Phytotoxic and Antimicrobial Properties of Cyclocolorenone from *Magnolia grandiflora* L.

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Cyclocolorenone, a sesquiterpene ketone of the aromadendrane class, was isolated from *Magnolia grandiflora* L. by bioassay-directed fractionation and its identity confirmed by UV, IR, NMR, and MS techniques. It showed good growth-inhibitory activity in an etiolated wheat coleoptile assay. Cyclocolorenone was also phytotoxic to greenhouse-grown corn, bean, and tobacco plants and displayed antimicrobial activity.

INTRODUCTION

Magnolia grandiflora L. is a tree widely distributed throughout the mid-Atlantic and southeastern United States. This species has been the object of exhaustive phytochemical research because of its long history of folk-medicinal usage [see Rao and Davis (1982a) for leading references] and has yielded a variety of natural products, including alkaloids (Nakano, 1954), terpenoids (Halim et al., 1984; Rao and Davis, 1982a; Doskotch and El-Feraly, 1969), lignan glycosides (Rao and Wu, 1978), and biphenyls (El-Feraly and Li, 1978). Some of these compounds show activity/toxicity in various animal-based bioassays (Chang et al., 1964; Doskotch and El-Feraly, 1969; Ogura et al., 1978; Rao and Davis, 1982b; Watanabe et al., 1975; Wiedhopf et al., 1973). However, despite the common observation that virtually no plant will grow beneath a magnolia tree, suggesting an allelopathic effect, none of the isolates appears to have been tested for phytotoxic properties. As part of a program aimed toward the identification and development of new, easily biodegraded agrochemicals from natural sources, we subjected an extract of *M. grandiflora* leaves to a bioassay-directed fractionation procedure based on the etiolated wheat coleoptile, with follow-up bioassays (greenhouse plants; antimicrobial disks) on one of the most active isolates, according to our established protocols (Cutler, 1984, 1986).

EXPERIMENTAL PROCEDURES

Materials and Equipment. Fallen, green leaves were collected in the spring from trees of *M. grandiflora* L. growing locally (Athens, GA). IR spectra were obtained with a Beckman IR 4210 spectrometer from thin films on KBr. The UV spectrum was obtained by using a Shimadzu UV-160U spectrometer. NMR spectra were determined on a Bruker AM 250 instrument; samples were dissolved in $CDCl_3$ with TMS as internal reference. EI mass spectra were obtained on a Hewlett-Packard 5985 B mass spectrometer. TLC was performed on Merck silica gel 60, F-254 plates. The C_{18} reverse-phase silica gel used in column chromatography was obtained from a Waters PrepPak 500.

Isolation and Purification. Partially dried magnolia leaves (~1.5 kg) were defatted with petroleum ether and then extracted with 80% aqueous ethanol by steeping at 25 ± 3 °C for several days. After evaporation of EtOH from the extract, the aqueous residue was acidified with dilute HCl and extracted with dichloromethane. The CH_2Cl_2 extract was evaporated to dryness and the residue chromatographed on C_{18} reverse-phase silica gel, eluting initially with 50:50 methanol–water and following this with 75:25 methanol–water. Fractions were monitored by TLC [using toluene–EtOAc–HCO_2H (5:4:1) as the developing solvent] and bioassay (etiolated wheat coleoptile), and chromatographically similar, active fractions were pooled, evaporated, and chromatographed further on silica gel, eluting with dichloromethane–acetone (95:5), acetone, and then methanol. Biologically active, like fractions were combined, evaporated, and subjected to chromatography by Chromatotron (silica gel plate), developing with hexane–acetone (8:2). The major component was obtained as a fragrant, colorless oil (108 mg).

Bioassays. The primary bioassay was the etiolated wheat coleoptile which was used throughout the isolation and purification process to determine biological activity. The assay was prepared by germinating wheat (*Triticum aestivum* L., cv. Wakeland) on moist sand in plastic boxes at 22 ± 1 °C for 4 days in the dark. Seedlings were removed and prepared under a safelight at 540 nm. Initially, the caryopses and roots were discarded, and then the etiolated shoots were fed into a Van der Weij guillotine, the apical 2 mm was cut and discarded, and the next 4 mm of the coleoptile was saved for bioassay. Ten 4-mm sections were placed in each test tube with 2 mL of phosphate–citrate buffer (pH 5.6) containing 2% sucrose and the pure compounds or the fraction to be tested (generally a 25-μL aliquot from a column chromatography cut that had been dried under nitrogen). The test tubes were placed in a roller tube apparatus that rotated 0.25 rpm for 20 h at 22 °C. Then, the coleoptiles were removed from solution, blotted on paper towels, and placed on a glass sheet, and their images (×3) were measured. All data were subjected to statistical analysis (Kurtz et al., 1965).

Microbial bioassays utilized Gram-positive and Gram-negative bacteria: *Bacillus subtilis* (+), *B. cereus* (+), *Mycobacterium thermosphactum* (+), *Escherichia coli* (−), *E. cloacae* (−), and *Citrobacter freundii* (−). Each organism was heavily seeded onto diagnostic sensitivity test agar (DST) in glass Petri dishes, to ensure a dense lawn, and to these were added 4-mm disks that had been impregnated with 500, 250, and 50 μg of cyclocolorenone, respectively. The compound was dissolved in acetone and added to the disks which were dried and then placed on the seeded agar surface. Plates were incubated at 37 °C for 18 h.

Antifungal assays were conducted with the following organisms: *Curvularia lunata*, *Chaetomium cochliodes*, *Chaetomium spinusum*, and *Aspergillus flavus*. Each fungus was inoculated onto potato dextrose agar in Petri dishes, and to these were added 4-mm paper disks, impregnated with specific amounts (50, 250, or 500 μg) of the test material. Plates were incubated at 25 °C and checked daily for fungal growth and inhibition, for a maximum of 7 days.

Cyclocolorenone was also tested for activity on greenhouse-grown plants. The sample (7.63 mg) was dissolved in 0.35 mL of acetone, and to this was added 3.15 mL of water containing

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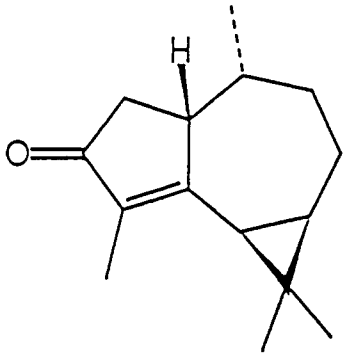

Figure 1. Cyclocolorenone (*M. grandiflora*).

0.1% Tween 20 to give a 10^{-2} M stock solution, from which 10^{-3} and 10^{-4} M dilutions were made with water. Six-week-old tobacco plants (*Nicotiana tabacum* L., cv. Hick's) were sprayed with 1 mL of each solution per plant. Nine-day-old bean plants (*Phaseolus vulgaris* L., cv. Black Valentine) were treated with 1 mL of each concentration per pot, each of which contained four bean plants. Nine-day-old corn plants (*Zea mays* L., cv. Norfolk Market White) were treated with 100 μL of each solution which was pipetted into individual leaf whorls. All experiments were done in triplicate. Observations were made daily for 2 weeks after treatment. Control plants were treated with solutions that contained the appropriate amounts of Tween 20, acetone, and water in all cases.

RESULTS AND DISCUSSION

Chemical. Cyclocolorenone was obtained as a colorless oil which was homogeneous by TLC. Spectroscopic data were as follows: UV λ_{max} (EtOH), 264 (log ϵ = 4.11); IR ν_{max} 2910, 1690 (s), 1625, 1450, 1385, 1335, 1305, 1235, 1120, 1060, 1015 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.97 (m, 1 H), 2.50 (dd, J = 6.5, 18.5 Hz, 1 H), 2.11–1.93 (m, 4 H), 1.73 (d, J = 1.7 Hz, 3 H), 1.70–1.35 (m, 3 H), 1.31–1.20 (m, 1 H), 1.27 (s, 3 H), 1.03 (s, 3 H), 0.80 (d, J = 7.0 Hz, 3 H); ^{13}C NMR ($CDCl_3$) 208.9, 177.1, 141.0, 43.1, 40.8, 33.1, 32.9, 32.3, 30.1, 29.1, 26.6, 21.8, 18.1, 17.2, 8.9 ppm. MS m/z 218 (M^+), 203, 189, 175, 161, 147, 133, 119, 105, 91 (100%), 77.

The apparent M^+ at m/z 218, together with the carbon and proton counts, leads to the tentative molecular formula $C_{15}H_{22}O$, consistent with a sesquiterpene.

The IR absorptions at 1690 and 1625 cm^{-1} suggested an enone system, and this assignment was supported by the ^{13}C resonances at 208.9, 177.1, and 141.0 ppm. The UV spectrum indicated extended conjugation of the enone system, but the absence of additional vinylic resonances in the ^{13}C spectrum suggested that the conjugation might be with a cyclopropyl group. Four C–CH_3 groups were evident from the ^1H spectrum, and two of these resonances (the singlets at δ 1.27 and 1.03) were consistent with *gem*-dimethyl substitution on a cyclopropane ring.

An examination of the literature revealed that the sesquiterpenoid ketone cyclocolorenone (Figure 1), incorporating a cyclopentenone with a cyclopropane β-substituent, had previously been isolated from *M. grandiflora* bark, as well as from a range of other plant species [Rao and Davis (1982a) and references cited therein]. The spectroscopic data from our compound were all in good agreement with those reported in the literature for cyclocolorenone (Rao and Davis, 1982a; Mikhailova et al., 1978), leading us to conclude that this was, in fact, the compound which we had isolated.

Biological. Growth of etiolated wheat coleoptiles was significantly ($P < 0.01$) inhibited by cyclocolorenone at 10^{-3} and 10^{-4} M, but there was no effect at 10^{-5} M (Table I). For comparison, Table I includes the activities of a herbicide/synthetic plant growth regulator [morphactin (*n*-butyl 9-hydroxyfluorene-9-carboxylate)], a naturally occurring plant growth regulator (abscisic acid), and an allelochemical (juglone). The actions of these compounds in vivo have been discussed at length (Schneider et al., 1986; Cutler, 1990; Ponder, 1987). These reference compounds were chosen so as to minimize chemical differences from cyclocolorenone, in the sense that none of the molecules contains nitrogen or halogens and functional groups are restricted to carbonyl-based functions, hydroxyls, and double bonds. Other examples of the activity of various natural products in the etiolated wheat coleoptile assay may be found in earlier publications from our laboratory [e.g., Cutler (1984)].

Table I. Percent Inhibition of Etiolated Wheat Coleoptile Growth Relative to Controls

	molar concn			
	10^{-3}	10^{-4}	10^{-5}	10^{-6}
cyclocolorenone	100	58	0	0
morphactin	100	36	(+)[a]	(+)[a]
juglone	100	100	0	0
abscisic acid	100	100	81	62

[a] Moderate growth enhancement relative to controls.

Gram-positive bacteria, *B. subtilis*, *B. cereus*, and *M. thermosphactum*, were inhibited at 250 μg/disk, whereas Gram-negative bacteria, *E. coli*, *E. cloacae*, and *C. freundii*, were only inhibited at 500 μg/disk. The fungi *C. lunata*, *C. spinusum*, and *C. cochliodes* were all susceptible to the agent at 250 μg/disk, with *C. cochliodes* showing marginal inhibition at 50 μg. *A. flavus* was not inhibited at concentrations up to 500 μg/disk.

Bean plants exhibited two types of response within 48 h of treatment with 10^{-2} M cyclocolorenone: irregular, necrotic lesions were present on leaf blades and veins and about 25% of the leaves were bent at the pulvinus, so that their surfaces were inverted. We have only observed the latter effect once previously, when bean plants were treated with prehelminthosporol (Cutler et al., 1982). In other respects, plant vigor did not appear to have been adversely affected. Lower concentrations of cyclocolorenone did not produce any significant effects. Exposure of corn plants to 10^{-2} M cyclocolorenone resulted in severe necrosis of the leaves and stem collapse, within 48 h. Plants appeared stunted even 2 weeks after treatment. At a concentration of 10^{-3} M, the agent produced only slight to moderate necrosis, localized in the area of application. Lower concentrations did not cause any significant effects. Tobacco leaves showed small, transparent lesions within 24 h of treatment with 10^{-2} M cyclocolorenone. Within 72 h, these spots became small, necrotic areas, but plant vigor appeared normal. Lower concentrations were without any effect.

Despite its isolation from plants with herbal-medicinal usage (e.g., *M. grandiflora*, *Ledum palustre*, and *Solidago canadensis*), cyclocolorenone has apparently never been subjected to biological testing, although the plant growth inhibiting activities of aromadendrane derivatives from *Ambrosia* and *Eucalyptus* spp. have been previously reported (Goldsby and Burke, 1987; Mizutani, 1989) and the plant growth regulating properties of other sesquiterpenes have been reviewed (Fischer, 1986). Antimicrobial activity for various terpenoids has been sporadically recorded in the literature [see, for example, Lee et al. (1977)].

Our findings demonstrate that cyclocolorenone has phytotoxic, antibacterial, and antifungal properties and suggest that this compound may serve as a template for

synthetic elaboration leading to useful new herbicides or antimicrobials. In view of its deleterious effects on plant growth in our bioassays, it is possible that cyclocolorenone plays an allelochemical role in the ecology of *M. grandiflora*, although further experiments are needed to establish this point. However, we have observed inhibitory effects on wheat coleoptile growth from other isolates of *M. grandiflora* leaves, and it is likely that a number of compounds may be involved in the suppression of plant growth around magnolia trees. These studies will be described in future publications.

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