Chemistry of Natural Compounds, Vol. 31, No. 2, 1995

ALKALOIDS AND THEIR PHOSPHORYLATED DERIVATIVES AS REGULATORS OF THE CATALYTIC ACTIVITY OF INSECT CHOLINESTERASES

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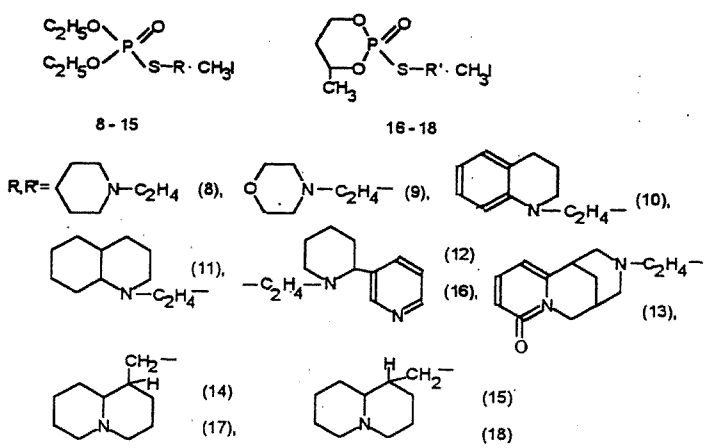
UDC 547.94.577.151.042

The results are given of structural–functional investigations of a number of alkaloids and their derivatives as regulators of the activity of the cholinesterases of various harmful insects.

At the present time, a tendency in the development of plant-protecting agents throughout the world consists in the search for natural plant substances — i.e., natural chemical structures — capable of exhibiting a toxic effect on various species of arthropods [1]. There are reports [2] on the insecticidal properties of compounds isolated from plants belonging to 28 families and being represented by substances of the classes of terpenoids, alkaloids, coumarins polyphenols, etc. In the opinion of a number of workers, the toxicity of insecticides is connected with their capacity for blocking the catalytic functions of hydrolytic enzymes, especially cholinesterase (CE). In this connection, it must be mentioned that the alkaloid eserine (physiostigmine) is the only inhibitor used at the present time in determining the classificational affiliation of hydrolases to the CE type [4]. The anticholinesterase activities of galanthamine, morphine, strychnine, brucine, cytisine, and ephedrine, and also of a number of tropolone alkaloids have been established [5]. Analysis of literature information permits the assumption that the specific nature of the structure of the alkaloids possibly serves as a basis in their binding to the active surface of the catalytic center of CE with which the natural substrate of CE — acetylcholine — usually binds. In order to elucidate this hypothesis, and also to establish the laws of the linkage of their structure with their action we have studied the kinetic parameters of the interaction of a number of commercially available alkaloids and their derivatives with the CEs of various insects.

For these purposes we have used methiodides of: piperidine (**1**), morpholine (**2**), anabasine (**3**), cytisine (**4**), salsoline (**5**), 2,4-lutidine (**6**), and 2,5-lutidine (**7**), and also their O,O-dialkyl phosphorothiolates (**8-15**) and their 4-methyl-1,3,2-dioxaphosphorinane-2-thiyl 2-oxide derivatives (**16-18**):



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0009-3130/95/3102-0151$12.50

TABLE 1. Anticholinesterase Activities ($K_i \times 10^{-5}$ M) of Alkaloids and Heterocyclic Compounds in Relation to Cabbage-fly CE and the ACE and BuCE of Warm-blooded Animals

Compound	Fly CE	Human ACE	Horse BuCE
1	3.2	24	160
2	100	170	1500
3	1.6	15	120
4	42	100	460
5	0.014	6.2	110
6	1.7	0.35	61
7	0.15	0.78	11

A comparison of the effect of anticholinesterase action as a function of the structure of the heterocyclic fragments in relation to the CE of the cabbage fly *Delia brassicae* have shown (Table 1) that salsoline methiodide is a weak inhibitor of this enzyme. The values of K_i for the enzyme of this insect are 20 times lower than the corrresponding value for ACE and 7000 times lower than for BuCE. With an increase in the hydrophobicity of the compounds under investigation and a complication of their structure on passing from morpholine methiodide to salsoline methiodide the sensitivity of the fly CE rose to a far greater degree (1400-fold) than the enzymes of mammals (41.3-fold for ACE and 20-fold for BuCE). These results indicate that the structure of the anionic moiety of the fly CE is more voluminous and accessible for the sorption of complex cyclic structures.

Among agents for protection from harmful insects and ticks the most widely used are organophosphorous inhibitors (OPIs) of CE. Particular interest is therefore presented by organophosphorus compounds (OPCs) including alkaloids in their molecules (Table 2). In this series of OPCs, the most powerful anticholinesterase activity was possessed by compound (**8**). The replacement of the piperidine in the structure of the OPC by morpholine (**9**) and by other heterocyclic residues led to a sharp (sometimes 400-fold) fall in the activity of the compound. A particularly pronounced fall was observed in relation to the enzymes of mammals and also of flies and the spider mite *Hemisarcoptes malus*. For the enzymes of other arthropods, the fall was 5- to 20-fold. The passage from (**12**) to (**15**), as compared with (**8**) led to a fall in the anticholinesterase effect of the latter in relation to the CEs of the insects studied. However, this decrease was expressed to different degrees. Thus, on passing from (**8**) to (**11**) the action of the last one on the CE of the spider mite fell 30-fold while in relation to the CEs of the greenbug *Schizaphis graminum*, the rice weevil *Sitophilus orizae*, and the mealybug *Pseudococcus gahani* it fell 2- to 5-fold. Compound (**13**) possessed a very weak action on all the CEs.

Differences in the sensitivity of the CEs in two species of moths and in human erythrocytes were found in a study of the anticholinesterase properties of compounds (**16**)-(**18**). In this series of compounds, the compounds with an N-β-hydroxyethylanabasine fragment (**16**) possessed pronounced inhibiting activity. The activity of this compound was most appreciable in relation to the ACEs of the erythrocytes and the turnip moth *Agrotes segetum*. The beet armyworm enzyme shows lesser sensitivity: in 697 times than in cases with human ACE and 189 times in proportion to turnip moth ACE. Compounds (**17**) and (**18**) were weak inhibitors of moth CEs. The replacement of the anabasine residue in the inhibitor by a salsoline residue caused a 200-fold fall in the sensitivity of human ACE and 113- and 28.9-fold falls in that of the moth ACEs, respectively.

The results given show that all the compounds studied exhibited a blocking effect on the hydrolysis of acetylcholine in the presence of the CEs of the insects investigated. What causes the block of the CE activity? An answer to this question has been given by a relative analysis of the structures of the inhibitors and of acetylcholine. All the substances, without exception, just like acetylcholine, contain an ammonium grouping capable of being protonated at physiological pH values. This grouping ensures the sorption of the inhibitor at the anionic point of the active surface of the CE, cutting off the catalytic center from possible interaction with the substrate.

In the case of compounds (**1**)-(**7**), there is the reversible type of inhibition of the activity of the cabbage-fly CE, while on interactions of the enzymes with (**8**)-(**18**) the irreversible type appears. While the first type of inhibitors blocks the oriented sorption of the substrate by screening the anionic point, in the second variant an interaction takes place (a covalent bond is formed between the P=O group of the inhibitor and the hydroxy residue of the amino acid serine, which is a component of the esterase point of the CE) of the inhibitors, apart from an anionic one, again with the esterase point of the CE, i.e., a phosphorylated enzyme loses its capacity for performing the act of hydrolysis of acetylcholine. However, the degree of the

TABLE 2. Anticholinesterase Activities (K_2, $M^{-1}\cdot min^{-1}$) of OPCs in Relation to the CEs of Various Insects

OPC	Source of cholinesterase					
	Greenbug	Spider mite	Mealybug	Rice weevil	Fly heads	Human ACE
8	20	91	32	1.4	5800	110
9	1.3	0.4	1.9	2.0	16.0	0.7
10	2.2	1.3	2.3	0.8	670	12
11	4.4	2.9	19.0	6.6	200	480
12	3.3	3.0	2.2	2.6	120	140
13	0.08	0.03	0.02	0.03	2.4	0.5
14	15	–	–	3.7	34000	2.2
15	10.3	–	–	3.7	1100	2.0

TABLE 3. Inhibiting Efficacy of Phosphorylated Derivatives of Anabasine, Lupinine, and Salsoline in Relation to Moth CEs and Human ACEs

Compound	K_2, M^{-1} min^{-1}		
	Turnip moth ACE	Beet armyworm ACE	ACE
16	5.87×10^5	3.1×10^3	2.16×10^7
17	5.54×10^3	9.43×10^3	
18	7.83×10^3	1.07×10^2	1.06×10^5

blocking effect of OPCs differs even when their phosphoryl moieties have the same form, which is explained by different structures of the ammonium groupings of these substances.

The anionic parts of the CEs are most complementary to those structures of the inhibitors the sorption of which is characterized by low values of K_i (in the case of the cabbage-fly CE, see Table 1) and high values on the irreversible inhibition of the CEs of other insects (see Tables 2 and 3). For the cabbage-fly, CE compound (**5**) is such an inhibitor, and for the ACE and BuCE of warm-blooded animals, (**6**) and (**7**), respectively (see Table 1). It must be mentioned that not all the compounds exhibit selectivity of their action in relation to the CEs from different insects (see Table 2). Compound (**8**) possesses the capacity for inhibiting the CEs of the greenbug, the spider mite, and the mealybug. Among the enzymes described, the maximum inhibiting effect was shown under the action of (**8**) with spider mite CE. OPI (**11**) inhibited the catalytic activity of the rice weevil and of human erythrocytes more strongly than the others, the degrees of inhibition differing by a factor of 76. A high sensitivity is characteristic for the CEs of fly heads on interaction with compound (**14**).

Thus, an investigation of the anticholinesterase properties of alkaloids, heterocyclic compounds, and their phosphorylated derivatives has shown that they have different degrees of activity in relation to insect CEs. The observed differences are due to the chemical specifics of the structures of natural compounds of plant origin the use of which makes it possible to regulate the activity of insect enzymes in the required direction and opens up prospects for obtaining selectively acting preparations for the needs of agriculture.

EXPERIMENTAL

The alkaloid methiodides were obtained as described by Orekhov [6].

Syntheses of phosphorylated derivatives of alkaloids and heterocyclic compounds were achieved according to [7]. The objects of investigation were the cabbage fly *Delia brassicae*, the greenbug *Schizaphis graminum* Rond., the spider mite *Hemisarcoptes malus* Shim., the mealybug *Pseudococcus gahani* Green., the rice weevil *Sitophilus oryzae* L., the turnip moth *Agrotis segetum* Schiff., and the small mottled willow moth *Spodoptera exigua* Hbn. grown at the base of the All-Russian Scientific–Research Institute of Plant Protection and SANIIZR [Central Asian Scientific–Research Institute of Plant Protection].

CE activities were determined by Ellman's colorimetric method as described previously [8].

The rate constants (K_2) of the interaction of the CEs with the inhibitors were determined from the equation

$$K_2 = \frac{2{,}3}{[I].t} \log \frac{V_0}{V_t},$$

where $[I]$ is the concentration of inhibitor, t is the time, and V_t and V_0 are the rates of enzymatic hydrolysis in the presence and in the absence of the inhibitor.

The parameters characterizing anticholinesterase properties were determined at 25°C and pH 7.5.

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