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## Note

# Hydroxyl reactivity—A kinetic approach is needed

JACOB LEHRFELD
*Northern Regional Research Laboratory*, Peoria, Illinois 61604 (U. S. A.)*
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The hydroxyl groups in a sugar molecule can and usually do react at different rates with the same reagent. Generally, product distribution has been used to determine the relative reactivities of these hydroxyl groups. When handled properly, this approach gives a fairly accurate assessment of reality. However, in multistep reactions, this approach can lead to completely erroneous conclusions regarding the relative reactivities of hydroxyl groups.

Partial acetylation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**1**) with acetic anhydride in pyridine gives methyl 3-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**2**) as the major product, with smaller amounts of methyl 2-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**3**) and of methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucopyranoside[1] (**4**). A superficial product-distribution analysis led to the conclusion that, in the initial stages of the reaction (**1**→**2** and **1**→**3**), the hydroxyl group on C-3 is more reactive toward acetic anhydride in pyridine than the hydroxyl group on C-2. This conclusion, however, is incorrect.

According to the scheme, $k_1'$, $k_2'$, $k_3'$, and $k_4'$ are the relative rate-constants for the corresponding reactions[†]. The numbers are their experimentally determined values. The small increase in reactivity of HO-3 in reaction **1** over that of HO-2 in reaction **2** cannot alone account for the extensive accumulation of **2**. The major contributing factor is the marked decrease in reactivity of the C-2 hydroxyl group in reaction **3**. The rate of reaction **3** is about one-sixth that of reaction **4**. Substitution of an acetyl group at O-3 markedly decreases the reactivity of the vicinal hydroxyl group (at C-2). When the hydroxyl group at C-2 is similarly substituted with an acetyl group, the reactivity of the vicinal hydroxyl group (at C-3) is slightly increased. Other compounds are being investigated in an attempt to explain this result.

From this example, it is obvious that the reactivity of a hydroxyl group in a sugar molecule can change during the course of a multistage reaction. Consequently, any conclusions about reactivity based only on product-isolation data can be erro-

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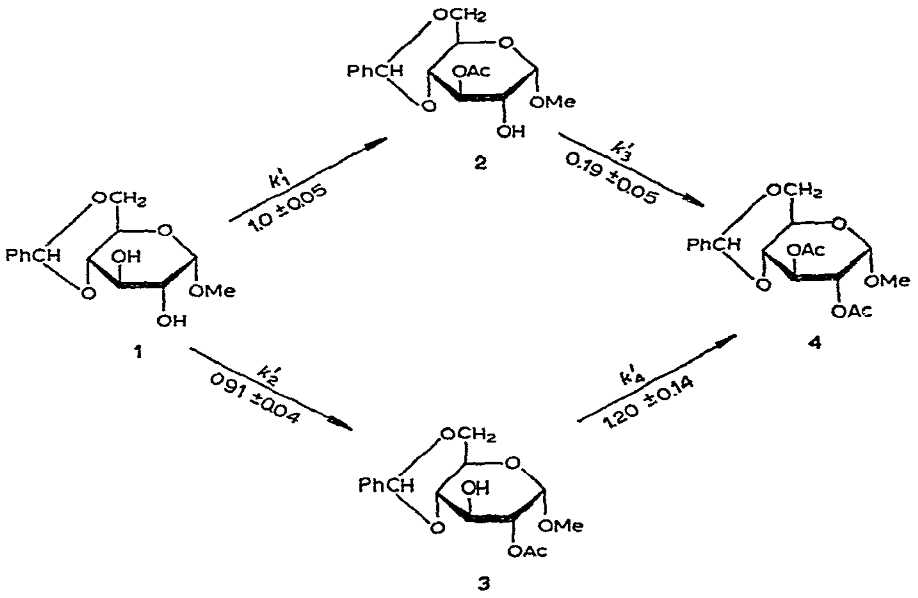
[*] Agricultural Research Service, U.S. Department of Agriculture. The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

[†] Editorial note. A similar kinetic approach has recently been used by J. Staněk, Jr., P. Chuchvalec, K. Čapek, K. Kefurt, and J. Jarý, *Carbohyd. Res.*, 36 (1974) 273–282.



Scheme 1.

neous. It is also obvious that generalizations, such as that the C-2 hydroxyl group in sugars is more reactive than the C-3 hydroxyl group, must be qualified not only by the reactants and solvent used, but also by the degree and type of substitution occurring elsewhere in the molecule.

## EXPERIMENTAL

*General.* — Glass plates coated with a 0.25-mm layer of Silica Gel G served for both qualitative and quantitative t.l.c. analyses. A 2:1 ethyl acetate–hexane solvent-system completely resolved mixtures containing **1**, **2**, **3**, and **4** (Scheme 1). For g.l.c. analysis, a mixture containing **1**, **2**, **3**, and **4** was trimethylsilylated following the procedure of Sweeley *et al.*[2] and then chromatographed on a 5-ft 3% OV-17 column, temperature-programmed from 190 to 215° at 2°/min. Compounds **1**, **2**, **3**, and **4** were

TABLE I

COMPOUNDS USED

| *Compound* | *M.p. (degrees)* | $[\alpha]_D^{23}$ *(degrees)* | $R_F$ | *G.l.c. retention time (min)* | *References* |
|---|---|---|---|---|---|
| **1** | 166–167 | +112 (*c* 0.8, chloroform) | 0.11 | 10.7[a] | 3 |
| **2** | 175–177 | +115 (c 0.44, chloroform) | 0.34 | 16.1[a] | 1 |
| **3** | 132–133 | +108 (c 0.83, chloroform) | 0.50 | 12.7[a] | 1 |
| **4** | 108–110 | +74 (c 0.95, chloroform) | 0.62 | 18.1 | 1 |

[a] $Me_3Si$ derivative.

prepared and isolated according to literature procedures (Table I). Purity was checked by g.l.c. and t.l.c.

The data were acquired by quantitative t.l.c. in an S-chamber. To maximize precision and reproducibility, the sample size was kept constant, and the distance traveled by the solvent was kept constant by scoring the plate[4–7]. A blank containing the four components **1**, **2**, **3**, and **4** in a pyridine–ethanol mixture was quantitated by t.l.c., stored in a freezer for 72 h, and requantitated by t.l.c. Because the relative amounts of the four components did not change, apparently neither solvolysis nor acetyl migration occurred during storage. Pure **2** and **3** were dissolved in a pyridine–ethanol mixture and chromatographed separately on t.l.c. plates. Because none of the other isomers or the starting material appeared when the spots on the plates were made visible, it was concluded that neither acetyl migration nor hydrolysis occurred during chromatography.

*Reaction.* — To an Erlenmeyer flask containing 0.300 g of compound **1**, 10 ml of dry pyridine was added. The flask was purged with nitrogen, sealed with a serum cap, and immersed in a constant-temperature bath maintained at 25.0°. From a freshly prepared 0.43M solution of acetic anhydride in dry pyridine, which had previously been equilibrated at 25.0°, 5 ml was rapidly added with a syringe, and the flask was shaken. A 1.0-ml sample was periodically removed, and the reaction quenched with 0.5 ml of ethanol. The samples were stored in a freezer until used for quantitation.

Pure **2** and **3** were separately acetylated similarly to confirm the computer-calculated rate-constants for reactions **3** and **4**.

*Quantitation.* — Densitometry of the t.l.c. spots was used for quantitating the data. Plates (20 × 20 cm) were coated with a 0.25-mm layer of Silica Gel G and air dried. Lanes 1.3 cm wide were scored on each plate. A 3.0 $\mu$l sample was spotted in each lane with a 10-$\mu$l Hamilton syringe. Samples from the reaction mixture were spotted in duplicate, whereas five different samples containing known amounts of **1**, **2**, **3**, and **4** were spotted singly. The weights of the spots ranged from 2–40 $\mu$g. The plates were developed for 16.6 cm, air dried, sprayed twice with 10% sulfuric acid in 85% ethanol, and then heated on a hot plate for 1 h at 200°. After the plates had been cooled for ~2 h, the densities of the spots were determined with a Photovolt t.l.c. densitometer Model 530. To minimize the coefficient of variations, the slit covering the photomultiplier tube was made smaller than the diameter of the spot being measured[8]. The signal from the photometer was recorded on the log scale of a Beckman Model 1005 linear-log recorder. Peaks were cut out and weighed. A standard curve was constructed from the known standards. The slopes for each of the curves and the standard deviation ($s$) of points from the least-squares line were: **1** 0.95, $s$ ±0.30; **2** 0.89, $s$ ±0.28; **3** 0.89, $s$ ±0.33; **4** 0.89, $s$ ±0.21. The amounts of **1**, **2**, **3**, and **4** in each sample were calculated from the standard curves. The average deviation for most components was ±3%. The data were tabulated into a form compatible with the digital-fitting program DRATE described by Butterfield[9] and processed on an IBM Model 1130 computer. DRATE requires that an initial guess be made for the rate constants. Several computer runs were made with widely different initial guesses. All of the runs converged onto the same approximate solution.

Incorporated into the program is a subroutine for plotting the data (Fig. 1).

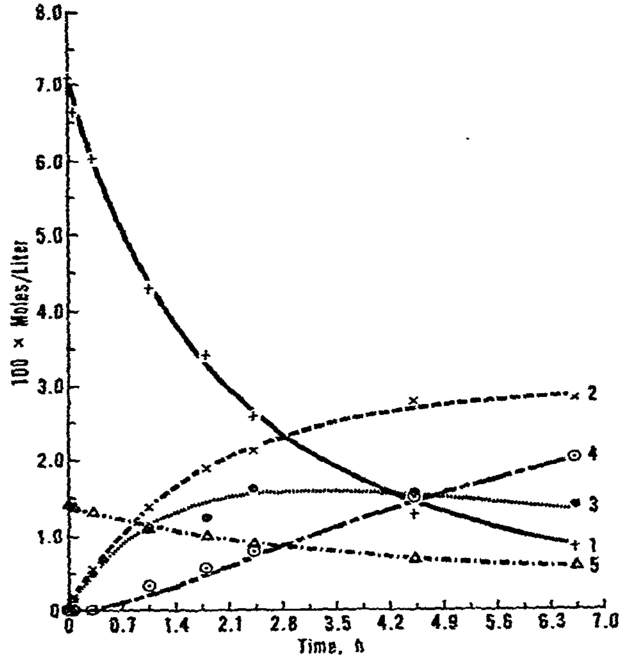


Fig. 1. Acetylation of 1 by acetic anhydride in pyridine: 1, +; 2, ×; 3, ●; 4, ⊙; 5, acetic anhydride, △. The concentration of acetic anhydride was decreased by a factor of 10 for plotting purposes.

The symbols are the experimentally determined values. The lines are the theoretical curves derived from the calculated rate-constants and the initial concentrations of the reactants.

## ACKNOWLEDGMENT

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