THE ISOLATION, STRUCTURE, AND ABSOLUTE CONFIGURATION OF U-43,795, A NEW ANTITUMOR AGENT

Sir:

Recently we described a novel antitumor antimetabolite, U-42,126[1], (NSC-163501) (Fig. 1). This communication describes a related antitumor agent, U-43,795, (NSC-176324) (Fig. 1) which, like U-42,126[2], has significant activity against L 1210 lymphoid leukemia in mice[3], a model system highly regarded by the National Cancer Institute as a predictive test for clinical activity. Extensive investigations on both U-42,126 and U-43,795 in other tumor systems are in progress at the National Cancer Insitute.

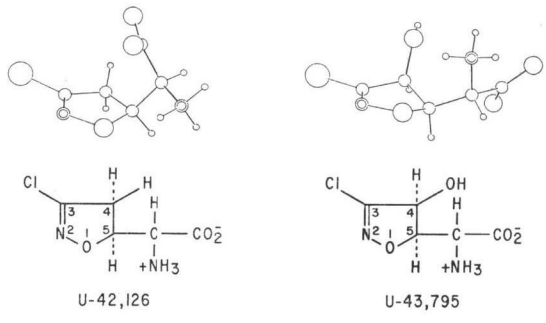
U-42,126 had originally been isolated from fermentation of *Streptomyces sviceus*[4] and purified by ion exchange chromatography, partition chromatography, and crystallization[1]. Before final purification had been achieved, gas chromatography-mass spectrometry studies on a silylated sample of U-42,126 indicated the presence of a minor component having a molecular ion at mass 410 consistent with a molecular weight of 194 plus 3 trimethylsilyl groups and suggestive of an oxygenated analog of U-42,126. The short supply of U-42,126 and suspected presence of potentially interesting compounds such as the oxygenated analog prompted upgrading mother liquors and less active chromatograph fractions on Amberlite IR 45 (OH^- form). Active eluates afforded additional pure U-42,126 after several recrystallizations and subsequent efforts to dissolve the resulting mother liquor residues in the solvent system used for partition chromatography[1] afforded a new crystalline solid melting with decomposition at 165°C after recrystallization from water.

Mass spectrometry of a silylated sample of these crystals indicated a molecular weight of 410 (relatively weak molecular ion with strong M-15 ion) suggesting that we probably had isolated the oxygenated analog. The 100 MHz NMR spectrum of a deuterium oxide solution of the new amino acid clearly indicated the presence of 3 non-exchangeable protons consistent with an amino acid methine proton and 2 methine protons on carbons bearing oxygen. The absence of the methylene protons of U-42,126, appearance of a new methine on a carbon bearing oxygen, and coupling pattern (a methine on oxygenated carbon coupled with the other 2 methines) was completely consistent with the 4-hydroxylated structure shown in Fig. 1. The IR spectrum of crystalline U-43,795 was consistent with amino acid and hydroxyl functions. The hydroxylated amino acid, unlike U-42,126, shows a tendency to associate with up to one equivalent of water which can be removed by drying under reduced pressure but may be reabsorbed from atmospheric moisture.

An X-ray crystallographic study of crystals of the monohydrate of U-43,795 confirmed the structure and rigorously established the stereochemistry as αS, $4S$, $5R$-α-amino-3-chloro-4-hydroxy-4, 5-dihydro-5-isoxazoleacetic acid. The crystals are orthorhombic, space group $P2_12_12_1$, with unit cell parameters: a=5.873Å, b=8.278Å, c=17.346Å. Three-dimensional intensity data (1006 reflections) were measured on an automated diffractometer using graphite-monochromated CuKα radiation. A trial structure was obtained using a computerized direct methods procedure with the automatic program DIREC developed in our laboratories. Anomalous dispersion techniques[5] were used to establish the absolute configuration; the R-factors for the 2 possible enantiomorphs (0.040 and 0.051), as well as all of the 15 specially measured sets, clearly indicated the αS, $4S$, $5R$ enantiomorph. Least-squares refinement converged with R=0.032 for all reflections.

The configuration of U-43,795 is like that of U-42,126[1] at the two asymmetric carbon

Fig. 1.



centers they have in common, but there are interesting differences between them in conformations found by X-ray. The orientation of the amino acid portion of the molecule relative to the isoxazoline ring is different as shown in Fig. 1. In the U-42,126 conformation, the U-43,795 molecule could not accommodate the hydroxyl group because of close approaches with the amino acid side chain. In both molecules, the isoxazoline ring is an envelope with C5 out of the plane of the other atoms; however, the direction of deviation is different and more pronounced in U-43,795. The ammonium nitrogen is 2.867 Å from the hydroxyl oxygen, close enough for an intramolecular hydrogen bond; however, the closest ammonium hydrogen is 2.47 Å from the hydroxyl oxygen, rather long for a hydrogen bond. A detailed account of the X-ray work will be published.

The presence of U-43,795 had originally been obscured by 2 factors. U-43,795 was less than 0.1 % as active as U-42,126 against the bacterial organism employed to monitor isolation and purification[2]; in effect, as little as 1 % of U-42,126 in mixed samples of the amino acids masked the presence of U-43,795 from detection by bioassay and bioautography techniques. A sensitive method for quantitating U-43,795 in mixtures was developed after the pure agent had been isolated. The method was based on the substantially different circular dichroism (CD) spectra of U-42,126 and U-43,795. Thus U-42,126 displayed a single positive CD band at 217 nm ($[\theta]=+12{,}600$) while U-43,795 showed CD bands at 222 nm ($[\theta]=+59{,}000$) and 202 nm ($[\theta]=-46{,}000$). At analytical wavelengths of 222 and 205 nm, as little as 1% of U-43,795 in samples of U-42,126 could be detected. The second factor contributing to the previous "invisibility" of U-43,795 was the similar chromatographic behavior of the 2 amino acids on many paper chromatography and thin-layer systems. It was therefore an exciting observation* that solvent systems comprised of methyl ethyl ketone, acetone, and water nicely resolved them on both paper and silica gel plates; chromatograms were readily visualized with ninhydrin spray. Equally important was the discovery that silica gel column chromatography with these solvents efficiently resolved the 2 amino acids on a larger scale in contrast to partition chromatography which was relatively inefficient. Improved purification procedures and greatly improved fermentation titres[3] have allowed preparation of relatively large quantities of U-42,126 and U-43,795 required for evaluation in other tumor systems. Successive processing of 16,000 liters of clarified fermentation broth with Dowex 50 (H^+ form) and then Amberlite IR 45 (OH^- form) afforded a mixture of the 2 agents. A relatively efficient resolution on silica gel afforded readily crystallized fractions of each component yielding (after recrystallization) over 150 g of each pure antitumor agent[6].

* The initial resolution of these amino acids on silica gel plates with these solvents was achieved by K.L. EBI and A.E. BERGER of these laboratories.

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