NADH Model Reaction. Importance of Hydroxy-groups in the Asymmetric Reduction of Ethyl Benzoylformate

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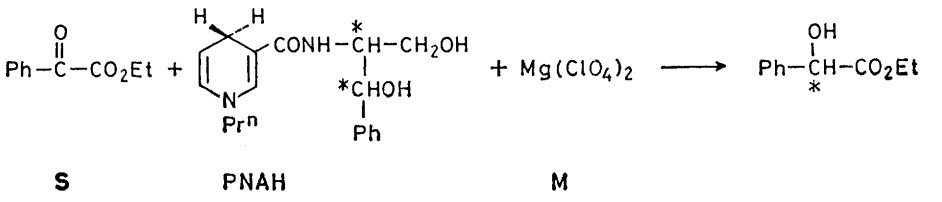
Ethyl benzoylformate has been reduced with an NADH analogue, *N*-{(1*S*)-2-hydroxy-1-[(*S*)-α-hydroxybenzyl]-ethyl}-1-propyl-1,4-dihydronicotinamide, to give (*S*)-mandelate in 2.9—28.5% e.e. The asymmetric yield was remarkably affected by the amount of magnesium perchlorate present and continuously changed throughout the reaction. The possible functions of hydroxy-groups in the model compound are discussed in the light of the resultant stereochemical outcome.

MANY biomimetic reactions for hydrogen transfer involving various dihydropyridine derivatives have been devised and investigated.[1] However, few stereochemical studies have been reported which simulate enzymic stereospecificity.[2] In a previous paper,[2c] we reported that the hydroxy-groups in a glucose moiety introduced into a dihydropyridine ring as the chiral source not only enhanced the reactivity, but also significantly affected the stereochemistry of the asymmetric reduction of iminium salt, presumably through electrostatic interaction between the substrate and reductants.



SCHEME 1

In order to explore the role of hydroxy-groups in more detail, we compared the reduction of an α-keto-ester both with a chiral reductant possessing hydroxy-groups on a dihydropyridine ring, using experimental conditions described in the literature,[3,4] and a reductant without such polar groups.

RESULTS AND DISCUSSION

The substrate, ethyl benzoylformate (S), was reduced in the presence of magnesium perchlorate (M), with *N*-{(1*S*)-2-hydroxy-1-[(*S*)-α-hydroxybenzyl]ethyl}-1-propyl-1,4-dihydronicotinamide (PNAH) (Scheme 1) in dry acetonitrile at room temperature under a nitrogen atmosphere for 72 h in the dark. The reaction afforded ethyl mandelate in 31—80% yield with a 2.9—28.5% enantioselectivity in favour of the *S*-isomer after hydrolysis of the reaction mixture. As can be seen from the results summarized in Table 1, the reduction was not effected without M and/or PNAH (runs 1 and 10). Furthermore, it was demonstrated that variation in the amount of M present affected the optical but not the chemical yield. When the molar ratio of M to PNAH was 0.5 (run 3 in Table 1), both chemical and optical yields were at a maximum; with higher ratios, only the stereoselectivity of the reduction was lowered. This resulted, however, not from racemisation of the product by an excess of magnesium, *i.e.* after the reduction for 72 h (run 3 in Table 1), since the presence in excess of M of two different ratios failed to alter either the chemical or optical yields (see Experimental section). To throw further light on the reaction run 3 (Table 1) was examined in detail (Table 2).

As Table 2 shows, the enantiomeric excess (e.e.)

TABLE 1

Dependence of e.e. on the ratio of M to PNAH [a]

Run [b]	PNAH [c]	M [c]	M/PNAH	Chemical [d] yield (%)	e.e. (%)	$[\alpha]_D^{25}$ (*c*) [e]	Config.
1	0 5	0	0	4			
2	2.0	0.5	0.25	31	2.9	+3.7 (1.07)	(*S*)
3	2.0	1.0	0.5	76	28.5	+36.0 (1.05)	(*S*)
4	2.0	2.0	1.0	70	17.9	+22.6 (1.02)	(*S*)
5	2.0	3.0	1.5	80	11.8	+14.9 (0.99)	(*S*)
6	2.0	4.0	2.0	72	8.3	+10.5 (1.04)	(*S*)
7	2.0	5.0	2.5	71	5.1	+6.5 (1.01)	(*S*)
8	2.0	6.0	3.0	66	3.7	+4.7 (1.01)	(*S*)
9	2.0	10.0	5.0	72	2.1	+2.6 (1.04)	(*S*)
10	PNA+ [f] 0.2	0.1		0			

[a] All reactions were conducted for 72 h. [b] In all runs equimolar amounts of S, PNAH (runs 1—9) or PNA+ (run 10) were used. [c] mmol. [d] Determined by v.p.c. [e] Measured in $CHCl_3$. [f] The oxidised form, PNA+; $^-I^+PrN{:}CH{\cdot}CH{:}CH{\cdot}CH{:}CH{\cdot}CONH{\cdot}\overset{*}{C}H(CH_2OH){\cdot}\overset{*}{C}H(OH){\cdot}Ph$, was used.

TABLE 2

Dependence of e.e. on the conversion of the reaction

Run [a]	PNAH [b]	M [b,c]	Reaction period (h)	Reaction [d] yield (%)	e.e. (%)	$[\alpha]_D^{25}$ (*c*) [e]	Config.
1	1.5	0.75	0.5	22	12.8	+16.2 (1.00)	(*S*)
2	1.0	0.5	1.0	32	21.0	+26.5 (1.05)	(*S*)
3	1.0	0.5	1.5	42	23.8	+30.0 (1.15)	(*S*)
4	1.0	0.5	2.0	46	29.1	+36.8 (1.04)	(*S*)
5	1.0	0.5	3.0	59	27.6	+34.8 (1.09)	(*S*)
6	1.5	0.75	12	69	26.3	+33.2 (1.17)	(*S*)

[a] Equimolar amounts of S and PNAH were used. [b] mmol. [c] Ratio of M to PNAH was 0.5 in all runs. [d] Determined by v.p.c. [e] Measured in $CHCl_3$.

TABLE 3

Effect of PNA^+ on e.e.[a]

Run [b]	PNA^+ [c]	$\frac{M}{PNAH}$ [d]	$\frac{PNA^+}{PNAH}$	Reaction [e] yield (%)	e.e. (%)	$[\alpha]_D^{25}$ (*c*) [f]	Config.
1	0.75	0.5	0.5	61	45.8	+57.8 (1.17)	(*S*)
2	1.5	0.5	1.0	54	51.5	+65.0 (1.20)	(*S*)
3	2.25	0.5	1.5	54	52.4	+66.1 (1.02)	(*S*)

[a] All reactions were conducted for 12 h. [b] S and PNAH were used in equal amount, 1.5 mmol, for all runs. [c] mmol. [d] 0.75 mmol of M was used in all runs. [e] Determined by v.p.c. [f] Measured in $CHCl_3$.

TABLE 4

Relief of the effect of PNA^+ by the addition of M [a]

Run [b]	M [c]	$\frac{M}{PNAH}$	$\frac{PNA^+}{PNAH}$	Reaction [d] yield (%)	e.e. (%)	$[\alpha]_D^{25}$ (*c*) [e]	Config.
1	1.5	1.0	1.0	59	26.5	−33.4 (0.82)	(*S*)
2	3.0	2.0	1.0	54	19.4	+24.5 (1.04)	(*S*)

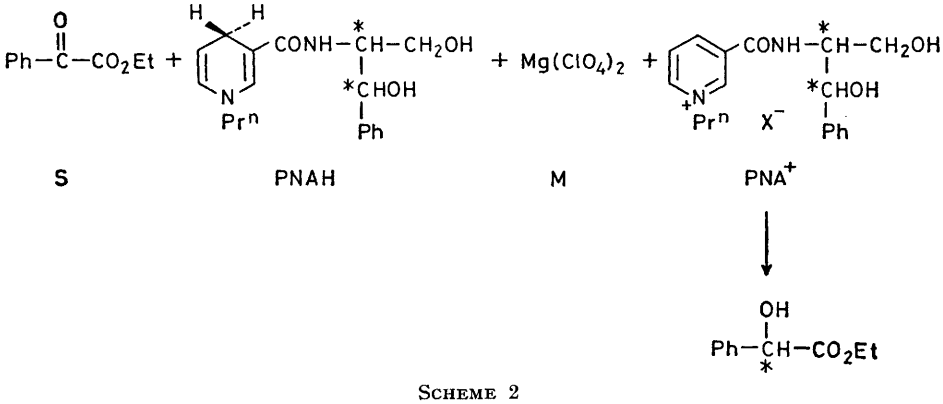
[a] All runs were conducted for 12 h. [b] Equimolar amounts (1.5 mmol) of S, PNAH, and PNA^+ were used in both runs. [c] mmol. [d] Determined by v.p.c. [e] Measured in $CHCl_3$.

increased with the reaction period. This can be rationalized only by taking into account the effect of other chiralities which are newly created as the reaction proceeds. In view of this we gave attention to the oxidised model compound (PNA^+), which accumulated during the reaction. Thus the stereochemical results listed in Table 3 reveal that addition of PNA^+ remarkably affected the steric course of the reduction.

These two features deserve comment as follows. (*i*) The asymmetric reduction involves two different processes. One involves S, PNAH, and M (Scheme 1) and the other, parallel process, involves the PNA^+ formed (Scheme 2). At an earlier stage of the reduction, the former process takes place exclusively, but the latter is of increasing importance as the reaction proceeds. The final asymmetric bias observed in the product arises, therefore, from both component processes. (*ii*) With a relatively small amount of M, the product with higher optical purity can be looked upon as resulting from the presence of a ternary species formed from PNAH, PNA^+, and M, which creates a new chiral environment in close proximity to the reaction site. In contrast, when an excess of M is available, PNA^+ will be saturated by the excess of M, which may eliminate the interaction between PNA^+ and PNAH and result in the destruction of the otherwise prevailing new chiral environment. It follows therefore that when the former conditions prevail ethyl mandelate is formed and the optical yield is low. The reaction rate, however, remains remarkably high.

Support for these arguments was provided by the following five experiments, (a)—(e).

(a) Dependence of the asymmetric reduction on magnesium and PNA^+ was clarified by an experiment in which an excess of M was added to the reaction mixture from run 2 (Table 3), and the reaction allowed to proceed for a further 12 h. The stereochemical results collected



SCHEME 2

in Table 4 indicate that an excess of M significantly lowered the asymmetric yield, but not the chemical yield. This implies that PNA^+ does not participate in the reaction because it is strongly bound with M. A comparison of these results with those of run 1 (Table 3) shows that the chirality-inducing effect of PNA^+ employed was apparently removed by the addition of an equimolar amount of M (compared with PNAH), and that further addition of M presumably kept even the PNA^+ which formed during the redox process away from the reaction site.

(b) In order to investigate the earlier stage of the reduction of run 2 (Table 3), the reaction was quenched after 1 h. The chemical and optical yields of (*S*)-(+)-mandelate were found to be 21 and 21.5% e.e. respectively. In a run without the addition of PNA^+, the corresponding values were recorded as 32 and 21.0% e.e. for the same enantiomer. This implies that the observed lowering in chemical yield and a rise, though slight, in enantiomeric enrichment may be attributed to the result of an interaction of M with PNA^+.

(c) Quenching the reaction (run 5 in Table 1) resulted in 37% chemical yield and 11.5% e.e. after 30 min, and 51 and 9.0% e.e. after 90 min respectively. From a comparison of these results with those for the same reaction period given in Table 2, it may be reasonably concluded that an increase in the amount of M caused not only the acceleration of the reaction but also the elimination of stereochemical effect of PNA^+, which may do much towards lowering the enantioselectivity.

(d) The substrate was subjected to the reduction with an equimolar PNAH and half an equivalent amount of M for 1 h; an equimolar amount of M (compared with PNAH) was added to the reaction mixture, and the reaction allowed to proceed for additional 1 h. The total amount of M used was 1.5 times that of the PNAH present. The chemical and optical yields found for the product were 63 and 22.4% e.e. respectively. These results, when compared with those in run 4 of Table 2, show that the addition of further M in the latter stage of the reaction accelerated the reaction. This led to a significant increase in chemical yield, by favouring the process illustrated in Scheme 1 and suppressing that of Scheme 2 which, in turn, led to a lowering of optical yield. This is in keeping with the above discussion.

(e) The apparent significance of hydroxy-groups led us to inquire whether these functions are so bound to the metal as to exert an important effect even in a protic solvent such as alcohol. Thus, the reaction was conducted in absolute alcohol in place of acetonitrile under the same conditions as those for the reactions in Table 2. Without addition of PNA^+, the reaction after 5 h gave (*S*)-ethyl mandelate in 33% yield with 13.3% e.e.; neither yield was improved by increasing the reaction time to 10 h. The reaction was then repeated in the presence of an equimolar amount of PNA^+ for 15 h, to afford the reduction product in 33% yield with 12.6% e.e. Here again, the chemical yield was unaffected even by the presence of an equimolar amount of M after 15 h. From these results it is clear that in an hydroxylic medium, PNA^+ did not affect the reaction because it no longer interacted with the reduced form of PNAH. The lower chemical yield may be ascribed to inactivation of the metal as a result of greater solvation with a protic solvent.

In conclusion, the stereochemical evidence presented above supports the view that enantioselectivity is substantially dependent on PNA^+ as a result of the intervention of M between PNA^+ and PNAH *via* some interaction such as $^-(OH)_2 \cdots M \cdots (OH)_2^-$. This is recognized to be closely related to the two hydroxy-groups. However, by comparing the reaction of run 2 (Table 1) with that of run 3 (Table 3), it is clear that it would be unwise to interpret the present results without taking into account the possibility of the formation of a charge-transfer type complex between the reduced and oxidised pyridine rings; this has been discussed by several investigators.[5-7]

EXPERIMENTAL

Instruments.—I.r. spectra were recorded with a Hitachi 215 spectrometer, and ^1H n.m.r. spectra with a Varian EM-360 spectrometer in [^2H]chloroform unless otherwise specified, with tetramethylsilane as internal standard. U.v. spectra were recorded in methanol with a Hitachi EPS-3 spectrophotometer. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. V.p.c. analyses were performed on a Hitachi K-53 instrument with 5% polyethylene glycol succinate (PEGS) on Chromosorb W.

Materials.—Commercial ethyl benzoylformate was purified by column chromatography (Kieselgel 60, 70—230 mesh, benzene), followed by distillation. The optically active amine, (+)(4*S*,5*S*)-5-amino-2,2-dimethyl-4-phenyl-1,3-dioxan, was generously supplied by Boehringer Gmbh Mannheim Co.

Optical and Chemical Yields.—Chemical yields were determined by quantitative comparison of v.p.c. chromatograms of the products with those of authentic samples. Optical yields were based on the reported maximum rotation of pure (+)-(*S*), (−)-(*R*)-ethyl mandelate, $\pm 126.2°$ in chloroform.[8]

Reducing Reagent (PNAH).—(+)-(4S,5S)-(*Nicotinoylamino*)-2,2-*dimethyl*-4-*phenyl*-1,3-*dioxan* (*Nicotinamide*).—A solution of nicotinic acid (23.5 g, 0.19 mol) and triethylamine (28 ml) in toluene (600 ml) was chilled at −5 to −10 °C and treated with ethyl chloroformate (25.0 g, 0.23 mol). After the mixture had been stirred for 30 min, a cold solution of (+)-(4*S*,5*S*)-5-amino-2,2-dimethyl-4-phenyl-1,2-dioxan (35.6 g, 0.16 mol) in chloroform (400 ml) was added dropwise below −5 °C, and the mixture was set aside overnight at room temperature. The solvent was evaporated, the residue was taken up in ethyl acetate, and the organic phase was washed successively with saturated aqueous sodium hydrogencarbonate (×2), saturated aqueous sodium chloride, and water, and then finally dried (Na_2SO_4). The filtrate was concentrated *in vacuo*, and the residue was crystallized from diethyl ether–n-hexane to give pale yellow crystals (42.4 g, 69%), m.p. 96—97 °C, $[\alpha]_D^{25}$ +107.1° (*c* 1.01 $CHCl_3$) (Found: C, 69.1; H, 6.5; N, 8.8. $C_{18}H_{20}N_2O_3$ requires C, 69.21; H, 6.45; N, 8.97%), $\nu_{max.}$ (KBr) 3 450, 1 700, 1 650, and 1 510 cm^{-1}; $\delta(CDCl_3)$ 1.58, 1.60 (6 H, s, 2 × C–CH_3), 3.81—4.41 (3 H, m, O–CH_2, N–CH), 5.23 (1 H,

d, *J* 2 Hz, CHPh), 6.6 (1 H, d, *J* 8 Hz, NH), 7.05—7.31 (6 H, m, Ph-5H, py H-4), and 7.6—7.8 (1 H, m, py H-2).

N-[2-*Hydroxy*-1-(α-*hydroxybenzyl*)*ethyl*]-1-*propylnicotinium Iodide*.—The nicotinamide formed in the preceding experiment (30.0 g, 0.10 mol) was treated with n-propyl iodide (50.0 g, 0.29 mol) in absolute ethanol (350 ml) under reflux for 5 h after which the solvent was evaporated off. The residue was crystallized from methanol–diethyl ether to give yellow crystals (20.0 g, 47.8%), m.p. 117—119 °C, $[\alpha]_D^{25}$ +52.6° (*c* 1.10 CH_3OH) (Found: C, 48.55; H, 5.35; N, 6.25. $C_{18}H_{23}N_2O_3I$ requires C, 48.88; H, 5.24; N, 6.33%), $\nu_{max.}$ (KBr) 3 400, 3 270, and 1 645 cm^{-1}; δ(CD_3OD) 1.02 (3 H, t, *J* 7 Hz, CH_3), 2.1 (2 H, m, *J* 8 Hz, CH_2Me), 5.0 (1 H, d, *J* 5 Hz, PhCH), 7.3br (6 H, m, aromatic and NH).

N-[2-*Hydroxy*-1-(α-*hydroxybenzyl*)]-1-*propyl*-1,4-*dihydronicotinamide* (*PNAH*).—To an aqueous solution of water (60 ml) containing sodium carbonate (6 g) and sodium dithionite (10 g), chloroform (100 ml) and a methanol (10 ml) solution of the above-mentioned quaternary salt (4.42 g, 0.01 mol) were added under a nitrogen atmosphere at room temperature. The mixture was stirred until the yellow colour of the aqueous layer had disappeared. After the mixture had been chilled in an ice-bath, the organic phase was separated, and work-up afforded a solid product (2.6 g, 81.3%), m.p. 110—112 °C (decomp.), $[\alpha]_D^{25}$ +156.3° (*c* 2.00 $CHCl_3$) (Found: C, 65.85; H, 7.35; N, 8.35. $C_{18}H_{24}N_2O_3 \cdot \frac{1}{2}H_2O$ requires C, 66.38; H, 7.37; N, 8.60%), $\lambda_{max.}$ (CH_3OH) 355 nm (ε 6 500); δ($CDCl_3$) 0.85 (3 H, t, *J* 7 Hz, CH_3), 1.5 (2 H, m, *J* 7 Hz, CH_2Me), 3.02br (2 H, 2 × 4-H), 4.62 (1 H, m, 5-H), 4.94 (1 H, d, PhCH), and 7.23 (5 H, aromatic).

General Experimental Procedure.—(*a*) *For those listed in Tables* 1 *and* 2. A mixture of ethyl benzoylformate, PNAH, and magnesium perchlorate in dry acetonitrile (10 ml/1 mmol of PNAH) was stirred at room temperature for the period specified in Tables under nitrogen atmosphere in the dark, after which water (1 ml) was added to the reaction mixture. The mixture was stirred for 5 min and then extracted with dichloromethane. The organic layer was evaporated *in vacuo*. The yield of the product was determined at this stage by v.p.c. The residue was purified by t.l.c. (Kieselgel G nach Stahl, Typ 60) with benzene; the optical rotation was then measured. The purity of the alcohol was confirmed by v.p.c. and ^1H n.m.r. spectroscopy. Both chemical and optical yields are summarized in Tables 1 and 2.

(*b*) *For those listed in Tables* 3 *and* 4. To the above mixture (see general procedure above) was added the nicotinium salt, PNA^+ (oxidised form of PNAH), in the stated amounts. The mixture was allowed to react under the same conditions as those for the described general procedure. After the reaction period specified in Tables 3 and 4, the mixture was worked up by the same procedure as described above. The chemical and optical yields are summarized in Tables 3 and 4.

Test for the Possibility of Racemization by an Excess of Magnesium Perchlorate.—A solution prepared from ethyl benzoylformate (534.6 mg, 3.0 mmol), PNAH (949.1 mg, 3.0 mmol), magnesium perchlorate (334.8 mg, 1.5 mmol), and dry acetonitrile (30 ml) was stirred for 72 h. After this time a portion (15 ml) of the reaction mixture was pipetted into a further flask and to this was added further magnesium perchlorate (167.4 mg, 0.75 mmol), while magnesium perchlorate (502.2 mg, 2.25 mmol) was dissolved in the residual solution. Both mixtures were allowed to react under the same conditions as those given in Table 1 for a further 24 h; each mixture was then worked up according to the previously outlined procedure to give ethyl mandelate. The former reaction gave 194.0 mg (72%) of the product, $[\alpha]_D^{25}$ +35.1° (*c* 1.08 $CHCl_3$), 27.8% e.e. and the latter, 231.5 mg (86%), $[\alpha]_D^{25}$ +33.7° (*c* 1.05 $CHCl_3$), 26.7% e.e.

Reaction in Ethanol.—A solution of ethyl benzoylformate (178.2 mg, 1.0 mmol), PNAH (316.4 mg, 1.0 mmol), magnesium perchlorate (111.6 mg, 0.5 mmol), and absolute ethanol (20 ml) was stirred for 5 h. Water (1 ml) was then added to the mixture which was stirred for a further 5 min and then evaporated to give an oily product. The residual oil was treated with dichloromethane, washed with water, and the organic layer analysed by v.p.c. (yield 59.4 mg, 33%). The product was purified by means of preparative t.l.c. (Kieselgel G nach Stahl, Typ 60, benzene), $[\alpha]_D^{25}$ +16.7° (*c* 1.04 $CHCl_3$), 13.3% e.e. A similar reaction carried out for 15 h gave identical chemical and optical yields. In a further experiment PNA^+ (663.4 mg, 1.5 mmol) was added to a reaction mixture prepared from ethyl benzoylformate (267.3 mg, 1.5 mmol), PNAH (474.6 mg, 1.5 mmol), magnesium perchlorate (167.4 mg, 0.75 mmol), and absolute ethanol (30 ml) under the reaction conditions described above. After the reaction period, the work-up gave the product (89.1 mg, 33%), $[\alpha]_D^{25}$ +15.9° (*c* 1.04 $CHCl_3$), 12.6% e.e.

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