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THE ISOLATION, STRUCTURE, AND ABSOLUTE CONFIGURATION OF U-42,126 A NOVEL ANTITUMOR ANTIBIOTIC

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In the course of screening for antimetabolites having potential use as antitumor agents, Hanka and Dietz of our laboratories isolated a culture, *Streptomyces sviceus*, producing an antimicrobial agent active *in vitro* against bacteria cultivated in synthetic media and fungi (1) In this preliminary report, we describe the isolation, structure, and absolute configuration of the antimetabolite antibiotic U-42,126, which significantly increased the life span of tumor-bearing (L1210 leukemia) mice at low drug levels with no demonstrable signs of toxicity to the hosts (2). Since L1210 lymphoid leukemia in mice is currently the model system regarded by the National Cancer Institute as the best predictive test for clinical activity, we are hopeful that this novel antimetabolite will prove to be a valuable addition to the arsenal of useful antitumor drugs

The isolation and purification of the active agent, formed in minute amounts, was greatly facilitated by bioassay and bioautography techniques described elsewhere (1) Approximately 250 l. of clarified fermentation broth containing 2.95 kgm. of low-grade solids, was passed through a cation exchange column (Dowex 50 x 16,H^+ cycle) Elution with 1 *N* NH_4OH afforded a fraction containing 244 gm of enriched antimetabolite having 11 times the *in vitro* potency of the crude solids

An aqueous solution of this fraction at neutral pH was passed through Amberlite IR45 (^-OH form) After washing the column with H_2O and aqueous MeOH, elution with dilute HOAc in aqueous MeOH afforded a fraction of 9 40 gm having 310 times the crude *in vitro* potency Partition chromatography, utilizing the upper phase of n-butanol, benzene, methanol, and water (2 1 1 1) as eluant and the lower phase supported on a medium porosity diatomite as stationary phase, of the ion exchange purified sample afforded 3 93 gm. of active antimetabolite having 900 times the crude *in vitro* potency Crystallization and recrystallization of this highly

active fraction from aqueous methanol afforded 433 mg. of pure crystalline antimetabolite having 3600 times the crude *in vitro* potency

The IR spectrum of crystalline U-42,126 was consistent with an amino acid and its NMR spectrum (D_2O) indicated the presence of four non-exchangeable protons consistent with a methylene group, an α amino acid residue, and an oxygen bearing carbon whose proton was coupled to the methine and the methylene protons A significant observation from mass spectrometry was the apparent presence of Cl patterns in some of the ion fragments Spectral data will be described in detail elsewhere (3) Elemental analysis on pure samples agreed well with the empirical formula $C_5H_7ClN_2O_3$

An x-ray crystallographic study, using crystals grown from water, served to determine the structure of the ring and the absolute stereochemistry of the molecule. The crystals are monoclinic with crystal data shown below

Space group C 2	$\underline{a}$ = 9 471 (2) Å	β = 97 33 (1)°
D_m = 1 609 g cm^{-3}	$\underline{b}$ = 5 420 (1)	F W = 178.6
D_c = 1.620 g cm^{-3}	$\underline{c}$ = 14 375 (4)	Z = 4

Three-dimensional intensity data (814 reflections) were collected using graphite-monochromated CuK radiation Ten reflections were monitored periodically during the data collection, considerable loss in intensity was noted especially in the latter part of the data collection Intensity data were corrected for deterioration using an isotropic time-dependent function obtained by least-squares fit of the deterioration data for the check reflections Trial positions for Cl and ten C, N, or O atoms were obtained by analysis of a three-dimensional Patterson function and two subsequent electron density calculations The elemental identity of the carboxyl O's and the adjacent amino-N soon became obvious from large drops in temperature factors during least-squares refinement and from hydrogen bonding considerations To establish the elemental identity (C, N, or O) at positions 1, 2, and 4 of the ring, seventeen possible ring systems were tested Six of these are consistent with the final elemental analysis, which was not available at the time the final x-ray work was done All ring systems were carried through preliminary refinement (without H's), evaluation of bond lengths and temperature factors, and location of H atoms by difference electron density maps One ring system refined to an R (0 061) significantly lower than the sixteen others (0 069 to 0 095) A 2-3 double bond, two H atoms off position 4, and no possible H atoms off position 2 resulted in all cases

Two test rings (both with N at position 1 and inconsistent with elemental analysis) gave peaks attributable to a H atom off position 1, in an attempted refinement of this H, it moved directly over the N at position 1 on the first iteration, effectively giving an O at 1 Thus the structure below could be unequivocally assigned to the molecule (4). Anomalous dispersion techniques (5) were used to establish the absolute configuration, all eighteen reflection pairs clearly indicated the αS,5S (6) enantiomorph shown in Figure 1. Least-squares refinement converged with R = 0.036 for all reflections. A complete difference electron density map verified that there was no extraneous electron density except along bonds between atoms.

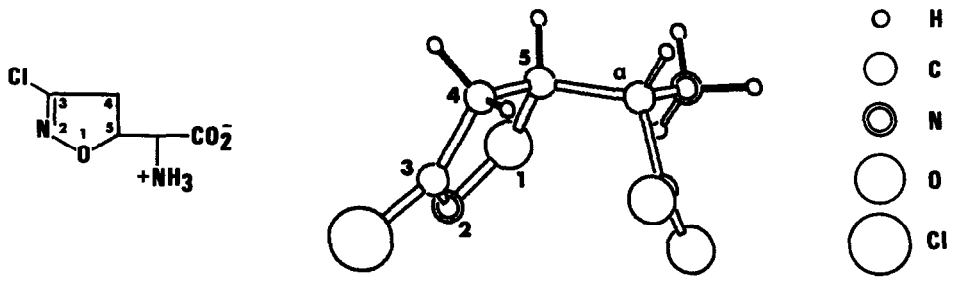

Figure 1
αS,5S-α-Amino-3-chloro-2-isoxazoline-5-acetic acid

The amino acid bond distances and angles of the glycine side chain agreed to within experimental error with the dimensions of the amino acid grouping given by Marsh and Donohue (7) in their review of amino acid structures. Remaining distances and angles are within expected ranges. The ring N and O do not participate in H bonding Extensive H bonding is found between the carboxylate O's and the ammonium groups ($-N\overset{+}{H}_3$) of symmetry-related molecules. The amino acid portion of the molecule, as in all other reported amino acids (7), exists as a zwitterion. A detailed account of the x-ray determination will be published (8) Synthesis of U-42,126 will be discussed at a later date.

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REFERENCES AND FOOTNOTES

1 L J. Hanka and A Dietz, Antimicrobial Agents and Chemotherapy, in press and references cited

2 L. J Hanka, D. G. Martin, and G L. Neil, Cancer Chemotherapy Reports, in press

3 D G Martin, G Slomp, S Mizsak, W C Krueger, L Baczynskyj and R J. Wnuk, manuscript in preparation

4 Earlier unpublished work by x-ray and spectroscopy on a small sample of impure U-42,126 resulted in incorrect elemental assignment at 1 and 2. The NMR spectrum showed a vinyl proton (now known to be from an impurity), and the earlier x-ray data were much poorer than that reported here (final R of 0 122) This study led to the assignment -- supported by H location in difference maps -- of C at 2 and N at 1 A study, in retrospect, showed that the earlier x-ray data could not distinguish between this assignment and the correct one At that time, had we done a thorough study of ring systems, as reported above, we would have known that more than one structure was consistent with the early x-ray data and would not have been misled into accepting an incorrect structure until availability of larger samples of pure material having better elemental analyses and a clean NMR forced a restudy

5 J M. Bijvoet, Endeavour *14*, 71 (1955)

6 R S Cahn, C Ingold, and V. Prelog Angew Chem. Internat Edit. *5*, 385 (1966).

7 R E Marsh and J Donohue Adv. in Prot. Chem. *22*, 235 (1967)

8. D J Duchamp, D. G Martin, and C G. Chidester, Acta Cryst., in press.