Selective Toxicity of N-Substituted Biscarbamoyl Sulfides

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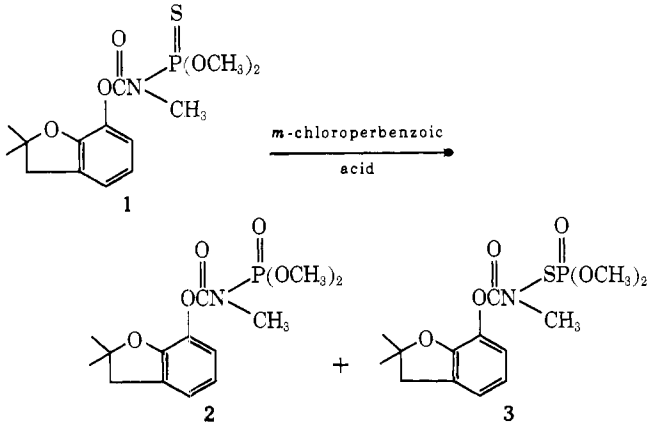
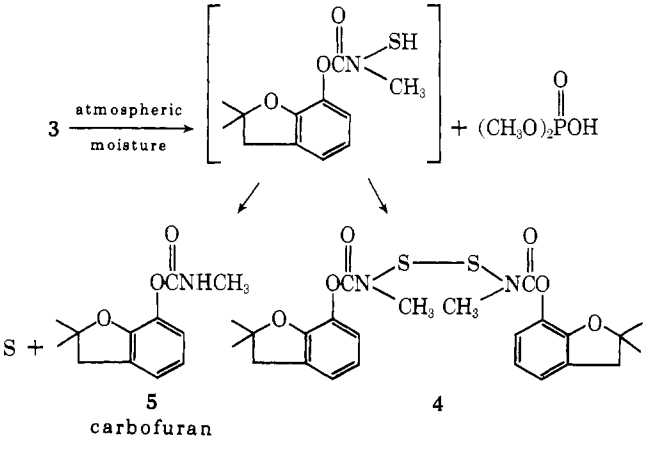
The reaction between sulfur monochloride and insecticidal methylcarbamate esters in the presence of pyridine gave as the principal product the corresponding N-substituted biscarbamoyl sulfide. Compared to the original methylcarbamate esters, the biscarbamoyl sulfides were substantially less toxic to the white mouse but were still highly effective insecticides, particularly against mosquito larvae. One of the compounds, bisaldicarb sulfide, was significantly superior to aldicarb as a systemic insecticide in greenhouse tests. In all cases, the biscarbamoyl sulfides were less effective in inhibiting both insect and mammalian cholinesterase than the methylcarbamate esters. A rationale for the selective action of these compounds is presented.

2,2-Dimethyl-2,3-dihydrobenzofuranyl-7 *N*-methyl-*N*-(dimethoxyphosphinothioyl)carbamate (1) is a highly effective selective insecticide, *i.e.*, it is toxic to insects but is substantially less toxic to mammals (Fahmy *et al.*, 1970). When 1 is treated with *m*-chloroperbenzoic acid in methylene chloride, the principal products observed are the expected desulfuration product 2,2-dimethyl-2,3-dihydrobenzofuranyl-7 *N*-methyl-*N*-(dimethoxyphosphinyl)carbamate (2) and an unexpected rearrangement product, the corresponding *N*-(dimethoxyphosphinylthio)carbamate (3) (Fahmy and Fukuto, 1972). The reaction is depicted below. 3 is unstable on silica gel thin-layer plates



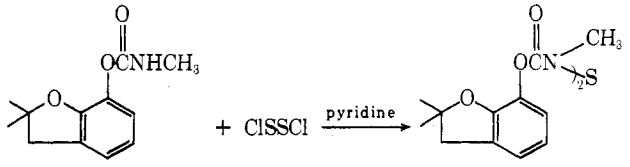
1 → 2 + 3

and decomposes to the disulfide 4 and to carbofuran 5 according to the equation below.



5 carbofuran; 4

In an attempt to prepare 4 by an independent method, 2 mol of carbofuran was reacted with 1 mol of sulfur monochloride in pyridine. To our surprise, the reaction did not afford 4 but gave the related biscarbamoyl sulfide (6) as the principal product. A subsequent surprise was



carbofuran; 6

the discovery that 6, although much less toxic to the white mouse, was still highly insecticidal, comparable in activity to carbofuran. Therefore, other N-substituted biscarbamoyl sulfides were prepared and examined. This report is concerned with the toxicological properties of these compounds, with particular emphasis on their selective action.

MATERIALS AND METHODS

The following methylcarbamate insecticides were obtained from their respective manufacturers as previously reported or were synthesized in this laboratory (Fahmy *et al.*, 1970): **5**, carbofuran (2,2-dimethyl-2,3-dihydrobenzofuranyl-7 methylcarbamate); **7**, carbaryl (1-naphthyl methylcarbamate); **9**, MIP (3-isopropylphenyl methylcarbamate); **11**, propoxur (2-isopropoxyphenyl methylcarbamate); **13**, aldicarb [2-methyl-2-(methylthio)propionaldehyde *O*-carbamoyloxime].

The N-substituted biscarbamoyl sulfides were prepared by treating 2 mol of the appropriate methylcarbamate ester with 1 mol of sulfur monochloride (Baker and Adamson Division, General Chemical Co.) in pyridine as a solvent. The following procedure for the preparation of *N,N'*-bis(3-isopropylphenyl methylcarbamoyl) sulfide (4) is typical. A mixture of 10 g of 3-isopropylphenyl methylcarbamate (3), 4 g of sulfur monochloride (S_2Cl_2), and 25 ml of pyridine was stirred overnight at 5°. The reaction mixture was diluted with water, the product was extracted into ether, and the ether phase was washed in turn with chilled 5% hydrochloric acid and water and dried over anhydrous sodium sulfate. Removal of the solvent gave a solid residue which was recrystallized from aqueous ethyl alcohol after filtration to remove sulfur. Elemental analyses (C. F. Geiger, Ontario, Calif.) and melting points are given in Table I.

Insecticidal activities were determined against the susceptible S_{NAIDM} strain of houseflies, *Musca domestica*, and 4th-instar mosquito larvae, *Culex pipiens quinquefasciatus*, according to usual procedures (Metcalf and March, 1949; Mulla *et al.*, 1966). The estimation of systemic insecticidal activity in cotton plants was carried out as previously described (Wustner and Fukuto, 1973). Mam-

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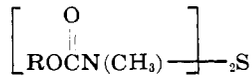
Table I. Analytical Data for N-Substituted Biscarbamoyl Sulfides



	R	Mp, °C	Elemental analysis Calcd	Found
6	2,2-Dimethyl-2,3-dihydrobenzofuranyl-7	129–129	C, 61.02 H, 5.93 S, 6.78	C, 61.55 H, 5.94 S, 7.00
8	1-Naphthyl-	141–142	C, 66.67 H, 4.63	C, 65.91 H, 4.56
10	3-Isopropylphenyl-	74–76	C, 63.46 H, 6.73 S, 7.69	C, 64.06 H, 7.24 S, 7.67
12	2-Isopropoxyphenyl-	77–79	C, 58.93 H, 6.25 S, 7.14	C, 58.54 H, 6.23 S, 7.02
14	$CH_3SC(CH_3)_2CH{=}N-$	88–91	C, 41.00 H, 6.34 S, 23.4	C, 41.55 H, 6.45 S, 23.76

malian toxicity was determined orally on Swiss white mice as described previously (Hollingworth *et al.*, 1967). Mortalities were recorded after 72 hr. Bimolecular rate constants (k_i) for the inhibition of housefly-head and bovine erythrocyte acetylcholinesterase (AChE) were determined at 30.0°, pH 7.0, by procedures previously established for phosphate esters (Aldridge, 1950) using acetylthiocholine as the substrate (Ellman *et al.*, 1961). The preparation of housefly-head AChE has been described (Fukuto and Metcalf, 1956) and bovine erythrocyte AChE was purchased from Sigma Chemical Co.

RESULTS

Insecticidal Activity. Toxicological properties of the various N-substituted biscarbamoyl sulfides are presented in Table II, along with those of the parent methylcarbamates. From examination of the data in the table, it is apparent that the N-substituted biscarbamoyl sulfides retained much of the insecticidal activity exhibited by the corresponding methylcarbamate esters. Although all of the biscarbamoyl sulfides, used alone or in combination with piperonyl butoxide (PB), were slightly less toxic to houseflies compared to the methylcarbamates, they were, however, significantly more toxic to mosquito larvae. Except for bisaldicarb sulfide (14), a five- to eightfold increase in larvicidal activity was observed with the biscarbamoyl sulfide.

The results obtained with mosquito larvae are similar to those reported previously for *N*-arylsulfenyl derivatives of insecticidal methylcarbamates where the derivatives were found to be substantially more toxic to larvae than the parent carbamate (Black *et al.*, 1973a). Increased larvicidal activity of the *N*-arylsulfenyl derivatives was attributed to their greater lipophilicity, a property which would promote rapid absorption into mosquito larvae from an aqueous phase (Leesch and Fukuto, 1972). The same explanation also may be applied to the high larvicidal activity of the biscarbamoyl sulfides.

Since aldicarb (13) is used primarily as a systemic insecticide, the corresponding bisaldicarb sulfide (14) also was examined for systemic activity in the cotton plant. Laboratory tests against the cotton aphid (*Aphis gossypii*), perforator (*Bucculatrix thurberiella*), mite (*Tetranychus cinnabarinus*), and salt marsh caterpillar (*Estigmene acrea*) revealed that 14 applied to soil containing cotton plants was significantly more effective in controlling the first three pests than aldicarb. For example, at the approximate dosage of 1.2 lb of actual material per acre, 14 gave virtually complete control of cotton aphids and perforator for over 21 weeks, as compared to 15 weeks for aldicarb. Aldicarb and 14 were about equal in their activity against the salt marsh caterpillar.

Biscarbofuran sulfide (6) also was examined as a cotton systemic insecticide and although it was outstandingly effective in controlling the cotton leaf perforator, giving 100% control for more than 20 weeks, it was almost totally ineffective against the other pests.

Mouse Toxicity. The oral mouse toxicity data presented in Table II show that, compared to the original methylcarbamates, the N-substituted biscarbamoyl sulfides are in most cases substantially less toxic to the white mouse. Depending on the compound, the biscarbamoyl sulfides were from 5- to 50-fold less toxic to the mouse than the parent methylcarbamate. The largest difference (50-fold) in mouse toxicity was observed between carbofuran (5) and biscarbofuran sulfide (6). Bisaldicarb sulfide (14) was approximately fivefold less toxic to mice than aldicarb (13). In general, improvement in mouse toxicity by conversion of the parent carbamates to the biscarbamoyl sulfides paralleled results obtained in other related derivatization studies (Black *et al.*, 1973a; Fahmy *et al.*, 1970).

Anticholinesterase Activity. Bimolecular rate constants (k_i) for the inhibition of housefly-head (HAChE) and bovine erythrocyte (BAChE) acetylcholinesterase by the methylcarbamate insecticides and N-substituted biscarbamoyl sulfides are presented in Table II. The data clearly indicate that on an overall basis the biscarbamoyl sulfides are less effective anticholinesterases than the parent methylcarbamates against both bovine erythrocyte and housefly-head AChE. Differences in anticholinesterase activity between respective biscarbamoyl sulfides and methylcarbamates were quite variable, ranging from 4.7-fold for aldicarb–bisaldicarb sulfide (13:14) against BAChE to 141-fold for carbofuran–biscarbofuran sulfide (5:6) against HAChE.

In the case of BAChE, *i.e.*, an AChE from a mammalian source, the reduction in anticholinesterase activity of the biscarbamoyl sulfide compared to the methylcarbamate is more or less in agreement with the reduction in mouse toxicity (*cf.* Table III). Although probably fortuitous, the correlation between mouse toxicity and BAChE inhibition by aldicarb (13) and bisaldicarb sulfide (14) is quite good. Correlation between the other pairs, particularly 9:10 and 11:12, however, is much less satisfactory.

On the other hand, differences in inhibition between the methylcarbamate–biscarbamoyl sulfide pairs against HAChE were far greater than their respective relative toxicities to houseflies. This suggests that factors other than cholinesterase inhibition must be considered to account for the comparatively high insecticidal activity of the biscarbamoyl sulfides relative to their anticholinesterase activity. This is particularly true in connection with mosquito larvae where the biscarbamoyl sulfides are observed to be more toxic than the methylcarbamates.

DISCUSSION

Although discovered essentially through a serendipitous route, the N-substituted biscarbamoyl sulfides appear to be closely related chemically and toxicologically to derivatives of methylcarbamates previously reported (Black *et al.*, 1973a; Fahmy *et al.*, 1970), in particular with the *N*-arylsulfenyl derivatives of insecticidal carbamates. By analogy with the derivatized methylcarbamates reported earlier, the favorable toxicological properties of the biscarbamoyl sulfides also may be attributed to opportunities which derivatization may provide in allowing additional metabolic detoxication and intoxication processes to take place in mammals and insects.

The rationale which may be suggested for the improvement in mammalian toxicity and the retention of insecti-

Table II. Toxicological Properties of Insecticidal Methylcarbamate Esters and Their Corresponding N-Substituted Biscarbamoyl Sulfides

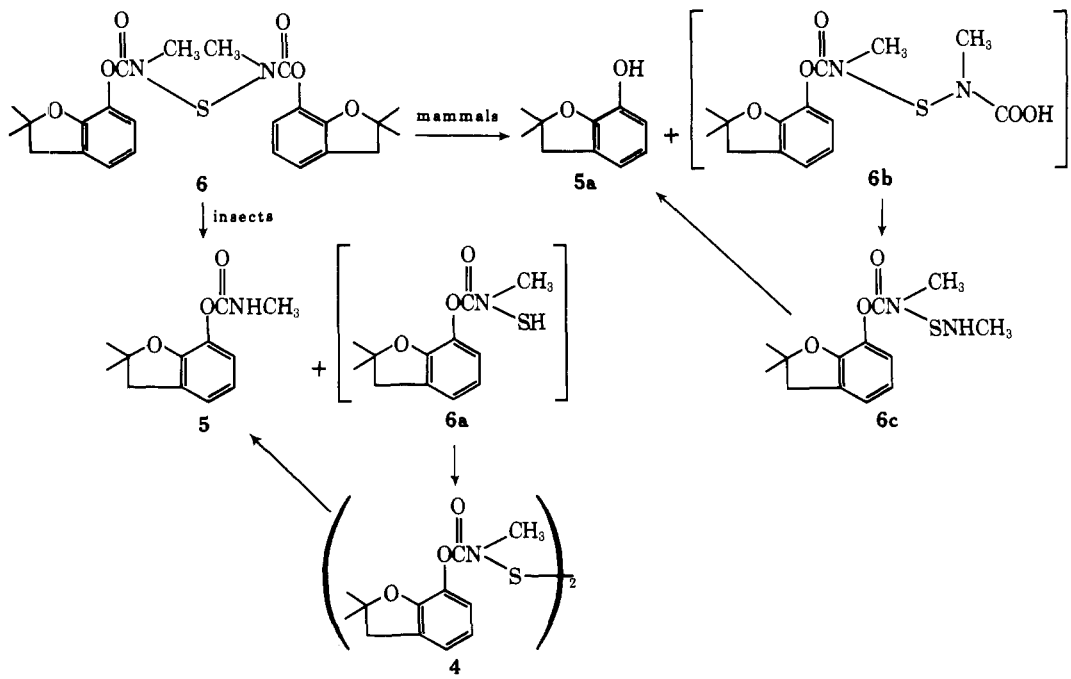
	Compound	LD_{50}, mg/kg				Anticholinesterase activity, k_i (M^{-1} min^{-1})	
		Housefly		Mosquito larvae	Mouse	Bovine erythrocyte	Fly-head
		Alone	+PB[a]				
5	Carbofuran	6.7	0.9	0.052	2	1.9×10^6	1.3×10^7
6	Biscarbofuran sulfide	19	1.2	0.007	50–100	2.5×10^4	9.2×10^4
7	Carbaryl	>500	12.5	1.0	560[b]	4.0×10^4	4.9×10^5
8	Biscarbaryl sulfide	>500	42.0	0.22	*c*	6.1×10^3	5.4×10^4
9	3-Isopropylphenyl methylcarbamate (MIP)	41	1.6	0.038	16	7.5×10^5	7.7×10^5
10	BisMIP sulfide	85	4.6	0.0056	200	2.7×10^4	2.3×10^4
11	Propoxur	22	0.9	0.33	24	4.3×10^4	1.2×10^5
12	Bispropoxur sulfide	35	2.5	0.041	700	4.6×10^3	2.8×10^4
13	Aldicarb	5.5	1.0	0.16	0.3–0.5	3.5×10^4	2.0×10^4
14	Bisaldicarb sulfide	8.5	3.2	0.17	1.6–2.5	7.4×10^3	2.1×10^3

[a] Piperonyl butoxide (PB) was applied at a constant dose of 40 μg/fly in combination with varying doses of insecticide. [b] Toxicity to the rat. [c] Compound was too insoluble in olive oil or propylene glycol for accurate evaluation.

Table III. Relative Values for the Inhibition of BAChE and HAChE and Toxicity to the Mouse and Housefly for the Indicated Pairs of Methylcarbamate and Biscarbamoyl Sulfides

Compounds	BAChE, k_i ratio	Mouse relative toxicity	HAChE, k_i ratio	Housefly relative toxicity
5:6	76	25–50	141	2.8
9:10	28	13	33	2.1
11:12	9.3	29	42	1.5
13:14	5.2	4.7	9.5	1.5

cidal activity for the biscarbamoyl sulfides is presented in the following metabolic scheme using biscarbofuran sulfide (**6**) as an example.



According to this hypothetical scheme, in insects **6** may be metabolically converted to carbofuran (**5**) and **6a** (or a conjugated derivative of it). The intermediate **6a** is probably unstable and couples to form **4** which, in turn, can be cleaved to generate additional carbofuran. Since carbofuran is a potent anticholinesterase, it would act as the agent responsible for intoxication. Support for this hypothesis is found in recent studies (Black *et al.*, 1973b) in which relatively large amounts of carbofuran were isolated from intoxicated houseflies after treatment with an *N*-arylsulfenyl derivative of carbofuran. Evidently, cleavage of the N–S bond occurs readily in the housefly.

Although reduced toxicity of the biscarbamoyl sulfides may be accounted for in part by their lower anticholinesterase activity, it is quite probable that they are detoxified before large amounts of the parent methylcarbamate are produced *in vivo* owing to the unlikelihood that a molecule such as **6** will pass through a mouse unchanged. Direct hydrolysis of the carbamoyl moiety to give the phenol (**5a**) and *N*-methylaminosulfenyl carbofuran (**6c**) probably constitutes a detoxication reaction, since **6c** also may be detoxified to the phenol. Detoxication reactions similar to this have been observed after treatment of mice with an

N-arylsulfenyl derivative of carbofuran (Black *et al.*, 1973b).

A study of the comparative metabolism of **6** in houseflies and mice is currently in progress.

Overall, the N-substituted biscarbamoyl sulfides presented in this study represent a promising group of carbamate derivatives which deserve further examination. Bisaldicarb sulfide (**14**) is an outstanding systemic insecticide in laboratory tests and is currently undergoing field tests.

ACKNOWLEDGMENT

The authors are indebted to Jon M. Fukuto for technical assistance.

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Received for review June 11, 1973. Accepted September 24, 1973. This investigation was supported by a Research Training Grant from The Rockefeller Foundation, New York, N. Y., and by a Research Grant from Cotton Incorporated, Raleigh, N. C.

Effects of Phenoxyacetic Acids on Rat Liver Tissues

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No drastic damaging effect was noted when male Long-Evans rats were given 2,4-dichlorophenoxyacetic acid (2,4-D) or 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) equivalent to 2–5 g/kilogram body weight over a 4- to 7-week feeding period. Response to herbicide treatment was dependent on animal age and on the duration of chemical feeding. Based on equal amounts of either chemical fed, animals showed stronger responses to 2,4,5-T than to 2,4-D. Feeding of 2,4,5-T caused an increase in liver fresh weight and dry weight per 100 g of body weight, whereas 2,4-D had little or no effect on liver weight. Herbicide-induced enlargement of the liver was associated with increases in most of the major cellular components on a per liver basis. Glycogen content was 50 to 100% higher in both 2,4-D- and 2,4,5-T-treated rats than in controls. Livers from 2,4,5-T treated animals but not 2,4-D treated animals contained increased quantities of RNA and protein. Total DNA was increased only 10% or less by the treatments, while nuclear DNA content was 15–45% lower in livers from 2,4,5-T-treated animals than those from control and 2,4-D-treated animals. Isolated liver nuclei from both 2,4-D- and 2,4,5-T-fed rats were 20–30% more active in *in vitro* RNA synthesis than control nuclei.

2,4-Dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) are considered to be strong auxins. They stimulate plant growth (at low concentration) by inducing cell division and/or cell enlargement (Key *et al.*, 1966; West *et al.*, 1960). Auxin treatment results in enhanced *in vivo* synthesis of nucleic acid and protein, as well as increased *in vitro* nuclear or chromatin RNA polymerase activity, both in intact and detached plant systems (Chrispeels and Hanson, 1962; Matthysse and Phillips, 1969; O'Brien *et al.*, 1968a,b; West *et al.*, 1960). Commercially the auxins have been used as selective herbicides and as defoliants (Johnson, 1971; Klingman, 1949). The mode of action of 2,4-D and 2,4,5-T as plant hormones has recently been discussed in relation to nucleic acid regulation (Cherry, 1970).

Phenoxyacetic acids have been shown to cause a number of abnormal conditions in animals. Effects on growth rate, survival values, and individual organ weights depend on concentration, degree of purity, and method of administration. Hemorrhagic gastrointestinal tracts and several abnormal hematological parameters have also been observed (Emerson *et al.*, 1971; Hansen *et al.*, 1971; Johnson, 1971; Radeleff, 1964; Rowe and Hymas, 1954; Sparschu *et al.*, 1971a).

Courtney *et al.* (1970) reported that 2,4,5-T was teratogenic, fetotoxic, and fetocidal in two strains of mice, and considerable concern has been expressed with regard to possible danger to humans. It has since been confirmed that the 2,4,5-T used in these studies was significantly contaminated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, a manufactural impurity. It currently appears that most but not all of the fetotoxic, fetocidal, and teratogenic effects observed are due to this impurity and not to 2,4,5-T *per se* (Johnson, 1971; Sparschu *et al.*, 1971b). The present study is concerned with some general effects of auxin herbicides on rats, as well as some specific biochemical effects on the liver.

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MATERIALS AND METHODS

Administration of the Herbicides. Unless otherwise stated, male Long-Evans rats (4-week-old or 7-week-old) were housed two per cage under a regulated cycle of 12 hr of light–12 hr of darkness. Animals were maintained on a diet of Wayne Lab Blox (Allied Mills, Inc., Chicago, Ill.), and were provided water *ad libitum*. Both 2,4-D and 2,4,5-T of the analytical standard grade (containing no 2,3,7,8-tetrachlorodibenzo-*p*-dioxin at a sensitivity of 0.05