Biological Activity as an Effect of Structural Changes in Aryl *N*-Methylcarbamates

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As part of a research program on control of destructive forest insects, 25 aryl *N*-methylcarbamates with a variety of substituents on both the ring and carbamyl portions were tested. Their biological activity was measured by topical application in acetone to spruce budworm and by oral administration to mice. Examples of biologically active carbamates were 4-dimethylamino-3,5-xylyl *N*-methylcarbamate, and 4-methylthio-3-tolyl *N*-methylcarbamate. They gave an LD_{50} for spruce budworm at 1 to 15 mg. per gram and an acute oral LD_{50} for mice at 20 to 50 mg. per kg. Addition of an *N*-acyl substituent to the carbamyl moiety usually maintained biological activity on spruce budworm, but decreased the toxicity to mice to the point where a dosage of 1 gram per kg. produced no visible effect.

The Insecticide Evaluation Project of the U.S. Forest Service is studying the biological activity of relatively nonpersistent insecticides as part of a program designed to control forest insect pests. Because carbamate insecticides show a great variation of activity on insects, a study was made to determine the most effective type of carbamate for control of spruce budworm [*Choristoneura fumiferana* (Clemens)], a highly destructive defoliating insect in North America. In our laboratory tests, the western form [*Choristoneura occidentalis* (Freeman)] (Freeman *et al.*, 1967) was utilized. An insecticide found to be among the most active in controlling spruce budworm was the carbamate Zectran (4-dimethylamino-3,5-xylyl *N*-methylcarbamate), a product of the Dow Chemical Co.

In recent years, numerous studies have been made of the structural relationship of aryl *N*-methylcarbamates on insects and their cholinesterase-inhibitory properties (Georghiou and Metcalf, 1962; Kaeding *et al.*, 1965, Metcalf *et al.*, 1964; Shorey *et al.*, 1962). These investigations revealed that the most effective carbamates were 3-alkyl, 3,5-dialkyl, or 2-alkoxy-substituted *N*-methylcarbamates. Metcalf and Fukuto (1965) and Kaeding *et al.* (1965) found that electron-donating substituents in the 4 position increased biological activity. Metcalf and Fukuto (1965) also suggested that structural variations of carbamates could result in compounds that were insecticidally active while being nontoxic or readily detoxified by mammals.

In 1965, a patent issued to Robertson *et al.* (1965) showed that reduced mammalian toxicity was a function of *N*-acylation of the carbamyl portion of insecticidal aryl *N*-methylcarbamates. Fraser *et al.* (1965) tested some of these acylated carbamates on both insects and rats and found, in some cases, a decrease in insecticidal activity and, in all cases, a large decrease in the acute oral toxicity to rats.

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MATERIALS AND METHODS

Many compounds tested in these studies have been described in the literature. Samples of some were available through commercial manufacturers. The methods of synthesis and elemental analysis are described for those not previously reported.

General Method for Synthesis. CARBAMATES. The carbamates were prepared by reaction of a suitable phenol with methyl isocyanate in ether, hexane, or benzene, using triethylamine or stannous octoate as a catalyst. Aminocarbamates were prepared by palladium (5 to 10%)-charcoal catalytic hydrogenation of the corresponding nitrocarbamates at atmospheric pressure in methanol solvent. Acylation was carried out with acetic anhydride at 100° to 110° C., using sulfuric acid as a catalyst. Details of this acylation were described by Robertson *et al.* (1965). Acylated aminocarbamates were prepared by reducing the corresponding acylated nitrocarbamates. The carbamates were purified by recrystallization from hexane or aqueous alcohol.

Structures of synthesized carbamates were confirmed by examination and identification of characteristic absorption peaks of their infrared spectra (Chen and Benson, 1966) and through elemental analysis (Tables I and II).

PHENOLS. For the synthesis of 5-amino-3-methylphenyl carbamate (IV), 3-methyl-5-nitrophenol was prepared from 4-chloro-3,5-dinitrotoluene (available from Sherwin Williams) by partial reduction and dehalogenation with ammonium sulfide according to the methods of Borsche and Rantscheff (1911) that gave 3-methyl-5-nitroaniline. The aniline was diazotized and hydrolyzed to 3-methyl-5-nitro-

Table I. Phenyl *N*-Methyl-*N*-acyl Carbamates

Compound No.	R Ring Position 3	4	5	R^1	Melting point, °C.	Elemental Analysis Calcd. C, %	Calcd. H, %	Found C, %	Found H, %
I[a]	H	H	H	H	82–84				
II	H	NH_2	H	H	132–135	57.82	6.07	58.49	6.10
III	CH_3	NH_2	H	H	127.5–128	59.98	6.71	59.80	6.61
IV	CH_3	H	NH_2	H	109.5–111.5	59.98	6.71	59.07	6.49
V[a]	CH_3	H	H	H	75.76				
VI[a]	CH_3	H	CH_3	H	100–102				
VII[b]	H	SCH_3	H	H	78–79				
VIII[b]	CH_3	SCH_3	H	H	81				
IX	—CH—CH—NH—		H	H	104.5–105	63.14	5.30	62.54	5.25
X[c]	CH_3	NH_2	CH_3	H					
XI[c]	CH_3	$NHCH_3$	CH_3	H					
XII[c]	CH_3	$N(CH_3)_2$	CH_3	H					
XIII[d]	CH_3	$N(CH_3)_2$	H	H					
XIV[e]	CH_3	OH	CH_3	H	161.5–164.5	61.52	6.71	61.93	6.64
XV	CH_3	NH_2	H	—$COCH_3$	88–89	59.44	6.35	59.03	6.08
XVI	CH_3	SCH_3	H	—$COCH_3$	36–37	56.89	5.97	57.60	6.12
XVII[f]	CH_3	$N(CH_3)_2$	CH_3	—$COCH_3$	37.5	63.61	7.63	63.79	7.74
XVIII	H	NH_2	H	—$COCH_2CH_3$	83–85	59.44	6.35	59.53	6.23
XIX	CH_3	NH_2	H	—$COCH_2CH_3$	72–74	61.00	6.83	60.59	6.71

[a] Prepared and identified according to Chen (1966).
[b] Prepared according to Farbenfabriken Bayer (1962).
[c] Courtesy of Dow Chemical Co.
[d] Courtesy of Chemagro Corp.
[e] Balba and Casida (1968).
[f] Prepared according to Robertson *et al.* (1965).

Table II. Naphthyl *N*-Methyl and *N*-Methyl-*N*-acyl Carbamates

Compound No.	Position of Carbamate	R Ring Position 2	3	4	6	R^1	Analysis Melting point, °C.	Calcd. C, %	Calcd. H, %	Found C, %	Found H, %
XX[a]	1	H	H	H	H	H					
XXI[b]	1	H	H	H	H	—$COCH_3$	102–104				
XXII	1	NO_2		H	H	H	130–32	49.49	3.11	49.57	3.44
XXIII	1	H	H	NH_2	H	H	178 dec.	66.65	5.60	66.31	
XXIV	1	H	H	H	NH_2	H	152	66.65	5.60	66.31	5.64
XXV[c]	2	H	H	H	H	H	113				
XXVI	1	H	NH_2	H	H	H	143–46	66.65	5.60	66.44	5.51

[a] Courtesy of Union Carbide Corp.
[b] Prepared according to Robertson *et al.* (1965).
[c] Prepared according to Siefken (1949).

phenol using the method of Nevile and Winter (1882). Nitro-1-naphthols were made from suitable nitronaphthalenes obtained from Fundamental Research, Berkeley, Calif. The 4-(methylthio)-*m*-cresol was obtained from Crown-Zellerbach, Chemical Products Division. Other phenols are available from chemical supply houses.

Insecticidal activity of these carbamates was determined by topical application to the dorsal thorax of the spruce budworm larvae. Most of the carbamates were dissolved in acetone, except for IX, XXIII, XXIV, and XXVI (Table I), which were dissolved in dimethyl sulfoxide (DMSO) and applied at the rate of 1.0 μl. per 100 mg. of weight using a calibrated, micrometer drive syringe. Mortality evaluations were made after 7 days.

Acute oral dosages for mice were determined in two ways. If the carbamate tested was a crystalline solid, a pellet was prepared by using a hydraulic press and die. These pellets were fed to mice without the use of additives. If the carbamate was in a liquid form, application was made directly into the mouse, again without additives, by capillary tube. The dosage was adjusted so as to obtain 50% mortality within one hour. Final mortality assessment, made after 30 days, showed virtually no change after the first hour assessment. Compounds with low toxicity to spruce budworm were not tested on mice.

RESULTS

Spruce budworm mortality was increased by adding a -3-methyl or -3,5-dimethyl substituent to the basic aryl-*N*-methylcarbamate (I). Compound I has an LD_{50} of 300 μg. per gram for spruce budworm, while the LD_{50} of -3-methyl (V) was 55, and of -3,5-dimethyl (VI) was 20. The addition of substituents in the 4 position, such as —NR_2 or —SCH_3, increased additionally the activity of the methylated compounds. Compounds III, VIII, X, and XIII show the decreased LD_{50} value produced by these substitutions. The electron-donating substituent, -4-hydroxy (XIV), did not provide activity.

Acetylation of the active carbamates (III, VIII, and XII) did not greatly decrease the toxicity to spruce budworm

Table III. Mortality in Spruce Budworm and Mice, by Compound Number

Compound No.	No. of Insects	Spruce Budworm Mortality, LD_{50}, μg./G.	Mouse Mortality, LD_{50}, Mg./Kg.
I	200	300	
II	220	230	160–190
III	160	4–5	20–30
IV	160	>1000	
V	200	55	800
VI	90	20	
VII	140	230	
VIII	200	75	20–40
IX	80	>1000	
X	40	1.6	
XI	120	2.6	
XII	350	1.2	30–50
XIII	90	1.2	
XIV	90	>1000	
XV	160	13	>1000
XVI	200	32.0	>1000
XVII	90	2.4	>1000
XVIII	160	>1000	
XIX	180	7.5	
XX	90	17	
XXI	90	>1000	
XXII	90	>1000	
XXIII	100	750	
XXIV	100	>1000	
XXV	140	>1000	
XXVI	240	>1000	

(Table III). This was not the case with mice, since no kill was observed at dosages of 1000 mg. per kg.

DISCUSSION

The foregoing data generally agree with results published by Metcalf and Fukuto (1965), Kaeding *et al.* (1965), and Fraser *et al.* (1965). In our studies there was not complete agreement on increased toxicity to the spruce budworm with all electron donors in the 4 position; the exception was the 4-hydroxy-3,5-xylyl *N*-methyl carbamate (XIV). We also found that not all carbamates are equally active, as also reported by Shorey *et al.* (1962) and Georghiou and Metcalf (1962). The 4-amino, 4-dimethylamino, and 4-thiomethyl, 3,5-methylated *N*-methyl carbamates were most toxic to spruce budworm. One of the most interesting aspects of this study was our findings that *N*-acylated carbamates did not greatly change insecticidal activity on spruce budworm, but decreased the acute oral toxicity to mice by a large factor. The most effective carbamate, Zectran, with an LD_{50} of 1.4 μg. per gram for spruce budworm and an acute oral toxicity of 30 to 50 mg. per kg. for mice, failed to kill mice at a dose of 1000 mg. per kg. after acetylation (compound XVII).

Table IV. Significant Changes in Biological Activity Produced by *N*-Acylation

Compound	Spruce Budworm Mortality, LD_{50}, μg./Gram	Mouse Mortality, LD_{50}, Mg./Kg.
III	4.5	20–30
Acetylated III (XV)	13.0	>1000
VIII	15.0	20–40
Acetylated VIII (XVI)	32.0	>1000
XII	1.4	30–50
Acetylated XII (XVII)	2.4	>1000

The data in Table IV showing changes in biological activity induced by *N*-acetylation are dramatic. These data merely correlate the decreased mammalian toxicity that occurs with *N*-acylation as reported by Fraser *et al.* (1965) and Robertson *et al.* (1965).

LITERATURE CITED

Balba, M. H. M., Casida, J. E., J. AGR. FOOD CHEM. **16,** 561 (1968).
Borsche, W., Rantscheff, D., *Ann.* **379,** 160 (1911).
Chen, J. T., Bensen, W. R., *J. Assoc. Offic. Agr. Chemists* **49,** 412 (1966).
Farbenfabriken Bayer, Brit. Patent **912,895** (Dec. 12, 1962).
Fraser, J., Clinch, G. P., Reay, R. C., *Sci. Food Agr.* **16,** 615–18 (1965).
Freeman, T. N., Stehr, G. W., Harvey, G. T., Campbell, I. M., *Can. Entomol.* **99,** 449–506 (1967).
Georghiou, G. P., Metcalf, R. L., *J. Econ. Entomol.* **55,** 125–7 (1962).
Kaeding, W. W., Shulgin, A. T., Kenagal, E. E., J. AGR. FOOD CHEM. **13,** 215–18 (1965).
Metcalf, R. L., Fukuto, T. R., J. AGR. FOOD CHEM. **13,** 220–31 (1965).
Metcalf, R. L., Fukuto, T. R., Frederickson, M., J. AGR. FOOD CHEM. **12,** 231–6 (1964).
Nevile, R. H. C., Winter, A., *Ber.* **15,** 2986 (1882).
Robertson, W. A. H., Fraser, J., Clinck, G. C. (to Bootes Pure Drug Co.), Brit. Patent **982.235** (Feb. 3, 1965).
Shorey, H. H., Reynold, H. T., Anderson, L. D., *J. Econ. Entomol.* **55,** 5–11 (1962).
Siefken, W., *Ann.* **562,** 75 (1949).

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