

apparently did not depend entirely on leaf area because, in the experiment with relatively mature plants, the retarded ones showed markedly greater tolerance to the salts although they had about the same leaf area as the unretarded ones.

Sacks (7) reported that the numbers of dividing cells in the terminal buds of chrysanthemum were reduced by Amo-1618. Such a reduction in cell number might result in reduced absorption of salts. In the present experiments, the relatively mature plants studied showed no outward evidence of retardation of growth, but plants that received the retardant exhibited increased tolerance to application of excessive fertilizer.

Although increased tolerance to excessive fertilizer did not obviously depend on reduced leaf area and growth rates, the outwardly apparent responses mentioned indicate that the retarded plants did develop marked chemical and

physical changes. Further evidence of chemical and physical changes that resulted from use of the retardants was supplied by the fact that mites, which are sucking insects, multiplied more rapidly on leaves of the unretarded plants than on the retarded ones.

Further research is needed to determine whether some retardants can be used to increase the resistance of certain crop plants to alkaline and saline soils.

Acknowledgment

Amo-1618 was supplied by the Rainbow Color & Chemical Corp., Northridge, Calif.; phosfon, by the Virginia-Carolina Chemical Corp., Richmond, Va.; and CCC, by the American Cyanamid Co., Stamford, Conn.

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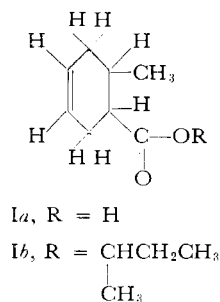
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INSECT ATTRACTANTS

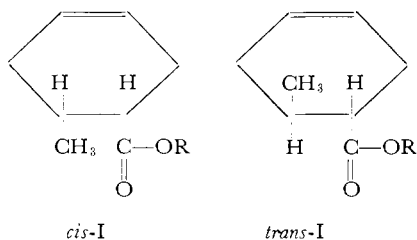
New Attractants for the Mediterranean Fruit Fly

INSECT ATTRACTANTS have proved to be invaluable tools for the control and eradication of certain insect species (7, 2, 7, 8). The important roles played by angelica seed oil (12) and *sec*-butyl 6-methyl-3-cyclohexene-1-carboxylate (Ib) (2, 4, 6).



now known as siglure, in the eradication of the Mediterranean fruit fly [*Ceratitis capitata* (Wied.)] or medfly, from Florida in 1956-57 have been recorded (7). These lures attract only the male of the species.

An observation that certain commercial lots of siglure gave inferior performance led to the discovery that *cis-trans*-isomerism affected the attractiveness of the product (11).



The *trans*-product was much superior to its *cis*-analog. Later, an infrared spectrophotometric method was developed to determine the *cis-trans*-content of the ester, and a means of preparing the desired all-*trans*-product was devised (6).

As part of this investigation, the authors studied the conditions that led to epimerization of the *trans*-acid, mainly to avoid such conditions in the course of the synthesis. An unexpected bonus accrued when a means was found to add the theoretical amount of hydrogen chloride to the double bond of the acid moiety of siglure. Past attempts to effect this addition by conventional means were unsuccessful. With the hydrochlorinated acid available, a series of esters was prepared and sent to

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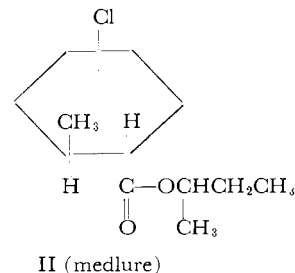
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Hawaii for testing as medfly lures. In laboratory and field trials, some of the esters were far superior to siglure in both attractiveness and lasting quality. The excellent performance of the hydrochlorinated products led to the preparation of the hydrogen bromide addition product of (Ia) and its esters.

Of all the esters prepared, the best were the *sec*- and *tert*-butyl esters of the hydrogen chloride adduct of (Ia) and the ethyl ester of the hydrogen bromide adduct.



sec-Butyl *trans*-4(or 5)-chloro-2-methylcyclohexane-1-carboxylate (II), now known as medlure, has already been produced commercially and is used by the U. S. Department of Agriculture,

Some new potent attractants for the male Mediterranean fruit fly [*Ceratitis capitata* (Wied.)] have been synthesized. The *sec*-butyl ester of *trans*-4(or 5)-chloro-2-methylcyclohexanecarboxylic acid, now known as medlure, is more attractive and longer-lasting than siglure, previously the best synthetic lure. Medlure can exist in eight isomeric forms, each of which may have a different degree of attractancy. Studies were undertaken to determine the conditions necessary to obtain the most attractive preparation of this compound and such conditions, which gave a good yield of product, are described. Of the 46 esters of 4(or 5)-chloro- and bromo-2-methylcyclohexanecarboxylic acid prepared, the most attractive was the *tert*-butyl analog of medlure, now called trimedlure. Although not quite as long-lasting as medlure, trimedlure will supplant medlure, if an economical means of synthesizing it can be devised. Besides being useful for survey purposes, the new lures (mixed with a toxicant) may be potent enough to eliminate male medflies and serve as direct control agents.

Table I. Physical, Chemical, and Attractancy Data for Esters of the Halogen-Containing Acids

Ester	Yield, %	Boiling Point			n_D^{25}	Halogen, %		Trap test ^a	Olfactometer Data		
		° C.	Mm. Hg	Calcd.		Found	Wick Test ^b				
							Fresh		15 days	30 days	
4(or 5)-Chloro-2-methylcyclohexanecarboxylic Acid											
Methyl	80	85-87	0.4	1.4661	18.6	17.4	9	5	
Ethyl	83	100-104	0.35	1.4621	17.3	16.1	79	85	0	...	
Propyl	83	106-109	0.30	1.4629	16.2	15.1	52	100	62	90	
Isopropyl	70	103-109	0.50	1.4577	16.2	15.0	70	100	21	...	
Allyl	69	98-101	0.35	1.4751	16.4	15.5	45	63	30	...	
Butyl	75	105-109	0.30	1.4630	15.2	15.7	14	10	
Isobutyl	79	106-110	0.30	1.4608	15.2	15.4	38	25	
<i>sec</i> -Butyl (medlure)	88	92-94	0.20	1.4589	15.2	15.0	72	100	85	32	
<i>tert</i> -Butyl (trimedlure)	45	107-113	0.60	1.4600	15.2	16.1	70	150	112	75	
Pentyl	70	104-108	0.40	1.4640	14.4	14.8	4	13	
Isopentyl	74	112-118	0.25	1.4629	14.4	14.6	5	13	
1,1-Dimethylpropyl	59	108-116	0.70	1.4679	14.4	14.3	52	125	100	113	
1-Ethylpropyl	67	100-107	0.30	1.4629	14.4	14.8	46	75	75	125	
Cyclopentyl	64	108-114	0.30	1.4821	14.5	13.6	17	13	
2-Ethylbutyl	74	108-113	0.30	1.4637	13.6	13.0	6	5	
2-Methyl-1-pentyl	73	112-119	0.40	1.4649	13.6	13.3	2	5	
2-Chloroethyl	72	105-107	0.35	1.4836	29.6	27.6	21	5	
Phenethyl	66	131-148	0.30	1.5153	12.3	11.3	1	5	
1-Methylbutyl	70	94-98	0.35	1.4596	14.4	14.0	56	33	
1,3-Dimethylbutyl	75	98-102	0.30	1.4591	13.6	13.9	46	0	
Benzyl	63	120-128	0.35	1.5235	13.3	12.3	4	0	
1-Methoxy-2-propyl	78	101-105	0.20	1.4624	15.8	14.9	49	50	
Propynyl	44	120-122	0.12	1.4882	50	25	
Cyclohexyl	50	109-111	0.25	1.4836	7	4	
2-Bromoethyl	66	111-113	0.03	1.4985	13	0	
2-(2-Butoxyethoxy)ethyl	68	133-134	0.03	1.4656	2	0	
2-Butoxyethyl	40	106-107	0.03	1.4671	7	0	
1-Ethylpentyl	71	105	0.03	1.4651	2	0	
Heptyl	93	107-109	0.07	1.4641	0	0	
Hexyl	70	93-95	0.03	1.4634	1	0	
3-Methoxybutyl	71	103-108	0.02	1.4649	0	0	
2-Methoxyethyl	54	83	0.03	1.4666	9	0	
1-Methylheptyl	70	110-112	0.08	1.4621	2	0	
Octyl	91	114-115	0.1	1.4642	2	0	
3-Phenylpropyl	89	143-144	0.08	1.5137	1	0	
Tetrahydro-2-furfuryl	64	118-120	0.07	1.4838	1	0	
<i>p</i> -Tolyl	87	127-129	0.07	1.5171	1	0	
4(or 5)-Bromo-2-methylcyclohexanecarboxylic Acid											
Methyl	81	73	0.3	1.4872	34.0	34.0	50	2	
Ethyl	77	78	0.3	1.4805	32.1	32.6	73	120	125	57	
	66	102-110	0.3	1.4831	
Propyl	83	86-87	0.35	1.4788	30.4	30.4	168	90	100	114	
Isopropyl	80	76-77	0.35	1.4743	30.4	30.6	75	120	106	57	
	62	100-108	0.4	1.4751	
Allyl	65	95-100	0.7	1.4881	30.6	29.0	32	38	100	...	
Butyl	77	98-99	0.35	1.4789	28.8	30.1	41	2	
	82	90-91	0.35	1.4750	
<i>sec</i> -Butyl	61	110-115	0.1	1.4760	28.8	...	30	90	106	86	
2-Methylallyl	53	106-109	0.7	1.4879	29.0	27.0	31	63	100	...	
Cyclohexyl	72	117-120	0.25	1.4989	26.4	27.2	6	2	

^a Number of times as attractive as water.

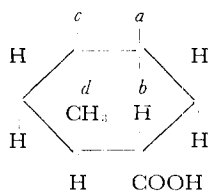
^b Fresh medlure = 100.

Plant Pest Control Division in Florida and the Florida State Plant Board. The *tert*-butyl ester, now called trimedure, is almost twice as attractive to the medfly as medlure. However, its yields, which the authors tried to improve, are low. Which of the three esters is chosen for eventual use may depend on the economics of their synthesis and potential usefulness as determined by more extensive field tests now under way in Hawaii.

Chemical Studies

The halogen-containing acids are best prepared by reacting *trans*-6-methyl-3-cyclohexene-1-carboxylic acid (Ia) (3) with aqueous concentrated hydrohalogen acid in the presence of a mutual solvent, dioxane. Attempts to hydrohalogenate (Ia) without the mutual solvent or in a nonpolar organic solvent with hydrogen chloride gas resulted in low yields due to incomplete addition.

The study of the halogen-containing acid (III) is much more complex than that of its unhalogenated analog (Ia), since it may theoretically exist as eight isomers, whereas there are only two isomers of the latter (6). If one assumes that the addition of hydrogen chloride to *trans*-(Ia) does not change its *trans*-configuration, the product may be one or more of four isomers depending on which of the four positions (*a, b, c, d*) the chlorine atoms occupy.



III $a, b, c, \text{ or } d = \text{Cl (or Br)}$

If the *trans*-configuration is changed to *cis*- during synthesis, four additional isomers are possible, making the theoretical total of eight.

To determine the most attractive ester of (III), 37 esters of the hydrogen chloride-acid adduct and nine esters of the hydrogen bromide-acid adduct were synthesized. Their physical, chemical, and attractant properties are given in Table I.

Consideration of the chemicals' performance as well as their cost of production led us to select the *sec*-butyl ester of the hydrochlorinated acid—that is, medlure (II)—for commercial production.

Mindful of the fact that the hydrogen chloride-acid adduct may exist in eight isomeric forms and that each may give a *sec*-butyl ester having a different attractancy for the medfly, the authors varied conditions of the acid synthesis (which will affect isomer content) to get the product that would yield the most attractive *sec*-butyl ester.

Table II. Studies on the Hydrochlorination of *trans*-6-Methyl-3-cyclohexene-1-carboxylic Acid (Ia)

<i>trans</i> - (Ia), grams	Reactants			Conditions			Product			Chlo- rine % found ^b
	Concd. ^a HCl, ml.	Di- oxane, ml.	Other	Heat- ing tempera- ture, ° C.	Heat- ing time, hours	Yield, % Stirred	B.P.		M.P., ° C.	
							° C.	Mm. Hg		
28	74	100	...	a	2	Yes	94-103	0.3	...	13.1
14	37	100	...	a	4	Yes	85	0.08	...	1.5
14	50	0	50 ml. H ₂ O	a	6	Yes	93-96	0.08	...	16.8
14	37	0	...	95-100	4	No	90-92	0.1	...	11.8
14	37	15	...	95-100	3	No	113-116	0.2	...	17.9
14	37	15	...	95-100	7.5	No	100-123	0.2-0.8	...	9.4
7	19	10	...	150	3	No	116-125	0.15	...	20.4
7	19	10	...	175	3	No	113-125	0.2	...	21.6
7	19	0	...	175	3	No	92	0.07	...	16.0
7	19	10	...	150	1.5	No	101-103	0.05	...	18.6
14	37	15	...	95-100	4	Yes	64-85	18.8
14	37	15	0.1 ml. Concd. HI	95-100	4	Yes
14	37	15	0.2 ml. Concd. HI	95	2	Yes	66-86	18.7
14	37	15	...	95	6	Yes	20.0
56	148	60	0.8 ml. Concd. HI	95	3	Yes	75-85	19.3
56	148	60	...	95	6	Yes	73-83	19.3

^a Refluxed at atmospheric pressure.

^b Calculated for C₈H₁₃ClO₂, Cl = 20.1%.

A summary of the more significant of these experiments is given in Table II. When the reactants were heated under reflux (first three experiments), hydrogen chloride escaped from the water-cooled condenser. If additional amounts of concentrated hydrochloric acid were added periodically to replenish the loss, the uptake of 1 mole of hydrogen chloride by the organic acid was still not attained, even after 16 to 20 hours of heating. Further experiments were therefore run in sealed containers, either in Carius tubes at 150° to 175° C. or in citrate of magnesia or soft drink bottles at 95° to 100° C.

It was believed that the addition of hydrogen chloride would be facilitated at 150° to 175° C. The hydrogen chloride and dioxane would develop high pressure at these temperatures, and since the addition of hydrogen chloride to the organic acid would tend to lower the pressure, the reaction should be forced to completion rapidly. Although this reasoning held true, synthesis of the compound commercially by this means would entail extra expense because high-pressure equipment would be required. The authors finally settled on heating the reactants for 6 hours at 95° C. with vigorous stirring. This procedure is easy to duplicate, yields are good, and conditions are sufficiently mild to prevent epimerization. Pressure is no problem because it remains below 20 p.s.i. Although dioxane is a mutual solvent for the reactants, two phases are present at 95° C. and vigorous stirring is essential to maintain high yields. The addition of catalytic amounts of hydriodic acid did speed up the addition of hydro-

gen chloride, but was not advantageous because it also cut the yield, presumably due to resin formation.

Peroxides are known to cause a reversal of the normal mode of addition of hydrogen halides. Therefore, hydrochlorination experiments were run in which hydrogen peroxide was added and in which dioxane with a high peroxide content was used as solvent. Physical properties of the resulting esters were nearly identical. None of these treatments resulted in a better attractant; usually the product was inferior.

The 4(or 5)-chloro-2-methylcyclohexanecarboxylic acid was converted to the acid chloride by reacting it with thionyl chloride under the mild conditions already described for the preparation of the *trans*-7-methyl-3-cyclohexene-1-carboxylic acid chloride intermediate of siglure (6)—i.e., the reaction product was stripped of its solvent in vacuo on a warm water bath and the acid chloride was not distilled. This precaution was indicated because the *trans*-acid chloride was shown to epimerize readily. The esters were prepared by reacting the acid chloride with the appropriate alcohol in the presence of pyridine and worked up in the usual manner (6). All the esters were clear, colorless liquids with pleasant fruity odors.

The medlure preparations that were obtained in reasonably good yield were tested for attractancy. The problems were simplified somewhat on finding that the procedure giving the best yield gave the most attractive product.

It would have been desirable to prepare and resolve the eight isomers of (III) and test each of the *sec*-butyl esters

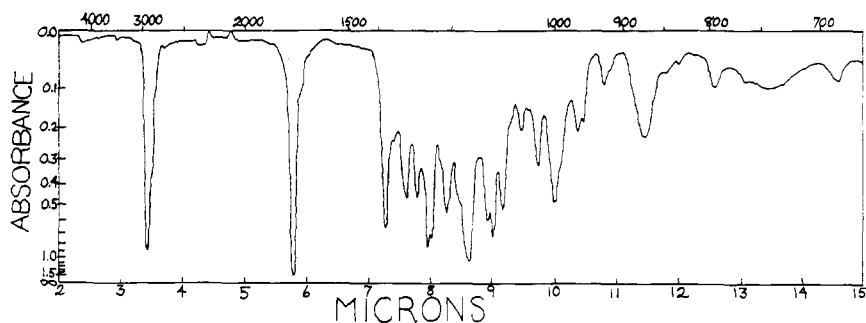


Figure 1. Infrared spectrum of medlure (II) in carbon disulfide, 25 mg. per ml., cell depth 0.4 mm.

Table III. Field Tests on Esters of 4(or 5)-Chloro (or bromo)-2-methylcyclohexanecarboxylic Acid in Comparison with Siglure

Ester	Number of Applications	Ml. per Application	Total Medfly Catch	Significance of Differences ^a	
Esters of 4(or 5)-chloro-2-Methylcyclohexanecarboxylic Acid (4-week test)					
Trimedlure	1	1	16,062		
Medlure	1	1	11,396		
1,1-Dimethylpropyl	1	1	10,316		
Isopropyl	1	2	10,238		
Ethyl	1	2	10,139		
Medlure	1	2	9,859		
2-Methylpentyl	1	2	7,390		
1-Ethylpropyl	1	1	7,279		
Propyl	1	2	6,709		
Siglure	2 ^b	2	6,119		
Isobutyl	1	2	3,982		
Allyl	1	2	2,430		
Esters of 4(or 5)-Bromo-2-methylcyclohexanecarboxylic Acid (6-week test)					
Ethyl	1	1.9	12,706		
Siglure	3 ^b	6	11,020		
Propyl	1	2	8,535		
Isopropyl	1	2	8,056		
sec-Butyl	1	2	6,353		

^a Duncan multiple range test. Lines connect treatments not significantly different at the 5% level. ^b Biweekly.

for attractancy, but such a task could be very time-consuming.

At first, the authors assumed that the retention of the *trans*-configuration of the methyl and carboxylic ester groups in the chlorinated product would give a more attractive product than its *cis*-analog because *trans*-siglure is more attractive than *cis*-siglure. This hypothesis was verified by comparing the *sec*-butyl esters prepared from the hydrochlorination products of an all-*trans*- and a *cis*-rich Ia. The results of an 8-week field test, given below, indicate that the *trans*-ester is superior to its *cis*-rich analog.

Cis, %	Trans, %	Number of Medflies Caught
0	100	16,884
66	34	12,101

The infrared spectrum of the *sec*-butyl *trans*-ester is shown in Figure 1.

Preparation Procedures

4(or 5)-Chloro-2-methylcyclohexanecarboxylic Acid (Table II, last experi-

ment). Fifty-six grams (0.40 mole) of *trans*-6-methyl-3-cyclohexene-1-carboxylic acid (6), 148 ml. of concentrated hydrochloric acid (ca. 1.8 moles), 60 ml. of peroxide-free dioxane, and a Teflon-covered magnetic stirring bar were introduced into a citrate of magnesia bottle. The stoppered bottle was heated in a 95° to 98° C. water bath for 6 hours, while the contents were stirred vigorously with the magnetic bar. After cooling to room temperature the reaction mixture was poured into 400 ml. of water and allowed to remain overnight in a refrigerator (ca. 5° C.). The crystalline product was filtered off, washed with water, and air dried. The crude, grayish crystals weighed 68.7 grams (97%) and melted at 73° to 83° C.

ANALYSIS, calcd. for C₈H₁₃ClO₂: Cl, 20.1%. Found: Cl, 19.3%.

4(or 5)-Bromo-2-methylcyclohexanecarboxylic Acid. It was prepared according to the directions given above for its chlorine-containing analog; b.p.

125° to 131°/0.8 mm. Hg; n_D²⁰ 1.5045.

ANALYSIS, calcd. for C₈H₁₃BrO₂: Br, 36.1%. Found: Br, 33.8%.

Esters of 4(or 5)-Chloro(or bromo)-2-methylcyclohexanecarboxylic Acid. The preparation of the esters from the hydrohalogenated acid (III) followed the mild acid chloride esterification route already reported (6). Their properties are detailed in Table I.

Entomological Tests

Olfactometer Trap Tests. In initial trials of candidate lures, glass-investigated traps and the cage olfactometer of Gow (5) were utilized to eliminate those chemicals having a low order of attractiveness. Surviving candidates were subjected to the wick test. The results of these tests are given in Table I.

Wick Test. A cotton dental roll 1½ inches long was impregnated with 0.5 ml. of the lure and attached with a cellulose tape to a piece of kraft paper in such a way as to prevent spreading of the lure to the paper. Six wicks were exposed simultaneously for 15 minutes on a slowly rotating 6-sided wheel in the olfactometer, and the number of flies congregating on or near such specimen was estimated. The wicks were then aged at room temperature and periodically exposed in the olfactometer as long as they were attractive. The duration of effectiveness in comparison with a standard lure gave a measure of the lure's persistence or lasting qualities. This procedure indicates which lures were of low persistence and therefore not worth testing in the field. Results are shown in Table I.

Field Tests. The effectiveness of the best lures as determined in the field is shown in Table III. Plastic traps (9) baited with the test lures were deployed in medfly-infested areas along with a standard lure. Ten or more replicates were usually employed, and the number of flies caught at intervals over a period of at least several weeks was determined.

Discussion

Because of its greater immediate as well as residual attraction and little difference in cost of production, medlure was chosen for large-scale manufacture to replace siglure. The ethyl ester of the hydrogen bromide-acid adduct was as attractive as medlure, but is more costly to make. Trimedlure [*tert*-butyl ester of (III)] catches more medflies than medlure, but is slightly less long-lasting. The medfly males do not feed as readily on the synthetic lures as on angelica seed oil. Instead, they congregate closely around treated wicks or surfaces. The insects undoubtedly do not approach closer because the concentration of lure becomes too high. (A lure may

become repellent when its concentration is too high.) However, flies may make direct contact with lures from which the output is weak through loss or a low application rate. Of all the synthetic lures discussed here, trimedlure seems to have the least repellency to medflies at close range. At cool temperatures (50° to 69° F.) trimedlure outperformed a high-grade angelica seed oil, previously the best low-temperature lure; thus in 12 weeks, 434 medflies were caught with angelica seed oil, 368 with medlure, and 1433 with trimedlure. Trimedlure is the most promising medfly lure developed thus far.

Few attempts to relate insect attractancy and chemical structure have been made. Unquestionably, a correlation may be made from the compounds of this study, since they are all structurally similar and many of them are attractive to the medfly, although few are sufficiently attractive to be considered useful. However, there is little that the authors can predict on the basis of these or past studies with siglure. Although the *tert*-butyl ester analog of siglure (*sec*-butyl ester) was much inferior to siglure (olfactometer ratings 8 and 87), trimedlure (*tert*-butyl ester) was much superior to medlure (*sec*-butyl ester). It does seem remarkable, however, that the medfly will exhibit a marked preference for one compound over some very closely related ones, such as homologs and positional isomers.

The ultimate criterion as to the value of a lure is based on field performance. Such variables as temperature, humidity, altitude, competing natural attractants, wind conditions, rainfall, insect popula-

tion, host plants, and even trap design may affect catches and some of these may have a bearing on which chemical is chosen.

Too high a volatility will cause the lure to lack persistence and possibly to issue in so high a concentration that it becomes repellent to an insect approaching the trap. Too low a volatility that cannot be compensated for by increasing wick surface and application rate is not good either, because not enough of the lure will volatilize to permit the insect to detect it from a distance. Most of the lures discussed herein differ in their most effective application rates, which can be determined only by field tests under a wide range of conditions. The most attractive of the chlorinated lures in this study were the branched-chain esters—specifically the isopropyl, 1,1-dimethylpropyl, *sec*-butyl, and *tert*-butyl esters. Of the brominated lures, only the ethyl ester showed up well; higher homologs probably were not sufficiently volatile.

The best of the new medfly lures may be sufficiently potent (when mixed with a toxicant) to eliminate males and serve as direct control agents, if used in accordance with the methods developed for the oriental fruit fly (*Dacus dorsalis* Hendel) (10).

Acknowledgment

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of synthesis of the chlorine-containing esters in high yield.

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HERBICIDE RESIDUES IN MILK

Form and Magnitude of 2,2-Dichloropropionic Acid (Dalapon) Residues in Milk

AFTER it had been established that residues may occur in forage crops sprayed with dalapon at dosages necessary for the control of noxious grass (7), it became important to know the magnitude of dalapon residues in milk from dairy cattle which ingest such forage. A series of related tests was conducted to accrue this information.

Dalapon could conceivably occur in milk in at least two forms—as the free acid (or more probably a salt) in the aqueous phase and as a glyceride in the fat. The first test, involving a single animal fed 200 p.p.m. of dalapon (as its sodium salt) for 8 weeks, was designed to determine the relative magnitudes of

dalapon residues in each of these forms. A second test wherein three cows were fed dalapon at 20, 50, and 100 p.p.m., respectively, for 4 weeks was conducted to establish the relation between residue and quantity of dalapon ingested. In a third test, this information was augmented by milk residue data from six cows, two each fed 100, 200, and 300 p.p.m. of dalapon for an 8-week period.

Test Procedure

The first test was conducted from March 8 to May 24, 1956, using a Hereford cow weighing 900 pounds and producing an average of 5.5 kg. of milk

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daily. The animal was confined to its stanchion during the entire test period. Control milk samples were collected for a 2-week period prior to feeding dalapon. The chemical was administered twice daily, morning and evening, by mixing with silage contained in a washtub. Each dose consisted of 1.13 grams of dalapon, as the sodium salt, in 50 ml. of H₂O, approximating 200 p.p.m. based on the average dry weight of feed consumed. The balance of the diet consisted of alfalfa hay.

Each day a suitable portion of milk from the morning's production was mixed with an equal amount from the preceding evening and 250-gram aliquots were