Synthesis of 1-(4-Thio-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide

Michael V. Pickering, Joseph T. Witkowski,* and Roland K. Robins

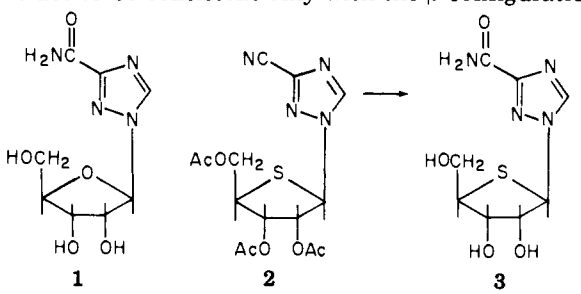
ICN Pharmaceuticals, Inc., Nucleic Acid Research Institute, Irvine, California 92715. *Received December 1, 1975*

The synthesis of 1-(4-thio-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (3) is described. The acid-catalyzed fusion procedure with 3-cyano-1,2,4-triazole and 1,2,3,5-tetra-*O*-acetyl-4-thio-D-ribofuranose provided 3-cyano-1-(2,3,5-tri-*O*-acetyl-4-thio-β-D-ribofuranosyl)-1,2,4-triazole (2) which was converted with NH_4OH to 3. In contrast to 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (1), the 4'-thio nucleoside 3 did not exhibit significant antiviral activity in vitro.

The synthetic nucleoside 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide[1] (1) has been shown by our laboratory[2-6] and others[7-9] to exhibit broad-spectrum activity against DNA and RNA viruses both in vitro and in vivo. Modifications of this nucleoside are of interest in determining structure–activity correlations and for providing information on the biochemical mechanism of action[10,11] of these antiviral agents. The syntheses of a number of nucleosides related structurally to 1 have been reported. These include 1,2,4-triazole nucleosides with modification of the heterocycle,[12,13] nucleosides of 1,2,-4-triazole with various glycosyl moieties,[14] and 1,2,3-triazole-4-carboxamide nucleosides.[15,16]

A number of 4'-thio analogues of naturally occurring and biologically active synthetic nucleosides have been reported.[17-19] These purine and pyrimidine 4'-thio nucleosides exhibited activity against certain bacteria and tumor cells.[17-19] The effect of substitution of sulfur for the ribofuranosyl ring oxygen on the biological activity of these nucleosides has been briefly reviewed.[17]

This account describes the synthesis of 1-(4-thio-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (3) which is the 4'-thio derivative of 1. The acid-catalyzed fusion procedure[20] using 3-cyano-1,2,4-triazole[12] has been shown[12,14] to be a convenient route to 1,2,4-triazole-3-carboxamide nucleosides. Fusion of 1,2,3,5-tetra-*O*-acetyl-4-thio-D-ribofuranose[21] with 3-cyano-1,2,4-triazole in the presence of an acid catalyst provided crystalline 3-cyano-1-(2,3,5-tri-*O*-acetyl-4-thio-β-D-ribofuranosyl)-1,2,4-triazole (2). Treatment of this blocked nucleoside 2 with aqueous ammonia removed the acyl groups and converted the cyano moiety to the carboxamide, affording 1-(4-thio-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (3). The anomeric configuration of 3 was established by formation of the 2',3'-*O*-isopropylidene derivative 4. The NMR spectrum of 4 exhibited a singlet for the anomeric proton and a difference in the δ values for the methyl groups of the isopropylidene moiety of 0.20. These values have been shown[22,23] in the case of ribofuranosyl nucleosides to be consistent only with the β configuration.



1 2 3

The effect of the nucleoside 3 on replication of type 1 herpes simplex, type 13 rhino, and type 3 parainfluenza viruses was determined in tissue culture as previously described.[24] In contrast to 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (1) which exhibited significant activity[1,2] against each of these viruses, the 4'-thio derivative 3 was slightly active only against herpes simplex virus with a virus rating[24] of 0.3, while the ribonucleoside 1 tested in parallel had a virus rating of 0.9.

Replacement of the ring oxygen of certain pyrimidine nucleosides with sulfur has been shown[19] by x-ray crystallography to result in a significant conformational change in the furanose ring. A similar change in conformation of the 4'-thio nucleoside 3 (vs. 1) could explain the lower rate of phosphorylation observed[25] for 3 compared with the efficient phosphorylation[26] of the ribonucleoside 1. This diminished rate of phosphorylation is consistent with the greatly reduced antiviral activity exhibited by the 4'-thio nucleoside 3.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. NMR spectra were obtained on a Hitachi Perkin-Elmer R20-A spectrometer using DSS or Me_4Si as an internal standard. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter at 25°. Elemental analyses were performed by Galbriath Laboratories, Inc., Knoxville, Tenn., and are within ±0.4% of the theoretical values. Thin-layer chromatography was performed on Woelm silica gel plates and components were visualized with a spray of 10% sulfuric acid in methanol followed by heating at ca. 110°. Silica gel for column chromatography was Woelm type 204. Evaporations were carried out in vacuo with the bath temperature below 35°.

3-Cyano-1-(2,3,5-tri-*O*-acetyl-4-thio-β-D-ribofuranosyl)-1,2,4-triazole (2). A mixture of 1,2,3,5-tetra-*O*-acetyl-4-thio-D-ribofuranose[21] (4.5 g, 13.5 mmol), 3-cyano-1,2,4-triazole[12] (1.68 g, 18.2 mmol), and bis(*p*-nitrophenyl) phosphate (15 mg) was heated in an oil bath at 150° under reduced pressure for 20 min. The mixture was cooled and dissolved in EtOAc (100 ml). The solution was washed with aqueous $NaHCO_3$ (three 50-ml portions) and with water. The EtOAc solution was dried (Na_2SO_4), treated with charcoal, filtered, and evaporated to dryness. The product was crystallized from Et_2O to give 1.48 g of 2 with mp 121–122°. Concentration of the filtrate gave an additional 0.32 g of product. The total yield was 36%. An analytical sample recrystallized from Et_2O had mp 124.5–125°: NMR (Me_2SO-d_6) δ 2.10 (s, 9, CH_3 of Ac), 3.7–4.6 (m, 3, H-4',5'), 5.6–5.85 (m, 2, H-2',3'), 6.42 (d, 1, $J_{1',2'}$ = 4 Hz, H-1'), 9.05 (s, 1, H-5). Anal. ($C_{14}H_{16}N_4O_6S$) C, H, N, S.

1-(4-Thio-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (3). A mixture of 3-cyano-1-(2,3,5-tri-*O*-acetyl-4-thio-β-D-ribofuranosyl)-1,2,4-triazole (1.0 g, 2.7 mmol) and concentrated NH_4OH (40 ml) was heated at 50–60° for 1 h. The resulting solution was evaporated to dryness and the residue was dissolved in MeOH. Silica gel (5 g) was added to the solution and the mixture was evaporated to dryness. The silica gel mixture was added to a column of silica gel (25 g) packed in EtOAc. Elution was with EtOAc (300 ml) followed by EtOAc–MeOH (10:1). Fractions containing the product were evaporated to dryness to give 0.5 g (71%) of 3 as amorphous material which crystallized slowly on standing. Recrystallization from EtOAc–MeOH gave an analytical sample with mp 124–125°: [α]D −69.1° (*c* 1, H_2O); NMR (Me_2SO-d_6) δ 5.8 (d, 1, $J_{1',2'}$ = 6 Hz, H-1'), 7.6 (br, s, 1, NH), 7.82 (br, s, 1, NH), 8.90 (s, 1, H-5). Anal. ($C_8H_{12}N_4O_4S$) C, H, N, S.

1-(2,3-*O*-Isopropylidene-4-thio-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (4). A suspension of 3 (200 mg) in

acetone (6 ml) and 2,2-dimethoxypropane (2 ml) was cooled in an ice bath and 70% perchloric acid (3 drops) was added. The mixture was stirred at 0° for 2 h and then was neutralized with 2 N aqueous KOH. Insoluble material was removed by filtration and the filtrate was evaporated to dryness. The residue was dissolved in MeOH; the solution was filtered and evaporated to dryness. Crystallization of the product from EtOAc gave 140 mg (61%) of 4 with mp 163–165°: NMR (Me_2SO-d_6) δ 1.33 (s, 3, CH_3), 1.53 (s, 3, CH_3), 6.24 (s, 1, H-1') 7.68 (br, s, 1, NH), 7.80 (br, s, 1, NH), 8.81 (s, 1, H-5). Anal. ($C_{11}H_{16}N_4O_4S$) C, H, N, S.

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Synthesis and Some Pharmacological Properties of Deamino[4-threonine,8-D-arginine]vasopressin and Deamino[8-D-arginine]vasopressin, Highly Potent and Specific Antidiuretic Peptides, and [8-D-Arginine]vasopressin and Deamino-arginine-vasopressin

Maurice Manning,* Lajos Balaspiri,[1] Jacqueline Moehring,

Department of Biochemistry, Medical College of Ohio, Toledo, Ohio 43614

Jaya Haldar, and Wilbur H. Sawyer

Department of Pharmacology, College of Physicians and Surgeons, Columbia University, New York, New York 10032.
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Deamino[4-threonine,8-D-arginine]vasopressin (dTDAVP), deamino[8-D-arginine]vasopressin (dDAVP), [8-D-arginine]vasopressin (DAVP), and deamino-arginine-vasopressin (dAVP) were synthesized by the solid-phase method and tested for their biological activities. dTDAVP has an antidiuretic potency of 793 ± 95 units/mg and undetectable vasopressor activity, <0.02 unit/mg. The antidiuretic–pressor (A/P) ratio of dTDAVP is greater than 39 000. dDAVP has an antidiuretic potency of 1200 ± 126 units/mg and a vasopressor potency of 0.39 ± 0.02; its A/P ratio is thus 3000. DAVP has an antidiuretic potency of 253 ± 44 units/mg, a vasopressor potency of 1.1 ± 0.04 units/mg, and an A/P ratio of 240. The A/P ratios of dDAVP and DAVP are much higher than those originally reported. dAVP has an antidiuretic potency of 1745 ± 385 units/mg, a vasopressor potency of 346 ± 13, and an A/P ratio of 5; values are in general agreement with those in the literature. Threonine substitution has thus brought about a significant enhancement in antidiuretic specificity, a finding entirely consistent with earlier observations that enhancement of lipophilicity at position 4 alone or in combination in arginine-vasopressin can lead to enhanced antidiuretic specificity.

An investigation of the structural changes in arginine-vasopressin (AVP) which modulate antidiuretic activity and specificity led to the synthesis of deamino-[4-valine,8-D-arginine]vasopressin (dVDAVP),[2a] a peptide possessing high antidiuretic activity, specificity, and increased duration of action. In a subsequent follow-up study[2b] it was shown that enhanced antidiuretic–pressor (A/P) specificity is governed chiefly by 8-D-arginine substitution. Enhanced lipophilicity at position 4 and deamination at position 1 also contribute but to much lesser degrees. We wished to explore further the effects of enhanced lipophilicity at position 4 in combination with