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Synthesis of alkyl glycosides through β-glucosidase-catalyzed condensation in an aqueous–organic biphasic system and estimation of the equilibrium constants for their formation

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Abstract

Hexyl, heptyl and octyl glucosides, galactosides and fucosides were separately synthesized through the β-glucosidase-catalyzed condensation of glucose, galactose and fucose with the corresponding alcohols in an aqueous–organic biphasic system, where one of the substrates, alcohol, was the organic phase, at various volume ratios. An equation for predicting the equilibrium yield of an alkyl glycoside was proposed, and the parameters involved in the equation were experimentally determined. The equilibrium constant K for the alkyl glycoside formation was then evaluated. Although the K value slightly depended on both the saccharide moiety and the alkyl chain length, it was approximately 2. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Alkyl glycoside; β-Glucosidase-catalyzed condensation; Aqueous–organic biphasic system

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1. Introduction

Alkyl β-D-glucosides are a group of nonionic surfactants and are used in cosmetics, pharmaceuticals, kitchen detergents etc. Although they are, under the present conditions, synthesized by chemical procedures, much attention has in this decade been focused on their enzymatic synthesis through β-glucosidase-catalyzed transfer or condensation reactions because of their regio- and stereoselectivity and simple and mild conditions without the protection and deprotection of the hydroxyl groups of glucose with a similar reactivity [1–7]. In the synthesis, an aqueous–organic biphasic system, where one of the substrates, the alcohol, itself is the organic phase, is usually used to shift the thermodynamically unfavorable reaction toward the synthesis by extracting a hydrophobic product, the alkyl glucoside, into the organic phase.

We proposed an equation for the prediction of the equilibrium yield of an alkyl β-D-glucoside via the

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β-glucosidase-catalyzed condensation in the biphasic system, and evaluated the equilibrium constants for the formation of hexyl to decyl β-D-glucosides [6]. We also showed that a heterogeneous system with a high glucose concentration could improve the equilibrium yield [8].

β-Glucosidase from almond can catalyze the hydrolysis not only of alkyl glucosides but also of alkyl galactosides and fucosides [9], and also their reverse hydrolysis (condensation). We reported the synthesis of alkyl β-D-fucosides through the enzyme-catalyzed condensation in a heterogeneous system, and examined their surfactant properties [10]. In the study [10], hexyl glucoside and galactoside were also synthesized for comparison. The equilibrium yields of the hexyl glycosides depended on the saccharide moiety and were in the order of fucoside > glucoside > galactoside.

In this study, the reason for the difference in the equilibrium yield among the glycosides was considered through estimation of the equilibrium constants of the hexyl to octyl glycosides in the biphasic system for the almond β-glucosidase-catalyzed condensation of the corresponding saccharide and 1-alcohol. The factors affecting the yield, such as the partition coefficient of the product to the organic (alcohol) phase and solubility of the alcohol in the aqueous phase, were observed, and it was found that both the coefficient and solubility significantly depended on the aqueous saccharide concentration. The equilibrium constants were evaluated by taking this dependence into consideration.

2. Materials and methods

2.1. Materials

β-Glucosidase (β-D-glucoside glucohydrolase, EC 3.2.1.21) from almond (14.8 units/mg) was purchased from Toyobo, Osaka, Japan, and used as received. Glucose, galactose, 1-hexanol, 1-heptanol, and 1-octanol were purchased from Wako Pure Chemical Industries, Osaka. Fucose was obtained from Nacalai Tesque, Kyoto. The hexyl, heptyl and octyl β-D-glucosides and octyl β-D-galactoside were purchased from Sigma, St. Louis, MO, USA.

2.2. Synthesis of alkyl glycosides

Fucose, glucose or galactose was dissolved with 0.2 mol/l acetate buffer, pH 5.5, pre-equilibrated with an alcohol, which was used as the organic phase, at a concentration of 2.5 mol/l. β-Glucosidase (15 mg) was weighed into a vial, and 1.0 ml of the saccharide solution and an appropriate volume of buffer-saturated alcohol were added to the vial. The vial was tightly screw-capped. The condensation was carried out at 50°C under vigorous magnetic stirring. At appropriate intervals, 15 μl of the organic (alcohol) phase was sampled and the concentration of the produced alkyl glycoside was determined by HPLC using a TMP-10 column (Ø 4.6 mm × 250 mm, Japan Organo, Tokyo) [11] and a Shodex RI SE-51 refractometer (Showa Denko, Tokyo). The eluents were methanol–water mixtures of 50:50, 55:45 and 60:40 in volume for the hexyl, heptyl and octyl glycosides, respectively. The flow rate was 1.0 ml/min.

2.3. Disaccharide formation

Fucose, glucose or galactose was dissolved in 0.2 mol/l acetate buffer, pH 5.5, and 5.0 ml of the saccharide solution (3.0 ml for fucose) was put into a vial. Ten milligrams of β-glucosidase (6 mg for fucose) were added to the vial to commence the condensation at 50°C with stirring. The initial concentrations of glucose and galactose were 0.5–2.5 mol/l, and the initial fucose concentration was in a range of 0.4–1.5 mol/l. A portion of the reaction mixture (15 μl) was sampled and mixed with the same volume of methanol. The amount of disaccharides formed was determined by HPLC using a YMC Pack ODS-AQ302 (Ø 4.6 mm × 250 mm, Kyoto) and RI detector. The eluent was degassed water and its flow rate was 0.8 ml/min.

2.4. Water concentration of saccharide solution

A given amount of the saccharide was weighed in a volumetric flask of 25 ml for glucose and galactose and of 2 or 5 ml for fucose. A 0.2 mol/l acetate buffer was then gradually added through a burette along with shaking the flask to dissolve the saccharide. The water concentration was calculated from the volume of buffer required to fill the flask.

2.5. Partition coefficient of saccharide to alcohol phase

Fucose, glucose or galactose was dissolved in 0.2 mol/l acetate buffer, pH 5.5, at a concentration of 0.5–1.5 mol/l (C_{S0}). Two milliliters of the saccharide solution and the same volume of the buffer-saturated alcohol were put into a vial. The mixture was magnetically stirred at 50°C for 1 h or for a longer period, and was allowed to stand to separate the two phases. A portion of the alcohol phase was sampled and the saccharide concentration in the phase, $C_{S,org}$, was determined by HPLC using the YMC Pack ODS-AQ302. The saccharide concentration in the aqueous phase, $C_{S,aq}$, was evaluated by $C_{S0} - \alpha C_{S,org}$ from the mass balance of the saccharide, where α is the volume ratio of alcohol to the aqueous phase. The partition coefficient, P_S, of the saccharide to the alcohol phase, which was defined as a ratio of the saccharide concentration in the alcohol phase, $C_{S,org}$, to that in the aqueous phase, was estimated from the slope for the plots of $C_{S,org}$ versus $C_{S,aq}$.

2.6. Partition coefficient of alkyl glycoside to alcohol phase and solubility of alcohol in aqueous phase

The partition coefficient of an alkyl glycoside to the alcohol phase was measured at various concentrations of the corresponding saccharide. An alkyl glycoside was dissolved in the corresponding alcohol saturated with the acetate buffer at a concentration of 25 mmol/l. Three milliliters of the solution (1.0 ml for fucoside) were put in a vial, and the same volume of saccharide solution, which had been prepared with alcohol-saturated buffer at a concentration of 0–2.5 mol/l, was then added. The mixture was kept at 50°C with stirring for 1 h. A portion of the aqueous phase was withdrawn after the two phases had separated, and the concentrations of the alkyl glycoside and alcohol were determined by HPLC. The analytical conditions were the same as in Section 2.2.

3. Theoretical considerations

3.1. Reaction scheme

In a previous paper [6], we proposed a reaction scheme for the alkyl glucoside formation in a biphasic system. Although no partition of glucose into the organic (alcohol) phase was assumed in the scheme, the assumption was not always satisfied as will be shown later. Therefore, the reaction scheme illustrated in Fig. 1 is considered in this paper. A saccharide, S, partitions into the organic phase with the partition coefficient, P_S. The aqueous phase is saturated with the alcohol, A, at its concentration of $C_{A,aq}$. The saccharide and the alcohol are condensed through the β-glucosidase-catalyzed reaction to yield an alkyl glycoside (product), P. The product partitions into the organic phase with the apparent distribution coefficient, P_P. In a previous paper [6], the formation of a dimeric aggregate in the organic phase was suggested. However, the formation is ignored in this paper because the effect of the aggregate formation on the P_P is not significant, but the saccharide concentration significantly affects the P_P as will be shown later. β-Glucosidase also catalyzes the formation of oligosaccharides through the condensation of the saccharide molecules [6,12–14]. Only the formation of disaccharides is taken into consideration, and no formation of tri- or higher oligosaccharides is assumed. Although some kinds of

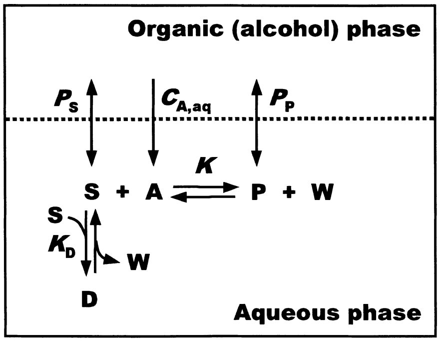

Fig. 1. Reaction scheme for alkyl glycoside formation in an aqueous–organic (alcohol) biphasic system. S, A, P, W and D indicate saccharide, alcohol, product (alkyl glycoside), water and disaccharide, respectively. K is the equilibrium constant for alkyl glycoside formation, and K_D is that for disaccharide formation.

disaccharides would be formed, they are grouped as D. The partition of D into the organic phase is not considered.

3.2. Disaccharide formation

The disaccharide formation is described as

$$S + S \overset{K_D}{\leftrightarrow} D + H_2O \tag{1}$$

where K_D is the equilibrium constant for the disaccharide formation and is defined by $C_D C_W / C_S^2$ where C_D, C_S and C_W are the equilibrium concentrations of the disaccharide, saccharide and water, respectively. The disaccharide formation is measured in the absence of alcohol, and the K_D can be evaluated from the mass balance of the saccharide residue by

$$C_D C_W = K_D (C_{S0} - 2C_D)^2 \tag{2}$$

where C_{S0} is the initial saccharide concentration.

3.3. Equilibrium yield of alkyl glycoside in a biphasic system

The alkyl glycoside formation is described by

$$S + A \overset{K}{\leftrightarrow} P + H_2O \tag{3}$$

where K is the equilibrium constant for the alkyl glycoside formation and is defined by

$$K = \frac{C_{P,aq} C_{W,aq}}{C_{S,aq} C_{A,aq}} \tag{4}$$

where $C_{i,aq}$ ($i =$ A, P, S or W) is the equilibrium concentration of component i in the aqueous phase. As mentioned above, the K_D is defined by

$$K_D = \frac{C_{D,aq} C_{W,aq}}{C_{S,aq}^2} \tag{5}$$

The apparent partition coefficient of the product, P_P, and the partition coefficient of the saccharide, P_S, are given by Eqs. (6) and (7), respectively:

$$P_P = \frac{C_{P,org}}{C_{P,aq}} \tag{6}$$

$$P_S = \frac{C_{S,org}}{C_{S,aq}} \tag{7}$$

The mass balance of the saccharide residue is expressed by Eq. (8):

$$V_{aq} C_{S0,aq} = V_{aq}(C_{S,aq} + 2C_{D,aq} + C_{P,aq}) + V_{org}(C_{S,org} + C_{P,org}) \tag{8}$$

Eqs. (4)–(8) are simultaneously solved to give Eqs. (9a) and (9b) for the equilibrium yield of an alkyl glycoside in a biphasic system, Y_{eq}, which is defined by Eq. (9a):

$$Y_{eq} = \frac{V_{aq} C_{P,aq} + V_{org} C_{P,org}}{V_{aq} C_{S0,aq}} \tag{9a}$$

$$Y_{eq} = \frac{K(1 + \alpha P_P) C_{A,aq}}{4 C_{S0,aq} K_D C_{W,aq}} \left[X^{1/2} - (1 + \alpha P_S) C_{W,aq} \right] \tag{9b}$$

where

$$X = (1 + \alpha P_S)^2 + 8 K_D C_{S0,aq} C_{W,aq} (1 - Y_{eq}) \tag{10}$$

In the derivation of Eq. (9b), the water concentration, $C_{W,aq}$, was assumed not to depend on the Y_{eq} value but to be constant for a given $C_{S0,aq}$. As will be shown later, both P_P and $C_{A,aq}$ depend on $C_{S,aq}$, that is, on Y_{eq}. Furthermore, Eq. (9b) includes the Y_{eq} value on the right-hand side as shown in Eq. (10). Therefore, since we cannot explicitly evaluate the K value, the value is estimated from the Y_{eq} value obtained at an α by a trial and error method.

4. Results and discussion

4.1. Effect of the volume ratio on the equilibrium yield of alkyl glycoside

Fig. 2 shows the transient changes in the yields of hexyl glucoside and galactoside at $\alpha = 10$ and $\alpha = 50$ and of hexyl fucoside at $\alpha = 3$ and $\alpha = 15$. Similar experiments were carried out at various α values, and also for the heptyl and octyl glycosides. Fig. 3(a)–(c) show the relationships between Y_{eq} and α for the hexyl, heptyl and octyl glycosides, respectively. For every glycoside synthesis, the higher Y_{eq} was obtained at the higher α. The Y_{eq} value of

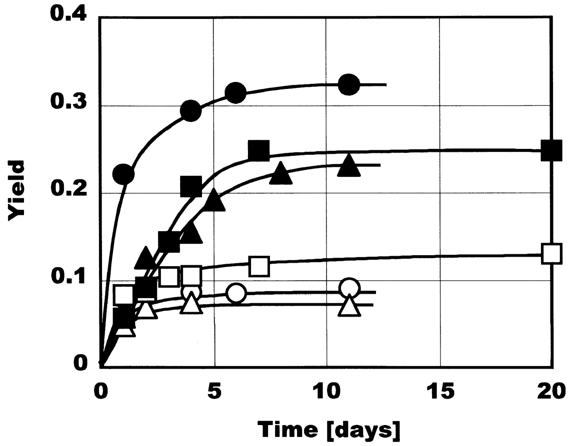

Fig. 2. Transient changes in yield of hexyl glucoside at the volume ratios of organic to aqueous phase α of (○) 10 and (●) 50, of hexyl galactoside at (△) $\alpha = 10$ and (▲) $\alpha = 50$, and of hexyl fucoside at (□) $\alpha = 3$ and (■) $\alpha = 15$ and at 50°C.

fucoside was the highest among the glycosides irrespective of the alkyl chain length, followed by the glucoside and galactoside.

4.2. Estimation of the equilibrium constants for disaccharide formation

The formations of glucobiose, galactobiose and fucobiose were separately observed at various C_{S0} in the absence of any alcohol. Fig. 4 shows the transient changes in the disaccharide concentration, C_D, at some C_{S0} values. Glucobiose was the most apt to be formed, while galactobiose was the least.

To evaluate the K_D value, the water concentration C_W at any C_S is required. Fig. 5 shows the relationships between C_W and C_S for glucose, galactose and fucose. The plots for glucose and galactose lay on the same line. This indicates that their molar volume is the same (0.113 l/mol), assuming that the additivity in volume holds for water and a saccharide. The plots for fucose also gave a line, and its molar volume was evaluated to be 0.110 l/mol.

According to Eq. (2), the $C_D C_W$ values are plotted versus the $(C_{S0}-2C_D)^2$ values in Fig. 6 in order to estimate the K_D values. For every disaccharide formation, the plots lay on a line passing through the origin. The K_D values for the glucobiose, galactobiose and fucobiose formation were evaluated to be 1.57, 0.0126 and 0.797, respectively, from the slopes of the lines.

4.3. Partition coefficients

Since a saccharide is a hydrophilic substance, the partition of the saccharide into an organic phase is usually ignored. However, if the saccharide partitions into the organic phase even with a low P_S,

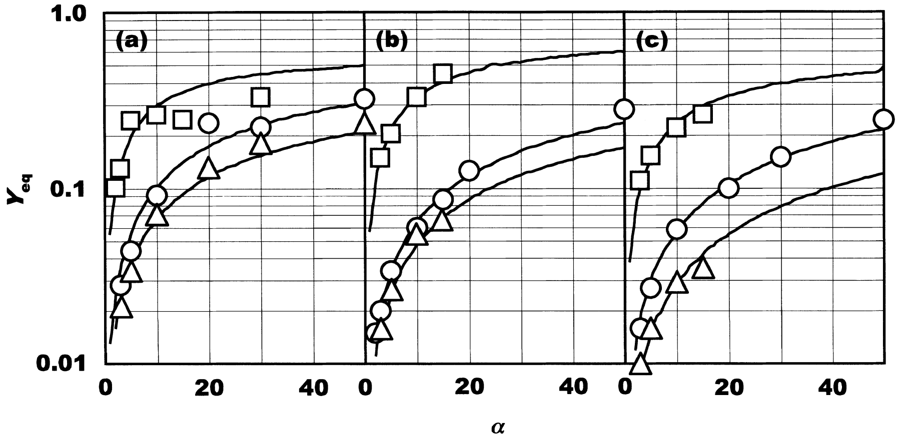

Fig. 3. Dependence of the equilibrium yield Y_{eq} on the volume ratio α of organic to aqueous phase for the formation of (a) hexyl, (b) heptyl and (c) octyl glycosides at 50°C. The symbols ○, △ and □ indicate glucoside, galactoside and fucoside, respectively. The solid curves were drawn using Eq. (9b), experimentally observed parameters and the equilibrium constant K for an alkyl glycoside which was evaluated in this study.

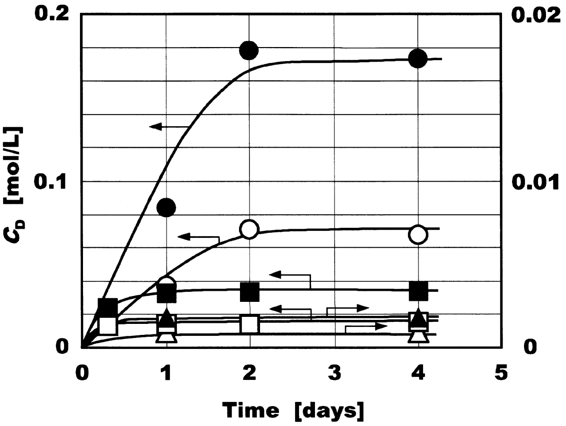

Fig. 4. Transient changes in disaccharide concentration through β-glucosidase-catalyzed condensation of glucose, galactose or fucose at 50°C. The initial concentrations of glucose were (○) 1.5 mol/l and (●) 2.5 mol/l, those of galactose were (△) 1.5 mol/l and (▲) 2.5 mol/l, and those of fucose were (□) 0.8 mol/l and (■) 1.5 mol/l.

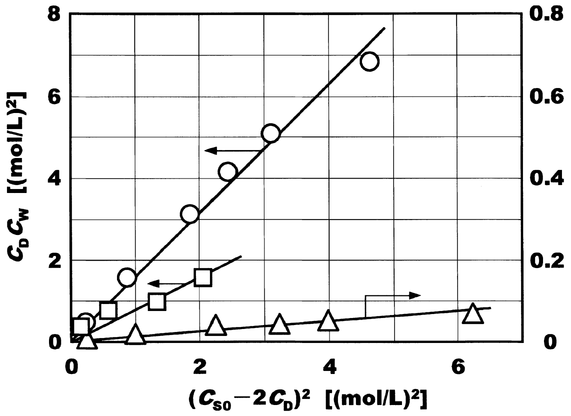

Fig. 6. Estimation of the equilibrium constants K_D for the formation of (○) glucobiose, (△) galactobiose and (□) fucobiose at 50°C.

there would be the possibility that the amount of the saccharide partitioned into the organic phase becomes significant at a high α value. Therefore, the P_S values of glucose, galactose and fucose to hexanol, heptanol and octanol were evaluated. Fig. 7(a)–(c) show the plots of $C_{S,org}$ versus $C_{S,aq}$ of the saccharides for hexanol, heptanol and octanol, respectively. The slope of each line gives the P_S value. The P_S values of glucose, galactose and fucose to hexanol were 0.0075, 0.0070 and 0.0327, those to heptanol were 0.0028, 0.0023 and 0.0131, and those to octanol were 0.0024, 0.0014 and 0.0136, respectively. Fucose had the largest P_S values among the tested saccharides for every alcohol. Fucose has no hydroxyl group at the C6 position and it would be more hydrophobic than glucose or galactose. This would be the reason for the high P_S value. There is also a tendency that the P_S value of any saccharide to alcohol with a shorter alkyl chain is larger.

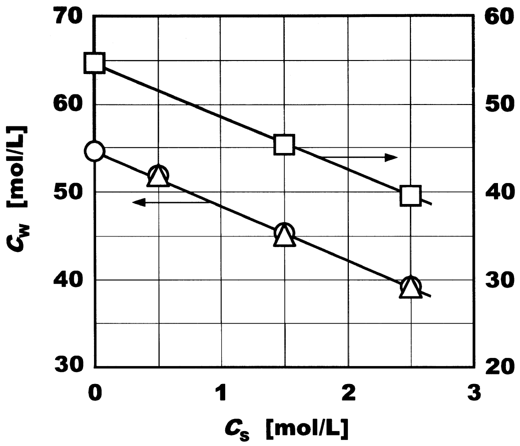

Fig. 5. Relationships between the saccharide and water concentrations, C_S and C_W, for (○) glucose, (△) galactose and (□) fucose at 50°C.

The partition of a product into the organic phase is the driving force to shift the condensation reaction (Eq. (3)) to synthesis. Therefore, the partition coefficient of the product is an important parameter in a biphasic system. The apparent distribution coefficients of all the alkyl glycosides considered here to the corresponding alcohol, P_P, were measured at various saccharide concentrations. Fig. 8(a)–(c) show the dependence of the P_P values of glucoside, galactoside and fucoside on the glucose, galactose and fucose concentrations, respectively. For every glycoside, the P_P value of a glycoside with a longer alkyl chain to the corresponding alcohol was larger. The P_P value significantly depended on the saccharide concentration and the value was larger at the higher saccharide concentration $C_{S,aq}$. As shown in Fig. 8, the dependence of P_P on $C_{S,aq}$ could empirically be expressed, for every alkyl glycoside, by $P_P = P_{P0}\exp(aC_{S,aq})$, where P_{P0} is the partition coefficient of the alkyl glycoside in the absence of the

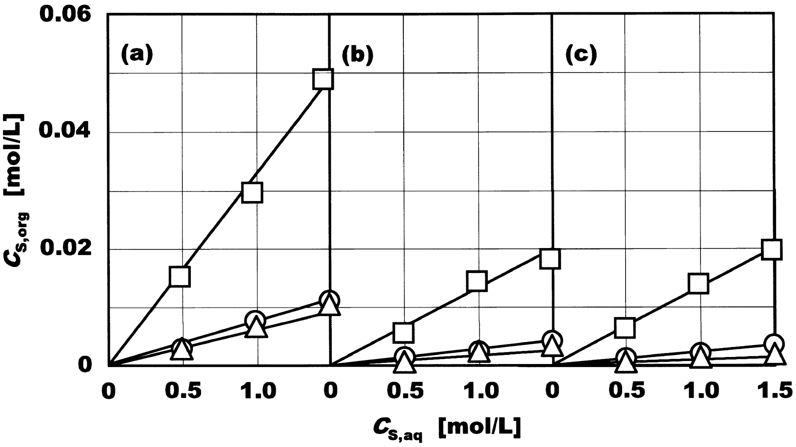

Fig. 7. Estimation of the partition coefficients P_S of (○) glucose, (△) galactose and (□) fucose to (a) 1-hexanol, (b) 1-heptanol and (c) 1-octanol at 50°C.

saccharide and a is a positive constant. The logarithm of P_{P0} was proportional to the alkyl chain length n, as shown in Fig. 9.

4.4. Alcohol concentration in aqueous phase

The β-glucosidase-catalyzed condensation between a saccharide and an alcohol would occur in the aqueous phase, and the alcohol concentration in this phase would be an important parameter. As mentioned above, the P_P value significantly depended on C_S. This suggested that the alcohol concentration in the aqueous phase, $C_{A,aq}$, would also depend on $C_{S,aq}$. Therefore, we measured the $C_{A,aq}$ of hexanol, heptanol and octanol at various C_S values of glucose, galactose and fucose (Fig. 8). The $C_{A,aq}$ became lower for higher $C_{S,aq}$ values in every case, and it could also be expressed as an expo-

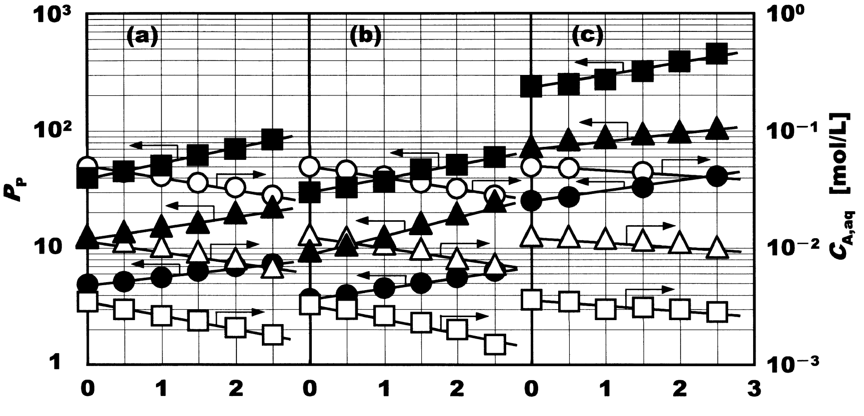

Fig. 8. Dependence of the apparent distribution coefficients P_P of (●) hexyl, (▲) heptyl and (■) octyl glycosides, and the concentrations of (○) hexanol, (△) heptanol and (□) octanol in the aqueous phase $C_{A,aq}$ on (a) glucose, (b) galactose and (c) fucose concentrations at 50°C.

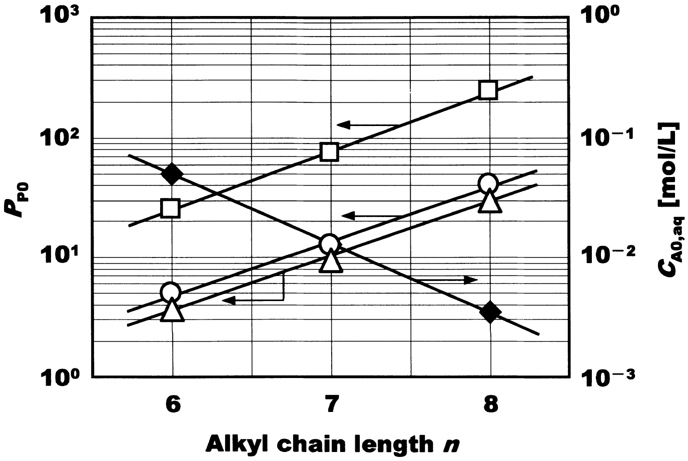

Fig. 9. Dependence of the partition coefficients P_{P0} of alkyl (○) glucoside, (△) galactoside and (□) fucoside, and of (◆) alcohol concentration in the aqueous phase $C_{A0,aq}$ on the alkyl chain length n in the absence of saccharide.

nential function of $C_{S,aq}$ with a negative slope. As shown in Fig. 9, the $C_{A,aq}$ in the absence of the saccharide, $C_{A0,aq}$, exponentially decreased with the n.

4.5. Estimation of the equilibrium constants for alkyl glycoside formation

The purpose of this paper is to evaluate the equilibrium constant, K, for various kinds of alkyl glycosides. As mentioned in Section 3, a trial and error method was used to estimate the K value from the experimental Y_{eq} at a specific α. The dependencies of P_P and $C_{A,aq}$ on $C_{S,aq}$ were empirically expressed by exponential functions, as mentioned above. The K value was then evaluated by the method for each set of Y_{eq} and α values. The K values for an alkyl glycoside were averaged and are plotted versus the n in Fig. 10. The plots indicate the average ± standard deviation. The K value depended on both the saccharide moiety and the alkyl chain length. The K value for the alkyl glucoside formation was the largest, and the K values for the alkyl fucoside and galactoside followed. There was a weak tendency that the K value for a longer alkyl chain was smaller. However, estimation of the K value used many experimental parameters, which more or less included errors although the experiments were done very carefully, and included many calculation steps. Therefore, the K value for any alkyl glycoside formation can, roughly speaking, be about 2.

Using the averaged K value for each alkyl glycoside formation, the dependence of Y_{eq} on α was calculated and is shown in Fig. 3 by the solid curves.

The equilibrium constant should be, in principle, defined based on the thermodynamic activities of substrates and products. However, we used the K value defined by Eq. (4) because it is not easy to estimate the activities in the present reaction system and the K value based on the concentrations has conveniently been used in many cases. As is known

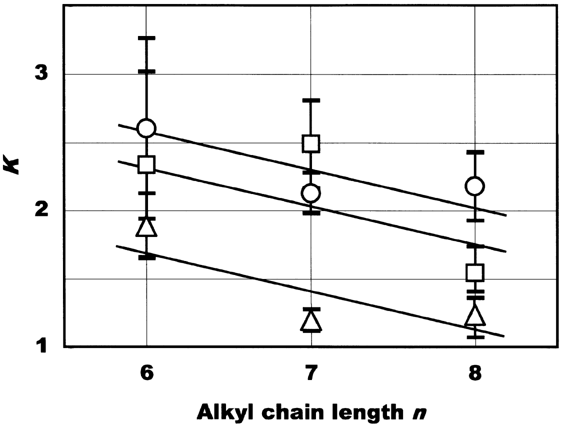

Fig. 10. Dependence of the equilibrium constant K for alkyl glycoside formation on the alkyl chain length n for (○) glucoside, (△) galactoside and (□) fucoside at 50°C.

well, the water activity decreases with an increase in saccharide concentration. Therefore, there was a possibility that the K value obtained here was more or less affected by the activity.

In this paper, the K value was estimated under the consideration of both the partition of a saccharide into alcohol phase and the dependencies of P_{P} and $C_{\mathrm{A,aq}}$ on $C_{\mathrm{S,aq}}$. The P_{S} of fucose to hexanol was the largest among the values obtained here. The effects of P_{S} and the $C_{\mathrm{S,aq}}$ dependencies of P_{P} and $C_{\mathrm{A,aq}}$ on the K value were examined for the synthesis of hexyl fucoside. The K value estimated here was 2.34. If the partition of fucose into hexanol was ignored, that is, $P_{\mathrm{S}} = 0$, but the dependence of P_{P} and $C_{\mathrm{A,aq}}$ on $C_{\mathrm{S,aq}}$ was considered, the K value was evaluated to be 1.87. When the dependence was also ignored, the K value was 1.81.

The K values were the same magnitude and roughly ca. 2 for all the alkyl glycosides. However, the Y_{eq} values for alkyl glucoside and galactoside decreased with an increase in the alkyl chain length n. On the other hand, the Y_{eq} value for the alkyl fucoside scarcely depended on n. This would be understood from the dependencies of P_{P} and $C_{\mathrm{A,aq}}$ on n. Since the K value was almost constant for every alkyl glycoside and the term $1 + \alpha P_{\mathrm{P}}$ in Eq. (9b) could be approximated by αP_{P} in most cases, Y_{eq} would be roughly proportional to the term $P_{\mathrm{P}} C_{\mathrm{A,aq}}$ at an α. For simplicity, let us consider the case where $C_{\mathrm{S,aq}} = 0$. The $P_{\mathrm{P0}} C_{\mathrm{A0,aq}}$ values for the hexyl, heptyl and octyl fucosides were 1.2, 0.94 and 0.86, respectively. The values for the glucosides and galactoside were 0.245, 0.157 and 0.140, and 0.185, 0.121 and 0.099, respectively. The values for the fucosides were much larger than those for the glucosides and galactosides, and their dependence on n was smaller for the fucoside than for the glucoside and galactoside. This would be the reason for the above-mentioned dependencies.

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