Table I. Efficacy of Avermectin Isomers against *T. urticae*

compd	mortality, % 6.25 ppm	1.25 ppm	0.25 ppm	0.05 ppm	0.01 ppm	LC_{90}, ppm
1				100	66	0.038
2	100	78	17	3		4.0
3	100	100	97	35		0.23

isomer 3 is 0.2 ppm, only one-fifth of the natural product.

Chromatograms corresponding to partial isomerization of the individual compounds are shown in Figure 1, and the reaction profiles are plotted in Figure 2, with peak areas normalized to that of the initial. The isomerization previously reported for ivermectin is confirmed here for avermectin B_{1a}. In addition, the behavior of the 2-epimer 2 is seen to be analogous: Initial epimerization to the natural product is followed by ultimate formation of the Δ^2 isomer 3. Both forward and reverse epimerizations appear to follow pseudo-first-order kinetics during the initial stage of the reaction with equal rate constants (~0.011 min^{-1} in 0.05 M hydroxide in 50% MeOH–H_2O), indicating that there is no significant energy difference between the two forms. This tendency toward formation of an equilibrium mixture of epimers is overridden, however, by conversion to the energetically favored conjugated isomer 3. Compound 3 is itself unstable in hydroxide, but there is no measurable conversion to either of the two epimeric Δ^3 compounds (1 or 2), a consequence of the lack of a proton sufficiently acidic for removal under these mild conditions. The kinetic profile shown in Figure 2B demonstrates that Fraser-Reid's specified reaction time of 1 h produces an optimum conversion of 20–25% for the specific conditions 0.05 M NaOH in 50% aqueous methanol at room temperature. Presumably the yield can be increased by establishing conditions where epimerization is kinetically more favored relative to the conjugation reaction and by isolating and recycling unepimerized 2 in a subsequent hydroxide-catalyzed equilibration.

Registry No. 1, 65195-55-3; 2, 106434-14-4; 3, 110415-68-4; avermectin B_1, 71751-41-2.

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[4 + 2] Cycloaddition of Conjugated Azomethines to Aryl Isothiocyanates and Fungitoxicity of the Resulting 6,7-Dihydro-1,3,4-oxadiazolo[3,2-*a*]-*s*-triazine-5-thiones

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[4 + 2] cycloaddition of conjugated azomethines, 5-aryl-2-[(*p*-fluorobenzylidene)amino]-1,3,4-oxadiazoles IIIa–d, to aryl isothiocyanates affords a novel class of compounds, 2,6,7-triaryl-6,7-dihydro-1,3,4-oxadiazolo[3,2-*a*]-*s*-triazine-5-thiones IVa–l. Condensation of 2-amino-5-aryl-1,3,4-oxadiazoles IIa–d with *p*-fluorobenzaldehyde furnished the requisite azomethines IIIa–d. The compounds IVa–l have been compared with Dithane M-45, a commercial fungicide, for their fugitoxic action against *Aspergillus niger* and *Fusarium oxysporium*, and the results correlated with their structural features.

Encouraged by the significant fungitoxicity displayed by some 1,3,4-oxa(thia)diazolo[3,2-*a*]-*s*-triazine-7-thiones reported in our earlier communications (Bhattacharya et al., 1982; Singh et al., 1981, 1983b), we considered it of interest to synthesize more compounds of this class with certain structural modifications. Thus, the title compounds with partial saturation in the *s*-triazine nucleus and thione function at position 5 instead of at position 7 have been synthesized and evaluated for their antifungal activity. The presence of the fluoroaryl moiety in these compounds is expected to enhance their fungitoxicity (Filler and Kobayashi, 1983). The investigation appeared

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Table I. Yields, Melting Points, Molecular Formulas, and Elemental Analyses of Compounds IVa–l

compd IV	R	yield, %	mp, °C	mol formula	found (calcd), % C	H	N
			Ar = C_6H_5				
a	C_6H_5	75	236	$C_{22}H_{15}FN_4OS$	65.82 (65.67)	3.55 (3.73)	14.12 (13.93)
b	4-ClC_6H_4	69	255	$C_{22}H_{14}ClFN_4OS$	60.37 (60.48)	3.50 (3.20)	13.00 (12.82)
c	2-$CH_3OC_6H_4$	69	185	$C_{23}H_{17}FN_4O_2S$	64.00 (63.88)	4.15 (3.93)	12.82 (12.96)
d	4-$CH_3OC_6H_4$	75	246	$C_{23}H_{17}FN_4O_2S$	63.75 (63.88)	3.82 (3.93)	13.12 (12.96)
			Ar = 2-$CH_3C_6H_4$				
e	C_6H_5	81	235	$C_{23}H_{17}FN_4OS$	66.26 (66.34)	3.98 (4.08)	13.28 (13.46)
f	4-ClC_6H_4	74	248	$C_{23}H_{16}ClFN_4OS$	61.35 (61.26)	3.45 (3.55)	12.53 (12.43)
g	2-$CH_3OC_6H_4$	70	165	$C_{24}H_{19}FN_4O_2S$	64.36 (64.57)	4.15 (4.26)	12.64 (12.55)
h	4-$CH_3OC_6H_4$	71	135	$C_{24}H_{19}FN_4O_2S$	64.62 (64.57)	4.18 (4.26)	12.48 (12.55)
			Ar = 4-$CH_3C_6H_4$				
i	C_6H_5	77	233	$C_{23}H_{17}FN_4OS$	66.36 (66.34)	4.15 (4.08)	13.52 (13.46)
j	4-ClC_6H_4	78	251	$C_{23}H_{16}ClFN_4OS$	61.15 (61.26)	3.62 (3.55)	12.25 (12.43)
k	2-$CH_3OC_6H_4$	72	182	$C_{24}H_{19}FN_4O_2S$	64.58 (64.57)	4.31 (4.26)	12.46 (12.55)
l	4-$CH_3OC_6H_4$	74	244	$C_{24}H_{19}FN_4O_2S$	64.47 (64.57)	4.48 (4.26)	12.75 (12.55)

Table II. Spectral Data of Compounds IVa–l

compd IV	IR (KBr), cm^{-1} C=N	C=S	^1H NMR ($CDCl_3$–DMSO-d_6), δ	MS/M^+, m/z
a	1605, 1630	1200	6.78 (1 H, s, NC*H*), 7.50–8.00 (14 H, m, aromatic *H*)	402
b	1610, 1635	1205	6.82 (1 H, s, NC*H*), 7.60–8.04 (13 H, m, aromatic *H*)	436, 438
c	1610, 1635	1195	3.74 (3 H, s, OCH_3), 6.76 (1 H, s, *nCH*), 7.46–7.94 (13 H, m, aromatic *H*)	432
d	1605, 1630	1195	3.74 (3 H, s, OCH_3), 6.74 (1 H, s, NC*H*), 7.46–7.96 (13 H, m, aromatic *H*)	432
e	1605, 1630	1200	2.34 (3 H, s, CH_3), 6.72 (1 H, s, NC*H*), 7.44–7.94 (13 H, m, aromatic *H*)	416
f	1605, 1630	1205	2.32 (3 H, s, CH_3), 6.80 (1 H, s, NC*H*), 7.56–8.02 (12 H, m, aromatic *H*)	450, 452
g	1610, 1635	1195	2.32 (3 H, s, CH_3), 3.74 (3 H, s, OCH_3), 6.72 (1 H, s, NC*H*), 7.42–7.92 (12 H, m, aromatic H)	446
h	1610, 1635	1195	2.32 (3 H, s, CH_3), 3.76 (3 H, s, OCH_3), 6.74 (1 H, s, NC*H*), 7.44–7.94 (12 H, m, aromatic *H*)	446
i	1615, 1630	1200	2.32 (3 H, s, CH_3), 6.74 (1 H, s, NC*H*), 7.46–7.94 (13 H, m, aromatic *H*)	416
j	1605, 1635	1205	2.34 (3 H, s, CH_3), 6.72 (1 H, s, NC*H*), 7.40–7.92 (12 H, m, aromatic *H*)	450, 452
k	1610, 1630	1195	2.32 (3 H, s, CH_3), 3.74 (3 H, s, OCH_3), 6.72 (1 H, s, NC*H*), 7.42–7.96 (12 H, m, aromatic *H*)	446
l	1610, 1635	1195	2.34 (3 H, s, CH_3), 3.76 (3 H, s, OCH_3), 6.74 (1 H, s, NC*H*), 7.40–7.96 (12 H, m, aromatic H)	446

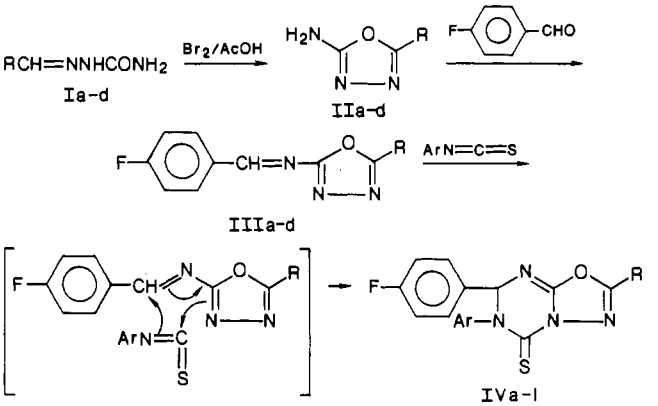
quite interesting as the fluorinated 2,6,7-triaryl-6,7-dihydro-1,3,4-oxadiazolo[3,2-*a*]-*s*-triazine-5-thiones IVa–l reported herein have been synthesized for the first time.

The reacton sequence leading to the formation of IVa–l is given in Scheme I. In the synthesis of IVa–l, a novel class of nitrogen-bridged heterocycles, aryl isothiocyanates, act as dienophiles and the conjugated azomethines IIIa–d as dienes (aza dienes). This is an interesting example of hetero Diels–Alder synthesis. The [4 + 2] cycloaddition of azomethines IIIa–d to aryl isothiocyanates was carried out in refluxing toluene to yield IVa–l in 69–81% yields (Table I). The required 2-amino-5-aryl-1,3,4-oxadiazoles IIa–d were prepared by oxidative cyclization of aldehyde semicarbazones with bromine (Gibson, 1962). Condensation of compounds II with *p*-fluorobenzaldehyde afforded the requisite azomethines III.

The structural assignments of the synthesized compounds were based on their elemental analyses, IR, ^1H NMR, and mass spectral data (Tables I and II).

The antifungal activities of compounds IVa–l were evaluated in vitro against *Aspergillus niger* and *Fusarium oxysporium* at three concentrations using Dithane M-45, a commercial fungicide, as standard (Table III). Compounds IVb, IVf, and IVj exhibited fungitoxicity almost equivalent to that of Dithane M-45 against both test fungi at 1000 ppm.

Scheme I[a]



[a] Key: a, R = C_6H_5; b, R = 4-ClC_6H_4; c, R = 2-$MeOC_6H_4$; d, R = 4-$MeOC_6H_4$.

EXPERIMENTAL SECTION

Melting points were determined in open glass capillaries and are uncorrected. IR spectra in KBr were recorded on

Table III. Fungicidal Screening Results of Compounds IVa–l

compd IV	av % inhibn against *A. niger* 1000 ppm	100 ppm	10 ppm	*F. oxysporium* 1000 ppm	100 ppm	10 ppm
a	65.8	36.6	20.3	65.0	35.4	19.5
b	97.6	57.8	40.6	96.8	53.3	40.0
c	69.7	41.0	24.2	71.1	42.4	25.9
d	67.0	39.4	22.7	68.7	41.0	24.1
e	71.4	42.1	29.8	69.6	39.9	26.2
f	100	65.0	44.5	98.8	63.2	43.1
g	75.6	46.6	33.2	76.7	47.2	35.0
h	72.1	42.8	30.3	74.2	45.1	33.3
i	68.5	39.4	28.2	67.4	38.6	21.1
j	98.6	62.4	43.0	97.9	59.3	42.3
k	71.0	42.5	29.6	72.7	43.9	31.1
l	69.2	40.3	24.1	70.0	42.1	26.6
Dithane M-45	100	80.8	66.5	100	85.3	68.2

a Perkin-Elmer 157 infrared spectrophotometer. ^{1}H NMR spectra were recorded on a EM-360L (60-MHz) NMR spectrometer in $CDCl_3$–DMSO-d_6 with TMS as internal reference; chemical shifts are expressed in δ. Mass spectra were recorded on a JEOL JMS-D 300 instrument.

2-Amino-5-aryl-1,3,4-oxadiazoles IIa–d. These were prepared by oxidative cyclization of apropriate aldehyde semicarbazones (Ia–d) with bromine in glacial acetic acid in the presence of anhydrous sodium acetate (Gibson, 1962). Compounds IIa–d agreed well with their analytical data already reported in literature (Gehlen and Moeckel, 1962; Gibson, 1962; Hoggarth, 1949).

5-Aryl-2-[(*p*-fluorobenzylidene)amino]-1,3,4-oxadiazoles IIIa–d. A mixture of 2-amino-5-phenyl-1,3,4-oxadiazole (IIa; 3.22 g, 0.02 mol) and *p*-fluorobenzaldehyde (2.48 g, 0.02 mol) in absolute ethanol (35 mL) was refluxed for 4 h and filtered while hot. The filtrate upon cooling furnished the desired product IIIa, which was recrystallized from ethanol as yellowish needles: yield 4.16 g (78%); mp 221–223 °C; IR, 1665 (exocyclic C=N), 1620 cm^{-1} (cyclic C=N). Anal. Found: C, 67.61; H, 3.80, N, 15.82. Calcd for $C_{15}H_{10}FN_3O$: C, 67.41; H, 3.74; N, 15.73.

The following compounds were similarly prepared and recrystallized from ethanol. IIIb: yield 85%; mp 228–230 °C; IR, 1660 (exocyclic C=N), 1625 cm^{-1} (cyclic C=N). Anal. Found: C, 59.49; H, 3.02; N, 14.12. Calcd for $C_{15}H_9ClFN_3O$: C, 59.70; H, 2.98; N, 13.93. IIIc: yield 90%; mp 233–235 °C; IR, 1670 cm^{-1} (exocyclic C=N), 1615 cm^{-1} (cyclic C=N); ^{1}H NMR, 3.76 (3 H, s, OCH_3), 7.50–8.22 (9 H, m, aromatic *H* and N=C*H*). Anal. Found: C, 64.52; H, 4.09; N, 14.21. Calcd for $C_{16}H_{12}FN_3O_2$: C, 64.64; H, 4.04; N, 14.14. IIId: yield 80%; mp 203–204 °C; IR, 1670 (exocyclic C=N), 1615 (cyclic C=N); ^{1}H NMr, 3.78 (3 H, s, OCH_3), 7.50–8.20 (9 H, m, aromatic *H* and N=C*H*). Anal. Found: C, 64.77; H, 4.10; N, 14.23. Calcd for $C_{16}H_{12}FN_3O_2$: C, 64.64, H, 4.34; N, 14.14.

2,6,7-Triaryl-6,7-dihydro-1,3,4-oxadiazolo[3,2-*a*]-*s*-triazine-5-thiones IVa–l. An equimolar mixture of the Schiff base III and appropriate isothiocyanate was refluxed for 4–6 h in dry toluene, and the solvent was distilled off under reduced pressure. The residue was washed with a small amount of ethanol followed by water, and the product was recrystallized from ethanol as shining yellowish needles. Yields, melting points, molecular formulas, and elemental analyses of the compounds (IVa–l) thus synthesized are recorded in Table I. The spectral data are given in Table II.

Fungicidal Screening. The fungicidal activity of compounds IVa–l was evaluated in vitro against *A. niger* and *F. oxysporium* by the usual agar plate technique (Horsfal, 1945) at 1000, 100, and 10 ppm concentrations as described earlier (Singh et al., 1983a). Dithane M-45, a standard commercial fungicide, was also tested under similar conditions for comparison. the antifungal activity displayed by the tested compounds IVa–l is summarized in Table III.

RESULTS AND DISCUSSION

It is evident from the screening data (Table III) that all the tested compounds, IVa–l, inhibited more than 65% growth of both test fungi at 1000 ppm concentration. Of these, the most active compounds, IVb, IVf, and IVj, exhibited fungicidal action almost equivalent to that of Dithane M-45 at 1000 ppm concentration and inhibited 40–44.5% growth of both the fungal species even at 10 ppm concentration.

Although some of the screened compounds (IVb, IVf, and IVj) were highly toxic to *A. niger* and *F. oxysporium* at higher concentration (1000 ppm), the overall results are not as encouraging as one would expect from the combined performance of the two biolabile nuclei, i.e. 1,3,4-oxadiazole and *s*-triazine. This might be attributed to the partial saturation in the *s*-triazine nucleus resulting in the loss of planarity of the oxadiazolo-*s*-triazine ring system. This presumption is supported by the earlier observations that compact size and planarity of a molecule often enhance its pesticidal activity (Chatt et al., 1956; Fischer and Summers, 1976; Rothwell and Wain, 1963; Singh et al. 1981, 1983a).

It is, however, noteworthy that the introduction of chloro, methoxy, and methyl groups in the aryl moiety of these compounds tends to augment the fungitoxicity and that the introduction of methoxy or methyl group at the ortho position is more effective than that at para position. Likewise, the introduction of chloro group was far more effective than that of methoxy and/or methyl group. The fungicidal activity varied marginally with the fungal species.

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Registry No. Ia, 1574-10-3; Ib, 5315-86-6; Ic, 5346-30-5; Id, 6292-71-3; IIa, 1612-76-6; IIb, 33621-61-3; IIc, 5711-59-1; IId, 5711-61-5; IIIa, 114719-70-9; IIIb, 114719-71-0; IIIc, 114719-72-1; IIId, 114719-73-2; IVa, 114719-74-3; IVb, 114719-75-4; IVc, 114719-76-5; IVd, 114719-77-6; IVe, 114719-78-7; IVf, 114719-79-8; IVg, 114737-74-5; IVh, 114719-80-1; IVi, 114719-81-2; IVj, 114719-82-3; IVk, 114719-83-4; IVl, 114719-84-5; *p*-FC_6H_4CHO, 459-57-4; PhNCS, 103-72-0; 2-$CH_3C_6H_4NCS$, 614-69-7; 4-$CH_3C_6H_4NCS$, 622-59-3.

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Coumaphos Degradation in Cattle-Dipping Vats

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Coumaphos [*O,O*-diethyl *O*-(3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl) phosphorothioate] is used for the control of cattle ticks. Cattle are dipped in large vats containing a solution of coumaphos at 0.1%–0.2% (ai). Recently, several vats (problem vats) have experienced unexplained and persistant losses of coumaphos. Experiments were initiated with cattle dip from five sites in Texas (four nonproblem and one problem vat) to obtain information on the fate of coumaphos. Aerobically, coumaphos was degraded in all vat samples. Anaerobically, coumaphos was reductively dechlorinated to potasan [*O,O*-diethyl *O*-(4-methyl-2-oxo-2*H*-1-benzopyran-7-yl) phosphorothioate] only in high-use vats (including the problem vat). Samples from the problem vat were also obtained biweekly over the lifetime of the vat. Initial concentrations of coumaphos decreased with time, while concentrations of potasan increased. Aerobically, coumaphos was degraded in all samples. Anaerobically, the rate of reductive dechlorination increased with vat age. Experiments with [*benzo ring*-U-^{14}C]coumaphos demonstrated that the aromatic portion of coumaphos was mineralized.

Microbial degradation of pesticides is an important process in the removal of these compounds from the environment. Compounds that are not readily degraded by soil and water microorganisms tend to accumulate and persist in the biosphere. Alternatively, biodegradation of a pesticide at too rapid a rate may cause a loss of efficacy for that particular pesticide and other structurally related compounds. Over the last several years, this rapid or enhanced metabolism has been documented in the "problem" soils phenomenon seen with soil-incorporated herbicides and insecticides (Harris et al., 1984; Read, 1983).

Recently, a loss of efficacy has been observed with the organophosphate pesticide coumaphos in cattle-dipping solutions. Coumaphos [*O,O*-diethyl *O*-(3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl) phosphorothioate] is used as an acaricide for the control of the southern cattle tick (*Boophilus microplus*) and the cattle tick (*Boophilus annulatus*) by the USDA Animal and Plant Health Inspection Service (APHIS) in its Tick Eradication Program. Annually, several hundred thousand cattle are dipped for tick control along the U.S.–Mexican border by APHIS, in one of 42 vats. Each of these vats contains approximately 12 000 L of a solution of Co-Ral flowable cattle insecticide (42% coumaphos, 58% inert ingredients) mixed with water to give a final concentration of 0.1%–0.2% (ai). The concentration of coumaphos in each vat is monitored weekly by onsite personnel using a colorimetric assay kit supplied by the manufacturer of coumaphos and is confirmed by APHIS' Analytical Chemistry Laboratory in Ames, IA. Normally, the levels of coumaphos in the vats are very stable over time such that any decrease in coumaphos concentration is due to removal by cattle. These vats are emptied, cleaned, and recharged annually because of fouling by soil and animal wastes. Recently, however, several vats (problem vats) have experienced unexplained and persistent losses of coumaphos that are probably due to microbial degradation. Since these vats are constantly monitored and the losses of pesticide are well documented over several years, they can serve as excellent experimental systems for the study of how microbial degradation affects the efficacy of a pesticide that must persist for a finite period of time.

The purpose of these experiments was to study the role of biological degradation in "problem vats" and determine the metabolic fate of coumaphos in several working dip vat solutions.

METHODS AND MATERIALS

Chemicals. Analytical-grade and formulated coumaphos, potasan [*O,O*-diethyl *O*-(4-methyl-2-oxo-2*H*-1-benzopyran-7-yl) phosphorothioate], and chlorferon (3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-ol) (Figure 1) were gifts from Animal Health Division, Mobay Corp. (Shawnee, KS 66201). [*benzo ring*-U-^{14}C]Coumaphos (sp act. 21.1 mCi/mmol) was also a gift from Mobay. Labeled

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