

are less than in any of the corresponding cyclopentadienyl complexes.

The short perpendicular distance of the cycloheptatrienyl ring from the metal atom suggests that π -cycloheptatrienyl-metal compounds would have carbon-carbon inter-ring contacts of about 3.1 Å. even if the two rings were staggered, resulting in considerable steric resistance to the formation of such compounds as, for example $(C_7H_7)_2Ti$ (effective atomic number = 36).

The authors are most grateful to Prof. F. G. A. Stone for calling their attention to this problem, and for supplying crystals for the X-ray study.

INSTITUTE FOR ATOMIC RESEARCH AND
DEPARTMENT OF CHEMISTRY
IOWA STATE UNIVERSITY
AMES, IOWA

GORDON ENGBRETTSON
R. E. RUNDLE

WORK WAS PERFORMED IN THE AMES LABORATORY OF THE U. S.
ATOMIC ENERGY COMMISSION. CONTRIBUTION No. 1261.

RECEIVED JANUARY 7, 1963

STEREOISOMERIC TRIPLET STATES OF AN α -DIKETONE¹ Sir:

In the accompanying communication¹ we have described chemical evidence from which we inferred that the lowest triplet states of conjugated dienes may exist in relatively stable *cis* and *trans* isomeric modifications. Since phosphorescence from diene triplets cannot be detected, at least one of the common means for characterization of triplet states cannot be used to shed further light on the problem. However, we might reasonably expect that there should be similar isomeric modifications of the lowest triplet states of α -diketones.

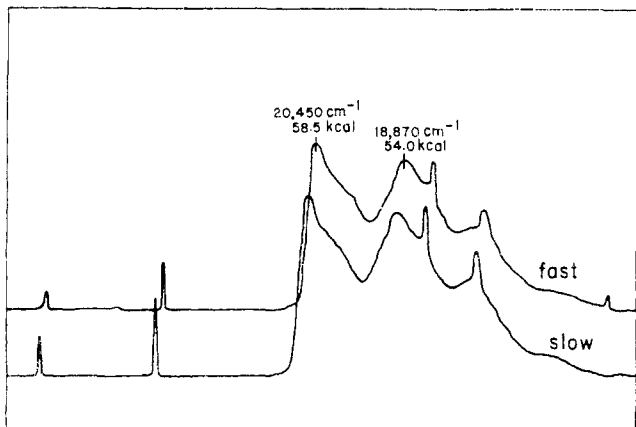


Fig. 1.—Phosphorescence spectrum of β -naphthil in EPA. Results with fast and slow chopping rates are shown. Sharp lines are scattered light from the source.

We have for some time been perplexed by curious features of the phosphorescence spectra of several α -diketones.² For example, the phosphorescence spectrum of benzil in an ether (5 parts)-isopentane (5 parts)-ethanol (2 parts) (EPA) glass at 77°K. is a broad band with a maximum at 20,040 cm^{-1} and a weak shoulder at about 21,200 cm^{-1} .³ However, we find that in a glass prepared from methylcyclohexane (5 parts) and isopentane (1 part) (MCIP) the phosphorescence spectrum shows two well resolved maxima

(1) Mechanisms of Photoreactions in Solution XIV. Part XIII is G. S. Hammond and R. S. H. Liu, *J. Am. Chem. Soc.*, **85**, 477 (1963).

(2) All spectra were taken at 77°K. on Kodak spectroscopic plates by means of a quartz prism spectrograph. Microdensitometer tracings, as shown in Fig. 1, 2, and 3, were recorded directly from the spectroscopic plates. A mercury lamp was used as the incident light source and mercury emission lines (the sharp peaks in the spectra) were used for calibration.

(3) G. N. Lewis and M. Kasha, *J. Am. Chem. Soc.*, **66**, 2100 (1944), reported the 0-0 phosphorescence band of benzil in EPA at 90°K. to be at 21,600 cm^{-1} .

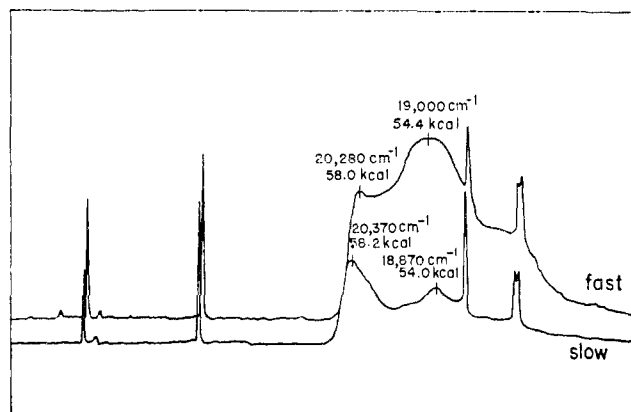


Fig. 2.—Phosphorescence spectrum of β -naphthil in MCIP. Results with fast and slow chopping rates are shown.

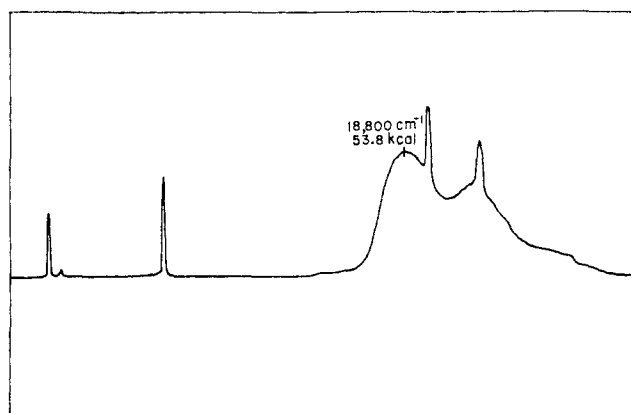
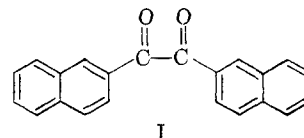


Fig. 3.—Phosphorescence spectrum of β -naphthil in isopentane-3-methylpentane.

at 18,800 and 17,240 cm^{-1} . While we have observed shifts in the phosphorescence spectra of many compounds as the solvent is changed from EPA to MCIP, the apparent shift for benzil is much larger than normal.

The spectrum of β -naphthil (I) in EPA shows clear resolution of three maxima at 20,450, 18,870 and 17,390



cm^{-1} (Fig. 1). The spectrum in MCIP (Fig. 2) also shows three slightly shifted maxima at 20,280, 19,000, and 17,400 cm^{-1} . However, the relative intensities of these bands do not correspond with those observed in EPA. Since a rotating sector was used to separate fluorescence from phosphorescence, it was very easy to change the delay time between excitation and measurement of the emission. As shown in Fig. 1 the rate of rotation of the sector does not affect the spectrum obtained in EPA. However, the relative intensities of the maxima in the MCIP spectrum change drastically when the rate of rotation of the sector is varied. Furthermore, the spectrum obtained in MCIP using a slow sector speed (Fig. 2) is remarkably similar to the spectrum obtained in EPA. It shows maxima at 20,370, 18,870, and 17,330 cm^{-1} . This result seems to indicate quite unequivocally that the emission in MCIP arises from two different triplets having different radiative lifetimes. Control experiments showed that none of the emission arises from the solvent.

While other explanations probably could be advanced to account for the results, we are strongly inclined toward the view that the two emitting species are *cis* and *trans* isomeric triplets of the diketone. Consequently

we assume that there are molecules in the glass having either cisoid or transoid configurations.⁴ Because there should be a large amount of steric strain in the *cis* configuration we were a little surprised by the evidence that both configurations were appreciably populated at 77°K. Since the setting temperature of MCIP is significantly higher than 77°K., it seemed possible that non-equilibrium configurations might have been "frozen in." It has been reported⁵ that a glass formed from isopentane (6 parts) and 3-methylpentane (1 part) has a setting temperature very close to 77°K. Therefore, we prepared a solution of β -naphthil in this glass with the thought that mobility would be maintained at lower temperatures and something closer to configurational equilibrium would be established in this system. As illustrated by Fig. 3, the phosphorescence spectrum of β -naphthil in this system has only two bands at 18,830 and 17,360 cm^{-1} . Probably only *trans* molecules are present in this glass. The experiment in the low setting glass also indicates that the "second" emission from β -naphthil in MCIP is not due to an impurity.

In light of the interpretation offered, we must presume that the emission from EPA solution arises essentially exclusively from *cis* triplets. While this leads to the conclusion that the ratio of *cis* to *trans* ground state configurations is greater in that solvent than in the hydrocarbons, it does not imply that the ratio is necessarily greater than unity. Since the lifetime of the *trans* triplet is obviously much shorter than that of the *cis* isomer, the decay process may have changed the *trans/cis* ratio by a large amount before emission is measured in any of our experiments.

Recently, evidence has been presented⁶ which supports the existence in methanol-methylcyclohexane solutions of two retinene triplets. It was suggested that the "second" triplet is a retinene-methanol complex. In view of our observations with β -naphthil, we would like to propose as an alternative explanation the existence of two stereoisomeric retinene triplets.

Acknowledgment.—We wish to thank Professor G. W. Robinson for the use of a phosphoroscope and wise counsel. This research was supported in part by a grant from the National Science Foundation.

(4) These need not be perfectly planar.

(5) W. J. Potts, Jr., *J. Chem. Phys.*, **21**, 191 (1953).

(6) W. Dawson and E. W. Abrahamson, *Spectrochim. Acta*, **18**, 1366 (1962); *J. Phys. Chem.*, **66**, 2542 (1962).

(7) National Science Foundation Predoctoral Fellow, 1961 to present.

CONTRIBUTION NO. 2661 WILLIAM G. HERKSTROETER⁷
GATES AND CRELLIN LABORATORIES OF CHEMISTRY

CALIFORNIA INSTITUTE OF TECHNOLOGY PASADENA, CALIFORNIA
JACK SALTIEL
GEORGE S. HAMMOND

RECEIVED DECEMBER 7, 1962

SUBSTRATE CONTROL OF CONFORMATION CHARACTERISTICS IN CHYMOTRYPSIN¹

Sir:

In a stimulating series of studies Vaslow and Doherty² found that although the equilibrium constant for the binding of virtual substrates to α -chymotrypsin (CT) was nearly independent of pH, the standard enthalpy and entropy underwent large variation. The occurrence of a significant variation in entropy change compensated by enthalpy changes suggested to them

(1) The contents of this note were presented at the 46th Federation meeting, R. Lumry and H. Parker, *Fed. Proc.*, **21**, 246 (1962), and at the 142nd National Meeting, American Chemical Society, Atlantic City, New Jersey, September, 1962, Abstract 48-C.

(2) D. Doherty and F. Vaslow, *J. Am. Chem. Soc.*, **74**, 931 (1952); F. Vaslow and D. Doherty, *ibid.*, **75**, 928 (1953); F. Vaslow, *Compt. rend. Trav. Lab. Carlsberg, Ser. Chim.*, **31**, 29 (1958).

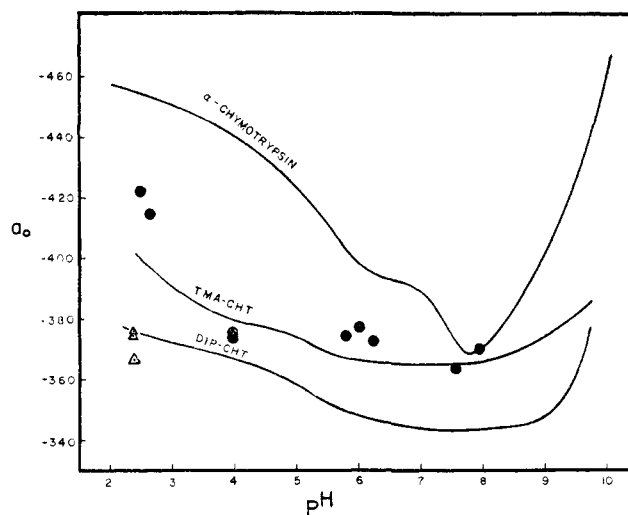


Fig. 1.—The value of the parameter a_0 for α -chymotrypsin and some derivatives. The solid circles are from α -chymotrypsin in the presence of 0.02 *M* hydrocinnamic acid; the open triangles from α -chymotrypsin in the presence of 0.007 *M* acetyl-L-tyrosine ethyl ester.

a modification in protein structure on substrate binding, with a maximal effect at about pH 7.5, the lowest pH of maximum catalytic activity. Schellman and Vaslow³ could find little or no substrate dependence of optical rotation though the experiments were not carried out under optimum conditions. Simpson, Jacobs and Lumry⁴ repeated the experiments of Vaslow and Doherty using trypsin, an enzyme known to be very similar to CT in its catalytic properties. Dialysis-equilibrium studies showed single-site binding with pH and temperature dependencies essentially identical with those reported for CT. Addition of virtual substrates at pH 8 produced no change in viscosity, specific rotation or tryptophan spectrum for trypsin solutions. Subsequently Brandts⁵ observed that the characteristic small substrate of CT, acetyl-L-tyrosine ethyl ester (ATEE) at pH 2 greatly reduced the entropy and enthalpy of the reversible denaturation of chymotrypsinogen. This observation coupled with the report by Rupley, Dreyer and Neurath⁶ of a pH-dependent change in specific rotation of CT lead us to suspect that conformation changes due to bound substrates might occur at low pH values rather than at high. Such was found to be the case in our early studies of the specific acylating reagents: diisopropylfluorophosphate (product DIPCT) and *p*-nitrophenyltrimethylacetate (product TMACT).¹ There were significant changes in protein physical parameters at low pH and also at high (a_0 in Fig. 1 for example) some of which were independently observed by Hess and co-workers.⁷ We have now found that real and virtual substrates produce quite as large changes in optical rotation as do the specific acylating reagents. The a_0 parameter computed from rotatory-dispersion data using the Moffitt equation⁸ is presented as a function of pH at 23° in Fig. 1. Hydrocinnamate ion (HC) is a good competitive inhibitor though a poor virtual substrate. The Moffitt b_0 parameter varies from form to form with pH

(3) Personal communication from F. Vaslow.

(4) J. Simpson and R. Lumry, 139th National Meeting, American Chemical Society, St. Louis, Missouri, March, 1961, Abstract 19-O.

(5) J. Brandts, Dissertation, "The Reversible Thermal Denaturation of Chymotrypsinogen," University of Minnesota, 1961.

(6) J. Rupley, W. Dreyer and H. Neurath, *Biochim. et Biophys. Acta*, **18**, 162 (1955); H. Neurath, J. Rupley and W. Dreyer, *Arch. Biochem. Biophys.*, **65**, 243 (1956).

(7) B. Havsteen, B. Labouesse and J. Mercouroff, *Fed. Proc.*, **21**, 229 (1962); B. Havsteen and G. Hess, *J. Am. Chem. Soc.*, **84**, 491 (1962).

(8) P. Urnes and P. Doty, *Advan. Protein Chem.*, **16**, 401 (1961).