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Sugar derivatives containing oxiranes and α,β-unsaturated γ-lactones as potential environmentally friendly insecticides

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Abstract: A range of novel sugar derivatives containing oxiranes or α,β-unsaturated γ-lactones in their structure were evaluated as potential insecticides with the added possible benefit of being benign in the environment. A number of arthropod species were chosen to represent those in the terrestrial, aerial and aquatic environments, covering target adult insects such as *Musca domestica* L (housefly) and *Trialeurodes vaporariorum* (Westwood) (glasshouse whitefly), which are public health and horticultural pests, *Drosophila melanogaster* Meig (fruitfly), both adult and larva, and a marine non-target crustacean, *Artemia salina* L. The tested compounds possessed efficacy and selectivity against these insect species, but were not toxic to brine shrimps, a reference organism in assays to evaluate the potential toxicity hazard to invertebrates in ecosystems.

Keywords: insecticides; non-toxic; sugar derivatives; *Musca domestica*; *Trialeurodes vaporariorum*; *Drosophila melanogaster*; *Artemia salina*

1 INTRODUCTION

In the search for new pesticides, among the demanding list of prerequisites is that any new molecules selected must be safe to the environment and have no propensity to cause pollution.[1] With this in mind, we have chosen to search for novel pesticides by focussing on substances that may be non-polluting, namely sugar derivatives. They all possess a lactone moiety, which is α,β-unsaturated in some of the tested compounds, and an oxirane ring is also present in one of the synthesised molecules. Epoxides are part of the range of compounds recognised as active principles with biological and pharmacological activities.[2,3] Reference can be made to senepoxide, which is known to play an important role in plants as an insecticide and antifeedant.[4]

Methods for the preparation of oxiranes use halohydrins as intermediate compounds[5] and also vicinal diols,[6] glycals[7] and carbonyl compounds.[8] Some epoxy sugars and α,β-unsaturated sugar lactones have shown important and selective insecticidal activity.[9] The synthesis and fungicidal activity of such lactones has been described previously.[6,10] α,β-Unsaturated γ-lactones have been reported as natural products that play an important role in chemical defence in insects.[11]

The commercial insecticides most closely related to the compounds evaluated in this work include spinosad, the avermectins and milbemycins, which are all microbial natural products but incorporate macrocyclic lactones and sugar moieties which are structurally different. These natural product insecticides possess high degrees of activity, but can also present hazards to mammals and crustacea amongst other taxa.[12] Novel derivatives of avermectin have been shown to have high toxicity to *Artemia salina* L.[13] The insecticide spinosad, which is an agonist of the nicotinic acetylcholine receptor, shows high insecticidal activity against the housefly, *Musca domestica* L, by topical treatment with quantities as low as 0.054 μg per adult insect.[14] Thus, compounds possessing lactone and sugar functions are capable of potent insecticidal activity.

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There are several methods for testing the insecticidal activity of this type of compound, namely topical treatment of adult flies or their larvae, ingestion (feeding) or foliar treatment. In this work we present an evaluation of toxic action on a number of insect species which represent important pest groups, and a marine cladosteran which is often used to determine aquatic ecotoxicological aspects.

2 EXPERIMENTAL

The topical bioassays used the fruitfly, *Drosophila melanogaster* Meig, and the housefly, *M domestica*, and the plant-based assay used glasshouse whitefly, *Trialeurodes vaporariorum* Westwood, adults. The immersion bioassay of brine shrimp (*A salina*) larvae was used to simulate the toxicity of compounds **1–8** (Fig 1) in the aquatic environment. These assays were chosen to allow topical treatment of adults of two dipteran species combined with the larval stage of one of the species. Within the constraints of limited supply of the individual compounds this was intended to explore structural–insecticidal relationships and cuticular uptake according to life stage. These constraints meant that not all compounds could be tested on all target species and stages. Uptake from the aqueous phase (brine) by an aquatic crustacean further explored toxicity that may be dependant on physical properties of the compounds.

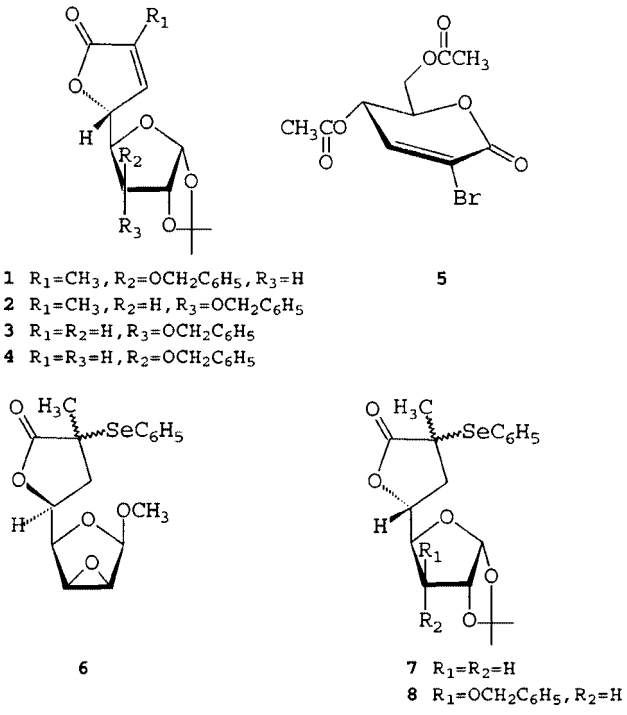

Figure 1. Structures of the tested compounds: 3-*O*-benzyl-6,7-dideoxy-1,2-*O*-isopropylidene-7-*C*-methyl-α-D-*gluco*-oct-6-enofuranurono-8,5-lactone (**1**); 3-*O*-benzyl-6,7-dideoxy-1,2-*O*-isopropylidene-7-*C*-methyl-α-D-*allo*-oct-6-enofuranurono-8,5-lactone (**2**); 3-*O*-benzyl-6,7-dideoxy-1,2-*O*-isopropylidene-α-D-*allo*-oct-6-enofuranurono-8,5-lactone (**3**); 3-*O*-benzyl-6,7-dideoxy-1,2-*O*-isopropylidene-α-D-*gluco*-oct-6-enofuranurono-8,5-lactone (**4**); 4,6-di-*O*-acetyl-2-bromo-2,3-dideoxy-D-*erythro*-hex-2-enono-1,5-lactone (**5**); methyl 2,3-anhydro-6,7-dideoxy-7-*C*-methyl-7-phenylselenyl-β-L-*gulo*-octofuranurono-8,5-lactone (**6**); 3,6,7-trideoxy-1,2-*O*-isopropylidene-7-*C*-methyl-7-phenylselenyl-α-D-*ribo*-octofuranurono-8,5-lactone (**7**); 3-*O*-benzyl-6,7-dideoxy-1,2-*O*-isopropylidene-7-*C*-methyl-7-phenylselenyl-α-D-*gluco*-octofuranurono-8,5-lactone (**8**).

2.1 Determination of the arthropod toxicity of compounds 1–8

2.1.1 Treatment of adults and larvae of Drosophila melanogaster

A culture of *D melanogaster* was used in the production of adults (body weight 0.23 mg). A wild-type strain (Blades Biological, Kent, UK) was reared on Drosophila Quickmix medium with yeast at 30 °C with a life cycle of about 7 days. Adult flies (both sexes) were randomly taken up to 1 day following eclosion for use in the bioassays. Serial dilutions of the compounds were prepared in acetone, and volumes of approximately 0.2 µl were applied to the ventral surface of each insect, using a calibrated PAX 100 microapplicator (Burkhard Scientific, Berkhamsted, UK) and a 1-ml SGE gas-tight syringe. The fruitflies, in small groups, were anaesthetised with carbon dioxide prior to treatment and afterwards placed in a vial to recover, and kept at 30 (±1) °C for observations of mortality at 1, 2, 3 and 24 h after treatment. For this purpose solutions of six concentrations of each compound and a control were employed in groups of 12 and 20 insects. Control mortalities were normally zero but occasionally rose to 5–10%. A similar method was used for the reference insecticide, imidacloprid, using technical grade (purity 99%) material.

For the topical treatment of *D melanogaster* larvae, these were separated from the growing medium and second and third instars were topically treated with acetone solutions (0.2 µl) of the compounds using a PAX microapplicator. Larvae were held in padded forceps for dosing and then placed on moistened filter paper supplemented with a small amount of food medium, in a Petri dish at 30 (±1) °C for observation at times zero, 1 and 24 h.

2.1.2 Treatment of Musca domestica *adults*

A culture of an insecticide-susceptible strain, Cooper, was established. Adult *M domestica* were fed on

sugar and milk and allowed to lay eggs on a larval medium made from organic wheat bran (250 g), skimmed milk powder (22 g), brewer's yeast (11 g) and deionised water (450 ml). The larval medium was replenished every 5 days; the containers with growing larvae were transferred to emergence cages and those adults emerging were pooled for selection for the bioassay. Temperature was a constant 25 °C with natural daylight. Three-day-old adult houseflies (body weight about 1.8 mg) were anaesthetised with carbon dioxide. Using the PAX microapplicator, an acetone solution (1 µl) of the test compound was applied to the dorsal cuticle, holding the fly in padded forceps. Groups of flies were placed into closed vials and kept at 29 (±1) °C for observation for 24 h.

2.1.3 *Foliar treatment of* Trialeurodes vaporariorum *adults*

Seedlings of tomato plants were infested with adult *T vaporariorum* collected from natural populations from a greenhouse of the Moorbank Botanic Gardens of Newcastle University. Selected leaves of the tomato plants were excised and carefully trimmed to three leaflets without disturbing the infestation. These were placed in glass tubes containing water and the leaflets were then sprayed on both sides with a small sprayer delivering for each leaflet a solution of the test compound in acetone + water (3 + 7 by volume; 200 µl). Controls were sprayed with the blank solvent alone.

Counts of insects on the individual leaflets were made immediately after spraying and then at 14 h following (temperature 24 °C).

2.1.4 *Immersion bioassay of* Artemia salina *larvae in brine*

Freshly hatched brine shrimps were prepared by adding aquarium brine shrimp eggs to salt water (15 g litre^{-1} sodium chloride in water) in a container with a large surface area. The following day hatchlings were separated from eggs and empty egg cases, using their phototropic movement and a Pasteur pipette.

Into each well of a 96-well microtitre plate was pipetted brine containing five shrimp larvae (195 µl), using a micropipette. The acetone solution (5 µl) of the test compound was added, the plate covered and kept at 30 (±1) °C, for observation for dead and alive shrimps at 1 and 24 h, using a binocular microscope.

Six concentrations and a control were used with 10 shrimps treated. A blank test was performed for comparison of the results.

2.2 Calculation of toxicity parameters

Dosages used in insect treatments were based upon the amount of compound applied to each insect. For the brine shrimps, the final concentrations of the compounds in the immersion brine were used.

The 24-h mortalities were used to calculate LD_{50}/LC_{50} values using regression analysis of the probability percentage mortality (probit) against log dose/concentration.[18] This was calculated using PoloPC software (LeOra Software, Berkeley, CA, 1994).

Where single dose treatments were used (in the case of *T vaporariorum* assays), no statistics were available and the results were expressed as percentage effect.

3 RESULTS AND DISCUSSION

3.1 Toxicity to *Drosophila melanogaster*

The results of assays with adults and larvae of *D melanogaster* are given in Table 1. For each compound a linear regression of percentage mortality (as probits) against $\log_{10}$ dosage has been made. From each of these has been obtained the LD_{50} value (and

Table 1. Toxicity parameters of compounds tested on fruitflies by topical treatment of adults and larvae

Compound	LD_{50} (µg per insect)	95% CI[a] (µg per insect)	χ^2 (*df*)[b]	Number of insects treated	Slope (±SE)	r^2	*g*[c]
Adults[d]							
1	2.00×10^{-5}	$0–11 \times 10^{-5}$	2.34 (6)	124	0.500 (±0.124)	0.91	0.24
2	2.27×10^{-6}	$0–20 \times 10^{-6}$	6.27 (9)	150	0.308 (±0.066)	0.82	0.18
3	5.02×10^{-6}	$0–80 \times 10^{-6}$	1.80 (6)	114	0.340 (±0.104)	0.84	0.36
4	1.20×10^{-4}	nd	10.68 (6)	114	0.385 (±0.109)	0.36	0.86
5	1.60×10^{-4}	$0–22.6 \times 10^{-4}$	1.45 (6)	114	0.296 (±0.096)	0.87	0.40
6	1.50×10^{-4}	$0–9.2 \times 10^{-4}$	9.81 (6)	114	0.696 (±0.155)	0.62	0.48
7	2.00×10^{-4}	$0–15.4 \times 10^{-4}$ (90%)	7.65 (6)	114	0.395 (±0.104)	0.73	0.53
8	3.70×10^{-4}	nd	14.94[e] (6)	114	0.453 (±0.115)	0.59	0.97
Imidacloprid	1.25×10^{-2}	$0.52–1.64 \times 10^{-2}$	1.05 (3)	75	2.218 (±0.911)	0.85	0.65
Larvae[d]							
2	Not toxic						
5	4.75	3.22–20.60 (90%)	0.20 (3)	75	1.602 (±0.674)	0.98	0.68
8	0.97	0.72–2.06	0.87 (3)	75	2.624 (±0.810)	0.94	0.37

[a] nd. data not available.
[b] For tabular critical (95%) values χ^2 test *df*: 3, 7.81; 6, 12.59; 9, 16.92;
[c] *g*: index of significance of potency, normally less than 0.4.
[d] Adult fruitfly (3 days old) weight 0.23 mg, larvae (2nd–3rd instar) 0.60 mg.
[e] Regression line not significant by χ^2 test.

confidence intervals at 95%), χ^2 and degrees of freedom (*df*), the slope (±SE) and the index *g* (of significance).[19] In addition the correlation coefficient r^2 is presented. For all compounds except one (**8**) the data fit the probit model, as χ^2 values are within the tabular critical values for 95% probability.[20] Thus, the data set (and most regression lines) for each bioassay provides a coherent description of the interaction between the compound and the animal.

The LD_{50} values show that all the compounds tested are very active against adult *D melanogaster*, with compounds **1, 2** and **3** extremely active. These are α,β-unsaturated lactones bonded to a protected furanose moiety, whereas the phenylselenyl derivatives are less active. All of them are much more active than the neonicotinoid imidacloprid, the reference insecticide chosen. Compared with the results obtained here on *D melanogaster*, avermectins also possess high topical toxicities (a wild-type strain had an LD_{100} value of 12 ng per fly).[13,21]

The slopes of the regression lines for the treatment of adults with all the compounds tested are similar, suggesting similar interactions with the insect, and much smaller than that for imidacloprid, indicating a different mode of action from this cholinergic receptor inhibitor. For most sugar compounds it is likely that the functional protecting groups provide the lipophilicity that facilitates their cuticular uptake into the adult insects.

The LD_{50} values obtained for compounds **5** and **8** against larvae are much higher than those obtained for the adults, and these compounds are therefore likely to be generally less toxic to larvae than to adult flies. Unfortunately, due to their limited availability, only these three compounds could be tested on the larvae. Compound **2** showed low activity at the highest dosage (13.3% mortality at 0.57 µg per insect). The slopes of the regression lines are higher than those for the adults, being closer to that for imidacloprid.

3.2 Toxicity to adult *Musca domestica*

Toxicity parameters for adult *M domestica* treated topically with compounds **5** and **8** are given in Table 2. The topical LD_{50} values are similar to those for *D melanogaster* larvae, even though the latter insects are approximately 8-fold larger. Thus, the compounds tested are much less toxic for this species than for *D melanogaster*, demonstrating a high degree of selectivity. However, the slopes are similar to those for adult *D melanogaster*, showing a related interaction.

Table 2. Toxicity parameters of compounds tested on housefly adults by topical treatment

Compound	LD_{50} (µg per insect)	χ^2 (*df*)[a]	Number of insects tested[b]	Slope (±SE)	r^2	*g*
5	1.065	0.17 (3)	40	0.228 (±0.146)	0.94	1.561
8	0.644	0.22 (3)	30	0.383 (±0.182)	0.95	0.863

[a] For tabular critical (95%) values χ^2 test *df*: 3, 7.81.
[b] Adult housefly (3 days old), weight 1.8 mg.

3.3 Insecticidal effect on adult *Trialeurodes vaporariorum*

The results of the bioassays of the insecticidal compounds on adult whiteflies *T vaporariorum* are given in Table 3. Five compounds were tested which were applied on the leaves of tomato plants infested with a known number of adult *T vaporariorum*. At 14 h after the spray application the number of dead insects (or eventually of insects that disappeared) was counted. From the data in Table 3 it is seen that only compound **4** shows insecticidal activity with this method of application. However, only a single low dose rate was evaluated, which limited the value of this assay.

3.4 Toxicity to *Artemia salina*

Toxicity parameters for assays in which *A salina* larvae are exposed for 24 h to the compounds in saline solution are given in Table 4. Regression analysis for each of these has provided the LC_{50} value (and

Table 3. Insecticidal effect against adult whitefly, assayed by spraying infested tomato leaves

Compound	Concentration (µg ml^{-1})	Control of whitefly (%)
3	1.3	0
4	2.5	50
6	1.3	0
7	4.8	0

Table 4. Toxicity parameters of compounds tested on brine shrimp larvae

Compound	LC_{50} (µg ml^{-1} in brine)	95% CI (µg ml^{-1})	χ^2 (*df*)[a]	Number of shrimps tested	Slope (±SE)	r^2	*g*
1	100.62	90.03–125.27	1.24 (3)	50	8.86 (±2.644)	0.91	0.34
2	64.30	57.75–77.28	1.05 (3)	50	9.22 (±2.638)	0.93	0.32
3	144.71	128.12–172.71	1.33 (3)	50	7.76 (±2.182)	0.99	0.30
4	358.92	324.89–400.42	3.01 (4)	60	9.77 (±2.057)	0.93	0.17
5	38.36	8.244–3258.70 (90%)	0.71 (2)	60	0.42 (±0.175)	0.95	0.66
6	125.48	113.08–139.97	1.65 (3)	50	10.28 (±2.421)	0.94	0.21
7	261.04	220.59–320.09 (90%)	3.54 (3)	50	8.60 (±2.195)	0.81	0.78
8	220.41	199.24–249.71	0.65 (3)	50	10.36 (±2.659)	0.98	0.25
Imidacloprid	0.0312	0.0239–0.0459	1.83 (3)	100	2.44 (±0.802)	0.85	0.42

[a] χ^2 tabular critical (95%) values: *df*, 2: 5.99, 3: 7.81, 4: 9.49.

confidence intervals at 95%), χ^2 and degrees of freedom (*df*), the slope (±SE) and the index *g* (of significance).[19] In addition the correlation coefficient r^2 is presented. For all compounds the data fit the probit model as χ^2 values are within the tabular critical values for 95% probability.

The LC_{50} values are much higher than for the commercial insecticide, imidacloprid, ($0.03\,\mu g\,ml^{-1}$ for imidacloprid compared with a range $38–358\,\mu g\,ml^{-1}$ for the sugar derivatives) indicating a low toxicity in aquatic milieux to crustacea. Large slopes were obtained for most compounds, even greater than that for imidacloprid, with the exception of compound **5** which was similar to the adult *D melanogaster* treatments. This compound was also unique, being a pyranoid α,β-unsaturated lactone. The use of regression line slopes in comparing the activities of the various compounds is illustrated in Fig 2, which compares the regression lines for this compound tested on all four arthropods/stages. The slopes of the lines for the two adult insects are similar, while those for the larvae and shrimps are different (note that for the shrimps concentration units are used in place of dose). A similar relationship is seen for compound **8** (Fig 3), another compound which was tested on all arthropods and stages. However, the slope for the treatment of *A salina* is even higher, along with the LC_{50} value, demonstrating that this sugar derivative has relatively safer potential environmental properties than compound **5**.

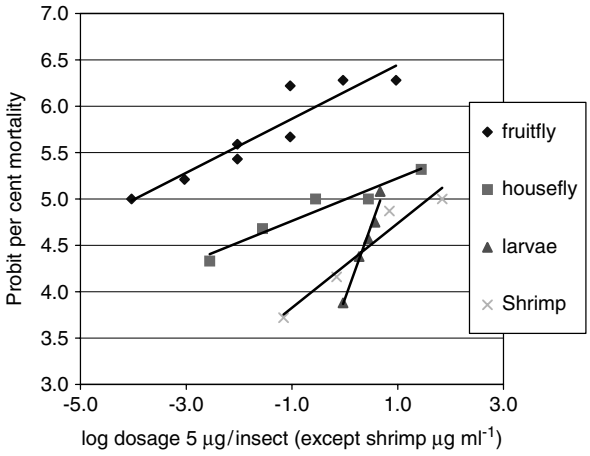

Figure 2. Mortality (probit) against log dosage following treatment of insects and shrimp with compound **5**.

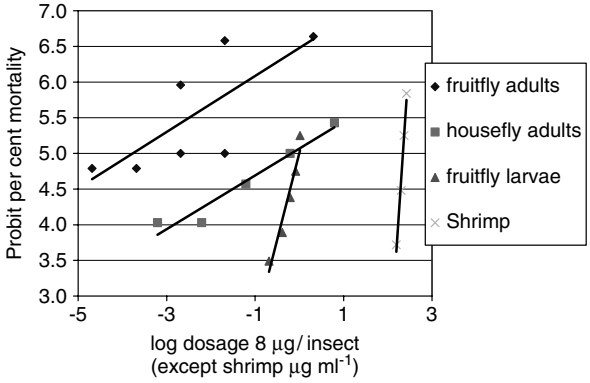

Figure 3. Mortality (probit) against log dosage following treatment of insects and shrimp with compound **8**.

4 CONCLUSION

Bioassays were performed using a range of compounds and treatment techniques on various species of arthropod. Adult *D melanogaster*, treated topically, showed high levels of sensitivity to the compounds tested, such that the LD_{50} values determined are much lower than that for the reference insecticide imidacloprid. However some variation was observed in the toxicity produced by the compounds. The slopes of the log-probit regression lines were generally small and much smaller than that for imidacloprid. Topical treatment of the larval stage had much less toxic effect but the regression line slope was uniformly higher than for the adults.

Some of the compounds were tested by topical application on adult *M domestica* and were much less toxic to this species than to *D melanogaster* adults. The slopes of the log-probit regression lines were similar to those obtained by the same type of treatment used in adult *D melanogaster*, suggesting a similar mechanism of action, although with less activity and some selectivity.

Contrary to the high insecticidal activity found, the compounds have a very low toxicity to the aquatic crustacean *A salina*. The high LC_{50} values are associated with steep regression lines. It can be concluded that these compounds show a very low toxicity towards this type of organism in a saline medium, potentially not producing toxicity in aquatic ecosystems.

In the test spraying some of the compounds onto leaves infested with adult *T vaporariorum* it was found that compound **4** showed promise of activity against this species. This compound also showed high toxicity against adult *D melanogaster*.

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