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## Note

# Acid-catalyzed hydrolysis of maltose and cellobiose 1-phenylflavazoles*

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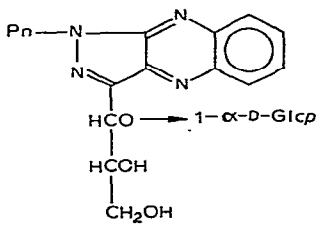
Investigations undertaken in an effort to find an oligosaccharide derivative that would allow the preferential removal of the reducing end-unit by hydrolysis and, thus, sequence determinations by a stepwise, peeling process have been reported[1,2]. The 1-phenylflavazole was investigated because of its stability in acid and its previous use in determining oligosaccharide sequence[1,3,4].

In one of these investigations, it was found that both the enthalpy and entropy of activation for the acid-catalyzed hydrolysis of maltose 1-phenylflavazole {3-(1-*O*-α-D-glucopyranosyl-D-*erythro*-trihydroxypropyl)-1-phenylpyrazolo[3,4-*b*]quinoxaline, **1**} were quite different from those of maltose (a model for the other linkages in an oligosaccharide)[1]. Hydrolysis of the former compound had a low $\Delta H^{\ddagger}$ value and a large negative $\Delta S^{\ddagger}$ value compared with those for other glycosides. This difference in thermodynamic parameters suggested a difference in mechanism of hydrolysis.

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One mechanism thought possible was that in which protonation of the glycosidic oxygen atom occurred *via* an intramolecular donation of a proton from the protonated ring-nitrogen atom, resulting in an ordered transition state in the rate-determining heterolysis step.

*Dedicated to Dr. Horace S. Isbell, in honour of his 75th birthday.

In the present investigation, hydrolysis of **1** was effected in 0.5, 1.0, 1.5, 2.0, 2.5, and 5.0M sulfuric acid, chosen because of its stability to changes in titer at 90°. A linear relationship between log $k$ and the Hammett acidity function ($H_0$) was found; this indicated a unimolecular decomposition of the conjugate acid without participation of water (A-1), though this evidence is equivocal[5]. More importantly, the fact that the rate constant was dependent on the activity of the added acid over the entire range suggested that intramolecular catalysis was not involved, for if it were, hydrolysis should have been independent of acid concentration at pH values at which the proton-donating group was completely protonated.

One of the groups appeared to have a p$K_a$ within the range of acid concentrations used in this experiment, as there was a definite color change from yellow to red on going from 1.5 to 2.0M sulfuric acid, without, however, any accompanying break in the log $k$ *vs.* $H_0$ curve. No p$K_a$ of any other group was indicated by scanning the visible and ultraviolet absorptions over a pH range of 1.0 to 10.8.

Another possible explanation involved steric considerations. Since the aglycon, a bulky 1-phenylpyrazolo[3,4-*b*]quinoxaline group, is in the axial ($\alpha$) position, there may be considerable strain in the molecule. Protonation of the glycosidic oxygen atom could bring about a further strain through a dipolar interaction, namely, the reverse anomeric effect described by Lemieux and Morgan[6]. According to this idea, a positively charged atom in axial orientation at the anomeric center meets a strong destabilizing effect arising from electrostatic interaction between the group attached to C-1 and the C-5–O bond when the groups on C-1 and C-5 are in a *gauche* relationship. The dipolar interactions that destabilize the $\alpha$-D anomer are strong enough to force such compounds as *N*-(tetra-*O*-acetyl-$\alpha$-D-glucopyranosyl)pyridinium bromide into the $^1C_4$ conformation[6]. Protonation of the glycosidic oxygen atom to form the conjugate acid could then force the D-glucopyranosyl unit into a more ordered, unstable conformation[7]. However, the hydrolyses of phenyl $\alpha$-D-glucosides show no such large changes in enthalpy and entropies of activation, even though axial–axial interactions would also be prevalent; and benzyl $\alpha$-D-glycosides, which are better models, behave very much like alkyl $\alpha$-D-glycosides[5].

If this explanation were correct, then hydrolysis of the corresponding $\beta$-D anomer (cellobiose 1-phenylflavazole) should give different results. So both maltose 1-phenylflavazole (**1**) and cellobiose 1-phenylflavazole {3-(1-*O*-$\beta$-D-glucopyranosyl-D-*erythro*-trihydroxypropyl)-1-phenylpyrazolo[3,4-*b*]quinoxaline, **2**} were subjected to acid-catalyzed hydrolysis under identical conditions. For **1**, hydrolysis rates were determined polarimetrically as before. With **2**, because the change in optical rotation was small, the standard error was large, and a determination of reducing power was used to follow the course of hydrolysis. The results are given in Table I.

These results point out two things. First, the results obtained in each of several runs for maltose 1-phenylflavazole differ from those reported previously[1]. Although the hydrolyses reported in this paper were done over a different temperature range, there is a considerable difference in the value of the rate constant at 80°. It is concluded that the earlier data were in error.

TABLE I

HYDROLYSIS DATA FOR **1** AND **2**

| *Compound* | $10^6$k ($sec^{-1}$)[a] | | | $E_a$ | $\Delta H^{\ddagger}$ | $\Delta S^{\ddagger}$ | $\Delta G^{\ddagger}$ |
|---|---|---|---|---|---|---|---|
| | *80°* | *85°* | *90°* | (*kcal.mole*$^{-1}$) | (*kcal.mole*$^{-1}$) | (*cal.mole*$^{-1}$.*deg*$^{-1}$) | (*kcal.mole*$^{-1}$) |
| **1**[1] | 25.8 | — | — | 12.4 | 11.7 | −46.7 | 27.3 |
| **1** | 5.87 | 14.8 | 39.5 | 48.6 | 47.9 | +52.7 | 29.0 |
| **2** | 14.5 | 17.0 | 90.0 | 46.3 | 45.6 | +47.6 | 28.6 |

[a]1:1 Water–2-methoxyethanol, M with respect to $H_2SO_4$.

The results agree with those for alkyl D-glucopyranosides, with the exception that the rate constants are smaller and the enthalpies, entropies, and free energies of activation are greater than are those of D-glucopyranosides of alkyl secondary alcohols. All of the values for maltose 1-phenylflavazole (**1**) and cellobiose 1-phenylflavazole (**2**) are in the same relationship to each other, as are the values for alkyl α- and β-D-glucopyranosides[7]. Thus, although investigations of this type suffer from the fact that kinetic measurements can be easily made over only a very narrow range of temperature, making significance of small differences in enthalpies or entropies of activation open to question[7], the results clearly indicate that both glucosides undergo hydrolysis by the same mechanism as do alkyl α- and β-D-glucopyranosides.

It is unlikely that there is an attack by solvent on the protonated intermediate (A-2 reaction) or that the transition state is less solvated than is the conjugate acid. However, Schaleger and co-workers[9] have shown that rate constants for acid-catalyzed reactions vary differently for different solutes with changes in water activity in glycerol–water mixtures and suggest this as a means of isolating from other effects the contribution of the hydration change occurring upon conversion of reactants into a transition state. Hence, this aspect needs further investigation.

The large gain in entropy on going to the product-like transition state[7] may be due to relief of steric restraints imposed by the bulky, ionic aglycon group.

## EXPERIMENTAL

*Sugar solutions.* — Maltose 1-phenylflavazole (**1**), prepared and recrystallized by published procedures[2,8], had m.p. 260–262°; lit.[10,4,2] 262–264°, 265°, 264°. A 0.5% solution of maltose 1-phenylflavazole in 2-methoxyethanol showed (t.l.c.) a single component.

Cellobiose 1-phenylflavazole (**2**), prepared in a similar manner[8], had m.p. 285–286°; lit.[10] 280–282°. A 0.5% solution of cellobiose 1-phenylflavazole in 2-methoxyethanol showed (t.l.c.) a single component.

*Determination of hydrolysis rates.* — (*a*) *Effect of* [$H^+$]. Aqueous sulfuric acid (2 ml) was added to 2.0 ml of 0.4% **1** in ethylene glycol, with stirring, to give a

solution of the appropriate molarity of acid. The resulting solution was injected into a preheated, water-jacketed, polarimeter cell of a Bendix ETL-NPL Automatic Polarimeter equipped with a mercury (546 nm) interference filter.

(*b*) *Effect of temperature.* For determination of thermodynamic values, 0.5% solutions of **1** and **2** in 2-methoxyethanol were mixed with equal volumes of 2.0M sulfuric acid. The progress of hydrolysis of **1** was followed polarimetrically, and the hydrolysis of **2** was followed by determining reducing power spectrophotometrically, using the Nelson reagent[11,12]. The amounts of sodium carbonate, Rochelle salt, and sodium hydrogen carbonate in the copper reagent were increased, and the amount of sodium sulfate was decreased to compensate for the sulfuric acid in the hydrolyzate and the sodium sulfate formed by neutralization.

Rate constants were determined by Guggenheim's method[13] by use of a computer to give a least-squares fit of the data.

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REFERENCES

1 J. N. BeMiller and R. K. Mann, *Carbohyd. Res.*, 2 (1966) 70.
2 J. N. BeMiller and D. R. Smith, *Carbohyd. Res.*, 6 (1968) 118.
3 P. Nordin and D. French, *J. Amer. Chem. Soc.*, 80 (1958) 1445.
4 J. E. Courtois and U. Ariyoshi, *Ann. Pharm. Franc.*, 16 (1958) 385.
5 J. N. BeMiller, *Advan. Carbohyd. Chem.*, 22 (1967) 25.
6 R. U. Lemieux and A. R. Morgan, *Can. J. Chem.*, 43 (1965) 2205.
7 J. N. BeMiller and E. R. Doyle, *Carbohyd. Res.*, 20 (1971) 23.
8 P. Nordin, *Methods Carbohyd. Chem.*, 2 (1963) 136.
9 L. L. Schaleger, C. N. Richards, and N. Watamori, *Chem. Commun.*, (1966) 381.
10 G. Neumuller, *Ark. Kemi*, 21A (1945) 1.
11 N. Nelson, *J. Biol. Chem.*, 153 (1944) 375.
12 J. E. Hodge and B. T. Hofreiter, *Methods Carbohyd. Chem.*, 1 (1962) 380.
13 E. A. Guggenheim, *Phil. Mag.*, 2 (1926) 538.