as preservative) was kept 15 hr at 50°. After cooling to room temperature, elution with chloroform afforded methyl 6-*O*-ethyl-3,4-di-*O*-methyl-2-*O*-(methylsulfonyl)-α-D-glucopyranoside (1.266 g, 21% yield) having $[\alpha]^{26}D$ +120° (*c* 2.0, chloroform).

*Anal.* Calcd for $C_{12}H_{24}O_8S$: C, 43.89; H, 7.37; S, 9.76. Found: C, 44.09; H, 7.30; S, 9.52.

Further elution with chloroform afforded a mixture of **1** (99 mg, 1%) and **2** (4.71 g, 67%) which was resolved by silica gel column chromatography.

**B. From 2.**—Compound **2** (0.104 g) in N,N-dimethylformamide (2 ml) with ethyl iodide (0.5 ml) and silver oxide (0.5 g) was stirred 3.5 hr when reaction was complete as judged by tlc using ether as solvent. The reaction mixture was worked up as described above for methylation reactions to give methyl 6-*O*-ethyl-3,4-di-*O*-methyl-2-*O*-(methylsulfonyl)-α-D-glucopyranoside (0.094 g, 83%) having $[\alpha]^{26}D$ +119° (*c* 2.4, chloroform). The infrared and nmr spectra were identical with those of the product from **1** described in A.

*Anal.* Calcd for $C_{12}H_{24}O_8S$: C, 43.89; H, 7.37; S, 9.76. Found: C, 43.87; H, 7.48; S, 9.62.

**Registry No.**—**2**, 16802-84-9; **3**, 16802-85-0; **4**, 7045-36-5; **6**, 16802-87-2; **7**, 16853-03-5; methyl 2,3-di-*O*-(methylsulfonyl)-α-D-mannopyranoside, 16802-88-3; methyl 2,3-di-*O*-(methylsulfonyl)-6-*O*-trityl-α-D-mannopyranoside, 16802-89-4; **9**, 16802-90-7; methyl 4,6-*O*-isopropylidene-β-D-glucopyranoside, 16802-97-4; methyl 4,6-*O*-isopropylidene-2,3-di-*O*-methyl-β-D-glucopyranoside, 16802-91-8; **11**, 16802-92-9; **12**, 16802-93-0; **13**, 16802-94-1; methyl 2,3,4-tri-*O*-methyl-6-*O*-(methylsulfonyl)-α-D-glucopyranoside 16802-95-2; methyl 6-*O*-ethyl-3,4-di-*O*-methyl-2-*O*-(methylsulfonyl)-α-D-glucopyranoside, 16802-96-3.

# Stereochemistry of the Anomers of Methyl 2-Deoxy-D-ribofuranoside. Synthesis of Methyl 5-(6-Aminopurin-9-yl)-2,5-dideoxy-α-D-ribofuranoside, a "Reversed" Nucleoside[1]

NELSON J. LEONARD, FRANK C. SCIAVOLINO, AND VASUDEWAN NAIR

*Department of Chemistry and Chemical Engineering, University of Illinois, Urbana, Illinois 61801*

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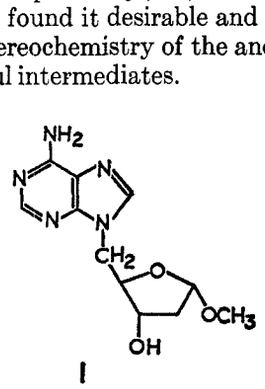
Methyl 2-deoxy-5-*O*-triphenylmethyl-α-D-ribofuranoside (**2**) and methyl 2-deoxy-5-*O*-triphenylmethyl-β-D-ribofuranoside (**3**) were synthesized and the stereochemistry of their anomeric centers was established unambiguously by chemical means and by complete analysis of their nmr spectra. The results are in agreement with those predicted by the Hudson isorotation rules. The syntheses of related ribofuranosides and of methyl 5-(6-aminopurin-9-yl)-2,5-dideoxy-α-D-ribofuranoside (**1**) are also described.

A route to the synthesis of ribose derivatives of adenine bonded at C-5 of the sugar moiety ("reversed" nucleosides) has been described[2] as part of a cooperative program with Professor Skoog at the University of Wisconsin[3] to determine the cytokinin activity[4,5] and chemical properties of compounds closely related to kinetin.[6,7] In providing a synthetic route to 2-deoxyribose derivatives of "reversed" nucleoside type, as exemplified by methyl 5′-(6-aminopurin-9-yl)-2′,5′-dideoxy-α-D-ribofuranoside (**1**), we found it desirable and also necessary to establish the stereochemistry of the anomeric centers for a series of useful intermediates.




**1**

A mixture of the α and β forms of methyl 2-deoxy-D-ribofuranosides[8] was treated with 1 equiv of triphenylmethyl chloride. Chromatography on silica gel afforded a separation of methyl 2-deoxy-5-*O*-triphenylmethyl-α-D-ribofuranoside (**2**) (28%), $[\alpha]^{26}D$ 64.4° (*c* 1.2, $CHCl_3$), and methyl 2-deoxy-5-*O*-triphenylmethyl-β-D-ribofuranoside (**3**) (24%), $[\alpha]D^{26}$ −43.8° (*c* 1.3, $CHCl_3$). The stereochemistry of the anomeric centers was temporarily assigned on the basis of Hudson's rules of isorotation[9] which correlate optical rotation and anomeric configuration. However, it has recently been discovered that several pyrimidine[10-12] and purine[13] 2-deoxy-D-ribonucleosides constitute exceptions to Hudson's rules. Although there is consistency among the rotations of a wide variety of 2-deoxy-D-ribofuranose esters and glycosides and there is no evidence currently available that Hudson's rules are not applicable to such substances,[14] it was desirable to confirm the assignments by further physical and chemical means. Accordingly, the configuraton of the anomeric center in **2** and **3** was rigorously established by an unambiguous chemical synthesis and by a complete analysis of their nmr spectra.

(1) The support of this work by a research grant (USPHS-GM-05829) from the National Institutes of Health, U. S. Public Health Service, is gratefully acknowledged.

(2) N. J. Leonard and K. L. Carraway, *J. Heterocycl. Chem.*, **3**, 485 (1966); see also J. Hildesheim, J. Cléophax, S. D. Géro, and R. D. Guthrie, *Tetrahedron Lett.*, 5013 (1967).

(3) F. Skoog, H. Q. Hamzi, A. M. Szweykowska, N. J. Leonard, K. L. Carraway, T. Fujii, J. P. Helgeson, and R. N. Loeppky, *Phytochemistry*, **6**, 1169 (1967).

(4) F. Skoog, F. M. Strong, and C. O. Miller, *Science*, **148**, 532 (1965).

(5) J. van Overbeek, *ibid.*, **152**, 721 (1966).

(6) F. M. Strong, "Topics in Microbial Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1958, p 98.

(7) C. O. Miller, *Ann. Rev. Plant Physiol.*, **12**, 395 (1961).

(8) R. E. Deriaz, W. G. Overend, M. Stacey, and L. F. Wiggins, *J. Chem. Soc.*, 2836 (1949).

(9) C. S. Hudson, *J. Amer. Chem. Soc.*, **31**, 66 (1909); C. S. Hudson, *Advan. Carbohyd. Chem.*, **3**, 1 (1948).

(10) M. Hoffer, R. Duschinsky, J. J. Fox, and N. Yung, *J. Amer. Chem. Soc.*, **81**, 4112 (1959).

(11) R. U. Lemieux and M. Hoffer, *Can. J. Chem.*, **39**, 110 (1961).

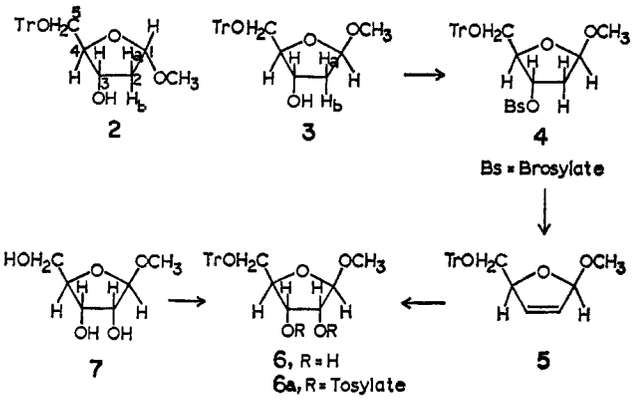
(12) T. R. Emerson and T. L. V. Ulbricht, *Chem. Ind.* (London), 2129 (1964).

(13) R. H. Iwamoto, E. M. Acton, and L. Goodman, *J. Med. Chem.*, **6**, 684 (1963).

(14) D. L. MacDonald and H. G. Fletcher, Jr., *J. Amer. Chem. Soc.*, **84**, 1262 (1962).

The chemical determination of the stereochemistry of the anomeric center consisted in the conversion of methyl 2-deoxy-5-*O*-triphenylmethyl-β-D-ribofuranoside (**3**) into a substance of known anomeric configuration, methyl di-2,3-*O*-*p*-toluenesulfonyl-5-*O*-triphenylmethyl-β-D-ribofuranoside (**6a**). Thus, treatment of **3** with *p*-bromobenzenesulfonyl chloride in pyridine provided the *p*-bromobenzenesulfonate **4**, which was transformed into the olefin **5** in 63% yield by means of excess sodium methoxide in anhydrous DMF (Scheme I).[15,16]

SCHEME I




Osmylation of **5** followed by alkali–mannitol hydrolysis afforded the diol **6** in 74% yield. It was predicted that the diol would have the ribose configuration since osmium tetroxide should attack the double bond of **5** from the less hindered side, *i.e.*, from the side opposite the trityl and methoxyl groups. The diol was converted into its crystalline ditosylate derivative **6a**. That **6** and consequently **6a** did have the ribose configuration was shown by the intersecting conversion of methyl β-D ribofuranoside (**7**),[17] of known configuration, into the 5-*O*-trityl compound **6** and thence to the ditosylate derivative **6a**. Identity of the samples of **6a** prepared by the separate routes was established by melting point and mixture melting point, infrared and nmr spectra, and optical rotation. Since the stereochemistry at the anomeric center of **7** was known,[17] the methyl 2-deoxy-5-*O*-triphenylmethyl-D-ribofuranoside with the negative specific rotation necessarily had the β configuration (**3**) and the dextrorotatory isomer had the α configuration (**2**).[18,19]

(15) This substance is crystalline and stable at room temperature, and the method offers a convenient route for the introduction of 2,3 double bonds into the pentofuranosides; *cf.* J. Hildesheim, J. Cléophax, and S. D. Géro, *Tetrahedron Lett.*, 1685 (1967).

(16) Compound **5** may be of related biochemical interest in view of the recent investigations on 2′,3′-unsaturated nucleosides: (a) DHFUDR, see T. A. Khwaja and C. Heidelberger, *J. Med. Chem.*, **10**, 1066 (1967); (b) blasticidin S, see N. Otake, S. Takeuchi, T. Endo, and H. Yonehara, *Tetrahedron Lett.*, 1411 (1965); (c) J. R. McCarthy, Jr., M. J. Robins, L. B. Townsend, and R. K. Robins, *J. Amer. Chem. Soc.*, **88**, 1549 (1966); (d) J. P. Horwitz, J. Chua, M. Noel, and J. T. Donatti, *J. Org. Chem.*, **32**, 817 (1967); (e) J. P. Horwitz, J. Chua, M. A. DaRooge, M. Noel, and I. L. Klundt, *ibid.*, **31**, 205 (1966); (f) J. P. Horwitz, J. Chua, J. A. Urbanski, and M. Noel, *ibid.*, **28**, 942 (1963); (g) J. P. Horwitz, J. Chua, I. L. Klundt, M. A. DaRooge, and M. Noel, *J. Amer. Chem. Soc.*, **86**, 1896 (1964); (h) J. J. Fox and N. C. Miller, *J. Org. Chem.*, **28**, 936 (1963); (i) P. Reichard, *J. Biol. Chem.*, **237**, 3513 (1962).

(17) R. Barker and H. G. Fletcher, Jr., *J. Org. Chem.*, **26**, 4605 (1961).

(18) Catalytic reduction of 2,3-didehydro-2,3-dideoxy compounds leads to dideoxyribose derivatives, which are of interest particularly in purine nucleoside combination.[19]

(19) (a) M. J. Robins and R. K. Robins, *J. Amer. Chem. Soc.*, **86**, 3585 (1964); (b) M. J. Robins, J. R. McCarthy, Jr., and R. K. Robins, *Biochemistry*, **5**, 224 (1966); (c) G. L. Tong, W. W. Lee, and L. Goodman, *J. Org. Chem.*, **30**, 2854 (1965).

The configuration at C-1 of the α and β anomers of methyl 2-deoxy-5-*O*-triphenylmethyl-D-ribofuranoside (**2** and **3**), predicted first on optical rotation and then based firmly on chemical interconversion, was correlated with the nmr spectra of these anomers by a full analysis for the C-1, C-2, and C-3 protons, which is in itself of interest. This study is relevant to correlations between nmr spectra and configuration of the anomeric proton reported by Jardetzky,[20] Lemieux,[11,21] Leonard and Laursen,[22] and Robins and Robins.[23] In particular, Jardetzky[20] and Robins and Robins[23] have suggested a correlation for a series of α and β anomers of 2′-deoxyribofuranosyl nucleosides based purely on the appearance of the resonance due to the anomeric proton, its peak width, and vicinal coupling constants abstracted on a first-order basis from these signals. Our purpose in presenting the full nmr analysis of this part of the molecule is to establish accurate values of vicinal coupling constants of the anomeric proton and to emphasize the variation, with configuration, of the chemical-shift difference between the C-2 protons in **2** and **3**.

Chemical-shifts and coupling constants of the furan ring protons are tabulated (see below). The chemical shift of the C-5 protons and the overlapping methyl signals of the methoxyls are included. Assignments of multiplets to protons on C-1, C-2, C-3, and C-5 were obvious from the relative chemical shifts, integrated areas, and amount of fine structure. Difficulties with the C-3 and C-4 protons included considerable overlapping in the case of the α anomer and complexity of splitting patterns in the β anomer.

The two compounds gave an ABMX system[24] for $H_{2a}$, $H_{2b}$ (AB part), $H_1$ (X), and $H_3$ (M), the M multiplet being further split by $H_4$. As usual, A is defined as the downfield part of the AB multiplet. The ABMX patterns can be analyzed by the general treatment of Pople and Schaefer[24] or by the procedure of Abraham and McLauchlan.[25] We chose to use the latter but with slight modifications. The AB part (showing 16 lines in the 100-Mc spectrum) was simplified by double irradiation at the position of the X resonance, which reduced this to an 8-line pattern (AB of ABM) which was analyzed by the general procedure for ABX analysis.[26] The 16-line AB part can be treated as the 8-line ABX pattern with each line doubled by M. The doublings, "$d_{AM}$" and "$d_{BM}$" (due to but not equal to $J_{AM}$ and $J_{BM}$ obtained in above analysis) are line separations in the M doublet of doublets. Subtraction of these doublings from the full AB part left the AB of ABX. The X part in both anomers appeared as a multiplet of four lines which gave $|J_{AX} + J_{BX}|$ for comparison with AB analysis. The parameters obtained from the analysis were used for the calculation of splitting patterns and intensities. In every case, excellent agreement was obtained between the calculated and the observed spectrum.

(20) C. D. Jardetzky, *J. Amer. Chem. Soc.*, **83**, 2919 (1961).

(21) R. U. Lemieux, *Can. J. Chem.*, **39**, 116 (1961).

(22) N. J. Leonard and R. A. Laursen, *Biochemistry*, **4**, 354 (1965).

(23) M. J. Robins and R. K. Robins, *J. Amer. Chem. Soc.*, **87**, 4934 (1965).

(24) J. A. Pople and T. Schaefer, *Mol. Phys.*, **3**, 547 (1960).

(25) R. J. Abraham and K. A. McLauchlan, *ibid.*, **5**, 195 (1962).

(26) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High-resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, pp 132–135.

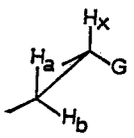
TABLE I

CHEMICAL-SHIFT AND COUPLING CONSTANT DATA FOR METHYL 2-DEOXY-5-*O*-TRIPHENYLMETHYL-D-RIBOFURANOSIDES

| Compound | Solvent | Chemical shifts[a] δ scale, ppm: X $H_1$ | A[b] $H_{2a}$ | B[b] $H_{2b}$ | M $H_3$ | $H_4$ | $H_5$ | Me | OH | $\delta_{AB}$, cps | Splitting patterns for $H_1$, $H_2$, $H_3$ | Coupling constants, cps: $J_{AB}$ | $J_{AX}$ | $J_{BX}$ | $J_{AM}$ | $J_{BM}$ | $J_{AX}/J_{BX}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| α anomer **2** | $CDCl_3$ TMS | 5.12 | 2.17 | 1.98 | 4.15 | 4.21 | 3.17 | 3.35 | 2.84 | 19.2 | ABMX | 13.4 | 4.8 | 0.7 | 5.8 | 1.3 | 6.85 |
| β anomer **3** | $CDCl_3$ TMS | 5.01 | 2.10 | 1.96 | 3.82–4.40 | | 3.25[c] | 3.25 | 2.10 | 13.6 | ABMX | 13.0 | 1.9 | 5.5 | 6.4 | 6.4 | 0.34 |

[a] Aromatic protons absorbed between δ 7.08 and 7.54. [b] Assignment of A and B between $H_{2a}$ and $H_{2b}$ discussed in text. [c] Approximate value.

Parameters were obtained above in terms of A, B, M, and X, and the problem that remained was the assignment of A and B to $H_{2a}$ and $H_{2b}$ or *vice versa*. We resorted to empirical analogy using rigid cyclic molecules containing the monosubstituted ethane fragment **8**. There are several examples of this where the proton




**8**

$H_b$, *cis* to and eclipsed by the substituent group G (such as Cl, Br, OH, CN, $N_3$), is upfield from $H_a$, *trans* to G.[27,28] Chemical-shift theory is at present inadequate to make such predictions with confidence. Chemical shifts for $H_{2a}$ and $H_{2b}$ in these two compounds have been assigned by selecting the upfield component as the proton *cis* to the hydroxyl group after consideration of possible conformations. In the α anomer this shielding will occur both from the hydroxyl group and the methoxyl group but in the β anomer the shielding effects from these groups are in opposition. Recourse had to be taken then in the values of the coupling $J_{AX}$ and $J_{BX}$ and an approach in terms of small and large $J_{vic}$ and the general Karplus equations.[29,30] Internal support for our assignment is discussed below (see Table I).

In the α anomer (**2**), the resonance of the C-1 proton was a clear doublet of doublets with $J_{AX} + J_{BX} = 5.5$ cps. The resonance of the C-3 proton was partly obscured by that of the C-4 proton but its splitting pattern was easily recognized in the overlapping sets of multiplets. The absorption of $H_4$ was a ragged doublet of doublets. Protons on C-5 were found to be magnetically equivalent and appeared as a clean doublet. The absorption of the C-2 protons (AB) appeared as a multiplet of 16 lines with some transitional degeneracies in the higher field part. The coupling constants $J_{AX} > J_{BX}$ and $J_{AM} > J_{BM}$ are of sufficient magnitude to use the ideas mentioned above on *cis* shielding by the hydroxyl and methoxyl groups and to assign $H_{2a}$ as A and $H_{2b}$ as B. An internal cross-check for self-consistency is provided by the larger observed value of $\delta_{AB}$ in this compound compared with that in the β anomer **3**.

In the spectrum of the β anomer the resonance of the C-1 proton appeared as a quartet with $J_{AX} + J_{BX} = 7.4$ cps. The signals due to $H_3$ and $H_4$ were a complex set of multiplets and were not analyzed. Analysis of the resonance of the C-5 protons was not possible because the methylene signal was obscured by the methoxyl group, but the former appeared to be magnetically nonequivalent. This is also the situation in the 2,3-didehydro compound **5**. The absorption of the methylene protons ($H_{2a}$, $H_{2b}$) appeared as a multiplet of 16 lines with little overlapping. The assignment of A and B as $H_{2a}$ and $H_{2b}$ was again made on the basis of the value of $\delta_{AB}$ and $J_{vic}$. The observation that $J_{AM} = J_{BM}$ is merely a reflection that changes in the conformation of the ring and orientation of substituents can produce gross changes in coupling constants.[31] In the two compounds the observed values of geminal coupling constants ($J_{AB}$) fit well their environment on both theoretical[32] and empirical grounds.[33] No sign determinations have been carried out but these values are presumed to be negative. The many factors which influence the magnitude of vicinal coupling constants in molecules of such complexity (in relation to their nmr spectra) cannot be dissected out in any quantitative fashion. Finally, the observed correlations between nmr spectra and configurations are as follows: (1) in the α anomer $J_{AX} > J_{BX}$, whereas in the β anomer $J_{AX} < J_{BX}$; (2) the value of $\delta_{AB}$ is larger in the α anomer.

Returning to the original goal, a series of five transformations converted the α anomer **2**, now of established configuration, into the "reversed" deoxynucleoside **1**. On treatment with *p*-bromobenzoyl chloride in pyridine, methyl 2-deoxy-5-*O*-triphenylmethyl-α-D-ribofuranoside was converted into methyl 3-*p*-bromobenzoyl-2-deoxy-5-*O*-triphenylmethyl-α-D-ribofuranoside (**9**). Aqueous acetic acid brought about detritylation. The resulting alcohol **10** was transformed by the action of *p*-bromobenzenesulfonyl chloride in pyridine into methyl 5-*p*-bromobenzenesulfonyl-3-*p*-bromobenzoyl-2-deoxy-α-D-ribofuranoside (**11**). The brosylate **11** reacted smoothly with sodium adenide in anhydrous DMF to give the blocked nucleoside, methyl 5-(6-aminopurin-9-yl)-3-*p*-bromobenzoyl-2,5-dideoxy-α-D-ribofuranoside (**12**), and methanolic ammonia transformed this into the "reversed" deoxynucleoside, methyl 5-(6-aminopurin-9-yl)-2,5-dideoxy-α-D-ribofuranoside (**1**) (Scheme II).

(27) (a) L. M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London, in press, Chapter 2-2; (b) A. D. Broom, M. P. Schweizer, and P. O. P. Ts'o, *J. Amer. Chem. Soc.*, **89**, 3612 (1967).

(28) For a full discussion of this point, see R. H. Andreatta, V. Nair, and A. V. Robertson, *Aust. J. Chem.*, **20**, 2701 (1967).

(29) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).

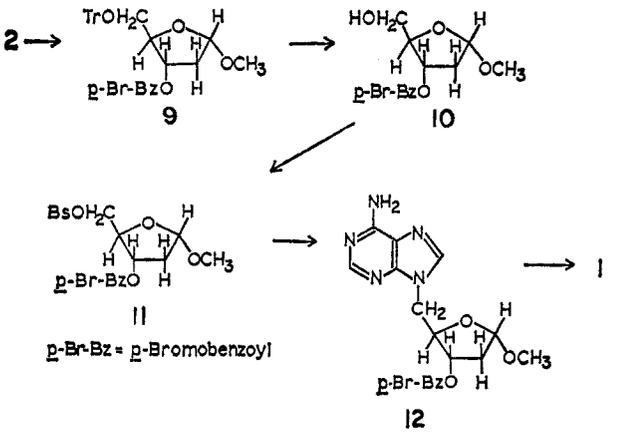
(30) M. Karplus, *J. Amer. Chem. Soc.*, **85**, 2870 (1963).

(31) It is of interest to note that no long range coupling was evident in the two spectra.

(32) A. A. Bothner-By, *Advan. Magnetic Resonance*, **1**, 195 (1965).

(33) R. C. Cookson, T. A. Crabb, J. J. Frankel, and J. Hudec, *Tetrahedron, Suppl.*, No. 7, 355 (1966).

SCHEME II




## Experimental Section[34]

**Methyl 2-Deoxy-5-*O*-triphenylmethyl-α- and -β-D-ribofuranosides (2 and 3).**—To a solution of 9.60 g (67.5 mmol) of 2-deoxy-D-ribose in 200 ml of dry methanol was added 4.0 ml of saturated methanolic hydrochloric acid. After standing at room temperature for 30 min, the pale yellow solution was neutralized with Dowex-1 ($HCO_3^-$), filtered, and evaporated to dryness *in vacuo*. The residual oil was dissolved in 50 ml of dry pyridine and evaporated to dryness under high vacuum. This process was repeated once and the residue was dissolved in 200 ml of dry pyridine and treated with 19.0 g (68.0 mmol) of triphenylmethyl chloride. The solution was stirred at room temperature for 3 days and then poured into 2 l. of ice-cold 5% hydrochloric acid. The pH was adjusted to 3 by the further addition of ice-cold 10% hydrochloric acid. The mixture ws extracted with 700 ml of ether, and the aqueous phase was separated and extracted again with 300 ml of ether. The combined organic layers were shaken with three 100-ml portions of 5% potassium bisulfate, three 100-ml portions of water, and two 100-ml portions of saturated sodium chloride solution and were dried over anhydrous sodium sulfate. The ether was evaporated under reduced pressure. The residue was dissolved in 25 ml of methanol and this was placed in the icebox for 3 days. The precipitated triphenylcarbinol was removed by filtration (3.10 g) and washed thoroughly with cold methanol, and the methanol solution was evaporated *in vacuo*. The residue (23.0 g) was dissolved in ether, 15 g of silica gel was added, and the mixture was evaporated to dryness. The solid was applied to the top of a column of 400 g of silica gel packed in pentane–ether (9:1). The progress of the column was conveniently followed by tlc using the solvent system with which the column was being eluted. Elution was continued with pentane–ether (9:1) until all the triphenylcarbinol was removed. Polarity was gradually increased to pentane–ether (6:4), and 7.3 g (28%) of methyl 2-deoxy-5-*O*-triphenylmethyl-α-D-ribofuranoside (**2**) was eluted, $[\alpha]^{26}D$ 64.4° (*c* 1.3, $CHCl_3$), as a colorless gum. The material was homogeneous by tlc but could not be induced to crystallize. Continued elution of the column with pentane–ether (6:4) afforded 6.3 g (24%) of methyl 2-deoxy-5-*O*-triphenylmethyl-β-D-ribofuranoside (**3**), $[\alpha]^{26}D$ −43.8° (*c* 1.2, $CHCl_3$). This material was also homogeneous by tlc but could not be induced to crystallize. Separation of the two anomers was practically quantitative; only three of the fractions contained mixtures and these were discarded.

**Methyl 3-*p*-Bromobenzenesulfonyl-2-deoxy-5-*O*-triphenylmethyl-β-D-ribofuranoside (4).**—A solution of 1.10 g (2.80 mmol) of methyl 2-deoxy-5-*O*-triphenylmethyl-β-D-ribofuranoside (**3**) in 25 ml of anhydrous pyridine was treated with 1.07 g (4.20 mmol) of *p*-bromobenzenesulfonyl chloride in one portion and the solution was stirred at room temperature for 24 hr. It was poured into 150 ml of 5% sodium bicarbonate solution and extracted with 300 ml of ether. The ether was washed with four 100-ml portions of 5% potassium bisulfate solution, water to neutrality, and saturated sodium chloride solution. The ethereal solution was dried over anhydrous sodium sulfate; then the ether was evaporated under reduced pressure. The residue crystallized from methyl alcohol–ethyl acetate (10:2) to give 1.65 g of crude product. Recrystallization from methanol gave 425 mg, mp 94–95°, of a first crop and 350 mg, mp 93–95°, of a second crop (45%) of **4**: $[\alpha]^{23}D$ −8.0° (*c* 1.3, $CHCl_3$); $\nu_{max}^{Nujol}$ 1580, 1555 $cm^{-1}$ (phenyl nuclei); nmr δ 7.76–7.15 (19 H, multiplet, aromatic protons), 5.16–4.88 (2 H, multiplet, $H_1$ and $H_3$), 4.30–4.05 (1 H, multiplet, $H_4$), 3.24 (3 H, singlet, $CH_3O$–), 3.10 (2 H, doublet, $J$ = 6 cps, $2H_5$) and 2.31–2.15 (2 H, multiplet, $2H_2$)

*Anal.* Calcd for $C_{31}H_{29}BrO_6S$: C, 61.08; H, 4.79; S, 5.25. Found: C, 61.18; H, 5.01; S, 5.57.

**Methyl 2,3-Didehydro-2,3-dideoxy-5-*O*-triphenylmethyl-β-D-ribofuranoside (5).**—A solution of 230 mg (0.01 g-atom) of sodium in 25 ml of anhydrous methanol was evaporated to a small volume under reduced pressure. The solution was diluted with 20 ml of anhydrous dimethylformamide and evaporation was continued for 30 min to ensure complete removal of the methanol. A solution of 1.00 g (1.65 mmol) of methyl 3-*p*-bromobenzenesulfonyl-2-deoxy-5-*O*-triphenylmethyl-β-D-ribofuranoside (**4**) in 10 ml of dry dimethylformamide was added dropwise during 5 min at room temperature. The solution was stirred for 45 min and poured into a two-phase mixture of 400 ml of water and 200 ml of ether. The aqueous phase was separated and extracted again with 100 ml of ether. The combined organic extracts were washed with water and saturated sodium chloride solution and dried over anhydrous sodium sulfate. Evaporation under reduced pressure afforded 500 mg of a colorless gum which was applied to the top of a column of 20 g of silica gel packed in pentane–ether (9:1). The column was eluted with pentane–ether (8:2) and 20 ml fractions were collected. Fractions 3–7 were combined, evaporated, and recrystallized from ether–pentane to give 386 mg (63%) of **5** as colorless needles: mp 82–83°; $[\alpha]^{23}D$ −72.2° (*c* 1.1, $CHCl_3$); $\nu_{max}^{Nujol}$ 1625 (C=C) and 1590 $cm^{-1}$ (phenyl nuclei); nmr δ 7.61–7.30 (15 H, multiplet, aromatic protons), 6.20–5.70 (3 H, multiplet, $H_1$, $H_2$, $H_3$), 5.01 (1 H, multiplet, $H_4$), 3.40 (3 H, singlet, $CH_3O$), and 3.20 (2 H, multiplet, $2H_5$).

*Anal.* Calcd for $C_{25}H_{24}O_3$: C, 80.61; H, 6.49. Found: C, 80.48; H, 6.49.

**Methyl 5-*O*-Triphenylmethyl-β-D-ribofuranoside (6). A. From Olefin 5.**—A solution of 1.46 g (3.93 mmol) of methyl 2,3-didehydro-2,3-dideoxy-5-*O*-triphenylmethyl-β-D-ribofuranoside (**5**) in 35 ml of anhydrous ether was treated with a solution of 1.00 g (3.93 mmol) of osmium tetroxide in 35 ml of the same solvent. A solution of 0.62 g (7.86 mmol) of anhydrous pyridine in 30 ml of dry ether was added, and the resulting solution was allowed to stand at room temperature for 24 hr. The light brown precipitate was collected by filtration, washed with ether, and dissolved in 50 ml of methylene chloride. A solution of 7.5 g of mannitol in 75 ml of 1% aqueous potassium hydroxide was added, and the two-phase system was stirred vigorously until the organic layer became colorless (*ca.* 5 hr). The methylene chloride layer was separated, washed with water, dried over anhydrous sodium sulfate, and evaporated. The resulting gum was filtered through a column of 20 g of silica gel with ether–pentane (4:1) and the eluate was evaporated under reduced pressure to give 1.18 g (74%) of **6** as a colorless glass: $[\alpha]^{23}D$ −18.8° (*c* 1.8, $CHCl_3$), homogeneous on tlc plates in solvent systems A, B, C, and D. It was characterized as the 2,3-ditosylate derivative.

A solution of 250 mg (0.61 mmol) of the compound described above in 10 ml of anhydrous pyridine, together with 285 mg (1.50 mmol) of *p*-toluenesulfonyl chloride, was stirred at room temperature for 5 days. The solution was poured into 100 ml of ice-cold 5% sodium bicarbonate, and the crystals were collected by filtration and washed well with water. One recrystallization from methanol–ethyl acetate gave 200 mg (46%) of methyl 2,3-di-*O*-*p*-toluenesulfonyl-5-*O*-triphenylmethyl-β-D-ribofuranoside (**6a**) as colorless needles: mp 140–141°; $[\alpha]^{23}D$ 63.8° (*c* 0.76' $CHCl_3$); $\nu_{max}^{Nujol}$ 1600 $cm^{-1}$ (phenyl nuclei); nmr δ 7.96–7.03 (23 H, multiplet, aromatic protons), 5.15–4.91 (3 H, multiplet,

(34) Melting points were determined on a Thomas–Hoover capillary melting point apparatus and are corrected. The infrared spectra were recorded on a Perkin-Elmer Model 337 grating spectrophotometer. The ultraviolet spectra were recorded on a Cary Model 15 spectrophotometer. Thin layer chromatography was performed on Eastman silica gel strips with a fluorescent indicator. Solvent system A refers to pentane–ether (1:1); B, to pentane–ether (1:4); C, to pentane–ethyl acetate (7:3); and D, to 4% methanol in chloroform. Routine nmr spectra were recorded on a Varian Associates A-60A or A-56/60 spectrometer at ordinary probe temperatures. Unless otherwise noted nmr spectra were run in $CDCl_3$. Nmr analyses were carried out on expanded traces of the decoupled and undecoupled spectra recorded on a Varian HA-100 instrument. Experimental errors are estimated at ±0.1 cps for coupling constants and ±0.01 ppm for chemical shifts. We are indebted to Mr. J. Nemeth and his associates at the University of Illinois for the microanalyses.

$H_1$, $H_2$, $H_3$), 4.46–4.16 (1 H, multiplet, $H_4$), 3.33 (3 H, singlet, $CH_3O$), and 2.45 and 2.36 (3 H each, singlets, $p$-$CH_3$).

*Anal.* Calcd for $C_{39}H_{38}O_9S_2$: C, 65.52; H, 5.35; S, 8.97. Found: C, 65.63; H, 5.38; S, 9.04.

**B. From Methyl β-D-Ribofuranoside (7).**[17]—A solution of 5.0 g (33 mmol) of D-ribose in 100 ml of anhydrous methanol was cooled to 0° and treated with 0.5 ml of concentrated sulfuric acid. The colorless solution was allowed to stand at 4° for 16 hr and was passed through a column of Amberlite IR-45 ($OH^-$). The filtrate was evaporated to a pale yellow oil under reduced pressure. The nmr spectrum of this oil in $D_2O$ showed the anomeric proton as a singlet at δ 4.90, indicating the β orientation of the methoxyl group at that center. The crude oil (4.5 g, 27.4 mmol) was dissolved in 25 ml of anhydrous pyridine and evaporated to dryness under reduced pressure. The process was repeated once, the residue was dissolved in 50 ml of anhydrous pyridine and treated with 7.6 g (27.4 mmol) of triphenylmethyl chloride, and the solution was stirred at room temperature for 3 days. It was poured into 500 ml of ice-cold water, and the aqueous solution was extracted with three 200-ml portions of ether. The ether was washed with four 100-ml portions of 5% potassium bisulfate, water to neutrality, and a saturated solution of sodium chloride. The ethereal solution was dried over anhydrous sodium sulfate; the ether was evaporated under reduced pressure to give 11.2 g of colorless gum. It was dissolved in ether, 5 g of silica gel was added, and the suspension was evaporated to dryness. The solid was applied to the top of a column of 100 g of silica gel packed in pentane–ether (9:1). Elution with the same solvent system removed the triphenylcarbinol present and increasing the polarity to pentane–ether (2:8) afforded 8.6 g (77%) of **6** as a colorless foam, $[\alpha]^{23}D$ −7.5° (*c* 1.9, $CHCl_3$). The material was homogeneous in solvent systems A, B, and C, but showed the presence of a very slight contaminant in system D. It was characterized as the 2,3-ditosylate derivative.

A solution of 500 mg (1.23 mmol) of the foam in 20 ml of anhydrous pyridine was treated with 570 mg (3.0 mmol) of $p$-toluenesulfonyl chloride and stirred at room temperature for 5 days. The solution was poured into 200 ml of ice-cold 5% sodium bicarbonate solution, and the crystals were collected by filtration and washed well with water. One recrystallization from methanol–ethyl acetate gave 425 mg (48%) of **6a** as colorless needles: mp 140–141°; $[\alpha]^{23}D$ 61.4° (*c* 0.64, $CHCl_3$). The infrared and nmr spectra were identical with the material prepared in section A. On admixture with a specimen from that section the mixture melted at 140–141°.

*Anal.* Calcd for $C_{35}H_{38}O_9S_2$: C, 65.52; H, 5.35; S, 8.97. Found: C, 65.38; H, 5.51; S, 9.25.

**Methyl 3-*p*-Bromobenzoyl-2-deoxy-5-*O*-triphenylmethyl-α-D-ribofuranoside (9).**—A solution of 3.1 g (7.95 mmol) of methyl of 2-deoxy-5-*O*-triphenylmethyl-α-D-ribofuranoside (**2**) in 25 ml of anhydrous pyridine was evaporated to dryness under reduced pressure. This process was repeated twice, and the resulting residue was dissolved in 25 ml of anhydrous pyridine, treated with 5.75 g (26.75 mmol) of freshly prepared $p$-bromobenzoyl chloride, and another 10-ml portion of anhydrous pyridine was added. The solution was stirred at room temperature for 18 hr, and the resulting pink suspension was poured into 200 ml of ice-cold 5% sodium bicarbonate solution. The precipitate was suspended in 200 ml of water, stirred for 1 hr, and filtered. The resulting light tan powder was suspended in 150 ml of ether, stirred for 1 hr, and filtered. The residue was extracted in the same manner with a second 150-ml portion of ether and filtered again. The combined ether extracts were washed with water and a saturated sodium chloride solution, dried over anhydrous sodium sulfate, and evaporated. The yellow semisolid was recrystallized from methanol–ethyl acetate (3:1) to give 3.9 g of **9** contaminated with a second component. The material was dissolved in 100 ml of ether, 10 g of silica gel was added, and the suspensions were evaporated to dryness and applied to the top of a column of 200 g of silica gel. Elution with pentane–ether (8.5:1.5) gave 3.5 g (77%) of **9** as a white crystalline solid, mp 123–126°, sufficiently pure for use in the preparation of **10**. An analytical specimen was recrystallized from methanol as colorless prisms: mp 125–126°; $[\alpha]^{26}D$ 101.3° (*c* 1.05, $CHCl_3$); $\nu_{max}^{KBr}$ 1720 (C=O) and 1595 $cm^{-1}$ (phenyl nuclei); nmr δ 8.01–7.10 (19 H, multiplet, aromatic protons), 5.55–5.15 (2 H, multiplet, $H_1$ and $H_3$), 4.53–4.25 (1 H, multiplet, $H_4$), 3.50–3.28 (5 H, multiplet, $CH_3O$ and $2H_5$), and 2.63–1.93 (2 H, multiplet, $2H_2$).

*Anal.* Calcd for $C_{32}H_{29}BrO_5$: C, 67.01; H, 5.09; Br, 13.93. Found: C, 66.98; H, 5.28; Br, 13.63.

**Methyl 5-*p*-Bromobenzenesulfonyl-3-*p*-bromobenzoyl-2-deoxy-α-D-ribofuranoside (11).**—A suspension of 10.25 g (0.018 mol) of methyl 3-*p*-bromobenzoyl-2-deoxy-5-*O*-triphenylmethyl-α-D-ribofuranoside (**9**) in 160 ml of glacial acetic acid was warmed on a steam bath until solution was complete, *ca.* 5 min. Water (25 ml) was added, and the solution was warmed for an additional 5 min. Water (15 ml) was added, and the warming was continued for 10 min. The colorless solution was cooled, and the solvents were evaporated *in vacuo*. The white crystalline residue was dissolved in 300 ml of ether, extracted with two 100-ml portions of 5% sodium bicarbonate solution, water, and saturated sodium chloride solution, and was then dried over anhydrous sodium sulfate. Evaporation of the ether under reduced pressure gave 12.5 g of semisolid residue which was suspended on 15 g of silica gel and applied to the top of a column of 225 g of silica gel packed in pentane–ether (9:1). Elution with pentane–ether (8:2) gave 6.10 g of triphenylcarbinol. The polarity was gradually increased to pentane–ether (2:8), and 2.85, g (48%) of **10** was obtained as a pale yellow syrup. The syrup (2.75 g, 8.30 mmol) was dissolved in 25 ml of anhydrous pyridine and the solution was evaporated to dryness *in vacuo*. This process was repeated once again, and the residue was dissolved in 50 ml of dry pyridine and treated with 3.18 g (12.15 mmol) of $p$-bromobenzenesulfonyl chloride in one portion. The solution was stirred at room temperature for 24 hr, poured into 400 ml of ice-cold 5% sodium bicarbonate solution, and stirred for 15 min, and the precipitate was collected by filtration. One recrystallization from methanol–ethyl acetate (1:1) gave 3.2 g (70%) of **11**, sufficiently pure for use in the preparation of **12**. An analytical specimen crystallized from methanol–ethyl acetate (1:1) as long colorless rods: mp 137–138° dec (insertion at 135°); $[\alpha]^{23}D$ 99.7° (*c* 0.71, $CHCl_3$); $\nu_{max}^{KBr}$ 1720 (C=O), 1600 and 1585 $cm^{-1}$ (phenyl nuclei); nmr δ 7.96–7.45 (8 H, multiplet, aromatic protons), 5.28–4.98 (2 H, multiplet, $H_1$ and $H_3$), 4.43–4.20 (3 H, multiplet, $H_4$ and $2H_5$), 3.33 (3 H, singlet, $CH_3O$), and 2.41–2.11 (2 H, multiplet, $2H_2$).

*Anal.* Calcd for $C_{19}H_{18}Br_2O_7S$: C, 41.47; H, 3.29; Br, 29.04. Found: C, 41.33; H, 3.46; Br, 28.67.

**Methyl 5-(6-Aminopurin-9-yl)-3-*p*-bromobenzoyl-2,5-dideoxy-α-D-ribofuranoside (12).**—A suspension of 162 mg (1.20 mmol) of adenine in 5 ml of anhydrous dimethylformamide was treated with 60 mg (*ca.* 1.20 mmol) of a 50% oil dispersion of sodium hydride, and the mixture was stirred at room temperature for 1 hr. It was warmed to 50°, maintained there for 30 min, and cooled to room temperature. A solution of 550 mg (1.00 mmol) of methyl 5-*p*-bromobenzenesulfonyl-3-*p*-bromobenzoyl-2-deoxy-α-D-ribofuranoside (**11**) in 15 ml of anhydrous dimethylformamide was added over a 10-min period, and the suspension was stirred at room temperature for 90 min. It was warmed to 50° and maintained at that temperature for 3 hr. After cooling to room temperature, the dimethylformamide was evaporated under high vacuum at a bath temperature of 40°. The white solid residue was extracted with two 25-ml portions of warm chloroform and the filtered chloroform extracts were combined, shaken with water, and dried over anhydrous sodium sulfate. Evaporation of the chloroform afforded 225 mg (42%) of **12**, mp 219–220°. Two recrystallizations from methanol afforded an analytical sample of **12** as small colorless rods: mp 221.5–222°; $[\alpha]^{23}D$ 142.9° (*c* 0.70, $CHCl_3$); $\nu_{max}^{KBr}$ 1715 (C=O), 1670 (purine nucleus), 1610 and 1575 $cm^{-1}$ (purine and phenyl nuclei); nmr δ 8.18 and 7.21 (1 H each, singlets, purine $H_2$ and $H_8$), 7.76 (4 H, broad singlet, phenyl protons), 5.41–5.06 (2 H, multiplet, $H_1$ and $H_3$), 4.76–4.38 (2.7 H, multiplet, $H_4$ and $-NH_2$), 3.32 (3 H, singlet, $CH_3O$), 3.28 (2 H, singlet, $2H_5$). The $2H_2$ protons are obscured by DMSO-$d_6$.

*Anal.* Calcd for $C_{18}H_{18}BrN_5O_4$: C, 48.22; H, 4.04; Br, 17.82; N, 15.62. Found: C, 48.50; H, 4.21; Br, 18.11; N, 15.36.

**Methyl 5-(6-Aminopurin-9-yl)-2,5-dideoxy-α-D-ribofuranoside (1).**—Methyl 5-(6-aminopurin-9-yl)-3-*p*-bromobenzoyl-2,5-dideoxy-D-ribofuranoside (**12**) (700 mg, 1.56 mmol) was dissolved in 600 ml of anhydrous methanol at room temperature, and ammonia was bubbled through the solution for 30 min. After 36 hr at room temperature, the solution was evaporated to dryness under reduced pressure. The solid was triturated with ether to remove the methyl $p$-bromobenzoate and was collected by filtration. One recrystallization from a small volume of methanol afforded 386 mg (93%) of **1** as a white microcrystalline solid: mp 200–201°; $[\alpha]^{23}D$ 97.6° (*c* 1.09, $CHCl_3$); $\lambda_{max}^{H_2O}$ 260 mμ (ε 14,600), $\lambda_{min}$ 227 (2200), $\lambda_{max}^{0.1\ N\ HCl}$ 258 (14,100), $\lambda_{min}$ 230 (2900), $\lambda_{max}^{0.1\ N\ NaOH}$ 260 (14,500), $\lambda_{min}$ 227 (2200); $\nu_{max}^{KBr}$ 1660,

1600, and 1585 $cm^{-1}$ (purine nucleus); nmr $\delta$ ($D_2O$)[34] (2 H, singlet, purine $H_2$ and $H_8$), 5.34 (1 H, quartet, $J_{AX} + J_{BX} = 7.5$ cps, $H_1$), 4.50–4.21 (3 H, multiplet, $H_4$ and $2H_5$), 3.45 (3 H, singlet, $CH_3O$), and 2.16–1.97 (2 H, multiplet, $2H_2$). The ultraviolet spectra confirmed 9 substitution on the adenine nucleus.[35]

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*Anal.* Calcd for $C_{11}H_{15}N_5O_3$: C, 49.80; H, 5.69; N, 26.40. Found: C, 49.46; H, 5.77; N, 26.10.

**Registry No.**—**1**, 16803-00-2; **2**, 16801-99-3; **3**, 16802-00-9; **4**, 16802-01-0; **5**, 16802-02-1; **6a**, 16802-03-2; **9**, 16802-04-3; **11**, 16802-05-4; **12**, 16802-06-5.

# A Kinetic Study of the Acid-Catalyzed Hydrolysis of Some Indolyl-β-D-glucopyranosides[1]

JEROME P. HORWITZ,[2] CHITTUR V. EASWARAN,[1c]

*Rollin H. Stevens Memorial Laboratory, Detroit Institute of Cancer Research Division of the Michigan Cancer Foundation, and Department of Oncology, Wayne State University School of Medicine, Detroit, Michigan*

AND LEON S. KOWALCZYK

*Department of Chemical Engineering, The University of Detroit, Detroit, Michigan*

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First-order rate coefficients have been determined along with energies ($E_a$), enthalpies ($\Delta H^{\pm}$), and entropies ($\Delta S^{\pm}$) of activation for the acid-catalyzed hydrolyses of 3-indolyl- (**1a**), 5-bromo-3-indolyl- (**1b**), and 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside (**1c**). It was demonstrated that the rate of oxidation of the intermediate indoxyl (**2**) to indigo (**3**) was not rate determining. No significant difference in $E_a$ is observed over a range of hydronium ion concentration. The relatively good agreement between the observed rate coefficients ($k'$) and the corresponding theoretical values ($k$) supports the conclusion that the hydrolysis step and not the oxidation of the intermediate indoxyl (**2**) to an indigo (**3**) is rate determining. The solvent isotope effect ($k'_{D_2O}/k'_{H_2O} \geqq 2$) indicates rapid preequilibrium protonation of the glucosides. The dependence of rate on acidity (Hammett–Zucker relationship and the Bunnett $w$ parameter) provides evidence for the unimolecularity of these hydrolyses. On the other hand, the values of $\Delta S^{\pm}$, which are narrowly positive, are consistent with several mechanistic possibilities which include the A1 mechanism.

Recent reports[3,4] from this laboratory described the syntheses of a number of indolyl-β-D-glycopyranosides (**1**) which have found application as agents for the histochemical localization of corresponding β-glycosidases in mammalian tissue.[4-6] The chromogenic reaction sequence underlying what has come to be known as "indigogenic staining"[7,8] is initiated by enzymic release of an intermediate indol-3-ol (indoxyl, **2**). The latter (*cf.* Scheme I) is rapidly and irreversibly transformed on air oxidation to an essentially insoluble (and highly colored) indigo (**3**) which is deposited at the sites of the activity.

The *O*-indoxyl derivatives, by virtue of the indigogenic principle, constitute a potentially useful group of substrates for kinetic studies of acid and base catalyzed, as well as enzymatic hydrolyses. The present study was undertaken to ascertain whether indolyl-β-D-glucopyranosides, as a consequence of a unique aglycon moiety, exhibit any unusual features when judged on the basis of the usual criteria (*vide infra*) employed in deciding the mechanism of the acid-catalyzed hydrolysis of relatively simple glycosides.

## Experimental Section

**Materials.**—3-Indolyl-β-D-glucopyranoside {mp 178–180° dec $[\alpha]^{27}D$ −65° (*c* 1.0, 50% aqueous DMF)} was purchased from the J. T. Baker Co. 5-Bromo-3-indolyl-β-D-glucopyranoside [mp 260–261° dec, $[\alpha]^{25}D$ −59° (*c* 1.0, 50% aqueous DMF)] and 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside {mp 240–243°, $[\alpha]^{23}D$ −89° (*c* 1.0, 50% aqueous DMF)} were prepared according to methods outlined in previous reports.[3,4] The purity of these pyranosides was checked by tlc on silica gel in butanol–water (86:14).

**Spectrophotometry.**—Spectrophotometric rates were determined using a Cary Model 11 recording spectrophotometer which was equipped with cell jackets thermostated by a Haake Type F constant-temperature bath. Both the jackets and the bath were joined in a series to a Thermo-Cool heat exchanger. This arrangement provided a temperature regulation of ± 0.02° over the desired range (47–65°). Rates were followed by observing the formation of **3a**, **b**, and **c** at 670, 600, and 660 mμ, respectively.

Beer–Lambert plots were utilized to ascertain the quantity of indigo (**3**) formed in the oxidation step. These plots, in turn, afforded a measure of the intermediate indoxyl (**2**) generated in the hydrolysis step. The procedure of Cotson and Holt[9] was adopted for the preparation of the plots which is based on the spectrophotometric measurement of the rate of appearance of the dyes. When such oxidations are carried out in aqueous solutions, the dyes initially form colloidal suspensions, the stabilities of which are not suitable for making reliable optical measurements. The dye sols can be stabilized by inclusion of 0.5% polyvinyl alcohol so that their optical properties do not vary over several hours, and certainly not for the duration of the kinetic measurements. It was found that the Beer–Lambert laws were obeyed by the polyvinyl alcohol stabilized sols of the indigo dyes over the concentration range encountered. Accordingly, it was possible to utilize optical densities directly in calculating velocity constants of the oxidation reactions.

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(2) To whom all correspondence should be addressed at the Detroit Institute of Cancer Research Division of the Michigan Cancer Foundation.

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