= H, R_3 = Cl), 89998-96-9; II (R = R_1 = H, R_2 = CH_3, R_3 = Cl), 89998-97-0; III (R = R_1 = R_2 = R_3 = H), 89998-98-1; III (R = R_1 = R_2 = H, R_3 = Cl), 89998-99-2; III (R = R_1 = R_2 = H, R_3 = Br), 89999-00-8; III (R = R_1 = Br, R_2 = H, R_3 = Cl), 89999-01-9; III (R = R_1 = H, R_2 = CH_3, R_3 = Cl), 90028-85-6.

LITERATURE CITED

Boell, W.; Volfratz, R.; Adolphi, H. Ger. *Offen.* 2 255 581, 1974; *Chem. Abstr.* **1974**, *81*, 37477*j*.

Brooke, G. M.; Furniss, B. S.; Musgrave, K. R. William. Brit. *Pat.* 1 227 352, 1971; *Chem. Abstr.* **1971**, *75*, 20177*q*.

Fuson, R. C.; Kaiser, E. W.; Speck, B. *J. Org. Chem.* **1941**, *6*, 845.

Grassberger, M.; Reinshagm, H. Swiss Pat. 570 933, 1975; *Chem. Abstr.* **1976**, *84*, 180026*d*.

Grinev, A. N.; Zotova, S. A.; Mildhailova, I. N.; Stolyarchuk, A. A.; Stepanyuk, G. I.; Matsak, V. V.; Sizova, T. N.; Pershin, G. N. *Khim.-Farm. Zh.* **1979**, *13* (8), 39.

Melnikov, N. N. "Chemistry of Pesticides"; Springer-Verlag: Berlin, 1971; p 5.

Metcalf, R. L. *J. Econ. Entomol.* **1948**, *41*, 875.

Saikawa, I.; Takano, S. Jpn. Pat. 7 029 816, 1970; *Chem. Abstr.* **1971**, *75*, 12985*r*.

Stoermer, R.; Chydenius, C. W.; Schinn, E. *Ber. Dtsch. Chem. Ges.* **1924**, *57*, 75.

Received for review July 1, 1982. Revised manuscript received June 7, 1983. Accepted February 21, 1984.

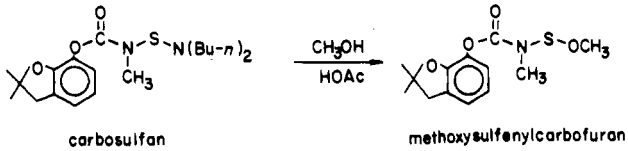
Formation of Alkoxysulfenyl Derivatives of Carbofuran by Acid-Catalyzed Alcoholysis of Carbosulfan

Noriharu Umetsu,[1] Takaaki Nishioka, and T. R. Fukuto*

The behavior of carbosulfan [2,3-dihydro-2,2-dimethylbenzofuran-7-yl *N*-[(dibutylamino)thio]-*N*-methylcarbamate] in alcohol–acid mixtures was examined. In methanol- or ethanol-containing acetic acid, carbosulfan was converted into a number of products, the major product being the solvolysis product in which the dibutylamino group is substituted by the methoxy or ethoxy moiety to give the respective alkoxysulfenyl derivative of carbofuran. The methoxysulfenyl derivative was also the principal alteration product of carbosulfan in methanolic hydrogen chloride, but in this case a number of polysulfide analogues of this derivative were observed. The alkoxysulfenyl derivatives showed good insecticidal activity against the house fly and were less toxic to the white mouse than carbofuran, the parent methylcarbamate.

Carbosulfan or 2,3-dihydro-2,2-dimethylbenzofuran-7-yl *N*-[(dibutylamino)thio]-*N*-methylcarbamate is a sulfenylated derivative of carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate), which is being developed as a broad-spectrum insecticide. Previous papers from this laboratory described the acid-catalyzed alteration of carbosulfan via N–S bond cleavage and formation of the polysulfide derivatives of carbosulfan and biscarbosulfan *N,N'*-disulfide, along with carbofuran and several other alteration products (Umetsu et al., 1981a,b; Umetsu and Fukuto, 1982). In these studies aprotic, inert solvents such as acetonitrile and dichloromethane were used.

A subsequent study of the behavior of carbosulfan in



carbosulfan methoxysulfenylcarbofuran

protic solvents, e.g., methanol–acetic acid (9:1), revealed solvent participation in the alteration reaction with significant amounts of the methoxysulfenyl derivative of carbofuran being formed, along with other alteration products. Examination of the toxicological properties of (methoxysulfenyl)carbosulfan revealed this compound to have insecticidal activity comparable to that of either carbosulfan or carbofuran. Because of our interest in new carbamate derivatives and the possible significance of acid-catalyzed solvolysis on the chemical and toxicological properties of carbosulfan, further probe into the nature of the alcoholysis reaction was made. This paper is concerned with the isolation, identification, and toxicological evaluation of the (alkoxysulfenyl)carbofuran derivatives formed from carbosulfan in alcohol-containing acid.

MATERIALS AND METHODS

Chemicals. [*carbonyl*-^{14}C]Carbosulfan (sp act. 25.20 mCi/mmol) was available from previous studies (Umetsu et al., 1979). The radiolabeled material was 99.5% pure and contained 0.2% carbofuran and 0.3% unknown components. Biscarbofuran *N,N'*-disulfide (CFS_2CF), biscarbofuran *N,N'*-polysulfide (CFS_nCF, $n \geq 3$), and polysulfide analogues of carbosulfan (CFS_nNBu_2, $n \geq 2$) were also available from previous studies (Umetsu et al., 1980, 1981a,b; Umetsu and Fukuto, 1982). Other chemicals were analytical reagent grade, and redistilled solvents were used.

Stability of Carbosulfan in Acidic Solvent Mixtures. In a preliminary examination of the stability of carbosulfan in different acidic solvents, samples of [*carbonyl*-^{14}C]carbosulfan (10.0 mg, 1.13 μCi) were dissolved in 0.2 mL of the following solvent mixtures and kept at 23 °C: acetonitrile–acetic acid (9:1), methanol–acetic acid (9:1), and ethanol–acetic acid (9:1). All solvent–acetic acid mixtures were prepared volume/volume. At different time intervals, duplicate 5-μL samples were removed and the contents were examined by thin-layer chromatography (TLC).

Acid-Catalyzed Alcoholysis of Carbosulfan. A mixture of 10.0 mg of purified carbosulfan (nonradioactive) and 18.6 μg of [*carbonyl*-^{14}C]carbosulfan (1.28 μCi) dissolved in 200 μL of methanol was added to 200 μL of a 4:1

Division of Toxicology and Physiology, Department of Entomology, University of California, Riverside, California 92521.

[1]Present address: Pesticide and Biological Science Research Laboratories, Otsuka Chemical Company, Ltd., Naruto, Tokushima-Ken 772, Japan.

0021-8561/84/1432-0765$01.50/0

methanol–acetic acid mixture. The same procedure was used for an ethanol–acetic acid mixture. For the examination of the alteration of carbosulfan in methanolic hydrogen chloride, [*carbonyl*-^{14}C]carbosulfan (9.3 mg, 0.025 mmol, 1.26 μCi) was dissolved in 250 μL of dried methanol and then added to 250 μL of dried methanol containing 0.05 mmol of hydrogen chloride. At predetermined time intervals, duplicate 5-μL samples were taken and examined by TLC.

The structures of the different solvolysis products were determined with products obtained by using a larger quantity of nonradioactive carbosulfan, as exemplified below for reaction in methanol. Carbosulfan (500 mg) was dissolved in 20 mL of methanol–acetic acid (9:1) and allowed to stand for 24 h at 23 °C. The methanol was removed under reduced pressure, and the residue was diluted with 20 mL of dichloromethane. The resulting solution was washed with water and dried over anhydrous sodium sulfate, and the products were subjected to preparative TLC using a total of six silica gel plates (1.0-mm thickness) and hexane–ether (7:3) as the solvent. The band containing I [(methoxysulfenyl)carbofuran] (R_f 0.34–0.41) was scraped, and I was extracted with dichloromethane. Removal of the solvent gave 161 mg of I as a light yellow gummy substance. In a similar manner, (trideuteriomethoxysulfenyl)carbofuran was prepared from 50 mg of carbosulfan and 1.8 mL of CD_3OD in the presence of 0.2 mL of acetic acid.

Analysis. Precoated silica gel GHLF plates (0.25 mm, Analtech, Inc.) were used for analytical TLC and silica gel 60 PF-254 plates (1.0 mm, EM Laboratories) were used for preparative TLC. $KC_{18}F$ reversed-phased TLC plates (0.2-mm thickness, Whatman, Inc.) were used for either analytical or preparative TLC. Location of spots on the plates was by iodine vapor or by ultraviolet detection. Location of radioactive spots on TLC plates and quantitation of radioactivity were by methods previously described (Umetsu et al., 1981a).

NMR spectra were recorded on a Varian EM 390 spectrometer using Me_4Si as the lock signal. Chemical ionization mass spectrometry (CIMS) was conducted with a Finnigan Model 3300 mass spectrometer, and electron impact mass spectrometry (EIMS) was conducted with a Finnigan Model 1015 mass spectrometer.

Toxicological Evaluation. The toxicity of the individual compounds to house flies was determined with the susceptible NAIDM strain according to March and Metcalf (1949). Mammalian toxicity of the products was determined by oral administration to Swiss white mice (Simonsen Laboratories, Gilroy, CA) using corn oil as the carrier. Compounds that were not soluble were suspended in the carrier.

RESULTS

Carbosulfan Alteration in Alcohol-Containing Acetic Acid. Preliminary experiments were carried out to determine the fate of carbosulfan in methanol or ethanol in the presence of acetic acid. As indicated earlier, carbosulfan was converted into a variety of polysulfide products in acetonitrile–acetic acid (Figure 1A). In either methanol- or ethanol-containing acetic acid (9:1), carbosulfan was also converted into a number of different products, but in this case the compound (I) isolated in greatest yield was one that was not formed in acetonitrile (see Figure 1B). TLC analysis showed that after 35 h, only 19.7% of the radiolabeled material was returned as carbosulfan, 33.1% as I, 27.1% as carbofuran, and 20.1% as a mixture of other products, i.e., CFS_nNBu_2, CFS_2CF, and CFS_nCF ($n \geq 3$). In the ethanol–acetic acid mixture, the

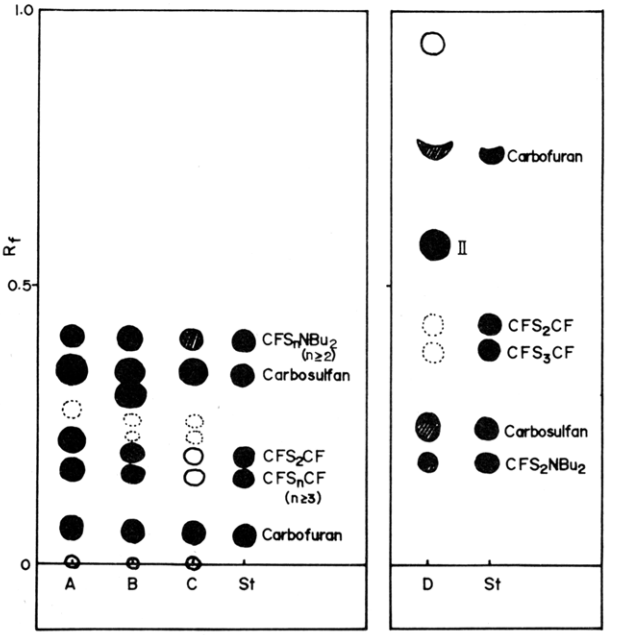
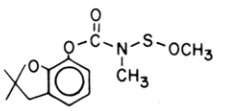


Figure 1. Silica gel (left) and KC_{18} reversed-phase (right) thin-layer chromatograms of [*carbonyl*-^{14}C]carbosulfan incubated in acetonitrile–acetic acid (9:1) (A), methanol–acetic acid (9:1) (B), and ethanol–acetic acid (9:1) (C and D) at 23 °C for 35 h; solvent = hexane–ether (7:3) (for silica gel TLC) and acetonitrile–water (9:1) (for KC_{18} reversed-phase TLC). St = standard.

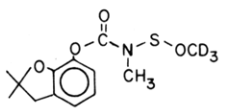
major alteration product was II, a compound that was not separable from carbosulfan by silica gel TLC (Figure 1C) but separable by KC_{18} reversed-phase TLC (Figure 1D).

Isolation and Identification of the Alcoholysis Products. I and II were expected to be the solvolysis products obtained from the reaction between the alcohol and carbosulfan, and their identification was achieved as follows.

The NMR spectrum of I (90 MH_z, $CDCl_3$) showed the



I (CF-S-OCH_3)



CF-S-OCD_3

following signals: δ 6.70–7.10 (m, 3 H, aromatic protons), 3.95 (s, 3 H, OCH_3), 3.55 (s, 3 H, N-CH_3), 3.02 (s, 2 H, CH_2), and 1.50 (s, 6 H, *gem*-di-CH_3). This spectrum revealed the absence of the *N*-dibutyl moiety and the presence of the *O*-methyl and the carbofuran moieties, indicating the structure of I to be the methoxysulfenyl derivative of carbofuran (CF–S–OCH_3). The mass spectrum of I confirmed this structure. Ions m/z 324 [$(M + 41)^+$], 312 [$(M + 29)^+$], and 284 [$(M + 1)^1$] satisfied the requirement for the molecular ions in methane CIMS. The molecular weight of I was determined as 283. EIMS (70 eV) showed the following major peaks: m/z (rel intensity) 284 (2.2), 283 (M^+, 9.8) 252 (1.5), 226 (5.7), 208 (1.5), 195 (2.6), 167 (5.9), 164 (13.2), 163 (100), 145 (7.5), 135 (39.2), 120 (14.2), 117 (10.3), 107 (26.3), 91 (21.6), 77 (13.2), 63 (33.1). The structure of I reasonably explained the EIMS fragmentation of I. The base peak ion (m/z 163) and the next intensive ion (m/z 135) are typical fragment ions of 2,3-dihydro-2,2-dimethylbenzofuran-7-ol (carbofuran phenol). Ion m/z 226 [$(M - 57)^+$] is a typical degradation fragment of an aromatic *N*-methylcarbamate, suggesting I is a derivative of carbofuran that is substituted at the nitrogen atom. Ion m/z 252 [$(M - OCH_3)^+$] and 226 [(sum of carbofuran phenol, 163, and $SOCH_3$, 63)$^+$] indicated that this substituent group is –S–OCH_3 but not –S(O)–CH_3.

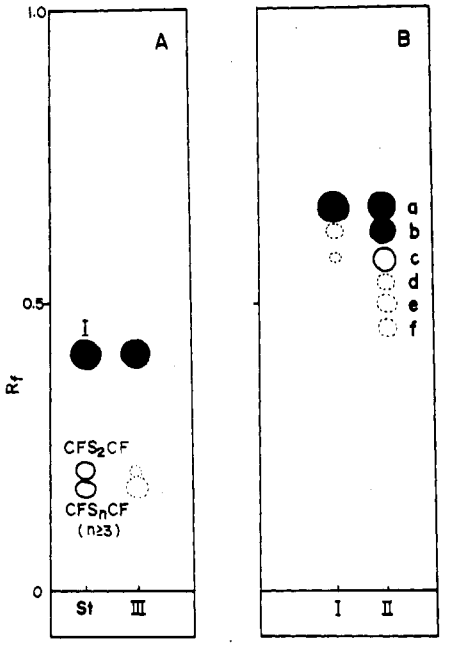


Figure 2. Thin-layer chromatograms of I and III. (A) Silica gel TLC using hexane–ether (7:3) as a solvent; (B) silica gel KC_{18} reversed-phase TLC using acetonitrile as a solvent. St = standard.

In order to confirm the tentative assignments for the O-CH_3 (3.95 ppm) and N-CH_3 (3.55 ppm) NMR signals of I, the trideuteriomethoxysulfenyl derivative of carbofuran (CF–S–OCD_3, 30 mg) was prepared from carbosulfan and CD_3OD. The NMR spectrum of CF–S–OCD_3 clearly indicated the presence of the N-CH_3 signal at 3.55 ppm. The EIMS spectrum of CF–S–OCD_3 was identical with that of I except for the 3 mass unit increase in the molecular ion and three major fragment ions [m/z 286 (M^+), 229, 123, 66].

Compound II (light yellow gum, 234 mg) was prepared from carbosulfan (740 mg) and ethanol (27 mL) in the presence of acetic acid (3 mL) following the procedure used to prepare I. The NMR spectrum of II showed the following signals: δ 6.70–7.10 (m, 3 H, aromatic protons), 4.20 (q, 2 H, OCH_2CH_3), 3.58 (s, 3 H, N-CH_3), 3.02 (s, 2 H, CH_2), 1.50 (s, 6 H, *gem*-di-CH_3), and 1.33 (t, 3 H, OCH_2CH_3). These data reveal that II is the ethoxysulfenyl derivative of carbofuran (CF–S–OC_2H_5). The mass spectrum of II reaffirmed the structure assignment.

Alcoholysis of Carbosulfan in Methanolic Hydrogen Chloride. Hydrogen chloride was used to investigate the effect of a stronger acid on the alcoholysis of carbosulfan. Carbosulfan (370 mg, 1.0 mmol) was dissolved in 12 mL of anhydrous methanol containing 2.0 mmol of hydrogen chloride. The reaction was completed virtually instantaneously and no carbosulfan was detected after 2 min, as determined by TLC analysis. The reaction mixture was worked up according to the procedure used to obtain I, yielding 61 mg of a light yellow gum (III), which showed an R_f value identical with that of CF–S–OCH_3 (I) on silica gel TLC plates. III appeared to be a single-component spot on silica gel plates with several different solvent systems except for the presence of trace amounts of CFS_2CF and CFS_nCF ($n \geq 3$), which probably were formed from III during the purification procedure (see Figure 2A). However, as shown in Figure 2B, III was separated into at least six components (a~f) by KC_{18} reversed-phase TLC using acetonitrile as the developing solvent. The R_f value of III-a, the most abundant component in III, was identical with that of I (CF–S–OCH_3).

The NMR spectrum of III (Figure 3) was similar to that of I except for the appearance of a pair of singlets each for

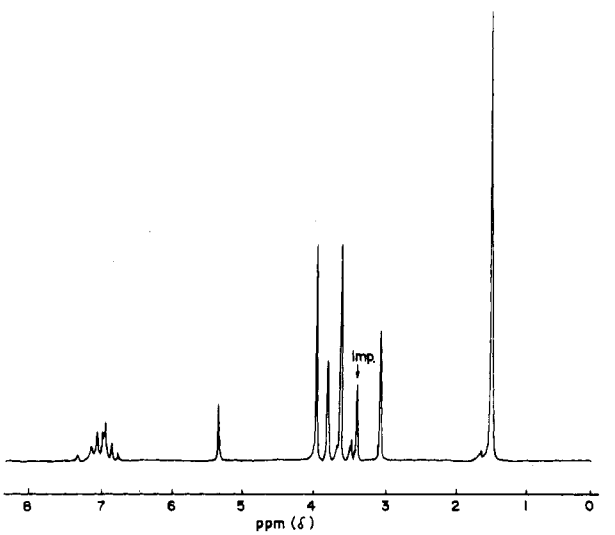
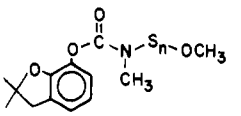


Figure 3. NMR spectrum of III. The impurity peak (Imp.) is due to the presence of trace amounts of CFS_nCF ($n \geq 2$).

O-CH_3 (3.95 and 3.80 ppm) and N-CH_3 (3.55 and 3.40 ppm). The signals for O-CH_3 at 3.95 ppm and N-CH_3 at 3.55 ppm corresponded to those of I. These results suggested that III was a mixture of I (CF–S–OCH_3) and the polysulfide analogues of I, i.e., CF–S_n–OCH_3 (n = 2~6).



polysulfide analogs of I

(CF-S_n-OCH_3, n=2-6)

The ratio of I to CF–S_n–OCH_3 was 65 to 35 based on peak heights of the singlets. CIMS (methane) of III measured at 40, 100, and 120 °C confirmed the presence of CF–S–OCH_3 and the individual components in CF–S_n–OCH_3 (n = 2–6). At 40 °C, only one set of ions satisfying the molecular ion requirement for CF–S–OCH_3 (I) appeared at m/z 324 [$(M + 41)^+$], 312 [$(M + 29)^+$], and 284 [$(M + 1)^+$]. At 100 °C four sets of ions assignable to CF–S–OCH_3, CF–S_2–OCH_3 (356, 344, and 316), CF–S_3–OCH_3 (388, 376, and 348), and CF–S_4–OCH_3 [380 for $(M + 1)^+$] appeared. At 120 °C five sets of ions assignable to CF–S_2–OCH_3, CF–S_3–OCH_3, CF–S_4–OCH_3, CF–S_5–OCH_3 [412 for $(M + 1)^+$], and CF–S_6–OCH_3 [444 for $(M + 1)^+$] were observed along with ions corresponding to CFS_2CF [505 for $(M + 1)^+$], CFS_3CF (537 for $(M + 1)^+$], and CFS_4CF [565 for $(M + 1)^+$]. The last three components were present as impurities in III or were formed from III by thermolysis.

Time Course for the Alcoholysis of Carbosulfan. [*carbonyl*-^{14}C]Carbosulfan was allowed to stand in 9:1 methanol–acetic acid for 120 h, and the breakdown of carbosulfan and formation of different products were monitored by TLC. A typical autoradiograph of a TLC plate showing products formed from [*carbonyl*-^{14}C]-carbosulfan is shown in Figure 1. Plots showing the relative amounts of each product formed at different time intervals are presented in Figure 4. The gradual disappearance of carbosulfan and concomitant formation of CF–S–OCH_3 and carbofuran are clearly indicated. The major product after 24 h was CF–S–OCH_3 (34.8%) and carbofuran (25.1%). However, significant amounts of CFS_nNBu_2 ($n \geq 2$, 4.4%), CFS_2CF (4.7%), and CFS_nCF ($n \geq 3$, 4.2%) were formed at the same time. The latter products are identical with those formed in acetonitrile–acetic acid. The amount of CF–S–OCH_3 reached a maximum at 24 h and then decreased. This observation sug-

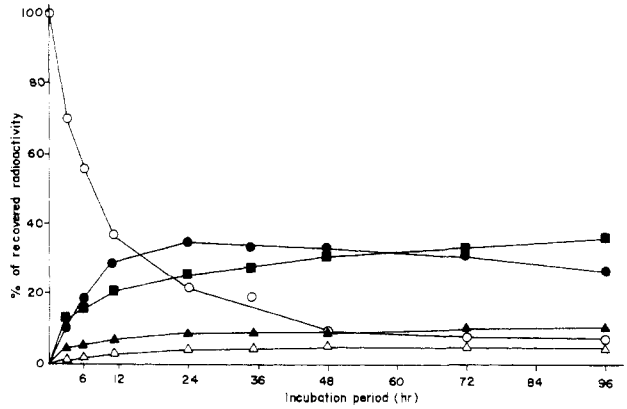


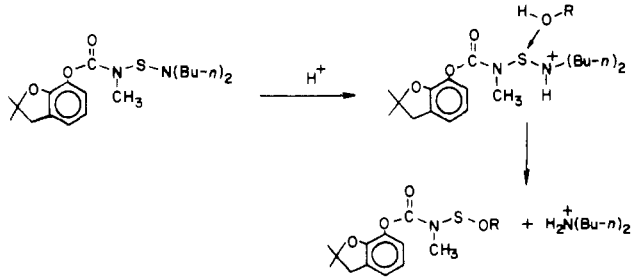
Figure 4. Graph showing the disappearance of [*carbonyl*-^{14}C]-carbosulfan and formation of CF–S–OCH_3 and other products in methanol–acetic acid (9:1). (○) Carbosulfan; (●) CF–S–OCH_3; (■) carbofuran; (▲) CFS_nCF ($n \geq 2$); (△) CFS_nNBu_2 ($n \geq 2$).

gests that CF–S–OCH_3 formed initially from carbosulfan was being converted into carbofuran and other products.

Toxicological Properties of the Alcoholysis Products. Data for the toxicity of the methoxy- and ethoxysulfenyl derivatives of carbofuran to the house fly and white mouse are given in Table I. Both compounds showed good insecticidal activity against the house fly, comparable in activity to that of carbofuran, and both compounds were less toxic to the white mouse than the parent methylcarbamate, carbofuran.

DISCUSSION

Previous studies showed that in dichloromethane– or acetonitrile–acetic acid solvent, carbosulfan was transformed into a number of products, including carbofuran, di-*n*-butylamine, and a mixture of biscarbofuran *N,N'*-polysulfides and the polysulfide analogues of carbosulfan (Umetsu et al., 1981a,b; Umetsu and Fukuto, 1982). In methanol– or ethanol–acetic acid, carbosulfan also was transformed into the same alteration products, but in this case solvolysis products were observed in greater abundance. These were the methoxysulfenyl (I, CF–S–OCH_3) and ethoxysulfenyl derivatives of carbofuran (II, CF–S–OC_2H_5), which were doubtlessly formed by the acid-catalyzed solvolysis of carbosulfan by the alcohols. The reaction probably takes place by attack of the alcohol oxygen on the sulfur atom of the protonated carbosulfan, resulting in displacement of the dibutylamine moiety:



Needless to say, other alcohols may participate in this reaction, and in fact, a number of other derivatives have also been prepared and their toxicological properties have been determined.

Substitution of the hydrogen atom on the carbamyl nitrogen of insecticidal methylcarbamate esters by different functional groups generally results in compounds of lower mammalian toxicity compared to the parent methylcarbamate and equal or improved insecticidal activity (Fukuto and Fahmy, 1981). The alkoxysulfenyl derivatives were no exception, and the methoxy- and ethoxysulfenyl derivatives of carbofuran showed insecticidal activity comparable to that of the parent methylcarbamate, carbofuran, along with slightly improved mammalian toxicity. These derivatives, along with those prepared from a wide variety of different alcohols, represent another series of sulfur-containing derivatives of insecticidal methylcarbamates that show promise as new insecticides with favorable toxicological properties (Kawata et al., 1983).

Table I. Toxicity of Methoxy- and Ethoxysulfenyl Derivatives of Carbofuran against House Fly and Mice

compound	toxicity: house fly LD_{50}, μg/g	toxicity: mouse LD_{50}, mg/kg
CF–S–OCH_3 (I)	15.5	39[a] (27–58)[c]
CF–S–OC_2H_5 (III)	15.8	43[a] (29–55)[c]
carbofuran	11.0	19[a] (16.2–24.3)[c]
		11.0[b] (9.8–12.4)[c]

[a] Corn oil was used as a carrier. [b] Propylene glycol was used as a carrier. [c] 95% confidence limit.

The formation of the polysulfide analogues of the methoxysulfenyl derivative of carbofuran (CF–S_n–OCH_3, n = 2–6) from carbosulfan in methanolic hydrogen chloride is of interest. The polysulfide analogues of carbosulfan (CFS_nNBu_2, $n \geq 2$) were previously observed to be formed from carbosulfan in a dichloromethane– or an acetonitrile–acetic acid (9:1) mixture (Umetsu et al., 1981a,b). As in the case of CFS_nNBu_2 it was not possible to determine the exact structure of the polysulfide chain in CF–S_n–OCH_3, although the composition of each component was established by NMR and mass spectral analysis. The mass spectra of these components provided information relating to the number of sulfur atoms but did not indicate the nature of the sulfur linkages, i.e., straight chain, branched, or part of a ring system.

Registry No. I, 86627-62-5; II, 86627-63-6; carbosulfan, 55285-14-8.

LITERATURE CITED

Fukuto, T. R.; Fahmy, M. A. H. In "Sulfur in Pesticide Action and Metabolism"; Rosen, J. D.; Magee, P. S.; Casida, J. E., Eds.; American Chemical Society: Washington, DC, 1981; ACS Symp. Ser. No. 158, pp 35–49.

Kawata, M.; Umetsu, N.; Fukuto, T. R. U.S. Patent 4 394 383, July 19, 1983.

March, R. B.; Metcalf, R. L. *Calif., Dep. Agric., Bull.* **1949**, *38*, 1.

Umetsu, N.; Fahmy, M. A. H.; Fukuto, T. R. *Pestic. Biochem. Physiol.* **1979**, *10*, 104.

Umetsu, N.; Fukuto, T. R. *J. Pestic. Sci.* **1982**, *7*, 117.

Umetsu, N.; Kuwano, E.; Fukuto, T. R. *J. Environ. Sci. Health, Part B* **1980**, *B15*, 1.

Umetsu, N.; Nishioka, T.; Fukuto, T. R. *J. Agric. Food Chem.* **1981a**, *29*, 711.

Umetsu, N.; Nishioka, T.; Fukuto, T. R. *J. Agric. Food Chem.* **1981b**, *29*, 1280.

Received for review October 17, 1983. Accepted March 16, 1984.