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A New Phenolic Compound from *Heracleum lanatum* Michx. var. *nippinicum* Hara. II[1)]

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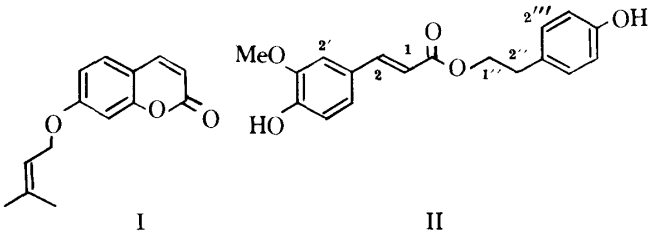
A new compound, *p*-hydroxyphenethyl *trans*-ferulate, was isolated together with 7-(3-methyl-2-butenyloxy)coumarin from the roots of *Heracleum lanatum* Michx. var. *nipponicum* Hara. Both compounds strongly inhibited the growth of the roots of *Brassica rapa* L. var. *pervidis* Bailey.

Keywords——*Heracleum lanatum* var. *nipponicum*; Umbelliferae; coumarins; 7-(3-methyl-2-butenyloxy)coumarin; phenolic compound; *p*-hydroxyphenethyl *trans*-ferulate; growth inhibitor

Heracleum lanatum Michx. var. *nipponicum* Hara (Japanese name: Hanaudo) is a large herbaceous plant growing in riverside places or forest edges in large thickets. Its root is used as an allied drug of Baizhi (白芷) or Duhuo (独活) in some districts of China. Junttila[2)] suggested that *H. lactiatum* Horn, a plant of the same genus, contained plant growth inhibitor(s) as allelopathic substance(s). The extract from the roots of *H. lanatum* var. *nipponicum* also inhibited the growth of *Brassica rapa* L. var. *pervidis* Bailey roots and *Cucumis sativus* L. hypocotyl cuttings. Further, the extract markedly increased the number of adventitious roots of hypocotyls of *C. sativus* cuttings.[3)] In our preceding paper,[1)] we reported the isolation of the following compounds as active substances from the root: seven furanocoumarins (pimpinellin, isopimpinellin, bergapten, isobergapten, sphondin, vaginidiol and apterin), two coumarins (scopoletin and umbelliferone) and two phenolic compounds (*trans*-ferulic acid and *p*-coumaric acid). We now report the structure elucidation of two chemical constituents with significant plant growth-inhibiting activity isolated from the same source.



I II

Chart 1

The concentrated methanol extract of the roots of *H. lanatum* var. *nipponicum* was reextracted with hot *n*-hexane, ether and hot ethyl acetate, successively. Compounds I and II were obtained from the *n*-hexane and ethyl acetate extracts, respectively, by silica gel chromatography.

Compound I, mp 66—68°C, $C_{14}H_{14}O_3$, was obtained as colorless needles, and was identified as 7-(3-methyl-2-butenyloxy)coumarin by direct comparison with an authentic synthetic sample.

Compound II, white powder, mp 165—166°C, was determined to have the composition $C_{18}H_{18}O_5$ by elemental analysis and mass spectroscopy. The ^1H nuclear magnetic resonance (NMR) spectrum revealed two adjacent methylene signals at δ 2.88 and 4.28, *trans*-alkene

signals at δ 6.31 and 7.54, and a methoxy group signal at δ 3.90. The other signals were assigned to two hydroxy groups and seven aromatic protons. Compound II, on hydrolysis with alkali, gave *trans*-ferulic acid and *p*-hydroxyphenethyl alcohol. These data suggest that II is *p*-hydroxyphenethyl *trans*-ferulate, and this was confirmed by direct comparison with an authentic synthetic sample.

Table I. Effect of Isolated Compounds on Growth of the Roots of *Brassica rapa* L. var. *pervidis* Bailey Seedlings

Concentration (ppm)	1	5	10
I	81	57[a)]	50[a)]
II	98	89	66[a)]

Each value represents the mean of the root lengths as a % of the control (42 ± 7.8 mm).
a) Significantly different from the control at the 5% level, *t*-test.

Compound II has not previously been reported as a naturally occurring substance, and I has been isolated from *Libanotis intermedia*,[4] *Seseli libanotis*[5] and *Haplopappus tenuisectus*.[6]

The effect of compound I and II on the growth of roots of *B. rapa* var. *pervidis* is shown in Table I. Both of them inhibited the growth of the roots at 10 ppm. The inhibiting activity of I was stronger than that of any other substance[1] isolated from the roots by us, and II also had strong inhibiting activity. At the concentrations used, I and II did not inhibit the germination of *B. rapa* var. *pervidis* seeds, and all the seeds germinated within 24 h. Therefore, it is unlikely that a delayed germination is responsible for the shorter roots. However, these compounds did not inhibit the growth of hypocotyl cuttings of *C. sativus*, or increase the number of adventitious roots in the hypocotyls at the concentration used.

Experimental

All melting points are uncorrcted. ^{1}H-NMR spectra were recorded at 100 MHz with tetramethylsilane (TMS) as an internal standard. Gas liquid chromatography (GLC) was carried out on OV-101 at 100—200°C.

Extraction and Separation——The roots of *Heracleum lanatum* Michx. var. *nipponicum* Hara were collected at Hino, Tokyo, in late April 1979, and dried at room temperature. The dried roots (3 kg) were extracted with hot MeOH (40 l). The extract, after removal of the solvent, was treated with hot *n*-hexane (4 l), ether (4 l) and hot ethyl acetate (4 l), successively.

The *n*-hexane extract afforded a precipitate when it was cooled. The supernatant of the extract was chromatographed on silica gel. The eluate with *n*-hexane–ethyl acetate (20: 1) was rechromatographed on silica gel with *n*-hexane–ethyl acetate (25: 1) to give 20 mg of compound I. The ethyl acetate extract was chromatographed on silica gel with *n*-hexane–$CHCl_3$ and $CHCl_3$–MeOH systems. The $CHCl_3$ fraction was subjected to silica gel chromatography followed by preparative thin-layer chromatography with $CHCl_3$–MeOH (50: 1) to give 15 mg of compound II.

7-(3-Methyl-2-butenyloxy)coumarin (I)——Colorless needles, mp 66—68°C (*n*-hexane), *Anal.* Calcd for $C_{14}H_{14}O_3$: C, 73.02; H, 6.13. Found: C, 72.93; H, 6.11. MS *m/z*: 230 (M^+), 162, 134, 69. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2920, 1720, 1610, 1110. ^{1}H-NMR ($CDCl_3$) δ: 1.68 and 1.70 (each 3H, s, $(C\underline{H}_3)_2C$=), 4.50 (2H, d, J=7 Hz, =CH–C$\underline{H}_2$–O–), 5.38 (1H, m, =C$\underline{H}$–CH_2–O–), 6.17 and 7.55 (each 1H, d, J=9 Hz, 3-H and 4-H), 6.76 (1H, d, J=2 Hz, 8-H), 6.78 (1H, dd, J=9, 2 Hz, 6-H), 7.28 (1H, d, J=9 Hz, 5-H). The melting point was not depressed on admixture with an authentic synthetic sample.

Synthesis of I——A mixture of umbelliferone (200 mg), 1-bromo-3-methyl-2-butene (0.2 ml) and K_2CO_3 in acetone (10 ml) was refluxed at 70°C for 1 h. After removal of acetone, the reaction mixture was diluted with H_2O (10 ml), and extracted with ether (3 × 20 ml). The ether extract was crystallized from *n*-hexane to give colorless needles (*ca.* 200 mg), mp 66—68°C. *Anal.* Calcd for $C_{14}H_{14}O_3$: C, 73.02; H, 6.13. Found: C, 72.92; H, 6.09.

***p*-Hydroxyphenethyl *trans*-Ferulate (II)**——White powder, mp 165—166°C ($CHCl_3$), *Anal.* Calcd for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: C, 68.44; H, 6.09. MS *m/z*: 314 (M^+), 194, 177, 145. IR ν_{max}^{KBr} cm^{-1}: 3350, 2890, 1710, 1620, 1580, 1230, 1130. ^{1}H-NMR (Me_2CO-d_6) δ: 2.88 and 4.28 (each 2H, t, J=7 Hz, 2″-H

and 1″-H), 3.90 (3H, s, $-OCH_3$), 6.34 and 7.54 (each 1H, d, $J=16$ Hz, 1-H and 2-H), 6.74 and 7.08 (each 2H, d, $J=8$ Hz, 3″,5″-H and 2″,6″-H), 6.83 (1H, d, $J=8$ Hz, 5′-H), 7.10 (1H, dd, $J=8$, 1.5 Hz, 6′-H), 7.28 (1H, d, $J=1.5$ Hz, 2′-H), 8.10 (2H, br, -OH). The melting point was not depressed with an authentic synthetic sample.

Hydrolysis of II——II (10 mg) in 5% KOH solution was allowed to stand at room temperature for 12 h. *p*-Hydroxyphenethyl alcohol and *trans*-ferulic acid were obtained from the hydrolysate and identified by GLC by comparison with authentic samples.

Synthesis of II——A mixture of *trans*-ferulic acid (100 mg), thionyl chloride (70 mg) and pyridine was stirred in ether (10 ml) at 0°C for 10 min. After removal of hydrochloride and thiosulfate by distillation under reduced pressure, the reaction mixture was added to *p*-hydroxyphenethyl alcohol (70 mg) and pyridine in ether (20 ml) and the whole was refluxed at 40°C for 30 min. After addition of H_2O (20 ml), the reaction mixture was treated with ether (4×30 ml). The ether extract was chromatographed on silica gel with $CHCl_3$–MeOH (25: 1) as an eluent to yield a white powder (100 mg), mp 166°C. *Anal.* Calcd for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: C, 68.62; H, 5.70.

Test Solution——Aqueous test solutions of I and II were prepared at three concentrations (1, 5 and 10 ppm), and H_2O was used as a control solution.

Bioassay with *Brassica rapa* L. var. *pervidis* Bailey——Twenty seeds of *B. rapa* var. *pervidis* were placed on filter paper moistened with 7 ml of the test solution in a Petri dish (9×1.5 cm). These Petri dishes were kept at 25°C in the dark for 72 h, and the lengths of the roots were measured. The experiment was carried out in triplicate.

Bioassay with *Cucumis sativus* L.——Seedlings of *C. sativus*, were grown at 25°C in the dark for 3—4 d. When the hypocotyls were *ca.* 4.5 cm long, they were excised at 3 cm below the cotyledons and dipped in small test tubes each containing one of the test solutions. After 9 d incubation at 25°C in the dark, the lengths of hypocotyls and the number of rootlets protruding were recorded. For each test solution, 6 hypocotyl cuttings were used. The experiment was carried out in triplicate.

Control (water): Length of hypocotyl. 50 ± 5.4 mm. Number of adventitious roots. 24.2 ± 3.0.

There was no significant difference between the lengths of the hypocotyls or between the numbers of adventititious roots in water and in the test solution.

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