Mechanism of Photoreduction of Diethyl Benzylidene Malonates by NAD(P)H Model and Comparison with Thermal Reaction

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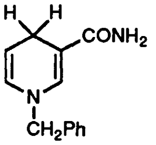
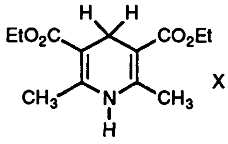
Photoreduction of diethyl benzylidene malonates by NAD(P)H model has been investigated. The reduction of the carbon–carbon double bond occurs and is enhanced by electron-withdrawing groups on the benzene ring. Deuteriation experiments in [$^2H_4$]methanol showed that the deuterium which originated from the solvent was mainly incorporated at the β-carbon. The mechanism of photoreduction is discussed in terms of sequential electron-hydrogen atom transfer from NAD(P)H model to the substrate in the solvent cage. On the other hand, thermal reduction of NAD(P)H model with the substrates in methan-[^{2}H]-ol and/or [$^2H_4$]methanol gave the reduced products with deuterium incorporated at the α-carbon. Reduction of diethyl benzylidene malonates with [4,4-$^2H_2$]-1-benzyl-1,4-dihydronicotinamide (BNAH) led to the direct transfer of deuterium from C-4 to the β-carbon. These results have been interpreted as support for the direct hydride transfer mechanism.

The mechanism by which NAD(P)H transfers hydrogen in redox reactions has been extensively examined in model systems. It is yet to be settled as to whether the transfer of a hydride equivalent from dihydropyridine to the substrate involves, (*a*) transfer of a hydride ion in a single step, (*b*) two-step transfer of an electron and a hydrogen atom, or (*c*) overall transfer of two electrons and a proton in three steps. The pioneering study of Abeles and co-workers[1] on the mechanism of biomimetic reduction of thiobenzophenone with 1-benzyl-1,4-dihydronicotinamide (BNAH) proposed that the reduction proceeds through one-step hydride transfer. However, Hamilton[2] argued that the possibility of proton and electron transfer should be considered for these reactions and since then many reactions have been investigated for signs of electron transfer mechanism.[3] Recently, Pandit[4] reported observations of specificity in the reduction of activated carbon–carbon double bonds by NAD(P)H models that are consistent with a direct hydride transfer mechanism. The results of Kreevoy[5] on measuring the rate and equilibrium constants of hydride transfer reactions also eliminated the possibility of a multi-step mechanism. However, Ohno[6] detected radical species in the ESR spectra for reduction of benzyls, and his work[7] on the kinetics and isotope effects for the reduction of substituted trifluoroacetophenone revealed that the reaction proceeds through a multi-step mechanism. Furthermore, photoinduced reductions of many substrates by dihydropyridines have been reported to proceed by a multi-step electron-proton-electron transfer process.[8] In a previous paper,[9] we reported that the nitro-group in diethyl nitrobenzylidene malonate (**1a**) can be reduced selectively with Hantzsch ester—an NAD(P)H model produced by an eosin-sensitized photoreaction, while the carbon–carbon double bond in the forementioned compound was reduced by metal ion catalysis in the dark. The results of photosensitized reduction can be easily explained by a multi-step electron–proton–electron transfer mechanism and a direct hydride transfer is proposed for the thermal reaction. The present paper deals with the studies of reduction of substituted and unsubstituted diethyl benzylidene malonates (**1d**) by direct irradiation and metal ion catalysis with Hantzsch ester (PyH_2) and/or BNAH. Deuteriation experiments were performed using [4-$^2H_1$]-BNAH and [4,4-$^2H_2$]-BNAH, methan-[^{2}H]-ol and/or [$^2H_4$]methanol to ascertain the site of transfer of the hydrogen (hydride equivalent). Different mechanistic implications for direct photoreduction and thermal reaction of NAD(P)H models in the dark with diethyl benzylidene malonates are briefly discussed on the basis of these observations.



BNAH



PyH_2

$X-PhCH=C(CO_2Et)_2$

(**1**)

a; X = NO_2, b; X = CN
c; X = Cl, d; X = H
e; X = CH_3, f; X = OCH_3

Experimental

Materials.—The diethyl benzylidene malonates were prepared according to the literature[10] and purified by recrystallization from aqueous alcohol. (**1a**) m.p. 91 °C (lit.,[10b] 92 °C); (**1b**) m.p. 73 °C; *m/z* 273 ($M^{+\cdot}$); ν_{max} (KBr) 2 236 (C≡N) and 1 732 (C=O) cm^{-1}; (**1c**) m.p. 25 °C (lit.,[10a] b.p. 71 °C/1 mmHg); (**1d**) b.p. 132 °C/2 mmHg (lit.,[10a] 164 °C/6 mmHg); (**1e**) m.p. 44 °C (lit.,[10] b.p. 240 °C/60 mmHg); (**1f**) b.p. 195 °C/3 mmHg, (lit.,[10a] 180 °C/11 mmHg). 1-Benzyl-1,4-dihydronicotinamide (BNAH) was prepared by a published method.[11] [4-$^2H_1$]-BNAH and [4,4-$^2H_2$]BNAH were synthesized as described in reference 12, and the ^{1}H NMR spectra obtained were consistent with the presence of deuterium only at the C-4 position. Hantzsch ester (PyH_2) was prepared by a known method.[13] Deuteriated solvents were commercially available (Beijing Chemical Factory). Acetonitrile was purified by being dried over phosphorus pentaoxide followed by distillation. Pyridine was refluxed over anhydrous sodium hydroxide and distilled before use. Magnesium perchlorate was thoroughly dried by being heated at 120 °C for 8 h.

Instruments and Methods.—Electronic absorption spectra

Table 1. Yields of products in the photoreaction of (**1**) (5×10^{-3} mol dm^{-3}) by PyH_2 (1×10^{-2} mol dm^{-3}) in pyridine–methanol (9:1) under irradiation $h\nu$ >420 nm.

X-BME	Time/h	Yield of (**2**) (%)[a]	m/z ($M^{+\cdot}$)
CN	5.5	79.3	275
Cl	6.0	75.0	284
H	8.0	81.5	250
CH_3	11.0	78.2	264
OCH_3	14.0	80.0	280

[a] Isolation yields based on (**1**) used.

were measured on a Hitachi 340 spectrometer. Fluorescence spectra were determined using a Perkin-Elmer LS-5 spectrofluorimeter with excitation wavelength 380 nm, excitation slit and emission slit 2.5 nm. ^1H NMR spectra were recorded on a Varian EM-360 spectrometer at 60 MHz, with $CDCl_3$ as solvent and TMS as internal standard. Mass spectra were obtained on a Finnigan 4021 quadruple GC–MS, 70 eV electron bombardment, direct insertion. Analytical VPC was carried out on a Shimadzu GC-9A machine with hydrogen flame ionization detector using a 300 × 3 mm column packed with 5% OV-7 on 101 (product of Shanghai No. 1 Reagent Factor, P.R. China). The redox potential was recorded using a cyclic voltammeter with L-23-204 XY function recorder (Shanghai Automatic Instrument No. 2 Factory). The three electrode system was applied; the reference electrode was Ag/AgCl, saturated with KCl solution, Pt was used as the counter electrode saturated with KCl solution and pyrolitic graphite was chosen as the working electrode with Et_4NClO_4 (0.1 mol dm^{-3}) as supporting electrolyte. Lifetime determination was made on a Model HORIBA NAES-1100 time-correlated single-photon counting instrument. ESR studies were carried out in a Bruker ESP-300 spectrometer.

Photoreduction.—The progress of the irradiation of a nitrogen purged pyridine–methanol (9:1) solution of diethyl benzylidene malonate (**1**) (5×10^{-3} mol dm^{-3}), Hantzsch ester (PyH_2) (1×10^{-2} mol dm^{-3}) at $h\nu$ >420 nm (500 W high pressure Hg lamp) where only PyH_2 was excited was followed by gas chromatography [5% OV-1 on 101, 200 °C, retention times: (**1b**) 12.1, (**1c**) 8.2, (**1d**) 4.1, (**1e**) 7.0, and (**1f**) 12.6 min]. On completion of the reaction the solvent was removed. The residue was dissolved in chloroform and washed several times with HCl (1 mol dm^{-3}) then with water. The chloroform layer was separated and evaporated to dryness in a rotary evaporator. The products were separated by column chromatography on silica gel and their structures were determined by ^1H NMR spectroscopy and mass spectral data. In order to ensure that there was no extensive exchange of $^2H/^1H$ in the deuteriated product during the isolation processes we dissolved the reduced product in 1:1 $CDCl_3$–^2H_2O and adjusted the solution to pH 1 with hydrochloric acid with stirring for 20 min. The ^1H NMR spectrum showed no exchange of $^2H/^1H$ after the treatment.

Thermal Reduction.—A mixture of PyH_2 and/or BNAH (2.5×10^{-2} mol dm^{-3}), (**1**) (1.25×10^{-2} mol dm^{-3}), and magnesium perchlorate (1.25×10^{-2} mol dm^{-3}) was refluxed in acetonitrile–methanol (9:1). The progress of the thermal reaction was followed spectrophotometrically by observing the decrease in the absorption of BNAH at 380 nm. The product was isolated as before and the structure was determined by ^1H NMR and mass spectral analysis.

Fluorescence Quenching Experiments.—Quenching of the PyH_2 fluorescence was carried out by using compounds (**1**) as quenchers. Relative fluorescence intensities of PyH_2 at 450 nm were measured for acetonitrile solutions containing PyH_2 (1.24×10^{-4} mol dm^{-3}) and the quencher at various concentrations (1.0×10^{-3} to 0.1 mol dm^{-3} for X = CN; 0.25 mol dm^{-3} for X = H; 0.3 mol dm^{-3} for X = CH_3; 0.65 mol dm^{-3} for X = OCH_3; up to 30% of the intensity was quenched). The absorption band due to PyH_2 was not changed by the addition of the quencher at high concentration. A change in the shape rather than the intensity of the fluorescence spectrum was shown on addition of (**1**). The Stern–Volmer relationship $I_0/I = 1 + K_q[Q]$ was obtained. The quenching rate constants k_q were calculated from the quenching constant K_q and the fluorescence life time τ_f (0.22 ns) by the following equation (1).

$$K_q = k_q \cdot \tau_f \qquad (1)$$

Spin-trapping Measurements.—A solution of PyH_2 (5×10^{-2} mol dm^{-3}), (**1b**) (2.5×10^{-2} mol dm^{-3}), and spin-trap 2,3,5,6-tetramethylnitrosobenzene (1×10^{-3} mol dm^{-3}) in benzene–pyridine (5:1) was introduced into the ESR tube and deoxygenated by bubbling with a slow stream of nitrogen for 20 min. It was then placed into the ESR cavity and photolysed with light of wavelength $h\nu$ >420 nm from a 500 W tungsten–iodine lamp for 20 min. The photolysis was monitored in the ESR cavity.

Results and Discussion

Products of Photoreduction.—Irradiation of the nitrogen

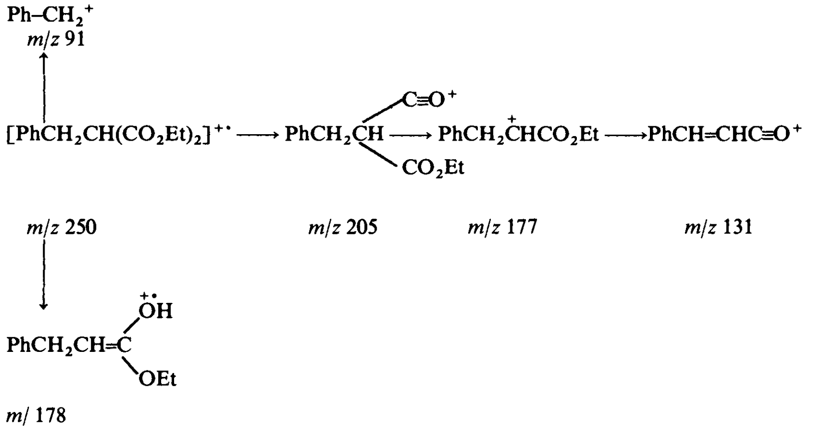


Figure 1.

Table 2. Relative amount of deuteriated products formed by direct irradiation of PyH_2 (3×10^{-2} mol dm^{-3}) and (**1**) (1×10^{-2} mol dm^{-3}) in pyridine–[$^2H_4$]methanol (4:1).

		(%)			
X	Time/h	A	B	C	D
CN	5.0	13.1	85.3	1.6	0.0
Cl	6.0	58.5	39.1	2.4	0.0
H	7.5	44.1	53.7	2.2	0.0
CH_3	8.0	54.8	42.0	3.2	0.0
OCH_3	9.0	42.4	56.4	1.2	0.0

Table 3. Rate constants k_q for quenching of PyH_2 (1.24×10^{-4} mol dm^{-3}) fluorescence by (**1**) in acetonitrile at room temperature.

X	CN	H	CH_3	OCH_3
$k_q{}^a \times 10^{-9}$ dm^3 mol^{-1} s^{-1}	22.39	8.47	7.56	2.54

^{a} Calculated from Stern–Volmer plot $\tau_f = 0.22$ ns.

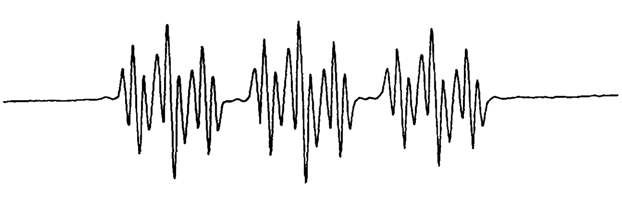
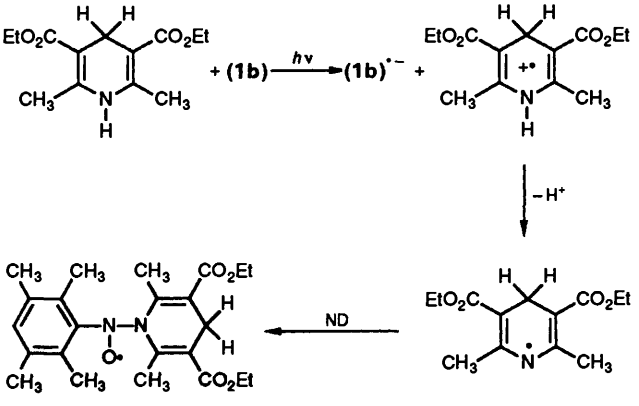


Figure 2. ESR spectrum after photolysis of PyH_2 (5×10^{-2} mol dm^{-3}), (**1b**) (2.5×10^{-2} mol dm^{-3}), ND (1×10^{-3} mol dm^{-3}) in benzene–pyridine (5:1) for 20 min.



Scheme 1.

purged pyridine–methanol solution of (**1**) consumed over 90% of PyH_2 to yield reduction products diethyl X-benzyl malonates. In the absence of pyridine, yields of the reduction products were significantly lower than those in the presence of pyridine suggesting that pyridine is essential for obtaining high yields. No reaction occurred in the dark. ^1H NMR spectral analysis of the products, with the exception of the reduction with (**1a**) which gave very complicated products, showed doublet peaks at *ca.* 3.3 ppm which are characteristic of α-methylenes. The yields of products are summarized in Table 1. In order to ascertain the site of transfer of the hydrogen from PyH_2 to (**1**), the reactions were performed in deuteriated solvent pyridine–[$^2H_4$]methanol (4:1). The position of the deuterium atom in the reduced products was established by mass spectrometry. The main fragmentation pattern in the electron-impact spectrum of diethyl benzylmalonate is as shown in Figure 1.

Using $M^{+\bullet}$ to assess total deuterium, upon incorporation of a deuterium atom in the benzylic methylene group X-Ph–$CH_2{}^+$, masses of the fragments carrying the latter moiety will be augmented by one unit after correction for the natural abundance of deuterium. This was observed in the mass spectra of (**2b–f**). The relative amount of deuteriated products [A: X-$PhCH_2CH(CO_2Et)_2$, B: X-$PhCH(D)CH(CO_2Et)_2$, C: X-$PhCH_2CD(CO_2Et)_2$, D: X-$PhCH(D)CD(CO_2Et_2)$] calculated from the peak area are listed in Table 2. From the results in Table 2 it is apparent that in the reduction of (**1**) by PyH_2 in pyridine:[$^2H_4$]methanol (4:1), the deuterium atom which is originated from the solvent is mainly incorporated at the β-carbon.

Fluorescence Quenching.—It was found that quenching of PyH_2 fluorescence (excited in the region 380–400 nm beyond the quencher absorption) by (**1**) obeys the Stern–Volmer relation, equation (2).

$$I_0/I = 1 + k_q\tau_f[Q] \qquad (2)$$

I_0/I represents the ratio of the fluorescence intensities measured in the absence and in the presence of quencher Q, τ_f is the fluorescence lifetime of PyH_2 for $Q = 0$. From the slope $k_q\tau_f$ of the straight line, taking $\tau_f = 0.22$ ns, the quenching rate constants k_q were calculated (correction coefficients $r > 0.99$) and listed in Table 3.

The quenching rate constants are close to the diffusion-controlled limit (K_{diff} 2.3×10^{10} dm^3 mol^{-1} s^{-1}) and decrease in the order (**1b**) $\gg$ (**1d**) $>$ (**1e**) $>$ (**1f**). Since the singlet excited energy of PyH_2 (E_s 3.44 eV) is lower than that of (**1**) (E_s >4.13 eV),* quenching of PyH_2 fluorescence by energy transfer is ruled out, and quenching by electron transfer is probable. It has been demonstrated that quenching of 1,4-dihydropyridine fluorescence by a variety of electron acceptors involves outer sphere electron transfer in which 1,4-dihydropyridine acts as an electron donor.[14] One electron transfer seems very likely as the primary process in the photoinduced reduction of diethyl fumarate by NAD(P)H model.[15]

Spin-trapping Measurement.—From the photolysis of a solution of PyH_2 (5×10^{-2} mol dm^{-3}) and (**1b**) (2.5×10^{-2} mol dm^{-3}) in benzene–pyridine (5:1) for 20 min using 2,3,5,6-tetramethylnitroso benzene (ND) (1×10^{-3} mol dm^{-3}) as spin trap, the resulting ESR spectrum, as indicated in Figure 2, is composed of 3 (1:1:1) × 3 (1:1:1) × 3 (1:2:1) = 27 lines. The spectrum is assigned to the spin-adduct of ND with pyridinyl radical PhH$^\bullet$ which is generated through photoinduced electron transfer from the singlet excited dihydropyridine to (**1**), followed by proton loss from the resulting dihydropyridine radical cation with hyperfine coupling constants ($a_N^\alpha = 10.18$, $a_N^\beta = 2.89$, $a_H^\gamma = 0.89$ G)[16] as shown in Scheme 1.

It has been established[17] by laser flash photolysis that photooxidation of PyH_2 by electron acceptors is initiated by one-electron transfer to obtain a 1,4-dihydropyridine radical cation $PyH_2{}^{+\bullet}$ followed by rapid proton loss giving a detectable neutral radical PyH$^\bullet$. On the basis of ESR spectroscopy, we believe that electron transfer from excited PyH_2 to (**1**) is responsible for the initiation of photoreduction.

Products of Thermal Reduction.—The products of the thermal

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* 1 eV = $1.602\,18 \times 10^{-19}$ J.

Table 4. Product yields for the reduction of (**1**) (1.25×10^{-2} mol dm^{-3}) with BNAH (2.5×10^{-2} mol dm^{-3}) and magnesium perchlorate (1.25×10^{-2} mol dm^{-3}) in acetonitrile–methanol (9:1).

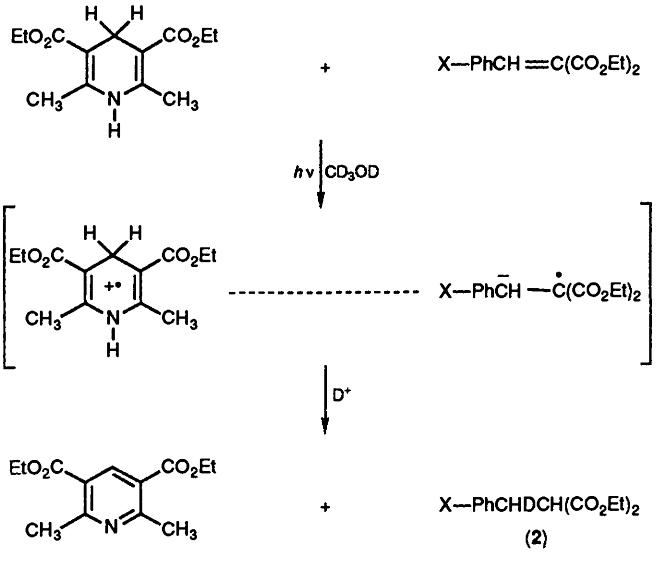
(**1**) X	Time/h	Yields[a] %	$-E(A^-/A)/V$[b] without Mg^{2+}	 with Mg^{2+}
NO_2	5.0	91.4	0.97	0.54
CN	5.5	90.1	1.26	0.69
Cl	6.0	98.0	1.52	0.75
H	6.5	82.3	1.63	0.64
CH_3	8.5	88.9	1.67	1.04
OCH_3	9.5	92.8	1.69	1.09

[a] Isolated yields. [b] Relative to the SCE.

Table 5. Deuterium content of reduced products from the thermal reduction of (**1**) (1×10^{-2} mol dm^{-3}), PyH_2 (2.5×10^{-2} mol dm^{-3}), and magnesium perchlorate (1.25×10^{-2} mol dm^{-3}).

(**1**) X	D:H/%* $CH_3CN:CH_3OD$ (9:1)	 $CH_3CN:CD_3OD$ (9:1)
NO_2	34.6	29.9
CH_3	30.0	30.0

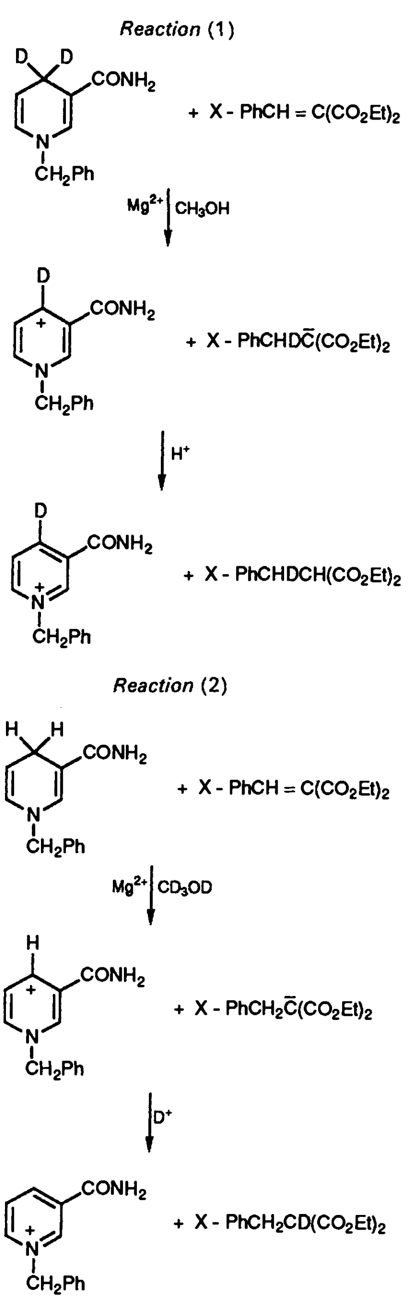
* Determined by ^1H NMR spectroscopy.



Scheme 2.

reaction were subjected to ^1H NMR spectroscopy and mass spectral analyses and the results indicate that the C=C double bond is reduced. The yields of the products are listed in Table 4.

Table 4 shows that the electron withdrawing group on the benzene ring of (**1**) enhances the reaction rate and the presence of magnesium ion remarkably affects the electrochemical behaviour of (**1**) and plays a specific role in the reduction reaction. When the reaction was carried out in acetonitrile:methan-[^2H]-ol and/or [$^2H_4$]methanol (9:1) it was found in all cases that the deuterium was located at the α-carbon to the dicarboxy group giving the reduced product X-$PhCH_2(CO_2Et)_2$ as indicated in Table 5. If 4-[^2H]BNAH and/or 4,4-[$^2H_2$]-BNAH was employed as the reducing agent, deuterium was incorporated at the β-carbon giving X-PhCH(D)CH$(CO_2Et)_2$ as the reduced product.



Scheme 3.

Mechanism of Photoreduction Compared to Thermal Reaction.—The present results provide evidence that photoreduction of (**1**) by PyH_2 proceeds *via* a sequential electron-hydrogen atom transfer mechanism. The first electron reduction appears to occur by electron transfer from excited PyH_2 to (**1**) giving the ion radical pair [$PyH_2^{+\bullet}\cdots(1)^{-\bullet}$]. Calculations using a modified PPP-CI method and standard parameters[18] showed that the α-carbon with respect to the dicarboxy group in (**1**) is more electron deficient than the α-carbon and should be the position for abstracting an electron from PyH_2. Therefore, the β-carbon receives an electron from PyH_2 forming an anion radical X-PhC–$\dot{C}(CO_2Et)_2$ which then picks up a hydrogen from the solvent and enters the product as a proton giving a neutral radical X-PhCH(D)–$\dot{C}(CO_2Et)_2$. As only one deuterium atom is incorporated into the reduced product, the involvement of an electron–proton–electron transfer to the substrate is eliminated.

Since the quenching of PyH_2 fluorescence by (**1**) is believed to occur by means of an electron transfer mechanism, the ion radical pair $[PyH_2^{+\bullet}\cdots(\mathbf{1})^{-\bullet}]$ formed, which has been confirmed by ESR measurement, might be readily deprotonated by a base (pyridine) and undergo hydrogen atom transfer simultaneously from $PyH_2^{+\bullet}$ to $(\mathbf{1})^{-\bullet}$ in a solvent cage, competitively with its dissociation into free radicals, giving reduced products as shown in Scheme 2.

The results of deuteriation experiments for the thermal reduction using methanol-[^{2}H]-ol and/or [$^2H_4$]methanol show that the deuterium is exclusively incorporated at the α-carbon giving $X\text{-}PhCH_2CD(CO_2Et)_2$ indicating that the hydroxylic proton of methanol is predominantly involved in the reduction pathway: the possibility of abstraction of a hydrogen atom from [$^2H_4$]methanol is eliminated [Scheme 3, *reaction* (2)]. When 4,4-[$^2H_2$]-BNAH was employed as the reducing agent, a direct transfer of deuterium as hydride ion from C-4 of BNAH to the β-carbon in (**1**) occurred, showing this to be the site susceptible to nucleophilic attack. Therefore, a mechanism involving direct hydride transfer is the best choice for thermal reduction by NAD(P)H model as indicated in Scheme 3.

Acknowledgements

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