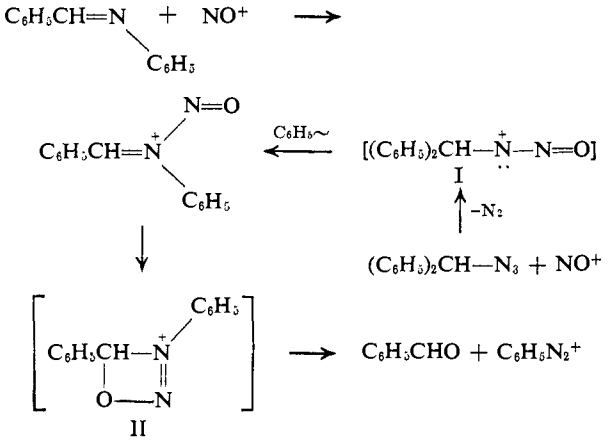
faster than the rate of loss of nitrous oxide, migration will occur.

The formation of benzaldehyde and benzenediazonium tetrafluoroborate can be rationalized by phenyl migration and has ample analogy with the protonic decomposition of benzhydryl azide.[12] We have observed that benzhydryl azide yields benzaldehyde and benzenediazonium tetrafluoroborate even when water and oxygen are rigorously excluded. This result, which was supported by our inability to effectively trap the expected benzylideneaniline intermediate by sodium borohydride reduction,[13] suggested that imines are labile to diazotization by the nitrosonium cation.

Treatment of *N*-benzylideneaniline with an equivalent amount of $NO^+BF_4^-$ in acetonitrile produces benzaldehyde and benzenediazonium tetrafluoroborate in nearly quantitative yields, as determined by chromatography and spectral properties. The pmr spectrum of benzylideneaniline before addition of the nitrosonium salt shows the C-hydrogen as a singlet at δ 8.51 relative to internal TMS.[14] Upon addition of $NO^+BF_4^-$ the C-hydrogen is observed at δ 9.37[15] which corresponds well to the change in chemical shift observed by Olah for the analogous protonated benzylideneaniline.[16] In addition, a new proton resonance due to benzaldehyde appears at δ 10.0 which increases in intensity with time at the expense of the δ 9.37 signal. These data support a mechanism in which nitrososation occurs prior to rearrangement (Scheme II). We speculate

Scheme II



that the production of aldehyde and diazonium ion is preceded by rate-limiting formation of an N-substituted oxadiazetine ring (II), but have at present no evidence to support the existence of such an unusual compound. In general, *N*-aryl derivatives of benzylideneimine react readily with nitrosonium salts to produce aryldiazonium ions and benzaldehyde (eq 3), and *N*-alkyl derivatives of benzylideneimine produce carbonium ions, nitrogen, and benzaldehyde (eq 4).

(12) C. H. Gudmundsen and W. E. McEwen, *J. Amer. Chem. Soc.*, **79**, 329 (1957).
(13) R. W. Layer, *Chem. Rev.*, **63**, 489 (1963).
(14) Spectra were taken on a Varian Associates Model A-60-A nmr spectrometer using acetonitrile as solvent and at a temperature of 35°.
(15) The expected change in position of phenyl patterns was also observed.[16]
(16) G. A. Olah and P. Kreienbuhl, *J. Amer. Chem. Soc.*, **89**, 4756 (1967).

$$C_6H_5CH{=}NAr + NO^+X^- \longrightarrow C_6H_5CHO + ArN_2^+X^- \quad (3)$$

$$C_6H_5CH{=}NR + NO^+X^- \longrightarrow C_6H_5CHO + N_2 + R^+X^- \quad (4)$$

Recently, Olah reported that alkyl and acyl sulfinylamines, isocyanates, and thioisocyanates react with nitrosonium salts to produce carbonium or oxocarbonium ions.[17] We have found that benzhydryl thioisocyanate and sulfinylamine react similarly, although at higher temperatures than we have used with the corresponding azides and without production of benzaldehyde. Adaption of the mechanism given in Scheme II to the reaction of nitrosonium salts with sulfinylamines, isocyanates, and thioisocyanates would explain the formation of carbonium ions, oxide gases, and nitrogen in those cases.

Production of benzophenone from benzhydryl azide occurs prior to quenching with water and may be explained by hydrogen migration or hydride abstraction from I. We have observed that benzhydrylidenimine reacts with $NO^+BF_4^-$ in anhydrous acetonitrile to produce an equal amount of benzophenone and nitrogen.

The reduction of alkyl azides with nitrosonium compounds represents a new and valuable route to the formation of carbonium ions. The cleavage of the carbon–nitrogen double bond of imines is also an efficient and useful method for diazotization. Whereas deamination of an amine requires a large excess of a particular nitrosonium compound[18] with resultant formation of water as a by-product, azides and imines react completely with 1 equiv of nitrosonium salt to produce carbonium ions. We are continuing investigations in these areas and into the reactions of nitrosonium compounds with other unsaturated systems.

Acknowledgment. We gratefully acknowledge the donors of the Research Corporation for partial support of this work.

(17) G. A. Olah, N. Friedman, J. M. Bollinger, and J. Lukas, *ibid.*, **88**, 5328 (1966).
(18) Benzhydrylamine, for example, requires more than 2 equiv of nitrosonium salt for complete reaction.
(19) National Science Foundation Undergraduate Research Participant, Summer 1969.
* To whom correspondence should be addressed.

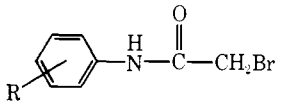
Michael P. Doyle,* Wendell Wierenga[19]
Department of Chemistry, Hope College
Holland, Michigan 49423
Received June 15, 1970

Nuclear Relaxation at the Active Site of α-Chymotrypsin

Sir:

In a recent paper we described the irreversible inhibition of the enzyme α-chymotrypsin by a series of trifluoromethyl-substituted α-bromoacetanilides (Ia–c) and alluded to our plans to examine the resulting derivatized proteins by fluorine nuclear magnetic resonance spectroscopy.[1] We now wish to report the results of preliminary experiments with these materials which provide, for the first time, direct data regarding nuclear transverse relaxation times (T_2) at the active site of this enzyme.

(1) E. W. Bittner and J. T. Gerig, *J. Amer. Chem. Soc.*, **92**, 2114 (1970).



Ia, R = *o*-CF_3
b, R = *m*-CF_3
c, R = *p*-CF_3

The modified enzymes ($\widehat{EI}$a–c) were examined in the native form as 2.5–3.0 × 10^{-3} *M* solutions in water while denatured samples of the proteins were prepared at the same concentration in 8 *M* urea solutions. The transverse relaxation time for the trifluoromethyl group in each sample was determined by the Carr–Purcell method,[2] incorporating the modification of Meiboom and Gill.[3,4] Our results are collected in Table I and, for comparison, relaxation times for the trifluoromethyl groups in the three inhibitor molecules (Ia–c) are also included there.

Table I. Transverse Relaxation Times for Trifluoromethyl-Substituted Enzymes and Inhibitors

	T_2, sec, for CF_3 group at position		
	ortho	meta	para
Derivatized α-chymotrypsin, native	0.04	0.016	0.006
Derivatized α-chymotrypsin, denatured	0.2	0.06	0.07
Irreversible inhibitor[a] (Ia–c)	1.4	1.0	0.95

[a] Acetone solutions 0.5 *M* in inhibitor were used.

The relaxation times for the inhibitors are not unusual and follow the same order, with respect to substitution pattern, as that found in various halogen-substituted benzotrifluorides.[5] We note a considerable decrease in the relaxation times of both the native and denatured forms of the enzymes when compared to the corresponding inhibitors; this decrease is consistent with the molecular size of these biopolymers and the concomitant slowing of the Brownian motions involved in the nuclear relaxation processes.[6] Our results suggest that the motion of the trifluoromethyl group is more restricted in the native enzymes than in the randomly constituted denatured enzymes and that this restriction of molecular motion becomes progressively more severe as this reporting group is placed at positions ortho, meta, and then, para relative to the acetamido linkage which holds the aromatic ring of the inhibitor to the enzyme.

The three trifluoromethyl-substituted α-bromoacetanilides probably react with the enzyme at the methionine-192 residue near the active site and the resulting proteins are devoid of enzymatic activity toward substrates constructed from amino acids with aromatic side chains.[1,7] Consideration of a molecular model of α-chymotrypsin indicates that the aromatic portion of the inhibitors Ia–c can be fitted into the region within the active site occupied by the tosyl group of the tosylated enzyme;[8] this conclusion is supported by the observation that *N*-formyl-L-tryptophan occupies this same location in the formyltryptophan–enzyme complex[9] and that enzyme $\widehat{EI}$b does not bind L-tryptophan, presumably because of a mutual exclusion effect.[10] The model also suggests that a trifluoromethyl group at the ortho position of the inhibitor can be relatively "exposed" while the same group at the para position is completely "buried" within the pocket which holds the tosyl or tryptophanyl moieties in these other systems. The interactions between the enzyme and the *p*-CF_3 group must be especially potent since a reduction of T_2 by a factor of six is realized when the group is located at this position relative to when this group occupies the ortho position.[11] We have previously noted the sensitivity of the kinetics of enzyme inhibition by various derivatives of I to substitution in the para position.[1]

If one assumes that fluorine–fluorine nuclear dipolar interactions are responsible for the major part of the relaxation effect and that the fluorine–fluorine internuclear distances are the same in solution as in the solid state, then the correlation time for motion of the *p*-CF_3 group within the active site of the enzyme may be estimated to be ~3.2 × 10^{-8} sec.[12–14] This correlation time is very close to that expected for the dimeric enzyme on the basis of hydrodynamic theory and agrees well with the correlation time data obtained by fluorescence spectroscopy.[15] It, therefore, appears that the trifluoromethyl groups in enzyme $\widehat{EI}$c and *m*-trifluoromethylbenzenesulfonylchymotrypsin have essentially stopped rotating about their local C_3 axes and that their rotational correlation time is determined to a large extent only by the overall Brownian motion of the protein. This conclusion may be modified when a more complete theory of nuclear relaxation is applied[16] and when the contributions of magnetic interactions between the nuclei of the amino acids within the active site and the trifluoromethyl group of the inhibitor can be evaluated.[17,18]

It will be interesting to see how these relaxation times change as a function of pH, temperature, electrolyte

(2) H. Y. Carr and E. M. Purcell, *Phys. Rev.*, **94**, 630 (1954).
(3) S. Meiboom and D. Gill, *Rev. Sci. Instrum.*, **29**, 688 (1958).
(4) A Bruker 321S pulsed-nmr spectrometer operating at 56.4 MHz was used. A Fabritek 1074 signal-averaging computer was used to enhance the signal-to-noise ratio to a usable level. Within experimental error, the relaxation process was exponential and did not appear to depend upon the spacing between the refocusing 180° pulses. The sample temperature was about 28°. The relaxation times quoted are averages of, typically, three determinations and are believed to be accurate to within 10%.
(5) A. S. Dubin and S. I. Chan, *J. Chem. Phys.*, **46**, 4533 (1967).
(6) J. G. Powles, *Polymer*, **1**, 219 (1960).
(7) H. J. Schramm and W. B. Lawson, *Z. Physiol. Chem.*, **97**, 332 (1963).
(8) D. M. Blow, *Biochem. J.*, **112**, 261 (1969).
(9) T. A. Seitz, R. Henderson, and D. M. Blow, *J. Mol. Biol.*, **46**, 337 (1969).
(10) J. T. Gerig, *J. Amer. Chem. Soc.*, **90**, 2681 (1968).
(11) The magnitude for T_2 when a trifluoromethyl group is within the active site and the suggestions made above concerning the location of the aromatic portion of the inhibitor molecule within the active site are confirmed by the observation that T_2 for *m*-trifluoromethylbenzenesulfonylchymotrypsin is 0.003 sec: J. T. Gerig, unpublished observations.
(12) O. Jardetzky, *Advan. Chem. Phys.*, **7**, 512 (1964).
(13) B. D. Sykes, P. G. Schmidt, and G. R. Stark, *J. Biol. Chem.*, **245**, 1180 (1970).
(14) An interfluorine distance of 2.1 Å was used, *cf.* M. R. Churchill and R. Mason, *Proc. Roy. Soc., Ser. A*, **292**, 61 (1966).
(15) R. P. Haugland and L. Stryer in "Conformations of Biopolymers," Vol. I, Academic Press, New York, N. Y., 1967, p 321.
(16) P. S. Hubbard, *J. Chem. Phys.*, **51**, 1647 (1969).
(17) S. I. Chan and G. P. Kreishman, *J. Amer. Chem. Soc.*, **92**, 1103 (1970).
(18) In an effort to support the interpretation of the trend in the transverse relaxation times, we have measured T_1 for the para-substituted enzyme and find a value (~0.07 sec) considerably less than would be expected on the basis of the single fluorine–fluorine relaxation mechanism discussed above. This discrepancy may indicate the presence of additional relaxation processes; we hope that frequency-dependence experiments in progress will clear up this question.

concentration, and the concentration of small organic molecules. Further work is in progress. It seems clear, however, that nuclear relaxation studies of appropriately constituted enzymes may offer a powerful probe into the dynamics of the interactions between protein active sites and substrate-like molecules.

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(19) NDEA Fellow, 1966–1969.
* To whom correspondence should be addressed.

E. W. Bittner,[19] J. T. Gerig*

Department of Chemistry, University of California
Santa Barbara, California 93106

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A New Type of Fluxional Molecule. Bis-μ-dimethylgermyl-dicobalt Hexacarbonyl

Sir:

We report here the observation of a type of intramolecular rearrangement which is rapid and interconverts equivalent nuclear configurations (*i.e.*, the molecule exhibiting it is fluxional) and which appears likely to have some scope and generality.

The molecule we have studied is $[(CH_3)_2Ge]_2Co_2(CO)_6$, **1**, which is one of several products isolated from reaction[1] of $(CH_3)_2GeH_2$ with $Co_2(CO)_8$ in toluene at −78°. The substance is characterized by satisfactory analyses for Co, C, and H, by its mass spectrum (*m/e* 491 (parent ion) and peaks corresponding to ions formed by loss of from one to six CO groups), ir spectrum (in CS_2 C–H stretches at 2960 (m) and 2900 (w) cm^{-1} and CO stretches at 2063 (s), 2028 (vs), 2002 (vs), 1990 (vs), 1960 (m, sh), and 1940 (w, sh, ^{13}C) cm^{-1}), as well as by its pmr spectrum described below.

Figure 1 shows the 100-MHz pmr spectrum of **1** at several temperatures. All methyl protons give a single sharp line at room temperature, but this line broadens, collapses, and is eventually replaced by two peaks of equal intensity which are sharp at −89°. Rates estimated from line widths follow the Arrhenius equation with $\Delta E = 15 \pm 1$ kcal/mol.

We propose that the molecule has structure I, which is analogous to that[3] of $Co_2(CO)_8$. The methyl groups on a given germanium atom lie in different environments, as emphasized by diagram II, which is similar to a Newman projection of an ethane-like structure. The low-temperature spectra are consistent with this structure and collapse of the two methyl peaks to a single one on raising the temperature shows that syn and anti methyl groups interchange rapidly at room temperature.

In a nonmechanistic sense the minimal required rearrangement steps can be described as II ⇄ III ⇄ IV, etc. From a more explicit or realistic point of view we consider traversal of the configuration V to be the most plausible of the various alternatives we have considered.[4,5] In each of its stable configurations,

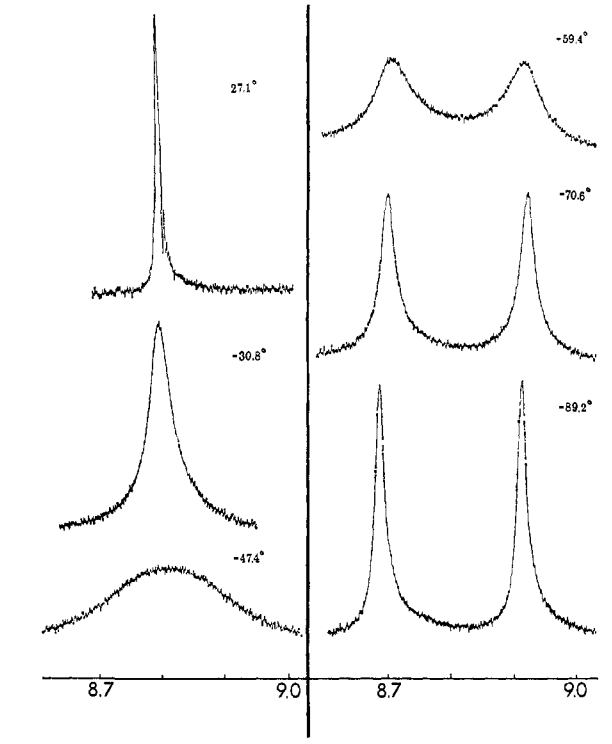
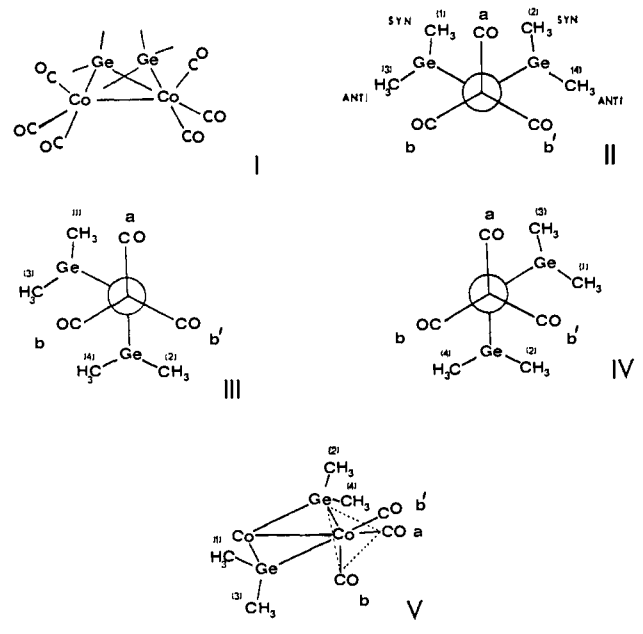


Figure 1. The pmr spectrum of $[(CH_3)_2Ge]_2Co_2(CO)_6$, **1**, at several temperatures. Recorded at 100 MHz in 2/1, v/v, CF_2Cl_2–CH_2Cl_2 solvent.



(1) Reaction[2] of $(C_6H_5)_2GeH_2$ with $Co_2(CO)_8$ apparently gave no digermyl product, but only $(C_6H_5)_2GeCo_2(CO)_7$.
(2) S. A. Fieldhouse, B. H. Freeland, and R. J. O'Brien, *Chem. Commun.*, 1297 (1969).
(3) G. G. Sumner, H. P. Klug, and L. E. Alexander, *Acta Crystallogr.*, 17, 732 (1964).

(4) Simple inversion of the four-membered Co–Ge–Co–Ge ring is inadmissible; it cannot lead to an equivalent configuration unless accompanied by some rearrangement of the $Co(CO)_3$ groups tantamount to a rotation about the local approximate threefold axis.
(5) A transition state or intermediate having nonbridging $(CH_3)_2Ge$ groups behaving as carbene analogs, in which rotation about an unbridged Co–Co bond might lead to the observed methyl group interchange, is considered unlikely, though it is not actually ruled out by present evidence. Rapid bridged-nonbridged-bridged interconversion