$C_9H_{12}(AgNO_3)_2$: C, 17.16; H, 1.92; Ag, 51.38. Found: C, 17.46; H, 2.06; Ag, 51.40). I was regenerated by treating the silver nitrate adduct with excess ammonia. Sublimation at room temperature (25 mm.) gave white needles (having a high vapor pressure), m.p. 50–51° cor. (*Anal.* Calcd. for C_9H_{12}: C, 89.94; H, 10.06. Found: C, 89.92; H, 10.12). The found molecular weight[8] was 120 (mass spectrometric determination). The conversion of VII to I by a given pyrolysis was 9–14% of the neutral pyrolysate as determined by gas–liquid chromatography (5% Carbowax 1500 on Fluoropak 80, 4 m. × 6 mm., 100° column).[9]

Infrared (vapor phase): strong absorptions at 3.30, 3.36, 3.40, 11.40, 13.85 μ; medium absorptions at 3.47, 5.94, 6.07, 6.72, 6.75, 6.80, 11.36 μ; ultraviolet: λ_{max} = 198 mμ (9,300), $\lambda_{shoulder}$ = 210 mμ (4,600) in cyclohexane at 7.4×10^{-5} mole/l.

Exhaustive hydrogenation of I gave cyclononane whose g.l.c. retention time and infrared spectrum were identical with those of an authentic sample.

Together these data show that the isolated hydrocarbon is a cyclononatriene. The possible isomers (other than I and *cis,cis,trans*-1,3,5-cyclononatriene[10]) that were considered are *cis,cis,cis*-1,3,5-, *cis,cis,cis*-1,3,6- and *cis,cis,trans*-1,4,7-cyclononatrienes. Any isomer containing a *trans* olefin is less plausible because the infrared absorptions (7.6–7.7, 10.4–10.5 μ) attendant with dialkyl *trans* olefins[11] are absent in the spectrum of the new hydrocarbon.

The n.m.r. spectrum[12] (Fig. 1) of the isolated compound provides evidence to assign its structure. The

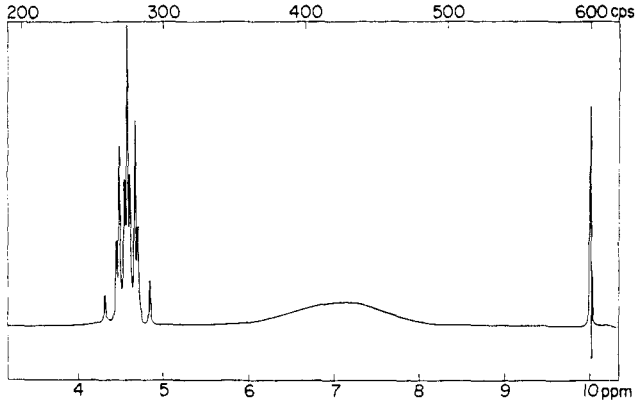

Fig. 1.—N.m.r. spectrum at 37° with tetramethylsilane as the internal reference standard (approx. 10% $CDCl_3$ solution).

symmetrical nonet (4.57 τ) is consistent with structure I wherein all the olefinic hydrogens are equivalent. The ratio of the two peak areas (4.57, 7.1 τ) are 1:1 (within 2%). The other cyclononatrienes are expected to show more complicated olefinic absorption patterns for the chemically, and presumably magnetically, non-equivalent olefinic hydrogens on adjacent double bonds. They should also exhibit more than one kind of methylene hydrogen due to chemical shift differences for doubly allylic, allylic and aliphatic hydrogens (note no absorption beyond a value of 8.2 τ).

cis,cis,cis-1,4,7-Cyclononatriene can assume different stereochemical forms, one being symmetric, I, and another less symmetric arrangement, Ia, which can exist in any of six equivalent conformations. The unusual absorption of the methylene hydrogens (Fig. 1, 7.17 τ) is the result of a dynamic equilibration. The appearance of this peak is extremely temperature dependent. Heating the sample causes the methylene absorption to narrow and sharpen up. Cooling causes the broad absorption to separate into two peaks of equal area which sharpen up with further cooling. Thus there is interconversion between two equivalent symmetrical conformers, I. N.m.r. spectra have been obtained at various temperatures ranging from −40 to 83.5°. These results are reported in a second paper.[13]

A completely rigid structure proof for I has been obtained by proton–proton spin decoupling experiments. These data along with a detailed analysis of the spectra will be reported.

Whether I shows aromatic character or not will be deferred until studies are completed on the "frozen" symmetrical conformer.[14] However, simple LCAO MO calculations have been carried out for I using angle parameters of 120° (sp^2 bonds) and 109°28″ (sp^3 bonds). The result thus obtained is a lower limit for a predicted absolute value for any delocalized energy the molecule might possess since these parameters ignore steric repulsion of the three inner methylene hydrogens. This repulsion is undoubtedly present and would cause the homoallylic overlap to be greater than that calculated. The calculations give a value of 25.0% as much 2,4-overlap as that of 1,2-overlap and predicts negligible (0.02%) delocalization energy with the six pi electrons occupying three delocalized orbitals (degenerate pair at -0.985β and one at -1.03β).

The author gratefully acknowledges the able technical assistance of Mr. D. J. Martin throughout this research and helpful discussions with Drs. A. A. Bothner-By and H. Conroy.

(8) Performed by G. A. Muccini, Mellon Institute, Radiation Research Laboratories, Pittsburgh, Pa.

(9) Performed by C. J. Lindemann, Mellon Institute.

(10) This cyclononatriene has been prepared by K. Alder and H. A. Dortmann, *Ber.*, **87**, 1905 (1954), and its properties differ markedly from those of I.

(11) L. J. Bellamy, "The Infra-red Spectra of Complex Molecules," John Wiley & Sons, Inc., New York, N. Y., 1958, p. 45.

(12) The n.m.r. spectrum was taken with a Varian Associates A-60 spectrometer.

(13) K. G. Untch and R. J. Kurland, *J. Am. Chem. Soc.*, **85**, 346 (1963).

(14) However, the infrared spectrum of V and a ferric chloride test showed no enol form present.

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RECEIVED NOVEMBER 19, 1962

THE CONFORMATIONAL EQUILIBRATION OF *cis,cis,cis*-1,4,7-CYCLONONATRIENE; A DETERMINATION OF ACTIVATION ENERGY AND ENTROPY BY N.M.R. II.

Sir:

The synthesis and properties of *cis,cis,cis*-1,4,7-cyclononatriene (I) have been reported.[1] We wish to present the results of a detailed investigation of the temperature dependence of the n.m.r. spectra of I. This behavior is displayed (Fig. 1) by the n.m.r. spectra taken at three representative temperatures.

As pointed out previously[1] this temperature dependence reveals that the two equivalent forms of the conformation (Ia), hereafter called the "crown," are interconverting. After the interconversion of one crown to its mirror image, the inner and outer methylene hydrogens are exchanged. I may also attain a less symmetrical conformation (Ib), hereafter called the "saddle."

Ia Ib

(1) K. G. Untch, *J. Am. Chem. Soc.*, **85**, 345 (1963).

The low temperature spectra ($<-25°$) correspond to the non-interconverting or "frozen" crown as shown by the following evidence: either methylene multiplet collapses into half an AX quartet when the olefin protons are decoupled[2] and the ratio of the areas under the two well separated patterns is 1:1.

The n.m.r. spectrum for I at a given interconversion rate could, in principle, be calculated according to the density matrix treatment proposed by Alexander.[3] However, this calculation is even more formidable than the complete analysis of the spectrum of the frozen crown. Our interest here is rather in obtaining values for the energy, $\Delta E^{\ddagger}$, and entropy, $\Delta S^{\ddagger}$, of activation for interconversion of I. Since these quantities depend logarithmically on the interconversion rate constant, the error in determining $\Delta E^{\ddagger}$ and $\Delta S^{\ddagger}$ will be considerably less than that in the interconversion frequency, k.

On the basis of sample calculations,[4] it is clear the appearance of the observed spectrum is well represented (for k/π between 25 and 90 c.p.s.) by this simple model: coupling constants and line widths (at $k = 0$) set equal to zero. These simplifying assumptions reduce Alexander's general treatment to one of the simpler cases considered by Gutowsky.[5] The k's can then be obtained explicitly as a function of Δ and δ, where Δ is the frequency separation between the peak maxima (taken after the fine structure washes out and the peaks begin to move together) and where δ is the chemical shift between the inner and outer methylene hydrogens. The equation for the interconversion frequency, k, is

$$k/\pi = \sqrt{(\delta^2 - \Delta^2)/2}$$

A useful check on the validity of the above assumptions is obtained by calculating α, the ratio of the intensity, taken midway between the peak maxima, to the intensity, taken at the peak maxima. The calculated α, given by the expression $1 - \Delta^2/\delta^2$, is compared to the observed values. Pertinent data are presented in Table I.

TABLE I

OBSERVED FREQUENCIES AND INTENSITY RATIOS AND CALCULATED PARAMETERS FROM THE N.M.R. SPECTRUM OF I AT SELECTED TEMPERATURES[a]

t (°C.)	ν_1[b]	$\bar{\nu}$[c]	ν_2[b]	Δ[d]	k/π[e]	α calcd.[f]	α obsd.[f]
−40.0	134.5		225.8	91.3			
−25.2	133.1		224.2	91.1			
− 4.5	133.6		224.2	90.6	8.0	0.02	0.00
8.1	133.1		222.2	89.1	14.1	0.09	0.08
16.8	136.6		219.5	82.9	27.1	0.32	0.19
19.0	139.4		217.9	78.5	33.0	0.45	0.30
25.0	144.3		213.9	69.6	41.8	0.66	0.55
27.0	148.7		208.3	59.6	48.9	0.82	0.74
27.8	151.3		208.1	56.8	50.5	0.85	0.83
31.8		176.0			64.6	1.00	1.00
46.7		175.6					
63.5		176.8					
78.0		177.1					

[a] The n.m.r. spectra were taken on a Varian DP-60 spectrometer; frequencies were measured by the usual side band technique with tetramethylsilane as internal reference; 10% $CDCl_3$ solution. [b] Center of separated methylene peaks from TMS in c.p.s. [c] Center of averaged methylene pattern from TMS in c.p.s. [d] Separation of methylene peak maxima. [e] Interconversion rate/π in c.p.s. [f] Intensity ratio, center to peak maxima.

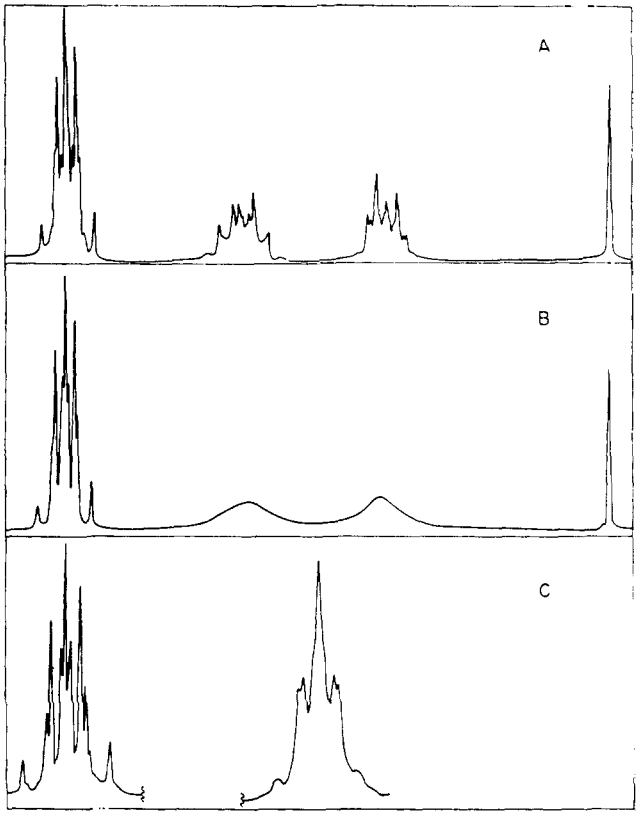

Fig. 1.—N.m.r. spectra of I at: A, 40°; B, 16.8°, C, 83.5°.

The activation energy, $\Delta E^{\ddagger}$, was obtained from a plot of ln k *vs.* $1/T$ over the temperature range −4.5 to 31.8°. The value of $\Delta E^{\ddagger}$ thus obtained is 9.78 kcal./mole.[6] The entropy of activation, $\Delta S^{\ddagger}$, was calculated from the Eyring rate equation[7] and found to be −16.0 ± 0.1 cal./°K./mole. The Helmholtz free energy of activation, $\Delta A^{\ddagger}$, is 14.6 ± 0.03 kcal./mole at 27°. Clearly, in this instance, the value of $\Delta E^{\ddagger}$ would be seriously in error were it equated to that of $\Delta A^{\ddagger}$, calculated at only one temperature under the assumption that $\Delta S^{\ddagger}$ is nearly zero, as has been done by some others for similar determinations.[8]

The crown–crown interconversion of I can be explained most simply by an ammonia-like inversion having a planar transition state. Another mechanism requires a transition state that closely resembles the saddle. This transition state goes to the saddle, which would rapidly equilibrate among six equivalent forms, and then reverts to the crown. Even though molecular models indicate that it would be much easier to go from crown to crown *via* the saddle intermediate than from crown to crown *via* the planar transition state, we favor the latter mechanism primarily on the basis of the large, negative value of ΔS. It may be that the ground state energy of the saddle is higher than the energy of the planar transition state.[9]

We are presently carrying out an analysis of the spectra according to Alexander's general scheme.[3] These results will provide a test of the approximations introduced in the calculations reported here.

(2) Unpublished data, this Laboratory.

(3) S. Alexander, *J. Chem. Phys.*, **37**, 967 (1962).

(4) Carried out for the AX case with chemical shift, $\delta_{AX} = 90$ c.p.s.; k/π taken in steps of $\delta_{AX}/10$ from 0 to δ_{AX}; coupling constant, $J_{AX} = 10, 0, 0$ c.p.s., respectively; line width (at $k = 0$), $\delta\nu = 0, 10, 0$ c.p.s. respectively.

(5) See J. A. Pople, W. G. Schneider and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, Chapter 10.

(6) The root-mean-square deviation of the plotted points from the straight line given by the equation, $\ln k = \Delta E^{\ddagger}/RT +$ constant, is 0.03 kcal./mole.

(7) $k = \kappa(k_B T/h) \exp(\Delta S^{\ddagger}/R - \Delta E^{\ddagger}/RT)$; κ assumed to be unity.

(8) *E.g.*, F. R. Jensen, D. S. Noyce, C. H. Sederholm and A. J. Berlin, *J. Am. Chem. Soc.*, **82**, 1256 (1960).

(9) A very large steric repulsion of the unique methylene hydrogen (easily seen from molecular models) which would be nearly embedded in the opposite double bond pi orbitals could give the saddle such an energy.

R. J. K. gratefully acknowledges the support of a National Science Foundation grant.

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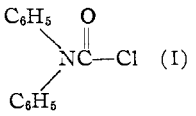
RECEIVED NOVEMBER 19, 1962

SPECIFIC INACTIVATION OF CHYMOTRYPSIN BY DIPHENYLCARBAMYL CHLORIDE

Sir:

We wish to report the inactivation of α-chymotrypsin by a reagent of high specificity: diphenylcarbamyl chloride (I). The inactivation, which occurs as a



result of a mole for mole reaction between I and the enzyme, is retarded by indole and can be reversed by nucleophilic agents.

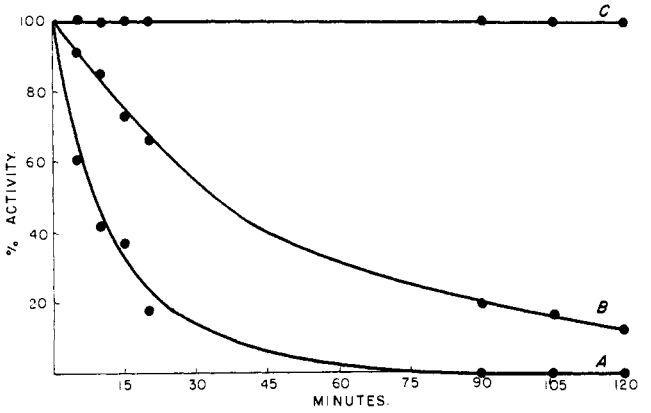


Fig. 1.—Effect of indole on the inhibition of chymotrypsin by diphenylcarbamyl chloride at pH 7.0, 25°. The reaction mixtures contained 1×10^{-6} M chymotrypsin, 2×10^{-6} M diphenylcarbamyl chloride, 9.5×10^{-3} M tris-(hydroxymethyl)-aminomethane–maleic acid–calcium chloride, 5% methanol. Curve A: in the absence of indole (the points are superimposed on a curve calculated from the second order rate constant); curve B, 2×10^{-3} M indole; curve C, 1×10^{-2} M indole.

In the experiments to be described, chymotryptic activity was determined using acetyl DL-phenylalanine β-naphthyl ester according to the procedure previously described[1]; trypsin assays used the chromogenic substrate benzoyl DL-arginine p-nitroanilide hydrochloride.[2]

Curve A of Fig. 1 shows the progress of the inactivation of a 10^{-6} M solution of α-chymotrypsin by a 2×10^{-6} M concentration of I (Distillation Products Industries, recrystallized from ethanol; m.p. 85°; lit.[3] 85°) at pH 7.0, 25°. The interaction under all conditions studied followed second order kinetics, its rate constant at pH 7.0 being shown in Table I. Also included in Table I are the rate constants for the inactivation of α-chymotrypsin by two other reagents, diisopropyl fluorophosphate(DFP)[4] and α-N-p-toluenesulfonyl-β-phenylalanylbromomethane (TBPK), the latter having been reported recently by Schoellmann and Shaw.[5] Even after making allowances for differences in the experimental conditions, I appears to be the most reactive of the three.

TABLE I

SPECIFIC RATE CONSTANTS OF INACTIVATION[a]

Inactivator	Chymotrypsin	Trypsin
I[b]	610	8.2
DFP[c]	317	...
TPBK[d]	2.37[e]	...

[a] l. mole^{-1} sec.$^{-1}$. [b] 0.01 M Tris-maleate, $CaCl_2$; 0.04 percentage acetone; pH 7.0; 25°. [c] 0.1 M phosphate, pH 7.7, 25°. [d] 0.1 M phosphate pH 6.0, 37°. [e] This value was calculated using the data for 100 minutes exposure as presented in Table I of ref. 5.

The inactivation of chymotrypsin by I was inhibited by indole as shown in curves B and C of Fig. I. Since indole is a competitive inhibitor of chymotrypsin,[6] its effect upon the inactivation process is good evidence for the reaction of I with a segment of the active center of α-chymotrypsin.

I was not reactive toward chymotrypsinogen, diethylphosphorylchymotrypsin or pepsin, as shown by subsequent addition of α-chymotrypsin and assay for chymotryptic activity after a suitable incubation period; controls lacking I were run simultaneously. In the case of chymotrypsinogen, following exposure to I, activation by trypsin was carried out as well. The formation of active chymotrypsin occurred exactly as with a chymotrypsinogen control.

I was capable of inactivating trypsin but at a rate that was approximately one-eightieth that of the inactivation of chymotrypsin (see Table I). The inhibition of trypsin was not an unexpected finding since, for example, acetyl L-tyrosine ethyl ester, a specific substrate of chymotrypsin, is also hydrolyzed by trypsin, the Km for the latter reaction at pH 8.0, 25°, being thirteen times that of the reaction with chymotrypsin,[7] I therefore exhibits a considerably higher specificity for chymotrypsin relative to trypsin than does acetyl-L-tyrosine ethyl ester and should prove to be useful for the inactivation of the small quantities of chymotrypsin always present in preparations of crystalline trypsin. The inhibition of trypsin by I is, incidently, additional evidence for the presence of a ring binding site at the active center of this enzyme, as was previously indicated by reactivation studies on diethylphosphoryltrypsin.[8]

Trypsinogen and diethylphosphoryl (DEP) trypsin are not affected by I.

The failure of I to react with DEP-chymotrypsin and DEP-trypsin may indicate, but certainly does not prove, that I is specific for the reactive serine present at the active centers of the esterases. This aspect is now under investigation. However, like the organophosphorus-inhibited enzymes in which the participation of the serine residue has been proven,[9] diphenylcarbamyl (DPC) chymotrypsin and DPC-trypsin can be completely reactivated by nucleophilic reagents. Chymotrypsin or trypsin (each at a concentration of 4×10^{-5} M) was exposed at 25° to an equimolar concentration of I in 0.1 M Tris buffer, pH 8.0, containing 0.1 M $CaCl_2$. After 30 minutes and 90 minutes, respectively, more than 99% inactivation of chymotrypsin and trypsin had occurred. To one part of the enzyme solution was added four parts of M isonitrosoacetone in the above buffer (final reactivator concentration, 0.8 M). Fifty per cent. reactivation of DPC-chymotrypsin occurred in 28.5 minutes; similar reactivation of DPC-trypsin required 63 minutes. After 18 hours both preparations showed complete reactivation.[10]

(1) W. Cohen and B. F. Erlanger, *J. Am. Chem. Soc.*, **82**, 3928 (1960); also correction, 6431 (1960).
(2) B. F. Erlanger, N. Kokowsky and W. Cohen, *Arch. Biochem. Biophys.*, **95**, 271 (1961).
(3) H. Erdmann and P. Huth, *J. prakt. Chem.*, [II] **56**, 7 (1897).
(4) A. J. J. Ooms, *Nature*, **190**, 533 (1961).
(5) G. Schoellmann and E. Shaw, *Biochem. Biophys. Res. Commun.*, **7**, 36 (1962).
(6) H. T. Huang and C. Niemann, *J. Am. Chem. Soc.*, **75**, 1395 (1953).
(7) T. Inagami and J. M. Sturtevant, *J. Biol. Chem.*, **235**, 1019 (1960).
(8) W. Cohen, M. Lache and B. F. Erlanger, *Biochem.*, **1**, 686 (1962).
(9) N. K. Schaffer, S. C. May, Jr., and W. H. Summerson, *J. Biol. Chem.*, **202**, 67 (1953).