Short Communication

Structure of Cladospolide A,
a Novel Macrolide from
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We wish to report the structural elucidation of Cladospolide A (**I**), a novel fungal metabolite, which was isolated from culture filtrate of *Cladosporium fulvum* FI-113 and showed the inhibitory activity to lettuce seedlings. The fungus was cultured at 30°C for 4 days in Czapek-Dox medium fortified with yeast extract. The culture filtrate was extracted with ethyl acetate. The neutral fraction was applied onto a silicic acid column and eluted with the benzene-ethyl acetate system. The eluate of 80% benzene was rechromatographed by use of silicic acid and benzene-ethyl acetate (9:1, v/v). Further, the fractions containing **I** were applied onto Sephadex LH-20 column (methanol) and crude **I** was obtained. Recrystallization from benzene afforded pure Cladospolide A as colorless needles. Cladospolide A, $C_{12}H_{20}O_4$, MS m/z 229.1436 ($(M+H)^+$)¹⁾ (Calcd. for $C_{12}H_{21}O_4$, 229.1438), mp 92~93°C, decolorized potassium permanganate. IR $\nu_{\max}^{KBr}$ cm^{-1} : 3470, 3340, 2925, 2850, 1706, 1640, 1460, 1270, 1160. UV $\nu_{\max}^{MeOH}$: 217 nm (ϵ 10, 650). In the 1H NMR spectrum (100 MHz, $CDCl_3$, TMS) of **I** a 1H double doublet ($J=16.5, 6.0$ Hz*) was observed at δ 6.81 ppm and also another 1H double doublet ($J=16.5, 1.5$ Hz) at δ 6.20 ppm. These

signals in 1H NMR spectrum and the absorption at 1706 cm^{-1} in IR spectrum indicated the presence of α,β -unsaturated ester and the stereochemistry of the double bond was *trans* configuration. The evidence that a 2H broad singlet at δ 2.6 ppm disappeared on treatment with D_2O suggested the presence of two hydroxyl groups. This was supported by fragment ions due to the loss of H_2O and $2H_2O$ in the mass spectrum (m/z 211 ($M+H-18$) and m/z 193 ($M+H-36$)), which was determined by high resolution mass spectrometry. Accordingly two of the four oxygens in the structure were in an ester group and other two in two hydroxyl groups. On irradiation to the olefinic proton at δ 6.81 ppm a double doublet ($J=3.5, 6.0$ Hz) at δ 4.55 ppm collapsed to a broad doublet ($J=3.5$ Hz) and also irradiation to a multiplet proton at δ 3.65 ppm ($J=10.0, 3.5, 1.5$ Hz) changed the double doublet at δ 4.55 ppm to the broad doublet ($J=6.0$ Hz). On the other hand irradiation to methyl protons at δ 1.28 ppm ($J=6.3$ Hz) changed the features of a ten-line one-proton system at δ 5.21 ppm ($J=6.3, 6.3, 3.3$ Hz) and conversely, irradiation at δ 5.21 ppm collapsed the doublet methyl protons to a singlet. From

the data above mentioned, the structure of Cladospolide A has been established as **I**, but the stereochemistry at C_4 , C_5 and C_{11} is unresolved. Furthermore ^{13}C NMR spectrum of **I** confirmed this structure. ^{13}C NMR (25 MHz) $\delta_{TMS}^{CDCl_3}$ ppm: 168.3 (s, $C=O$), 146.3, 122.0 (each d, olefinic carbon), 74.7, 73.2, 73.0 (each d, $-CH-O-$), 32.5, 30.0, 28.0, 25.1, 22.6 (each t, $-CH_2-$), 19.0 (q, $-CH_3$).

In the bioassay using lettuce seedlings, Cladospolide A inhibited the root elongation about 50% at a concentration of 100 ppm.

Cladospolide A is a 12-membered fungal macrolide which is probably a hexaketide-

* Coupling constants in this communication are all determined in 400 MHz 1H NMR spectrum.

derived compound. Among more than twenty fungal macrolides so far reported, only re-cifeiolide²⁾ (II) belongs to the same group as I does.

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