without increasing the hazard to man, domestic animals, and the beneficial fauna. Such knowledge may also be used to modify certain toxicants so that they will be activated by the very detoxicative mechanisms responsible for resistance to other insecticides, thus utilizing the principle of "negative correlation."

Literature Cited

(1) Barker, R. J., *J. Econ. Entomol.* **50,** 499–500 (1957).
(2) Barnes, J. H., Chapman, M. V. A., McCrea, P. A., Marshall, P. G., Walsh, P. A., *J. Pharm. Pharmacol.* **13,** 39–48 (1961).
(3) Busvine, J. R., *Entomol. Exptl. Appl.* **2,** 61 (1959).
(4) Drummond, R. O., Moore, B., Wrich, M. J., *J. Econ. Entomol.* **53,** 953–5 (1960).
(5) Fukuto, T. R., Metcalf, R. L., Winton, M. Y., Roberts, P. A., *Ibid.*, **55,** 341–5 (1962).
(6) Georghiou, P. G., Metcalf, R. L., *Ibid.*, **55,** 125–7 (1962).
(7) Goodman, L. S., Gilman, A., "The Pharmacological Basis of Therapeutics," 2nd ed., p. 438, Macmillan, New York, 1958.
(8) Hood, J. J., Hargett, L. T., J. AGR. FOOD CHEM. in press.
(9) Hoyt, S. C., *J. Econ. Entomol.* **55,** 639–41 (1962).
(10) Kolbezen, M., Metcalf, R. L., Fukuto, T. R., J. AGR. FOOD CHEM. **2,** 864–70 (1954).
(11) Kraemer, P., *J. Econ. Entomol.* **52,** 1195–9 (1959).
(12) Kriegler, P. J., *Deciduous Fruit Grower* **10,** 302–3 (1960).
(13) Lichtenstein, E. P., Casida, J. E., J. AGR. FOOD CHEM. **11,** 410–15 (1963).
(14) Metcalf, R. L., Fuertes-Polo, C., Fukuto, T. R., *J. Econ. Entomol.* **56,** 862–4 (1963).
(15) Metcalf, R. L., Fukuto, T. R., Winton, M. Y., *Ibid.*, **53,** 828–32 (1960).
(16) *Ibid.*, **55,** 889–94 (1962).
(17) Moorefield, H. H., *Contrib. Boyce Thompson Inst.* **19,** 501–7 (1958).
(18) Moorefield, H. H., Tefft, E. R., *Ibid.*, **20,** 293–8 (1959).
(19) Moorefield, H. H., Weiden, M. H. J., *Ibid.*, **22,** 425–33 (1964).
(20) Nachmansohn, D., "Chemical and Molecular Basis of Nerve Activity," chap. X, Academic Press, New York, 1959.
(21) Smith, F., Henneberry, T. J., Boswell, A. L., *J. Econ. Entomol.* **56,** 274–8 (1963).
(22) Tombes, A. S., Forgash, A. J., *J. Insect. Physiol.* **7,** 216–23 (1961).
(23) Weiden, M. H. J., Moorefield, H. H., *World Rev. Pest Control* **3,** 102–7 (1964).

Received for review October 5, 1964. Accepted January 29, 1965. Division of Agricultural and Food Chemistry, Symposium on Carbamate Insecticides, 148th Meeting, ACS, Chicago, Ill., September 1964.

CARBAMATE INSECTICIDES

Photodecomposition of Carbamate Insecticides

DONALD G. CROSBY, ERIKS LEITIS, and WRAY L. WINTERLIN

Agricultural Toxicology and Residue Research Laboratory, University of California, Davis, Calif.

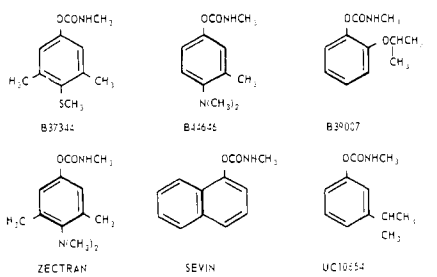
The effect of sunlight and of laboratory ultraviolet light on six *N*-methylcarbamate insecticides has been determined by an improved method which combines thin-layer chromatography and enzyme inhibition. With the exception of Bayer 39007, each of the compounds decomposed to give unidentified cholinesterase inhibitors as well as other substances.

During the past five years *N*-methylcarbamate esters have become an important class of insecticides. Although, like the organophosphorus insecticides, the carbamates are inhibitors of the enzyme acetylcholinesterase, they generally exhibit much lower mammalian toxicities than most of the phosphates. In 1955, Cook (*1*) developed a method for the detection of acetylcholinesterase inhibitors on paper chromatograms. With it, he was able to demonstrate that several phosphate insecticides were decomposed in the presence of ultraviolet light to unidentified products which themselves inhibited the enzyme to a significant degree.

Although several other investigators (*2, 4*) have improved on Cook's procedure, paper chromatographic methods are not readily applied to the isolation of most organic substances because of limited adsorptive capacity and the introduction of impurities during elution. We have developed a variation of Cook's method which employs thin-layer chromatography (TLC) and have demonstrated that the carbamates, too, decompose under the influence of ultraviolet light to series of inhibitors.

Experimental

Materials and Methods. CARBAMATES. 3,5-Dimethyl-4-(methylthio)-phenyl *N*-methylcarbamate (Bayer 37344), 3-methyl-4-(*N,N*-dimethylamino)phenyl *N*-methylcarbamate (Bayer 44646), and 2-isopropoxyphenyl *N*-methylcarbamate (Bayer 39007) were analytical reference standards provided by the Chemagro Corp., Kansas City, Mo. 1-Naphthyl *N*-methylcarbamate (Sevin), 3-isopropylphenyl *N*-methylcarbamate (UC 10854), and 3,5-dimethyl-4-(dimethylamino)phenyl *N*-methylcarbamate (Zectran) were supplied as technical products by the Union Carbide Corp. Chemicals Division and the Dow Chemical Co., their respective manufacturers; they were purified by repeated recrystallization from ethanol and from benzene until sharp melting points were attained.



IRRADIATION. Solutions of the carbamates in absolute ethanol or redistilled hexane were subjected to irradiation in the laboratory for 1 to 3 hours. Two types of ultraviolet source were used, both of which produced peak radiation at about 254 mμ: (1) a Multiray short wavelength laboratory lamp (G. W. Gates and Co., Franklin Square, N. Y.)

consuming less than 15 watts of power; and (2) a 360-watt Uviarc high-pressure mercury arc lamp (420-watt power consumption) (G. W. Gates and Co.).

The solutions, generally containing about 1 mg. per ml. of the insecticide, were irradiated in open 9-cm. Petri dishes at a distance of about 2 cm. from the weaker lamp. Although the dishes could be covered with Saran or Mylar film without apparent effect on the results, it was thought that full exposure to air and atmospheric moisture was desirable in the experiments described here. Very little rise in temperature or evaporation of solvent was observed.

When the stronger source was used, the dishes were held at a distance of about 10 cm. and, because of the intense heat, they were floated in an ice bath during the irradiation. Sunlight irradiation was conducted outdoors in open Petri dishes for periods of 1 to 3 hours about noon during the months of June, July, and August. Although the solutions remained relatively cool, rapid evaporation necessitated frequent additions of fresh solvent.

CHROMATOGRAPHY AND DETECTION. Aluminum oxide G (Brinkmann Instruments, Inc., Great Neck, N. Y.) (25 grams) was mixed with 100 ml. of 50% aqueous methanol to form an even slurry, filtered through a 50-mesh screen to remove any coarse particles, and applied immediately to the surface of 200- × 200-mm. glass plates with a commercial spreader. A layer thickness of 500 microns generally was found to be satisfactory. The plates were allowed to stand for about 15 minutes in a level position and then were dried in an oven at 110° C. for about 40 minutes, allowed to cool, and stored in a desiccating cabinet. Plates coated with Brinkmann silica gel H were prepared in the same way.

Aliquots (1 to 10 μl.) of the irradiated or reference solutions were applied as spots with a microliter syringe about 2 cm. from the lower edge of the plate and at least 3 cm. apart. The chromatograms were developed in the ascending direction in the usual way. Acetone-toluene-pentane (1:1:3) and acetone-hexane (1:5) were found to be good all-purpose mobile phases.

For the detection of acetylcholinesterase inhibition, the developed plates were placed flat on a horizontal surface in a borosilicate glass baking dish, and the solvent was permitted to evaporate. The plates were sprayed with a mixture of 1 part (by volume) of human plasma and 3 parts of 0.1% cresol red indicator in 0.01*N* aqueous sodium hydroxide solution. Saturation of the adsorbent caused its surface to appear glossy, and care was exercised to avoid spraying past this point.

The baking dish was covered with a glass plate or baking dish cover and held at room temperature for 20 to 30 minutes. During this period, spots may be formed by either strongly acidic or strongly alkaline substances, and the location of such spots should be carefully noted. After this incubation period, an 8- × 8-inch sheet of Whatman No. 1 filter paper, freshly dipped in an 0.2*M* aqueous solution of acetylcholine bromide, was laid on top of the developed chromatoplate and immediately became soaked with indicator solution. Within 15 minutes, the acetic acid liberated during the enzyme-catalyzed hydrolysis of the ester caused the filter paper to take on a uniform yellow color, except where cholinesterase inhibitors on the plate gave rise to red spots of unchanged indicator on the yellow background. The spots were marked or photographed; they disappeared within a few days.

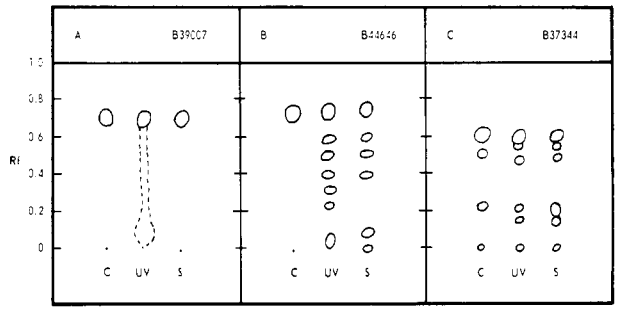


Figure 1. Photodecomposition of Bayer 39007, Bayer 37344, and Bayer 44646

C. Control
S. Sunlight irradiation
UV. Ultraviolet irradiation

Results and Discussion

The combination of TLC with an assay for acetylcholinesterase provides a very sensitive and specific means for the detection of inhibitors of this enzyme. Although the experiments described here employed human plasma cholinesterase, cholinesterase preparations from other sources may be used effectively to examine differential inhibition. The similar application of other enzymes is under investigation.

Thin-layer chromatography affords the means for isolation of the detected inhibitors as well as for facile sample purification in many cases. Sensitivities generally are good—enzyme and substrate levels may be kept high and sufficient material may be chromatographed to permit the detection of even small amounts of inhibitors. However, because of the variation in inhibition constants, insecticides may vary greatly in their effect on the enzyme. In the present case, for instance, 0.01 μg. of Sevin could be detected while only about 0.5 μg. of Zectran was clearly evident.

Water-soluble inhibitors present a problem because their diffusion after application of the enzyme solution causes excessively large spots. Likewise, the high degree of sensitivity restricts the amounts of inhibitor which will provide optimum results. In general, 1 to 20 μg. of inhibitor appears to represent the most satisfactory range. Care also must be exercised to avoid prolonged contact of certain compounds, at least, with the TLC plate. A homogeneous spot of Sevin could readily be shown to contain its hydrolysis product 1-naphthol after remaining on a plate for a few hours.

A number of adsorbents, chromatographic solvents, and indicators were examined, and those described here provided very satisfactory results. Acidic adsorbents, such as silicic acid, obviously were found to be unsuited for sensitive determinations.

The applicability of the method to the examination of photochemical decomposition products of common carbamate insecticides is indicated in Figure 1. The analytical standards of Bayer 39007 and Bayer 44646 appeared to be homogeneous in acetone-toluene-pentane, while the Bayer 37344 sample was a mixture (perhaps due to the formation of the sulfoxide and sulfone during storage). Bayer 39007 was not converted to other inhibitors by exposure to sunlight for 3 hours, and 37344 formed few inhibitory decomposition products. Bayer 44646, however, was extensively decomposed.

The atmosphere is very effective in preventing light of wavelengths less than about 290 mμ from reaching the earth's surface. To observe the effect of these shorter wavelengths as well as the effect of more intense irradiation, solutions of the carbamates also were exposed to the 15-watt short-wavelength lamp for 3 hours. The stable Bayer 39007 was only slightly affected, and Bayer 37344 produced additional inhibitory spots identical in R_f values to those observed after exposure to sunlight. Bayer 44646 produced at least two more inhibitory spots.

The purity of standard samples may influence strongly the interpretation of photodecomposition data. For example, an analytical sample of UC10854 which had been recrystallized several times from different solvents still contained so many cholinesterase-inhibiting impurities that unambiguous determination of the effects of irradiation was impossible (Figure 2*A*). The standard sample was purified by thin-layer chromatography, the major inhibitor was eluted, and a definite ultraviolet breakdown pattern

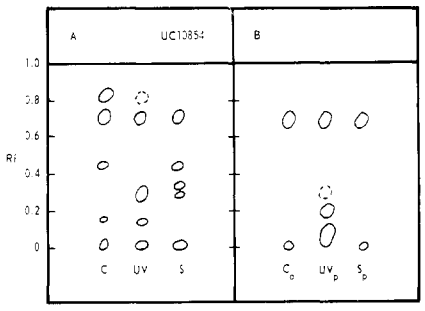


Figure 2. Photodecomposition of UC 10854

C. Control
S. Sunlight irradiation
UV. Ultraviolet irradiation
P. Use of purified UC 10854

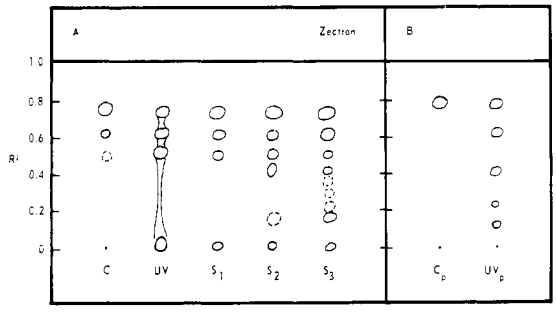


Figure 3. Photodecomposition of Zectran

C. Control
S. Sunlight irradiation for 1, 2, and 3 hours
UV. Ultraviolet irradiation
P. Use of purified Zectran

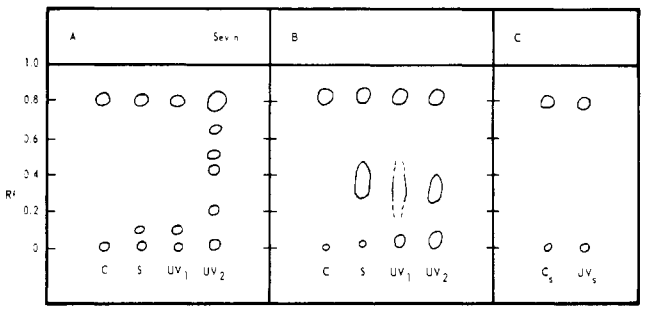


Figure 4. Photodecomposition of Sevin

C. Control
S. Sunlight irradiation
UV. Ultraviolet irradiation with (1) weak and (2) strong light
S. Use of solid formulation

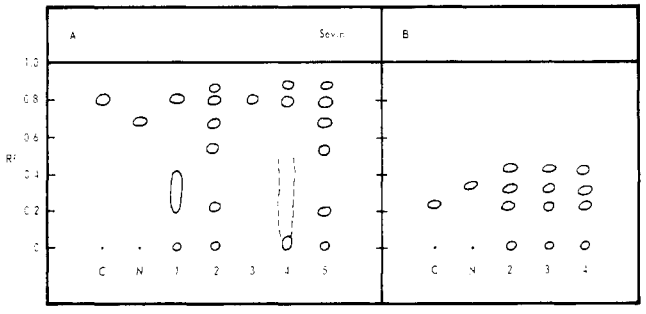


Figure 5. Color tests on ultraviolet-irradiated Sevin

C. Control
N. 1-Naphthol
Test 1. Cholinesterase inhibition
Test 2. 4-Nitrobenzenediazonium fluoborate
Test 3. Trinitrofluorenone
Test 4. UV fluorescence
Test 5. Potassium permanganate

was established with the pure 3-isopropylphenyl *N*-methylcarbamate (Figure 2*B*). The ability to study independently the behavior of the several components of such a mixture demonstrates an important advantage of our detection procedure.

The insecticide Zectran is very similar in structure to Bayer 44646. As shown in Figure 3*A*, an analytical sample of Zectran contained two major and one minor cholinesterase inhibitors. An ethanol solution was exposed to sunlight and sampled at three hourly intervals (S_1, S_2, and S_3). The third sample contained so many cholinesterase inhibitors that an exact count could not be made, but nine distinct spots were present. Likewise, exposure to ultraviolet light resulted in a smear of activity on the chromatogram.

To clarify the breakdown pattern, Zectran was purified by thin-layer chromatography to produce only a single spot in acetone-toluene-pentane (Figure 3*B*). Irradiation of an ethanol solution of the pure compound for 3 hours resulted in five distinct inhibitory spots.

From a photochemical viewpoint, there is no question of the importance of pure starting materials if the mechanism of breakdown is to be elucidated. However, from the practical aspects of agriculture and public health, the total spectrum of biologically active compounds assumes considerable significance.

The present experiments were restricted to a convenient maximum of 3 hours and so may not present an accurate picture of the appearance and disappearance of decomposition products under field conditions. Under conditions comparable to those employed with the other carbamates, Sevin produced only one minor cholinesterase-inhibiting decomposition product (Figure 4*A*) in sunlight or weak ultraviolet light. Exposure to intense ultraviolet light, however, gave rise to five inhibitory substances and suggests that extended periods of irradiation in the field may produce more rather than fewer decomposition products.

Again for convenience, most of these irradiations were conducted in homogeneous ethanol solution. Identical results were obtained with solutions in methanol or acetone. In practice, however, the use of hydrocarbon solvents is much more common. We found that formulation may have a significant effect on photodecomposition. For example, control samples of Sevin generally were homogeneous, although they occasionally gave rise to an inhibitory spot at the origin of thin-layer chromatograms (Figure 4 *A*). Both sunlight and weak ultraviolet light caused the formation of an additional inhibitor, while strong ultraviolet light caused extensive formation of inhibitors. Irradiation in hexane solution-suspension (Figure 4*B*) produced very different results. Irradiation of either solid Sevin or a 50% wettable powder did not result in additional inhibitors even with prolonged exposure to intense ultraviolet light (Figure 4*C*).

Our detection of cholinesterase-inhibiting photodecomposition products of carbamate insecticides should not be construed to mean that these are the only substances formed during irradiation. Most of the inhibitors undoubtedly have undergone substitution or displacement on the aromatic ring but retain the carbamate ester moiety intact. Probably the majority of breakdown products which are not carbamate esters would not be detected by cholinesterase inhibition.

Several additional tests were applied to the irradiated carbamates after thin-layer chromatography. Typical results are shown in Figure 5*A* for an ethanol solution of Sevin, irradiated with weak ultraviolet light and chromatographed on alumina with acetone-toluene-pentane. Unlike the control Sevin, the irradiated sample contained two major cholines-

terase inhibitors, and weak inhibition could be detected at the origin (Test 1). Treatment with an alkaline solution of 4-nitrobenzenediazonium fluoborate (Test 2), however, indicated six "phenolic" compounds to be present. One of these had the same chromatographic and color characteristics as 1-naphthol, and others may well be polyhydroxylated naphthalenes. Strongly oxidizing conditions (potassium permanganate solution, Test 5) revealed the same six substances.

Trinitrofluorenone (TNF) (Test 3) has been found effective in locating certain types of substituted aromatic compounds on paper chromatograms (*3*). In this case, only a single spot, corresponding to Sevin in color and R_f, could be detected. Several fluorescent spots and much streaking could be detected under ultraviolet light (Test 4).

Chromatography on silica plates with acetone-hexane solvent (Figure 5*B*) provided somewhat different results upon application of the color reagents. Although the cholinesterase inhibition test could not be successfully applied, both Sevin and 1-naphthol were shown to be present. Another substance also was present in significant amounts, and its isolation from the plate as the TNF complex will be described in another communication.

Acknowledgment

We are indebted to Herman F. Beckman for helpful discussions and to Connie Fleischer for technical assistance. The technical sample of Sevin was provided by W. J. Entley, Chemical Division, Union Carbide Corp.

Literature Cited

(1) Cook, J. W., *J. Assoc. Offic. Agr. Chemists* **38,** 150 (1961).
(2) Getz, M. E., Friedman, S. J., *Ibid.*, **46,** 707 (1963).
(3) Gordon, H. T., Haraux, M. J., *Anal. Chem.* **31,** 302 (1959).
(4) McKinley, W. P., Read, S. I., *J. Assoc. Offic. Agr. Chemists* **45,** 467 (1962).

Received for review September 18, 1964. Accepted February 10, 1965. Division of Agricultural and Food Chemistry, Symposium on Carbamate Insecticides, 148th Meeting, ACS, Chicago, Ill., August 1964.

CARBAMATE INSECTICIDES

Synthesis of Dithiolane Oxime Carbamate Insecticides

ROGER W. ADDOR

Chemical Research and Development Laboratories, Agricultural Division, American Cyanamid Co., Princeton, N. J.

The methylcarbamate of 2-oximino-1,3-dithiolane (I) is a novel, broad-spectrum insecticide. Its preparation involved a study of the reaction of ethanedithiol with cyanogen chloride to give 2-imino-1,3-dithiolane hydrochloride. Conversion of the imino hydrochloride to the oxime was accomplished in good yield using one equivalent of hydroxylamine. The oxime could also be prepared from a 2-alkylimino-1,3-dithiolane and one equivalent of hydroxylamine hydrochloride. Methyl isocyanate converted the oxime to the desired methylcarbamate. Analogous compounds in which a ring sulfur atom is replaced by an oxygen atom or in which the ring is expanded by another methylene group were also prepared.

THE insecticidally active carbamates reported until very recently were derived from either phenols (1-naphthyl methylcarbamate) or enols (1-isopropyl-3-methyl-5-pyrazolyl dimethylcarbamate). They all contain ring systems—the phenol derivatives by definition, the enolic ones possibly because the ring systems used lend stability to the *O*-carbamylated form.

Some *N*-alkylcarbamates of dicyclopropyl ketoneoxime were reported in 1959 to be toxic to animals (*4*). Aryl carbamates of some simple ketoximes—e.g., acetone—have been described as herbicides (*9*), and alkyl and aryl carbamates of a series of hydroxamic acid chlorides have been reported to be fungicides (*3*). These compounds were not claimed to be insecticidal.

Recently, workers at Union Carbide reported a series of methylcarbamates of oximes derived from cycloaliphatic and bicyclic ketones (*7*). The limited data given show these to be active against a number of insect species.

Dithiolane Oxime Carbamates

Work with heterocyclic oximes in these laboratories has led to a new group of carbamate insecticides. An example is 2-methylcarbamoyloxyimino-1,3-dithiolane (I) (Figure 1). Carbamate (I) and analogs in which a ring-sulfur atom is replaced by an oxygen atom—e.g., compound V (Figure 1)—have been shown in the laboratory to be broad-spectrum insecticides (*1*, *14*). Toxicity to mice and rats has also proved to be high, however. Examples of other active compounds (*1*)—i.e., II, III, and IV—are also shown in Figure 1.

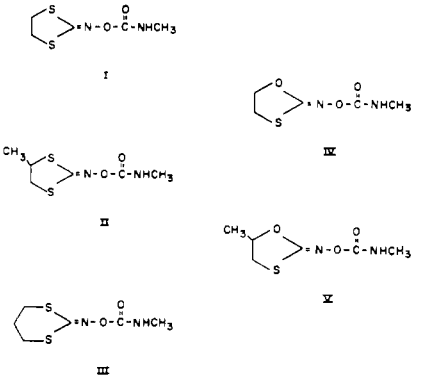


Figure 1. Oxime carbamates

Discussion

The method of preparation of carbamate (I) and its analogs is outlined in Equations 1 to 3 (Figure 2). Details of the reaction shown to obtain 2-imino-1,3-dithiolane hydrochloride (VI) have been described (*2*). Although oxime (VII) was known prior to this work (*12*), the oxathiolane and dithiane analogs had not been reported. Conversion of VI to VII goes in high yield if one equivalent of hydroxylamine hydrochloride and one equivalent of a base such as sodium acetate are used. Under these conditions, the free imine ($pK_B = 8.2$) (*2*) of VI should remain at least partially