Spectrophotometric Analyses of Ribose and 2-Deoxy-D-ribose Alone and in Mixtures

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The rates of production of the chromophore for ribose (R) and the maximum absorbance of the chromophore for 2-deoxy-D-ribose (DR), produced under thermal and acidic conditions are directly related to their sugar concentrations. These factors are the bases for qualitative and quantitative analysis of these sugars. The $\lambda_{max.}$ 261 mμ chromophore of degraded DR is stable for at least 15 hr. after 5 hr. of treatment at 80.0° with 1.0 *M* HCl. The $\lambda_{max.}$ 277 mμ chromophore of degraded R increases continuously during the same period at an apparent zero-order rate. These phenomena serve as bases for a sensitive spectrophotometric assay of both sugars, alone and in mixtures. These acid-generated chromophores when made alkaline generate other absorption bands with specific properties which permit additional methods of differentiation between the sugars.

Analyses of sugars in mixtures constitute a broad field of investigation. Sugar mixtures have been separated by paper or column chromatography with further analyses of the effluents (1–3). Methods of separation by gas chromatography of methylated sugars are available (4, 5). Differences in color development after reaction with specific reagents have been another approach (6). Dialysis based on differential kinetics (7) and combinations of different methods (8) have been proposed. However, some of these techniques are nonselective and are insensitive to small sugar concentrations.

The phenomenon of chromophore production when sugars are heated with mineral acids is well known. Correlations between absorbances of those chromophores and the sugar concentration have served as bases for sugar analyses (9–11). Limited attempts have been made to analyze sugars in mixtures by these procedures. The extremely strong conditions in acid concentration and temperature which have been used sometimes do not permit the attainment of reproducible results and are not specific for individual sugars.

If a sugar is subjected to well-defined and mild conditions of acid concentration, temperature, and time, definite chromophores with well-defined properties can result (12–14). For example, when 2-deoxy-D-ribose (DR) is heated 5 hr. in 1.0 *M* HCl at 80.0°, a chromophore $\lambda_{max.}$ 261 mμ (12) is produced as shown in Fig. 1 and is stable under those conditions for at least 15 hr. (Fig. 2). Ribose (R) under the same conditions generates a chromophore $\lambda_{max.}$ 277 mμ (Fig. 1) which increases continuously during the same period at an apparent zero-order rate (Fig. 2). Such differences can provide sensitive assay methods for these sugars, alone and in mixtures. The properties of these acid-generated chromophores were also investigated.

EXPERIMENTAL

Materials and Equipment—D-Ribose (R) and 2-deoxy-D-ribose (DR) were obtained from Calbiochem (analytical reagent grade) and furfural from the Fisher Scientific Co. (reagent grade). All other chemicals used were of analytical reagent grade. A Beckman model DU spectrophotometer, slit width 0.1 mm., was used. Complete absorption spectra were obtained on a Cary model 15 spectrophotometer.

Spectrophotometric Procedures for Calibration Curves of 2-Deoxy-D-ribose (DR)—A calibration curve for DR was prepared using concentrations between $1 \times 10^{-4}M$ and $7 \times 10^{-4}M$ in 1.0 *M* HCl.

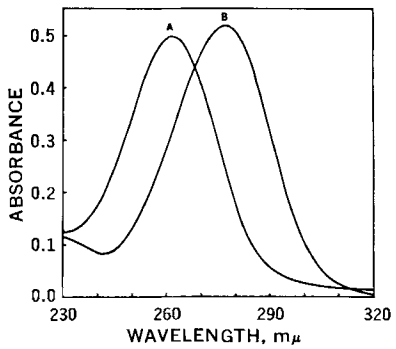

Fig. 1—Ultraviolet spectrum of the products of sugar degradation in 1.0 M HCl at 80.0°. Curve A is for 3.75 × 10⁻⁴ M 2-deoxyribose after 5 hr. of degradation. Curve B is for 8.00 × 10⁻⁴ M ribose after 6.5 hr. of degradation and is also the spectrum of furfural in 1.0 M HCl.

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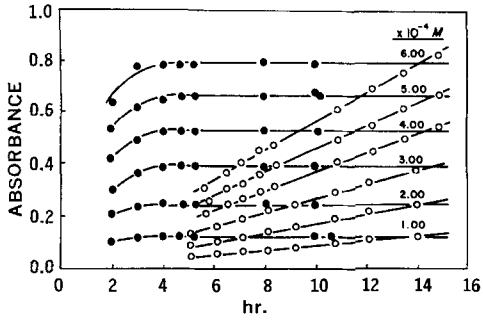

Fig. 2—Effect of time on the absorbances of (○) *1.0* M *HCl acid-degraded ribose measured at 277 mμ, and* (●) *deoxyribose measured at 261 mμ, at 80.0°.*

The samples were maintained at 80.0° in a thermostatted bath. Aliquots were taken between 5 and 20 hr. of degradation. On cooling, the absorbances were read at $\lambda_{max.}$ 261 mμ. A plot of these absorbances against the respective sugar concentration resulted in the calibration curve for DR as shown in Fig. 3.

Calibration Curves of D-Ribose (R)—Samples of R between $1 \times 10^{-4}M$ and $6 \times 10^{-4}M$ were degraded under the same conditions as specified for DR. Samples were taken between 5 and 20 hr. of degradation. The cooled aliquots were read at 277 mμ against a blank solution of 1.0 *M* HCl. The plots of absorbances against time for each R concentration are given in Fig. 2. The apparent zero-order rate constants, the slopes of such lines, are plotted against the respective R concentration in the calibration curve for R (Fig. 3).

Assay of Ribose and 2-Deoxy-D-ribose—Solutions of the sugars were prepared so that the absorbance between 200 and 400 mμ did not exceed 1.50 after heating at 80.0° for 20 hr. in 1.0 *M* HCl. For greater absorbances the solution may be diluted with known volumes of 1.0 *M* HCl. To assay DR, the absorbance at 261 mμ was measured between 5 and 20 hr. of degradation. This value was used to obtain the DR concentration from Fig. 3, the calibration curve. To assay R, samples between 5 and 20 hr. were removed from the reaction flask, and the absorbances of the cooled aliquots were measured at 277 mμ. A plot of absorbance against time was made and the apparent rate constant was measured. This value was used to obtain the R concentration from Fig. 3, the calibration curve. Seven or eight aliquots at 1.0 or 1.5 hr. intervals will provide an accurate value for the rate constant. However, two measurements at 5 and 20 hr. are sufficient to estimate the apparent zero-order rate constant for any R concentration.

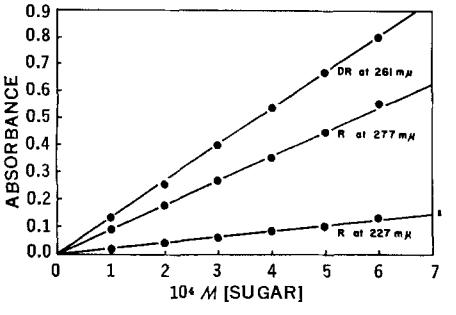

Fig. 3—Calibration curves for the spectrophotometric analyses of acid-degraded ribose and deoxyribose. The ordinate represents the absorbance after 5 hr. for deoxyribose (DR) and the rate constants in 10 × absorbance units/hr. at 277 mμ for ribose (R) as obtained at 80.0° in 1.0 M *HCl.*

Anaylsis of Ribose and 2-Deoxy-D-ribose in Mixtures—Solutions containing the mixture were degraded in 1.0 *M* HCl at 80.0°. Samples were taken between 5 and 20 hr. of degradation. The absorbances at 261 mμ and 277 mμ were measured and plotted against time. In the plot at 277 mμ, the slope was determined and the R concentration obtained from the calibration curve, Fig. 3. The intercept of the plot of absorbances of the mixtures at 261 mμ against time corresponds to the absorbance due to degraded DR. The pertinent DR concentration was read from its calibration curve, Fig. 3.

Investigation of Properties of Furfural—The spectrum of furfural was obtained in 1.0 *M* HCl and followed as a function of time in 0.25 *M* NaOH at 45.0°. The extraction of furfural and the chromophore of acid-degraded ribose from 1.0 *M* HCl by equal volumes of chloroform was followed by observing the decreases in the $\lambda_{max.}$ 277 mμ absorbance in the aqueous phases.

RESULTS AND DISCUSSION

Kinetic Studies—When 2-deoxy-D-ribose (DR) is degraded in 1.0 *M* HCl at 80.0° a chromophore at 225 mμ is generated by an apparent first-order process. The apparent first-order rate constants are proportional to the hydrogen ion activity, a_{H^+}, at a given temperature (12). The compound with this chromophore is the precursor of 5-methyl-3(2*H*)-furanone (13) with a $\lambda_{max.}$ 261 mμ (Fig. 1) which is also generated by an apparent first-order process. The 261 mμ absorbance reaches a constant value A_∞ after 5 hr. of heating in 1.0 *M* HCl, which is maintained for at least 15 hr. (Fig. 2). The A_∞ is directly proportional to DR concentration, and the apparent first-order rate constants are also proportional to the hydrogen ion activity. A complete discussion of the properties of acid-degraded DR, 5-methyl-3(2*H*)-furanone and the chemical nature of the degraded products has been reported (12, 13).

When ribose (R) is degraded under the same conditions, a chromophore at $\lambda_{max.}$ 277 mμ is generated (Fig. 1). After a slight induction period, this chromophore appears at apparent zero-order rates between 5 and 25 hr. after initiation of the degradation (Fig. 1). However, the over-all reaction may not be strictly a zero-order process. As the degradation proceeds, the rate of appearance of the 277 mμ absorbance band decreases, but this deviation from zero order is not significant until after 25 hr. A final absorbance $\lambda_{max.}$ 277 mμ with an apparent molar absorptivity of 7,160 based on the initial ribose concentrations is obtained after 200 hr. at 80.0° in 1.0 *M* HCl.

Concomitant with the appearance of the 277 mμ absorbance band, another at 227 mμ $\lambda_{max.}$ is also observed to arise by an apparent zero-order rate process. The 227 mμ chromophore is not preferred for analytical purposes due to its lessened absorptivity.

The effect of the R concentration on the rate of generation of the chromophores was studied. Concentrations of R ranging from $1 \times 10^{-4}M$ to $6 \times 10^{-4}M$ were degraded in 1.0 M HCl at 80.0°. The resultant absorbances, A, were measured at 227 and 277 mμ and plotted against time, t, as shown in Fig. 2 for the 277 mμ values. The values for the slopes, k_o, of the lines between 5 and 20 hr. of degradation were obtained (Fig. 1) in accordance with the equation:

$$A = k_o t \qquad \text{(Eq. 1)}$$

The apparent intercepts are not significantly different from zero for ribose concentrations less than $6 \times 10^{-4}M$. The k_o values are directly related to the ribose concentration as shown in Fig. 3 where:

$$k_{277} = \alpha_{277}\,\mathrm{R};\ \alpha_{277} = 2.49 \times 10^{-2}\ \text{absorbance units} \times \text{L./mole/sec.} \qquad \text{(Eq. 2)}$$

where the percent of standard deviation of α_{277} is 1.8% and the standard deviation of k_{277} about regression on R is 1.87×10^{-7}, so that at $3.5 \times 10^{-4}M$ ribose the percent of standard deviation of k_{277} is 2.1%. The percent of standard deviation is often referred to as the coefficient of variation Also,

$$k_{227} = \alpha_{227}[\mathrm{R}];\ \alpha_{227} = 5.92 \times 10^{-3}\ \text{absorbance units} \times \text{L./mole/sec.} \qquad \text{(Eq. 3)}$$

The dependency of the apparent zero-order rate constant, k_{277}, at 277 mμ on the HCl concentration was also studied for $1 \times 10^{-3}M$ ribose solution degraded at 80.0°. The values of k_{277} were determined (Table I) and plotted against the respective hydrogen ion activity, $a_{H+} = f[\mathrm{HCl}]$, where f is the activity coefficient obtained from the literature (15). From the plot of k_{277} against a_{H+} (Fig. 4), at 80.0° at $10^{-3}M$ ribose:

$$k_{277} = k_{H+}a_{H+};\ k_{H+} = 3.2 \times 10^{-5}\ \text{absorbance units} \times \text{L./mole/sec.} \qquad \text{(Eq. 4)}$$

Alternatively, in terms of HCl concentration:

$$k_{277} = k_{\mathrm{HCl}}[\mathrm{HCl}];\ k_{\mathrm{HCl}} = 2.3 \times 10^{-5}\ \text{absorbance units} \times \text{L./mole/sec.} \qquad \text{(Eq. 5)}$$

Thus, at 80.0°:

$$\alpha_{277} = [\beta_{277}]_{H+}a_{H+} = (k_{277}/[\mathrm{R}])\,a_{H+};\ [\beta_{277}]_{H+} = 3.2 \times 10^{-2}\ \text{absorbance units} \times \text{L.}^2/\text{mole}^2/\text{sec.} \qquad \text{(Eq. 6)}$$

$$\alpha_{277} = [\beta_{277}]_{\mathrm{HCl}}[\mathrm{HCl}] = (k_{277}/[\mathrm{R}])[\mathrm{HCl}];\ [\beta_{277}]_{\mathrm{HCl}} = 2.3 \times 10^{-2}\ \text{absorbance units} \times \text{L.}^2/\text{mole}^2/\text{sec.} \qquad \text{(Eq. 7)}$$

For a given hydrogen ion activity or HCl concentration, the appropriate value at 80.0° can be determined for the 277 mμ absorbance of degraded ribose (Eqs. 6 and 7). Knowledge of the experimental zero-order rate constant at that acid concentration or hydrogen ion activity permits calculation of the ribose concentration from the rearranged Eq. 2.

The rate dependency on the absolute temperature, °C. + 273, was determined from the appropriate plot (Fig. 5, curve A) of the Arrhenius equation from data obtained at several temperatures (Table I) for $10^{-3}M$ ribose in 1.0 M HCl:

$$\log k_{277} = -(\Delta H_a/2.303\ \mathrm{R})\,1/T + \log P \qquad \text{(Eq. 8)}$$

The apparent heat of activation calculated from the slope of this plot was $\Delta H_a = 28.6$ Kcal./mole, and the log P value was 13.1. Since $k_{\mathrm{HCl}} = k_{277}$ at 1.0 M HCl (Eq. 5), the Arrhenius parameters are the same for log k_{HCl} at 277 mμ. Also, the Arrhenius parameters for log $[\beta_{277}]_{\mathrm{HCl}}$ are $\Delta H_a = 28.6$ Kcal./mole and log $P = 10.1$ so that $[\beta_{277}]_{\mathrm{HCl}}$ may be calculated for various temperatures from the Arrhenius equation (Eq. 8). Substitution of this value and a given HCl concentration in Eq. 7 determine the α_{277} value to be used in Eq. 2 for determining ribose concentration.

Assay of Ribose and 2-Deoxy-D-ribose in Mixtures—The 261 mμ absorbance of degraded DR increases to a stable value after 4 hr., whereas the 277 mμ absorbance of degraded R continues to increase linearly with time (Fig. 6). When a mixture of R and DR is subjected to thermal acid degradation the apparent zero-order rates of increase of the absorbances in the region of 277 mμ after 5 hr. are identical with the rates for the same concentration of ribose alone under the same conditions of acid and temperature (Figs. 2, 3, and

Table I—Apparent Zero-Order Rate Constants[a] for the Appearance of the 227 and 277 mμ Absorbances from Acid-Degraded Ribose at 80°

°C.	[HCl]	10^4 [Ribose]	10^7k_{227}	10^6k_{277}
80.0	1.00	1.00	3.89	2.47
80.0	1.00	2.00	11.4	4.86
80.0	1.00	3.00	16.1	7.50
80.0	1.00	4.00	22.8	9.80
80.0	1.00	5.00	28.6	12.5
80.0	1.00	6.00	37.2	15.4
80.0	1.00	10.00	...	25.7
80.0	0.75	10.00	...	18.9
80.0	0.50	10.00	...	10.7
80.0	0.13	10.00	...	2.47
70.0	1.00	10.00	...	7.31
60.0	1.00	10.00	...	2.11

[a] In absorbance units/second and obtained between 5 and 20 hr. after initiation of reaction.

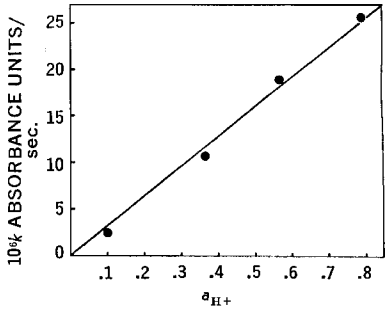

Fig. 4—Dependence of the apparent zero-order rate constant, k, in absorbance units/sec., on the hydrogen ion activity, a_{H+}, for the degradation of 1.0 × 10^{-3} M ribose at 80.0° as determined from the rate o appearance of the 277 mμ chromophore.

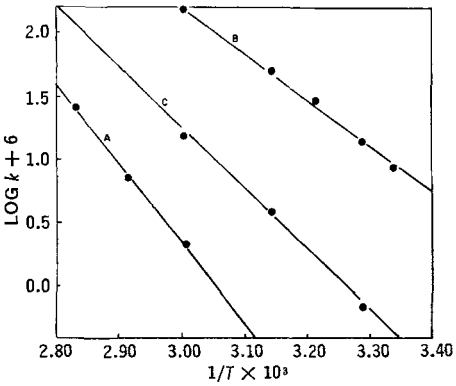

Fig. 5—Arrhenius plots for the apparent zero-order degradation of ribose in 1.0 M HCl (k *in absorbance units/sec.) as measured by the appearance of a* $\lambda_{max.}$ *277 mμ absorbance* (A) *and for the 0.20 M NaOH degradation of acid-degraded ribose as measured by the apparent first-order disappearance of* $\lambda_{max.}$ *277 mμ* (B) *and appearance of* $\lambda_{max.}$ *324 mμ* (C), k *in sec.*$^{-1}$.

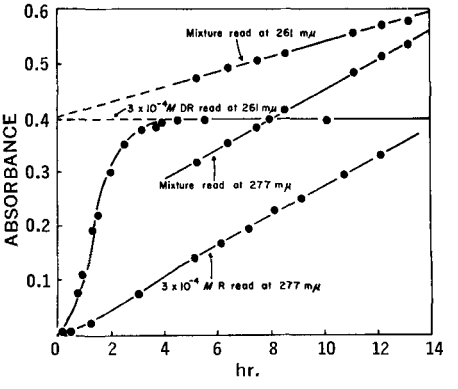

Fig. 6—Examples of analyses of mixtures of ribose (3×10^{-4} M) *and deoxyribose* (3×10^{-4} M) *degraded in 1.0 M HCl at 80.0°. The combined absorbances are read at 261 mμ to determine the intercept value related to the deoxyribose concentration which is obtained by extrapolation of the linearity observed after 5 hr. The combined absorbances are read at 277 mμ to determine the linear slope after 5 hr. which is related to the ribose concentration. The absorbances with time at these* $\lambda_{max.}$ *values are also given for the acid degradation of the separate sugars.*

6). The slope of the absorbance *versus* time plot after 5 hr. for such a mixture is a function only of the R concentration which can be obtained from the calibration curve for R (Fig. 3). The zero time intercept for this linear segment after 5 hr. when absorbance at 261 mμ is plotted against time is only due to the absorbance of the degraded DR (Fig. 6). This intercept is related to the concentration of DR present in the mixture by the appropriate calibration curve (Fig. 3).

This technique was applied to the resolution of several mixtures of both sugars in the concentration ranges from $1 \times 10^{-4}M$ to $6 \times 10^{-4}M$. The results are given in Table II. The estimated percent of standard deviations of each assay in such mixtures is 1.87% for deoxyribose and 2.93% for ribose.

TABLE II—RESULTS OF THE ASSAY OF MIXTURES OF RIBOSE (R) AND 2-DEOXYRIBOSE (DR)

Comp. of the Mixtures		Found by Assay	
10^{-4} *M* (DR)	10^{-4} *M* (R)	10^{-4} *M* (DR)	10^{-4} *M* (R)
1.00	1.00	1.00	1.15
2.00	1.00	2.00	0.78
3.00	1.00	3.00	1.00
4.00	1.00	3.91	1.00
1.00	2.00	1.00	1.96
2.00	2.00	2.02	2.02
3.00	2.00	3.03	2.02
4.00	2.00	4.03	1.94
1.00	3.00	1.03	2.96
2.00	3.00	2.06	3.03
3.00	3.00	3.03	3.06
4.00	3.00	4.10	2.92
5.00	3.00	5.15	3.08
1.00	4.00	1.02	4.05
2.00	4.00	2.00	3.98
3.00	4.00	3.03	4.00
4.00	4.00	3.95	3.95
6.00	4.00	6.10	4.25
1.00	5.00	1.00	5.00
2.00	5.00	1.90	5.00
3.00	5.00	3.03	4.97
4.00	5.00	4.03	4.93
5.00	5.00	5.00	4.85
1.00	6.00	1.00	6.00
2.00	6.00	1.98	5.97
3.00	6.00	3.12	6.25
4.00	6.00	4.00	5.88
5.00	6.00	5.20	5.92

Alkaline Degradation of the Acid-Generated Chromophore of Ribose—The 261 mμ chromophore of acid-degraded DR can be destroyed in mildly alkaline solutions. The 261 mμ absorbance upon alkalinization shifts to a $\lambda_{max.}$ 293 mμ, which absorbance band disappears rapidly by a first-order process (12). When acid-degraded ribose is made alkaline by addition of NaOH, the $\lambda_{max.}$ 277 mμ absorbance is lost by a first-order process to a small residual absorbance (Fig. 7). The loss in absorbance is accompanied by a gradual hypsochromic shift to a residual absorbance with a $\lambda_{max.}$ about 260 mμ. Concomitant with the 277 mμ absorbance disappearance, a band $\lambda_{max.}$ 324 mμ arises by an apparent first-order process and approaches an asymptotic value. This band subsequently disappears by an apparent first-order process (Fig. 7). The initial isosbestic point at 302 mμ indicates a 1:1 transformation for the first reaction in the apparent sequence, *i.e.*, $A \rightarrow B$, with an apparent first-order rate constant k'_{277}. The subsequent slower transformation, $B \rightarrow C$, of an apparent first-order rate constant k_{324} is accompanied by a loss of the isosbestic point. The respective k'_{277} and k_{324} values at various temperatures and concentrations of alkali are given in Table III. These apparent rate constants at 45.0° when plotted against the respective NaOH concentrations show a linear dependency (Fig. 8) and:

$$k_i = k_{NaOH}[NaOH] \qquad \text{(Eq. 11)}$$

where k_{NaOH} for the disappearance of the 277 mμ chromophore and appearance of the 324 mμ chromophore at 45.0° is 2.58×10^{-4} L./mole/sec., and the k_{NaOH} for the disappearance of the 324 mμ chromo-

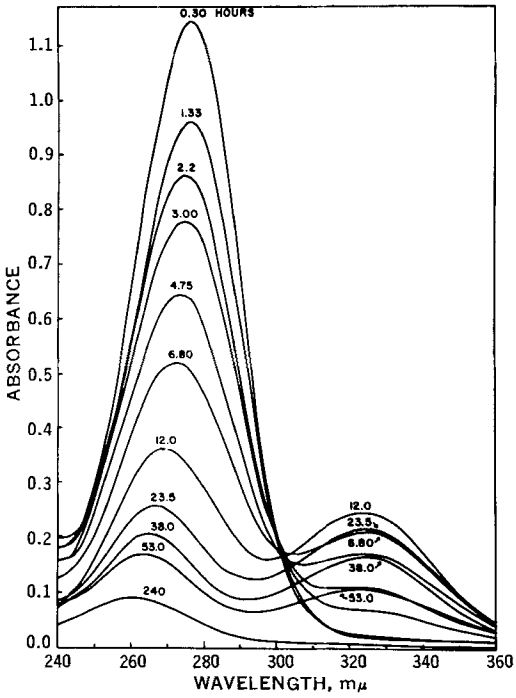

Fig. 7—Ultraviolet spectra of acid-degraded ribose and furfural at various times in 0.26 M NaOH at 45.0°.

TABLE III—APPARENT FIRST-ORDER RATE CONSTANTS FOR THE DISAPPEARANCE IN ALKALI OF THE 277 mμ CHROMOPHORE OF DEGRADED RIBOSE (FURFURAL), k'_{277} IN SEC.$^{-1}$, AND FOR THE SLOWER DISAPPEARANCE OF A RESULTANT 324 mμ CHROMOPHORE, k_{324}

°C.	[NaOH]	$10^5 k'_{277}$	$10^6 k_{324}$
60.0	0.20	15.4	15.3
45.0	0.26	7.09	4.92
45.0	0.20	5.15	3.83
45.0	0.13	3.41	2.47
45.0	0.066	1.42	1.36
38.0	0.20	2.95	...
31.0	0.20	1.40	0.666
25.0	0.20	0.852	...

phore is 1.92×10^{-5} L./mole/sec. The Arrhenius parameters, (Eq. 8) ΔH_a and log P, for the apparent first-order rate constants for the transformation of the acid-degraded ribose chromophores in 0.20 *M* NaOH were 16.5 Kcal./mole and 7.0 for the loss of the $\lambda_{max.}$ 277 mμ and 21.3 Kcal./mole and 9.1 for the loss of the resultant $\lambda_{max.}$ 324 mμ.

Correlation of the Chromophore of Acid-Degraded Ribose with Furfural—It is well-known that furfural is the major product of acid degradation of ribose (16). The ultraviolet spectrum of furfural in 1.0 *M* HCl is exactly that of acid-degraded ribose (Fig. 1). The bands at $\lambda_{max.}$ of 277 mμ and 227 mμ have a ratio of absorbances for furfural A_{277}/A_{227} =

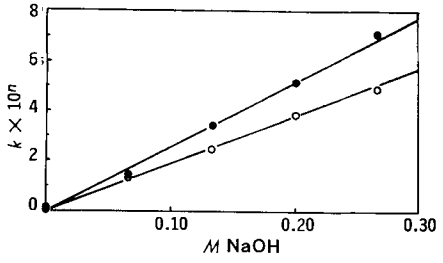

Fig. 8—Dependence of the apparent first-order rate constant, k in sec.$^{-1}$, on the NaOH concentration for the disappearance of the chromophores of the products of acid-degraded ribose at 45.0° where n = 5 for $\lambda_{max.}$ 277 mμ (●) and n = 6 for $\lambda_{max.}$ 324 mμ (○).

3.78, whereas the spectrum of acid acid-degraded ribose of an equivalent absorbance, A_{277} = 1.5, has a ratio of absorbances of 3.68. Furfural in 0.28 *M* NaOH at 45.0° loses its chromophore at 277 mμ with a slight hypsochromic shift. Concomitantly a less intense band at $\lambda_{max.}$ 324 mμ arises with a clear isosbestic at 301 mμ. These are exactly the properties of the chromophore of acid-degraded ribose (Fig. 7). The molar absorptivity of furfural ϵ_f at 277 mμ is 14,962 in agreement with the literature value of 14,800 (17). Thus the ultimate yield of furfural after 200 hr. from acid degradation of ribose at 80.0° in 1.0 *M* HCl is 7,160/14,962 = 47.8%.

Both furfural and the chromophore of acid-degraded ribose are readily extracted from 1.0 *M* HCl by chloroform. When equal volumes of chloroform and 1.0 *M* HCl were used, 94.4% of the 277 mμ absorbance was extracted from the aqueous phase as compared to 94.5% of the 277 mμ absorbance of acid-degraded ribose.

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