7'-Hydroxyseiridin and 7'-Hydroxyisoseiridin, Two New Phytotoxic #-Butenolides from Three Species of Seiridium Pathogenic to Cypresses

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7′-HYDROXYSEIRIDIN AND 7′-HYDROXYISOSEIRIDIN, TWO NEW PHYTOTOXIC $\Delta^{\alpha,\beta}$-BUTENOLIDES FROM THREE SPECIES OF *SEIRIDIUM* PATHOGENIC TO CYPRESSES

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ABSTRACT.—Two new phytotoxic $\Delta^{\alpha,\beta}$-butenolides, 7′-hydroxyseiridin [**1**] and 7′-hydroxyisoseiridin [**2**], were isolated from culture filtrates of three species of *Seiridium* (*S. cardinale, S. cupressi,* and *S. unicorne*). These fungi are associated with the canker diseases of cypress trees (*Cupressus sempervirens*) in the Mediterranean area. The structures of butenolides **1** and **2** were established using spectroscopic and chemical methods in comparison with seiridin [**5**] and isoseiridin [**6**], two phytotoxic metabolites produced in higher concentration by the same fungal species. Necrotic and chlorotic symptoms were produced on cuttings of both host and non-host plants by absorption of 100 or 50 μM solutions, respectively, of compounds **1** and **2**.

Three species of *Seiridium,* namely, *S. cardinale, S. cupressi*, and *S. unicorne*, are associated with the canker diseases of cypress (*Cupressus sempervirens* L.) (1–3).

Five phytotoxic metabolites were isolated from the *t*-BuOMe extract of the in vitro culture filtrates of all three fungi and characterized as two butenolides, seiridin [**5**] and isoseiridin [**6**] (4,5), and three sesquiterpenes, named seiricardines A (6), B, and C (7). In addition, cyclopaldic acid (8) and the 10-macrolide, seiricuprolide (9), were produced by *S. cupressi* only.

The organic extracts of the culture filtrates of the above fungi contained at least two other phytotoxic metabolites, present in very low concentrations. They were more polar compounds structurally related to seiridin and isoseiridin.

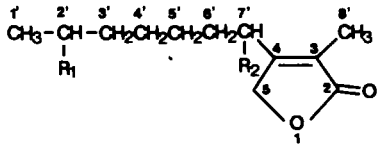
This paper describes the isolation, chemical characterization, and biological activity of these two new toxic $\Delta^{\alpha,\beta}$-butenolides, named 7′-hydroxyseiridin[1] [**1**] and 7′-hydroxyisoseiridin[1] [**2**].

Culture filtrates of *S. cardinale* grown as described previously (4) were extracted with *t*-BuOMe and the organic extract was fractionated by means of SiO_2 column chromatography (see Experimental). From the less polar fractions collected, as described previously (4,6,7), seiridin [**5**], isoseiridin [**6**], and the seiricardines were isolated, while the new toxic butenolides, named 7′-hydroxyseiridin (**1**, 0.8 mg/liter) and 7′-hydroxyisoseiridin (**2**, 0.2 mg/liter), were obtained as pure oils from the more polar fractions following combined SiO_2 cc and prep. tlc separations.

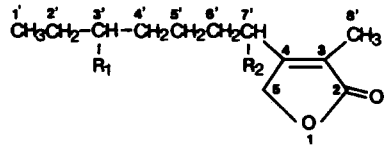
Using the same procedure, culture filtrates of *S. cupressi* and *S. unicorne* yielded smaller amounts (<0.1 mg/liter) of both **1** and **2**.

Symptoms of phytotoxicity were recorded when **1** and **2** were assayed on severed twigs of cypresses (*C. macrocarpa* Hartw., *C. sempervirens* L., and *C. arizonica* Gr.), as well as on cuttings of germlings of tomato (*Lycopersicon esculentum* L. cv. Marmande) and mung bean (*Phaseolus vulgaris* L. var. *aureus*). 7′-Hydroxyseiridin [**1**] tested at 100 μM caused the die-back syndrome on *C. macrocarpa* and *C. sempervirens* within two weeks after absorption of 3 ml of each toxic solution,

[1]Nomenclature: 7′-hydroxyseiridin [**1**]: 3-methyl-4-(1,6-dihydroxyheptyl)-2(5H)furanone; 7′-hydroxyisoseiridin [**2**]: 3-methyl-4-(1,5-dihydroxyheptyl)-2(5H)furanone.



1 $R_1=R_2=OH$
3 $R_1=R_2=OAc$
5 $R_1=OH$, $R_2=H$



2 $R_1=R_2=OH$
4 $R_1=R_2=OAc$
6 $R_1=OH$, $R_2=H$

whereas it produced leaf necrosis on *C. arizonica*. 7′-Hydroxyisoseiridin [**2**] (100 μM) caused necrosis of the apical leaves in *C. macrocarpa* and *C. sempervirens* and leaf yellowing and stem browning in *C. arizonica*. Tomato or mung bean germlings treated with 50 μM of 7′-hydroxyseiridin [**1**] showed several confluent necrotic spots on the leaf surfaces. The treatment of the same germlings with 50 μM of 7′-hydroxyisoseiridin [**2**] caused the formation of a few necrotic spots on the leaves. It is interesting to point out that the appearance of symptoms on the cypresses took place earlier when treated with a 100 μM concentration of butenolide **1** or **2**, and lower than when treated with 150 μM of either of the seiridins (**5** and **6**). The uptake of both toxic solutions containing **1** or **2** was also more rapid than those containing the seiridins (**5** and **6**). The increased polarity of **1** and **2**, with respect to **5** and **6**, respectively, probably plays an important role in their translocation.

7′-Hydroxyseiridin [**1**] and 7′-hydroxyisoseiridin [**2**] showed uv absorption maxima at 213 and 212 nm, respectively, typical of α,β-unsaturated-γ-lactones (10), and also gave evidence for the presence of hydroxy and butenolide carbonyl groups from their ir spectra (11). Both toxins exhibited a molecular formula of $C_{12}H_{20}O_4$ as deduced from their hrms. This suggested that **1** and **2** were structurally related to each other and to seiridin [**5**] and isoseiridin [**6**], respectively.

The ^{1}H-nmr spectrum of 7′-hydroxyseiridin [**1**] (Table 1) showed the presence of an AB system at δ 4.86 and 4.74 attributable to the methylene (CH_2-5) of the γ-lactone, which in **5** was observed as a sharp quartet at a very similar chemical shift value of δ 4.62 (5); moreover, the methyl group at C-3 of the same ring resonated as a sharp triplet at δ 1.86. Another substantial difference between **1** and **5** was observed by comparing the signal systems of the aliphatic side-chain attached to C-4. As in **5**, the secondary terminal methyl (CH_3-1′) and the proton of the secondary alcohol (H-2′) yielded a doublet and a triple quartet at δ 1.19 and 3.80, respectively, while the broad triplet observed in **5** at δ 2.39, and assigned to CH_2-7′, was absent in **1**. Moreover, the presence of a double doublet resonating at δ 4.76 and attributed to the proton of another secondary alcohol was observed in **1**. This signal was assigned to H-7′ due to its multiplicity. Additionally, in comparison with **5**, the CH_2-6′ (m, δ 1.68) resonance was shifted downfield (Δδ 0.18), while signals of the other three methylene groups (CH_2-3′, CH_2-4′, and CH_2-5′) of the side-chain remained substantially unchanged. Moreover, the presence of a hydroxy group attached to C-7′ explained the different chemical shift value of CH_2-5 in **1** compared with **5** (Table 1).

These results were consistent with the ^{13}C-nmr spectrum of **1** (Table 2), which, when compared to that of **5**, only differed in the absence of the C-7′ triplet at δ 27.0 which appeared as a doublet at δ 68.0 in **1**. Furthermore, the C-6′ signal was shifted downfield (Δδ 8.7) to δ 36.2. The chemical shift values reported for **5** and **6** in Table 2 provide reassignments for the C-3 and C-4 chemical shifts, compared with published values (5).

TABLE 1. ^{1}H-Nmr Data of Compounds **1–6**.[a]

Proton	Compound					
	1	**2**	**3**	**4**	**5**	**6**
H-5A	4.86 dq (17.5, 1.8)	4.87 dq (17.5, 1.8)	4.75 dq (17.5, 1.8)	4.74 dq (17.5, 1.8)	4.62 q (2H) (1.8)	4.62 q (2H) (1.8)
H-5B	4.74 dq (17.5, 1.8)	4.74 dq (17.5, 1.8)	4.63 dq (17.5, 1.8)	4.64 dq (17.5, 1.8)	—	—
CH_3-1′	1.19 d (6.2)	0.95 t (7.3)	1.20 d (6.2)	0.99 t (7.3)	1.16 d (6.3)	0.89 t (7.4)
H-2′	3.80 tq (6.2, 6.2)	1.45 m (2H)	4.84 ddq (6.2, 7.3, 4.8)	1.55 m (2H)	3.76 tq (6.3, 6.3)	1.42 m (2H)
H-3′	1.45 m (2H)	3.56 m	1.80–1.40 m (2H)	4.79 m	1.38 m (2H)	3.47 tt (6.7, 4.5)
H_2-4′	1.60–1.40 m	1.45 m	1.80–1.40 m	1.80–1.40 m	1.38 m	1.42 m
H_2-5′	1.60–1.40 m	1.70–1.40 m	1.80–1.40 m	1.80–1.40 m	1.38 m	1.42 m
H_2-6′	1.68 m	1.70–1.40 m	1.80–1.40 m	1.75 m	1.50 tt (7.4, 7.4)	1.48 m
H-7′	4.76 dd (6.4, 4.5)	4.78 m	5.67 dd (8.0, 6.2)	5.66 t (7.3)	2.39 br t (2H) (7.4)	2.38 br t (2H) (7.4)
CH_3-8′	1.86 t (1.8)	1.87 t (1.8)	1.90 t (1.8)	1.92 t (1.8)	1.79 t (1.8)	1.77 t (1.8)
CH_3-CO	—	—	2.08 s	2.09 s	—	—
CH_3-CO	—	—	2.02 s	2.04 s	—	—

[a]Chemical shifts in δ values (ppm) from TMS; coupling constants (*J*) in parentheses in Hz.

TABLE 2. ^{13}C-Nmr Data of **1**, **2**, **5**, and **6**.[a]

Carbon	Compound			
	1[b]	**2**[b]	**5**	**6**
C-2	176.3 s	175.8 s	175.5 s	175.5 s
C-3	122.5 s	122.0 s	122.9 s	122.7 s
C-4	161.4 s	162.8 s	160.4 s	160.4 s
C-5	69.5 t	73.0 t	71.3 t	71.3 t
C-1′	23.7 q	9.9 q	23.5 q	9.7 q
C-2′	67.8 d	30.4 t	67.8 d	30.2 t
C-3′	38.9 t	69.8 d	38.9 t	72.8 d
C-4′	25.4 t[c]	35.8 t[c]	29.4 t	36.2 t
C-5′	25.2 t[c]	21.4 t	25.3 t	25.4 t
C-6′	36.2 t	36.0 t[c]	27.5 t	27.5 t
C-7′	68.0 d	67.4 d	27.0 t	27.0 t
C-8′	8.8 q	8.8 q	8.4 q	8.3 q

[a]Chemical shifts are in δ values (ppm) from TMS.

[b]Multiplicities were determined by DEPT spectra (18). Assignments made in comparison to ^{13}C-nmr data of **5** and **6** (5).

[c]Interchangeable signals.

The structure of **1** as 7′-hydroxyseiridin was corroborated by its hreims. Besides the molecular ion at *m/z* 228.1501 ($C_{12}H_{20}O_4$), peaks diagnostic for the presence of a 1,6-dihydroxyheptyl side-chain, as well as those of an α,β-unsaturated γ-lactone ring and due to the loss of H_2O, methyl, CO_2, and H_2CO residues, were present at *m/z* 213, 195, 180, and 151 (12,13). Moreover, the molecular ion, as already reported for **5**, **6**, and other 3,4-dialkyl-$\Delta^{\alpha,\beta}$-butenolides (5,14), through the probable cleavage of the C-5–C-6 or the C-6–C-7 bond of the side-chain yielded the ions at *m/z* 141, 128, and 127 (base peak).

Structure **1** was confirmed by acetylation to produce the 2′,7′-*O*,*O*′-diacetyl derivative **3** ($[MH]^+ = m/z$ 313 by eims). In the ir spectrum of **3**, hydroxy group absorptions were not observed, while the bands of the lactone carbonyl at 1754 cm^{-1} and those attributable to acetyl groups at 1745 and 1735 cm^{-1} (11) were present. The ^{1}H-nmr spectrum of **3** differed from that of **1** in the downfield shifts (Δδ 1.04 and δ 0.91) of H-2′ and H-7′, respectively, appearing as a quartet of double doublets and as a double doublet at δ 4.84 and 5.67, and as a result of the two methyl singlets resonating at δ 2.08 and 2.02 for the acetyl groups (Table 1).

7′-Hydroxyisoseiridin [**2**], with the same molecular formula as **1** ($C_{12}H_{20}O_4$), had ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2, respectively) very similar to those of isoseiridin [**6**] (5). In addition, the spectra of **2** as compared to those of **6** showed the same differences as observed in the comparison of **1** and **5**. Thus, the ^{1}H-nmr spectrum of **2** differed from that of **6** in the absence of the CH_2-7′, a broad triplet present in **6** at δ 2.38, and in the presence of the H-7′ signal in **2** that resonated as a multiplet at δ 4.78. In addition, the H_2-5′ signal appeared as a sharp quartet at δ 4.62 for **6**, and as an AB system at δ 4.87 and 4.74 in **2**. The ^{13}C-nmr spectrum of **2** lacked the C-7′ aliphatic methylene signal at δ 27.0 observed for **6**, but showed a signal for a secondary oxygenated carbon resonating at δ 67.4 (C-7′) and a downfield shift (Δδ 8.5) for CH_2-6′ at δ 36.0. Thus, toxin **2** was tentatively assigned as 7′-hydroxyisoseiridin.

The hreims of **2** exhibited a molecular ion at *m/z* 228.1364 ($C_{12}H_{20}O_4$) and fragmentation pathways very similar to those observed for **1**. Thus, the peaks

typical for the presence of a 1,5-dihydroxyheptyl side-chain, as well as those of an α,β-unsaturated γ-lactone ring and due to the loss of H_2O, ethyl, CO_2, and H_2CO residues, were present at *m/z* 210, 199, 180, and 137 (12,13). Moreover, as already observed in **1**, **5**, **6**, and other 3,4-dialkyl-$\Delta^{\alpha,\beta}$ butenolides (5,14), the molecular ion produced by the probable cleavage of the C-5′–C-6′ or the C-6′–C-7′ bonds of the side-chain the ions at *m/z* 141, 128, and 127 (base peak).

Acetylation of 7′-hydroxyisoseiridin gave a 3′,7′-*O*,*O*′-diacetyl derivative [**4**] ($[MH]^+$ = *m/z* 313 by eims). Its ir spectrum lacked hydroxyl absorption but showed bands at 1758 cm^{-1} (lactone carbonyl), and 1744 and 1733 cm^{-1} (ester carbonyl) (11).

The ^{1}H-nmr spectrum of **4** differed from that of **2** essentially in the downfield shifts (Δδ 1.23 and δ 0.88) of H-3′ and H-7′ which appeared as a multiplet and a triplet at δ 4.79 and 5.66, respectively, and in the presence of the singlets of the two acetyl groups at δ 2.09 and 2.04 (Table 1).

$\Delta^{\alpha,\beta}$-Butenolides (15) are relatively common as natural products (16,17), but 3,4-dialkyl substituted derivatives, such as **1** and **2**, are rare fungal metabolites (14,17).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were carried out in MeCN on a Perkin-Elmer 550S spectrophotometer; ir spectra were recorded neat on a FT 1760X Perkin-Elmer instrument; ^{1}H- and ^{13}C-nmr spectra were recorded in $CDCl_3$ at 270 and/or 300 MHz and at 67.92 and/or 75.47 MHz, respectively, on Bruker spectrometers. ^{13}C-Nmr multiplicities were determined by DEPT spectra (18), using Bruker microprograms. Eims and hreims were taken on MS-30 AEI and MS-50 Kratos spectrometers, respectively, operating with an ionization energy of 70 eV. Analytical and prep. tlc were performed on SiO_2 plates (Merck, Kieselgel 60 F_{254}, 0.25 and 0.50 mm, respectively). The spots were visualized by exposure to uv and by spraying with 10% H_2SO_4 in MeOH and then with 5% phosphomolybdic acid in EtOH followed by heating at 110° for 10 min. Cc was carried out on SiO_2 (Merck, Kieselgel 60, 0.063–0.20 mm). The petroleum ether used had a bp range of 40–70°.

BUTENOLIDE PRODUCTION AND BIOASSAYS.—Methods used for the preparation of cultures of *S. cardinale*, *S. cupressi*, and *S. unicorne*, as well as for the evaluation of phytotoxicity of the two novel butenolides [**1** and **2**] on both host (*Cupressus sempervirens* var. *pyramidalis*, *C. arizonica*, and *C. macrocarpa*) and non-host (*Lycopersicon esculentum* cv. Marmande and *Phaseolus vulgaris* var. *aureus*) cuttings have been described previously (4,6,9).

EXTRACTION AND ISOLATION.—Culture filtrates (15 liters) of *S. cardinale*[2] were adjusted to pH 4 with 0.1 N HCl and extracted with *t*-BuOMe (4×3.8 liters). The combined extracts were dried (Na_2SO_4) and evaporated under reduced pressure to produce a brown oily residue (2.5 g). This was fractionated by cc on SiO_2 using $CHCl_3$-*i*-PrOH (9:1) as eluent to yield 13 groups of homogeneous fractions. Tlc analysis of the residues obtained from the 9th–11th groups showed the presence of two uv-absorbing metabolites with R_f values of 0.24 and 0.42 and 0.18 and 0.39 on SiO_2 plates (petroleum ether-Me_2CO, 7:3; and $CHCl_3$-*i*-PrOH, 9:1, respectively). Further fractionation of these combined residues (211 mg) by two successive cc steps on SiO_2 (petroleum ether-Me_2CO, 6:4) produced a less polar metabolite, 7′-hydroxyisoseiridin [**2**], as a homogeneous oil (2.2 mg) and a fraction (17.6 mg) containing a mixture of two metabolites. Two successive prep. tlc separations of the mixture on SiO_2 (petroleum ether-Me_2CO, 6:4; and $CHCl_3$-*i*-PrOH, 9:1, respectively) yielded 0.9 mg of **2** (total 3.1 mg, 0.2 mg/liter) and the most polar butenolide, 7′-hydroxyseiridin [**1**], also as a pure oil (11.8 mg, 0.8 mg/liter).

When the same fractionation procedure was used to process the oily residue (2.44 and 0.64 g, respectively) obtained from the *t*-BuOMe extract of culture filtrates (8.0 and 2.4 liters, respectively) of the other two species of *Seiridium* (*S. cupressi* and *S. unicorne*) the same pure compounds **1** and **2** were obtained, but the yields were lower (both <0.1 mg/liter) than those from *S. cardinale*.

7′-Hydroxyseiridin [**1**].—Oil; uv λ max (log ε) 213 (3.95) nm; ir ν max 3608 (OH), 3441 (OH), 1751 (C=O, lactone), 1677 (C=C), 1080, 1038 cm^{-1}; ^{1}H and ^{13}C nmr, see Tables 1 and 2, respectively; hreims *m/z* $[M]^+$ 228.1501 (calcd 228.1362) (4), 213 (8), 195 (7), 192 (3), 180 (4), 163 (15), 151 (21), 141 (9), 128 (30), 127 (100), 124 (26), 111 (10), 110 (34).

7′-Hydroxyisoseiridin [**2**].—Oil; uv λ max

[2]Isolate No. 1025, collection of the Department of Plant Pathology, University of Bari, Italy.

(log ε) 212 (4.14) nm; ir ν max 3392 (OH), 1748 (C=O, lactone), 1672 (C=C), 1093, 1031 cm^{-1}; ^1H and ^{13}C nmr, see Tables 1 and 2, respectively; hreims *m/z* $[M]^+$ 228.1364 (calcd 228.1362) (1), 210 (5), 199 (10), 192 (12), 181 (65), 180 (7), 163 (10), 155 (3), 141 (9), 137 (17), 128 (32), 127 (100), 124 (31), 111 (17), 110 (41).

PREPARATION OF 2′,7′-*O*,*O*′-DIACETYLHYDROXYSEIRIDIN [**3**].—7′-Hydroxyseiridin (**1**, 3.6 mg) was acetylated with Ac_2O (200 μl) and pyridine (200 μl) at room temperature. After 12 h the reaction was stopped with MeOH at 0° and pyridine was removed by azeotrope formation on addition of C_6H_6. The residue was purified by prep. tlc (SiO_2, petroleum ether-Me_2CO, 8:2) to give **3** as a pure oil (4.0 mg): uv λ max (log ε) 210 (sh) nm; ir ν max 1754 (C=O, lactone), 1745 (C=O, acetyl), 1735 (C=O, acetyl), 1680 (C=C), 1250 (O-CO) cm^{-1}; ^1H nmr, see Table 1; eims *m/z* 313 $[MH]^+$ (4), 270 (22), 252 (29), 210 (47), 192 (63), 127 (36), 125 (54), 110 (42), 43 (100).

PREPARATION OF 3′,7′-*O*,*O*′-DIACETYLHYDROXYISOSEIRIDIN [**4**].—7′-Hydroxyisoseiridin (**2**, 4.0 mg) was converted into the corresponding 3′,7′-*O*,*O*′-diacetyl derivative [**4**] by treatment with Ac_2O (200 μl) and pyridine (200 μl) according to the procedure used to prepare **3** from **1**. The crude residue obtained from the reaction workup was purified by prep. tlc (SiO_2, petroleum ether-Me_2CO, 8:2) to give **4** as a pure oil (4.5 mg): uv λ max (log ε) 210 (4.10) nm; ir ν max 1758 (C=O, lactone), 1744 (C=O, acetyl), 1733 (C=O, acetyl), 1682 (C=C), 1241 (O-CO) cm^{-1}; ^1H nmr, see Table 1; eims *m/z* 313 $[MH]^+$ (34), 270 (11), 253 (63), 210 (18), 192 (58), 127 (13), 125 (13), 110 (11), 43 (100).

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