Note

Isolation and Identification of Aucuparin as a Phytoalexin from *Eriobotrya japonica* L.Keisuke WATANABE, Yukio ISHIGURI,
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Previously, we reported on the production of a phytoalexin in the cortical layer of the loquat shoot (*Eriobotrya japonica* L.) when it was inoculated with *Colletotrichum lindemuthianum*.¹⁾ In this communication, isolation and identification of the phytoalexin are described. The antimicrobial properties of the phytoalexin are also briefly mentioned.

The cortical layer of a loquat shoot (4 kg, 3 cm in length, each) was peeled off and the inoculum droplets (0.3~0.6 ml, each) containing spores of *C. lindemuthianum* were put on each cortical layer. After incubation at 25°C

for 24 hr, the droplets were collected and centrifuged at 14,000 rpm for 20 min. The supernatant layer was adjusted to pH 3 with HCl and extracted with petroleum ether. On evaporation of the solvent, 35 mg of crude antimicrobial material was obtained. A TLC (Merck Kieselgel 60 GF₂₅₄, 0.25 mm) analysis of the crude material developed with *n*-hexane-ethyl acetate-methanol (60:40:1 v/v), indicated the presence of one major spot at *R_f* 0.62~0.65 when viewed under UV light (254 nm). The major spot was not detected in the corresponding extract of a healthy cortical layer.²⁾ A TLC bioautography of the same material against *Cochiobolus miyabeanus* revealed that the above spot was antimicrobial. The crude material was purified on TLC plates to give the pure compound (I), 2.0 mg, mp 101~101.5°C. The mass spectrum of I gave a molecular ion peak at *m/z* 230 (100%), corresponding to C₁₄H₁₄O₃ and fragments at 215 (M⁺-CH₃, 25%), 187 (M⁺-CH₃-CO, 25%); UV λ_{max}^{EtOH} nm (log ε) 273 (4.18) and IR ν_{nujol} cm⁻¹: 3420, 1610, 1380, 1340, 1120. In its PMR spectrum, I showed a multiplet of mono substituted phenyl ring protons at δ_{TMS}^{CDCl₃} 7.30 (5H) and a singlet at δ 3.85 (6H) due to two methoxy groups. A broad singlet at δ 5.30 (1H) indicated the presence of one hydroxyl group in the molecule, supported by the IR spectrum. In its UV spectrum, the absorption at 273 nm was diagnostic for a biphenyl system with bathochromic substituents on 3, 4 and 5 positions in one phenyl ring.³⁾ Equivalence of the two methoxy groups whose PMR signal appeared as a 6 proton singlet at δ 3.85 indicated that the whole molecule should be symmetrically substituted. On the basis of the above spectroscopic data, it was reasonably predicted that the phytoalexin seemed to be aucuparin (I) (4-hydroxy-3,5-dimethoxybiphenyl), originally isolated from the

TABLE I. ANTIMICROBIAL ACTIVITIES OF NATURAL AND SYNTHETIC AUCUPARIN (I) AGAINST *Colletotrichum lindemuthianum* AND *Pestalotia funerea* (24 and 48 hr after inoculation)

Fungi	Aucuparin conc. (ppm) ^a	Germination rate (%) ^b				Germ tube length (μm) ^c			
		Natural		Synthetic		Natural		Synthetic	
		24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
<i>C. lindemuthianum</i> (non-phytopathogenic)	0*	90	100	89	99	500	500	500	500
	10	38	42	41	41	24	29	31	31
	50	5	6	3	4	8	9	5	5
	100	—	—	0	0	—	—	0	0
<i>P. funerea</i> (phytopathogenic)	0*	97	100	93	100	252	500	210	500
	10	72	100	76	100	75	213	80	220
	50	63	85	60	88	72	185	69	180
	100	—	—	30	66	—	—	5	60

^a In water-ethanol (98:2, v/v).^b 600 spores were counted.^c Mean length of germ tubes of 50 spores selected from 600 spores in sequence of their germ tube length.

* Blank (treated with 2% ethanol only).

healthy heartwood of *Sorbus aucuparia*.⁴⁾ The phytoalexin from the cortical layer was identical in all respects (PMR, UV, MS and GLC t_R) with a synthetic specimen prepared by the authors according to the reported procedures.⁵⁾ Since the natural phytoalexin was isolated in a small quantity, the antimicrobial properties of the synthetic aucuparin were studied in comparison with the natural specimen.

FIG. 1. The Structure of Aucuparin (I).

The natural and synthetic aucuparin (I) revealed similar activities against *Colletotrichum lindemuthianum*, a non-phytopathogenic fungus for loquat, and *Pestalotia funerea*, a phytopathogenic fungus of loquat gray leaf spot (see Table I). Spore germination of *C. lindemuthianum* was more severely inhibited by 50 ppm of aucuparin (I) than that of *P. funerea* at 24 hr after inoculation. Then, after 48 hr, the germ tube growth of *P. funerea* recovered significantly, indicating that aucuparin was probably in-

activated by *P. funerea*. The recovery and insensitivity of *P. funerea* to aucuparin would account for the phytopathogenic character of the fungus against loquat.

Although a number of phytoalexins have been isolated from various kinds of plants, no phytoalexins of the biphenyl type have been reported so far. This is the first report of the isolation of the substituted biphenyl, aucuparin (I), as the phytoalexin of the cortical layer of loquat shoot. A TLC bioassay of the extract indicated the presence of other minor components of the phytoalexins which are the subject of further investigations.

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