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SYNTHESIS, INSECTICIDAL AND ANTI-ACETYLCHOLINESTERASE ACTIVITY OF A NEW CLASS OF HETEROCYCLIC METHANESULFONATES

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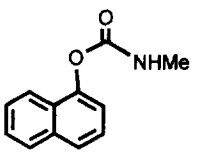
Abstract: A series of 3-methanesulfonyloxy-2-pyrazolines were prepared by mesylation of the corresponding pyrazolidin-3-ones. The compounds exhibited strong insecticidal and anti-acetylcholinesterase activity.

Acetylcholinesterase inhibitors are the single most important class of insecticide, with the nearly one hundred different commercially available carbamates (eg Carbaryl **1**) and organophosphates (eg Dichlorvos **2**) accounting for almost 60% of all insecticide sales. It is well established that the activity of these insecticides results from de-activation of the acetylcholinesterase enzyme by slowly-reversible carbamoylation or irreversible phosphorylation of the active site serine.[1]

In recent years there has also been enormous interest in the possibility of using acetylcholinesterase inhibitors as a therapy for Alzheimers disease.[2] The reversible acetylcholinesterase inhibitor tacrine is now in use and there are also several other acetylcholinesterase inhibitors in clinical trials,[3] including some carbamates and the organophosphate compound metrifonate **3**, which is converted to dichlorvos **2** *in vivo*.



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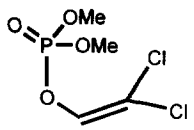

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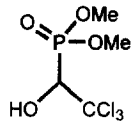

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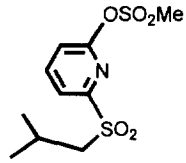

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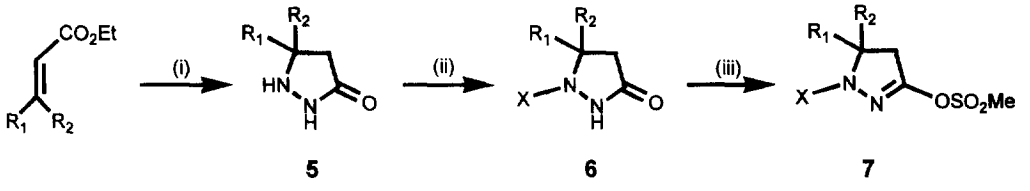
It has been known for more than forty years that methanesulfonyl fluoride is a potent and irreversible inhibitor of acetylcholinesterase, probably through mesylation of the active site serine.[4] In recent years methanesulfonyl fluoride has also been investigated as a possible treatment for Alzheimers disease and in animal experiments it gives promising prolonged memory enhancement effects with no chronic side effects.[5] Methanesulfonates of certain phenols and hydroxy aromatic heterocycles,[6-7] such as the pyridine **4**,[8] show anti-acetylcholinesterase and insecticidal activity, and there is good evidence that these compounds also cause mesylation within the enzyme active site.[9] Nevertheless, there are no commercial insecticides with the methanesulfonate type structure.

As part of a program to explore the insecticidal activity of novel heterocyclic methanesulfonates we have discovered some highly insecticidal pyridine[10] and pyrazole[11] methanesulfonates. We are also interested in the possibility of identifying methanesulfonates which are potential candidates for the treatment of Alzheimers disease, and in particular, compounds which are potent inhibitors of acetylcholinesterase, but which have minimal peripheral side effects. We now report the synthesis and biological properties of the pyrazoline 3-methanesulfonates **7** which are the first examples of insecticidal non-aromatic methanesulfonates.[12]

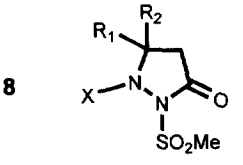
Synthesis:

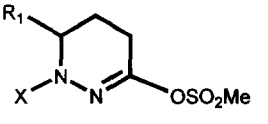
The pyrazolidin-3-ones **5** were prepared by condensation of the appropriate α,β-unsaturated ester with hydrazine hydrate, generally in refluxing ethanol.[13] Reaction of the pyrazolidin-3-ones **5** with various sulfamoyl chlorides, sulfonyl chlorides, acyl chlorides and isocyanates took place at the more nucleophilic N1 nitrogen atom to give the derivatives **6** in good yields (Scheme 1). Mesylation of the compounds **6** was carried out in dichloromethane, with one equivalent of triethylamine added first and one equivalent of methanesulfonyl chloride added slowly with stirring at about 5°.



Scheme 1. Reagents: (i) H_2NNH_2, EtOH reflux; (ii) RNCO, Toluene RT or RCOCl, K_2CO_3, Acetone RT or RSO_2Cl, Pyridine RT; (iii) Et_3N, $MeSO_2Cl$, CH_2Cl_2 5°.

The mesylates **7** were usually formed in high yield, but often there was also a small amount (5-10%) of the corresponding N-methanesulfonyl compound **8** which could be removed by column chromatography on silica gel. The position of mesylation in compounds **7** and **8** was best identified from the infrared spectra which showed either a weak C=N absorption around 1640 cm^{-1} or a strong C=O absorption around 1720 cm^{-1} respectively. In an analogous manner we have also prepared a series of tetrahydropyridazine 3-methanesulfonates **9**. The ready formation and stability of the non-aromatic methanesulfonates **7** and **9** is somewhat surprising given the fact that amides, including lactams[14] and the pyroglutamic acid analogs[15] of compounds **7**, do not form isolable methanesulfonates.



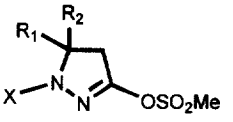


Biological results and Discussion:

Each new compound was tested for inhibition of bovine erythrocyte acetylcholinesterase (AChE) and for insecticidal activity against Southern Corn Rootworm (SCRW) and the blowfly *Lucilia cuprina*. A summary

of the results is shown in Table 1. It can be seen that all of the 3-alkylcarbamoyl pyrazoline methanesulfonates (entries 3-9) show good activity on both insect species and the 3-alkylsulfamoyl compounds (entries 14-17) are also somewhat insecticidal. The 3-acyl and 3-alkylsulfonyl substituted compounds (entries 10-13) are essentially inactive on the insect screens which is in contrast to the previously reported highly insecticidal alkylthio and alkylsulfonyl pyridines such as compound **4**.[8] The N-methanesulfonyl pyrazolidinones **8** show no biological activity and the tetrahydropyridazines **9** display a similar pattern of activity on SCRW to the corresponding pyrazolines.

TABLE 1 Structures and activity of pyrazoline methanesulfonates **7**



Entry	X	R_1	R_2	IC_{50} of AChE inhibition (μM)[a]	SCRW LD_{50} (ppm)[b]	Lucilia cuprina LD_{50} (mg/Kg)[c]
1	H_2NCO	Me	Me	> 1000	> 1000	> 100
2	C_6H_5NHCO	Me	Me	> 1000	> 1000	> 100
3	AllylNHCO	Me	Me	149	1.6	6.84
4	i-PrNHCO	Me	Me	714	1.0	7.14
5	t-BuNHCO	Me	Me	> 1000	12	75.90
6	CyclohexylNHCO	Me	Me	284	> 1000	2.64
7	i-PrNHCO	Ph	H	40.6	< 0.5	34.41
8	i-PrNHCO	Me	H	28.6	< 0.5	20.96
9	i-PrNHCO	i-Pr	H	602	5	25.11
10	EtOCO	Me	Me	> 1000	> 1000	> 100
11	i-PrCO	Me	Me	> 1000	120	> 100
12	$MeSO_2$	Me	Me	> 1000	> 1000	> 100
13	n-$PrSO_2$	Ph	H	62.5[d]	> 1000	> 100
14	i-$PrNHSO_2$	Me	Me	1269	60	36.96
15	n-$PrNHSO_2$	Me	Me	> 1000	55	40.93
16	i-$PrNHSO_2$	Ph	H	> 1000	> 1000	> 100
17	Me_2NSO_2	Ph	H	> 1000	> 1000	> 100
Carbaryl **1**				57.4	< 25	117.5
Dichlorvos **2**				599	n.d.	1.31
Tacrine				0.55	> 1000	n.d.
Metrifonate **3**				> 1000	n.d.	21.80
Pyridine **4**				60.5	< 0.5	> 100

[a]The test for inhibition of bovine erythrocyte AChE was carried out following the method of Ellman[16] with a 1 hour incubation time and each value is the mean of at least 6 replicates. [b]The activity on SCRW (Diabrotica undecimpunctata howardi) was determined following the reported method[10] and each value is the mean of three determinations. [c]The toxicity to Lucilia cuprina was determined following the previously described method[17] and the values are the mean of 2 to 4 replicates. [d]Incubation time 10 min..

The overall general agreement between the acetylcholinesterase inhibition data and the insecticidal activity shown by the pyrazoline methanesulfonates **7** indicates that acetylcholinesterase is the probable site of action for these compounds. To test the reactivity of the methanesulfonates **7**, one of the compounds (entry 4) was

heated with excess piperidine and a good yield (70%) of N-methanesulfonylpiperidine was isolated, confirming that nucleophiles attack the mesylate sulfur atom rather than reacting at the ring carbon and displacing the mesylate group.

Several of the pyrazoline methanesulfonates were tested for toxicity to white mice (*Mus musculus*) using intraperitoneal injection and it was found that they have relatively high levels of mammalian toxicity (ALD's 5-50 mg/Kg), consistent with acetylcholinesterase inhibition. In the same assay tacrine was found to have a similar level of toxicity (ALD 25 mg/Kg). We have not tested the activity of these new methanesulfonates on butyrylcholinesterase. Thus, although compounds **7** and **9** show high levels of insecticidal activity, we believe it will be necessary to identify derivatives with reduced mammalian toxicity before these methanesulfonates can find practical use as insecticides or as potential candidates for the treatment of Alzheimers disease.

Acknowledgement

We thank the DuPont Agricultural Products Insecticide Primary Evaluation Team for carrying out the Southern Corn Rootworm tests and Kevin Kranis for calculation of the SCRW LD_{50} data.

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