Reduction by a Model of NAD(P)H. 36. First and Direct Evidence for the Multi-step Mechanism

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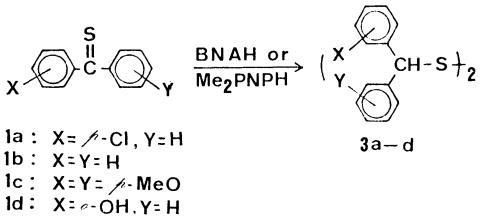
Reductions of thiobenzophenone derivatives with a model compound of NAD(P)H, 1-benzyl-1,4-dihydronicotinamide (BNAH) or *N*-(α-methylbenzyl)-1-propyl-2,4-dimethyl-1,4-dihydronicotinamide (Me_2PNPH), in a solvent containing an *O*-deuterated alcohol afforded products partially deuterated at their methine positions. The deuterium content depends both on the character of substituents in the substrate and on the polarity of solvent. In contrast, no incorporation of deuterium into the product was observed in the reduction of thiopivalophenone with BNAH or Me_2PNPH under the same conditions. These results can be accounted for in terms of an electron transfer followed by a proton transfer from the model to substrate. This is the first and direct evidence for the ion radical-pair intermediacy for reductions by model compounds of NAD(P)H.

Although many investigators have studied the mechanism of reductions with model compounds of NAD(P)H, several points are still ambiguous. Of these, the mechanism of transfer of a "net hydride" from a model compound to substrate has frequently been subjected to discussion. In 1957, Abeles *et al.*[1] examined reductions of thiobenzophenone derivatives with 1-benzyl-1,4-dihydronicotinamide (BNAH) and concluded that a hydride would transfer directly from a model to substrate. On the other hand, a multi-step mechanism including at least one intermediate was proposed by Steffens and Chipman[2] in order to explain a discrepancy between kinetic isotope effect and isotopic ratio in products found in a reduction of α,α,α-trifluoroacetophenone with 1-substituted 1,4-dihydronicotinamide-4-*d*. They suggested that the intermediate in this reaction would be a non-covalent charge-transfer complex generated through an electron transfer from the dihydropyridine derivative to the substrate. Thereafter, such discrepancies have been reported for reductions of substituted α,α,α-trifluoroacetophenones,[3] 1,10-phenanthroline-2-carbaldehyde,[4] *N*-methylacridinium ion,[5,6] and 2-acylisoquinolines.[7] We detected radical species on ESR spectra for reductions of benzil[8] and thiobenzophenone[9] with BNAH. These facts strongly support existence of an ion radical-pair intermediate in reduction with model compounds of NAD(P)H.

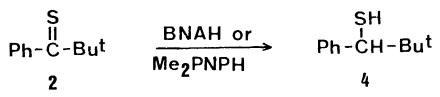
The spectroscopic detection of an ion radical, however, cannot necessarily be an unambiguous proof for its intermediacy. One can argue that at least part of hydrogen atoms incorporated in products should come from solvents or some sources other than NAD(P)H-models, if an ion radical-pair is an intermediate in the reduction. The fact that no such observations have yet been reported may be taken to be an indication that the spectroscopically detected ion radicals might be abortive species. On the other hand, one can also propose that hydrogen transfer between reactive ion radicals might take place so fast within a cage of solvent that no incorporation of hydrogen might be brought about from the solvent into product. The latter argument necessarily predicts that out-of-cage reactions may be detected chemically when one employs such substrates as are capable of affording relatively unreactive ion radical-pairs as intermediates. Thus, we tried for such reduction systems and found that a hydrogen atom from solvent is incorporated into products in some reductions of certain thiocarbonyl compounds.

In this paper we report that reductions of *p*-chlorothiobenzophenone (**1a**), thiobenzophenone (**1b**), *p,p'*-dimethoxythiobenzophenone (**1c**), or *o*-hydroxythiobenzophenone (**1d**) with BNAH or N-(α-methylbenzyl)-1-propyl-2,4-dimethyl-1,4-dihydronicotinamide (Me_2PNPH) afford products whose methine protons partially originate from the solvent and that the hydrogen atom at the 4-position of the model compound transfers directly to thiopivalophenone (**2**). Contrary to the conclusion by Abeles *et al.*,[1] this result clearly indicates an existence of intermediate in the reductions.



1a : X=*p*-Cl, Y=H
1b : X=Y=H
1c : X=Y=*p*-MeO
1d : X=*o*-OH, Y=H

3a–d



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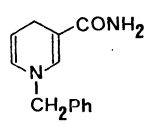
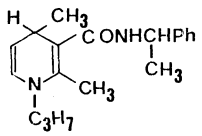


BNAH



Me_2PNPH

Results

One of substrates **1** was allowed to react with BNAH in a DMSO–methanol mixture at room temperature in the dark under a nitrogen atmosphere. For each run, after usual work-up, the product was isolated and assigned by NMR and mass spectrometries to the corresponding dibenzhydryl disulfide derivatives (**3**). No other products except the pyridinium salt were detected on TLC, indicating quantitative forma-

Table 1. Reaction with BNAH[a]

S[b]	Q_s[c] mmol	Q_{BNAH}[d] mmol	Reaction time	Solvent[e]	C_D/%[f] NMR	C_D/%[f] MS
1a	0.15	0.17	3 d	DMSO(1)-CD_3OD(0.5)	84	—
	0.13	0.13[g]	5 d	DMSO(1)-CH_3OH(0.5)	<10	—
	0.31	0.33	4 d	CH_2Cl_2(1)-CD_3OD(1)	<35	—
1b	0.30	0.28	0.5 h	CD_3OD(1)	31	32
	0.30	0.28	24 h	DMSO(1)-CD_3OD(0.5)	38	32
	0.15	0.16[g]	24 h	DMSO(0.5)-CH_3OH(0.25)	43	—
	0.31	0.37	20 h	MeCN(0.5)-CD_3OD(0.5)	43	48
	0.30	0.28	24 h[h]	DMF(0.5)-CD_3OD(0.5)	38	36
	0.31	0.37	20 h	$PhCH_3$(1)-CD_3OD(0.5)	31	—
	0.31	0.36	41 h	CD_3CN(2)	0	
1c	0.20	0.24	6 d	DMSO(1)-CD_3OD(0.5)	15	15
	0.16	0.17[g]	6 d	DMSO(1)-CH_3OH(0.5)	36	41
	0.21	0.23	5 d	DMF(1)-CD_3OD(0.5)	12	—
	0.21	0.23	7 d	$PhCH_3$(1)-CD_3OD(2)	6	6
1d	0.65	0.67	15 h	DMSO(1)-CD_3OD(0.5)	0	0
	0.55	0.55	19 h	C_2H_5OD(2.5)	0	0
	0.30	0.34[g]	23 h	DMSO(1)-CH_3OH(0.5)	≈100	≈100
2	0.26	0.28	2.5 h	DMSO(1)-CD_3OD(0.5)	0	0
	0.13	0.14[g]	4 h	DMSO(0.5)-CH_3OH(0.25)	≈100	≈100

a) At room temperature under a nitrogen atmosphere in the dark. b) S stands for substrate. c) Q_s is the quantity of S used. d) Q_{BNAH} is the quantity of BNAH used. e) The figures in parentheses indicate quantities in cm^3. f) C_D is the D content in a product. g) Reaction with BNAH-4,4-d_2.[10] h) At 273 K.

Table 2. Reaction with Me_2PNPH[a]

S[b]	Q_s[c] mmol	Q_M[d] mmol	Reaction time	Solvent[e]	C_D/%[f]
1a	0.15	0.18	29 h	DMSO(1)-CD_3OD(0.5)	>89
1b	0.32	0.39	33 h	DMSO(1)-CD_3OD(0.5)	92
1c	0.20	0.23	2 d	DMSO(1)-CD_3OD(0.5)	43
	0.18	0.21	2 d	DMSO(1)-C_2H_5OD(0.5)	35
1d	0.35	0.41	23 h	DMSO(2)-CD_3OD(1)	34
	0.18	0.21	41 h	DMSO(1)-C_2H_5OD(0.5)	59
2	0.16	0.18	4 d	DMSO(1)-CD_3OD(0.5)	0

a) At room temperature under a nitrogen atmosphere in the dark. b) S stands for substrate. c) Q_s is the quantity of S used. d) Q_M is the quantity of Me_2PNPH used. e) The figures in parentheses indicate quantities in cm^3. f) C_D is the D content in a product measured on NMR.

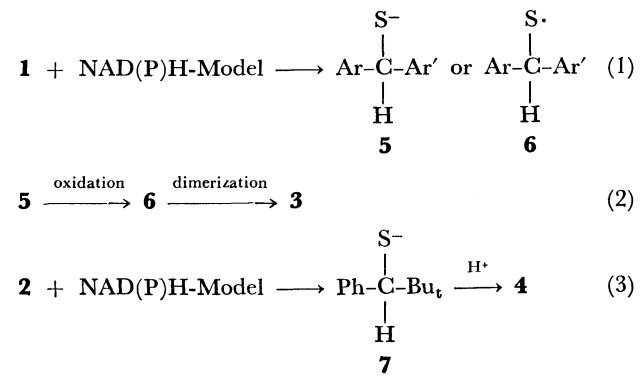
tion of **3**. In contrast, reaction of **2** with BNAH or Me_2PNPH afforded 1-phenyl-2,2-dimethyl-1-propanethiol (**4**) quantitatively.

When the reduction was carried out in a DMSO–methanol-d_4 mixture, the isolated product contained a deuterium atom in its methine position except in the case with **1d**. It is apparent that this deuterium atom originates from the solvent. Analyses by NMR and mass spectrometries gave deuterium contents 84%, 35±3%, and 15%, for **3a**, **3b**, and **3c**, respectively. Solvent effect also was examined by carrying out the reduction in several solvent systems containing methanol-d_4 or other deuterated compounds. Reductions of **1d** in DMSO–methanol-d_4 or ethanol-*O*-*d* and of **2** in DMSO–methanol-d_4 gave no deuterated products. On the other hand, reductions of these substrates with BNAH-4,4-d_2 afforded products having wholly deuterated methine groups. Results obtained are summarized in Table 1 together with those for reductions under other conditions.

A more dramatic hydrogen-incorporation from solvent into product was seen in reductions with Me_2PNPH in DMSO–methanol-d_4 or DMSO–ethanol-*O*-*d*. Even **1d** afforded a considerably deuterated product. Results obtained are listed in Table 2.

Discussion

In the reaction course leading to product **3** it is sure that **1** will be reduced by the model compound in the first stage (Eq. 1) to afford, as the primary reduction product, a thiolate anion (**5**) or thiyl radical (**6**), which will then dimerize to yield **3** during work-up or *in situ* (Eq. 2).[1,9] On the other hand, **2** is known to be reduced with the model compound to a thiolate anion (**7**),[11] which is protonated upon the



sulfur atom during work-up (Eq. 3). Here, we should recall the fact that the hydrogen on the methine position of **3** does not exchange with any hydrogens from solvents even under basic conditions.[12] Moreover, the dimerization of **5** or **6** to **3** is not likely to be accompanied by hydrogen exchange. Therefore, there is no doubt that in any cases the incorporation of hydrogen from solvent into product does occur during the first reduction step, Eq. 1, which implies involvement of at least one intermediate in this reduction step.

That the hydrogen picked up from the solvent has entered the product as a proton is obvious from the fact that no deuterium is incorporated into the product in the reduction in DMSO–acetonitrile-d_3, whereas deuterium is incorporated into the product when the reduction is carried out in ethanol-*O*-*d*. Thus, the intermediate which undergoes the out-of-cage reaction should be anionic in character. The anionic character of the intermediate is further confirmed by the following evidence. Table 1 indicates that the deuterium incorporation becomes deeper as the substituent on substrate becomes more electron-withdrawing and the solvent becomes more polar. The reduction with Me_2PNPH, as listed in Table 2, results in a deeper deuterium incorporation than that with BNAH. Me_2PNPH has two methyl groups on its dihydropyridine ring and a methylbenzyl moiety on its carbamoyl group, so that the electron-releasing power of Me_2PNPH is stronger than that of BNAH.[13] On the other hand, the presence of the 4-methyl group and less abundant reacting hydrogens makes Me_2PNPH less reactive toward proton releasing than BNAH. Consequently, the intermediate in the reduction with Me_2PNPH has more chance to escape from the solvent cage than the one in the reduction with BNAH; note that even **3d** contains deuterium from the solvent in a considerable amount.

The above discussion has revealed that the intermediate should be an anion radical, in agreement with previous evidence from kinetic,[3,11,14,15] ESR,[8,9,16] and several other studies.[5,17–19]

An anomaly with reaction of **1d** has been recognized[1] that the reduction of **1d** with BNAH takes place faster than expected from the simple electronic substituent effect. This phenomenon was interpreted in terms of general acid catalysis by *o*-hydroxyl proton on the basis of the "hydride transfer" mechanism. However, with the idea of "multi-step" mechanism,

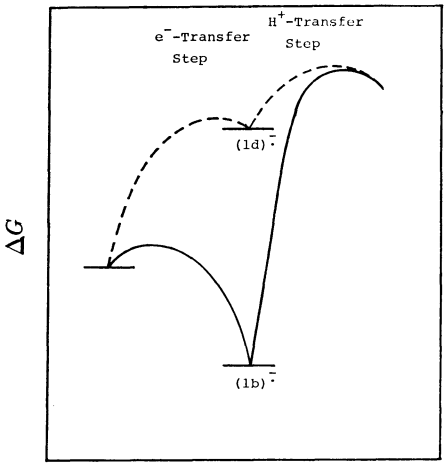
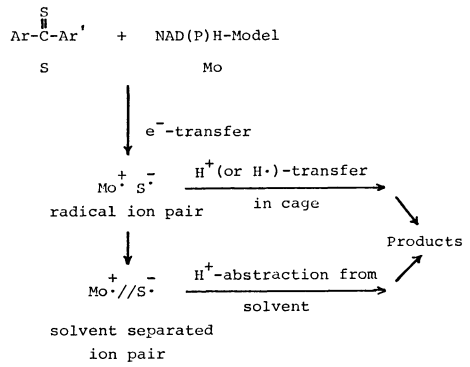


Fig. 1. Schematic illustration of energy diagrams for the reduction of thiobenzophenone (——) and *o*-hydroxythiobenzophenone (----).



Scheme 1.

this phenomenon may be accounted for in terms of the stability of the intermediate anion radical. Reduction of **1b** and some other substrates is retarded by the presence of bivalent metal ion.[11,19] The retardation is due to an extraordinary stability of the cation-radical intermediate; a stable cation radical necessarily requires high transition energy for proton-accepting reaction.[3,6,19] In **1d**, the hydroxyl proton chelates on the thiocarbonyl sulfur by hydrogen bonding, which can be recognized by its abnormal low-field shift of proton (δ=12.7 in $CDCl_3$) in NMR spectrum and by its low-frequency shifts of ν_{OH} (3430 cm^{-1}) and $\nu_{C=S}$ (1228 cm^{-1})(in 2.5% CCl_4) in IR spectrum. Thus, the thiocarbonyl group in **1d** is different in character from the one in **1b**. The electron-accepting power of the former thiocarbonyl group is, therefore, weaker than that of the latter, and the initial electron-transfer process requires more energy for **1d** than for **1b**. In other words, the energy barrier for the proton-transfer process is less for **1d** than for **1b**, resulting in more opportunity for **1d** than for **1b** to undergo the in-cage reaction. Figure 1 visualizes energy diagrams for the reduction schematically. As mentioned above, Me_2PNPH is more electron-releasing but less proton-releasing than BNAH. Therefore, the energy diagram for the reduction with Me_2PNPH may be represented by a full line (**1b**-like relationship)

in Fig. 1. Since anion radical from **2** is the least stable of all, it has no chance to escape from the solvent cage to pick up a proton from the solvent even in the reduction with Me_2PNPH.

In conclusion, the reduction of a substrate by models of NAD(P)H proceeds *via* an intermediate of ion radical-pair. The anion radical can pick up a proton from the solvent when it has life-time long enough to escape from the solvent cage as shown in Scheme 1. Thus, "direct transfer of a hydrogen from a model to a substrate" is valid only for those substrates which will afford unstable anion radicals.

In enzymic reactions, a substrate is kept in a pocket of enzyme, and it has no chance to escape from the pocket into the bulk of solvent. It should also be kept in mind that all substrates susceptible to reduction by NAD(P)H-dependent dehydrogenases are such as afford unstable intermediates. Therefore, it is reasonable that "direct transfer of a hydrogen" is observed in all enzymic reactions.

Experimental

Materials. *p*-Chlorothiobenzophenone (**1a**), thiobenzophenone (**1b**), *p,p'*-dimethoxythiobenzophenone (**1c**), and *o*-hydroxythiobenzophenone (**1d**) were prepared from their corresponding ketones according to literature procedures,[1] and were purified by column chromatography using Florisil and carbon tetrachloride eluent (for **1a** and **1d**) or with recrystallization from ethanol (for **1b** and **1c**). Preparations of 1-benzyl-1,4-dihydronicotinamide (BNAH),[20] BNAH-4,4-d_2,[11,21] *N*-(α-methylbenzyl)-1-propyl-2,4-dimethyl-1,4-dihydronicotinamide (Me_2PNPH),[22] and thiopivalophenone (**2**)[23,21] were described previously. Spectral data of each material were satisfactory. The deuterium content of BNAH-4,4-d_2 was 99.0±1.0%. Each non-deuterated solvent was distilled and dried according to the usual method before use. Deuterated solvents were commercially available (Commissariat a l'Energie Atomique).

Product Analysis. In a vessel equipped with a Silicone-rubber stopper, a mixture of **1** or **2** and a model compound in an appropriate solvent was stirred under a nitrogen atmosphere in the dark. When the reaction was complete (on TLC), water was added to the mixture and the product was extracted three times with dichloromethane. The organic layer was washed twice with water, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The resulting residue was purified by HPLC using silica gel and benzene eluent (for **1a** and **1d**), with recrystallization from ethanol (for **1b** and **1c**), or by column chromatography using silica gel and hexane eluent (for **2**). Identification of isolated product was based on NMR (JASCO JNM-FX 100 FT NMR Spectrometer), MS (Hewlett-Packard 18947A GC/MS), and IR (Hitachi EPI-S-2 Spectrometer) spectra. Deuterium contents for products from reactions in deuterated solvent were estimated through comparison with those of authentic samples obtained from corresponding reactions in non-deuterated solvent.

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