NAD(P)$^+$–NAD(P)H Models. 82. Effect of Magnesium Ion on the Stereospecificity and Conformations at the Ground and Transition States of the Reaction

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(Received October 5, 1992)

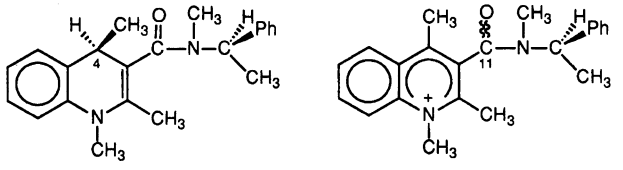
Conformations of 3-[methyl(α-methylbenzyl)carbamoyl]-1,2,4-trimethyl-1,4-dihydroquinoline ((4*R*)-Me_3MQPH) in the presence and absence of magnesium ion have been studied. The major conformation at the ground state of Me_3MQPH changes largely when this molecule forms a complex with a magnesium ion. The conformations at the ground and transition states will be discussed in relation to the stereospecificity of the reaction of this compound with quinones.

3-[Methyl(α-methylbenzyl)carbamoyl]-1,2,4-trimethyl-1,4-dihydroquinoline (Me_3MQPH) is oxidized stereospecifically by quinones of various reactivity to form 3-[methyl(α-methylbenzyl)carbamoyl]-1,2,4-trimethylquinolinium ion (Me_3MQP^+) (Chart 1).[1—4] The corresponding dihydropyridine derivative reacts similarly. The stereospecificity depends on the reactivity of quinone subjected to the reaction.

In a previous paper of the series,[5] it was reported that the stereospecificity is influenced by the presence of magnesium ion, and the conformation at the ground state of the reagent plays an important role to determine the *syn*- and *anti*-conformations at the transition state, hence, the (*S*)- and (*R*)-configurations of the final product. On the other hand, in some reactions, the conformation of transition state is not affected by the conformation at the ground state.

It is well known that zinc ion is involved in the active site of several NAD(P)H-dependent dehydrogenases.[6—8] Zinc and other metal ions are also known to promote the reactions of many NAD(P)H analogs.[9—23] However, no detailed study has been reported on the role of a metal ion in stereospecificity of the reaction.

In order to obtain more detailed insight into the mechanism which exerts the stereospecificity of the reaction, we studied the conformation of Me_3MQPH in the presence and absence of magnesium ion. The present report will discuss the relationship between the conformations at the ground and transition states of the reaction in the presence and absence of magnesium ion. It will, then, be concluded that magnesium ion forms a complex with Me_3MQPH and the conformations at the ground state play a crucial role to determine the *R*/*S* configuration of the final product, Me_3MQP^+. Gibbs energies of activation from different conformers at the ground state that undergo the reaction are not much different and the energy difference between the ground-state conformers remains at the transition state, then is reflected into the *R*/*S* ratio in the product.



(4*R*)-Me_3MQPH Me_3MQP^+

Chart 1.

Experimental

Instruments. ^{1}H NMR spectra were recorded at 200 and 400 MHz on a Varian VXR-200 and a JEOL GX-400 FT-NMR spectrometers, respectively, in CD_3CN or THF-d_8 with TMS as an internal standard. UV-vis spectra were obtained on a Hitachi U-3210 spectrophotometer with a Hitachi SDR-30 temperature controller. Kinetic measurements were performed with a Union Giken RA-401 Rapid Reaction Analyzer equipped with a Union Giken K2R temperature controller and a Union Giken System 77 microcomputer.

Materials. Acetonitrile was distilled over calcium hydride and used immediately. Anhydrous magnesium perchlorate and lithium perchlorate were purchased from Wako Pure Chemicals Industries, Ltd. New bottles were opened everytime in a dry box before the use. Me_3MQPH was prepared according to the literature procedures.[1,3] Quinones were purchased from Nacalai Tesque Inc. and purified by repeated recrystallization.

Measurement of Association Constant. The association constant between Me_3MQPH and magnesium ion in acetonitrile, K_{ass}, was measured on a UV-vis spectrometer as reported by Creighton.[24]

Measurement of Kinetics. Kinetics in the absence of magnesium ion were followed under the pseudo-first-order conditions with 10 fold excess quinone by observing the increase in the intensity at λ_{max} of respective radical anion of the quinone. The procedure is equivalent to following the formation of the final product.[25—27] Rate constants were calculated by the least-squares curve-fit using a personal computer. All measurements gave excellent pseudo-first-order rate correlations.

In the presence of magnesium ion, however, the hydride-transfer reaction only was detectable and the radical anion was unable to be detected.[20,27]

Measurement of Stereospecificity. In a 50 ml round-

bottomed flask equipped with a magnetic stirrer, filled with argon and sealed with a serum cap, 0.03 mmol of 1,4-benzoquinone or its derivative dissolved in 27 ml of anhydrous acetonitrile was injected through a syringe. The solution was equilibrated in a thermostated bath at 293±0.1 K. To the solution, 3 ml of thermostated solution of acetonitrile containing 0.03 mmol of Me_3MQPH and an appropriate amount of anhydrous magnesium (or lithium) perchlorate, if necessary, was injected through a syringe. Then, the reaction mixture was stirred for 1 h in the dark. After evaporation of the solvent under reduced pressure, the organic materials were extracted with dichloromethane. The crude product obtained after evaporation of the dichloromethane was dissolved into CD_3CN and subjected to ^1H NMR spectroscopy to elucidate the diastereomer ratio in the product as well as the chemical yield. It was confirmed that the diastereomer ratio in the products does not change at room tempemperatute within the experimental period. The ^1H NMR spectra of Me_3MQP^+ thus obtained was the same as those reported.[1,3]

Conformational Analysis. The conformations of Me_3MQPH was studied in CD_3CN by means of 400 MHz ^1H NMR spectroscopy. Variable temperature NMR technique was applied to this system in the temperature range from 183 (THF-d_8 as the solvent) to 333 K. The NOE experiment was performed at 193 K in THF-d_8.

Results

^1H NMR Spectra and Assignment of Signals. The methyl group on the carbamoyl nitrogen of (4*R*)-Me_3MQPH (Me_{carb}) exerts one singlet signal in a ^1H NMR spectrum of this compound in CD_3CN at room temperature: i.e., it is obvious that various conformers of the molecule in this solution are in rapid equilibrium at room temperature within the time-scale of ^1H NMR spectroscopy. When the solution is cooled to 243 K, however, the signal splits into three, which indicates that the molecule is conformationally freezed at this temperature. Signals from the other methyl groups as well as the benzylic methine-proton also split into three at this low temperature. A part of ^1H NMR spectrum of Me_3MQPH at 243 K is shown in Fig. 1.

Inspection of a CPK model of the molecule reveals that there are three possibly stable conformations, **1** (*anti-Z*), **2** (*anti-E*), and **3** (*syn-Z*), out of four plausible ones shown in Scheme 1.[28] Since the fourth conformation, *syn-E*, suffers from large steric hindrance, this conformation is unlikely to exist stably.

The conformation analysis is supplemented by an NOE experiment at 193 K in THF-d_8:[29] 11% interaction was observed between a signal from the C_4-proton and one of three signals from Me_{carb} (at δ=2.58), which was safely assigned as the one from the conformation **1** (*anti-Z*). This conformer has Me_{carb} and C_4-proton in the closest neighbor among three. The corresponding signal from the benzylic methine-proton in this conformer appears at δ=5.94. Inspection of the intensities of signals reveals that the singlet at δ=2.65 is paired by the signal at δ=5.23 and that at δ=2.80 corresponds

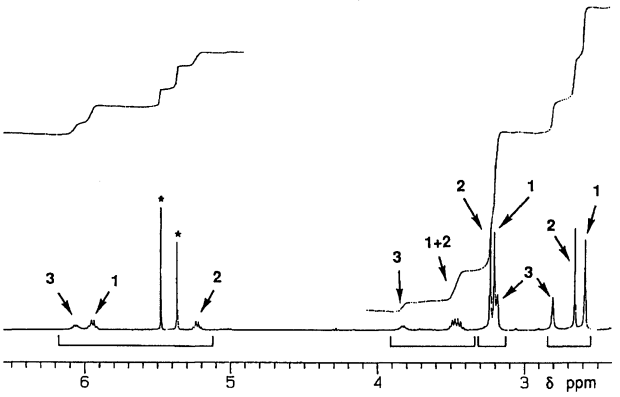
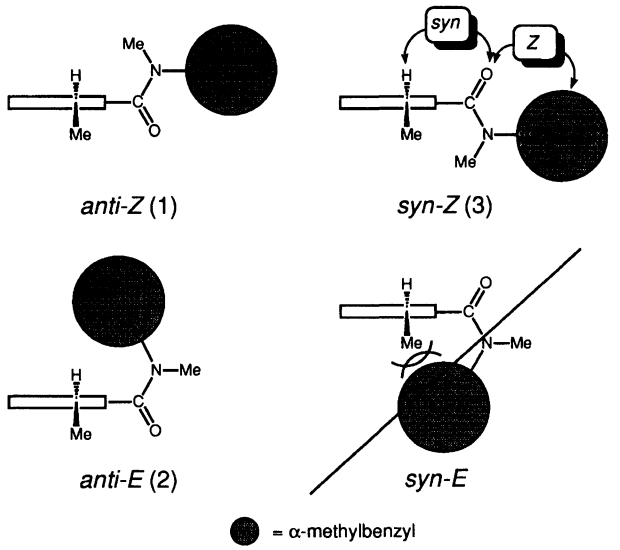


Fig. 1. ^1H NMR spectrum (CD_3CN, 243 K) of Me_3MQPH showing the existence of three rotational species, **1**, **2**, and **3**. Signals from the solvent are denoted by *.



Scheme 1.

to the one at δ=6.05. Since it is reasonable to expect that the chemical shift of a benzylic methine-proton is more sensitive to the rotation around the $C_{carbonyl}$–N_{amide} bond than that around the C_3–$C_{carbonyl}$ bond, the signal at δ=5.23, then the one at δ=2.65 ppm, was assigned to the conformation **2** (*anti-E*). This benzylic signal appears in an upfield region apart from the others, and the conformations **1** and **3** have *Z*-orientation with respect to the carbonyl oxygen and the α-methylbenzyl group, whereas **2** has the *E*-orientation. Finally, the remaining signals at δ=2.80 and 6.05 were assigned to the conformation **3** (*syn-Z*).

The assignment described above, accepts correctly the anisotropic deshielding effect of the carbonyl group on chemical shifts of nearby protons. All signals from the C_4–Me in **1** and **2**, the C_4–H in **3**, and the benzylic C–H in **1** and **3** appear more downfield than the corresponding signals from the other conformer(s) supporting the validity of the assignment described above. Since the protons in the Me_{carb} are a subject of variable

deshielding effect from both the carbonyl and aromatic (or dihydroaromatic) groups depending on the conformation, their chemical shifts are unpredictable.

Kinetics and Thermodynamics. The computer simulations of the ^{1}H NMR spectra were performed by means of an original program[30,31] on which the rates of interconversion between rotational isomers at various temperatures were calculated from the Eyring equation. Kinetic parameters ($\Delta H^{\neq}$ and $\Delta S^{\neq}$) of activation were obtained directly by a least squares method[32] concerning the peak positions, relative intensities and half-height widths of signals of all the ^{1}H NMR spectra obtained at various temperatures. The results are listed in Table 1. Figure 2 demonstrates the identity of the observed and simulated spectra at principal temperatures.

The presence of magnesium ion also freezes the conformations even at higher temperatures. The freezing is the result of the formation of a complex between the magnesium ion and Me_3MQPH.[5,16—18] The complexation shifts the equilibria of the conformers in favor of the *syn*-position, **3**, which seems to suggest that the major site of complexation in Me_3MQPH is the carbonyl oxygen: Only the conformation **3** keeps the carbonyl oxygen in the least clouded syn-position with respect to the C_4–hydrogen. Table 1 also lists kinetic parameters calculated for the systems with and without magnesium ion. Definition of rate constants used in Table 1 is shown in Scheme 2.

The complexation constant, K_{ass}, between Me_3MQPH and magnesium ion in acetonitrile at 298 K measured by UV-vis spectroscopy[14,24,32] is $(1.18\pm0.6)\times10^3$ M (1 M = 1 mol dm^{-3}). Table 2 lists apparent (sum of complexed and uncomplexed) relative amounts of **1**, **2**, and **3**. The values listed in Table 2 predict that 38% of the Me_3MQPH molecules are complexed by magnesium ion in a solution which contains 1 mM each of the reagent at 293 K, which is a normal reaction condition for observing the *R*/*S* ratio in the product. More than 99% of the molecules form the complex under the conditions where the variable-temperature ^{1}H NMR spectra were observed. Relative intensities of the signals in ^{1}H NMR spectra of (4*R*)-Me_3MQPH in the absence and presence of 10 times excess magnesium ion reveals, respectively, that the *syn*/*anti* ratios in the free and complexed Me_3MQPH molecules, i.e., $\mathbf{3}^{free}/(\mathbf{1}^{free}+\mathbf{2}^{free})$ and $\mathbf{3}^{complexed}/(\mathbf{1}^{complexed}+\mathbf{2}^{complexed})$, are 25/75 and 70/30, respectively (Scheme 3).

The apparent stereospecificities (*R*/*S* ratios in the product, Me_3MQP^+) in the reactions with chloranil and 1,4-benzoquinone under various proportions of complexed molecules were also studied and the results are summarized in Table 3.

Table 1. Kinetic Parameters for Interconversions Among Three Rotational Isomers of Me_3MQPH at 273 K[a]

Kinetic parameter[b]	Uncomplexed	Complexed by Mg^{2+}
$k_{12}s^{-1}$	0.83	0.24
$k_{21}s^{-1}$	1.02	0.35
$k_{23}s^{-1}$	44.7	0.48
$k_{32}s^{-1}$	50.6	0.092
$k_{31}s^{-1}$	105	0.077
$k_{13}s^{-1}$	75.1	0.28
$\Delta H^{\neq}_{12}$/kcal mol^{-1}	11.88±0.43	12.84±0.30
$\Delta S^{\neq}_{12}$/cal mol^{-1} deg^{-1}	−15.25±1.19	−14.20±0.76
$\Delta H^{\neq}_{23}$/kcal mol^{-1}	13.65±0.12	17.40±1.00
$\Delta S^{\neq}_{23}$/cal mol^{-1} deg^{-1}	−0.85±0.52	3.90±0.44
$\Delta H^{\neq}_{31}$/kcal mol^{-1}	10.64±0.61	19.71±0.12
$\Delta S^{\neq}_{31}$/cal mol^{-1} deg^{-1}	−10.15±0.19	8.70±0.42

a) Subscripts correspond to the conformers represented in Schemes 1 and 2. b) Rate constants were calculated from the corresponding kinetic parameters using the Eyring equation and the following relationships: $p_1k_{12}=p_2k_{21}$, $p_2k_{23}=p_3k_{32}$, $p_3k_{31}=p_1k_{13}$, $p_1:p_2:p_3=34:41:26$ (Uncomplexed) and 13:19:68 (Complexed by Mg^{2+}).

Discussion

Kinetic Parameters. The entropies of activation for the rotation from $\mathbf{2}^{complexed}$ to $\mathbf{3}^{complexed}$ and from $\mathbf{3}^{complexed}$ to $\mathbf{1}^{complexed}$ are positive, whereas those for the other processes are negative. It should be noted that the former processes involve rotation of the C_3–$C_{carbonyl}$ bond in which the carbonyl oxygen is chelated by a magnesium ion. Thus, the positive entropy can be interpreted as the result form the release of a magnesium ion at the transition state of the rotation of this bond. Since the chelated oxygen is so bulky to flip, the complexed species can interconvert between these conformers quite hardly.

It is interesting to point out that the rate constants for the interconversions from **1** to **2** are much smaller than the others in the absence of magnesium ion. The fact suggests that the property of the $C_{carbonyl}$–N_{amide} bond in these conformers are similar to those of aliphatic amides, because the π-orbitals in the carbonyl groups of these conformers are nearly perpendicular to those in the ring[33—35] and partial π-character arises between the $C_{carbonyl}$ and N_{amide} atoms resulting in higher energy barrier for the rotation of this bond.

The retardation of rates for bond rotation on addition of magnesium ion is most significant in the interconversions between **3** and **1** and between **3** and **2**. The observation is in accord with the result obtained from the ^{1}H NMR spectroscopy that the *syn*-orientation is the most stable conformation for the complexed Me_3MQPH because the magnesium ion coordinates on the carbonyl oxygen making this moiety bulky.

The Role of Magnesium Ion. It was suggested in the previous paper[5] that the conformation at the transition state in the oxidation of Me_3MQPH with chloranil in the absence of magnesium ion is casted by the conformation at its ground state. At the same time, it

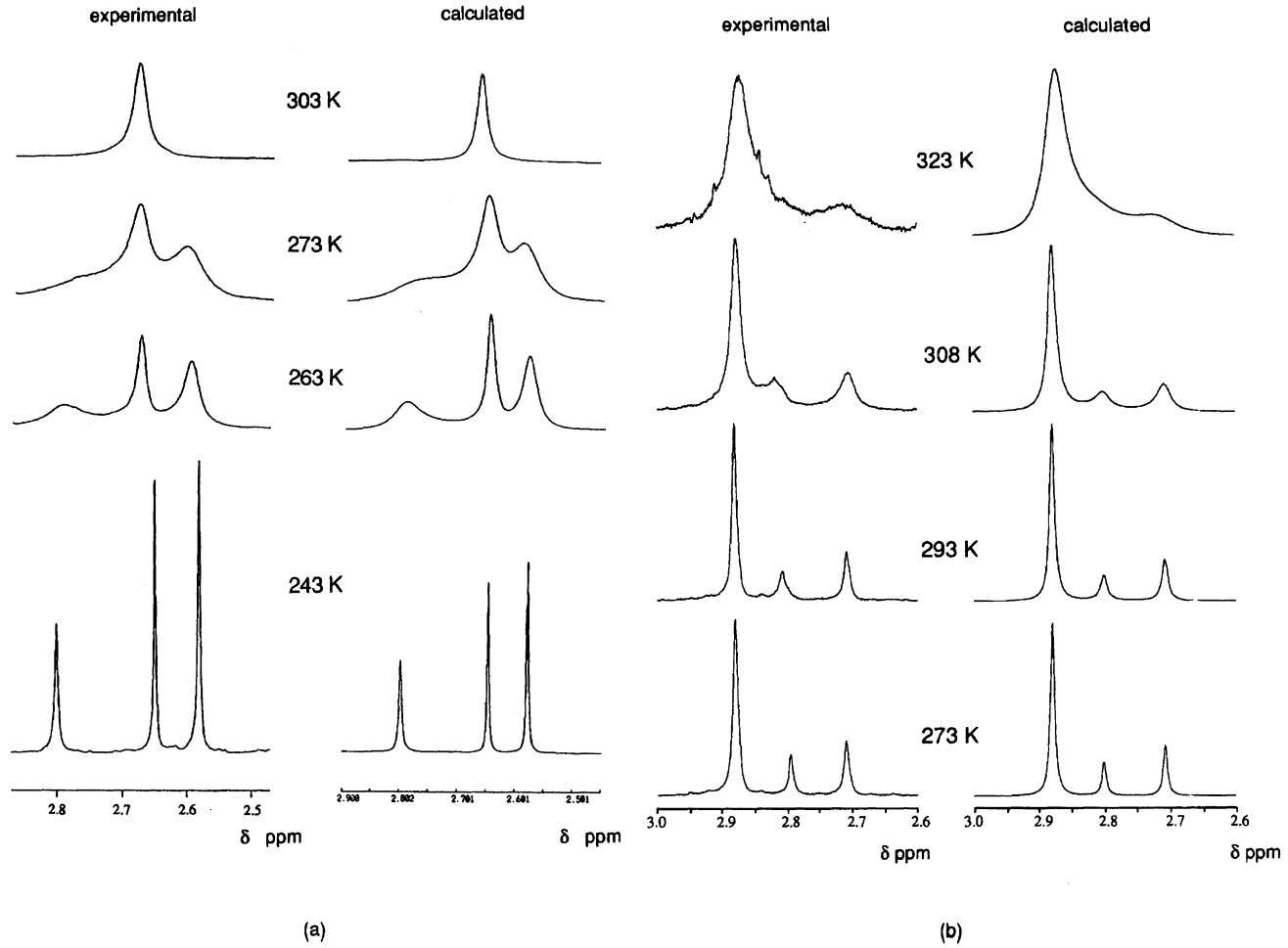
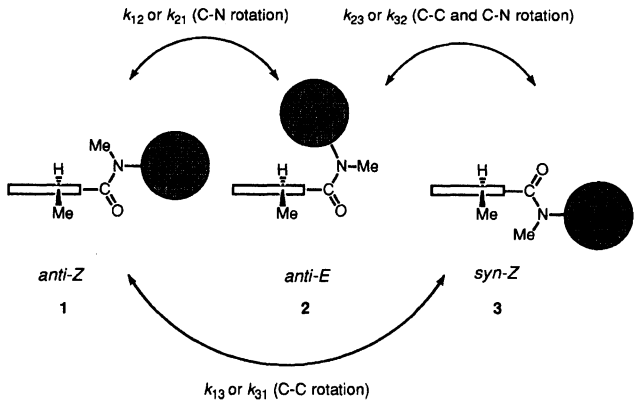


Fig. 2. Experimental and calculated ^{1}H NMR spectra (CD_3CN, 400 MHz) of Me_3MQPH at principal temperatures: (a) in the absence of magnesium ion. (b) in the presence of magnesium ion. The rates for interconversion at various temperatures were calculated from the corresponding kinetic parameters shown in Table 1 using the Eyring equation.



Scheme 2.

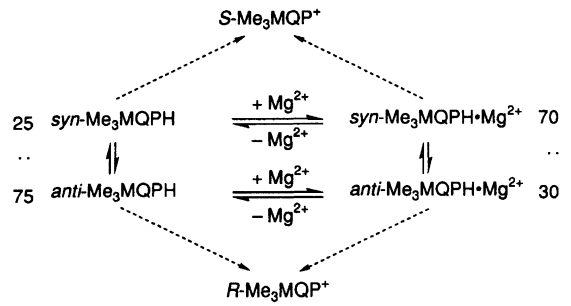
Table 2. Equilibrium Composition of Rotational Isomers as a Function of $[Mg^{2+}]/[Me_3MQPH]$ Ratio

$[Mg^{2+}]$/mM	0	14	62	41	106	400
$[Me_3MQPH]$/mM	40	58	121	57	104	42
$[Mg^{2+}]/[Me_3MQPH]$	0	0.24	0.51	0.71	1.0	9.5
Complex[a,b]/%	0	23	50	68	92[c]	100
1 (*anti-Z*)[a,d]/%	34	24	16	12	14	13
2 (*anti-E*)[a,d]/%	41	29	19	19	16	19
3 (*syn-Z*)[a,d]/%	26	47	65	69	70	68

a) Errors are estimated to be ±5%. b) Complex %=$[Me_3MQPH–Mg^{2+}]/([Me_3MQPH–Mg^{2+}]+[Me_3MQPH])$. c) Under the normal reaction conditions, where $[Me_3MQPH]=[Mg^{2+}]=1$ mM, 38% of the Me_3MQPH molecules are complexed. d) Total of complexed and uncomplexed species.

was found that the *anti/syn* difference in Gibbs energy at the tranasition state for this oxidation is not affected by the presence or absence of magnesium ion. The suggestion means that the reaction proceeds through the uncomplexed molecule and the *R/S* ratio in the product is directly determined by the *syn/anti* ratio in the ground-state conformation of uncomplexed Me_3MQPH (**3**/(**1**+**2**)) even in the presence of magnesium ion.

As is seen in Table 3, the *R/S* ratio in the product is 1.3/1, whereas the *anti/syn* ratio is 75/25 (or 3.0/1) which is different largely from the *R/S* ratio. When one looks at the conformation **2**, it might be recognized that the reacting C_4–hydrogen in this conformer



Scheme 3.

Table 3. R/S Ratio in Me_3MQP^+ Obtained from the Oxidation of (4R)-Me_3MQPH with Quinones in the Presence of Magnesium Perchlorate

Quinone	$[Mg^{2+}]/[Me_3MQPH]$	T/K	R/S Ratio
Chloranil	0	293	1.28/1
	0.5	293	1.16/1
	1	293	1.33/1
	2.5	293	1.50/1
	5	293	1.52/1
	100	293	1.80/1
	50	255	1.83/1
1,4-Benzoquinone	0	293	No reactn.
	1	293	1/7.61
	100	293	1/10.8

is set in a different situation from those in the other conformers: steric interference by a large α-methylbenzyl group. That is, it is unprobable to expect that the conformers **1** and **2** exert the same reactivity toward the oxidation by a quinone, although they have the same *anti*-conformation.[36] The relative abundance of uncomplexed *anti-Z* to *syn-Z*, [**1**]/[**3**], is 34/25=1.3/1 from Table 2. The value is exactly the same with that observed as the R/S ratio in the product. Is this a fortuitous coincidence? Of course, since the bond rotation in an uncomplexed molecule takes place faster than the oxidation of Me_3MQPH molecule at 293 K,[27] the relative abundance of the conformers of uncomplexed molecules always stays constant even in the presence of magnesium ion.

In the presence of an equivalent amount of magnesium ion, the R/S ratio does not change appreciably. Even in its presence of 100 times excess, the value, 1.8/1, is within the limit of experimental error when one takes into consideration large salt effect by such concentrated magnesium ion. The present observation reveals that the reaction with chloranil, a reactive oxidizing reagent, always proceeds from free molecules of Me_3MQPH. In other words, the complex is abortive and magnesium ion plays an inhibitory role in this reaction where the oxidizing reagent is highly reactive.[17,22]

Since 1, 4- benzoquinone does not react with Me_3MQPH in the absence of magnesium ion, there remains no doubt that magnesium ion plays a role of catalysis for this reaction with less reactive oxidizing reagent. Here, the reactivity of the complexed *syn*-conformer, **3**, is calculated to be about 1.5 times as large as that of the complexed *anti*-conformer, **1**. The conclusion is in good agreement with that previously reported:[5] The *syn*-conformer is more reactive than the other because of the assistance by the carbonyl dipole. However, on the other hand, fifty-percent larger reactivity may be less than error and the complexed *syn-Z* conformer may have similar reactivity to the complexed *anti-Z* conformer as was elucidated in the uncomplexed system (vide infra). We cannot guarantee the accuracy of the value at present. This topic will be discussed again in the last part of this discussion.

Effect of Lithium Ion on the R/S Ratio and Kinetics. The results mentioned above predict that magnesium ion is not involved in the reaction with chloranil except for exerting small salt effect on the R/S ratio in the product. Since kinetics of the reaction with chloranil in the presence of magnesium ion has difficulty to be followed because of spectral complexity, the reaction was studied under the coexistence of lithium ion in place of magnesium ion to confirm the salt effect. Both the ratios and second-order kinetic constants are summarized in Table 4.

It is known that lithium ion forms no complex with an NAD(P)H analog.[16,37] Nevertheless, Table 4 shows that the R/S ratio in the product changes slightly on addition of large excess lithium perchlorate. The change is comparable to that caused by magnesium ion. The rate constant also changes a little bit. Thus, it is confirmed that both the R/S ratio and rate constant are the subject of salt effect as discussed above and the variation in the R/S ratio listed in Table 3 is safely attributable to the salt effect by magnesium ion.

Energy Diagram of the Reaction. The discussion in the present and previous papers has revealed that the *syn*-conformer of Me_3MQPH at the ground state assumes the *syn*-conformation at the transition state of the reaction and the *anti*-conformer at the ground state carries its history up to the transition state to assume the *anti*-conformation. The difference in Gibbs energy of activation between the *syn*- and *anti*-conformers was reported in the previous paper,[5] and the difference in Gibbs energy between the *syn*- and

Table 4. R/S Ratio in Me_3MQP^+ Obtained from the Oxidation of (4R)-Me_3MQPH and Rate Constant for the Oxidation with Chloranil in the Presence of Lithium Perchlorate at 293 K

$[Li^+]/[Me_3MQPH]$	R/S	$k_2/M^{-1}\,s^{-1}$ [a]
0	1.28/1	123±6
1	1.38/1	—
50	1.50/1	110±6
500	—	106±5

a) Studied with 3-(α-methylbenzylcarbamoyl)-1,2,4-trimethyl-1,4-dihydroquinoline (Me_2MQPH).

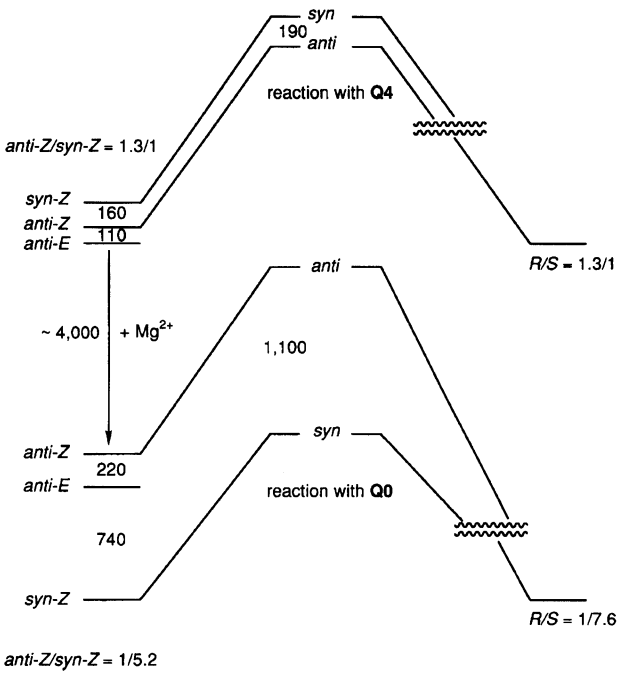


Fig. 3. Energy diagram for reactions of Me_3MQPH with chloranil (**Q4**) and 1,4-benzoquinone (**Q0**) in the presence of magnesium ion (unit, cal).

anti-conformers at the ground state has been elucidated by the present research. Therefore, now, we are able to draw a quantitative reaction diagram for the oxidations of *syn*- and *anti*-Me_3MQPH with 1,4-benzoquinone derivatives. Examples are illustrated in Fig. 3.

Figure 3 demonstrates that the environmental conditions determine the most stable conformation at the ground state of the reaction and relative stability of conformations casts the R/S ratio in the product. Energy difference at the ground state is taken up to the transition state without changing its value appreciably.

This conclusion is quite important for considering enzyme chemistry. Stereochemistry associated with an enzyme reaction has to be very close to 100% in purity. No other enantiomer (or diastereomer) is allowed to be produced. If the stereochemistry were determined at the transition state, the process has to start to move along the reaction coordinate regardless it is the route to the correct or wrong transition state. Since the activation energy to the transition state of wrong conformation is so higher than that to the correct one, the former process comes back to the ground state before it reaches the transition state. In other words, there is unnecessary movement of atoms, and, therefore, unnecessary consumption of energy. In addition, it is another energy consuming and difficult process to produce a large energy difference (more than 10 kcal mol^{-1}) at the transition state by the difference in molecular arrangement only.

On the other hand, when the conformation at the ground state determines the stereochemistry of the product, the only one reaction along the correct process may proceed, which is perfectly economic in energy. Furthermore, the idea of ground-state template suggests that once an enzyme fixes the orientation of carbonyl dipole at the ground state, the stereochemistry of the product is already defined at this stage without constructing walls at the vicinity of the coenzyme.[38] This is again economic in constructing an enzyme, and the idea is interesting from the viewpoint of evolution of enzymes.

The present work partially supported by Grant-in-Aid for Scientific Research Nos. 03854057 (M.O.), 04303002 (A.O.), 04403006 (A.O.), and 1068 (Y.M.) from the Ministry of Education, Science and Culture.

Computation time was provided by the Supercomputer Laboratory, Institute for Chemical Research, Kyoto University.

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