Phytotoxic Melampolides from Leaf Extracts of *Samallanthus sonchifolius**

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Introduction

Yacon [*Smallanthus sonchifolius* (Poepp. and Endl.) H. Robinson; Compositae], originally cultivated in the Andean highlands, was introduced in Japan via New Zealand in 1985. In the Andean region, the tubers are served as food and the aerial parts are utilized as fodder for animals⁷⁾. Yacon has recently become very famous because of the high concentration of oligofructans¹⁾ in its tubers. We have been interested in yacon in another respect because it is easily cultivated without pesticides.

We earlier isolated antifungal compounds including a novel antifungal melampolide, sonchifolin³⁾ from yacon leaves. We speculated that the plant may contain other bioactive compounds as agrochemicals because Compositae is well known to be rich in useful bioactive compounds. Here, we report the isolation and properties of

phytotoxic compounds from yacon leaves.

Materials and Methods

Plant material

Yacon (*S. sonchifolius*) was cultivated at the Experimental Farm of Ibaraki University in Ami.

Bioassay

Samples in methanol were put on filter paper (5.5 cm, diameter) in a petri dish. After methanol had evaporated, 3 ml of distilled water was added. Four germinated seeds of lettuce (*Lactuca sativa*, L.) and barnyardgrass (*Echinochloa oryzicola* Vasing.) were placed on the filter paper and kept at 25°C under fluorescent light. After 7 days, the radicle length of the lettuce and barnyardgrass was measured and the inhibition percentage was calculated against control. The experiment was repeated twice.

Isolation of the active compound

Crude extract was obtained from 800 g of fresh yacon leaves as described³⁾ and the crude extract was loaded on a silica gel column (eluted with EtOAc:Hex=2:1) to afford the active fractions. The active fractions were concentrated and dissolved in a small volume of MeOH and kept at 5°C for 2 days to give crystals of the active compound (40 mg), which appeared as one peak by HPLC analysis (Cosmosil 5C18AR, CH₃CN: H₂O=1:1) detected at 210 nm.

Hydrogenation of enhydrin

A solution of enhydrin (5 mg) and 5% Pd/C (5 mg) in ethanol (2 ml) was stirred overnight

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under H_2 at room temp. After removal of Pd/C by filtration, solvent was removed to give hydrogenated enhydrin quantitatively.

Results and Discussion

We purified the active compound by following the activity against lettuce. Properties of the active compound were as follows: IR (v max, neat) cm^{-1} : 1771, 1715, 1230, 1120. FD-MS m/z 465 (M+H), 349, 99, 88. ^1H -NMR (500 MHz $CDCl_3$); δ 1.17(3H, d, $J=5.5$ Hz), 1.45(3H, s), 1.71(3H, s), 2.05(3H, s), 2.35(1H, m), 2.46(1H, m), 2.67(1H, d, $J=10.0$ Hz), 3.00(3H, m), 3.83(3H, s), 4.28(1H, t, $J=10.0$ Hz), 5.84(1H, d, $J=3.3$ Hz), 5.87(1H, d, $J=9.1$ Hz), 6.34(1H, d, $J=3.5$ Hz), 6.71(1H, dd, $J=9.1$, 1.3 Hz), 7.15(1H, dd, $J=10.8$, 7.5 Hz). ^{13}C -NMR(125MHz, $CDCl_3$); δ 13.6, 17.4, 19.1, 20.2, 24.7, 39.4, 45.5, 51.8, 58.9, 59.1, 59.5, 62.7, 70.7, 71.3, 75.8, 121.7, 130.3, 134.4, 147.4, 165.5, 167.9, 168.5, 170.2.

These ^1H and ^{13}C -NMR, MS, and IR spectra revealed that the active compound was enhydrin **1**⁴). Enhydrin **1** inhibited radicle elongation of lettuce seedling 50% at 100 ppm.

Sonchifolin **3**³), polymatin B^{4⁸), uvedalin **5**²) and enhydrin **1** have already been isolated from yacon leaves as fungicidal compounds.}

Among these melampolides, sonchifolin **3** showed the most effective fungicidal activity against *Pyricularia oryzae* followed by polymatin B**4**, uvedalin **5** and enhydrin **1** in this order³).

We compared the phytotoxic activities among these melampolides and found that enhydrin **1** was most effective against lettuce and uvedarin **5** was most effective against barnyardgrass. Enhydrin **1** was more active against lettuce than against barnyardgrass, while sonchifolin **3**, polymatin B**4** and uvedalin **5** were more active against barnyardgrass. These results are summarized in the Table 1. Negligible activity was shown against the aerial part of these plants. To our best knowledge, this is the first report on the phytotoxic activities of these melampolides including enhydrin **1**.

We modified this α -methylene- γ -lactone moiety of enhydrin **1** by hydrogenation with Pd/C to prepare derivative **2**, because this moiety is present in many of the isolated natural melampolides, and has been proposed as one of the factors which can serve their allelopathic activities⁶). In ^1H -NMR spectrum of derivative **2**, new signals came up at δ 1.27 (3H, d, $J=5.5$ Hz) and δ 2.98 (1H, m) instead of two olefinic protons at δ 6.71 and δ 5.87 ppm, suggesting that hydrogenation

Table 1. Phytotoxic Activities of Enhydrin (Compound **1**) and its Related Compounds **2-5**.

Compound	Inhibitory activity (%) against radicle growth*			
	Lettuce		Barnyardgrass	
	100 ppm	200 ppm	100 ppm	200 ppm
1	50	77	—**	72
2	—**	—**	—**	—**
3	—**	5	77	86
4	26	47	73	81
5	—**	23	95	91

*Average of eight seeds against control.

**No inhibition.

took place at this moiety. ^1H -NMR of derivative **2** was as follows: (500 MHz, CDCl_3) δ ; 1.16 (3H, d, $J=7.5$ Hz), 1.27 (3H, d, $J=5.5$ Hz), 1.48 (3H, s), 1.68 (3H, s), 2.00 (3H, s), 2.32 (1H, m), 2.42 (1H, m), 2.55 (2H, m), 2.28 (1H, m), 2.98 (1H, m), 3.05 (1H, m), 3.80 (3H, s), 4.48 (1H, t, $J=10.5$ Hz), 5.80 (1H, d, $J=7.5$ Hz), 6.30 (1H, d, $J=7.5$ Hz), 7.08 (1H, dd, $J=11.5, 6.5$ Hz). Derivative **2** showed no inhibitory activity against either lettuce or barnyardgrass at 200 ppm. Thus, this α -methylene- γ -lactone moiety in enhydrin **4** was suggested to be important for the phytotoxic activity.

Recently Macias⁵⁾ reported that melampolide is one of the most potential natural allelochemicals from higher plants. Yacon is a very useful plant which contains these potential natural agrochemicals.

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