exposed in hemispherical screen cages to the bait soaked onto cotton wads in waxed paper containers 3.5 cm. in diameter. The aphids were reared and held at 68 ± 5° F. and 50 ± 10% RH; the others at 80 ± 5° F. and 50 ± 10% RH. Mortality counts were made in 24 hours, 5 to 7 days, 72 hours, 72 hours, and 24 hours for the aphids, mites, armyworm, bean beetle, and fly, respectively. Armyworm and bean beetle larvae were considered dead if unable to move the length of their body when prodded. Results for aphids and mites were corrected for check mortality by means of Abbott's formula. Dosage mortality curves were plotted on log-probit paper and the LD_{50} read from an eye-fitted line.

Anticholinesterase Determination

Fly-head cholinesterase molar I_{50}'s were measured by the manometric method as described by Moorefield and Tefft (12). Final ACh concentration was 0.02M.

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Literature Cited

(1) Adams, D. H., Biochim. Biophys. Acta 3, 1 (1949).
(2) Blatt. A. H., "Organic Syntheses," Collective Vol. II, p. 313, Wiley, New York, N. Y., 1943.
(3) Butterbaugh, D. J., U. S. Patent 2,496,198 (Jan. 31, 1950).
(4) Dornow, A., Jordan, H. D., Chem. Ber. 94, 76 (1961).
(5) Hauser, C. R., Jordan, E., J. Am. Chem. Soc. 58, 1772 (1936).
(6) Heath, D. F., "Organophosphorus Poisons," pp. 227, 245, Pergamon Press, New York, N. Y., 1961.
(7) Hopkins, A. R., Taft, H. M., J. Econ. Entomol. 58, 746 (1965).
(8) Kolbezen, M. J., Metcalf, R. L., Fukuto, T. R., J. AGR. FOOD CHEM. 2, 864 (1954).
(9) Lemieux, R. U., Nagabhushan, T. L., O'Neill, I. K., Tetrahedron Letters 29, 1909 (1964).
(10) Lustig, E., J. Phys. Chem. 65, 491 (1961).
(11) Metcalf, R. L., Fukuto, T. R., J. AGR. FOOD CHEM. 13, 220 (1964).
(12) Moorefield, H. H., Tefft, E. R., Contrib. Boyce Thompson Inst. 19, 295 (1958).
(13) Niemczyk, H. D., J. Econ. Entomol. 58, 423 (1965).
(14) Ogloblin, K. A., J. Gen. Chem. (U.S.S.R.) 22, 2175 (1952).
(15) Payne. L. K., U. S. Patent 3,217,-036 (Nov. 9, 1965).
(16) Payne, L. K., Weiden, M. H. J., Ibid., 3,217,037 (Nov. 9, 1965).
(17) Phillips, W. D., Ann. N. Y. Acad. Sci. 70, 816 (1958).
(18) Pritzkow, W., Schaefer, H., Pabst, P., Ebenroth, A., Beger, I., J. Prakt. Chem. 29, 123 (1965).
(19) Ridgway, R. L., Gorzycki, L. J., USDA, ARS 33-106, 6 pp., June 1965.
(20) Weiden, M. H. J., Moorefield, H. H., Payne, Jr., L. K., J. Econ. Entomol. 58, 1954 (1965).
(21) Werner, E. A., J. Chem. Soc. 117, 1946 (1920).
(22) Wilson, I. B., in "The Enzymes," 2nd ed., Vol. 4, Chap. 30, Academic Press, New York, N. Y., 1960.
(23) Wilson, I. B., Harrison, M. A., Ginsburg, S., J. Biol. Chem. 236, 1498 (1961).
(24) Wilson, I. B., Hatch, M., Ginsburg, S., Ibid., 235, 2312 (1960).
(25) Wright, H. B., Moore, M. B., J. Am. Chem. Soc. 70, 3865 (1948).
(26) Yakubovich, A. Ya., Lemke, A. L., J. Gen. Chem. (U.S.S.R.) 19, 607 (1949).

STRUCTURE AND TOXICITY

The Fungitoxicity of Compounds Containing a Trichloromethylthio-Group

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The R-group can affect fungitoxicity of compounds of the type R—SCCl$_3$ in two areas, that of permeation of fungal cells, and that of chemical reactivity of the R—SCCl$_3$ molecule with cellular constituents. Potential hydrophilicity and ionization of the R-group may decrease permeation while potential lipophilicity of the R-group may increase permeation. High levels of fungitoxicity of compounds of the type R—SCCl$_3$ necessitate a reactive sulfur linkage. The R-group may influence reactivity at this site and direct the mode of chemical reactions of compounds of the type R—SCCl$_3$ with cellular constituents.

FUNGITOXICITY has been reported for a wide variety of compounds containing the trichloromethylthio-group (—SCCl$_3$) (2, 1, 5, 8, 9, 18–21). Captan [(N-trichloromethylthio)-4-cyclo-hexene-1,2-dicarboximide, Figure 1, C-5] is a highly successful agricultural fungicide. Because many compounds containing the —SCCl$_3$ group are fungitoxic, the —SCCl$_3$ group has been considered to be the toxic reaction center or toxophore. However, not all compounds of the type R—SCCl$_3$ are fungitoxic. A few are weak toxicants and each varies in its specificity toward fungi. In addition to

altering fungitoxicity, the R-group controls other chemical and physical properties. An attempt will be made to relate these effects to biological activity.

Some compounds of the type R—SCCl$_3$ have been reported nontoxic simply because testing procedures did not allow for volatility or instability. According to Block (7), fungitoxicity of trichloromethylthiomethane sulfonate (Figure 1, A-4) was missed by Johnston et al. (8) because the compound vaporized from their testing system. Uhlenbroek and Koopmans (19) may have reported trichloromethyl-methyl disulfide (Figure

1, A-1) as nontoxic for the same reason.

O-n-butyl trichloromethane sulfenate (Figure 1, A-3) may be unstable. Sosnovsky (18) reported it as nontoxic. Many simple amine derivatives of trichloromethylmercaptan decompose on standing (3, 7). The isobutyl analog is toxic to certain fungi and boils at a lower temperature than does O-n-butyl trichloromethane sulfenate. Hence, the lack of activity of the n-butyl analog is not due to volatility.

Some compounds containing two —SCCl$_3$ groups have been reported as nontoxic. Analogs of 1,4-di(trichloro-
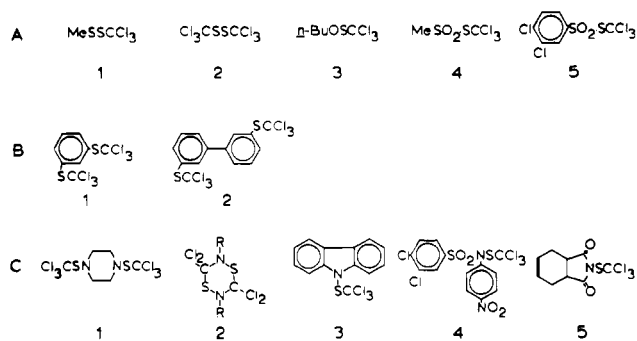
Figure 1. Structures of miscellaneous compounds of the type R—SCCl₃

methylthio)piperazine (Figure 1, C-1) (18), 1,3-di(trichloromethylthio)benzene (Figure 1, B-1), and 3,3′di(trichloromethylthio) diphenyl (Figure 1, B-2) (8) are nontoxic. However, Block (7) found activity in bis(trichloromethyl)disulfide (Figure 1, A-2).

Sosnovsky (18) found a series of diathiadiazines nontoxic. Diathiadiazines (Figure 1, C-2) may be considered derivatives of trichloromethylmercaptan in which two primary amine-SCCl₃ molecules are fused to form a six-membered heterocyclic ring. With the diathiadiazines, lack of activity may result from reduced reactivity of the sulfur atom when a chlorine atom of the trichloromethyl group is replaced by nitrogen on cyclization. N-(trichloromethylthio)-carbazole (Figure 1, C-3) is nontoxic, and it reacts slowly with biological constituents (12).

Apparently, to retain high levels of fungitoxicity, compounds of the type R—SCCl₃ must contain a sulfur linkage that is stable, but reactive with nucleophilic groups (12). Unstable sulfur linkages may cause the compounds to decompose before they reach their sites of action. However, sulfur linkages that are too stable may prevent the compounds from reacting with the biological groups that are associated with fungitoxicity.

The sulfur linkage is destroyed during hydrolysis. The primary products are unknown. The rate of hydrolysis of trichloromethylthio-3, 4-dichlorobenzene sulfonate (Figure 1, A-5 and hereafter referred to as sulfonate-SCCl₃) is eight times faster than that of captan, and captan is considerably the more fungitoxic (12). On the other hand, folpet [N - (trichloromethylthio) phthalimide], Figure 2A, which hydrolizes slightly faster than captan, is slightly more fungitoxic. Ease of hydrolysis of the sulfur linkage may be a measure of reactivity and stability of compounds containing the —SCCl₃ group. Maximum fungitoxicity is associated with an optimum stability of this bond.

Under physiological conditions, the sulfur atom of the —SCCl₃ compounds reacts with thiols (Reaction 1).

$$R—S—CCl_3 + 2R'S—H \rightarrow R—H +$$
$$Cl—C:S—Cl + R'S—SR' + HCl \quad (1)$$
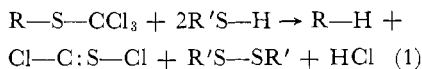
This reaction has been invoked as a mechanism of the fungitoxicity of captan (13, 14) and as a mechanism for biological detoxication of the fungicide (11, 16). In the process, captan is reduced to tetrahydrophthalimide and thiophosgene, as the thiol is oxidized to the corresponding disulfide. Lukens et al. (12) have shown that highly fungitoxic compounds of the type R—SCCl₃ react quickly with thiols, while nontoxic ones react slowly with thiols—e.g., N - (trichloromethylthio) carbazole (Figure 1, C-3). The Reaction 2 with thiols may involve the chlorine atoms of N-(trichloromethylthio)carbazole rather than sulfur.
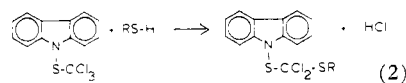
Of the fungitoxic compounds of the type R—SCCl₃, N'-(trichloromethylthio)-N - (4 - nitrophenyl) - 4 - dichlorobenzenesulfonamide (Figure 1, C-4 and hereafter referred to as sulfonamide-SCCl₃) is unique in its specificity for a few fungi (12). The —SCCl₃ compound is less fungitoxic than the free sulfonamide against Monilinia fructicola and Stemphylium sarcinaeforme. Thiols reduce the —SCCl₃ group to thiophosgene, but thiophosgene has not been detected when the compound reacts with yeast cells. When released from the —SCCl₃ group by cellular thiols, the sulfonamide may compete with cellular constituents for thiophosgene. A suspension of thiophosgene in water evolves CS₂ as a hydrolytic product. A mixture of thiophosgene with the sulfonamide evolves less CS₂ than thiophosgene in water does.

The sulfonamide-SCCl₃ illustrates what can happen when two potential toxophores are combined in a single molecule. In this case, the potential toxophores, once released, may react with each other rather than with biological constituents. Thus the molecule containing both toxophores is less fungi-

toxic than molecules containing either one alone.

Undoubtedly, the R-group determines the mode of fungitoxicity of compounds of the type R—SCCl₃. Lukens et al. (12) found differences in response of yeast cells to toxic doses of four compounds of the type R—SCCl₃. Fungitoxicity of the three highly reactive compounds (Figure 1, A-5, C-4, and C-5) is destroyed when they are mixed with thiols prior to adding yeast. Fungitoxicity to yeast is reversed by adding thiols to cells poisoned with the sulfonamide-SCCl₃, but fungitoxicity to yeast is not reversed by adding thiols to cells poisoned with captan or the sulfonate-SCCl₃. Toxicity was partially reduced by thiols in all sequences of mixing carbazole-SCCl₃ (Figure 1, C-3, the less reactive compound), thiol, and yeast cells. The three highly reactive compounds were fungicidal, while the carbazole-SCCl₃ was a mild fungistat. Either captan and the sulfonate-SCCl₃ are converted by reacting with thiol to more fungitoxic molecules that react with cellular constituents or the intact fungicide reacts directly with cellular constituents other than thiols. Apparently, the toxicity of the sulfonamide-SCCl₃ involves oxidation of cellular thiols to disulfides. Cells can be revived by adding excess thiols. The mode of action of the carbazole-SCCl₃ has not been determined. The compound may form addition products with cellular thiols as illustrated in Equation 2. However, the compound appears to be loosely bound to cellular constituents because treated cells resume normal growth when washed free of ambient toxicant.

It is difficult to demonstrate the distinction between the fungitoxicity of the two imide-SCCl₃ compounds, captan and folpet (Figure 2, B and A). However, action on powdery mildew fungi— i.e., Sphaerotheca rosae—is the exception to this rule. These fungi are susceptible to the action of folpet, while captan has no effect on them.

The importance of the aromatic ring of phthailmide, the imide of folpet, to fungitoxicity can be demonstrated with the free imides—i.e., those lacking the —SCCl₃ group. Phthalimide and other aromatic (cyclic) imides are more toxic to spore germination of Stemphylium sarcinaeforme than tetrahydrophthalimide and other aliphatic (cyclic) imides (10). The aromatic ring raises the standard free energy of phthalimide. The higher melting point and lower water solubility of phthalimide as compared with those of tetrahydrophthalimide are consistent with this view. With other things being equal, the higher standard free energy enables phthalimide to react with cellular constituents with greater ease than does tetrahydrophthalimide. The aromatic ring also draws electrons away from the imide-N and reduces the
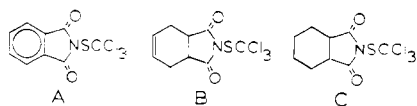
Figure 2. Structures of N—imide—SCCl₃ compounds

electronegativity of the latter structure. A reduction in the charge on >N⁻ causes the free imide to be a stronger acid and causes the imide-SCCl₃ compound to be more reactive in nucleophilic reactions.

Among the imide-SCCl₃ compounds, N-(trichloromethylthio)cyclohexane-1,2-dicarboximide (Figure 2C) is exceptional. This aliphatic (cyclic) imide-SCCl₃ compound controls powdery mildew pathogens (*Erysiphe graminis*) and hydrolizes at a faster rate than captan [17]. However, the free imide possesses fungitoxicity and acidity on a level with that of tetrahydrophthalimide, the imide of captan. Thus, the increase in reactivity of N-(trichloromethylthio)-cyclohexane - 1,2 - dicarboximide over that of captan must be a result of the condensation rather than be ascribed to the sum of the standard free energies of the two moieties and resonance in the imide moiety. Since NMR spectra have shown no rearrangement of hydrogen atoms of the cyclohexane ring during the condensation reaction, the increase in reactivity is not due to a change of hydrogen atoms on positions 1 and 2 from cis to trans relationship. The source of the increase in activity has yet to be determined.

Organic toxicants permeate fungal cells rapidly. To do so, the compounds must possess an oil-water partition coefficient suitable for penetrating the lipoid layers of cell membranes. Undoubtedly, the trichloromethyl group of trichloromethylthio compounds contributes to an oil-water partition coefficient suitable for these compounds to permeate fungal cells [6, 15].

The lipophilic properties of compounds of the type R—SCCl₃ may be altered by change in the R-group. The higher level of standard free energy of phthalimide over that of tetrahydrophthalimide may contribute to the 10-fold increase in oil-water partition coefficient of folpet over that of captan [15]. The increase in lipophilicity with change in imide group may be responsible, in part, for the greater fungitoxicity of folpet over that of captan to mildew pathogens. That carboxyl substitution to the benzene ring of trichloromethylthiobenzene sulfonate reduces fungitoxicity [20] suggests that lipophilicity of the carboxylated derivatives is reduced. The reduction in lipophilicity can occur from the hydrophilicity and ionization of the carboxyl substituent.

Literature Cited

(1) Block, S. S., *Conn. Agr. Exptl. Sta. Bull.* **663**, 114 (1963).
(2) Cannon, W. N. (to Eli Lilly & Co.), U. S. Patent **2,888,462** (May 26, 1959).
(3) Connolly, J. M., Dyson, G. M., *J. Chem. Soc.* **1937**, p. 827.
(4) Desmoras, J., LaCrois, L., Metivier,
J., Fifth International Pesticide Congress, London, p. 38, 1963.
(5) Fawcett, C. H., Spencer, D. M., Wain, R. L., *Ann. Appl. Biol.* **46**, 651 (1958).
(6) Horsfall, J. G., "Principles of Fungicidal Action," pp. 73–74, Ronald Press Co., New York, 1956.
(7) Johnson, T. B., Hemingway, E. H., *J. Am. Chem. Soc.* **38**, 1860 (1916).
(8) Johnston, R. P., Rueggeberg, H. C., Block, S. S., J. AGR. FOOD CHEM. **5**, 672 (1957).
(9) Kittleson, A. R., *Ibid.*, **1**, 677 (1953).
(10) Lukens, R. J., Horsfall, J. G., *Phytopathology* **55**, 1066 (1965).
(11) Lukens, R. J., Rich, S., *Ibid.*, **49** 228 (1959).
(12) Lukens, R. J., Rich, S., Horsfall, J. G., *Ibid.*, **55**, 658 (1965).
(13) Lukens, R. J., Sisler, H. D., *Ibid.*, **48**, 235 (1958).
(14) Owens, R. G., Blaak, G., *Contrib. Boyce Thompson Inst.* **20**, 459 (1960).
(15) Richmond, D. V., Somers, E., *Ann. Appl. Biol.* **50**, 33 (1962).
(16) *Ibid.*, **52**, 327 (1963).
(17) Richmond, D. V., Somers, E., Zaracovitis, C., *Nature* **204**, 1329 (1964).
(18) Sosnovsky, C., *J. Chem. Soc.* **1956**, pp. 3139–3141.
(19) Uhlenbroek, J. H., Koopmans, M. J., *Rec. Trav. Chim.* **76**, 657 (1957).
(20) Uhlenbroek, J. H., Koopmans, M. J., Huisman, H. O., *Ibid.*, p. 129.
(21) Waeffler, R., Gasser, R., Margot, A., Gysin, H., *Experientia* **11**, 265 (1955).

THALLIUM ASSAY

X-Ray Emission Spectrographic Determination of Thallium in Biologic Materials

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An x-ray emission method for the determination of trace concentrations of thallium in biologic materials is described. The ratio of emission intensity at the Tl Lβ line to an adjacent background line was constant in dried biologic materials whose scattering efficiency varied. In the presence of added Tl, the net peak-to-background ratio was linearly related to Tl concentration over a 5- to 1000-p.p.m. range. Nondestructive Tl determinations can be completed in about 10 minutes on dried biologic materials with a precision of ±18 p.p.m. for single determinations.

THE usefulness of x-ray emission to determine thallium in geologic specimens has been demonstrated [3]. This method has been adapted to study thallium toxicosis as a part of an ecological evaluation of this metal in the control of nuisance birds and provides for the rapid, nondestructive determina-

tion of Tl with minimal sample preparation.

Experimental

Apparatus and Reagents. The instrumental settings on the Norelco Universal x-ray spectrograph utilized in these studies are summarized in Table I.

The measurement of the emission intensity of the L-characteristic lines of thallium is within the range of energies available using a LiF analyzing crystal and a tungsten target tube powered by a 50 KVP x-ray generator. The qualitative nature of the Tl spectrum in a light biologic matrix is shown in Fig-