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REACTION OF DERIVATIVES OF 1,4-DIHYDROPYRIDINE WITH THE PEROXYNITRITE ANION

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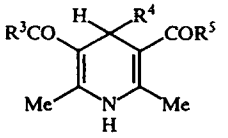
The reaction of derivatives of 1,4-dihydropyridine (1,4-DHP) with peroxynitrite anion is an oxidation reaction; no products of nitration were detected. It was found that some 1,4-DHP increase the lifetime of the peroxynitrite anion and the yield of hydroxyl radical in decomposition of the peroxynitrite anion.

The peroxynitrite anion ($ONOO^-$) is a strong oxidant and is formed from nitrogen superoxide and oxide both in model systems and in the body [1, 2]. On protonation, the peroxynitrite anion forms unstable peroxynitrous acid (ONOOH) which spontaneously decomposes with the formation of hydroxyl radical ($HO^{\cdot}$) and ($NO_2^{\cdot}$) [3]. The physiological role of the peroxynitrite anion is not yet clear, but it is a strong bactericidal compound whose intense formation damages surrounding tissues in the region of phagocytosis. The role of the peroxynitrite anion as a participant in the secondary alarm system in the cell is discussed in [4].

1,4-DHP has radioprotective, antiaggregation, and other types of physiological activity in addition to the well-known cardiovascular action [5, 6]. Derivatives of 1,4-DHP not substituted in position 4 are antioxidants [7] and have high reactivity with respect to free radicals [8].

The reaction of some water-soluble derivatives of 1,4-DHP (I), including derivatives of 1,4-dihydroisonicotinic acid, with the peroxynitrite anion and the products of this reaction and the effect of derivatives I on the lifetime of the peroxynitrite anion and formation of hydroxyl radical from the anion were investigated to study the reactivity of physiologically active derivatives of 1,4-DHP with biological oxidants.



Ia-g

The analysis of the products of the reaction of 1,4-DHP with the peroxynitrite anion (Table 1) showed that in the conditions used (phosphate buffer, pH 7.4; temperature of 20 ± 2°C), oxidation or decomposition of 1,4-DHP primarily takes place. For 1,4-DHP Ia, oxidative hydrolysis of the ester substituent in positions 3 and 5 also occurs. For compound Ib containing a phenyl substituent in position 4, neither hydroxylation nor nitration of the phenyl ring was found. Derivatives of 1,4-dihydroisonicotinic acid Ic and Id containing a carboxyl group in position 4 undergo oxidative decarboxylation, previously observed in the effect of another oxidant on these compounds — sodium nitrite in acetic acid [9]. It was surprisingly found that derivatives Ia and Ib increase the lifetime of the peroxynitrite anion while Ib also increases the yield of hydroxyl radical (intensifies hydroxylation of benzoic acid). This property of increasing the lifetime (stability) of the peroxynitrite anion is characteristic of the structure of 1,4-DHP, since the pyridine derivative corresponding to Ia — 2,6-dimethylpyridine-3,5-dicarboxymethylcarboxylic acid disodium salt — does not have this property. The peroxynitrite anion could form an adduct with compound Ia which is more stable than the peroxynitrite anion itself. The maximum increase in the lifetime of the peroxynitrite anion in this adduct is observed for a peroxynitrite anion—Ia molar ratio of 1:1. Formation of a similar adduct

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TABLE 1. Effect of 1,4-DHP Derivatives on the Lifetime $(\tau/\tau_0)^*$ of the Peroxynitrite Anion ($ONOO^-$) and Formation of Hydroxyl Radical $(F/F_0)^{*2}$

Compound	R_3 = R_5	R_4	τ/τ_0	F/F_0	Products of the reaction (HPLC data)
Ia	OCH_2COONa	H	1,45	0,89	2,6-Dimethylpyridine-3,5-dicarboxymethylcarboxylic acid disodium salt (85%); 2,6-dimethylpyridine-3,5-dicarboxylic acid disodium salt (15%)
Ib	OCH_2COONa	C_6H_5	1,15	1,57	100% Ib
Ic	CH_3	COONa	0,34	0,69	2,6-Dimethyl-3,5-diacetylpyridine (100%)
Id	OC_2H_5	COONa	0,25	0,34	2,6-Dimethyl-3,5-diethoxycarbonylpyridine (100%)
Ie	OC_2H_5	$CONH(CH_2)_2SO_3Na$	0,95	1,00	Ie (95%) and two unidentified products
Ig	OC_2H_5	$CONHCHCOONa$ $\vert$ $(CH_2)_2COONa$	0,77	1,00	Ig (95%) and four unidentified products
2,6-Dimethylpyridine-3,5-dicarboxymethylcarboxylic acid disodium salt			1,0	1,0	Starting substance (100%)

*τ) Lifetime (sec) of the peroxynitrite anion in the presence of the substance studied, τ_0) in the absence of the substance.

*^2F) Relative intensity of fluorescence of hydroxybenzoic acids (products of hydroxylation of benzoic acid) in the presence of the substance studied; F_0) in the absence of the substance.

of the peroxynitrite anion with ascorbic acid was noted in [10]. 1,4-DHP containing electron-acceptor substituents in position 4 (Ib, e, h) and a pyridine derivative — 2,6-dimethylpyridine-3,5-dicarboxymethylcarboxylic acid disodium salt — do not react with the peroxynitrite anion or with the hydroxyl radical formed from it. Since the hydroxyl radical reacts energetically with almost all organic compounds, we can hypothesize that in the given case, another softer oxidant is formed from the peroxynitrite anion — the cryptohydroxyl radical. The possible existence of cryptohydroxyl radicals has been noted repeatedly (see, e.g., [11]). In all probability, the cryptohydroxyl radical is formed in decomposition of ONOOH, since at pH > 11, oxidation takes place very slowly or does not take place at all.

1,4-DHP derivatives containing electron-donor groups in the molecule thus oxidize the peroxynitrite anion (or other products of its transformation), basically with formation of the corresponding pyridine derivatives, and in the case of 1,4-dihydroisonicotinic acid derivatives, oxidative decarboxylation takes place.

EXPERIMENTAL

The derivatives of 1,4-DHP, 1,4-dihydroisonicotinic acid, and pyridine were synthesized at the Latvian Institute of Organic Synthesis (concentration of basic substance of no less than 98%) and the remaining reagents were from Aldrich.

The peroxynitrite anion was generated with the method in [1] from solutions of $NaNO_2$, HCl, and H_2O_2. Equimolar quantities of the peroxynitrite anion and the investigated compounds in 0.1 M phosphate buffer (pH 7.4) was mixed in the standing jet installation of a Hitachi 557 spectrophotometer. The concentration of the peroxynitrite anion was followed by the absorption at 302 nm. The concentration of the substances (~0.25 mmole) was selected as a function of the concentration of the peroxynitrite anion determined immediately before the experiment. The lifetime of the peroxynitrite anion in a control experiment was 1.52 sec.

Formation of the hydroxyl radical was determined fluorometrically with hydroxylation of benzoic acid according to [12]. Then 5 mmole of a solution of benzoic acid and 0.1 mmole of a solution of the compound investigated in 0.1 M phosphate buffer (pH 7.4) was rapidly mixed with a solution of the peroxynitrite anion (concentration of ~0.25 mmole) at 20 ± 2°C in the three-sided cuvette of a Hitachi 850 fluorimeter positioned at an angle of 45° to the exciting beam and the relative flu-

orescence was measured at 430 nm after 5 min (excitation of 300 nm). The average data for (τ/τ_0) and (F/F_0) from 5-8 measurements are reported in Table 1; the root-mean-square error did not exceed $\pm 10\%$.

The products of the reaction with the peroxynitrite anion were analyzed by HPLC on a Gilson 302 (UV detector) using a 4.6 × 250 mm column with Zorbax C_8 stationary phase. The mobile phase was 20-40% acetonitrile in 0.1 mole phosphate buffer.

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