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THE COUMARIN HERACLENOL AS A GROWTH INHIBITOR IN PARSLEY SEEDS

TADAHIRO KATO*, MASAKI KOBAYASHI, NOBUKI SASAKI, YOSHIO KITAHARA and NORINDO TAKAHASHI†

Department of Chemistry, Faculty of Science, Tohoku University, Sendai 980, Japan

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Key Word Index—*Petroselinum crispum*; Umbelliferae; parsley; seeds; germination inhibitor; furanocoumarin; heraclenol.

Abstract—By the aid of germination assay with lettuce seeds, the germination inhibitors in the parsley seeds were investigated. One of the inhibitors was revealed to be heraclenol.

INTRODUCTION

It is well known from agricultural experience that if parsley seeds are washed with water before sowing, they germinate faster than untreated seeds. It is also known that weeds are relatively difficult to germinate and grow in fields of parsley. These facts suggest that parsley seeds contain water soluble growth inhibitor(s). We have been much interested in the investigation of germination and growth inhibitors in higher plants [1] and we describe here the structural elucidation of one of the germination inhibitors contained in parsley seeds.

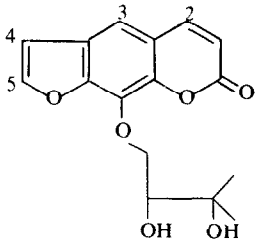
RESULTS

Parsley seeds (*Petroselinum crispnum*) were immersed for one week in water at room temperature and the solution was acidified with aq. HCl, left a further 3 days and then extracted with ethyl acetate. A bioassay with lettuce seeds [1a] demonstrated that the germination inhibitors were transferred into the organic layer, probably as aglycone liberated by hydrolysis. The solvent soluble part was successively stirred with hexane, C_6H_6, and then CH_2Cl_2. The most active components were found to be present in CH_2Cl_2 extracts which were separated into acidic and neutral portions, both of which exhibited the strong inhibitory activity toward the germination of lettuce seeds.

The neutral portion was submitted to SiO_2 column chromatography and the resultant active mixture was further separated by HPLC and then recrystallization to obtain pale yellow crystals, mp 122–125° (decomp.). The germination of lettuce seeds were completely inhibited by this compound at 1000 ppm after 24 hr.

The crystals analyzed for $C_{16}H_{16}O_6$, ν_{max}^{KBr} 3490 (OH), 1712, 1689 (conjugated δ-lactone), 1620, 1578, 1560, and 1543 cm^{-1} (double bonds including aromatics). The MS showed, in addition to the molecular ion at *m/e* 304, base peak at *m/e* 202 ($M-C_5H_{10}O_2$) indicating the presence of $C_5H_{10}O_2$ grouping in the molecule. The 100 MHz NMR spectrum as well as the above physical evidence revealed the structure of the compound as **1**. In the NMR spectrum, there appeared two pairs of AB quartet. One appeared at 8.13 (*bd*) and 6.22 (*d*) ppm with the coupling constant of 9.8 Hz which were assigned to C_2- and C_1-protons, respectively. The another AB signals were observed at 6.95 (C_4-H, *dd*, 2.5 and 0.9 Hz) and 7.57 (C_5-H, 2.5 Hz) ppm, respectively. The presence of long range couplings of C_2- and C_4-protons with C_3-proton was confirmed by the irradiation at 7.09 (*bd*, 0.9 Hz) ppm due to C_3-proton, resulting in the simultaneous change of the former two protons to a clear doublet. The $-OCH_2CH(OH)C(OH)Me_2$ grouping was indicated by the observation of two singlets due to the tertiary methyls at 1.30 and 1.35 ppm and three protons at 3.89 (1H, *dd*, 7.0 and 3.3 Hz), 4.45 (1H, *d*, 7.0 Hz) and 4.51 (1H, *d*, 3.3 Hz) ppm.

The compound (**1**) may be identical with heraclenol isolated previously from *Heracleum candicans* [2] although direct comparison was not carried out. Heraclenol may exist in parsley seeds as its glucoside which



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is hydrolyzed by the HCl treatment as evident from the bioassay experiments, the water soluble extracts contain other active compounds and their structural elucidation will be carried out in future.

EXPERIMENTAL

Extraction of parsley seeds Parsley seeds (2.7 kg) was immersed in H_2O (15 l.) for a week at room temp. and filtered off. The extraction was repeated 5 times and the combined H_2O layers were evapd *in vacuo* to obtain 800 g of a jelly like extract. The extract was dissolved in 2N HCl (2.5 l.) and kept 3 days at room temp. The mixture was then extracted with EtOAc. From the EtOAc layer was obtained 13 g of a gel which showed 95%

*To whom all correspondence should be addressed.

†Present address: Institute for Agricultural Research, Tohoku University, Sendai 980, Japan.

inhibition of the germination of lettuce seeds at 1000 ppm. The EtOAc soluble part was successively stirred with 1 l. each of hexane, C_6H_6, and then CH_2Cl_2. The each organic soluble part was separated into acidic and neutral fractions. The hexane extracts gave 110 mg, 45% germination (neutral) and 440 mg, 8% germination (acidic); C_6H_6 extracts gave 570 mg, 33% germination (neutral) and 1.43 g, 0% germination (acidic) and and CH_2Cl_2 gave 440 mg, 0% germination (neutral) and 1.49 g, 0% germination (acidic) fractions, respectively. The neutral portion of CH_2Cl_2 soluble part was eluted on SiO_2 (30 g) column with a mixed solvent of C_6H_6–EtOAc (1:1). The resultant active fraction was further separated by HPLC [column; μ-porasil, solvent; $CHCl_3$–EtOH (100:3)] to isolate pale yellow crystals (**1**). Recrystallization from C_6H_6–$CHCl_3$ afforded a pure specimen (10 mg) mp 122–125° (decomp). The germination assay with lettuce seeds showed that the (**1**, heraclenol) inhibited completely the germination of lettuce seeds at 1000 ppm after after 24 hr, while *ca* 50% of the seeds germinated at 100 ppm.

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PRODUCTION OF AN AURONE BY BRYOPHYTES IN THE REPRODUCTIVE PHASE

KENNETH R. MARKHAM and LAWRENCE J. PORTER

Chemistry Division, D.S.I.R., Petone, New Zealand

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Key Word Index—*Marchantia*; *Conocephalum*; Hepaticae; aurone; aureusidin glucuronide; reproduction.

Abstract—The aurone, aureusidin 6-*O*-glucuronide, has been isolated from the antheridiophores of two liverworts, *Marchantia berteroana*, *M. polymorpha* and from *Conocephalum supradecompositum*. It occurs only in these organs in *Marchantia*. The appearance of this aurone in bryophyte reproductive structures suggests parallel evolution within the angiosperms and the bryophytes.

INTRODUCTION

The flavonoid chemistry of bryophytes has been studied intensively in recent years and a wide range of structures are now known in these plants. Flavones [1], flavonols [2], dihydroflavones [3], 3-deoxyanthocyanins [4] and biflavonyls [5] have all been identified in one or more species but chalcones, isoflavones and 3-hydroxylated anthocyanins have not. The aurone, bracteatin has recently been identified in the moss, *Funaria hygrometrica* [6] and we now report the occurrence of another aurone, aureusidin 6-*O*-glucuronide, in three liverworts.

RESULTS

In the course of our recent study of the liverwort *Marchantia berteroana* [1] we found both quantitative and qualitative seasonal variations in the flavonoid constituents. A further experiment using a single genotype of this liverwort showed that marked changes took place as the plant moved into its reproductive phase. Perhaps the most spectacular of these changes is the production of aureusidin 6-*O*-glucuronide as it appears, on PC in UV light, as a distinctive yellow fluorescent spot which turns reddish-orange in NH_3. Its absorption spectrum and reagent induced shifts were found to be identical with those of 4,6,3′,4′-tetrahydroxyaurone 6-*O*-glucoside (aureusin) isolated from *Antirrhinum majus* flowers. PC comparison in the solvents TBA and HOAc also indicated their apparent identity, but in H_2O the liverwort aurone is more mobile, which is characteristic for flavonoid glycosiduronic acid derivatives. Its identity as a glucuronide was substantiated by its resistance to acid hydrolysis, relative ease of enzymatic hydrolysis with β-glucuronidase, and GLC analysis of the sugar produced on hydrolysis. The aglycone was shown to be 4,6,3′,4′-tetrahydroxyaurone (aureusidin), thereby defining the natural product as aureusidin 6-*O*-β-D-glucuronide.

Aureusidin 6-*O*-glucuronide was found only in the antheridiophores of male plants and not in the associated thallus or in female plants. It is present in the plant for only 4–8 weeks, the life span of these reproductive structures. The same aurone was also found in the antheridiophores of *Marchantia polymorpha* and in a sample of *Conocephalum supradecompositum*.*

*Antheridiophores in this species are imbedded in the thallus in contrast to those of *Marchantia* which are erect.