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Isolation and Structure of Lettucenin A, a Novel Guaianolide Phytoalexin from *Lactuca sativa* var. *capitata* (Compositae)

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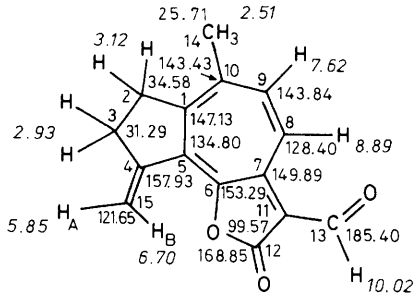
Inoculation of lettuce (*Lactuca sativa* var. *capitata*) leaves with the pathogenic bacterium *Pseudomonas cichorii* induced the production of two antifungal sesquiterpenes, costunolide and a new compound named lettucenin A, whose structure has been elucidated on the basis of spectroscopic studies.

The important role of phytoalexins in plant disease resistance has been suggested.[1] Although the Compositae is one of the largest families of flowering plants, only two phytoalexins have been isolated from a composite plant.[2,3] Recently, one of us described the production of phytoalexin-like substance(s) in lettuce (*Lactuca sativa* var. *capitata*, Compositae) leaves inoculated with the bacterium *Pseudomonas cichorii*.[4] We now report the isolation of two phytoalexins from the lettuce leaves.

Bioassay-directed fractionation† of the acetone extracts from the inoculated leaves gave a new compound, named lettucenin A (**1**), and costunolide (**2**)[5]‡ in 0.00084 and 0.012% yields (from the dried leaves), respectively; the latter was identified as a phytoalexin for the first time.§

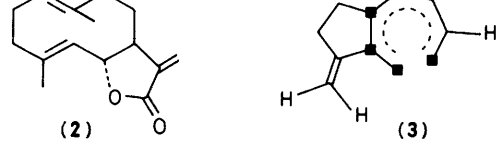
Lettucenin A (**1**), $C_{15}H_{12}O_3$, unstable yellow crystals,¶ m.p. 175—176 °C, showed the following spectral data: *m/z* 240.0792 (M^+) and 212.0814 (base peak, $M^+ - CO$); $\lambda_{max.}$ (MeOH) 233 (ε 25 600), 255 (15 600), 329 (25 000), and 446 nm (32 000); i.r. ($CHCl_3$) $\nu_{max.}$ 1755 (α,β-unsaturated γ-lactone) and 1650 cm^{-1} (conjugated formyl).

The ^{13}C n.m.r. data of (**1**) (Figure 1), aided by off-resonance decoupling, indicated that (**1**) has the following 15 carbon atoms: CH_3–, –CHO, CH_2=, –C(=O)–O–, two –CH_2–, two –CH=, and seven > The ^1H n.m.r. data (Figure 1), together with decoupling studies, revealed the presence of the following moieties: –CH=CH– [δ 7.62 and 8.89 (each d, *J* 11.2 Hz, 9-H and 8-H] and CH_2=C(–C■)CH_2CH_2C■ [δ 5.85 (dd, *J* 2.4 and 2.4 Hz, 15-H_A), 6.70 (dd, *J* 2.4 and 2.4 Hz, 15-H_B),



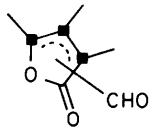
(**1**)

Figure 1. N.m.r. spectral parameters for (**1**) (^1H in italics; ^{13}C assigned by LSPD experiments). δ Values for solutions in $CDCl_3$.



(**2**)

(**3**)



(**4**)

† Each fraction was tested for antifungal activity against *Bipolaris leersiae*.

‡ Data for (**2**): *m/z* 232.1456 (M^+, $C_{15}H_{20}O_2$); R_f (diethyl ether, silica gel) 0.46; m.p. 107—108 °C (lit.[5] 106 °C); $[\alpha]_D^{26}$ +121° (*c* 1.0, $CHCl_3$; lit.[5] +128°); superimposable i.r. ($CHCl_3$)[5] spectra.

§ The compounds (**1**) and (**2**) completely inhibited spore germination of *Ceratocystis fimbriata* at concentrations of 2 and 32 μg/ml, respectively. Antimicrobial spectra of these compounds will be reported in the near future.

¶ The compound (**1**) showed a greenish yellow fluorescent spot of R_f (diethyl ether, silica gel) 0.16 under u.v. light (365 nm).

2.93 (dddd, *J* 8, 8, 2.4, and 2.4 Hz, 2 × 3-H), and 3.12 (dd, *J* 8 and 8 Hz, 2 × 2-H)]. The latter moiety must form a five-membered ring with an exocyclic methylene group by comparing respective proton signals of (**1**) with those of 1-methyleneindan.[6] Measurement of nuclear Overhauser enhancement difference spectra of (**1**) indicated the presence of partial structures (**3**) and (**4**).

Irradiation at δ 2.51 (10-CH_3) caused clear enhancements of signals due to 2-CH_2 and 9-H, while irradiation at δ 3.12 (2-CH_2) enhanced signals due to both 10-CH_3 and 3-CH_2. Irradiation at δ 10.02 (11-CHO), however, did not affect any other proton signals. Long range selective proton decoupling (LSPD) experiments resulted in combination of (**3**) and (**4**) to the whole structure (**1**). Irradiation at δ 10.02 (11-CHO) and 8.89 (8-H) caused the unusually high field sp^2 carbon signal at δ 99.57 (dd, *J* 26 and 3 Hz, C-11) to simplify to a doublet with *J* 3 Hz and a doublet with *J* 26 Hz, respectively. The structure (**1**) was further supported by similar chemical shifts** in 3-methoxycarbonyl-2*H*-cyclohepta[*b*]furan-2-one[7] and by co-occurrence of costunolide (**2**) with (**1**).

Lettucenin A is the first guaianolide phytoalexin containing a unique 2*H*-cyclohepta[*b*]furan-2-one ring system.

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** The compound showed high carbon [δ 96.05, C-3, corresponding to C-11 in (**1**)] and low proton [δ 8.80, d, *J* 11.2 Hz, 4-H, corresponding to 8-H in (**1**)] chemical shifts in $CDCl_3$.

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