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Algicidal and antifungal compounds from the roots of *Ruta graveolens* and synthesis of their analogs

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Abstract

Bioassay-guided fractionation of the ethyl acetate extract of *Ruta graveolens* roots yielded rutacridone epoxide with potent selective algicidal activity towards the 2-methyl-isoborneol (MIB)-producing blue-green alga *Oscillatoria perornata*, with relatively little effect on the green alga *Selenastrum capricornutum*. The diol-analog of rutacridone epoxide, gravacridondiol, which was also present in the same extract, had significantly less activity towards *O. perornata*. Rutacridone epoxide also showed significantly higher activity than commercial fungicides captan and benomyl in our micro-bioassay against the agriculturally important pathogenic fungi *Colletotrichum fragariae, C. gloeosporioides*, *C. acutatum*, and *Botrytis cineara* and *Fusarium oxysporium.* Rutacridone epoxide is reported as a direct-acting mutagen, precluding its use as an agrochemical. In order to understand the structure–activity relationships and to develop new potential biocides without toxicity and mutagenicity, some analogs containing the (2-methyloxiranyl)-dihydrobenzofuran moiety with an epoxide were synthesized and tested. None of the synthetic analogs showed comparable activities to rutacridone epoxide. The absolute stereochemistry of rutacridone was determined to be 2′(R) and that of rutacridone epoxide to be 2′(R), 3′(R) by CD and NMR analysis. Published by Elsevier Ltd.

Keywords: *Ruta graveolens*; Rutaceae; *Oscillatoria perornata*; *Selenastrum capricornutum*; Algicide; Fungicide; Acridone alkaloids; *Colletotrichum fragariae*; *C. gloeosporioides*; *C. acutatum*; *Botrytis cinearea*; *Fusarium oxysporium*

1. Introduction

Many *Ruta* species are sources of diverse classes of natural products with biological activities including antifungal, phytotoxic, and antidotal activities (Aliotta et al., 1994, 2000; Oliva et al., 2003; De Feo et al., 2002; Sallal and Alkofahi, 1996). Previously, the presence of fungicidal constituents against some agriculturally important fungi in the ethyl acetate extract of *Ruta graveolens* L. leaves has been demonstrated (Oliva et al., 2003). In the present study, we investigated the ethyl acetate extract of the roots of *R. graveolens* for algicidal activity against the 2-methyl-isoborneol (MIB) producing blue-green alga, *Oscillatoria perornata*, a pest in commercial catfish (*Ictalurus punctatus*) production ponds in the southeastern United States. MIB accumulates in the flesh of catfish, giving them a ''musty'' off-flavor and thereby rendering them unpalatable. The agents currently approved by United States Environmental Protection Agency to control off-flavor compound-producing blue-green algae, copper-based products and diuron (3-[3,4-dichlorophenyl]-1,1-dimethylurea), are limited in their usefulness due to their persistence in the environment, lack of selectivity towards noxious blue-green algae and little margin of safety between phytotoxic and ichthyotoxic concentrations. As an alternative to these synthetic algicides, we are screening natural compounds and extracts from plants to discover selective and environmentally safe algicides for use in catfish aquaculture.

Preliminary bioassays of the ethyl acetate extract indicated the presence of constituents with selective toxicity towards *O. perornata*, when compared to *Selenastrum capricornutum*, a green alga that is found in fish production ponds and considered to be one beneficial type of

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phytoplankton. Bioassay-guided fractionation of this extract led to the isolation of active compounds. These compounds also showed potent fungicidal activity against several agriculturally important pathogenic fungi *Colletotrichum fragariae, C. gloeosporioides*, *C. acutatum*, and *Botrytis cineara* and *Fusarium oxysporium*. This paper describes the isolation, structure elucidation, synthesis, and structure–activity studies of the bioactive constituents from the ethyl acetate extract of *R. graveolens* roots.

2. Results and discussion

The ethyl acetate extract of roots of *R. graveolens* showed moderate toxicity towards *O. perornata* (LCIC = 10 ppm) and at least an order of magnitude greater toxicity compared to *S. capricornutum* (LCIC > 100 ppm). Bioassay-guided fractionation of this extract on silica gel afforded several highly active fractions. Further chromatographic separation of the most active fractions on C-18 silica gel, followed by crystallization, led to the isolation of rutacridone epoxide **(1)** as the most active algicidal compound (lowest-complete inhibition concentration, LCIC = 0.1 ppm, 0.3 μM) (Table 1). The structure of (**1**) was confirmed by direct comparison of NMR and mass spectral data reported in the literature (Nahrstedt et al., 1981).

The IC_{50} of **1** was determined to be 9×10^{-3} μM, which is among the most active natural products against *O. perornata* that we have identified in our algicide-screening program (Fig. 1). The IC_{50} for the green alga *S. capricornutum* was found to be 173×10^{-3} μM, further indicating a high degree of selective toxicity towards *O. perornata*.

The separation of a weakly active column fraction, led to the isolation of another active constituent, which was identified as gravacridondiol (**2**) by analysis of its NMR and mass spectra (Paulini et al., 1991; Bergenthal et al., 1979). The results of the bioassay indicated that **2** was 100× less active than **1** against *O. perornata* (Table 1). Both compound **2** and its possible biosynthetic precursor **1** have been previously isolated from *R. graveolens* (Rozsa et al., 1976; Reisch et al., 1976; Baumert et al., 1987; Nahrstedt et al., 1981, 1985). Rutacridone (**3**), a possible biosynthetic precursor of compound **2**, was isolated from the same extract and had no activity in this bioassay. It has been recently reported that gravacridondiol glucoside (glucoside analog of **2**) as the dominant metabolite and **3** as the major acridone alkaloid of the root tips of *R. graveolens* (Kuzovkina et al., 2004).

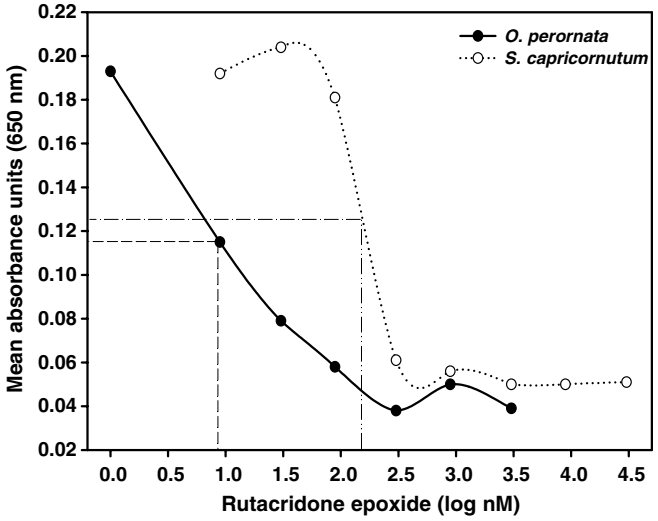

Fig. 1. Rutacridone epoxide (**1**) 96-h IC_{50}. Dotted lines represent IC_{50} intersects of absorbance curves.

These rutacridone analogs were also evaluated for their antifungal activity using bioautography against *C. fragariae*. Compound **1** showed potent antifungal activity at 1 mg/mL, whereas compounds **2** and **3** had no activity against *C. fragariae* at the same concentration. This is in agreement with the observation that rutacridone epoxide (**1**) is a phytoalexin biosynthesized and accumulated from compound **3** in *R. graveolens* suspension cultures after elicitation with dead or live fungi (Eilert et al., 1984; Wolters and Eilert, 1982, 1983; Baumert et al., 1991). LC-MS analysis of the ethyl acetate extract of *R. graveolens* roots indicated that **1** and **3** are present in approximately equal amounts. Compound **1** was further evaluated for fungal growth inhibition (GI) in a micro-bioassay against the plant pathogenic fungi *C. fragariae*, *C. gloeosporioides*, *C. acutatum*, and *Botrytis cinearea*, and *Fusarium oxysporium*. The commercial fungicides captan and benomyl were used as positive controls (Fig. 2). The results of the antifungal micro-bioassay indicated that **1** was significantly more active against *C. fragariae*, *C. gloeosporioides*, and *C. acutatum*, than either captan or benomyl at 2 μM. Rutacridone epoxide (**1**) was the most active compound and demonstrated some selectivity against the three *Colletotrichum* species with an IC_{50} between 0.125 and 1.0 μM. Compound **1** produced 100% GI of *C. fragariae* and *C. gloeosporioides* at 0.5 μM, and was slightly less active against the benomyl resistant species *C. acutatum* (100% GI at 1.0 μM). *Botrytis cinerea* was less sensitive to **1** and, even at the highest concentration, **1** did not cause more that 80% GI. *Fusarium oxysporium* was insensitive to **1** in the concentration range

Table 1
Algicidal activity of the isolated compounds and synthetic analogs

Test compound	Test organism	
	Oscillatoria perornata LCIC (ppm)	*Selenastrum capricornutum* LCIC (ppm)
(**1**)	0.1	1
(**2**)	10	>100
(**3**)	>100	>100
(**7**)	>100	100
(**8**)	>100	>100
(**9**)	100	100
(**10**)	>100	>100
(**11**)	>100	>100
(**12**)	>100	>100