BIOORGANIC CHEMISTRY 10, 233–242 (1981)

Consideration of a New Catalytic Role for the Thiazolium Sulfur Atom of the Coenzyme Thiamine Pyrophosphate

JOHN L. HOGG

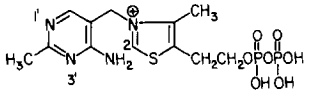
Department of Chemistry, Texas A&M University, College Station, Texas 77843

Received March 6, 1980

A review of the mechanism of action of thiamine pyrophosphate is presented in brief detail along with a new proposal for the role of the sulfur atom of the thiazolium ring of the coenzyme. Evidence is presented to support the idea that sulfur plays a catalytic role in stabilizing negative charge developing in the transition states of the biological reactions.

INTRODUCTION

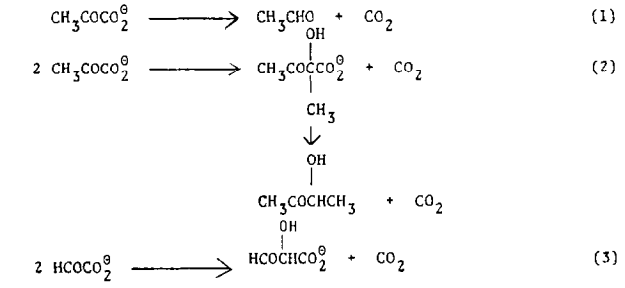
Thiamine pyrophosphate (**1**), abbreviated hereafter as TPP, is the active coenzyme for a number of single enzyme and multienzyme systems which



1

catalyze the cleavage of a carbon–carbon bond adjacent to a carbonyl group. (*1–4*) Among the enzymes for which TPP is a coenzyme are pyruvic decarboxylase, acetolactate synthetase, and glyoxalate carboligase. These enzymes are involved in nonoxidative decarboxylations to yield aldehydes, as shown in Eq. [1] for conversion of pyruvate to acetaldehyde, or their condensation products, as shown in Eq. [2] for the synthesis of acetolactate from pyruvate. This is followed by a decarboxylation to acetoin. Glyoxalate carboligase catalyzes the condensation–decarboxylation reaction of glyoxalate shown in Eq. [3]. In an oxidative sense,

(a) Nonoxidative Decarboxylations



(1)

(2)

(3)

0045-2068/81/030233-10$02.00/0

(b) Oxidative Decarboxylations

$$CH_3COCO_2^{\ominus} \xrightarrow[-2e^{\ominus}]{CoASH} CH_3COSCoA + CO_2 \quad (4)$$

$$CH_3COCO_2^{\ominus} \xrightarrow{-2e^{\ominus}} CH_3CO_2^{\ominus} + CO_2 \quad (5)$$

SCHEME 1

TPP is involved along with electron acceptor molecules in the conversion of pyruvate to acetyl-CoA, Eq. [4], or to acetate ion, Eq. [5]. These equations represent the reactions catalyzed by the multienzyme complex, pyruvate dehydrogenase, and the single enzyme, pyruvate oxidase, respectively, and are representative of the enzyme or enzymatic systems catalyzing such reactions. TPP is also involved in ketol transfers, where it serves much the same function as in the previous examples given. The enzymes transketolase and phosphoketolase are representative of this process.

Many of the reactions catalyzed by TPP-requiring enzymes or enzyme complexes are catalyzed to some extent by TPP in the absence of the enzyme, and many model systems have been developed to probe the catalytic mechanism. The historical development of the mode of TPP catalysis has been well documented (*1*). Recent efforts have been directed at investigating the role of the thiazolium ring in these catalytic mechanisms (*5–17*). Carbanion formation at C-2 of the thiazolium ring has been shown to be a key step in these mechanisms, and considerable controversy has arisen over the exact nature of the "carbanion." The necessity of having a thiazolium ring (**2a**) as opposed to an oxazolium ring (**2b**) or an imidazolium ring (**2c**) has been demonstrated in many studies (*6, 7, 18, 19*).

2a X = S
2b X = O
2c X = NH

The role of the sulfur of the thiazolium ring of TPP has been probed by a variety of methods, but there is still no general agreement on the subject. A proposal is put forth in this paper which, in light of data available in the literature, suggests a new and potentially very important catalytic role for the sulfur atom of the coenzyme TPP. It is suggested that sulfur, through the use of its 3d-orbitals, can stabilize negative charges built up in the transition state structures of the reactions catalyzed by TPP. A brief review of the previous roles considered for the sulfur atom will be followed by a discussion of this potential new catalytic role. Evidence gleaned from the literature will be offered in support of this theory.

BACKGROUND AND PROPOSAL

There has been a great deal of speculation concerning the role of the sulfur atom of the thiazolium ring of TPP and of the relative importance of the resonance

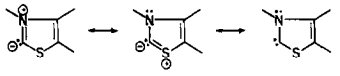


FIGURE 1

structures (Fig. 1) in stabilizing the ylid formed by loss of a proton from C-2 of TPP (*9, 11*). Early model studies had shown that **2a-c** ($R_1 = R_2 = CH_3$; $R_3 = H$) underwent deuterium exchange at C-2 (*5, 7, 9, 19*). Haake and co-workers determined the relative rates of deuterium exchange to be $10^{5.5}$, $10^{3.5}$, and 1 for the oxazolium, thiazolium, and imidazolium compounds, respectively (*9*). The oxazolium compound which underwent C-2 deuterium exchange most readily failed, however, to catalyze the benzion condensation and similar reactions catalyzed by the thiazolium and imidazolium compounds. It was subsequently shown that the oxazolium compounds underwent a ring-opening reaction which accounted for their lack of catalytic activity (*10, 20, 21*). Thus, attention again centered on the stabilization of the ylid (Fig. 1) via participation of the 3*d* orbitals of sulfur. It appears that although stabilization of the ylid by any mechanism is certainly important, not enough attention has been focused on stabilization of the *transition states* for reactions catalyzed by TPP.

Stabilization of *transition states* for enzymatic reactions is generally recognized as an important factor in catalysis (*22*). Evidence will be presented to support a new proposal: *the sulfur atom of thiamine pyrophosphate utilizes its 3d orbitals to stabilize negative charge which develops on the carbonyl oxygen in the transition state for formation of the active aldehyde intermediate, and the stabilization of the tetrahedral species shifts the equilibrium between it and the active aldehyde in favor of the tetrahedral species* (Fig. 2). It is reasonable to expect that the transition state for attack of the C-2 carbanion on a substrate molecule carbonyl group would involve formation of a tetrahedral species localizing considerable negative charge on the former carbonyl oxygen, as suggested in Fig. 2. The available evidence suggests that the thiazolium sulfur atom of TPP can offer significant stabilization of such a transition state. The influence of the tautomeric prototropic equilibrium between the suggested transition state and active aldehyde will be considered below.

SUPPORTING EVIDENCE

There have been several reviews of the ability of bivalent sulfur to utilize its 3*d* orbitals for bonding (*23–28*). Bivalent sulfur has been shown to stabilize a negative charge on an α carbon, although the mechanism by which it does this is

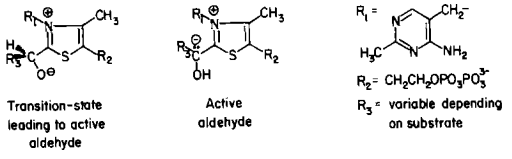
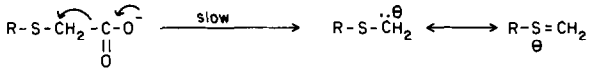


FIGURE 2

$(PhS)_2CHCO_2H$ $(PhO)_2CHCO_2H$

3 4

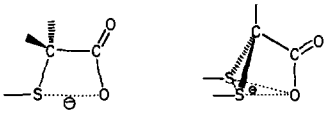
not agreed on (*23–28*). For example, α-mercaptocarboxylic acids, of which **3** is an example, undergo decarboxylation as much as 8.8×10^3 times faster than the corresponding oxygen analogs (e.g., **4**) at 160°C in 50% aqueous ethanol (*25, 29, 30*). Oae and co-workers attributed this kinetic acceleration to 3*d*-orbital resonance stabilization of the incipient carbanion formed in the rate-determining step of the reaction as shown in



[4]

Recognizing an electron-withdrawing inductive effect for the heteratoms, one would have predicted a faster rate for the oxygen analogs, since the electronegativities of oxygen and sulfur are 3.5 and 2.5, respectively (*31*). A significant amount of data is presented by Oae and co-workers in support of their idea.

Ionization constants and hydrolysis data can also be used to support 3*d*-orbital stabilization of negative charge by sulfur. In Table 1 are collected some data for the ionization of α-mercapto- and α-alkoxyacetic acids in two different solvent systems (*32*). In water the pk_a of the sulfur derivatives is lower than that of the methylene analog (i.e., 4.11 vs 4.84). Thus, in spite of the fact that carbon and sulfur have the same electronegativity (*31*), the α-mercaptoacetic acid is a stronger acid than the methylene analog. Presumably, the carboxylate anions can be stabilized as in **5** or **6** as previously suggested (*32*).



5 6

In water the α-alkoxy acids are stronger acids than the sulfur analogs. As the solvent is changed to a less polar one a reversal of relative acidities between the oxygen and sulfur analogs is now seen. This could be due to an increased importance of structures like **5** and **6** in solvents where the carboxylate anion is not as readily stabilized by solvent interactions. It is noted here that the sulfur atom and oxygen anion bear a 1,4 relationship in these structures. This is the same as would be found in many of the TPP-catalyzed reactions (e.g., the transition state structure proposed in Fig. 2).

Some pertinent hydrolysis data have been collected in Table 2. Initial inspection reveals that in all cases the oxygen analogs hydrolyze approximately twice as fast as the sulfur analogs. Since oxygen is more electronegative than sulfur the inductive effect alone would tend to favor the hydrolysis of the oxygen compounds even in the presence of 3*d*-orbital stabilization of the transition state for

TABLE 1

IONIZATION CONSTANTS AT 25°C

Carboxylic acid	pK_a in water[a]	pK_a in 50% EtOH–H_2O[a]
$EtOCH_2CO_2H$	3.84 ± 0.03	4.92 ± 0.03
$EtSCH_2CO_2H$	4.11 ± 0.05	4.75 ± 0.02
$EtCH_2CH_2CO_2H$	4.84[b]	—
$(EtO)_2CHCO_2H$	—	4.82 ± 0.06
$(EtS)_2CHCO_2H$	—	4.52 ± 0.05

[a] From Ref. (*32*) unless otherwise specified.
[b] Ref. (*33*).

hydrolysis. Although not originally considered in the interpretation of these data, a case can be made for 3*d*-orbital stabilization along the following lines. Since the electronegativities of sulfur and carbon are the same, a comparison of the sulfur and methylene analogs should allow an estimate of the importance of 3*d*-orbital stabilization.

The hydroxide-promoted hydrolysis of the sulfur analog is significantly enhanced (note the temperature difference) relative to the methylene analog. Stabilization by sulfur of the negative charge building up on the carbonyl oxygen in a tetrahedral intermediate could account for this rate difference. One could speculate that the sulfur analog reacts slightly more slowly than the oxygen analog because the sulfur stabilizes the tetrahedral intermediate to such an extent that breakdown of the intermediate now becomes partially rate determining for this compound. This stabilization of a full tetrahedral intermediate could actually shift the tautomeric prototropic equilibrium between the tetrahedral structure proposed in Fig. 2 and the active aldehyde. This conceivably impedes subsequent condensation reactions of the active aldehyde to some extent but should favor loss of aldehyde due to the equilibrium effect.

TABLE 2

RATES OF ESTER HYDROLYSIS

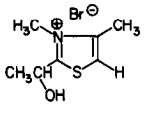
Ester	Catalyst	Solvent	Temperature (°C)	k
				$(M^{-1}\ min^{-1})$[a]
$EtOCH_2CO_2Et$	OH^-	80% EtOH–H_2O	10.0°	2.56
$EtSCH_2CO_2Et$	OH^-	80% EtOH–H_2O	10.0°	1.44
$EtCH_2CH_2CO_2Et$	OH^-	80% EtOH–H_2O	25°	0.115[b]
				$\times 10^5\ (M^{-1}\ sec^{-1})$[a]
$EtOCH_2CO_2Et$	H^+	70% Acetone–H_2O	25°	2.57
$EtSCH_2CO_2Et$	H^+	70% Acetone–H_2O	25°	1.42
$EtCH_2CH_2CO_2Et$	H^+	70% Acetone–H_2O	25°	1.79[c]

[a] From Ref. (*32*) unless otherwise specified.
[b] Ref. (*34*).
[c] Ref. (*35*).

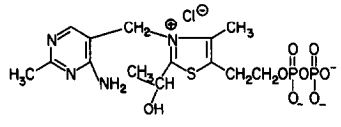
The acidic hydrolysis data are also consistent with this proposal. The sulfur and methylene analogs hydrolyze at almost exactly the same rate under acid-catalyzed conditions. This is to be expected since the acid-catalyzed hydrolysis probably involves prior protonation of the carbonyl oxygen. Thus, no significant amount of negative charge builds up, and stabilization by sulfur does not play a large role. One could again speculate that the tetrahedral intermediate is stabilized to an extent by sulfur that makes breakdown at least partially rate limiting. The interaction of interest is again a 1,4 interaction in the hydrolysis reactions. Oae and co-workers have also shown that favorable $3d$-orbital interactions are enhanced in certain cyclic compounds due to conformational restraints (*32*).

X-RAY STRUCTURAL SUPPORT

In the past several years the X-ray crystal structures of several thiamine derivatives and model compounds have been determined. These structures add further, convincing support to a theory that the sulfur atom of a thiazolium ring could significantly interact with developing negative charge on a carbonyl oxygen atom in TPP-catalyzed reactions. The crystal structures of 2-(α-hydroxyethyl)-3,4-dimethylthiazolium bromide (**7**) and 2-(α-hydroxyethyl)thiamine chloride hydrochloride (**8**) were determined by Sax and co-workers (*36*). In both structures there is a significant interaction between the sulfur atom and the hydroxyethyl oxygen atom of the C-2 side chain. The sum of the van der Waals radii is 3.25 Å but the S . . . O distance is only 2.852 Å in **7** and 2.901 Å in **8**. Thus, there is a



7



8

considerable shortening of the S . . . O distance in these molecules. This attraction, which has been attributed to an electrostatic interaction between a partial positive charge on sulfur with the unshared electrons on oxygen, has a great deal of influence on the conformation of the side chain. The O–C–C–S torsion angles agree to within 2.2° and are 20.60° and 18.42° for **7** and **8** respectively (Fig. 3). Compound **8** represents the isolable protonated form of the intermediate in reactions catalyzed by TPP, so this interaction is very important (*3, 37–39*). Deprotonation of **8** at the hydroxyl group has been proposed as the first

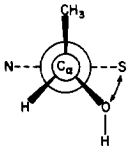
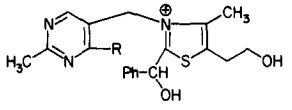


FIGURE 3

step in the elimination of acetaldehyde. The resultant oxygen anion should be significantly stabilized by a similar S . . . O interaction. In fact, this stabilization has been used to explain the relatively low pk_a (11.4 at 25° and 0.5 *M* ionic strength) for this hydroxyl proton (*37*). The exact same interaction should be important in stabilizing the oxygen anion resulting from attack of the C-2 carbanion on the carbonyl carbon in the transition state for the reaction in the forward direction. Thus, the most important role of the proposed stabilization may be in α-decarboxylation reactions where sulfur can stabilize the transition states leading to tetrahedral oxyanion intermediates and exert its influence of the tautomeric prototropic equilibrium between this intermediate and the α-carbanion or active aldehyde to assist in loss of aldehyde.

The crystal structures of DL-2-(α-hydroxybenzyl)thiamine chloride hydrochloride trihydrate (**9**) and DL-2-(α-hydroxybenzyl)oxythiamine chloride hydrochloride trihydrate (**10**) have also been determined (*40, 41*). In both structures there is again a significant S . . . O interaction. The S . . . O distances are 2.764 Å and 2.749 Å in **9** and **10**, respectively, with the corresponding O–C–C–S torsion angles being 8.4° and 11.7°.



9 R = $-NH_2$
10 R = $-OH$

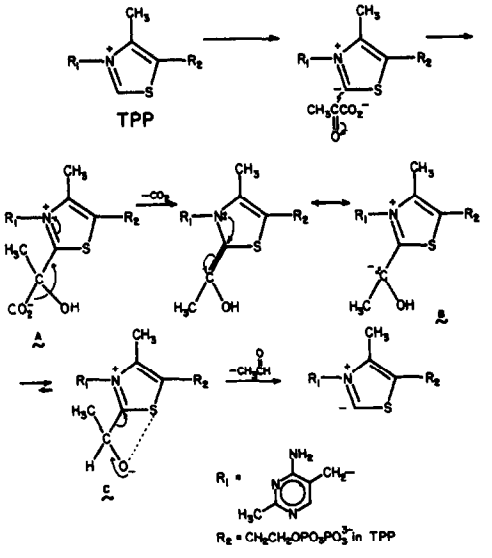
Oxythiamine, whose crystal structure has also been determined, is a thiamine pyrophosphate antagonist as its pyrophosphate ester (*42*). It binds at the binding site of pyruvate decarboxylase and reacts with pyruvate to form the C-2 adduct, but the acetaldehyde product is not released (*39*). An earlier proposal attributed this failure to release product to its inability to form an intramolecular hydrogen bond between the 4′-amino group and the hydroxyl group proton. This hydrogen bond was proposed to stabilize the adduct and assist in the proton removal. This idea has not been supported by the crystal structures or any other evidence. Perhaps, according to the theory being put forth in this paper, a significantly enhanced S . . . O interaction stabilizes the C-2 adduct to such an extent that it prevents release of the acetaldehyde product. Based on S . . . O distances in **10**, the strongest interaction is for oxythiamine. This factor, coupled with the other conformational and electronic differences, may then be used to explain the antagonist properties of the pyrophosphate ester of **10**. The X-ray structures indicate that the oxygen atom at the 4 position of the pyrimidine ring actually exists in the keto form (*42*). It is difficult to predict which nitrogen the proton is on. Conformational studies of **8** have been conducted by Mieyal *et al.* (*2*). Their data indicate that it is the α-carbanion form of a C-2 adduct which is the active aldehyde intermediate in TPP-catalyzed reactions. The molecular conformation of 2-(α-hydroxyethyl)thiamine determined in their studies is consistent with the X-ray structure.

Jordon's theoretical calculations of the conformational and electronic properties of thiamine and 2-(α-hydroxyethyl)thiamine support the existence of a significant S . . . O interaction in intermediates found in TPP-catalyzed reactions (*17, 43*). His electron density calculations indicate the sulfur atom to be positive. This is supported by the X-ray crystal structure data. His results appear to allow for an electrostatic interaction between sulfur and oxygen but seem to rule out a long-range bonding interaction in the stable molecules. The interaction to be expected in a transition state is certainly open to question. He does suggest that the $\overset{\delta-}{C}(2)-\overset{\delta+}{S}$ bond polarity may be important in aligning the $\overset{\delta+}{C}=\overset{\delta-}{O}$ dipole of the reactant for nucleophilic attack by the C-2 carbanion. An extension of this idea to stabilization of negative charges developing in the transition state is a logical one.

This interaction between sulfur and oxygen has been noted several times (*36–43*) and has been suggested to have some mechanistic significance (*38, 40*). Most emphasis has been placed on the ability of the thiazolium sulfur to facilitate sterically and electronically deprotonation of the hydroxyl group of the initial adduct (*40*) and to provide a significant electrostatic coenzyme-substrate interaction suggested as a binding force (*38*) in the nonpolar environment of the active site of the enzyme (*44, 45*). Stabilization of transition state structures along with supporting data has not been previously considered as a major role for the thiazolium sulfur atom of TPP.

MECHANISM OF PYRUVATE DECARBOXYLATION

In scheme 2 is shown a reasonable mechanistic sequence for the reaction of TPP with pyruvate (*3*). One can readily identify several reaction steps which might



SCHEME 2

have transition state structures that could benefit from 3*d*-orbital stabilization of the type described above. The transition state for the formation of **A** from the C-2 carbanion probably involves the formation of an oxyanion which could certainly benefit from sulfur stabilization. The decarboxylation step to give **B** could also derive some stabilization of negative charge in the transition state leading to the active aldehyde intermediate. All of the intermediates having a hydroxyl group of the α carbon are presumably stabilized to some extent by this interaction. The influence of this interaction on the tautomeric equilibrium between **B** and **C** in Scheme 2 should be to favor formation of **C** and thus facilitate loss of aldehyde.

CONCLUSION

This potential catalytic role for the sulfur atom of TPP has not been previously considered. However, evidence has been presented which suggests the potential importance of 3*d*-orbital stabilization of negative charge on oxygen in a 1,4 relationship with sulfur. This source of catalytic power in thiamine pyrophosphate should be seriously considered in light of the available data. The data suggest a possible new role in *transition state stabilization* for the sulfhydryl groups present at the active site of other enzymes or coenzymes.

ACKNOWLEDGMENTS

Support provided by the Robert A. Welch Foundation to the author is gratefully acknowledged. The critical comments of Kenneth R. Davis have significantly improved the manuscript as have those of an anonymous referee.

REFERENCES

1. For an excellent review see: T. C. BRUICE AND S. BENKOVIC, "Bioorganic Mechanisms," Vol. 2, Chap. 8. Benjamin, New York, 1966.
2. A. A. GALLO, J. J. MIEYAL, AND H. Z. SABLE, "Bioorganic Chemistry" (E. E. van Tamelen, Ed.), Vol. 4, pp. 147–177. Academic Press, New York, 1978.
3. L. O. KRAMPITZ, *Annu. Rev. Biochem.* **38**, 213 (1969).
4. C. WALSH, "Enzymatic Reaction Mechanisms," pp. 682–700. Freeman, San Francisco, 1979.
5. R. BRESLOW, *J. Amer. Chem. Soc.* **79**, 1762 (1957).
6. P. HAAKE AND W. B. MILLER, *J. Amer. Chem. Soc.* **85**, 4044 (1963).
7. W. HAFFERL, R. LUNDIN, AND L. L. INGRAHAM, *Biochemistry* **2**, 1298 (1963).
8. P. HAAKE AND L. P. BAUSHER, *J. Phys. Chem.* **72**, 2213 (1968).
9. P. HAAKE, L. P. BAUSHER, AND W. B. MILLER, *J. Amer. Chem. Soc.* **91**, 1113 (1969).
10. P. HAAKE AND J. M. DUCLOS, *Tetrahedron Lett.* 461 (1970).
11. P. HAAKE, L. P. BAUSHER, AND J. P. MCNEAL, *J. Amer. Chem. Soc.* **93**, 7045 (1971).
12. H. C. SORENSEN AND L. L. INGRAHAM, *J. Heterocycl. Chem.* **8**, 551 (1971).
13. R. A. COBURN, J. M. LANDESBURG, D. S. KEMP, AND R. A. OLOFSON, *Tetrahedron* **26**, 685 (1970).
14. A. STREITWEISER, JR. AND P. J. SCANNON, *J. Amer. Chem. Soc.* **95**, 6273 (1973).
15. R. BRESLOW, *Ann. N.Y. Acad. Sci.* **98**, 445 (1962).
16. B. PULLMAN AND C. SPANJAARD, *Biochem. Biophys. Acta* **46**, 576 (1961).

17. F. JORDAN, *J. Amer. Chem. Soc.* **96**, 3623 (1974).
18. T. UKAI, T. DOKAWA, AND S. TSUBOKAWA, *Yakugaku Zasshi* **64**(7A), 3 (1944).
19. R. BRESLOW, *J. Amer. Chem. Soc.* **80**, 3719 (1958).
20. D. G. OTT, N. HAYES, AND V. N. KERR, *J. Amer. Chem. Soc.* **78**, 1941 (1956).
21. T. OZAWA, M. NODA, AND I. TOMITA, *Chem. Parm. Bull. (Japan)* **16**, 1858 (1968).
22. R. D. GANDOUR AND R. L. SCHOWEN (Eds.), "Transition States of Biochemical Processes." Plenum, New York, 1978.
23. G. CILENTO, *Chem. Rev.* **60**, 147 (1960).
24. C. C. PRICE AND S. OAE, "Sulfur Bonding." Ronald Press, New York, 1962.
25. Y. YANO AND S. OAE, *Mech. Sulfur Reactions* **4**, 167 (1969).
26. A. STREITWEISER, JR. AND S. P. EWING, *J. Amer. Chem. Soc.* **97**, 190 (1975).
27. A. STREITWEISER AND J. E. WILLIAMS, *J. Amer. Chem. Soc.* **97**, 191 (1975).
28. F. BERNARDI, I. G. CSIZMADIA, A. MANGINI, H. B. SCHLEGEL, M.-H. WHANGBO, AND S. WOLFE, *J. Amer. Chem. Soc.* **97**, 2209 (1975).
29. K. UNEYAMA, W. TAGAKI, I. MINAMIDA, AND S. OAE, *Tetrahedron* **24**, 5271 (1968).
30. S. OAE, W. TAGAKI, K. UNEYAMA, AND I. MINAMIDA, *Tetrahedron* **24**, 5283 (1968).
31. L. PAULING, "The Nature of the Chemical Bond," 3rd ed. Cornell Univ. Press, Ithaca, N.Y., 1960.
32. I. MINAMIDA, Y. IKEDA, K. UNEYAMA, W. TAGAKI, AND S. OAE, *Tetrahedron* **24**, 5293 (1968).
33. Z. RAPPOPORT, "Handbook of Tables for Organic Compound Identification," 3rd ed. The Chemical Rubber Co., Cleveland, Ohio, 1967.
34. G. DAVIS AND D. P. EVANS, *J. Chem. Soc.* 339 (1940).
35. D. P. EVANS, J. J. GORDON, AND H. W. WATSON, *J. Chem. Soc.* 1439 (1938).
36. M. SAX, P. PULSINELLI, AND J. PLETCHER, *J. Amer. Chem. Soc.* **96**, 155 (1974).
37. J. CROSBY, R. STONE, AND G. E. LIENHARD, *J. Amer. Chem. Soc.* **92**, 2891 (1970).
38. J. CROSBY AND G. E. LIENHARD, *J. Amer. Chem. Soc.* **92**, 5707 (1970).
39. A. SCHELLENBERGER, *Angew. Chem. Int. Ed. Engl.* **6**, 1024 (1967).
40. J. PLETCHER, M. SAX, G. BLANK, AND M. WOOD, *J. Amer. Chem. Soc.* **99**, 1396 (1977).
41. W. SHIN, J. PLETCHER, AND M. SAX, *J. Amer. Chem. Soc.* **101**, 4365 (1979).
42. W. SHIN, J. PLETCHER, M. SAX, AND G. BLANK, *J. Amer. Chem. Soc.* **101**, 2462 (1979).
43. F. JORDAN, ibid., **98**, 808 (1976).
44. J. ULLRICH, *Angew. Chem. Int. Ed. Engl.* **8**, 82 (1969).
45. J. H. WITTORF AND C. J. GUBLER, *Eur. J. Biochem.* **14**, 53 (1970).