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The kinetics of the reduction of the lipophilic quinone avarone by *n*-alkyl-1,4-dihydronicotinamides of various lipophilicities

MARIO ZLATOVIĆ, DUŠAN SLADIĆ and MIROSLAV J. GAŠIĆ

Faculty of Chemistry and Center for Chemistry, ICTM, University of Belgrade, Studentski trg 14, Belgrade, Yugoslavia

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Several NADH model compounds, *N*-alkyl-1,4-dihydronicotinamides, some of them possessing amphiphilic properties, have been synthesized, and the kinetics of their reaction with a biologically active liphophilic quinone, avarone, has been studied in a protic solvent both in the presence and absence of cationic, anionic or non-ionic surfactants. In the absence of micellar agents, the medium- and long-chain *N*-dodecyl (**3**) and *N*-heptadecyl (**4**) derivatives show a significant increase in the reaction rates compared to other model compounds, due to the stabilization of the semiquinone intermediate. Anionic surfactants retard the reaction, non-ionic surfactants slightly accelerate the reaction with the short-chain derivatives, and retard the reaction with the medium- and long-chain derivatives, and the cationic surfactants increase the reaction rate with all derivatives except the long-chain **4**. The results support the *e-p-e* mechanism of the reduction of lipophilic quinones by NADH models in protic medium.

Key words: avarone, quinone, NADH models, surfactants, kinetics.

The redox reactions of compounds containing a quinone/hydroquinone moiety have been extensively examined[1] but still, for particular cases, simplified or inconclusive presentations of the mechanism are occasionally offered. The ambiguities in the interpretation are usually related to cases in which it was experimentally difficult to distinguish between stepwise proton-electron-proton transfers[2] and one-step two-electron (hydride transfer) processes.[3] Consequently, effective extrapolations of the results of chemical experiments to biological systems is hampered both by the complexity of these systems and an apparent susceptibility of the reaction course of structurally different quinoid compounds to the reaction conditions.[4,5] Nevertheless, the established importance of pyridine nucleotide coenzymes in enzymatic redox reactions prompted the investigation of the mechanism of the oxidation of NADH and related compounds as electron donors with simple quinones. The currently growing interest for this reaction is also instigated by the fact that many physiologically active compounds of natural or synthetic origin contain quinoid moieties in a redox equilibrium.

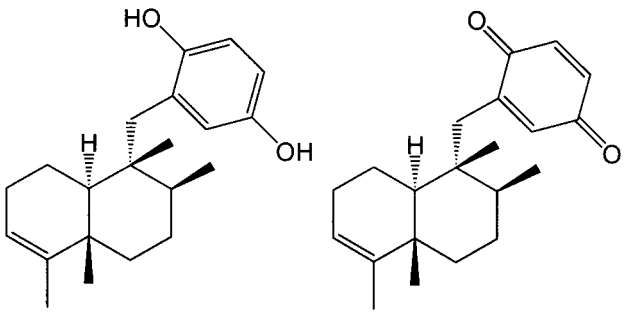


Fig. 1. The sesquiterpenoid hydroquinone/quinone couple, avarol and avarone

For some of quinoid compounds it has been well established that they undergo direct nucleophilic addition in a biological medium; for others, particularly those with lipid properties, redox activation in the cell membranes seems to be the crucial step for the subsequent reaction sequence. One such redox couple, the sesquiterpenes avarol and avarone[6] (Fig. 1), has ween the subject of intensive investigation by several groups.[7,8] This work was initiated by our finding of the pronounced antitumor activity of this redox couple.[9] On the basis of complementary investigations, we have suggested that this couple undergoes reversible, one electron transfer with the formation of quinone radical anions which, in reaction with molecular oxygen, produce superoxide radicals, the species responsible for the observed bioactivity.[10,11] This proposal was substantiated by kinetic experiments on the oxidation of BNAH with avarone and a series of structurally related quinones in different protic and non-protic solvents.[12] Since the avarol/avarone redox couple has lipid properties, it is quite likely that in living cells, the redox activation of this couple takes place in biological membranes. Therefore, to approximate biological conditions, kinetic and electrochemical experiments were carried out in cationic, anionic and neutral micellar systems, yielding results which confirmed the formation of the proposed quinone anion radical intermediate in the oxidation of BNAH.[12,13]

In this work, this approach was extended by following the course of the reduction of avarone with *N*-alkyl-1,4-dihydronicotinamide derivatives of different chain length, some of them possessing amphiphilic properties, in a protic solvent, both in the presence and absence of surfactans. These amphiphilic compounds can be considered as good NADH models since no significant change in the redox potential, resulting from differences in the alkyl chain length, has been observed.[14]

RESULTS AND DISCUSSION

In this work, four alkyl derivatives, *N*-butyl(**1**), *N*-octyl(**2**), *N*-dodecyl(**3**) and *N*-heptadecyl-1,4-dihydronicotinamide **(4)** (Fig. 2) were synthesized by alkylation of nicotinamide and subsequent sodium dithionite reduction of the *N*-alkyl-3-carbamoylpyridinium halide (Scheme 1).

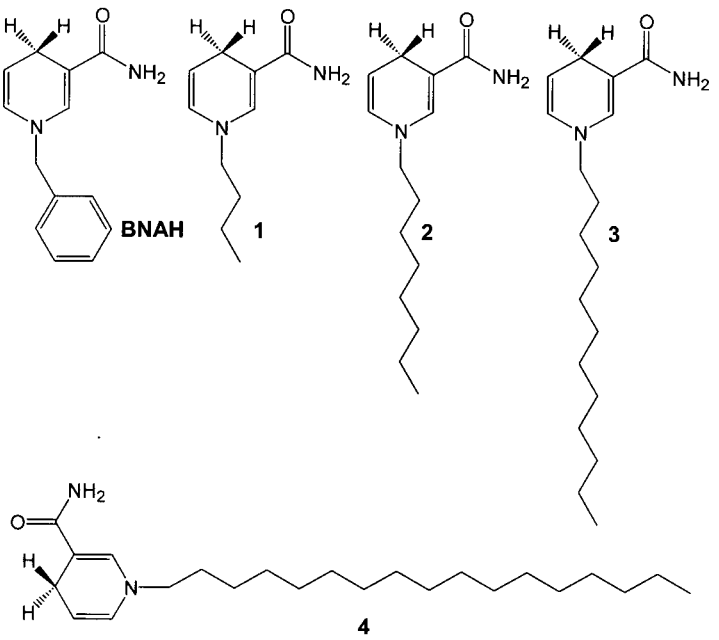
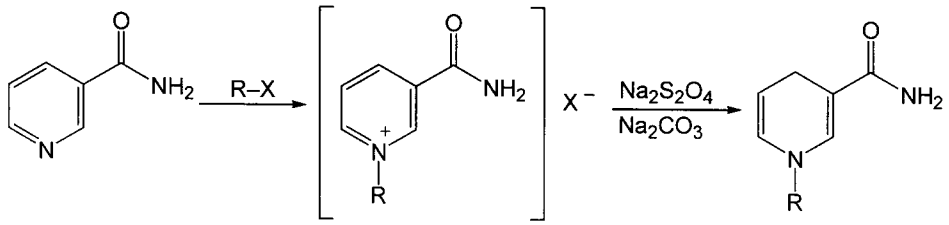


Fig. 2. Synthesized derivatives of 1,4-dihydronicotinamide

The reaction rates for the reduction of avarone by the NADH models were determined in a protic polar solvent (H_2O : EtOH, 1:1, v/v), both in the presence and absence of surfactants (SDS, CTAB, Tween 80). The solution was buffered with 0.02M sodium phosphate buffer solution (pH 6.98). The concentration of both reactants was 1×10^{-4} M. The reaction was carried out under an inert atmosphere (argon, < 3 ppm O_2).



Scheme 1.

The reaction rates were followed by monitoring the changes in the UV absorption at λ=350 nm (characteristic absorption maximum for the 1,4-dihydronicotinamide functionality).

The results are given in Table I, as relative second order rate constants in regard to the rate constant of the reaction of BNAH with avarone (k=0.67 mol^{-1} dm^3 s^{-1}).

In the absence of micellar agents, the rate of the lipophilic quinone avarone reduction by the NADH model compounds depends on the number of carbon atoms

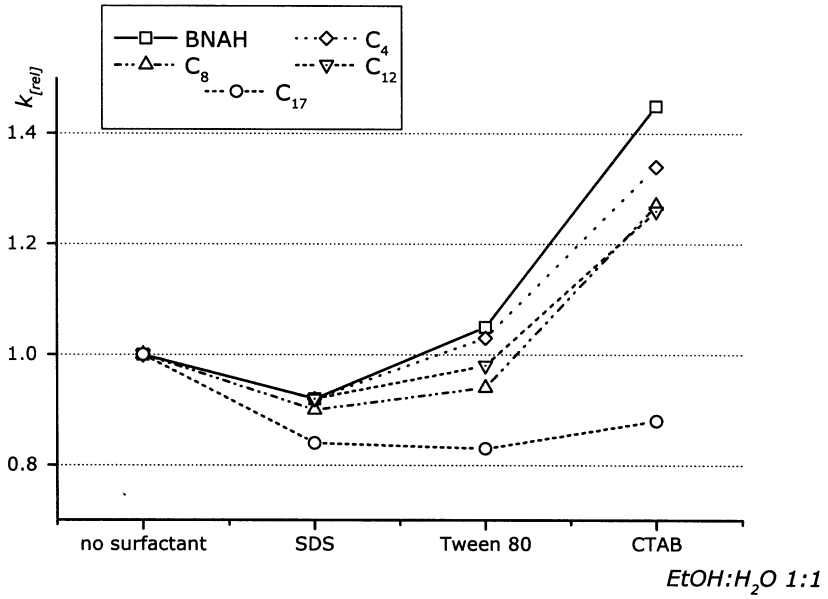


Fig. 3. The relative reaction rate constants of the reduction of avarone by various NADH derivatives in ethanol-water 1:1 (v/v), pH 6.98, with and without surfactants. The relative reaction rate constants were calculated with respect to the rate of reaction in the absence of surfactant for a particular derivative.

in the *N*-alkyl chain. The reaction was slowest with the *N*-butyl derivative, and fastest with the *N*-heptadecyl derivative. The significant increase in the reaction rate with the *N*-dodecyl and the *N*-heptadecyl derivatives is in accordance with our previously proposed mechanism.[11] As step I (Scheme 2) is the rate determinig step, the formation of a tightly bound ion pair in step II, stabilized by both ionic and hydrophobic interactions, should result in an increase in the reaction rate with increasing alkyl chain length of the derivatives. In this respect, the long-chain *N*-alkyl derivatives are taking on the role of CTA^+, which accelerates the reaction of avarone with the NADH model compounds by stabilizing the semiquinone intermediate. However, they are even more effective, since they directly participate in the formation of the ion pair in step II, and also by increasing the local concentrations of the reacting species. In the proposed *e-p-e* mechanism, steps III, IV and V are very fast, so that additional acceleration of these steps has no significant effect on the total reaction rate.

The effect of surfactants on the reaction rates is shown is Table I and Fig. 3. In Fig. 3, for each NADH model compound, the reaction rate without added surfactant is taken as unity and the rates in the presence of a surfactant are given relative to it. As expected, the anionic surfactant SDS deccelerates the reaction, due to the destabilization of the semiquinone intermediates. The nonionic surfactant Tween 80 slightly accelerates the reaction with BNAH and the *N*-butyl derivative by bringing together the reacting species, but decreases the reaction rate with the

$$\underset{\text{I}}{\text{R–NADH + Q}} \rightleftarrows \underset{\text{II}}{[\text{R–NADH,Q}]} \rightleftarrows \underset{\text{III}}{[\text{R–NADH}^{+\bullet}, \text{Q}^{-\bullet}]} \rightleftarrows$$

$$\rightleftarrows \underset{\text{IV}}{[\text{R–NAD}^{\bullet}, \text{QH}^{\bullet}]} \rightleftarrows \underset{\text{V}}{[\text{R–NADH}^{+},\text{QH}^{-}]} \rightleftarrows \text{R–NAD}^{+} + \text{QH}^{-}$$

$$\text{R} = \text{–CH}_2\text{–Ph}, \text{–C}_4\text{H}_9, \text{–C}_8\text{H}_{17}, \text{–C}_{12}\text{H}_{25}, \text{–C}_{17}\text{H}_{35}$$

Scheme 2.

medium- and long-chain derivatives, probably due to their automicellization, so that there is a concurrent distribution of the lipophilic quinone between the Tween 80 micelles, and the micelles of the lipophilic NADH model. In this way, the effective concentration of the quinone is lowered, resulting in a decrease in the reaction rate. As expected, this effect is most pronounced with the *N*-heptadecyl derivative. The cationic surfactant CTAB accelerates the reaction with BNAH and short- and medium-chain *N*-butyl, *N*-octyl and *N*-dodecyl derivatives by stabilization of the semiquinone intermediate. However, the reaction of the *N*-heptadecyl derivative is deccelerated in the presence of the cationic surfactant. In addition, there is a strong acceleration of the reaction in the presence of CTAB, compared to the reaction in the presence of Tween 80 for all the NADH models except the *N*-heptadecyl derivative. The reason for the different behaviour of the short- and medium-chain derivatives *vs*. the long-chain *N*-heptadecyl derivative might lie in the difference in their solubilization in CTAB micelles. Namely, electrochemical studies have shown that BNAH is not solubilized in CTAB micelles.[13] On the other hand, kinetic studies indicate that the uncharged NADH model 1-hexadecyl-4-cyano-1,4-dihydronicotinamide is well solubilized in CTAB micelles, contrary to the positively charged oxidized froms.[15] Thus, different effects of CTAB can be observed depending on the solubilization of the NADH derivative in the micelle. Since avarone is solubilized in CTAB micelles, then, if there is no solubilization of the NADH derivative, an increase of the reaction rate is expected, due to the stabilization of the semiquinone derivative, assuming that the reaction takes place in the Stern region of the micelle. Such an effect is observed with BNAH and the short- and medium-chain derivatives. On the other hand, if the NADH model is solubilized in the CTAB

TABLE I. The relative reaction rate constants of reduction of avarone by various NADH derivatives in ethanol-water: 1:1 (v/v), pH 6.98, with and without surfactants

Type and conc. of surfactant	*N*-alkyl-1,4-dihydronicotinamide				
(M)	BNAH	**1**	**2**	**3**	**4**
0	1.00	0.79	1.08	2.02	2.62
SDS, 1.5×10^{-3}	0.92	0.73	0.97	1.85	2.19
Tween 80, 1.5×10^{-3}	1.05	0.81	1.02	1.98	2.18
CTAB, 1.5×10^{-3}	1.45	1.06	1.37	2.54	2.31

micelles, as expected for the *N*-heptadecyl derivative, then the positively charged one-electron oxidation product (radical cation) would be destabilized in the positively charged micelle, resulting in a decrease in the reaction rate.

Our results support the *e-p-e* mechanism of the reduction of lipophilic quinones by BNAH and its derivatives in a protic medium. However, the hydride two-electron reduction mechanism cannot be excluded in reactions of less lipophilic quinones and under different reaction conditions.

EXPERIMENTAL

Physical measurements

^{1}H-NMR spectra were recorded on a Varian FT-80A instrument using TMS as an internal standard; UV/VIS spectra were recoreded on a Beckman D-25 spectrophotometer; melting points were determined in a Thiele apparatus and are uncorrected; molar absorption coefficient and λ_{max} were determined in ethanol-water mixture at pH 6.98, using 1×10^{-4} M concentrations.

Avarol was isolated[16] from the marine sponge *Dysidea avara* and avarone was obtained by oxidation of avarol with silver(I) oxide as described earlier.[6]

1-Benzyl-3-carbamoylpyridinium chloride: 0.6 g of nicotinamide and 1.0 g of benzylchloride (Fluka) were refluxed in 12 ml of MeOH. After 3 h the MeOH was partially evaporated and the solution was left in a cold place for the quaternary ammonium salt to crystallize. After filtration, the crude product (1.2 g) was recrystallized from MeOH. The yield of white 1-benzyl-3-carbamoyl-pyridinium chloride was 0.9 g, (74%), m.p.220 °C; microanalysis: calcd. for $C_{13}H_{13}ClN_2O$: C 62.78%, H 5.27%, N 11.26%; found: C 62.05%, H 5.45%, N 11.68%.

N-alkyl derivatives of nicotinamide (general procedure): 10 mmol of nicotinamide was dissolved in 15 ml of 1-pentanol and a solution of 12 mmol of the *n*-alkyl halide in 1-pentanol was added. The mixture was refluxed for 3-4 h. After cooling the product solidified and occluded almost all of the solvent. The content of the flask was transferred onto a Büchner funnel, filtered and washed with a small amount of cold 1-pentanol and light petroleum. The crystals were dried for several hours *in vacuo* at 60 °C. Dried salt was used for subsequent reduction without the need for additional purification. The derivatives obtained by this procedure are:

1-Butyl-3-carbamoylpyridinium bromide: yield 80.5%, m.p. >220 °C, *microanalysis:* calcd. for $C_{10}H_{15}BrN_2O$: C 46.35%, H 5.83%, N 10.81%; found: C 46.36%, H 5.99%, N 10.71%.

1-Octyl-3-carbamoylpyridinium bromide: yield 65.7%, m.p. >220 °C, *microanalysis:* calcd. for $C_{14}H_{23}BrN_2O$: C 53.34%, H 7.35%, N 8.89%; found: C 52.76%, H 7.17%, N 9.44%.

1-Dodecyl-3-carbamoylpyridinium bromide: yield 68.2%, m.p. > 220 °C, *microanalysis*: calcd. for $C_{18}H_{31}BrN_2O$: C 58.22%, H 8.41%, N 7.54%; found: C 58.77%, H 8.48%, N 7.64%.

1-Heptadecyl-3-carbamoylpyridinium bromide: yield 60.3%, m.p. >220 °C, *microanalysis:* calcd. for $C_{23}H_{41}BrN_2O$: C 62.57%, H 9.36%, N 6.35%; found: C 61.95%, H 9.36%, N 6.42%.

The reduction of the quaternary ammonium salts was carried out according to the classical Westheimer procedure.[17]

N-butyl-1,4-dihydronicotinamide: 5 mmol of 1-butyl-3-carbamoylpyridinium bromide dissolved in 10 ml of water was added dropwise into a mixture of 1.38 g anhydrous Na_2CO_3 and 2.61 g fresh $Na_2S_2O_4$ (Fluka, 85%) in 15 ml of water at 40°–50 °C. After 10–12 min the oily yellowish dihydro derivative separates. The reaction mixture was allowed to cool for 5 min and then extracted with $CHCl_3$. The extract was washed with water, dried over anh. Na_2CO_3, and the $CHCl_3$ evaporated. After recrystallisation from ethanol and drying in a vacuum desiccator over P_2O_5, 65% of the yellow dihydroderivative was obtained; m.p. 135 °C (decomp.); λ_{max} 352 nm; ε_{350} 6720; ^{1}H-NMR ($CDCl_3$): δ 7.35 (1H, *s*), 7.00 (1H, *s*), 5.68 (2H, *m*), 4.70 (1H, *m*), 3.10 (2H, *t*), 1.03 (4H, *s*), 0.90 (3H,*t*).

N-Alkyl derivatives of 1,4-dihydronicotinamide (general procedure): 5 mmol of the quaternary ammonium salt dissolved in 10 ml of solvent was added dropwise into a mixture of 1.38 g anhydrous Na_2CO_3 and 2.61 g fresh $Na_2S_2O_4$ (Fluka, 85%), in 15 ml of solvent kept at 40–50 °C. After 10–12 min, the yellow dihydro derivative separates. The reaction mixture was allowed to cool for 5 min and then the solid product was filtered, washed with cold water, recrystallized from ethanol, dried in vacuum desiccator over P_2O_5 and used immediately. The derivatives obtained by this procedure are:

N-Benzyl-1,4-dihydronicotinamide: solvent: water; yield 61%; m.p. 110 °C (decomp.); λ_{max} 354 nm; ε_{350} 6950; ^{1}H-NMR ($CDCl_3$): δ 7.12 (5H, *m*), 5.70 (1H, *d*), 5.25 (1H, *s*), 4.70 (1H, *m*), 4.20 (2H,*s*), 3.10 (2H, *s*).

N-Octyl-1,4-dihiydronicotinamide: solvent: water-ethanol (9:1); yield 70%; m.p. 120 °C (decomp.); λ_{max} 352 nm; ε_{350} 6812; ^{1}H-NMR ($CDCl_3$): δ 7.31 (1H, *s*), 7.00 (1H, *s*), 5.67 (2H, *m*), 4.62 (1H, *m*), 3.09 (2H, *t*), 1.26 (12H, *s*), 0.90 (3H, *t*).

N-Dodecyl-1,4-dihydronicotinamide: solvent: water-ethanol (9:1); yield 81%; m.p. 100 °C (decomp.); λ_{max} 350 nm; ε_{350} 6834; ^{1}H-NMR ($CDCl_3$): δ 7.26 (1H, *s*), 7.03 (1H, *d*), 5.72 (2H, *m*), 5,.23 (2H, *s*), 4.72 (1H, *q*), 3.08 (2H, *t*), 1.25 (20H, *s*), 0.90 (3H, *t*).

N-Heptadecyl-1,4-dihydronicotinamide: solvent: water-ethanol (9:1); yield 62%; m.p. 94 °C (decomp.); λ_{max} 354 nm; ε_{350} 6806^{-1}H-NMR ($CDCl_3$): δ 7.21 (1H, *s*), 7.00 (1H, *d*), 5.78 (2H, *m*), 5.30 (2H, *s*), 4.68 (1H, *m*), 3.10 (2H, *t*), 1.20 (30H, *s*), 0.90 (3H, *t*).

Determination of the reaction rates. The reduction reactions of avarone by the dihydronicotinamide derivatives were performed in quartz cells with hermetic Teflon stoppers. The concentrations of avarone and 1,4-dihydronicotinamide derivatives were 1.00×10^{-4}. M. The solutions were made in ethanol-water 1:1 (v/v) with 0.02 M sodium phosphate buffer pH 6.98. The CTAB, SDS and Tween 80 surfactants were commercial products (Serva) and were used in 1.50×10^{-3} M concentrations. All solutions were purged with argon (< 3 ppm O_2) prior to use and all work was doen under an argon atmosphere. The reactions were monitored on a Beckman D-25 spectrophotometer, using the change in absorption at λ=350 nm (characteristic absorption maximum for the 1,4-dihydronicotinamide functionality).

И З В О Д

КИНЕТИКА РЕДУКЦИЈЕ ЛИПОФИЛНОГ ХИНОНА АВАРОНА *N*-АЛКИЛ-1,4-ДИХИДРОНИКОТИНАМИДИМА РАЗЛИЧИТЕ ЛИПОФИЛНОСТИ

МАРИО ЗЛАТОВИЋ, ДУШАН СЛАДИЋ и МИРОСЛАВ Ј. ГАШИЋ
Хемијски факултет, Студентски трг 14, Београд и Центар за хемију ИХТМ-а, Његошева 12, Београд

Синтетисано је неколико *N*-алкил-1,4-дихидроникотинамида, модел-једињења NADH, од којих неки имају амфифилне особине, и проучавана је кинетика њихове реакције са биолошким активним, липофилним хиноном авароном у протичном растварачу у присуству катјонских, анјонских или нејонских површински активних супстанци и без њих. Без додатих мицеларних агенаса, *N*-додецил-дериват **3** (дериват средње дужине низа) и дуголанчани *N*-хептадецил-дериват **4** показују значајно повећање брзине реакције у поређењу са другим модел-једињењима, услед стабилизације семихинонских интермедијера. Анјонски површински активни агенси успоравају реакцију, нејонски агенси доводе до слабог убрзања са дериватима кратког алкил-низа, а успоравају реакцију са дериватима средњег и дугог низа, док катјонски агенси убрзавају реакцију са свим дериватима, сем са дуголанчаним **4**. Резултати указују на механизам *e-p-e* редукције липофилних хинона моделима NADH у протичном медијуму.

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