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SPIN-LABELED ANALOGS OF ACETYLCHOLINESTERASE

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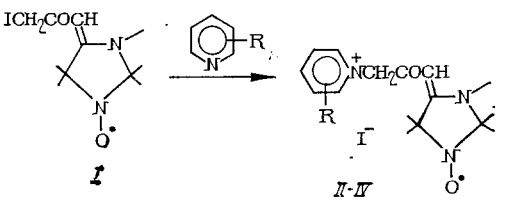
It is well known that alkylpyridinium aldoximes [5] and compounds with structures analogous to the structure of isonitrosoacetone [4] are effective acetylcholinesterase reactivators when it is inhibited by organophosphorus compounds. Since nitroxyl radicals can be detected by EPR spectroscopy at extremely low concentrations (10^{-9}-10^{-7} M) [2], analogs of acetylcholinesterase reactivators that contain a nitroxyl radical center could prove to be useful for the study of the mechanism of the action of such compounds on inactivated acetylcholinesterase.

We have previously shown [1] that a pyridinium salt (II) is formed in the reaction of a nitroxyl radical (I) with pyridine. This reaction was used for the synthesis of spin-labeled pyridinium aldoxime salts. In the reaction of I with 2-oximinomethylpyridine one might have expected the formation of products of alkylation at both the oxime group and at the pyridine nitrogen atom; however, the reaction gives only one compound, the spectral characteristics of which are similar to those of pyridinium salt II [1], on the basis of which the 2-oximinomethyl-1-[3-(2,2,3,5,5-pentamethyl-1-oxylimidazolidin-4-ylidene)-2-oxopropyl]-pyridinium iodide (III) structure was assigned to it. Under similar conditions the reaction of I with 4-oximinomethylpyridine leads to 4-oximinomethyl-1-[3-(2,2,3,5,5-pentamethyl-1-oxylimidazolidin-4-ylidene)-2-oxopropyl]pyridinium iodide (IV).



II: R = H; III: R = *o*-CH=NOH; IV: R = P-CH=NOH.

In order to obtain a spin-labeled analog of isonitrosoacetone we treated pyridinium salt II with p-nitroso-N,N-dimethylaniline under the conditions of the Kröhnke reaction, as a result of which N-[4-(2-oxo-1-propyliden-3-ylidene)-2,2,3,5,5-pentamethylimidazolidinyl-1-oxyl]-p-N,N-dimethylaminophenylamine N-oxide (V) is formed. Treatment of V with hydroxylamine hydrochloride leads to the formation of VI, which has an EPR spectrum in the form of the characteristic (for nitroxyl radicals) triplet with hyperfine interaction constant α_N = 14.0 Oe. Absorption bands at 1610 and 1550 cm^{-1}, which are characteristic for an enamino ketone grouping [3], are observed in the IR spectrum of this compound; absorption with λ_{max} 343 nm (log ε = 4.26) is observed in the UV spectrum. On the basis of the spectral characteristics and the results of elementary analysis we assigned the 2,2,3,5,5-pentamethyl-4-(3-oximino-2-oxopropylidene)imidazolidine-1-oxyl structure — a structural analog of isonitrosoacetone — to VI.

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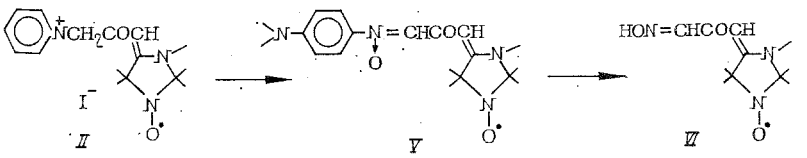
TABLE 1. Synthesized Compounds

Compound	Yield, %	mp, °C	Found, %				Empirical formula	Calculated, %			
			C	H	I	N		C	H	I	N
III	100	179—181[a]	44,7	5,4	27,4	12,7	$C_{17}H_{24}IN_4O_3$	44,5	5,2	27,7	12,3
IV	100	176—8[a]	45,0	5,5	27,4	12,2	$C_{17}H_{24}IN_4O_3$	44,5	5,2	27,7	12,3
V	48	185—6[b]	63,8	7,8	—	15,9	$C_{19}H_{27}N_4O_3$	63,5	7,5	—	15,6
VI	30	180—2[c]	55,0	7,5	—	17,5	$C_{11}H_{18}N_3O_3$	55,0	7,5	—	17,5
VII	100	185—6[d]	55,1	7,7	—	17,2	$C_{11}H_{19}N_3O_3$	54,9	7,9	—	17,5

Note. The compounds were purified: [a]by reprecipitation from methanol by means of dry ether and by recrystallization, [b]from ethanol, [c]from benzene, and [d]from ethyl acetate—benzene (1:1).



The structure of VI is confirmed by the PMR spectrum of its diamagnetic analog, viz., 1-hydroxy-2,2,3,5,5-pentamethyl-4-(3-oximino-2-oxopropylidene)imidazolidine (VII), which is formed by treatment of VI with hydroxylamine. Singlets of two geminal dimethyl groups at 1.22 ppm (6H) and 1.43 ppm (6H), a singlet of protons of a methyl group attached to a nitrogen atom at 2.25 ppm (3H), a singlet of a proton attached to a C—C bond at 5.3 ppm (1H), a singlet of a proton attached to the carbon atom of an oxime group at 7.34 ppm (1H), and a signal at 7.90 ppm (1H) of a proton of a hydroxy group. Oxidation of VII with lead dioxide leads to nitroxyl radical VI.

The synthesized compounds — spin-labeled analogs of pyridinium aldoxime salts (III and IV) and isonitrosoacetone (VI) — displayed moderate activity as acetylcholinesterase reactivators and can therefore be used to study the mechanisms of their action on inactivated acetylcholinesterase by means of EPR spectroscopy.

EXPERIMENTAL

The IR spectra of KBr pellets of the compounds (0.25% of the compound in the pellet) were recorded with a UR-20 spectrometer (East Germany). The UV spectra were recorded with a Specord UV-vis spectrophotometer. The PMR spectrum was obtained with a Varian A-56-60A spectrometer. The EPR spectra of solutions in methanol were recorded with a Sibir'-3 spectrometer. The results of elementary analysis, the melting points, and the yields of the synthesized compounds are presented in Table 1.

2-Oximinomethyl-1-[3-(2,2,3,5-pentamethyl-1-oxylimidazolidin-4-ylidene)-2-oxopropyl]-pyridinium Iodide (III). A solution of 0.3 g (0.89 mmole) of I and 0.08 g (0.6 mmole) of 2-oximinomethylpyridine in 5 ml of dry DMF was allowed to stand for 30 h, after which it was diluted with 20 ml of dry ether. The precipitated product crystallized upon trituration. The precipitated III was removed by filtration. IR spectrum (KBr), ν, cm^{-1}: 1635, 1530 (O=C—C=C). UV spectrum (ethanol), λ_{max}, nm (log ε): 317 (4.60).

4-Oximinomethyl-1-[3-(2,2,3,5,5-pentamethyl-1-oxylimidazolidin-4-ylidene)-2-oxopropyl]-pyridinium Iodide (IV). This compound was similarly obtained from I and 4-oximinomethylpyridine. IR spectrum (KBr), ν, cm^{-1}: 1640, 1535 (O=C—C=C). UV spectrum (ethanol), λ_{max}, nm (log ε): 316 (4.60).

N-[4-(2-Oxo-1-propylidene-3-ylidene)-2,2,3,5,5-pentamethylimidazolidinyl-1-oxyl]-p-N,N-dimethylaminophenylamine N-Oxide (V). A solution of 13.7 g (8.9 mmole) of the pyridinium salt II in 200 ml of methanol was added to a solution of 1.38 g (8.9 mmole) of p-nitroso-N,N-dimethylaniline in methanol, the mixture was cooled to 0°C, and a solution of 10 ml of 2 N NaOH in 40 ml of methanol was added dropwise with stirring. The mixture was then maintained at 0°C for 1 h, after which it was diluted with 100 ml of cold water, and the precipitated

V was removed by filtration, dried, and recrystallized from alcohol. IR spectrum (KBr), ν, cm^{-1}: 1610, 1545. UV spectrum (ethanol), λ_{max}, nm (log ε): 249 (4.27), 318 (4.05), and 470 (4.30).

2,2,3,5,5-Pentamethyl-4-(3-oximino-2-oxopropylidene)imidazolidine 1-Oxide (VI). A 0.194-g (2.79 mmole) sample of hydroxylamine hydrochloride in methanol solution was added to a solution of 0.2 g (0.556 mmole) of nitrone V in 10 ml of methanol, and the reaction mixture was evaporated. Compound VI was isolated by chromatography with a column packed with silica by elution with chloroform—methanol (30:1) and purified by refluxing with activated charcoal in benzene with subsequent recrystallization.

1-Hydroxy-2,2,3,5,5-pentamethyl-4-(3-oximino-2-oxopropylidene)imidazolidine(VII). A solution of 0.1 g (0.42 mmole) of VI, 0.29 g (4.2 mmole) of hydroxylamine hydrochloride, and 0.17 g (4.2 mmole) of NaOH in 10 ml of methanol was allowed to stand for 2 h, after which it was evaporated, and the residue was diluted with water. The aqueous mixture was neutralized to pH 7.0 with 5% hydrochloric acid, and the precipitated VII was removed by filtration, washed with water, dried, and recrystallized from ethyl acetate—benzene.

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