# Deuterium Exchange at the 7-Methyl Group in 6,7,8-Trimethyl-lumazine [6,7,8-Trimethylpteridine-2(1*H*),4(3*H*)-dione]. An Unusual Example of General Acid–General Base Catalysis

By **Ross Stewart** * and **J. M. McAndless,** Department of Chemistry, University of British Columbia, Vancouver, Canada

The rate of exchange of the hydrogen atoms in the 7-methyl group of 6,7,8-trimethyl-lumazine (I) at 34·5° has been followed by means of ^{1}H n.m.r. spectroscopy. The reaction is subject to both general acid and general base catalysis, with Brönsted coefficients $\alpha = 0.46$ (12 acids) and $\beta = 0.45$ (14 bases). The rate of the spontaneous reaction is extremely low and there is no evidence of a product term, [HA][A$^-$], for the catalysis. The catalytic constants k_{HA} and k_{A^-} are approximately equal when the acid–base pair used as buffer has a pK_a of 2·8. The acid- and base-catalysed routes differ only by the presence of an extra proton in the former. This permits a comparison to be made of the ability $[k_{(SH^+ + A^-)}]$ of the buffer anion A$^-$ to abstract a proton from the protonated substrate relative to its ability $[k_{(S + A^-)}]$ to do so from the neutral substrate. The value of $k_{(SH^+ + A^-)}/k_{(S + A^-)}$ is 10^2 in the case of compound (I), and since $\alpha + \beta$ for this reaction is close to unity, is almost independent of the buffer used. Other prototropic reactions that are also subject to general acid–base catalysis are analysed in the same way and it is shown that the value of $k_{(SH^+ + A^-)}/k_{(S + A^-)}$ found in the present work is unusually low.

ISOTOPIC exchange of the protons at the 7-methyl group of several lumazine (pteridine-2,4-dione) derivatives has been demonstrated recently.[1-3] The exchange is subject to general acid–base catalysis and exchange mechanisms in both acidic and basic media have been suggested.[2,3] In the present study, the effectiveness of a series of acids and their conjugate bases in catalysing the exchange of 6,7,8-trimethyl-lumazine (I) in D$_2$O is examined. Trimethyl-lumazine serves as a model compound for the

(I) (II)
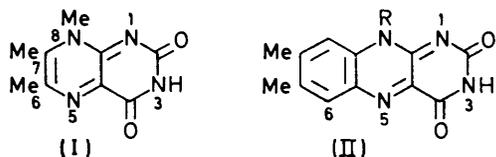
naturally occurring 8-substituted 6,7-dimethyl-lumazines and for flavin nucleotides having the general structure (II). Hydrogen–deuterium exchange has also been observed for the activated 8-methyl groups in the latter system.[4]

EXPERIMENTAL

Materials.—6,7,8-Trimethyl-lumazine (I) was prepared as previously described.[2,5] The carboxylic acids, obtained from commercial sources, were purified by recrystallization to constant m.p. or by fractional distillation and then were stored under dry nitrogen.

Appropriate buffer solutions (0·075M) in deuterium oxide were prepared and adjusted to the desired acidity with deuterium chloride or sodium deuterioxide. (Measurements of acidity were not corrected to give the corresponding pD values.) Any adjustments to the ionic strength of the buffer solutions were made by the addition of sodium chloride.

Kinetic Measurements.—A Varian T-60 60 MHz spectrometer (probe temperature 34·5°) was used to monitor the exchange reaction. The exchange took place in deuterium oxide solutions of compound (I) (0·07M) dissolved in the appropriate nitrogen-flushed buffer. After preparation, the

reaction mixture (0·35 ml) was introduced into a rubber-capped n.m.r. tube by means of a syringe. The tube was then placed into the spectrometer probe and the reaction was monitored in its entirety.

Since the 8-methyl protons of the lumazine (I) did not exchange under the reaction conditions, the signal for these protons was used as reference. The extent of exchange was determined by comparing the areas of the peaks for the 7- and 8-methyl protons at various times. After the exchange was complete the pH of the reaction solution was checked by use of a Radiometer model 26 pH meter equipped with semimicro glass and calomel electrodes.

The exchange followed first-order kinetics for most of the reaction. The observed first-order rate constant k_{obs} was obtained by plotting the data according to equation (1)

$$\ln (I_m) = k_{obs} t \qquad (1)$$

where $I_m = [(M/R)_\infty - (M/R)_0]/[(M/R)_\infty - (M/R)_t]$, M is the average area of exchanging peak, R is the average area of reference peak, and the subscripts refer to the reaction time. A value of 1·0 is assigned to $(M/R)_0$.

RESULTS

The exchange of the 7-methyl protons of trimethyl-lumazine (I) was examined in the range pH 1—7·5, in which the substrate exists predominately in the neutral form.

A typical kinetic run using a deuterium solution buffered at two pH values is illustrated in Figure 1. The observed exchange rate constant k_{obs} is a composite value at a given pH [equation (2) where k_0 is the uncatalysed exchange rate

$$k_{obs} = k_0 + k_{H^+}[H^+] + k_{HA}[HA] + k_{A^-}[A^-] \qquad (2)$$

constant (very small compared to the other terms [2]) and the other coefficients are specific rate constants referring to the species indicated by the subscripts]. Measurements of k_{obs} at two different pH values combined with a knowledge of the magnitude of k_{H^+} (1·2 1 mol^{-1} min^{-1}) [2] allows k_{HA} and k_{A^-} to be calculated. The catalyst concentrations at a given pH are calculated from the pK_{HA} value (35°) of the acid used in the buffer in each case.

[1] T. Paterson and H. C. S. Wood, *Chem. Comm.,* 1969, 290.
[2] J. M. McAndless and R. Stewart, *Canad. J. Chem.,* 1970, **48,** 263.
[3] R. L. Beach and G. W. E. Plaut, *Biochemistry,* 1970, **9,** 760.
[4] F. J. Bullock and O. Jardetzky, *J. Org. Chem.,* 1965, **30,** 2056.
[5] A. J. Birch and C. J. Moye, *J. Chem. Soc.,* 1958, 2622.

For the weaker monoacids the values of k_{A^-} were obtained from kinetic plots obtained for reactions under conditions for which the $k_{H^+}[H^+]$ and k_{HA} terms are relatively small (Figure 2). The intercept serves as a check on the calculated k_{HA} value in each case.
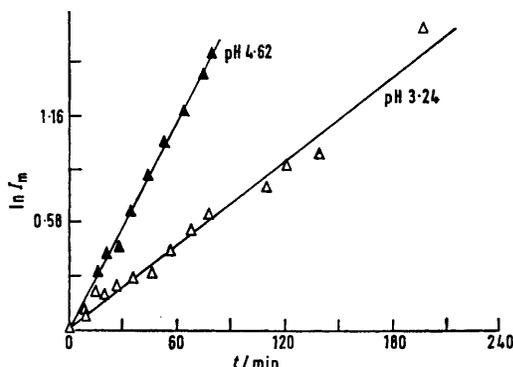

FIGURE 1 Exchange kinetics for 6,7,8-trimethyl-lumazine (I) in 0·075M-glycolic acid buffer in D_2O; $T = 34·5°$

The specific rate constants for the buffers used to catalyse the exchange of compound (I) are recorded in Table 1. The effectiveness of the acids and their conjugate bases in catalysing the exchange can be determined by applying the
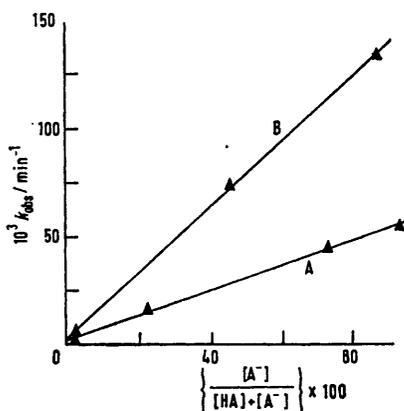

FIGURE 2 Plots of observed first-order rate constants against percentage conjugate base concentration for A, acetic acid (slope $= 0·61$ min^{-1}, intercept $= 1·75 \times 10^{-3}$ min^{-1}) and B, trimethylacetic acid (slope $= 1·65$ min^{-1}, intercept $= 1·85 \times 10^{-3}$); $T = 34·5°$

Brönsted relationship.[6] The data in Table 1 are plotted according to equations (3) and (4). Statistical corrections[7]

Acids (Figure 3a) $pk_{HA} = \alpha pK_{HA} + C$ (3)

Conjugate bases (Figure 3b) $pk_{A^-} = -\beta pK_{HA} + C$ (4)

have been applied in the cases where the catalyst has more than one ionizable proton.

For the 12 acids that were used, the data fit the relationship $pk_{HA} = 0·46pK_{HA} - 0·471$. For the conjugate anions, the relationship $pk_{A^-} = -0·45pK_{HA} + 2·088$ describes the data. The amphoteric ions $H_2PO_4^-$ and $HO_2C·CH_2·CO_2^-$ are believed to act as bases rather than as acids since their catalytic constants (after statistical

[6] J. N. Brönsted and K. J. Pedersen, *Z. phys. Chem.*, 1924, **108**, 185.

O

TABLE 1

Specific catalytic constants [a] for acids used to catalyse hydrogen–deuterium exchange in trimethyl-lumazine

Acid	pK_{HA} [b]	$10^2 k_{HA}/$ l mol^{-1} min^{-1}	$10^2 k_{A^-}/$ l mol^{-1} min^{-1}	
Dichloroacetic	1·49	53·9	5·31	
Cyanoacetic	2·45	26·0	7·02	
Chloroacetic	2·89	14·4	13·0	
Bromoacetic	2·94	18·0	14·0	
Mandelic	3·42	12·5	85·4	
Formic	3·76	5·68	22·1	
Glycolic	3·83	4·24	28·3	
Phenylacetic	4·32	2·90	107	
Acetic	4·76	2·11	85	
Trimethylacetic	5·05	1·20	215	
Other acids				$10^2 k_{A^{2-}}/$ l mol^{-1} min^{-1}
Malonic	2·86	13·2	25·0	231
	5·73			
Phosphoric	2·18	59·0	21·3	5460
	7·19			

[a] Determined at 34·5°.   [b] 35·0° in H_2O.

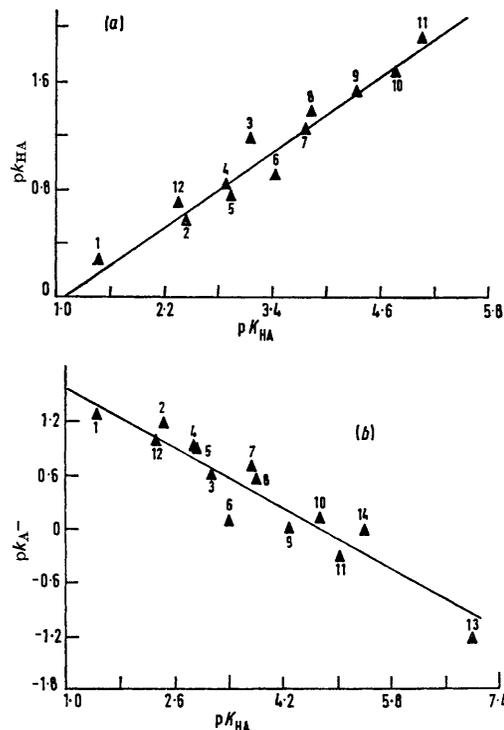corrections) are correlated well by equation (4) and poorly by equation (3).

FIGURE 3 Catalytic constants for exchange reaction as functions of the strength of buffer acid; (a) pk_{HA} against pK_{HA} of catalysing acid, (b) pk_{A^-} against pK_{HA} of conjugate acid of catalysing base. 1, $Cl_2CH·CO_2H$; 2, $NC·CH_2·CO_2H$; 3, $CH_2(CO_2H)_2$; 4, $ClCH_2·CO_2H$; 5, $BrCH_2·CO_2H$; 6, $PhCH(OH)·CO_2H$; 7, HCO_2H; 8, $HO·CH_2·CO_2H$; 9, $PhCH_2·CO_2H$; 10, $MeCO_2H$; 11, $Me_3C·CO_2H$; 12, H_3PO_4; 13, $H_2PO_4^-$; 14, $HO_2C·CH_2CO_2^-$. The plotted values of pk or pK for polyprotic acids have been statistically corrected[7]

Mandelic acid and its conjugate base are slightly better catalysts than expected from a consideration of its acidity

[7] K. J. Laidler, ' Reaction Kinetics,' Pergamon, London, 1963, vol. 2, pp. 63—65.

constant and they are nearly as effective as the phosphate species. Whether this is due to some structural effect in the acid is not known. Glycolic acid, which also contains an α-hydroxy-group, shows no such effect. The effect cannot be due to an incorrect value of pK_{HA} being used. Any alteration in the value of K_{HA} causes a greater deviation in either k_{HA} or k_{A^-}.

Varying the ionic strength of the solution has very little effect on the exchange rate (Table 2).

TABLE 2

Effect of ionic strength (μ) on exchange rate of compound (I) at 34·5°

Buffer[a] pH	μ[b]	$10^3 k_1/\text{min}^{-1}$
4·02	0·075	12·40 ± 0·42
3·96	0·196	11·92 ± 0·37
3·98	0·458	12·06 ± 0·39

[a] Formic acid–sodium formate in D_2O, $M = 0.075$. [b] Controlled by addition of sodium chloride.

DISCUSSION

A mechanism for general acid- and general base-catalysed exchange is presented in the Scheme, where HA is the acid used in buffering the system. Both the intermediate ions (IH⁺) and (IV) are extensively stabilized by resonance and this is doubtless the reason for the ready exchange by either route. The Scheme resembles those generally accepted for the prototropic


SCHEME
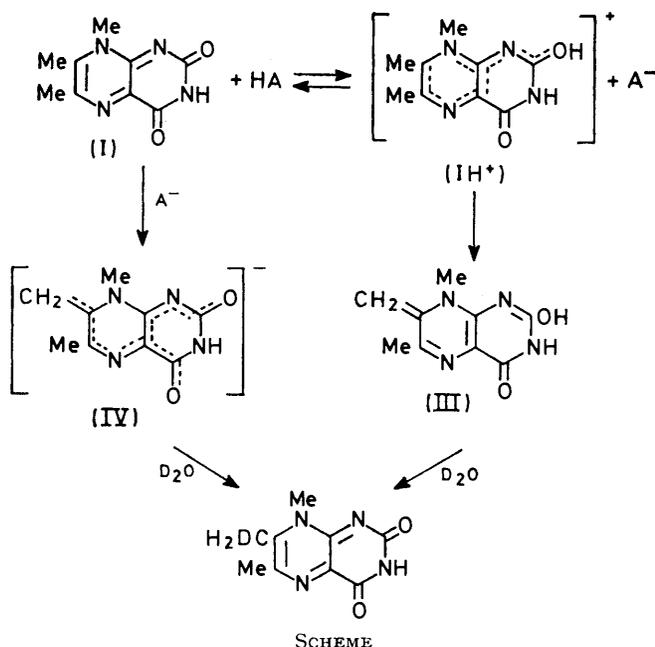
reactions of ketones and hemiacetals. [The intermediates (III) and (IV) correspond to enol and enol-anion, respectively, in ketone enolization.] No product term [A⁻][HA] was detected in the present work, suggesting that a concerted path is not important.

The rate expressions for the general acid and general base routes are as follows, where $K_{(IH^+)}$ represents the

dissociation constant of the conjugate acid of compound (I).

General Acid Catalysis.—For a given buffer acid HA,

$$(I) + H^+ \underset{}{\overset{1/K_{(IH^+)}}{\rightleftarrows}} (IH^+)$$

$$(IH^+) + A^- \xrightarrow{k_{[(IH^+)+A^-]}} \text{Exchangeable intermediate}$$
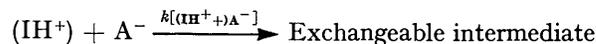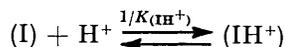
the rate $= k_{[(IH^+)+A^-]}[(IH^+)][A^-]$. Substitution using the expressions for $K_{(IH^+)}$ and K_{HA} gives rate $= k_{[(IH^+)+A^-]}[(I)][HA]K_{HA}/K_{(IH^+)}$. For a given acid the catalytic coefficient k_{HA} is thus equal to $k_{[(IH^+)+A^-]}K_{HA}/K_{(IH^+)}$, assuming that little of the substrate (I) is in the protonated form.

General Base Catalysis.—For a given base A⁻, the

$$(I) + A^- \xrightarrow{k_{[(I)+A^-]}} \text{Exchangeable intermediate}$$

rate $= k_{[(I)+A^-]}[(I)][A^-]$; the catalytic coefficient k_{A^-} is thus equal to $k_{[(I)+A^-]}$.

It is apparent that the general acid route differs from the general base route only by the presence of an additional proton in the former. We are thus in a position to compare the ability of a base A⁻ to remove a proton from the cation (IH⁺) or from the neutral molecule (I). The former is the rate-controlling step in the acid-catalysed route, the latter the rate-controlling step in the base-catalysed route.

For the pair acetic acid–acetate (HOAc–⁻OAc) the bimolecular catalytic constants, given in Table 1, are 0·0211 and 0·85 l mol⁻¹ min⁻¹, respectively. Substituting for the appropriate values of $K_{(IH^+)}$ and K_{HOAc} (0·14 and $1·8 \times 10^{-5}$) gives $k_{[(IH^+)+⁻OAc]} : k_{[(I)+⁻OAc]} = 2 \times 10^2$. This means that an acetate ion's ability to remove a proton from the 7-methyl group of compound (I) is raised by a factor of roughly 200 when the lumazine (I) is protonated.

In general, the ratio of effectiveness of an anion A⁻ reacting with a protonated substrate SH⁺ and neutral substrate S is given by equation (5), where k_{HA} and k_{A^-} are the usual catalytic coefficients, K_{SH^+} is the dissociation constant of the protonated substrate, and K_{HA} is the dissociation constant of the buffer acid HA.

$$k_{(SH^+ + A^-)}/k_{(S + A^-)} = (k_{HA}/k_{A^-})(K_{SH^+}/K_{HA}) \quad (5)$$

With a given substrate the relative effectiveness of the two routes will be independent of the buffer pair provided that $\alpha + \beta$ equals unity. This condition is approximately met in the present case, and, in fact, the ratio $k_{[(IH^+)+A^-]}/k_{[(I)+A^-]}$ does not vary greatly for the acid-base pairs shown in Table 1. (Although acetic acid-acetate is a convenient pair to choose for purposes of comparison with data for other prototropic systems, the value of 200 is higher than the average for all the buffers examined herein.)

What is the magnitude of the reactivity ratio with other substrates? Taking the iodination of acetone as an example, the data of Bell and Lidwell [8] show that the

[8] R. P. Bell and O. M. Lidwell, *Proc. Roy. Soc.*, 1940, *A*, **176**, 88.

ratio of these catalytic coefficients for acetic acid and acetate ion is 0·42. Substitution of the appropriate values of K_{HA} and K_{SH+} (4×10^7) [9,10] in equation (5) gives the value $1·0 \times 10^{12}$ for the reactivity ratio $k_{(SH+ + ^-OAc)}/k_{(S + ^-OAc)}$. (Leinhard and Anderson [11] have come to a similar conclusion regarding the effect of protonation on the rate of proton abstraction from acetone. They calculated a value of 3×10^{11} for the relative rates of reaction of acetate ion with protonated and protonated acetone. The difference between this figure and our value of $1·0 \times 10^{12}$ is due largely to our using a corrected value for the basicity of acetone.[10])

The enormous difference, shown in Table 3, in the ratio of $k_{(SH+ + ^-OAc)}/k_{(S + ^-OAc)}$ for compound (I) and acetone may be explained by their abilities to disperse

TABLE 3

Reactivity ratios, basicities, and acidities of various compounds

Compound	$k_{(SH+ + ^-OAc)}/$ $k_{(S + ^-OAc)}$	pK_{SH+}	pK_a	ΔpK $(pK_a -$ $pK_{SH+})$
6,7,8-Trimethyl-luma-zine (deuterium exchange)	2×10^2	$0·9^a$	11^b	10
Glucose (mutarotation)	$3 \times 10^{7\,c}$	$-4·5^d$	$12·4^e$	17
Acetone (enolization)	1×10^{12}	$-7·6^f$	20^g	28

^{a} Ref. 12. ^{b} The pK_a of compound (I) is 9·90,[12] corresponding to proton loss from the N-3 atom. Upon methylation at N-3 the pK_a rises to 10·34, corresponding either to hydroxide addition at C-7 [12] or proton loss to give the methyl analogue of the anion (IV). Structural considerations suggest that the pK_a values of these two processes will not differ greatly. ^{c} Calculated from equation (5) using the rate data of J. N. Brönsted and E. A. Guggenheim, *J. Amer. Chem. Soc.*, 1927, **49**, 2554, and an estimate of $-4·5$ for pK_{BH+} of glucose based on basicity data of other acetals ($-4·6$ and $-4·1$ for dimethoxymethane and diethoxymethane, respectively, reported by A. Kankaanpura, *Acta Chem. Scand.*, 1969, **23**, 1723). ^{d} See footnote c. ^{e} J. Thamsen, *Acta Chem. Scand.*, 1952, **6**, 270. ^{f} Refs. 9 and 10. ^{g} R. P. Bell, *Trans. Faraday Soc.*, 1943, **39**, 253.

positive and negative charge as indicated by their pK_{SH+} and pK_a values. (To avoid confusion we use pK_a for the pK_{HA} value of the substrates.) The $k_{(S+A^-)}$ value must be greatly influenced by the stability of the anion of the substrate S, that is by its pK_a. Similarly, the value of $k_{(SH+ + A^-)}$ will be influenced by the stability of the ion SH^+, that is, by its pK_{SH+}.*

We might expect a substrate with a low tendency both to gain and to lose protons (large negative pK_{SH+} and large positive pK_a) to show a very large discrimination factor between the two routes; that is $k_{(SH+ + A^-)}/k_{(S+A^-)}$ should be large. Conversely, a substrate which is protonated and deprotonated readily might be expected

* The pK_{SH+} values with respect to proton loss to give the tautomers, $CH_3 \cdot C(OH)=CH_2$ in the case of acetone, would provide a better basis for comparison but this information is not available in all cases. Provided there is not an enormous difference between the tautomeric equilibrium constants the conventional pK_{SH+} values are perfectly adequate for the purpose.

[9] H. J. Campbell and J. T. Edward, *Canad. J. Chem.*, 1960, **38**, 2109.

to have a low value of this ratio. [The ratio for a particular substrate varies with the buffer used unless $\alpha + \beta$ equals unity, a condition that is not met exactly by either of the systems under discussion. Such variations in $k_{(SH+ + A^-)}/k_{(S+A^-)}$ are relatively small, however, and do not seriously affect the conclusions drawn herein.]

The data in Table 3 show that the acidities and basicities of acetone are widely separated, by *ca.* 28 powers of ten, and that this compound has an extremely large value for its reactivity ratio. Compound (I) has a much smaller reactivity ratio and we believe this can be traced to its ability to stabilize both a positive and a negative charge, that is, to the relatively small difference between its pK_{SH+} and pK_a values.

The rate of base-catalysed deuterium exchange at the 5-position of 4-methylisothiazole (V) is increased by a factor of 3×10^6 by alkylation at the 2-position, as in the ion (VI).[13] This effect is analogous to those described earlier that result from protonation. Although the discrimination factor is much smaller than that found for acetone enolization it is still larger by several powers of ten than that observed for compound (I). Unfor-

(V)   (VI)
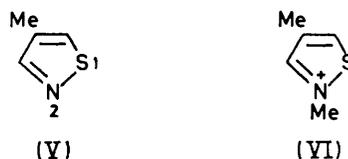
tunately, the necessary acidity constants for the isothiazole (V) (and other thiazoles and diazoles that undergo exchange) are not available, so that we are not able to determine if the discrimination factor reflects the difference between the compound's pK_{SH+} and pK_a values.

Another reaction whose reactivity ratio is midway between those for the lumazine (I) and acetone is the mutarotation of glucose (Table 3). It is interesting that the ΔpK for glucose is also midway between the values for compound (I) and acetone. We hesitate to regard this as confirmation of the hypothesis put forward earlier, since mutarotation is not a simple prototropic reaction like the others; carbon-oxygen bond scission also takes place.

[10] E. M. Arnett, R. P. Quirk, and J. W. Larsen, *J. Amer. Chem. Soc.*, 1970, **92**, 3977.

[11] G. E. Lienhard and F. H. Anderson, *J. Org. Chem.*, 1967, **32**, 2229.

[12] W. Pfleiderer, J. W. Bunting, D. D. Perrin, and G. Nübel, *Chem. Ber.*, 1966, **99**, 3503.

[13] R. A. Olofson and J. M. Landesberg, *J. Amer. Chem. Soc.*, 1966, **88**, 4263; R. A. Olofson, J. M. Landesberg, K. N. Houk, and J. S. Michelman, *ibid.*, p. 4265. See also P. Haake, L. P. Bausher, and W. B. Miller, *J. Amer. Chem. Soc.*, 1969, **91**, 1113 and references therein.