Short Communication

Oleuropein, a Secoiridoid Glucoside from Olive, as a Feeding Stimulant to the Olive Weevil (Dysceerus perforatus)

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From olive [Olea europaea LINNE; Oleaceae], a feeding stimulant to the olive weevil [Dysceerus perforatus (ROELFOE); Coleopetera; Curculionidae] was extracted with methanol and identified as oleuropein, a secoiridoid glucoside, by spectroscopic methods.

In Japan, the olive weevil, Dysceerus perforatus is the most serious insect pest for olive, Olea europaea belonging to Oleaceae. This weevil is indigenous to Japan and widely distributed in the islands of Honshu, Shikoku, and Kyushu. The original host plants are two species of wild oliveaceous type, Ligustrum japonicum Thunb. and L. obtusifolium Sieb. et Zucc. This weevil is known to have begun attacking olive trees immediately after they were introduced into Japan.1) Although the population density of this weevil on the original host plants is very low (published data), the density on olive trees is much higher, the adult density sometimes exceeding one hundred insects per tree.2) Once the insects begin to infest olive trees, they increase their population very rapidly and the trees subsequently die. The reason why the insects damage olive trees so seriously prompted us to conduct the present study. One possible mechanism for such a serious infestation by D. perforatus is their utilization of the surface chemicals of the olive tree. Tracing the ecological aspects of the weevil from the chemical viewpoint is, of course, very important for developing a novel control method against the insect. Thus, the aim of the present study is to trace the host-plant components responsible for this serious infestation. Since the adults of the weevil feed on the leaf and bark of the olive tree,3) branches (12 g) were extracted with methanol (50 ml) for 7 days, and the methanol extract was concentrated and used for further purification.

The adult insects collected from infested olive trees were reared in a plastic cup (100 mm dia. × 70 mm depth) containing a piece of a young branch of an olive tree (50 mm length, 5 mm dia.) and wet cotton. A methanol solution of the test sample (1 mg/10 μl) and an aqueous solution of sucrose (5 mg/10 μl) were directly applied to a filter paper (Advantec Toyo No. 2, 7 mm dia.) which was then dried under reduced pressure (20 mmHg) in a desiccator. After the disk had been placed on a Petri dish (40 mm dia.), a starved weevil was introduced into the dish. A control test was performed with 10 μl of methanol applied to the disk instead of the sample solution. Only one disk was exposed to a weevil in each replicate, and three replicates were made in this experiment. After the dishes had been kept at 27°C in darkness for 24 h, the feeding activity was evaluated. In this no-choice experiment, we expected that if the sample had any feeding stimulant activity, the weevils would bite the paper disk, and the area of the bitten surface could be regarded as an assessment for the activity of the sample. Thus, the assessment is expressed by a score from 0 to +3, in which zero is the case of no biting, +1 for less than 20% of the area bitten, +2 for 20–50% bitten, and +3 for more than 50%. The activity was evaluated by using the mean scores in each treated and control disks, and the results of the test were analyzed by Students’ t-test.

To the crude methanol extract (0.9 g) of the olive tree was added water (50 ml), and the solution was successively extracted with hexane (3 × 50 ml), ethyl acetate (3 × 50 ml) and butanol (3 × 50 ml). The ethyl acetate-soluble fraction was chromatographed in a silica gel column (eluting with hexane-ethyl acetate at 1:1, ethyl acetate, acetone, and methanol) to afford fractions No. 1–5. The assay just described showed that the activity of fraction No. 4 (+2.7) was higher than those of the other fractions (+0.3–1.0). As the next step, isolation of the active compound in this fraction was conducted and, after several stages of silica gel chromatography, a pure active compound (8 mg) as an amorphous solid was obtained that exhibited significant activity (more than +2) even at a dose of 0.05 mg/disk.

The positive FABMS data of the active compound gave a molecular ion peak at m/z 541 (M + H)+, and negative FABMS also exhibited a prominent molecular ion peak at m/z 539 (M – H)+ as well as a fragment ion peak at m/z 377 (M–H–Glc)–. The molecular formula of C22H25O13 was established by HR-FABMS (found, m/z 563.1749 (M + Na)+; calcd., m/z 563.1741). The FT-IR spectrum showed the presence of ester carbonyls (1708 cm−1 and 1630 cm−1) and hydroxy groups (3400 cm−1). From the 1H-NMR spectrum of the acetylated compound, the molecule was found to have 6 hydroxy groups. This spectrum also suggested that the molecule contained a glucose moiety with a β-glucosidic bond (the chemical shift and coupling constant of the anomeric proton were δ 5.02 ppm and J = 8.0 Hz, respectively). A methoxy proton signal in ester was observed at δ 3.75 ppm as a singlet. The spectrum also showed the presence of a methyl group (δ 1.68 ppm) on an olefinic carbon, two olefinic protons (δ 5.98 ppm and 7.46 ppm) and aromatic protons (3H, δ 7.04–7.13 ppm) substituted with three groups at positions 1, 3, and 4. The 13C-NMR (δ 168.7 ppm and 173.2 ppm) spectrum of the active compound suggested the presence of two carbonyl carbons.

A literature survey of biologically active substances isolated from olive led us to find a report giving the same data for the 1H-NMR and IR spectra as those of our compound. Therefore, the compound was identified as...
oleuropein, a secoiridoid glucoside which was first discovered by Panizzi et al. \textsuperscript{51} It has previously been reported that oleuropein exhibited a molluscidal property \textsuperscript{60} against the South American snail, \textit{ Biomphalaria glabrata\textit{,} and it is also known as one of the antimicrobial compounds \textsuperscript{71} from plants. In the report, \textsuperscript{41} Kubo et al. described an interesting possibility that the olive tree is resistant to insect and microbe attack and that the secoiridoid glucosides, oleuropein and ligstroside, are involved in the defense mechanism, oleuropein being considered as a phytoalexin precursor. Our present study has revealed oleuropein to be a feeding stimulant to the olive weevil, and our study on specific plant-insect interaction may contribute to developing novel pest control methods and integrated pest management.

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\textbf{References}