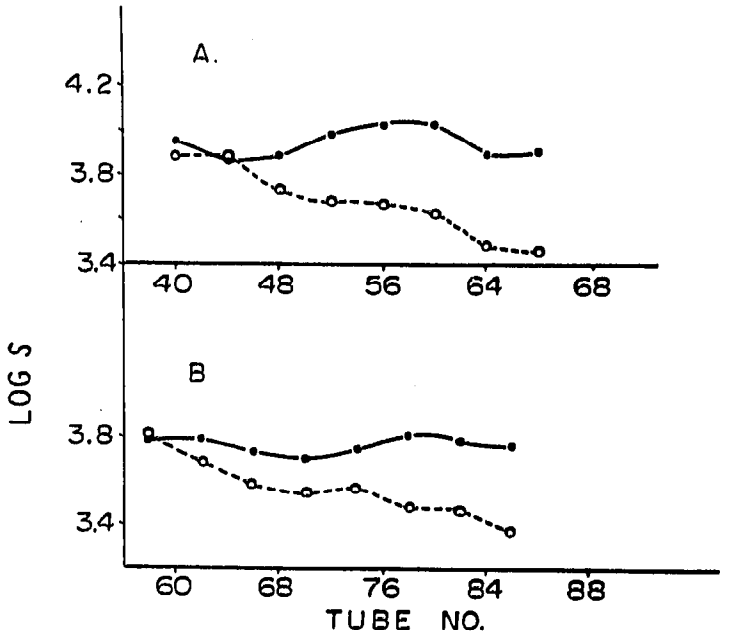
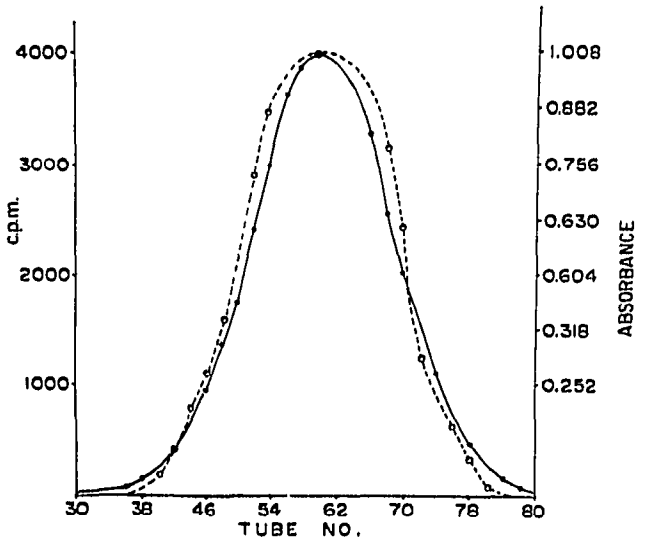


Fig. 2. Plots of log specific activity, log S, against tube number, X, in accordance with $\ln S = [(M_1 - M_2)X/\sigma^2] + [(M_2^2 - M_1^2)/2\sigma^2]$ derived from the ratio of the curves (absorbance, ^{14}C-activity) assuming the normal distribution and that they have the same standard deviation 6, but M_1, the mean of the absorbance curve, differs from M_2, the mean of the ^{14}C activity curve; thus the slope of the line is the index of resolution for the distribution. (A) Solid line: methyl α-D-arabinoside-1-^{14}C after 500 transfers along with the pentose from it on hydrolysis; dotted line, after 590 transfers. (B) Solid line: methyl β-D-arabinoside-1-^{14}C after 600 transfers along with the pentose from it after 800 transfers.

the presence of an isotope effect that is characterized by a decrease in specific activity across the countercurrent zone will appear as a straight line with a negative slope. While the scattering of the points in Fig. 2 does not permit straight lines, lines drawn through the most points in A and B would show a slope for the glycoside that is compatible with the view that the slopes for the two are unlike. Thereby, the

influence of the labeled carbon on the mobility of the pentose during the distribution differs from its effect on the glycoside. With respect to arabinose-1-^{14}C, the observations for the pentose—not the derivative—are similar to those previously reported[3] and add support, since the initial glycoside gave evidence of reasonable purity, to the earlier interpretations of the data, namely, that impurities could not have accounted for the isotope effect during the distribution of this pentose.

Methyl α-mannoside showed no isotope effect during countercurrent distribution (Fig. 3). When mannose from the hydrolyzed glycoside was distributed in the cyclohexane–ethanol system, the data (Fig. 4) from the dissymmetry of the curves suggest a greater heterogeneity than was observed with the glycoside. Indeed, this



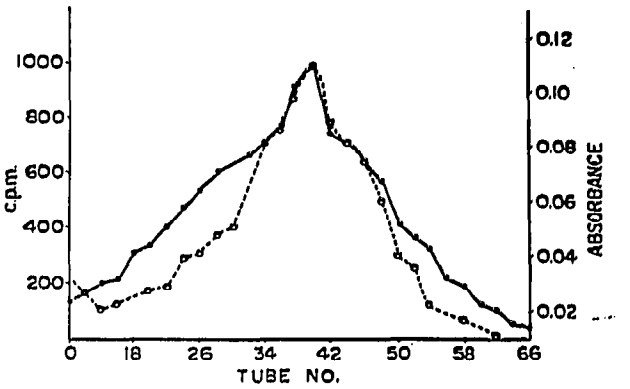


Fig. 3. Five hundred transfer countercurrent distribution of methyl α-D-mannopyranoside-1-^{14}C. The solvent system was an equilibrated mixture of 2 parts of cyclohexane with 1 part 95% ethanol at 22°. The upper phase composition, by volume in percent of water, ethanol and cyclohexane, was 0.8, 14.5 and 84.5 as determined by matching spectra of synthetic mixtures of the components using the Perkin-Elmer infrared spectrophotometer Model 21. Solid line, counts/min; dotted line, absorbance at 370 mμ of the mannosylamine.

Fig. 4. Five hundred transfer countercurrent distribution of mannose-1-^{14}C from methyl α-D-mannopyranoside-1-^{14}C. Solid line, counts/min; dotted line, absorbance. Except that the aliquot for the absorbance was one half that used for Fig. 3, all other conditions were the same.

heterogeneity is reminiscent of that earlier found for arabinose. While the data for mannose-1-^{14}C suggest from the left side of the curve of Fig. 4 that the labeled molecules have a slightly lower K than the unlabeled pentose population, the plot of the right side of the band lends no support to an isotope effect. Commercial mannose-1-^{14}C that had not undergone glycosidation and hydrolysis showed similar apparent heterogeneity when the dissymmetry of the plots of the countercurrent distribution zone was the index.

The data allow the possibility that the anomerization that contributes to molecular heterogeneity can be influenced by the observed isotope effect previously reported for arabinose-1-^{14}C. The most reasonable explanation for this overlooked relationship would be that the isotope contributes to the formation of one anomer more than the other during mutarotation. Such an influence is compatible with the

earlier suggestion[5] that a difference in electronegativity between carbon-12 and carbon-14 could account for such isotope effects. Through this suggested mechanism an inductive effect would be expected to influence the non-bonded interaction between groups of the pentose with the consequence that the stability of one anomer more than the other would be augmented.

SUMMARY

The isotope effect observed during the countercurrent distribution of arabinose-1-^{14}C did not appear with the methyl glycoside of this labeled pentose. This observation along with the difference in the symmetry of plots for methyl α-D-mannoside and D-mannose were considered in the discussion of such isotope effects.

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