myosin length measurements is too great, particularly since any hydration would make the reported lengths too large.

We may safely conclude that all of the data on both the meromyosins and intact myosin can be explained, within the experimental errors, by the sub-unit arrangement LLH and that the molecule is probably of this form.

There is some further, fragmentary evidence supporting this choice of model. Myosin has been observed to aggregate in a completely side to side fashion.[15] If the LHL arrangement were correct, side to side aggregation would leave the mass distribution and, consequently, $P(\theta)$ unchanged. In the LLH case, however, so long as the cross-linking groups are not all on the heavier unit, one can have dimers of the form $\left\{\begin{matrix}\text{LLH}\\\text{HLL}\end{matrix}\right\}$ as well as $\left\{\begin{matrix}\text{LLH}\\\text{LLH}\end{matrix}\right\}$. The side to side aggregation would then tend to homogenize the mass distribution and thus to straighten out $P(\theta)^{-1}$. The latter is in fact the case. Scattering envelopes of aggregated samples always show measurably less downward curvature at high angles than monomeric myosin. (See Fig. 4).

It has become increasingly clear in recent years that the derivation of shape information from light scattering data is a hazardous affair. The increasing number of variables known to affect the scattering curve appear to make it more and more difficult to interpret in terms of any model. It should be pointed out, however, that these factors can actually add immeasurably to the strength of the method. If polydispersity has been shown to affect the shape of the curve, it must also be kept in mind that, coupled with the theory, that same curve can now serve to inform us on the nature of the polydispersity. The same could be said of the effect of inhomogeneous mass distributions, as we have tried to show here in a particular case.

NEW HAVEN, CONN.

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA AND THE INSTITUTE OF MOLECULAR PHYSICS OF THE UNIVERSITY OF MARYLAND]

A Simple Model for Barriers to Internal Rotation. II. Rotational Isomers[1]

BY MAURICE M. KREEVOY AND EDWARD A. MASON

RECEIVED APRIL 8, 1957

The energy differences between rotational isomers in substituted ethanes and between *cis–trans* isomers in substituted ethylenes have been calculated from a model which assumes spherical symmetry for atoms in molecules. Force laws are taken by analogy with known forces between similar atoms or groups of atoms which are not a part of a larger molecule, with allowances made for any residual electrical charges. No disposable parameters are involved in the calculations. Good agreement is obtained for the substituted ethanes. For the substituted ethylenes the *cis* form is always found to be more stable than calculated, and the difference is attributed to resonance involving a partial double bond between the substituent and the ethylenic carbon.

Introduction

A simple model for interactions between non-bonded atoms or groups of atoms has recently been applied to the calculation of symmetrical barriers to internal rotation about single bonds.[2] The model is now extended to the calculation of energy differences between *trans* and *gauche* forms of substituted ethanes, and energy differences between *cis* and *trans* disubstituted ethylenes. For the substituted ethanes the agreement with experiment is quite good and it is felt that these energy differences are now substantially understood. To explain the results for the dihaloethylenes something more than steric and electrostatic effects is required. A resonance effect such as that proposed by Pitzer and Hollenberg[3] seems likely. No disposable parameters are involved in any of the present calculations.

The Model.—The present simplified model estimates the van der Waals repulsions between non-bonded atoms or groups of atoms in molecules by analogy with known repulsions between atoms or groups of atoms which are not part of a larger molecule. For example: the interactions between two fluorine atoms bonded to different parts of a larger molecule are assumed to be the same as the interactions between two isolated neon atoms; the interactions between two non-bonded chlorine atoms the same as those between two isolated argon atoms. The force laws describing these interactions are taken from well-founded quantum mechanical calculations or from experimental results, so that no disposable parameters are involved in the procedure.

The force laws to be used in the present calculations already have been given,[2] with the exception of the bromine and the iodine interactions, which are taken by analogy with krypton and xenon, respectively. Unfortunately, the potential functions for these systems are not as well known experimentally as for the other systems. From a number of potential functions proposed for interactions between krypton atoms,[4,5] we have selected the following as a suitable representative

$$\varphi(r) = (4.694 \times 10^4)\exp(-2.76r) - 3888/r^6 \text{ kcal./mole} \quad (1)$$

where r is the separation distance in Å. This potential function was derived from measurements on second virial coefficients at high temperatures,[6]

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[1] This research was supported in part by Contract Nonr 595(02) of the U. S. Office of Naval Research at the University of Maryland.

[2] E. A. Mason and M. M. Kreevoy, THIS JOURNAL, **77**, 5808 (1955).

[3] K. S. Pitzer and J. L. Hollenberg, *ibid.*, **76**, 1493 (1954).

[4] E. A. Mason and W. E. Rice, *J. Chem. Phys.*, **22**, 843 (1954).

[5] E. Whalley and W. G. Schneider, *ibid.*, **23**, 1644 (1955).

[6] E. Whalley and W. G. Schneider, *Trans. Am. Soc. Mech. Engrs.*, **76**, 1001 (1954).

and agrees moderately well with a potential valid at very small separation distances obtained from the scattering of beams of fast krypton atoms in krypton gas.[7] A similar situation exists for the case of xenon; we have used the representative potential function[5]

$$\varphi(r) = (6.342 \times 10^4)\exp(-2.50r) - 9473/r^6 \text{ kcal./mole} \quad (2)$$

which is derived from measurements on high temperature second virial coefficients[5,8] and is in fair agreement with a potential valid at small distances obtained from the scattering of xenon beams in xenon gas.[9]

The effects of any partial charge caused by the electronegative halogen atoms have been roughly taken account of by adding to equations 1 and 2 a coulomb term in $1/r$, as described in connection with the calculations of symmetrical barriers to internal rotation.[2] The coefficient of the $1/r$ term in any particular molecule is determined by the magnitudes of the partial charges in the molecule, and these in turn can be calculated by distributing partial charges on the constituent atoms in accord with accepted bond polarizabilities, screening constants, covalent bond radii, and electric dipole moments, in the manner of Smith, Ree, Magee and Eyring.[10] The assumption of spherical symmetry of each atom in a molecule is implied in the methods of calculating both the van der Waals repulsions and the effects of electrostatic charges, so the model being used is at least simple and self-consistent.

Given the geometry of a substituted ethane or ethylene and the force laws between its nonbonded atoms it is a straightforward procedure to calculate the steric contribution to ΔE_0^0 The present calculations are a good deal less sensitive to the precise values of bond distances than are the calculations of the heights of barriers hindering internal rotation.[2] The effect of the zero-point energy has been neglected as insignificant throughout.

Results and Discussion

Substituted Ethanes.—For substituted ethanes all carbon atoms were assumed to be tetrahedral and the bond lengths were taken as follows[11]: C–H, 1.09 Å; C–C, 1.54 Å.; C–F, 1.33 Å.; C–Cl, 1.76 Å.; C–Br, 1.91 Å.; C–I 2.12 Å. The internal rotation angle between the two substituted methyl groups was taken as 60° for both the *trans* and the *gauche* isomers, although this assumption is rigorously correct only for the *trans* form. The error involved was checked by making an accurate calculation of the energy of the *gauche* form of 1,2-dichloroethane. The potential energy minimum for internal rotation was actually found at 64.3°, but the difference in the energy from the 60° form was only 0.07 kcal./mole, not a significant difference. The calculated and experimental energy differences between the *trans* and the *gauche* isomers are shown in Table I. Most of the experimental values are ΔH^0 at around 25° rather than ΔE_0^0 to which the calculations pertain, but these two quantities should not be very different. All the tabulated values are *gauche* energy minus *trans* energy.

Table I is not exhaustive, but it contains enough examples to illustrate the utility and limitations of the method. For the halogen substituted ethanes the agreement, in general, is excellent. For the chloro and fluoro compounds the principal source of the energy differences between the stable isomers is electrostatic interactions. Nevertheless, the height of the barrier which must be passed to go from *trans* to *gauche* is affected very strongly by the van der Waals repulsions. For instance, in 1,2-dichloroethane this height according to the present model is 3.43 kcal./mole, of which 1.75 kcal./mole is contributed by the van der Waals interactions. Our quantitative calculations thus confirm the views of Mizushima, Morino and Shimanouchi,[12,13] who concluded that the van der Waals forces are important in determining which configurations are stable, but do not contribute so strongly to the energy difference between the stable forms. For the bromo compounds the contributions of the van der Waals forces are about as large as those of the electrostatic forces, and for iodo compounds and for hydrocarbons the van der Waals repulsions are the dominant factors, again confirming previous qualitative views.[14]

TABLE I

ENERGY DIFFERENCES BETWEEN ROTATIONAL ISOMERS

Compound	ΔE_0^0, kcal./mole: van der Waals	Electrostatic	Total	Exptl.
CH_2F–CH_2F	0.23	2.00	2.23	. . .
CH_2Cl–CH_2Cl	−0.28	1.27	0.99	1.21[a] 1.03[b] 1.10[c] 1.3[d] 1.48[e]
CH_2Br–CH_2Br	0.61	0.97	1.58	1.45[b] 1.77[f] 1.4[a] 2.0[e]
CH_2I–CH_2I	2.45	0.99	3.44	Large[g]
CH_2Cl-CH_2Br	−0.06	1.10	1.04	1.43[e] 1.85[e]
CH_2Br–CF_2Br	0.41	−0.93	−0.52	±0.71 ± 0.05[h]
$CH_2(CH_3)$–$CH_2(CH_3)$	2.56	.00	2.56	.77 ± .09[i]
$CH_2(CH_3)$–CH_2Cl	−0.03	− .08	−0.11	− .05 ± .15[j]
$CH_2(CH_3)$–CH_2Br	0.77	− .06	0.71	− .1 ± .2[j]

[a] I. Watanabe, S. Mizushima and Y. Morino, *Sci. Papers Inst. Phys. Chem. Res. Tokyo*, **39**, 401 (1942). [b] T. Shimanouchi, H. Tsuruta, and S. Mizushima, *ibid.*, **42**, 51 (1944). [c] H. J. Bernstein, *J. Chem. Phys.*, **17**, 258 (1949). [d] W. D. Gwinn and K. S. Pitzer, *ibid.*, **16**, 303 (1948). [e] J. Powling and H. J. Bernstein, THIS JOURNAL, **73**, 1815 (1951). [f] H. J. Bernstein, *J. Chem. Phys.*, **18**, 897 (1950). (g) C. P. Smyth, "Dielectric Behavior and Structure," McGraw-Hill Book Company, Inc., New York, N. Y., 1955, p. 366. [h] R. E. Kagarise, *J. Chem. Phys.*, **24**, 1264 (1956); this author favors the negative value. [i] G. J. Szasz, N. Sheppard and D. H. Rank, *ibid.*, 16, 704 (1948); K. S. Pitzer, *ibid.*, **8**, 711 (1940). [j] C. Komaki, I. Ichishima, K. Kuratani, T. Miyazawa, T. Shimanouchi and S. Mizushima, *Bull. Chem. Soc. Japan*, **28**, 330 (1955).

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(13) S. Mizushima, "Structure of Molecules and Internal Rotation," Academic Press, Inc., New York, N. Y., 1954, p. 64.

(14) See ref. 13, pp. 42, 66.

The present model sometimes permits a more reliable interpretation of existing data to be made. Thus for 1,2-dibromo-2,2-difluoroethane Kagarise[15] was unable to make a conclusive assignment as to which was the more stable isomer. The present calculations indicate that the *gauche* is the stable configuration, confirming Kagarise's tentative assignment. For 1,1,2-trichloroethane and 1,1,2,2-tetrachloroethane the model predicts the same small contribution to $\Delta E_0{}^0$ from the van der Waals interactions as for 1,2-dichloroethane, -0.28 kcal./mole. The predicted electrostatic contribution to $\Delta E_0{}^0$, however, falls off from $+1.27$ kcal./mole in dichloroethane to $+0.97$ in trichloroethane and $+0.75$ in tetrachloroethane. Thus differences in the $\Delta E_0{}^0$ values for these three molecules[16,17] are apparently explainable on the basis of differences in the electrostatic forces due to inductive effects.[10] For 1,1,2-trichloroethane the model would seem to preclude a high value of the energy difference, in agreement with the views of Sheppard and Szasz.[18]

The relative stabilities of the rotational isomers of the hydrocarbons and the monohalogen hydrocarbons are governed largely by van der Waals repulsions. On the basis of the present model, which assumes the methyl group to be spherically symmetrical, the energy differences between the rotational isomers of 2-methylbutane and of 2,3-dimethylbutane are predicted to be the same as for *n*-butane, 2.56 kcal./mole. The fact that they are not the same[19,20] provides evidence that the methyl groups cannot be considered spherical and that "cogwheeling" is important. Very likely this is also the reason that the discrepancies between calculated and observed values of $\Delta E_0{}^0$ for *n*-butane and *n*-propyl bromide are rather large. The calculated and observed values of $\Delta E_0{}^0$ for *n*-propyl chloride check because the van der Waals interactions between the methyl group and the chlorine atom are small in any event.

Szasz's conclusion[21] that the methyl groups and the chlorine atom in molecules such as *n*-propyl chloride and isobutyl chloride must strongly attract each other seems to be unjustified. The present model predicts the observed small energy [differences between the rotational isomers without the necessity of invoking any such attraction. Furthermore, any model which assumes the methyl group and the halogen atom to be spherically symmetrical will predict the isobutyl halides to have the *same* energy differences between their rotational isomers as do the corresponding *n*-propyl halides, provided that electrostatic effects are relatively constant or of secondary importance, as they indeed seem to be. Consequently it is not necessary to postulate any new forces to explain the observed results.

Substituted Ethylenes.—The following bond distances and bond angles were used for substituted ethylenes[11,22]; C=C, 1.34 Å.; C–H 1.09 Å.; C–Cl, 1.67 Å.; C–Br, 1.93 Å.; C–I, 2.04 Å.; $C-CH_3$, 1.54 Å.; < C=C–R,[23] 122–123°. Table II shows the calculated and experimental energy differences between *trans* and *cis* disubstituted ethylenes. The calculations on the 2-butenes were made using both the spherically symmetrical methyl–methyl potential function and the hydrogen–hydrogen potential function and they are in good agreement. For the calculations involving the hydrogen–hydrogen interactions the stable methyl rotational positions were established by calculation and these are shown in Fig. 1, so cogwheeling has been taken into account. To calculate the electrostatic part of the energy differences the γ_{XC} parameters[10] for vinyl-X were needed, and these were calculated from the dipole moments of the vinyl halides in the manner outlined by Smith, Ree, Magee and Eyring.[10] The value of γ_{HC} for vinyl-H was assumed to be 0.00. Otherwise the calcula-

TABLE II

ENERGY DIFFERENCES BETWEEN *cis–trans* ISOMERS

Compound	$\Delta E_0{}^0$, kcal./mole van der Waals	Electrostatic	Total	Exptl.
CHCl=CHCl	−0.249	0.623	0.374	−0.445 ± 0.020[a]
CHBr=CHBr	0.618	.378	0.996	−0.13 ± 0.30[b]
CHI=CHI	4.70	.28	4.98	1.8 ± 1.0[c]
$(CH_3)CH=CH(CH_3)$	2.53[d]	.00	2.53	1.0[f]
	2.52[e]	.00	2.52	
$(CH_3)CH=CHBr$	0.51[d]	.00	0.51	0.0[g]

[a] K. S. Pitzer and J. L. Hollenberg, THIS JOURNAL, **76**, 1493 (1954). [b] R. M. Noyes and R. G. Dickinson, *ibid.*, **65**, 1427 (1943). [c] R. M. Noyes, R. G. Dickinson and V. Schomaker, *ibid.*, **67**, 1319 (1945); this value was measured in solution rather than in the gas phase, and is probably too low. [d] Calculated with a spherical average methyl potential. [e] Calculated with hydrogen–hydrogen potential alone. [f] American Petroleum Institute Research Project 44, "Selected Values of Physical and Thermodynamic Properties of Hydrocarbons and Related Compounds," Table 8n (Part 1), 1953. [g] K. E. Harwell and L. F. Hatch, THIS JOURNAL, **77**, 1682 (1955).

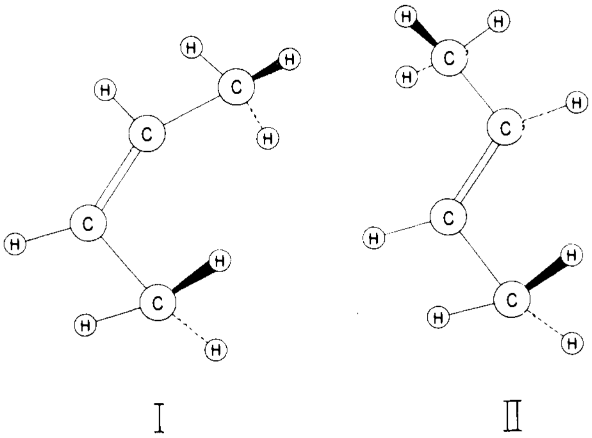

Fig. 1.—Configurations assumed for *cis*(I) and *trans*(II)-2-butene for the calculation of H–H interactions. Wedges represent bonds projecting above the plane of the paper, while dotted lines represent bonds below the plane of the paper. Other bonds are in the plane of the paper.

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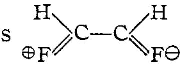
tions were made in the same manner as for the ethanes. Here, as with the ethanes, most of the experimental values are ΔH^0 at around room temperature rather than ΔE_0^0.

It has been recognized that the energy differences between *cis* and *trans* isomers of certain substituted ethylenes are not in accord with the expectation of a repulsion between the substituents.[3] Table II bears out this conclusion strikingly, and shows that even where the expected isomer is the more stable the difference is not as large as expected. A plausible explanation of this fact has been offered by Pitzer and Hollenberg[8] in terms of attraction between formal charges on the chlorine atoms in certain resonance structures which have double bonds between the substituents and the carbon atoms. Table II indicates that this sort of resonance is general for substituted olefins, being of the hyperconjugation type when methyl groups are involved.

By providing a better standard of comparison for the "non-resonating" molecule, the present calculations also permit a better evaluation of the fractional double bond character than was previously possible.[3] From the equations of Pitzer and Hollenberg a 4% double bond character is calculated for the C–Cl bonds in 1,2-dichloroethylene. This estimate is in excellent agreement with recent theoretical[24] and empirical[25] calculations, which yield about 6 and 4%, respectively.

It is noteworthy that the *cis* form of 1,2-difluoroethane cannot be stabilized by the same sort of resonance. Although fluorine can double bond to carbon, it cannot do so by taking up extra electrons because it lacks the d-orbitals of the other halogens, and resonance forms such as  are excluded on energy grounds. It is therefore predicted that the *trans* form of 1,2-difluoroethane will be more stable than the *cis* form by at least several hundred small calories per mole.

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MINNEAPOLIS, MINN.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, FLORIDA STATE UNIVERSITY]

A Spectrophotometric Method for the Determination of the Catecholase Activity of Tyrosinase and Some of its Applications[1]

BY MOHAMED ASHRAF EL-BAYOUMI[2] AND EARL FRIEDEN

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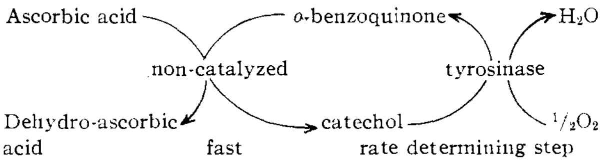
A spectrophotometric method is described for determining the catecholase activity of tyrosinase. Ascorbic acid is used to reduce the *o*-benzoquinone formed from the interaction of catechol with tyrosinase. The enzymatic activity is determined by measuring the decrease in optical density at 265 mμ, the absorption maximum for ascorbate ion. Spectral changes during the reaction are described. Effects of varying substrate and enzyme concentration and *p*H are reported. Applications of the method to tyrosinase inhibition and proteolysis studies are given. The use of this method and an analogous method employing ascorbic acid oxidase for ascorbic acid analysis in biological materials such as orange juice is also described.

Introduction

Available methods for the study of the catalytic activity of the enzyme tyrosinase leave much to be desired.[3] Conventional Warburg technique using catechol or other phenols as substrate results in the formation of highly reactive products, which lead to a highly complex reaction mechanism.[3] While the determination of initial rates would obviate this difficulty, the Warburg method is poorly adapted for such rate studies. A satisfactory method is the "chronometric" method of Miller and Dawson[4] which employs ascorbic acid as an inert reductant[5a] to reduce continuously and instantaneously the quinone back to catechol.



Net reaction: $\text{Ascorbic acid} + {}^1/_2\text{O}_2 \rightleftarrows \text{Dehydroascorbic acid} + \text{H}_2\text{O}$

The maintenance of the catechol in the reduced state effectively eliminates the possibility of complicating secondary reactions involving the quinone. However, the technique used to follow the disappearance of the ascorbate was cumbersome. In addition, this method is not easily adaptable to numerous kinetic studies of tyrosinase because of the complexity of the rate equations.[4] We have, therefore, adapted this method to a highly sensitive and rapid determination of the catecholase ac-

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