Polyfluoro 1,3-Diketones as Systemic Insecticides

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A series of aryl polyfluoro 1,3-diketones were examined for systemic ectoparasiticidal activity in cattle. The compounds demonstrated efficacy against several economically important species of insects and acarina. At dosages of 5 mg/kg ×1 or 0.35 mg/kg per day intraruminally, activity was observed against blowfly larvae (*Phormia regina*), adult stable fly (*Stomoxys calcitrans*), and lone star tick (*Amblyomma americanum*). In vivo activity was not directly related to in vitro activity, showing a stronger dependence on perfluoroalkyl-chain length and aryl-group substitution.

Polyfluoro 1,3-diketones (Figure 1) have been shown to be insecticides.[1-3] These compounds are members of a broad class of weak organic acids which have in common a 1,3-dioxo functionality as depicted in the structures in Figure 2. These compounds function as metal-chelating agents[4-6] and have been shown to act as "hydrogen ionophores", disrupting intracellular pH gradients. This results in an uncoupling of mitochondrial oxidative phosphorylation, thus disrupting energy production.[7,8,13] Whether by reason of their effect as oxidative phosphorylation uncouplers (OPU) or their metal chelating properties, these molecules are, in general, highly active in biological systems. Various members of this class of compound have been shown to exhibit antibacterial,[9] antiviral,[10] anthelmintic,[11] fungicidal,[12] and/or herbicidal[13] activity. In spite of the breadth of activity, adequate specificity can be observed in specific cases to warrant development as therapeutic agents.[11,14]

In light of the continuing need for effective means of control of external parasites in agronomically important animals as well as companion animals,[15] we were interested in determining whether this series of compounds could be of value in the systemic control of insects and acarina. With the exception of a report on a mouse assay using a small number of diketones,[2] no studies have been reported on the evaluation of aryl polyfluoro diketones, or other compounds in this general class, as systemic insecticides in animals. Our objective was to determine the feasibility of using these compounds systematically in cattle and to monitor for overt effects on the host.

Results and Discussion

Diketones. The polyfluoro diketones used in this study were prepared by slight modification of published procedures[16-18] (Figure 3). Generally, the appropriate acetophenones were treated under basic conditions with a perfluorinated ester. Standard workup, including an acid wash, produced the free acids as low-melting solids or oils which were often difficult to purify. Workup without the acid wash afforded the sodium salts as stable, high-melting powders. Analysis indicated these powders, homogeneous by TLC, to be a mixture of monomeric, dimeric, and trimeric forms, sometimes containing a small percentage of free acid. In vitro dose-titration studies measuring insecticidal activity of sera spiked with between 10 and 50 ppm (in increments of 5 ppm) of free diketones and their sodium salts showed the salt form to be about 25% more active against adult stable flies (ASF) than the corresponding free acid, presumably due to improved solubility characteristics (McGowan, unpublished data). In theory, it would be anticipated that at the physiological pH the two forms should exist in equilibrium with each other, so that the form used in vivo would be immaterial. As a practical consideration, the high-melting salts also proved much easier to handle and formulate; hence, most of the work was done using the sodium salts.

Based on dose titrations of 10–100 ppm in bovine sera, the majority of the diketones examined in vitro showed similar activity (Table I). All caused >50% mortality in both test species [ASF and larval blowfly (LBF)] at rates of between 15 and 40 ppm. The three insecticide reference compounds, avermectin, biphenate, and carbofuran, were

Table I. In Vitro Insecticidal Activity of Diketones in Bovine Serum vs Adult Stable Fly and Blowfly Larvae

compound no.	phenyl substituents	R_f	lowest effective concentration,[a] ppm ASF	LBF
1	3,5-dichloro	C_3F_7	25	15
2	3,5-dichloro	C_2F_5	20	40
3	3,5-dichloro	C_2F_4H	20	25
4	3,5-dichloro	CF_3	25	20
5	3,4-dichloro	C_3F_7	25	30
6	3,4-difluoro	C_3F_7	25	30
7	3,5-dibromo	C_3F_7	25	25
8	4-chloro	C_3F_7	25	50
9	3-trifluoromethyl	C_3F_7	35	40
10	4-trifluoromethyl	C_3F_7	30	35
11	avermectins		10	0.5
12	biphenate (pyrethroid)		25	5
13	carbofuran (carbamate)		1	5

[a] Lowest effective concentration represents the lowest rate at which >50% mortality is observed.

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Table II. Comparison of Percent Mortalities[a] of Adult State Flies and Blowfly Larvae following 24- and 48-h Exposure of Insects to Sera from Cattle Treated with 5 mg/kg of Compound 2

species	time of exposure, h	day after treatment							
		0	1	2	3	4	5	6	7
ASF	24	0	20	55	45	20	0	0	10
ASF	48	b			100	100	80	30	
LBF	24	0	0	0	0	0	0	0	0
LBF	48				100	50	0	0	

[a] ≥50% mortality is active and adjusted to mortalities in nontreated controls. [b] 48-h readings were not taken prior to day 3.

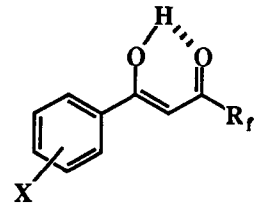

Figure 1. General structures of aryl polyfluoro 1,3-diketones (R_f is a polyfluoroalkyl group).

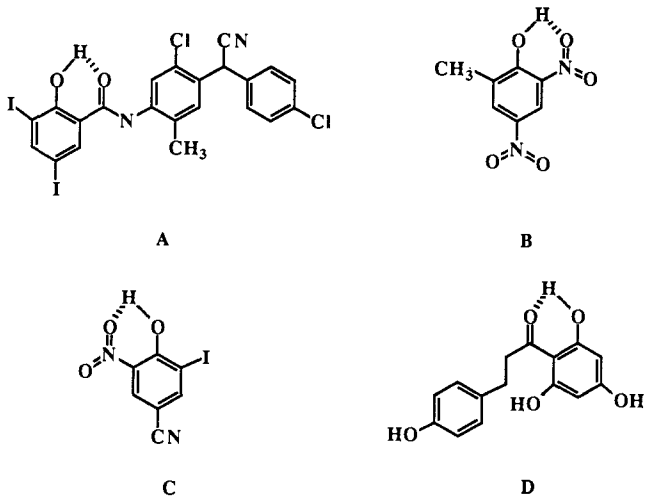

Figure 2. Representative examples of oxidative phosphorylation uncouplers which have in common a 1,3-dioxo functionality: (A) closantel (anthelmintic), (B) DNOC (herbicide), (C) nitroxynil (anthelmintic), (D) phloretin (herbicide).

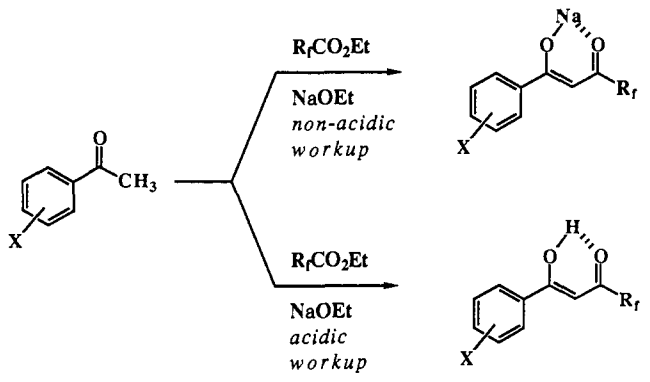

Figure 3. Synthesis of diketones. Acidic or basic workup delivers the neutral or basic derivatives, respectively.

active between 0.1 and 25 ppm. These data suggested testing in cattle, the target animal, to differentiate compound activity. When cattle were treated via intramuscular, subcutaneous, or intraperitoneal injection with a diketone, sera collected up to six days postinjection showed no insecticidal activity. However, when cattle were treated orally or intraruminally (ir), the active ingredient was absorbed rapidly into the blood stream. Serum samples obtained from cattle treated with a single ir dose of 5 mg/kg of compound 1 were insecticidally active (>50% insect mortality in both ASF and LBF) beginning 24–48 h after injection and continuing until up to 7 days after treatment. Though the three insecticide reference compounds were active in vitro against ASF and LBF, they do not exhibit systemic insecticidal activity against ASF in cattle.

In contrast to the dose titrations described above, systemic activity showed a marked dependence on perfluoroalkyl-chain length. Maximum activity was observed with compounds which contained the heptafluoropropyl (C_3F_7) chain; the analogous pentafluoroethyl derivative (compound **2**) was inactive against LBF at 5 mg/kg and showed considerably diminished ASF activity (2 days). The corresponding tetrafluoroethyl or trifluoromethyl analogues (compounds **3** and **4**) were inactive even at 10 mg/kg.

Systemic activity also showed a surprising dependence on aryl-group substitution. Diketones containing aryl groups with 3,4- or especially 3,5-dihalo substitution patterns were considerably more active against both ASF and LBF than were monosubstituted derivatives. Thus, at the 5 mg/kg dose only compound 1 caused >50% mortality against LBF, and none of the other substitution patterns (compounds **5–10**) resulted in stable fly activity lasting longer than 3 days.

It was observed in some of the experiments that the blowfly larvae exhibited apparent arrested development after 24 h exposure to sera. After retaining larvae an additional 24 h, the affected larvae eventually died. Further examination revealed this to be more general: with both LBF and ASF, in vitro activity readings were consistently higher following a 48-h exposure to treated sera. Longer periods were less conclusive due to increased mortality within the nontreated serum (control) groups. In Table II, a comparison was made between mortality percentages 24 and 48 h after initial exposure to sera. The higher overall activity and apparently longer duration of activity are indicative of a slow onset of activity. Boisvenue and Hair[20] conducted experiments involving another OPU and observed a similar increase in stable fly activity with successive feedings. While the 48-h data might portend somewhat greater activity for these compounds under actual field conditions, the duration of the test had no material effect on the relative rankings of the compounds.

At rates higher than 5 mg/kg, some toxic effects were observed in the treated animals. These consisted primarily of lethargy and reduced feed consumption. All treated and untreated animals lost some weight during these tests, apparently as a result of the testing conditions (i.e., a fixed amount of feed). At rates of 10 mg/kg or lower, all animals returned to normal within 10 days posttreatment.

Daily Dosing. To simulate the effects of continuous insecticidal control under pasture conditions, cattle were

(20) Although lice were not specifically tested, it was noted that in one case an animal which was louse-infested prior to administration of the compound was cleared of adult lice on treatment with a single 10 mg/kg intraruminal dose of compound **6** (McGowan, unpublished data).

Table III. Comparison of in Vitro and in Vivo Activity of Diketones following Daily Intraruminal Administration

compd no.	dose, mg/kg per day	day of onset of ASF in vitro[a] act.	day of onset of ASF in vivo[b] act.	day of onset of LBF act.	% tick act.[b]
1	1.00	3[c]	3[c]	5[c]	100
1	0.50	4	4	8	NT
1	0.35	9	none	none	NT
1	0.20	none	none	none	NT
2	1.50	2	2	2	NT
2	1.00	3[c]	3[c]	5[c]	100
2	0.75	4	4	4	NT
2	0.50	6	6	6	NT
2	0.30	*d*	none	none	NT
3	1.00	5	none	none	NT

[a] Serum samples were collected daily from treated calves. [b] ASF and ticks placed on treated curves. [c] Activity ratings were made following 24-h in vitro exposure to treated sera. Otherwise, onset of activity was based on 48-h exposure. [d] Activity was observed, although mortality never reached 50%. NT = not tested.

treated with daily intraruminal injections of the active diketones. Within 2–3 days after beginning a 1 mg/kg per day dosing regimen of compound 1 (Table I) in a manner previously described, in vitro insecticidal activity of cattle serum was observed. Excellent control of all insects was observed within 4 days (Figure 4, parts a and b), and this activity continued for at least 6 days after treatment ended on day 18. The pentafluoroethyl derivative (compound 2) was also active at this rate, although blowfly activity was markedly less. Shorter perfluoroalkyl chains (compounds 3 and 4 in Table I) showed considerably reduced activity against ASF and LBF. In addition to serum testing, results from animals infested directly with ASF or ticks demonstrated similar activity. Adult and nymphal ticks as well as ASF were controlled at the 1 mg/kg per day rate with either compound 1 or compound 2 (Table III).

Toxic effects in the cattle paralleled activity. Compound 1 (Table I) at 1 mg/kg per day caused reduced feed consumption and lethargy 15 days into the test, although the effects were likely exacerbated by the confining conditions and the limited diet. Toxic symptoms were not observed with the other compounds at this rate.

At rates below 1 mg/kg per day, compound 1 had no apparent deleterious effect on the cattle. The lower limit of activity under these conditions was 0.35 mg/kg per day for compound 1 and 0.50 mg/kg per day for compound 2 (Table III).

The differences between in vitro and in vivo activity for compounds of this series appear to be related to lipophilicity and compound stability, i.e., the rate at which the compounds can be eliminated from the host. The sodium salts of these compounds are highly ether soluble. The effect of increasing both perfluoroalkyl-chain length and phenyl-group substitution was to further enhance lipophilicity. The observed differential toxicity to parasites is likely related to its strong binding with plasma proteins in the blood stream of the host, as observed with other parasiticides capable of chelation,[19] thus attenuating the activity until digestion by the parasite.

Two factors may account for the slow onset of activity for this series. First, these compounds exhibit some antifeedant activity, and some of the flies may not have consumed a toxic dose of the compound after 24 h. Secondly, the mode of action is thought to be one of energy depletion and not a central nervous system effect; thus, the onset of mortality may be delayed. Experience with testing tick-infested cattle further confirms the antifeedant effects observed alone. When ticks were placed on the backs of a treated animal, we observed a delay in tick attachment and once attached, the ticks would often succumb to apparent desiccation rather than ingestion of a full blood meal containing a toxic dosage of compound.

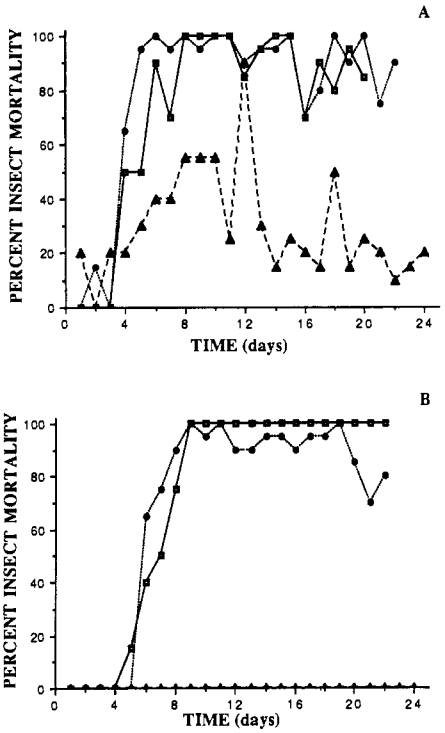

Figure 4. Comparison of systemic insecticidal activity of diketones as a function of perfluoroalkyl-chain length: (A) insecticidal activity, measured against adult stable fly, of sera obtained from the blood of calves treated intraruminally with daily 1 mg/kg injections of (—) compound 1 (···) compound 2, and (– –) compound 3 (treatment ended on day 17); (B) insecticidal activity, measured against larval blowfly, of sera obtained from cattle treated as described above.

This study has demonstrated that the application of specifically substituted polyfluorinated diketones can be an effective method for the systemic control of a limited range of external parasites. The antifeedant effects observed with these compounds coupled with the delayed activity suggest that somewhat lower dosages may be effective in cattle herds under actual field conditions, although it is uncertain whether this could offset the low therapeutic ratio observed under our experimental conditions. It would also be of interest to determine the activity of these compounds under conditions of continuous payout, rather than single daily injections. It has not been determined why these compounds show no activity by alternative routes of administration, how these compounds are eliminated from the host, or whether they exist predominantly in the blood stream or elsewhere. Future work will involve better defining the lowest effective dosages, expanding the spectrum of insects tested,[20] and

determining the most effective method of administration.

Experimental Section

All acetophenones used as precursors to diketones were available commercially, and were generally used without further purification. NMR data were obtained on a Bruker 250-MHz spectrometer, using DMSO-d_6 as solvent with TMS as internal standard. Melting points were taken on a Fischer MelTemp apparatus and are uncorrected. Field-desorption mass spectra (FMDS) were obtained on a Varian-MAT 731 spectrometer.

General Procedure for Preparation of Aryl Polyfluoro Diketone Sodium Salts. Preparation of 5,5,6,6,7,7,7-Heptafluoro-1-(3,5-dichlorophenyl)-1-hydroxyhex-1-en-3-one, Sodium Salt (1). Into a three-necked round-bottom flask equipped with mechanical stirrer, N_2 inlet, and addition funnel were placed 100 mL of dry ether and 27.0 g (0.25 mol) of absolute ethanol. Sodium hydride (28.0 g of a 50% dispersion of oil, 0.58 mol) was added over a period of 15 min. The solution was cooled by means of an ice bath, and ethyl heptafluorobutyrate (76 g, 0.31 mol) was added dropwise. After addition was complete, a solution of 3,5-dichloroacetophenone (53.74 g, 0.285 mol) in 50 mL of ether was added dropwise. Stirring and cooling were maintained for 3 h, whereupon the reddish solution was poured onto 200 g of crushed ice and extracted into ether. The organic layer was washed with 2 × 200 mL of brine and then dried over $MgSO_4$ overnight. The light yellow solution was concentrated and crystallized by trituration with hexane. The product was isolated by filtration and air-dried. There was obtained 75 g of a white powder: mp 140–150 °C; NMR (DMSO-d_6) δ 6.3 (1 H), 7.5–7.9 (m, 3 ArH). Anal. ($C_{12}H_4Cl_2F_7O_2Na$) C, H.

4,4,5,5,5-Pentafluoro-1-(3,5-dichlorophenyl)-1-hydroxypent-1-en-3-one, Sodium Salt (2). Following the procedure described above, a white solid was isolated: mp 140–160 °C; NMR (DMSO-d_6) δ 6.33 (s, 1 H), 7.7–7.9 (m, 3 ArH). Anal. ($C_{11}H_4Cl_2F_5O_2Na$) C, H.

4,4,5,5-Tetrafluoro-1-(3,5-dichlorophenyl)-1-hydroxypent-1-en-3-one, sodium salt (3): mp 136–149 °C; NMR (DMSO-d_6) δ 6.3 (s, 1 H), 6.6 (tt, 1 H), 7.4–7.8 (m, 3 ArH); FDMS m/z 656 (dimer), 991 (trimer).

4,4,4-Trifluoro-1-(3,5-dichlorophenyl)-1-hydroxybut-1-en-3-one, sodium salt (4): mp 264–266 °C; NMR (DMSO-d_6) δ 6.35 (s, 1 H), 7.7–7.9 (m, 3 ArH); FDMS m/z 590 (dimer).

4,4,5,5,6,6,6-Heptafluoro-1-(3,4-dichlorophenyl)-1-hydroxyhex-1-en-3-one, sodium salt (5): mp 137–145 °C; NMR (DMSO-d_6) δ 6.35 (s, 1 H), 7.6–8.0 (m, 3 ArH).

4,4,5,5,6,6,6-Heptafluoro-1-(3,4-difluorophenyl)-1-hydroxyhex-1-en-3-one, sodium salt (6): mp 146–154 °C; NMR (DMSO-d_6) δ 6.3 (s, 1 H), 7.5–7.9 (m, 3 ArH). Anal. ($C_{12}H_9Cl_4F_{14}O_4Na$) C, H.

4,4,5,5,6,6,6-Heptafluoro-1-(3,5-dibromophenyl)-1-hydroxyhex-1-en-3-one, sodium salt (7): mp 131–134 °C; NMR (DMSO-d_6) δ 6.3 (s, 1 H), 8.0 (m, 3 Ar H); FDMS m/z 965 (dimer).

4,4,5,5,6,6,6-Heptafluoro-1-(3-chlorophenyl)-1-hydroxyhex-1-en-3-one, sodium salt (8): mp 220–225 °C; NMR (DMSO-d_6) δ 6.3 (s, 1 H), 7.5 (d, J = 8 Hz, 2 H), 7.9 (d, J = 8 Hz, 2 H). Anal. ($C_{12}H_5ClF_7O_2Na$) C, H.

4,4,5,5,6,6,6-Heptafluoro-1-[3-(trifluoromethyl)phenyl]-1-hydroxyhex-1-en-3-one, sodium salt (9): mp 140–150 °C; NMR (DMSO-d_6) δ 6.35 (s, 1 H), 7.6–8.1 (m, 4 ArH). Anal. ($C_{13}H_5F_{10}O_2Na$) C, H.

4,4,5,5,6,6,6-Heptafluoro-1-[4-(trifluoromethyl)phenyl]-1-hydroxyhex-1-en-3-one, sodium salt (10): mp 160–180 °C; NMR (DMSO-d_6) δ 6.35 (s, 1 H), 6.23 (s, 1 H), 7.8 (d, J = 7.5 Hz, 2 H), 8.05 (d, J = 7.5 Hz, 2 H). Anal. ($C_{13}H_5F_{10}O_2Na$) C, H.

Efficacy Testing. Larval Blowfly, *P. regina*. The primary screen for potential ectoparasiticide activity utilized the LBF as the test organism.[21] This screen has been shown to detect known classes of insecticides at rates of between 1 and 20 ppm. A clump of approximately 50 first instar blowflies was deposited onto the center of a compound/bovine serum saturated dental wick placed upright in the bottom of a 6.5-mL plastic, disposable test tube. The tubes were loosely plugged with a cotton ball and placed in a test tube rack 30 cm beneath a fluorescent strip light at a temperature of 27 °C and 55% relative humidity for 24 h. Compounds or preparations exhibiting less than 50% mortality were considered inactive. Initially, compounds were tested at 100, 50, and 25 ppm. Then compounds active at the lowest in vitro concentration were titrated to 1 ppm using increments of 5 ppm. Each ppm increment had two replicates.

Adult Stable Fly, *S. calcitrans*. Five-day-old, hungry, adult stable flies were utilized in this test. Twenty adult stable flies were placed, after chilling, in a covered petri dish and exposed to the test compound in a saturated dental wick as described above. The dish was placed into an incubator and fly mortality was assessed according to the above method following 24- or 48-h exposure to compound/serum baits.

Cattle Testing. Calves used in this study weighed between 150 and 350 kg and were housed in stanchions equipped with feed and watering troughs. Animals were fed approximately 2.5 kg of feed per day and water was available ad libitum. A minimum of four calves were used in each evaluation test. Two animals were treated with a single administration of the appropriate diketone, formulated as a solution in 5 mL of PEG-200. A third animal was treated with 5 mL of PEG-200 alone, and one animal was untreated. Daily intraruminal dosing experiments were conducted in the same manner; for convenience, treatments were administered via a plastic tube surgically implanted into the rumen prior to the start of the test. For in vitro insecticidal determinations, blood samples were taken prior to injection and then 2, 5, and 24 h after injection. Thereafter, samples were collected daily until no further insecticidal activity was observed. Serum samples were harvested from the whole blood and fed to LBF and ASF in the manner described above. An in vivo test consisted of placing six adult stable flies per day in chambers attached to the calves' backs and assessing fly mortality after 24 h. Forty (20 female and 20 male) adult lone star ticks (*A. americanum*) were placed on each animal in muslin containers. Animals were infested with adult ticks three days prior to treatment to allow time for attachment.[22] Ticks were allowed to feed to repletion and engorged ticks were collected daily and held at 27 °C and 96% relative humidity to assess the compound efficacy on tick viability.

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