

Isolation, Characterization, and Biological Activity of Naphthoquinones from *Calceolaria andina* L.

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Two compounds recognized as responsible for the insecticidal activity of extracts of *Calceolaria andina* L. (Scrophulariaceae) have been isolated and characterized as 2-(1,1-dimethylprop-2-enyl)-3-hydroxy-1,4-naphthoquinone and the corresponding acetate, 2-acetoxy-3-(1,1-dimethylprop-2-enyl)-1,4-naphthoquinone. Their activities against 29 pest species and 9 beneficial species of arthropod from a total of 11 orders have been determined. Activities against homopteran and acarine species are of the same order as those of established pesticides, and, significantly, no cross-resistance is observed for strains resistant to established classes of insecticide. Mammalian toxicities are low.

Keywords: *Naphthoquinones; Calceolaria; insecticides; botanical; resistance-defeating*

INTRODUCTION

One of the major limiting factors to global food production is damage by pests, during both growth and storage stages. The most effective method of pest control at present, and for the foreseeable future, is the use of synthetic pesticides (Copping and Hewitt, 1998). However, the effectiveness of such chemicals is continually eroded by the development of resistance in the economically important pests (Denholm et al., 1998). Therefore, there is a continuing need for novel classes of pesticides with alternative modes of action to be identified and developed. Lead structures for subsequent optimization are chiefly sought by random screening of synthetic compounds or natural products, primarily of microbial or plant origin. Important aspects of the bioassay used to evaluate candidate compounds are the sensitivity of the test, the range of species tested, and the use of well-characterized strains that are either susceptible or resistant to established classes of pesticide to establish the presence or absence of cross-resistance.

In the case of insecticides, the plant kingdom was the starting point for the discovery of both pyrethroids and carbamates, two of the three major classes currently in use. As part of a program searching for further active compounds from plant sources, we identified insecticidal activity in extracts of the slipper plant, *Calceolaria*

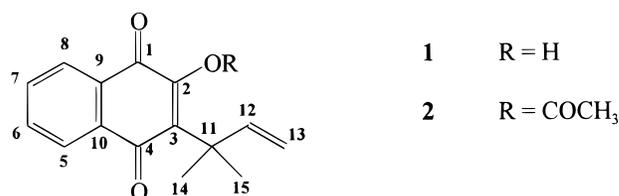


Figure 1. Structures of compounds **1** and **2**.

andina L. (Scrophulariaceae) (Khambay et al., 1995, 1997b). This paper details the isolation, characterization, and biological activities of the two compounds (Figure 1) responsible for such activity. The selection of species for bioassay screening was aimed at examining both beneficial and pest species across a broad spectrum of insects/acarids.

EXPERIMENTAL PROCEDURES

Plant Material. Aerial parts of the shrub *C. andina* L. (Scrophulariaceae) (Royal Botanic Gardens, Kew, Accession No. 1995-1009) were collected from the rocky slopes of the San Francisco river valley, ~2000 m above sea level, 33° 14' S, 70° 21' W, in Chile.

Isolation of Pesticidal Naphthoquinones. Comminuted plant material (450 g, leaves and stems) was extracted with hexane (2 × 1500 mL) using microwave irradiation (Panasonic NN-6452B, 800 W, 3 min) and filtered, and the filtrate was evaporated to dryness under reduced pressure to yield a green oil (10.8 g). This residue was chromatographed on silica gel

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Table 1. NMR Shifts for Isolated Compounds

atom no.	compound 1		compound 2	
	¹ H	¹³ C	¹ H	¹³ C
1		182.2		178.6
2	7.89 (OH)	152.9		150.7
3		128.4		143.9
4		184.8		185.1
5	8.05 (d) ^a	127.0	8.03 (d)	126.8
6	7.74 (dt)	135.2	7.73 (dt)	134.3
7	7.65 (dt)	132.6	7.69 (dt)	133.4
8	8.03 (d)	125.8	8.03 (d)	126.1
9		128.2		133.4
10		134.1		133.0
11		41.0		41.5
12	6.29 (dd)	148.1	6.19 (dd)	147.2
13	4.96 (d)	109.6	4.99 (d)	109.5
	4.98 (d)		4.96 (d)	
14, 15	1.57	28.1	1.53	27.6
16				168.3
17			2.32	20.7

^a d, doublet; t, triplet.

(60H, Merck 7736) eluted with petroleum ether/ether (2:1). Monitoring using the *Bemisia* bioassay identified fractions that gave >30% mortality counts at a concentration of 1%. These (4.7 g) were rechromatographed on silica gel (60H, Merck 9385) eluted with petroleum ether/ether (4:1) to give a major fraction (3.81 g), which NMR showed to contain two major components. A solution of this fraction (1.76 g) in ether (50 mL) was extracted with saturated aqueous sodium carbonate solution (4 × 50 mL). Acidification of the aqueous fraction to pH 5 with 2 M hydrochloric acid was followed by extraction with ether (3 × 40 mL). The combined extracts were washed with water (2 × 25 mL) and saturated sodium chloride solution (25 mL), then dried (MgSO₄), and evaporated under reduced pressure to give a red solid (226 mg), mp 60 °C, identified by NMR as compound 1 (see Table 1).

The organic layer remaining from the above base extraction procedure was similarly washed, dried, and evaporated to yield a red residue (1.53 g), which was rechromatographed on silica gel (60H, Merck 7736) eluted with petroleum ether (4:1). The major product (1.14 g) from this, mp 55 °C, was identified by NMR as compound 2 (see Table 1).

Spectroscopy. ¹H and ¹³C NMR spectra were measured in CDCl₃ with a JEOL GX-400 spectrometer. Shifts (in parts per million) are relative to internal tetramethylsilane. C–H correlations to confirm assignments were established using a waiting time of 1.9 ms (for 1-bond couplings) or 40 ms (for long-range couplings).

Bioassays. General Procedure. Strains of insect were from field populations or laboratory cultures, some of which were well-characterized for the presence of particular resistance mechanisms. Microdroplet application was via an Arnold microapplicator or a Rainin edp 2 electronic pipet. Unless otherwise stated, the test solutions of the compound were in 40% acetone-distilled water. After treatment, organisms were confined in controlled environment rooms at ~21 °C for 48 h (unless otherwise stated). Observed mortalities were corrected for control mortality using Abbott's formula. For brevity, only the most pertinent details of bioassays are presented below. Further details of all test protocols are available from the corresponding author on request.

Species (See Table 2 for Taxonomic Affiliations). *Lucilia sericata* Meig. (*Blowfly*). Adults (5 per batch, 2 batches per concentration) retrieved from carcasses were cooled and treated topically with the test compound in acetone (1 μL) and then kept in untreated vials in the presence of honey-impregnated cotton wool for 72 h.

Musca domestica L. (*Housefly*). Anesthetized 2–4-day-old adults (15 per batch, 2 batches per concentration) were treated topically with the test compound in acetone (0.5 μL) as described previously (Elliott et al., 1987). Bioassays were conducted against the standard susceptible Cooper strain and against strain 579sel expressing target-site resistance ("knock-

down resistance") on the order of 6–35-fold to pyrethroids and 20-fold to DDT (Farnham et al., 1987).

Callosobruchus maculatus Fab. (*Cow-pea Weevil*), *Diabrotica virgifera virgifera* L. and *D. undecimpunctata* Barber (*Corn Rootworms*), and *Tribolium castaneum* Herbst. (*Red Flour Beetle*). Insects (adults and also third-instar larvae for the *Diabrotica* species) (5 per batch, 2 batches per concentration) were treated topically with the test solution (1 μL) in acetone, transferred to containers with food (or the oviposition substrate, cowpea seeds, in the case of *C. maculatus*), and kept for 72 h.

Phaedon cochleariae Fab. (*Mustard Beetle*). Adult insects (20 per batch, 2 batches per concentration) were treated topically with the test solution (0.5 μL) in acetone and held in Petri dishes as described previously (Elliott et al., 1987).

Stegobium paniceum L. (*Drugstore Beetle*). Adult insects (10 per batch, 2 batches per concentration) were treated topically with the test solution (1 μL) and allowed to feed on a diet of wheat germ, glycerol, and yeast in Petri dishes.

Aphis gossypii Glover (*Cotton Aphid*). Apterous adults, cultured on chrysanthemums (var. White Fresco), were transferred to the adaxial surface of a chrysanthemum leaf disk (4.25 cm diameter) and left to settle for 2 h. The insects (10 per batch, 4 batches per concentration) were then drenched with 1 μL of the test solution.

Bemisia tabaci Genn. (*Cotton Whitefly*). Adults were retained for 2 h in a treated scintillation vial and then transferred to untreated cotton leaf disks in Petri dishes as described previously (Cahill et al., 1991). Results for a reference susceptible strain (SUD-S) are compared with data for a composite strain (NED) originating in several glasshouses in The Netherlands. Most individual strains from which the NED was derived exhibited very high (>100-fold) resistance to pyrethroids and high (50–100-fold) resistance to several organophosphates (Cahill et al., 1996a). Up to 50-fold resistance to the insect growth regulator buprofezin has also been documented in some of these strains (Cahill et al., 1996b).

Myzus persicae Sulzer. (*Peach–Potato Aphid*). Batches of 10–15 apterous adults (2 batches per concentration) on disks (35 mm diameter) cut from Chinese cabbage were dosed with microdroplets (0.25 μL) of the test compound in acetone and confined for 72 h. Results for a reference susceptible clone (USIL) are compared with those for a clone (794J) exhibiting very high (>100-fold) resistance to pyrethroids and organophosphates and weaker (~10-fold) resistance to pirimicarb conferred largely by overproduction (to R₃ levels) of insecticide-detoxifying carboxylesterases (Devonshire et al., 1982).

Planococcus citri Risso (*Citrus Mealy Bug*), *Frankliniella occidentalis* Pergande (*Western Flower Thrips*), and *Thrips tabaci* Lindeman (*Onion Thrips*). Insects (third-instar nymphs for *P. citri*, adults for thrips; 10 insects per batch, 2 batches per concentration) were transferred individually from their host plant to a glass slide with a fine camel hair brush and then treated with the test compound in acetone (0.1 μL). They were then transferred to a piece of absorbent paper and finally to host plant material (chrysanthemum for *F. occidentalis*, French dwarf bean leaf for *T. tabaci*, and sprouting potatoes for *P. citri*). Mortality was assessed after 72 h.

Trialeurodes vaporariorum Westwood (*Greenhouse Whitefly*). Adults cultured on mixed hosts (including tomato, dwarf French bean, and tobacco) were anesthetized with CO₂ and transferred with a soft, fine brush to a leaf disk (4.5 cm diameter) cut from dwarf French bean that had been dipped in the test solution, dried on absorbent paper, and placed on damp matting in a 4.5 cm diameter Petri dish. Insects were treated in batches of 10, with 4 batches per concentration.

Nephotettix virescens Distant (*Green Rice Leafhopper*). Adults cultured on rice (var. TN-1) were transferred using an aspirator to a clear delipot, lined with damp capillary matting through which a single rice seedling was inserted so that the roots were held under the matting. The ricehoppers (5 per batch, 4 batches per concentration) were then anesthetized with CO₂ and treated on the ventral surface with the test solution (1 μL).

Table 2. Summary of Laboratory Contact Bioassays

species	activity ^a		
	compound 1	compound 2	standard ^b
Pests			
Diptera			
<i>Lucilia sericata</i> (Calliphoridae)	23%/2000 ng	25%/2000 ng	4.8 ng
<i>Musca domestica</i> (Muscidae)	3400 ng	3000 ng	4 ng ^c
Coleoptera			
<i>Callosobruchus maculatus</i> (Bruchidae)	23%/2000 ng	21%/2000 ng	1.8 ng
<i>Diabrotica undecimpunctata</i> (Chrysomelidae)			
adults	35%/2000 ng	47%/2000 ng	1.2 ng
larvae	35%/2000 ng	34%/2000 ng	0.6 ng
<i>Diabrotica virgifera virgifera</i> (Chrysomelidae)			
adults	35%/2000 ng	35%/2000 ng	0.8 ng
larvae	140 ng	160 ng	0.14 ng
<i>Phaedon cochleariae</i> (Chrysomelidae)	700 ng	3000 ng	3.3 ng ^c
<i>Stegobium paniceum</i> (Anobiidae)		0%/500 ppm	45%/1 ppm
<i>Tribolium castaneum</i> (Tenebrionidae)			
adults	34%/2000 ng	23%/2000 ng	1.0 ng
larvae	60 ng	24%/2000 ng	0.8 ng
Homoptera			
<i>Aphis gossypii</i> (Aphididae)	45%/10 ng	32%/10 ng	67%/1 ng
<i>Bemisia tabaci</i> (Aleyrodidae)	7 ppm	5 ppm	0.5 ppm
<i>Myzus persicae</i> (Aphididae)	60 ng	250 ng	15 ng ^d
<i>Nephotettix virescens</i> (Cicadellidae)		60%/1000 ng	88%/1 ng
<i>Nilaparvata lugens</i> (Delphacidae)		42%/500 ppm	47%/10 ppm
<i>Planococcus citri</i> (Pseudococcidae)	190 ng	10 ng	9.4 ng
<i>Trialeurodes vaporariorum</i> (Aleyrodidae)	10 ppm	30 ppm	100%/0.1 ppm
Lepidoptera			
<i>Helicoverpa armigera</i> (Noctuidae)	45%/2000 ng	180 ng	1.2 ng
<i>Mamestra brassicae</i> (Noctuidae)		5%/1000 ng	
<i>Plutella xylostella</i> (Yponomeutidae)	50%/10000 ng	10%/5000 ng	4.4 ng ^c
<i>Spodoptera littoralis</i> (Noctuidae)	120 ng	100 ng	1.0 ng
<i>Spodoptera litura</i> (Noctuidae)	24%/2000 ng	140 ng	0.6 ng
<i>Spodoptera frugiperda</i> (Noctuidae)	28%/2000 ng	160 ng	0.08 ng
Orthoptera			
<i>Locusta migratoria</i> (Acrididae)	250 ng	24 ng	1.0 ng
Thysanoptera			
<i>Frankliniella occidentalis</i> (Thripidae)	170 ng	60 ng	3.6 ng
<i>Thrips tabaci</i> (Thripidae)	90 ng	7%/2000 ng	8.6 ng
Dictyoptera (Orthoptera)			
<i>Blatta orientalis</i> (Blattidae)	4%/2000 ng	32%/2000 ng	12 ng
<i>Periplaneta americana</i> (Blattidae)	3%/2000 ng	45%/2000 ng	16 ng
Acari			
<i>Tetranychus urticae</i> (Tetranychidae)	80 ppm	30 ppm	50 ppm
<i>Dermanyssus gallinae</i> (Dermanyssidae)	12 ppm	790 ppm	0.22 ppm ^e
<i>Ixodes ricinus</i> (Ixodidae)	120 ng	50 ng	22 ng ^e
Beneficial Species			
Hymenoptera			
<i>Aphidius rhopalosiphon</i> (Aphididae)	58%/100 ppm	56%/100 ppm	88%/1 ppm
<i>Encarsia formosa</i> (Aphelinidae)	33%/100 ppm	c.100 ppm	100%/0.1 ppm
<i>Trichogramma cacoeciae</i> (Trichogrammatidae)	77%/100 ppm	86%/100 ppm	35%/0.1 ppm
Coleoptera			
<i>Aleochara bilineata</i> (Staphylinidae)		0%/1000 ng	10%/10 ng
<i>Coccinella septempunctata</i> (Coccinellidae)		10%/1000 ng	100%/1 ng
Diptera			
<i>Episyrphus balteatus</i> (Syrphidae)	0%/500 ng	13%/500 ng	30%/10 ng
Neuroptera			
<i>Chrysoperla carnea</i> (Chrysopidae)		0%/1000 ng	0%/10 ng
Acari			
<i>Phytoseiulus persimilis</i> (Phytoseiidae)	5%/10 ppm	24%/10 ppm	100%/1 ppm
<i>Typhlodromus pyri</i> (Phytoseiidae)		0%/500 ppm	76%/10 ppm

^a Results are given as LD₅₀ or percent kill at a given dose in nanograms per insect for species assayed by a topical application procedure or as LC₅₀ or percent kill at a given concentration in parts per million for species assayed otherwise. ^b Bifenthrin, unless stated otherwise. ^c Bioresmethrin. ^d Pirimicarb. ^e Carbaryl.

Nilaparvata lugens Stal. (Brown Rice Leafhopper). Adults cultured on rice (var. TN-1) (10 per batch, 4 batches per concentration) on a damp filter paper in a 9 cm diameter Petri dish were sprayed with test solution at an application rate equivalent to 22 L/ha in a Potter laboratory spray tower (Burkard Manufacturing Co. Ltd., Rickmansworth, U.K.). The adults were then transferred into a clear delipot containing a rice seedling.

Helicoverpa armigera Hubner (American Bollworm), *Spodoptera littoralis* Boisduval (Egyptian Leafworm), *S. litura* Fab.

(Tobacco Cutworm), and *S. frugiperda* J. E. Smith (Fall Armyworm). Fifth-instar larvae, 6–12 h after molting, were treated topically with the test solution in acetone (2 μ L) and then kept in a Petri dish with artificial diet (Simmonds et al., 1992) for 72 h; 10–20 insects were treated per concentration.

Mamestra brassicae L. (cabbage moth) second- and third-instar larvae, cultured on an artificial diet, were transferred to the abaxial surface of a leaf disk (9 cm diameter) cut from cabbage (var. Greyhound) placed on a filter paper in a Petri dish (9 cm diameter). After settling, the larvae (10 per batch,

Table 3. Results from Bioassays against Resistant Strains

species	compound 1		RF ^b	compound 2		RF
	LC ₅₀ or LD ₅₀ ^a			LC ₅₀ or LD ₅₀		
	susceptible strain	resistant strain		susceptible strain	resistant strain	
<i>Musca domestica</i>	3400 ng	4000 ng	1.2	3000 ng	3000 ng	1.0
<i>Bemisia tabaci</i>	7 ppm	n/a ^c		5 ppm	3.5 ppm	0.7
<i>Myzus persicae</i>	60 ng	25 ng	0.4	250 ng	500 ng	2.0
<i>Tetranychus urticae</i>	80 ppm	44 ppm	0.6	30 ppm	33 ppm	1.1

^a See footnote a, Table 2. ^b Resistance factor, obtained by dividing LC₅₀(R) by LC₅₀(S). ^c Not available.

4 batches per concentration) were treated dorsally with 1 μ L of the test solution.

Plutella xylostella L. (diamondback moth) fifth-instar larvae (10 per batch, 3 batches per concentration) were treated topically with 0.5 μ L of the test compound in acetone and then retained in a Petri dish containing a cabbage leaf for 5 days. Failure to pupate was used as the assessment of mortality.

Locusta migratoria L. (desert locust) fifth-instar nymphs (5 per batch, 2 batches per concentration) were treated topically with the test compound in acetone (2 μ L), and mortality was recorded after 72 h of holding.

Blatta orientalis L. (oriental cockroach) and *Periplaneta americana* L. (American cockroach) 7–10-day-old adults (5 per batch, 2 batches per concentration) were treated topically with the test compound in acetone (2 μ L) and then kept in Petri dishes with pieces of carrot as food. Mortality was assessed 72 h after treatment.

Tetranychus urticae Koch (Two-Spotted Spider Mite). The microimmersion test (Dennehy et al., 1993), in which the test solution is in a 1:4 solution of acetone/water, was used. Results for a standard susceptible strain (UK-S) are compared with those for a composite population (NYR) originating from intensively sprayed apple orchards in New York state and exhibiting very strong resistance to chlorpyrifos (350-fold) and bifenthrin (200-fold) (Farnham et al., 1992).

Dermanyssus gallinae De Geer (Poultry Red Mite). The test solution (1.0 mL) was applied to the center of a filter paper (Whatman No. 1, 9.0 cm diameter) and allowed to dry. Two such papers, one with a square hole (20 \times 20 mm) cut in it, were sandwiched between microscope slides. Batches of 10 adult mites (3 batches per concentration) were confined in the resulting cell for 24 h.

Ixodes ricinus L. (Pasture Tick). Nymphal ticks were treated topically with microdroplets (0.25 μ L) of the compound in acetone, and mortality was assessed after 24 h. Ten individuals were treated per concentration.

Aphidius rhopalosiphii De Stephani (a Parasitoid of Aphids). Adults (cultured on *Metopolophium dirhodum* Walker) were held in a cold room (1–4 $^{\circ}$ C) and then transferred with a fine brush into a glass vial (14 mL volume) that had been treated with the test solution (0.15 mL) by swirling with evaporation until all of the inside was coated. The wasps (10 per batch, 2 batches per concentration) were confined by covering the mouth of the vial with fine meshed netting. Cotton wool soaked with a 1:3 solution of honey/water was provided for food.

Encarsia formosa Gahan (a Parasitoid of Whiteflies). Adults of both sexes (supplied by Biological Crop Protection, West Wittering, West Sussex) were anesthetized and assayed as described above for *T. vaporariorum*.

Trichogramma cacoeciae March (an Egg Parasitoid of Lepidoptera). Newly emerged adults supplied by Wuhrens Nützlingsservice, Germany (10 per batch, 2–3 batches per concentration), were placed in a vial (14 mL volume) that had been coated with the test solution and then confined there in the presence of a piece of filter paper containing honey/agar.

Aleochara bilineata Gyllenhal (a Predatory Beetle). Adults supplied by De Groene Vlieg, The Netherlands (5 per batch, 1 batch per concentration), in a Petri dish (9 cm diameter) lined with three damp filter papers were treated topically with 1 μ L of the test solution and then fed with a supply of cat food (Delicat).

Coccinella septempunctata L. (seven-spot ladybird) midinstar larvae (2 per batch, 5 batches per concentration) cultured on

a mixture of aphid species were treated topically with the test solution (1 μ L) and confined in Petri dishes containing vetch aphids, *Megoura viciae* Buckton, as food.

Episyrphus balteatus De Geer (a Hoverfly). Larvae (1 per batch, 10 batches per concentration) cultured on *Megoura viciae* were placed in 3-cm Petri dishes, treated topically with the test solution (1 μ L), and confined with *M. viciae* as food.

Chrysoperla carnea Stephens (Green Lacewing). Midinstar larvae (8 per batch, 1 batch per concentration) from a culture fed on mixed aphid species were treated topically on a filter paper in a 4.5 cm diameter Petri dish with the test solution (1 μ L). Treated insects were fed with pea (*Acyrtosiphon pisum* Harris) and black bean (*Aphis fabae* Scopoli) aphids.

Phytoseiulus persimilis Atkias-Henriot (a Predatory Mite). Mixed-sex adults cultured on *Tetranychus urticae* (supplied by Biological Crop Protection) (10 per batch, 2 batches per concentration) were transferred using a fine brush to the abaxial surface of a dwarf French bean leaf that had been treated with test solution as described for *T. vaporariorum*. The mites were confined by floating the leaf, adaxial surface down, on tap water, in a 9 cm diameter Petri dish.

Typhlodromus pyri Scheuter (a Predatory Mite). Adults cultured on dwarf broad bean pollen (var. The Sutton) (10 per batch, 2 batches per concentration), retained dorsally on double-sided adhesive tape mounted on a microscope slide, were submerged in the test solution and then left to dry. Mortality was assessed as a lack of response of the legs when the mites were touched lightly with a soft brush.

RESULTS AND DISCUSSION

The work reported here is based on the hexane extract from *C. andina* (Scrophulariaceae), using chromatographic fractionation directed by bioassays with whiteflies as the test species. The active compounds, **1** and **2** (see Table 1), were isolated from the crude extracts of leaves and stems, but extracts from the flowers were much less active, the reverse situation to that for the natural pyrethrin insecticides.

NMR shifts listed for compounds **1** and **2** are essentially identical to those recorded for these compounds isolated from *Calceolaria sessilis* L. by Chamy et al. (1993). The assignments indicated in Table 1 were confirmed by the use of 2D NMR, specifically one-bond and long-range CH correlation experiments. They differ from those listed previously (Chamy et al., 1993) for the aromatic region and C-3 in both compounds. Compound **1** has also been synthesized previously to investigate its possible involvement in the biosynthesis of dunnione (Inoue et al., 1982). Recognition of the activity against arthropods of compounds **1** and **2** is novel, although many other types of naphthoquinones are known to show such activity (E. I. Dupont de Nemours & Co., 1977; Bayer AG, 1989; Jacobsen et al., 1986). Naphthoquinones as a class display many other types of biological activity (Thompson, 1997; Medentsev and Akimenko, 1996; Fry and Pudney, 1996) including fungicidal and antimalarial.

The results from testing the two compounds against 29 pest species from 9 orders and 9 beneficial species

Table 4. Acute Mammalian Toxicities of Compounds 1 and 2 in Standard Acute Oral [OECD 401/ECBI (Rat)] and Acute Dermal [OECD 402/ECBI (Rat)] Tests

compound	LD ₅₀ (mg kg ⁻¹)	
	oral	dermal
1	1366	>2000
2	1072	>2000

from 5 orders are summarized in Table 2. For agricultural pests, activity was particularly high against homopteran and acarine species. The former includes sucking pests of cereals, cotton, vegetables, and ornamentals, and the latter includes fruit-destroying mites. Activity against both classes is of a similar order to that observed for a range of established pesticides (see Table 2). In addition, both compounds are generally only weakly active against beneficial species. Of the two livestock pests examined, one (the cattle tick, *Ixodes ricinus*) was particularly susceptible to compound 2.

Bioassays against susceptible and multiresistant strains of four representative species of dipteran, homopteran, and acarine pests yielded no evidence of cross-resistance to naphthoquinones (Table 3). This finding is of particular significance in cases (e.g., for *M. persicae* and *B. tabaci*) where resistance to conventional insecticides is known to be based at least partly on nonspecific metabolic mechanisms conferring strong (>100-fold) and broad-spectrum protection between as well as within chemical groups.

Compounds 1 and 2, when tested by standard procedures, have low oral and dermal mammalian toxicities (see Table 4) unlike many other naturally occurring naphthoquinones [e.g., Munday et al. (1995)].

CONCLUSION

Compounds 1 and 2 are highly active toward some commercially important pests, especially whiteflies and mites. They show no evidence of cross-resistance and exhibit low mammalian toxicities. They offer exciting opportunities both as lead structures for analogue synthesis (Khambay et al., 1997a) and as new botanical pesticides (Khambay et al., 1997b).

The use of the compounds as pesticides has been patented (Khambay et al., 1995) by BTG International Ltd.

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