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SYNTHESIS AND PROPERTIES OF NICOTINIC ACID DERIVATIVES CONTAINING A 2,2,6,6-TETRAMETHYLPIPERIDINE 1-OXYL RESIDUE

N. L. Lifshits, N. I. Mal'tsev, V. A. Yakovlev, E. A. Zorontsov, and N. S. Zakharova

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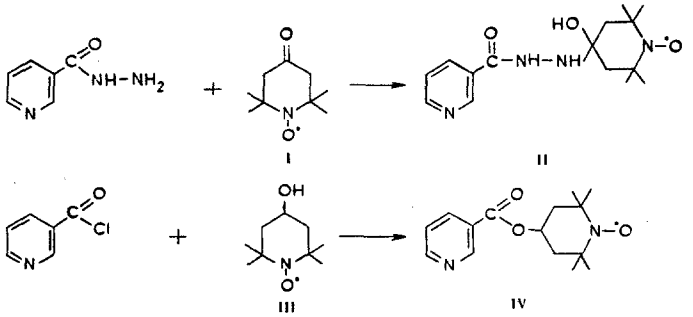
N^1-(Nicotinoyl)-N^2-4-(2,2,6,6-tetramethyl-4-hydroxypiperidine-1-oxyl)hydraxine was obtained by condensation of nicotinoyl hydrazide with 2,2,6,6-tetramethyl-4-oxopiperidine 1-oxyl. Acylation of 2,2,6,6-tetramethyl-4-hydroxypiperidine 1-oxyl with nicotinoyl chloride gives nicotinic acid 2,2,6,6-tetramethyl-1-oxyl 4-piperidyl ester. A spin-labeled analog of nicotinamide was obtained by condensation of nicotinoyl azide with 4-amino-2,2,6,6-tetramethylpiperidine 1-oxyl. The synthesis of 1-N-(β-D-ribofuranoside)-3'-N[4-(2,2,6,6-tetramethylpiperidine-1-oxyl)]pyridinecarboxamide from 2,2,6,6-tetramethyl-4-nicotinoylaminopiperidine 1-oxyl and 2,3,5-tri-O-benzoyl-β-D-ribofuranosyl bromide proceeds without damage to the iminoxyl radical. The preparation of the corresponding spin-labeled nucleotide is hindered by destruction of the iminoxyl radical during ion-exchange chromatography.

A number of authors has shown the possibility of the synthesis of various compounds containing a stable iminoxyl radical [1]. The synthesis of spin-labeled derivatives of a biologically active substance such as nicotinic acid seems of undoubted interest. Compounds II and IV were synthesized by traditional methods in 33 and 64.5% yields, respectively.



I II III IV

The starting compound for the synthesis of IV is 2,2,6,6-tetramethyl-4-aminopiperidine 1-oxyl (V), which is obtained by the method in [2]. Replacement of the steam-distillation step in the isolation of 2,2,6,6-tetramethyl-4-aminopiperidine by extraction with ether from a saturated alkaline solution made it possible to considerably shorten the synthesis time and raise the yield of the product from 29% to 70%.

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TABLE 1. Properties of Spin-Labeled Compounds

No.	Compound	IR spectrum, cm^{-1}	UV spectrum, $\varepsilon \cdot 10^{-3}$ (λ, nm; solvent)	R_f (system)	mp, °C	Yield, %
II	Nicotinoyl hydrazide	1510 (—CONH) 1690 (—CONH) 3350—3400 (—NH—NH—) 3600 (—OH)	12,6 (262; water) 9,05 (270; 0,1 *N* NaOH)	0,30 (a)	162	33
IV	Nicotinic acid ester	1280 (O—CO—) 1350—1370 (CH_3—) 1720 (CO) 1590 (—C=C—)		0,65 (a)	123—124	64,5
VI	Nicotinamide	1550 (—CONH) 1670 (CONH) 1350 (> CH_2) 3050 (γCH)	5,6 (262; water) 5,6 (270; 0,1 *N* NaOH)	0,58 (a)	144—146	81
VIII	Riboside	—	6,0 (262; water)	0,40 (b)	116—117	39

TABLE 2. Inhibitory Properties of Spin-Labeled (SL) Nicotinic Acid Derivatives in the Lactate Dehydrogenase-Catalyzed Oxidation of Lactate

No.	Compound	$K_i \cdot 10^{-2}$ M
1	Nicotinamide	3.2
2	SL-Nicotinamide (VI)	1.07
3	Nicotinoyl hydrazide	6.0
4	SL-Nicotinoyl hydrazide (II)	0.46
5	SL-Riboside (VII)	5.0

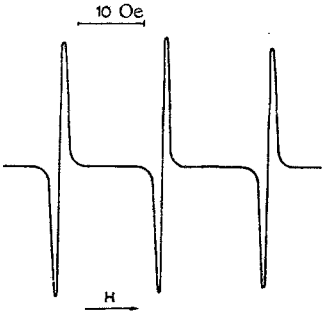


Fig. 1. ESR spectrum of VIII in water.

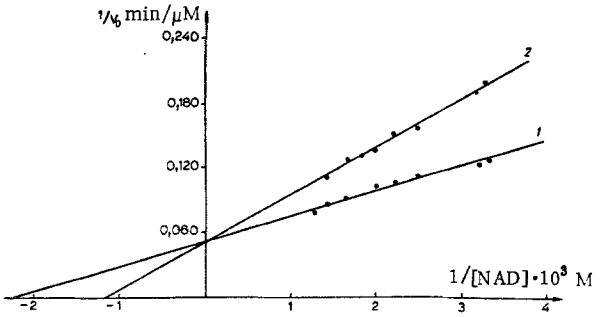
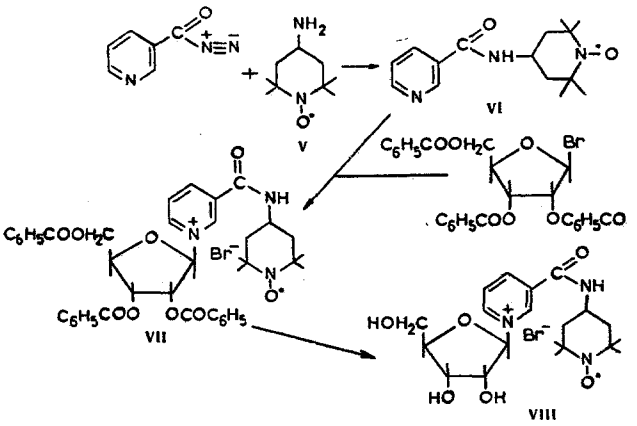


Fig. 2. Dependence of the lactate dehydrogenase-M_4-catalyzed reduction of NAD on the NAD concentration in the presence of sodium lactate and 1-N-(β-D-ribofuranoside)-3-N-[4-(2,2,6,6-tetramethylpiperidine-1-oxyl)]pyridinecarboxamide (VIII). The NAD concentration was varied from $8 \cdot 10^{-4}$ to $2 \cdot 10^{-4}$ M, the sodium lactate concentration was $4 \cdot 10^{-2}$ M, and the lactate dehydrogenase concentration was $1.4 \cdot 10^{-9}$ M: 1) without an inhibitor; 2) in the presence of $3.2 \cdot 10^{-3}$ M inhibitor (VIII).

The acylation of amine V with nicotinoyl chloride gives the product in low yield (10%) because of considerable decomposition of amine V during the reaction. We therefore developed a method for the azide condensation of nicotinic acid with amine V.

Nicotinoyl azide [3] was added gradually to an aqueous solution of amine V while maintaining the pH of the solution at ≈10. The condensation product was easily isolated in pure form in 84% yield.



In the synthesis of nicotinamide mononucleotide [4], nicotinamide is condensed with 2,3,5-tri-O-benzoyl-β-D-ribofuranosyl chloride, but condensation with the chloride does not take place in the case of spin-labeled nicotinamide VI. However, the corresponding bromide does react with nicotinoyl iminoxyl VI to give spin-labeled riboside VII. The debenzoylation of riboside VII by the action of ammonia in dry methanol proceeds in stepwise fashion. According to the results of thin-layer chromatography (TLC) on cellulose, 5-10 h after the start of the process the reaction mixture contains four reaction products. A study of the debenzoylation process made it possible to find the optimum reaction time (36 h at 0° C) and to obtain chromatographically homogeneous 1-N-(β-D-ribofuranosyl)-3'-N-[4-(2,2,6,6-tetramethyl-4-aminopiperidine-1-oxyl)]pyridinecarboxamide (VIII). Compound VIII gives a characteristic triplet in its ESR spectrum (Fig. 1).

The ability of the synthesized spin-labeled nicotinic acid derivatives to inhibit the catalyzed [by swine muscle lactate dehydrogenase (LDG-M_4)] oxidation of lactate was studied. Competitive [with respect to nicotinamide adenine dinucleotide (NAD)] character of the inhibition was detected during an investigation of the dependence of the initial reaction rate on the NAD concentration at constant inhibitor concentration. The K_i values determined by the Lineweaver–Burk method are presented in Table 2.

As seen from Table 2, the spin-labeled compounds are tied up approximately just as effectively as nicotinamide, and this makes it possible to conclude that there are no unfavorable interactions of the piperidine iminoxyl residue in the bonding portion of LDG-M_4. Inhibition by ester IV and benzoylated riboside VII was not studied because of the low solubilities of these compounds in water.

EXPERIMENTAL

The IR spectra of mineral oil suspensions of the spin-labeled nicotinic acid derivatives were recorded with a UR-10 spectrometer. Thin-layer chromatography (TLC) was carried out on cellulose and on Silufol plates in the following systems: a) water-saturated butanol; b) butanol–25% ammonium hydroxide (10:1); c) butanol–acetic acid–water (5:2:3); d) chloroform–methanol (15:1). The kinetic experiments were carried out with an 8600-A LKB reaction-rate analyzer (Sweden) at 35°. Commercial NAD-H and swine muscle lactate dehydrogenase from the Reanal company (Hungary) were used in the experiments. The azide condensation was carried out with a TTT-11 titrator (Radiometer, Denmark).

N^1-(Nicotinoyl)-N^2-4-(2,2,6,6-tetramethyl-4-hydroxypiperidine-1-oxyl)hydrazine (II). A solution of 0.4 g (2.32 mmole) of nicotinoyl hydrazide and 0.5 g (2.82 mmole) of keto radical I in 11 ml of methanol was refluxed for 7 h, after which it was evaporated to dryness with a rotary evaporator. The oily residue was treated with chloroform, and the insoluble portion was removed by filtration. The filtrate was washed with water and dried over $CaCl_2$. The solvent was evaporated, and the resulting crystalline residue was crystallized from methanol to give 0.3 g of hydrazine II with mp 162° and R_f 0.3 (system a). Found: C 59.3; H 7.2; N 17.9%. $C_{15}H_{23}N_4O_3$. Calculated: C 58.6; H 7.3; N 18.0%.

2,2,6,6-Tetramethyl-1-oxyl-4-piperidyl Nicotinate (IV). A 2.36-g (13.3 mmole) sample of alcohol radical III was dissolved in 21 ml of absolute pyridine, and 3.81 g (21.4 mmole) of nicotinoyl chloride was

added in portions with stirring. The mixture was then stirred at 25° for 16 h, after which it was evaporated with a rotary evaporator. Traces of pyridine were removed by repeated evaporation with toluene. The oily residue was dissolved in 30 ml of water, and the solution was extracted with four 20-ml portions of chloroform. The chloroform extract was washed successively with 10% $NaHCO_3$ solution (two 10-ml portions) and water, dried over $MgSO_4$, and evaporated. The crystalline residue was recrystallized from methanol to give 2.45 g of ester IV with mp 123-124° and R_f 0.65 (system a). Found: C 64.8; H 7.7; N 10.4%. $C_{15}H_{21}N_2O_3$. Calculated: C 65.0; H 7.6; N 10.5%.

N-(2,2,6,6-Tetramethyl-4-piperidyl)nicotinamide 1-Oxyl (VI). A solution of 1.93 g (11 mmole) of amine V in 52 ml of a 0.1 N solution of NaOH was placed in the potentiometric cell of a titrator, and 2 g (13.5 mmole) of nicotinoyl azide was added in portions. The solution was stirred at 25° for 2 h while maintaining the pH of the reaction mixture at 10 during the entire reaction by automatic addition of a saturated Na_2CO_3 solution. The reaction was complete when consumption of the titrant ceased. The precipitated VI was removed by filtration, and the filtrate was washed with chloroform and dried over Na_2SO_4. The solvent was removed by distillation, and the oily residue was crystallized from cyclohexane to give a certain additional amount of VI. The total yield of VI, with mp 144-146° (from water) and R_f 0.58 (system d), was 2.6 g. Found: C 65.2; H 7.8; N 15.0%. $C_{15}H_{22}N_3O_2$. Calculated: C 65.2; H 8.0; N 15.2%.

1-N-(β-D-Ribofuranosidyl)-3-N-[4-(2,2,6,6-tetramethylpiperidine-1-oxyl)]pyridinecarboxamide (VIII). A solution of 1.1 g (4 mmole) of nicotinoylaminooxyl VI in 10 ml of acetonitrile was added dropwise to a cooled (to −5°) solution of 1-bromo-2-3-5-tri-O-benzoyl-β-D-ribofuranose (VII), obtained from 1.5 g (3 mmole) of 1-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose by the method in [5], in 10 ml of absolute acetonitrile. The mixture was then stirred at 0° for 96 h. The resulting precipitate was removed by filtration, and the filtrate was evaporated with a rotary evaporator. The residue was treated with 10 ml of water and 10 ml of chloroform, and the chloroform extract was washed with three 3-ml portions of water, dried over Na_2SO_4, and evaporated to a volume of 2 ml. The concentrated solution was added dropwise to 50 ml of absolute ether, and the mixture was allowed to stand at 0° for 12 h. The resulting precipitate was removed by filtration, washed with ether, and dissolved in 24 ml of absolute methanol. The solution was saturated with ammonia and allowed to stand at 0° for 40 h. The solvent was then removed by distillation, and the oily residue was dissolved in 1 ml of methanol. The solution was added dropwise to 50 ml of ethyl acetate, and the resulting precipitate was separated by centrifugation at 1800 rpm and −5°. It was then dried in a vacuum desiccator over P_2O_5 at 0° to give 1.8 g of VIII with mp 116-117° and R_f 0.75 (system c). Found: C 46.7; H 6.9; N 7.9%. $C_{20}H_{31}BrN_3O_6$. Calculated: C 46.2; H 6.4; N 7.6%.

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