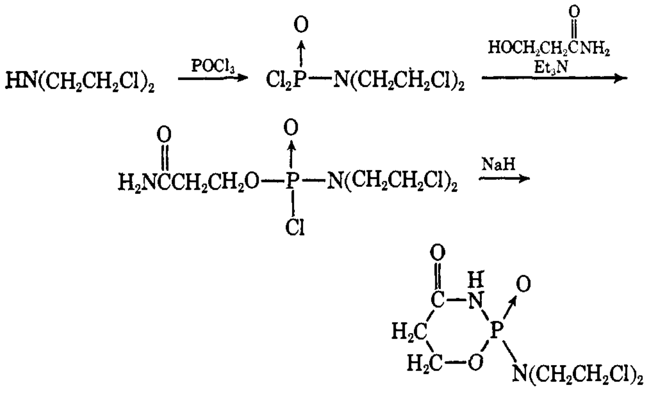
These observations suggested 4-ketocyclophosphamide (II) as the structure of the metabolite. The infrared spectral behavior can be summarized as shown above.

The mass spectrum of the metabolite is clearly different from mass spectra of N^3-methylcyclophosphamide,[3] which we synthesized for comparison purposes, and 6-methylcyclophosphamide.[4] The melting point of the metabolite is significantly higher than that of either 6-methyl or N^3-methylcyclophosphamide and suggests increased polar character.

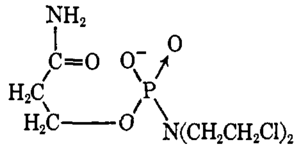
In order to confirm the structural assignment, 4-ketocyclophosphamide (II) was synthesized by the following route. The synthetic product's melting point, infrared spectrum, and mass spectrum were identical with those of the metabolite. In addition, a mixture melting point of the metabolite and the synthetic product gave no depression. The synthetic product gave satisfactory elemental analyses.



II

In subsequent experiments, 4-ketocyclophosphamide (II) was obtained consistently by ether extraction of fresh urine from catheterized dogs. Mass spectral analysis proved the extracts to be mixtures of II and cyclophosphamide (I) contaminated with other components of urine. From these extracts, the metabolite II was purified by DEAE-Sephadex column chromatography and crystallization.

Further evidence that C$_4$ of cyclophosphamide is oxidized by the dog comes from the fact that β-hydroxypropionamide was isolated as a fragment of the metabolite present in the third peak from the DEAE-Sephadex A-25 column. In this case, the urine came from a dog treated as before, except that the cyclophosphamide (0.03 μCi/mg) was labeled in position 6 of the ring. To obtain this fragment, the metabolite was treated with 0.1 *N* HCl at room temperature for 24 hr. After neutralization of this solution, alkaline phosphatase was added. The material was again placed on the DEAE-Sephadex A-25 column, and the radioactive compound washed from the column with 0.02 *M* NH$_4$HCO$_3$ was deionized by passage through a Sephadex G-10 column. The mass spectral pattern and the infrared spectrum of the fragment were identical with those of an authentic sample of β-hydroxypropionamide. This amide can arise from cyclophosphamide only by oxidation of C$_4$. We surmise that the third peak contained the ring-opened compound derived from II. Its structure is likely to be the following.



V

It seems probable that 4-ketocyclophosphamide is either the active form of cyclophosphamide or a precursor of the active form. 4-Ketocyclophosphamide (II) inhibits clone formation of H. Ep. 2 cells by 50% at 1 μg/ml. Under identical conditions, 50 μg/ml of cyclophosphamide (I) is required for comparable inhibition. Synthesis of the ring-opened compounds III, IV, and V, as well as C$_4$-hydroxy and C$_5$- and C$_6$-keto and -hydroxy analogs, is in progress.

Acknowledgments. We are grateful to Dr. L. B. Mellett and Dr. S. El Dareer for supplying urine samples from cyclophosphamide-treated dogs. This work was supported by Contracts PH43-66-29, PH43-64-51, and PH43-65-654 with Chemotherapy, National Cancer Institute, National Institutes of Health.

---

(3) H. Arnold, F. Bourseaux, and N. Brock, *Arzneim. Forsch.*, **11**, 143 (1961).

(4) Obtained from the Cancer Chemotherapy National Service Center, National Institutes of Health, Bethesda, Maryland.

(5) Affiliated with the Sloan-Kettering Institute for Cancer Research, New York, N. Y.

Donald L. Hill, Marion C. Kirk, Robert F. Struck
Kettering-Meyer Laboratory,[5] Southern Research Institute
Birmingham, Alabama 35205
Received December 31, 1969

The Crucial Role of Dynamic Effects in the Hydrogen–Iodine Reactions[1]

Sir:

Mechanistic details of chemical reactions follow from the application of dynamic principles to nuclei and electrons of the reaction complex. This problem is usually separated into three parts: (1) motion of the electrons in the field of the nuclei clamped at successive positions[2] (this leads to the concept of a potential energy surface (PES)); (2) motion of the nuclei in regions of the PES corresponding to reactants, products, and transition state[3a] (this leads to the concept of internal energy levels for these species); (3) momentum-conserving motions of the nuclei[3b] required to pass from reactants to transition state to products (this leads to what we henceforth call *dynamic effects*[4]).

Powerful techniques for probing chemical mechanisms have generally concentrated upon parts 1 (*e.g.*, the Woodward–Hoffmann rules[5]) and 2 (*e.g.*, the transi-

---

(1) Supported by Oklahoma State University Research Foundation, PHS Grant No. GM 13253, and by Los Alamos Scientific Laboratory of the University of California.

(2) M. Born and J. R. Oppenheimer, *Ann. Phys.*, **84**, 457 (1927).

(3) (a) H. Eyring, *J. Chem. Phys.*, **3**, 107 (1935); H. Pelzer and E. Wigner, *Z. Phys. Chem., Abt. B*, **19**, 445 (1932); S. Glasstone, K. J. Laidler, and H. Eyring, "The Theory of Rate Processes," McGraw-Hill Book Co., Inc., New York, N. Y., 1941; (b) explicit representation of such nuclear motions on a PES appears to have first been made by J. Hirschfelder, H. Eyring, and B. Topley, *J. Chem. Phys.*, **4**, 170 (1936).

(4) As we use the term, dynamic effects do not include the "ponderal" effects discussed by P. B. D. de la Mare, L. Fowden, E. D. Hughes, C. K. Ingold, and J. D. H. Mackie, *J. Chem. Soc.*, 3200 (1955). The latter pertain to nuclear motions giving rise to internal energy levels of the transition state (part 2), and are thus included in a transition-state theory treatment.

tion-state theory of Eyring[3a]) of the problem. In transition-state theory, part 3 is circumvented by the quasi-equilibrium hypothesis; corrections for dynamic effects appear in the transmission coefficient.

Computational difficulties with part 3 and success of transition-state theory have led chemists generally to ignore dynamic effects and consider only energetics in their mechanistic investigations. However, theoretical techniques based upon classical trajectory analysis can now explicitly consider such effects for reactions of small molecules.[6] For some reactions, these effects are crucial in determining mechanism. The results of our application of classical trajectory analysis to the hydrogen-iodine reactions strikingly illustrate this point.

Experiments show that the hydrogen-iodine exchange reaction proceeds by an atomic mechanism, while the bimolecular process

$$H_2 + I_2 \longrightarrow 2HI \tag{1}$$

makes negligible contribution.[7] Nozzle beam studies[8] indicate that the reverse reaction

$$HI + HI \longrightarrow H_2 + I_2 \tag{2}$$

is extremely slow for internally "cold" HI molecules.

If the transition-state properties are similar for two mechanisms, transition-state theory distinguishes them primarily by different transmission coefficients. While the classical trajectory treatment might be considered as a means of calculating the transmission coefficient for a reaction, no factorizability of the rate constant expression into dynamic and statistical factors is possible, nor was such an assumption made in our calculations. Semiempirical calculations indicate a plausible PES for (1) to have a 42.04 kcal/mol barrier relative to $H_2 + I_2$.[9] From energetics alone, the activation energy for reaction 1 should essentially equal this barrier height. Using this value and other transition-state parameters evaluated from the PES, we obtain transition-state theory rate constants (assuming transmission coefficient unity) of 0.71×10^5 and 1.56×10^5 l.^{2}/(mol^2 sec) at 633 and 738°K, respectively,[10] only an order of magnitude less than the values 9.38×10^5 and 18.5×10^5 observed[7] for HI production. The experiments,[7] however, indicate a rate of reaction 1 about an order of magnitude smaller than the transition-state theory results, thus necessitating a much smaller transmission coefficient for (1) than for the atomic process. A similar transition-state theory treatment shows that the (2) transmission coefficient is also unusually small.

Using the same PES as for the transition-state theory calculations, we employed classical trajectory analysis to investigate extensively the dynamics of the H_2–I_2 system and, by appropriate statistical averaging over reactant states, made *a priori* dynamical calculations of the rate constants for the elementary processes.

The calculated rate constants of (1) are 1.78 and 14.3 l.^{2}/(mol^2 sec) at 633° and 738°K, respectively (about 10^5 smaller than the experimental values for HI production[7]). The theoretical Arrhenius plot yields an *activation energy* for (1) of 53.6 kcal/mol, compared with the *energy barrier* of 42 kcal/mol.[9] Thus dynamic effects raise the effective activation energy by 11.6 kcal/mol; this provides a firm theoretical basis for the observation[7] that (1) is negligible in the production of HI.

Similar classical trajectory investigations with internally "cold" HI reactants show (2) to be extremely slow as a result of dynamic effects, even with relative translational energy up to 66 kcal/mol.

Moreover, our classical trajectory treatment of the H_2–I_2 system reveals an exchange mechanism involving iodine atoms and hydrogen molecules that is compatible with the reaction energetics, the dynamic effects, and with Sullivan's experimental results.[7] Let I_2^d represent two colliding I atoms with energy above the dissociation energy at an internuclear separation comparable to that existing in a bonded molecule, which we shall represent by I_2^b. A trajectory analysis of reaction of H_2 with I_2^d shows that for the plausible PES we have used, only $H_2I^b + I$ or $H_2 + I_2^b$ are formed as products, where H_2I^b is a slightly bound complex.[11] Similar investigations for reactions of H_2I^b with I show the reaction products to be either 2HI or $H_2 + I_2^d$. Thus, the classical trajectory treatment yields the following mechanism.

$$I_2^d + H_2 \xrightarrow{k_1} H_2I^b + I \tag{3}$$

$$I_2^d + H_2 \xrightarrow{k_{\mathrm{recomb}}} H_2 + I_2^b \tag{4}$$

$$H_2I^b + I \xrightarrow{k_2} 2HI \tag{5}$$

$$H_2I^b + I \xrightarrow{k_d} H_2 + I_2^d \tag{6}$$

The *a priori* classical trajectory rate constants and experimental H_2 and I_2 concentrations give a steady-state H_2I^b concentration of 1.7×10^{-10} *M*, within 25% of the equilibrium statistical mechanical value. Furthermore, for (4) the calculated values for k_{recomb} are 0.9×10^9 and 0.5×10^9 l.^{2}/(mol^2 sec) at 633 and 1000°K, respectively. The extrapolated experimental values are 1.6×10^9 and 0.8×10^9 l.^{2}/(mol^2 sec).[12] *The theory predicts that the recombination does not involve a stable intermediate H_2I^b complex,* since H_2I^b + I collisions do not produce stable I_2^b molecules. This is supported by the failure of an earlier H_2I intermediate complex treatment of I recombination.[13]

From the predicted mechanism and steady-state conditions for H_2I^b, the classical trajectory treatment

---

(5) R. B. Woodward and R. Hoffmann, *Angew. Chem., Int. Ed. Engl.*, **8**, 781 (1969); for an application to the present system, see R. Hoffmann, *J. Chem. Phys.*, **47**, 3739 (1968); L. M. Raff and R. N. Porter, *ibid.*, **51**, 4701 (1969); R. N. Porter and L. M. Raff, *ibid.*, **50**, 5216 (1969).

(6) F. T. Wall, L. A. Hiller, Jr., and J. Mazur, *ibid.*, **29**, 255 (1958); **35**, 1284 (1961); N. C. Blais and D. L. Bunker, *ibid.*, **37**, 2713 (1962); F. T. Wall and R. N. Porter, *ibid.*, **39**, 3112 (1963); M. Karplus, R. N. Porter, and R. D. Sharma, *ibid.*, **40**, 2033 (1964); **43**, 3259 (1965); **45**, 3871 (1966); M. Karplus and L. M. Raff, *ibid.*, **41**, 1267 (1964); L. M. Raff and M. Karplus, *ibid.*, **44**, 1212 (1966); D. L. Bunker and N. C. Blais, *ibid.*, **41**, 2377 (1964); P. J. Kuntz, E. M. Nemeth, J. C. Polanyi, S. D. Rosner, and C. E. Young, *ibid.*, **44**, 1168 (1966); M. Godfrey and M. Karplus, *ibid.*, **49**, 3602 (1968); N, Blais, *ibid.*, **49**, 9 (1968); P. J. Kuntz, E. M. Nemeth, and J. C. Polanyi, *ibid.*, **50**, 4607 (1969).

(7) J. H. Sullivan, *ibid.*, **46**, 73 (1967).

(8) S. B. Jaffe and J. B. Anderson, *ibid.*, **51**, 1057 (1969).

(9) L. M. Raff, L. Stivers, R. N. Porter, D. L. Thompson, and L. B. Sims, *ibid.*, in press.

(10) The rate of (1) is $d(HI)/dt = 2k(H_2)(I_2) = kK^{-1}(H_2)(I)^2$, where *K* is the iodine molecule-atom equilibrium constant. We and Sullivan list the "termolecular" rate constant $k' = kK^{-1}$.

(11) For a discussion of such I atom complexes see G. Porter and J. A. Smith, *Nature*, **184**, 446 (1959).

(12) R. Engleman and N. R. Davidson, *J. Amer. Chem. Soc.*, **82**, 4770 (1960).

(13) D. L. Bunker and N. R. Davidson, *ibid.*, **80**, 5090 (1958).

yields 20×10^5 l.^{2}/(mol^2 sec) for the overall rate constant for HI production at 633°K, whereas Sullivan measures 9.38×10^5 l.^{2}/(mol^2 sec).[7]

Thus, the present investigations give a detailed explanation for Sullivan's experimental observation that the molecular mechanism is forbidden, but that an atomic mechanism is allowed, as had previously been suggested by Semenov[14] and by Eyring.[15] Noyes' suggestion[16] that (1) might be forbidden as a result of the trajectory restrictions imposed by the dynamic effect of momentum conservation is essentially confirmed. A detailed report is in preparation.

[14] N. N. Semenov, "Some Problems in Chemical Kinetics and Reactivity," Vol. 2, Princeton University Press, Princeton, N. J., 1959, pp 73 and 74.

[15] H. Eyring and F. W. Cagle, Jr., in "Treatise on Analytical Chemistry," Vol. 11, Part 2, I. M. Koltoff and P. J. Elving, Ed., Interscience Publishers, New York, N. Y., 1965, p 45, and private communication.

[16] R. M. Noyes, *J. Chem. Phys.*, **48**, 323 (1968); *ibid.*, **49**, 3741.

[17] Alfred P. Sloan fellow.

R. N. Porter,[17] D. L. Thompson, L. B. Sims
Department of Chemistry, University of Arkansas
Fayetteville, Arkansas 72701

L. M. Raff
Department of Chemistry, Oklahoma State University
Stillwater, Oklahoma 74074

Received March 3, 1970

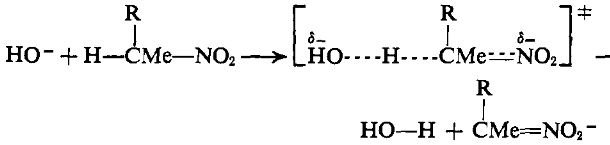
Deviant Brønsted Relations[1]

Sir:

The exponent α of the Brønsted relation is generally thought to measure the position of the transition state along the reaction coordinate of a proton-transfer process.[2] It is a necessary consequence of this view that α should lie between the limits zero and unity, and yet Brønsted relations with exponents greater than 1 and less than 0 have recently been reported.[3] I wish to show how these unusual Brønsted relations may be understood on a molecular level and to point out some implications of this on the use of Brønsted exponents as measures of transition-state structure.[4]

It is easily demonstrated that the Brønsted relation correlates rate constants of a proton-transfer reaction with equilibrium constants for the same process and that the Brønsted exponent is equal to the substituent effect on $\Delta F^{\ddagger}$ divided by the substituent effect on $\Delta F°$; in the symbolism of ref 2, $\alpha = \delta_R\Delta F^{\ddagger}/\delta_R\Delta F°$. It follows then that a Brønsted exponent greater than unity indicates a reaction in which the substituent effect on $\Delta F^{\ddagger}$ is greater than the substituent effect on $\Delta F°$. However such a situation goes against the notion that structural changes occurring during a chemical reaction take place continuously as the initial state changes into the final state and that the interaction of a substituent with such a system must also change continuously between initial- and final-state limits and must not pass through extrema outside these limits. It is possible, however, to retain the essential features of this idea and still permit $\delta_R\Delta F^{\ddagger}$ to exceed $\delta_R\Delta F°$ by recognizing that new interactions, absent from initial or final states, can be present in transition states. Such new interactions exist, for example, in the transition state of every bimolecular proton transfer reaction: union of the reactants creates forces not present before the reacting molecules come together or after they separate.

Consider the process for which α was recently found to be equal to 1.4: reaction of substituted nitroalkanes with hydroxide ion (eq 1).[3] The effect of the substituent R on $\Delta F°$ for this reaction, $\delta_R\Delta F°$, may be assigned to the electrical interaction of R with a negatively charged nitro group in the final state, I_{R,NO_2^-}; *i.e.*, $\delta_R\Delta F° = I_{R,NO_2^-}$. There will be a corresponding interaction in the transition state whose magnitude depends on the fraction of an electronic charge transferred from the hydroxide ion, x; *i.e.*, this contribution to $\delta_R\Delta F^{\ddagger}$ will be equal to xI_{R,NO_2^-}. In addition, there will be another effect in the transition state contributing to $\delta_R\Delta F^{\ddagger}$: the interaction of R with the partly charged hydroxide ion; this effect will be equal to $(1 - x)\cdot I_{R,HO^-}$, where I_{R,HO^-} is the interaction of R with a fully charged hydroxide ion situated in its transition-state position. Since this hydroxide ion is remote from R in the initial state, and since it no longer exists in the final state, the initial- and final-state limits of this effect will be zero. Thus, this is a transition-state interaction which is absent from initial and final states.



$$\mathrm{HO^- + H\!-\!\overset{\overset{R}{|}}{C}Me\!-\!NO_2 \longrightarrow \left[\overset{\delta-}{HO}\cdots H\cdots\overset{\overset{R}{|}}{C}Me\,\text{=\!-}\,\overset{\delta-}{NO_2}\right]^{\ddagger} \longrightarrow HO\!-\!H + \overset{\overset{R}{|}}{C}Me\!=\!NO_2^-} \quad (1)$$

This model leads directly to values of α greater than unity whenever the substituent interacts more strongly with a fully charged hydroxide ion than with a fully charged nitro group; *i.e.*, $\alpha = [xI_{R,NO_2^-} + (1 - x)\cdot I_{R,HO^-}]/I_{R,NO_2^-}$ is greater than 1 when $I_{R,HO^-} > I_{R,NO_2^-}$. This is likely to be the case in the present reaction, for geometrical considerations show that the distances between R and the centers of negative charge in the hydroxide ion and the nitro group will be comparable at the transition state, but the effective dielectric constant in the direction of the nitro group should exceed that in the direction of the hydroxide ion.

Deviant behavior in the series nitromethane, nitroethane, and 2-nitropropane can also be explained in a similar way. The acidity of these substances increases in the order given,[5] but their rates of reaction with hydroxide ion decrease in the same sequence;[6] combination of these two sets of data gives $\alpha = -0.7$.[3] The methyl groups successively introduced along this series will decrease acidity through their inductive effect but raise acidity by hyperconjugative stabilization of the nitronate ion (**1**). The latter effect predominates as it usually does in electron release to a positive center; this makes $\delta_R\Delta F°$ negative. Both of these interactions will be only partly realized at the transition state, but their sum will nevertheless still amount to net stabi-

---

[1] This research was supported by a grant (GP 9253) from the National Science Foundation to the Illinois Institute of Technology.

[2] See, for example, J. E. Leffler and E. Grunwald, "Rates and Equilibria of Organic Reactions," Wiley, New York, N. Y., 1963, p 235.

[3] F. G. Bordwell, W. J. Boyle, Jr., J. A. Hautala, and K. C. Yee, *J. Amer. Chem. Soc.*, **91**, 4002 (1969).

[4] For a discussion of this along somewhat different lines, see R. A. Marcus, *ibid.*, **91**, 7224 (1969).

[5] D. Turnbull and S. Maron, *ibid.*, **65**, 212 (1943); G. W. Wheland and J. Farr, *ibid.*, **65**, 1433 (1943).

[6] R. P. Bell and D. M. Goodall, *Proc. Roy. Soc., Ser. A*, **294**, 273 (1966).