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AN INVESTIGATION OF THE EQUILIBRIA BETWEEN AQUEOUS RIBOSE, RIBULOSE, AND ARABINOSE

Y.B. TEWARI and R.N. GOLDBERG

Chemical Thermodynamics Division, Center for Chemical Physics, National Bureau of Standards, Gaithersburg, MD 20899, U.S.A.

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The thermodynamics of the equilibria between aqueous ribose, ribulose, and arabinose were investigated using high-pressure liquid chromatography and microcalorimetry. The reactions were carried out in aqueous phosphate buffer over the pH range 6.8–7.4 and over the temperature range 313.15–343.75 K using solubilized glucose isomerase with either $Mg(NO_3)_2$ or $MgSO_4$ as cofactors. The equilibrium constants (K) and the standard state Gibbs energy (ΔG°) and enthalpy (ΔH°) changes at 298.15 K for the three equilibria investigated were found to be:

ribose(aq) = ribulose(aq) $K = 0.317$, $\Delta G^\circ = 2.85 \pm 0.14$ kJ mol^{-1}, $\Delta H^\circ = 11.0 \pm 1.5$ kJ mol^{-1};

ribose(aq) = arabinose(aq) $K = 4.00$, $\Delta G^\circ = -3.44 \pm 0.30$ kJ mol^{-1}, $\Delta H^\circ = -9.8 \pm 3.0$ kJ mol^{-1};

ribulose(aq) = arabinose(aq) $K = 12.6$, $\Delta G^\circ = -6.29 \pm 0.34$ kJ mol^{-1}, $\Delta H^\circ = -20.75 \pm 3.4$ kJ mol^{-1}.

Information on rates of the above reactions was also obtained. The temperature dependencies of the equilibrium constants are conveniently expressed as $R \ln K = -\Delta G^\circ_{298.15}/298.15 + \Delta H^\circ_{298.15}[(1/298.15)-(1/T)]$ where R is the gas constant (8.31441 J mol^{-1} K^{-1}) and T the thermodynamic temperature.

1. Introduction

This investigation was undertaken as a continuation of earlier research [1,2] on the thermodynamics of the reactions catalyzed by glucose isomerase (EC 5.3.1.5). Here, the aim was to study the conversion of aqueous ribose to ribulose and to gain additional insight into the reactions catalyzed by glucose isomerase. However, during the course of the study, it was found that arabinose was also formed in solution, a fact which apparently had not been previously reported. The investigation was then extended to include the equilibria involving all three carbohydrates:

ribose(aq) = ribulose(aq) (A)

ribose(aq) = arabinose(aq) (B)

ribulose(aq) = arabinose(aq) (C)

We report herein the results of high-pressure liquid chromatography (HPLC) measurements which lead to values of the equilibrium constants (K), and standard Gibbs energy (ΔG°) and enthalpy (ΔH°) changes for the above processes. Microcalorimetric measurements were also performed which lead to a value of ΔH° for process A. The interpretation of the microcalorimetric measurements also necessitated a study of the rates of conversion of ribose to ribulose and to arabinose.

2. Experimental

The materials used in this investigation and their sources * were: crystalline D-ribose from Pfanstiehl Laboratories; crystalline D-(−)-arabi-

nose and D-ribulose (a viscous syrup) from Sigma; $Mg(NO_3)_2$ and $MgSO_4$ from Fisher Scientific; and KH_2PO_4 and Na_2HPO_4 were Standard Reference Materials Nos. 186-I-c and 186-II-c from the National Bureau of Standards. The solubilized glucose isomerase was from *Streptomyces olivochromogenes* and was provided by Corn Products. Analytical information on the enzyme preparation has been reported in an earlier paper [2].

The water contents of ribose, arabinose and ribulose were determined by Karl Fischer titration and found to be 0.14, 0.03 and 8.5 mass percent, respectively. The moisture contents of ribose and arabinose were applied as corrections to both the HPLC and heat measurements.

Heat measurements were performed [1,2] by mixing two separate solutions, designated as enzyme and substrate solutions, respectively, in the microcalorimeters. Heats of mixing of enzyme solutions with a (blank) 'substrate' solution containing zero substrate were also determined at each temperature at which measurements were performed [2] and applied as corrections to the measured heats.

The HPLC techniques have been described [1,2]. In these measurements the flow rate of the mobile phase (water) was 0.5 ml min^{-1}. The retention times of arabinose, ribulose and ribose were 17.2, 24.1 and 26.3 min, respectively. The samples of arabinose and ribose were analyzed by HPLC and showed no detectable impurities. However, HPLC analysis of the ribulose sample indicated the presence of significant amounts of both arabinose and ribose.

The response factors for arabinose and ribose were determined each day in separate control experiments. Since ribulose was not available in sufficiently pure form, a mass balance method was used to determine the response factor for the ribulose. Thus, the response factor was calculated from the ratio of the ribulose concentration, as determined from the starting substrate concentration after adjusting for the concentration of ribose and arabinose, and the area corresponding to the ribulose peak. The average of the ribulose response factors calculated from the first eight measurements taken at 30 min intervals was used for the calculation of the concentrations of ribulose.

3. Results and discussion

3.1. HPLC results

The results * of the HPLC experiments are given in table 1. Independent experiments were performed at all but the lowest temperature (313.15 K) starting with either ribose or arabinose in solution. At 313.15 K, even after a 13 day equilibration, and at 325.25 K after a 7 day equilibration, equilibrium was not obtained for those processes which involve arabinose. The average of the values of K_B and K_C at 325.25 K starting from both ribose and arabinose was found to be very nearly in agreement with the data at the other temperatures where the attainment of equilibrium was clearly demonstrated; consequently, the results at 325.25 K were utilized in the final treatment of the data.

Fitting the values of K_A, K_B and K_C to the model of Clarke and Glew [3] leads to the following values at 298.15 K: for process A, $\Delta G^\circ = 2852 \pm 143$ J mol^{-1} and $\Delta H^\circ = 11\,971 \pm 1496$ J mol^{-1}; for process B, $\Delta G^\circ = -3436 \pm 303$ J mol^{-1} and $\Delta H^\circ = -9753 \pm 2981$ J mol^{-1}; and for process C, $\Delta G^\circ = -6200 \pm 506$ J mol^{-1} and $\Delta H^\circ = -20\,866 \pm 4979$ J mol^{-1}. The uncertainties given here and throughout this paper refer to 95% confidence limits. The equilibrium data were not precise enough to justify the calculation of values of the standard heat capacity change (ΔC_p°) for any of the processes of interest. Thus, ΔC_p° and its temperature derivatives were taken as equal to zero in performing these calculations. In the model of Clarke and Glew, the temperature dependence of

* Certain commercial materials and products are identified in this paper to specify adequately the experimental procedures. Such identification does not imply recommendation or endorsement by the National Bureau of Standards.

* Detailed tables giving the results of individual measurements are available from the authors.

Table 1

Equilibrium constants for the interconversion of ribose, ribulose, and arabinose: K_A = (ribulose)/(ribose), K_B = (arabinose)/(ribose) and K_C = (arabinose)/ribulose)

All measurements were performed in phosphate buffer at pH 7.4. The concentration of the buffer was 8.7 and 30.3 mmol l^{-1} for KH_2PO_4 and Na_2HPO_4, respectively. The concentration of $Mg(NO_3)_2$ was in the range 10–16 mmol l^{-1}. Substrate concentrations were in the range 70–126 mmol (kg solution)$^{-1}$. The enzyme concentration was in the range 96–345 g (kg solution)$^{-1}$. All uncertainties refer to 95% confidence limits.

T (K)	Equilibrium time (days)	Number of measurements	K_A	K_B	K_C
313.15	13 [a]	6	0.3908 ± 0.043		
320.25	8 [a]	5	0.4461 ± 0.0040	3.041 ± 0.066	6.816 ± 0.133
	8 [b]	5	0.4461 ± 0.0038	3.059 ± 0.050	6.857 ± 0.105
325.25	7 [a]	5	0.4846 ± 0.0024	2.651 ± 0.071	5.470 ± 0.165
	7 [b]	5	0.4832 ± 0.0069	3.040 ± 0.103	6.291 ± 0.187
328.15	8 [a]	4	0.4880 ± 0.1194	2.860 ± 0.166	5.863 ± 0.426
	8 [b]	3	0.4890 ± 0.0306	2.913 ± 0.089	5.940 ± 0.635
331.95	2 [a]	5	0.5206 ± 0.0026	2.615 ± 0.046	5.024 ± 0.093
	2 [b]	5	0.5207 ± 0.0705	2.623 ± 0.042	5.038 ± 0.032
338.15	2 [a]	4	0.5599 ± 0.0139	2.495 ± 0.049	4.457 ± 0.141
	2 [b]	4	0.5650 ± 0.0213	2.472 ± 0.085	4.375 ± 0.139
343.75	2 [a]	4	0.5936 ± 0.0153	2.401 ± 0.038	4.045 ± 0.145
	2 [b]	4	0.5885 ± 0.0081	2.394 ± 0.051	4.069 ± 0.034

[a] Starting substrate: ribose.
[b] Starting substrate: arabinose.

the equilibrium constant is

$$R \ln K = -\Delta G^\circ/298.15 + \Delta H^\circ[(1/298.15) - (1/T)] + \Delta C_p^\circ[(298.15/T) - 1 + \ln(T/298.15)] \quad (1)$$

where ΔG°, ΔH° and ΔC_p° refer to a reference temperature of 298.15 K, R is the gas constant (8.31441 J mol^{-1} K^{-1}) and T the absolute temperature. As in our earlier investigations [1,2], the standard state is the hypothetical ideal solution of unit molality, and we have assumed ideal behavior for the three carbohydrates in solution on the basis of isopiestic data for similar nonelectrolytes [4,5]. Izatt et al. [6] report pK values for ribose and arabinose of 12.22 and 12.54, respectively, at 298.15 K. On the basis of structural similarity, ribulose should have a similar pK value. Thus, if equilibrium or heat measurements are performed at pH values less than 9, ionization corrections become negligible (less than 0.1%).

3.2. Rates of conversion of ribose to ribulose and to arabinose

The treatment of the calorimetric data (section 3.3) required a knowledge of the rates of conversion of ribose to ribulose and to arabinose. Thus, HPLC was used to determine amounts of ribose, ribulose and arabinose in solution as a function of time under conditions very similar to those used in the calorimetric experiments (see table 2). The rate data showed that equilibrium was achieved between ribose and ribulose within 1 h at temperatures from 320.25 to 338.15 K. At 313.15 K, 3 h were required to attain this equilibrium. The percent conversion of ribose to arabinose as a function of time is shown in fig. 1.

3.3. Calorimetric measurements

At the initiation of the calorimetric measurements only ribose was present in solution. Due to the long equilibration times needed, it was not

Table 2

Calorimetric data for the isomerization of ribose to ribulose and arabinose

The measured heat (q_{meas}) corrected for blank heat effects yields the values of q_{corr}.

Enzyme solution				Substrate solution [mmol (kg solution)$^{-1}$]				q_{meas} (mJ)	q_{corr} (mJ)	n_{ribose} (start) (μmol)	ξ_1 (μmol)	ξ_2 (μmol)	ΔH_A (J mol^{-1})
KH_2PO_4 [mmol (kg solution)$^{-1}$]	Na_2HPO_4 [mmol (kg solution)$^{-1}$]	$MgSO_4$ [mmol (kg solution)$^{-1}$]	Enzyme [g (kg solution)$^{-1}$]	Ribose	KH_2PO_4	Na_2HPO_4	$MgSO_4$						
Duration of experiment, 1.0 h; T = 320.15 K													
4.24	14.8	6.9	509.7	218.5	9.28	32.3	15.12	−376.98	−375.89	117.79	36.9	1.531	11056
4.24	14.8	6.9	509.7	218.5	9.28	32.3	15.12	−369.62	−368.53	117.28	36.7	1.525	10901
4.24	14.8	6.9	509.7	218.5	9.28	32.3	15.12	−320.61	−319.52	102.65	32.1	1.334	10806
4.24	14.8	6.9	509.7	218.5	9.28	32.3	15.12	−357.11	−356.02	111.00	34.8	1.443	11108
Average $\Delta H_A = 10968 \pm 164$ J mol^{-1}													
Duration of experiment, 1.0 h; T = 325.35 K													
4.24	14.8	6.9	509.7	218.5	9.28	32.3	15.12	−428.69	−428.99	121.11	40.5	2.810	12033
4.24	14.8	6.9	509.7	218.5	9.28	32.3	15.12	−349.80	−350.10	115.50	38.6	2.680	10506
4.24	14.8	6.9	509.7	218.5	9.28	32.3	15.12	−350.83	−351.13	107.80	36.1	2.501	11181
4.24	14.8	6.9	509.7	218.5	9.28	32.3	15.12	−415.59	−415.89	119.95	40.1	2.783	11809
Average $\Delta H_A = 11382 \pm 807$ J mol^{-1}													
Duration of experiment, 1.0 h; T = 331.65 K													
1.72	6.0	2.8	800.5	218.2	7.89	27.5	12.68	−392.86	−377.41	111.87	41.8	6.265	12144
1.72	6.0	2.8	800.5	218.2	7.89	27.5	12.68	−389.78	−374.33	112.33	42.0	6.290	12034
1.72	6.0	2.8	800.5	218.2	7.89	27.5	12.68	−387.97	−372.52	112.78	42.2	6.316	11956
1.72	6.0	2.8	800.5	218.2	7.89	27.5	12.68	−388.17	−372.72	110.92	41.5	6.212	12108
1.72	6.0	2.8	800.5	218.2	7.89	27.5	12.68	−405.54	−390.09	113.62	42.5	6.363	12304
Average $\Delta H_A = 12109 \pm 125$ J mol^{-1}													
Duration of experiment, 0.42 h; T = 338.15 K													
4.94	17.2	7.1	428.7	190.9	4.85	16.9	6.96	−349.44	−341.04	87.35	32.8	2.795	12163
4.94	17.2	7.1	428.7	190.9	4.85	16.9	6.96	−353.08	−344.68	88.20	33.2	2.822	12173
4.94	17.2	7.1	428.7	190.9	4.85	16.9	6.96	−345.74	−337.34	86.12	32.4	2.756	12197
4.94	17.2	7.1	428.7	190.9	4.85	16.9	6.96	−358.33	−349.93	87.43	32.9	2.798	12425
4.94	17.2	7.1	428.7	190.9	4.85	16.9	6.96	−353.95	−345.55	87.45	32.9	2.798	12288
Average $\Delta H_A = 12249 \pm 105$ J mol^{-1}													

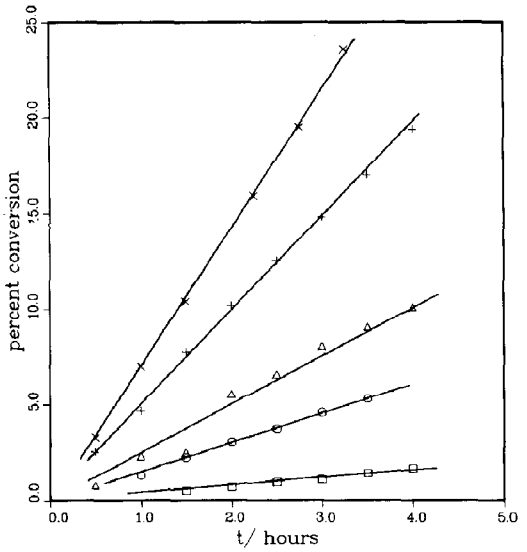

Fig. 1. Percent conversion of ribose to arabinose as a function of time and at the temperatures 313.15 K (□), 320.25 K (○), 325.25 K (△), 331.95 K (+) and 338.25 K (×).

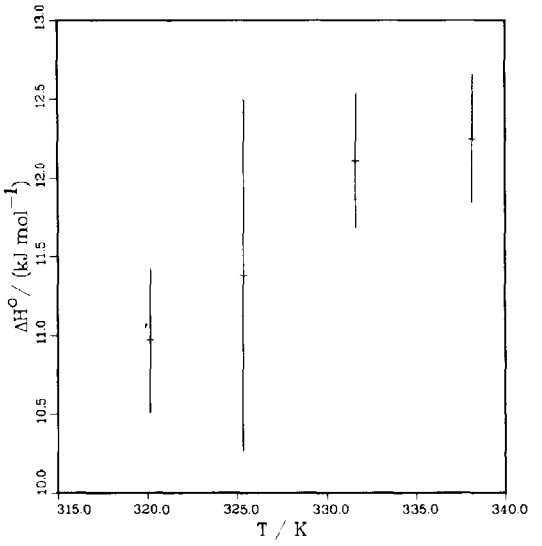

Fig. 2. Measured enthalpies of reaction for process A as a function of temperature.

possible to perform experiments where equilibrium was achieved for the formation of arabinose. Therefore, it was necessary to use the rate of conversion data (fig. 1) in order to obtain a correction for the amount of arabinose formed during an experiment to calculate a value ΔH_A° from the calorimetric data. The treatment of the calorimetric data is now described.

There are n_s moles of ribose present in solution at the start of an experiment. At the time at which an experiment is concluded there are $(n_s - \xi_1)$ moles of ribose, $(\xi_1 - \xi_2)$ moles of ribulose and ξ_2 moles of arabinose present in solution. The extent of reaction variables (ξ_1 and ξ_2) can be calculated from the percent conversion data and from the value of K_A at the temperature of interest. Thus

$$\xi_2 = [\%(\text{arabinose})/100]\, n_s \tag{2}$$

and

$$K_A = (\xi_1 - \xi_2)/(n_s - \xi_1) \tag{3}$$

The enthalpy of the solution at the start of an experiment is

$$H_s = n_s H_{\text{ribose}} + \left(\sum n_i H_i\right)_s \tag{4}$$

where $(\sum n_i H_i)_s$ represents the contribution to the enthalpy from the water, buffer, cofactor, and enzyme at the start of an experiment. At the conclusion of an experiment, the enthalpy of the solution is

$$H_f = (n_s - \xi_1) H_{\text{ribose}} + (\xi_1 - \xi_2) H_{\text{ribulose}} + (\xi_2) H_{\text{arabinose}} + \left(\sum n_i H_i\right)_f \tag{5}$$

Previous experiments [1] have demonstrated that glucose isomerase makes a negligible contribution to the enthalpy of conversion of glucose to fructose. Since this is a very similar reaction and since the water, buffer and cofactors are essentially unaffected by the conversion of ribose to ribulose and arabinose, $(\sum n_i H_i)_s$ is taken equal to $(\sum n_i H_i)_f$. Eqs. 4 and 5 can then be combined to yield

$$\Delta H = H_f - H_s = \xi_1 \Delta H_A^\circ + \xi_2 \Delta H_C^\circ \tag{6}$$

Thus, ΔH_A° can be calculated from the calorimetric data, values of ξ_1 and ξ_2 calculated from eqs. 2 and 3 and the percent conversion data, and the value of ΔH_C° of -20.9 ± 5.0 kJ mol^{-1} determined from the HPLC data. The calorimetric data, calculated values of ξ_1 and ξ_2, and enthalpy change for process A are given in table 2.

Table 3

Summary of HPLC and microcalorimetric results

HPLC results				Microcalorimetric results	
T (K)	K_A	K_B	K_C	T (K)	ΔH_A^o (J mol^{-1})
313.15	0.3908	–	–	313.15	–
320.25	0.4461	3.050	6.837	320.15	10968 ± 164
325.25	0.4839	2.846	5.881	325.35	11382 ± 807
328.15	0.4885	2.887	5.902	331.65	12109 ± 125
331.95	0.5207	2.619	5.031	338.15	12249 ± 105
338.15	0.5625	2.484	4.416		
343.75	0.5911	2.398	4.057	average $\Delta H_A^o = 11677 \pm 713$	

For process A: $\Delta G_A^o = 2852 \pm 143$ J mol^{-1}; $\Delta H_A^o = 11971 \pm 1496$ J mol^{-1}
For process B: $\Delta G_B^o = -3436 \pm 303$ J mol^{-1}; $\Delta H_B^o = -9753 \pm 2981$ J mol^{-1}
For process C: $\Delta G_C^o = -6200 \pm 506$ J mol^{-1}; $\Delta H_C^o = -20866 \pm 4979$ J mol^{-1}

The uncertainties given in table 2 refer to 95% confidence limits but do not include any possible systematic errors which might arise from the corrections applied using eqs. 2–6. We estimate that these 95% confidence limits should be increased by at least 300 J mol^{-1} to reflect these possible errors. These enthalpies and their associated uncertainties are shown in table 3. From the temperature derivative of these enthalpies the following values are calculated at 298.15 K: $\Delta H_A^o = 9.37 \pm 1.86$ kJ mol^{-1} and $\Delta C_p^o = 75 \pm 59$ J mol^{-1} K^{-1}. An average of the enthalpies at the four different temperatures leads to $\Delta H_A^o = 11.68 \pm 0.71$ kJ mol^{-1} while the value of ΔH_A^o calculated from the HPLC data was 11.97 ± 1.5 kJ mol^{-1}. For a final value of ΔH_A^o we adopt an average value of 11.0 ± 1.5 kJ mol^{-1}. This value, with its uncertainty interval, overlaps all of the aforementioned values of ΔH_A^o and their associated uncertainty intervals.

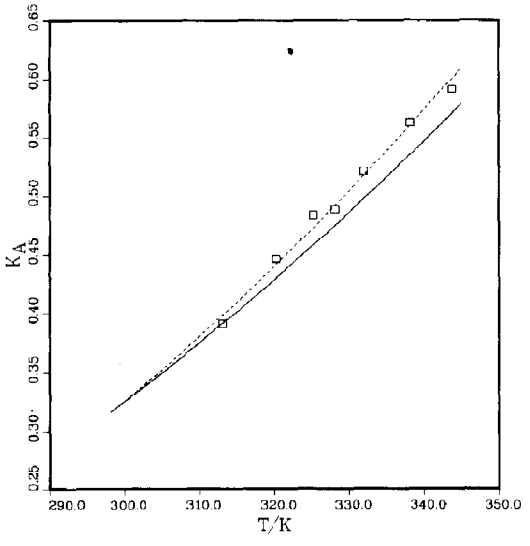

Fig. 3. The equilibrium constant for process A, the conversion of ribose to ribulose, as a function of temperature. (□) Measured values; (———) calculated using eq. 1 with $\Delta G^o = 2.85$ kJ mol^{-1} and $\Delta H^o = 11.0$ kJ mol^{-1}; (— — —) calculated using $\Delta G^o = 2.85$ kJ mol^{-1} and $\Delta H^o = 12.0$ kJ mol^{-1}.

3.4. Summary of results

For process A, the conversion of ribose to ribulose, we have determined $\Delta G^o = 2.85 \pm 0.14$ kJ mol^{-1} and $\Delta H^o = 11.0 \pm 1.5$ kJ mol^{-1} at 298.15 K. For process B, the conversion of ribose to arabinose, the HPLC data led to $\Delta G^o = -3.44 \pm 0.30$ kJ mol^{-1} and $\Delta H^o = -9.75 \pm 3.0$ kJ mol^{-1} at 298.15 K. Combination of these results for processes A and B led to the following values for process C at 298.15 K: $\Delta G^o = -6.29 \pm 0.34$ J mol^{-1} and $\Delta H^o = -20.75 \pm 3.4$ kJ mol^{-1}. Note that these latter values are slightly different from those determined directly from the HPLC data and that the uncertainties are also smaller. The corresponding values of the equilibrium constants are 0.317, 4.00 and 12.6 for processes A, B and C, respectively, at 298.15 K.

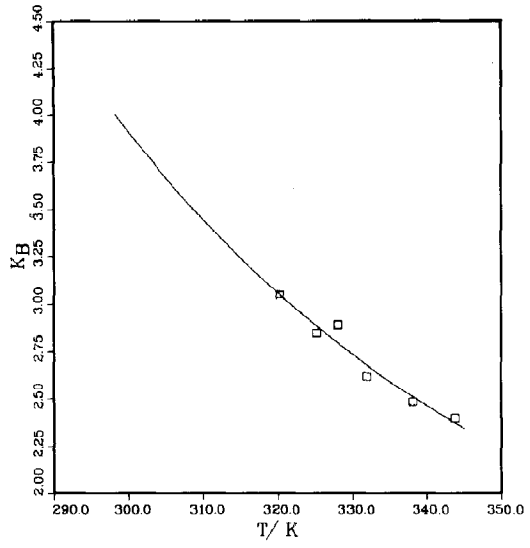

Fig. 4. The equilibrium constant for process B, the conversion of ribose to arabinose, as a function of temperature. (□) Measured values; (———) calculated using eq. 1 with $\Delta G^\circ = -3.44$ kJ mol^{-1} and $\Delta H^\circ = -9.8$ kJ mol^{-1}.

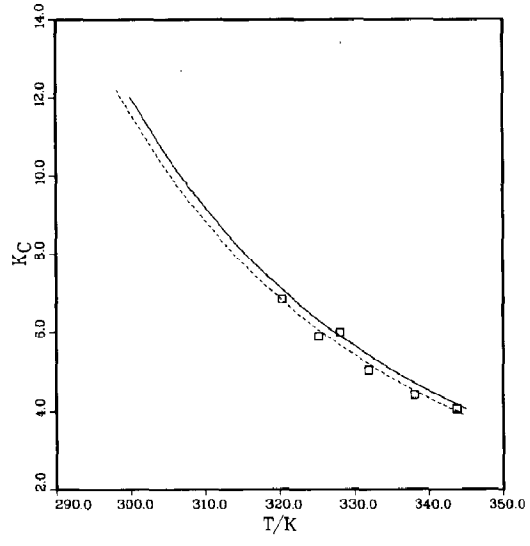

Fig. 5. The equilibrium constant for process C, the conversion of ribulose to arabinose, as a function of temperature. (□) Measured values; (———) calculated using eq. 1 with $\Delta G^\circ = -6.29$ kJ mol^{-1} and $\Delta H^\circ = -20.75$ kJ mol^{-1}; (— — —) calculated using $\Delta G^\circ = -6.20$ kJ mol^{-1} and $\Delta H^\circ = -20.87$ kJ mol^{-1}.

Values of the equilibrium constants calculated using the above results are shown as solid lines in figs. 3–5 together with the experimentally determined equilibrium constants. A summary of the results is given in table 4.

3.5. Comparisons with literature data

The enthalpy of formation data for D-arabinose(c) and D-ribose(c) quoted in the review by Domalski [7] together with the enthalpies of solution of these compounds in water determined by Jasra and Ahluwalia [8] lead to a value of $\Delta H^\circ_B = -6.8$ kJ mol^{-1}. We estimate it to be uncertain by at least 3 kJ mol^{-1}. Colbert (National Bureau of Standards, personal communication, Jan. 1985) has recently performed heat of combustion experiments which lead to an enthalpy of formation of -1046.44 ± 0.83 kJ mol^{-1} for crystalline ribose at 298.15 K. Use of this value in the thermochemical cycle calculation instead of the value given in Domalski's earlier review leads to a value of $\Delta H^\circ_B = -11.2$ kJ mol^{-1}. Both of these values are in agreement with the value of -9.75 ± 3.0 kJ mol^{-1} determined in this investigation.

Jasra and Ahluwalia also measured enthalpies of solution of D-ribose and D-arabinose in water as a function of temperature and calculated values of ΔC_p° for the solution process. They then used heat capacity data on the solids to calculate values of the standard state partial molar heat capacities of the aqueous solutes. Use of these values leads to $\Delta C_p^\circ = 24$ J mol^{-1} K^{-1} for process B which we estimate to be uncertain by at least 20 J mol^{-1} K^{-1}. Thus, the value of 0 J mol^{-1} K^{-1} for process B which we implicitly used to treat the HPLC data is not unreasonable.

The only other available data on ribose and arabinose are the Gibbs energy and enthalpy of ionization data of Izatt et al. [6]. We are aware of no other thermodynamic information in the literature on ribulose.

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