$C_{16}H_{27}NO$ (HRMS on the parent ion at M^+ 249.2090, $C_{16}H_{27}NO$ requires 249.2094). ^{1}H-NMR spectrum contained signals at δ 8.14 (1 H, d, J = 13.0 Hz) and 3.63 (1 H, broad) indicative of a HCONH- group, and at δ 5.36 (1 H, bs) attributable to the vinylic proton of a trisubstituted double bond. Resonances due to three secondary [δ 0.72, 0.92 and 0.98 (3 H each, d's, J = 7.0 Hz)] and one vinylic methyl [δ 1.69 (3 H, bs)] are also present. These data coupled with the presence in the IR spectrum of a band at ν_{max} 3440 cm^{-1}, suggested the compound could be the formamide corresponding to **4** and **5**. This was established by way of its synthesis, starting from isonitrile **4**. The ^{1}H-NMR spectrum shows that compound **6**, which thus differs from the other formylamino sesquiterpenes which are generally mixtures of the two rotational isomers of the formamide group, exists almost exclusively as the *trans* isomer, as indicated by the J value (13 Hz) of the signal at δ 8.14.

The structural similarity (including relative stereochemistry at C-7 and C-10) between the series **1–3** and **4–6** and their co-occurrence in the same sponge point to the biogenetic pathway reported in the scheme. The carbonium ion A, through a hydride shift, could generate the key intermediate B. The genesis of compounds **4–6** requires only introduction of the nitrogenous functions on carbonium ion B, whereas its rearrangement, through the cyclopropane-containing ion C, could account for the production of the spiro-axane ion D which is positively charged, just at the carbon atom carrying the functionalities in **1–3**.

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New phytotoxic butenolides produced by *Seiridium cardinale*, the pathogen of cypress canker disease[1]

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Summary. Two new butenolides, seiridin and *iso*-seiridin, were isolated from culture filtrates of *Seiridium cardinale*, the pathogen of cypress canker, a destructive disease of *Cupressus* and related *Coniferae*. These metabolites were characterized as 3-methyl-4-(2-hydroxyheptyl)-2(5H)-furanone and its 4-(3-hydroxyheptyl) isomer, respectively. Chlorotic and necrotic symptoms were produced on leaves of either host or non-host test plants by absorption of 0.3 mg/ml solutions of either compound. These also showed antibacterial activity.

Key words. Seiridium cardinale; cypress canker; seiridin; *iso*-seiridin; butenolides; phytotoxins; antibiotics.

Since its first introduction in Europe[2], the canker caused by the imperfect fungus *Seiridium cardinale* (Wag.) Sutt. et Gibs. *(Coelomycetes)* has become the major disease of the Mediterranean cypress (*Cupressus sempervirens* L.) and other species of *Cupressaceae*. This cypress canker, which had been previously reported in the U.S.A.[3] and later in other parts of the world, is a destructive disease that kills the affected trees. Over the last two decades, heavy losses have been caused in Italy and in other Mediterranean countries to the nursery industry, the cypress plantations used for afforestation and wind-breaks, and to ornamental cypresses[4,5].

The foliage of infected twigs and branches first shows a diffuse yellowing and later turns brown or reddish as the die-back progresses. Cankers develop on the live bark of branches and stem around the sites of infection of the pathogen. A cardinal-red discoloration and a necrotic browning of the infected bark tissues occur, and a flow of resin exudes from cracks formed on these cankers. Extensive infection leads to the dying of branches and eventually of the whole tree.

The nature and appearance of the damage caused by *S. cardinale* to its host suggest that necrotic toxins are produced in the infected tissues and are possibly involved in pathogenesis.

Early work[6,7,8] provided preliminary information on the toxicity of culture filtrates of *S. cardinale* on test plants and on the molecular weight of the toxins involved. In a previous paper[9], the in vitro production of phytotoxins by the pathogen under various cultural conditions, and the first attempts to separate the active substances from culture filtrates, were reported.

This paper briefly reports on the production, isolation, structure determination and biological activity of two new phytotoxins from culture filtrates of *S. cardinale*. Details of this study and further information will be presented elsewhere.

The single-spore strain of *S. cardinale* used in this study was isolated from an infected cypress tree *(C. sempervirens)* in Italy and was grown in tubes of Czapek-corn meal agar. Czapek's medium with the addition of 2% corn meal was dispensed in 1 l Roux flasks (150 ml/flask) and used for stationary cultures. Each flask was seeded with 2 ml of a suspension of the homogenate of two 15-day-old culture tubes in 50 ml sterile water. The flasks were incubated at 23 °C for 30 days in the dark.

After removal of the mycelial mat, the culture filtrate was adjusted to pH 4 with 0.1 N HCl and subsequently extracted 4 times with one fourth its volume of *t*-butyl-methyl-ether. The pooled organic extract was evaporated under reduced pressure to give an oily residue, which was fractionated on a silica gel column using chloroform – *iso*-propanol (9:1, v:v) as an eluent system. The fractions were tested for toxicity (see later) and examined by thin layer chromatography (TLC) using various solvent systems. The TLC plates developed with petroleum ether (b.p. 40–70 °C) – acetone (6:4, v:v) showed that the phytotoxic activity was associated with two substances having R_f values of 0.51 and 0.56. The fractions containing these substances were combined and evaporated under reduced pressure. Purification of the residue on a silica gel column, followed by TLC of the phytotoxic fractions (both the column and plates were run with the petroleum ether – acetone mixture mentioned above), afforded two pure oily substances which were named seiridin (**1**) (49.5 mg/l) and *iso*-seiridin (**2**) (17.4 mg/ml).

Seiridin had a molecular formula $C_{12}H_{20}O_3$ as shown by high resolution mass spectral data, m/z 212.1413 (calc. 212.1413), and an optical rotation $[\alpha]_D^{25} = -4.80°$ (c = 1.48 $CHCl_3$). IR and UV absorption frequencies were typical for a $\Delta^{\alpha,\beta}$ butenolide [2(5H) furanone])[10].

These findings were confirmed and further extended by NMR

studies. The ^1H NMR spectrum of (1) showed a quartet at δ 4.62 (J = 1.8 Hz) indicating the presence of a $OC(5)H_2$ group, a triplet at δ 1.79 (J = 1.8 Hz) assigned to $CH_3C(3)$ = group, and signals in agreement with the protons of a 2-hydroxyheptyl side chain. The ^{13}C NMR spectrum of (1) exhibited 12 carbon atoms possessing complexity and chemical shifts in agreement with the proposed structure. The compound (1) was reduced to the corresponding dihydroderivative by catalytic hydrogenation on Pd/$BaSO_4$ and to a trihydroxy olefin by $LiAlH_4$. These results were in agreement with the presence of an α,β-unsaturated γ-lactone. The formation of a monoacetyl derivative accounts for the presence of a hydroxy group.

Iso-seiridin also had the molecular formula $C_{12}H_{20}O_3$ from high resolution mass spectral data, m/z 212.1399 (calc. 212.1413) and an optical rotation $[\alpha]_D^{25} = -6.28°$ (c = 3.04 $CHCl_3$). Its ^1H and ^{13}C NMR spectra were similar to those of (1). In addition, the inspection of the corresponding three derivatives prepared from (1) showed that (2) is a structural isomer of (1) carrying a 3-hydroxyheptyl side chain. The feature which differentiates (1) from (2) was further supported by ^1H NMR decoupling experiments in the presence of the shift reagent $Eu(fod)_3$. Furthermore, the occurrence of a peak at m/z 197.1185 (M^+-CH_3, $C_{11}H_{17}O_3$) in the high resolution mass spectrum of (1) and at m/z 183.1028 (M^+-CH_2CH_3, $C_{10}H_{15}O_3$) in that of (2) is consistent with the position of the hydroxy group in the side chain of (1) and (2).

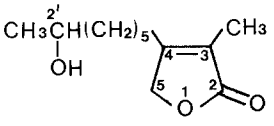
The phytotoxicity of culture filtrates, extracts and pure substances was tested on severed twigs of cypresses (*C. sempervirens, C. arizonica* Gr. and *C. macrocarpa* Hartw.), as well as on cuttings of young tomato (*Lycopersicum esculentum* L.) and basil (*Ocymum basilicum* L.) plants[9]. Samples of culture filtrate were assayed after 1:1000 dilution; crude extracts and pure substances were tested at a concentration of 3.0 and 0.3 mg/ml, respectively. The test plants were placed for 24 h in the assay solution and then transferred into water. Extensive leaf chlorosis and subsequent necrosis occurred within 4 days on tomato and basil cuttings, and within 14 days on excised cypress twigs; the controls, which had absorbed either an equivalent dilution of the culture medium or distilled water, showed no symptoms.

Seiridin appeared to be 2–3 times more toxic than *iso*-seiridin. The highest symptom intensity was reached when a mixture of the two toxins (0.3 mg/ml each) was supplied to test plants.

In preliminary experiments with species of *Pseudomonas* Mig. and *Bacillus* Cohn, both substances showed significant antibacterial activity.

Among but-3-enolides, which are relatively common as natural products, several are produced by fungi[11], but 3,4-disubstituted derivatives are rare. To our knowledge, the 3-butyl-4-methyl-furan-2(5H)-one[12] produced by *Hypoxylon serpens* (Pers. ex Fr.) Kicks is the fungal metabolite closest to seiridin.

The results of this study strongly support the view that fungal toxins are involved in the syndrome of cypress canker disease.



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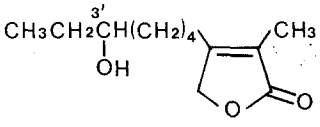

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Announcements

Courses

In the series 'Current Advances in Laboratory Techniques' the Royal Postgraduate Medical School of the University of London is organizing courses on the following topics:
Endocrine Pathology, 24–28 November 1986;
Monoclonal Antibodies, 1–5 December 1986;
Immunocytochemistry in Cytopathology: Methods and Applications, 8–12 December 1986;
Immunolabeling for Electron Microscopy, 26 January–6 February 1987;
Modern Immunocytochemistry, 16–27 February 1987;
Techniques in Human Molecular Genetics, 30 March–3 April 1987;
Hybridization Histochemistry, 6–10 April 1987.
Details and application forms are available from:
Professor Julia M. Polak, Histochemistry Unit, or the appropriate Course Organizers at the Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0HS, U.K.

Italy

International congress on 'The biological and clinical aspects on phygocyte function'

Pavia, September 7–10, 1986

The scientific program covers the following topics:
- Leukocyte random locomotion, chemotaxis, chemokinesis;
- Membrane metabolism;
- Pharmacological manipulation and immunomodulation of phagocyte function;
- Genetic control of phagocyte function;
- The role of phagocyte in inflammation;
- Leukotrienes, prostaglandins, chemotaxis and phagocyte function;
- Membrane chemotactic and phagocyte function;
- Clinical aspects of phagocyte disorders.

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