N-ACYLATION OF *N*-METHYLCARBAMATE INSECTICIDES AND ITS EFFECT ON BIOLOGICAL ACTIVITY

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N-Acylated *N*-methylcarbamates have been prepared and tested for contact/stomach poison and systemic insecticidal activities, mammalian toxicity and *in vitro* anticholinesterase activity. The *N*-acyl derivatives in general have lower mammalian toxicities and somewhat lower insecticidal activities than the corresponding unacylated *N*-methylcarbamates and are relatively poor inhibitors of bee cholinesterase. Tentative reasons are given for these effects.

Introduction

Stedman,[1] following earlier work on the nature of the alkaloid physostigmine, synthesised a number of aryl *N*-methylcarbamates with anticholinesterase activity from phenols containing quaternary ammonium substituents. These derivatives were not insecticidal and Kolbezen *et al.*[2] concluded that, because of their highly polar nature, such compounds are unable to penetrate the lipoid sheath of insect nerves. They synthesised a number of non-ionised *N*-substituted carbamates which were shown to have insecticidal properties. The present communication deals with the *N*-acylation of certain *N*-methylcarbamates and its effect on biological activity.

Experimental

(1) *Chemical*

N-Acylcarbamates[3] (**I**; R = aryl, R″ = CH_3) were synthesised initially as possible insecticide precursors which might be degraded in plants or animals, including insects, to active *N*-methylcarbamates.

$$RO{\cdot}CO{\cdot}NR''{\cdot}CO\ R'$$

(**I**)

Ethyl *N*-methylcarbamate may be acetylated with either acetyl chloride,[4] or acetic anhydride in the presence of zinc chloride.[5] Aryl *N*-methylcarbamates, however, do not react when boiled under reflux with acetyl chloride, but are readily acetylated with acetic anhydride in the presence of a strong acid. Treatment of 1-naphthyl *N*-methylcarbamate (carbaryl) with acetic anhydride and a catalytic amount of conc. sulphuric acid at 100° for 10 min. gives a quantitative yield of the *N*-acetyl derivative, m.p. 102–103° (Found: C, 69·1; H, 5·5; N, 6·1. $C_{14}H_{13}NO_3$ requires C, 69·1; H, 5·3; N, 5·8%). Omission of the acid catalyst results in the formation of a large amount of 1-naphthyl acetate. Acetylation of carbamates possessing basic groups requires sulphuric acid in excess of that needed to form the *acid* sulphate of the base. A steric effect is noted with bulky acyl groups: for complete reaction between isobutyric anhydride and 3-isopropylphenyl *N*-methylcarbamate it is necessary to heat the mixture to 140° for 3 h.

Acylation is also accomplished with acid chlorides at higher temperatures, preferably in the presence of 1 mole of a tertiary base such as dimethylaniline; yields are usually inferior to those of the anhydride method. The structure of 1-naphthyl *N*-acetyl-*N*-methylcarbamate was confirmed by alternative syntheses involving either methylation of the acetylcarbamate (**I**; R = 1-naphthyl, R′ = CH_3, R″ = H) or condensation of 1-naphthyl chloroformate with *N*-methylacetamide.

N-Alkanoyl-*N*-methylcarbamates are oils or low-melting solids and are generally more soluble in organic solvents than the corresponding methylcarbamates. Acyl derivatives have greater thermal stability than the parent methylcarbamates and can be distilled under reduced pressure without detectable decomposition even when the boiling point exceeds 200°. They are

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relatively stable in neutral aqueous media; 1-naphthyl *N*-methylcarbamate decomposes much more rapidly than the acetyl derivative in aqueous suspension at 50°. At low pH, hydrolysis to methylcarbamates occurs, but under alkaline conditions rapid degradation to the phenols is observed.

(2) *Biological*

Five insect species were used throughout the tests. They were chosen since they are easily reared in large numbers under laboratory conditions and because they represent genera of economic importance.

(*a*) *Cydia pomonella* Linn. A non-diapausing strain of Codling moth was maintained under constant conditions at 21° and 65% R.H. Apple thinnings of uniform size (3–4 cm. dia.) were dipped in the test solutions, allowed to dry and each infested with five, newly emerged, larvae. Mortality was estimated after 3 days.

(*b*) *Plutella maculipennis* Curtis. Each replicate comprised ten 4th instar larvae of the Diamond-back moth, placed in small (5 × 2·5 cm.) glass tubes. Approximately 6·5 sq. cm. of cabbage leaf was dipped into the test solution, allowed to dry and placed in the tube. After 24 h., untreated cabbage was provided for food and mortality estimated after a further 24 h.

(*c*) *Phaedon cochleariae* Fabr. Ten imagines were used in a test similar to that for *Plutella*. Both species were maintained at 21° and 65% R.H. during the test period.

(*d*) *Megoura viciae* Kalt. Vetch aphid apterae were used in tests under glasshouse conditions. To investigate *contact aphicidal activity*, broad bean plants were grown singly in pots and when 5–7 cm. high, infested with 35–40 *Megoura*. The infested plants were sprayed to run-off in a specially designed chamber,[6] and mortality assessed after 24 h. In the determination of *systemic activity*, broad bean plants 5–7 cm. high were infested with aphids immediately after 25 ml. of the test solution had been added to the soil in each of the (3 in.) pots. The soil surface was covered with cardboard and mortality recorded after 3 days.

(*e*) *Apis mellifera* Linn. Worker bees (English-Italian hybrids) of foraging age were dosed on the mid-ventral abdomen with the test compound in 10 μl. of ' Analar ' acetone using an Agla micrometer syringe. They were held by a vacuum device during dosage to avoid the use of anaesthetics. Each replicate comprised 25 bees and there were at least 3 replicates per treatment. Mortality was measured after 24 h. during which time the bees were held at 31° and provided with water and sucrose solution.

Except for *Apis*, dilutions of test compounds were prepared from a stock of 5% w/v of active ingredient in a mixture of 90% Cellosolve (2-ethoxyethanol) and 10% 'Empilan A' as emulsifier. The concentration of adjuvants was kept constant throughout the dilutions. Median lethal concentrations were derived from plots of percentage mortality/dosage data.

For the *in vitro enzyme inhibition studies*, cholinesterase was obtained from bee brains which were homogenised and extracted in batches of 50. The principle used was to titrate acetic acid produced by the reaction of the enzyme and acetylcholine bromide, using a pH meter to determine balance points. Times at which balance points occurred were noted as were corresponding titres. The plotting of titre versus time yielded a straight line which was a measure of enzyme activity. A comparison was made between activity in the presence of insecticide and in the presence of solvent only, the test compound being added to the preparation 10 min. before the addition of substrate.

Results and discussion

The results are given in Tables I, II and III; relevant mammalian toxicity data obtained by the Biology Division are also included.

Examination of the activity of almost 250 acylcarbamates derived from more than 100 phenols has failed to indicate any obvious relationship between the nature of the acyl group and contact/stomach poison activity: examples are given in Table I. Some general conclusions can, however, be drawn. Unacylated members are usually more active than acyl derivatives

Table I

Contact/stomach-poison activity of some N-*acylcarbamates*

I, R'' = CH_3		M.L.C.* (% active ingredient)				Oral
R	R'	*C. pomonella*	*P. cochleariae*	*M. viciae*	*P. maculipennis*	$L.D._{50}$, m.g./kg., to mice
1-Naphthyl	Unacylated†	0·0025	0·03	0·0125	0·02	700
1-Naphthyl	CH_3	0·01	0·0175	0·0175	>0·5	>3000
1-Naphthyl	C_2H_5	0·01	0·05	0·005	>0·5	3000
1-Naphthyl	$(CH_2)_2{\cdot}CH_3$	>0·01	0·05	0·1	>0·5	>3000
4-Dimethyl-amino-3,5-xylyl	Unacylated**	0·0015	0·06	0·01	0·06	12
4-Dimethyl-amino-3,5-xylyl	CH_3	0·001	0·005	0·125	0·05	550
4-Dimethyl-amino-3,5-xylyl	C_2H_5	0·0015	0·015	0·125	0·035	400
4-Dimethyl-amino-3,5-xylyl	$(CH_2)_2{\cdot}CH_3$	0·008	0·02	0·05	0·045	150
2,3,5-Trimethylphenyl	Unacylated	0·01	0·01	0·05	>0·5	90
2,3,5-Trimethylphenyl	CH_3	0·01	0·006	0·07	>0·5	1350
2,3,5-Trimethylphenyl	C_2H_5	>0·01	0·0125	0·10	>0·5	1350
2,3,5-Trimethylphenyl	$(CH_2)_2{\cdot}CH_3$	>0·01	0·0125	0·025	>0·5	700

* Median lethal concentration † Carbaryl (' Sevin ', Union Carbide Corp.) ** ' Zectran ' (Dow Chemical Co.)

and in no case is an acylated compound active against a particular species if the corresponding unacylated member is completely inactive. Of the acyl groups, acetyl confers the highest activity, with certain notable exceptions.

The 2-isopropoxyphenyl and the 2-isopropylphenyl *N*-methylcarbamates have high systemic insecticidal activity against *M. viciae*. Whereas acylation of these has only a small effect on contact aphicidal activity, it greatly effects systemic toxicity. In each case the unacylated member has the greatest systemic effect and in general this is reduced as the length of the acyl chain increases. However, the butyryl side chain is associated with greater systemic activity than the propionyl, and in the 2-isopropoxyphenyl series, the isobutyryl more than the butyryl. It should be noted that 3-isopropoxyphenyl and 3- and 4-isopropylphenyl *N*-methylcarbamates lack both contact and systemic activity against this species.

Following Kolbezen *et al.*,[2] several authors[7,8] have suggested that insecticidal carbamates inhibit insect cholinesterase. Consequently several of the present range of *N*-acylated *N*-methylcarbamates were tested for their anti-cholinergic properties (see Table III).'

1-Naphthyl *N*-methylcarbamate is a very potent *in vitro* inhibitor of cholinesterase extracted from bee-heads but this activity is greatly reduced on acetylation (Table III). There is, however, an increase in activity with higher homologues of acetyl but not up to the level of the

Table II

Systemic and contact toxicities of some N-*acylcarbamates to* Megoura viciae

I, R'' = CH_3		M.L.C. (% active ingredient)*		Oral
R	R'	Systemic toxicity	Contact toxicity	$L.D._{50}$, mg./kg., to mice
2-Isopropoxyphenyl	Unacylated†	+++++	+++++	24
2-Isopropoxyphenyl	CH_3	++++	++++	370
2-Isopropoxyphenyl	C_2H_5	+	++++	350
2-Isopropoxyphenyl	$(CH_2)_2{\cdot}CH_3$	++	+++++	500
2-Isopropoxyphenyl	$CH(CH_3)_2$	++++	++++	370
2-Isopropylphenyl	Unacylated	++++	++++	350
2-Isopropylphenyl	CH_3	+++	++++	1850
2-Isopropylphenyl	C_2H_5	++	++++	1850
2-Isopropylphenyl	$(CH_2)_2{\cdot}CH_3$	+++	++++	1850
2-Isopropylphenyl	$(CH_2)_4{\cdot}CH_3$	+	+++++	>3000
2-Isopropylphenyl	$(CH_2)_{10}{\cdot}CH_3$	+	++++	>3000

* M.L.C. % 0·2–0·1 +; <0·1–0·025 ++; <0·025–0·01 +++; <0·01–0·00125 ++++; <0·00125 +++++

† Bayer 39007

Table III

Comparison of insecticidal and in vitro *anticholinesterase activities of a group of* N-*acylcarbamates*

I, R″ = CH_3		* I.C.$_{50}$	† L.D.$_{50}$
R	R′		
1-naphthyl	Unacylated	5×10^{-8}	0·75
1-naphthyl	CH_3	5×10^{-6}	1·3
1-naphthyl	C_2H_5	9×10^{-7}	1·9
1-naphthyl	$(CH_2)_2 \cdot CH_3$	$7{\cdot}5 \times 10^{-7}$	2·5

* Molar concentration for 50% bee-brain cholinesterase inhibition
† Median lethal dose, μg. per bee

unacylated parent. In the 1-naphthyl group the acetyl member is less inhibitory by a factor of ×100 but in the 2-isopropoxyphenyl series the comparable reduction is about ×1000. However, Table III shows that there is only a difference of ×2 between the contact toxicity to bees of 1-naphthyl *N*-methylcarbamate and that of its *N*-acetyl derivative. Other series have been tested in this way and the greatest difference in contact toxicity found to date between acetylated and unacetylated members is only ×10.

The possible significance of this quantitative difference between *in vitro* enzyme inhibitory power and contact toxicity may be partially and tentatively explained thus. *N*-Acylcarbamates may be converted inside insects either to the corresponding unacylated member or to a common active metabolite. Preliminary work with bees has shown that there is a marked decrease in brain cholinesterase activity *in vivo* for *both* types of compound after topical application, thus supporting the toxification hypothesis. The large reduction in the oral toxicity of acylated compounds to mice may be due to their inability to effect this metabolic change. These hypotheses are being investigated and results will be published in due course.

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