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α-OXOTHIOHYDROXIMATE S-ESTERS. PREPARATION AND PHYSIOLOGICAL ACTIVITY

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The synthesis of thiohydroximate esters has opened up a new pathway to central cholinesterase reactivators [1, 2, 4, 5, 7]. A distinguishing feature of this approach is that the thiohydroximate esters offer extensive possibilities for the goal-oriented construction of novel molecules with a given type of physiological activity, namely cholinesterase reactivators. If the thiohydroximate ester molecule is arbitrarily split into acid and ester moieties, the construction of novel molecules can be carried out in two main directions, viz., introduction of changes into the structure of the acid or the ester part of the molecule.

The acid components can be hydroximoyl chlorides of the most diverse structure (aliphatic, aromatic with a variety of substituents in the benzene ring [1, 5, 8], and heterocyclic [2, 7]).

The ester component can be provided by aminothiols of different types, or aminoalcohols, to give the corresponding thiohydroximate or hydroximate esters:

$RC(=NOH)Cl + HA(CH_2)_nNR'_2 \rightarrow RC(=NOH)A(CH_2)_nNR'_2 \cdot HCl$, where R is alkyl, aryl, or hetaryl; NR'_2 is a dialkyl or cycloalkylamino-group; A is S or O, and n = 2, 3, 4, or more.

In order to develop further this approach to the generation of novel central cholinesterase reactivators using α-chloro-α-oximinoketones as the hydroximoyl chlorides, we have prepared the α-oxothiohydroximate esters [3] of general formula:

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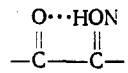
$R(C{=}O)C(=NOH)SCH_2CH_2A \cdot X$

I—XIII

R=CH_3 (I, Ia, II, IIa), $(CH_3)_3C$ (III, IV, IVa), C_2H_5O (V), C_6H_5 (VI, VIa, VII), 4-$C_2H_5C_6H_4$ (VIII), 4-$CH_3OC_6H_4$ (IX), 4-BrC_6H_4 (X, Xa, XI, XII), 2-thienyl (XIII); A = $N(C_2H_5)_2$ (I, Ia, III, V, VI, VIa, VIII—X, Xa, XI, XIII), piperidino (II, IIa, IV, IVa), $N(C_3H_7)_2$ (VII, XII); X=HCl (I—X, XII, XIII), $0{,}5H_2SO_4$ (XI).

Cholinesterase reactivators of this type are of interest from two points of view.

Firstly, the oxo-group in the thiohydroximate ester could play a part in the sorption of the reactivator at the active site of the cholinesterase as a result of the additional charge on the oxygen arising as a result of polarization of the C=O bond. Secondly, the oxo-group, being adjacent to the oximino-group, could participate in the formation of a hydrogen bond:



which should facilitate ionization of the =NOH group to give reactive $=NO^-$ ions. This intramolecular interaction in the reactivator molecule could facilitate and accelerate the reactivation of the phosphorylated cholinesterase.

Synthesis of α-oxothiohydroximate esters as reactivators has been carried out not only by ourselves [3], but also by A. Kenley et al., who have carried out extensive studies with compounds of this type [9, 10, 14].

EXPERIMENTAL (CHEMISTRY)

α-Chloro-α-oximinoketones were obtained as described in [11, 13, 15], and aminothiols as described in [12]. IR spectra were obtained on a Perkin-Elmer 325 (USA) in KBr disks. Melting points were determined on a Boetius hot plate (East Germany).

S-Diethylaminoethyl 2-Oxo(4-bromophenyl)ethane-1-thiohydroximate Hydrochloride (X). A solution of 5.24 g (0.002 mole) of α-chloro-α-isonitroso-4-bromoacetophenone [11] in 10 ml of methanol was treated with 2.66 g (0.02 mole) of 2-diethylaminoethanethiol, followed by 15 ml of ether and 10 ml of light petroleum (40-70°C). The precipitate was filtered off, washed with ether with the addition of alcohol, and dried in a vacuum desiccator over calcined calcium chloride to give 6.33 g of a colorless crystalline powder, which was crystallized from water. Its properties are shown in Table 1.

Compounds (I-IV), (VI-IX), (XII), and (XIII) were obtained similarly.

S-Diethylaminoethyl 2-Oxo-2-(4-bromophenyl)ethane-1-thiohydroximate (Xa). A solution of 0.39 g (0.001 mole) of (X) in 9 ml of water was treated with 0.07 g (0.001 mole) of potassium hydroxide, and the mixture stirred. The precipitate was centrifuged, washed three times with water, and dried in a vacuum desiccator. Pale yellow, finely-crystalline powder, yield 0.23 g. Its properties are shown in Table 1.

Compounds (Ia), (IIa), (IVa), and (VIa) were obtained similarly.

S-Diethylaminoethyl 2-Oxo-2-(4-bromophenyl)ethane-1-thiohydroximate Sulfate (XI). A suspension of 1.8 g (0.005 mole) of (Xa) in 10 ml of propan-2-ol was treated dropwise with 0.25 g (0.0025 mole) of 98% sulfuric acid. The crystalline precipitate coagulated to a viscous mass, which was heated for 10 min on the water bath at 50-60°C. On cooling, the mass crystallized, and was filtered off, washed with propan-2-ol, and dried in a vacuum desiccator to give 1.92 g of a colorless powder. Its properties are given in Table 1.

S-Diethylaminoethyl α-Oximino-α-thioethyl acetate Hydrochloride (V). A solution of 3.03 g (0.02 mole) of α-chloro-α-oximinoethyl acetate [15] in 10 ml of acetone was treated dropwise with 2.66 g (0.02 mole) of 2-diethylaminoethanethiol with water cooling. Ether (10 ml) was added, and the mixture kept in the refrigerator overnight. The solid was filtered off, washed with 10 ml of ether, and dried in vacuo to give a colorless powder, yield 5.4 g, which was recrystallized from acetone—alcohol. Its properties are shown in Table 1.

TABLE 1. Physicochemical Properties of α-Oxothiohydroximate S-Esters

Compound	Yield, %	mp, °C (solvent)	Empirical formula	IR spectra, cm^{-1}		
				C=O	C=N	C=C
I	44,3	155—7 (ac.-ip.)	$C_9H_{18}N_2O_2S \cdot HCl$	1690	1548	—
Ia	24,0	—	$C_9H_{18}N_2O_2S \cdot$	...	...	
II	36,5	144—6 (ac.-ip.)	$C_{10}H_{18}N_2O_2S \cdot HCl$	1692	1560	—
IIa	50,0	85—9 (lp.-c.)	$C_{10}H_{18}N_2O_2S$	...	...	
III	29,6	150—3 (ac.-ip.)	$C_{12}H_{24}N_2O_2S \cdot HCl$	1690	1578	—
IV	35,0	189—95 (ip.-et.)	$C_{13}H_{24}N_2O_2S \cdot HCl$	1690	1582	—
IVa	32,8	184—5 (w.)	$C_{13}H_{24}N_2O_2S$	...	...	
V	95,0	119—21 (ac.-et.)	$C_{10}H_{20}N_2O_3S \cdot HCl$	1710	1570	—
VI	63,7	134—6 (ch.-et.)	$C_{14}H_{20}N_2O_2S \cdot HCl$	1670	1590	1570
VIa	46,5	82—3 (e.-lp.)	$C_{14}H_{20}N_2O_2S$	1652	1593	1570
VII	58,0	116—8 (ip.-e.)	$C_{16}H_{24}N_2O_2S \cdot HCl$	1670	1595	1578
VIII	28,4	137—9 (w.)	$C_{16}H_{24}N_2O_2S \cdot HCl$	1652	1600	1566
IX	86,0	130—1 (ip.)	$C_{15}H_{22}N_2O_3S \cdot HCl$	1646	1595	1574
X	80,0	164—6 (w.)	$C_{14}H_{19}BrN_2O_2S \cdot HCl$	1656	1582	1565
Xa	64,0	99—101	$C_{14}H_{19}BrN_2O_2S$	1655	1582	1564
XI	94,0	128—9 (ip.)	$C_{14}H_{19}BrN_2O_2S \cdot {}^1/_2H_2SO_4$	...	...	...
XII	72,5	154—6 (ch.-et.)	$C_{16}H_{23}BrN_2O_2S \cdot HCl$	1660	1580	—
XIII	85,0	122—4 (ip.)	$C_{12}H_{18}N_2O_2S_2 \cdot HCl$	1625	1566	1505

Notes. ac., acetone; ip., propan-2-ol; lp., light petroleum; c., carbon tetrachloride; w., water; et., ethanol; ch., chloroform; e., diethyl ether. The elemental analyses of the compounds were in satisfactory agreement with the calculated values.

EXPERIMENTAL (BIOLOGY)

In investigating the reactivating activity of the thiohydroximate esters in vitro, the activity of the cholinesterases was measured by continuous potentiometric titration at constant pH [6]. The enzyme preparations used were dry serum cholinesterase (CE, EC 3.1.1.8) of specific activity 9.5 U/mg, human erythrocyte cholinesterase (ACE, EC 3.1.1.7) of specific activity 0.8 U/mg, and brain tissue propionylcholinesterase (PCE, EC 3.1.1.8) of specific activity 7.5 U/mg, produced by the Perm Research Institute for Vaccines and Sera. The organophosphorus cholinesterase inhibitor used was methylsulfomethylate O-ethyl-S-β-ethylthioethyl methylthiophosphinate (GD-42). The reaction was carried out at 25 ± 0.2°C and pH 7.5 ± 0.05. The concentrations of the reagents in the reaction mixture (in moles) were: acetylcholine $1 \cdot 10^{-2}$, inhibitor GD-42, $1.0 \cdot 10^{-8}$ and $4.0 \cdot 10^{-8}$, and reactivator $1.05 \cdot 10^{-4}$. The amounts of enzyme (in mg) were: CE 0.3, ACE 1.0, and PCE 0.05. Titration was carried out with 0.02 N sodium hydroxide. pH values were measured with a pH-340 pH meter—millivoltmeter, and for the caustic alkali titration, a B-701 automatic laboratory buret-dispenser was used.

The results obtained for the reactivating activity of the test compounds are shown in Table 2.

The antidote and therapeutic activity of the α-oxothiohydroximate S-esters was examined in rats poisoned by dichlorfos (DDVP) in a dose of 2 LD_{50}. A 2% aqueous emulsion of DDVP in a dose of 100 mg/kg (0.454 mmole, 2 LD_{50}) was introduced into the stomachs of mongrel white rats of both sexes weighing 150-200 g. After 1-2 min, when the first symptoms of poisoning appeared, the animals were given a single dose of the thiohydroximate ester as

TABLE 2. Extent of Reactivation (%) of CE by Thiohydroximate Esters

Compound	CE reactivation, 60 min	PCE reactivation, 15 min	ACE reactivation, 60 min
I	50,8	34,9	11,2
II	36,4	5,4	24,3
III	36.4	43,3	...
IV	20,3	11,7	30,2
V	29,3	3,7	7,5
VI	50,5	68,9	2,1
VII	21.7	65,9	13,0
VIII	15.4	...	...
IX	42,3	...	16,1
X	43,3	76,0	0,5
XI	3,0	...	16,5
XII	22,3	62,5	...
XIII	32,5	...	11,6

TABLE 3. Antidote and Therapeutic Activity of Thiohydroximate Esters in Rats Poisoned with DDVP in a dose of 100 mg/kg (0.454 mole, 2 LD_{50})

Compound	Dose, mg/kg (0.027 mmole)	Therapeutic effect, % *
I	6,9	30
Ia	5,9	66
II	7,2	50
IIa	6,2	16
III	8,0	40
IV	8,4	20
IVa	7,4	33
VI	8,6	50
VIa	7,6	50
VII	9,3	33
VIII	9,3	60
IXa	9,3	20
X	10,7	20
Xa	9,7	66
XI	11,0	35
XII	11,4	50
XIII	8,7	40

*The death rate in the controls was 100%.

a 0.5% solution in doses of 5.9-11.4 mg/kg (0.027 mmole) into the thigh muscle. The salts were dissolved in water, and the bases in dimethyl sulfoxide. The antidote and therapeutic properties of the compounds were assessed by the numbers of animals surviving poisoning. The results are given in Table 3.

All the thiohydroximate esters showed reactivating activity and antidote-therapeutic properties. The levels of these properties were, however, dependent on the structures of the compounds. The number of compounds was insufficient to establish relationships between the structures of the compounds and their physiological activity, but the general outline of these relationships is clearly apparent.

It has previously been shown that esters of aliphatic thiohydroximic acids reactivate serum CE only to the extent of 5.5-5.9% [6]. An analog of one of these with an oxo-group (I) reactivates CE under similar experimental conditions by 50.8% (Table 2). High reactivating activity is also shown by other aliphatic α-oxothiohydroximates (II-V). Consequently, the introduction of a C=O group in the α-position to the oximino-group in aliphatic thiohydroximates increases their reactivating ability by nearly an order of magnitude. In aromatic thiohydroximates, which are active reactivators [6], the introduction of a C=O group has less effect in increasing their physiological activity.

It is noteworthy that the different types of CE show selectivity towards the structures of the reactivators. Of the three CE which we have examined, the least selective with respect to the structure of the reactivator is serum CE, which is reactivated to approximately the same extent by nearly all the compounds (Table 2). Brain tissue PCE is more selective towards the structure of the reactivators, some of the thiohydroximates (VI, VII, X, XII) reactivating it to the extent of 62-76% very rapidly (within 3-15 min), while others (II, V) reactivate it only by 3-5%. Erythrocyte ACE is the most difficult to reactivate with thiohydroximates. It appears that its reactivation requires a reactivator containing a positively charged quaternary nitrogen. Attention is drawn to the fact that compounds which readily reactivate PCE (VI, X) activate ACE to a much lesser extent, while conversely compounds which poorly reactivate PCE (II, IV) are the best reactivators for ACE. These observations suggest that each type of CE requires a reactivator with a particular structure, and it is apparently impossible to produce a universal reactivator which is equally effective with all types of CE. It is therefore necessary, to obtain the maximum activity, to use several activators with different structures.

Antidote and therapeutic activity was shown by all the test compounds (Table 3) despite the fact that we chose a very severe model of intoxication. The dose of DDVP used (100 mg/kg,

0.454 mmole) was 17 times greater than that of the reactivator (0.027 mmole), which was given in a single dose without a cholinolytic. Despite the 17-fold excess of the toxin over the antidote, some of the thiohydroximates permitted 40-60% of the intoxicated animals to survive. S-Esters of α-oxothiohydroximic acids are therefore of considerable interest for the generation of novel compounds in the search for highly active and selective antidotes for organophosphorus compounds.

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INVESTIGATION OF THE ANTIHYPERTENSIVE ACTIVITY AND BRADYKININ-POTENTIATING PROPERTIES OF AMINO ACID DERIVATIVES OF PHOSPHONOCARBOXYLIC ACIDS

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One of the new trends in the pharmacotherapy of arterial hypertension is the use of drugs whose mechanism of action is associated with inhibition of angiotensin-1-converting enzyme (ACE, dipeptidyl-carboxypeptidase, kininase II, EC 3.4.15.1) [5, 8]. Two drugs of this group are now being used in medical practice: captopril (I) and enalapril (II) [9, 11]. However, both captopril and enalapril are not without side effects, in view of which new active inhibitors of ACE, possessing antihypertensive activity, are being sought.

The purpose of this work was to study the antihypertensive activity of a number of amino acid derivatives of phosphonocarboxylic acids with the general formula III (see scheme on following page), representing phosphone or thiophosphone analogs of captopril (compounds IIIa-d), as well as possessing elements of similarity to enalapril in their structure (compounds IIIe-f) and, as was shown earlier, exhibiting an inhibitory influence on ACE [2, 13].

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