
 Communications to the editor

 STRUCTURE OF CC-1065 (NSC-298223),
 A NEW ANTITUMOR ANTIBIOTIC

Sir:

In the course of screening for soil cultures producing agents displaying both cytotoxic activity against L1210 cells in culture and *in vivo* activity against P388 leukemia in mice, a culture, *Streptomyces zelensis*, producing the agent CC-1065 was isolated; its production, *in vitro* biological activity, microbiological assays, and taxonomy were recently described¹. In spite of low initial titers of only 200 $\mu\text{g/liter}$, the agent was isolated by extraction and chromatography monitored by *in vivo* assay. Improved monitoring systems were then developed which allowed substantial improvements in its production and isolation². CC-1065 displayed remarkable potency against L1210 cells in culture; its ID_{50} and ID_{90} values were approximately 1×10^{-5} and 4×10^{-5} $\mu\text{g/ml}$, respectively (3-day growth, continuous drug contact)¹. The *in vivo* potency was equally impressive against a spectrum of mouse tumors including P388 leukemia, L1210 leukemia, and B16 melanoma where significant activity was observed with doses from 1 to 50 $\mu\text{g/kg}$ ³.

Characterization data on the new antitumor agent² did not readily lead to its structure. In the ultraviolet, CC-1065 displayed strong end absorption with shoulders at 230 and 258 nm and a maximum at 364 nm. The infrared spectrum was consistent with the presence of NH and OH groups, amide-type carbonyls, and unsaturation. The ¹³C NMR spectrum indicated 11 aliphatic and 26 unsaturated carbons. The aliphatic carbons were consistent by off-resonance decoupling with a methyl, 3 methylenes, a methine, a quaternary carbon, 3 methylenes on nitrogen, and 2 O-methyls; of the 26 unsaturated carbons only 4 carried hydrogens and 8 carried oxygens. The proton NMR spectrum displayed 7 exchangeable protons and confirmed the presence of 4 protons on unsaturated carbons, 3 methylenes on nitrogen, 2 O-methyls, and a methyl on an unsaturated carbon. Some important features were obscured in the proton spectrum; half the signal for a cyclopropyl methylene was hidden under the methyl signal, the aliphatic methylene signals were hidden

under the DOH peak, and the methine signal was buried under DMSO signals.

Numerous crystallization studies on CC-1065 yielded needles unsuitable for X-ray crystallography. Efforts to prepare a crystalline derivative for crystallography invariably generated complex mixtures; CC-1065 degraded under basic or acidic reaction conditions. The crystalline degradation product shown in Fig. 1 was isolated from the complex mixture obtained by reacting CC-1065 with ethyl isocyanate in pyridine and its structure determined by X-ray crystallography⁴. The nucleus of this derivatized fragment was compatible with some of the NMR signals generated by CC-1065 and represented about one third of the CC-1065 molecule. A closely related compound, PDE I (Fig. 2), was isolated in the course of screening fermentations for inhibitors of cyclic adenosine-3',5'-monophosphate phosphodiesterase^{5,6}. PDE I was reported⁵ nontoxic intraperitoneally in mice at 200 mg/kg; in contrast, CC-1065 was toxic intraperitoneally in mice at 0.1 mg/kg.

Fig. 1. Structure of fragment from CC-1065.

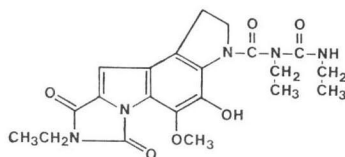
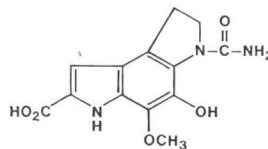
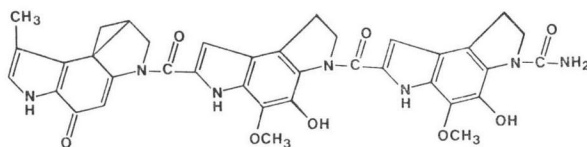


Fig. 2. Structure of PDE I.



Crystallization efforts finally produced a solvated granular crystalline form of CC-1065 which gave usable diffraction data; X-ray calculations coupled with spectroscopic data established that the intact antitumor agent had the structure shown (Fig. 3) in its keto form. Details of the isolation and structure determination will be described in separate publications in preparation.

Fig. 3. The structure of CC-1065.



Acknowledgments

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