for the iodides in Tables II and III. Whereas the dimethylammonium and diethylammonium salts display the maximum limiting equivalent conductances in the methyl and ethyl series, the monobutylammonium salt is most conducting in the butyl series.

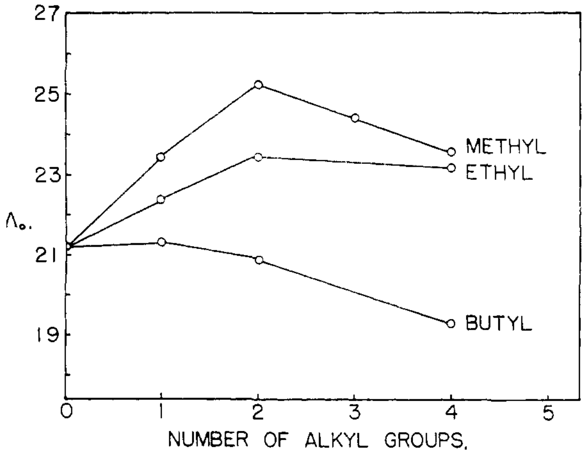

Fig. 4.—Limiting equivalent conductances of substituted ammonium chlorides.

Other aspects of the conductance behavior of the substituted ammonium salts are normal. For isomeric cations, the relative conductances increase with increasing symmetry of the cation as shown by the following examples

$$BuNH_3^+ < Et_2NH_2^+ < Me_4N^+$$
$$Bu_2NH_2^+ < Et_4N^+$$
$$EtNH_3^+ < Me_2NH_2^+$$

For similarly substituted cations, the conductances increase with decreasing formula weight of the cation

$$BuNH_3^+ < EtNH_3^+ < MeNH_3^+$$
$$Bu_2NH_2^+ < Et_2NH_2^+ < Me_2NH_2^+$$
$$Bu_4N^+ < Pr_4N^+ < Me_3PhN^+ < Et_4N^+ < Me_4N^+$$

The following series of decreasing relative conductances of the symmetrically substituted quaternary ammonium ions shows that the difference (in Λ units) between the conductances of the Me_4N^+ and Et_4N^+ ions is very small in comparison to those for the other two cases.

$$Me_4N^+ \overset{0.35}{>} Et_4N^+ \overset{2.50}{>} Pr_4N^+ \overset{1.39}{>} Bu_4N^+$$

Similar results for the same series of cationic conductances in nitrobenzene have been reported by Taylor and Kraus[10] who postulated that the greater charge density on the Me_4N^+ ion causes appreciable ion–dipole interaction resulting in additional retardation of the mobility of that ion.

[10] E. G. Taylor and C. A. Kraus, This Journal, **69**, 1731 (1947).

Lexington, Kentucky

[Contribution from the Department of Chemistry, University of Utah]

The Mass Spectra of Four Deuterated Butanes

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The mass spectra of *n*-butane-2-*d*, *n*-butane-1,1,1-d_3, *n*-butane-1,1,1,3,3-d_5 and *n*-butane-1,1,1,2,2,3,3-d_7 using 80 volt electrons at 100° are compared with that of *n*-butane. The results are discussed with respect to the isotope effect, rearrangement phenomena, and activated complexes. The rate of H–D exchange in the parent molecule–ions is slower than the rate of C–C bond break, but faster than the rate of loss of H_2 or HD. Such exchange occurs between atoms on adjacent carbons and on carbons 1–3 and 1–4.

I. Introduction

A theory of mass spectra of polyatomic molecules, with reference primarily to saturated hydrocarbons, has been presented.[3–5] The mass spectra are assumed to arise from the quasi-equilibrium decomposition of the parent molecule–ion through a sequence of competing unimolecular reactions. Using thermal data combined with mass spectral appearance potentials, Wahrhaftig and co-workers obtained a set of reactions and activation energies for the decomposition of propane. The detailed application of these permitted the satisfactory calculation of the major aspects of the mass spectra of propane and propane-2,2-d_2, including the metastable transition peaks of propane.[6] However, on attempting to make similar calculations for butane and isobutane, it was found that more information was needed on the reaction scheme than could be obtained from a study of the mass spectra of the normal compounds. In order to gain further information on the rearrangements preceding and accompanying the decomposition reactions, three new deuterated butanes have been prepared and studied.

II. Experimental

Apparatus.—All mass spectra reported in this paper were obtained on a mass spectrometer constructed at the University of Utah. This machine is of the usual sector field type developed by Nier, with 90° deflection at an 8 inch radius. The details of operation were as follows.

Background pressure in the main tube was between 2.5 × 10^{-6} and 4.5 × 10^{-6} mm. The gas samples were stored at 20 mm. pressure and were introduced into the ionization region at the rate of approximately 0.5 cc./min. An electron accelerating potential of 80 volts was used in all runs. Total electron emission was in the range of 35–60 micro-

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[1] From a dissertation in partial fulfillment of the requirements for the Ph.D. degree, University of Utah, 1954. Now at the Department of Chemistry, Cornell University.

[2] The construction of the mass spectrometer used in this research was initiated under a grant from the University of Utah Research Fund. This research was assisted by the U. S. Atomic Energy Commission under Contract AT(11-1)-82, Project No. 5.

[3] H. M. Rosenstock, M. B. Wallenstein, A. L. Wahrhaftig and Henry Eyring, *Proc. Natl. Acad. Sci. U. S.*, **38**, 667 (1952).

[4] M. B. Wallenstein, Ph.D. Thesis, University of Utah, 1951.

[5] H. M. Rosenstock, Ph.D. Thesis, University of Utah, 1952.

[6] Allen Kropf, Ph.D. Thesis, University of Utah, 1954.

amperes while the current through the ionizing region was maintained at 17 microamperes. The temperature of the ionization chamber, as measured with a chromel–alumel thermocouple, was 100 ± 5°. The positive ion beam was drawn from this region by a potential of 5 volts and accelerated into the analyzer section by a potential of 3500 volts. Magnetic scanning was employed. The ion current arriving at the collector, amplified with a negative feedback amplifier, was recorded on a Brown Electronik Recordng Potentiometer. Thirty minutes were required to sweep the range $m/e = 12$ to $m/e = 60$.

Preparation of Deuterated Butanes.—The four deuterated butanes studied were *n*-butane-2-*d*, *n*-butane-1,1,1-d_3, *n*-butane-1,1,1,3,3-d_5 and *n*-butane-1,1,1,2,2,3,3-d_7 (hereafter referred to as butane-d_1, butane-d_3, butane-d_5 and butane-d_7, respectively). The standard organic procedures used are summarized in the equations

$$CH_3CH_2CHBrCH_3 \xrightarrow[\text{Tetrahydrofuran (THF)}]{\text{LiD, LiAlD}_4\text{ (catalyst)}} \text{butane-}d_1$$

$$CH_3CH_2CH_2COCl \xrightarrow[\text{2, HCl, H}_2\text{O}]{\text{1, LiAlD}_4\text{, ether}} \underset{\text{I}}{CH_3CH_2CH_2CD_2OH}$$

$$\text{I} \xrightarrow[\text{bromobenzene}]{PBr_3} \underset{\text{II}}{CH_3CH_2CH_2CD_2Br}$$

$$\text{II} \xrightarrow[\text{THF}]{\text{LiD, LiAlD}_4\text{ (catalyst)}} \text{butane-}d_3$$

$$CH_3COCH_2CH_3 \xrightarrow[\text{K}_2\text{CO}_3\text{ catalyst}]{\text{D}_2\text{O (excess)}} \underset{\text{III}}{CD_3COCD_2CH_3}$$

$$\text{III} \xrightarrow[\text{2, HCl, H}_2\text{O}]{\text{1, LiAlH}_4\text{, ether}} \underset{\text{IV}}{CD_3CHOHCD_2CH_3}$$

$$\text{IV} \xrightarrow[\text{bromobenzene}]{PBr_3} \underset{\text{V}}{CD_3CHBrCD_2CH_3}$$

$$\text{V} \xrightarrow[\text{THF}]{\text{LiAlH}_4} \text{butane-}d_5$$

$$\text{III} \xrightarrow[\text{2, HCl, H}_2\text{O}]{\text{1, LiAlD}_4\text{, ether}} \underset{\text{VI}}{CD_3CDOHCD_2CH_3}$$

$$\text{VI} \xrightarrow[\text{bromobenzene}]{PBr_3} \underset{\text{VII}}{CD_3CDBrCD_2CH_3}$$

$$\text{VII} \xrightarrow[\text{THF}]{\text{LiD, LiAlD}_4\text{ (catalyst)}} \text{butane-}d_7$$

The heavy water was provided by the Stewart Oxygen Company and certified by the National Bureau of Standards to be 99.5% D_2O. LiD and $LiAlD_4$ were obtained from Metal Hydrides, Inc., and reportedly contain less than a few thousandths of 1% active hydrogen. The other materials were obtained as reagent grade chemicals and were distilled through a small column, with the usual rejected forerun and residue, before use.

In all manipulations extreme caution was exercised to eliminate possible contamination from atmospheric moisture. All glassware was flame dried under vacuum; where necessary, solvents were distilled from a slurry of $LiAlH_4$ through a suitable spray trap directly into the reaction vessel. The boiling chips and K_2CO_3 used were heated to red heat for one hour and stored over P_2O_5. Before use they were flame dried *in vacuo*.

The equilibrations of 2-butanone with D_2O to give product III were carried out in a manner similar to that described by Condon[7] for the preparation of acetone-d_6. The mole ratio of D_2O to ketone was three to one. In order to conserve D_2O, the equilibrations were performed in four columns in such a fashion that the residue from column one was used in column two, that from column two in column three, and so on. The number of equilibrations in each column was one greater than theoretically required to give a sample in which 99.4% of the replaceable hydrogens are deuteriums.

Conversion to the alkyl halide with PBr_3 was used in preference to the use of concentrated hydrochloric acid and zinc chloride which reportedly causes some exchange between deuterium and acid hydrogen.[7] The final reduction to the butane with LiD and a catalyst of $LiAlD_4$, as suggested by the experiments of Johnson, Blizzard and Carhart,[8] avoided the disproportionation and olefin formation encountered using a Grignard method. Tetrahydrofuran (THF) was used as the solvent with the expectation that simple distillation would separate the butane. As an azeotrope formed, the evolved butane was purified by bubbling it through 36 *N* H_2SO_4 at 0° followed by a spray trap of solid NaOH.

III. Results

Table I presents the mass spectra of butane and the prepared deuterated butanes obtained using the mass spectrometer described. The relative ion currents, normalized to total ion current equal to 100, are the averages of three runs appropriately corrected for background. Background runs were made before and after admitting each isotopic species to the mass spectrometer. The major background peaks were at mass to charge ratios of 18, 28 and 32, with small contributions over the regions 40–44 and 55–57. Of these, only the corrections at 55, 56 and 57 were a significant fraction of the peaks observed so that uncertainty in the correction introduces a 50% uncertainty into the intensities for these masses. At the other masses, the relative corrections were much smaller. No changes in background ascribable to the deuterated butanes were observed.

The mass spectra tabulated have not been corrected for C^{13} content. Data have not yet been obtained at low electron voltages to permit analyses for isotopic purity by the method of Stevenson.[9] Simple comparison of the C_4 region mass spectra for the deuterated butanes with the spectrum for the normal compound indicates the absence of large amounts of impurities containing a number of deuterium atoms different from that stated.

IV. Discussion

From arguments based upon appearance potentials, relative abundances and metastable ion peaks, Rosenstock[5] concluded that the major peaks in the mass spectrum of butane with electron voltage 50 to 100 volts result from the set of reactions indicated in Fig. 1. Attempts by Rosenstock and others in this Laboratory to calculate the mass spectrum of butane, assuming the reaction scheme shown in Fig. 1, indicated more data were necessary to resolve ambiguities. It seemed probable that

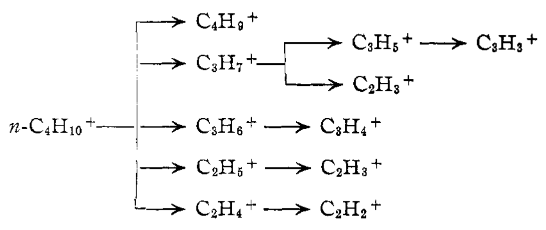

Fig. 1.—Simplified reaction scheme for production of ions from *n*-butane. In each case, the neutral fragment not listed is assumed to be in its most stable configuration, *e.g.*, H_2, CH_4, C_2H_6.

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(7) Francis E. Condon, THIS JOURNAL, **73**, 4675 (1951).

(8) J. E. Johnson, R. H. Blizzard and H. W. Carhart, *ibid.*, **70** 3664 (1948).

(9) D. P. Stevenson and C. D. Wagner, *ibid.*, **72**, 5612 (1950).

TABLE I

MASS SPECTRA OF DEUTERATED BUTANES[a]

m/e	d_0	d_1	d_3	d_5	d_7
66					0.30
65					6.89
64				0.26	1.04
63				5.77	0.69
62			0.36	1.21	.12
61			7.74	0.47	.21
60		0.39	1.70	.31	.17
59	0.39	8.50	0.45	.21	.14
58	9.02	1.28	.33	.19	.08
57	1.23	0.36	.24	.18	.16
56	0.31	.37	.17	.17	.09
55	.28	.12	.23	.14	.05
54	.06	.18	.17	.11	.10
53	.32	.21	.16	.14	.12
52	.09	.15	.19	.14	.13
51	.27	.24	.18	.11	.74
50	.19	.14	.08	.05	20.3
49				.76	3.11r
48				21.2	3.83
47			0.75	3.89	17.6
46			23.2	4.34	8.15
45		1.29	3.72	14.9	3.19
44	1.16	36.2	4.68	5.22	3.06
43	33.6	6.52	18.4	3.62	1.36
42	5.37	7.70	4.12	2.66	2.06
41	11.0	3.18	4.56	1.92	1.38
40	0.61	1.82	1.42	1.53	0.84
39	4.11	2.10	2.18	0.67	.19
38	0.35	0.21	0.31	.16	.01
37	0.12		0.11	.05	
35					.06
34				0.06	3.18
33			0.09	.54r	1.05r
32			3.27	3.92	3.17
31		0.51	1.61	4.61	3.76
30	0.26	5.47	3.16	7.01	6.81
29	12.5	9.38	5.98	5.98	2.68
28	9.25	7.35	5.46	4.81	1.73
27	8.05	5.62	3.68	1.65	0.39
26	0.77	0.58	0.51	0.20	.07
25					
18			.35	.35	.31
17			.09r	.09r	.07r
16			.07r	.08r	.10r
15	0.71	0.57	.36	.31	.29

[a] Relative abundances given as per cent. of total ion current

several of these reactions, such as the loss of methane to yield $C_3H_6^+$, could proceed *via* several different activated complexes. Consideration of the mass spectra of the monodeuterobutanes[10] leads to the same conclusion. It was expected that this study would permit the determination of the relative importance of various reactions yielding the same mass peak. Rearrangements, discussed in the next section, increase the complexity of the mass spectra observed with 80 volt electrons to the extent that no simple conclusions can be drawn regarding reactions other than the simple bond break to yield the deuterated equivalents of $C_3H_7^+$ and $C_2H_5^+$. It is hoped that further studies with low voltage electrons[11] will permit the elucidation of the reaction scheme.

Rearrangements.—The existence of rearrangements in hydrocarbon mass spectra is well known[12] and has been indicated by previous work on deuterated compounds.[7,13,14] For the deuterated propanes studied by Condon, *et al.*, rearrangement fragments were observed which indicated an exchange between hydrogen and deuterium on adjacent carbons. The exchange noted by Stevenson and Wagner with butane-d_1 could be attributed either to a 1–2 or a 2–4 rearrangement. With the present data on polydeuterated butanes, exchanges have been noted that indicate 1–3, 1–4 and adjacent carbon rearrangements and permit an estimation of the extent to which each contributes to the mass spectrum of a given compound.

In the mass spectra of butane-d_3, butane-d_5 and butane-d_7, nine peaks were observed that could be attributed solely to hydrogen–deuterium exchanges (indicated by "r" in Table I). For all three of these compounds, ions are detected at mass to charge ratios of 16 and 17 which correspond, in part, to the m/e 16 peak obtained by Stevenson and Wagner with butane-d_1.[15] However, the polydeuterated compounds reveal considerable about the mechanism of these rearrangements. Assuming that multiple exchange and double ionization can be neglected, the m/e 16 peak from butane-d_3 and m/e 17 peak from butane-d_7 must be a result of a rearrangement across carbon atoms 1 and 4. The m/e 17 and 16 peaks for butane-d_3 and butane-d_7, respectively, can be due to 1–2, 1–3 or 1–4 exchanges and, although the difference is small, it is significant to note that they are larger than those that could occur only by a 1–4 path. For butane-d_5, these ions could arise from either 1–2 or 1–4 rearrangement.

It has here been assumed that CH_3^+ and analogs result from a single bond break in the butane. An alternative assumption is that the CH_3^+ is formed by the sequence $C_4H_{10}^+ \rightarrow C_2H_5^+ \rightarrow CH_3^+$. Then, simple shift of a hydrogen from one carbon to the other should be very rapid in the ethyl radical ion. In a deuterated molecule this will produce a nearly statistical distribution of hydrogens and deuteriums. This assumption, too, is consistent with the data.

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[10] D. P. Stevenson and C. D. Wagner, *J. Chem. Phys.*, **19**, 11 (1951).

[11] D. O. Schissler, S. O. Thompson and John Turkevich, *Disc. Faraday Soc.*, **10**, 46 (1951).

[12] A. Langer, *J. Phys. Colloid Chem.*, **54**, 618 (1950).

[13] Francis E. Condon, H. L. McMurray and V. Thornton, *J. Chem. Phys.*, **19**, 1010 (1951).

[14] D. P. Stevenson and C. D. Wagner, *ibid.*, **19**, 11 (1951).

[15] In the present work, the noise level at the time butane-d_1 was analyzed was too high to definitely establish the appearance of this peak. However a small indication was noted (not reported in Table I).

No such ambiguity in interpretation exists for several other peaks. The mass spectrum of butane-d_7 has reasonably large peaks at masses 33 and 49 which can only be due to an H–D interchange preceding simple bond break.[16] Only an H–D exchange between any carbon positions followed by loss of CH_2D will yield mass 49, while only a 1–3 or 1–4 exchange can yield mass 33, $C_2D_4H^+$. Corresponding to the exchange processes giving rise to the ions of mass 33 and 49, there must be exchange products at masses 32 and 48 coming from the other end of the molecule. Although the rearranged fragments are not the sole possible contributors at these mass values, it is possible to calculate the approximate fraction of ion current due to other fragments. Consider the mass 48 ion. Aside from rearrangement, this ion will result from loss of CH_3D by the parent. For *n*-butane, loss of CH_4 is about 15% as great as loss of $\cdot CH_3$ and a crude calculation can be made by assuming that in the -d_7 compound loss of CH_3D is about 15% as probable as loss of $\cdot CH_3$ which yields the mass 50 peak. Thus, the relative ion current at mass 48 would be about 3.0. The discrepancy between this and the observed value is satisfactorily explained by the existence of the rearrangement ion when one remembers that only a 1–4 rearrangement will contribute. Similarly the mass 32 peak resulting from loss of CD_3CH_3 from the parent ion would have an intensity of 2.2. The difference between calculated and observed intensity, 1.0, is attributed to loss of C_2D_4H from the rearranged parent ion and since the rearrangement ions at masses 32 and 33 both come from the same paths (1–3 and 1–4) the agreement is extremely satisfying. In Table II the expected peak height, neglecting exchange contribution, is compared with the observed value for those mass peaks for which this simple calculation is applicable; in all cases the observed ion current is higher than that calculated neglecting exchange.

In these calculations, the isotope effect has been ignored. The effect of isotopic substitution is complex, and is certainly different for reactions involving loss of CH_4 or C_2H_6 as contrasted with loss of $\cdot CH_3$ or $\cdot C_2H_5$. However, the internal consistency exhibited in Table II, along with the fact that three of the peaks shown can occur only as a result of rearrangement, is strong evidence for the approximate validity of the calculations.

TABLE II

CONTRIBUTION OF FRAGMENTATION FOLLOWING EXCHANGE TO THE MASS SPECTRA OF DEUTERATED BUTANES

m/e	Ion		Expected ion current no exchange	Expected exchange contribution	Relative ion current obsd.	Type of exchange
	No exchange	With exchange				
			Butane-d_7			
49	....	C_3D_6H	0	..	3.11	1–2, 1–3, 1–4
48	C_3D_6	$C_3D_5H_2$	3.0	1.0	3.83	1–4
33	....	C_2D_4H	0	..	1.05	1–3, 1–4
32	C_2D_4	$C_2D_6H_2$	2.2	1.0	3.17	1–3, 1–4
			Butane-d_5			
47	C_3D_5H	$C_2D_4H_3$	.90	2.1	3.89	1–2, 1–4
46	$C_3D_4H_2$	$C_3D_3H_4$	2.2	2.1	4.34	1–2, 1–4
33	....	C_2D_4H	0	..	0.54	2–3
			Butane-d_3			
45	$C_3D_3H_3$	$C_3D_2H_5$	2.1	1.0	3.72	1–4
44	$C_3D_2H_4$	C_3DH_6	3.2	3.1	4.68	1–2, 1–3, 1–4
31	C_2D_3H	$C_2D_2H_3$	0.95	1.0	1.61	1–3, 1–4
30	$C_2D_2H_2$	C_2DH_4	1.4	1.0	3.16	1–3, 1–4
			Butane-d_1			
43	C_3DH_5	C_3H_7	5.0	1.0	6.52	(1–2, 2–4) × 1/2

Activated Complexes.—The appearance potentials for the more abundant ions in the *n*-butane mass spectrum can be interpreted in terms of a set of reactions only if it is assumed that in general hydrogens come off pairwise as hydrogen molecules. The comparison of energies of activation taken as differences of appearance potentials and energies of reaction calculated from thermal data and ionization potentials leads to an excess of $\Delta H^{\ddagger}$ over ΔH of about 20 kcal./mole for loss of H_2.[5] This small value requires that as the two C–H bonds are broken, the H–H bond is formed and the hydrocarbon ion is stabilized by the formation of a double bond or by resonance. If an ion is in essentially the activated complex configuration, but with the excitation in the partially bonded hydrogens perpendicular to the translation along the reaction coordinate, the hydrogens will exchange. Thus, the activation energy for H–D exchange in the deuterated butanes should be somewhat less than the activation energy for HD loss. In *n*-butane, following ionization by 80 volt electrons, the ratio of probability of H_2 loss to primary C–C bond break is given roughly by the ratio of peak heights of masses 56 and 43, and is 0.01. However, the ratio of probability of H–D exchange to primary C–C bond break in butane-d_7 is given roughly by the ratio of peak heights of masses 49 and 50, and is 0.15. Thus, the rate of HD exchange is rapid enough to greatly affect the mass spectrum, but is not fast enough to yield a statistical distribution of hydrogen and deuterium. The activated complexes proposed are sketched in Fig. 2; in each case, the rotation indicated by arrows (a) will give exchange while the translation (b) will give loss of a hydrogen molecule. From the calculations of the previous section it appears that all four exchanges indicated take place. While direct evidence is lacking, it seems only reasonable that all four structures also contribute to H_2 loss, and that in similar fashion the three structures indicated in Fig. 3 would all be important in the production of mass 42 in butane-d_0.

Isotope Effect.—The influence of deuterium substitution on the breakdown pattern of a hydrocarbon molecule, the so-called "isotope effect," has been a subject of considerable interest since the first observation of this phenomenon in 1938 by Delfosse and Hipple.[17] An empirical approach suggested by Evans, Bauer and Beach[18] has been

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[16] As was previously mentioned, the amount of hydrogen on deuterated positions is believed to be small. Furthermore these rearrangements are necessary to explain other anomalous features in the spectra of all three of the polydeuterated butanes.

[17] J. Delfosse and John A. Hipple, *Phys. Rev.*, **54**, 1060 (1938).

[18] Marjorie W. Evans, N. Bauer and J. Y. Beach, *J. Chem. Phys.*, **14**, 701 (1946).

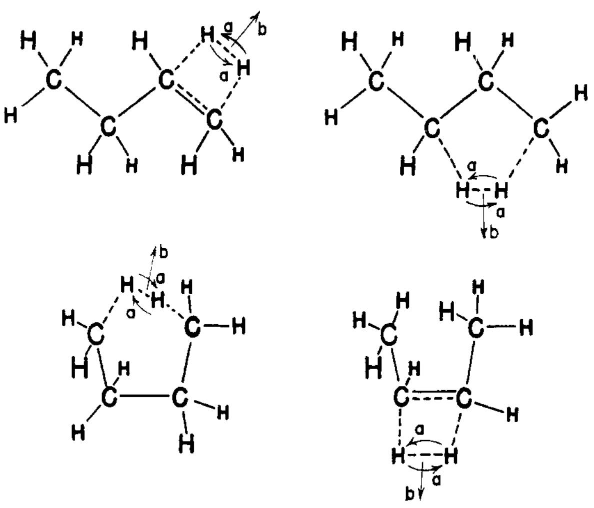

Fig. 2.—Activated complexes for (a) hydrogen exchange and (b) loss of a hydrogen molecule from butane ion.

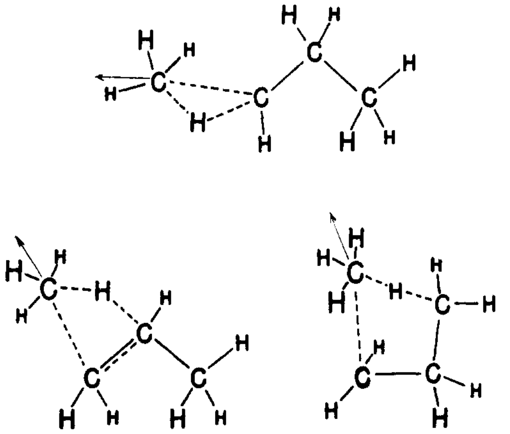

Fig. 3.—Activated complexes for loss of methane from butane ion.

used to predict the mass spectra of substituted methanes and ethanes, but Stevenson and Wagner[14] have demonstrated that for larger molecules the contributing factors are too complex to be explained by this simple two parameter theory. In the present work the influence of deuterium substitution on the mobility of remaining hydrogens is well illustrated by comparing the intensities of the parent ions with those showing loss of one hydrogen. For the series butane-d_0, butane-d_1, butane-d_3, butane-d_5 and butane-d_7 the ratios (parent minus H)/(parent) are 0.13, 0.15, 0.22, 0.21 and 0.15. The ratios calculated, assuming statistical removal of H and D, are 0.13 (assumed), 0.12, 0.09, 0.06 and 0.04.

Stevenson and Wagner have discussed the isotope effect for simple reactions in terms of the dissociation of a molecule ABA and its isotopically substituted counterpart, ABA′, where ABA + e $\rightarrow$ ABA$^+$ + 2e $\rightarrow$ AB$^+$ + A + 2e. They state that in the dissociation of ABA′, the increased zero point energy in AB$^+$ compared to the heavier counterpart A′B$^+$ will make the relative abundance of A′B$^+$ greater than that of AB$^+$. The greater zero point energy in ABA$^+$ compared to ABA′$^+$ will make the yield of AB$^+$ from ABA greater than the yield of AB$^+$ from ABA′, while the yield of A′B$^+$ cannot be predicted. The quasi-equilibrium theory of Rosenstock, *et al.*,[3] permits in principle the quantitative calculation of the isotope effect for all reactions. Such calculations have been made for propane-2,2-d_2.[6] In this theory, isotopic substitution will change the density of energy levels both in the ground state and in the activated complex, and also by changing zero point energies will change the effective energies of activation for a reaction. The rate of reaction does not depend on the energy of the final state but on the energy difference between the initial state and the activated complex. However, for simple bond break, the case discussed by Stevenson, *et al.*, $\Delta H^{\neq}$ and ΔH are essentially equal and the effect of isotopic substitution upon energy level densities is approximately the same for normal and activated complex states. Consequently their rules are those predicted by the quasi-equilibrium theory. For a reaction involving a "tight" activated complex resulting in loss of H_2, CH_4 or C_2H_6, the calculation is more involved; the final effect of isotopic substitution results from the interplay of too many factors to permit a simple, qualitative picture. Stevenson and Wagner also discuss the effect of isotopic substitution in changing the average amplitude of vibration of atoms in the parent molecule and so changing the distribution of configurations of the molecule ion resulting from vertical ionization. We believe that only a small fraction of the energy required for the several reactions of the parent ion can come from vibrational excitation due to vertical ionization; most energy must come from electronic excitation which will be independent of isotopic substitution. Parent ion relative abundance is sensitive to small changes in energy distribution and so is markedly affected by temperature and by isotopic substitution. The relative abundances of the fragment ions generally are not sensitive to small changes in energy distribution of the parent ions.

Stevenson and Wagner showed that their rules for the reactions of ABA and ABA′ were confirmed by their data on monodeuterated propane, *n*-butane and isobutane. Before the mass spectra of the polydeuterated butanes can be considered in this fashion, it is necessary to take into account increase and decrease of intensity at a given mass value due to rearrangement products as described in a previous section. For example, it was shown that in the mass spectrum of butane-d_7 the intensity of the ion $CD_3CD_2CD_2^+$, $m/e = 50$, is decreased because of the existence of the ion $C_3D_6H^+$, $m/e = 49$, from rearrangement. As a result some uncertainty exists in the assignment of intensities to the heavier and lighter equivalent ions. Nevertheless, with this factor taken into account the ion intensities of the two possible propyl ions from all isotopic species are in reasonable agreement with Stevenson's prediction (Table III).

It is noted that the intensity of the heavier propyl is in each case greater than one-half the intensity of the butane-d_0 propyl and the intensity of the lighter propyl less than one-half (within the limits of the arbitrary assignment). For the ethyl ion, it was not possible to assign individual values to each

TABLE III

INTENSITIES OF PROPYL IONS FOR *n*-BUTANE AND FOUR DEUTERATED BUTANES

Butane-d_0		Butane-d_1		Butane-d_3		Butane-d_5		Butane-d_7	
Ion	I^a	Ion	I	Ion	I	Ion	I	Ion	I
$CH_3CH_2CH_2^+$	34	$CH_3CDHCH_2^+$	36	$CH_3CH_2CH_2^+$	18	$CH_3CD_2CH_2^+$	15	$CH_3CD_2CD_2^+$	19
		$CH_3CH_2CDH^+$		$CD_3CH_2CH_2^+$	24	$CD_3CH_2CD_2^+$	23	$CD_3CD_2CD_2^+$	23

[a] Intensities or relative abundances as percentage of total ion current, corrected for exchange contribution indicated in Table II.

of the heavier and lighter ions. However, the sum of these was less than the intensity of the ethyl ion from butane-d_0.

One further point of interest should be noted regarding the effect of isotopic substitution. In Table IV the total relative intensities of the C_2, C_3 and C_4 groups for *n*-butane and four isotopic butanes are given. It is noted that the relative intensity of the C_2 group for butane-d_5 shows a considerable increase over that expected with a corresponding decrease in the C_3 and C_4 groups. This may be attributed to the close similarity of the two possible ethyl ions resulting from a simple bond break or, oppositely, to the greater dissimilarity of the two groups immediately adjacent to the bond. Future work on symmetrically substituted molecules such as $CD_3CH_2CH_2CD_3$ and $CH_3CD_2CD_2CH_3$ is indicated.

TABLE IV

TOTAL RELATIVE INTENSITIES FOR THE C_2, C_3 AND C_4 GROUPS FOR *n*-BUTANE AND FOUR DEUTERATED BUTANES

Group	Butane-d_0	Butane-d_1	Butane-d_3	Butane-d_5	Butane-d
C_2	30.6	28.9	23.8	28.8	22.9
C_3	56.3	59.0	63.4	60.9	65.8
C_4	12.2	11.9	12.0	9.5	10.3

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[CONTRIBUTION FROM THE NAVAL MEDICAL RESEARCH INSTITUTE]

Charge Distribution in Protein Molecules. I

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Assuming that ion (*e.g.*, H^+) binding sites are distributed throughout a spherical protein molecule and that electrolyte solution can penetrate somewhat into the protein molecule, it is possible to calculate the non-uniform radial distribution of bound ions at equilibrium. In the numerical cases worked out, there is some tendency, but not an overwhelming one, for the bound ions to concentrate near the surface. The titration curve (or binding isotherm) is computed and compared with more conventional titration curves. The net charge (bound charge plus electrolyte) radial distribution is also calculated. The total net charge turns out to be about half of the total bound charge, in the examples chosen.

I. Introduction

When charge interactions are taken into account in work on ion binding, titration curves, etc., of proteins, it is generally assumed that the charges on the protein molecule are distributed over the surface. This assumption is difficult to test experimentally, so we examine the question theoretically here and in a second paper to be published later. In the present paper we restrict ourselves to the case of one type of dissociable group (*e.g.*, $-NH_3^+ \rightarrow H^+ + -NH_2$). The second paper will consider a mixed case (*e.g.*, $-NH_3^+$ and $-COOH$).

A protein surface charge distribution is strictly possible only if all dissociable groups (or binding sites) are on the surface. A more reasonable assumption (see below) would appear to be that the binding sites (*e.g.*, $-NH_2$) are distributed throughout the protein but that the sites nearest the surface bind preferentially (to give, for example, $-NH_3^+$) because this results in a lower electrostatic free energy than a more or less uniform charge distribution throughout the molecule.

There might be some tendency for the binding sites themselves to be built in predominantly near the surface of the protein on synthesis (polymerization of amino acids), since this would decrease the free energy of synthesis. However, this suggestion seems rather unlikely since (1) the protein presumably folds up, forming a surface, *after* the polymerization is accomplished and (2) restricting, on synthesis, the order of amino acids along the chain (or chains), so that the binding sites will appear at or near the surface after folding, places a very severe limitation (statistically) on the possible arrangements of the amino acids along the chain (or chains).

As a model, we take a rigid, slightly expanded (hydrated), spherical protein molecule with a radially symmetrical distribution of binding sites. For a given total number of bound ions (*e.g.*, H^+), we find that distribution of bound ions among sites which minimizes the total (electrostatic plus binding) free energy. An alternative but equivalent statement is that the required distribution of bound ions among sites is such as to make the electrochemical potential of the bound ions the same at all points in the protein molecule. Having found the charge distribution, we then note the extent to which the charges are actually concentrated near the surface, according to this model.

In an earlier paper,[1] we attempted to treat the apparent swelling of bovine serum albumin at low *p*H. In the electrostatic calculation, a uniform degree of ionization throughout the protein was assumed. Here we remove this assumption, but do not allow (variable) swelling. Both swelling and a

(1) T. L. Hill, *J. Phys. Chem.*, **60**, 358 (1956).