Tetrahedron Vol. 42, No. 4, pp. 961 to 973, 1986
Printed in Great Britain.

0040-4020/86 $3.00 + .00
Pergamon Press Ltd.

NAD(P)$^+$-NAD(P)H MODELS. 57. STEREOCHEMISTRY IN (NET) HYDRIDE TRANSFER FROM AND TO NAD(P)$^+$-NAD(P)H MODELS: CHIRALITY SINK

Atsuyoshi OHNO,* Masanori KASHIWAGI, Yuji ISHIHARA,[1]

Satoshi USHIDA, and Shinzaburo OKA

Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, JAPAN

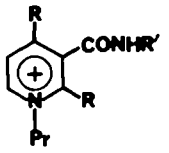
(*Received in USA* 23 *May* 1985)

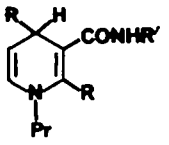
Abstract—NAD(P)H/NAD(P)$^+$ and related compounds have intrinsic chirality against the axis along the C_3-$C_{carbonyl}$ single bond. A model compound which has a stable conformation with respect to this chirality was synthesized and the conformational relationship between the carbonyl dipole and the reacting hydrogen was studied. It has been concluded that there is a relationship between conformations of these groups both in reduction and in oxidation provided the substrate is a neutral species. The result is discussed in relation with the hypothesis proposed previously on the basis of quantum chemical calculations.

INTRODUCTION

When a dehydrogenase catalyzes the reduction of a substrate with NADH or NADPH, two hydrogens on the achiral carbon atom at the 4-position of dihydropyridine ring of the coenzyme are sterically discriminated. For example, alcohol dehydrogenase (EC 1.1.1.1) catalyzes the transfer of *pro-R* hydrogen of the coenzyme (A-specific),[2] whereas phosphogluconate dehydrogenase (EC 1.1.1.44) utilizes the *pro-S* hydrogen (B-specific).[3] However, the A,B-specificity seems to have no relationship with the stereochemistry of reduction product.[4]

It is easily expected for an enzymic reaction that either side of the dihydropyridine ring may be blocked by a wall of enzyme protein when NAD(P)H associates with a dehydrogenase and, as a result, either *pro-R* or *-S* hydrogen is forced to face against the substrate in the active site so that only one hydrogen is available for the reaction. On the other hand, it was a surprise that optically active N-α-methylbenzyl-1-propyl1,4-dihydronicotinamide (PNPH), a model of NAD(P)H, which has a chiral center separated by five atoms from the reaction center, reduced ethyl benzoylformate and other substrates in about 20% e.e.[5-7]





PNA^+ : R = R' = H

PNP^+ : R = H, R' = $\begin{matrix}Me\\Ph\end{matrix}$>CH-

Me_2PNP^+ : R = Me, R' = $\begin{matrix}Me\\Ph\end{matrix}$>CH-

PNAH : R = R' = H

PNPH : R = H, R' = $\begin{matrix}Me\\Ph\end{matrix}$>CH-

Me_2PNPH : R = Me, R' = $\begin{matrix}Me\\Ph\end{matrix}$>CH-

Recently, Buck and his co-workers proposed an interesting hypothesis for the stereospecificity associated with the reaction of dihydronicotinamide derivative.[8,9] Based on MINDO/3 and STO-3G calculations, they came to a conclusion that the conformation in which the carbonyl dipole in dihydronicotinamide points toward the substrate corresponds to the reaction of low enthalpy of activation, whereas the conformation in which the dipole points away from the substrate is associated with high enthalpy of activation. That is, the C-4 hydrogen in the face pointed by the carbonyl dipole is transferred more rapidly than the other (Fig. 1). The difference amounts to 33 kJ/mole for the

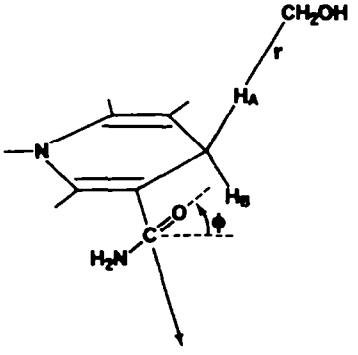


Fig. 1. Transition state for the transfer of H_A. Calculations have been done in relation with the tortional angle ϕ and bond length r.

3-carbamoylpyridinium-methanol system. It has also been elucidated that the X-ray crystal structure of N-α-methylbenzyl-1-propyl-2,4-dimethyl-1,4-dihydronicotinamide (Me_2PNPH) sets its C_4-H and C=O bonds in *syn*-configuration,[10] which may provide a part of supporting evidence for their calculations.

Although the hypothesis was extended to explain the A,B-specificity in enzymic system,[10] factors that govern the specificity in an enzymic system would be much more complicated than the model subjected to the calculation. The coenzyme in an active site of enzyme is strongly influenced by environmental potentials and the orientation of the carbonyl dipole may have just a minor importance, if any. Indeed, it was reported that 3-cyanopyridine adenine dinucleotide, which has no carbonyl group, exerts the identical stereospecificity to that of NAD^+ in enzymic reaction system.[11] Moreover, when the coenzyme binds to an enzyme, it locates close to the wall of active site cleft,[12-15] as mentioned above, and the substrate can find no chance to choose one of two C-4 hydrogens with equal probability.

Nevertheless, the hypothesis is quite attractive in the sense that it includes an interesting suggestion for the mechanism of asymmetric induction in organic chemistry and is worth to be tested experimentally. At the same time, if the three-dimensinal structure of the enzyme has been constructed in the process of billions of years of biological evolution so as to promote the reduction most efficiently, the stereochemical trick employed by the enzyme may also be an important suggestion for understanding the mechanism of catalysis.

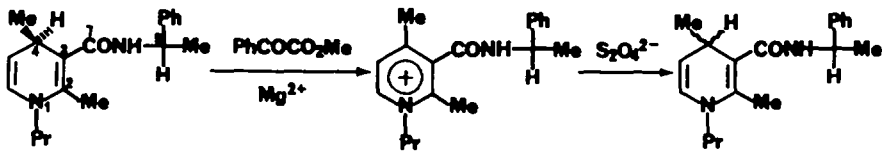
Since the unsubstituted carbamoyl group in 3-carbamoyl-1,4-dihydropyridine/pyridinium ion system can rotate freely in solution, it is necessary to design a compound to have a stable conformation with respect to the "axial chirality at the carbonyl group" (ACCG) for testing the validity of the hypothesis from the viewpoint of chemical reactions. We synthesized several model compounds and found that one of them has reasonably stable conformation to be subjected to the study. In this paper, we will describe the results obtained from the reaction with this particular model compound in relation with the hypothesis proposed by Buck and his co-workers.

RESULTS AND DISCUSSION

A Model with Stable Conformation

Although X-ray crystallography has revealed that Me_2PNPH in crystalline state has *syn*-conformation with respect to the C_4-H and C=O bonds,[10] the conformation is not stable in solution; 4*R*,9*R*-Me_2PNPH reduced methyl benzoylformate into methyl *R*-mandelate in almost perfect enantiospecificity,[16] and 9*R*-Me_2PNP^+ (the oxidized form of 9*R*-Me_2PNPH) can be recovered almost quantitatively from the reaction mixture. If Buck's hypothesis operates for this reaction, the 9*R*-Me_2PNP^+ recovered from the reaction mixture has to be one of two enantiomers with respect to ACCG, because the hydrogen transferred onto the substrate departed only from the B-side of this dihydropyridine derivative. In ^{1}HNMR spectrum obtained from a $CDCl_3$ solution, signals from methyl protons at the 2- and 4-positions of the Me_2PNP^+ appeared broad, which may suggest that the rotation around the C_3-$C_{carbonyl}$ single bond is restricted but not inhibited completely within a time scale of NMR spectroscopy. 9*R*-Me_2PNP^+ isolated from the reaction mixture as described above was reduced by sodium dithionite in the ordinary procedure, but the 9*R*-Me_2PNPH thus obtained was found to be racemic with respect to the carbon atom at the 4-position (Scheme 1). The result

Scheme 1



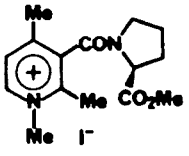
racemic

indicates either the orientation of carbonyl dipole does not exert an influence on the stereochemistry of approaching hydrogen[17,18] or free rotation is allowed for the C_3-$C_{carbonyl}$ single bond. One possible explanation for the free rotation is that, in the reduced form of the model, the carbon atom at the 4-position has sp^3-configuration, which implies that the substituent on this position sticks out of the plane of dihydropyridine ring so that steric interference by this substituent against the rotation of the carbonyl group in the

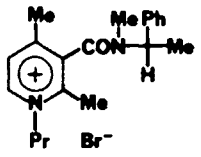
carbamoyl moiety becomes less important than the interference by the corresponding substituent in the oxidized form. When the conformation with respect to ACCG in the oxidized form is stable, however, the conformation in the transition state of (net) hydride transfer has to remain in the oxidized form even if the carbonyl group can rotate freely in the reduced form. Therefore, the result obtained here suggests the possibility that the conformation is not sufficiently frozen in Me_2PNP^+ in accordance with the result from NMR spectroscopy.

In order to improve the situation, model compounds that possess di-substituted amide group were synthesized (*e.g.*, $Me_2MNPr^+I^-$ and $Me_3PNP^+Br^-$). The conformations of these salts were so stable that they were able to be separated into each diastereomer.[19] However, unfortunately, these pyridinium salts resisted reduction to their corresponding 1,4-dihydro derivatives.

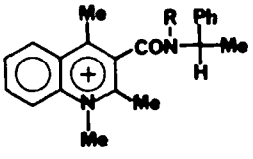
3-(N-α-Methylbenzyl)carbamoyl-1,2,4-trimethyl-1,4-dihydroquinoline (Me_2MQPH) reduces methyl benzoylformate and other compounds in the presence of aluminum chloride.[20] Stereospecificity associated with the reduction by 4*S*,11*S*-Me_2MQPH is also excellent; benzaldehyde-1-*d* was reduced into *R*-benzyl alcohol-α-*d* in 88% e.e. Since the reaction center in a quinoline-type model compound is sterically more hindered than the corresponding position in a pyridine-type compound which has no *peri*-hydrogen, the conformational stability with respect to ACCG in Me_2MQP^+ (oxidized form of Me_2MQPH) seems to be higher than that in Me_2PNP^+. As a matter of fact, in ^{1}HNMR spectrum obtained from a $CDCl_3$ solution, the signals



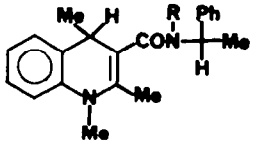
$Me_2MNPr^+I^-$



$Me_3PNP^+Br^-$



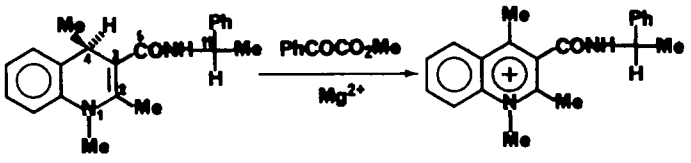
Me_2MQP^+ : R = H
Me_3MQP^+ : R = Me



Me_2MQPH : R = H
Me_3MQPH : R = Me

from methyl protons at the 2- and 4-positions in Me_2MQP^+ appeared to be two discrete singlets, respectively, in contrast to those in Me_2PNP^+. However, the conformation with respect to ACCG in the corresponding reduced form, Me_2MQPH, was found to be not stable enough to afford discernible isomers. Methyl benzoylformate was reduced by 4*R*,11*R*-Me_2MQPH into methyl *R*-mandelate in the presence of magnesium perchlorate in quantitative chemical yield with 95% e.e. The corresponding oxidized form was isolated from the reaction mixture (Scheme 2). This oxidized form was confirmed, by ^{1}HNMR spectroscopy, to be a mixture of equivalent amounts of two isomers with respect to ACCG, which was also witnessed by the fact that the reduction of this Me_2MQP^+ with 1-propyl-1,4-dihydronicotinamide (PNAH) or sodium dithionite afforded racemic Me_2MQPH with respect to the hydrogen at the 4-position. Thus, the conformation of Me_2MQPH/Me_2MQP^+ system is still labile under the reaction condition.

Scheme 2



racemic

Finally, a quinoline-type model compound which possesses a di-substituted amide structure was examined. 11*R*-3-(N-MethylN-α-methylbenzyl)carbamoyl-2,4-dimethylquinoline had rather stable conformation with respect to ACCG and both diastereoisomers were able to be separated on column chromatography. Since we do not know the absolute conformation of this quinoline derivative with respect to ACCG at present, the isomer which was eluted earlier will be hereafter referred to as the 9*X*,11*R*-isomer and the isomer eluted later will be denoted as

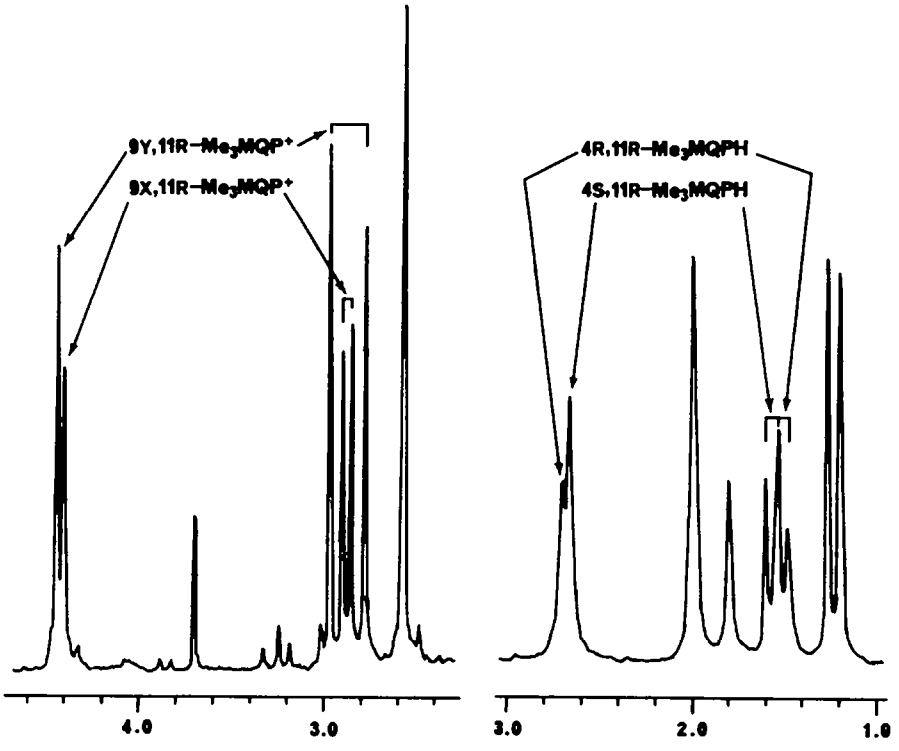
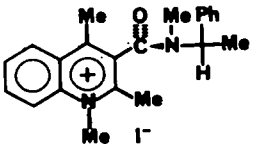
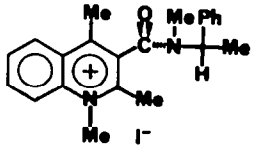


Fig. 2. 100 MHz ^{1}HNMR spectra from diastereomers of (a) Me_3MQP^+ and (b) Me_3MQPH.



9*X*(or *Y*),11*R*-Me_3MQP^+



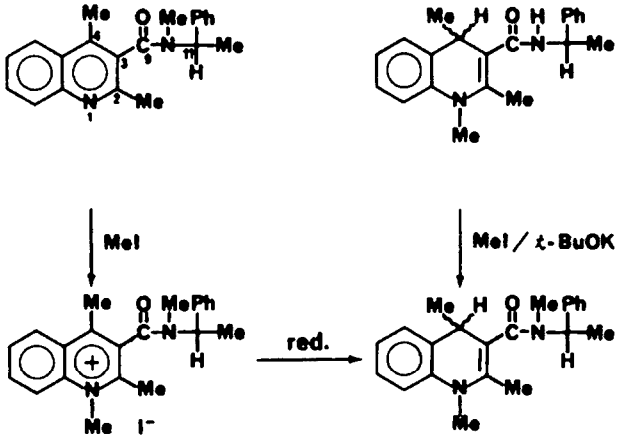
9*Y*(or *X*),11*R*-Me_3MQP^+

9*Y*,11*R*-isomer.

Treatment of the diastereomers with methyl iodide gave the corresponding quinolinium salts ($Me_3MQP^+I^-$) without any effect on ACCG. The quinolinium salts afforded the corresponding diastereomers of 1,4-dihydro derivatives (Me_3MQPH), respectively, on reduction with PNPH or with sodium dithionite. In Me_3MQPH, however, the ACCG is not stable and the C_3-$C_{carbonyl}$ bond seems to be subject to free rotation.

It was also found, as will be described in the experimental section, that methylation of Me_2MQPH under an alkaline condition results in the formation of Me_3MQPH without influencing the configurations at the 4-position and the benzylic carbon (Scheme 3). Characteristic signals in ^{1}HNMR spectra from diastereomers of Me_3MQP^+ and Me_3MQPH are illustrated in Fig. 2.

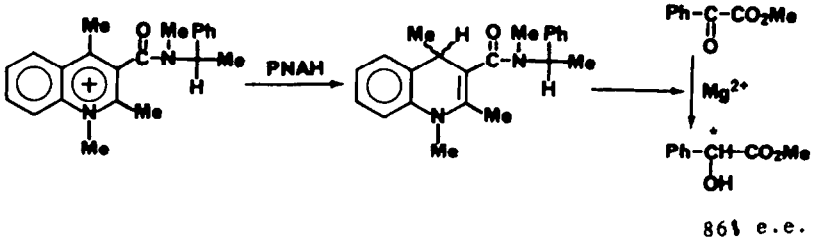
Scheme 3



Reduction of Me_3MQP^+

11*R*-Me_3MQPH was obtained by the reduction of 9*X*,11*R*-Me_3MQP^+ with sodium dithionite in a biphasic solvent consisting of dichloromethane and aqueous alkaline buffer. The ratio of diastereomers in this Me_3MQPH was determined by the ^{1}HNMR spectrum to be 4*R*,11*R* : 4*S*,11*R* = 33 : 67. Although Buck and his co-workers tried to apply the hypothesis to negatively charged hydridic substrates such as BH_4^-, AlH_4^-, HSO_2^-,[10] the value is not consistent with the prediction. On the other hand, a neutral reductant, PNAH, in methanol afforded 4*S*,11*R*-Me_3MQPH predominantly. Since the ^{1}HNMR spectrum of this product showed no signal corresponding to the 4*R*,11*R*-isomer, the enantiomer excess in this product could not be elucidated at this stage. Therefore, this product was subjected to the reduction of methyl benzoylformate and it was found that the enantiomer excess in the product, methyl *S*-mandelate, was 86% (Scheme 4). That

Scheme 4



is, the diastereomeric ratio in the Me_3MQPH obtained by the reduction with PNAH can be estimated to be better than 4*R*,11*R* : 4*S*,11*R* = 7 : 93. Taking into account the fact that the stereospecificity of the reduction of methyl benzoylformate with Me_3MQPH is about 99%, the ratio may be corrected to about 6 : 94.

Similarly, the other diastereomer, 9*Y*,11*R*-Me_3MQP^+ (more than 95% diastereomeric purity), was reduced by PNAH. Thus obtained Me_3MQPH was subjected to the reduction of methyl benzoylformate and the enantiomer excess in the resulted methyl *R*-mandelate was elucidated to be 88%, which revealed that the diastereomeric ratio in the Me_3MQPH obtained was, after the correction for 95% purity and 99% stereospecificity of the reaction, 4*R*,11*R* : 4*S*,11*R* = 94 : 6. The value is in excellent agreement with that obtained from 9*X*,11*R*-Me_3MQPH.

Consequently, it is concluded that the *X*- and *Y*-conformations with respect to ACCG in Me_3MQP^+ corresponds to 4*S*- and 4*R*-configurations in Me_3MQPH, respectively, in more than 94% stereospecificity when PNAH is employed as a reductant. However, the reduction with an anionic species does not hold the stereospecificity.

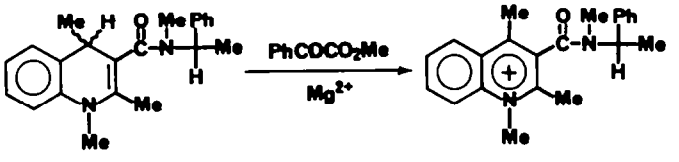
Oxidation of Me_3MQPH

Since Me_3MQPH would not crystallize and is susceptible to decomposition, it was difficult to obtain this compound in diastereomerically pure state.

4*S*,11*R*-Me_3MQPH in 93% diastereomeric purity was oxidized by methyl benzoylformate, and the resulted Me_3MQP^+ was found, by ^{1}HNMR spectroscopy, to be a mixture of 9*Y*,11*R* : 9*X*,11*R* = 10 : 90 or, after correction to the purity, 3 : 97. Similarly, 4*R*,11*R*-Me_3MQPH in 97% purity afforded the oxidized form in the ratio of 9*Y*,11*R* : 9*X*,11*R* = >95 : <5 or, after the correction to the purity, better than 98 : 2 in excellent agreement with the result obtained from the former isomer (Scheme 5). At the same time, the diastereomeric ratios observed here coincide quite well with those observed in the reduction of Me_3MQP^+.

On the other hand, oxidation of 4*S*,11*R*- and 4*R*,11*R*-Me_3MQPH with ferricyanide, an anionic oxidant, afforded racemic Me_3MQP^+ with respect to ACCG. Here again an anionic species exerted no stereospecificity in agreement with the reverse reaction.

Scheme 5



96% e.e.

Correlation with the Carbonyl Dipole

As mentioned above, the *X*- and *Y*-conformations in Me_3MQP^+ have been correlated specifically with the 4*S*- and 4*R*-configurations in Me_3MQPH, respctively, by both reduction and oxidation. Therefore, it is evident now that the reacting hydrogen in the reaction of dihydronicotinamide derivative has conformational relationship with the carbonyl dipole provided the substrate is a neutral species. The result seems in agreement with the hypothesis proposed by Buck and his co-workers. However, unfortunately, since we have no evidence on

the absolute conformation of 9*X*- and/or 9*Y*-Me_3MQP^+, we cannot discuss whether the reacting hydrogen has the *syn*-configuration in relation with the carbonyl dipole or it is in *anti*-configuration.

Another factor which should be taken into account is that the oxidation of Me_3MQPH is mediated by a magnesium ion. Since this bivalent ion can coordinate both the model and oxidant,[21] the orientation of carbonyl dipole may possibly restricted during the reaction in the presence of this metal ion. The present oxidation, however, did not proceed without magnesium ion and the situation without magnesium ion could not be studied. At least, the reduction process is free from the influence of magnesium ion.

In addition, the present model compound is substituted by a bulky α-methylbenzyl group on the carbamoyl group. Therefore, it is possible that the reaction is affected by the steric bulk of this α-methylbenzyl group; CPK-models for Me_3MQPH/Me_3MQP^+ show that the carbonyl-oxygen prefers to point *anti*-face with respect to this bulky substituent and the substrate approachs much easier from the face pointed by the carbonyl dipole than from the other face, which means that the reacting hydrogen is always set in the *syn*-position with respect to the carbonyl dipole regardless the amount of enthalpy of activation. The fact that small species such as dithionite and ferricyanide ions cannot keep the stereospecificity may be a support for the steric block instead of the enthalpic preference. If this is the case, the situation in the present mimetic system is quite similar to that exerted by an enzyme. That is, one face of the (dihydro)nicotinamide ring is intrinsically blocked by a bulky residue and the reaction takes place only in the face pointed by the carbonyl dipole.

In spite of these uncertainties, the present result has provided a positive evidence for the relationship between the conformation of carbonyl dipole and the stereochemistry of the reacting hydrogen, whatever the origin is. The present model system involves self-immolative transfer of the axial chirality at the 9-position of the oxidized form into the central chirality at the 4-position of the corresponding reduced form and *vice versa*. In this sense, the present reactions may be claimed as asymmetric inductions of brand-new type (chirality sink).[22]

It is also interesting to note that the reacting hydrogen in the reaction with Me_3MQPH/Me_3MQP^+ system leaves and arrives from the same face of the (dihydro)pyridine ring. Therefore, the diastereomers of Me_3MQP^+ are once resolved, their conformations and/or configurations might be kept unchanged throughout the redox reactions due to the effect of chirality sink. That is, a compound of this type may be used as a catalyst for asymmetric reactions without further resolution. The idea has been tested using PNAH as a reductant and methyl benzoylformate as an oxidant. The methyl mandelate obtained was in 99% e.e. in the first cycle and was in 90% e.e. in the second cycle. The value from the second cycle sounds reasonable taking into account the stereospecificity in the reaction with PNAH. Further effort to find a proper design to freeze the ACCG and the reactions of higher stereospecificity may supply an excellent catalyst for this purpose.

EXPERIMENTAL

Instruments

All melting and boiling points were not corrected. ^{1}HNMR spectra were recorded at 60, 100, and 400 MHz on a Varian T-60, a JEOL JNM-FX-100 Fourier

transform, and a JEOL GX-400 Fourier transform NMR spectrometers. Gas chromatographic data were recorded on a Yanaco G-1800F gas chromatogram. HPLC was performed with a Hitachi 655 liquid chromatograph. UV spectra were recorded on a Union Giken SM401 spectrophotometer. Elemental analyses were performed with a Yanaco MT-3 elemental analyzer.

Anhydrous Solvents and Lewis Acids

Tetrahydrofuran and ether were distilled over sodium benzophenone ketyl. Acetonitrile, benzene, dichloromethane, and pyridine were distilled over calcium hydride. Acetone was dried over Drierite and distilled. These solvents were distilled just prior to the use.

Magnesium perchlorate was powdered, dried at 100°C under reduced pressure in the presense of phosphorous pentoxide, placed in a sealed tube, and stored in a descicator.

Materials

Methyl benzoylformate[7], 1-propyl-1,4-dihydronicotinamide (PNAH)[23] and 4*R*,9*R*-N-α-methylbenzyl-2,4-dimethyl-1-propyl-1,4-dihydronicotinamide (4*R*,9*R*-Me_2PNPH)[16] were prepared according to the literature procedures.

2,4-Dimethyl-3-carboxyquinoline

In a 200 ml three-necked flask equipped with a magnetic stirrer, a reflux condenser, and a dropping funnel, was placed 31 g of ethyl 3-aminocrotonate in 50 ml of anhydrous ethanol. The agitated solution was warmed in an oil bath to about 50°C. Therein 27 g of *o*-aminoacetophenone in 50 ml of glacial acetic acid was added dropwise. Then the solution was heated to reflux for 24 h. After the evaporation of ethanol and glacial acetic acid under reduced pressure, the residue was distilled to give 37.5 g of 2,4-dimethyl-3-carboethoxyquinoline as a pale yellow oil in 82% yield; bp 140°C (0.1 mmHg).

^{1}HNMR ($CDCl_3$): δ^{TMS} 1.44 (t, J=7, 3H), 2.66 (s, 3H), 2.71 (s, 3H), 4.49 (t, J=7, 2H), and 7.5-8.05 (m, 4H).

The ester obtained was hydrolyzed in aqueous sodium hydroxide and, after the usual work-up, crude 2,4-dimethyl-3-carboxyquinoline was isolated in 99% yield based on the ester; mp 238-239°C.

^{1}HNMR ($CDCl_3$): δ^{TMS} 2.64 (s,3H), 2.69 (s, 3H), 4.00 (s, 3H), and 7.2-8.1 (m, 4H).

11R-3-(N-α-Methylbenzyl)carbamoyl-2,4-dimethylquinoline

In a 200 ml three-necked flask equipped with a magnetic stirrer, a reflux condenser, and a dropping funnel, were placed 8.04 g of 2,4-dimethyl-3-carboxyquinoline and 3.69 g of cyanuric chloride[24] in 60 ml of anhydrous acetone. With vigorous stirring, 5.6 g of triethylamine was added carefully drop by drop at room temperature. After the addition, the mixture was stirred for additional 4 h at room temperature. Then 4.84 g of *R*-α-methylbenzylamine in 10 ml of anhydrous acetone was added slowly and the mixture was stirred for 3 h at room temperature. After the filtration, the filtrate was condensed, and the residue was chromatographed on a column (50 cm x 2.6 cm ϕ) of silica gel with benzene-ethyl acetate (5:1 v/v) as an eluent to give 7.77 g of 11*R*-3-(N-α-methylbenzyl)carbamoyl-2,4-dimethylquinoline in 64% yield; mp 157.5-158.5°C.

^{1}HNMR ($CDCl_3$): δ^{TMS} 1.69 (d, J=7, 3H), 2.37 (s, 3H), 2.57 (s, 3H), 5.42 (dq, J=7, 1H), 6.80 (bd, J=7, 1H), and 7.26-7.86 (m, 9H).

11R-3-(N-α-Methylbenzyl)carbamoyl-1,2,4-trimethyl-1,4-dihydroquinoline

Into a teflon tube, 0.82 g of 11*R*-3-(N-α-methylbenzyl)carbamoyl-2,4-dimethylquinoline in 5 ml of ethanol and 3.6 g of methyl iodide were placed. The reaction was carried out under high pressure (10,000 kgw/cm^2) for 7 days at

room temperature. After the evaporation of ethanol and methyl iodide, the product (11*R*-Me_2MQP$^+$) was obtained quantitatively and used for the following reaction without any purification; mp 196-198-202°C. This quinolinium salt was a mixture of two diastereomers.

^{1}HNMR ($CDCl_3$): δ^{TMS} 1.61 (d, J=7, 3H), 2.68, 2.77 (s x 2, 3H), 2.98, 3.09 (s x 2, 3H), 4.47, 4.53 (s x 2, 3H), 5.31 (q, J=7, 1H), and 7.25-8.6 (m, 10H).

In a 200 ml flask fitted with a magnetic stirrer, a reflux condenser, and a dropping funnel, was placed 0.82 g of the quinolinium salt in 20 ml of methanol. To the solution, 1.6 g of sodium dithionite dissolved in 130 ml of 0.05 M-borate buffered aqueous solution was added dropwise. After the addition, the reaction was run overnight at room temperature in the dark under an atmosphere of nitrogen and a pale yellow precipitate was collected by filtration. The precipitate was dissolved in dichloromethane and dried over sodium sulfate. After the removal of the solvent under reduced pressure, the residue was chromatographed on a column (20 cm x 2.6 cm ϕ) of silica gel with benzene-ethyl acetate (20-10:1 v/v) as an eluent to give 0.268 g of 11*R*-3-(N-α-methylbenzyl)-carbamoyl1,2,4-trimethyl-1,4-dihydroquinoline (11*R*-Me_2MQPH) as a pale yellow solid in 46% yield. This is a mixture of two diastereomers.

^{1}HNMR ($CDCl_3$): δ^{TMS} 1.14, 1.15 (d x 2, J=6.8, 3H), 1.53 (d, J=6.8, 3H), 2.26, 2.30, (s x 2, 3H), 3.27 (s, 3H), 3.65 (q, J=6.8, 1H), 5.17, 5.23 (dq x 2, J=6.8, 6.8, 1H), 5.75 (bd, J=6.8, 1H), and 6.81-7.48 (m, 9H).

The product was dissolved into the smallest possible volume of methanol at 30-40°C. To this methanol solution, water was added dropwise until crystals began to appear. The flask was kept in the dark at room temperature overnight. The pale yellow precipitate appeared was collected on a filter and washed with water, then the precipitate was dried over calcium chloride under reduced pressure. After repeated recrystallizations several times, one of the two diastereomers was obtained as pale yellow needles (4*R*,11*R*-Me_2MQPH); mp 169-170 °C. $[\alpha]_D^{18}$ -192.2 ($CHCl_3$, c=0.100). CD [θ] = -190 x 10^4 (297 nm) and -1.77 x 10^4 (325 nm). $\lambda_{max}(CH_3CN)$ = 313 nm (ε = 1.05 x 10^4).

Anal. Calcd for $C_{21}H_{24}N_2O$: C, 78.71; H, 7.55; N, 8.74%. Found : C, 77.47; H, 7.51; N, 8.97%.

The 4*S*,11*S*-diastereomer was prepared similary starting from *S*-α-methylbenzylamine.

$[\alpha]_D^{27}$ +210.3 ($CHCl_3$, c=0.203).

11R-3-(N-Methyl-N-α-methylbenzyl)carbamoyl-1,2,4-trimethyl-1,4-dihydroquinoline

The corresponding quinolinium salt (11*R*-Me_3MQP$^+$) was prepared by the reaction of N-methyl-*R*-α-methylbenzylamine and 2,4-dimethyl-3-carboxyquinoline followed by methylation under high pressure. The whole procedure was essentially the same as described for the preparation of Me_2MQPH.

When 11*R*-3-(N-methyl-N-α-methylbenzyl)carbamoylquinoline was subjected to column chromatography on silica gel with benzene-ethyl acetate (7:3 v/v), two diastereomers were eluted separately. The diastereomer eluted earlier (9*X*,11*R*-isomer) was a pale yellow oil and partially solidified in a refrigerator.

^{1}HNMR ($CDCl_3$): δ^{TMS} 1.64 (d, J=6.9, 1H), 2.40 (s, 3H), 2.60 (s, 3H), 2.62 (s, 3H), 6.35 (q, J=6.9, 1H), and 6.9-8.05 (m, 9H).

The later fraction (9*Y*,11*R*-isomer) was a pale yellow oil.

^{1}HNMR ($CDCl_3$): δ^{TMS} 1.63 (d, J=7.1, 1H), 2.40 (s, 3H), 2.55 (s, 3H), 2.68 (s, 3H), 6.34 (q, J=7.1, 1H), and 6.9-8.05 (m, 9H).

These two diastereomers isomerized each other slowly at room temperature and the contamination of the other isomer became recognizable on a ^{1}HNMR spectrum after a week.

9*X*,11*R*-Me_3MQP$^+I^-$; yellow crystal.

^{1}HNMR ($CDCl_3$); δ^{TMS} 1.70 (d, J=7.2, 3H), 2.87 (s, 6H), 3.03 (s, 3H), 4.59 (s, 3H), 6.23 (q, J=7.2, 1H), 7.3-7.5 (m, 5H), and 7.75-8.35 (m, 4H).

Anal. Calcd for $C_{22}H_{25}N_2OI$: C, 57.40; H, 5.47; N, 6.08%. Found : C, 57.26; H, 5.43; N, 6.06%.

9*Y*,11*R*-Me_3MQP$^+I^-$, a yellow solid, was obtained only in crude state because this salt was quite deliquescent.

^{1}HNMR ($CDCl_3$) of the perchlorate: δ^{TMS} 1.67 (d, J=7.2, 3H), 2.72 (s,3H), 3.03 (s, 3H), 4.52 (s, 3H), 6.23 (q, J=7.2, 1H), 7.1-7.5 (m, 5H), and 7.8-8.35 (m, 4H).

Me_3MQPH was obtained in three ways but its optical data have not been obtained yet in convincing accuracy. Its chemical as well as diastereomeric purity was confirmed on NMR spectra.

Method A. In a 10 ml fask equipped with a magnetic stirrer and sealed with a serum cap, 139 mg (0.30 mmol) of 9*X*,11*R*Me_3MQP$^+I^-$ and 101 mg (0.60 mmol) of 1-propyl-1,4-dihydronicotinamide (PNAH) were placed and the atmosphere inside the flask was replaced by nitrogen. Then 3 ml of methanol was injected into the flask through a syringe and the mixture was stirred for 14 h at room temperature in the dark under an atmosphere of nitrogen. After evaporation of the solvent, the residue was dissolved into dichloromethane, washed three times with water, dried over sodium sulfate, and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with benzene-ethyl acetate (9:1 v/v) as an eluent to give 73 mg (73% yield) of 4*S*,11*R*-Me_3MQPH as a pale yellow viscous oil. Since 100 MHz ^{1}HNMR spectrum did not exibit the signals from the other diastereomer, diastreomeric purity of this product could not be determined by this method. The value, however, was obtained from the enantiomeric excess associated with the reduction of methyl benzoylformate by this compound.

^{1}HNMR ($CDCl_3$): δ^{TMS} 1.24 (d, J=6.8, 3H), 1.57 (d, J=6.8, 3H), 2.01 (s, 3H), 2.67 (s, 3H), 3.23 (s, 3H), 3.35-3.65 (m, 1H), 5.7-6.3 (m, 1H), and 6.75-7.35 (m, 9H).

λ_{max}(EtOH) = 307 nm (ε = 1.0 x 10^4).

Method B. In a 30 ml flask equipped with a magnetic stirrer, 46.4 mg (0.10 mmol) of 9*X*,11*R*-Me_3MQP$^+I^-$ was placed with 10 ml of 0.05 M borate buffer, 10 ml of dichloromethane, and 1 ml of methanol. Then, nitrogen gas was bubbled through the solution. To the solution, 140 mg (0.8 mmol) of sodium dithionite was added with vigorous stirring, then the flask was sealed with a serum cap immediately. The mixture was stirred vigorously for 24 h at room temperature in the dark under an atmosphere of nitrogen. The organic layer was separated and the aqueous layer was extracted twice with dichloromethane. The combined organic layer was dried over sodium sulfate, passed through a silica pad, and the solvent was evaporated to obtain the corresponding reduced product. The diastereomeric ratio determined on a ^{1}HNMR spectrum was 4*R*,11*R* : 4*S*,11*R* = 33 : 67. The residue was purified by preparative TLC on silica gel with benzene-ethyl acetate (7:3 v/v) as an eluent to obtain pure material in 22.1 mg was a 1 : 2 mixture of 4*R*,11*R*- and 4*S*,11*R*-Me_3MQPH.

Method C. In a 20 ml flask equipped with a magnetic stirrer and sealed with a serum cap, 128 mg of 4*R*,11*R*-Me_2MQPH and 227 mg of potassium *tert*-butoxide were placed and the atmosphere inside the flask was replaced by nitrogen. Then 20 ml of anhydrous tetrahydrofuran was injected into the flask through a syringe. The mixture was stirred for a while, then 0.125 ml of methyl iodide

was injected dropwise into the mixture with continuous stirring. The mixture was stirred for 12 h at room temperature in the dark under an atmosphere of nitrogen. The precipitate was filtered off and the filtrate was concentrated by evaporation of the solvent. The residue was dissolved into dichloromethane and the solution was washed twice with water, dried over sodium sulfate, passed through a silica pad, and the solvent was evaporated to obtain 4*R*,11*R*-Me_3MQPH in 130 mg (98% yield) as a highly viscous and almost colorless oil. Diastereomeric purity of this product was determined as mentioned in Method A.
^{1}HNMR ($CDCl_3$): δ^{TMS} 1.23 (d, J=6.9, 3H), 1.52 (d, J=6.9, 3H), 1.99 (s, 3H), 2.70 (s, 3H), 3.21 (s, 3H), 3.35-3.65 (m, 1H), 5.9-6.2 (m, 1H), and 6.7-7.4 (m, 9H).
λ_{max}(EtOH) = 306 nm ($\varepsilon = 1.1 \times 10^4$).

It was confirmed that no hydrogen exchange took place on Me_2MQPH during the reaction except for the one on the amide-nitrogen: a reaction mixture of Me_2MQPH and potassium *tert*-butoxide in tetrahydrofuran was quenched by deuterium oxide and found that no deuterium was incorporated into the recovered Me_2MQPH except for the one at the amide-nitrogen. This experiment reveals that the configurations at the 4-position and benzylic carbon in Me_2MQPH are not affected under the reaction condition.

Oxidation of Me_3MQPH with Methyl Benzoylformate

In a test tube (1.8 cm ϕ x 13 cm) equipped with a magnetic stirrer and sealed with a serum cap, 33.5 mg (0.10 mmol) of 4*R*,11*R*-Me_3MQPH and 25 mg (0.11 mmol) of anhydrous magnesium perchlorate were placed. Then, the atmosphere inside the tube was replaced by nitrogen and 2 ml of anhydrous acetonitrile and 16.5 mg (0.10 mmol) of methyl benzoylformate were injected into the tube through a syringe, successively. The mixture was stirred for 24 h at room temperature in the dark under an atmosphere of nitrogen. It was determined by gas chromatography (OV330, 1 m, 156°C, β-methylnaphthalene as an internal standard) that the conversion of the ester was 97% and the yield of methyl mandelate was 74% based on the consumed substrate. After evaporation of the solvent at room temperature, the residue was extracted with benzene several times. The crude Me_3MQP^+ salt was obtained as a residue which was insoluble to benzene. The diasteromeric composition in this salt was determined by 100 MHz ^{1}HNMR analysis to be more than 97% of 9*Y*,11*R*-isomer and less than 3% of 9*X*,11*R*-isomer.

The enantiomer excess in the afforded alcohol was 99% with predominancy of the *R*-configuration, which was determined by 400 MHz ^{1}HNMR analysis of the corresponding ester of (+)-α-methoxy-α-trifluoromethylphenylacetyl chloride according to the Mosher's procedure.[25]

Starting from 4*S*,11*R*-Me_3MQPH, 9*X*,11*R*-Me_3MQP^+ was isolated in more than 95% of diastereomeric predominancy together with methyl *S*-mandelate.

REFERENCES AND NOTES

1 Present Adress: Takeda Chemical Industries, Co., Ltd., 2-17-85 Juso-honmachi, Yodogawa-ku, Osaka 532, JAPAN.

2 Loewus, F. A.; Westheimer, F. H.; Vennesland, B., *J. Am. Chem. Soc.*, **1953**, *75*, 5018-5023.

3 Stern, B. K.; Vennesland, B., *J. Biol. Chem.*, **1960**, *235*, 205-208.

4 Everse, J.; Kaplan, N. O., *Advan. Enzymol.*, **1973**, *37*, 61-133.

5 Ohnishi, Y.; Kagami, M.; Ohno, A., *J. Am. Chem. Soc.*, **1975**, *97*, 4766-4768.

6 Ohnishi, Y.; Numakunai, T.; Kimura, T.; Ohno, A., *Tetrahedron Lett.*, **1976**, 2699-2702.

7 Ohno, A.; Kimura, T.; Kim, S. G.; Yamamoto, H.; Oka, S.; Ohnishi, Y., *Bioorg. Chem.*, **1977**, *6*, 21-29.

8 Donkersloot, M. C. A.; Buck, H. M., *J. Am. Chem. Soc.*, **1981**, *103*, 6549-6554.

9 Donkersloot, M. C. A.; Buck, H. M., *J. Am. Chem. Soc.*, **1981**, *103*, 6554-6558.

10 van Lier, P. M.; Donkersloot, M. C. A; Koster, A. S.; van Hooff, H. J. G.; Buck, H. M., *Recl. Trav. Chim. Paye-Bas*, **1982**, *101*, 119-120.

11 Biellmann, J.-F.; Hirth, C. G.; Jung, M. J.; Rosenheimer, N.; Wrixon, A. D., *Eur. J. Biochem.*, **1974**, *41*, 517-524.

12 Eklund, H.; Samama, J.-P.; Wallén, L.; Brändén, C.-I. *J., Mol. Biol.*, **1981**, *146*, 561-587.

13 Grau, U. M.; Trommer, W. E.; Rossmann, M. G., *J. Mol. Biol.*,**1981**, *151*, 289-307.

14 Walker, J. E.; Carne, A. F.; Runswick, M. J.; Bridgen, J.; Harris, J. I., *Eur. J. Biochem.*, **1980**, *108*, 549-565.

15 Biesecker, G.; Harris, J. I.; Thierry, J. C.; Walker, J. E.; Wonacott, A. J., *Nature*, **1977**, *266*, 328-333.

16 Ohno, A.; Ikeguchi, M.; Kimura, T.; Oka, S., *J. Am. Chem. Soc.*, **1979**, *101*, 7036-7040.

17 The calculations reported by Buck and his co-workers dealt with the C-H stretching mode only. However, it is reported that the bending mode is important as well. See Husky, W. P.; Schowen, R. L., *J. Am. Chem. Soc.*, **1983**, *105*, 5704-5706.

18 Hermes, J. D.; Morrical, S. W.; O'Leary, M. H.; Cleland, W. W., *Biochemistry*, **1984**, *23*, 5479-5488.

19 Cf. van Lier, P. M.; Meulendijks, G. H. W. M.; Buck, H. M., *Recl. Trav. Chim. Paye-Bas*, **1983**, *102*, 337-338.

20 Ohno, A.; Ishihara, Y.; Ushida, S.; Oka, S., *Tetrahedron Lett.*, **1982**, *23*, 3185-3188.

21 Cf. Ohno, A.; Kimura, T.; Yamamoto, H.; Kim, S. G.; Oka, S., *Bull. Chem. Soc. Jpn.*, **1977**, *50*, 1535-1538.

22 One-way transfer of the chirality has been reported. Meyers A. I.; Wettlaufer, D. G., *J. Am. Chem. Soc.*, **1984**, *106*, 1135-1136.

23 Ohno, A.; Yasui, S.; Nakamura, K.; Oka, S., *Bull. Chem. Soc. Jpn.*, **1978**, *51*, 290-293.

24 Venkataraman, K.; Wagle, D. R., *Tetrahedron Lett.*, **1979**, 3037-3040.

25 Dale, J. A.; Dull, D. L.; Mosher, H. S., *J. Org. Chem.*, **1969**, *34*, 2543-2549.