The ^{18}O Isotope Shift in ^{13}C Nuclear Magnetic Resonance Spectroscopy. 13. Oxygen Exchange at the Anomeric Carbon of D-Glucose, D-Mannose, and D-Fructose[1]

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The ^{18}O isotope shift in ^{13}C NMR spectroscopy was used to study the kinetics of the oxygen exchange reaction at the anomeric carbon atoms of D-glucose, D-mannose, and D-fructose. The exchange reactions follow apparent first-order kinetics and increase with temperature. The anomeric forms of each of these sugars undergoes oxygen exchange at approximately the same rate. The oxygen exchange reactions of these monosaccharides are catalyzed by acid and by base, and a water reaction is also observed. Detailed pH-rate profiles are presented. The rate of oxygen exchange in D-fructose at 25 °C agrees closely with the mannose exchange rate from pH 2 to 6; however above pH 7 the rate for fructose is approximately 5 times the rate for mannose. At 26 °C the rate of oxygen exchange in D-mannose is approximately 5 times the rate for D-glucose over the pH range 2-10. Comparisons between the hydration rates for these sugars and for simple aldehydes and ketones are made. High effective molarities of neighboring hydroxyl groups may be estimated, consistent with the rapid mutarotation processes. There is no need to invoke novel pseudo-acyclic intermediates in order to explain the low (apparent) rate of oxygen exchange compared to mutarotation.

Introduction

In aqueous solution the anomeric carbon atom of a monosaccharide undergoes an oxygen exchange reaction as well as an anomerization reaction. In contrast to the large body of kinetic data characterizing the anomerization process,[2] there are relatively few quantitative data describing the oxygen exchange reaction.[3] Thirty years ago Rittenberg and Graff demonstrated the oxygen exchange kinetics of D-glucose at a number of pH values and temperatures.[4] Surprisingly, there has been no comparable investigation for other sugars. Limited measurements of oxygen exchange rates for sugars have appeared sporadically in the literature, but the studies were done using differing experimental conditions, thus making meaningful comparisons between sugars quite difficult.[5] Such measurements were necessary as part of the mechanistic investigations involving isotopic labeling in our recent elucidation of the position of bond cleavage in the acid-catalyzed hydrolysis of sucrose.[1] We sought to extend these measurements because they would facilitate careful comparisons of structure and reactivity in the monosaccharide area.

The ^{18}O isotope shift in ^{13}C NMR spectroscopy[6] may be used to study oxygen exchange reactions in monosaccharides.[3] In this paper we present our investigation of the kinetics for the oxygen exchange reaction of the biologically important monosaccharides D-fructose and D-mannose. We have also reinvestigated the oxygen exchange reaction of D-glucose since the results from an earlier study[4] could not be readily extrapolated to different reaction conditions. Here, we present extensive pH-rate profiles. The results are also compared with trends previously established for the hydration kinetics of carbonyl compounds.

Experimental Section

Materials. D-[2-^{13}C]Fructose, D-[1-^{13}C]mannose and D-[1-^{13}C]glucose were obtained from Omicron Biochemicals. A sample of D-[2-^{13}C]fructose was also obtained from the Los Alamos National Laboratory. [^{18}O]Water (98 atom % ^{18}O) was from Merck, and deuterium oxide (99.8 atom % D) was purchased from Aldrich. Glass-distilled, deionized water and analytical grade reagents were used in the preparation of the solutions.

Solution Preparation. The exchange reactions were observed either by following the incorporation of ^{18}O into the sugar dissolved in an [^{18}O]water medium (in-exchange reaction)[3] or by following the loss of ^{18}O from an ^{18}O-labeled sugar into normal water (out-exchange reaction).[7]

In-Exchange. Samples were prepared by dissolving enough ^{13}C-enriched sugar in 60% [^{18}O]water (60% $H_2{}^{18}O$, 20% D_2O, 20% H_2O) to make a 50 mM mannose or 96 mM fructose solution. Traces of solid sodium phosphate (monobasic or dibasic) or sulfuric acid were used to adjust the sample pH.

Out-Exchange. Enough ^{18}O-labeled sugar (prepared by dissolving the ^{13}C-enriched sugar in approximately 0.1 mL of acidic [^{18}O]water (pH ~1) and allowing the solution to react at 37 °C for several days) was dissolved in 4 mL of water (20% D_2O/80% H_2O or 99.8% D_2O) to make 50 mM mannose, 20 mM fructose, or 30 mM glucose solutions. The solution pH was adjusted using concentrated HCl or NaOH solutions. For pH values greater than 6, sodium phosphate buffers (≤5 mM) were used.

Quantitative Analysis of Isomeric Forms. Enough ^{13}C-enriched sugar was dissolved in water (30% D_2O, 70% H_2O) to make 81 mM fructose or 50 mM mannose solutions. The solution pH was adjusted using trace amounts of sodium phosphate or sulfuric acid.

Measurements of pH were made at room temperature using a Corning Model 130 pH meter calibrated using buffer standards containing normal water. The data from a kinetic run were accepted only if the measured pH before and after an exchange experiment agreed to better than 0.2 units. Since pH measurements of the 2 M NaCl solutions gave anomalous results,[8] measurements for these samples were made on solutions diluted 10:1 in H_2O. For each sample at least two 10:1 dilutions wee prepared; the pH of the original sample was taken as 1 pH unit less than

(1) Paper no. 12: Mega, T. L.; Van Etten, R. L. *J. Am. Chem. Soc.* **1988**, *110*, 6372-6376.
(2) (a) Pigman, W.; Isbell, H. S. *Adv. Carbohydr. Chem.* **1968**, *23*, 11-57. (b) Isbell, H. S.; Pigman, W. *Adv. Carbohydr. Chem. Biochem.* **1969**, *24*, 13-65.
(3) Risley, J. M.; Van Etten, R. L. *Biochemistry 1982*, *21*, 6360-6365.
(4) Rittenberg, D.; Graff, C. *J. Am. Chem. Soc.* **1958**, *80*, 3370-3372.
(5) (a) Kohlbrenner, W. E.; Nuss, M. M.; Fesik, S. W. *J. Biol. Chem.* **1987**, *262*, 4534-4537. (b) Butler, W.; Serif, G. S. *Biochim. Biophys. Acta* **1985**, *829*, 238-243. (c) Varki, A.; Sherman, W.; Kornfield, S. *Arch. Biochem. Biophys.* **1983**, *222*, 145-149. (d) Sherman, W. R.; Loewus, M. W.; Pina, M. Z.; Wong, Y. H. *Biochim. Biophys. Acta* **1981**, *660*, 299-305. See also the references in ref 3.
(6) Risley, J. M.; Van Etten, R. L. *J. Am. Chem. Soc.* **1979**, *101*, 252-253.
(7) Risley, J. M.; Van Etten, R. L. *J. Am. Chem. Soc.* **1981**, *103*, 4389-4392.
(8) Bates, R. G. *Treatise on Analytical Chemistry*; Kolthoff, I. M., Elving, P. J., Eds.; Wiley: New York, 1978; Part I, Vol. 1, pp 821-863.

0022-3263/90/1955-0522$02.50/0

Table I. Relative Proportions (%) of the Tautomeric Forms of D-Fructose and D-Mannose at Equilibrium[a]

			pyranose		furanose		
sugar	temp, °C	pH[b]	α[c]	β	α	β	open chain
D-fructose[d]	21	7.0	3.0	69.6	5.7	21.1	0.5
	40	7.0	5.3	60.1	6.5	26.9	1.2
	50	7.0	3.9	58.5	8.1	28.2	1.3
	21	4.9	4.1	68.1	5.1	22.0	0.6
	50	4.9	4.0	58.1	8.2	28.4	1.3
D-mannose[e]	21	7.0	68.0	32.0			
	42	7.0	65.7	34.3			
	50	7.0	65.8	34.2			
	50	4.5	63.9	36.1			

[a] For solutions in 30% D_2O. [b] pH values represent direct pH meter readings. [c] Estimated by peak height measurements (see Experimental Section). [d] 81 mM fructose. [e] 50 mM mannose. Only the pyranose forms could be detected.

the reading for the diluted sample. Determining the pH in this way gave good results using control solutions containing no salt.

NMR Measurements. Spectra were obtained using an NTC-200 spectrometer fitted with a 12-mm probe operating at 50.3 MHz. Solutions were equilibrated at the probe temperature for a minimum of 20 min prior to data acquisition. Probe temperatures were measured using a chemical thermometer[9] or, in a few cases, using a custom-built digital thermometer equipped with a thermocouple which was designed by Dr. Gary W. Kramer of this department. Broadband ^1H decoupling was achieved using 1–2 W and a Waltz-16 decoupling scheme.[10] The joule heating effects[11] caused by the interaction between the ^1H decoupler and the solutions containing 2 M NaCl were calibrated as follows: a 5-mL solution of 1 M sucrose + 2 M NaCl in 30% D_2O with a few drops of dioxane was placed in the NTC-200, and the chemical shift difference between the dioxane resonance and the sucrose(fructosyl) C-3′ resonance[12] was measured as a function of temperature. Two series of measurements were made: one with the decoupler on constantly, and one with the decoupler on during the acquisition period (1.7 s) but off during the delay period (30 s). A calibration curve was made, and the results were used to estimate the sample temperature for the fructose solutions containing 2 M NaCl.

Oxygen Exchange Reactions. In a typical experiment 100–300 scans were collected every 10–60 min using 45° pulses spaced 5–10 s apart, a sweep width of 220 Hz, an acquisition time of 4.5–9.3 s, and a 2–4K block size. For very slow reactions ($t_{1/2}$ > 3 days) the samples were placed in a water bath at 25 °C or 26 °C (±1 °C), and spectra were acquired periodically using a QE-300 spectrometer fitted with a 5-mm probe operating at 75.6 MHz. Broadband ^1H decoupling was achieved using decoupler power of 1 W and an MLEV sequence.[13] The probe temperature in the QE-300 was measured using a chemical thermometer[9] and established to be 24-25°.

Quantitative Analysis of Isomeric Forms. Gated ^1H decoupling was used to suppress the nuclear Overhauser enhancement.[14] For the mannose solutions the spectra were acquired using 40–60 pulses spaced 7.8 s apart, a 55° pulse angle, a 600-Hz sweep width, and an 8K block size. For the fructose solutions the spectra were acquired using 140–540 pulses spaced 27–34 s apart using a 55° pulse angle, a 600-Hz sweep width and an 8–16K block size. To detect the carbonyl form, 600–860 pulses were used spaced 81 s apart, with a 68° pulse angle, a 10000-Hz sweep width, and a 16K block size. In order to minimize dynamic range effects arising from the simultaneous presence of strong and weak signals, the accumulated FID was block-averaged and processed in floating point form.

Data Analysis. Oxygen Exchange Kinetics. The accumulated FIDs were zero-filled, and a line-broadening factor of 0.01–0.2 Hz was applied prior to Fourier transformation. In a few cases the FIDs were apodized for resolution enhancement by a double exponential multiplication routine prior to Fourier transformation. The percentage, P, of ^{18}O in the anomeric species at a given time, t, was estimated by direct peak height measurements. The slope of a plot of ln $(1-(P/P_\infty))$ vs t (in-exchange[3] or ln P vs t (out-exchange)[7] is $-k_{ex}$, where P_∞ is the percentage of ^{18}O at equilibrium and k_{ex} is the pseudo-first-order rate constant describing the exchange process.

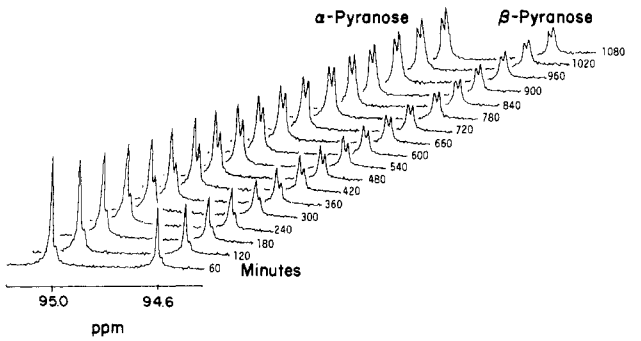


Figure 1. Oxygen exchange (in-exchange) at the anomeric carbon atom of α- and β-D-[1-^{13}C]mannose at 50 °C and pH 7.0. Signals for the ^{18}O isotopomers are shifted upfield relative to the ^{16}O species by 0.018 ppm for the α-anomer and 0.017 ppm for the β-anomer.

Quantitative Analysis of Isomeric Forms. A line-broadening factor of 1–3 Hz was applied to the accumulated FIDs prior to Fourier transformation. The relative proportions of the isomeric forms were estimated by electronic integration of the peak areas. To estimate the proportion of α-fructopyranose, a line-broadening factor of only 0.05–0.7 Hz was applied, and direct peak height measurements, rather than electronic integrations, were used.

Results

The application of the ^{18}O isotope-induced shift in ^{13}C NMR to follow the exchange reaction at the anomeric carbon atom of monosaccharides is illustrated in Figure 1 for D-mannose. Since the presence of an ^{18}O atom that is bonded directly to the anomeric carbon atom results in an upfield shift of the ^{13}C NMR signal (relative to that of the ^{16}O species), the oxygen exchange reaction may be monitored by observing the change in relative intensities of the two signals with time.[6] In this study the following isotope shifts were measured: α-glucopyranose, 0.019 ppm; β-glucopyranose, 0.017 ppm; α-fructofuranose, 0.022 ppm; β-fructofuranose, 0.021 ppm; α-fructopyranose, 0.021 ppm; β-fructopyranose, 0.023 ppm; α-mannopyranose, 0.018 ppm; β-mannopyranose, 0.017 ppm. The error in this measurement was less than 0.002 ppm. Within experimental error no difference was found in the magnitude of the isotope shift at the various temperature and pH values used in the kinetic runs for this study.

The monosaccharide oxygen exchange reaction follows apparent first-order kinetics and, as can be seen in Figure

(9) Van Geet, A. L. *Anal. Chem.* **1968**, *40*, 2227–2229.
(10) Shaka, A. J.; Keeler, J.; Freeman, R. *J. Magn. Reson.* **1983**, *53*, 313–340.
(11) Led, J. J.; Petersen, S. *J. Magn. Reson.* **1978**, *32*, 1–17.
(12) Morris, G. A.; Hall, L. D. *J. Am. Chem. Soc.* **1981**, *103*, 4703–4711.
(13) Levitt, M. H.; Freeman, R.; Frenkiel, T. *J. Magn. Reson.* **1982**, *47*, 328–330.
(14) Schoolery, J. N. *Prog. Nucl. Magn. Reson. Spectrosc.* **1977**, *11*, 79–93.

Table II. Oxygen Exchange Rates for D-Glucose, D-Mannose, and D-Fructose[a]

sugar	temp, °C	pH[b]	10^7k_{ex}, s^{-1}	sugar	temp, °C	pH[b]	10^7k_{ex}, s^{-1}
D-glucose[c]	26	1.8	37	D-fructose[h]	25	1.7	640
		2.0	12			2.9	40
		3.1	2.7			3.5	7.4
		4.0	1.5			4.4	4.9
		5.0	1.3			5.3	5.3
		6.0	1.3			6.2	6.6
		7.0	2.8			7.0	37
		7.0[d]	7.9			7.0[i]	50
		7.0[e]	21			7.0[j]	250
		8.4	14			8.1	240
		9.8	470			9.7	4200
					40	2.9	120
					60	2.9	480
					70	2.9	1300
D-mannose[f]	26	1.8	160		21	7.0	20
		2.3	100		30	7.0	52
		3.1	15		40	7.0	120[g,k]
		3.8	7.7		50	7.0	450[g,k]
		5.0	6.9		60	7.0	1100
		6.0	6.7		50	3.5	65[g,k]
		7.1	12			4.9	73[g,k]
		8.5	140		29	2.3[l]	150
		9.8	1700			2.5[l]	120
	42	7.0	200[g]		25	2.3[m]	200
	50	7.0	370[g]		35	2.3[m]	520
		4.5	250[g]		20	2.4[m]	95
					40	2.4[m]	1000
					50	2.4[m]	3200
					60	2.4[m]	6900

[a] Unless otherwise noted the rate constants are for out-exchange of the ^{18}O-labeled sugar into solutions containing 20% D_2O (as an NMR lock) and, for pH values greater than 6, 5 mM phosphate buffer. The kinetic plots typically gave correlation coefficients better than 0.98. [b] pH values are direct pH meter readings unless otherwise noted. [c] 30 mM D-glucose solutions. [d] 50 mM phosphate buffer. [e] 200 mM phosphate buffer. [f] 50 mM D-mannose solutions. [g] In-exchange measurements. [h] 15–20 mM D-fructose solutions unless otherwise noted. [i] 50 mM phosphate buffer. [j] 500 mM phosphate buffer. [k] 96 mM D-fructose solutions. [l] 50 mM D-fructose + 2 M NaCl in 99.8% D_2O. pH values were estimated by dilution as explained in the Experimental Section. [m] 50 mM D-fructose in 99.8% D_2O. pH values listed are 0.4 units greater than the pH meter reading (i.e., pD: ref 8).

1 the different anomers of a given sugar exchange oxygen at approximately the same rate, regardless of the relative proportions of the different isomers for different sugars.[3] In this study, at least 85% of each of the sugars is present in two isomeric forms[15] (Table I); only these predominant forms could be accurately analyzed to obtain rate data. The rate constants that were obtained upon analysis of either of the predominant forms for a given sugar were identical within experimental error. Consequently, these values were averaged to give the appropriate rate constants. The line widths corresponding to the furanose forms of D-fructose broadened significantly at high pH values, and this precluded observation of the isotope shift.[15c] However, because similar line-broadening effects were not observed for the β-pyranose form, rate data could still be obtained at high pH.

The data in Table II detail the temperature and pH dependencies of the monosaccharide exchange reaction. Arrhenius plots of the data for D-fructose give energies of activation (E_a) of 20 kcal/mol at pH 7.0, 15 kcal/mol at pH 2.9, and 21 kcal/mol in D_2O at pD 2.4. For D-glucose at pH 7.0 Rittenberg and Graff[4] found E_a = 23 kcal/mol based upon measurements at 40, 50, and 61 °C. (However, if our data at 26 °C were to be included, a value of 33 kcal/mol would be obtained; see also the Discussion). For D-galactose, Anderson and Garver[16] found E_a = 23 kcal/mol, and for D-erythrose, Risley and Van Etten[3] found E_a = 12.1 kcal/mol (in these last two studies the specific solution pH was not noted).

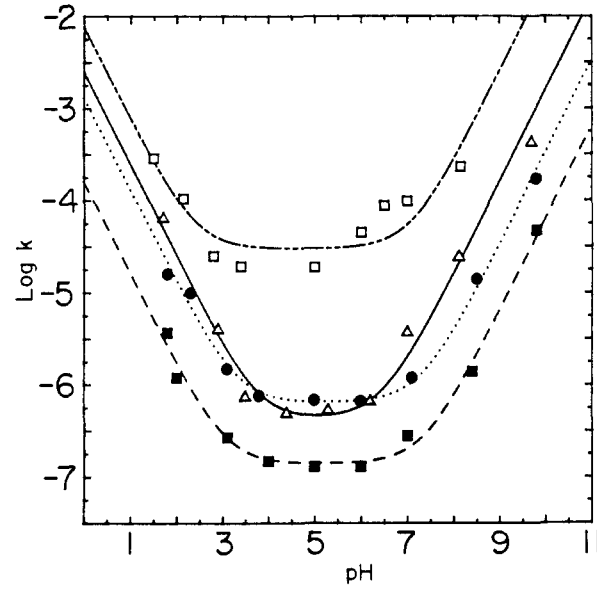


Figure 2. pH dependence for the oxygen exchange rates of (■) D-glucose (26 °C), (●) D-mannose (26 °C), (△) D-fructose (25 °C), and (□) D-glucose (61 °C, ref 4). Curves are based on a nonlinear, least-squares fit of the data to eq 1 (see Table III).

Figure 2 illustrates the pH dependence of the oxygen exchange reaction for D-glucose and D-mannose at 26 °C and D-fructose at 25 °C. The data of Rittenberg and Graff[4] at 61 °C are also included in Figure 2. The exchange data were fitted to eq 1. Here, k_{ex} is the observed exchange

$$k_{ex} = k_{H_2O} + k_H[H^+] + k_{OH}[OH^-] \quad (1)$$

(15) (a) Angyal, S. J. *Adv. Carbohydr. Chem. Biochem.* **1984**, *42*, 15–68. (b) Cockman, M.; Kubler, D. G.; Oswald, A. S.; Wilson, L. *J. Carbohydr. Chem.* **1987**, *6*, 181–201. (c) Goux, W. J. *J. Am. Chem. Soc.* **1985**, *107*, 4320–4327.

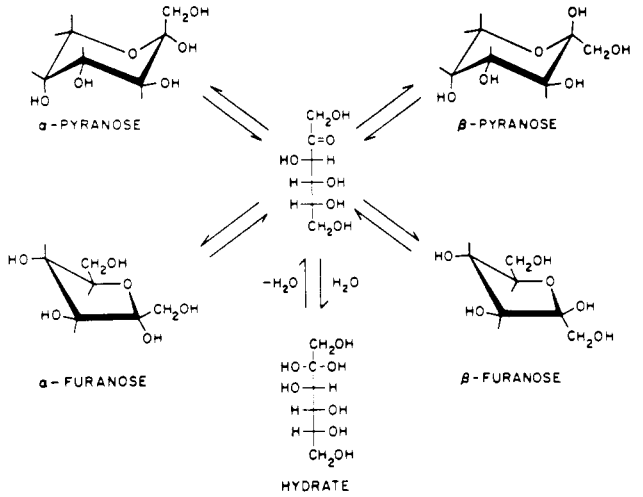
(16) Anderson, L.; Garver, J. C. *Adv. Chem. Ser.* **1973**, No. 117, 20–38.

Table III. Estimated Rate Parameters for the Oxygen Exchange and Hydration Reactions of Various Carbonyl-Containing Compounds

		oxygen exchange[a]			hydration[b]		
compound	temp, °C	10^4k_H, M^{-1} s^{-1}	$10^7k_{H_2O}$, s^{-1}	k_{OH}, M^{-1} s^{-1}	k_H, M^{-1} s^{-1}	$10^3k_{H_2O}$, s^{-1}	$10^{-4}k_{OH}$, M^{-1} s^{-1}
D-glucose[c]	61	76 (42)[d]	300 (96)	250 (150)	150 (83)	600 (190)	500 (300)
D-glucose[e]	26	1.6 (0.3)	1.4 (0.2)	0.65 (0.13)	11 (2.1)	10 (1.4)	4.6 (0.9)
D-mannose[f]	26	13 (2.0)	6.5 (0.9)	3.4 (0.6)	36 (5.5)	18 (2.5)	9.3 (1.6)
D-fructose[g]	25	25 (7.6)	4.3 (1.4)	16 (4.3)	0.59 (0.18)	0.10 (0.03)	0.38 (0.10)
acetone[h]	27	165000	≤100	55	33	≤0.02	0.011
acetaldehyde[i]	25				560	4.7	4.8

[a] Exchange rate parameters were obtained by a nonlinear least-squares fit of the exchange data to eq 1 by the KINFIT program.[17] [b] Hydration rate parameters for the open chain forms of the sugars were obtained via eq 4, assuming that the mole % of open chain forms remained constant over the pH range studied and were as follows: D-glucose (61 °C), 0.010%; D-glucose (26 °C), 0.0028%; D-mannose (25 °C), 0.0073%; D-fructose (25 °C), 0.85%.[21] [c] From the data of Rittenberg and Graff: 1.1 M glucose in 5 mM phosphate buffer. [d] Values in parentheses represent the standard deviations of the parrameter estimates generated by the KINFIT program. [e] 30 mM glucose in 20% D_2O. [f] 50 mM mannose in 20% D_2O. [g] 20 mM fructose in 20% D_2O. [h] Data from exchange studies of 0.1–0.2 M acetone at 27 ± 2 °C.[19a] [i] 0.1–0.2 M acetaldehyde.[19a,25]

Scheme I



rate, and k_{H_2O}, k_H, and k_{OH} are terms corresponding to the pH-independent reaction, the hydronium ion dependent reaction, and the hydroxide ion dependent reaction, respectively. The program KINFIT[17] was used to fit the data to eq 1 using a nonlinear regression routine and assuming that the hydronium ion and hydroxide ion activity coefficients were equal to 1. The resulting parameters are listed in Table III. The smooth curves in Figure 2 were drawn based upon these parameters.

Discussion

Isotopic labeling has proved to be an important mechanistic tool in the study of reactions involving the formation of or cleavage of carbon–oxygen bonds. In many biologically related organic molecules the carbon–oxygen functional group is often sufficiently reactive to undergo attack by the solvent water and thereby undergo oxygen exchange. Solutions of reducing carbohydrates, in particular, contain small percentages of open-chain carbonyl forms which, at least in simpler compounds, are known to undergo relatively rapid, reversible hydration reactions.[18] (cf. Scheme I: D-fructose tautomerization.) Thus, one prerequisite to any mechanistic study using ^{18}O labeling

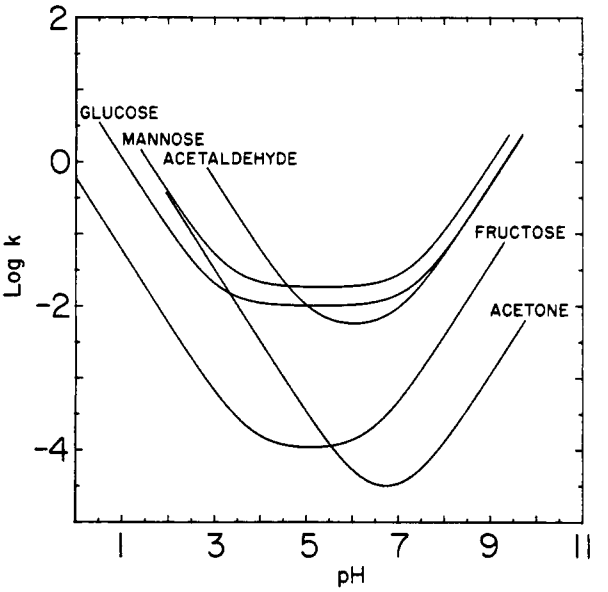


Figure 3. pH dependence of the hydration rates for D-glucose (26 °C), D-mannose (26 °C), D-fructose (25 °C), acetaldehyde (25 °C), and acetone (27 °C). Curves are based upon the parameters listed in Table III.

to trace the course of carbohydrate bond cleavage or formation is to understand the oxygen exchange reactions that take place between the sugars and the aqueous medium.

The reversible hydration of simple aldehydes and ketones has been well-studied, and, in some cases, oxygen exchange has been employed to measure indirectly the hydration rate.[18,19] Suppose that the ^{18}O-labeled carbonyl compound, *C, is placed in a large molar excess of ordinary water and a rapid equilibrium is established with its hydrate *H (eq 2). Then the pseudo-first-order rate constant

$$*C \rightleftarrows *H \rightarrow C \qquad (2)$$

for the hydration reaction, k_{hy}, is twice the value of the observed rate constant for oxygen exchange, k_{ex}.[19a] For

$$*C \xrightarrow{k_{ex}} C \qquad (3)$$

simple sugars only a small fraction of the sugar is present as the carbonyl species. Thus, the rate of oxygen exchange is further decreased by this factor and where N_c represents the mole fraction of the sugar present as the carbonyl

(17) Knack, I.; Röhm, K.-H. *Hoppe-Seyler's Z. Physiol. Chem.* **1981**, *362*, 1119–1130.

(18) (a) Bell, R. P. *Adv. Phys. Org. Chem.* **1966**, *4*, 1–29. (b) Pocker, Y.; Meany, J. E. *J. Phys. Chem.* **1967**, *71*, 3113–3120. (c) Bell, R. P.; Critchlow, J. E. *Proc. R. Soc. London A* **1971**, *325*, 35–55. (d) Buschmann, H.-J.; Dutkiewicz, E.; Knoche, W. *Ber. Bunsenges. Phys. Chem.* **1982**, *86*, 129–134. (e) Sørensen, P. E.; Jencks, W. P. *J. Am. Chem. Soc.* **1987**, *109*, 4675–4690.

(19) (a) Greenzaid, P.; Luz, Z.; Samuel, D. *Trans. Faraday Soc.* **1968**, *64*, 2780–2786. (b) Greenzaid, P.; Luz, Z.; Samuel, D. *Trans. Faraday Soc.* **1968**, *64*, 2787–2793.

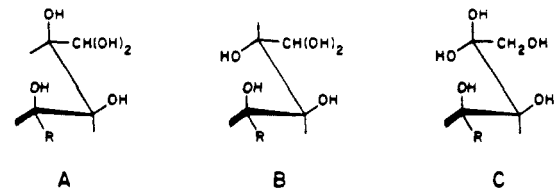
$$k_{ex} = N_c k_{hy}/2 \quad (4)$$

species.[20] Equation 4 may be used to calculate hydration rates from exchange data provided that values for N_c are known. We used the data of Hayward and Angyal[21] (which are in good agreement with other reports[15,22]) since "the relative values, when comparing different sugars, can be regarded as reliable".[15a] We have assumed that dilute acids or bases do not significantly alter the value of N_c.[15a] This seems reasonable since these sugars have pK_a values[23] greater than 12. (However, there is some limited evidence supporting a pH dependence for N_c.[15c,24]) The resulting pH–rate profiles for the hydration of glucose, mannose, and fructose are compared to those of acetaldehyde[19a,25] and acetone[19a] in Figure 3. As expected, the hydration rates for aldehydes are generally much faster than the rates for ketones.[18] Although the exchange rate in D-fructose is several times faster than the exchange rate in D-glucose (Figure 2), the opposite relationship actually holds for their hydration rates since the value of N_c for fructose (0.0085) is over 2 orders of magnitude greater than the value of N_c for glucose (0.000 028).[21]

The value of N_c for simple sugars increases markedly with temperature, and the temperature dependence is different for different sugars.[21] Because the exchange rate is directly proportional to N_c (eq 4), the apparent energy of activation (E_a) for the exchange reaction may be expected to vary with temperature as well. The interpretation of the E_a values reported to date is further complicated since most measurements have been made at pH 7.0, a point in the pH–rate profile (Figure 2) where catalysis by both water and base contribute to the exchange rate. Clearly, extrapolation of the oxygen exchange rates of sugars to different temperatures must be made with caution.

The solution pH in the basic region was controlled using sodium phosphate buffers (≤5 mM). Although phosphate buffers catalyze the hydration of simple aldehydes[18b] and ketones,[19a] the effect has been found to be insignificant at buffer concentrations of less than 10 mM.[18d] To assess the contribution of phosphate buffer catalysis in this study, experiments with D-glucose and D-fructose were carried out at pH 7.0 using increasing buffer concentrations (Table II). A plot of the results indicated that catalysis by phosphate does not contribute significantly to the exchange rate at a concentration of 5 mM.

There is also a small effect of sodium chloride on the rate of fructose exchange in the acidic region. At a concentration of 2 M NaCl the fructose exchange rate decreases approximately 2-fold. Similar reductions in the rate of acetic acid oxygen exchange have been observed in the presence of sodium chloride and sodium perchlorate.[7] However, a small catalytic effect by potassium chloride and lithium chloride has been observed in the acid-catalyzed hydration of propanal.[26]



A B C

Figure 4. Zig-zag conformations for the hydrates of the open-chain forms of (A) D-glucose, (B) D-mannose, and (C) D-fructose. The carbon backbones are drawn to resemble the right half of a cyclohexane chair conformation. This emphasizes the analogy between the 1,3-parallel interactions between the hydroxy groups at C2 and C4 and the corresponding steric interactions in cis-1,3-diaxially substituted cyclohexane derivatives (R = CH(OH)CH_2OH).

The oxygen exchange rates would be expected to be independent of the total sugar concentration as well as the method (in- or out-exchange) used to observe the reaction. The in- and out-exchange measurements for D-fructose at pH 7.0, in which the sugar concentration varies by a factor of 5, are consistent with these expectations. This result is also in agreement with the data of Risley and Van Etten[3] who, employing an in-exchange experiment with a 34-fold lower glucose concentration, reproduced the out-exchange measurement of Rittenberg and Graff.[4] The hydration kinetics for aliphatic aldehydes[18d] and ketones[19a] have also been found to be independent of substrate concentration.

The mechanism for the hydration of carbonyl compounds has been the subject of considerable attention.[18,19,26,27] Ketones are generally hydrated to a much smaller extent than are aldehydes, and the difference in reactivity in a homologous series can largely be explained in terms of steric and inductive effects. The reaction is catalyzed by general acids and by general bases. The formation of the carbon–oxygen bond between the attacking water molecule and the carbonyl carbon is thought to be concerted with a rate-determining proton transfer to the carbonyl oxygen involving two to four water molecules in a cyclic transition state.

Substituent inductive effects influence the susceptibility of the carbonyl oxygen toward protonation as well as the tendency of the carbonyl carbon to be attacked by water. If carbonyl protonation is rate-limiting the electron-withdrawing substituents adjacent to the carbonyl moiety will decrease the hydration rate. If carbonyl attack is rate-limiting then electron-withdrawing substituents will increase the rate of hydration. Using these criteria Buschmann et al.[18d] concluded that for simple aldehydes and ketones carbonyl protonation is rate-limiting in the region of acid catalysis, while attack at the carbonyl carbon is rate-limiting in the uncatalyzed (water) region. Analysis of Table III leads to the same conclusions for the open-chain forms of glucose, mannose, and fructose, which can be considered as polyhydroxy derivatives of acetaldehyde and acetone.

The possible influence of steric effects upon the kinetics of hexose hydration is more difficult to assess since the preferred conformations of the open-chain forms of simple sugars are not always well established.[28] Studies of acyclic

(20) Wertz, P. W.; Garver, J. C.; Anderson, L. *J. Am. Chem. Soc.* **1981**, *103*, 3916–3922.
(21) Hayward, D. L.; Angyal, S. J. *Carbohydr. Res.* **1977**, *53*, 13–20.
(22) (a) Los, J. M.; Simpson, L. B.; Wiesner, K. *J. Am. Chem. Soc.* **1956**, *78*, 1564–1568. (b) Maple, S. R.; Allerhand, A. *J. Am. Chem. Soc.* **1987**, *109*, 3168–3169.
(23) deWit, G.; Kieboom, A. P. G.; van Bekkum, H. *Recl. Trav. Chim. Pays-Bas* **1979**, *98*, 355–361.
(24) (a) Pigman, W.; Anet, E. F. L. J. *The Carbohydrates: Chemistry and Biochemistry*; Pigman, W., Anet, E. F. L. J., Eds.; Academic Press: New York, 1972; Vol. IA, p 171. (b) Wolfrom, M. L. *Ibid.* p 384. (c) Cantor, S. M.; Peniston, Q. P. *J. Am. Chem. Soc.* **1940**, *62*, 2113–2121. (d) Lippich, F. *Biochem. Z.* **1932**, *248*, 280–308.
(25) Bell, R. P.; Rand, M. H.; Wynne-Jones, K. M. A. *Trans. Faraday Soc.* **1956**, *52*, 1093–1102.
(26) Knoche, W.; Lopez-Quintela, M. A.; Weiffer, J. *Ber. Bunsenges Phys. Chem.* **1985**, *89*, 1047–1050.
(27) (a) Bell, R. P. *The Proton in Chemistry*; Cornell University Press: New York, 1973; pp 183–190. (b) Critchlow, J. E. *J. Chem. Soc., Faraday Trans. 1* **1972**, *68*, 1774–1792. (c) Eigen, M. *Disc. Faraday Soc.* **1965**, *39*, 7–15.
(28) (a) Durette, P. L.; Horton, D. *Adv. Carbohydr. Chem. Biochem.* **1971**, *26*, 49–125. (b) Angyal, S. J.; Greeves, D.; Mills, J. A. *Aust. J. Chem.* **1974**, *27*, 1447–1456. (c) Grindley, T. B.; Gulasekharam, V.; Tulshian, D. B. *Abstr. Pap. Joint Conf. 2nd*, CIC/ACS, Montreal, 1977, CARB 12.

Table IV. Comparison of Ring-Closing Rates and Hydration Rates for Various Sugars

sugar	ring form	k_{cl},[a] s^{-1}	$10^4 k_{hyd}$,[b] M^{-1} s^{-1}	effective molarity,[c] M
D-glucose	α-pyranose	50[d]	10[e]	50000
	β-pyranose	30	10	30000
D-galactose	α-pyranose	0.87[f]	1.6[g]	5400
	β-pyranose	1.7	1.6	11000
	α-furanose	1.7	1.6	11000
	β-furanose	3.0	1.6	19000
D-threose	α-furanose	2.2[h]	<27[h]	>810
	β-furanose	5.3	<27	>2000
D-fructose	β-pyranose	<20[i]	1.7[j]	<120000
	α-furanose	80	1.7	470000
	β-furanose	500	1.7	2900000
D-fructose-1,6-diphosphate	α-furanose	70[k]	7.1[l]	99000
	β-furanose	1450	7.1	2000000

[a] Unimolecular ring-closing rate constants. [b] The pseudo-first-order hydration rate constants, k_{hy} (s^{-1}), were calculated from exchange data (using eq 4), and the values were divided by 55.5 M to approximate the second-order hydration rate constants, k_{hyd} (M^{-1} s^{-1}). [c] Calculated from k_{cl}/k_{hyd}. For other examples of effective molarity calculations involving neighboring hydroxyl groups, see Kirby.[41] [d] 0.7 M glucose at 25 °C and pH 6.9 extrapolated to 50 mM phosphate buffer.[22a] [e] 30 mM glucose + 50 mM phosphate buffer (20% D_2O) at 26 °C and pH 7.0 (Tables II and III). [f] 0.3 M galactose + 2 mM maleate buffer at 25 °C and pH 6.2.[20] [g] 0.5 M galactose in unbuffered water extrapolated to 25 °C.[16,20] [h] 0.1 M threose + 50 mM acetate buffer in D_2O at pD 5.0 and 55 °C.[35a] [i] 1.5 M fructose in unbuffered water (10% D_2O) at 27 °C and pH 8.4.[15c] [j] 20 mM fructose + 5 mM phosphate buffer (20% D_2O) at 25 °C extrapolated to pH 8.4 (Table III). [k] 0.3 M fructose-1,6-diphosphate + 5 mM EDTA in 10% D_2O at 25 °C and pH 7.2.[38a] [l] 0.3 M fructose-1,6-diphosphate + 20 mM phosphate buffer at pH 7.0 with 0.1% BSA and 1 mM EDTA at 25 °C.[38b]

sugar derivatives and unsubstituted alditols suggest that the conformation in which the backbone carbon atoms are arranged in an approximately planar zig-zag with substituents above and below the plane is energetically favored unless 1,3-parallel interactions between substituents result.[15a,28] The 1,3-parallel interactions are analogous[29] to the steric effects which disfavor the chair conformation in cis-1,3-diaxially substituted cyclohexane derivatives (Figure 4). At 37 °C the hydration equilibrium constant for 2,3,4,5-tetra-*O*-methyl-D-mannose ($K_{eq} \simeq 2$), which can adopt a zig-zag conformation, is approximately 4-fold greater than the hydration equilibrium constant for 2,3,4,5-tetra-*O*-methyl-D-glucose ($K_{eq} \simeq 1/2$), for which the zig-zag conformation is not favored due to 1,3-parallel interactions between the substituents on C2 and C4.[15a,28c] At 26 °C the hydration rates for mannose (Table III) are 2–3-fold greater than those for glucose, suggesting that perhaps kinetics follow equilibria[19a] for these epimeric hexoses. However, this pattern is not supported by the existing data for D-galactose. The hydration equilibrium constants for the 2,3,4,5-tetra-O-methylated derivatives of D-mannose and D-galactose (which can also adopt the zig-zag conformation) are the same ($K_{eq} \simeq 2$),[15a,28c] whereas the hydration rate for D-galactose (approximately 0.009 s^{-1} based upon values extrapolated to 26 °C of $k_{ex} = 1 \times 10^{-6}$ s^{-1} and $N_c = 0.000\,22$)[20,21] is closer to that of D-glucose (approximately 0.010 s^{-1} in the water region). Further experimentation will be necessary to explain the apparently increased hydration rate of mannose compared to other aldohexoses.

Rittenberg and Graff's original study[4] demonstrated that the rate of oxygen exchange at the anomeric carbon of D-glucose was slow relative to the mutarotation rate. To explain this result Isbell et al.[30] proposed the existence of a conformation for the open-chain form of the sugar resembling a pyranose ring that has been "opened momentarily" (a "pseudoacyclic intermediate") which could undergo anomerization without passing to the free aldehyde form. Subsequently, pseudoacyclic intermediates have been advanced to explain the base-catalyzed mutarotation of D-glucose,[31,32] D-mannose,[31] D-fructose,[31,33] D-ribose,[33] and 5-thio-D-glucose,[32] as well as the base-catalyzed enolization and isomerization of D-glucose, D-mannose, and D-fructose.[34] However, attempts to observe pseudoacyclic intermediates by NMR techniques have failed,[15c,35] and calculations indicate that even if such intermediates exist for D-glucose, they are not involved in a significant pathway for mutarotation.[36] The idea that anomerization products are favored over oxygen exchange products because of the existence of such a "pseudoacyclic intermediate" is also inconsistent with the Curtin–Hammett principle.[37]

Isbell et al.'s original proposal of the pseudoacyclic intermediate was apparently based on the assumption that the hydration rate for the completely "free" aldehyde form of the sugar should be fast relative to the anomerization reactions. As can be seen in Table III, the data do not support this assumption. On the contrary, because the anomerization process is so fast, it appears that the oxygen exchange reactions observed for the ring forms of the sugar (Figure 1) merely assay the hydration kinetics of the open chain species (Scheme I). Furthermore, the oxygen exchange rate for D-glucose does not appear to be anomalously slow. In the uncatalyzed (water) region the rate of hydration for glucose is somewhat greater than that of acetaldehyde (Table III).[39] As noted above, the pH dependence of glucose hydration is as expected for a polyhydroxy aldehyde.

(29) Dempster, A. B.; Price, K.; Sheppard, N. *J. Chem. Soc., Chem. Commun.* **1968**, *1457*–1458.
(30) Isbell, H. S.; Frush, H. L.; Wade, C. W. R.; Hunter, C. E. *Carbohydr. Res.* **1969**, *9*, 163–175.
(31) deWit, G.; Kieboom, A. P. G.; van Bekkum, H. *Tetrahedron Lett.* **1975**, 3943–3949.
(32) Grimshaw, C. E.; Whistler, R. L.; Cleland, W. W. *J. Am. Chem. Soc.* **1979**, *101*, 1521–1532.
(33) Maier, G. D. *Carbohydr. Res.* **1977**, *53*, 1–11.
(34) deWit, G.; Kieboom, A. P. G.; van Bekkum, H. *Carbohydr. Res.* **1979**, *74*, 157–175.
(35) (a) Serianni, A. S.; Pierce, J.; Huang, S.-G.; Barker, R. *J. Am. Chem. Soc.* **1982**, *104*, 4037–4044. (b) Pierce, J.; Serianni, A. S.; Barker, R. *J. Am. Chem. Soc.* **1985**, *107*, 2448–2456.
(36) Grimshaw, C. E. *Carbohydr. Res.* **1986**, *148*, 345–348.
(37) Eliel, E. L. *Stereochemistry of Carbon Compounds*; McGraw Hill: New York, 1962; pp 151–2.
(38) (a) Midelfort, C. F.; Gupta, R. K.; Rose, I. A. *Biochemistry* **1976**, *15*, 2178–2185. (b) Model, P.; Ponticorvo, L.; Rittenberg, D. *Biochemistry* **1968**, *7*, 1339–1347.
(39) It has been reported that the hydration rate for acetaldehyde in the water region is intermediate between the hydration rates for D-galactose and D-erythrose (ref 35b). In fact, the hydration rate for acetaldehyde in this region (ref 18b,d,e, 19a, 25, 27b, 40) is smaller than the hydration rates for these sugars (ref 3, 20). The correct value for the rate of hydration of acetaldehyde in the water region at 25 °C is 0.0047 s^{-1} (smaller than the reported value (ref 3, 20, 35b) by about a factor of 10).
(40) Bell, R. P.; Evans, P. G. *Proc. R. Soc. London A.* **1966**, *291*, 297–323.

The relatively complex mutarotation and oxygen exchange of sugars result from a combination of more fundamental reaction processes. Thus, perhaps more appropriate than a comparison of mutarotation and oxygen exchange would be an examination of the relationship between ring-closing and hydration for the open-chain forms of the sugars. By comparing the rates for these two reactions the effective molarity[41] of the relevant backbone hydroxyl group may be estimated (Table IV). The magnitudes of the effective molarities listed in Table IV are in the range previously observed for cyclization reactions involving the hydroxyl group.[41] The values of the effective molarities for the two epimeric aldohexoses glucose and galactose are comparable, as are the values for fructose and its 1,6-diphosphate ester. For ring-closing reactions the effective molarity has generally been interpreted to be a measure of the entropic advantage enjoyed by intramolecular processes compared to intermolecular processes. This principle provides a reasonable explanation for the increased rate of mutarotation over oxygen exchange, thus eliminating the need to introduce novel acyclic intermediates into the tautomerization scheme.

The similarity between the kinetics and mechanism of the mutarotation of glucose and the hydration of acetaldehyde has been noted by several investigators.[18a,18b,42] Oxygen exchange studies can provide a more direct comparison, namely, with the actual hydration of glucose itself. Much of the attention in studies of glucose mutarotation and acetaldehyde hydration/dehydration has been focused on the question of whether proton transfer is coupled to carbon-oxygen bond formation/cleavage.[42] Nielsen and Sorensen have recently proposed that the degree of coupling between these two processes in the general base-catalyzed mutarotation of glucose changes with increasing basicity of the catalyst.[43] A related examination of the general base-catalyzed hydration of glucose might now be indicated.

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Registry No. ^{18}O, 14797-71-8; ^{16}O, 7782-44-7; D-fructose, 57-48-7; D-mannose, 3458-28-4; α-D-fructopyranose, 10489-81-3; β-D-fructopyranose, 7660-25-5; α-D-fructofuranose, 10489-79-9; β-D-fructofuranose, 470-23-5; α-D-mannopyranose, 7296-15-3; β-D-mannopyranose, 7322-31-8; D-glucose, 50-99-7; α-D-glucopyranose, 492-62-6; β-D-glucopyranose, 492-61-5; α-D-glucofuranose, 36468-84-5; β-D-glucofuranose, 30412-16-9; acetone, 67-64-1; acetaldehyde, 75-07-0; D-fructose 1,6-diphosphate, 488-69-7; D-threose, 95-43-2; D-galactose, 59-23-4; 2,3,4,5-tetra-*O*-methyl-D-glucose, 4261-26-1; 2,3,4,5-tetra-*O*-methyl-D-mannose, 95120-16-4; 2,3,4,5-tetra-*O*-methyl-D-galactose, 69502-91-6.

(41) Kirby, A. J. *Adv. Phys. Org. Chem.* **1980**, *17*, 183-278.
(42) Huang, H. H.; Robinson, R. R.; Long, F. A. *J. Am. Chem. Soc.* **1966**, *88*, 1866-1872.
(43) Nielsen, H.; Sørensen, P. E. *Acta Chem. Scand. A* **1984**, *38*, 309-326.

Synthesis of (±)-15-Deoxybruceolide and Conversion of (−)-15-Deoxybruceolide into (−)-Bruceantin: Total Synthesis of Bruceantin

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A total synthesis of bruceantin (1) using 15-deoxybruceolides **9** and **43** derived from naturally occurring brusatol as relay compounds was achieved. Previously reported ABCE tetracyclic compound **16** was converted into 11,12-*cis*-diol **30** bearing a suitable oxygen function at C-7. Ring D (δ-lactone) was formed by oxidation of a hydroxyl group at C-16 and selective removal of a C-7 hydroxyl protecting group to give **36**. Inversion of the stereochemistry of the hydroxyl group at C-11 was done by selective oxidation and reduction to give **12**. Enolate oxidation of the C-3 carbonyl group of **40**, which was derived from **12**, followed by bismuth trioxide oxidation yielded (±)-15-deoxybruceolide derivative **42**, which was further converted into acetate (±)-**43** and TBS ether (±)-**9**. Authentic specimens of **9**, **42**, and **43** were derived from naturally occurring brusatol (**3**) by using a radical-mediated deoxygenation of phenyl thiocarbonate **46**. Oxygenation at C-15 was achieved by oxidation of vinyl ethers **52** and **67**. Esterification of the C-15 hydroxyl group followed by acid-catalyzed hydrolysis of protecting groups at C-3, -11, and -12 gave bruceantin (1).

A wide spectrum of biological properties for the quassinoids, bitter principles isolated from the *Simaroubaceae* plants, has enormously increased interest in these highly oxygenated degraded triterpenes in recent years.[1] Among a large number of quassinoids,[1,2] some of those, which have been isolated from the genus *Brucea* and called bruceolides, display marked antileukemic activity. Bruceantin (**1**, Scheme I) and bruceantinol (**2**), isolated from *Brucea antidysenterica* Mill. by Kupchan and co-workers,[3] exhibited remarkably high antitumor activity. The quassinoids have many contiguous chiral centers and highly

(1) Polonsky, J. *Forschr. Chem. Org. Naturst.* **1973**, *30*, 101; **1985**, *47*, 221. Polonsky, J. Chemistry and Biological Activity of the Quassinoids. In *The Chemistry and Chemical Taxonomy of the Rutales*; Waterman, P. G., Grundon, M. F., Eds.; Academic Press: New York, 1983; p 247. Lidert, Z.; Wing, K.; Polonsky, J.; Imakura, Y.; Okano, M.; Tani, S.; Lin, Y.-M.; Kiyokawa, H.; Lee, K.-H. *J. Nat. Prod.* **1987**, *50*, 442.
(2) Connolly, J. D.; Hill, R. A. *Nat. Prod. Rep.* **1986**, *3*, 421, and references therein.
(3) (a) Kupchan, S. M.; Britton, R. W.; Ziegler, M. F.; Sigel, C. W. *J. Org. Chem.* **1973**, *33*, 178. (b) Kupchan, S. M.; Britton, R. W.; Lacadie, J. A.; Ziegler, M. F.; Sigel, C. W. *Ibid.* **1975**, *40*, 648.

0022-3263/90/1955-0528$02.50/0