Pergamon

0031-9422(95)00023-2

Phytochemistry, Vol. 39, No. 4, pp. 845–848, 1995
Copyright © 1995 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0031-9422/95 $9.50 + 0.00

ANTIFUNGAL MELAMPOLIDES FROM LEAF EXTRACTS OF *SMALLANTHUS SONCHIFOLIUS*

ATSUSHI INOUE, *SHIGERU TAMOGAMI, HIDEKI KATO, YUMIKO NAKAZATO, MASAKI AKIYAMA, OSAMU KODAMA,† TADAMI AKATSUKA and YASUYUKI HASHIDOKO‡

Laboratory of Bioorganic and Pesticide Chemistry, School of Agriculture, Ibaraki University, Ami, Ibaraki 300-03, Japan; ‡JRDC Plant Ecochemicals Project, 1-1 Megumino Kita 3-Chome, Eniwa, Hokkaido 061-13, Japan

(*Received in revised form* 17 *November* 1994)

Key Word Index—*Smallanthus sonchifolius* (Poepp. and Endl.) H. Robinson; Compositae; Yacon; antifungal melampolide; sesquiterpene lactone; sonchifolin.

Abstract—A new antifungal melampolide, 8-angeloyl-1(10),4,11(13)-germacuratrien-12,6-olid-14-oic acid methyl ester, named sonchifolin, as well as three known melampolides, polymatin B, uvedalin and enhydrin, were isolated from leaf extracts of Yacon [*Smallanthus sonchifolius* (Poepp. and Endl.) H. Robinson]. Sonchifolin exhibited the highest fungicidal activity against *Pyricularia oryzae*, a fungus causing rice blast disease and the ED_{50} value for the spore germination was 22 ppm. This is the first report of these melampolides as fungicidal compounds.

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 used as a food and the aerial parts are utilized as fodder for animals [1]. Recently, Ohyama *et al.* [2] reported that the Yacon tubers contained a high concentration of oligofructans and the plant drew attention as a natural source for the supply of oligofructans. We speculated that the aerial part should contain some antifungal and pesticidal compounds, as it is not necessary to use pesticides in the cultivation of Yacon. Therefore, we first investigated the antifungal compounds against *Pyricularia oryzae* from leaf extracts of Yacon.

Recently, Kakuta *et al.* [3] isolated some kaurene-type diterpenoids from the glandular trichome exudate and leaf extracts of *S. sonchifolius* and suggested that these constituents were defensive substances. However, they did not report the bioactivity of the constituents.

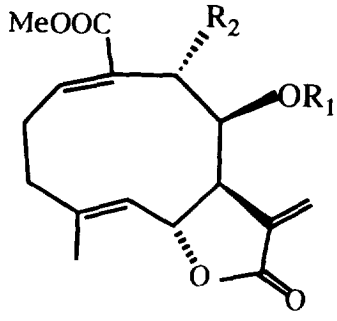
We now report the isolation and structure elucidation of a new fungicidal melampolide (**1**) which we have named sonchifolin, together with three known melampolides, polymatin B (**2**), uvedalin (**3**) and enhydrin (**4**) from leaf extracts of *S. sonchifolius*. The relation between the fungicidal activities and the structures is also discussed.

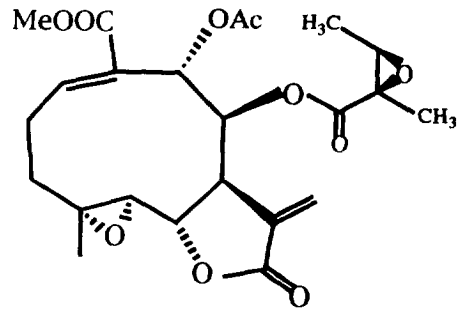
*Present address: Agricultural Technology and Food Technology, Institute of Hokuren, Sapporo, Hokkaido 060, Japan.

†Author to whom correspondence should be addressed.

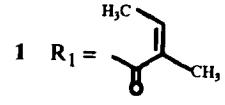
RESULTS AND DISCUSSION

The 70% methanol extract of yacon leaves was subjected to column chromatography to afford antifungal compounds **1**–**4**. Compound **1**, which had highest antifungal activity had the molecular formula $C_{21}H_{26}O_6$. It was identified as 8-angeloyl-1(10),4,11(13)-germacuratrien-12,6-olid-14-oic acid methyl ester. Assignments of the ^{1}H NMR and ^{13}C NMR spectra and the relative stereochemistry of sonchifolin were made on the basis of a standard ^{13}C–^{1}H COSY, HMBC and NOE experiments. The ^{13}C NMR and ^{1}H NMR data are shown in Table 1 and the ^{13}C–^{1}H COSY experiment showed the C–H connectivities. The ^{13}C NMR spectrum of the compound showed peaks for 21 carbons (Table 1) and the multiplicity of signals was established using the DEPT experiment. The DEPT experiments also established the presence of all 26 protons bonded to those carbons. Interpretation of the ^{13}C NMR data indicated that three carbonyl carbons at δ169.5 (C-12), 167.0 (C-14), 166.2 (C-1′) and eight olefinic carbons at δ142.0 (C-1), 137.8 (C-4), 125.5 (C-5), 131.2 (C-10), 135.2 (C-11), 120.0 (C-13), 127.0 (C-2′), 139.0 (C-3′) were present. Typical signals at 127.0 (C-2′), 139.0 (C-3′), 15.5 (C-4′), and 20.5 (C-5′) in the ^{13}C NMR spectrum and protons at 6.10 (H-3′, *br q*, J = 7.5 Hz) and at 1.85 (H-5′, *br s*) were characteristic of an angeloyloxy group. The position of the angeloyloxy group was deduced to be at C-8 (δ66.4) by the cross peak between H-8 (δ6.31) and C-1′ (δ166.2) in the HMBC spectrum (Fig. 1). Two typical doublets in the ^{1}H NMR spectrum at 5.56 (H-13, *br d*, J = 3.5 Hz) and 6.23 (H-13, *br d*, J = 3.5 Hz) and 2.61 (H-7, *m*), and

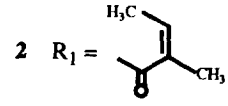




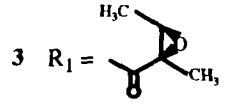
4



1 R_1 = R_2 = H



2 R_1 = R_2 = OAc



3 R_1 = R_2 = OAc

Table 1. ^{13}C–^{1}H heterocosy of **1**

Position	δ_C	δ_H
1	142.0 (1)*	6.83 (*br ddd*, J = 10.0 Hz, 7.5 Hz and 1.6 Hz)
2	25.4 (2)	2.12 (*br t*, J = *ca* 12.0 Hz) 2.36 (*m*)
3	37.0 (2)	2.06 (*br t*, J = *ca* 10.0 Hz) 2.31 (*m*)
4	137.8 (0)	
5	125.5 (1)	5.09 (*m*)
6	75.8 (1)	5.11 (*m*)
7	49.5 (1)	2.61 (*m*)
8	66.4 (1)	6.31 (*br ddd*, J = *ca* 9.8 Hz, *ca* 7.5 Hz and *ca* 1.0 Hz)
9	30.0 (2)	2.03 (*br t*, J = *ca* 10.4 Hz)
		2.86 (*m*)
10	131.2 (0)	
11	135.2 (0)	
12	169.5 (0)	
13	120.0 (2)	5.56 (*br d*, J = 3.5 Hz)
		6.23 (*br d*, J = 3.5 Hz)
14	167.0 (0)	
15	52.0 (3)	1.89 (*s*)
16	17.0 (3)	3.78 (*s*)
1′	166.2 (0)	
2′	127.0 (0)	
3′	139.0 (1)	6.10 (*br q*, J = 7.5 Hz)
4′	15.5 (3)	1.98 (*br d*, J = *ca* 7.5 Hz)
5′	20.5 (3)	1.85 (*br s*)

*The numbers in parentheses indicate the number of hydrogens attached to the corresponding carbon and were determined by DEPT experiments.

absorbance at 1771 cm^{-1} in the IR spectrum, suggested the presence of an α-methylene-γ-lactone moiety. The correlations between these carbons, C-13 (δ120.0) and C-7 (δ49.5), and their neighbouring carbons in HMBC validated the assignment of this α-methylene-γ-lactone moiety, to be C-6–C-7–C-11–C-12 and C-11–C-13 as shown in Fig. 1. Other connectivities of proton bearing carbons, C-1–C-2–C-3 and C-5–C-6–C-7–C-8–C-9 were easily deduced. Moreover, correlations between the quaternary carbons, C-4 (δ137.8) and C-10 (δ131.2), and their neighbours made clear the total skeleton of sonchifolin as shown in Fig. 1. NOE observed between H1

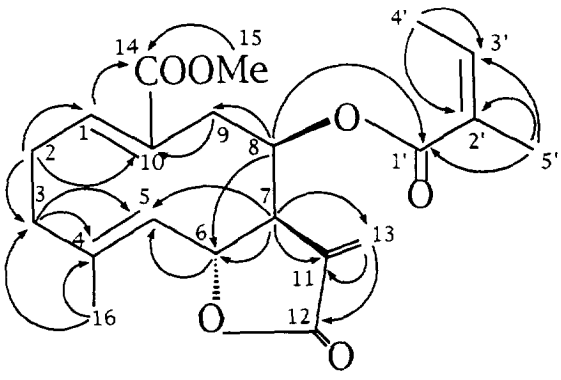


Fig. 1. Correlation observed in the HMBC spectrum of **1**.

(δ6.83) and H15 (δ1.98), H6 (δ5.11) and H16 (δ3.78), H3′(δ6.10) and H5′(δ1.85) established the geometry of the three double bonds at C-1 = C-10, C-4 = C-5, C-2′ = C-3 = , as *E*, *E* and *Z*, respectively. The NOEs observed between H-7 (δ2.61) and H-8 (δ6.31), H-5 (δ5.09) and H-7 (δ2.61), and H-6 (δ5.11) and H-16 (δ3.78) established the conformation of the lactone ring and the orientation of the angeloyloxy group as shown in Fig. 1. Assignments of stereochemistry for all the proposed structures relied upon results of NOE experiments of the four compounds **1**–**4**. Further investigations will be needed to confirm the stereochemistry of the compounds, and these are now in progress.

Compound **2** was characterized as an acetoxy derivative at C-9 of sonchifolin and the HRMS showed a molecular formula $C_{23}H_{28}O_8$. This compound was also identified in *Smallanthus maculata* and named polymatin B [5]. Compound **3** had a molecular formula $C_{23}H_{29}O_9$ and the spectral data were closely related to compound **2**. Differences were observed in the values of the signals corresponding to the epoxidized angeloyloxy group. This compound named uvedalin was also identified in *Smallanthus uvedalia* [6]. Compound **4** had a molecular formula $C_{23}H_{28}O_{10}$ and the spectral data was similar to **3**. Differences were observed in the signals which were indicative of an additional epoxide group to **3**. Compound **4** has also been identified in another plant, *Enhydra fluctuans*, and named enhydrin [7]. Epoxidation of **3** with *m*-CPBA gave an epoxide derivative, identical with **4**. The ^{13}C NMR spectra of compounds **1**–**4** are shown in Table 2.

Fungicidal activities of sonchifolin (**1**), polymatin B (**2**), uvedalin (**3**) and enhydrin (**4**) against *P. oryzae* are shown in Table 3. Among these four melampolides, the sonchifolin exhibited the most potent activity. Enhydrin showed antifungal activity only against the germ tube growth at 500 ppm. Among the four melampolides, enhydrin showed the lowest activity, therefore the 4,5 epoxide of enhydrin is not responsible for the activity. The activity of polymatin B is much higher than that of uvedalin. Therefore, the angeloyloxy group rather than the epoxyangeloyloxy group is necessary for fungicidal activity. As the activity of sonchifolin is much higher than that of polymatin B, the acetyl group at the C-9 position of enhydrin, uvedalin and polymatin B does not seem to be required for the activity. As the order of elution of enhydrin, uvedalin, polymatin B and sonchifolin, on a C18 HPLC column correlated with the activities, the polarity of these compounds appear to be very important for the activity.

Table 2. ^{13}C NMR spectral data of **1**–**4** ($CDCl_3$, TMS)

Position	1	2	3	4*
1	142.0	148.3	148.1	147.4
2	25.4	26.1	26.1	24.7
3	37.0	37.0	36.8	39.4
4	137.8	138.6	131.1	58.9
5	125.5	126.3	126.9	62.7
6	75.8	75.5	74.9	75.8
7	49.5	51.0	51.1	45.5
8	66.4	69.1	71.3	71.3
9	30.0	71.3	71.1	70.7
10	131.2	130.7	135.5	130.3
11	135.2	134.6	137.9	134.4
12	169.5	169.4	168.6	168.5
13	120.0	121.7	120.6	121.7
14	167.0	164.8	165.7	165.5
15	17.0	17.0	16.5	17.4
16	52.0	52.3	51.8	51.8
1′	166.2	166.3	168.7	167.9
2′	127.0	126.9	59.2	59.1
3′	139.0	139.5	59.6	59.5
4′	15.5	15.8	13.7	13.6
5′	20.5	20.5	19.3	19.1
OAc	—	170.2	170.0	170.2
	—	20.8	20.3	20.2

*Compound **4** was measured in C_6D_6.

Table 3. Antifungal activities of **1**–**4** against spore germination of *P. oryzae*

	Inhibitory activity (%)					
	Concentration (ppm)					
Compound	20	25	50	100	250	500
1	29.0	97.5	99.2	100	100	100
2	0	5.0	24.4	69.1	100	100
3	0	0	0	4.0	70.2	100
4	0	0	0	0	5.1	5.1

EXPERIMENTAL

General. ^{1}H NMR: 500 MHz; ^{13}C NMR: 125 MHz, $CDCl_3$ with TMS as int. standard; IR: KBr pellet.

Plant material. Fresh leaves of *S. sonchifolius* (Poepp. and Endl.) H. Robinson were supplied by Experimental Farm of Ibaraki University in Ami. The tubers possessing young buds were transplanted into the farm in May and the leaves were collected in October.

Extraction and isolation. Yacon leaves (1 kg) were cut and homogenized in 70% MeOH with an ultrahomogenizer (Physcotron) and extracted for one day using a

shaker at room temp. After centrifugation at 3900 *g* for 15 min, the extract was concd *in vacuo* to remove the MeOH. The concentrate was further extracted with EtOAc (× 3). The combined extract was evaporated *in vacuo*. The residue dissolved in MeOH was applied on a silica gel column with C_6H_6–EtOAc (5:1) to afford 3 fungicidal frs (Fr. 1–Fr. 3). Fr. 1 was applied onto a silica gel column and eluted by C_6H_6–EtOAc (12:1) to afford 2 fungicidal frs. These 2 frs where then subjected to BOND ELUT C18 cartridge using 70% MeOH. The eluates were further purified by HPLC (× 2) on a Cosmosil 5C18AR using MeOH–H_2O (7:1) and then MeCN–H_2O (1:1). Fr. 1 yielded **1** (15 mg) and **2** (12 mg). Frs 2 and 3 were applied on a BOND ELUT C18 cartridge using 60% MeOH and ODS HPLC on Cosmosil 5C18AR using MeOH–H_2O (6:4). Finally, Fr. 2 and Fr. 3 yielded **3** (30 mg) and **4** (40 mg), respectively.

Sonchifolin (**1**). Oil; molecular formula: $C_{21}H_{26}O_6$ (found 374.1704, calcd 374.1728); UV λ_{max}^{MeOH} nm: 219; $[\alpha]_D^{25}$ + 33.0° ($CHCl_3$; *c* 4.1). IR ν_{max}^{KBr} cm^{-1}: 1771, 1715, 1439, 1304, 1304, 1231, 1142. EIMS *m/z* (rel. int.): 374 (3.1), 356 (1.2), 343 (11.2), 314 (34.6), 292 (17.3), 274 (75.3), 242 (100), 215 (60.4), 169 (19.7), 105 (48.1). ^1H and ^{13}C NMR: see Tables 1 and 2.

Polymatin B (**2**). ^1H NMR (500 MHz, $CDCl_3$): δ7.01 (1H, *dd*, *J* = 10.3 Hz, 8.3 Hz), 6.68 (1H, *dd*, *J* = 8.6 Hz, 1.3 Hz), 6.28 (1H, *d*, *J* = 3.0 Hz), 6.08 (1H, *dddd*, *J* = 7.1 Hz, 1.5 Hz), 5.81 (1H, *d*, *J* = 3.0 Hz), 5.41 (1H, *d*, *J* = 8.6 Hz), 5.11 (1H, *t*, *J* = 10.0 Hz), 3.81 (3H, *s*), 2.78 (1H, *m*), 4.96 (1H, *d*, *J* = 10.8 Hz), 2.41 (2H, *m*), 1.96 (3H, *s*), 1.95 (3H, *dd*, *J* = 7.1 Hz, 1.3 Hz), 1.83 (3H, *br s*). ^{13}C NMR: see Table 2.

Uvedalin (**3**). ^1H NMR (500 MHz, $CDCl_3$): δ7.05 (1H, *dd*, *J* = 10.3 Hz, 7.5 Hz), 6.66 (1H, *dd*, *J* = 1.3 Hz, 8.5 Hz), 6.26 (1H, *d*, *J* = 3.3 Hz), 5.73 (1H, *d*, 3.3 Hz), 5.41 (1H, *d*, *J* = 8.5 Hz), 5.11 (1H, *t*, *J* = 10.0 Hz), 4.95 (1H, *br d*, *J* = 10.8 Hz), 3.81 (3H, *s*), 3.02 (1H, *ddd*, *J* = 5.3 Hz), 2.79 (1H, *m*), 2.43 (2H, *m*), 2.01 (3H, *s*), 2.01 (3H, *s*), 1.47 (3H, *s*), 1.19 (3H, *d*, *J* = 5.3 Hz); ^{13}C NMR: see Table 2.

Enhydrin (**4**). ^1H NMR (500 MHz, $CDCl_3$): 7.15 (1H, *dd*, *J* = 10.8 Hz, 7.5 Hz), 6.71 (1H, *dd*, *J* = 9.1 Hz, 1.3 Hz), 6.34 (1H, *d*, *J* = 3.0 Hz), 5.87 (1H, *d*, *J* = 9.1 Hz), 5.84 (1H, *d*, *J* = 3.3 Hz), 4.28 (1H, *dd*, *J* = 10.0 Hz), 3.83 (3H, *s*), 3.00 (1H, *m*), 3.00 (1H, *m*), 2.67 (1H, *d*, *J* = 10.0 Hz), 2.46 (1H, *m*), 2.35 (1H, *m*), 2.05 (3H, *s*), 1.71 (3H, *s*), 1.45 (3H, *s*), 1.17 (3H, *d*, *J* = 5.0 Hz); ^{13}C NMR: see Table 2.

Assay of antifungal activity. The antifungal activity was estimated using a spore suspension of *P. oryzae*, according to a previous method [4].

Acknowledgements—We thank Professor T. Tsukihashi, Ibaraki University for providing the plant material. This work was supported in part by a grant from Hokuren.

REFERENCES

1. Tsukihashi, T., Yoshida, T., Miyamoto, M. and Suzuki, N. (1989) *Japanese J. of Farm Work Res.* **24**, 32.
2. Ohyama, T., Ito, O., Yasuyosi, S., Ikarashi, T., Minamisawa, K., Kubota, M., Tsukihashi, T. and Asami, T. (1990) *Soil Sci. Plant Nutr.* **36**, 167.
3. Kakuta, H., Seki, T., Hashidoko, Y. and Mizutani, J. (1992) *Biosci. Biotech. Biochem.* **56**, 1562.
4. Akatsuka, T., Kodama, O., Sekido, H., Kono, Y. and Takeuchi, S. (1985) *Agric. Biol. Chem.* **49**, 1689.
5. Van, N. L. and Fischer, N. H. (1979) *Phytochemistry* **18**, 851.
6. Herz, W. and Bhat, S. V. (1970) *J. Org. Chem.* **35**, 2605.
7. Joshi, B. S., Kamat, V. N. and Fuhrer, H. (1971) *Tetrahedron Letters* **26**, 2373.