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SEIRICUPROLIDE, A NEW PHYTOTOXIC MACROLIDE FROM A STRAIN OF *SEIRIDIUM CUPRESSI* INFECTING CYPRESS*

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Key Word Index—*Cupressus sempervirens*; Cupressaceae; cypress; *Seiridium cupressi*; cypress canker disease; phytotoxins; macrolides; seiricuprolide; seiridins.

Abstract—A strain of *Seiridium cupressi*, a fungus causing a canker disease of cypress (*Cupressus sempervirens*) in Greece, produces several phytotoxins in culture. Two of them were identified as seiridin and *iso*-seiridin, the butenolides previously isolated from another cypress pathogen, *S. cardinale*. A third phytotoxin, which is present in small amounts in the culture filtrates of *S. cupressi* and is not produced by *S. cardinale*, was identified as a new macrolide which we have called seiricuprolide. Its structure was established by spectroscopic analysis of the metabolite and of some key derivatives.

INTRODUCTION

In recent decades, the canker disease of cypress (*Cupressus sempervirens* L.) and other Cupressaceae, caused by the imperfect fungus *Seiridium cardinale* (Wag.) Sutt. *et* Gibs. has spread almost everywhere in the Mediterranean area and caused heavy losses and the death of millions of trees [1, 2]. A similar disease, recently reported from the island of Kos (Greece) [2, 3], is caused by a fungus that may be considered as a strain of *S. cupressi* (Guba) Boesew., a species found in other parts of the world (Africa, Australia, Oceania) [2–4].

Previous work showed that *S. cardinale* produced in culture several phytotoxic substances responsible for some of the symptoms of the disease. Two major toxic compounds, seiridin (**1**) and *iso*-seiridin (**2**), were subsequently identified as new butenolides [5–7].

This paper is the first contribution to our knowledge of the phytotoxins produced in culture by the above mentioned strain of *S. cupressi*. It describes the occurrence of seiridin and *iso*-seiridin and the isolation of a new toxic macrolide, seiricuprolide.

RESULTS AND DISCUSSION

The strain of *S. cupressi* used in this study was grown in stationary culture on a Czapek's-corn meal medium, at 23° in the dark, for one month. The culture filtrate was acidified and extracted with *t*-BuOMe. The organic extract was evaporated to give a solid mixed with an abundant oily residue. The extract was resolved into a highly phytotoxic solid and 12 groups of homogenous fractions of which ten proved to be toxic to test plants.

The residue obtained from the third group of fractions contained seiridin (**1**) and *iso*-seiridin (**2**) (TLC, silica gel, solvent A). Further fractionation on a silica gel column with solvent B afforded **1** (33.7 mg/l crude filtrate) and **2** (21.7 mg/l) as pure oils identical to authentic samples of the two $\Delta^{\alpha,\beta}$-butenolides previously isolated from culture filtrates of *S. cardinale* [6, 7].

The fifth group of fractions contained a major phytotoxic component which was purified by applying the same method used to separate **1** from **2**. This procedure yielded a group of homogeneous fractions which, after evaporation of the solvent, gave a crystalline solid (1.7 mg/l) which we have named seiricuprolide (**3**). Recrystallization from ethyl acetate–petrol afforded white needles. When assayed on test plants (severed twigs of cypress or tomato and mung bean cuttings, at concentrations of 0.5 or 0.4 mg/ml, respectively) seiricuprolide produced a diffuse yellowing followed by browning on host plants, or chlorosis and necrosis on non-host plants.

High resolution MS and elemental analysis showed that seiricuprolide (**3**) has a molecular formula of $C_{14}H_{20}O_5$. Its IR spectrum showed absorption bands characteristic for hydroxy, carbonyl, olefinic and ester functions, while its UV spectrum exhibited only end absorption.

Detailed examination of the ^{1}H NMR spectrum (Table 1) revealed signals which resolved into an approximately first order pattern, except for the region of the methylene groups. This, integrated by the information yielded by a number of spin decoupling measurements, allowed the assignment of the chemical shift values and multiplicities to each proton.

In particular, the spectrum showed two double doublets at δ6.14 and 6.84 attributed to the protons of the 2,3-

*Part 3 of the series 'Phytotoxins produced by species of *Seiridium* causing canker diseases of cypress'. For parts 1 and 2 see refs [6, 7].

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Table 1. ^{1}H NMR spectral data of seiricuprolide (**3**), and its derivatives **4**–**6**

H	3*	4†‡	6†	H	5*
2	6.14 *dd*$_{AM}$	6.18 *dd*$_{AM}$	6.27 *dd*$_{AM}$	2	2.67 *ddd*$_{AB}$
3	6.84 *dd*$_{AM}$	6.83 *dd*$_{AM}$	6.87 *dd*$_{AM}$	2′	2.37 *ddd*$_{AB}$
4	4.32 *ddd*	5.36 *ddd*	4.64 *ddd*	3 (2H)	1.82 *m*
5	3.23 *dd*$_{AM}$	3.32 *dd*$_{AM}$	4.54 *dd*$_{AM}$	4	3.64 *ddd*
6	3.01 *dd*$_{AM}$	3.07 *dd*$_{AM}$	3.62 *dd*$_{AM}$	5	2.97 *dd*$_{AB}$
7	4.23 *dd*	5.43 *dd*	4.45 *dd*	6	3.06 *dd*$_{AB}$
8	5.37 *ddd*$_{AB}$	5.35 *ddd*$_{AB}$	5.56 *ddd*$_{AB}$	7	3.47 *ddd*
9	5.54 *ddd*$_{AB}$	5.57 *ddd*$_{AB}$	5.51 *ddd*$_{AB}$	8 (2H)	1.20–1.62 *m*
10	2.43 *m*	2.49 *m*	2.22 *m*	9 (2H)	1.20–1.62 *m*
10′	2.07 *m*	2.10 *m*	1.99 *m*	10 (2H)	1.20–1.62 *m*
11	1.78 *m*	1.78 *m*	1.63 *m*	11 (2H)	1.20–1.62 *m*
11′	1.23 *m*	1.21 *m*	1.13 *m*	12 (2H)	1.20–1.62 *m*
12	1.86 *ddd*	1.85 *ddd*	1.81 *m*	13	4.91 *ddq*$_{A_3X}$
12′	1.44 *ddd*	1.47 *ddd*	1.50 *m*	14 (3H)	1.21 *d*
13	4.91 *ddq*$_{A_3X}$	5.05 *ddq*$_{A_3X}$	5.04 *ddq*$_{A_3X}$		
14 (3H)	1.26 *d*	1.26 *d*	1.31 *d*		

J (Hz): **3**, **4**, **6**: 2,3 = 15.4; 2,4 = 1.5; 3,4 = 6.1; 8,9 = 11.0; 8, 10′ = 2.6; 9, 10 = 9.6; **3**, **4**: 4,5 = 6.3; 5,6 = 4.4; 6,7 = 7,8 = 8.5; 9,10′ = 3.3; 10,10′ = 11,11′ = 12,12′ = 13.6; 10,11 = 4.8; 11,12 = 10.6; 11′,12′ = 7.4; **3**, **5**: 12,13 = 2.5; 12′,13 = 7.4; **4**, **6**: 12, 13 = 3.3; 12′, 13 = 8.8; **3**, **4**, **5**, **6**: 13,14 = 6.6; **5**: 2,2′ = 14.7; 2,3 = 10.5; 2,3′ = 2.8; 2′, 3 = 2.1; 2′,3′ = 7.7; 3,4 = 11.2; 4,3′ = 2.1; 4,5 = 6, 7 = 9.1; 5,6 = 4.2; 7,8 = 10.3; 7,8′ = 1.5; **6**: 4,5 = 3.1; 5,6 = 6.1; 6,7 = 2.4; 7,8 = 7.3; 9,10′ = 4.9.

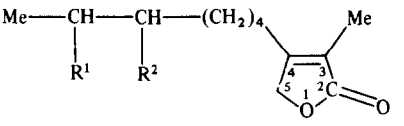
*270 and 500 MHz.

†270 MHz.

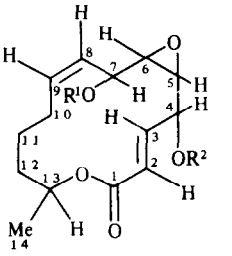
‡Signals (*s*) of the two acetyl groups appeared at δ2.13 and 2.07, respectively.

trans-oriented double bond ($J_{2,3}$ = 15.4 Hz) [8, 9]. The other double bond, located between C-8 and C-9, gave rise to two doublets of double doublets at δ5.37 and 5.54 (H-8 and H-9, respectively) and was *cis*-oriented ($J_{8,9}$ = 11.0 Hz) [8, 9]. The unexpected complexity of H-2 and H-8 was due to their allylic coupling [8, 9] with H-4 ($J_{2,4}$ = 1.5 Hz) and H-10′ ($J_{8,10'}$ = 2.6 Hz), respectively. Irradiation of H-3, the A part of an AMX system, simplified the signal of H-2 (the M part) into a doublet ($J_{2,4}$ = 1.5 Hz) and converted the doublet of double doublets at δ4.32, the X part attributed to H-4, into a double doublet ($J_{2,4}$ = 1.5 Hz and $J_{4,5}$ = 6.3 Hz). As expected, irradiation of H-2 transformed the H-3 signal into a doublet ($J_{3,4}$ = 6.1 Hz) and the H-4 signal into a double doublet ($J_{3,4}$ = 6.1 Hz and $J_{4,5}$ = 6.3 Hz). Similarly, on irradiation of H-9 (the A part of an ABXMM′ system), the doublet of double doublets of H-8 was converted into a double doublet ($J_{7,8}$ = 8.5 Hz and $J_{8,10'}$ = 2.6 Hz) and the two complex systems due to H-10 and H-10′ centred at δ2.43 and 2.07, respectively, were simplified. Irradiation of the doublet of double doublets of H-8 (the B part of the above system) changed the double doublet at δ4.23 (the X part assigned to H-7) into a doublet ($J_{6,7}$ = 8.5 Hz) and converted the H-9 into a double doublet ($J_{9,10}$ = 9.6 Hz and $J_{9,10'}$ = 3.3 Hz); in the same experiment the H-10′ system appeared simplified. As expected for secondary alcoholic protons, the signals of H-7 and H-4 showed a downfield shift (Δδ 1.20 and 1.04, respectively) in the ^{1}H NMR of diacetylseiricuprolide (**4**) (Table 1). Moreover, the ^{1}H NMR spectrum of **3** showed the signals of an AMXY system; the AM part appeared as two double doublets centred at δ3.23 and 3.01 which were attributed to H-5 (the A part) and H-6 (the M part), respectively. The signal of H-5 collapsed into a doublet ($J_{5,6}$ = 4.4 Hz) on irradiation of H-4 (the X part); at the same time the signals of H-2 and H-3 were converted into two doublets ($J_{2,3}$ = 15.4 Hz). On irradiation of H-7 (the Y part) the H-6 signal became a doublet ($J_{5,6}$ = 4.4 Hz); and the H-8 signal changed to a double doublet ($J_{8,9}$ = 11.0 Hz and $J_{8,10'}$ = 2.6 Hz). These results and the typical chemical shift values suggested the presence of a symmetrically disubstituted epoxide ring with oxygen bridging C-5 and C-6. The coupling constant between H-5 and H-6 (4.4 Hz) is fully consistent with a *cis*-substitution for the epoxide ring [10]. The H-13 attached to the fifth oxygen-carrying carbon is the X part of an A_3XMM' system as indicated by a quartet of double doublets at δ 4.91. This proton was coupled to the secondary methyl group Me-14 (the A_3 part) resonating as a doublet ($J_{13,14}$ = 6.6 Hz) at δ1.26. Irradiation of this latter group simplified the complex system of H-13 into a double doublet ($J_{12,13}$ = 2.5 Hz and $J_{12',13}$ = 7.4 Hz) by residual coupling with H_2C-12 (the MM′ part). Irradiation of H-13 collapsed the doublet of Me-14 into a singlet and simplified the complex systems of the H_2C-12 centred at δ1.86 and 1.44. The other two methylene groups yielded complex signals at δ 2.43 and 2.07, attributed to H_2C-10, and at δ1.78 and 1.23, attributed to H_2C-11.

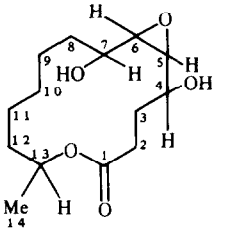
The finding that the chemical shift value of H-13 was unchanged after acetylation of **3**, together with the absence of hydroxy group absorptions in the IR spectrum of diacetylseiricuprolide (**4**), suggested that the oxygen on C-13 was involved in the closure of a large lactone ring. This situation was corroborated by the chemical shift (δ166.0) of O=C-1 in the ^{13}C NMR spectrum of **3** (Table 2) and its downfield shift (δ 176.3; Δδ7.6) in the ^{13}C NMR spectrum of tetrahydroseiricuprolide (**5**); in fact these chemical shift values are typical for a conjugated and a saturated carbonyl ester group, respectively [11, 12]. Seiricuprolide (**3**) was quantitatively hydrolysed in *ca* two hr by treat-



1 R^1 = OH, R^2 = H
2 R^1 = H, R^2 = OH



3 R^1 = R^2 = H
4 R^1 = R^2 = Ac



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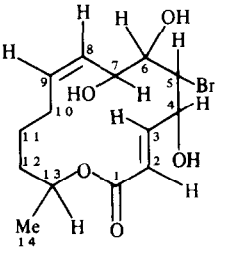

6

ment with ethanolic sodium hydroxide. All attempts to isolate the reaction products from the acidified solution failed. Great difficulties also arose for the purification of the lithium aluminium hydride reduction products of **3**.

On this ground, and taking into account both the chemical shift and the coupling constants of each proton and the evidence resulted from the extensive decoupling experiments, structure **3** for seiricuprolide was proposed.

The ^{13}C NMR spectrum of **3** (Table 2) corroborated this structure. In addition to the signal at δ 166.0, assigned to O=C-1, those of two olefinic groups were observed. The double bond conjugated to the lactone group gave signals at δ123.8 and 142.9 (C-2 and C-3, respectively), while the carbons of the other olefinic group resonated at δ127.4 and 135.5 (C-8 and C-9, respectively). In the region of the oxygenated carbons were present signals at δ71.9, 73.1 and 64.4 for C-4, C-13 and C-7, respectively; these latter signals appeared as doublets in the SFORD (single frequency off-resonance decoupled) and APT (attached proton test) spectra. The carbons of the epoxide ring appeared at δ 62.6 and 58.9 (C-5 and C-6, respectively), while the signal of Me-14 was recorded at δ19.8. The assignments of the secondary carbons were confirmed by the collapse into a singlet of the signal of every carbon observed in a series of SFSD (single frequency selective decoupled) spectra (Table 2), performed by irradiation of the corresponding bonded proton. Moreover, the signals of the three methylene groups were at δ33.5. 28.8 and 25.1 for C-12, C-10 and C-11, respectively.

Analysis of the high resolution EI mass spectrum provided further information consistent with the structure assigned to **3**. Besides the molecular ion at *m/z* 268.1425 ($C_{14}H_{20}O_5$), significant peaks derived from intermolecular hydrogen migration were observed at *m/z* 269.1375 ($C_{14}H_{21}O_5$) and 267.1262 ($C_{14}H_{19}O_5$). Peaks at *m/z* 251.1412 ($C_{14}H_{19}O_4$) and 233.1352 ($C_{14}H_{17}O_3$) clearly arose by successive losses of a hydroxy group and water from the molecular ion. Loss of CO and H_2CO from the ion at *m/z* 233.1352 gave the ions at *m/z* 205.1396 ($C_{13}H_{17}O_2$) and 203.1047 ($C_{13}H_{15}O_2$), respectively. The first of them eliminated water yielding the fragment at *m/z* 187.1153 ($C_{13}H_{15}O$). An ion at *m/z* 167.1128 ($C_{10}H_{15}O_2$) probably originated from the molecular ion (*m/z* 268.1425) by a McLafferty rearrangement which resulted in the elimination of the OOC–HC=CH–CH_2–OH moiety. Finally, this ion yielded the ions at *m/z* 149.0892 ($C_{10}H_{13}O$) and 131.0919 ($C_{10}H_{11}$) by two successive losses of water.

Table 2. ^{13}C NMR data of seiricuprolide (**3**) and tetrahydroseiricuprolide (**5**)

C	3*	5†
1	166.0 *s*	173.6 *s*
2	123.8 *d*‡	25.7 *t*§
3	142.9 *d*‡	30.3 *t*§
4	71.9 *d*‡	68.6 *d*
5	62.6 *d*‡	62.7 *d*
6	58.9 *d*‡	61.5 *d*
7	64.4 *d*‡	68.0 *d*
8	127.4 *d*	30.3 *t*§
9	135.5 *d*‡	29.1 *t*§
10	28.8 *t*§	21.4 *t*§
11	25.1 *t*§	22.9 *t*§
12	3.5 *t*§	35.9 *t*§
13	73.1 *d*‡	71.5 *d*
14	19.8 *q*	20.7 *q*

*Multiplicities determined by SFORD and APT spectra.

†Multiplicities obtained by APT spectra.

‡Assignments made also by SFSD spectra.

§Assignment in agreement with available literature data [9, 12].

An overall evaluation of the spectroscopic findings led to assignment of a large α,β-unsaturated lactone (**3**) to seiricuprolide. Support for the structure assigned to seiricuprolide was obtained by preparation, starting from **3**, of some appropriate derivatives.

Treatment of **3** with acetic anhydride in pyridine afforded the expected diacetyl derivative **4** ($[MH]^+ = m/z$ 353 by CIMS). Its ^{1}H NMR spectrum (Table 1) was very similar to that of **3**, except for the presence of two singlets at δ 2.13 and 2.07, due to the acetyl groups, and the previously mentioned downfield shift of H-4 and H-7.

Catalytic hydrogenation of **3** with platinum on charcoal afforded, with high yield, the tetrahydroseiricuprolide **5** ($[MH]^+ = m/z$ 273 by CIMS). The ^{1}H NMR spectrum of **5** (Table 1) showed, when compared to that of **3**, the disappearance of all olefinic signals and greater complexity in the signals for aliphatic protons. In particular, two doublets of double doublets (an AB system) were noticed at δ 2.67 and 2.37. These values are typical for methylene protons alpha to a O=C group and were assigned to H_2C-2. Another multiplet, attributed to H_2C-3, was observed at δ1.82. Moreover, an upfield shift ($\Delta\delta$ 0.68 and 0.78, respectively) was noted for the signals of H-4 and H-7, both appearing as doublet of double doublets, and a more complex system was observed for H_2C-10. The ^{13}C NMR spectrum of **5** (Table 2), compared to that of **3**, showed the above reported downfield shift of O=C-1 ($\Delta\delta$ 7.6), characteristic for the carbonyl group of a saturated lactone [11, 12]. Moreover, the absence of signals of olefinic carbons and the appearance of those of four new CH_2 groups were observed. These methylene groups appeared at δ30.3, 30.3, 29.1 and 25.7 and were attributed to C-3, C-8, C-9 and C-2, respectively.

The occurrence of an epoxide group ring in **3** was confirmed by the conversion of seiricuprolide to *trans*-bromohydrin **6**. This derivative was prepared by treating **3** with dilithium tetrabromonickelate(II) complex (Li_2NiBr_4) in dry THF, a procedure recently reported for the regioselective opening of epoxide rings [13]. The location of the bromine on C-5 was deduced from the chemical shift of H-5 and H-6 and the evidence obtained by a series of spin proton decoupling experiments carried out on **6**. An inspection of Dreiding models, as well as the mechanism of the reaction [13], suggests a *trans*-configuration between the hydroxy groups on C-4 and C-7 and a *cis*-configuration between that on C-4 and the epoxide ring in 3. In this situation C-5 is sterically less hindered than C-6 and consequently must correspond to the point of attachment of the bromine ion. The EI mass spectrum of the bromohydrin **6** was typical of a monobromide [14], showing a doublet in the ratio 1:1 approximately at m/z 351 ($[MH+2]^+$) and 349 ($[MH]^+$). Comparison of its ^{1}H NMR spectrum with that of **3**, showed a downfield shift of H-5 and H-6 ($\Delta\delta$ 1.32 and 0.61, respectively). Moreover, the variation of dihedral angles, due to the opening of the epoxide ring, changed the coupling constants between H-5 and both H-4 and H-6, as well as between H-6 and H-7.

In conclusion, the macrolide structure of seiricuprolide appears to be satisfactorily demonstrated. The elucidation of the stereostructure will be performed by an X-ray crystallographic analysis.

Macrolides are quite common as naturally occurring substances and some are biologically active [15–17]. Fungi too, are known to produce macrolides [18].

Seiricuprolide seems to be a minor toxin of *S. cupressi* both in terms of the small amounts produced *in vitro* and its lower toxicity towards plants. Nevertheless, as a component of the mixture of fungal metabolites, it may contribute to the overall toxic activity of the pathogen.

EXPERIMENTAL

Analytical methods. Optical rotations: $CHCl_3$; mps: uncorr.; IR and UV: $CHCl_3$ and MeCN respectively; ^{1}H NMR: 270 and/or 500 MHz in $CDCl_3$, using TMS as internal standard; ^{13}C NMR: 67.88 and/or 50.3 MHz in $CDCl_3$, using TMS as internal standard; EIMS and high-resolution EIMS: 70 eV; CIMS: 200–300 eV, using *iso*-butane as reagent gas; analytical and prep. TLC: silica gel (Merck, Kieselgel 60, F_{254}) 0.25 and 2.0 mm, respectively or on reverse phase plates (Stratocrom C-18, Whatman) 0.2 mm; the spots were visualized by exposure to UV radiation and by spraying first with 10% H_2SO_4 in MeOH and then with 5% phosphomolybdic acid in MeOH followed by heating at 110° for 10 min. CC: silica gel (Merck, Kieselgel 60, 0.63–0.20 mm). Solvent systems: (A) $CHCl_3$–*iso*-PrOH (9:1); (B) petrol–Me_2CO (7:3); (C) petrol–Me_2CO (4:1); (D) H_2O–MeCN (1:1). The petrol used had bp 40–70°.

Phytotoxin production. The single-spore isolate of *S. cupressi* used in this study was a subculture of a colony isolated by Dr S. Xenopoulos from an infected cypress tree (*C. sempervirens*) on the island of Kos (Greece). It was grown in tubes of potato–sucrose–agar at 25°. Czapek's medium with the addition of 2% corn meal (pH 5.7) (the addition of an homogenate obtained from young twigs of *C. arizonica* gave poor results) was dispensed in 1 l Roux flasks (150 ml for flask). Each flask was seeded with 3 ml of a suspension of the homogenate of three 10-day-old culture tubes in 50 ml sterile H_2O. The flasks were incubated at 23° for 30 days in the dark, without shaking.

Phytotoxin bioassay. The toxic activity of organic extracts of culture filtrates and pure substances was tested on severed twigs of cypresses (*C. sempervirens* var. *pyramidalis*; *C. arizonica* Gr. and *C. macrocarpa* Hartw.), and on cuttings of young tomato (*Lycopersicum esculentum* L. cv. Marmande) and mung bean (*Phaseoulus aureus* L.) plants. Samples of culture filtrates were assayed after 1:1000 dilution; crude extracts were tested at concentrations of 2, 1 and 0.5 mg/ml. Fractions collected from chromatographic columns and pure substances were tested at concentrations of 1, 0.5, 0.4 and 0.3 mg/ml. The test plants were placed in the assay solution at 23° (non-host plants for 48 hr; host plants for 72 hr) and then transferred to dist. H_2O. Symptoms developed within 4 days on tomato and bean cuttings, and within 10 days on excised cypress twigs. The control, which had absorbed either an equivalent dilution of the culture medium or distilled water, showed no symptoms.

Extraction and purification of phytotoxins. After removal of the mycelial mat by filtration of 11 l of liquid culture through Whatman no. 54 paper, the filtrate (8.5 l) was frozen at −20° for 24 hr and then defrosted at room temp. The liquid was filtered through Whatman no 1 paper, adjusted to pH 4 with 0.1 M HCl, and extracted (×4) with *t*-BuOMe (0.5 vol.) The combined organic extracts, dried over Na_2SO_4, were concd under red. pres. to give a solid suspension in an abundant oily residue (5.38 g). Repeated washes of the mixture with $CHCl_3$, in which the solid was poorly soluble, removed the oil, leaving a white amorphous compound (254 mg). The $CHCl_3$ washes were combined and concd under red. pres.; the resulting brown oil (5.0 g) was chromatographed on a silica gel column, eluted with solvent system A to give 12 groups of fractions. The pooled fractions between the groups 2–11 showed phytotoxic activity. The third

group contained, as shown by TLC analysis (silica gel, solvents A and B), seiridin (**1**) and *iso*-seiridin (**2**) as its main components. Further fractionation of this mixture (779 mg) by CC, using solvent B, afforded both toxin **1** (287 mg, 33.7 mg/l) and **2** (184 mg, 21.7 mg/l) as pure oils. The fifth group of toxic fractions contained essentially the toxin having R_f values of 0.45 and 0.21 on TLC (silica gel) with solvent systems A and B, respectively. The oily residue, left by evaporation of the solvent from this latter group of fractions, was fractionated by CC eluted with solvent B to yield **3** as crystalline compound (14 mg, 1.7 mg/l). Seiricuprolide (**3**) was recrystallized from EtOAc–petrol: mp 128–130°; elemental analysis found: C, 62.44; H, 7.76%. $C_{14}H_{20}O_5$, requires: C, 62.68; H, 7.46; $[\alpha]_D^{25}+67.2°$ (*c* 1.45); UV λ_{max} nm: < 220; IR ν_{max} cm^{-1}: 3680, 3590, 3400 (OH), 1720 (C=O), 1660 (C=C), 1250; ^1H and ^{13}C NMR: Tables 1 and 2; EIMS, *m/z* (rel. int.): 268.1425 ($[M]^+$, calcd. 268.1311) (80.4), 251 (20), 233 (17), 215 (10), 205 (20), 203 (6.6), 197 (2.6), 187 (22), 179 (9.2), 167 (42), 161 (19.6), 149 (48), 131 (24), and 43 (100).

4,7-*Diacetylseiricuprolide* (**4**). Acetylation of **3** (6.7 mg) with Ac_2O/pyridine afforded the expected 4,7-diacetyl derivative. The usual work-up of the mixture reaction, followed by prep. TLC (silica gel, eluent C) gave **4** as a pure oil (8.0 mg): $[\alpha]_D^{25}-3.6°$ (*c* 0.11); UV λ_{max} nm < 220; IR ν_{max} cm^{-1}: 1750, 1740, 1720, (C=O), 1600 (C=C), 1260; ^1H NMR: Table 1; CIMS *m/z* (rel. int.): 353 $[MH]^+$ (43), 310 (15), 293 (100), 251 (55), 233 (67), 215 (50) and 205 (42).

2,3,8,9-*Tetrahydroseiricuprolide* (**5**). Seiricuprolide (5.5 mg) dissolved in dist. THF (1.5 ml) was added to a suspension of presaturated 5% Pt–C (5 mg) in 1.5 ml of the same solvent. The hydrogenation was performed at room temp. and at atmospheric pressure, with stirring. After 48 hr the reaction was stopped by filtration, and the clear soln evapd under vacuum. The residue (5.0 mg) was purified by prep. TLC (silica gel, solvent system B) to give **5** as a pure oil (4.3 mg): $[\alpha]_D^{25}+5.0$ (*c* 0.20); UV λ_{max} nm < 220; IR ν_{max} cm^{-1}: 3690, 3600, 3460 (OH), 1720 (C=O), 1600 (C=C), 1270; ^1H and ^{13}C NMR: Tables 1 and 2; CIMS *m/z* (rel. int.) 273 $[MH]^+$ (53), 255 (79), 237 (50), 219 (32), 205 (16), 153 (68) and 127 (100).

Conversion of seiricuprolide into 5,6-*bromohydrin derivative* (**6**). Seiricuprolide (6.2 mg) dissolved in dry THF (2 ml) was treated with a soln of 0.4 M Li_2NiBr_4 in dry THF (2.4 ml) prepared according to ref. [13]. The reaction was performed at room temp. with stirring and in the dark. After 3 hr, **3** was completely transformed and the reaction was stopped by addition of K-Pi buffer, pH 7.5 (20 ml). The aq soln was extracted with CH_2Cl_2. The organic extracts were combined, washed with H_2O, dried (Na_2SO_4) and evapd under red. pres. to give a crude residue (8.0 mg). Purification by prep. TLC (reverse phase, solvent D) afforded **6** as a pure oil (6.8 mg). This showed: $[\alpha]_D^{25}-16.0$ (*c* 0.15); UV λ_{max} nm < 220; IR ν_{max} cm^{-1}: 3690, 3570, 3400 (OH), 1720 (C=O), 1600 (C=C), 1260; ^1H NMR data are listed in Table 1; EIMS *m/z* (rel. int.): 351 $[MH+2]^+$ (4.6), 349 $[MH]^+$ (4.8), 333 (27), 331 (25), 315 (9), 313 (7), 297 (2.3), 295 (2.5), 251 (29) and 125 (100).

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