Fluorolipids as Targeted Termiticides and Biochemical Probes

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Alkyl diacylglycerols and triacylglycerols containing strategically located ω-fluoroalkyl or ω-fluoroacyl groups have been prepared and tested as delayed-action termiticides by using the Eastern subterranean termite *Reticulitermes flavipes*. Slow hydrolysis of ω-fluoroalkoxy moieties provides increased delay in toxicity at doses equivalent to those achieved with ω-fluoroacyl groups. Termites are killed by 20–200 ng of fluorolipid per individual for most compounds tested, with toxicity delays of 24–72 h and complete kill in 48–180 h. Thus, a mature colony of 60 000 termites could be eliminated with 1–10 mg of a properly administered fluorolipid. In addition to their potential economic importance, fluorolipids provide new insight into acylglycerol metabolism and transport in a primitive eusocial insect.

In the preceding paper (Prestwich et al., 1981), we presented the preparation of (ω-fluoroalkyl)- and (ω-fluoroacyl)glycerides, and we described the rationale for their potential use as targeted, delayed-action toxicants in the bait block method of control for subterranean termites (Beard, 1974; Esenther and Beal, 1974, 1978). We report here the results of laboratory toxicity tests employing these fluorolipids and others (Scheme I) with the Eastern subterranean termite *Reticulitermes flavipes*. The latent toxicity of these synthetic fluorolipids provides (1) an economical alternative to Mirex in the bait block technique and (2) experimental data on the absorption and catabolism of acyl(alkyl)glycerols in termites in vivo.

MATERIALS AND METHODS

Synthetic Fluorolipids. The 1-*O*-alkyl-2,3-*O*-diacylglycerols (**5**, **6**, and **10**), the parent (*E*)- and (*Z*)-16-fluoro-9-hexadecenoic acids (**1** and **2**), the (*E*)-16-fluoro-9-hexadecen-1-ol (**8**), the synthetic intermediate **9**, byproduct **11**, and ether **12** were prepared as described by Prestwich et al. (1981). In addition, for this study we required the triacylglycerol derivatives **4** and **7** and intermediate **3**, for which synthetic procedures and spectral data are provided below.

1-O-[(E)-16-Fluoro-9-hexadecenoyl]-2,3-O-isopropylidene-rac-glycerol (**3**). A solution of 750 mg (2.76 mmol) of fluoro acid **1** in 15 mL of dry, distilled dichloromethane containing 43 mg (0.35 mmol) of 4-(dimethylamino)pyridine (Aldrich) and 457 mg (3.46 mmol) of solketal (Aldrich, 2,3-*O*-isopropylidene-*rac*-glycerol) was cooled to 0 °C with stirring under N_2, while 625 mg (3.03 mmol) of dicyclohexylcarbodiimide (Aldrich) was added in one portion (Neises and Steglich, 1978). The solution was stirred 5 min at 0 °C and overnight at 25 °C. The precipitated urea was removed by filtration, and the CH_2Cl_2 solution was washed with 0.5 N HCl and saturated $NaHCO_3$ solution, dried ($MgSO_4$), and concentrated in vacuo to give 669 mg (61%) of crude **3**. A portion (150 mg) was chromatographed on Florisil (100–200 mesh) by elution with 5% (v/v) ethyl acetate in hexane to give 125 mg of **3** as a clear oil which was homogeneous by GLC and TLC (R_f 0.30): IR (film) 1735 cm^{-1} (C=O), 1380, 1370 (*gem*-dimethyl), 975 (*trans*-HC=CH); NMR ($CDCl_3$) δ 5.38 (vinylic, br t, 3 Hz, 2 H), 4.38 ($-CH_2F$, dt, 48 Hz, 6 Hz, 2 H), 4.27 (glyceryl H-2, quintet, 6 Hz, 1 H), 4.12 (glyceryl H-1, m, 2 H), 3.98 (glyceryl H-3a, d, 6 Hz, 1 H), 3.69 (H-3b, dd, 8 Hz, 6 Hz, 1 H), 2.33 ($-CH_2CO-$, t, 7 Hz, 2 H), 1.95 (allylic, m, 4 H), 1.44, 1.37 (*gem*-dimethyl, s, s, 6 H). Anal. Calcd for $C_{22}H_{39}O_4F$: C, 68.36; H, 10.17; F, 4.91. Found: C, 68.50; H, 10.39; F, 4.78.

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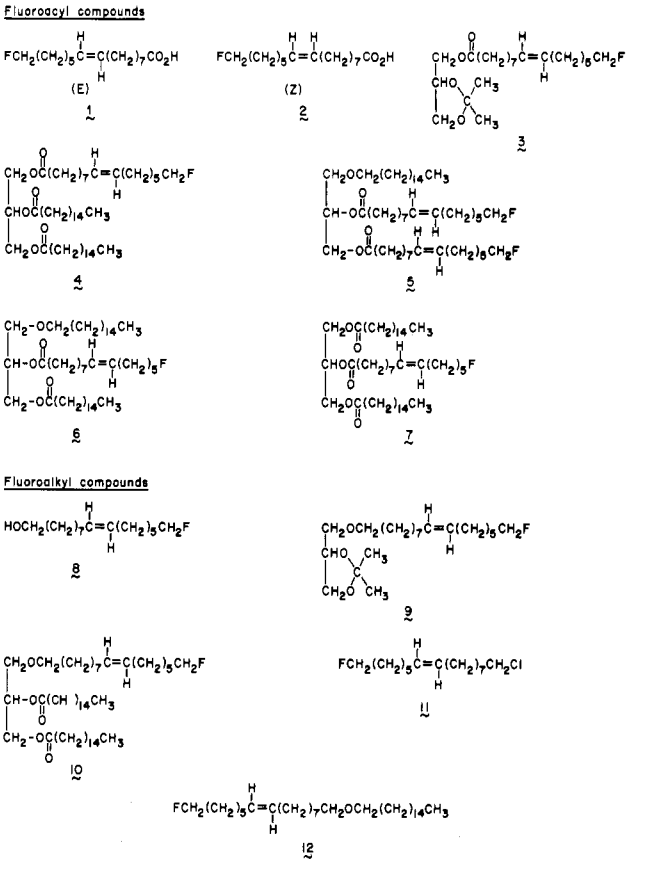
Scheme I. Synthetic Fluorolipids for Bioassays



1-O-[(E)-16-Fluoro-9-hexadecenoyl]-2,3-O-dihexadecanoyl-rac-glycerol (**4**). Following the methods outlined by Jensen and Pitas (1976), the protecting group was removed by controlled acid hydrolysis; the hexadecenoyl groups were introduced by using the DMAP/DCC procedure of Neises and Steglich (1978) as described above. Thus, a solution of 250 mg of crude **3** in 2 mL of ether was cooled to −15 °C and treated with 2 mL of concentrated HCl, also at −15 °C. The mixture was allowed to stand at this temperature for 30 min with occasional agitation, poured into ice-cold ether (20 mL), washed with four portions of ice water, dried ($MgSO_4$), and concentrated in vacuo to give an oily solid. Crystallization from hexane at −10 °C gave white microcrystals of the 1-*O*-[(*E*)-16-fluoro-9-hexadecenoyl]-*rac*-glycerol, mp 43–46 °C, which showed the loss of the *gem*-dimethyl group in the NMR spectrum and R_f 0.01 on TLC.

The recrystallized monoacylglycerol (92 mg, 0.23 mmol) was acylated with 166.7 mg (0.65 mmol) of palmitic acid (Sigma grade II) in the presence of DCC (134 mg, 0.65 mmol) and 4-DMAP (8 mg, 0.07 mmol) in 10 mL of dry

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CH_2Cl_2 as described above to give 188 mg of crude **4**. Chromatography on Florisil with 5% (v/v) ethyl acetate in hexane gave 109 mg (60%) of the TLC homogeneous (R_f 0.39) **4** as a white solid: mp 49.5–50.5 °C; IR (film) 1740 (C=O), 975 cm^{-1} (*trans*-HC=CH); NMR ($CDCl_3$) δ 5.38 (vinylic, br t, 3 Hz, 2 H), 5.12 (glyceryl H-2, m, 1 H), 4.38 ($-CH_2F$, dt, 48 Hz, 6 Hz, 2 H), 4.2–3.9 (glycerol H-1 and H-3, m, 4 H), 2.32 ($-CH_2CO-$, t, 7 Hz, 6 H), 1.95 (allylic, m, 4 H), 0.90 ($-CH_3$, br t, 6 Hz, 6 H). Anal. Calcd for $C_{51}H_{95}O_6F$: C, 74.40; H, 11.63; F, 2.31. Found: C, 74.17; H, 11.63; F, 2.15.

1,3-O-Dihexadecanoyl-2-O-[(E)-16-fluoro-9-hexadecenoyl]-rac-glycerol (**7**). 1,3-Dipalmitin (Sigma grade I, less than 2% of 1,2 isomer) (100 mg, 0.18 mmol), was condensed with fluoro acid **1** (54 mg, 0.2 mmol) in the presence of DCC (36 mg, 0.18 mmol) and DMAP (1 mg, 0.04 mmol) as described above to give the crude triacylglycerol **7**. Chromatography on Florisil with 5% (v/v) ethyl acetate in hexane gave 98 mg (65%) of the TLC homogeneous (R_f 0.48) **7** as a white solid: mp 52.0–52.5 °C; IR (film) 1740 (C=O), 970 cm^{-1} (*trans*-HC=CH); NMR ($CDCl_3$) 5.37 (vinylic, br t, 3 Hz, 2 H), 5.12 (glyceryl H-2, m, 1 H), 4.38 ($-CH_2F$, dt, 48 Hz, 6 Hz, 2 H), 4.2–3.9 (glyceryl H-1 and H-3, m, 4 H), 2.30 ($-CH_2CO-$, t, 7 Hz, 6 Hz), 1.94 (allylic, m, 4 H), 0.90 ($-CH_3$, br t, 6 Hz, 6 H). Anal. Calcd for $C_{51}H_{95}O_6F$: C, 74.40; H, 11.63; F, 2.31. Found: C, 74.68; H, 11.43; F, 2.32.

Bioassay Experiments. Synthetic fluorolipids were dissolved in dry diethyl ether, and an aliquot of a hexane solution of either [16-^{14}C]palmitic acid (PA) or [*carboxyl*-^{14}C]tripalmitin (TP), both purchased from New England Nuclear, was added to the test solution. An aliquot of the labeled test solution was counted on an Intertechnique scintillation counter by using an Omnifluor–toluene–ethylene glycol monomethyl ether (4 g:500 mL:500 mL) cocktail. Counting was 85% efficient for ^{14}C. Quench curves were determined when needed. All volumetric transfers were made with ±5% accuracy by using a Gilson variable-volume Pipetteman. A 1.0-mL portion of a solution of the test compound in anhydrous ether (Fisher) was pipetted onto a sterile 47-mm cellulose pad (Gelman) (Howard and Haverty, 1979). The ether was allowed to evaporate for 10 min, 1.0 mL of doubly distilled deionized water was added to the pad, and the pad was placed in a tight-sealing 50 mm diameter plastic Petri dish (Gelman).

Termites (*R. flavipes* Kollar) were collected from rotten stumps on the Stony Brook campus. Colonies were kept in trash cans in an insectary (85% relative humidity, 27 °C) when not in use. Incubation experiments were performed in the insectary or in an incubator at +28 °C. Undifferentiated larvae above the third instar ("workers") were used in toxicity tests unless otherwise noted. Termites were transferred by using mouth aspirators, as handling with forceps was found to cause additional mortality. Twenty termites per dish were added to the radioisotopically labeled, lipid-treated, moist pads, and the number dead was recorded at daily intervals. Dead termites were removed from test dishes and frozen at daily intervals; when all termites in a given replicate had died, the corpses were homogenized (Sorvall Omnimixer microassembly; 5 min at 6000 rpm) in 2.0 mL of 1:1 methanol–chloroform, and 1.0 mL of this solution was counted to provide an estimate of the lethal quantity of lipid ingested per termite. Each treatment was performed in six replicate dishes; data for mortality and lethal dose are reported as means ± standard error of the mean.

Finally, experiments were performed to correlate the amount of food (cellulose pad) consumed per day with the amount of radioactivity incorporated per day for each of the two labeled tracers employed. Thus 6 replicates of 20 termites each were held at 28 °C on pads containing only 1 mL of H_2O, 1 mg of palmitic acid, and a known quantity of TP or PA. One replicate was frozen each day for 6 days. Termites were divided into four groups in a given dish (to obtain a measure of variance) and then homogenized and counted as described above. The data were subjected to least-squares regression to determine whether incorporation of the radiolabels into lipids (i.e., quantity of food consumed) was occurring at a constant rate.

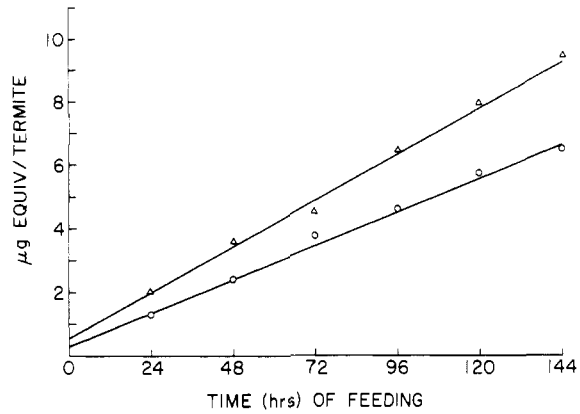

Figure 1. Feeding occurs at a constant rate. The radioactivity of termite lipid extracts (1:1 chloroform–methanol) was monitored as a function of feeding time. Open circles represent the rate of feeding as measured with [16-^{14}C]palmitic acid (PA), and open triangles indicate feeding as measured with [*carboxyl*-^{14}C]tripalmitin. Each point represents the mean of four replicates. Regression lines both give correlations of r = 0.96.

RESULTS AND DISCUSSION

Consumption of cellulose pads treated with [16-^{14}C]-palmitic acid and [*carboxyl*-^{14}C]tripalmitin proceeded at a constant rate during the 1-week observation period (Figure 1). For a 1-mg dose per pad, this rate was 1.45 $\mu g\ day^{-1}$ $termite^{-1}$ for TP and 0.99 $\mu g\ day^{-1}$ $termite^{-1}$ for PA for the level of radioactivity employed in this experiment (~40 000 dpm/pad). The quantity of fluorolipids ingested by a given group of termites was calculated by determining the incorporation of labeled PA or TP into the termites which had died as a result of ingestion of a given latent poison. The values obtained in this fashion for compounds in Scheme I are presented in Table I and represent the mean lethal doses for ingestion by *R. flavipes* workers. It is important to note that in general the quantity of fluorolipid ingested prior to death quantitatively reflects the amount of fluorolipid present in the test pad rather than a fixed minimum lethal dose. That is, the termites feed ad libitum on the impregnated cellulose pad, and they can easily ingest 10–20-fold the lethal dose before toxicity occurs. Moreover, comparison of the ingested quantities of fluorolipids with the 1 mg/day rate determined for PA-tracer experiments shows that the feeding is not deterred during the lag-time period of 1–3 days prior to the onset of toxicity. Feeding slows considerably after several days as the effect of fluoroacetate on the termite metabolism becomes evident in the lethargic behavior and ataxic movements of the poisoned individuals.

Preliminary experiments showed that low doses of ω-fluoro fatty acids would not be suitable for termite control, since feeding must continue over a 2-day period for a 0.2-mg dose to give complete kill in 1 week. Higher doses resulted in more rapid and complete intoxication, but the delay time seemed to be insufficient to allow adequate food sharing between foragers and other colony members

Table I. Toxicity of Fluorolipids Measured by Radioisotope Incorporation into Dead Termites[a]

compd	dose, mg/dish	tracer	lethal dose, μg/termite
1	0.2	PA	0.23 ± 0.04
	2	PA	2.29 ± 0.35
2	2	PA	2.69 ± 0.45
3	1	TP	0.16 ± 0.02
4	1	TP	0.24 ± 0.10
	0.2	PA	0.23 ± 0.06*
5	5	TP	1.53 ± 0.09
	1	TP	0.21 ± 0.04
6	5	TP	2.73 ± 0.64
	1	TP	0.34 ± 0.06
	0.2	PA	0.33 ± 0.03*
7	1	PA	1.10 ± 0.15
	0.2	PA	0.26 ± 0.07
8	0.2	PA	0.37 ± 0.02
	0.02	PA	0.023 ± 0.004
9	1	TP	0.42 ± 0.06
10	1	TP	0.33 ± 0.10
	0.2	PA	0.28 ± 0.11*
11	5	PA	2.46 ± 0.33
	1	TP	0.13 ± 0.02
	0.2	PA	0.25 ± 0.03*
12	1	PA	1.35 ± 0.40*
	0.2	PA	0.22 ± 0.08*

[a] Values shown represent the mean quantity consumed per termite ± 1 standard deviation for six replicates. TP indicates that [*carboxyl*-^{14}C]tripalmitin was the tracer, and PA indicates that a [16-^{14}C]palmitic acid tracer was employed. A single asterisk indicates incomplete (<50%) mortality after 1 week.

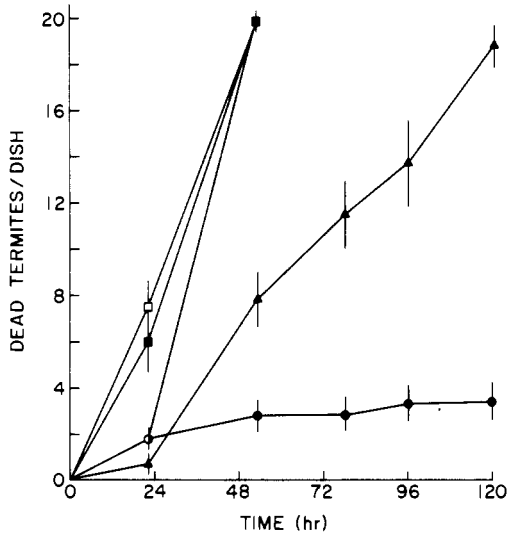

Figure 2. Toxicity of ω-fluoro acids and alcohols. Mortality (mean for six replicates ± SE) is plotted vs. time of feeding for the H_2O control (filled circles), 2 mg of *E* acid 1 (open circles), 2 mg of *Z* acid 2 (open squares), 0.2 mg of alcohol 8 (filled squares), and 0.02 mg of alcohol 8 (filled triangles). These parent compounds exhibited insufficient delay times. The 10-fold higher toxicity of the alcohol was surprising.

(Beard, 1974). For determination of what lipid analogues would be most efficacious in termite control, 12 fluorolipids were tested at different dosages, and the time course of mortality (Figures 2–6) as well as the quantity of food consumed at death (Table I) were determined.

First, comparison of the simple fluoro acids 1 and 2 (2 mg/pad) and fluoro alcohol 8 (0.2 and 0.02 mg/pad) confirms the problem of achieving high toxicity over a large dose range with an adequate delay time (Figure 2). Fluoro acids kill too fast at 2 mg/mL and the fluoro alcohol kills too fast at 0.2 mg/pad. At the lower dosage (0.02 mg/pad),

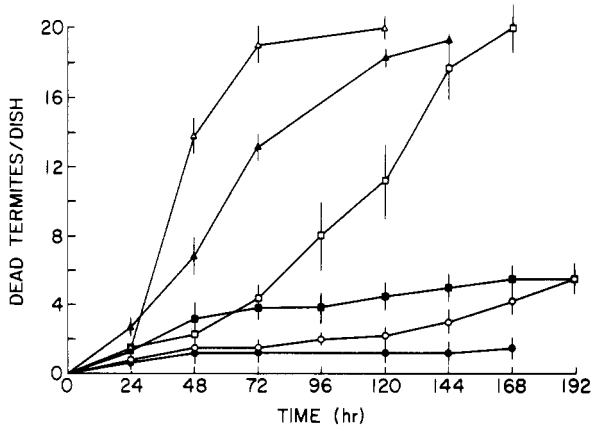

Figure 3. Toxicity of ω-fluoroacyl lipids. Mortality (mean for six replicates ± 1 SE) is plotted vs. time of feeding for 1.0 mg of 2,3-*O*-isopropylidene-*rac*-glycerol as the control (filled circles), 0.2 mg of 2-*O*-ω-fluoroacyl lipid 7 (open circles), 0.2 mg of 1-*O*-ω-fluoroacyl lipid 4 (filled squares), 1.0 mg of lipid 7 (open squares), 1.0 mg of lipid 4 (filled triangles), and 1.0 mg of the synthetic intermediate 3 (open triangles).

fluoro alcohol 8 required over 1 week to achieve total kill, and mortality increased in a linear fashion during this time. However, the higher toxicity of fluoro alcohol 8 relative to fluoro acids 1 and 2 is noteworthy; for analogous saturated C_{12} to C_{18} ω-fluoro compounds, Pattison et al. (1956) and Pattison (1959) found no difference in the LD_{50} in mice between the ω-fluoro acids and their corresponding ω-fluoro alcohols.

Incorporation of ω-fluoroalkyl and -acyl groups into glycerol derivatives should alter the timing of their absorption and metabolism. Most insects studied thus far transport glycerol derivatives through the hemolymph as specific lipoprotein complexes of 1,2-*O*-diacyl-*sn*-glycerols (Chino and Downer, 1979; Downer and Matthews, 1976; Gilbert, 1967; Gilbert and Chino, 1974), and lipolysis of the 2-acyl moiety is slowest. Introduction of a toxic fluoroacyl group in this position seemed likely to provide an increased lag time for the onset of toxicity, allowing more time for trophallactic food exchange of the latent poison with other colony members. It is possible that absorption, transport, and uptake by the fat body could all occur without the hydrolysis of the 2-acyl moiety.

The time course of mortality for the 1-*O*-(ω-fluoroacyl)-2,3-dipalmitoyl-*rac*-glycerol 4 and 2-*O*-(ω-fluoroacyl)-1,3-dipalmitoyl-*rac*-glycerol 7 is summarized in Figure 3. At 0.2 mg/pad, neither of these substrates resulted in substantial mortality after 1 week. At 1.0 mg/pad, the delay for 50% mortality was 120 h for the 2-*O*-(ω-fluoroacyl) derivative and 60 h for the 1(3)-*O*-(ω-fluoroacyl) derivative. Neither showed any mortality at 1 day and both gave complete kill in 1 week. These compounds now possess useful delay times but only marginally acceptable (5-fold) dosage ranges. In addition, this experiment provides in vivo evidence in support of the slower hydrolysis of the secondary acyl group relative to the primary acyl group. Finally, the rapid toxicity of synthetic intermediate 3 is noteworthy, indicating that a nonphysiological substrate may be more susceptible to rapid catabolism in vivo.

The time course of mortality for racemic 1-*O*-hexadecyl-2- and 2,3-*O*-bis(ω-fluoroacyl) lipids is presented in Figure 4. The choice of the hexadecyl ether moiety in the *rac*-1 position exploits the findings of Paltauf et al. (1974) that alkyldiacylglycerols are convenient models for triacylglycerols for studying lipase stereospecificity. At 0.2 mg/pad, 2-*O*-(ω-fluoroacyl) compound 6 achieved only 50% mortality after 1 week. However, at 1.0 mg/pad, the

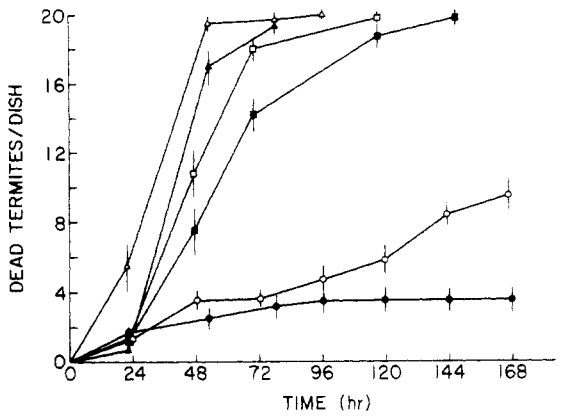

Figure 4. Toxicity of 1-*O*-hexadecyl-2,3-diacyl lipids. Mortality (mean for six replicates ± SE) is plotted vs. time of feeding for 1.0 mg of 1-*O*-hexadecyl-*rac*-glycerol as control (filled circles), 0.2 mg of 2-*O*-ω-fluoroacyl lipid **6** (open circles), 1.0 mg of lipid **6** (filled squares), 1.0 mg of di(fluoroacyl) lipid **5** (open squares), 5.0 mg of lipid **6** (filled triangles), and 5.0 mg of lipid **5** (open triangles).

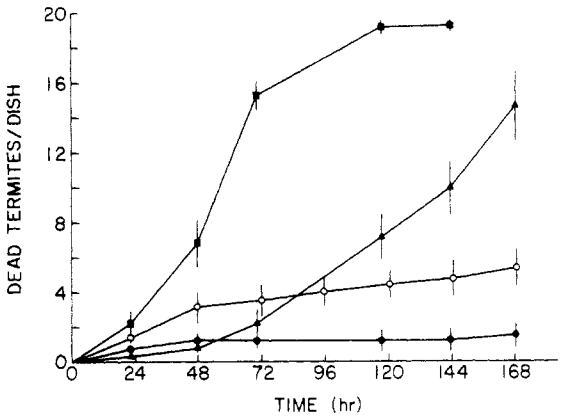

Figure 5. Toxicity of ω-fluoroalkyl ethers. Mortality (mean for six replicates ± 1 SE) is plotted vs. time of feeding for 1.0 mg of solketal as the control (filled circles), 1.0 mg of ω-fluoroalkyl hexadecyl ether **12**, (open circles), 1.0 mg of the ω-fluoroalkyl intermediate **9** (filled triangles), and 1.0 mg of the ω-(fluoroalkyl)dipalmitoylglycerol **10** (filled squares).

2-*O*-(ω-fluoroacyl)glycerol derivative **6** was 60% lethal in 3 days and 100% lethal in 5 days, while the 1,2-*O*-bis(ω-fluoroacyl) compound **5** gave 50% kill in 2 days and 100% kill in 4 days. At higher doses (5 mg/pad of **5** or **6**), this difference becomes negligible as mortality occurs too rapidly.

We feld that glyceryl ethers (alkoxy lipids) (Mangold, 1979) derived from ω-fluoro alcohols were attractive synthetic targets based on the slower cleavage of the ether linkage in vivo (Snyder et al., 1973). This cleavage, which may involve cis dehydrogenation of the 1-*O*-alkyl ether to the 1-*O*-alk-1′-enyl ether (Stoffel and LeKim, 1971) followed by hydrolysis of the vinyl ether to the ω-fluoro fatty aldehyde, might allow still greater delay in toxicity at dosages analogous to those for the corresponding ω-fluoroacyl compounds. Moreover, slow hydrolysis of alkyl chlorides (Pattison, 1959) to alcohols in vivo could provide a slow-release form of the highly toxic fluoro alcohol **8**.

The time course of mortality for a selection of ω-fluoroalkoxy compounds is shown in Figure 5. Our expectations that toxicity would be delayed were fully justified for the nonphysiological glycerol ether **9**. Poor adsorption of dialkyl ether **12** probably accounts for the low incorporation into termite lipids as well as the low toxicity of this compound. The ω-fluoroalkoxy lipid **10** showed a mortality time profile similar to that of the 2-*O*-(ω-

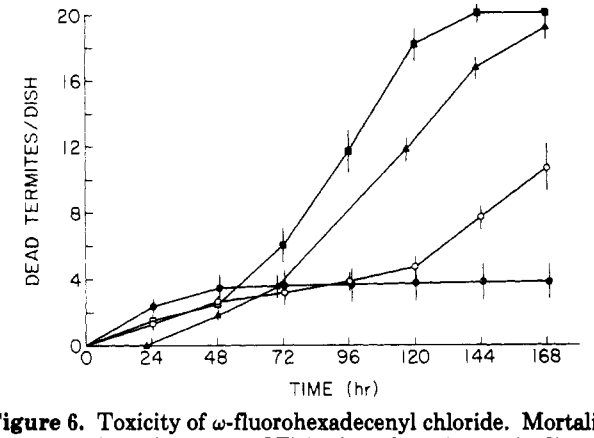

Figure 6. Toxicity of ω-fluorohexadecenyl chloride. Mortality (mean for six replicates ± 1 SE) is plotted vs. time of feeding for 1.0 mg of 1-*O*-hexadecyl-*rac*-glycerol as the control (filled circles), 0.2 mg of ω-fluoroalkyl chloride **11** (open circles), 1.0 mg of **11** (filled triangles), and 5.0 mg of **11** (filled squares). A 3-day toxicity lag is observed over a 25-fold range of concentrations, with complete mortality at 1 week over a 10-fold range.

fluoroacyl) compound **6** (see Figure 4), suggesting that 1-*O*-alkoxyl oxidative cleavage and 2-*O*-acyl hydrolysis may occur at comparable rates in vivo in termites. We are examining the relative rates of cleavage of these compounds by vertebrates in vivo to see if this relatively facile ether dealkylation may provide an increased insect/vertebrate toxicity ratio (Hollingworth, 1976).

Finally, the most promising candidate for use in bait blocks appears to be the (*E*)-1-chloro-16-fluoro-9-hexadecene **11**, which exhibits both a substantial time delay and a wide dosage range (Figure 6). At doses of 5, 1, and 0.2 mg/pad, no significant mortality occurred in the first 3 days of feeding. After 1 week, the higher doses gave complete kill while the lower dose had reached 50% mortality, with the remainder dying on days 8–10.

In evaluating chemicals for use as effective bait toxicants, Stringer et al. (1964) established three criteria: (a) delayed toxicity (less than 15% after 24 h and more than 90% mortality after 14 days) over a 10–100-fold range of doses, (b) easy transferrability from one social insect to another and resulting in mortality of the recipient, and (c) nonrepellency to the foragers. Over 5000 chemicals have been tested by the U.S. Department of Agriculture to replace Mirex in fire ant control, and a diminishingly small number have passed these criteria, other than chlorocarbons or organophosphous insecticides which are environmentally or otherwise unacceptable (Van der Meer, 1980). We believe that the fluorolipids described herein may provide a viable alternative to Mirex in termite and fire ant control by bait techniques, since they do satisfy all three criteria described above.

We have demonstrated that the incorporation of ω-fluoroacyl or ω-fluoroalkyl group into glycerol derivatives provides compounds of potential use in control of social (food-sharing) insects. Manipulation of the position and oxidation states of the toxic moiety can alter the latency period and therefore the rate at which the pesticidal compound is metabolized in vivo. The similarity of these latent poisons to normal physiological substrates, the triacylglycerols, precludes repellency; noningestion of the poisons is therefore not a problem. Low water solubility of the lipid compounds tested would enable their use in bait blocks in the soil with little problem of the toxic agents leaching out. Finally, the low dosage theoretically sufficient for complete kill of a mature termite colony implies that fluorolipids may be economical and environmentally acceptable termiticidal agents. We are actively pursuing

both the biochemical manifestations of our results using chiral, radiolabeled fluorolipids and the translation of our laboratory studies to control of termites in field situations.

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Metabolites of Diuron, Linuron, and Methazole Formed by Liver Microsomal Enzymes and Spinach Plants

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Mouse liver microsomal oxidases convert diuron [ArNHC(O)N$(CH_3)_2$; Ar = 3,4-dichlorophenyl] to seven metabolites modified only at the dimethylamino moiety [–N$(CH_3)CH_2OH$, –N$(CH_3)CHO$, –N$(CH_2OH)_2$, –NHCH_3, –NHCH_2OH, –NHCHO, and –NH_2] and to an *N*-hydroxy derivative [ArN(OH)C(O)NHCH_3]. Linuron [ArNHC(O)N$(OCH_3)CH_3$] in this system yields the corresponding –N$(OCH_3)CH_2OH$, –NHCH_3, and –N$HOCH_3$ derivatives. Microsomal metabolism of methazole [ArNC(O)N(CH_3)C(O)] requires NADPH and gives primarily desmethylmethazole under aerobic conditions and ArNHC(O)NHCH_3 under nitrogen. Most of these metabolites are also detected in spinach leaves treated with [^{14}C]diuron and [^{14}C]methazole. Some 3-hydroxy-1-methylurea from methazole metabolism in spinach is reduced to the 1-methylurea while the remainder is conjugated as the N–O-β-D-glucoside and its 6-*O*-malonyl ester (identified by ^{1}H and ^{13}C NMR). Synthesis procedures, spectroscopic data, and potencies as Hill reaction inhibitors are given for many of the metabolites and derivatives which are useful in their identification. Methazole appears to be a proherbicide while diuron and linuron act directly and via metabolites as photosynthetic inhibitors.

Three substituted urea herbicides share a common metabolite in both plants and mammals. 3-(3,4-Dichlorophenyl)-1-methylurea (DCPMU) is formed from diuron by oxidative N-demethylation, from linuron by oxidative N-demethoxylation, and from methazole by reduction of an intermediate *N*-hydroxy metabolite (Geissbühler et al., 1975; Suzuki and Casida, 1980). DCPMU and its 3-hydroxy derivative are potent Hill reaction inhibitors, as are diuron and linuron but not methazole (Suzuki and Casida, 1980). Several of the other liver microsomal oxidase metabolites of diuron and linuron are also Hill reaction inhibitors (Suzuki and Casida, 1980).

The present investigation considers the metabolic chemistry of diuron, linuron, and methazole in microsomal enzyme systems, the applicability of these findings to plants, and the identity of two conjugated methazole metabolites in spinach.

MATERIALS AND METHODS

Spectroscopy. Proton nuclear magnetic resonance (NMR) spectra were determined with a Perkin-Elmer Model R 32-B spectrometer at 90 MHz or a Nicolet NT-180 spectrometer at 180 MHz by using $CDCl_3$ for nonpolar compounds and acetone-d_6 for polar compounds with tetramethylsilane as the internal standard (δ = 0).

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