STRUCTURE OF GLIOCLADIC ACID

Sir:

As reported in a previous paper, heptelidic acid, later found to be identical with avocettin, was isolated from the culture broths of three different strains of fungi identified as Gliocladium virens SANK 12679, Chaetomium globosum SANK 13379 and Trichoderma viride SANK 13479. In addition to this antibiotic, gliocladic acid, a new antitumor substance, was isolated as a minor component from the fermentation broths of the said fungi. In the present paper we wish to report the structure determination and antitumor activity of gliocladic acid. Gliocladic acid (1) was indicated to possess a molecular composition of C₁₄H₂₂O₃ from its CMR and elemental analysis. The PMR spectrum of 1 showed signals due to two methyl groups at δ 0.91 (d, J = 6.0) and δ 0.80 (d, J = 6.0), two hydroxymethyl groups at δ 4.20 (b-s) and δ 3.78 (b-s) and two olefinic protons at δ 6.44 (d, J = 11.0) and δ 5.17 (b-s). The IR spectrum of 1 exhibited absorptions at 3300 - 2300 and 1690 cm⁻¹ corresponding to a carboxyl group and at 1375 and 1385 cm⁻¹ to an isopropyl group, respectively. The above spectral data suggest that the following moieties (Fig. 1) are present in the chemical structure of 1.

Reaction of 1 with diazomethane gave a methyl ester (2). The PMR spectrum of 2 showed a signal at δ 3.73 (s) corresponding to the methyl ester. In the MS spectrum of 2, a molecular ion peak for C₁₅H₂₄O₄ appeared at m/z 268. It is strongly suggested that gliocladic acid is a nor-sesquiterpenoid compound.

Oxidation of 2 with manganese dioxide in CHCl₃ yielded an aldehyde (3). The PMR spectrum of 3 showed a singlet at δ 9.39 and a broad singlet at δ 6.40 attributed to an olefinic proton on the double bond conjugated with the aldehyde. Signals of one hydroxymethyl group at δ 4.41 and one olefinic proton at δ 6.60 (d, J = 10.0) conjugated with a carboxyl group still remain unsolved in 3.

Treatment of 2 with meta-chloroperbenzoic acid (m-CPBA) in CHCl₃ solution gave an epoxide compound (4, C₁₅H₂₄O₅) and an ether compound (5, C₁₅H₂₄O₅). A broad-singlet signal at δ 5.17 attributed to an olefinic proton in 1 disappeared from the PMR spectrum of 4. However, a doublet signal arose at δ 3.15 (J = 2.0) coupling with a methine proton at δ 2.84 which coupled with the β-proton of α,β-unsaturated carboxylic acid at δ 6.82 (J = 10.5) and in addition with another methine proton (J = 9.0).

From these assignments described above, the following partial structure (Fig. 2) was deduced for 1.

Acetylation of 4 with acetic anhydride in pyridine gave the diacetate (6). In the PMR spectrum of 6, two hydroxymethyl signals at δ 3.61 and δ 4.48 of 4 shifted downfield to δ 4.10 and δ 4.89, respectively. Acetoxymethyl signal at δ 4.89 in the latter is coupled with the β-proton of α,β-unsaturated carboxylic acid by long range coupling. Therefore, the partial structure of 1 was further defined as in the following structure (Fig. 3).

On the other hand, in the PMR spectrum of 5, an oxidation product of 2 with m-CPBA, the β-proton of α,β-unsaturated carboxylic acid shifted to downfield at δ 7.36 and appeared as a multiplet signal. Acetylation of 5 with acetic anhydride in pyridine gave a monoacetate (7, m/z 326). The IR spectrum of 7 showed a hydroxyl signal at 3350 cm⁻¹. These results indicated the presence of unacetylated hydroxyl group in compound 7. In the PMR spectrum of 7, a hydroxyl signal ap-
peared as a singlet at δ 3.15, and a broad-singlet signal of hydroxymethyl at δ 3.68 of 5 shifted to δ 4.12 but a signal at δ 4.41 remained unchanged. From these results, the partial structure of 5 was surmised as shown in the above chemical scheme. In addition, 1 was assumed to be a monocyclic compound from its molecular formula and also to possess an isopropyl group and two methylene carbon atoms from its CMR spectrum. Finally, the total structure of gliocladic acid was elucidated as 1. The stereochemistry of six membered ring and double bond of 1 were strongly supported by the following results. The stereochemistry of substituted group (isopropyl and α-hydroxymethyl α,β-unaturated carboxylic acid) were both equatorial by the fact observed above mentioned coupling constant between H-4 and H-5 in the PMR spectrum of 4. Geometry of double bond at C-2 and C-3 might be assigned by affording the ether compound 5 to E-configuration. It may be reasonable to assume that 1 is biogenetically derived from heptelidic acid by decarbonylation.

Sarcoma-37 tumor cells (2 × 10^6/mouse) were implanted subcutaneously to the axially region of ICR/JCR mice (female, 7 weeks old, 5 mice in a group) and 1 was administered intraperitoneally once a day for 8 days on the 1st to 4th and 7th to 10th day after implantation. On the 21st day after transplantation, the tumor diameters were measured and compared with those of the control group to evaluate the suppression of tumor growth. Gliocladic acid exhibited a moderate inhibition effect (46% reduction) on Sarcoma-37 at a dose of 3 mg/kg/day. Mice treated with an intraperitoneal administration of 1 at 200 mg/kg showed no ill effects.

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References