Identification of Plant Growth Inhibitory Principles in *Ailanthus altissima* and *Castela tortuosa*

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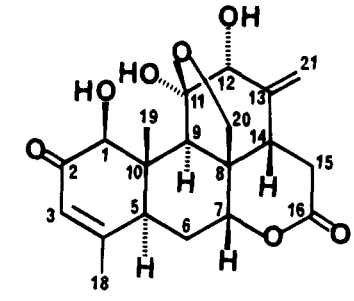
A methanol extract of *Ailanthus altissima* and a methylene chloride extract of *Castela tortuosa* exhibited plant growth inhibitory activity against *Brassica juncea*, *Eragrostis tef*, and *Lemna minor*. Ailanthone and chaparrinone were identified as the active constituents in *A. altissima* and *C. tortuosa*, respectively. In the bioassays with *B. juncea*, *E. tef*, and *L. minor* ailanthone inhibited growth by 50% (I_{50}) at 0.9, 2.6, and 21 μM, respectively, and the I_{50} values for chaparrinone in these three bioassays were 0.9, 2.1, and 4.2 μM, respectively. In a greenhouse test ailanthone exhibited inhibitory activity against several weed species, and it was more effective postemergent than preemergent.

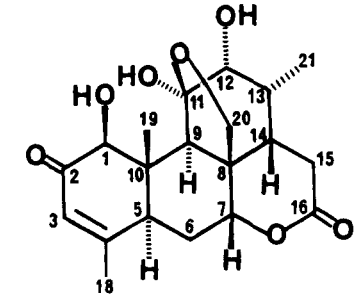
Keywords: *Phytotoxic activity; quassinoids; ailanthone; chaparrinone*

INTRODUCTION

Quassinoids, bitter principles of the Simaroubaceae, have been reported to possess diverse biological activities including anticancer, antimalarial, and amoebicidal, antiviral, insecticidal, antiinflammatory (O'Neill et al., 1985), and phytotoxic activities (Hoffmann et al., 1992). Heisey (1990) and Lawrence et al. (1991) reported that extracts of various parts of *Ailanthus altissima* (Mill.) Swingle had phytotoxic activity, although the active principle(s) was (were) not identified. In our search for potential herbicides from natural sources, we found that the methanol extract of *A. altissima* and the methylene chloride extract of *Castela tortuosa* had strong phytotoxic activity in our bioassays. We used bioassay-guided fractionation to determine the source of the phytotoxic activity. This led to the isolation of ailanthone (Figure 1) as the active principle, a major quassinoid of *A. altissima* (Polonsky and Fourrey, 1964). Chaparrinone (Figure 1) was identified to be the active constituent in *C. tortuosa*.



ailanthone



chaparrinone

Figure 1. Structures of ailanthone and chaparrinone.

MATERIALS AND METHODS

Plant Material. The stem bark of *A. altissima* was collected from San Jose, CA, in January 1994. The bark was collected from 2–3-year-old trees, with most of the bark being of first- and second-year growth and a small portion being of third-year growth. *C. tortuosa*, chaparro amargosa, was purchased from a market in Mexico City and the plant identified by G. Peiser. The material consisted of the bark and stem tissue with stem diameter of approximately 7 mm or less.

Extraction. The air-dried stem bark (2.7 kg) of *A. altissima* was ground into powder and extracted with methylene chloride followed by methanol in a Soxhlet apparatus. Extraction with methanol proceeded for 48 h to afford a methanol extract (215 g). The coarse powder (91 g) of the stems of *C. tortuosa* was extracted with methanol at ambient temperature.

Isolation of Ailanthone. The methanol extract (215 g) was subjected to silica gel column chromatography (J. T. Baker, Inc., 40 μm; 3 kg). Elution was initiated with 8% methanol in methylene chloride followed by 10% and 15% methanol in methylene chloride. A total of eight column fractions were collected. Column fraction 6 (2.52 g), which exhibited the strongest plant growth inhibitory activity in *Brassica juncea* and *Eragrostis tef* bioassays, described below, was further chromatographed over a silica gel column. Elution of the column with 5% methanol in methylene chloride followed by 6%, 8%, 10%, and 15% methanol in methylene chloride afforded 11 column fractions. The activity was mainly at fraction 7 (2.07 g). Recrystallization of this column fraction in methanol yielded colorless needles which displayed data comparable to those reported for ailanthone (Naora et al., 1983), except for NMR data that was obtained in a different solvent (pyridine-d_5). The proton magnetic resonance (^{1}H NMR) and carbon-13 magnetic resonance (^{13}C NMR) of this isolate recorded in $CDCl_3$ are reported here: ^{1}H NMR (300 MHz) δ 7.95 (1H, bs, OH), 6.12 (1H, bs, H-3), 5.38 (1H, bs,

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Table 1. Probit Analysis for Dose–Response Experiments To Determine the Concentration of Ailanthone and Chaparrinone Required To Inhibit Growth by 50% (I_{50}) in the *L. minor*, *B. juncea*, and *E. tef* Bioassays

		L. minor			*B. juncea*			*E. tef*		
		I_{50} (μM)	95% fiducial limits	slope ± SE	I_{50} (μM)	95% fiducial limits	slope ± SE	I_{50} (μM)	95% fiducial limits	slope ± SE
ailanthone	light	21	16–29	1.12 ± 0.05	0.9	0.8–1.1	1.78 ± 0.08	2.6	2.3–2.8	1.85 ± 0.07
	dark	nd[a]	nd	nd	1.9	1.2–3.0	2.31 ± 0.10	4.7	4.0–5.6	2.58 ± 0.12
chaparrinone	light	4.2	3.2–5.4	0.62 ± 0.05	0.9	0.7–1.2	3.75 ± 0.25	2.1	1.6–2.7	2.61 ± 0.15

[a] Not determined.

OH), 5.32 (1H, s, H_2-21), 5.22 (1H, s, H_2-21), 4.49 (1H, t, J = 2.7 Hz, H-7), 4.11 (1H, s, H-1), 3.99 (1H, s, H-12), 3.88 (1H, d, J = 8.5 Hz, H_2-20), 3.50 (1H, d, J = 8.6 Hz, H_2-20), 3.06 (1H, dd, J = 15.2, 5.2 Hz, H_2-15), 2.84 (1H, m, H-5), 2.84 (1H, s, H-9), 2.70 (1H, dd, J = 15.2, 5.6 Hz, H_2-15), 2.69 (1H, m, H-14), 2.28 (1H, m, H_2-6), 2.02 (1H, m, H_2-6), 1.99 (3H, s, H_3-18), 1.14 (3H, s, H_3-19); ^{13}C NMR (75 MHz) δ 195.90 (s, C-2), 169.23 (s, C-16), 164.25 (s, C-4), 142.52 (s, C-13), 124.36 (d, C-3), 121.63 (t, C-21), 108.43 (s, C-11), 82.84 (d, C-1), 79.34 (d, C-12), 77.99 (d, C-7), 71.64 (t, C-20), 47.08 (d, C-14), 45.00 (s, C-10), 44.81 (s, C-8), 43.98 (d, C-9), 41.90 (d, C-5), 34.30 (t, C-15), 25.62 (t, C-6), 23.05 (q, C-18), 9.51 (q, C-19); MS (EI, rel int), m/z 376 (M^+, 3), 264 (2), 248 (4), 231 (3), 219 (2), 191 (5), 189 (6), 179 (6), 177 (5), 175 (6), 165 (9), 151 (17), 125 (30), 123 (24), 111 (46).

Isolation of Chaparrinone. The concentrated methanol extract of *C. tortuosa* was suspended in water and partitioned with pentane, methylene chloride, and ethyl acetate sequentially to yield pentane (0.83 g), methylene chloride (0.26 g), ethyl acetate (0.22 g), and aqueous extract (2.82 g). The methylene chloride extract was fractionated by a Sephadex LH-20 column (MeOH–CH_2Cl_2 = 1:1 v/v) to give four fractions. Fraction 3 (82.5 mg) was separated by preparative HPLC (Asahipak GS-310P column, gel filtration, 20 × 500 mm; solvent, methanol; flow rate, 4 mL/min) to give an active fraction (12.8 mg). Purification of this active fraction under the same HPLC condition afforded chaparrinone (5.5 mg), which exhibited the following NMR data: ^{1}H NMR (300 MHz, $CDCl_3$–CD_3OD = 10:1) δ 6.15 (1H, bs, H-3), 4.48 (1H, bs, H-7), 4.07 (1H, s, H-1), 3.96 (1H, d, J = 8.7 Hz, H_2-20), 3.68 (1H, d, J = 8.8 Hz, H_2-20), 3.56 (1H, d, J = 4.4 Hz, H-12), 2.85 (1H, m, H-5), 2.79 (1H, m, H_2-15), 2.65 (1H, m, H_2-15), 2.71 (1H, s, H-9), 2.30 (1H, m, H-13), 2.30 (1H, m, H_2-6), 2.02 (1H, m, H_2-6), 2.02 (3H, s, H_3-18), 2.01 (1H, m, H-9), 1.19 (3H, s, H_3-19), 1.05 (3H, d, J = 7.1 Hz, H_3-21); ^{13}C NMR (75 MHz, $CDCl_3$–CD_3OD = 10:1) δ 196.42 (s, C-2), 170.81 (s, C-16), 164.13 (s, C-4), 124.52 (d, C-3), 108.90 (s, C-11), 82.94 (d, C-1), 78.39 (d, C-12), 78.11 (d, C-7), 70.95 (t, C-20), 45.45 (s, C-10), 44.83 (s, C-8), 43.67 (d, C-9), 41.97 (d, C-5), 41.97 (d, C-14), 30.50 (d, C-13), 29.60 (t, C-15), 25.53 (t, C-6), 22.80 (q, C-18), 12.30 (q, C-21), 9.46 (q, C-19); MS (CI, rel int), m/z 378 (M^+, 29), 361 (19), 219 (16), 179 (14), 165 (20), 151 (26), 125 (33), 111 (47).

Bioassays. Bioassays with three different plant species, *Lemna minor* (duckweed), *B. juncea* (mustard), and *E. tef* (a grass), were used to follow the plant growth inhibitory activity. The bioassay with *L. minor* was conducted as previously described (Einhellig et al., 1985). In brief, the assay was conducted aseptically in 24-well tissue culture plates. Extract dissolved in DMSO was added to the wells, and its effect upon the growth of *L. minor* under continuous fluorescent light (90 μEinstein m^{-2} s^{-1}) at 29 °C was determined after 7 days. The bioassays for *B. juncea* and *E. tef* were also conducted aseptically in 24-well tissue culture plates. One milliliter of liquid agar was placed in each well, and before it solidified, 10 μL of extract dissolved in DMSO was uniformly mixed with the agar. After the agar had cooled, surface-sterilized seed of *B. juncea* and *E. tef* were placed on the agar in separate wells. Growth, as measured by average shoot height compared to controls, was evaluated after 5 days at 29 °C and continuous fluorescent light (45 μEinstein m^{-2} s^{-1}). Two commercial herbicides, metribuzin (Mobay Chemical Corp.) and trifluralin (Treflan, DowElanco), were tested for comparison in the *L. minor*, *B. juncea*, and *E. tef* bioassays. These were conducted as described above except appropriate dilutions of metribuzin and trifluralin were added in place of the plant extracts. Experiments in the dark were conducted identically with those in the light except that the bioassays were conducted in complete darkness. Experiments were repeated three times for each bioassay with three replications in each experiment. Dose–response data were used to determine the I_{50} (concentration that gave 50% growth inhibition) for each compound in the three bioassays using statistical probit analysis (Finney, 1971). The data from all three experiments were combined in the probit analysis. The 95% fiducial limits and slope with standard errors were also determined.

Greenhouse Tests. One preemergence and postemergence test was conducted with ailanthone. For both tests flats were seeded in soil to provide 20–30 seedlings of the grass species *Setaria viridis* (green foxtail), *Braccharia platyphylla* (broadleaf signalgrass), and *Echinochloa crus-galli* (barnyard grass) and 4–6 seedlings of the broadleafed species *Abutilon theophrasti* (velvetleaf), *Cassia obtusifolia* (sicklepod), and *Ipomea hederacea* (ivyleaf morningglory). Ailanthone was dissolved in acetone–water, 1:1, and applied with a small hand sprayer to the top of the soil after seeding in the preemergence test and to 11-day-old seedlings in the postemergence test. For the preemergence test the flat was watered regularly by applying water to the top of the soil. Acetone–water, 1:1, without ailanthone was applied to the controls. The preemergence test was evaluated 2 weeks after seeding and treatment, and the postemergence test was evaluated 2 weeks after treatment. There was no replication for either test.

RESULTS AND DISCUSSION

Using bioassay-guided fractionation, we isolated a plant growth inhibitory compound from *A. altissima* that was identified as ailanthone. It was very active in all three plant bioassays with I_{50} values of 21, 0.9, and 2.6 μM in the *L. minor*, *B. juncea*, and *E. tef* bioassays, respectively (Table 1). In comparison to the activity of two commercial herbicides, metribuzin and trifluralin, ailanthone had greater activity than these two herbicides in the *B. juncea* and *E. tef* bioassays, though weaker activity than these in the *L. minor* bioassay. The I_{50} values for metribuzin in the *L. minor*, *B. juncea*, and *E. tef* bioassays were 0.3, 500, and 50 μM and for trifluralin they were 2, 150, and 15 μM, respectively. It is interesting that ailanthone was almost 10 and 20 times more active in the *E. tef* and *B. juncea* bioassays, respectively, than in the *L. minor* bioassay. This is somewhat unusual as *L. minor* is generally more sensitive to phytotoxic compounds than most other plants, such as *B. juncea* and *E. tef* (G. Peiser, F. Karasina, K. Mathias, unpublished results; Grossman et al., 1992). With respect to this difference in activity, we followed the UV spectrum of ailanthone in *L. minor* growth medium for 4 days but observed no change in the spectrum (data not shown), indicating that ailanthone was stable in the growth medium.

We isolated a very closely related quassinoid, chaparrinone, from *C. tortuosa* which was also phytotoxic.

Table 2. Weed Control[a] by Ailanthone in a Preemergence and Postemergence Greenhouse Test

		weed species[b]					
test	rate appld (kg/ha)	SETVI	BRAPP	ECHCG	ABUTH	CASOB	IPOHE
preemergence	0.125	0	0	0	0	0	0
	0.5	100	50	20	30	20	0
	1.0	100	60	60	60	30	20
	2.0	100	80	60	80	60	20
postemergence	0.125	100	0	20	30	100	30
	0.25	100	50	20	40	100	30
	0.5	100	85	50	60	100	80
	1.0	100	95	80	100	100	90
	2.0	100	95	100	100	100	100

[a] Weed control indicates the percent injury or growth inhibition compared to the control. [b] Species: SETVI, *S. viridis* (green foxtail); BRAPP, *B. platyphylla* (broadleaf signalgrass); ECHCG, *E. crus-galli* (barnyard grass); ABUTH, *A. theophrasti* (velvetleaf); CASOB, *C. obtusifolia* (sicklepod); IPOHE, *I. hederacea* (ivyleaf morningglory).

In the *L. minor*, *B. juncea*, and *E. tef* bioassays chaparrinone had I_{50} values of 4.2, 0.9, and 2.1 μM, respectively (Table 1). The activities of chaparrinone and ailanthone were essentially the same in the *B. juncea* and *E. tef* bioassays conducted in the light, 0.9 μM for both ailanthone and chaparrinone in the *B. juncea* bioassay and 2.6 and 2.1 μM for ailanthone and chaparrinone, respectively, in the *E. tef* bioassay. Like ailanthone, chaparrinone was more active in the *B. juncea* and *E. tef* bioassays than in the *L. minor* bioassay, although chaparrinone was 4 times more active than ailanthone in the *L. minor* bioassay.

Chaparrinone has been previously isolated, although it has not been reported to have phytotoxic activity (Polonsky, 1973; Mitchell et al., 1971; Kubo et al., 1992). This compound differs from ailanthone only at C-21, having a methyl instead of a methylene group. Hoffmann et al. (1992) isolated holacanthone from *Castela emoryi* and reported that it had phytotoxic activity on grape seedlings. Holacanthone is identical to chaparrinone except that it has an acetyl group at C-15. It is difficult to compare the activities of chaparrinone and holacanthone since different bioassays were used. However, we suggest that chaparrinone is more active than holacanthone since the reported activity of holacanthone was 100 ppm (240 μM) on grape seedlings. The fraction containing chaparrinone was the most active in our bioassays, and no other strongly active fraction was observed. Therefore, we do not know whether holacanthone is not produced in *C. tortuosa* or if its concentration was simply too low for us to easily follow its activity.

Ailanthone was tested in a preliminary greenhouse experiment, conducted only one time with one replication, and it was found to have both pre- and postemergence activity on both grasses and broadleafed weeds (Table 2). Postemergence activity of ailanthone on all species was greater than its preemergence activity. In the preemergence test, *S. viridis* (green foxtail) was the most sensitive species, while most other weeds were only partially controlled at the highest application rate, 2.0 kg/ha, and *I. hederacea* (ivyleaf morningglory) had only slight injury at this rate. In the postemergence test *S. viridis* and *C. obtusifolia* (sicklepod) were completely controlled even at the lowest application rate of 0.125 kg/ha. The other four species tested were less sensitive to ailanthone than *S. viridis* and *C. obtusifolia*, though all were controlled at the highest application rate. This level of activity with ailanthone is comparable with the rates of application of some commercial herbicides. Heisey (1990) reported that the root bark extract from *A. altissima* also had pre- and postemergence activity on some of these weeds. This suggests that all or part of this phytotoxic activity in the root bark extract (Heisey, 1990) may be attributed to ailanthone. We did not conduct a greenhouse test on chaparrinone since a limited amount of this compound was available.

Only minimal information was obtained about the mode of action of ailanthone. When dose–response experiments were conducted in the dark with *B. juncea* and *E. tef*, the I_{50} values were only slightly higher than those found in experiments conducted in the light (Table 1). This indicates that ailanthone does not act by inhibiting photosynthesis. It is unclear why ailanthone was slightly more active in the light than in the dark. This dark experiment could not be conducted with *L. minor* since *L. minor* was grown photoautotrophically, without sucrose, and essentially no growth occurred in the dark. With concentrations of ailanthone that gave minimal growth of *E. tef*, the shoots and roots were very short and swollen. This type of growth was similar to that we observed with trifluralin, a herbicide that disrupts the mitotic process (Ashton and Crafts, 1981). When ailanthone and trifluralin were tested in the same experiment with *E. tef*, the patterns of growth for both compounds were similar, but not identical. Further experiments are necessary to determine if ailanthone acts as a mitotic disruptor.

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