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DIRECT SYNTHESIS OF THE ANTITUMOR AGENT ERYTHRO-α-AMINO-3-BROMO-4,5-DIHYDROISOXAZOLE-5-ACETIC ACID

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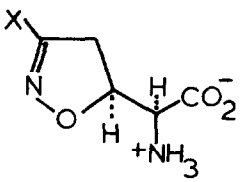
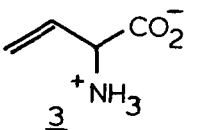
Summary: Generation of bromonitrile oxide in the presence of vinylglycine leads to the formation of the title compound 2 and its threo isomer in good yield.

Streptomyces sviceus produces, in very small amounts, (αS,5S)-α-amino-3-chloro-4,5-dihydroisoxazole-5-acetic acid (1, AT-125)[1]. Besides exhibiting significant antibacterial activity, this chloroisoxazoline possesses antitumor properties both in vitro and in vivo[1,2]. Of considerable interest is the report[2b] that 1 prolongs the life of rats implanted with human mammary tumors. In the course of an elegant synthesis of 1, Kelly, Wierenga and coworkers[3] prepared the analogous bromoisoxazoline 2 (U60096). Preliminary testing of this compound reveals antibacterial potency against B. subtilis "essentially identical" (on a molar basis) to that of 1, as well as antifungal and antitumor activity[3,4]. We are pleased to report a direct, if stereorandom, synthesis of racemic 2 by the cycloaddition of bromonitrile oxide to vinylglycine 3.



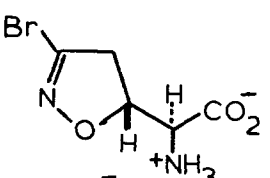
1 X = Cl
2 X = Br



3

$Br_2C{=}NOH$

4



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An attempted synthesis of chloroisoxazoline 1 by addition of chloronitrile oxide to vinylglycine (or derivatives) was unsuccessful[5]. We have obtained very good yields of 3-bromoisoxazolines by HBr elimination of dibromoformaldoxime 4[6] in the presence of various alkenes. As the yields are much higher than for the corresponding chloro compounds (eg., with styrene, 70% vs 6%[5]), we were encouraged to try this direct approach to 2. Addition of solid dibromoformaldoxime in small portions to a vigorously stirred aqueous solution of racemic vinylglycine (Calbiochem, "A grade") and sodium bicarbonate or acetate led to the disappearance of 3 and the formation of two amino acids of higher R_f. Approximately three equivalents of the oxime was required for complete consumption of 3. Extraction with ether removed the byproduct dibromofuroxan formed by dimerization of the nitrile oxide; removal of inorganic ions by ion exchange, followed by lyophilization of the ninhydrin-positive fractions, gave a good yield (60-80%) of

a mixture of the desired amino acid 2 and its threo isomer 5, which were barely separable by tlc. Comparison with an authentic sample of 2 showed it to be the less mobile isomer on silica gel using butanol-water-acetic acid (60:25:15) for development; with methyl ethyl ketone-acetone-water-acetic acid (60:20:15:5), 2 has the slightly higher R_f. Comparison of the nmr spectrum of our mixture with that of pure 2 permitted quantitation. In D_2O solution, the α-proton of 2 appeared as a doublet (J= 3.5 Hz) at δ3.94 (lit.[3], δ3.92, d, J= 4 Hz), while the corresponding signal for the epimer 5 appears at somewhat higher field (δ3.79, d, J= 7.4 Hz). The other signals (ca δ3.4, 2H, overlapping doublets with J= ca 9 Hz; ca δ5.2, 1H, complex) could not be analyzed for contributions of each isomer. Integration of the α-proton signals gave 2:5 ratios near 1/3 for both the bicarbonate and acetate reactions[7].

We are presently attempting separation of these isomers on a preparative scale, and examining other reaction conditions (specifically, buffered reaction over a range of pH) in an attempt to improve the stereoselectivity. We are also studying dipolar additions of bromonitrile oxide to various protected forms of 3 and to other, more readily available compounds properly functionalized for elaboration of the amino acid side chain; we also hope to be able to replace the bromine with chlorine, thereby avoiding the low reactivity of chloronitrile oxide.

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References and Notes

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3. R. C. Kelly, I. Schletter, S. J. Stein and W. Wierenga, J. Am. Chem. Soc., 101, 1054 (1979).

4. R. C. Kelly, private communication.

5. J. E. Baldwin, C. Hoskins and L. Kruse, J. Chem. Soc., Chem. Commun., 795 (1976).

6. I. DePaolini, Gazz. chim. ital., 60, 700 (1930); cf. C. A., 25, 1488 (1931). A modification of this procedure which permits fairly large scale preparations will be published later.

7. In addition to the tlc and nmr evidence reported, the presence of amino acid 2 in the product mixture was demonstrated by paper chromatography using several standard solvent systems, by chromatography on polyamide, and by the colors (and rates of coloration) shown upon reaction with ninhydrin, both in solution and on the developed chromatograms.

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