spectrum of insecticidal activity, their use on plant insects is limited by phytotoxicity. They are active against a variety of household and grain pests and may be useful in such applications.

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CHEMOSTERILANTS

Structure-Activity Relationships in Apholate Analogs

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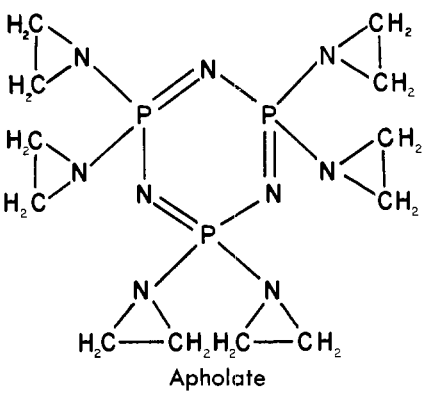
Twenty-five analogs of apholate [2,2,4,4,6,6-hexakis (1-aziridinyl)-2,2,4,4,6,6-hexahydro-1,3,5,2,4,6-triazatriphosphorine] were evaluated for chemosterilant activity. A minimum of two aziridinyl substitutions were required in the dimethylamino analogs for effective sterilization of houseflies. As aziridinyl substitutions were increased from two to five, more highly active chemosterilants were obtained. A minimum of four aziridinyl groups were required, however, in the chloro analogs for effective housefly sterilization. This difference between the two series may be related to water solubility. All of the aziridinyl substituted dimethylamino analogs were water-soluble, whereas water solubility in the chloro analogs did not occur until at least four aziridinyl groups were present. Monosubstituents in the apholate molecule other than chlorine or dimethylamine did not alter activity. Substitutions on the aziridinyl groups of apholate reduced chemosterilant activity. The tetrameric analog of apholate containing eight aziridinyl groups, instead of the six in apholate, did not improve activity.

APHOLATE, 2,2,4,4,6,6-hexakis (1-aziridinyl) - 2,2,4,4,6,6 - hexahydro-1,3,5,2,4,6-triazatriphosphorine is one



Apholate

of the more promising chemosterilants containing the aziridinyl group. Although first synthesized by Rätz and Grundmann in 1954 and patented in 1958 (*8*), it was the discovery of its chemosterilant activity by LaBrecque of the U. S. Department of Agriculture in 1960 (*3, 4*) that stimulated interest in compounds of this type and led to the synthesis of several analogs (*5, 6, 9*). The biological evaluation of these analogs is reported in this paper.

Methods and Materials

Chemosterilant activity has been assessed in the authors' laboratory by two different methods. In the first, newly emerged houseflies were fed a granular sugar diet containing 0.5% of the chemosterilant. Eggs subsequently laid in a milk-food cup were removed from the cellucotton with tweezers and floated in a Syracuse watch glass. Approximately 200 eggs were distributed with an eye dropper onto two green blotter tabs. These paired tabs were placed in covered Petri plates and were incubated at 78° F. for 24 hours (Figure 1). The eggs were then observed with a microscope, and the unhatched eggs were expressed as per cent nonviable or sterile (Figure 2).

A second rapid in vitro screening test employed was an existing Squibb Institute cytotoxicity test (*7*) which utilized mouse fibroblast cells grown in tissue culture. Although results from the cytotoxicity method demonstrate that active chemosterilants were not missed, these data also show that the cells are not sufficiently sensitive to separate the highly active chemosterilants from each other.

Results and Discussion

Because apholate is usually made by the substitution of aziridinyl groups for chlorine atoms in trimeric phosphonitrilic chloride, one of the first chemical series studied biologically was the chloro-substituted analogs. Significant housefly sterility or cytotoxicity was not attained in this series until four aziridinyl

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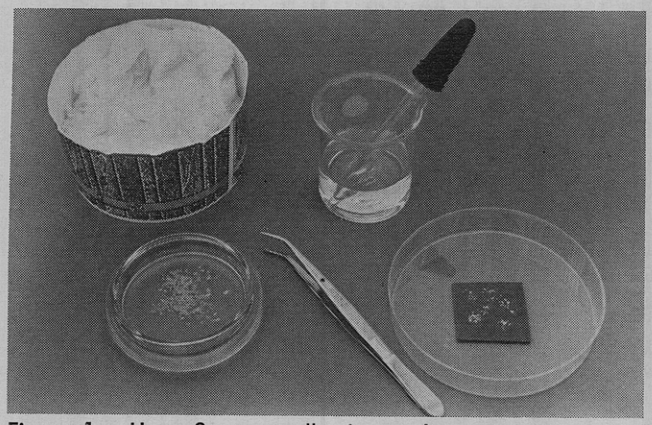

Figure 1. Housefly egg collection and counting apparatus

Figure 2. Hatched (left) and unhatched-sterile (right) housefly eggs

groups were substituted for the chlorine atoms (Table I). The monochloro pentakis aziridinyl analog was about twice as active as apholate against houseflies in the sterilant test and was twice as toxic against mice in an acute oral test.

A second series studied was the dimethylamino-substituted analogs of apholate. In this series, housefly sterility or cytotoxicity first occurred when at least two aziridinyl groups were substituted for dimethylamino atoms. As aziridinyl substitutions were increased from two to five, more highly active chemosterilants were obtained (Table II). The hexakis dimethylamino analog of apholate was inactive at concentrations as high as 5% on sugar baits. This lack of activity is in contrast with that reported recently (*2*) for the dimethylamino analog of aphoxide (HMPA) which, in the authors' laboratory, was active in 1% concentration on sugar bait.

Comparisons are also given in Table II for two bis-dimethylamino-tetra aziridinyl structural isomers of apholate. Both were highly active in the initial housefly and cytotoxicity screens. However, dose response comparisons against the housefly indicate that the 6,6-bis-dimethylamino isomer is a more active housefly chemosterilant than is the 4,6-bis-dimethylamino isomer.

In brief, when the chloro- and dimethylamino-substituted apholate analogs are compared, high sterilant activity occurs with fewer aziridinyl groups in the dimethylamino series. One explanation for this may be the difference in water solubility that exists between the two series (Table III). This experience with apholate analogs confirms Borkovec's suggestion (*1*, *2*) about the importance of water solubility for the aziridine contained chemosterilants. Indeed, the insolubility of the hexakis dimethylamino analog of apholate may explain its inactivity since HMPA, the active dimethylamino analog of aphoxide, is water soluble.

Since the monochloro pentakis

Table I. Structure-Activity Relationships of Chloro-Substituted Apholate Analogs

Substituents —C	Substituents —N<	Housefly Sterility[a] 0.5%	Housefly Sterility[a] SC_{90}, p.p.m.	Cytotoxicity[b] ED_{50}, P.P.M.	Acute Oral Toxicity Mouse LD_{50}, Mg./Kg.
6	0	5	...	...	...
5	1	13	...	40	...
4	2	23	...	20	...
3	3	16	...	>50	...
2	4	99	1500	5.0	...
1	5	99	200	4.0	70 ± 4
0	6	99	500	2.0–9.0	110–190
Apholate					

[a] Feeding on treated sugar.
[b] Tissue culture of mouse fibroblast cells.

Table II. Structure-Activity Relationships of Dimethylamino-Substituted Apholate Analogs

Substituents —N(CHt)t	Substituents —N<	Housefly Sterility[a] 0.5%	Housefly Sterility[a] SC_{90}, p.p.m.	Cytotoxicity[b] ED_{50}, P.P.M.	Acute Oral Toxicity Mouse LD_{50}, Mg./Kg.
6	0	7	...	>40	330 ± 22
5	1	27	...	>50	...
4	2	67	...	1–2.5	575 ± 52
3	3	99	2000	1	115 ± 6
2	4[c]	99	850	0.5–5.0	100 ± 7
2	4[d]	99	500	3.0–5.0	185 ± 9
1	5	99	450	2.5–4.0	130 ± 7
0	6	99	500	2.0–9.0	110–190
Apholate					

[a] Feeding on treated sugar.
[b] Tissue culture of mouse fibroblast cells.
[c] 4,6-Bis-dimethylamino-tetra aziridinyl isomer (*5*).
[d] 6,6-Bis-dimethylamino-tetra aziridinyl isomer (*5*).

Table III. Relationships between Dimethylamino- and Chloro-Substituted Apholate Analogs

—Cl or —$N(CH_3)_2$	—N<	Housefly Sterility[a] —$N(CH_3)_2$	Housefly Sterility[a] —Cl	Cytotoxicity[b] —$N(CH_3)_2$	Cytotoxicity[b] —Cl	Water Solubility[c] —$N(CH_3)_2$	Water Solubility[c] —Cl
6	0	7	5	40	...	I	I
5	1	27	13	50	40	Sol.	I
4	2	67	23	1–2.5	20	Sol.	I
3	3	99	16	1.0	50	Sol.	I
2	4	99	99	0.5–5.0	5.0	Sol.	Sl. Sol.
1	5	99	99	2.5–4.0	4.0	Sol.	Sol.
0	6	99		2.0–9.0		Sol.	
Apholate							

[a] Feeding on 0.5% sugar baits.
[b] Tissue culture ED_{50}, p.p.m.
[c] I = Compounds less than 1% soluble in water.

Table IV. Structure-Activity Relationships of Mono-Substituted Apholate Analogs

Substitution	Housefly Sterility[a] 0.1%	Housefly Sterility[a] SC_{90}, p.p.m.	Cytotoxicity[b] ED_{50}, P.P.M.	Acute Oral Toxicity Mouse LD_{50}, Mg./Kg.
—Cl	99	200	4.0	70 ± 4
—NH_2	99	...	0.6	...
—$NHNH_2$	5 71[c]	...	2.5	...
—$NHCH_3$	89	...	2.0	...
—$N(CH_3)_2$	99	500	2.5–4.0	130 ± 7
—OCH_3	89	350	1.0	125 ± 10
—SCH_3	74	...	2.0	...
—N(apholate)(apholate)	99	500	2.0–9.0	110–190

Apholate

[a] Feeding on treated sugar.
[b] Tissue culture of mouse fibroblast cells.
[c] Liquid bait without sugar.

Table V. Structure-Activity Relationships of Miscellaneous Apholate Analogs

Substitution (all six positions)	Housefly Sterility[a] 0.1%	0.5%	1.0%	Cytotoxicity[b] ED_{50}, P.P.M.
—N(apholate)(apholate) apholate	99	99	...	2.0–9.0
—N(apholate)(CH_3) methyl apholate	...	42	58	12.0–15.0
—N(apholate)(CH_2O-C6H5)	...	...	0[c]	>40
—N(apholate) + $CuSO_4$ or $ZnCl_2$	...	5[c]	...	>30
—NHCON(apholate)	...	...	2[c]	>30
—NHCSN(apholate)	...	...	0[c]	>30
Tetrameric analog of apholate	69	100	...	0.5–5.0

[a] Feeding on treated sugar.
[b] Tissue culture of mouse fibroblast cells.
[c] Less than 1% soluble in water.

aziridinyl analog was more active than apholate as a housefly chemosterilant, a third series of mono substituted analogs of apholate was also biologically evaluated (Table IV). All of these analogs were water soluble and, with the exception of the hydrazino analog, were highly active housefly chemosterilants. High sterilant activity for the monohydrazino analog was attained only when higher concentrations on sugar or aqueous liquid baits were used thus confirming indications of activity from the cytotoxicity screen. It would appear, therefore, that the biological activity of this analog was affected by sugar.

Seven other miscellaneous apholate analogs were also evaluated (Table V). The reduction in activity for the 2-methyl aziridinyl or the 2-phenoxymethyl aziridinyl analog of apholate is consistent with reduced activities previously reported by Borkovec (*1*) for aziridine ring substituted chemosterilants. Formamido, thioformamido aziridinyl analogs, and metal complexes of apholate were water insoluble, inactive chemosterilants. The tetrameric analog of apholate containing eight aziridinyl groups was less active than apholate as a housefly chemosterilant.

In summary, these results with twenty-five apholate analogs show the following structure-activity relationships: insect sterility was related to the number of aziridines on the phosphonitrilic ring, differences in biological activity between the chloro and dimethylamino apholate analog series may be related to water solubility, and significant differences in sterilant activity between two bis-dimethylamino structural isomers of apholate were observed.

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