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STRUCTURE DETERMINATION OF SEIRIDIN AND ISOSEIRIDIN, PHYTOTOXIC BUTENOLIDES FROM CULTURE FILTRATE OF *SEIRIDIUM CARDINALE*

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ABSTRACT.—Seiridin (**1**) and isoseiridin (**2**) are two new phytotoxic $\Delta^{\alpha,\beta}$-butenolides isolated from culture filtrates of *Seiridium cardinale*, the causal agent of the cypress canker disease. Spectroscopic data of the two metabolites and of some key derivatives and the application of Mosher's method provided the complete stereostructure of both products.

In a preliminary paper, the isolation, structure determination, and phytotoxic and antibacterial activities of seiridin[1] (**1**) and isoseiridin[1] (**2**), two new metabolites of *Seiridium cardinale* (Wag.) Sutt. et Gibs., have been briefly reported (1).

This fungus causes cypress canker, a highly destructive disease that appeared first in the United States (2) and later in other parts of the world. Since its introduction to Europe, the pathogen has caused heavy damage in Italy and in other Mediterranean countries (3,4).

This paper reports the methods used for the isolation and stereostructure determination of the two phytotoxic compounds produced in culture by *S. cardinale*.

RESULTS AND DISCUSSION

Column chromatography on SiO_2, monitored by tlc, of *t*-BuOMe extracts of the acidified *S. cardinale* culture filtrates afforded thirteen fractions; three of them were active in the bioassay (1). Further purification of the most active of these fractions by column chromatography and tlc on SiO_2, yielded two phytotoxic compounds as colorless oils, homogeneous in various preparative tlc systems. The major compound (49.5 mg/liter) had a Rf value of 0.51 (tlc on SiO_2, petroleum ether-Me_2CO, 6:4) and was named seiridin (**1**). The other compound (17.4 mg/liter), named isoseiridin (**2**), had a Rf value of 0.56 in the above tlc system.

Seiridin had a molecular formula $C_{12}H_{20}O_3$ as deduced from its hrms. The nature of the oxygen atoms was shown by its ir spectrum, which contained absorption bands characteristic for hydroxy and conjugated carbonyl groups and ester functions. The ir bands at 1750 and 1680 cm^{-1} suggested the presence of an α,β-unsaturated-γ-lactone ring (5,6), which was further corroborated by the characteristic uv absorption (7) at 215 nm.

The ^{1}H-nmr spectrum of **1** (Table 1) confirmed these molecular features and also yielded some more information. A quartet at δ 4.62, attributed to an $OCH_2C=$ group, and a triplet at δ 1.79, attributed to a CH_3-C=C group, were present. The broadening of both signals was due to further coupling ($J<1$ Hz) with the CH_2-7′. This group appeared at δ 2.39 as a broad triplet further coupled to a triplet of triplets centered at δ 1.50 and assigned to CH_2-6′. This signal corresponds to a methylene group (CH_2-6′) that is part of the 2-hydroxyheptyl side chain. Further features of this chain were re-

[1]Nomenclature, seiridin: 3-methyl-4-(2-hydroxyheptyl)-2(5H)-furanone, (2*R*); isoseiridin: 3-methyl-4-(3-hydroxyheptyl)-2(5H)-furanone, (3*R*).

TABLE 1. ^{1}H-nmr Data of Seiridin (**1**), Isoseiridin (**2**) and the Corresponding Dihydroderivatives **5** and **6** [Chemical Shifts are in δ-Values (ppm) from TMS]

Compounds			Compounds		
Atom	**1**[a]	**2**[a]	Atom	**5**	**6**
2H-5	4.62 q	4.62 q	H-2 . . .	2.67 dq	2.66 dq
3H-1′	1.16 d	0.89 t	H-3 . . .	2.48 tddd	2.47 tddd
H-2′	3.76 tq	1.42 m (2H)	2H-4 . . .	4.27 dd AB	4.26 dd AB
H-3′	1.38 m (2H)	3.47 tt		4.00 dd	3.99 dd
2H-4′	1.38 m	1.42 m	3H-1′ . . .	1.17 d	0.92 t
2H-5′	1.38 m	1.42 m	H-2′ . . .	3.78 tq	1.44 m (2H)
2H-6′	1.50 tt	1.48 m	H-3′ . . .	1.33 m (2H)	3.50 m
2H-7′	2.39 br t	2.38 br t	2H-4′ . . .	1.33 m	1.44 m
3H-8′	1.79 t	1.77 t	2H-5′ . . .	1.33 m	1.44 m
			2H-6′ . . .	1.33 m	1.44 m
			2H-7′ . . .	1.33 m	1.44 m
			3H-8′ . . .	1.15 d	1.15 d

[a]Run at 270 and 500 MHz.

J (Hz), **1**: 5,8′=1.8; 1′,2′=2′, 3′=6.3; 5′,6′=6′, 7′=7.4; **2**: 5,8′=1.8; 1′,2′=6′, 7′=7.4; 2′,3′=6.7; 3′,4′=4.5; **5**,**6**: 2, 3=2, 8′=3, 7′=7.4; 3,4A=6.6; 3,4B=5.5; 4A,4B=9.2; **5**: 1′,2′=2′, 3′=6.3; **6**: 1′,2′=7.4.

sponsible for the complex signal system centered at δ 1.38 (CH_2-3′, CH_2-4′, and CH_2-5′), of a triplet of quartets at δ 3.76 (HOCH-2′) and of a doublet at δ 1.16 (CH_3-1′).

These results were consistent with the assignment to seiridin of a 3,4-disubstituted $\Delta^{\alpha,\beta}$-butenolide nucleus (8). $\Delta^{\alpha,\beta}$-Butenolides are quite common as natural products (9,10), but their corresponding 3,4-alkyldisubstituted derivatives are rare fungal metabolites (10,11).

The partial structure proposed for seiridin was corroborated by the data of the proton noise decoupled (PND), the single frequency off-resonance decoupled (SFORD), and the attached proton test (APT) ^{13}C-nmr spectra of **1** (Table 2). The carbonyl group

TABLE 2. ^{13}C-nmr Data of Seiridin (**1**) and Its Derivatives **5**,**7**, and **11** [Chemical Shifts are in δ-Values (ppm) from TMS]

	Compounds			
Atom	**1**[a]	**5**[a]	**7**[b]	**11**[a]
C-1	—	180.2 s	62.3 t	—
C-2	175.5 s[c]	39.1 d	134.0 s	138.9 d
C-3	160.4 s	37.7 d	137.8 s	120.0 s
C-4	122.9 s	70.8 t	64.0 t	125.5 s
C-5	71.3 t	—	—	139.2 d
C-1′	23.5 q[d]	23.5 q	23.5 q	23.5 q
C-2′	67.8 d	68.0 d	68.1 d	68.1 d
C-3′	38.9 t[d]	39.1 t	39.2 t	39.3 t
C-4′	29.4 t[d]	29.6 t	29.6 t	29.4 t
C-5′	25.3 t[d]	25.5 t	25.6 t	25.6 t
C-6′	27.5 t[d]	27.2 t	28.4 t	29.3 t
C-7′	27.0 t[d]	27.0 t	32.1 t	23.4 t
C-8′	8.4 q[c,d]	10.0 q	17.6 q	8.1 q

[a]Multiplicities were determined by SFORD and APT spectra.

[b]Multiplicities were determined by APT spectrum.

[c]Attribution made also by the long-range ^{1}H-^{13}C couplings observed in the "gated decoupling" ^{13}C-nmr spectrum of seiridin.

[d]Assignments made also by SFSD spectra.

of the α,β-unsaturated lactone resonated at δ 175.5, the carbons of the tetrasubstituted double bond gave singlets in the SFORD and APT spectra at δ 160.4 and 122.9. The signal of the hydroxylated carbon (C-2′), a doublet in the SFORD and APT spectra, appeared at δ 67.8 while the signals of the two methyl groups at δ 23.5 and 8.4 were attributed respectively to C-1′ and C-8′. Experiments of single frequency selective decoupled (SFSD) ^{13}C nmr, carried out on **1**, allowed assignment of the chemical shift (Table 2) of each of the five methylene carbons of the side chain.

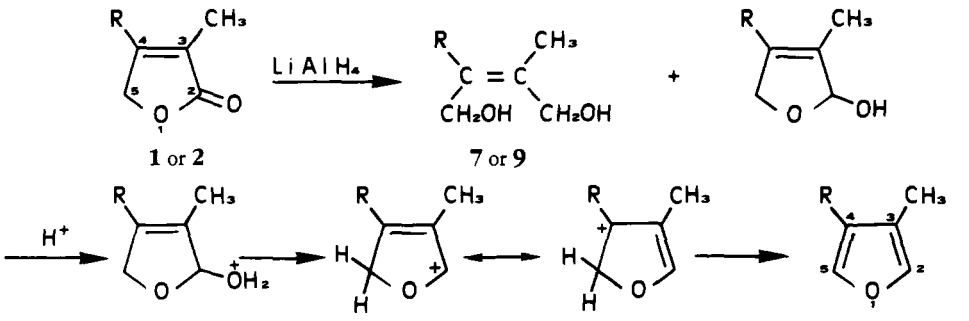
The relative position of the two substituents in the γ-lactone ring of **1** was deduced by comparison of the ^1H-nmr spectrum of **1** with respect to that of its dihydroderivative (**5**) (Table 1). In the spectrum of **5**, the presence of a new secondary methyl group (CH_3-8′) was observed at δ 1.15 and a doublet of quartets, due to H-2, at δ 2.67. This latter proton was coupled to the triple doublet of double doublets at δ 2.48 attributed to H-3, the X part of an ABX system. The AB part (CH_2-4) appeared as two doublets of doublets at δ 4.27 and 4.00. Irradiation of the doublet at δ 1.15 (CH_3-8′) converted the doublet of quartets of H-2 at δ 2.67 into a doublet (J=7.4 Hz) while irradiation of H-2 collapsed the doublet of CH_3-8′ into a singlet and simplified the complex system due to H-3. The two doublets of doublets of H-4A and H-4B at δ 4.27 and 4.00 became two doublets (J_{AB}=9.2 Hz), and the signal of H-2 appeared as a quartet (J=7.4 Hz) at δ 2.67 by irradiation of H-3. Similar results were obtained by performing the same proton decoupling experiments on **6**.

The absolute configuration of C-2′ in **1** was established using Mosher's method (12,13). Seiridin was converted into the diastereomeric *R*-(+)-α-methoxy-α-trifluorophenylacetate (MTPA) (**13**) and *S*-(−)-MTPA (**15**) esters; on both derivatives accurate ^1H-nmr studies were carried out. The comparison of the ^1H-nmr data of **15** with respect to those of **13** (Table 3) showed a downfield shift (Δ δ 0.08) of CH_3-1′ along with an upfield shift (Δ δ 0.09) of CH_2-3′. These findings, according to the literature data (12-14), allowed us to assign the *R*-configuration to C-2′. From the above results, the structure of (R)-3-methyl-4-(2-hydroxyheptyl)-2(5H)-furanone (**1**) is proposed for seiridin. Such a structure is in agreement with the data obtained from high resolution eims of **1**. Besides the molecular ion (*m/z* 212.1413), ions at *m/z* 197.1185 ($C_{11}H_{17}O_3$) and 194.1311 ($C_{12}H_{18}O_2$) corresponding to losses of a methyl and of H_2O from the molecular ion, respectively, were consistent with the presence in **1** of a secondary hydroxy group. The ions appearing at *m/z* 165.1282 ($C_{11}H_{17}O$) and 149.1329 ($C_{11}H_{17}$) were probably formed on fragmentation of the *m/z* 194.1311 ion through processes described for other α,β-unsaturated-γ-lactones (15). The base peak at *m/z* 125.0604 ($C_7H_9O_2$) and the peak at *m/z* 112.0524 ($C_6H_8O_2$) might derive from the molecular ion by cleavage of the C(5′)-C(6′) and C(6′)-C(7′) bonds, respectively, the latter fragmentation occurring with a rearrangement mechanism (11).

TABLE 3. ^1H-nmr Data of the α-Methoxy-α-trifluorophenylacetate (MTPA) Esters of Seiridin (**13** and **15**) and Isoseiridin (**14** and **16**) (Chemical Shifts Are in δ-Values (ppm) from TMS [270 MHz, $CDCl_3$])

Compounds	Ph	OCH_3	CH_3-1′	H-2′	CH_2-3′	
13[a]	7.53-7.40 m	3.52 br s	1.26 d	5.13 tq	1.36 m	
15[a]	7.54-7.39 m	3.55 br s	1.34 d	5.14 tq	1.27 m	
	Ph	OCH_3	CH_3-1′	CH_2-2′	H-3′	CH_2-4′
14[b]	7.54-7.40 m	3.52 br s	0.83 t	1.62 m	5.02 tt	1.68 m
16[b]	7.55-7.40 m	3.55 br s	0.92 t	1.69 m	5.02 tt	1.59 m

[a,b]The other proton resonances were very close to those of **1** and **2**, respectively.
J (Hz), **13**,**15**: 1′,2′=2′, 3′=6.3; **14**,**16**: 1′,2′=7.4; 2′,3′=6.3.



1 or **2** **7** or **9** **11** or **12**

1,**7**,**11** R=$CH_3CH(OH)CH_2CH_2CH_2CH_2CH_2$ **2**,**9**,**12** R=$CH_3CH_2CH(OH)CH_2CH_2CH_2CH_2$

FIGURE 1. Mechanism of transformation of **1** and **2** into the furan derivatives **11** and **12**, respectively, by $LiAlH_4$ reduction.

Confirmation of the structure assigned to **1** was obtained through the analysis of some derivatives. Treatment of **1** with Ac_2O in pyridine afforded acetyl seiridin (**3**) (M^+=*m/z* 254), which had a ^{1}H-nmr spectrum showing, on comparison with that of **1**, the expected downfield shift ($\Delta\,\delta$1.11) of the signal attributed to CH-2′. Catalytic hydrogenation of **1** gave a *cis*-dihydroderivative (**5**) (M^+=*m/z* 214). The significant signals of its ^{1}H-nmr spectrum have been described above. Reduction of seiridin with $LiAlH_4$ afforded a mixture that contained, as main products, the trihydroxy olefin **7** and the 3,4-disubstituted furan **11**. The latter compound was probably formed in consequence of the acid treatment during the workup of the reaction; its formation might be accounted for by the mechanism reported in Figure 1. Compound **7** had a ^{1}H-nmr spectrum (Table 4) very similar to that of **1** except for the presence of a broad singlet at δ 4.16 due to CH_2-1 and CH_2-4, and the absence of the quartet and the triplet attributed to the H_2C-O-CO and CH_3C=C groups, respectively. The triacetyl derivative of **7** (**8**) (MH^+=*m/z* 343 by chemical ionization) had a ^{1}H-nmr spectrum which exhibited, as compared to that of **7**, the downfield shifts ($\Delta\,\delta$: 0.47, 0.47, and 1.08) of the signals due to CH_2-1, CH_2-4, CH_2-2′, respectively. Compound **11** (M^+=*m/z* 196) exhibited in its ^{1}H-nmr spectrum (Table 4) the typical furanoid signals at δ 7.12 (a doublet of triplets) and 7.14 (a doublet of quartets), assigned to CH-5 and CH-2, respectively.

TABLE 4. ^{1}H-nmr Data of Derivatives **7**, **11** and **9**, **12**, Obtained by $LiAlH_4$ Reduction of Seiridin and Isoseiridin, Respectively [Chemical Shifts Are in δ-Values (ppm) from TMS]

Compounds			Compounds		
Atom	**7**	**9**	Atom	**11**	**12**
2H-1	4.16 br s	4.14 br s	H-2 . . .	7.14 dq	7.14 dq
2H-4	4.16 br s	4.14 br s	H-5 . . .	7.12 dt	7.12 dt
3H-1′	1.18 d	0.93 t	3H-1′ . . .	1.18 d	0.94 t
H-2′	3.79 tq	1.44 m (2H)	H-2′	3.79 tq	1.49 m (2H)
H-3′	1.40 m (2H)	3.52 m	H-3′ . . .	1.39 m (2H)	3.53 m
2H-4′	1.40 m	1.44 m	2H-4′ . . .	1.39 m	1.49 m
2H-5′	1.40 m	1.44 m	2H-5′ . . .	1.39 m	1.49 m
2H-6′	1.40 m	1.44 m	2H-6′ . . .	1.54 m	1.55 m
2H-7′	2.16 br t	2.17 br t	2H-7′ . . .	2.33 td	2.35 td
3H-8′	1.81 br s	1.81 br s	3H-8′ . . .	1.94 d	1.95 d

J (Hz), **7**: 1′,2′=2′, 3′=6.3; 6′,7′=7.0; **9**: 1′,2′=7.4; 6′,7′=7.0; **11**,**12**: 2,5=1.8; 2,8′=5,7′=1.5; 6′,7′=7.4; **11**: 1′,2′=2′,3′=6.3; **12**: 1′,2′=7.4.

The other proton resonances were very close to those observed for **1**, whereas different multiplicities, due to long-range coupling, were observed for the signals of CH_3-8′ and CH_2-7′, which now appeared as doublet and triplet of doublets, respectively.

Isoseiridin (**2**) had a molecular formula $C_{12}H_{20}O_3$, as deduced from the hrms. The significant ir bands at 3680, 3480, 1750, and 1680 cm^{-1} and the intense uv absorption at 216 nm were very similar to those of **1**. The ^{1}H- and the ^{13}C-nmr spectra of **2** (Tables 1 and 5, respectively) were close to those of **1**, thus suggesting that isoseiridin is also a 3,4-alkyldisubstituted $\Delta^{\alpha,\beta}$-butenolide (8).

TABLE 5. ^{13}C-nmr Data of Isoseiridin (**2**) and Its Derivatives **6**,**9**, and **12** [Chemical Shifts Are in δ-Values (ppm) from TMS]

Atom	Compounds			
	2[a]	**6**[b]	**9**[b]	**12**[b]
C-1	—	180.7 s	62.3 t	—
C-2	175.5 s	39.1 d	134.3 s	138.9 d
C-3	160.4 s	37.7 d	137.6 s	119.9 s
C-4	122.7 s	70.8 t	64.1 t	125.4 s
C-5	71.3 t	—	—	139.3 d
C-1′	9.7 q[c]	10.0 q	9.9 q	9.9 q
C-2′	30.2 t[c]	30.3 t	30.3 t	30.2 t
C-3′	72.8 d	73.1 d	73.1 d	73.2 d
C-4′	36.2 t[c]	36.6 t	36.5 t	36.7 t
C-5′	25.4 t[c]	25.7 t	25.4 t	25.5 t
C-6′	27.5 t[c]	27.3 t	28.3 t	29.4 t
C-7′	27.0 t[c]	27.1 t	32.2 t	23.4 t
C-8′	8.3 q[c]	9.9 q	17.6 q	8.1 q

[a]Multiplicities were determined by SFORD and APT spectra.
[b]Multiplicities were determined by APT spectrum.
[c]Assignments made also by SFSD spectra.

The ^{1}H-nmr spectrum had a triplet at δ 0.89 that was assigned to the terminal methyl of the side chain (CH_3-1′). Thus, isoseiridin must be a structural isomer of **1**, differing from it only in the hydroxyheptyl side chain.

The hydroxy group was located at C-3′ on the following grounds. The ^{1}H-nmr spectrum of a $CDCl_3$ solution of **2** containing Eu(fod)$_3$ showed a downfield shift of CH_2-2′ and CH-3′ (Δ δ 0.55, doublet of quartets, and 1.00, multiplet, respectively) while the other resonances remained substantially unchanged. Irradiation of the multiplet at δ 4.47 (CH-3′) converted the doublet of quartets at δ 1.97 (CH_2-2′) into a quartet (J=7.4 Hz), while irradiation of these latter protons simplified the multiplet of CH-3′ into a very broad singlet and collapsed into a singlet the triplet present at δ 1.27 assigned to CH_3-1′. Moreover, the doublet of quartets of CH_2-2′ became a doublet (J=5.9 Hz) on irradiation of CH_3-1′.

The hrms of **2** was in agreement with the above reported results. In fact, the significant peak recorded at *m/z* 183.1028 ($C_{10}H_{15}O_3$) was produced from the molecular ion (*m/z* 212.1399) by loss of an ethyl radical, according to a known fragmentation process of the secondary alcohols, as well as the loss of a molecule of H_2O which yielded the peak at *m/z* 194.1299 ($C_{12}H_{18}O_2$). In addition, the ions at *m/z* 165.1278 ($C_{11}H_{17}O$), 149.1330 ($C_{11}H_{17}$), 125.0602 ($C_7H_9O_2$), and 112.0520 ($C_6H_8O_2$) were observed. They were formed from the molecular ion by means of fragmentation mechanisms similar to those described for **1**.

The configuration of C-3′ in **2** was also derived by Mosher's method (12,13). As re-

ported above, for **1**, isoseiridin (**2**) was converted into the diastereomeric *R*-(+)-MTPA and *S*-(−)-MTPA esters (**14** and **16**, respectively). The ^{1}H-nmr data reported in Table 3 showed a downfield shift of CH_3-1′ ($\Delta\ \delta$ 0.09) and of CH_2-2′ ($\Delta\ \delta$ 0.07) along with an upfield shift of CH_2-4′ ($\Delta\ \delta$ 0.09) when **16** was compared to **14**. This result suggested, according to literature data (12-14), an *R*-configuration for C-3′. These findings assigned to isoseiridin the structure of (R)-3-methyl-4-(3-hydroxyheptyl)-2(5H)-furanone (**2**).

The chemical and the spectroscopic analysis of derivatives **4**, **6**, **9**, **10**, and **12**, prepared from **2** by the same procedures used for **1**, further indicated that the isoseiridin is a structural isomer of **1**. In fact, the consequence of the side chain isomerization was an upfield shift in the ^{1}H-nmr spectrum of the signal attributed CH_3-1′, on passing from **1**, **3**, **5**, **7**, **8**, and **11** to **2**, **4**, **6**, **9**, **10**, and **12** ($\Delta\ \delta$: 0.27, 0.33, 0.25, 0.25, 0.33, and 0.24, respectively). The same structural difference also justified the downfield shift in the ^{13}C-nmr spectrum of the signal of C-4′ on passing from **1**, **5**, **7**, and **11**to **2**, **6**, **9**, and **12** ($\Delta\ \delta$: 6.8, 7.0, 6.9, and 7.3, respectively).

The elucidation of the structure of both toxins is a prerequisite for studying the role of seiridin and isoseiridin in the pathogenesis of cypress canker disease.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were recorded on a Perkin-Elmer 141 polarimeter in $CHCl_3$ solutions; uv spectra were carried out in MeCN on a Varian-Cary 210 spectrophotometer; ir spectra were recorded on a Perkin-Elmer 684 instrument for solution in $CHCl_3$; ^{1}H-nmr spectra were recorded at 270 MHz on a Bruker instrument for solutions in $CDCl_3$. ^{13}C-nmr spectra were obtained, in $CDCl_3$, at 67.88 and/or 50.30 MHz on a Bruker or a Varian XL-200 spectrometer, respectively. Mass spectra and hrms were taken on a MS-30 AEI and on a MS-50 Kratos spectrometer, respectively, operating with an ionization energy of 70 eV; cims was recorded on a MS-80 Kratos spectrometer with the sample introduced through a direct evaporation rod using isobutane as reagent gas; the electron energy for ionization of reagent gas was 70 eV. Analytical and preparative tlc were performed on SiO_2 plates (Merck, Kieselgel 60 F_{254}, 0.25 and 2 mm, respectively). The spots were visualized by exposure to uv radiation and by spraying first with 10% H_2SO_4 in MeOH and then with 3% phosphomolybdic acid in MeOH followed by heating at 110° for 10 min. Column chromatography was carried out on SiO_2 (Merck, Kieselgel 60,0.063-0.2 mm). The petroleum ether used for chromatography had bp 40-70°. Eu(fod)$_3$ [Europium (111)-tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octaolionate)] was purchased from C. Erba, Italy. The *R*-(+)-α-methoxy-α-trifluorophenylacetic (MTPA) and the *S*-(−)-MTPA acids were purchased from Fluka AG Buschs, Switzerland. The (+)-MPTA-Cl and the (−)-MTPA-Cl were obtained from the corresponding acid by reaction with $SOCl_2$ and then distilled as previously reported (14).

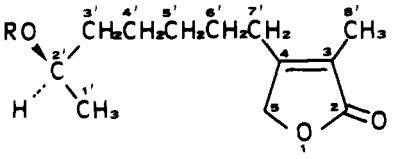
TOXIN PURIFICATION.—Culture filtrates (10 liters) of *S. cardinale*[2] (1) were adjusted at pH 4 with 0.1N HCl and extracted with *t*-BuOMe (4×2.5 liters). The combined extracts were dried (Na_2SO_4) and evaporated under reduced pressure to afford a brown oily residue (2.0 g). This was fractionated by column chromatography on SiO_2 using $CHCl_3$-iPrOH (9:1) as eluent. After inspection by tlc, homogeneous fractions were pooled and assayed for their phytotoxicity (1). Three groups of fractions displayed activity, the most potent containing two products with Rf value of 0.51 and 0.56 on tlc run with petroleum ether-Me_2CO (6:4). Separation of the products was achieved by chromatography of the mixture (736 mg) on a SiO_2 column run with the same solvent system; the products were finally purified by preparative tlc with the same solvent system to afford seiridin (**1**) (495 mg, 49.5 mg/liter) and isoseiridin (**2**) (174 mg, 17.4 mg/liter) as pure compounds.

Seiridin (**1**).—Seiridin obtained as a colorless oil had: $[\alpha]^{25}D$ −4.8° (c=1.48); uv λmax nm (log ε) 215 (4.21); ir νmax 3680, 3610, 3480, 1750, 1680, 1080, 1040 cm^{-1}; ^{1}H- and ^{13}C-nmr spectra see Tables 1 and 2, respectively; ms *m/z* (rel. int.) 212.1413 (M^+, calcd. 212.1413) (21), 197 (21), 194 (13), 165 (13), 149 (15), 125 (100), 112 (64).

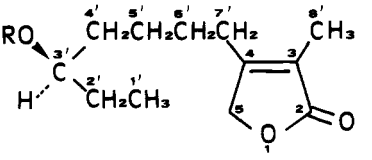
Isoseiridin (**2**).—Isoseiridin obtained as colorless oil had: $[\alpha]^{25}D$ −6.3° (c=3.04); uv λmax nm (log ε) 216 (4.24); ir νmax 3680, 3610, 3480, 1750, 1680, 1080, 1040 cm^{-1}; ^{1}H- and ^{13}C-nmr spectra see Tables 1 and 5, respectively; ms *m/z* (rel. int.) 212.1399 (M^+, calcd. 212.1413) (8.5), 194 (16), 183 (51), 179 (2), 165 (12), 149 (16), 125 (100), 112 (95).

[2]Collection of Department of Plant Pathology, University of Bari, Bari, Italy.

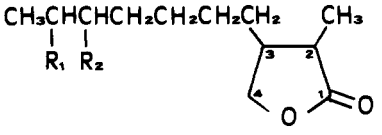
2′-O-acetylseiridin (**3**).—Acetylation of **1** (14.7 mg) with Ac_2O/pyridine afforded the *2′-O*-acetyl derivative. The usual workup of the reaction mixture, followed by preparative tlc (petroleum ether-Me_2CO, 8:2) gave **3** as an oil (14.3 mg, 83%): $[\alpha]^{25}D$ +1.7° (*c*=1.10); uv λmax nm (log ε) 215 (4.17); ir νmax 1750, 1740, 1680, 1250 cm^{-1}; ^1H-nmr, δ 4.87 (tq, *J*=6.3, 6.3 Hz, H-2′), 2.01 (s, 3H, MeCO); the other proton resonances were very similar to those of **1**; ms *m/z* (rel. int.) 254 (M^+) (4.2), 194 (27), 165 (17), 149 (17), 125 (54), 112 (46), 43 (100).



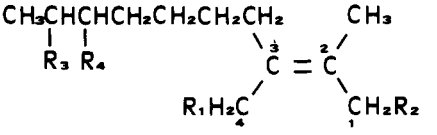
1 R=H
3 R=Ac
13 R=*R*-(+)-MTPA*
15 R=*S*-(−)-MTPA*



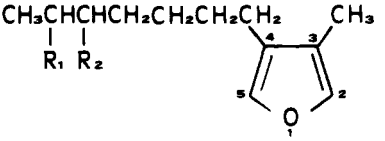
2 R=H
4 R=Ac
14 R=*R*-(+)-MTPA*
16 R=*S*-(−)-MTPA*



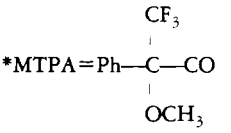
5 R_1=OH,R_2=H
6 R_1=H,R_2=OH



7 R_1=R_2=R_3=OH,R_4=H
8 R_1=R_2=R_3=OAc,R_4=H
9 R_1=R_2=R_4=OH,R_3=H
10 R_1=R_2=R_4=OAc,R_3=H



11 R_1=OH,R_2=H
12 R_1=H,R_2=OH



3,4-Dihydroseiridin (**5**).—Seiridin (29.6 mg) in MeOH (10 ml) was added to a suspension of presaturated 10% Pd on charcoal (30 mg) in MeOH (10 ml). The hydrogenation was performed at room temperature and atmospheric pressure, under stirring. After 48 h, the reaction was stopped by filtration, and the clear solution was evaporated under reduced pressure. The residue (28 mg) was purified by column chromatography (petroleum ether-Me_2CO, 6:4) to give a pure oil (26.8 mg, 91%): $[\alpha]^{25}D$ −6.0° (*c*=2.02); uv λmax nm (log ε) 213 (2.30); ir νmax 3680, 3610, 3500, 1780, 1180 cm^{-1}; ^1H- and ^{13}C-nmr spectra see Tables 1 and 2, respectively; ms *m/z* (rel. int.) 214 (M^+) (0.4), 213 (1.1), 199 (25), 196 (2.2), 170 (40), 155 (10), 99 (80), 97 (100).

LiAlH₄ Reduction of **1**.—$LiAlH_4$ (45 mg) was added to a solution of **1** (69 mg) in dry Et_2O (25 ml) at 0°; the reaction was carried out at room temperature under stirring. After 2 h, the reaction was stopped by addition, drop by drop of H_2O (2 ml) at 0°. The mixture was adjusted to pH 5, with 0.1 N HCl, and then extracted with Et_2O (4×50 ml). The combined ethereal extracts were washed with H_2O, dried (Na_2SO_4), and evaporated under reduced pressure. Tlc ($CHCl_3$-iPrOH, 85:15) of the residue (59.6 mg) showed the presence of at least five compounds. Column chromatography ($CHCl_3$-iPrOH, 85:15) separated the mixture essentially into two main fractions. The first eluted fraction (38.8 mg) contained **1** and **11**, as shown by tlc run with petroleum ether-Me_2CO (6:4). This solvent system was used for the further purification by column chromatography, which gave compound **11** as an homogeneous oil (7.4 mg, 11%): $[\alpha]^{25}D$ −5.3° (*c*=0.80); uv λmax nm (log ε) 215 (3.58); ir ν max 3680, 3600, 3460, 1600 cm^{-1}; ^1H- and ^{13}C-nmr data are reported in Tables 4 and 2, respectively; ms *m/z* (rel. int.) 196 (M^+) (31), 181 (7.5), 96 (100), 95 (66). The second fraction (13 mg), obtained from the column run with $CHCl_3$-iPrOH, contained **7** and another product as shown by tlc analysis with $CHCl_3$-iPrOH (85:15). This solvent was used for the column chromatography fractionation of the mixture, yielding the trihydroxy olefin **7** as a pure compound (7.4 mg, 11%): $[\alpha]^{25}D$ −7.0° (*c*=0.64); uv λmax nm (log ε) < 200; ir νmax 3680, 3610, 3440, 1600 cm^{-1};

^{1}H- and ^{13}C-nmr data are reported in Tables 4 and 2, respectively; ms *m/z* (rel. int.) 198 (M^+-H_2O) (10), 180 (60), 165 (100), 149 (90).

1,4,2'-O,O',O''-*Triacetyl* **7** (**8**).—Acetylation of **7** (10.6 mg) with Ac_2O/pyridine afforded the triacetyl derivative. The usual workup of the reaction mixture, followed by column chromatography (petroleum ether-Me_2CO, 8:2), gave **8** as an oil (12.0 mg, 71%): $[\alpha]^{25}D$ +0.6° (*c*=1.00); uv λmax nm (log ε) < 200; ir νmax 1730, 1600, 1225 cm^{-1}; ^{1}H nmr δ 4.87 (tq, *J*=6.3, 6.3 Hz, H-2'), 4.63 (br s, 4H, H-1 and H-4), 2.04 (s, 3H, MeCO), 2.04 (s, 3H, MeCO), 2.02 (s, 3H, MeCO), 1.61 (m, H-3'); the other proton resonances were very close to those of **7**; ms *m/z* (rel. int.) 282 (M^+-CH_3COOH) (25), 240 (45), 180 (65) 43, (100).

3'-O-*Acetyl-isoseiridin* (**4**).—Isoseiridin (19.5 mg) was acetylated with Ac_2O/pyridine; the usual workup of the reaction mixture followed by preparative tlc purification (petroleum ether-Me_2CO, 8:2) gave a pure oil (21.2 mg, 92%): $[\alpha]^{25}D$ +5.0° (*c*=1.80); uv λmax nm (log ε) 215 (4.04); ir νmax 1750, 1740, 1680, 1250, cm^{-1}. ^{1}H nmr δ 4.78 (tt, *J*=6.3 and 6.3 Hz, H-3'), 2.02 (s, 3H, MeCO); the other proton resonances were very similar to those of **2**; ms *m/z* (rel. int.) 254 (M^+) (7.6), 194 (38.5), 183 (54), 179 (3.8), 165 (27), 125 (73), 112 (73), 43 (100).

3,4-Dihydroisoseiridin (**6**).—Isoseiridin (23 mg) was hydrogenated with 10% Pd on charcoal under the same conditions reported for the hydrogenation of **1**. The reaction afforded an oily residue that was purified by column chromatography (petroleum ether-Me_2CO, 6:4) to give pure **6** (18.6 mg, 81%): $[\alpha]^{25}D$ −7.7° (*c*=1.84); uv λmax nm (log ε) 213 (2.40); ir νmax 3680, 3600, 3500, 1770, 1180 cm^{-1}; ^{1}H- and ^{13}C-nmr data are summarized in Tables 1 and 5, respectively; ms *m/z* (rel. int.) 214 (M^+) (2.5), 213 (4.8), 197 (24), 185 (100), 156 (24), 99 (85), 97 (28).

Compounds **9** *and* **12**.—Reduction of isoseiridin (61.0 mg) with $LiAlH_4$ was carried out under the same conditions used to convert **1** into **7** and **11**. The workup of the reaction afforded a complex mixture (47.4 mg) which was fractionated by column chromatography eluted with $CHCl_3$-iPrOH (85:15). The fractions containing **12** were combined and evaporated under reduced pressure. The residue (24.4 mg) was purified by further column chromatography (petroleum ether-Me_2CO, 6:4) to give **12** as an homogeneous oil (5.3 mg, 9.6%): $[\alpha]^{25}D$ −9.2° (*c*=0.53); uv λmax nm (log ε) 215 (3.58); ir νmax 3690, 3600, 1600 cm^{-1}; ^{1}H- and ^{13}C-nmr data are summarized in Tables 4 and 5, respectively; ms *m/z* (rel. int.) 196 (M^+)(17), 167 (6.6), 149 (20), 96 (93), 95 (70), 83 (100). The fractions obtained from the first column run with $CHCl_3$-iPrOH containing **9** were combined and evaporated under reduced pressure. The residue (8.8 mg) was further fractionated by column chromatography ($CHCl_3$-iPrOH, 85:15) to yield **9** as a pure oil (4.5 mg, 10%): $[\alpha]^{25}D$ −10.8° (*c*=0.45); uv λmax nm (log ε) < 200; ir νmax 3680, 3600, 3410, 1600 cm^{-1}; ^{1}H- and ^{13}C-nmr data are listed in Tables 4 and 5, respectively; ms *m/z* (rel. int.) 198 (M^+-H_2O (4.3), 180 (86), 167 (91), 165 (91), 151 (100), 149 (41).

1,4,3'-O,O',O''-*Triacetyl* **9** (**10**).—The triacetyl derivative **10** was obtained from **9** (13.2 mg) as described for preparing **8** from **7**. Purification of the crude triacetyl derivative by column chromatography (petroleum ether-Me_2CO, 8:2) yielded **10** as a pure oil (12.0 mg, 70%): $[\alpha]^{25}D$ +4.8° (*c*=1.20); uv λmax nm (log ε) < 200; ir νmax 1730, 1600, 1225 cm^{-1}; ^{1}H nmr δ 4.79 (m, H-3'), 4.60 (br s, 4H, H-1 and H-4), 2.04 (s, 3H, MeCO), 2.03 (s, 3H, MeCO), 2.03 (s, 3H, MeCO), 1.51 (m, H-2'); the other proton resonances were very similar to those of **9**; ms *m/z* (rel. int.) 282 (M^+-CH_3COOH) (5.4), 240 (47), 222 (9.4), 180 (85), 43 (100).

R-(+)-α-*Methoxy*-α-*trifluorophenylacetate (MTPA) ester of seiridin* (**13**).—To seiridin (10 mg), dissolved in dry pyridine (200 μl) and dry CCl_4 (200 μl), was added distilled (+)-MTPA-Cl (20 μl). The mixture was allowed to stand at room temperature under stirring. After 12 h, the reaction was complete, and ice cold H_2O was added. The resulting aqueous solution was extracted with Et_2O. The combined ether extracts, after washing successively with 1 N HCl, saturated Na_2CO_3 solution, and H_2O, were dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue (27.2 mg) was purified by preparative tlc (petroleum ether-Me_2CO, 8:2) affording **13** (19.1 mg, 94%) as a pure oil: $[\alpha]^{25}D$ +18.8° (*c*=1.76); uv λmax nm (log ε) 260 (2.34), 209 (4.18); ir νmax 1745, 1680, 1600, 1495, 1270, 1170, cm^{-1}; ^{1}H-nmr spectrum is reported in Table 3; ms *m/z* (rel. int.) 428 (M^+) (5), 408 (3), 398 (1.5), 359 (0.7), 235 (0.3), 195 (61), 189 (100).

S-(−)-*MTPA ester of seiridin* (**15**).—To seiridin (10.2 mg), dissolved in dry pyridine (200 μl) and dry CCl_4 (200 μl), was added (−)-MTPA-Cl (20 μl). The reaction was carried out under the same condition used for preparing **13** from **1**. Purification of the crude residue (30 mg) by preparative tlc (petroleum ether-Me_2CO, 8:2) yielded **15** as a pure oil (19.5 mg, 96%): $[\alpha]^{25}D$ −45.1° (*c*=1.71); ^{1}H-nmr spectrum is reported in Table 3; uv, ir, and ms spectra were very similar to those of **13**.

R-(+)-*MTPA ester of isoseiridin* (**14**).—The ester derivative **14** was obtained from **2** (6.7 mg) as des-

cribed for preparing **13** from **1**. The pure oily **14** (13.0 mg, 96%) had: $[\alpha]^{25}D$ +22.9° (c=1.30); ^{1}H-nmr data are summarized in Table 3; uv, ir, and ms spectra were very close to those of **13**.

S-(−)-*MTPA ester of isoseiridin* (**16**).—The ester derivative **16** was obtained from **2** (9.1 mg) as reported for preparing **15** from **1**. The pure oily **16** (17.1 mg, 93%) showed: $[\alpha]^{25}D$ −36.9° (c=1.41); ^{1}H-nmr data are summarized in Table 3; uv, ir, and ms spectra were very close to those of **13**.

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