effect should be pH dependent. However, it is noteworthy that within experimental error the observed isotope effect is invariant with pH in a range where the maximum velocity of the decarboxylation increases, goes through a maximum, and decreases again.[11a]

The magnitude of the carbon isotope effect on the enzymatic decarboxylation indicates clearly that decarboxylation is at least partially rate determining. In this case it is possible to estimate the value of k_3^{12}/k_3^{13} by a different method. The rate of carbonyl oxygen exchange of acetoacetic acid catalyzed by acetoacetate decarboxylase has been measured by Hamilton.[21] At pH 6.5 the ratio of the rates of decarboxylation and exchange, k_d/k_e, is approximately unity. This partitioning is related to eq 1 by eq 7. The term on the right side of

$$\frac{k_d}{k_e} = \frac{k_3}{k_{-2}}\left(1 + \frac{k_2}{k_{-1}}\right) \qquad (7)$$

eq 7 appears also in eq 5, and enables us to calculate that k_3^{12}/k_3^{13} is about 1.034. However, the uncertainty in the oxygen exchange measurement is on the order of ± 0.5, so the uncertainty in k_3^{12}/k_3^{13} is large. Thus the isotope effect on the decarboxylation step may be slightly smaller than that observed in the amine-catalyzed decarboxylation.

The decarboxylation of acetoacetic acid catalyzed by acetoacetate decarboxylase is more than a 1000-fold faster than that catalyzed by aminoacetonitrile.[5] In both of these reactions the decarboxylation step is partially, but not entirely, rate limiting. The enzyme apparently accelerates the decarboxylation step to very nearly the same extent as it accelerates the other steps. The similarity of the isotope effects obtained in the two cases makes it likely that the transition states have similar structures in the two cases. Since the starting states are the same (free acetoacetate) and therefore have the same energy, it follows that the energy of the transition state for decarboxylation has been changed by a considerable amount without any large change in structure. The means by which this is accomplished is not clear, but one attractive possibility is the polarity effect demonstrated by Crosby, *et al.*, for a closely related decarboxylation.[22]

(21) G. A. Hamilton, Ph.D. Dissertation, Harvard University, Cambridge, Mass., 1959.

Experimental Section

Materials. Acetoacetate decarboxylase isolated from *Cl. acetobutylicum* was kindly provided by Professor F. H. Westheimer and J. V. Connors.[23] Aminoacetonitrile sulfate was recrystallized three times from ethanol–water. Lithium acetoacetate was prepared as described by Hall.[24] Doubly distilled deionized water was used for all buffers. The following buffers were used: pH 3.58, sodium chloroacetate; pH 4.07 and 5.02, sodium acetate; pH 5.31, pyridinium sulfate; pH 6.00, sodium phosphate; pH 7.18, *N*-ethylmorpholine sulfate.

Kinetic Measurements. All kinetic measurements were carried out on a Cary 15 spectrophotometer at 270 nm at 30°. The absorbance change corresponding to 100% reaction was measured by allowing solutions of acetoacetate with either enzyme or amine present to decarboxylate for at least ten half-lives.

Isotope Effect Measurements. These procedures have been described previously.[12] All solutions were degassed by bubbling prepurified CO_2-free N_2 through them for at least 30 min. The enzyme was purified by chromatography on Sephadex G-25 using degassed buffer immediately before use. Reactions were quenched by the addition of sufficient H_2SO_4 to lower the pH to 1.0, and the flasks were immediately frozen to prevent any trace of acid-catalyzed decarboxylation. Approximately 0.01–0.02 *M* acetoacetate was used for all measurements. The aminoacetonitrile concentration was 0.15–0.4 *M*.

The decade settings given in Tables I and II are appreciably different from the actual isotope ratios for *m/e* 45:44 because of the design of the electronics in the isotope-ratio mass spectrometer. As discussed by Nier,[25] the observed ratios are directly proportional to the actual abundances and can thus be used directly in the calculation of isotope effects.

Acknowledgment. We are grateful to Professor F. H. Westheimer for providing us with acetoacetate decarboxylase and for permission to quote unpublished results. We are also grateful to Dr. J. Peter Guthrie and Dr. W. W. Cleland for many helpful discussions.

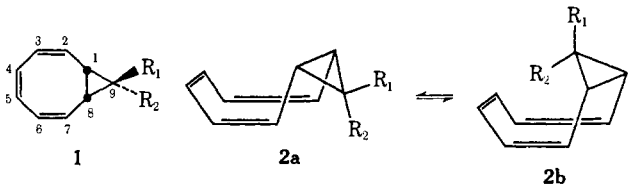
(22) J. Crosby, R. Stone, and G. E. Lienhard, *J. Amer. Chem. Soc.*, **92**, 2891 (1970).
(23) F. H. Westheimer, *Methods Enzymol.*, **14**, 231 (1969).
(24) L. M. Hall, *Biochem. Prepn.*, **10**, 1 (1963).
(25) A. O. Nier, *Phys. Rev.*, 77, 789 (1950).

Communications to the Editor

The Stereochemistry of Uniparticulate Electrophilic Additions to *cis*-Bicyclo[6.1.0]nonatrienes[1]

Sir:

The *cis*-bicyclo[6.1.0]nonatriene system **1** is an intriguing chemical entity due chiefly to: (a) its possible conformational flexibility which interchanges in a very fundamental way the spatial relationship of the cyclopropane ring to the nonplanar triene unit (*cf.* **2a** and **2b**); (b) the distinctly different alignment of $p\pi$ and cyclopropane orbitals particular to these individual conformations; and (c) the latent potential of **1** for a variety of thermal and photochemical pericyclic changes. The advent of orbital symmetry theory has caused considerable attention to be focused currently on bicyclo-

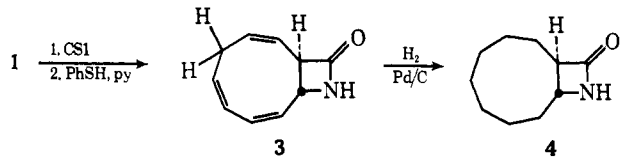


[6.1.0]nonatriene rearrangements in an attempt to unravel which of the many alternative pathways open to these polyenes is actually operative under a variety of conditions.[2] By contrast, electrophilic additions

(1) Unsaturated Heterocyclic Systems. LXXXVI. For paper LXXXV, see L. A. Paquette, L. B. Anderson, J. F. Hansen, S. A. Lang, Jr., and H. Berk, *J. Amer. Chem. Soc.*, in press.

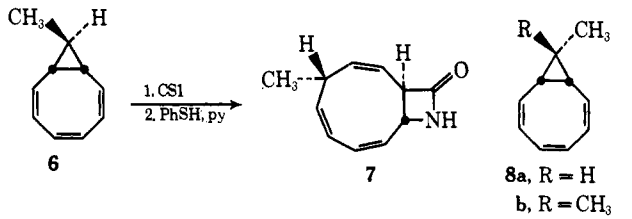
to **1** have been little evaluated.[3,4] We now detail the results of key experiments, involving use of the highly reactive uniparticulate electrophile[5] chlorosulfonyl isocyanate (CSI), which provide definitive information about the conformational and stereochemical consequences of electrophilic additions to **1**.

Admixture of equimolar quantities of **1** and CSI in methylene chloride solution at 25° [6] gave rise during 35–40 hr to a lone *N*-chlorosulfonyl β-lactam, mp 117–118° (60%; $\nu_{max}^{CHCl_3}$ 1825 cm^{-1}). Reduction of this product with thiophenol and pyridine in acetone at 0° led quantitatively to β-lactam **3**: mp 103–103.5°;[7]



3 4

$\nu_{max}^{CHCl_3}$ 1750 cm^{-1}; $\lambda_{max}^{C_2H_5OH}$ 224 nm (ε 6850). These data, in conjunction with the nmr spectrum, attest to the absence of a cyclopropane ring in **3**. The structural assignment, particularly the trans ring fusion, was substantiated by catalytic hydrogenation of **3** to **4**, mp 62.5–64°.[6] which was prepared independently by treatment of *trans*-cyclononene (**5**) with CSI and subsequent dechlorosulfonylation. To gain additional stereochemical insight into this reaction, the behavior of the 9-methyl derivatives of **1** was next examined. Treatment of **6** with CSI as before led to the isolation of



6 7 8a, R = H b, R = CH_3

a single β-lactam (**7**): mp 123.5–124.5°;[7] $\nu_{max}^{CHCl_3}$ 1750 cm^{-1}; $\lambda_{max}^{C_2H_5OH}$ 240 nm (ε 4230). Although the nmr spectrum of **7** was similar in many respects to that of **3**, no definitive stereochemical information could be derived therefrom. Accordingly, a single-crystal X-ray analysis was performed. The final X-ray model (Figure 1) clearly reveals the trans fusion of the four- and nine-membered rings; the dihedral angle of the butadiene portion is 42° and the dihedral angles of all the double bonds are 180 ± 2°. Full details relating to all distances and angles, which agree well with generally accepted values, will be given later.

Meaningfully, the syn 9-methyl isomer (**8a**) did not react with CSI at the reflux temperature of CH_2Cl_2 after 72 hr (70–80% recovery of **8a**). A similar lack of reactivity was noted with the 9,9-dimethyl derivative (**8b**, 65–70% recovery).

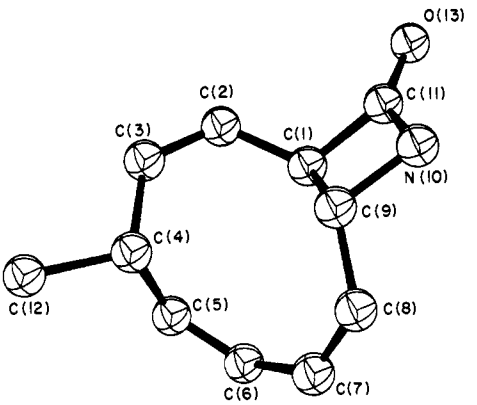
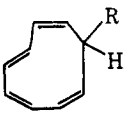


Figure 1. A structural view of **7** as determined by X-ray analysis showing the conformation of the molecule.

In terms of mechanism, the trans ring fusion in adducts **3** and **7** could be viewed as arising from ($_{\pi}2_s$ + $_{\pi}2_s$) cycloaddition of CSI to the trans double bond of the respective 1,3,5,7-*cis*^{2},*trans*,*cis*-cyclononatetraene (**9**).[2b] Such a conclusion would seem warranted on the basis of the well-established ease with which **1** and **6** (but not **8a** and **8b**) can attain the folded tub conformation **2b**, an apparent prerequisite for valence isomerism to **9**.[2b,8] However, the uniqueness of positional attack and high level of stereoselectivity encountered in the formation of these β-lactams does not appear entirely reconcilable with this mechanistic proposal. If **9** were involved, **1** would be expected



9

to give rise to two isomeric *trans*-β-lactams;[2b] similarly, **6** with its additional methyl group should reasonably serve as the precursor to four such adducts. In the several examples studied to date, no more than one β-lactam has been isolated from a given anti 9-substituted bicyclo[6.1.0]nonatriene.[9]

Rather, we advance for consideration the alternative rational possibility that electrophilic additions to *cis*-bicyclononatrienes occur *via* transient dipolar 1,3-bishomotropylium ions. In brief, the capability of these polyenes for participation in these reactions appears also to be governed by the innate ability of the structure to attain folded conformation **2b**. This is because initial bonding of an electrophile to C_3 of **2b** results in nearly perfect alignment of the vacant p orbital at C_2 with the internal cyclopropyl bond (*cf.* **10**).[10] Conversely, similar attack on conformation **2a** produces a carbonium ion **11**, the vacant orbital of which bisects the three-membered ring[10] thereby en-

(2) (a) J. C. Barborak, T.-M. Su, and P. v. R. Schleyer, *J. Amer. Chem. Soc.*, **93**, 279 (1971); (b) A. C. Anastassiou and R. C. Griffith, *ibid.*, **93**, 3083 (1771), and pertinent references cited in these papers.

(3) P. Warner and S. Winstein, *ibid.*, **93**, 1284 (1971).

(4) (a) W. H. Okamura and T. W. Osborn, *ibid.*, **92**, 1061 (1970); (b) C. S. Baxter and P. J. Garratt, *ibid.*, **92**, 1062 (1970).

(5) L. A. Paquette, G. R. Allen, Jr., and M. J. Broadhurst, *ibid.*, **93**, 4503 (1971).

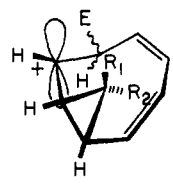
(6) Conditions under which all the *cis*-bicyclo[6.1.0]nonatrienes examined are otherwise stable.

(7) Satisfactory elemental analyses were obtained for all new compounds.

(8) (a) A. G. Anastassiou and R. C. Griffith, *Chem. Commun.*, 1301 (1971); (b) P. Radlick and W. Fenical, *J. Amer. Chem. Soc.*, **91**, 1560 (1969).

(9) The yields in these cycloadditions customarily range from 45 to 60%. In principle, therefore, other β-lactams could have escaped isolation. In practice, we have carefully examined (nmr and ir) the viscous syrupy by-product in each instance and have found no evidence for the presence of additional adducts of monomeric structure.

(10) These conclusions have been derived from various types of molecular models in which the proton at C_2 has been perfectly staggered between the substituents (E and H) at C_3.



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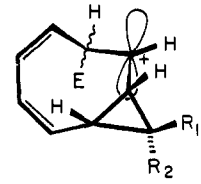

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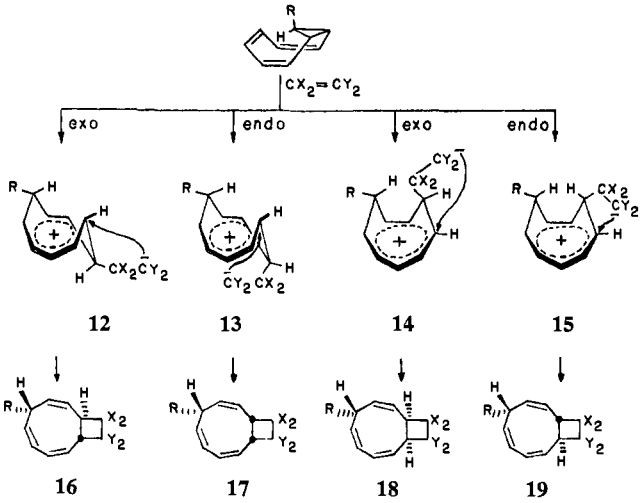
countering destabilization of the cationic center.[11] In this frame of reference, then, **8a**, **8b**, and other syn 9-substituted derivatives of **1** are expected to react slowly, if at all, in the midst of discriminating electrophiles.

Addition as depicted in **10** can ultimately lead with a small readjustment of bond angles to 1,3-bishomotropylium ion intermediates. In view of the established "aromatic" nature of these entities,[3] a driving force in this direction is certainly present. The results with **6** are now convincingly and perhaps uniquely accommodated by that pathway from among the four mechanistic options (Scheme I) in which exo bonding of CSI

Scheme I

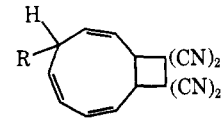


12 13 14 15

16 17 18 19

to C_3 occurs initially, perhaps for steric reasons. The stereochemical features of **7** necessitate further that this interesting cationic intermediate possess trans-disposed methylene bridges as in **12**.[12] Collapse of this zwitterion with C–N bond formation leads ultimately to trans-fused heterobicyclo[7.2.0]undecatriene **16**.

Such reactions appear to be entirely general. For example, tetracyanoethylene condenses with **1** and **6** in tetrahydrofuran solution at *ambient temperature*[6] to furnish uniquely the adducts **20a**, mp 140.5–141°, $\lambda_{max}^{C_2H_5OH}$ 225 nm (ϵ 7250),[4] and **20b**, mp 152–153°,[6] $\lambda_{max}^{C_2H_5OH}$ 231 nm (ϵ 11,240), respectively. Trienes **8a** and **8b** are again unreactive to this reagent under the conditions employed. In keeping with the mechanistic scheme outlined herein, both **20a** and **20b** should be trans fused and not of cis stereochemistry.[2b] X-Ray crystal-structure analyses of these substances are in progress.



20a, R = H
b, R = CH_3

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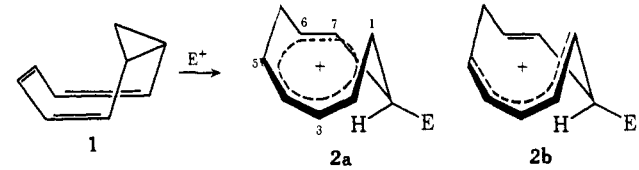
Substituent Effects on the Nonconcerted Cycloaddition of Chlorosulfonyl Isocyanate to *cis*-Bicyclo[6.1.0]nonatriene Derivatives[1]

Sir:

Available experimental evidence indicates that *cis*-bicyclo[6.1.0]nonatriene (**1**) and its anti 9-substituted derivatives undergo impressively stereocontrolled electrophilic additions.[2] As noted in the preceding paper,[2] electrophilic attack at C_3 of the syn conformer of **1** should be kinetically favored, for this process uniquely gives rise to a delocalized cation **2a** which initially must necessarily have a trans orientation. However, because **2a** is a double Möbius bishomotropylium ion, the 6,7 double bond requires significant twisting to achieve satisfactory overlap. As a result, the question of actual charge delocalization in **2a** gains significance and the possible alternative involvement of the noninteracting structure **2b** with a simple pentadienyl unit and



1 2a 2b

an isolated double bond must be considered. In an effort to understand more completely the factors which govern the intriguing reactivity of such polyenes, we have examined the cycloaddition of chlorosulfonyl isocyanate (CSI) to several derivatives of **1** bearing substituents on the medium ring.

Addition of dichloromethane to a liquid ammonia solution of methylcyclooctatetraenyl dianion proceeded to give all four possible methyl-*cis*-bicyclo[6.1.0]-nonatrienes (**3**–**6**). These were separated and purified by preparative scale vpc (15% PPGA on Chromosorb P at 65°) and assigned individual structures on the basis

(11) (a) J. C. Martin and B. R. Ree, *J. Amer. Chem. Soc.*, **91**, 5882 (1969); **92**, 1660 (1970); (b) P. Schleyer and V. Buss, *ibid.*, **91**, 5880 (1960); V. Buss, R. Gleiter, and P. von R. Schleyer, *ibid.*, **93**, 3927 (1971).

(12) Warner and Winstein[3] have examined the protonation of **1** and have concluded that the resulting 1,3-bishomotropylium ion is cis disposed. It remains to determine if the same cations are involved in the two studies (work in progress).

(1) Unsaturated Heterocyclic Systems. LXXXVII. The preceding paper in this series is given in ref 2.

(2) L. A. Paquette, M. J. Broadhurst, C. Lee, and J. Clardy, *J. Amer. Chem. Soc.*, **94**, 630 (1972).