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THE SEARCH FOR A VETERINARY INSECTICIDE II.*-Carbamates active against sheep blowfly

By J. FRASER, D. GREENWOOD, I. R. HARRISON and W. H. WELLS

Many aryl N-methylcarbamates and their N-acyl derivatives have been prepared and tested against larvae of the sheep blowfly, Lucilia sericata. Mammalian toxicities and persistences on sheep fleece were also determined. The factors which led to the selection of Butacarb (3,5-di-t-butylphenyl N-methylcarbamate) as a new veterinary insecticide are discussed.

Introduction

Greenwood & Harrison¹ outlined the requirements for compounds to be used for the control of sheep blowfly, Lucilia sericata (Meig.) It was shown that a number of compounds, active against the larval stage of this parasite when they were screened in the laboratory, were useless because of their lack of persistence on sheep fleece. The third requirement of any promising compound was stated to be low mammalian toxicity, and this has recently become of paramount importance.

The present paper gives details of a large number of carbamate compounds which were prepared and screened against the sheep blowfly. Although carbamate insecticides have been widely known for many years, only one has been used to any extent against veterinary parasites. This is Carbaryl (1-naphthyl N-methylcarbamate), which has been used for the control of the cattle tick,² and poultry parasites.^{3,4} Carbaryl has been tested extensively on sheep, where it has been found to control the sheep louse, but unfortunately it causes a yellow staining of the fleece and this prevents it use for this purpose. Dimetilan 2-(N,N-dimethylcarbamoyl)-3methylpyrazol-5-yl N.N-dimethylcarbamate has been used as a fly bait, and has also been tested for the control of sheep blowfly, but its high mammalian toxicity prevents its widespread use for this purpose although it will persist for several weeks on the fleece.

Certain substituted phenyl N-methylcarbamates (e.g. Zectran) are also too toxic to be used on sheep. However, N-acylation in some cases reduces mammalian toxicity without necessarily impairing the insecticidal potency.⁵ A range of N-acyl-N-methylcarbamates was therefore included in the screening programme.

Experimental

The methods of rearing sheep blowfly, testing the compounds in the laboratory, and determining their presistence on sheep fleece are fully described in the previous paper,¹ and the same techniques were employed for the testing of the carbamates listed in the table.

Preparation of compounds

RO·CO·N(CH₃)R'

(I)

N-methylcarbamates (I, $\mathbf{R}' = \mathbf{H}$) were obtained by standard procedures, involving either condensation of phenols with phosgene and treatment of the resulting chloroformates with methylamine, or reaction between phenols and methyl isocyanate in the presence of a basic catalyst.

N-Acetylcarbamates and their homologues (I, $\mathbf{R}' = acetyl$, propionyl, etc.) were prepared by treatment of the N-methylcarbamates with acid anhydrides in the presence of a trace sulphuric acid.5,13

Phenyl N-acetyl-N-methylcarbamate

A mixture of phenyl N-methylcarbamate (15 g), acetic anhydride (50 ml) and concentrated sulphuric acid (2 drops) was boiled under reflux for 20 min. An excess of anhydrous sodium acetate (\sim 1g) was added to neutralise the sulphuric

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^{*}Part I-J. Sci. Fd Agric., 1965, 16, 293

Attempts to formulate *N*-methylcarbamates directly were unsuccessful, but treatment of the sodium salt of *N*-methylformamide with aryl chloroformates gave the required *N*formyl derivatives.

1-Naphthyl N-formyl-N-methylcarbamate

N-methylformamide $(5 \cdot 9 \text{ g})$ and sodium $(2 \cdot 5)$, refluxed and stirred in xylene (100 ml) for $1\frac{1}{2}$ h, formed a thick precipitate of the sodium salt. The mixture was cooled, and 1-naphthyl chloroformate $(15 \cdot 0 \text{ g})$ was gradually added. After 30 min, the solution was clarified by filtration and the filtrate was washed with water, dried and distilled to dryness *in vacuo*. Addition of ethanol (15 ml) to the residue precipitated a byproduct, m.p. 126–128°, identified as 1-naphthyl carbonate. After removal of this, distillation yielded the *formylcarbamate*, $9 \cdot 3 \text{ g}$.

Introduction of phenoxyacetyl, methoxyacetyl, methylthioacetyl, chloroacetyl, ethoxalyl, and methoxycarbonylpropionyl groups was effected using the appropriate acyl chlorides.

2-Isopropoxyphenyl N-methyl-N-(methylthio)acetylcarbamate

A mixture of 2-isopropoxyphenyl *N*-methylcarbamate (75 g) (methylthio)acetyl chloride (63 g) and dry toluene (100 ml) was boiled under reflux until evolution of hydrogen chloride ceased (24 h). Distillation gave the product as a liquid, b.p. $178-182^{\circ}/2$ mm (58 g).

Carbamates containing substituted acetyl groups were in some cases formed by treating corresponding *N*-chloroacetylcarbamates with nucleophilic reagents, such as potassium thiocyanate, triethylamine, or sodium benzenesulphinate. Examples illustrating the use of the first two reagents are given below, together with various other miscellaneous reactions.

2-isopropoxyphenyl N-methyl-N-thiocyanatoacetylcarbamate

2-Isopropoxyphenyl N-chloroacetyl-N-methylcarbamate (28.6 g) was added portionwise during 20 min to a refluxing mixture of potassium thiocyanate (19.4 g) and absolute alcohol (100 ml). The mixture was boiled under reflux for 24 h, cooled, poured into water, and extracted with ether.

Distillation of the dried (Na₂SO₄) ethereal extract afforded a viscous oil (29.2 g), b.p. $202-208^{\circ}/2.5$ mm.

2-Isopropylphenyl N-(diethylaminoacetyl)-N-methylcarbamate ethochloride

2-Isopropylphenyl *N*-chloroacetyl-*N*-methylcarbamate (6 g), methylene chloride (6 ml), and triethylamine $(3 \cdot 3 \text{ g})$ were mixed and allowed to stand for 24 h at room temperature. Dry ether (70 ml) was added to precipitate the product, the supernatant liquid was decanted, and the viscous residue was dried *in vacuo* to give a light brown deliquescent solid (1 · 38 g).

2-Isopropoxyphenyl N-methyl-N-methylsulphonylacetylcarbamate

An excess of a solution of monoperphthalic acid in ether (0.39 M, 75 ml) was added portionwise with ice-cooling to a solution of 2-isopropoxyphenyl *N*-methyl-*N*-(methylthio)-acetylcarbamate (3.13 g) in ether (40 ml). The mixture was kept at 0° for 72 h, washed with aqueous sodium hydrogen

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carbonate and water, and dried (Na_2SO_4) . Evaporation afforded the required sulphone as an oil (3.18 g).

2-Isopropylphenyl N-(α , β -dibromobutyryl)-N-methylcarbamate

A solution of bromine $(4 \cdot 5 \text{ g})$ in carbon tetrachloride (40 ml) was added gradually to a solution of 2-isopropylphenyl *N*-crotonoyl-*N*-methylcarbamate (7 \cdot 3 g) in carbon tetrachloride (60 ml). The pale yellow solution was distilled, and the fraction, b.p. 188–192°/2 mm (5 · 9 g), was collected.

Attempted formation of a N-(hydroxyacetyl)carbamate

Hydrogenolysis of 2-isopropoxyphenyl N-benzyloxyacetyl-N-methylcarbamate using palladised charcoal as catalyst gave, instead of the expected hydroxyacetyl derivative, the cyclisation product 3-methyloxazolidine-2,4,dione, together with 2-isopropoxyphenol.

(A comparable cyclisation occurred on hydrogenolysis of 2benzyloxyphenyl *N*-acetyl-*N*-methylcarbamate, giving *O*phenylene carbonate and *N*-methylacetamide.)

Results and Conclusions

Table I lists the carbamates synthesised during the course of this work, their chemical data and their approximate LD_{50} to blowfly larvae. In some cases, their persistence on the sheep fleece and their oral toxicities to mice are also included. The latter are only rough approximations as in most cases only two mice were used for each determination. The code letters are explained at the bottom of the table.

Generally, only compounds active at 30 ppm or less were tested on sheep, but in several cases compounds of lower activities were tested because of their interesting chemical structures. Other carbamates which were expensive to prepare, or which had mammalian toxicities greater than 50 mg/ kg were not tested extensively.

The compounds fell into three broad categories. A high proportion of the compounds exhibited little persistence on the fleece. The second group similar to Carbaryl persisted for 6–7 weeks, and a third small group gave persistences of more than nine weeks. It should be pointed out, however, that two factors are involved in the duration of protection against blowfly strike. These are the intrinsic activity of the compound against first instar larvae and the persistence of the chemical on the fleece. As we are judging the persistence on the fleece by its biological activity, a compound of high activity may appear to last longer than a similar compound of lower activity. For this reason, differences in persistence of 1–2 weeks are not significant. It has not been possible during the course of this work to correlate structure with the property of persistence.

The figures in the table illustrate how Butacarb¹⁴ (3,5-di-tbutylphenyl-*N*-methylcarbamate) satisfied the three requirements for a successful veterinary insecticide. Thus, it is highly active in the laboratory screening, it shows outstanding persistence (14–16 weeks) on the sheep fleece, and it possesses a low mamalian toxicity. Although a number of other carbamates tested have comparable laboratory activities, none of these showed the same overall combination of desirable properties. For example, 3,5-di-isopropylphenyl *N*-methylcarbamate is also active, but has a much greater oral toxicity to mice than Butacarb.

Butacarb is a powerful inhibitor *in vitro* of acetylcholinesterase.¹¹ However, it possesses little general insecticidal activity apart from its pronounced toxicity to blowfly larvae. The reason for this extreme selectivity is not known at present.

~	R	Formula	Found, %	Required, %	M.p., °c or b.p., °c/mm	Approx. LD50 to blowfly larvae ppm	Persistence on sheep (weeks) 0.1% 0.05% Concn.	Approx. oral toxicity to mice
	H MeCO	Ref. 6 Ci ₀ H ₁₁ NO ₃	N,7·5	N.7·3	85-86 120-122/2·5	250 500		
	MeCO MeCO MeCO	CuH13NO3 CuH13NO3 CigH15NO3	ZZ 201	N,6.8 N,6.8 N,6.3	124-128/2·5 120-123/1·5 126-129/2	1,000 500		шшш
	MeCO	ClitH15NO3 Ref. 6	N.6-7	N,6·3	132 - 134/2 94-96.5	1,000	6	i U
	HCO MeCO	C ₁₂ H ₁₅ NO ₃ C ₁₃ H ₁₇ NO ₃	N,5.5,5.5 N,6.3	N,6-3 N,6-0	130-140/2 128-130/2	88	7 1	ш
	EtCO PrCO	C ₁₄ H ₁₉ NO ₃ C ₁₅ H ₂₁ NO ₃	C,67 •4;H,7 • 5 N,5 •6	C,67 · 5;H,7 · 6 N,5 · 3	138-140/2 · 5 150/2 · 5	34	3 7	шш
	Me(CH ₂) ₄ CO Me(CH ₂) ₁₀ CO	C17H25NO3 C23H37NO3	N.5.2 N.4.0	N,4 8 N,3 7	164-168/2 214-216/2	40 > 2,500	0	ы
	Crotonyl Me(CHBr)23CO	C ₁₅ H ₁₉ NO ₃ C ₁₅ H ₁₉ Br ₂ NO ₃	N.5.6 N.3.7	N.S.4 N.3-3	150-154/2 188-192/2	500 100	£9.	ц
	MeOCH _a CO MeSCH _a CO	C14H19NO4 C14H19NO3S	N,5·7 C,59·3;H,6·6	N,5·3 C,59·8;H,6·8	154-158/2 170-174/2	2 <u>8</u> 9	-0 :	ലല
	PhOCH ₂ CO CICH ₂ CO	C19H21NO4 C13H16CINO3	X.4.3	N,5-2	80-81 37-39	€ \$8;	۶.	щО
	4-CI · C ₆ H40CH2CO NCS · CH2CO McOOC(CH2)·CO	C19H20CINO4 C14H16N2O3S C12H26NO2	CI,9-75 S,11-0 N 4-2	CI.9-8 S.11-0 N 4-4	102104 160-166/0-1 180-200/0-4	88 <u>7</u>	44 3	
	H	(Union Carbide	7.1.1	F + F 51	1 0/007-001	027		•
	MeCO	ClaH ₁₇ NO3	0.9'N	0.9'N	136-140/2	28 <u>3</u>	4	¢0
	Prco	C14H19NO3 C15H21NO3	N.5.5	N.5.6	146-148/2 150-151/2	00 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0	A0;
	Me(CH ₁)4CO CICH ₂ CO	C17H25NO3 C13H16CINO3	4 0 0	N.4-8	168-172/2 148-152/1	38 V V	0	щœ
	MeCO	CIaH17NO3 CI4H19NO3	N,5.9	N,5.6	140142/2 138140/2	2,500 60	4	пп
	MeCO MeCO	Cl4H19NO3	N.5-9	N.5.6	134-138/2	<u>8</u> 9	, ,	â
	4-CIC6H40CH2CO	CanNa2CINO4	0.4X	N. 20	92–93 192–196/2	;8 <u>8</u>	10 16	щц
	CICH ₂ CO	C14HisCINO3	N,5.05	N,5.05	157-158/1.6	0.00		ы
	MeCO H	CitHisNO3 CitHisNO3 Ref 8	N,6·0	N,5·6	135-137/2 58_60	<2,500		Щ <
	MeCO	Ci4H10NO3	N.5.8 N.5.2	N.5.6	140-143/1 · 5	388 /	y Y	(m)
	PhOCH CO	C20H23NO4	Z,44		67-69	; ? ?)))))	ц
	CICH2CO	CisH1NO3S Ci4H18CINO3	N,5-0	N,5.0	184-190/2.5	281	4	ושמ
	MeCO	Ket. b C14H19NO3	C,68-0;H,7-4	C,67.5;H,7.6	145-144-5	350	T	ומנ
	Prco	C16H21NO3 C16H23NO3	N,5.6 N,5.3	N.5.1	156-160/2	200 500	3	υш
	MeOCH ₂ CO 4-CIC ₆ H ₄ OCH ₂ CO	C15H21NO4 C20H20CINO4	N.4-9	N.5.0	150-160/1 123-125	100		щ
	EtOOC CO	CleH21NO5	C,62,1;H,7-1	C,62,5;H,6-8	71-73	99		шС
	MeCO	ClsH19NO3	N.5.3	N.5.4	168-180/2	50		D
	H	Ref. 6	1.c.N	4.0.N	100-101	000 ⁺ 7 <	1	щ
	HCO MeCO	C16H19NO3 C16H21NO3	N,4·8 C,70·0;H,7·5	N,5·4 C,69·8;H,7·6	165-175/2 172-174/3	250 350		щ
	PhOCH ₂ CO MeCO	C22H25NO4 C16H21NO3	N.5.5	N.3.8 N.5.1	90-91 · 5 69 · 5-70 · 5	150 > 2,500		щ
	H MeCO	C ₁₁ H ₁₃ NO ₂ C ₁₃ H ₁₅ NO ₃	N.7.5 N.6.3	N.7.3	69-70 134/1	30	, S	Ош
	CICH ₂ CO	C ₁₃ H ₁₄ CINO ₃	Cl,13-0;N,5-2	C.,13-3;N,5-2	153156/1-2	< 30	4	ш
	MeCO	C15H17N03 C12H15N03 C12H15N03	Z,5.8	NX.5.4	158-160/2 132-134/1·5	350 350	0 0	шц
	MeCO	ClaH15NO3	N,6.4	N,6·3	129-133/2	1,500		цЩ

TABLE I ROOC·NMe·R'

Fraser et al.: Veterinary Insecticides. II

Approx. oral toxicity to mice	аслава ва в в составление и полова состава. Вбали и полова и полова состава и полова и полов Вбали и полова и поло Вбали и полова и полов Вбали и полова и полов Полова и полова и поло	
Persistence on sheep (weeks) 0.1% 0.05% Concn.	ر م م م م م م م م م م م م م م م م م م م	
Approx. LDse to blowfly larvae ppm		
M.p., °c or b.p., °c/mm	120-122 52-54 53-54 160-165/3 160-165/3 160-165/3 160-165/3 160-165/3 160-165/3 160-165/3 160-165/3 160-165/3 160-107 - 5 122-128 102-103 102-103 168-167/2 148-152/1 - 5 133/0 - 08 160-104-105 173-182/2 173	ch tube) LDso 00 mg/kg 250 1,000
Required, %	N. 6. 3 N. 5. 6 N. 5. 5 N. 5. 6 N. 5 N. 5 N. 5 N. 5 N. 5 N. 5 N. 5 N. 5	imations by stoms imations 0-60-2 B 500-1 C 500-1 C 500-1 F
Found, %	N, 5-9 N, 5-9 N, 5-9 N, 5-9 N, 5-9 N, 5-9 N, 5-9 N, 4-8 N, 4-8 N, 5-9 N, 5-9 N, 5-9 N, 5-9 N, 5-1 N, 5-2 N, 5-4 N, 5-2 N, 5-4 N,	an toxicities (Mice: 4 Approx
Formula	Ref. 6 Control of the second	Mammali
R,	H HCO MECO MECO MECO MECO MECO MECO MECO ME	
R	2,3,5-MesCaH2 	

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Other unacylated carbamates which showed a noteworthy level of activity in the laboratory screening were those substituted in the 3-position by an alkyl group of moderate size (C_3 - C_4) and the sterically equivalent¹² 2-alkoxy derivatives. 3-Alkoxy compounds were less active, and the 4-alkyl derivatives were completely inactive. However, some of the 2-alkyl compounds were active.

N-Acylation in many cases reduced larvicidal potency as well as mammalian toxicity, although not always to the same extent. The acetyl derivative of Butacarb was slightly less active than the parent carbamate, and had no advantage over the latter with respect to mammalian toxicity. However, in most of the series investigated, the parent carbamates were more toxic to mammals than the acyl derivatives.

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ROUTINE DETERMINATION OF CALCIUM AND MAGNESIUM IN SOIL EXTRACTS BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

By W. S. G. MACPHEE and D. F. BALL

Experiments using a commercial atomic absorption spectrophotometer show that accurate routine determinations of calcium and magnesium in extracts of soil in N/2 acetic acid or neutral N ammonium acetate can be made, employing 1600 ppm Sr (as $SrCl_2$ 6H₂O) as an additive to suppress interferences due to phosphate and other ions. The technique may be applied also to determinations of cation exchange capacity which require measurement of magnesium in solution.

Introduction

Atomic absorption spectrophotometry¹ and its analytical applications have been the subject of several recent reviews.²⁻⁴ David⁵ first described the use of this technique for determination of exchangeable cations in soil extracts. Problems in determination of exchangeable cations by standard methods are most marked for calcium and magnesium, especially at low concentrations. This note reports experiments using a commercial instrument in the determination of exchangeable calcium and magnesium. The application of the magnesium procedure to cation exchange capacity determination is also reported.

Experimental and Results

Exchangeable calcium and magnesium

It is known⁵ that phosphate, aluminium and silicate interfere with determination of calcium and magnesium in soil extracts by atomic absorption spectrophotometry, and that such interferences can be suppressed by addition of high concentrations of strontium or lanthanum. David⁵ reported that strontium at 1500 ppm concentration in solution was effective in suppressing these interferences. Although recently proposed methods⁶ have suggested that lanthanum is preferable to deal with high concentrations of phosphate.

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