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DEPARTMENT OF CHEMISTRY
UNIVERSITY OF NORTH CAROLINA
CHAPEL HILL, NORTH CAROLINA

WILLIAM E. HATFIELD
JOHN S. PASCHAL

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Activation Energy of Deuterium-Hydrogen Exchange in Amides

Sir:

When it was found that deuterium exchange rates with protein N-H groups fall roughly into two classes, rapid and slow,^{1,2} it seemed reasonable to assign the fast group to unfolded portions of the polypeptide chain and the slow group to helical sections.³ On this basis one could ascribe the measured heat of activation of 20 kcal./mole for the slow exchange to the need to open three adjacent hydrogen bonds in order to unfold a helical segment,³ each hydrogen bond presumably requiring 6-8 kcal. It was assumed in this connection that unfolded sections of the polypeptide chain exchanged hydrogens with a greatly reduced heat of activation.

As far as we can find no actual measurements have been made of the activation energy for deuterium-hydrogen exchange in a simple, solvated N-H group. In the course of studies of catalytic effects on such exchanges,⁴ we have examined, therefore, the effect of temperature on the kinetics of deuterium exchange in a model amide, N-methylacetamide, $\text{CH}_3\text{CONHCH}_3$, when it is entirely in the monomeric state.

Exchange rates were followed for solutions of 1 M N-methylacetamide in D_2O by observation of changes in absorbance at 1.43μ in the near-infrared region of the spectrum. It was found immediately at room temperature that the rate of exchange was acid- and base-catalyzed, in agreement with the report of Nielsen.⁵ Experiments were then extended to lower and higher temperatures, over the pD range 4.5-6.5.

In the range pD 4.5-5.5, the exchange was acid-catalyzed at each of three temperatures, 12, 24, and 32° . The activation energy (Fig. 1), calculated from the temperature coefficient of the rate constant at pD 5.0, is 17 kcal./mole. The rate reaches a minimum at approximately pD 5.5. Above pD 5.5 it is base-catalyzed, and rate constants were measured over the range pD 5.5-6.5. Data at pD 6.3 (Fig. 1) were used to calculate the activation energy, and a value of 23 kcal./mole was obtained. Essentially the same energies would be obtained for other pD's in the acid or basic range, respectively. The observed rates at pD values more than 0.5 unit below or above the pD of the minimum rate were greater than 90% acid- or base-catalyzed, respectively.

It seems clear, therefore, that at least in this model of a peptide group, $\text{CH}_3\text{CONHCH}_3$, a substantial activation energy (near 20 kcal./mole) is essential for exchange with deuterium even when the N-H is not hydrogen bonded to a $\text{C}=\text{O}$. If additional energy

(1) H. Lenormant and E. R. Blout, *Nature*, **172**, 770 (1953).

(2) A. Hvidt, G. Johansen, K. Linderstrom-Lang, and F. Vaslow, *Compt. rend. trav. lab. Carlsberg, Ser. chim.*, **29**, 129 (1954).

(3) K. Linderstrom-Lang, Special Publication No. 2, The Chemical Society London, 1955, p. 1.

(4) I. M. Klotz and B. H. Frank, *Science*, **138**, 830 (1962).

(5) S. O. Nielsen, *Biochim. Biophys. Acta*, **37**, 146 (1960).

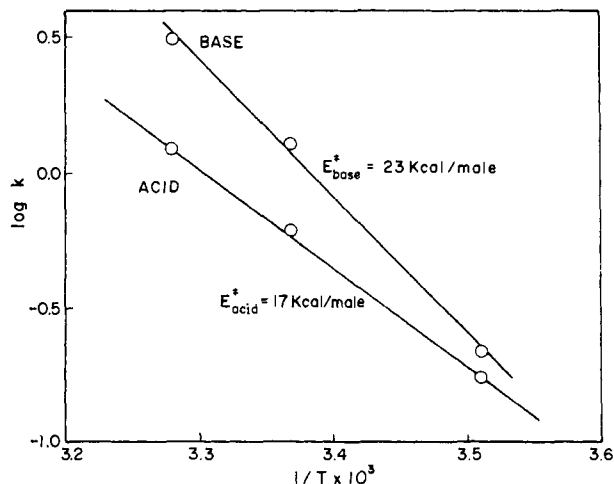


Fig. 1.—Logarithm of the observed rate constant (in min.^{-1}) for the deuterium exchange of D_2O with the N-H hydrogen of $\text{CH}_3\text{CONHCH}_3$, as a function of temperature. Acid constants at pH 5.0; base constants at pH 6.3.

is needed to break hydrogen bonds in a helix before exchange occurs, then the observed activation energy for the over-all exchange process ought to be substantially greater than 20 kcal./mole.

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(7) Predoctoral Fellow, National Institute of General Medical Sciences, 1963-1964.

DEPARTMENT OF CHEMISTRY⁶
NORTHWESTERN UNIVERSITY
EVANSTON, ILLINOIS

IRVING M. KLOTZ
BRUCE H. FRANK⁷

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X-Ray Structure Determination of Cubane¹

Sir:

We wish to present the results of a high-precision X-ray analysis as absolute confirmation of the structural assignment of the recently synthesized hydrocarbon cubane.²

The space group of cubane³ is $R\bar{3}$ with the cell constants $a = 5.340 \pm 0.002 \text{ \AA}$. and $\alpha = 72.26 \pm 0.05^\circ$. There is one molecule per unit cell.

Three-dimensional X-ray data were collected with Cu $K\alpha$ radiation on a GE XRD-5 instrument with a scintillation counter employed as a detector. The intensities were collected using the moving counter-moving crystal technique. The 97 independent structure amplitudes were obtained by averaging the three reflections related by the crystal's threefold axis.

A body diagonal carbon and the atom related to it by the center of symmetry are at special positions on a threefold axis at x, x, x and $\bar{x}, \bar{x}, \bar{x}$. The hydrogens attached to these carbons also lie on this special position. The other carbons and hydrogens, six of each, occupy the general positions of the space group. The coordinates of the independent atoms and the temperature factors for the atoms are given in Tables I and II.

(1) This research was supported by a Public Health Service Grant.

(2) P. Eaton and T. Cole, *J. Am. Chem. Soc.*, **86**, 3157 (1964).

(3) (a) The crystals were grown by sublimation with material supplied by P. Eaton; (b) No. 148, International Tables for X-ray Crystallography.

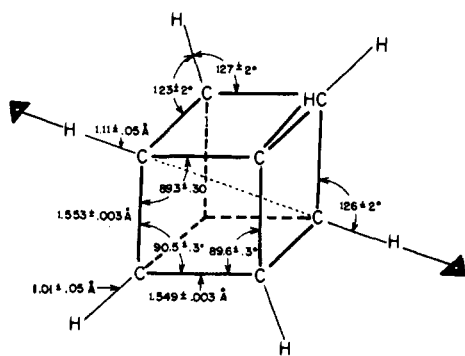


Fig. 1.—Bond parameters for the cubane molecule; the back CH group was omitted for clarity.

The structure was refined by least-squares techniques using the Hughes weighting scheme.⁴ The refinement was carried out varying all independent parameters; anisotropic temperature factors were used for the carbon atoms and isotropic for the hydrogens. The final disagreement factor, R , was 7.3%. As seen from Table II the carbon atoms are vibrating almost isotropically.

TABLE I
FRACTIONAL COORDINATES AND STANDARD DEVIATIONS OF CUBANE ATOMS^a

Atom	x	y	z
C _{gen}	-0.18711	0.19519	0.10706
σ	0.00060	0.00061	0.00062
C _{spec}	0.11546	0.11546	0.11546
σ	0.00040	0.00040	0.00040
H _{gen}	-0.3246	0.3468	0.1848
σ	0.0085	0.0081	0.0080
H _{spec}	0.2100	0.2100	0.2100
σ	0.0060	0.0060	0.0060

^a σ is the standard deviation as given by the least-squares program.

TABLE II
TEMPERATURE FACTORS FOR CUBANE

Atom	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
C _{gen}	0.0410 ^a	0.0425	0.0450	-0.0042	-0.0051	-0.0142
C _{spec}	0.0468	0.0468	0.0468	-0.0143	-0.0143	-0.0143
C ^b	0.0415	0.0415	0.0415	-0.0097	-0.0097	-0.0097
H _{gen}	4.0 ^c					
H _{spec}	3.6 ^c					

^a $\sigma_{av} = 0.002$. ^b Carbon with isotropic vibration for comparison. ^c Isotropic temperature factor.

The independent bond distances and angles for the cubane molecule are shown in Fig. 1. The molecule has cubic symmetry to within the errors of the determination. The carbon-carbon bond distances in cubane are similar to those obtained by microwave and electron diffraction for cyclobutane derivatives (1.548 ± 0.003 Å. in cyclobutane⁵ and 1.548 ± 0.003 Å. in bromocyclobutane⁶).

(4) M. J. Beurger, "Crystal-structure Analysis," John Wiley and Sons, Inc., New York, N. Y., 1960, Chapter 22.

(5) A. Almendinger, O. Bastiansen, and P. N. Skancke, *Acta Chem. Scand.*, **15**, 711 (1961).

(6) W. G. Rothschild and B. P. Dailey, *J. Chem. Phys.*, **36**, 2931 (1962).
GEORGE HERBERT JONES LABORATORY EVERLY B. FLEISCHER
CHEMISTRY DEPARTMENT
UNIVERSITY OF CHICAGO
CHICAGO 37, ILLINOIS

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Kinetic and Tracer Studies¹ on the Alkaline Hydrolysis of $\text{Co}(\text{NH}_3)_5\text{O}_2\text{CCF}_3^{+2}$

Sir:

The experiments we have been doing on the alkaline hydrolysis of $\text{Co}(\text{NH}_3)_5\text{O}_2\text{CCF}_3^{+2}$ reveal some unusual effects which are not anticipated by earlier work in this subject area.²

The rate law for the hydrolysis has the form

$$\frac{-d \ln (\text{complex})}{dt} = k_1(\text{OH}^-) + k_2(\text{OH}^-)^2$$

At 25.0° and $\mu = 1.0$ (NaCl), $k_1^3 = 1.6 \times 10^{-2} M^{-1} \text{sec.}^{-1}$ and $k_2 = 0.50 M^{-2} \text{sec.}^{-1}$; ΔH_1^* and ΔS_1^* are 27 kcal. mole⁻¹ and 24 cal. mole⁻¹ deg.⁻¹; ΔH_2^* and ΔS_2^* are 7.5 kcal. mole⁻¹ and -35 cal. mole⁻¹ deg.⁻¹. The term second order in (OH^-) is novel for ammine complexes; our observations suggest that it appears when the carboxyl group is strongly activated by an electronegative group. Thus, it is featured also by $\text{Co}(\text{NH}_3)_5\text{C}_2\text{O}_4^{+}$ but not by $\text{Co}(\text{NH}_3)_5\text{O}_2\text{CH}^{+2}$.

Oxygen isotopic experiments show that on complete hydrolysis at 0° and 1.00 M NaOH, the product $\text{Co}(\text{NH}_3)_5\text{OH}^{+2}$ derives only 1.3% of its oxygen from the solvent, while the product CF_3CO_2^- derives 49.3%. Thus it is clear that under these conditions bond breaking is entirely (>98%) at the C-O position, and there is little exchange of oxygen between the reacting complex and the solvent (exchange would lead to the incorporation of more than one solvent oxygen into the products for each act of hydrolysis). It should be noted that under the conditions specified, >99% of the reaction takes place by the k_2 path.

Oxygen exchange between the complex and solvent does become significant when the temperature is raised, especially when the concentration of alkali is reduced. That exchange takes place has been shown by examining the oxygen isotopic composition of the residual complex after partial hydrolysis. The reaction mixture was quenched by acidifying, and the trifluoroacetate complex was separated from the product aquo using $\text{Cr}^{+2}(\text{aq})$ which, under selected conditions, reacts more rapidly with the aquo than with the trifluoroacetate complex. Isotopic analysis of the trifluoroacetate complex was accomplished taking advantage of the clean isotopic course of the hydrolytic reaction at 0° and 1 M NaOH. The experiments show that Co-O-C oxygen at 25° and 0.10 M OH^- exchanges with solvent at a half-life of 60 ± 10 sec. and that the half-life varies inversely with (OH^-) .

The isotopic composition of the hydroxo product on complete hydrolysis as a function of (OH^-) can be accounted for by allowing for exchange as described above, and by assuming that by the k_1 path only Co-O bond scission takes place while by the k_2 path there is only C-O bond scission. The results are, however, not very sensitive to the assumption about bond breaking by the k_1 path, because over the range of (OH^-) covered, the k_2 path is dominant and as much

(1) This work was supported by the A.E.C. under Contract No. AEC AT(04-3)-326. The funds for the purchase of the spectrophotometer were acquired under N.S.F. Grant No. NSF GP-2406.

(2) C. A. Bunton and D. R. Llewellyn, *J. Chem. Soc.*, 1692 (1953).

(3) In the kinetic work of F. Basolo, J. G. Bergmann, and R. G. Pearson, *J. Phys. Chem.*, **56**, 22 (1952), the concentration of OH^- was so low (0.004 M) that only the k_1 path contributed to the rate they measured. Our value of k_1 is consistent with theirs ($0.73 \times 10^{-2} M^{-1} \text{sec.}^{-1}$) at lower ionic strength.