tailed study (including medium effects) will be given at a later date.[8]

(8) Note Added in Proof. Recent pH-metric studies lead to dissociation rates k_{-1} of the order of 10^{-5} and 10^{-4} sec^{-1} for the barium and strontium cryptates, respectively, and to log K_s values of the order of 10 for these complexes. More accurate values will be given in the final account of this work.

J. M. Lehn, J. P. Sauvage, B. Dietrich
Institut de Chimie, 1 rue Blaise Pascal
Strasbourg, France

Received January 26, 1970

On the Volume of Diels–Alder Transition States

Sir:

We wish to report the first case of a bimolecular reaction for which the transition state is smaller than the product. The Diels–Alder additions of three different dienes to maleic anhydride have activation volumes which are significantly more negative than the volume changes on reaction.

Several authors[1] have previously measured activation volumes for Diels–Alder reactions, but there has been considerable controversy concerning the accuracy of these data.[2] In particular, the data for the dimerization of isoprene have been interpreted to support either a two-step diradical mechanism[1b] or a concerted multicenter molecular mechanism[2a] for the Diels–Alder reaction. This situation has led to skepticism regarding any interpretation of these data in recent reviews on the mechanism of the Diels–Alder reaction.[3]

The new data for activation volumes come from precise determinations of reaction rate constants at elevated pressures, using an improved technique involving *in situ* mixing to avoid errors due to heat of compression. This technique[4] should yield rates appreciably more accurate than those which could be achieved in previous high-pressure kinetic studies of Diels–Alder reactions. Reactant and product volumes were measured as partial molal volumes by an accurate dilatometric method, similar to that used by McCabe, *et al.*[5]

The reactions studied included maleic anhydride with isoprene, with 1,3-cyclohexadiene, and with *trans*-1-methoxy-1,3-butadiene, and dimethyl acetylenedicarboxylate with cyclopentadiene. Rate constants based on mole fractions were measured at 50, 2500, 5000, 7500, 10,000, 15,000, and 20,000 psi. Initial reactant concentrations were approximately 0.01 mol fraction. Activation volumes at zero pressure were determined from a least-squares fit to the equation $\ln k = a + bP + cP^2$ and from the relationship

$$\left(\frac{\partial \ln k}{\partial P}\right)_{T,P=0} = -\frac{\Delta\bar{v}_0^{\ddagger}}{RT} = b$$

which is rigorously correct only when rate constants are based on pressure-independent concentration units.[6] The activation volumes and the differences in partial molal volumes between reactants and products are listed in Table I.[7]

Comparison of the activation volumes and volume changes on reaction indicates that the transition state is smaller than the product for the three maleic anhydride reactions. Measurements of activation volumes and volume changes in eight additional solvents for the maleic anhydride–isoprene reaction showed that the transition state was consistently smaller than the product, indicating that neither electrostriction nor specific solute–solvent interactions were responsible for the effect.

Table I. Comparison of Activation Volumes with the Volume Change on Reaction for Several Diels–Alder Reactions

Reaction	Solvent	Temp, °C	Actn vol, $\Delta\bar{v}_0^{\ddagger}$, cc/g mol	Vol change on reactn,[a] cc/g mol
Maleic anhydride–isoprene	Acetone	35	−39.0 ± 0.8	−35.9 ± 0.9
Maleic anhydride–*trans*-1-methoxy-1,3-butadiene	1,2-Dichloroethane	35	−43.9 ± 2.0	−30.4 ± 0.9
Maleic anhydride–1,3-cyclohexadiene	Dichloromethane	35	−39.6 ± 0.8	−30.3 ± 0.9
Dimethyl acetylenedicarboxylate–cyclopentadiene	Ethyl acetate	10	−30.2 ± 0.7	−33.8 ± 0.8

[a] $\bar{v}^{\infty}$(product) − $\bar{v}^{\infty}$(diene) − $\bar{v}^{\infty}$(dienophile).

If the diene and maleic anhydride are coplanar in the transition state, some increase in volume might be expected after the new bonds are completely formed, due to conformational changes in the product. A similar difference in position of the anhydride entity is found between the *endo* and *exo* adducts of maleic anhydride and cyclopentadiene. The *exo* adduct was prepared by the method of Craig[8] and the partial molal volumes of the *endo* and *exo* adducts were measured in nitromethane and dichloromethane. The partial molal volumes at infinite dilution are shown in Table II. The volume difference between the two is an order of

---

(1) (a) B. Raistrick, R. H. Sapiro, and D. M. Newitt, *J. Chem. Soc.*, 1761 (1939); (b) C. Walling and J. Peisach, *J. Amer. Chem. Soc.*, **80**, 5819 (1958); (c) C. Walling and H. J. Schugar, *ibid.*, **85**, 607 (1963).

(2) (a) S. W. Benson and J. A. Berson, *ibid.*, **84**, 152 (1962); (b) C. Walling and D. T. Tanner, *ibid.*, **85**, 612 (1963); (c) S. W. Benson and J. A. Berson, *ibid.*, **86**, 259 (1964).

(3) (a) J. Sauer, *Angew. Chem.*, **79**, 76 (1967); (b) S. Seltzer, *Advan. Alicyclic Chem.*, **2**, 1 (1968).

(4) R. A. Grieger and C. A. Eckert, *AIChE J.*, in press.

(5) J. R. McCabe, R. A. Grieger, and C. A. Eckert, *Ind. Eng. Chem. Fundamentals*, **9**, 156 (1970).

(6) W. J. LeNoble, *Progr. Phys. Org. Chem.*, **5**, 207 (1967).

(7) As a check on these results, the alternate scheme of Benson and Berson[2a] for getting activation volumes from a modification of the Tait equation was applied. Because of a numerical approximation in the development, this method is applicable only to points taken at pressures above about 2000 atm. The rate constants have been measured to 6000 atm for the maleic anhydride–isoprene reaction and for the dimethyl acetylenedicarboxylate–cyclopentadiene reaction, and the activation volumes found from the high-pressure points by this method are within experimental error of the activation volume from the quadratic fit of the low-pressure points.

(8) D. Craig, *J. Amer. Chem. Soc.*, **73**, 4889 (1951).

magnitude too small to account for the volume difference between the transition states and products of the maleic anhydride reactions.

Table II. Partial Molal Volumes of *endo*- and *exo*-5-Norbornene-2,3-dicarboxylic Anhydride at Infinite Dilution

	Solvent	
Adduct	Nitromethane	Dichloromethane
endo, cc/g mol	126.9 ± 0.4	123.7 ± 0.4
exo, cc/g mol	127.4 ± 0.4	124.1 ± 0.4

According to Hoffmann and Woodward,[9] the general rule for preferential formation of *endo* Diels–Alder adducts can be explained by secondary π-electron interactions at nonbonding atoms. Such a concept may also explain the abnormally small volumes of the transition states for the maleic anhydride reactions. For the acetylenic dienophile, with which secondary interactions cannot occur, the transition state is somewhat *larger* than the product, as expected.

In contrast with previous high-pressure studies, the results support a concerted four-center mechanism for the reactions examined.

Acknowledgment. The authors gratefully acknowledge the financial support of the U. S. Army Research Office—Durham. They also thank Professor Peter Beak for helpful discussion.

---

[9] R. Hoffmann and R. B. Woodward, *J. Amer. Chem. Soc.*, **87**, 4388 (1965).

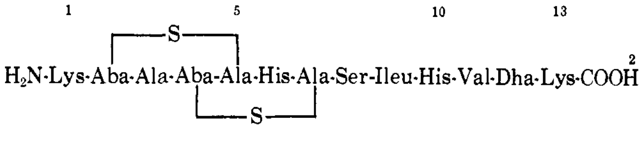
R. A. Grieger, C. A. Eckert
Department of Chemistry and Chemical Engineering
University of Illinois, Urbana, Illinois
Received February 14, 1970

Nisin. The Assignment of Sulfide Bridges of β-Methyllanthionine to a Novel Bicyclic Structure of Identical Ring Size

Sir:

The presence of the rarely encountered amino acids lanthionine, β-methyllanthionine, dehydroalanine, and β-methyldehydroalanine in the antibiotic nisin[1] required a number of approaches new to the structural elucidation of peptides. Sulfide bridges of β-methyllanthionine had hitherto not been determined in peptides and, previously, it had not been necessary to distinguish between genuine alanine residues and those resulting from the desulfurization of β-methyllanthionine.

The antibiotically active tridecapeptide of the following structure was isolated from nisin after cleavage with cyanogen bromide.



H_2N-Lys-Aba-Ala-Aba-Ala-His-Ala-Ser-Ileu-His-Val-Dha-Lys-COOH

The peptide was degraded to the greatest possible extent prior to chemical modification of the sulfide bridges. Desulfurization was a prerequisite for the determination of the linear sequence. Conversion of the peptide to the tetrasulfonate and selective cleavage of the aminoacyl bond of serine were critical factors in the assignment of the sulfide bridges.

Chymotryptic digestion of the tridecapeptide liberated the tripeptide Val-Dha-Lys. Aminopeptidase cleaved the valyldehydroalanine bond and caused the release of pyruvyllysine.[3]

Carboxypeptidase A removed histidine and isoleucine from the decapeptide at nearly equal rates. The indication that histidine occupied the terminal position was confirmed by the tritium labeling technique.[4]

Edman degradation of the octapeptide removed lysine in the first cycle. Differential amino acid analysis of the peptide from the second cycle showed that none of the monoamino acids had been removed. It demonstrated, however, that one residue of β-methyllanthionine had been destroyed, presumably *via* β elimination since cysteine and cystine were present in the hydrolysate. The amino acid analysis after the third cycle did not indicate any additional change. The phenylthiocarbamyl derivative of the β-methyllanthionine residue will only cyclize, and subsequently decompose, under the more stringent conditions of hydrolysis.

Desulfurization of the octapeptide with Raney nickel W-6[5] was followed by five cycles of stepwise degradation providing the H_2N-terminal pentapeptide sequence. The positions of the remaining amino acids were established by a time study of their release by carboxypeptidase A.

The **original alanine residue** was assigned to position 3 following Edman degradation of the tetrasulfonic acid derivative of the undecapeptide amide. This amide is formed as the result of the loss of the terminal dipeptide due to the oxidation and decomposition of dehydroalanine during the preparation of the tetrasulfonate.

The tridecapeptide was oxidized with performic acid to form the bissulfone. Treatment of the bissulfone with 1 *N* sodium bisulfite solution for 7 hr at 105° resulted in β elimination and the addition of bisulfite to the newly formed α,β-unsaturated amino acids. Performic acid treatment converted the sulfinic acids to sulfonic acids.

The heptapeptide comprising residues 2–8 was esterified by treatment with 2.5 *N* methanolic hydrogen chloride for a period of 28 hr at room temperature. Complete release of serine methyl ester was demonstrated by amino acid analysis. The selective removal of the serine residue presumably proceeds *via* N→O acyl shift and transesterification. The resulting methyl ester was reduced to the alcohol by treatment with sodium borohydride in 0.5 *N* trisacetate buffer (pH 8.5) at 0° for 23 hr.

The peptide alcohol was allowed to react with phenylisothiocyanate and the addition product was hydrolyzed. The hydrolysate contained one residue each of alanine, histidine, and β-methyllanthioninol. In agreement with the earlier observation made during the attempted stepwise degradation of residue 2, the β-methyllanthionine residue was destroyed during hydrolysis. The absence of β-methyllanthionine from the

---

(1) E. Gross and J. L. Morell, *FEBS Lett.*, **2**, 61 (1968).

(2) Aba = aminobutyric acid; Dha = dehydroalanine.

(3) Free dehydroalanine is not stable and decomposes with the formation of ammonia and pyruvic acid; *cf.* E. Gross and J. L. Morell (*J. Amer. Chem. Soc.*, **89**, 2791 (1967)), for the formation of pyruvyllysine from nisin.

(4) H. Matsuo, Y. Fujimoto, and T. Tatsuno, *Biochem. Biophys. Res. Commun.*, **22**, 69 (1966).

(5) H. R. Billick and H. Adkins, *Org. Syn.*, **3**, 176 (1955).