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Acetylcholinesterase Inhibition by Alkaloids of the Ant's Venom Monomorium minutum

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Abstract : 2,5-Dialkylpyrrolidines and pyrrolines **1**, **2**, **3** and **4** are *in vitro* competitive inhibitors of acetylcholinesterase. These compounds are present in ant's venom *Monomorium minutum*.

Alkaloids of the series of 2,5-dialkylpyrrolidines and pyrrolines are the major constituents of ant's poison gland of the species *Monomorium minutum* [1]. These ants are important predators of European termite societies of the genus Reticulitermes. The topical application of the venom by ants leads to a rapid death of termites after typical symptoms of an acute intoxication of the central or peripherical nervous system. These symptoms are similar to those occuring in contact insecticides as carbamates or organophosphates which are well known as acetylcholinesterase inhibitors at cholinergic synapses level [2]. That's why, we have measured *in vitro* anticholinesterase activity of the pyrrolidines **1**, **2** and the pyrrolines **3** and **4** which are present in the ant's venom. These compounds are substituted by two long alkyl or alkenyl chains in position 2,5 (Table). This substitution is the origin of their large hydrophobicity. The LogP (P partition coefficient water/octanol) calculated by increments's Rekker method [3], have remarkably high values (Table). This property allowed this toxines to penetrate rapidly the termite's cuticle.

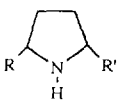
These compounds are provided by Professor G.Lhommet in the Laboratory of Heterocyclic Chemistry of our University. The synthesis which has been described previously, leads for 2,5-dialkylpyrrolidines, to a 50/50 mixture of *E and Z* isomers [4][5], while the pyrrolidines of the venom are the *E* isomers [1].

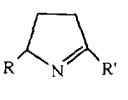
Enzymatic activity measures are made with Electric Eel acetylcholinesterase (EC 3.1.1.7) by Ellman procedure using acetylthiocholine as substrate [6]. The studied compounds are insoluble in water and are solubilised in methanol at concentrations about 100μM. The final concentration of methanol in the reaction medium is not over 1% and the enzyme kinetic constants are the same as in water [7] as we have shown in precedent papers [8][9]. We have studied the inhibition of compounds **1**, **2**, **3** and **4** by the graphical method of Dixon [10]. The results indicate clearly that inhibition is competitive as shown, for exemple, in figure for pyrrolidine **1**. The dilution of the reaction medium restores the native enzyme activity. The affinity constants Ki of pyrrolidines **1** and **2**, which represent more than 88% of the venom composition, have notable values about 2μM. The pyrroline **3** (4% of the venom) is less active (Ki = 5μM), while the pyrroline **4**, present in small trace, is about 10-fold less active than **2** (Table).

These compounds are basic and are protonated in the reaction medium (phosphate buffer pH 7,4). An interaction between the positively-charged nitrogen and the anionic site of the acetylcholinesterase is probable [11], while the hydrophobic character of the long alkyl chains results in an interaction with the aromatic surface of the protein in the deep gorge where is found the active site [12]. These compounds are not as potent inhibitors as Tacrine [11] (Ki = 10nM) but are comparable to the activity of Neostigmine or Eserine [11] (Ki = 1μM). Their anticholinesterasic activity as competitive inhibitors, combined with their large hydrophobicity deserve to be recognized.

Table

Pyrrolidines 1, 2 Pyrrolines 3, 4

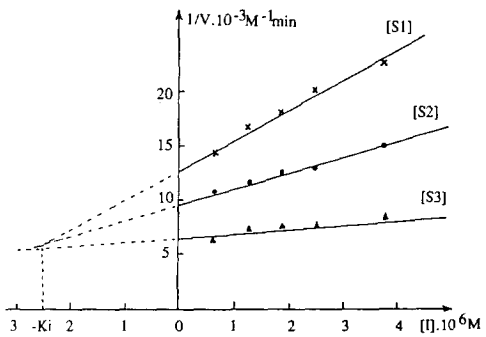




Compounds		% in venom	Ki(μM)	LogP
1	R : $(CH_2)_7$-CH=CH_2	29,8	2,5±0,3	7,32
	R' : $(CH_2)_4$-CH=CH_2			
2	R : $(CH_2)_8$-CH_3	58,7	1,5±0,2	7,68
	R' : $(CH_2)_4$-CH=CH_2			
3	R : $(CH_2)_8$-CH_3	4,4	5±0,5	8,39
	R' : $(CH_2)_4$-CH=CH_2			
4	R : $(CH_2)_4$-CH=CH_2	0,2	13±1,5	8,02
	R' : $(CH_2)_7$-CH=CH_2			

Figure : Dixon plot of pyrrolidine **1**

[S1],[S2],[S3] = 1Km,2Km,3Km



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[7] Kinetic constants are measured at 25°C in 100 mM sodium phosphate buffer, pH 7,4 : Km = 0,85±0,12 10^{-4}M (n=11). All the assays are made with [E] = 0,13 UE/ml. (Electric Eel Sigma Type VI S). In this conditions, the Ki of Neostigmine determined by the graphical method of Dixon is 1,5μM ± 0,2 (n=6). This value is in good agreement with literature data [11].

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