Thermodynamics of Proton Dissociation in Dilute Aqueous Solution. V. An Entropy Titration Study of Adenosine, Pentoses, Hexoses, and Related Compounds[1a, b]

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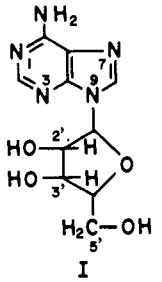
Abstract: A thermometric titration study of proton ionization from adenine, adenosine, ribose, adenosine 5′-monophosphate, adenosine 3′,5′-cyclic phosphate, adenosine 2′(3′)-monophosphate, 2′-deoxyadenosine, 3′-deoxyadenosine, 2′-O-methyladenosine, and 2-deoxyribose indicates that the acidity of adenosine is associated with the ribose portion of the molecule and that the presence of both the 2′- and 3′-hydroxyl groups in the case of adenosine results in a marked acidity increase compared to similar compounds lacking vicinal OH groups. A similar study was made of proton ionization from lyxose, xylose, arabinose, mannose, fructose, glucose, galactose, 2-deoxyglucose, glucose 6-phosphate, glucose 1-phosphate, ribose 5-phosphate, methyl-β-D-glucoside, and methyl β-L-arabinopyranoside. A comparison of the resulting data for these monosaccharides and their derivatives indicates that the acidity observed in the monosaccharides is associated with proton ionization from the 1 position. The thermodynamic pK, $\Delta H°$, and $\Delta S°$ values for proton ionization from adenosine, adenosine 5′-monophosphate, ribose, 2-deoxyribose, xylose, lyxose, arabinose, mannose, fructose, glucose, galactose, 2-deoxyglucose, glucose 6-phosphate, and ribose 5-phosphate are calculated from individual thermometric titration curves for these substances by the entropy titration method.

Adenosine (I) is known to be acidic with a reported[2] pK value of 12.5. The site of this acidity has not been established, but is of interest for several reasons.



I

An important step in protein synthesis is the action of soluble RNA in transporting amino acids from various locations in the cytoplasm of the cell to the microsomes where protein manufacture takes place.[3] The terminal group on soluble RNA is adenosine,[4] and several investigators have shown[5-7] that the amino acid involved in protein synthesis is attached to the soluble RNA through linkage with the adenosine portion of the molecule. The site of this attachment has also been investigated. Raacke[8] has suggested an ester linkage between the amino acid and the 2′ position. However, Feldman and Zachau[9] postulate that the amino acid is attached to the 3′ position. McLaughlin and Ingram[10] state that acylation takes place at both the 2′ and 3′ positions and that there is a rapid transfer of acyl groups between these two positions. A knowledge of the site of acidity in adenosine should be helpful in elucidating the point of attachment of the amino acids in these cases.

Harkins and Freiser[11] accounted for the formation of copper(II)–adenine complexes by postulating that the acidic group in adenine is involved in the chelation. However, they were not able to explain the existence of stable copper(II)–adenosine complexes because they were unable to locate an acidic group in adenosine. Quantitative information concerning the acidic site in adenosine should help elucidate its interaction with cupric ion and other metal ions.

Several possible locations have been suggested for the acidic site in adenosine. Michelson[12] states that the 2′-hydroxyl group in ribonucleosides is known to be more acidic than the 3′ position. A recent study by Broom and Robins[13] also suggests that the 2′ position is the acidic site. Zamecnik[14] postulates an intramolecular hydrogen bond bridging the 2′ and 3 positions in his explanation of the biosynthesis of proteins. However, the work of several other researchers suggests that the acidity may be related to the 3′- as well as to the 2′-hydroxyl group.[9,10] Further, it is known[15] that DNA (2′-OH replaced by H) and RNA differ in their acid–base properties, DNA being more resistant to alkali.

It occurred to us that some insight concerning the location of the acidic site on adenosine might be

(1) (a) Supported by National Institutes of Health Grant No. RG 9430-04; presented in part at the 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1965; (b) published in part as a preliminary communication: R. M. Izatt, L. D. Hansen, J. H. Rytting, and J. J. Christensen, *J. Am. Chem. Soc.*, **87**, 2760 (1965); (c) to whom inquiries should be directed; (d) National Defense Education Act Predoctoral Fellow, 1962–1965, Brigham Young University.

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obtained by a systematic determination of pK, $\Delta H°$, and $\Delta S°$ values for adenosine, related nucleoside derivatives, and constituent monosaccharides and their derivatives. Other than the one reported pK value for adenosine, no pK or ΔH values appear to have been determined for any of the nucleoside derivatives included in the present study. The pK values for many monosaccharides have been determined;[16-19] however, ΔH and ΔS values have been determined only in the case of glucose.[20-22]

In the present study proton ionization from the following substances in the pH region 11.5–13.0 have been investigated: adenine, ribose, adenosine, xylose, adenosine 5′-monophosphate, 2-deoxyribose, 2′-deoxyadenosine, 3′-deoxyadenosine, 2′-O-methyladenosine, adenosine 3′,5′-cyclic phosphate, adenosine 2′(3′)-monophosphate, lyxose, arabinose, mannose, fructose, glucose, galactose, 2-deoxyglucose, glucose 6-phosphate, glucose 1-phosphate, ribose 5-phosphate, methyl β-L-arabinoside, and methyl β-D-glucoside. The entropy titration method[23,24] is used to calculate thermodynamic pK, $\Delta H°$, and $\Delta S°$ values for those substances having pK values in this pH region.

Experimental Section

Materials. The chemicals used were of the highest purity available from California Biochemical Corp. (A grade adenine, adenosine, xylose, adenosine 5′-monophosphate, 2-deoxyribose, lyxose, 2-deoxyglucose, glucose 6-phosphate, glucose 1-phosphate, ribose 5-phosphate, methyl β-D-glucoside, methyl β-L-arabinopyranoside, adenosine 3′(2′)-phosphate, adenosine 3′,5′-cyclic phosphate), Sigma Chemical Co. (Sigma grade 2′-deoxyadenosine, ribose), Eastman (White Label ribose, arabinose, glucose, mannose, fructose), and Matheson Coleman and Bell (reagent grade galactose), or were synthesized (2′-O-methyladenosine and 3′-adenosine).[25] All solutions were prepared, stored, and used under a pure nitrogen atmosphere.

Procedure. The determinations were made with a precision thermometric titration calorimeter having accuracy comparable to that of a conventional solution calorimeter. The calorimeter together with its operation and calibration[26] have been described. Solutions ~0.002 F in 2′-O-methyladenosine, ~0.004 F in 3′-deoxyadenosine, and ~0.01 F in the remaining substances were titrated with ~0.6 F NaOH.

Calculations.[27] The entropy titration procedure[23,24] used to determine pK and $\Delta H°$ values in this study is a calorimetric method involving the relative amounts of the reacted and unreacted species. The thermogram obtained for an incomplete reaction is a function of both the equilibrium constant and enthalpy change for the reaction. Therefore, the thermometric titration data alone enable one to calculate simultaneously both the thermodynamic equilibrium constant and enthalpy change. Calculations show that the entropy titration method can be used to determine pK and $\Delta H°$ values under the conditions prevailing in this study provided that the pK is between 11.5 and 13.0 and the heat of reaction is sufficiently large. Our experience indicates that heat changes in the calorimeter of less than approximately 0.2 cal fall within the uncertainty caused by heat of dilution of the titrant, heat losses from the calorimeter, heat input from stirring, and possible CO_2 contamination. However, the precision between determinations using aliquots of solutions containing the same compounds is approximately 0.05 cal.

The method of data analysis has been described.[24] The calculations were made using an IBM 7040 computer. The heat of dilution data for NaOH used in making the calculations are those determined by Sturtevant.[28] Values for the heat of ionization (13.34 kcal/mole) and ion product (1.004×10^{-14}) of water are taken from Hale, *et al.*,[29] and Harned and Owen,[30] respectively.

Results

In Table I are presented, as a function of millimoles of NaOH titrant added, the heats of reaction corrected for heat of stirring and heat losses from the calorimeter.

The pK, $\Delta H°$, and $\Delta S°$ values valid at 25° and μ = 0 calculated from the data in Table I are given in Table II for those substances found to be acidic in the pH region studied. In each case the thermometric titration data have been analyzed assuming one dissociable proton.

Determinations were made for several other substances besides those listed in Table II; however, in these cases the quantity of heat involved was too small to calculate thermodynamic values under the conditions of this study. These substances can be divided into two categories: (a) those which show heat changes less than 0.2 cal, which is about the limit of resolution under the conditions of this study (*i.e.*, sodium adenate, 2′-O-methyladenosine, adenosine 3′(2)-monophosphate, glucose 1-phosphate, methyl β-L-arabinopyranoside, and β-methyl D-glucoside), and (b) those which show very small, but definitely measureable heat effects (*i.e.*, 2′-deoxyadenosine, 3′-deoxyadenosine, and adenosine 3′,5′-cyclic phosphate). Of the substances in b, 3′-deoxyadenosine and 2′-deoxyadenosine show a corrected heat change of 0.4 cal, while adenosine 3′,5′-cyclic phosphate shows a corrected heat change of 0.8 cal.

Discussion

The pK value of 12.5 determined in 1925 by Levene, *et al.*,[2] for proton ionization from adenosine is in good agreement with the value of 12.35 reported in this paper. In 1913, Michaelis[16] determined pK values for several sugars. The values obtained by him and also those obtained by Urban and Shaffer,[18] Hirsch and Schlage,[17] and Thamsen[19] are compared to the values reported in this paper in Table II. The lack of agreement between the results of the present study and many of those reported in the literature is probably a result of differences in the temperature and μ values of the several studies. The $\Delta H°$ value reported in Table II for proton ionization from glucose falls between the values

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Table I

Heats of Reaction,[a] $-Q$, (cal.), for Interaction of NaOH with Sugars and Sugar Derivatives[b,c]

Titrant Delivered	Lyxose	Arabinose	Mannose	Fructose	Glucose	2-Deoxyglucose	Galactose	2'-Deoxyadenosine
mmoles x 10³	*(0.01000)*	*(0.01073)*	*(0.01006)*	*(0.01020)*	*(0.01143)*	*(0.01037)*	*(0.01072)*	*(0.007898)*
0.2827[d]	0.6595	0.3376	0.6890	0.4990	0.4294	0.3580	0.2466	0.1019
0.5653	1.1939	0.6515	1.2641	0.9211	0.8502	0.6647	0.6079	0.1603
0.8480	1.6610	0.9379	1.7550	1.3282	1.2271	0.9370	0.8648	0.2097
1.1306	2.0559	1.1893	2.1603	1.6706	1.5559	1.1847	1.1037	0.2569
1.4133	2.3848	1.4264	2.5104	1.9614	1.8535	1.4075	1.3137	0.2983
1.6959	2.6749	1.6347	2.8116	2.2138	2.1158	1.6156	1.4981	0.3392
1.9786	2.9258	1.8137	3.0702	2.4344	2.3495	1.7945	1.6563	0.3778
2.2612	3.1366	1.9845	3.2819	2.6225	2.5611	1.9652	1.8096	0.4158
2.5439	3.3140	2.1435	3.4713	2.7849	2.7541	2.1203	1.9398	0.4514
2.8265	3.4829	2.2904	3.6379	2.9356	2.9172	2.2742	2.0793	0.4863
3.1092	3.6322	2.4213	3.7850	3.0962	3.0717	2.4121	2.1952	0.5169

Titrant Delivered	Glucose-6-phosphate	Adenosine-5'-monophosphate	Ribose-5-phosphate	Adenosine-3'(2')-phosphate	Adenosine-3',5'-cyclicphosphate	Methyl-β-D-arabinoside	β-methyl-D-glucoside	Glucose-1-phosphate
	(0.005585)	*(0.009962)*	*(0.006831)*	*(0.01134)*	*(0.01049)*	*(0.005830)*	*(0.009667)*	*(0.009879)*
0.2827[d]	1.5837	0.4804	0.2440	0.0676	0.1611	0.0715	0.0585	0.0824
0.5653	1.7284	0.5755	0.4666	0.1015	0.2717	0.1201	0.0942	0.0968
0.8480	1.8614	0.6653	0.6603	0.1327	0.3699	0.1488	0.1298	0.1090
1.1306	1.9824	0.7445	0.8317	0.1577	0.4624	0.1716	0.1632	0.1223
1.4133	2.0877	0.8253	0.9984	0.1800	0.5350	0.1812	0.1945	0.1332
1.6959	2.1948	0.9060	1.1494	0.2031	0.6053	0.1952	0.2219	0.1434
1.9786	2.2966	0.9792	1.2881	0.2234	0.6696	0.2101	0.2488	0.1512
2.2612	2.3894	1.0504	1.4180	0.2373	0.7278	0.2187	0.2716	0.1546
2.5439	2.4802	1.1212	1.5425	0.2554	0.7799	0.2317	0.2975	0.1735
2.8265	2.5618	1.1899	1.6434	0.2779	0.8402	0.2420	0.3210	0.1899
3.1092	2.6376	1.2545	1.7458	0.3011	0.8906	0.2531	0.3439	0.1983

Titrant Delivered	Ribose	2-deoxyribose	Xylose	Adenosine	3'-deoxyadenosine	2'-O-methyladenosine	Sodium adenate
	(0.009871)	*(0.01024)*	*(0.01029)*	*(0.01019)*	*(0.003726)*	*(0.001543)*	*(0.01011)*
0.3058[e]	0.5550	0.3220	0.5237	0.3581	0,0976	0.0695	0.1956
0.6116	1.0465	0.6203	0.8897	0.6504	0.1658	0.1087	0.2508
0.9173	1.4735	0.8908	1.3512	0.8926	0.2216	0.1383	0.2825
1.2231	1.8315	1.1367	1.6880	1.1164	0.2790	0.1652	0.3064
1.5289	2.1302	1.3612	1.9724	1.3107	0.3167	0.1823	0.3205
1.8347	2.3862	1.5673	2.2213	1.4851	0.3627	0.2036	0.3336
2.1404	2,6027	1.7442	2.4341	1.6378	0.4102	0.2221	0.3474
2.4462	2.7968	1.9165	2.6390	1.7684	0.4555	0.2377	0.3476
2.7520	2.9574	2.0842	2.8070	1.8917	0.4948	0.2504	0.3557
3.0577	3.1167	2.2252	2.9702	2.0047	0,5355	0.2637	0.3663
3.3635	3.2491	2.3612	3.1283	2.1079	0.5701	0.2814	0.3758

[a] Typical runs corrected for stirring and heat losses from the calorimeter. [b] Initial molar concentrations of the titrated substances are given in parentheses. [c] Initial volume in calorimeter: 100.0 ml. [d] Titrant concentration: 0.5513 *F*. [e] Titrant concentration: 0.5964 *F*.

reported by Hendricks and Steinbach[20] and Kilde and Wynne-Jones.[21]

The possible transformation of the sugars to decomposition products (*e.g.*, saccharinic acids) in the alkaline solutions (pH <13[1b]) during the titration (~10 min) was considered and rejected as a possible source of error. The bases for this rejection were the reproducibility and precision of our results together with the fact that sugar decomposition in alkaline solutions is a slow process.[18]

The $\Delta H°$ values in Table II are approximately 2–3 kcal/mole larger than corresponding values for proton ionization from phenol, *m*- and *p*-cresol, and several xylenols.[31] A comparison of $\Delta S°$ values shows them to be similar in both cases (~ −30 eu).

The results obtained in this study confirm the conclusion of Hendricks and Steinbach[20] that the 1 position is the acidic site for the hexoses. This is indicated by the fact that substitution of a nonacidic group on the 1 position of the hexose (*i.e.*, glucose 1-phosphate, methyl β-D-glucoside) effectively eliminated the acidity. Since methyl β-L-arabinopyranoside is not acidic while arabinose is, we conclude that the site of acidity in the pentoses is also the 1 position. The relatively constant p*K*, $\Delta H°$, and $\Delta S°$ values given in Table II for proton ionization from the pentoses and hexoses indicate that either rearranging the hydroxyl groups on the molecule or increasing the carbon chain length by one carbon atom has little effect on the measured thermodynamic quantities except in the case of ribose 5-phosphate, where the $\Delta H°$ and $\Delta S°$ values are markedly different from those of related species (*e.g.*, glucose 6-phosphate). Possible reasons for these differences will be discussed in a later section. Substitution of a hydrogen for a hydroxyl group (*i.e.*, 2-deoxyribose, 2-deoxyglucose) also appears to have little effect upon the measured thermodynamic quantities.

The results obtained with the pentoses and hexoses would lead one to expect that substitution of the base adenine on the 1 position of ribose would result in a nonacidic molecule, and that derivatives formed by substitution on the 2′, 3′, or 5′ positions would also be nonacidic. It is, therefore, surprising that the acidity of adenosine (Table II) is comparable to that of the pentoses and hexoses. In addition, the acidic site in adenosine must be different from that in ribose. The acidity of adenosine is especially interesting in view of

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Table II. pK, $\Delta H°$, and $\Delta S°$ Values Valid at 25° and $\mu = 0$ for Proton Ionization from Several Pentoses, Hexoses, and Their Derivatives[a]

Compound	pK	$\Delta H°$, kcal/mole	$\Delta S°$, eu	No. of runs
		Pentoses		
		$HA = H^+ + A^-$		
Lyxose	12.11 ± 0.03	8.0 ± 0.2	−28.6 ± 0.7	4
Ribose	12.22 ± 0.04	8.1 ± 0.3	−28.7 ± 1.1	9
2-Deoxyribose	12.67 ± 0.04	7.7 ± 0.5	−32.1 ± 1.9	5
Xylose	12.29 ± 0.03	8.2 ± 0.2	−28.7 ± 0.7	6
	(12.14)[c]			
Arabinose	12.54 ± 0.04	8.3 ± 0.3	−29.6 ± 1.3	11
	(12.43)[c]			
		Hexoses		
		$HA = H^+ + A^-$		
Mannose	12.08 ± 0.01	7.9 ± 0.1	−28.9 ± 0.3	8
	(11.96)[c]			
Fructose	12.27 ± 0.01	8.2 ± 0.2	−28.6 ± 0.5	4
	(12.06)[c]			
	(11.693)[d]			
	(11.68)[e]			
	(12.07)[i]			
	(11.99)[j]			
	(12.67)[l]			
Glucose	12.46 + 0.05	7.7 ± 0.3	−31.3 ± 1.4	4
	(12.18)[c]			
	(12.107)[d]	(8.0)[g]	(−26.3, −19.9)[m]	
	(12.09)[e]	(7.3)[h]		
	(12.43)[f]	(9.2, 10.7)[m]		
	(12.34)[h]			
	(12.22)[i]			
	(12.23)[j]			
	(12.24)[k]			
	(12.87)[l]			
	(12.46, 12.17)[m]			
	(12.96)[n]			
2-Deoxyglucose	12.52 ± 0.04	8.2 ± 0.2	−29.7 ± 0.6	3
Galactose	12.48 ± 0.04	9.0 ± 0.3	−26.9 ± 1.2	4
	(12.28)[c]			
	(12.37)[i]			
		Sugar Derivatives		
		$HA^{2-} = H^+ + A^{3-}$		
Adenosine	12.35 ± 0.03	9.7 ± 0.2	−24.0 ± 0.7	16
	(12.5)[b]			
Glucose 6-phosphate	11.71 ± 0.10	8.4 ± 0.4	−25.0 ± 2.0	4
Adenosine 5′-monophosphate	13.06 ± 0.1	10.9 ± 0.4	−23.3 ± 1.5	6
Ribose 5-phosphate	13.05 ± 0.2	6.1 ± 1.0	−39.4 ± 5.0	4

[a] The values are reported as the average of the various runs with the uncertainties expressed as standard deviations between runs. [b] Reference 2, $\mu = 0.1$; value not corrected for activity. [c] Reference 16, 17–18°, $\mu = 0.3$, 0.1 M KCl. [d] Reference 17, $\mu = 0.25$. [e] Reference 18, $\mu = 0.3$. [f] Reference 19, 18°, $\mu = 0.2$. [g] Reference 20, $\mu = 0.1$. [h] Reference 21, $\mu = 0.1$. [i] A. E. Stearn, *J. Phys. Chem.*, **35**, 226 (1931), 23°, $\mu = 0.5 - 2$. [j] T. Madsen, *Z. Physik. Chem.*, **36**, 290 (1901). [k] H. T. S. Britton, *J. Chem. Soc.*, **127**, 1896 (1925), 18°, $\mu = 0.5$. [l] N. A. Ramaiak and S. S. Katiyar, *Proc. Ann. Conv. Sugar Technol. Assoc. India*, **29**, 77 (1961), $\mu = 0.2$. [m] Reference 22. The first value is for the α anomer and the second for the β anomer. [n] P. Souchay and R. Schaal, *Bull. Soc. Chim. France*, 819 (1950).

the fact that the adenosine derivatives in groups a and b show very small corrected heat changes. The question now arises whether these small heat changes for the adenosine derivatives are a result of a pK increase resulting in less product formed or a $\Delta H°$ increase, making the heat change measured in the calorimeter (*i.e.*, the difference between the heat of formation of H_2O from its ions and the reaction in question) very small. The second of these alternatives is unlikely since the $\Delta H°$ values given in Table II for the pentoses, hexoses, and their derivatives included in the present study are relatively unaffected by substitution on the molecules; *e.g.*, compare ribose and 2-deoxyribose, glucose and 2-deoxyglucose, glucose and glucose 6-phosphate. Therefore, we conclude that the small heat changes observed for the adenosine derivatives in groups a and b compared to that of adenosine are a result of higher pK values in these substances.

Titration of 2′-O-methyladenosine with NaOH showed a very small corrected heat change (<0.2 cal). This, together with the fact that adenosine 5′-phosphate and adenosine 3′,5′-cyclic phosphate are acidic, is evidence that the 5′ position is not the acidic site in adenosine. These results suggest that the acidity in adenosine is associated with the 2′ position. This conclusion is confirmed by the fact that the heat change in the titration of 2′-deoxyadenosine with NaOH is much less than that observed in the case of adensoine. Titration of 3′-deoxyadenosine surprisingly yielded the same result as that for 2′-deoxyadenosine leading to the conclusion that not one or the other, but *both* the 2′- and 3′-hydroxyl groups are required for the increased

acidic character of adenosine. This conclusion is confirmed by the fact that a thermometric titration of adenosine 3′(2′)-monophosphate with NaOH shows no evidence for proton ionization.

There are at least two possible explanations for the fact that two adjacent hydroxyl groups are a necessary structural feature for acidity in adenosine and its derivatives. First, the combined inductive effect of the vicinal 2′- and 3′-hydroxyl groups could induce the acidity. Second, the anion may be stabilized by a hydrogen bonded ring. Actually, both effects could contribute to the observed acidity. Work is presently in progress to determine the relative importance of these two effects.

The fact that both the 2′- and 3′-hydroxyl groups are necessary for the acidity of adenosine is undoubtedly related to the reactivity of these positions and should aid in the explanation of the differences between the biological roles of adenosine and deoxyadenosine in DNA and RNA. This requirement for acidity in adenosine is also in good agreement with the observations of McLaughlin and Ingram[10] since their research indicates that acylation takes place at both the 2′ and 3′ positions. Our results also support the proposition that the 2′ and 3′ positions are associated with the attachment of the amino acid to the soluble-RNA in protein synthesis.[8-10]

In glucose 6-phosphate, the $\Delta S°$ value is 11–12 eu lower than one would predict for proton ionization from a dinegative ion.[32,33] The fact that the entropy change for glucose 6-phosphate is similar to that for the ionization of a proton from the neutral pentoses and hexoses in this study rather than like that for a dinegative ionization (*e.g.*, $H_2P_2O_7^{2-}$ ionization[32,33]) indicates that the ionization occurs from a site on the ion which is relatively unaffected by the dinegative charge which is consistent with the previous conclusion that proton ionization occurs from the 1 position in the hexoses. However, the $\Delta S°$ value obtained for ionization from ribose 5-phosphate is very similar to that which one would expect from a dinegative ion, leading one to suspect that in the case of ribose 5-phosphate the dinegative charge is sufficiently close to influence proton ionization. This may be due to the fact that there is one less carbon atom in ribose 5-phosphate or there may be a configurational difference to account for the result.

Acknowledgments. The authors acknowledge several helpful discussions with Dr. H. Smith Broadbent, Dr. Roland K. Robins, and Dr. John H. Mangum.

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Hydrogen Atom Transfer between Free Radicals and Their Diamagnetic Precursors

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Received February 3, 1966

Abstract: Magnetic resonance techniques have been used to study the kinetics of hydrogen atom exchange between a series of hydroxylic compounds and their corresponding oxy free radicals. In some cases it was possible to determine the lifetime of a short-lived complex formed during the reaction. The rate constant for exchange was determined in each case. Deuterium was substituted for the hydroxylic proton in two cases and deuterium isotope effects were examined. The temperature dependence of the rate of exchange was examined for each set of compounds.

Rates of chemical processes in systems at equilibrium may often be determined from the dependence of the shapes of magnetic resonance lines on the chemical composition of the systems. The spectra provide a chronicle of the motion of the spins among the various sites which they may occupy. In this paper we describe studies of a set of reactions in which hydrogen atoms move from hydroxylic compounds to the corresponding oxy free radicals. In all cases the spectra yield the over-all rate of hydrogen atom transfer. In certain favorable instances mean lifetimes of short-lived complexes between reactants may be determined from the spectra.

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Theoretical Background

Consider the reaction

$$\mathrm{ROH} + \cdot\mathrm{OR} \underset{k_1}{\overset{k_1}{\rightleftharpoons}} \mathrm{RO}\cdot + \mathrm{HOR} \qquad (1)$$

where ROH is an even-electron substance. RO· is then necessarily an odd-electron substance. k_1 is the rate constant for the reaction in which the hydroxylic hydrogen atom is transferred to a different molecule from the one on which it started.

A mixture of ROH and RO· exhibits nuclear spin resonance from the nuclei in ROH and electron spin resonance from the unpaired electrons in RO·. The spectra are affected by process 1. The nuclear reso-