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Essential fatty acids and phenolic acids from extracts and leachates of southern cattail (*Typha domingensis* P.)

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Abstract

We have been able to isolate several phytotoxic compounds from aqueous extracts and leachates of cattails (*Typha domingensis*) using activated charcoal as an absorbant, followed by successive extraction with organic solvents, analysis by GC/MS, and structural elucidation by NMR spectroscopy when possible. The phytotoxins were identified as essential fatty acids (linoleic acid and α-linolenic acid) and phenolic compounds of known phytotoxic activity (caffeic acid from the aqueous extracts; caffeic, *p*-coumaric, and gallic acid from the leachates). Both extracts and the phytotoxins in the extracts have the potential of inhibiting the growth and chlorophyll production of several ecologically relevant species.

Keywords: *Typha domingensis*; Typhaceae; Cattail; Phytotoxins; Fatty acids; Phenolics

1. Introduction

Cattails make up the genus *Typha* of the family *Typhaceae*, and are probably the most familiar of all wetland plants in the world. Different species of cattail occur commonly in wet soil, marshes, swamps, and shallow fresh and brackish waters throughout the world. The genus consists of ten species, three of which can be found in Florida: *Typha angustifolia* L., *Typha latifolia* L., and the southern cattail *Typha domingensis* P. (Long and Lakela, 1976).

There is ample evidence regarding the presence of a number of biologically active substances in cattail tissue (Gallardo et al., 1998). Eleven kinds of phenolic compounds were detected in female flowers of *T. latifolia* (Ozawa and Imagawa, 1988). Ishida and co-workers (1988) isolated a new flavonol glucoside from extracts of *T. latifolia* pollen. Aliotta and co-workers (1990) successfully isolated three steroids and three fatty acids (α-linolenic, linoleic, and an unidentified $C_{18:2}$) from dried *T. latifolia*. Several free and acylglucosylated stigmasterols were also found in *T. latifolia* (Della Greca et al., 1990c). The structure of (20*S*)-4α-methyl-24-methylenecholest-7-en-3β-ol, another sterol isolated from *T. latifolia,* was reported (Della Greca et al., 1990a). Also, two carotenoid-like compounds were also isolated from methanolic extracts of *T. latifolia* (Della Greca et al., 1990d), and a different set of compounds has been isolated from ether extracts of dry *T. latifolia*: 5β,8β-epidioxyergosta-6,22-dien-3β-ol, 5α,8α-epidioxyergosta-6,9(11),22-trien-3β-ol, and 5α,8α-epidioxyergosta-6,22-trien-3β-ol (Della Greca et al., 1990b).

Previous research (Prindle and Martin, 1996) dealing with aqueous extracts of *T. domingensis* noted its allelopathic properties; 2-chlorophenol and salicylaldehyde were found in the extracts (Prindle et al., 1996). These compounds have been found to be present in sediments in the *T. domingensis* growth front but not in sediments 2 m away from the cattail infestation (Albalat et al., 1997).

The present paper describes the isolation and characterization of two essential fatty acids and several biologically active phenolic compounds from aqueous extracts and leachates of the southern cattail, *T. domingensis*.

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2. Results and discussion

A bioassay-driven separation of cattail aqueous extracts led to the isolation of several compounds that were identified as unsaturated fatty acids and phenolic compounds. Compounds were identified on the basis of their mass spectra. The quantities isolated from the extracts were insufficient for IR or NMR studies and structural assignments were made on the basis of mass spectral data and co-elution with authentic samples. The compounds isolated from the cattail extracts are well-known phytotoxic agents, such as linoleic and α-linolenic acids (Aliotta et al., 1990). Benzoic acid, acetophenone, caffeic acid and coumarin, which are present in trace amounts, have been described previously as phytotoxic agents (Harborne and Baxter, 1993).

Cattail leachates were studied in order to obtain an accurate representation of the bioavailability of secondary metabolites from cattail in the aquatic ecosystem. Cattail leachates collected in the manner described and extracted with activated charcoal were used in a bioassay-driven fractionation. Only the diethyl ether fraction was found to be phytotoxic, containing several fractions that possessed distinct phytotoxic activity against Black Seeded Simpson lettuce (Table 1). After column chromatography and analysis by GC, and GC/MS of the active fractions, several bioactive compounds were identified (Table 2). The two essential fatty acids (linoleic and α-linolenic acid) were the major components of the cattail leachates, comprising more than 80% of the total isolated material. The total amount of leachate processed was 24 l and 983 mg of active organic materials was recovered. From this crude mixture, after purification by sequential column chromatography, enough linoleic and α-linolenic acid were present for facile isolation. Only traces of the phenolic acids were present. From the analysis of the spectral data, the structures of the fatty acids were elucidated. Co-injection with authentic standards corroborated the proposed structures.

Table 1
Effect of fractions of the ethyl ether extract of cattail leachate on the germination of lettuce

Fraction	% Germinated seeds after 3 days
100% Hexane	90
99% Hexane/1% diethyl ether	100
98% Hexane/2% diethyl ether	90
97% Hexane/3% diethyl ether	90
96% Hexane/4% diethyl ether	90
95% Hexane/5% diethyl ether	90
94% Hexane/6% diethyl ether	100
93% Hexane/7% diethyl ether	100
92% Hexane/8% diethyl ether	100
91% Hexane/9% diethyl ether	90
90% Hexane/10% diethyl ether	90
89% Hexane/11% diethyl ether	90
88% Hexane/12% diethyl ether	20
87% Hexane/13% diethyl ether	30
86% Hexane/14% diethyl ether	20
85% Hexane/15% diethyl ether	20
84% Hexane/16% diethyl ether	50
83% Hexane/17% diethyl ether	90
82% Hexane/18% diethyl ether	80
81% Hexane/19% diethyl ether	10
80% Hexane/20% diethyl ether	10
Control	100
10 ppm Phenol control	40

Table 2
Bioactive compounds isolated from cattail leachates

Compound	% Crude extract	Concentration in the leachate
Linoleic acid	20.3	8 mg/l
α-Linolenic acid	14.2	5.8 mg/l
Caffeic acid	Less than 1	–
p-Coumaric acid	Less than 1	–
Gallic acid	Less than 1	–

These essential fatty acids found in cattail extracts were also found in *Typha latifolia* tissue by Aliotta and co-workers (1990). Their phytotoxic properties are well known and understood. There is evidence of the allelopathic properties of long-chain fatty acids on algae reported by Proctor (1957) and confirmed by Kroes (1972). Among the active fatty acids previously isolated from cattails, the most effective against algae growth was α-linolenic acid (Aliotta et al., 1990).

3. Experimental

3.1. Physical methods

Infrared spectra of neat liquids of interest were obtained in a Perkin-Elmer Paragon 1000 FT-IR spectrometer. ^{1}H NMR, ^{13}C NMR, DEPT and COSY were acquired in $CDCl_3$ in a Brüker 360 NMR spectrometer.

All GC/MS studies were done using a Finnigan MAT INCOS series MS coupled with a Varian 3400 GC. Data were processed in an INCOS XL computer system. The column used was a J&W DB-1.30 m long, 0.25 mm i.d.

3.2. Plant material

Fully mature cattail samples were taken in the summer of 1997 near the University of South Florida campus in Tampa, FL. Dr. Richard Wunderlin, USF Herbarium, identified the samples as *T. domingensis*. After collection, the samples were rinsed with deionized water and maintained in half-strength Hoagland's medium (Steward and Elliston, 1973) in a controlled environment room (Environmental Growth Chambers, Chagrin Falls, OH)

for a month prior to use. The conditions in the growth chamber were as follows: constant temperature (26 °C), 12 h photoperiod with a light intensity of 190 μE m^{-2} s^{-1} at ground level and 80% relative humidity. The plants were set in plastic trays (59 cm L×40.5 cm W×17 cm H) and acid-washed sand was used as a solid support.

3.3. Cattail extracts

Mature cattail plants were divided in three sections: root systems, stems (taken from just above the root to the part where the leaves start separating), and leaves. Root and stem sections were weighed and extracted with deionized water (10 ml per 1.0 g of fresh material) for 3 min at room temperature using a blender. Leaves were discarded. The resulting crude extracts were then filter-sterilized, and germination inhibition bioassays were performed using lettuce seeds (*Latuca sativa* var. Black Seeded Simpson).

The filtered extracts were mixed with solvent-cleaned activated charcoal and agitated. The proportion of activated charcoal to aqueous extract was 1.5 g per 10 ml. After a 24-h period, the charcoal was filtered out. The aqueous phase was tested for phytotoxic activity against lettuce seeds and found to be inactive (no inhibition of germination was detected). The activated charcoal was extracted sequentially in a Soxhlet apparatus with diethyl ether, chloroform, and methanol. The organic fractions were dried over anhydrous Na_2SO_4 and, after evaporation of the solvent, each fraction was redissolved in dichloromethane (10 μl/mg). A 1-ml aliquot was taken from each fraction and placed on a disk of filter paper in a small Petri dish. The dish was left uncovered briefly to allow the solvent to evaporate. A control dish containing only dichloromethane on the paper dish was also prepared. Once dry, the filter paper was moistened with 2 ml of deionized water and ten lettuce seeds were placed on top of the filter paper. A 10-ppm phenol solution was used as a positive control. The Petri dish was then covered and placed under lights. After 3 days, the activity of the extracts was assessed. The extracts were considered to be active if any inhibition of germination was detected by the end of the third day. The diethyl ether fraction, which exhibited potential inhibitory activity, was further fractionated by column chromatography (silica gel 0.063–0.2 mm, 20 g), using hexane containing increasing percentages of diethyl ether as eluent, and tested in a second lettuce seed bioassay. The fractions that were found to be active were then esterified (excess 14% BF_3 in methanol), dried, and analyzed by GC/MS.

3.4. Cattail leachates

In order to collect the cattail leachate in a non-disruptive manner, a mature cattail harvested from the growth chamber was placed in an inverted, capped amber bottle that had the bottom removed. A hole in the cap allowed for insertion of a small-diameter hose that was clamped to prevent drainage. The system was placed in the environmental growth chamber, under the previously mentioned conditions of light and temperature. The container was filled with Hoagland's medium, and water losses due to evaporation were replenished with deionized water on a daily basis. After 2 weeks of acclimation, the growing medium was replaced with fresh Hoagland's, and this new solution was collected after 24 h. New medium was replenished and collected every day for 3 days. The collected leachate was then extracted with activated charcoal and fractionated and analyzed by GC/MS in the manner previously described.

3.5. Structural assignments

The isolated fatty acids were identified as the corresponding methyl esters. Methyl linoleate had a molecular ion at m/z 294 in its mass spectrum. Its ^{1}H NMR had four olefinic protons at δ 5.36, an ester methyl group at δ 3.86, a methylene α to two double bonds at δ 2.72, a methylene adjacent to the ester group at δ 2.30, two allylic methylenes at δ 2.08, a methylene at δ 1.86, seven methylenes at δ 1.60, and a methyl at δ 0.89. Double bond positions were estimated based on DEPT and COSY experiments. GLC comparison with an authentic sample (Sigma, St. Louis, MO) confirmed the structural assignment. Methyl α-linolenate had a molecular ion at m/z 292 in its mass spectrum. Its ^{1}H NMR showed six olefinic protons at δ 5.37, an ester methyl group at δ 3.66, two methylene groups α to two double bonds at δ 2.81, a methylene adjacent to the ester group at δ 2.30, two allylic methylenes at δ 2.09, a methylene at δ 1.62, four methylenes at δ 1.50, and a methyl at δ 0.98. Double bond positions were estimated based on DEPT and COSY experiments. GLC comparison with an authentic sample (Sigma, St Louis, MO) confirmed the structural assignment.

Phenolic compounds were present in the extracts and leachates in trace amounts, insufficient for NMR analysis, and were identified based on their mass spectrum and GLC comparison with authentic samples (Sigma, St Louis, MO).

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