THE TAUTOMERIZATION AND MUTAROTATION OF β-L-ARABINOPYRANOSE. PARTICIPATION OF BOTH FURANOSE ANOMERS

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ABSTRACT

Both the α- and the β-furanose forms have been identified in aqueous solutions of L-arabinose by g.l.c., p.m.r., and optical rotation measurements on the trimethylsilylated sugar. The equilibrium composition in water at 25° is: α-pyranose, 57; β-pyranose, 30.5; α-furanose, 8; and β-furanose, 4.5%. In the tautomerization of β-L-arabinopyranose in water the rate constants for approach to the equilibrium level appear to be nearly the same for all components: $t_{1/2} = 6.8 \pm 0.4$ min at 25°. Thus the complex optical mutarotation of β-L-arabinopyranose remains unexplained.

INTRODUCTION

Efforts to analyze the changes in composition underlying the complex mutarotation of certain sugars were made during the 1920's and 1930's. These investigations, in which measurements were made of the changes in molar volume[1,2], refractive index[1,2], and solubility[3] of the mutarotating sugar, substantiated the idea that at least one tautomeric form other than the well-known α- and β-pyranose is involved. But they furnished no reliable clues to the number, identity, or proportion of the additional form(s). Fruitful work on these questions had to await the development of methods that could permit direct measurement of the proportion of each tautomer in a mutarotating sugar solution.

Following the advent of procedures for the gas–liquid chromatography (g.l.c.) of sugars[4,5], Acree *et al.* used this technique for a comprehensive study of the tautomerization of α-D-galactopyranose[6,7], and p.m.r. spectroscopy was similarly used by Lemieux and the present authors to examine 2-deoxy-β-D-*erythro*-pentopyranose[8]. These studies demonstrated the presence of all four of the possible ring-forms of the respective sugars in the tautomerizing solutions, and accomplished the measurement of the kinetics of appearance (or disappearance) of the individual tautomers.

We have used the g.l.c. method to study the tautomerization of β-L-arabinopyranose (**2a**). The results are presented in this paper.

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RESULTS

Optical mutarotation. — β-L-Arabinopyranose has long been known to have a complex mutarotation[9], which could be described by a three term, exponential equation:[10,11]

$$[\alpha]_D = A \times 10^{-m_1 t} + B \times 10^{-m_2 t} + C$$

At 20°, the values of the exponential constants found by Isbell and Pigman[11] are m_1, 0.0300, and m_2, 0.138.

In the present work, the mutarotation of β-L-arabinopyranose was followed at 25° with the aid of a flow system for filling the polarimeter cell. This made it possible for the instrument's recorder to register the rotation within 30 sec after the solvent (water) first contacted the sugar. The logarithmic plot obtained is shown in Fig. 1. It verifies the biphasic nature of the mutarotation. The value of m_1, equal to the slope of the later portion of the curve, is 0.045; the value of m_2, calculated as described by Isbell and Pigman[11], is 0.140. The initial specific rotation, $[\alpha]_D^{25}$, corrected for the small amounts of other tautomers apparently present in the sample, is +187.4°, as compared with $[\alpha]_D^{20} = +190.6°$ recorded by Isbell and Pigman.[11].

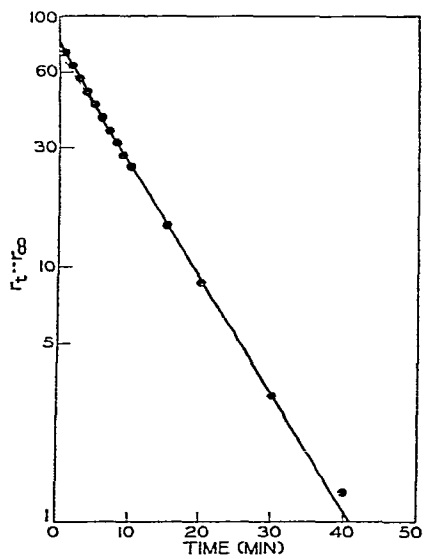
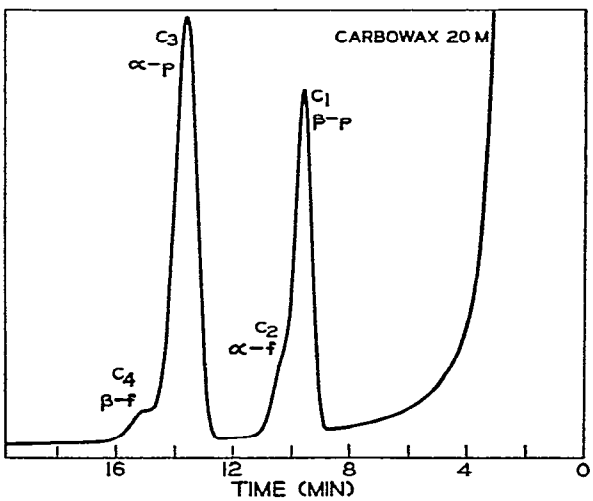


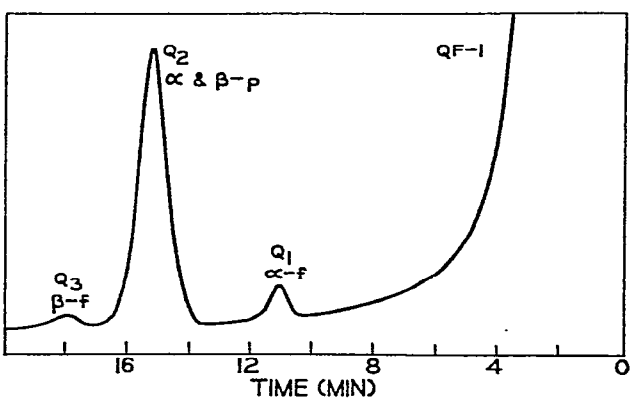
Fig. 1. Mutarotation of β-L-arabinopyranose in water at 25°. r_t and r_∞ are specific rotations at time t and at equilibrium, respectively. Each point is the average of values from three separate runs.

Composition at equilibrium. — Sweeley *et al.*[4] reported that trimethylsilylated arabinose showed three peaks by g.l.c. on columns of SE-52 and poly(ethylene glycol succinate), and investigations by p.m.r. have likewise revealed three signals for anomeric protons in equilibrated arabinose solutions[12,13]. Sawardeker and Slone-

ker[14], however, found evidence for four components by g.l.c. of the arabinose trimethylsilyl (Me_3Si) ethers over Carbowax 20M on Chromosorb W.

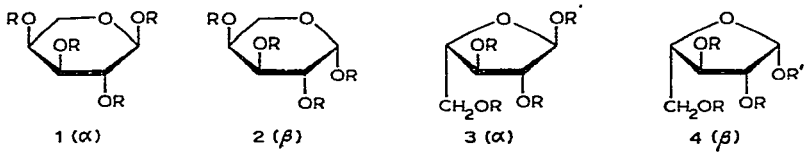
In our hands, a column packed with Carbowax 20M on Anakrom A gave the results shown in Fig. 2a. The major peaks, C_1 and C_3, were readily shown, by comparison of their retention times with those of Me_3Si ethers prepared from the pure anomers, to correspond with the β-pyranose and α-pyranose forms, respectively. The minor components, represented by shoulders C_2 and C_4 on the major peaks, were separated cleanly from the pyranose derivatives on a column packed with QF-1 on Anakrom A (Fig. 2b). On this column the pyranoses traveled as a single peak (Q_2). By preparative g.l.c. over QF-1, and chromatography on Carbowax of each of the fractions corresponding to peaks Q_1–Q_3, it was shown that peak Q_1 corresponds to shoulder C_2, and Q_3 to C_4.





Figs. 2a, b. Gas–liquid chromatograms of L-arabinose equilibrated in water at 25°, freeze dried, and trimethylsilylated.

For the characterization of the minor peaks, the mixed Me_3Si ethers were prepared from a sample of β-L-arabinopyranose tautomerized in warm pyridine to enhance the proportion of furanose forms. The approximate composition of the mixture was α-pyranose, 30; β-pyranose, 30; component Q_1, 30; and component Q_3, 10%. The p.m.r. spectrum of this mixture (Fig. 3) shows signals for anomeric protons (total intensity 1 proton) in the range τ 4.9–5.6. The doublets at τ 5.00 ($J_{1,2} = 1.3$ Hz) and 5.60 ($J_{1,2} = 5.4$ Hz) were readily shown to correspond to tetrakis-*O*-trimethylsilyl-β-L-arabinopyranose (**2b**) and -α-L-arabinopyranose (**1b**), respectively, by comparison with the spectra of the authentic pyranose Me_3Si ethers. The remaining, low-field lines are thus due to the other two tautomers, and comprise two overlapping doublets.



1a, 2a, R = H
1b, 2b, R = Me_3Si

3a, 4a, R = R' = H
3b, 4b, R = R' = Me_3Si
3c, 4c, R = H, R' = Me
3d, 4d, R = Me_3Si, R' = Me

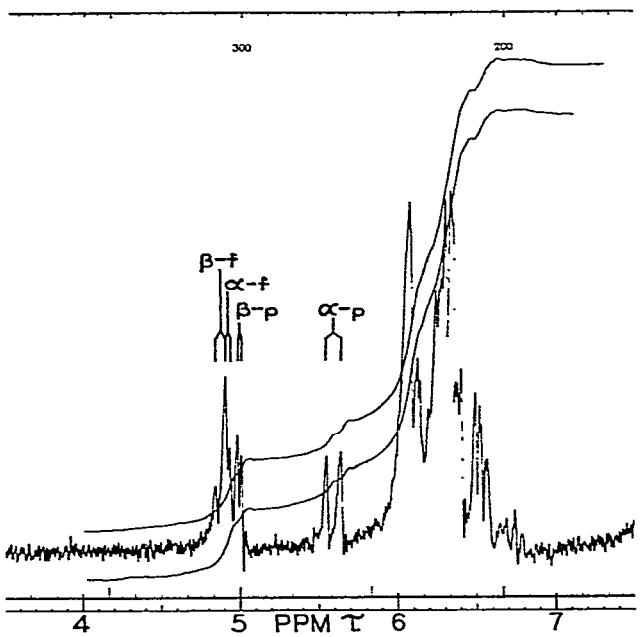


Fig. 3. P.m.r. spectrum, at 60 MHz, of a mixture of the four tetrakis-*O*-trimethylsilyl-L-arabinoses, enhanced in the furanose forms. Solvent, $CDCl_3$; external tetramethylsilane as reference.

Examination of the $J_{1,2}$ values for arabinofuranose derivatives of known anomeric configuration (Table I) leads to the assignment of the doublet at lowest field (τ 4.88, $J_{1,2} = 3.8$ Hz) to tetrakis-*O*-trimethylsilyl-β-L-arabinofuranose (**4b**), and the remaining signal (τ 4.92, $J_{1,2} = 1.7$ Hz) to the α-furanose anomer (**3b**). The possibility that one of the non-pyranose components is the hexakis-Me_3Si ether of the open-chain aldehydrol form seems remote in view of the large proportion of these components formed in anhydrous pyridine, and the similarity of their chromatographic mobilities to those of the pyranose derivatives. Since the doublet for the α-furanose is the more intense of the two furanose signals, it must correspond to component Q_1, and Q_3 must be the β-furanose.

TABLE I

$J_{1,2}$ VALUES FOR ARABINOFURANOSE DERIVATIVES

Substituents and their positions				$J_{1,2}$ (*Hz*)	*Ref.*
5	*3*	*2*	*1*		
α-Arabinofuranose derivatives (3 or enantiomer)					
H	H	H	Me	1.0	15
Bz	Bz	Bz	Me	<0.5	16
Me_3Si	Me_3Si	Me_3Si	Me	2.0	this work
β-Arabinofuranose derivatives (4 or enantiomer)					
H	H	H	Me	4.0	15
Bz	Bz	Ms	Bz	4.5	16
Me_3Si	Me_3Si	Me_3Si	Me	4.0	this work

Substantiation of these assignments was provided by optical-rotation measurements. Two mixtures containing different proportions of the several arabinose tautomers were prepared by keeping pyridine solutions of the β-pyranose for 3 h at room temperature and for 1 h at 75°, respectively. The Me_3Si ethers of these mixtures were isolated, and their compositions and specific rotations determined. The specific rotations of tetrakis-*O*-trimethylsilyl-α-L- and β-L-arabinopyranose were also measured. Solution of a pair of simultaneous equations then gave $[\alpha]_D^{25} = -45°$ for component Q_1 and $[\alpha]_D^{25} = +37°$ for Q_3. These values are not highly accurate because the ratio of the two furanoses was nearly the same in the two mixtures. However, they do show that component Q_1 is levorotatory, as expected for tetrakis-*O*-trimethylsilyl-α-L-arabinofuranose, and Q_3 is dextrorotatory, as expected for the β-anomer.

With components Q_1 (C_2) and Q_3 (C_4) thus identified, it was possible to establish the composition of solutions of L-arabinose at equilibrium in water at 25° as that shown in Table II.

Kinetics of tautomerization. — When the tautomerization of freshly prepared solutions of β-L-arabinopyranose was monitored by g.l.c. at 25°, the data shown in

TABLE II

RATE CONSTANTS AND EQUILIBRIUM VALUES FOR THE TAUTOMERIZATION OF β-L-ARABINOPYRANOSE IN WATER AT 25°

Component	k ± *S.E.* (*min*$^{-1}$)[a]	$t_{1/2}$ (*min*)[b]	*Equilibrium proportion* (% ± *S.E.*)
α-Pyranose	0.102 ± 0.005	6.8	56.7 ± 0.9
β-Pyranose	0.102 ± 0.004	6.8	30.7 ± 0.8
α-Furanose	0.108 ± 0.007	6.4	7.9 ± 0.2
β-Furanose	0.096 ± 0.013	7.2	4.7 ± 0.2

[a]First-order rate-constant for the approach of the component to its equilibrium proportion. Calculated with logarithms to the base *e*. S.E. = standard error. [b]For approach of the component to its equilibrium proportion.

Fig. 4 were obtained. It will be seen that α-pyranose, α-furanose, and β-furanose are formed simultaneously at the expense of the β-pyranose. The curves show no evidence of lag phases or maxima, but appear to follow a simple exponential course.

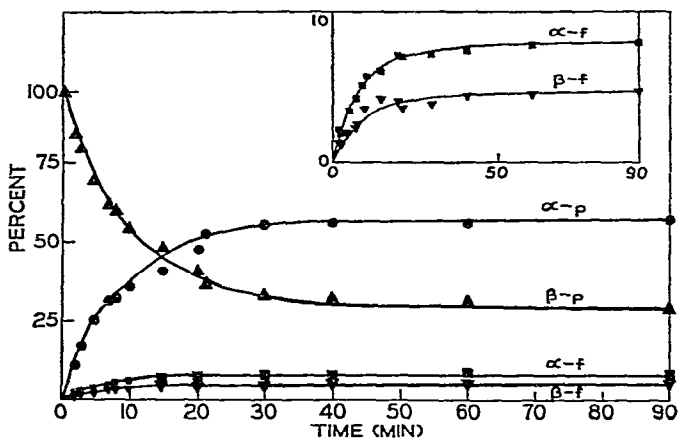


Fig. 4. Progress curves of the tautomerization of β-L-arabinopyranose in water at 25°. Each point is the average of values from three separate runs.

In view of the apparent exponential form of the progress curves, the data for each tautomer were replotted in logarithmic form, and the best straight lines were fitted to the data by a least-squares procedure* (Fig. 5). The slopes of these lines then give the rate constants for the approach of the individual tautomers to their equilibrium levels. These constants, and the associated half-times, are recorded in Table II.

*Devised by Prof. W. W. Cleland.

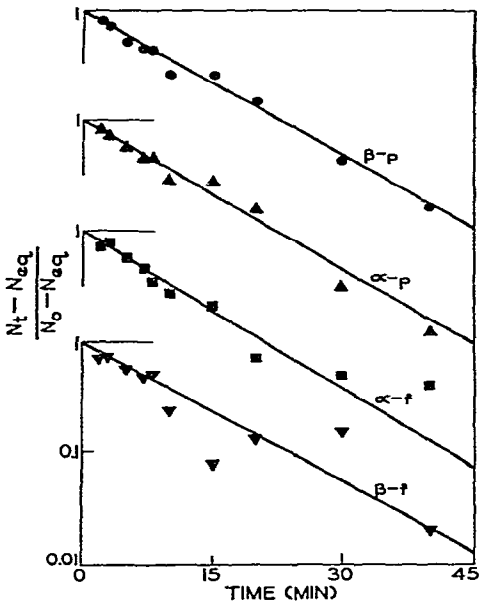


Fig. 5. Logarithmic replots of the progress curves. N_0, N_t, and N_{eq} are mole fractions at time zero, time t, and equilibrium, respectively.

DISCUSSION

It is clear from the examination by g.l.c. described here that when β-L-arabinopyranose is dissolved in water it is converted into a mixture containing three additional tautomeric forms of the sugar. The preponderant tautomer in the equilibrium mixture, as is well known, is the α-pyranose, and a substantial proportion of β-pyranose is also present. Evidence was adduced that the two minor tautomers are the α- and β-furanoses, which together account for about 13% of the total sugar at 25°. This value for the furanose content is substantially higher than that obtained by Angyal and Pickles[12] from p.m.r. measurements. The finding that the α-furanose is present in higher proportion than the β is in accord with the predictions of conformational analysis: the β-furanose is less stable because of its 1,2-*cis* configuration.

In our study, by g.l.c., of the kinetics of tautomerization of β-L-arabinopyranose, we found no evidence that the furanose forms approach their equilibrium levels more rapidly than the pyranoses. This is surprising in view of the complex mutarotation of the sugar, which is indicative of two or more simultaneous or successive processes going on with different rates. It has been generally supposed that the rapid initial phase of the complex mutarotation represents a period of furanose formation, with the slower, later phase corresponding to a period of pyranose–pyranose interconversion[17]. Indeed, the recent studies on D-galactose[7,18] and 2-deoxy-D-*erythro*-pentose[8] show that, in tautomerizing solutions of these sugars,

the progress toward equilibrium of the furanoses is somewhat more rapid than that of the pyranoses. Although it is not clear that the biphasic optical mutarotations of these sugars are quantitatively accounted for by the higher rates of progress of the furanoses[18], it is intuitively evident that a biphasic mutarotation-plot will *not* be generated by a set of four simultaneous processes with nearly identical rate-constants. This deduction has been confirmed by a computer simulation of the arabinose system[18].

There appear to be two possible explanations for the discrepancy between the observed optical rotatory changes and the changes in composition of tautomerizing solutions of arabinose. One is that the g.l.c. analyses are in error, perhaps because of changes in the composition of the samples during freeze drying. Evidence against this explanation is that, when the procedure was applied to pure anomers, only one component was detected. Also, one would expect increased proportions of furanose forms to result from tautomerization in the syrup phase[19], whereas the relative amounts of furanoses found were less than expected.

Another possibility is the rapid initial formation of an as yet undetected fifth component of the mixture. This would necessarily be the open-chain *aldehydo* form or its hydrate. Further experiments will have to be done to settle these points.

EXPERIMENTAL

Materials. — Commercial *β-L-arabinopyranose* (**2a**) was recrystallized from ethanol; m.p. 160° (lit.[20], 160°); $[\alpha]_D$, see *Optical rotation.* G.l.c. analysis of a trimethylsilylated sample showed the presence of *ca.* 1% of α-pyranose and 0.5% of each of the furanoses. *α-L-Arabinopyranose*·$CaCl_2 \cdot 4H_2O$ was prepared by the method of Montgomery and Hudson, crystallization being accomplished by slow evaporation of the solvent; m.p. 206° (lit.[9], 204°). G.l.c. analysis showed the presence of *ca.* 5% (total) of other anomers.

Tetrakis-O-trimethylsilyl-β-L-arabinopyranose (**2b**), liquid, $[\alpha]_D^{25}$ +72° ($CHCl_3$), p.m.r. ($CDCl_3$) τ 4.99 ppm (d, 1, $J = 1.5$ Hz, H-1), and *tetrakis-O-trimethylsilyl-α-L-arabinopyranose* (**1b**), liquid, $[\alpha]_D^{25}$ +14° ($CHCl_3$) (*ca.* 5% of other anomers), p.m.r. ($CDCl_3$) τ 5.58 ppm (d, 1, $J = 5.4$ Hz, H-1), were prepared by treating suitable portions of the crystalline sugars with the silylating reagent. Excess reagent was evaporated off in vacuum, the residue was taken up in chloroform, the mixture was filtered to remove ammonium chloride, and the chloroform was evaporated off in vacuum. Samples for rotation measurements were distilled under high vacuum.

Methyl α-L-arabinofuranoside (**3c**) (syrup) and *methyl β-L-arabinofuranoside* (**4c**) (syrup) were prepared as described by Augestad and Berner[21]. They were converted into their tris-*O*-trimethylsilyl derivatives, **3d** and **4d** respectively, by the procedure used for the pyranose sugars.

Optical rotation. — Mutarotation was measured at 589 nm with a Perkin–Elmer model 141 polarimeter equipped with a Leeds and Northrup 25-cm Speedomax W Azar recorder. Sugar (0.2–0.4 g) was placed on the sintered-glass disc *c* of the

three-bulb dissolving chamber shown in Fig. 6, and bulb *A* was charged with 25 ml of distilled water. Bulbs *A* and *B* were then stoppered, tubing was attached to *C*, and the chamber was immersed in a water bath thermostatted at 25°. After thermal equilibration the water was drawn over the sugar into bulb *C* by application of vacuum at *a*. After a few seconds of stirring (magnetic) the sugar solution was drawn by suction into the polarimeter cell (10 cm path length) through a water-jacketed tube attached at *b*. The concentration of the sugar solution was determined from its equilibrium rotation.

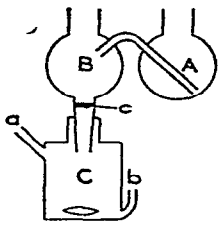


Fig. 6. Dissolving chamber. Each of the bulbs *A*, *B*, and *C* has a volume of *ca.* 50 ml.

Gas–liquid chromatography. — For the kinetic studies, samples of the thermostatted sugar solution, containing 4–8 mg of sugar, were discharged at the desired times into vials that were kept in the water bath. The temperature was 25°. Each vial was immediately removed and the contents quickly frozen by swirling the vial in a Dry Ice–acetone bath. After freeze drying for 1–2 h the samples were promptly trimethylsilylated.

The silylation reagent consisted of pyridine (stored over KOH), chlorotrimethylsilane, and hexamethyldisilazane in the ratio 7:1:2 (*v/v/v*).

The instrument used was an Aerograph Hy-Fi model 600D gas chromatograph equipped with a hydrogen flame-ionization detector and a Disc integrator. The Carbowax column (11% Carbowax 20M on Anakrom A, 3.7 m × 3.2 mm o.d., aluminum) was run at 150°. The QF-1 column (5.5% QF-1 on Anakrom A, 1.8 m × 3.2 mm o.d., aluminum) was run at 130°. The carrier gas was nitrogen at 25 ml per min. The areas under the peaks were determined either from the integrator trace or by multiplying width at half-height by height. It was assumed that the isomeric Me_3Si-derivatives all gave the same detector response per unit weight. The percentages of the furanose forms were measured on the QF-1 chromatograms, and these were subtracted from the corresponding values for the two peaks of the Carbowax chromatograms to give the percentages of pyranoses.

ACKNOWLEDGMENTS

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