Carbohydrate Research, 40 (1975) 235–253

MATHEMATICAL ANALYSIS OF THE MUTAROTATION OF SUGARS

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(Received October 28th, 1973; accepted in revised form, September 1st, 1974)

ABSTRACT

The tautomerism of sugars showing complex mutarotation has been analyzed mathematically based on a scheme of three components. More-complete but complex schemes are shown to be reducible to the three-component scheme, because the supposed intermediates of interconversion can be neglected in the kinetics when their equilibrium contents are very small and because two furanose tautomers can be regarded as one component under appropriate conditions. The kinetics of the three-component scheme have been fully studied and are shown to explain successfully the tautomerism of most of the sugars. The tautomerism of D-galactose has been analyzed in especial detail. From the polarimetric data and the equilibrium content of α-pyranose, the kinetic rate-constants for D-galactose were determined and found consistent with all other experimental data. The slow and fast processes of complex mutarotation of D-galactose are shown to correspond approximately to pyranose–pyranose and pyranose–furanose interconversions, respectively.

INTRODUCTION

The mutarotation of a sugar is the polarimetric change in a freshly prepared solution, and it reflects the first-order interconversions between several tautomers of the sugar. Several monosaccharides such as glucose, gulose, and mannose show "simple mutarotation", as expressed by Eq. *1*:

$$[\alpha]_t = Ae^{-mt} + [\alpha]_\infty \qquad (1),$$

where $[\alpha]_t$ and $[\alpha]_\infty$ are respectively the specific rotation of the sugar at time t and at equilibrium, m is the rate constant of mutarotation, and A is a constant coefficient.

Solutions of sugars in this group are known to contain two anomeric forms, α- and β-pyranoses, with only small proportions of other tautomers[1,2]. The kinetics of interconversion can be fully described by Scheme I and Eq. *1*:

$$X \underset{k_{yx}}{\overset{k_{xy}}{\rightleftarrows}} Y$$

Scheme I

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where X and Y denote α- and β-pyranose forms, respectively, and

$$m = k_{xy} + k_{yx} \tag{2}$$

On the other hand, many monosaccharides, such as galactose, arabinose, and altrose, show "complex mutarotation" which is approximately expressed by an equation[1] having two exponential terms, namely by Eq. *3*:

$$[\alpha] = Ae^{-m_1 t} + Be^{-m_2 t} + [\alpha]_\infty \tag{3}$$

Smith and Lowry[3] analyzed the "complex mutarotation" of D-galactose based on the Scheme II,

$$X \rightleftarrows \mu \rightleftarrows Y,$$

Scheme II

where μ is the (presumed) intermediate open-chain form (aldehyde form) of the sugar. The equilibrium relative-content of μ estimated by Smith and Lowry was 12%, which is larger by far than the experimental value of 0.082% obtained polarographicically by Cantor and Peniston[4]. Riiber and Minsaas[5] analyzed the dilatometric change of freshly dissolved solutions of α- and β-galactopyranose, also on the basis of Scheme II, and obtained 27.35% for the relative content of μ at equilibrium. This value agreed neither with the estimate by Smith and Lowry[3] nor with the experimental value.

The foregoing results show that Scheme II is not adequate for analyzing complex mutarotation. It is now well known, that equilibrium solution of many sugars, especially those showing complex mutarotation, contain considerable proportions of furanose forms as well as pyranoses[6,8]. Several experimental approaches to relate the slow and fast rate-constants m_1 and m_2 in Eq. *3* to pyranose–pyranose and pyranose–furanose interconversions, respectively, have been made[7,8], but no successful theoretical interpretations have been given. Anderson and Garver[9] recently performed computer simulations of complex mutarotations, based on more-realistic schemes that included furanoses (Schemes IV and V in Section A). They determined many sets of kinetic constants that can explain the change of tautomers with time observed by the gas–liquid chromatographic (g.l.c.) technique. However, they were unable to explain at the same time both optical rotation and g.l.c. data without introducing components other than pyranoses, furanoses, and an open-chain form.

In this paper, we show that the three-component system of Scheme III can successfully explain the complex optical rotation experimentally observed, the change of tautomers observed by g.l.c. and by the dilatometric changes of solutions of galactose, and that the analysis based on this scheme:

$$\begin{tikzcd}
X \arrow[rr, shift left] \arrow[dr, shift left] & & Y \arrow[ll, shift left] \arrow[dl, shift left] \\
& Z \arrow[ul, shift left] \arrow[ur, shift left] &
\end{tikzcd}$$

Scheme III

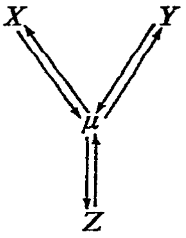
where Z stands collectively for the two furanose forms, can be applied to most of the monosaccharides.

In the first Section (A), it is shown that the intermediates whose equilibrium contents are negligibly small can be neglected in the kinetics of mutarotation. In the next Section (B), the conditions under which the more-general four-component scheme can be reduced to a three-component scheme are investigated. In Section C, full mathematical descriptions of Scheme III are presented. In Section D, the formula expressing optical rotation is given. Next, in Section E, the kinetic behavior of three tautomers of D-galactose is analyzed from the polarimetric data and the equilibrium content of one component measured by g.l.c.[8]. It is shown that the equilibrium content of other components agree with the experimental data and that slow and fast processes of complex mutarotation in the solution of D-galactose corrrespond approximately to pyranose–pyranose and pyranose–furanose interconversions, respectively.

RESULTS AND DISCUSSION

A. Omission of intermediate open-chain forms

We start with Scheme IV, namely:



Scheme IV

where μ is the intermediate open-chain form of a sugar. Indicating, for example, the first-order kinetic constant of the reaction, $X \rightarrow \mu$ as $k_{x\mu}$, the differential equations of Scheme IV can be written as:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = -k_{x\mu}\cdot x + k_{\mu x}\cdot \mu \qquad (4a),$$

$$\frac{\mathrm{d}y}{\mathrm{d}t} = -k_{y\mu}\cdot y + k_{\mu y}\cdot \mu \qquad (4b),$$

$$\frac{\mathrm{d}z}{\mathrm{d}t} = -k_{z\mu}\cdot z + k_{\mu z}\cdot \mu \qquad (4c),$$

and $$\frac{\mathrm{d}\mu}{\mathrm{d}t} = -(k_{\mu x}+k_{\mu y}+k_{\mu z})\cdot \mu + k_{x\mu}\cdot x + k_{y\mu}\cdot y + k_{z\mu}\cdot z \qquad (4d),$$

where x, y, z, and μ denote the concentrations of the components X, Y, Z, and μ, respectively. We assume that the equilibrium value of μ (indicated by μ_∞) is small enough in comparison with x_∞, y_∞ and z_∞, that is to say:

$$\frac{\mu_\infty}{x_\infty} = \frac{k_{x\mu}}{k_{\mu x}} \ll 1, \quad \frac{\mu_\infty}{y_\infty} = \frac{k_{y\mu}}{k_{\mu y}} \ll 1, \quad \text{and} \quad \frac{\mu_\infty}{z_\infty} = \frac{k_{z\mu}}{k_{\mu z}} \ll 1. \tag{5}$$

Under these conditions, μ rapidly attains a steady-state value in a characteristic time $T = 1/(k_{\mu x} + k_{\mu y} + k_{\mu z})$ (see the last paragraph of Appendix I), which is too short to detect. Thus, we can equate the right-hand side of Eq. *4d* to zero and obtain:

$$\mu = \frac{1}{k_{\mu x} + k_{\mu y} + k_{\mu z}} (k_{x\mu} \cdot x + k_{y\mu} \cdot y + k_{z\mu} \cdot z) \tag{6}$$

Introducing Eq. *6* into Eqs. *4*, we obtain:

$$\frac{dx}{dt} = -(k_{xy} + k_{xz})x + k_{yx} \cdot y + k_{zx} \cdot z \tag{7a}$$

$$\frac{dy}{dt} = k_{xy} \cdot x - (k_{yx} + k_{yz})y + k_{zy} \cdot z \tag{7b}$$

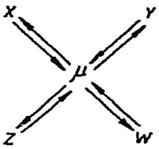
and
$$\frac{dz}{dt} = k_{xz} \cdot x + k_{yz} \cdot y - (k_{zx} + k_{zy})z \tag{7c}$$

where we put

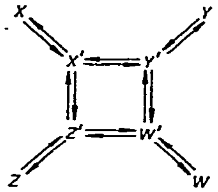
$$\left.\begin{aligned}
k_{xy} &= \frac{k_{x\mu} \cdot k_{\mu y}}{k_{\mu x} + k_{\mu y} + k_{\mu z}} \\
k_{yx} &= \frac{k_{y\mu} \cdot k_{\mu x}}{k_{\mu x} + k_{\mu y} + k_{\mu z}} \\
k_{zx} &= \frac{k_{z\mu} \cdot k_{\mu x}}{k_{\mu x} + k_{\mu y} + k_{\mu z}} \\
k_{xz} &= \frac{k_{x\mu} \cdot k_{\mu z}}{k_{\mu x} + k_{\mu y} + k_{\mu z}} \\
k_{yz} &= \frac{k_{y\mu} \cdot k_{\mu z}}{k_{\mu x} + k_{\mu y} + k_{\mu z}} \\
k_{zy} &= \frac{k_{z\mu} \cdot k_{\mu y}}{k_{\mu x} + k_{\mu y} + k_{\mu z}}
\end{aligned}\right\} \tag{8}$$

Differential equations *7a–7c* are just the same as those for Scheme III. It is thus concluded that Scheme IV reduces to Scheme III, when the equilibrium value of μ is negligibly small. A stricter mathematical proof of this is given in Appendix I.

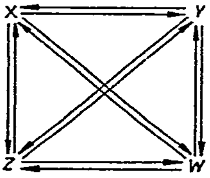
The result obtained here can easily be extended to the more-complicated schemes. Both Scheme V, which shows interconversion of four tautomers (two pyranoses and two furanoses) through an intermediate μ, and Scheme VI, which was proposed by Isbell and Pigman[10] (where X', Y', Z', and W' are the intermediates of interconversion), reduce to Scheme VII, if the equilibrium content of the intermediate μ in Scheme V (or X', Y', Z', and W' in Scheme VI), are negligibly small.



Scheme V



Scheme VI



Scheme VII

The equilibrium content of open-chain forms, which have been considered to be intermediates of interconversion of sugars, are generally found to be very small (less than 0.5%), except for a few sugars such as D-ribose and L-allose[4]. Therefore, open-chain forms or other intermediates may be neglected in the analysis of mutarotations of the most of sugars.

B. The four-component scheme and conditions for its reduction to the three-component scheme

The differential equations of Scheme VII are given as:

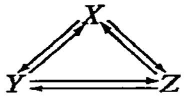
$$\frac{\mathrm{d}x}{\mathrm{d}t} = -(k_{xy}+k_{xz}+k_{xw})x + k_{yx}\cdot y + k_{zx}\cdot z + k_{wx}\cdot w \qquad (9a),$$

$$\frac{\mathrm{d}y}{\mathrm{d}t} = k_{xy}\cdot x - (k_{yx}+k_{yz}+k_{yw})y + k_{zy}\cdot z + k_{wy}\cdot w \qquad (9b),$$

$$\frac{\mathrm{d}z}{\mathrm{d}t} = k_{xz}\cdot x + k_{yz}\cdot y - (k_{zx}+k_{zy}+k_{zw})z + k_{wz}\cdot w \qquad (9c),$$

and

$$\frac{\mathrm{d}w}{\mathrm{d}t} = k_{xw}\cdot x + k_{yw}\cdot y + k_{zw}\cdot z - (k_{wx}+k_{wy}+k_{wz})w \qquad (9d).$$

At equilibrium, there must be no circular flow in each of four triangular circuits such as  in Scheme VII, and thus:

$$\frac{k_{xy}}{k_{yx}}=\frac{y_\infty}{x_\infty},\quad \frac{k_{zx}}{k_{xz}}=\frac{x_\infty}{z_\infty},\quad \text{and}\quad \frac{k_{yz}}{k_{zy}}=\frac{z_\infty}{y_\infty} \qquad (10).$$

By multiplying the foregoing together and simplifying, we obtain:

$$k_{xy}\cdot k_{yz}\cdot k_{zx}=k_{xz}\cdot k_{zy}\cdot k_{yx} \qquad (11a).$$

Two more, independent relations are obtained in the same way:

$$k_{xy}\cdot k_{yw}\cdot k_{wx}=k_{xw}\cdot k_{wy}\cdot k_{yx} \qquad (11b),$$

and $$k_{yz}\cdot k_{zw}\cdot k_{wy}=k_{yw}\cdot k_{wz}\cdot k_{zy} \qquad (11c).$$

Thus, nine out of twelve kinetic-constants in Eqs. *9* are independent. The solution of Eqs. *9* contain three exponential terms and the three rate-constants are given as the roots of a cubic equation.

On the other hand, experimental data for complex mutarotation can all be approximately described by two exponential terms, and the third exponential term is, within experimental error, not necessary[1]. Moreover, the rate constants determined by starting from the α-pyranose are approximately the same as those from the β-pyranose[1]. These results strongly suggest that each optically active tautomer existing in nonnegligible amount in the mutarotating solution can be described approximately by the same two exponential terms.

The conditions under which Scheme VII is expressed by two exponential terms are the following three cases.

The first (case I): one of the four components (possibly, one of the two furanoses) is negligible at equilibium. In this case, Scheme VII reduces to Scheme III in the same way as described in section A.

The second (case II): four components exist in appreciable proportion at equilibrium but proper relations hold between kinetic constants. From consideration of the differential equations *9*, it is determined that necessary and sufficient conditions under which kinetics of every one of the four components is described by the same two exponential terms, starting from either of the two pyranoses (designated as *X* and *Y*, respectively) or from a mixture of the two, are the following relations given in Eqs. *12* and *13* or in Eqs. *12* and *14*:

$$\left.\begin{aligned} k_{zx} &= k_{wx} \\ k_{zy} &= k_{wy} \end{aligned}\right\} \qquad (12),$$

$$k_{xz}=k_{xw} \qquad (13),$$

and $$k_{xz}=k_{yz}=k_{wz} \qquad (14).$$

The proof of this is not difficult, but is rather tedious. An outline is given in Appendix II.

The third (case III): one of the three rate-constants (for example, m_3) is too high to detect experimentally. However, if we assume that the kinetic constants of furanose–furanose interconversions are of the same order as those of pyranose–furanose interconversions, based on several experimental results[1], this situation is unlikely to occur.

The conditions of cases I and II, under which Scheme VII is reduced to Scheme III, might be approximately satisfied in many sugars showing complex mutarotation because their mutarotation curves are almost completely expressed by two exponential terms. In case II, the third component z in Scheme III can be regarded as the sum of two furanose tautomers. In the latter sections, we will deal with two furanoses as one component, z.

C. *Kinetics of the three-component scheme*

In this section, the solutions of Eqs. *7a*, *b*, and *c* for Scheme III are given under the condition that the total amount of sugar in the solution is constant. The constant amount is put to be equal to 1 for convenience in calculation, so that:

$$x+y+z=1 \qquad (15).$$

The solutions have the following forms when the two rate-constants are not equal[15] ($m_1 \neq m_2$):

$$x = A_1 \cdot \mathrm{e}^{-m_1 t} + A_2 \cdot \mathrm{e}^{-m_2 t} + x_\infty \qquad (16a),$$

$$y = B_1 \cdot \mathrm{e}^{-m_1 t} + B_2 \cdot \mathrm{e}^{-m_2 t} + y_\infty \qquad (16b),$$

and $$z = C_1 \cdot \mathrm{e}^{-m_1 t} + C_2 \cdot \mathrm{e}^{-m_2 t} + z_\infty \qquad (16c),$$

where the rate constants m_1 and m_2 are the roots of the next equation:

$$m^2 - Pm + Q = 0 \qquad (17),$$

where

$$P = m_1 + m_2 = k_{xy} + k_{yx} + k_{zx} + k_{xz} + k_{yz} + k_{zy} \qquad (18),$$

and $$Q = m_1 \cdot m_2 = k_{xy} \cdot k_{yz} + k_{xy} \cdot k_{zx} + k_{xy} \cdot k_{zy} + k_{xz} \cdot k_{yx} + k_{xz} \cdot k_{yz} + k_{xz} \cdot k_{zy} + \\ + k_{yx} \cdot k_{zx} + k_{yz} \cdot k_{zy} + k_{yx} \cdot k_{zx} \qquad (19).$$

The equilibrium value of each component (x_∞, y_∞, and z_∞) is given as:

$$x_\infty = \frac{1}{Q} (k_{yx} \cdot k_{zx} + k_{yx} \cdot k_{zy} + k_{yz} \cdot k_{zx}) \qquad (20a),$$

$$y_\infty = \frac{1}{Q} (k_{xy} \cdot k_{zx} + k_{xy} \cdot k_{zy} + k_{xz} \cdot k_{zy}) \qquad (20b),$$

and $$z_\infty = \frac{1}{Q}(k_{xy}\cdot k_{yz}+k_{xz}\cdot k_{yx}+k_{xz}\cdot k_{yz}) \qquad (20c).$$

From the condition of no circulation at equilibrium, Eq. *11a* in Section A, is obtained. Thus, among six kinetic-constants, five are independent. The coefficients A_i, B_i, and C_i ($i=1, 2$) are determined when the initial conditions are given. Here, we adopt the most simple and useful case; only one anomeric from is present at first:

$$x_0 = 1, \quad \text{and} \quad y_0 = z_0 = 0 \qquad (21).$$

Under these initial conditions, coefficients A_1 and A_2 are obtained by differentiating Eq. *16a* with respect to time, and by using *16a* and the resulting equation as simultaneous equations in A_1 and A_2. In both equations, t is set equal to zero, and the value of $\mathrm{d}x/\mathrm{d}t$ at $t=0$ is found from Eq. *7a*. The B_i and C_i values are found similarly, giving,

$$A_1 = \frac{1}{m_2-m_1}\{(y_\infty+z_\infty)m_2-(k_{xy}+k_{xz})\} \qquad (22a)$$

$$A_2 = \frac{-1}{m_2-m_1}\{(y_\infty+z_\infty)m_1-(k_{xy}+k_{xz})\} \qquad (22b),$$

$$B_1 = \frac{-1}{m_2-m_1}(y_\infty\cdot m_2-k_{xy}) \qquad (22c),$$

$$B_2 = \frac{1}{m_2-m_1}(y_\infty\cdot m_1-k_{xy}) \qquad (22d),$$

$$C_1 = \frac{-1}{m_2-m_1}(z_\infty\cdot m_2-k_{xz}) \qquad (22e),$$

and $$C_2 = \frac{1}{m_2-m_1}(z_\infty\cdot m_1-k_{xz}) \qquad (22f).$$

A more-general solution is given elsewhere[14].

Summing Eqs. *16a*, *b*, and *c* and using relation *15*, we obtain a relation of coefficients at any initial conditions as:

$$A_i+B_i+C_i=0 \qquad (i=1, 2) \qquad (23).$$

When $m_1 = m_2 \equiv m$, that is, the condition

$$P^2-4Q=0$$

holds, a special solution is obtained which is included in Eqs. *16* and *22* as the limit of $(m_1-m_2 \to 0)$.

D. Optical rotation

The specific rotation of a sugar solution at time t ($[\alpha]_t$) is given by

$$[\alpha]_t = x \cdot \alpha_x + y \cdot \alpha_y + z \cdot \alpha_z \qquad (24),$$

where α_x, α_y, and α_z are respectively the specific rotation of pure solutions of x, y, and z.

Substituting Eqs. *16* into Eq. *24* and then Eqs. *22* into the resulting equation, and simplifying, gives:

$$[\alpha]_t = \frac{1}{m_2 - m_1}\{(\alpha_x - \alpha_y)(y_\infty \cdot m_2 - k_{xy}) + (\alpha_x + \alpha_z)(z_\infty \cdot m_2 - k_{xz})\}\, e^{-m_1 t} -$$

$$- \frac{1}{m_2 - m_1}\{(\alpha_x - \alpha_y)(y_\infty \cdot m_1 - k_{xy}) + (\alpha_x - \alpha_z)(z_\infty \cdot m_1 - k_{xz})\}\, e^{-m_2 t} +$$

$$+ [\alpha]_\infty \qquad (25),$$

where

$$[\alpha]_\infty = x_\infty \cdot \alpha_x + y_\infty \cdot \alpha_y + z_\infty \cdot \alpha_z \qquad (26).$$

E. Mutarotation of D*-galactose*

Based on the results of the foregoing sections, there is analyzed here the mutarotation of D-galactose, which shows complex mutarotation and is one of the sugars investigated most intensively. From the experimental data and the condition *11a*, six kinetic-constants of Scheme III will be obtained, and equations *16* for each component will be reconstructed.

As experimental data, we employed two mutarotation curves (at 20°) starting from α-galactopyranose (denoted X) and β-galactopyranose (Y), respectively, obtained by Isbell and Pigman[11]. Although the slow and fast rate-constants (m_1 and m_2) starting from the α-pyranose were somewhat different from those starting from the β-pyranose, we have adopted the average of the two and define m_1 and m_2 as 0.0187 and 0.193 (min^{-1}), respectively. The specific rotation of the α-pyranose (α_x), β-pyranose (α_y), and that of the solution at equilibrium ($[\alpha_\infty]$) were given as +150.7°, +52.8°, and +80.2°, respectively. Two coefficients of mutarotation, A and B of Eq. *3* were given[11] as 64.9° and 5.6°, respectively. Hence,

$$\frac{1}{m_2 - m_1}\{(\alpha_x - \alpha_y)(y_\infty \cdot m_2 - k_{xy}) + (\alpha_x - \alpha_z)(z_\infty \cdot m_2 - k_{xz})\} = 64.9° \qquad (27a),$$

and
$$\frac{-1}{m_2 - m_1}\{(\alpha_x - \alpha_y)(k_\infty \cdot m_1 - k_{xy}) + (\alpha_x - \alpha_z)(z_\infty \cdot m_1 - k_{xz})\} = 5.6° \qquad (27b).$$

Similarly, the coefficients starting from the β-pyranose were given[11] as −32.2° and 4.9°. By deriving the A_i, B_i, and C_i values for the condition $y_0 = 1$, and $x_0, z_0 = 0$, an equation similar to Eq. *25* can be written. Eqs. *28a*, and *b* may then be derived in the same way as *27a* and *b*.

$$\frac{1}{m_2-m_1}\{(\alpha_y-\alpha_x)(x_\infty\cdot m_2-k_{yx})+(\alpha_y-\alpha_z)(z_\infty\cdot m_2-k_{yz})\} = -32.3^\circ \qquad (28a),$$

$$\frac{-1}{m_2-m_1}\{(\alpha_y-\alpha_x)(x_\infty\cdot m_1-k_{yx})+(\alpha_y-\alpha_z)(z_\infty\cdot m_1-k_{yz})\} = 4.9^\circ \qquad (28b).$$

The equation obtained by adding Eq. *27a* to Eq. *27b* or the one obtained by adding Eq. *28a* to Eq. *28b* are essentially the same as Eq. *26* equated to 80.2°, and so only three equations are independent among five Eqs. *27a* and *b*, *28a* and *b*, and *26* equated to 80.2°. There are seven unknown quantities: six k_{ij} values and α_z, and the experimentally obtained independent relations are five; namely the values of α_x, α_y and $[\alpha]_\infty$ and Eqs. *27a* and *28a*. As Eq. *11a* holds between k_{ij} values, we need one more relation to solve the problem completely.

Recently, Acree *et al.*[8] analyzed by g.l.c. the *O*-trimethylsilyl derivatives of D-galactose from a mutarotating solution, and they determined the equilibrium content of each tautomer at 25° and the kinetic properties at 15°. From their data, we estimated the equilibrium compositions x_∞, y_∞, and z_∞ (sum of furanoses) at 20°, as 0.321, 0.642, and 0.037 (β-furanose: 2.7% and α-furanose: 1.0%), respectively.

We tentatively adopt the value of x_∞ as an additional experimental datum to solve the problem. The value of y_∞ and z_∞ will be derived theoretically and be compared with the experimental values to check the consistency of the theory. The outline for solving the equations is given in Appendix III. The results obtained are as follows,

$$\left.\begin{aligned} z_\infty &= 0.0313 \\ y_\infty &= 0.6477 \end{aligned}\right\} \qquad (29),$$

$$\alpha_z = -76.01^\circ \qquad (30),$$

and

$$\left.\begin{aligned} k_{xy} &= 0.8274\times10^{-2}\ \text{min}^{-1} \\ k_{yx} &= 0.4100\times10^{-2}\ \text{min}^{-1} \\ k_{xz} &= 0.6543\times10^{-2}\ \text{min}^{-1} \\ k_{zx} &= 6.720\times10^{-2}\ \text{min}^{-1} \\ k_{yz} &= 0{,}5798\times10^{-2}\ \text{min}^{-1} \\ k_{zy} &= 12.015\times10^{-2}\ \text{min}^{-1} \end{aligned}\right\} \qquad (31).$$

The equilibrium contents of furanose (z_∞) and β-pyranose (y_∞) obtained in Eq. *29* are in rather good agreement with the values experimentally estimated, but the theoretical content of furanoses is about 0.6% smaller than the experimental value. Taking into account experimental errors, such as those included in the measurement of the area of the small g.l.c. peak for the α-furanose, the foregoing small difference is not significant.

The specific rotation of the furanoses (α_{-}) is strongly levoratatory, and the pure β-furanose is the more strongly levorotatory.

It is worth noting that the rate constants for pyranose–pyranose interconversion, $k_{xy}+k_{yx}$ (1.24×10^{-2}) are much smaller than those for pyranose–furanose interconversions; $k_{xz}+k_{zx}$ (7.37×10^{-2}) or $k_{yz}+k_{zy}$ (12.6×10^{-2}).

From Eqs. *31* it is possible to reconstruct the equations of interconversion *16a*, *b*, and *c* starting from the α-pyranose ($x_0 = 1$ and $y_0 = z_0 = 0$):

$$\left.\begin{aligned} x &= 0.6667\cdot e^{-0.0187t}+0.0123\cdot e^{-0.193t}+0.3210 \\ y &= -0.6695\cdot e^{-0.0187t}+0.0218\cdot e^{-0.193t}+0.6477 \\ z &= 0.0028\cdot e^{-0.0187t}-0.0341\cdot e^{-0.193t}+0.0313 \end{aligned}\right\} \quad (32).$$

The curves of $(x-x_\infty)/(1-x_\infty)$, y/y_∞, and z/z_∞ are plotted against time in Fig. 1, comparing with slow and fast, simple, exponential processes of mutarotation having

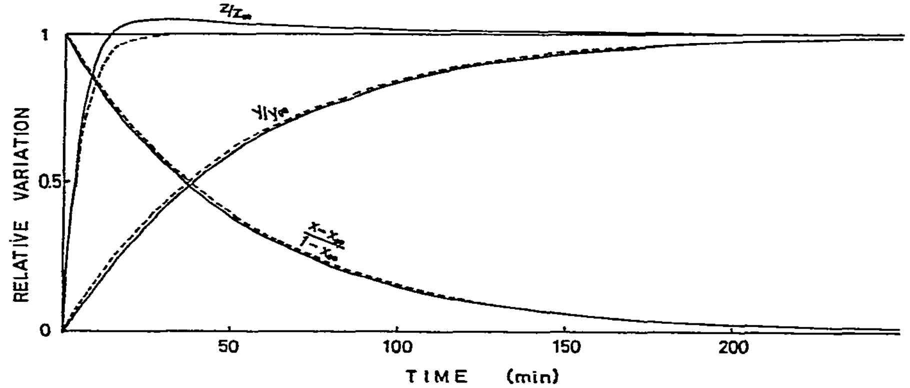


Fig. 1. Calculated time-course of the normalized variation of each tautomer of D-galactose, starting from the α-pyranose. Concentration of α-pyranose, β-pyranose, and furanose are denoted by x, y, and z, respectively, and x_∞, y_∞, and z_∞ designate their equilibrium values. Dotted lines show slow and fast processes of mutarotation, having rate-constants m_1 and m_2, respectively.

the rate constants m_1 and m_2. Disappearance of the α-pyranose and production of β-pyranose are approximately expressed by the slow processes, whereas production of "furanose" is nearly described by the fast processes. This is also in good agreement with the recent g.l.c. data of Acree *et al.*[8] giving direct measurement of interconversions of each component.

Equations starting from the β-pyranose ($y_0 = 1$, $x_0 = z_0 = 0$) are obtained in the same way, from Eq. *31* and the appropriate expressions for A_i, B_i, and C_i.

$$\left.\begin{aligned} x &= -0.3318\cdot e^{-0.0187t}+0.0108\cdot e^{-0.193t}+0.0321 \\ y &= 0.3332\cdot e^{-0.0187t}+0.0191\cdot e^{-0.193t}+0.6477 \\ z &= -0.0014\cdot e^{-0.0187t}-0.0299\cdot e^{-0.193t}+0.0313 \end{aligned}\right\} \qquad (33).$$

The time-course of normalized variation of each component is given in Fig. 2. Again, changes of the pyranoses are approximated by the slow process, whereas the production of furanose is nearly equal to the fast process.

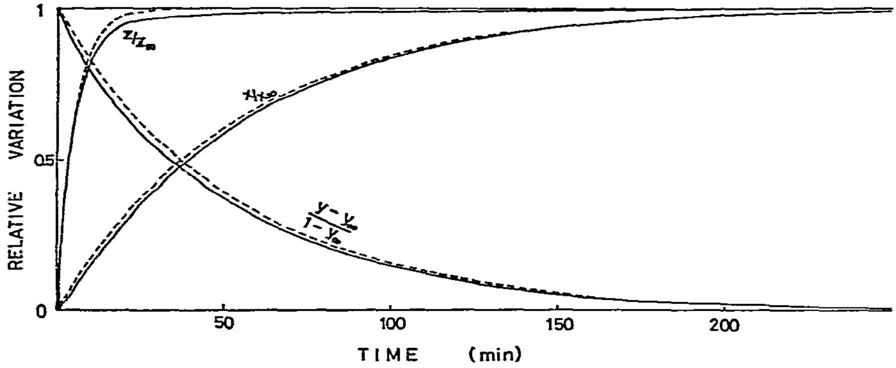


Fig. 2. The same as Fig. 1, but starting from the β-pyranose.

The problem as to when these approximations are generally valid is too lengthy to describe here, and we only point out the results. Two conditions, namely, (*a*) $k_{xz} \doteqdot k_{yz}$, and (*b*) kinetic constants of furanose to pyranose conversion (k_{zx}, k_{zy}), are much larger than others. Both of these are satisfied by D-galactose (*cf.* Eq. *31*), and make the foregoing approximations valid.

Isbell and Pigman[7] investigated several properties of m_1 and m_2 of galactose. They showed that m_1 has the same properties as the single, slow rate-constant of D-glucose, which represented pyranose–pyranose interconversion, and that m_2 has the same character as the furanose–pyranose interconversion rate-constant of D-fructose. We can also give a theoretical basis to this experiment by showing that m_1 and m_2 are approximately expressed by linear combinations of kinetic constants of pyranose–pyranose and pyranose–furanose interconversions, respectively, when (*a*) or (*b*) is valid.

We examined next whether or not Eqs. *32* and *33* could explain the dilatometric change of D-galactose in solution, as investigated by Riiber and Minsaas[5]. Their experiment showed that the dissolution of α-galactopyranose in water at 20° gave rise to an expansion during the first 15 min, followed by a contraction, which became simple exponential only after the elapse of 50 min.

We assumed the additivity of "molecular solution volume" of each component as defined by Riiber[12]. Then, we obtained the molecular solution-volume of the components x, y, and z (V_m^x, V_m^y, and V_m^z) from Eqs. *32* and the dilatometric data[5] starting from the α-pyranose, as 109.63, 109.28, and 119.78 (ml/mol), respectively, at a concentration of 10% (w/v). This result shows that the volume of the furanose form in solution is about 9.5% larger than those of pyranoses. From this result, we can anticipate the dilatometric change of a freshly dissolved solution of β-galactopyranose. The molecular solution-volume [$V_m^\beta(t)$] starting from the β-pyranose is calculated from Eqs. *33* and the foregoing values of V_m^x, V_m^y, V_m^z, as

$$V_m^\beta(t) = -0.13\cdot e^{-m_1 t} - 0.31\cdot e^{-m_2 t} + V_m^\infty \qquad (34).$$

On the other hand, the time-course of $V_m^\beta(t)$ could be calculated from the data of the meniscus change of the dilatometer as given by Riiber and Minsaas[5]. The curve theoretically expected, as obtained from Eq. (*34*) and the experimental values estimated from the data of Riiber and Minsaas[5], are shown in Fig. 3. Both are in fairly good agreement with each other. An accurate reinvestigation of the dilatometric data would be desirable to explain the discrepancy observed at times shorter than 20 min.

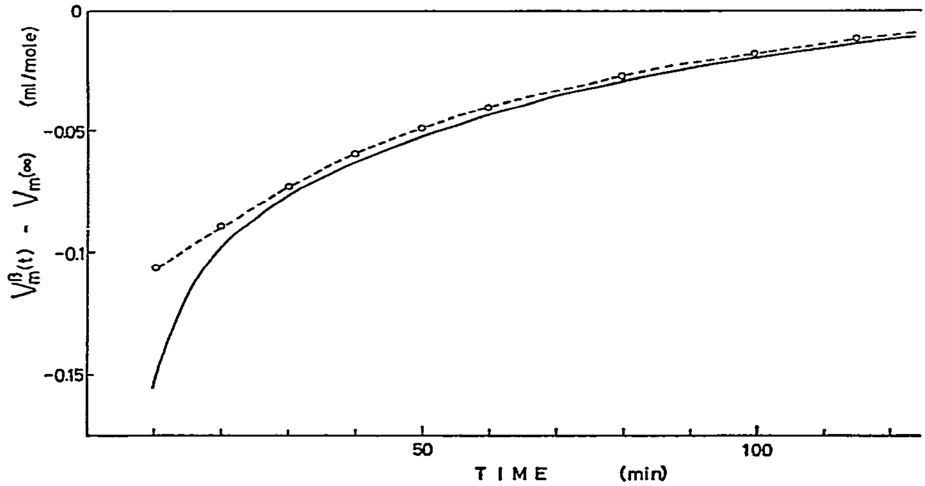


Fig. 3. Dilatometric change of a freshly dissolved solution of β-galactopyranose at 20° ($c \doteqdot 10$) Deviation of the molecular solution-volume from the equilibrium value is plotted on the ordinate The solid line is the theoretical curve calculated on the basis of Scheme III and dilatometric data starting from the α-pyranose. The dotted line through circles is an estimate from the experimental data of Riiber and Minsaas[5].

In Section B, we determined conditions under which a four-component scheme reduces to a three-component one and suggested, based on the experimental data, that these conditions might be approximately satisfied by sugars showing complex mutarotation. We examine here which of these conditions holds for D-galactose. The equilibrium contents of α- and β-furanose, estimated from the data of Acree *et al.*, were 1.0% and 2.7%, respectively. Both are much smaller than the content of pyranoses, but the content of α-furanose is not small enough to be neglected in comparison with the content of β-furanose. It is thus uncertain as to whether case I in

Section B is the main cause for D-galactose being approximated by Scheme III. Eqs. *12* and *13* in case II are not satisfied, because the relation $k_{zw} = k_{wz}$ (compare Appendix II, Eq. *A15*) holds under these conditions and the equilibrium content of the two furanoses must be equal. Whether Eqs. *12* and *14* hold or not cannot be determined by the data available at present. However, these conditions may be approximately satisfied, because the relation:

$$k_{xz} = k_{yz} \qquad (35),$$

is approximately satisfied in this case (compare Eqs. *31*), which corresponds to the first part of Eqs. *A17* in Appendix II in a four-component scheme.

F. Discussion and conclusions

One of the attempts thus far, to improve the analysis of complex mutarotation by Smith and Lowry[3], was that of Ivanov *et al.*[13]. They adopted Scheme IV, but considered z as an orthoaldehyde–open-chain form of the sugar and that both z_∞ and μ_∞ were negligibly small. Their idea that the introduction of a negligibly small component z to the scheme of Smith and Lowry (Scheme II) permits complex mutarotation (such as that of D-galactose) to be explained is shown, by extending the principle described in Section A, to be incorrect. However, the proof of this is not so simple in this instance. As both z_∞ and μ_∞ are negligibly small, the last condition of Eq. *5* does not hold. However, it can be shown that change of x and y is approximately expressed by a single rate-constant $m = k_{xy} + k_{yx}$, and z is always negligible, when starting from the initial condition $z_0 = 0$. Component z in Ivanov's scheme is a labile intermediate, so that $z_0 = 0$ is usually valid. It is concluded that Scheme IV, with negligible values of μ_∞ and z_∞, are described by Eq. *1* and cannot explain complex mutarotation.

Anderson and Garver's recent work[9] on computer simulation of complex mutarotation, based on Schemes IV and V, have led to some interesting results. They found infinite sets of kinetic constants that can explain the g.l.c. data showing how each tautomer varies with time. That is quite natural, because there is one degree of freedom in assigning kinetic constants on the right-hand side of Eq. *8*, when those on the left-hand side are determined. They also found that the value assigned to the equilibrium concentration of intermediate μ can be varied over wide limits—from vanishingly small to over 2%—with little effect on the progress of the ring forms to equilibrium. This seems to be a confirmation of our principle described in Section A, but the reason why they could not find a set of kinetic constants that would explain both optical rotation and g.l.c. data, based on the Schemes IV or V, is not clear.

In this paper, it is shown that analysis of the tautomerism of a freshly dissolved sugar, based on the three-component Scheme III, is successful not only in the analysis of experimental data but also has a sound theoretical basis. The analysis presented here may be useful for many kinds of first-order, reversible reactions between several components.

ACKNOWLEDGMENT

The authors thank Prof. L. Anderson, University of Wisconsin, for reading the manuscript and for many useful discussions and suggestions.

APPENDIX I

Reduction of Scheme IV to Scheme III when μ_∞ is sufficiently small

The kinetic constants of Scheme IV are set as follows:

$$k_{x\mu} = \varepsilon \cdot k'_{x\mu}, \quad k_{y\mu} = \varepsilon \cdot k'_{y\mu}, \quad k_{z\mu} = \varepsilon \cdot k'_{z\mu} \tag{A1}$$

where $k'_{x\mu}$, $k'_{y\mu}$, and $k'_{z\mu}$ are taken to be of the same order of magnitude as $k_{\mu x}$, $k_{\mu y}$, and $k_{\mu z}$, so that from the conditions of Eq. *5* we obtain:

$$\varepsilon \ll 1 \tag{A2}$$

A variable v is introduced instead of μ, and it is defined as:

$$v = \mu - \varepsilon \cdot \frac{k'_{x\mu} \cdot x + k'_{y\mu} \cdot y + k'_{z\mu} \cdot z}{k_{\mu x} + k_{\mu y} + k_{\mu z}} \tag{A3}$$

The differential equations *4a–d* can be written with the variable v as:

$$\left.\begin{aligned}
\frac{\mathrm{d}x}{\mathrm{d}t} &= -\varepsilon \cdot \frac{k'_{x\mu}(k_{\mu y} + k_{\mu z})}{k_{\mu x} + k_{\mu y} + k_{\mu z}} \cdot x + \varepsilon \cdot \frac{k'_{y\mu} \cdot k_{\mu x}}{k_{\mu x} + k_{\mu y} + k_{\mu z}} \cdot y + \varepsilon \cdot \frac{k'_{z\mu} \cdot k_{\mu x}}{k_{\mu x} + k_{\mu y} + k_{\mu z}} \cdot z + k_{\mu x} \cdot v \\
\frac{\mathrm{d}y}{\mathrm{d}t} &= \varepsilon \cdot \frac{k'_{x\mu} \cdot k_{\mu y}}{k_{\mu x} + k_{\mu y} + k_{\mu z}} \cdot x - \varepsilon \cdot \frac{k'_{y\mu}(k_{\mu x} + k_{\mu z})}{k_{\mu x} + k_{\mu y} + k_{\mu z}} \cdot y + \varepsilon \cdot \frac{k'_{z\mu} \cdot k_{\mu y}}{k_{\mu x} + k_{\mu y} + k_{\mu z}} \cdot z + k_{\mu y} \cdot v \\
\frac{\mathrm{d}z}{\mathrm{d}t} &= \varepsilon \cdot \frac{k'_{x\mu} \cdot k_{\mu z}}{k_{\mu x} + k_{\mu y} + k_{\mu z}} \cdot x + \varepsilon \cdot \frac{k'_{y\mu} \cdot k_{\mu z}}{k_{\mu x} + k_{\mu y} + k_{\mu z}} \cdot y - \varepsilon \cdot \frac{k'_{z\mu}(k_{\mu x} + k_{\mu y})}{k_{\mu x} + k_{\mu y} + k_{\mu z}} \cdot z + k_{\mu z} \cdot v
\end{aligned}\right\} \tag{A4}$$

$$\text{and} \quad \frac{\mathrm{d}v}{\mathrm{d}t} = \varepsilon^2 \cdot f - K \cdot v \tag{A5}$$

where f is a linear combination of x, y, and z as:

$$\begin{aligned}
f = -\frac{1}{(k_{\mu x} + k_{\mu y} + k_{\mu z})^2} [&k'_{x\mu}\{(k_{\mu y} + k_{\mu z}) \cdot k'_{x\mu} - k_{\mu y} \cdot k'_{y\mu} - k_{\mu z} \cdot k'_{z\mu}\}\, x + \\
&+ k'_{y\mu}\{(k_{\mu x} + k_{\mu z}) \cdot k'_{y\mu} - k_{\mu x} \cdot k'_{x\mu} - k_{\mu z} \cdot k'_{z\mu}\}\, y + k'_{z\mu}\{(k_{\mu x} + k_{\mu y}) \cdot k'_{z\mu} - \\
&- k_{\mu x} \cdot k'_{x\mu} - k_{\mu y} \cdot k'_{y\mu}\}\, z]
\end{aligned} \tag{A6}$$

$$\text{and} \quad K = k_{\mu x} + k_{\mu y} + k_{\mu z} + \varepsilon \cdot \frac{k_{\mu x} \cdot k'_{x\mu} + k_{\mu y} \cdot k'_{y\mu} + k_{\mu z} \cdot k'_{z\mu}}{k_{\mu x} + k_{\mu y} + k_{\mu z}} \doteqdot k_{\mu x} + k_{\mu y} + k_{\mu z} \tag{A7}$$

The differential equation *A5* is solved formally as follows:

$$v(t) = v(0)\,\mathrm{e}^{-Kt} + \varepsilon^2 \int_0^t \mathrm{d}\tau\, f(\tau)\,\mathrm{e}^{-K(t-\tau)} \qquad (A8).$$

The magnitude of v reduces to the order of ε^2 for $t > T = 1/K$ whatever the initial value $v(0)$ may be. This means from Eq. *A3* that μ is accurately described by Eq. *6*, for $t > T$, whatever the initial value $\mu(0)$ may be. The terms containing v in Eqs. *A4* can, therefore, be neglected for $t > T$ and Eqs. *A4* reduce to Eqs. *7* if the kinetic constants are given as Eqs. *8*.

APPENDIX II

Proof of the theorem that Eqs. 12 *and* 13 *or Eqs.* 12 *and* 14 *are necessary and sufficient conditions under which each of four components of Scheme VII is described by the same two exponential terms at the initial condition* $x_0 + y_0 = 1$, *and* $z_0 = w_0 = 0$

Under the conditions *12*, differential equations *9* are reduced to:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = -(k_{xy} + k_{xz} + k_{xw})x + k_{yx}\cdot y + k_{zx}(z+w) \qquad (A9a),$$

$$\frac{\mathrm{d}y}{\mathrm{d}t} = k_{xy}\cdot x - (k_{yx} + k_{yz} + k_{yw})y + k_{zy}(z+w) \qquad (A9b),$$

$$\frac{\mathrm{d}}{\mathrm{d}t}(z+w) = (k_{xz} + k_{xw})x + (k_{yz} + k_{yw})y - (k_{zx} + k_{zy})(z+w) \qquad (A9c),$$

and

$$\frac{\mathrm{d}}{\mathrm{d}t}(z-w) = (k_{xz} - k_{xw})x + (k_{yz} - k_{yw})y - (k_{zw} - k_{wz})(z+w) - $$

$$- (k_{zx} + k_{zy} + k_{zw} + k_{wz})(z-w) \qquad (A9d).$$

Eqs. *A9a, b,* and *c* are essentially the same as Eqs. *7a, b,* and *c* of Scheme III, if we identify $z+w$, $k_{xz}+k_{xw}$, and $k_{yz}+k_{yw}$ of Eqs. *A9* with z, k_{xz}, and k_{yz} of Eqs. *7*, respectively. Furthermore, only under the condition *12* do differential equations *9a, b, c,* and *d* reduce to the form of Eqs. *A9a, b,* and *c*, and x and y are described by two exponential terms, whatever the initial conditions may be. Thus the conditions *12* are sufficient for mathematical analysis of the four-component system, based on Scheme III.

Eq. *A9d* is solved by introducing the solutions of *A9a, b,* and *c*, as:

$$z - w = \mathrm{e}^{-m_3 t} \int_0^t f\cdot \mathrm{e}^{m_3 \tau}\,\mathrm{d}\tau + c\cdot \mathrm{e}^{-m_3 t} \qquad (A10),$$

where

$$f = (k_{xz}-k_{xw})x+(k_{yz}-k_{yw})y-(k_{zw}-k_{wz})(z+w)$$

$$m_3 = k_{zx}+k_{zy}+k_{zw}+k_{wz} \qquad (A11),$$

and c is a constant.

When the mutarotation is started from either of the two pyranoses (x_0 or $y_0 = 1$) or from their mixtures, the initial condition $(z-w)_0 = 0$ holds, so that $c = 0$. The solutions of Eqs. *A9a, b*, and *c* are of the same form as described in Section C (see Eqs. *16, 22*, and *23*). Thus, f is written as:

$$f = D_1 \cdot \mathrm{e}^{-m_1 t}+D_2 \cdot \mathrm{e}^{-m_2 t}+f_\infty \qquad (A12),$$

and

$$\left.\begin{aligned} D_1 &= (k_{xz}-k_{xw})A_1+(k_{yz}-k_{yw})B_1+(k_{zw}-k_{wz})(A_1+B_1) \\ D_2 &= (k_{xz}-k_{xw})A_2+(k_{yz}-k_{yw})B_2+(k_{zw}-k_{wz})(A_2+B_2) \\ f_\infty &= (k_{xz}-k_{xw})x_\infty+(k_{yz}-k_{yw})y_\infty+(k_{zw}-k_{wz})(z_\infty+w_\infty) \end{aligned}\right\} \qquad (A13),$$

where m_1 and m_2 are the two rate-constants, and A_i and B_i ($i = 1, 2$) are coefficents of the solutions of Eqs. *A9a* and *b*.

From Eqs. *A10* and *A12*, $z-w$ is written as:

$$z-w = \frac{D_1}{m_3-m_1} \cdot \mathrm{e}^{-m_1 t} + \frac{D_2}{m_3-m_2} \cdot \mathrm{e}^{-m_2 t} - \left(\frac{D_1}{m_3-m_1} + \frac{D_2}{m_3-m_2} + \frac{f_\infty}{m_3}\right) \mathrm{e}^{-m_3 t} + \frac{f_\infty}{m_3} \quad (m_3 \neq m_1,\ m_3 \neq m_2) \qquad (A14a),$$

and

$$z-w = D_1 \cdot t \cdot \mathrm{e}^{-m_1 t} + \frac{D_2}{m_3-m_2} \cdot \mathrm{e}^{-m_2 t} - \left(\frac{D_2}{m_3-m_2} + \frac{f_\infty}{m_3}\right) \mathrm{e}^{-m_1 t} + \frac{f_\infty}{m_3} \quad (m_3 = m_1) \qquad (A14b).$$

The coefficients of the third exponential term, $\mathrm{e}^{-m_3 t}$ in Eq. *A14a*, or that of the term $t \cdot \mathrm{e}^{-m_1 t}$ in Eq. *A14b*, must vanish for $z-w$ to be described by two exponential terms $\mathrm{e}^{-m_1 t}$ and $\mathrm{e}^{-m_2 t}$. Taking into account the fact that A_i and B_i ($i = 1, 2$) in Eq. *A13* are varied with initial conditions x_0 and y_0, we obtain the conditions for the foregoing:

$$k_{xz} = k_{xw}, \quad k_{yz} = k_{yw}, \quad k_{zw} = k_{wz} \qquad (A15),$$

or

$$m_3 = m_1, \quad \text{or} \quad m_3 = m_2 \qquad (A16a),$$

and

$$k_{xz}-k_{xw} = k_{yz}-k_{yw} = k_{wz}-k_{zw} \qquad (A16b).$$

Using the conditions *12* and the conditions of no circulation *11*, the latter two equations in Eqs. *A15* may be obtained from the first one. From these Eq. *13* is obtained.

From Eq. *A16b, 11* and *12,* Eq. *14* or Eq. *13* is derived. Inversely, from Eq. *11, 12* and Eq. *14,* Eq. *A16b* is obtained. Thus, Eq. *A16b* reduces to Eq. *13* or *14.*

When Eqs. *11, 12,* and *14* hold, the relationships:

$$k_{xw} = k_{yw} = k_{zw}$$

also hold. Summing up the foregoing and Eq. *14,* there is obtained:

$$k_{xz} + k_{xw} = k_{yz} + k_{yw} = k_{zw} + k_{wz} \qquad (A17).$$

Under these conditions, m_1 and m_2 are readily obtained as:

$$\left.\begin{aligned} m_1 &= k_{xz} + k_{xw} + k_{xy} + k_{yx} \\ m_2 &= k_{xz} + k_{xw} + k_{zx} + k_{zy} \end{aligned}\right\} \qquad (A18).$$

This shows, therefore, that:

$$m_3 = m_2.$$

Thus, the condition *A16a* is always satisfied when Eqs. *11, 12,* and *14* hold. In conclusion, under the conditions of Eqs. *12* and *13*, or Eqs. *12* and *14,* four components are all expressed by two exponential terms having the rate constants (m_1, m_2), and the reverse is also valid at the initial conditions, $x_0 + y_0 = 1$ and $z_0 + w_0 = 0$.

APPENDIX III

To solve the seven simultaneous equations: Eq. *18, 19, 20a, 11a, 26, 27a,* and *28a,* the three relations of Eq. *10* are used instead of Eq. *11a.* This allows elimination from the foregoing equations of three unknown k_{ij} values, namely, k_{xy}, k_{zx} and k_{zy}, but z_∞ is used as a parametric, unknown quantity.

Eq. *19* and *20a* reduce to the same equation, and α_z is given from Eq. *26* by parameter z_∞ as:

$$\alpha_z = \frac{L}{z_\infty} + \alpha_y \qquad (A19),$$

where

$$L = [\alpha]_\infty - \alpha_y - (\alpha_x - \alpha_y) \cdot x_\infty \qquad (A20).$$

The remaining four equations concerning the unknowns, k_{yx}, k_{xz}, k_{yz}, and z_∞ are readily solved. To check the consistency, we have intentionally not used the values of z_∞, y_∞. For obtaining k_{ij} values only, we can solve more readily by using them and omitting Eq. *19* and *20a.*

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