duction to approximately half the normal concentration in a single heterozygote. The total lack of reaction between the homozygous serums and antiserum to C'2 precludes the presence of an antigenically related, hemolytically inactive analog of C'2 protein in these serums. Since all deficient individuals tested do possess residual C'2 activity (*1–5*), it must be concluded that the deficiency of C'2 protein is not absolute, and that there are small amounts of protein in the abnormal serums, which, however, are too small to be detected by the methods used in this study.

Note added in proof: Since this paper was submitted a similar observation was made by Dr. M. Klemperer and presented as an oral communication to the Complement Workshop, Boston.

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Pyrethroid-Like Biological Activity of Compounds Lacking Cyclopropane and Ester Groupings

Abstract. *The following new insecticidal compounds respond to synergism by piperonyl butoxide and block nerve excitability in the same manner as the insecticide allethrin: 1-(4-allethronyl)-acetyl-2,2-dimethyl-3-isobutenylcyclopropane (the ketone analog of allethrin) and the esters of 5-benzyl-3 furylmethanol with 2,2,3,3-tetramethylcyclopropanecarboxylic acid, 2,2,3,3-tetramethylaziridinecarboxylic acid, and* N,N-*diisopropylcarbamic acid. Therefore pyrethroid-like activity is not restricted to esters of cyclopropanecarboxylic acids.*

Pyrethrum and a few of its many synthetic analogs play important roles in insect control because they are highly insecticidal (especially if used with a synergist), have low toxicity to mammals, and leave no hazardous or persistent residues on crops or food. Their use is limited by high cost, relative to that of most insecticides, resulting from either a limited botanical source (pyrethrum, for example) or, in the case of the synthetic analogs, costly preparation of compounds of such complex structures.

During the past 50 years, many workers have tried to expand the uses for pyrethroids and to elucidate the relations between their structures and activities; they have gained much information regarding the requirements for insecticidal activity by certain analogs of pyrethrin I, the most active natural pyrethroid, and of allethrin, the most important synthetic compound of this type, which contains an allyl group in place of the pentadienyl group of pyrethrin I (*1*). Insecticidal activity is improved when the respective alcohol component, pyrethrolone or allethrolone, is replaced by 5-benzyl-3-furylmethanol (*2*). Replacement of the acid component, chrysanthemumic acid, by 2,2,3,3-tetramethylcyclopropanecarboxylic acid changes insecticidal activity very little (*3*).

Although many compounds related to cyclopropanecarboxylic acid esters have been tested, no known highly insecticidal compounds of this type lack either the cyclopropane ring or the ester group. So far all studies indicate that the spatial configuration of the groupings in both the acid and alcohol moieties, and (in particular) a cyclopropane ring containing geminal dimethyl substituents, are important structural features.

Three compounds (Table 1) prepared by us and of special interest are related in structural topography to allethrin (A) but lack either the ester function or the cyclopropane ring. They include the ketone analog (B) of allethrin and the carbamates (D and E) related to compound C, all of which are insecticidal. The finding that the new compounds are insecticidal raises the question of whether or not their mode of action is the same as that of pyrethrin I or allethrin. Our present knowledge of the mode of action of pyrethroids allows this question to be answered only in terms of electrophysiological parameters. Allethrin acts on the giant axons of the cockroach (*Periplaneta americana* L.) to give slight depolarization, increased magnitude and prolongation of the negative afterpotential and eventual blockage of conduction (*4*). The increased negative afterpotential, which is sometimes accompanied by repetitive afterdischarges followed by blockage of conduction, is best explained in terms of sodium and potassium conductances across the nerve membrane (*5*).

Allethrin (A) is obtained by treating ±-*trans*-chrysanthemumoyl chloride with ±-allethrolone in the presence of pyridine (*1*). The ketone analog of allethrin (B) is prepared by a substitution reaction of ±-4-chloroallethrone (*6*) at the methyl group of the acetyl side chain of ±-*trans*-1-acetyl-2,2-dimethyl-3-isobutenylcyclopropane (*7*). This substitution is achieved by way of the ethoxycarbonyl derivative in the presence of sodium ethoxide (*8*). 5-Benzyl-3-furylmethanol (*2*) is converted to the various esters by reaction with the acid chloride of 2,2,3,3-tetramethylcyclopropanecarboxylic acid (*3*), in the presence of pyridine, to give compound C; or with phosgene to give the chloroformate, followed by either 2,2,3,3-tetramethylaziridine (*9*) to give compound D, or diisopropylamine to give compound E (*10*).

Toxicity studies involved treatment of susceptible adult female houseflies (*Musca domestica* L.) and of adult male and female milkweed bugs (*Oncopeltus fasciatus* Dallas) on the dorsum of the thorax and on the ventrum of the abdomen, respectively, with 1 μl of acetone containing each test compound in solution. In certain studies with houseflies, 5 μg of the synergist piperonyl butoxide was applied in 1 μl of acetone solution to the abdomen 0.5 to 1.0 hour before treatment with the insecticide. Mammalian toxicity studies involved intraperitoneal administration of the compounds in dimethylsulfoxide solution to male white mice. Symptoms of poisoning were recorded, but only-24 hour LD_{50} (lethal dose, 50

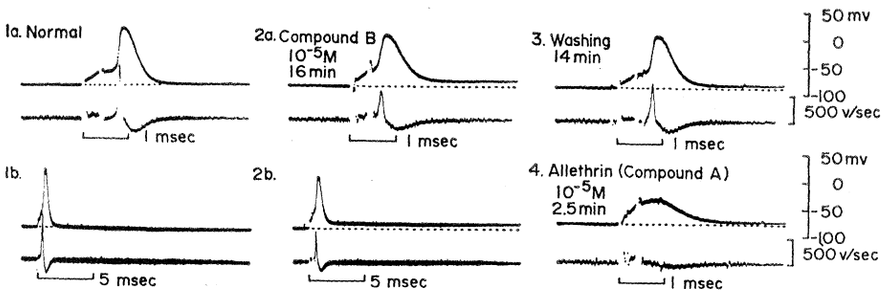


Fig. 1. Action potentials (top) and their derivatives (bottom) recorded from the giant axon of a crayfish when immersed in normal Van Harreveld solution (1a and 1b), after exposure to $10^{-5}M$ compound B for 16 minutes (2a and 2b), after washing with normal Van Harreveld solution for 14 minutes (3), and after exposure to $10^{-5}M$ allethrin (compound A) for 2.5 minutes (4). Measurements followed the sequence of the numbering. Dotted lines represent the base lines. Note that the negative afterpotential is increased by compound B, and that allethrin is more potent than compound B in blocking the action potential.

percent effective) values were determined.

Electrophysiological studies involved observations of membrane resting and action potentials from giant axons of the crayfish (*Procambarus clarkii* G.). The potentials were recorded from a giant axon in the circumesophageal connective, immersed in Van Harreveld solution (*11*), with an intracellular microelectrode filled with $3M$ KCl, while electrical pulses of currents were applied across the nerve membrane by means of another microelectrode inserted near the recording electrode. Since the rate of rise of the action potential is proportional to the inward ionic current at the moment when it is maximum under such experimental conditions (*12*), the maximum rate was taken as a measure of excitability. The test compounds as stock solutions in ethanol were injected into the Van Harreveld solution to give the desired final concentrations, ethanol alone having no effect on the nerve at the concentrations used.

Each of the test compounds (Table 1, A–E) is of low to moderate toxicity to mammals and is remarkably enhanced in toxicity to houseflies by pretreatment with piperonyl butoxide; the marked synergism by the latter suggests that each compound is metabolized in living flies by the microsome mixed-function oxidase system, and that this detoxification is a factor limiting its insecticidal activity. Considerable species-specificity in toxicity also is evident.

Further evidence of selective toxicity, not tabulated in Table 1, is the finding that allethrin (A) is seven times more toxic than its ketone analog (B) to adult German cockroaches (*Blattella germanica* L.), but is only twice as toxic to larvae of the salt-marsh caterpillar [*Estigmene acrea* (Drury)]. Rapid knockdown and paralysis of houseflies occur only with the cyclopropanecarboxylic acid esters (A and C); the very low dose necessary for both knockdown and mortality with compound C is particularly noteworthy.

Although differing in rate of action, each of the five compounds (Table 1) shows the characteristic symptoms, in both houseflies and milkweed bugs, of intense excitement followed by paralysis; this symptomatology differs markedly from that produced by any other important type of insecticide such as chlorinated hydrocarbons, organophosphates, and carbamates.

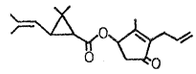
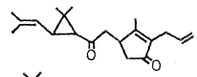
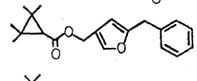
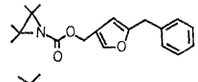
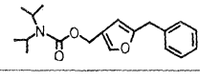
Allethrin and compounds B–E exert essentially the same effect on crayfish axons as allethrin does on cockroach axons (*4*); they depolarize the nerve membrane slightly (by 2 to 10 mv) and progressively (2 to 8 minutes), and increase and prolong the negative afterpotential; eventually they block the action potential. Sometimes, repetitive afterdischarges are superimposed on the increased negative afterpotential. These effects, which are distinct from those of most other nerve poisons, are slowly and poorly reversed after washing with the normal saline solution.

An example of the changes in action potential is illustrated (Fig. 1). Quantitative comparisons of the test compounds (A–E) show that the potency in blockage of the action potential follows the same order as does the insecticidal activity (Table 1): compound C is more potent than allethrin (compound A) by a factor of 1.6; compounds B and D are less potent than allethrin by factors of 6 and 9, respectively; and compound E is the weakest, being less potent than allethrin by a factor of 50.

It is noteworthy that although compounds D and E are carbamates their action on the axon membrane is completely different from that of the insecticidal carbamate compounds carbaryl and isolan; the latter two are cholinesterase inhibitors and exert no effect whatsoever on the action potential of the crayfish giant axon. Despite the rather drastic changes in chemical structure and potency involved, each of the new test compounds (B–E) acts on the nerve in the same manner as does allethrin.

The insecticidally active chemicals described, resulting from a series of modifications of the structure of the

Table 1. Chemical structures and biological activities of allethrin and four structurally related compounds. Potency: nanomolar level to decrease maximum rate of rise of the action potential of crayfish giant axons to 50 percent of normal.

Compound	Structure	Toxicity, 24-hour LD_{50} (mg/kg)				Effect on nerve, potency (nM)
		Mouse, intraperitoneal	Milkweed bug, topical	Housefly, topical		
				Without synergist	With synergist	
A		41	30	21	0.8	2.6
B		>500	200	171	5	17
C		25	9	0.9	0.1	1.6
D		500	300	228	13	24
E		>500	800	750	30	130

natural product pyrethrin I, contain few of the functional groups of the original molecule. However, although their structures are greatly different, their mode of action appears to be the same. It is clear that combination with the active site in the insect nerve, to give a functional lesion, is not absolutely dependent on either an ester group or a cyclopropane ring. The configuration of the molecule, relative to appropriate size and shape to interact with the receptor site in the nerve, seems to be the critical factor for pyrethroid-like activity.

Since the change in excitability produced by allethrin is explicable in terms of conductance changes of the nerve membrane (*5*), the receptor can be visualized as the site in the nerve membrane that controls the conductance changes. Compounds A and C, and the chrysanthemumate analog (*2*) of compound C, must fit this receptor exceptionally well. However, the nature of the receptor and the manner in which it is affected by pyrethroids remain to be explored. One way toward explanation of these relations is further study of structure and activity in terms of both insecticidal activity and disturbance of conductance.

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Echinoderms: An Autoradiographic Study of Assimilation of Dissolved Organic Molecules

Abstract. *In a holothurian and an ophiuroid, tritiated glucose and glycine in great dilution are removed from seawater by uptake through the skin. Cells differ in their competence to metabolize specific nutrients, an indication that there are specialized cellular responses to exogenous organic molecules. Embryonic ophiuroid tissues have an exceptional capacity for assimilation.*

There is substantial evidence that many marine invertebrates, with the exception of the arthropods, can absorb and metabolize free organic molecules of low molecular weight dissolved in ambient seawater (*1*). The echinoderms have attracted much attention in this regard (*2*). These studies have usually used techniques of isotope counting and consequently provide little information on specific sites of cellular uptake. However, an autoradiographic study of three asteroid species (*3*) has reported that nutrients labeled with C^{14} are concentrated in the epidermis, particularly in the tube feet. To determine specific sites of assimilation and to gain information about their use in the cell, we have autoradiographically compared the uptake of tritium-labeled dissolved organic materials (DOM) in two echinoderms, the holothurian *Cucumaria lactea* and the ophiuroid *Amphipholis squamata*, collected from the Northumberland coast.

Experimental animals were immersed for 3 hours in 50-ml quantities of $6 \times 10^{-6}M$ glycine-2-H^3 (specific activity, 11.8 mc/mg) or $10^{-5}M$ D-glucose-6-H^3 (specific activity, 2.8 mc/mg) dissolved in filtered seawater (33 to 34 parts per thousand) at a temperature approximating that of local surface seawater. After being rinsed in clean seawater, some animals were fixed immediately (0-day animals); others were returned to holding tanks and fixed after intervals of 1, 3, and 7 days. Animals treated with glycine were fixed in Bouin's; those treated with glucose were fixed in Bouin's or Rossman's fluids. Autoradiographs were prepared from 8-μ paraffin sections by dipping them in liquid Ilford K2 nuclear emulsion; they were developed after 14 days, an optimum exposure determined empirically. The sections were stained in Mayer's hemalum through the emulsion. Rough estimates of assimilation were obtained by counting silver grains with the aid of a squared-graticule ocular. With a $\times$ 100 oil-immersion objective, each ocular square (field) represented 5.76 μ^2. The expression of number of grains per field for a specific tissue is based on the mean grain count for 60 representative fields, less a correction for background grains.

Since free glucose is washed out of tissues during histological processing, glucose labeling is attributable to glucose-6-H^3 incorporated metabolically into large, fixable molecules such as glycogen. When sections were incubated in 0.5 percent malt diastase (*p*H 6.0) before dipping, all labeling due to glucose-6-H^3 was abolished, showing that most, if not all, glucose labeling is associated with sites of synthesis or deposition of desmoglycogen.

Free glycine is also removed from tissues during processing. Therefore, visible labeling marks sites where this amino acid has been synthesized into a macromolecule, most probably a polypeptide. To test this, we added an inhibitor of protein synthesis to the labeled medium. An analog of transfer-RNA, chloramphenicol (4 mmole/liter), which interferes with the transfer of amino acids into peptide linkages at ribosomal sites (*4*), was added to the seawater together with $6 \times 10^{-6}M$ glycine-2-H^3. After a 3-hour exposure, the experimental animals were fixed and autoradiographs were prepared. The tissues appeared normal and healthy after this treatment. However, compared to controls lacking chloramphenicol, labeling was substantially reduced. A comparison of grain counts of identical tissues from experimental and control animals showed that protein synthesis was inhibited by 86.8 percent for *Cucumaria* and by 82.7 percent for *Amphipholis*. The focal position of glycine in metabolic pathways leaves the possibility that some was transformed and fixed into large, nonpeptide molecules. However, we conclude that most of the labeling is due to glycine