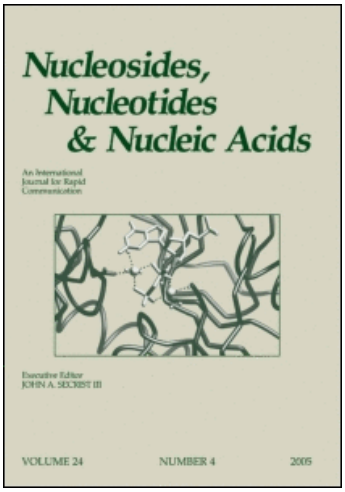

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SYNTHESIS AND X-RAY CRYSTAL STRUCTURE OF 2-β-D-RIBOFURANOSYLTHIAZOLE-4-CARBOXAMIDE-N_3-OXIDE

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ABSTRACT: 2-β-D-Ribofuranosylthiazole-4-carboxamide-N_3-oxide (3) has been prepared by oxidation of tiazofurin (2) with hydrogen peroxide in the presence of trifluoroacetic acid. The absolute structure of 3 has been determined by X-ray diffraction techniques employing Mo Kα radiation. The thiazole ring in 3 is planar and the glycon moiety is in the ^3E configuration. The N3-C4 bond in 3 is significantly larger (1.412 Å) than that found in tiazofurin (1.376 Å). Similar to tiazofurin, the S...O4' intramolecular contact distance is considerably shorter than the sum of the van der Waals radii, which favors the anti conformation of 3.

The chemical synthesis of a carbon-linked azole nucleoside 2-β-D-ribofuranosylthiazole-4-carboxamide (tiazofurin, 2) has been reported from our laboratory[1], and has shown significant in vitro antiviral activity against a number of DNA and RNA viruses.[1-3] Although tiazofurin exhibited a narrower antiviral spectrum than the broad-spectrum antiviral agent ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, 1)[4], tiazofurin is exceptionally active in vitro against Japanese encephalitis (JE), yellow fever (YF) and Hantaan virus, the causative agent of Korean hemorrhagic fever (KHF).[2] Combinations of ribavirin and tiazofurin were synergistic against YF and JE (fractional inhibitory concentrations, 0.41 and 0.48, respectively) but showed additive effects against KHF virus.[3] Tiazofurin exhibited remarkable activity against Lewis lung carcinoma and good activity against L1210 and P388 leukemias in vivo.[5] Recent testing[6] of tiazofurin against L1210 leukemia in mice has shown a T/C of 284 at 512 mg/kg/day x 9.

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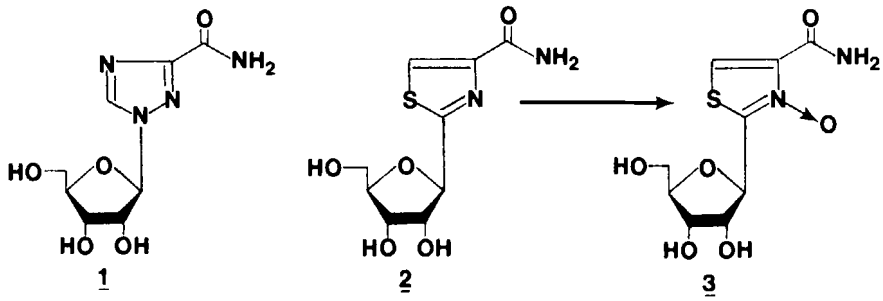
Tiazofurin is also significantly active against 6-mercaptopurine and 6-thioguanine resistant L1210, as well as several resistant strains of P388 leukemias in vivo.[6] The mechanism of antitumor action of tiazofurin has recently been studied.[7-9] It has been shown that tiazofurin is phosphorylated enzymatically to the 5'-monophosphate, which in the presence of ATP and Mg++ is further converted to the analog of coenzyme NAD which is a potent inhibitor of IMP dehydrogenase.[7-10]

A recent single crystal X-ray diffraction study[11] of tiazofurin has shown that in the solid state, the molecule is in the anti conformation with the sulfur atom in rather close proximity to the furanose oxygen and the C2' carbon exhibits an endo pucker. The distance between the thiazole sulfur and the furanose oxygen is less than the sum of their van der Waals radii. Results from molecular orbital calculations suggest that the thiazole sulfur has a net positive charge.[12,13] Thus, the S....O contact observed represents an electrostatic attraction between the positive thiazole sulfur and the lone pair of electrons on the furanose oxygen. In an effort to study the effect of N-oxide on such electrostatic attraction, we have now prepared 2-β-D-ribofuranosylthiazole-4-carboxamide-N_3-oxide (3). Moreover, compound 3 may act as a depot form of the drug tiazofurin and have different substrate binding characteristics toward certain nucleic acid enzymes.



Oxidation of tiazofurin[1] (2) with hydrogen peroxide in trifluoroacetic acid at ambient temperature gave the desired 2-β-D-ribofuranosylthiazole-4-carboxamide-N_3-oxide (3) in 54.3% yield. The absolute structural assignment of 3 was made by single crystal X-ray analysis.

Single-Crystal X-ray Diffraction Analysis of 3. Slow crystallization of 3 from MeOH:H_2O (1:1) gave X-ray quality crystals. A

suitable crystal (0.02 x 0.20 x 0.40 mm) was mounted on a Nicolet P3 auto-diffractometer and the diffraction data for the determination of both the lattice parameters and the structural study were collected using Mo Kα graphite monochromated radiation (λ = 0.71069 A). Compound 3 crystallizes in the monoclinic space group $P2_1$ with a = 4.847 (4), b = 7.230 (7), c = 15.669 (13) A; β = 93.28 (7)°, volume = 548.2 (8) A^3 and Z = 2. The lattice parameters were obtained using a least-squares technique involving 15 centered 2θ values. Single crystal X-ray data were collected to a $\sin\theta/\lambda$ limit of 0.65 utilizing a θ-2θ scan procedure. A variable scan rate was employed with total background time being equal to the scan time. The three standard reflections measured every 97 reflections showed no significant change throughout data collection indicating crystal and electronic stability. A total of 1590 reflections were merged to 1338 unique non-zero reflections. Of these 166 were considered unobserved as $I < 2\sigma(I)$ and the remaining 1172 reflections were used in the structure determination and refinement process.

The structure was solved using the direct methods and refined with a full-matrix least-squares procedure.[14] All hydrogen atoms were found in difference maps. With nonhydrogen atoms refined anisotropically and hydrogen atoms refined isotropically the final residual value was R = 0.042. Unit weights were used in refinement. The largest peak in the final difference map was 0.31e A^{-3}. The largest shift/esd value in the final refinement calculation, excluding hydrogen atoms, was 0.07 for the y value for the carboxamide oxygen.

RESULTS AND DISCUSSION

The structural formula, conformation, atom labels and the interatomic bond lengths of compound 3 are shown in FIGURE 1. The estimated standard deviations on all bond lengths are between 0.005 and 0.007 A. The hydrogens of the carbohydrate moiety are omitted for clarity. The nucleoside exists in the β-anomeric configuration. The presence of the N-oxide function causes some of the bond lengths in the thiazole ring to vary from similar bonds in unsubstituted tiazofurin. This is particularly evident for N3-C4 where the value of 1.412 A is significantly larger than 1.376 A found in tiazofurin.[11] The thiazole ring in 3 is

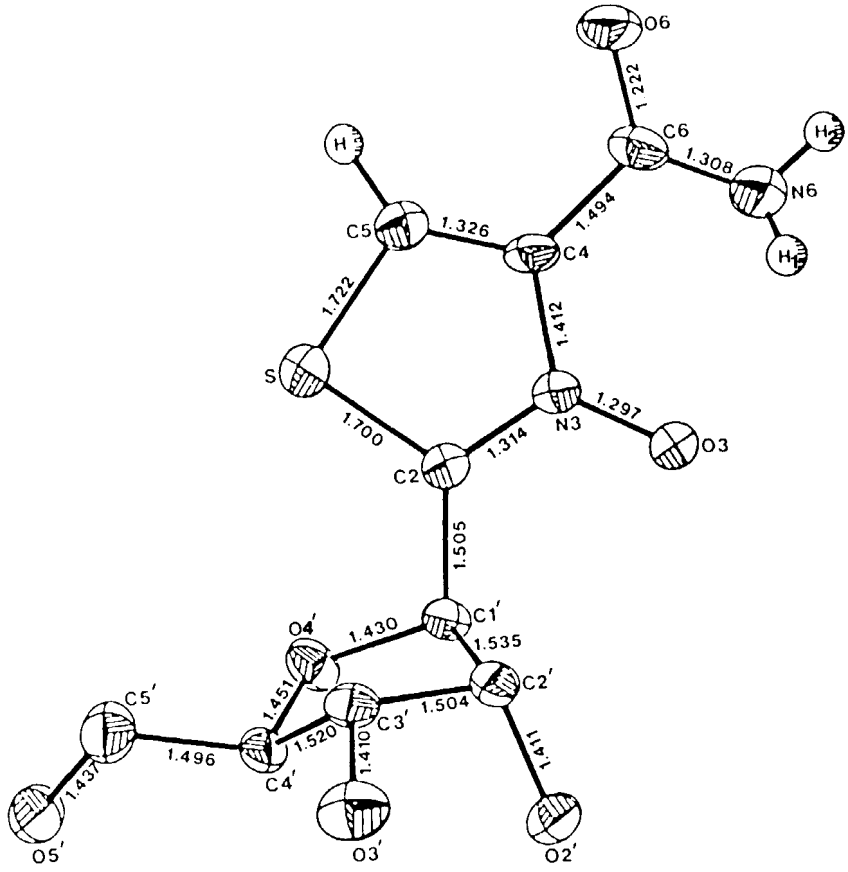


FIGURE 1. ORTEP drawing with the atom labels and bond lengths of compound 3.

planar with the largest deviation of any atom from the least-squares plane of the aglycon being -0.0062 A(C2). The largest deviation from this plane of an atom bonded to the ring is 0.0522 A (C6). The plane of of the carboxamide group makes a dihedral angle of 7.4° with the plane of aglycon. The near coplanarity is probably caused by the presence of the intramolecular hydrogen bond N6-N6H_1....O3. The hydrogen bond data for this, as well as the intermolecular hydrogen bonds are listed in TABLE 1.

The glycon moiety is in the C3' endo (^{3}E) configuration. The conformation about the C4'-C5' is gauche, trans. Values for the selected torsion angles are listed in TABLE 2. The torsion angle O4'-C1'-C2-S

TABLE 1

Hydrogen Bond Data

D....H....A†			D....A (A) distance	H....A (A) distance	D-H....A (°) angle	Symmetry of A
N6	$N6H_1$	O3	2.68	2.17	118	x,y,z
N6	$N6H_2$	O6	2.86	1.91	171	-x,0.5+y,2-z
O5'	O5'H	O3	2.87	1.96	163	1+x, -1+y, z
O2'	O2'H	O3'	2.67	1.96	162	1-x, 0.5+y, 1-z
O3'	O3'H	O5'	2.88	2.40	146	2-x, 0.5+y, 1-z

† D, donor atom; H, hydrogen atom; and A, acceptor atom

TABLE 2

Selected Torsion Angles in 3

A - B - C - D	Torsion Angle (°)
O5' -C5' -C4' -C3'	81.4
O5' -C5' -C4' -O4'	161.6
O4' -C1' -C2 -S	8.3
N3 -C4 -C6 -N6	5.5

has a value of 8.3°, which is significantly smaller than the value found in tiazofurin. Similar to tiazofurin, the S....O4' intramolecular contact distance [2.918(3) A] is considerably shorter than the sum of the van der Waals radii. This short contact distance is clearly visible in the space filling model of 3 (FIGURE 2), which also shows the close proximity of the N_3-oxide atom (O3) to the carboxamide hydrogen atom ($N6H_1$). Such interaction favors the anti form and is discussed in considerable detail by Goldstein et al.[11]

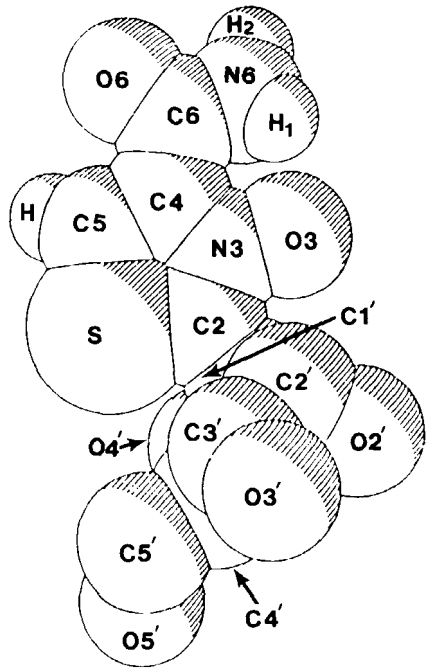


FIGURE 2. Space Filling Model of 3

In addition to the intramolecular hydrogen bond mentioned above, there is an extensive intermolecular hydrogen bonding network. All three alcoholic hydrogens of the glycon, as well as $N6H_2$ are involved in intermolecular hydrogen bonds. The acceptor atoms are O6 and O3 of the heterocycle and O3' and O5' of the carbohydrate moiety. The N-oxide atom (O3) is therefore involved in two hydrogen bonds, one intramolecular and the other intermolecular (See TABLE 1). Similar intermolecular hydrogen bonding to form a three dimensional network in bredinin (4-carbamoyl-1-β-D-ribofuranosylimidazolium-5-olate) has been observed,[15] in which all the available groups including the water of crystallization, participate in the hydrogen bonds. The hydrogen bonding seen in the solid state of 3 is also present in solution (Me_2SO) as shown by the large difference in chemical shift (1.74 ppm) of the amide protons in the NMR spectrum. This phenomenon has recently been observed[16] in our laboratory with several 4-substitutedpyrazolo[3,4-d]-pyrimidine-3-carboxamide and 4-substitutedpyrrolo[2,3-d]pyrimidine-5-carboxamide ribonucleosides.

EXPERIMENTAL SECTION

Melting point was determined on a Thomas-Hoover capillary melting point apparatus. Nuclear magnetic resonance (NMR) spectrum was recorded on a Jeol FX-90 Q spectrometer. The chemical-shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. Infrared spectrum (IR) was obtained on a Beckman Acculab 2 spectrophotometer and expressed in reciprocal centimeters. Ultraviolet spectrum (UV; sh=shoulder) was recoreded on a Cary Model 15 spectrophotometer. Elemental analysis was performed by Robertson Laboratory, Florham Park, New Jersey.

2-β-D-Ribofuranosylthiazole-4-carboxamide-N_3-oxide (3). To a solution of 2-β-D-ribofuranosylthiazole-4-carboxamide[1] (2, 2.60g. 10 mmol) in trifluoroacetic acid (15.0 ml) was added H_2O_2 (30%, 4 ml) and the reaction mixture was stirred at room temperature for 18 hr. Thin-layer chromatography (silica gel 60 F-254, EM Reagents, solvent - $CHCl_3$: MeOH, 6:1, v/v) indicated completion of the reaction. The reaction mixture was cooled to 0°C and carefully neutralized with conc. NH_4OH to the final pH of 8. The resulting solution was diluted with an equal volume of MeOH. After standing overnight, the crystallized material was collected by filtration, washed with cold MeOH (3 x 10 ml) and dried to give the title compound. Recrystallization from aqueous MeOH gave analytical sample, 1.50g (54.3%); mp 214-215°C; IR (KBr) ν 1685 (C=O of amide), 3070-3310 (OH, NH_2)cm^{-1}; UV λmax (pH1) 275 (sh) nm (ε 1,500); λmax (pH7) 275 (sh) nm (ε 800); λmax (pH11) 277 (sh) nm (ε 1,800); NMR (Me_2SO-d_6) δ 5.20 (d,1,$J_{1',2'}$ = 3.58 Hz, $C_1'\underline{H}$), 8.51 (s,1,$C_5\underline{H}$), 8.12 and 9.86 (2br s, 2, $CON\underline{H}_2$), and other sugar protons. Anal. Calcd for $C_9H_{12}N_2O_6S$: C, 39.13; H, 4.38; N, 10.14; S, 11.60. Found C, 39.04; H, 4.35; N, 10.18; S, 11.85.

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REFERENCES

1. Srivastava, P.C.; Pickering, M.V.; Allen, L.B.; Streeter, D.G.; Campbell, M.T.; Witkowski, J.T.; Sidwell, R.W.; Robins, R.K. J. Med. Chem. (1977), 20, 256-262.
2. Kirsi, J.J.; North, J.A.; McKernan, P.A.; Murray, B.K.; Canonico, P.G.; Huggins, J.W.; Srivastava, P.C.; Robins, R.K. Antimicrob. Agents Chemother. (1983), 24, 353-361
3. Huggins, J.W.; Robins, R.K.; Canonico, P.G. Antimicrob. Agents Chemother. (1984), 26, 476-480.
4. Sidwell, R.W.; Revankar, G.R.; Robins, R.K. Pharmac. Ther. (1985), in press.
5. Robins, R. K.; Srivastava, P.C.; Narayanan, V.L.; Plowman, J.; Paull, K.D. J. Med. Chem. (1982), 25, 107-108.
6. Robins, R.K.; Revankar, G.R. Med. Res. Rev. submitted.
7. Jayaram, H.N.; Dion, R.L.; Glazer, R.I.; Johns, D.G.; Robins, R.K.; Srivastava, P.C.; Cooney, D.A. Biochem. Pharmacol. (1982), 31, 2371-2380.
8. Kuttan, R.; Robins, R.K.; Saunders, P.P. Biochem. Biophys. Res. Commun. (1982), 107, 862-868.
9. Cooney, D.A.; Jayaram, H.N.; Gebeyehu, G.; Betts, C.R.; Kelley, J.A.; Marquez, V.E.; Johns, D.G. Biochem. Pharmacol. (1982), 31, 2133-2136.
10. Gebeyehu, G.; Marquez, V.E.; Cott, A.V.; Cooney, D.A.; Kelley, J.A.; Jayaram, H.N.; Ahluwalia, G.S.; Dion, R.L.; Wilson, Y.A.; Johns, D.G. J. Med. Chem. (1985), 28, 99-105.
11. Goldstein, B.M.; Takusagawa, F.; Berman, H.M.; Srivastava, P.C.; Robins, R.K. J. Am. Chem. Soc. (1983), 105, 7416-7422.
12. Lalitha, S.; Kannappan, V.; Nanjan, M. Indian J. Chem., Sect. A (1981), 20A, 714-716.
13. Metzger, J.; Vincent, E. Chem. Heterocycl. Compd. (1979), 34 (Pt 1), 26-41, 132-134.
14. Sheldrick, G.M. SHELX-76 "A Program for X-Ray Crystal Structure Determination", (1976), University of Cambridge, England.
15. Yoshioka, H.; Nakatsu, K.; Hayashi, M.; Mizuno, K. Tetrahedron Lett. (1975), 4031-4034.

16. Dalley, N.K.; Petrie III, C.R.; Revankar, G.R.; Robins, R.K. J. Am. Chem. Soc. submitted.

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