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PREPARATION AND CONFORMATION OF α-L-ARABINOFURANOSYL-PYRIDINIUM SALTS, AND HYDROLYSIS OF THE 4-BROMOISOQUINOLINIUM COMPOUND

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ABSTRACT

Tri-*O*-benzoyl-α-L-arabinofuranosylpyridinium salts can be made in acceptable yields and high stereochemical purity by the reaction of 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl bromide and the pyridine in the presence of tetrabutylammonium bromide. Analysis of the ^{1}H-n.m.r. signals of the sugar reveals that the benzoylated compounds adopt largely the E_2 conformation whereas the debenzoylated compounds are largely in the $^0T_1$ conformation. The α-L-arabinofuranosyl-4-bromoisoquinolinium ion hydrolyses by both pH-independent and base-catalysed pathways, complicated by the reversible formation of an inert pseudo-base in alkali. The comparatively low rate of the pH-independent reaction is discussed in terms of the acid-lability of furanosides.

INTRODUCTION

Glycopyranosylpyridinium salts have proved very informative about enzymic[1–4] and non-enzymic[5,6] glycoside hydrolysis, by virtue of the absence of any possibility of acidic assistance to the departure of the aglycone, and of the conformational preferences dictated by the reverse anomeric effect of the pyridinium moiety. The preparation of glycofuranosylpyridinium salts and investigation of their hydrolytic behaviour therefore seemed likely to be fruitful. Departure of nicotinamide from the ribofuranosyl ring of NAD^+ is important biologically in the mono-[7] and poly-ADP-ribosylation of proteins[8], and simple NAD^+-glycohydrolases are also known and have been studied mechanistically[9,10]. The C–N cleavage of NAD^+ has been subject to some mechanistic investigations[11,12]; strangely, it is accelerated by anionic buffers, especially phosphate, and, in principle, is complicated by base stacking and the lability of the pyrophosphodiester group. We therefore selected α-L-arabinofuranosylpyridinium salts for investigation, since their spontaneous hydrolysis would not present these problems, yet in all probability[1–4] they would be substrates for α-L-arabinofuranosidases, which are widely distributed.

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RESULTS AND DISCUSSION

Preparation (Table I). — Crystalline tri-*O*-benzoyl-α-L-arabinofuranosyl bromide is conveniently obtainable from L-arabinose[13], and was used as starting material. Reaction with various pyridine derivatives at room temperature gave, for the most part, anomeric mixtures of the pyridinium salts, although low yields of the 4-bromoisoquinoline and 3,5-dimethylpyridine compounds were crystallised anomerically pure. By carrying out the reaction in the presence of tetrabutylammonium bromide, however, pure, crystalline α salts were obtained. Rough quantification of the effect of tetrabutylammonium bromide was obtained as follows. When tri-*O*-benzoyl-α-L-arabinofuranosyl bromide (0.1 g) was allowed to react in [U-^{2}H]pyridine (0.6 mL) overnight at room temperature, a 1:1 mixture of the anomeric pyridinium salts was produced, as estimated from the anomeric proton resonances and the complexity of the H-2,3,4 region [the H-1 resonance was a singlet superimposed on a doublet (J ~3 Hz) of approximately equal intensity]; in the presence of tetrabutylammonium bromide (0.07 g), only the clean spectrum of the α salt was discernible. A related phenomenon is the reported increase in the proportion of α-*N*-glycosyl derivative obtained when 2,3,5-tri-*O*-benzyl-α-L-arabinofuranosyl bromide reacts with deazaguanine and tetra-alkylammonium chlorides under phase-transfer conditions[14].

These results are reminiscent of those of Lemieux and Morgan[15] with tetra-*O*-acetyl-α-D-glucopyranosyl bromide, and their basic explanation, that apparently "retained" pyridinium salt arises from "inverted" glycosyl halide, itself produced by the action of bromide ion on the starting halide, clearly also applies in this case (Fig. 1) (in the absence of added bromide ion, the bromide ion from first-formed "inverted" pyridinium salt achieves the interconversion of the glycosyl halide). However, in the pyranose series, with halide-ion catalysis, 1,2-*cis*-glycosylpyridinium salts are formed exclusively, whereas in this case the 1,2-*trans* compounds are formed exclusively.

Although CPK models of the β pyridinium compounds can be constructed and are not inordinately strained, it is likely that a factor contributing to the exclusive formation of α salts when the two glycosyl halides are equilibrated is the steric clashes consequent upon having a bulky pyridine, a benzoyloxy group, and a benzoyloxymethyl group on the β face of the furanosyl ring. This factor must over-ride the ion–dipole interactions that seem to account for observed reactivities in the pyranosyl series[6].

Debenzoylation of the benzoylated pyridinium salts proved exceedingly tricky, and success was achieved only with ice-cold methanolic ammonia[16]. Crystalline salts suitable for kinetic studies have been obtained only with the 4-bromoisoquinoline and 3,5-dimethylpyridine aglycones. There is no doubt as to their anomeric configuration, since they are hydrolysed by the α-L-arabinofuranosidase III of *Monilinia fructigena*[17].

Conformation. — The ^{1}H-n.m.r. data for the sugar moieties of the benzoyl-

TABLE I

YIELDS AND CHARACTERISATION DATA FOR α-L-ARABINOFURANOSYLPYRIDINIUM SALTS

2,3,5-Tri-O-benzoyl compounds

Aglycone	*Yield (%)*	*M.p. (degrees)*	$[\alpha]_D^{25}$ *($CHCl_3$, c 1) (degrees)*	*Empirical formula*	*Found (%)*				*Calc. (%)*			
					C	*H*	*Br*	*N*	*C*	*H*	*Br*	*N*
4-Bromoisoquinoline	44	158–159	−84	$C_{35}H_{27}Br_2NO_7$	57.72	3.78	21.93	1.56	57.31	3.71	21.79	1.91
3,5-Dimethylpyridine	38	178–179	−16	$C_{33}H_{30}BrNO_7$	62.72	4.67	12.61	2.37	62.66	4.78	12.63	2.21
4-Methylpyridine	36	168–170	−5	$C_{32}H_{28}BrNO_7$	62.10	5.41	13.1	2.38	62.14	5.41	12.92	2.65
3-Bromopyridine	36	148–150	−23	$C_{31}H_{25}Br_2NO_7$	54.39	3.45	23.92	2.23	54.48	3.68	23.38	2.05
Pyridine	30	153–154	+11	$C_{31}H_{26}BrNO_7$	61.37	4.36	13.37	2.31	61.59	4.33	13.22	2.31

Debenzoylated compounds

Aglycone	*Yield (%)*	*M.p. (degrees)*	$[\alpha]_D^{25}$ *(H_2O, c 1) (degrees)*	*Empirical formula*	*Found (%)*				*Calc. (%)*			
					C	*H*	*Br*	*N*	*C*	*H*	*Br*	*N*
4-Bromoisoquinoline	59	103–104	−53	$C_{14}H_{15}Br_2NO_4$	39.46	3.47	36.75	3.08	39.93	3.57	37.6	3.33
3,5-Dimethylpyridine	39	146–147	−48	$C_{12}H_{18}BrNO_4$	44.87	5.59	29.46	4.23	45.01	5.66	24.95	4.37

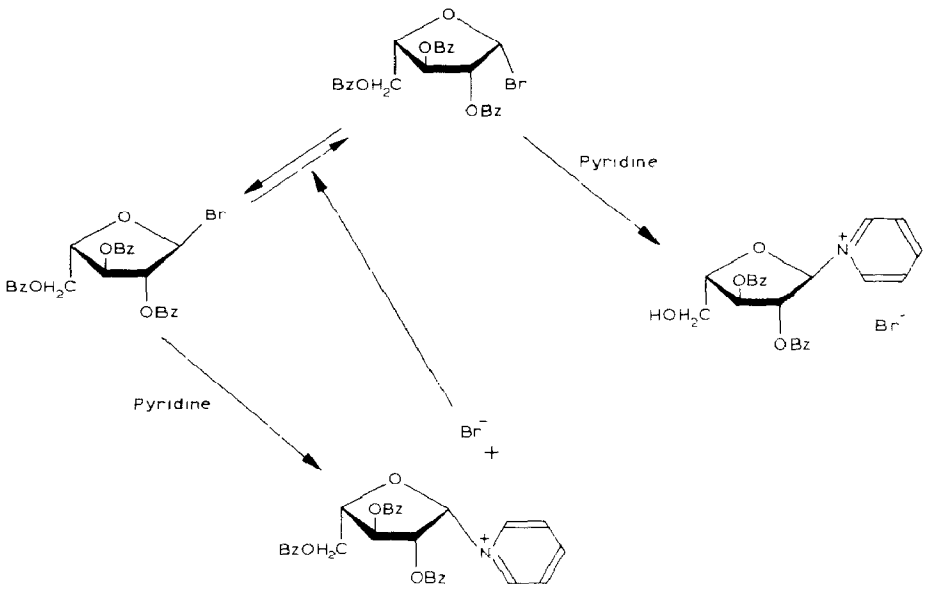
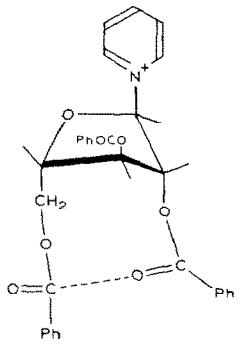


Fig. 1. Reaction of pyridine with 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl bromide.

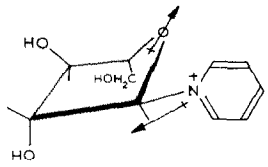
ated pyridinium salts are given in Table II. Dihedral angles were calculated from the ^3J values, using the modified Karplus equation, $J = 10.1 \cos^2 \Phi - 1.2 \cos \Phi$, proposed[18] for use in furanoid rings with nitrogen attached to C-1. However, this equation is parameterised on data for nucleosides and nucleotides, and there is the probability that, with a quaternary nitrogen attached to C-1, the estimated angles are too small.

Since the furanoid ring is flexible, observed couplings may be the time-average of several conformations. However, the E_2 conformation (**1**) can account for observed couplings of the benzoylated derivatives; indeed, if $J_{1,2}$ and $J_{2,3}$ are zero, this conformation is occupied exclusively. The contrast between splittings observed for this compound and for 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl fluoride is noteworthy[19]. Here, occupation of the V_3(D) [$\equiv {}^3E$(L)] conformation was mixed with appreciable occupation of other parts of the pseudo-rotational itinerary, V_1(D) [$\equiv {}^1E$(L)] and ${}^4T_3$(D). The occupation of the former conformation by the fluoride is readily explained by the anomeric effect; the reverse anomeric effect, expected to be operative with the pyridinium salts, would strongly disfavour it. In the E_2 conformation, the C–N^+ dipole nearly eclipses one of the lone pairs on the ring oxygen atom (if these are regarded as in equivalent sp^3 orbitals).

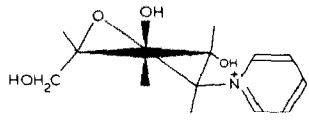
When these compounds are debenzoylated, all couplings increase. This is consistent with population of other conformers on the pseudo-rotational itinerary of the furanoid ring, particularly those in which the favourable dipolar interactions of the reverse anomeric effect can be maximised [*e.g.*, ${}^oE$ (**2**)]. However, again one conformation [${}^oT_1$ (**3**)] can account by itself for the observed splittings, and this is



$^1E_2$



$^2{}^oE$



$^3{}^oT_1$

adjacent to the ^oE conformation (**2**) on the pseudo-rotational itinerary. As with the benzoylated compound, conformational preferences are the reverse of those exhibited when the glycone–aglycone dipole is in the other sense; methyl α-L-arabinofuranoside adopts the E_0 conformation[20].

This change of the position of the conformation of a glycosylpyridinium salt on the pseudo-rotational itinerary of a flexible ring, consequent upon deacylation, is reminiscent of the behaviour of α-D-glucopyranosylpyridinium salts; the acetylated compounds in $CDCl_3$ adopt the $B_{2,5}$ conformation[21] and the deacetylated compounds in D_2O adopt[6] the $^1S_3$ conformation. It is possible that attractive interactions between acyloxy substituents[22] (see **1**) are one reason for the change.

Hydrolysis. — Table III gives measured first-order rate constants for the hydrolysis of the α-L-arabinofuranosyl-4-bromoisoquinolinium ion at various pH values and temperatures; data extrapolated to 25.0° are displayed as a function of pH in Fig. 2. The observed data can be quantitatively accounted for by three processes: (*a*) a pH-independent S_N1 hydrolysis of the salt similar to that observed for other glycosylpyridinium salts[5,6], (*b*) a specific base-catalysed pathway, and (*c*) the reversible formation of an inert pseudo-base at high pH.

These three processes give rise to a rate expression of the form

$$k_{obs} = \frac{k_o + k_B/[H^+]}{1 + K_a/[H^+]},$$

and the solid line of Fig. 2 is drawn with $k_o = 2.6 \times 10^{-9}\ s^{-1}$, $k_B = 6 \times 10^{-15}$ M.s^{-1}, and $K_a = 3.2 \times 10^{-11}$ M. The value of K_a, describing the formation of an inert pseudo-base, is that measured (as a pK_a of 10.5) in a separate, non-kinetic experiment. The behaviour of the α-L-arabinofuranosyl derivative in forming a pseudo-base is very similar to that of the β-D-galactopyranosyl derivative, in which the ionisation is governed[23] by a pK_a of 10.1. This similarity of pK_a values is an indication that the two pseudo-bases are being formed by the direct addition of hydroxide ion, rather than of the ionised HO-2 of the sugar, to the heteroaromatic system. Were tetracyclic structures being formed, the much higher strain of two *trans*-fused

TABLE II

CHEMICAL SHIFTS AND COUPLING CONSTANTS OF THE SUGAR PROTONS OF BENZOYLATED AND DEPROTECTED α-L-ARABINOFURANOSYLPYRIDINIUM BROMIDES

Aglycone	*Chemical shift (δ)*[a]						*Coupling constants (Hz)*				
	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-5'*	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5} = J_{4,5'}$	$J_{5,5'}$
Benzoylated salts (in CD_3CN)											
3-Bromopyridine	6.96	5.97	5.91	5.58	4.80	4.80	—	—	1.8(111°)[b]	5.9	—
4-Bromoisoquinoline	—	6.03	(multiplet	~5.9)	4.84	4.84	—	—	—	6.1	—
Pyridine	6.96	5.94	5.88	5.54	4.82	4.82	—	—	—	5.3	—
4-Methylpyridine	6.97	5.91	5.88	5.53	4.80	4.80	—	—	2.0(113°)	5.8	—
3,5-Dimethylpyridine	6.84	5.94	5.84	5.58	4.80	4.80	—	—	1.5(108°)	5.8	—
Debenzoylated salts (in CD_3OD)											
4-Bromoisoquinoline	6.43	4.51	4.25	4.80	3.91	3.86	1.5(108°)	3.0(118°)	2.4(115°)	4.1	12.2
3,5-Dimethylpyridine	6.17	4.34	4.19	4.63	3.85	3.79	2.0(113°)	3.0(118°)	3.4(122°)	4.4	12.0

[a]Relative to tetramethylsilane [b]Dihedral angles calculated from $J = 10.1 \cos^2\Phi - 1.2 \cos\Phi$.

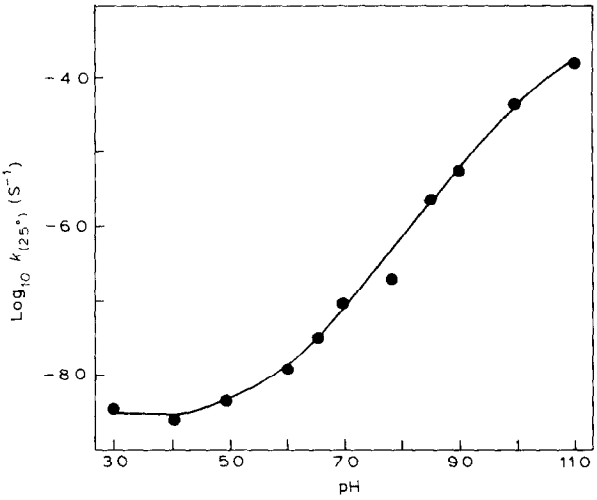


Fig. 2. pH-Dependence of the rate of hydrolysis of α-L-arabinofuranosyl-4-bromoisoquinolinium ion in 1.0M $NaClO_4$.

five-membered rings would be expected to result in a much higher pK_a for the α-L-arabinofuranosyl derivative.

At both pH 3.02 and 9.06, L-arabinose (76% and 69%) and 4-bromoisoquinoline (90% and 85%) are the products; kinetically significant quantities of 1,5-anhydro-L-arabinose are not formed. Therefore, the base-catalysed pathway probably involves participation by ionised HO-2 in the normal mechanism for base-catalysed glycoside hydrolysis (Fig. 3). No buffer catalysis could be detected; values of k_{obs} at 79.8° of 6.46×10^{-4} s^{-1} in 50mM phosphate (pH 7.04) and 5.34×10^{-4} s^{-1} in 150mM phosphate buffer (pH 6.91) were obtained. The reaction is therefore specific base catalysed. The specific base-catalysed pathway is much more important in the present case than with analogous β-D-galactopyranosyl[5], α-D-xylopyranosyl, or α-D-glucopyranosyl[6] compounds, in the hydrolysis of which base-catalysed pathways are important only above pH 9. The 109° H-1/H-2 dihedral angle for α-L-arabinofuranosyl-4-bromoisoquinolinium ion (Table II) implies that the HO-2/N^+ dihedral angle is 131°. Such a ground-state dihedral angle permits much readier nucleophilic participation by HO-2 at C-1 than the 60° dihedral angle of β-D-galactopyranosyl derivatives: hence, the much greater importance of the base-catalysed pathway. For much the same reason, the base-catalysed hydrolysis of NAD^+ is relatively more important than its spontaneous hydrolysis[11]. Likewise, methyl α-L-arabinofuranoside is much more base-labile than the pyranoside; indeed, small quantities of the pyranoside accumulate during base-catalysed hydrolysis[24], implying that, when the leaving groups at C-1 have comparable nucleofugacity (both being alkoxide ions), the ring-opening pathway, *via* an acyclic epoxide, is important. When the exocyclic leaving-group, as in the present case, has a much higher nucleofugacity, the endocyclic pathway is unlikely to be important.

TABLE III

KINETIC DATA FOR HYDROLYSIS OF α-L-ARABINOFURANOSYL-4-BROMOISOQUINOLINIUM ION IN 1.0M $NaClO_4$

pH	*Temperature (degrees)*	10^5 k/s^{-1}	*Rate constant extrapolated to 25°*	$\Delta H^{\ddagger}$(*kcal.mol*$^{-1}$)	$\Delta S^{\ddagger}$(*cal.mol*$^{-1}$.K^{-1})
3.02	101.6 ±0.1	105.4 97.8	$2.8_5 \times 10^{-9}$	36.5 ±0.8	25 ±2
	80.1 ±0.4	5.11 4.62			
4.03	101.2 ±0.1	110.4 112.9			
	91.2 ±0.1	27.8 30.1	$2.4_8 \times 10^{-9}$	37.4 ±0.7	30 ±2
	80.1 ±0.4	5.17 5.30			
4.95	101.2 ±0.15	106.0 101.6			
	90.3 ±0.1	32.6 34.6	$4.2_0 \times 10^{-9}$	35.7 ±2.5	23 ±9
	80.5 ±0.4	5.77 5.94			
6.01	90.2 ±0.1	56.4 59.4			
	80.3 ±0.3	16.06 15.27	1.14×10^{-8}	34.6 ±0.6	22 ±2
	70.1 ±0.3	3.10 3.21			
6.55	80.6 ±0.3	47.0 44.1	$3.1_3 \times 10^{-8}$	34.0 ±0.5	22 ±2
	64.2 ±0.1	3.79 3.61			
7.04	79.7 ±0.2	67.6 61.6			
	70.2 ±0.1	17.87 17.70	9.0×10^{-8}	33.3 ±0.6	21 ±2
	60.4 ±0.1	3.83 3.94			
7.95	70.2 ±0.2	75.9 75.4			
	60.4 ±0.1	17.48 17.87	$2.1_9 \times 10^{-7}$	36.4 ±0.8	33 ±3
	50.4 ±0.1	2.74 2.76			

TABLE III *(continued)*

pH	*Temperature (degrees)*	10^5 k/s^{-1}	*Rate constant extrapolated to 25°*	$\Delta H^{\ddagger}$*(kcal.mol*$^{-1}$*)*	$\Delta S^{\ddagger}$*(cal.mol*$^{-1}$*.K*$^{-1}$*)*
8.55	63.6 ±0.2	77.2 78.8	2.33×10^{-6}	29.5 ±0.5	15 ±2
	45.4 ±0.1	5.72 6.08			
9.06	50.7 ±0.2	26.7 32.0	5.6×10^{-6}	28.0 ±0.6	12 ±2
	39.8 ±0.3	6.35 6.11			
	30.3 ±0.2	1.48 1.42			
10.04	25.1 ±0.2	4.55 4.46	4.50×10^{-5}		
11.14	25.1 ±0.2	16.15 16.47	1.63×10^{-4}		

α-D-Xylopyranosyl and α-D-glucopyranosyl derivatives have no *trans* HO-2, and their base-catalysed hydrolysis involves other processes[6].

The rate of the pH-independent hydrolysis, which extrapolates to 2.6×10^{-9} s^{-1} at 25°, is in the range for various pyranosyl derivatives[4,5]. To avoid the errors inherent in long extrapolations, it is better, for purposes of more detailed comparisons, to use interpolated or extrapolated data at 80°; we then find values of 10^5 k/s^{-1} of 5.2 for α-L-arabinofuranosyl-, 6.4 for α-D-xylopyranosyl-, 1.39 for β-D-xylopyranosyl-, 14.5 for α-D-glucopyranosyl-, and 0.36 for β-D-glucopyranosyl-4-bromoisoquinolinium ions[6]. From literature data, it is possible to calculate that, at 39°, the spontaneous departure of phenolate from aryloxytetrahydrofurans is only twice as fast as from aryloxytetrahydropyrans[25]. It is thus apparent that the rate of leaving-group departure is determined much more by the substitution pattern on the ring than by ring size *per se*. The all-equatorial substitution pattern of the β-D-glucopyranosyl derivative disfavours formation of the half-chair conformation of the oxocarbonium ion intermediate; this restraining influence is not present in the α-L-arabinofuranosyl derivative where the interactions on going from the $^{o}T_1$ conformation of the salt to the ^{3}E or E_3 conformation of the oxocarbonium ion are not obviously unfavourable.

This result has a bearing on the long-recognised higher rate of acid-catalysed hydrolyses of alkyl and aryl furanosides than of pyranosides. This difference was originally thought to arise from the adoption of ring-opening pathways by the furanosides, but more recent work by Lönnberg *et al.* has indicated this is not al-

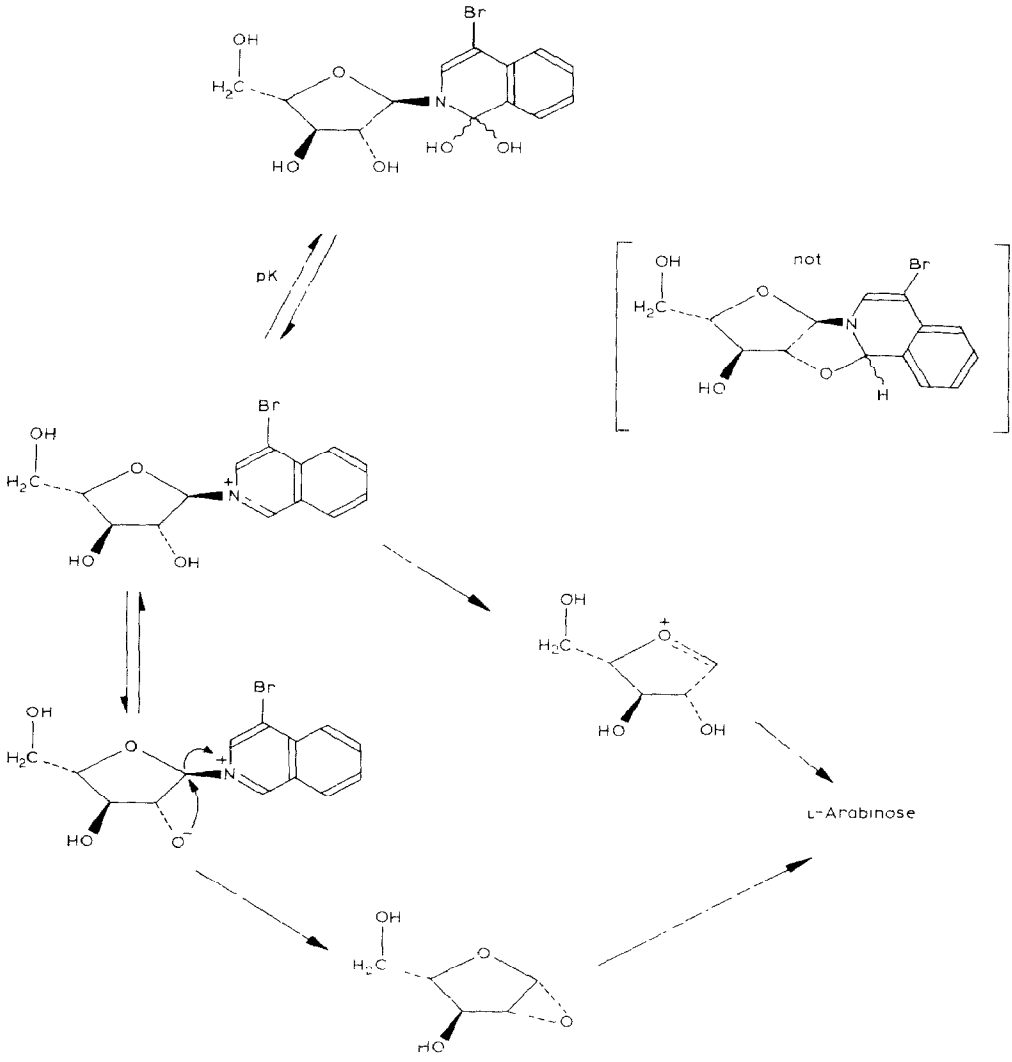


Fig. 3. Pathways of hydrolysis of α-L-arabinofuranosyl-4-bromoisoquinolinium ion.

ways so, since the pathway adopted depends on the pattern of substitution of the glycone and on the acidity of the aglycone, acidic aglycones tending to lead to reaction *via* aldofuranosyl cations[26–29]. However, aldofuranosides reacting via aldofuranosyl cations are still hydrolysed faster than are pyranosides. Thus, Lönnberg and Kulonpää[27] showed, on the basis of entropies of activation and solvent effects, that methyl α-D-arabinofuranoside reacted *via* an aldofuranosyl cation, yet its hydrolysis rate (3.19×10^{-3} M^{-1}.s^{-1} in aqueous perchloric acid at 79.45°) was still some 70 times greater than that of methyl β-D-glucopyranoside*.

*Calculated from the rate of hydrolysis[30] in 2M HCl at 79.7° and a value[31] of h_0 of 4.9 for 2M HCl.

An approximately similar figure was obtained[32] from a comparison of the acid-catalysed hydrolyses of the *p*-nitrophenyl glycosides; a rate[32] of 1.60×10^{-2} M^{-1}.s^{-1} in aqueous perchloric acid at 80° for the α-L-arabinofuranosyl glycoside compares with one of 1.39×10^{-4} M^{-1}.s^{-1} at 78.2° for the β-D-glucopyranosyl compound[33], and, for the furanoside, a primary ^{18}O kinetic isotope effect[32], $k_{16}/k_{18} = 1.023 \pm 0.003$, establishes the site of bond cleavage directly.

The rates of hydrolysis of α-L-arabinofuranosyl- and β-D-glucopyranosyl-4-bromoisoquinolinium ions differ by a factor of 14.4. If it is assumed that the reactivities of these ions parallel those of the protonated glycosides, there is thus still a factor of 5–6 in the greater acid-lability of furanosides that remains unaccounted for.

EXPERIMENTAL

N-(2,3,5-Tri-O-benzoyl-α-L-arabinofuranosyl)pyridinium bromides. — 2,3,5-Tri-*O*-benzoyl-α-L-arabinofuranosyl bromide[13] (2.5 g) and tetrabutylammonium bromide (dried *in vacuo* over P_2O_5; 1.0 g) were dissolved in dry acetone (5 mL). The dry pyridine derivative (2.5 g) was added and the solution was stirred overnight at room temperature. Crystalline product was collected; if the product did not crystallise, dry ether was added to turbidity, and the solution was stirred at 4° until crystallisation occurred. The products were recrystallised from dry ethanol.

Debenzoylation. — To dry methanol (70 mL), saturated at 22° with ammonia and then cooled to 0°, was added the benzoylated salt (0.7 g). After 18 h at 0°, the mixture was concentrated, and a solution of the residual yellow gum in dry methanol (5 mL) was poured into dry ethyl acetate (250 mL). The precipitate was collected by centrifugation, and crystallised and recrystallised from methanol–ethyl acetate.

Buffer solutions. — These were made up using AnalaR reagents. Buffering systems were EDTA–HCl (pH 3.02), acetic acid–NaOH (pH 4.03 and 4.95), NaH_2PO_4 (pH 6.01, 6.55, 7.04, and 7.95), EDTA–NaOH (pH 8.55, 9.06, and 10.04), and Na_2HPO_4–NaOH (pH 11.12). To a 50mM solution of the first component in M sodium perchlorate was added a highly concentrated solution of the second component until the correct pH was registered (Radiometer PHM62 pH Meter, equipped with a standard combination electrode which had just been calibrated with BDH standard buffers.

Kinetic techniques. — These have been described[6]; however, rates below 50° were measured using a Pye–Unicam SP8-200 kinetic system.

Product analyses. — For each pH value, 3.02 and 9.06, duplicate solutions (5 mL) of the substrate in the buffer used for kinetics were heated at 100° for 10 half-lives, then neutralised, and, for the determination of 4-bromoisoquinoline, extracted with dichloromethane (3 × 2 mL). The combined extracts were concentrated using a stream of nitrogen, the residue was dissolved in ethanol (1 mL), and the 4-bromoisoquinoline was determined from its u.v. absorbance.

L-Arabinose was determined enzymically, using β-D-galactopyranose dehydrogenase (Boehringer), in a rate assay as described by Melrose and Sturgeon[34].

The pK_a of α-L-arabinofuranosyl-4-bromoisoquinolinium bromide was measured from the change in u.v. absorbance, essentially as described earlier[23]; the value reported pertains to 1.0M sodium perchlorate.

^{1}H-N.m.r. spectra were recorded at 200 MHz on a JEOL FX200 Fourier-transform instrument.

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