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PLANT GROWTH REGULATORS FROM *HERACLEUM LANATUM*

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Key Word Index—*Heracleum lanatum*; Umbelliferae; coumarins; furanocoumarins; phenolic compounds; growth inhibitor; root formation.

Abstract—The plant growth regulating substances, pimpinellin, isopimpinellin, bergapten, isobergapten, vaginidiol, sphondin, scopoletin, umbelliferone, ferulic acid, *p*-coumaric acid and apterin were isolated from root of *Heracleum lanatum*. Scopoletin and umbelliferone were highly inhibitory to the growth of Chinese cabbage seedlings, and bergapten and sphondin to the growth of hypocotyl cuttings of cucumber seedlings. Bergapten and sphondin accelerated root formation of cucumber hypocotyl cuttings.

INTRODUCTION

Our previous paper reported that an extract from roots of *Heracleum lanatum* Michx. var. *nipponicum* Hara markedly increased the number of adventitious roots of hypocotyls of cucumber cuttings, and that it inhibited the growth of Chinese cabbage roots and cucumber cutting hypocotyls. The extract accelerated adventitious root formation, more powerfully than any of 108 crude drug extracts and nine medicinal plant extracts tested [1].

Junttila [2] reported on allelopathic effect by *H. lacimiatum* Horn. We presume that *H. lanatum* var. *nipponicum* which often grows in large thickets in riverside places also possesses allelopathic effects.

In the present investigation, various components derived from root of *H. lanatum* var. *nipponicum* were tested for the effect on growth of Chinese cabbage roots and cucumber hypocotyls and on adventitious root formation of cucumber cuttings.

RESULTS AND DISCUSSION

Separation of active components

The methanol extract (methanol and aqueous extracts possessed the same activity) of dried roots of *H. lanatum* gave pimpinellin (**1**), isopimpinellin (**2**), bergapten (**3**), isobergapten (**4**), vaginidiol (**5**), sphondin (**6**), scopoletin (**7**), umbelliferone (**8**), ferulic acid (**9**), *p*-coumaric acid (**10**) and apterin (**11**). Of these nine coumarins and two phenolic compounds, **1–4**, **6** and **8** had been isolated from the roots of this plant by Fujita and Furuya [3]. The presence of compounds **5**, **7**, and **9–11** in this plant had not been reported previously.

The optical rotation (+230°) of our apterin is opposite to the value given in the lit. (−229°) [4]. In this regard, Dr. Steck told us that the sign had been misprinted in ref. [4] and that the true optical rotation was +229°.

Physiological activities.

The effect of these compounds on the growth of Chinese cabbage roots is shown in Table 1. All the compounds inhibited root growth. The effects of scopoletin (**7**) and umbelliferone (**8**) were the most pronounced, while those of the other compounds were rather mild. At the concentrations used, non of the compounds inhibited the germination of Chinese cabbage seeds, and all the seeds germinated within 24 hr.

As shown in Table 2, compounds **1**, **3** and **6–11** inhibited the growth of hypocotyl cuttings of cucumber seedlings with bergapten (**3**) and sphondin (**6**) the most active. It is considered that **2**, **4** and **5**, which are poorly soluble in water, will bring about a greater inhibition of the growth of the hypocotyls at higher concentrations than the test solutions.

The effect of these compounds on adventitious root formation of hypocotyl cuttings of cucumber seedlings is summarized in Table 3. Compounds **1**, **3**, **4**, **6**, **8** and **10** increased the number of roots. Thus, bergapten (**3**) and sphondin (**6**) effectively inhibited the growth of hypocotyl cuttings, and at the same time, accelerated their root formation. It should be noted that the acceleration of root formation by these compounds at 10 ppm was more intense than shown by IAA at 25 ppm. Similarly, the root-forming activity of **8** at 100 ppm was higher than that of IAA at 25 ppm. However, **3** and **6** at 10 ppm and **8** at 100 ppm somewhat inhibited root formation at the lower part of the hypocotyls. Coumarin [5] and umbelliferone [6] have been reported as rooting promoters, but the effects of **3** and **6** were stronger than that of umbelliferone.

Junttila suggests that the seeds of *H. lacimiatum* contain substance(s) inhibitory to lettuce seed germination [2]. Most of the nine coumarins and two phenolic acids isolated from *H. lanatum* var. *nipponicum* in the present study, were found to show growth inhibitory effects on Chinese cabbage seedlings and an accelerating effect on root formation of cucumber cuttings.

A close relationship between IAA-oxidase activity and adventitious root formation has been reported [7–12], but the interaction between coumarins and IAA or other growth regulators on root formation has not

Table 1. Effect of isolated compounds on growth of roots of Chinese cabbage seedlings

Treatment (ppm)	1	5	10	50	100
Pimpinellin (1)	92	88	74*	—	—
Isopimpinellin (2)	101	97	79*	—	—
Bergapten (3)	96	85	69*	—	—
Isobergapten (4)	95	90	71*	—	—
Vaginidiol (5)	96	91	80*	—	—
Sphondin (6)	98	98	72*	—	—
Scopoletin (7)	86	—	52*	44*	24*
Umbelliferone (8)	98	—	51*	15*	7*
Ferulic acid (9)	95	—	82	71*	66*
p-Coumaric acid (10)	101	—	103	86	44*
Apterin (11)	104	—	84	77*	62*

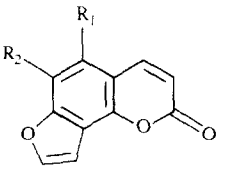
Each value represents the mean of the root lengths as a percentage of control (45 ± 8.2 mm).
*Significantly different from control at 5% level, *t*-test.

Table 2. Effect of isolated compounds on growth of hypocotyls of cucumber cuttings

Treatment (ppm)	1	5	10	50	100
Pimpinellin (1)	110	101	79*	—	—
Isopimpinellin (2)	101	105	86	—	—
Bergapten (3)	112	70*	52*	—	—
Isobergapten (4)	120	101	88	—	—
Vaginidiol (5)	106	98	88	—	—
Sphondin (6)	115	71	62*	—	—
Scopoletin (7)	101	—	91	76*	72*
Umbelliferone (8)	107	—	111	77*	67*
Ferulic acid (9)	93	—	96	92	69*
p-Coumaric acid (10)	99	—	92	79*	72*
Apterin (11)	102	—	92	76*	71*

Each value represents the mean of the hypocotyl lengths as a percentage of control (50 ± 5.6 mm).
*Significantly different from control at 5% level, *t*-test.

been elucidated. On the other hand, it was reported that the inhibition of seedling growth by coumarins was accompanied by an increase in IAA-oxidase or IAA-oxidation[13–15], but Svensson[16] mentioned that coumarins directly inhibited growth, and IAA-oxidase was not involved. We are now studying the mechanism of root formation by coumarins.



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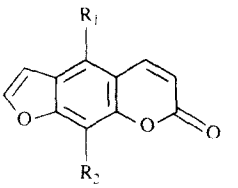
4 R_1 = OMe, R_2 = H
6 R_1 = H, R_2 = OMe



2 R_1, R_2 = OMe
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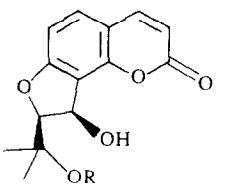

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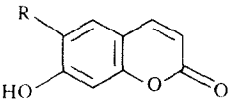
11 R = 1-β-D-glucosyl



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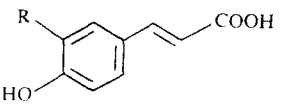

9 R = OMe
10 R = H

Table 3. Effect of isolated compounds on adventitious root formation of cucumber cuttings

Treatment (ppm)	Number of roots (>0 mm)*				
	1	5	10	50	100
Pimpinellin (1)	27.8 ± 2.9	35.0 ± 4.4	38.0 ± 4.0	—	—
Isopimpinellin (2)	23.3 ± 1.3	27.3 ± 3.3	31.5 ± 2.8	—	—
Bergapten (3)	35.8 ± 3.3	49.6 ± 3.4	66.9 ± 7.2	—	—
Isobergapten (4)	26.6 ± 3.5	27.3 ± 5.4	36.8 ± 4.4	—	—
Vaginidiol (5)	25.6 ± 5.7	24.9 ± 2.6	29.5 ± 5.6	—	—
Sphondin (6)	30.3 ± 4.0	49.5 ± 3.7	56.6 ± 3.7	—	—
Scopoletin (7)	30.0 ± 2.5	—	31.7 ± 8.2	31.0 ± 5.6	33.2 ± 5.4
Umbelliferone (8)	28.3 ± 3.4	—	39.4 ± 5.3	46.0 ± 10.5	65.1 ± 10.8
Ferulic acid (9)	25.7 ± 5.1	—	26.6 ± 4.7	25.4 ± 2.5	22.8 ± 5.6
p-Coumaric acid (10)	33.6 ± 5.5	—	29.5 ± 6.8	33.4 ± 3.2	51.1 ± 6.2
Apterin (11)	22.5 ± 2.2	—	26.1 ± 2.5	26.1 ± 2.9	24.4 ± 2.1

*Control (H_2O) 24.2 ± 3.0; reference (IAA, 25 ppm) 48.6 ± 4.0.

EXPERIMENTAL

^{1}H NMR: 100 MHz, TMS as int. standard; GC: OV-101, 100–200°.

Test solution. Aq. solns of **7–11** were prepared at concns of 1, 10, 50 and 100 ppm, and those of **1–6**, which are sparingly soluble in water, at 1, 5 and 10 ppm. H_2O was used as control soln, and 25 ppm IAA soln as the reference soln.

Bioassay with Chinese cabbage seedlings. A piece of filter paper, impregnated with 7.0 ml test soln was placed in a Petri dish (9 × 1.5 cm). Twenty seeds of Chinese cabbage (*Brassica rapa* L. var. *pervidis* Bailey) were placed on the filter paper and kept at 25° in the dark. After 3 days, the lengths of the roots were measured. The expt was carried out in triplicate.

Bioassay with cucumber cuttings. Seedlings of cucumber (*Cucumis sativus* L.) were grown at 25° in the dark for 3–4 days, when the hypocotyls were *ca* 4.5 cm long. Hypocotyls were excised 3 cm below the cotyledons and were dipped in small test tubes each containing one of the test solns. After 9 days incubation at 25° in the dark, the length of the hypocotyls and the number of rootlets protruding were recorded. For each test soln, four hypocotyl cuttings were used. The expt was done in triplicate.

Extraction. Roots of *H. lanatum* were collected at Hino, Tokyo, in late April 1979 and dried at room temp. The dried root (3 kg) was extracted with hot MeOH (40 l). The extract, after removal of the solvent, was treated with hot *n*-hexane (4 l.), Et_2O (4 l.) and EtOAc (4 l.), successively to give an *n*-hexane extract, an Et_2O extract, an EtOAc extract and 'residue'.

Isolation of compounds from the n*-hexane and* EtOAc *extracts.* The ppt produced by cooling the *n*-hexane extract was chromatographed on Si gel. Elution with *n*-hexane–$CHCl_3$ (5:1) gave bergapten (**3**) $C_{12}H_8O_4$ (270 mg), mp 118–119°, and then isopimpinellin (**2**) $C_{13}H_{10}O_5$ (50 mg), mp 146–148°. The supernatant of the *n*-hexane extract was chromatographed over Si gel. Elution with *n*-hexane–EtOAc (20:1) yielded isobergapten (**4**) $C_{12}H_8O_4$ (800 mg), mp 219–220°, and then pimpinellin (**1**) $C_{13}H_{10}O_5$ (1.1 g), mp 116–118°. CC of the EtOAc extract over Si gel eluted with mixtures of *n*-hexane–$CHCl_3$ and $CHCl_3$–MeOH gave sphondin (**6**) $C_{12}H_8O_4$ (1.3 g), mp 189–191° in the *n*-hexane–$CHCl_3$ (5:1) fraction, and scopoletin (**7**) $C_{10}H_8O_4$ (50 mg), mp 202–203° in the $CHCl_3$ fraction. The $CHCl_3$–MeOH fraction was subjected to CC on Si gel followed by prep. TLC (Si gel) with $CHCl_3$–MeOH (10:1) to give umbelliferone (**8**) $C_9H_6O_3$ (20 mg), mp 224–225°, ferulic acid (**9**) $C_{10}H_{10}O_4$ (140 mg), mp 166–167°, and *p*-coumaric acid (**10**) $C_9H_8O_3$ (30 mg), mp 210–212°. These compounds were identified by comparison UV, IR, ^{1}H NMR and mmp with authentic samples.

Isolation of vaginidiol (**5**) *from the* Et_2O *extract.* The Et_2O extract was subjected to CC on Si gel eluted with *n*-hexane–$CHCl_3$ and $CHCl_3$–MeOH systems. The $CHCl_3$ fraction was rechromatographed on a Si gel column with *n*-hexane-EtOAc to give **5** (10 mg).

Vaginidiol (**5**), prisms, mp 166–167° (C_6H_6), $C_{14}H_{14}O_5$, $[\alpha]_D^{20}$ +230° (EtOH; *c* 0.2). MS (*m/z*): 262 $[M]^+$ 213, 187, 160; IR ν_{max}^{KBr} cm^{-1}: 3400, 2920, 1700, 1605, 1260, 1060, 835; ^{1}H NMR (Me_2CO–d_6): δ 1.45 and 1.54 (6H, gem dimethyl), 4.56 (1H, *d*, *J* = 6.0 Hz, H-8), 5.74 (1H, *d*, *J* = 6.0 Hz, H-9), 6.18 (1H, *d*, *J* = 9.0 Hz, H-3), 6.86 (1H, *d*, *J* = 8.0 Hz, H-6), 7.58 (1H, *d*, *J* = 8.0 Hz, H-5), 7.88 (1H, *d*, *J* = 9.0 Hz, H-4). The mp was not depressed on admixture with authentic vaginidiol.

Isolation of apterin (**11**) *from 'residue'.* The 'residue' was passed through a charcoal column using H_2O–MeOH as eluent. The eluate was then chromatographed over Si gel with $CHCl_3$–MeOH (9:1) as eluent to yield **11** (320 mg).

Apterin (**11**), $C_{20}H_{24}O_{10}$, $[\alpha]_D^{20}$ +230° (EtOH; *c* 0.1), UV $\lambda_{max}^{95\%EtOH}$ nm: 325; MS (*m/z*): 424 $[M]^+$ 262, 245, 227, 186; IR ν_{max}^{KBr} cm^{-1}: 3300, 2900, 1700, 1615, 1590, 1260; ^{1}H NMR (MeOH–d_4): δ 1.61 (6H, gem dimethyl), 4.51 (1H, *d*, *J* = 6.5 Hz, H-8), 5.61 (1H, *d*, *J* = 6.5 Hz, H-9), 6.22 (1H, *d*, *J* = 10.0 Hz, H-3), 6.84 (1H, *d*, *J* = 8.5 Hz, H-6), 7.50 (1H, *d*, *J* = 8.5 Hz, H-5), 7.82 (1H, *d*, *J* = 10.0 Hz, H-4).

Hydrolyses of apterin (**11**). When heated with 10% HCl at 100° for 1 hr, **11** gave D-glucose, which was identified by GC, and an aglycone which was identified as oloselone, $C_{14}H_{10}O_3$, mp 176–178°, (IR and ^{1}H NMR).

To **11** in acetate buffer (pH 5.0), β-glucosidase was added and the mixture was kept at 37°. After 3 days the hydrolysate was treated with Et_2O. The Et_2O extract was concd and the residue was crystallized from C_6H_6 to give the aglycone, vaginidiol as colorless needles, mp 166–167°, (IR and ^{1}H NMR). The mp was not depressed on admixture with authentic vaginidiol.

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