Short Communication

Structure of Cladospolide A, a Novel Macrolide from Cladosporium fulvum

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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]^+)(Calcld. for C_{12}H_{21}O_{4}, 229.1438), mp 92~93°C, decolorized potassium permanganate. IR\nu cm\(^{-1}\): 3470, 3340, 2925, 2850, 1706, 1640, 1460, 1270, 1160. UV\nu\text{MeOH}: 217 nm (ε 10, 650). In the \textsuperscript{1}H NMR spectrum (100 MHz, CDC\textsubscript{13}, TMS) of I a 1H double doublet (J = 16.5, 6.0 Hz) was observed at δ 6.81 ppm and another 1H double doublet (J = 16.5, 1.5 Hz) at δ 6.20 ppm. These signals in \textsuperscript{1}H NMR spectrum and the absorption at 1706 cm\(^{-1}\) in IR spectrum indicated the presence of \alpha,\beta-unsaturated ester and the stereochemistry of the double bond was \textit{trans} configuration. The evidence that a 2H broad singlet at δ 2.6 ppm disappeared on treatment with D\textsubscript{2}O suggested the presence of two hydroxyl groups. This was supported by fragment ions due to the loss of H\textsubscript{2}O and 2H\textsubscript{2}O 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\textsubscript{4}, C\textsubscript{5} and C\textsubscript{11} is unresolved. Furthermore \textsuperscript{13}C NMR spectrum of I confirmed this structure. \textsuperscript{13}C NMR (25 MHz) δ\textsuperscript{13}C 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\textsubscript{2}-), 19.0 (q, -CH\textsubscript{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-
derived compound. Among more than twenty fungal macrolides so far reported, only re-cifeiolide\(^2\) (II) belongs to the same group as I does.

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**REFERENCES**

1) While the molecular ion was not observable, the \((M+H)^+\) ion was abundant, which was given by an ion-molecule reaction: F. W. McLafferty, *Anal. Chem.*, 29, 1782 (1957).