7.0 Hz), 3.4 (m, methylene H at C(4), 2 H), 3.6 (m, methylene H at C(1), 2 H), 3.68 (s, OCH_3, 3 H), 4.91 (m, olefinic H, 1 H), 7.0–8.0 (m, aromatic H, 4 H).

20d: ^1H NMR ($CDCl_3$) δ 6.0 (dd, olefinic H, 2 H).

1,2-Dihydrophenanthrene (21). This compound was synthesized by known methods:[20] ^1H NMR ($CDCl_3$) δ 2.2–2.5 (m, methylene H at C(2), 2H), 2.8–3.0 (m, methylene H at C(1), 2 H), 6.1–6.4 (m, olefinic H at C(3), 1 H), 7.2–7.3 (m, olefinic H at C(4), 1H), 7.2–8.2 (7, aromatic H, 6 H); UV (methanol) λ_{max} (log ϵ) 336 nm (3.59), 329 (3.69), 314 (3.88), 301 (3.79), 293 (3.79), 282 (3.62), 275 (3.60), 236 (4.66).

3,4-Dihydrophenanthrene (22) was synthesized by known methods:[20] ^1H NMR ($CDCl_3$) δ 2.3–2.6 (m, methylene H at C(3), 2 H), 3.1–3.3 (m, methylene H at C(4), 2 H), 6.0–6.2 (m, olefinic H at C(2), 1 H), 6.5–6.7 (m, olefinic H at C(1), 1H), 7.2–8.2 (m, aromatic H, 6 H); UV (methanol) λ_{max} (log ϵ) 345 nm (sh, 2.53), 323 (3.68), 311 (3.82), 299 (3.74), 259 (4.71), 249 (4.65), 241 (4.42), 233 (4.27), 212 (4.40).

3-Methyl-1,4-dihydrophenanthrene (24a) and 6-Methyl-1,4-dihydrophenanthrene (24b). An argon-flushed 10^{-3} M solution of *trans-p*-methylstilbene in *n*-propylamine was irradiated at 300 nm for 18 h. After evaporation of the solvent, the NMR spectrum of the crude reaction mixture revealed the presence of two isomers which were identified by comparison with the NMR spectrum of 3,5-dimethyl-1,4-dihydrophenanthrene[9] as **24a** and **24b**.

24a: ^1H NMR ($CDCl_3$) δ 1.92 (s, CH_3, 3 H), 3.3–3.7 (m, methylene H, 4 H), 5.73 (br s, olefinic H, 1 H), 7.1–8.0 (m, aromatic H, 6 H).

24b: ^1H NMR ($CDCl_3$) δ 2.54 (s, CH_3, 3 H), 3.3–3.7 (m, methylene H, 4 H), 6.06 (br s, olefinic H, 2 H), 7.1–8.0 (m, aromatic H, 5 H).

1,2-Dihydronaphthalene (33) was synthesized from α-tetralone following the procedure by which **31** and **32** were synthesized.[27] It was purified by bulb-to-bulb distillation.

1,4-Dihydronaphthalene (34). This compound was synthesized according to the procedure of Nieuwstad and van Bekkum.[33] Protonation of the anion to generate **34** was made by the addition of ethanol.

(33) Nieuwstad, Th. J.; van Bekkum, H. *Recl. Trav. Chim. Pays*-Bas **1972**, *91*, 1069.

Acknowledgment. Part of this investigation was carried out under the auspices of The Netherlands Foundation for Chemical Research (SON) with financial support from the Netherlands Organization for the Advancement of Pure Research (ZWO).

Registry No. (*E*)-**1**, 103-30-0; (*Z*)-**1**, 645-49-8; **6**, 20508-11-6; **9**, 69103-81-7; **10a**, 13054-95-0; **10b**, 14770-93-5; **11a**-d_2 (isomer 1), 94484-29-4; **11a**-d_2 (isomer 2), 94484-30-7; **11b**-d_2 (isomer 1), 94484-31-8; **11b**-d_2 (isomer 2), 94484-32-9; **13**, 20244-28-4; **13**-d_2 (isomer 1), 94484-38-5; **13**-d_2 (isomer 2), 94484-39-6; **14**, 776-35-2; **14**-d_2 (isomer 1), 94498-88-1; **15**, 2633-08-1; **16**, 7427-84-1; **17**, 28124-10-9; (*Z*)-**18**, 1657-53-0; (*E*)-**18**, 1694-19-5; **19a**, 1485-98-9; **19b**, 93370-99-1; **19c**, 93371-00-7; **20a**, 94484-21-6; **20a**-*d* (isomer 1), 94484-33-0; **20a**-*d* (isomer 2), 94484-34-1; **20a**-*d* (isomer 3), 94484-35-2; **20a**-*d* (isomer 4), 94484-36-3; **20b**, 94484-22-7; **20c**, 94484-23-8; **20d**, 94484-24-9; **21**, 56179-83-0; **21**-*d* (isomer 1), 94484-40-9; **21**-*d* (isomer 2), 94484-41-0; **21**-*d* (isomer 3), 94484-42-1; **21**-*d* (isomer 4), 94484-43-2; **22**, 38399-10-9; **23**, 4714-21-0; **24a**, 94484-25-0; **24b**, 94484-26-1; **25a**, 2039-70-5; **25b**, 94484-37-4; **26a**, 80663-26-9; **26b**, 80663-27-0; **33**, 447-53-0; **34**, 612-17-9; CD_3OD, 811-98-3; D_2O, 7789-20-0; $CH_3CH_2CH_2CHDND_2$, 94484-18-1; $NaOCH_3$, 124-41-4; *n*-PrSNa, 6898-84-6; *n*-PrSH, 107-03-9; CH_3OD, 1455-13-6; *t*-BuOK, 865-47-4; *t*-BuOH, 75-65-0; *N,N*-dideuterio-*n*-butylamine, 17529-81-6; tetraarsenichexabutylimide, 3690-32-2; cyclobutyldiphenylcarbinol, 4404-60-8; cyclobutyl(4-(trifluoromethyl)phenyl)phenylcarbinol, 94484-19-2; cyclobutyl(4-methoxyphenyl)phenylcarbinol, 94484-20-5; *n*-propylamine, 107-10-8; *trans-p*-methylstilbene, 1860-17-9; α-tetralone, 529-34-0; 3-methoxy-1,4-DHP, 94484-27-2; 3-methyl-9,10-DHP, 94484-28-3; cyclobutyl phenyl ketone, 5407-98-7; cyclobutanecarboxylic acid, 3721-95-7; cyclobutanecarbonyl chloride, 5006-22-4; benzene, 71-43-2; cyclohexane, 110-82-7; stilbene, 588-59-0; *tert*-butylamine, 75-64-9; methanol, 67-56-1; cyclohexaneamine, 108-91-8; naphthalene, 91-20-3.

Structural Studies of a New Antitumor and Antiviral Agent: Selenazofurin and Its α Anomer

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Contribution from the Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111, and the Cancer Research Center, Department of Chemistry, Brigham Young University, Provo, Utah 84602. *Received June 6, 1984*

Abstract: The crystal and molecular structures of the new antitumor and antiviral agent selenazofurin (2-β-D-ribofuranosylselenazole-4-carboxamide, $C_9H_{12}N_2O_5Se$ (I)) and its α anomer (2-α-D-ribofuranosylselenazole-4-carboxamide, $C_9H_{12}N_2O_5Se$ (II)) have been determined by using single-crystal X-ray diffraction techniques employing Cu Kα radiation. I crystallizes in space group $P2_12_12_1$ with cell dimensions a = 5.1284 (4) Å, b = 13.083 (1) Å, c = 16.536 (1) Å, and Z = 4. The structure was refined to a conventional R value of 0.049 for all data based on 2359 reflections. The α anomer crystallizes in space group $P2_12_12_1$ with cell dimensions a = 5.4545 (2) Å, b = 9.3964 (4) Å, c = 21.656 (1) Å, Z = 4 and was refined to R = 0.031 for all 1263 independent reflections. The absolute configuration of each structure was confirmed by refinement of the corresponding enantiomorph and application of Hamilton's significance test. The selenazole ring in each structure is close to planar and shows some conjugation. The sugar moiety in selenazofurin shows a C2′ endo pucker, and a C2′ exo pucker is seen in the α-anomer. In each structure the conformation about the C-glycosyl bond is such that the selenazole Se forms a close intramolecular contact with the furanose ring oxygen O1′. These results are compared to those obtained for the thiazole analogue tiazofurin, and a model for drug activity is discussed.

Selenazofurin (2-β-D-ribofuranosylselenazole-4-carboxamide,[1] NSC 340847 (I)) is the selenium analogue of the antitumor agent tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide, NSC 286193[5]). In this analogue the sulfur atom in the heterocyclic base is replaced by a selenium atom yielding a C-glycosyl selenazole. Selenazofurin is five times more cytotoxic than tiazofurin against P388 and L1210 cells in vitro and is about equally effective against Lewis lung carcinoma at slightly lower doses.[1,2,3] It is

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(1) Srivastava, P.; Robins, R. *J. Med. Chem.* **1983**, *26*, 445–448.
(2) Streeter, D.; Robins, R. *Biochem. Biophys. Res. Commun.* **1983**, *115*, 544–550.

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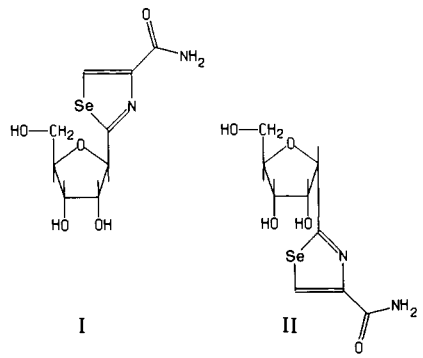
Table I. Crystal and Refinement Data

	I	II
space group	$P2_12_12_1$	$P2_12_12_1$
a, Å	5.1284 (4)	5.4545 (2)
b, Å	13.083 (1)	9.3964 (4)
c, Å	16.536 (1)	21.656 (1)
Z	4	4
M_r	307.2	307.2
V, Å^3	1109.5	1109.9
ρ(calcd), g/cm^3	1.839	1.838
no. of observations	2359	1263
no. of variables (n)	203	203
S	1.29	1.33
R, %	4.92	3.07
R_w	3.42	2.85
R_w (enantiomorph)	4.06	4.48

as active as tiazofurin against in vivo L1210 leukemia at one-tenth the dose and is effective against lines of leukemia made resistent to methotrexate, 5-fluorouracil, cisplatin, and other antineoplastic agents.[33] Further, selenazofurin exhibits broad spectrum antiviral activity against both DNA and RNA viruses.[4] It is in fact more effective than either tiazofurin or ribavirin, a related nucleoside analogue which is currently undergoing clinical evaluation as an antiviral agent. In contrast, the α anomer of selenazofurin (2-α-D-ribofuranosylselenazole-4-carboxamide (II)) is inactive as either an antitumor or antiviral agent.[6]

We present here the results of single-crystal X-ray diffraction studies of both selenazofurin and its α anomer. These are compared with results obtained in a recent structural study of the thiazole nucleoside tiazofurin, its 2′-deoxy derivative (2′-deoxytiazofurin), and its α anomer (α-tiazofurin).[7]



I II

Experimental Section

X-ray Data Collection. (a) 2-β-D-Ribofuranosylselenazole-4-carboxamide (I). Crystals were obtained as described in ref 1. Precession photographs and a preliminary rapid data scan by diffractometer revealed an orthorhombic crystal system. The only observed systematic absences were $h00$ for $h = 2n + 1$, $0k0$ for $k = 2n + 1$, and $00l$ for $l = 2n + 1$, indicating unambiguously space group $P2_12_12_1$. Cell dimension and intensity data were collected at room temperature from a colorless rectangular plate of approximate dimensions 0.15 × 0.10 × 0.02 mm mounted roughly parallel to the a axis. An Enraf-Nonius CAD-4 diffractometer was employed with a graphite monochromator and Cu Kα radiation. Lattice constants were obtained by least-squares refinement of the angular settings of 24 reflections in the range $\theta = 25$–$30°$. These are indicated in Table I. The lattice constants obtained for selenazofurin were close to those obtained for tiazofurin,[8] suggesting that the two crystals were isomorphous. This was confirmed by the final structure. Reflections were measured in the range $2° < \theta < 70°$ with use of the ω–2θ scan method with a variable scan width $\Delta\omega = (1.67 + 0.15 \tan\theta)°$, this angle being extended 25% on each side for background measurements. The scan rate varied between 0.6 and 2.0 deg/min depending upon the value of $\sigma(I)/I$ for each reflection. Data were collected in two octants ($-h,k,\pm l$). Only identical redundantly measured intensities were averaged, yielding a total of 2359 reflections. Of these, 152 had values of $|F_o|^2 < 0.2\sigma(F_o^2)$, where $\sigma(F_o^2) = [\sigma^2(I) + (0.02|F_o|^2)^2]^{1/2}$, and were reset to $0.2\sigma(F_o^2)$. All data were used in the subsequent analysis and refinements.

Three standard reflections measured every 2 h of X-ray exposure time showed an approximate 2% decline in intensity over the course of the data collection. A separate linear decay correction was applied to the set of reflections collected within each 2-h time period. Corrections for Lorentz and polarization factors were applied and, in addition, data were corrected for absorption with use of the semiempirical ψ-scan technique.[9] A single ψ scan was employed, consisting of 36 measurements of a standard reflection in 10° steps of ϕ with the crystal in an approximate equi-inclination setting.

(b) 2-α-D-Ribofuranosylselenazole-4-carboxamide (II). Colorless rod-shaped crystals were grown at room temperature by slow evaporation from a 7.5 mM aqueous solution of II. Preliminary photographic and counter measurements indicated an orthorhombic system with systematic absences $h00$ for $h = 2n + 1$, $0k0$ for $k = 2n + 1$, and $00l$ for $l = 2n + 1$, again defining the space group as $P2_12_12_1$. Cell dimension and intensity data were obtained from a crystal of approximate dimensions 0.35 × 0.05 × 0.05 mm mounted roughly parallel to the a axis. The CAD-4 diffractometer was used with graphite monochromatized Cu Kα radiation. Lattice constants were obtained as described above and are listed in Table I. Intensity data were collected by using the parameters and methods described above with the exception that the variable scan width was $\Delta\omega = (1.33 + 0.15 \tan\theta)°$; 2628 reflections (including standards) were measured in the two equivalent but non-centrosymmetrically related octants (h,k,l) and ($-h,-k,l$). In this case the data were averaged, yielding 1263 unique reflections, of which 13 had values of $|F_o|^2 < 0.2\sigma(F_o^2)$ and were reset to $0.2\sigma(F_o^2)$. All unique data were used in the subsequent analysis. Standard reflections measured as above revealed no decline in intensity over the course of the data collection. Data were corrected for absorption as described above.

Structure Solutions and Refinements. Initial non-hydrogen atom coordinates for I were obtained from the tiazofurin structure, with the sulfur atom replaced by a selenium atom. The selenium position in II was determined from a Patterson map, and the positions of the remaining non-hydrogen atoms were obtained from subsequent least-squares refinements and Fourier maps. In both cases the positions of all hydrogen atoms were obtained from successive difference Fourier maps, employing only low-angle data [$(\sin\theta)/\lambda < 0.4$ Å^{-1}].

Both structures were refined with use of full-matrix least-squares techniques. The function minimized was $\sum w(\Delta F)^2$ where $\Delta F = |F_o| - |F_c|$ with weights $w = 1/\sigma^2$. In the early refinements, values of σ were calculated according to $\sigma = \sigma(F_o^2)/2|F_o|$, where $\sigma(F_o^2)$ is defined as above. In the later refinements, weights $w = 1/\sigma_{new}^2$ were used where $\sigma_{new}^2 = [\sigma^2 + 0.5A|F_o|^2 + 0.5B[(\sin\theta)/\lambda]^2]^{1/2}$. Values of A and B were obtained by a least-squares minimization of the function of $|\Delta F|^2 - \sigma_{new}^2$ for 20 seperate segments in $|F_o|$ and $(\sin\theta)/\lambda$ for each data set. Non-hydrogen atoms were refined anisotropically. Positional parameters of all hydrogen atoms were refined with isotropic temperature factors. The later refinements of each structure included a type I isotropic extinction correction and utilized all data. Final refinements converged to the values of $R = \sum|\Delta F|/\sum|F_o|$ and $R_w = [\sum w(\Delta F)^2/\sum w|F_o|^2]^{1/2}$ listed in Table I. Also listed for each structure is the discrepancy factor $S = [\sum w(\Delta F)^2/(m - n)]^{1/2}$. The largest final parameter shift observed in both structures was 0.07σ. Atomic scattering factors for the non-hydrogen atoms and anomalous dispersion corrections for the selenium atoms were from ref 10. Scattering factors for the hydrogen atoms were those of Stewart et al.[11] Crystallographic programs developed at ICR were used throughout.[12]

Absolute Configuration Determinations. The enantiomorphs of structures I and II were also refined, using the same techniques described above. The values of R_w thus obtained are listed in Table I. Application

(3) Jayaram, H.; Ahluwalia, G.; Dion, R.; Gebeyehu, G.; Marquez, V.; Kelley, J.; Robins, R.; Cooney, D.; Johns, D. *Biochem. Pharmacol.* **1983**, *32*, 2633–2636.

(4) Kirsi, J.; North, J.; McKernan, P.; Murray, B.; Canonico, P.; Huggins, J.; Srivastava, P.; Robins, R. *Antimicrob. Agents Chemother.* **1983**, *24*, 353–361.

(5) Srivastava, P.; Pickering, M.; Allen, L.; Streeter, D.; Campbell, M.; Witkowski, J.; Sidwell, R.; Robins, R. *J. Med. Chem.* **1977**, *20*, 256–262.

(6) Robins, R.; Srivastava, P., unpublished data.

(7) Goldstein, B.; Takusagawa, F.; Berman, H.; Srivastava, P.; Robins, R. *J. Am. Chem. Soc.* **1983**, *105*, 7416–7422 and references therein.

(8) Lattice constants for tiazofurin are $a = 5.1053$ (5) Å, $b = 13.182$ (1) Å, $c = 16.268$ (1) Å.

(9) North, A.; Phillips, D.; Mathews, F. *Acta Crystallogr., Sect A* **1968**, *A24*, 351–359.

(10) "International Tables for X-ray Crystallography", 3rd ed.; Kynoch Press: Birmingham, England, 1974; Vol. IV, pp 99 and 149.

(11) Stewart, R.; Davidson, E.; Simpson, W. *J. Phys. Chem.* **1965**, *42*, 3175–3187.

(12) Takusagawa, F. "Crystallographic Computing System:DNA"; The Institute for Cancer Research: Fox Chase, PA, 1981.

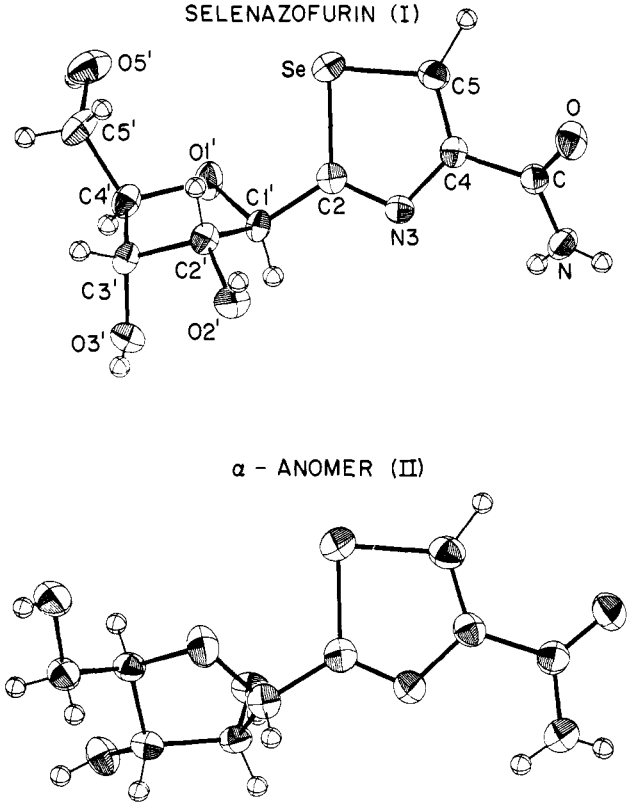


Figure 1. Conformations of the selenazofurin molecule (top) and its α anomer (bottom). Non-hydrogen atoms are represented by thermal ellipsoids at the 50% probability level.

of Hamilton's significance test[13] to each of the weighted R factor ratios confirmed the original absolute configuration assignments at the 99.5% significance level.

Results

The conformations of the two structures are illustrated in Figure 1. The crystal structure of selenazofurin is isomorphous with that of tiazofurin. α-Selenazofurin is approximately isostructural with α-tiazofurin. Atomic coordinates for I and II are listed in Table II. Bond lengths involving non-hydrogen atoms are listed in Table III. Selected bond and torsion angles are given in Tables IV and V, respectively. Corresponding values for tiazofurin and α-tiazofurin from ref 7 are listed in Tables III–V for comparison.

Selenazole Rings. As far as the authors are aware, these are the first crystal structure determinations of true selenazole rings. Bond lengths and angles in the two rings are similar, most agreeing within 3σ. Se–C bond lengths in both I and II are less than the unconjugated Se–C(sp^2) bond length seen in a selenazolidine derivative (1.909 (9) Å)[14] as well as that estimated for a single Se–C(sp^2) bond (ca. 1.93 Å).[15] They are within a standard deviation of the mean Se–C(sp^2) bond length observed in five well-refined ($R < 0.10$) selenophene derivatives (1.87 (2) Å).[16] Thus, the selenazole rings display some conjugation. In each structure the difference in bond length between Se–C5 and Se–C2 suggests greater contributions from resonance forms containing the fragment C5=Se^+—C2 rather than C5—Se^+=C2. Of interest is that the C5–Se–C2 bond angles in both structures are significantly smaller than that observed in the selenazolidine structure (88.6 (4)°)[14] or the mean C5–Se–C2 bond angle observed in the five selenophene derviatives (87 (1)°).[16]

The C2–N3, N3–C4, and C4–C5 bond lengths observed in selenazofurin and α-selenazofurin are close to those observed in the corresponding thiazole compounds, most agreeing within 3σ. Despite the variations in Se–C bond lengths discussed above, each Se–C bond is 0.141–0.151 Å longer than the corresponding S–C bond in tiazofurin or α-tiazofurin. In addition, the endocyclic bond angles about Se in I and II are both 5.1° smaller than those observed about S in tiazofurin and α-tiazofurin (89.4 (1)° and 89.6 (2)°, respectively). In both selenazole rings, the C5–C4–N3 and C4–N3–C2 bond angles are significantly larger than the

Table II. Atomic Coordinates and Isotropic Thermal Parameters (for Non-H Atoms $B = (4/3)\sum_i\sum_j\beta_{ij}\mathbf{a}_i\cdot\mathbf{a}_j$)

atom	x	y	z	B
	(a) Selenazofurin (I)			
SE	−0.02266 (9)	0.29412 (4)	0.06751 (3)	2.48
C2	0.2495 (9)	0.3635 (4)	0.1201 (3)	1.92
N3	0.2902 (8)	0.4561 (3)	0.0961 (2)	2.05
C4	0.1119 (9)	0.4848 (3)	0.0386 (3)	1.97
C5	−0.0684 (9)	0.4171 (4)	0.0128 (3)	2.33
C	0.1253 (9)	0.5925 (4)	0.0074 (3)	2.07
O	−0.0770 (7)	0.6381 (3)	−0.0117 (2)	2.84
N	0.3599 (9)	0.6332 (4)	0.0019 (3)	2.48
C1′	0.3967 (9)	0.3137 (3)	0.1876 (3)	1.85
C2′	0.2649 (9)	0.3234 (4)	0.2707 (3)	1.96
C3′	0.3977 (9)	0.2354 (4)	0.3151 (3)	2.00
C4′	0.4104 (9)	0.1533 (4)	0.2502 (3)	2.22
C5′	0.1935 (9)	0.0778 (4)	0.2561 (4)	3.03
O1′	0.3994 (7)	0.2063 (3)	0.1734 (2)	2.66
O2′	0.3141 (8)	0.4193 (3)	0.3070 (2)	2.45
O3′	0.6563 (7)	0.2626 (3)	0.3370 (2)	2.37
O5′	0.1775 (9)	0.0098 (3)	0.1885 (3)	3.69
HC5	−0.22 (1)	0.419 (4)	−0.035 (3)	5.3
H1N	0.50 (2)	0.595 (5)	0.009 (3)	6.0
H2N	0.37 (1)	0.692 (4)	−0.009 (3)	4.0
HC1′	0.58 (1)	0.340 (4)	0.189 (3)	2.7
HC2′	0.073 (9)	0.306 (3)	0.268 (2)	1.5
HC3′	0.300 (7)	0.213 (3)	0.364 (2)	1.0
HC4′	0.57 (1)	0.117 (4)	0.250 (3)	3.0
H1C5′	0.18 (1)	0.045 (4)	0.308 (3)	4.6
H2C5′	0.05 (2)	0.119 (5)	0.255 (3)	5.2
HO2′	0.19 (1)	0.437 (5)	0.311 (4)	2.7
HO3′	0.655 (9)	0.295 (3)	0.374 (2)	1.2
HO5′	0.29 (1)	−0.025 (5)	0.183 (4)	4.5
	(b) α-Selenazofurin (II)			
SE	−0.82049 (6)	0.32355 (3)	0.18262 (1)	3.19
C2	−0.6197 (5)	0.4765 (3)	0.2047 (1)	2.52
N3	−0.6562 (5)	0.5275 (3)	0.2594 (1)	2.64
C4	−0.8428 (6)	0.4557 (3)	0.2900 (1)	2.48
C5	−0.9543 (6)	0.3476 (3)	0.2603 (1)	2.88
C	−0.9003 (6)	0.5013 (3)	0.3545 (1)	2.71
O	−1.0690 (6)	0.4476 (3)	0.3841 (1)	3.94
N	−0.7589 (7)	0.6041 (3)	0.3760 (1)	3.56
C1′	−0.4347 (6)	0.5317 (3)	0.1602 (1)	2.56
C2′	−0.4984 (5)	0.6717 (3)	0.1283 (1)	2.18
C3′	−0.3444 (5)	0.6582 (3)	0.0702 (1)	2.12
C4′	−0.3727 (5)	0.5002 (3)	0.0535 (1)	2.21
C5′	−0.1513 (6)	0.4406 (3)	0.0222 (1)	2.58
O1′	−0.4106 (5)	0.4287 (2)	0.1116 (1)	3.41
O2′	−0.7514 (3)	0.6764 (3)	0.1141 (1)	2.67
O3′	−0.4232 (5)	0.7517 (2)	0.0236 (1)	2.83
O5′	−0.1912 (6)	0.2925 (2)	0.0094 (1)	3.46
HC5	−1.103 (8)	0.288 (5)	0.275 (2)	3.4
H1N	−0.64 (1)	0.633 (5)	0.354 (2)	4.3
H2N	−0.80 (1)	0.632 (6)	0.406 (3)	4.9
HC1′	−0.295 (8)	0.540 (4)	0.181 (2)	3.0
HC2′	−0.454 (8)	0.746 (4)	0.151 (2)	2.6
HC3′	−0.168 (7)	0.678 (4)	0.082 (2)	2.0
HC4′	−0.515 (8)	0.481 (4)	0.027 (2)	2.8
H1C5′	−0.121 (8)	0.484 (4)	−0.016 (2)	3.0
H2C5′	−0.012 (8)	0.456 (5)	0.046 (2)	3.3
HO2′	−0.783 (9)	0.751 (6)	0.116 (2)	3.2
HO3′	−0.31 (1)	0.766 (7)	0.000 (3)	5.1
HO5′	−0.07 (1)	0.266 (7)	−0.002 (3)	4.7

(13) Hamilton, W. *Acta Crystallog.* **1965**, *18*, 502–510.
(14) Delugeard, Y.; LePouezat-Guinier-Manach, Y.; Meinnel, J. *Cryst. Struct. Commun.* **1974**, *3*, 387–390.
(15) Hauge, S. *Acta Chem. Scand., Ser A.* **1979**, *33*, 317–322.
(16) (a) Aurivillius, B. *Chem. Scr.* **1971**, *1*, 21–29. (b) Nardelli, M.; Fava, G.; Giraldi, G. *Acta Crystallogr.* **1962**, *15*, 737–746. (c) Hope, H.; Knobler, C.; McCullough, J. *Acta Crystallogr., Sect B* **1970**, *B26*, 628–640. (d) Campsteyn, H.; Dupont, L.; Lamotte, J.; Vermeire, M. *Cryst. Struct. Commun.* **1977**, *6*, 639–643. (e) Ruban, G.; Zobel, D.; Kossmehl, G.; Sgustav, I. *Chem. Ber.* **1981**, *114*, 818–821.

Table III. Select Bond Distances (Å)

	selenazofurin (I) X = Se	tiazofurin X = S	α-selenazofurin (II) X = Se	α-tiazofurin X = S
C2–N3	1.292 (6)	1.301 (2)	1.292 (4)	1.311 (4)
N3–C4	1.371 (6)	1.376 (2)	1.390 (4)	1.385 (4)
C4–C5	1.350 (7)	1.356 (2)	1.348 (4)	1.350 (5)
C5–X	1.861 (5)	1.711 (2)	1.848 (3)	1.707 (3)
X–C2	1.878 (5)	1.727 (2)	1.869 (3)	1.720 (3)
C2–C1′	1.497 (7)	1.490 (2)	1.489 (4)	1.502 (4)
C4–C	1.501 (7)	1.489 (2)	1.493 (4)	1.482 (4)
C–O	1.238 (6)	1.239 (2)	1.230 (4)	1.244 (4)
C–N	1.319 (7)	1.316 (2)	1.321 (4)	1.317 (6)
C1′–C2′	1.536 (7)	1.526 (2)	1.526 (4)	1.520 (5)
C2′–C3′	1.526 (7)	1.533 (2)	1.519 (4)	1.530 (4)
C3′–C4′	1.519 (7)	1.528 (2)	1.536 (4)	1.512 (5)
C4′–C5′	1.491 (8)	1.499 (2)	1.494 (4)	1.510 (5)
C4′–O1′	1.448 (6)	1.439 (2)	1.442 (4)	1.442 (4)
O1′–C1′	1.424 (6)	1.426 (2)	1.435 (4)	1.447 (4)
C2′–O2′	1.413 (6)	1.413 (2)	1.415 (3)	1.424 (5)
C3′–O3′	1.420 (6)	1.417 (2)	1.406 (3)	1.412 (4)
C5′–O5′	1.431 (8)	1.433 (2)	1.435 (3)	1.423 (6)

Table IV. Select Bond Angles (deg)

	selenazofurin (I) X = Se	tiazofurin X = S	α-selenazofurin (II) X = Se	α-tiazofurin X = S
C2–X–C5	84.3 (2)	89.4 (1)	84.5 (1)	89.6 (2)
X–C5–C4	109.1 (3)	109.4 (1)	110.4 (2)	110.0 (2)
C5–C4–N3	119.7 (4)	116.3 (1)	117.9 (2)	116.0 (3)
C4–N3–C2	111.3 (4)	109.7 (1)	111.8 (2)	109.5 (3)
N3–C2–X	115.5 (3)	115.1 (1)	115.4 (2)	114.9 (2)
C1′–C2–N3	123.8 (4)	123.1 (1)	124.6 (3)	124.7 (3)
C1′–C2–X	120.6 (3)	121.6 (1)	119.9 (2)	120.2 (2)
C–C4–N3	117.7 (4)	119.0 (1)	117.5 (2)	122.3 (3)
C–C4–C5	122.6 (4)	124.6 (1)	124.6 (3)	121.5 (3)
C4–C–O	120.2 (4)	120.2 (2)	121.8 (3)	119.5 (3)
C4–C–N	116.4 (4)	116.4 (1)	114.6 (3)	117.5 (3)
O–C–N	123.4 (5)	123.4 (1)	123.6 (3)	123.0 (3)

Table V. Select Torsion Angles and Sugar Conformations (deg)

	selenazofurin (I) X = Se	tiazofurin X = S	α-selenazofurin (II) X = Se	α-tiazofurin X = S
κ(N–C–C4–N3)	34.2 (6)	33.1 (1)	−2.0 (4)	0.6 (2)
ϕ(O5′–C5′–C4′–C3′)	170.6 (4)	172.3 (5)	178.8 (2)	53.9 (3)
χ(O1′–C1′–C2–X)	30.5 (5)	30.67 (7)	−13.6 (3)	−20.8 (3)
τ_0(C4′–O1′–C1′–C2′)	−28.9 (5)	−30.4 (1)	19.1 (3)	15.1 (2)
τ_1(O1′–C1′–C2′–C3′)	42.3 (4)	42.6 (1)	−36.4 (3)	−36.7 (2)
τ_2(C1′–C2′–C3′–C4′)	−39.3 (5)	−38.4 (1)	39.3 (2)	44.2 (2)
τ_3(C2′–C3′–C4′–O1′)	23.5 (5)	21.8 (1)	−29.3 (3)	−36.7 (2)
τ_4(C3′–C4′–O1′–C1′)	3.4 (5)	5.2 (1)	6.4 (3)	13.7 (2)
τ_m	43.5	43.4	40.7	45.0
P	157.4	154.9	−9.2	−0.7

angles about the same bonds in the thiazole structures. This may be compensation for the longer heteroatom bond length observed in the selenazole rings.

Deviations from the least-squares planes of the selenazole rings are listed in Table VI. The ring in II is planar within experimental error. The ring in I shows a small but significant pucker, the C2 atom lying above the ring plane with the flanking Se and N3 atoms lying below the plane. This is consistent with the bending of the C2–C1′ bond implied by the 0.128 (5) Å deviation of C1′ above the ring plane. A similar distortion was observed in the tiazofurin and 2′-deoxytiazofurin structures and is probably due to crystal packing.

Carboxamide Groups. C4–C, C–O, and C–N bond lengths in I and II are similar. The C–C4 bond lengths in both structures are comparable with those observed in tiazofurin (1.489 (2) Å) and α-tiazofurin (1.482 (4) Å), suggesting little conjugation across this bond[7]. The C4–C bonds in both I and II are bent slightly out of the selenazole ring plane indicated by the 0.04-Å deviations of C from the least-squares plane in both structures. In the case of II, this bending is significantly less than that observed in the thiazole analogue (0.143 (7) Å deviation in C from the α-thiazole L.S. plane). This is probably the result of packing forces, as the carboxamide group in α-selenazofurin participates in fewer hydrogen bonds than does the group in α-tiazofurin (see below).

Table VI. Deviations from Selenazole Ring Least-Squares Planes (Å)

	I	II
Se[a]	−0.009 (1)	0.001 (1)
C2[a]	0.016 (5)	−0.002 (3)
N3[a]	−0.015 (4)	0.002 (3)
C4[a]	0.005 (5)	0.000 (3)
C5[a]	0.004 (5)	−0.001 (3)
C	0.040 (5)	0.040 (3)
C1′	0.128 (5)	−0.019 (3)

[a] Used in calculations of LS planes.

Rotation of the carboxamide group relative to the heterocyclic ring is indicated by the torsion angle κ(N–C–C4–N3), listed in Table V. The carboxamide group in selenazofurin shows a larger rotation about the C–C4 bond than is seen in α-selenazofurin.

Table VII. Hydrogen Bond Distances and Angles

D—H···Å	D···A, Å	H···A, Å	D-H-A, deg	symmetry of acceptor A
		I		
N—H1N···O	2.898 (6)	2.29 (8)	127 (5)	$1 + x, y, z$
N—H2N···O	3.013 (6)	2.26 (5)	158 (5)	$^1/_2 + x, {}^3/_2 - y, -z$
O2′—HO2′···O5′	2.787 (6)	2.12 (6)	172 (6)	$-x, {}^1/_2 + y, {}^1/_2 - z$
O3′—HO3′···O	2.848 (5)	2.12 (4)	166 (5)	$^1/_2 - x, 1 - y, {}^1/_2 + z$
O5′—HO5′···O2′	2.865 (6)	2.14 (7)	159 (6)	$1 - x, -{}^1/_2 + y, {}^1/_2 - z$
		II		
N—H2N···O5′	3.061 (4)	2.37 (5)	155 (6)	$-1 - x, {}^1/_2 + y, {}^1/_2 - z$
O2′—HO2′···O	2.730 (4)	2.02 (5)	170 (5)	$-2 - x, {}^1/_2 + y, {}^1/_2 - z$
O3′—HO3′···O3′	2.912 (3)	2.19 (7)	150 (6)	$^1/_2 + x, {}^3/_2 - y, -z$
O5′—HO5′···O5′	2.871 (4)	2.13 (7)	164 (6)	$^1/_2 + x, {}^1/_2 - y, -z$

This difference was seen between tiazofurin and α-tiazofurin, and it is also likely due to packing forces. The carboxamide group in I participates in a larger number of hydrogen bonds than does the group in II (see below). This may also account for the differences in bond angles about C and C4 seen between I and II.

In both selenazofurin and the α anomer, the carboxamide amino group is generally cis to the selenazole nitrogen N3 and the carboxamide oxygen cis to HC5 (Figure 1). This geometry was also observed in the three thiazole nucleosides. Its appearance in the two selenazole structures supports the suggestion that this is a low-energy conformation, allowing electrostatic interactions between one amino hydrogen (H1N) and the lone pair electrons of N3 and between the carboxyl oxygen and the C5 ring hydrogen (HC5).[7]

Sugar Moieties. Bond lengths in the ribose moieties of I and II are within the range of those seen in other β and α nucleosides[7,17] and are generally in good agreement with those found in the ribose moieties of tiazofurin and α-tiazofurin, respectively. The largest difference is found in the C3′–C4′ bond length in II which is 0.024 Å (~3.7σ) longer than that observed in α-tiazofurin (1.512 (5) Å).

The amplitude τ_m and phase angle P of pseudorotation[18] for each structure are given in Table V. Selenazofurin adopts a C2′ endo (^{2}E) pucker. This conformation is seen in tiazofurin and is common in β nucleosides.[7,19] The conformation of the α anomer is close to a C_2' exo ($_2$E) pucker, approximately enantiomeric to that seen in I. This is very similar to the C3′ endo–C2′ exo ($^3_2$T) pucker seen in α-tiazofurin (P = −0.7°). Sugar conformations in in this range are seen in other α ribonucleosides (e.g., P = −3°, −19°).[17b,c]

Conformations about the C4′–C5′ bond, defined by the torsion angle φ(O5′–C5′–C4′–C3′), are shown in Table V for I and II. Both fall within the range gauche–trans (t). This conformation was observed in tiazofurin and is more common in β nucleosides.[7,19] It is also seen in several α ribonucleosides.[17c,d] The conformation seen in α-tiazofurin was gauche–gauche (g+, φ = 53.9 (3)°). This represents the major difference in molecular geometry between α-selenazofurin and α-tiazofurin.

C–Glycosyl Bonds: Conformational Stabilization Revisited. The lengths of the C–glycosyl bonds C1′–C2 are similar to the mean values seen for other C–glycosyl nucleosides [1.50 (1) Å],[7] although somewhat shorter than analogous bonds in the one selenophene derivative with sp^3-hybridized carbons on the C2 and C5 positions [1.52 (2), 1.53 (2) Å, respectively].[16a]

The conformations about the C–glycosyl bonds are defined by the torsion angles χ(O1′–C1′–C2–Se) listed in Table V. In both structures these conformations are such that the selenium atom is cis to the furanose oxygen O1′ (Figure 2). In each case the

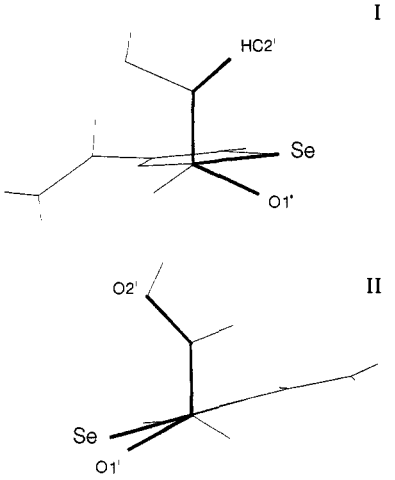


Figure 2. Views down the C–glycosyl bonds of the selenazofurin molecule (top) and its α anomer (bottom). Heavy bonds connect atoms involved in observed or potential close intramolecular contacts. Only part of each sugar moiety is shown for clarity.

distance between the Se and O1′ atoms (3.012 (3) Å in I, 2.888 (3) Å in II) is significantly less than the sum of their van der Waals radii (3.40 Å). Quite similar conformations with close heteroatom oxygen contacts were observed in tiazofurin, 2′-deoxytiazofurin, and α-tiazofurin. However, the absolute value of χ seen in α-selenazofurin is smaller than that observed in any of these structures.

Close intramolecular Se···O contacts have been noted in α-selenophenecarboxylic acid (3.046 Å)[16b] and selenoindigo (3.20 Å).[20] In addition, a large number of close intermolecular selenium contacts have been recently surveyed.[21] Most common among these are contacts between selenium and various nucleophiles, including oxygen. It has been suggested that nucleophilic atoms interact with an antibonding σ* orbital centered on selenium. These atoms approach Se approximately in the plane of the selenium ligands and along the back sides of these bonds.[21] There are some similarities between this geometry and that of the intramolecular Se···O1′ contacts observed here. In I and II, the vectors from Se to O1′ make angles of approximately 9 and 6°, respectively, with the C2–Se–C5 plane, although intramolecular constraints prevent O1′ from lying along the extension of the C5–Se bond.

Close heteroatom–O1′ contacts have now been observed in four thiazole nucleosides[7,32] and two selenazole nucleosides, suggesting that these interactions may stabilize the conformation about the C–glycosyl bonds in these structures. As in the thiazole nucleosides, rotation of the ring so as to minimize the heteroatom–O1′ interaction results in additional close contacts. In selenazofurin

(17) (a) Armstrong, V.; Dattagupta, J.; Eckstein, F.; Saenger, W. *Nucle. Acids Res.* **1976**, *3*, 1791–1810. (b) Post, M.; Birnbaum, G.; Huber, C.; Shugar, D. *Biochim. Biophys. Acta* **1977**, *479*, 133–142. (c) Rohrer, D.; Sundaralingam, M. *J. Am. Chem. Soc.* **1970**, *92*, 4950–4955. (d) Ruzic-Toros, Z. *Acta Crystallog., Sect. B* **1979**, *B35*, 1277–1280.

(18) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1972**, *94*, 8205–8211.

(19) Saenger, W. "Principles of Nucleic Acid Structure"; Springer-Verlag: New York, 1984; Chapter 4.

(20) (a) von Eller, H. *Bull. Soc. Chim. Fr.* **1955**, *106*, 1429–1433. (b) Susse, P.; Kunz, G.; Luttke, W. P. *Naturwissenschaften* **1973**, *60*, 49–50.

(21) (a) Ramasubbu, N.; Parthasarathy, R. In "Collected Abstracts: Conversation in Biomolecular Stereodynamics III"; Sarma, H., Ed.; Institute of Biomolecular Stereodynamics: Albany, 1983; pp 134 and 135. (b) Parthasarathy, R., private communication.

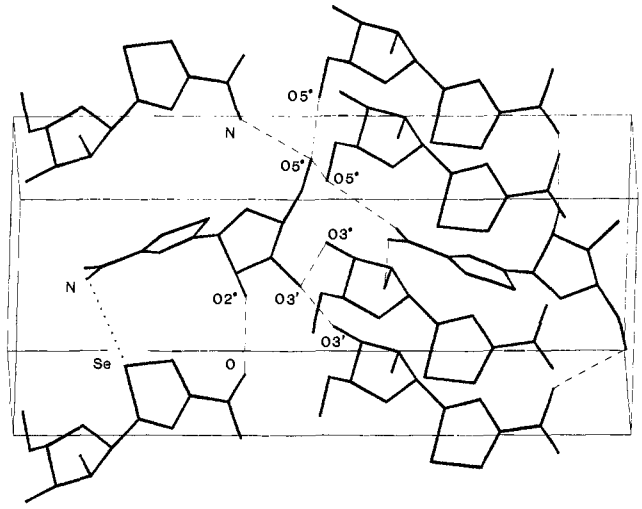


Figure 3. Molecular packing and hydrogen bonding in the α anomer viewed approximately down the crystallographic *a* axis. Thin dashed lines are hydrogen bonds. Hydrogen atoms and part of the unit cell contents are omitted for clarity. The dotted line illustrates the close Se···N contact.

(I), rotation of the ring to $\chi \simeq 94°$ (counterclockwise in Figure 2) produces a short contact of 2.69 Å between the selenium atom and the furanose hydrogen HC2′. In α-selenazofurin (II), rotation of the ring to $\chi \simeq -99°$ (clockwise in Figure 2) results in a very short contact of 2.50 Å between Se and O2′. Thus, free rotation about the C-glycosyl bonds may also be hindered by steric effects.

Molecular Packing and Hydrogen Bonding. Hydrogen bond distances and angles are listed in Table VII. The crystal packing in selenazofurin is isomorphous to that seen in tiazofurin and is not illustrated.[22] As seen before, all hydroxyl oxygens in I and II act as hydrogen bond donors and/or acceptors. In selenazofurin (I), the carboxamide oxygen is again capable of acting as an acceptor in three hydrogen bonds with the donor atoms in a distorted tetrahedral arrangement around the oxygen. Both carboxamide hydrogen atoms in I also act as hydrogen donors.

Although there are no selenazole ring stacking interactions in either I or II, there is a short Se···Se contact (3.591 (1) Å) in I. The vector connecting the two atoms is approximately normal to the C2–Se–C5 plane in one selenazole ring and approximately along the extension of the C2–Se bond in the other. This geometry is consistent with that seen in a survey of 24 structures containing 70 Se···Se contacts.[21] Analogous although longer S···S contacts are seen in tiazofurin (3.596 (1) Å) and α-tiazofurin (3.666 (1) Å), consistent with the results of a similar survey of S···S contacts.[23]

Crystal packing in II is illustrated in Figure 3. The carboxamide group in this structure participates in only two hydrogen bonds, H2N acting as a donor and O acting as an acceptor in one bond apiece. H1N does not participate in any intermolecular H bonding. There are no close Se···Se contacts in this structure. However, there is an interesting close Se···N contact of 3.335 (3) Å between Se and the carboxamide nitrogen of a neighboring molecule. The vector connecting these two atoms is approximately normal to the H1N–N–H2N plane and approximately along the extension of the C2–Se bond. This suggests that the nonhybridized p orbital on N is interacting with a Se antibonding orbital in the manner of a nucleophile.[21]

Discussion

The fact that selenazofurin and tiazofurin give isomorphous crystal structures indicates that, despite differences in bond lengths and angles noted above, the overall steric and hydrogen-bonding properties of the selenazole- and thiazole-4-carboxamide groups can be quite similar. Thus, it is not surpising that the two drugs show similar biochemical behavior. Like tiazofurin, selenazofurin

(22) See Figure 3, ref 7.
(23) Guru Row, T.; Parthasarathy, R. *J. Am. Chem. Soc.* **1981**, *103*, 477–479.

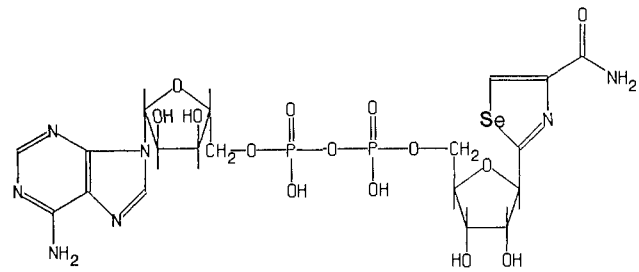


Figure 4. The NAD analogue SAD.

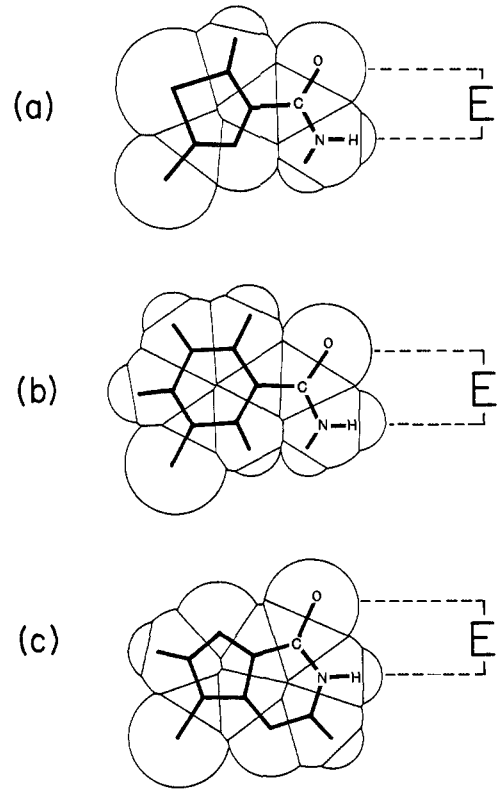


Figure 5. Comparison of the selenazole-4-carboxamide ring (a) with the nicotinamide ring (b)[29] and the base in inosine (c).[30] The potential similarity in IMPd (E) binding between the three moieties is illustrated schematically. All atoms are drawn at their van der Waals radii.[31] The carboxamide group torsion angle (κ) in parts a and b has been set to 0° for comparison.

is thought to exert its cytotoxic effects via inhibition of inosine monophosphate dehydrogenase (IMPd), resulting in a depletion of guanine nucleotides.[2,3] The primary IMPd inhibitor is a dinucleotide anabolite of selenazofurin, SAD. This compound is an analogue of nicotinamide adenine dinucleotide (NAD) in which the nicotinamide ring is replaced by the selenazole-4-carboxamide group (Figure 4).[2,3] SAD is thus the selenium analogue of TAD, the dinucleotide anabolite of tiazofurin.[2,3] Like TAD, SAD has much greater efficacy as an IMPd inhibitor ($K_i \sim 0.02$–0.06 μM) than either selenazofurin or its 5′-phosphate intermediate ($K_i \sim 140$ μM).[2,3]

The structural similarities between the selenazole-4-carboxamide group and the nicotinamide ring (Figure 5) suggest that SAD, like TAD, may be able to mimic NAD(H) binding to IMPd (although the finding that IMPd inhibition by TAD and SAD is non-competitive with respect to NAD[2,3] indicates that these dinucleotide analogues may be able to bind the enzyme at sites other than the regular cofactor binding site). Similarly, comparison of the selenazole-4-carboxamide group with the base in inosine (Figure 5) suggests that selenazofurin 5′-monophosphate (SMP) may also act as an analogue of the IMPd substrate inosine 5′-monophosphate. A similar mechanism has been proposed as one basis for the antiviral activity of the nucleoside analogue ribavirin.[24–26]

It has been suggested that the conformation about the C-glycosyl bond in the selenazole nucleosides may be restricted. If this occurs in solution, it would be expected to influence the stereochemistry of binding of SAD and/or SMP to IMPd.[7] Such restrictions could also affect the binding of selenazofurin and its 5'-phosphate to the enzymes involved in SAD synthesis. The inactive α anomer may have the same conformational restrictions as the active drug. However, the overall configuration of the α anomer, hence the location of its potential hydrogen-bonding groups, will remain distinct from that of the β form. Thus, it is likely that α-selenazofurin is either not converted to α-SAD or, if converted, the α-dinucleotide analogue cannot bind IMPd.[7]

Although selenazofurin behaves in many ways like its thiazole analogue, there are clearly differences between the two compounds and their anabolites. SAD is approximately three times more effective than TAD in inhibiting IMPd and is produced in greater quantities by P388 cells.[1-3] Most interesting is the greater efficacy of selenazofurin vs. tiazofurin as an antiviral agent.[4] The differences in size and conformation between the thiazole and selenazole rings are probably not by themselves sufficient to account for the differences in activity observed between the two drugs. However, the selenazole ring is also more reactive than the thiazole moiety. It is, for example, more susceptible to electrophilic substitution at the 5-position and is cleaved more easily.[27,28] Such differences in chemical reactivity may well contribute to the variations seen in drug activity.

Acknowledgment. This research was supported by the National Institutes of Health through Grants GM 21589, RR 05539, CA 06927, and CA 09035 and through an appropriation by the Commonwealth of Pennsylvania.

Registry No. I, 83705-13-9; II, 83705-14-0; tiazofurin, 60084-10-8; α-tiazofurin, 61502-38-3.

Supplementary Material Available: Anisotropic thermal parameters and listings of observed and calculated structure amplitudes for both structures (Tables VIII and IX, respectively) (20 pages). Ordering information is given on any current masthead page.

(24) Streeter, D.; Witkowski, J.; Khare, G.; Sidwell, R.; Bauer, R.; Robins, R.; Simon, L *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 1174-1178.
(25) Prusiner, P.; Sundaralingam, M. *Nature (London), New Biology* **1973**, *244*, 116-117.
(26) For an alternative mechanism, see: Goswami, B.; Borek, E.; Sharma, O.; Fujitaki, J.; Smith, R. *Biochem. Biophys. Res. Commun.* **1979**, *89*, 830-836.
(27) Bulka, E. In "Organic Selenium Compounds: Their Chemistry and Biology"; Klayman, D., Gunther, W., Eds.; Wiley-Interscience: New York, 1973; pp 470-478.
(28) Guglielmetti, R. *Chem. Heterocycl. Compd.* **1979**, *34* (*Part 3*), 239-248.
(29) Voet, D. *J. Am. Chem. Soc.* **1973**, *95*, 3763-3770.
(30) Brown, E.; Bugg, C. *Acta Crystallogr., Sect. B.* **1980**, *B36*, 2597-2604.
(31) Bondi, A. *J. Phys. Chem.* **1964**, *68*, 441.
(32) Close S···O contacts have recently been observed in two independent molecules of the xylofuranosyl analogue of tiazofurin. Goldstein, B.; Mao, D.; Marquez, V. In "Abstracts of Papers"; 189th National Meeting of the American Chemical Society, Miami, FL, 1985; in press.
(33) Burchenal, J.; Pancoast, T.; Carroll, A.; Elslager, E.; Robins, R. *Proc. Am. Assoc. Cancer Res.* **1984**, *25*, Abstract No. 1375.

Conformational Analysis of a Cyclic Pentapeptide by One- and Two-Dimensional Nuclear Overhauser Effect Spectroscopy

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Abstract: Detailed conformational analysis of the cyclic pentapeptide cyclo(D-phenylalanyl-L-prolylglycyl-D-alanyl-L-prolyl) in chloroform was carried out by one- and two-dimensional proton nuclear magnetic resonance spectroscopy. Two-dimensional *J*-resolved and spin-echo correlated spectroscopy were performed in order to verify the previous proton line assignments obtained for this peptide. The solution conformation was determined from measurement of one- and two-dimensional nuclear Overhauser effects. Quantitative interproton distances were determined from the time dependence of transient nuclear Overhauser effects, and these distances were compared to those obtained from the buildup rate of cross-peak intensities in a series of two-dimensional nuclear Overhauser effect spectra obtained with different mixing times. There was excellent agreement between the distances obtained by the two methods, and both methods yielded interproton distances with an average uncertainty of 0.2 Å. Furthermore, striking agreement was observed between interproton distances obtained for the peptide in solution and those for the crystal structure of this peptide as solved by X-ray diffraction. This indicates that the crystal structure, which contains one transannular hydrogen bond in a β turn and one in a γ turn, is essentially retained in solution.

Proton nuclear magnetic resonance (NMR) spectroscopy is a powerful tool for investigating the solution conformation of peptides and proteins. While much information can be obtained from chemical shifts and coupling constants, one of the best tools for conformational analysis is the nuclear Overhauser effect (NOE).[1] Conventional (1-D) steady-state and transient NOE experiments have been applied extensively to the conformational analysis of peptides[2-5] and proteins,[6-10] and two-dimensional nuclear Overhauser effect (2-D NOE) spectroscopy has been used to study the general conformational features of peptides[11,12] and

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(1) Smith, J. A.; Pease, L. G. *CRC Crit. Rev. Biochem.* **1980**, *8*, 315.
(2) Balaram, P.; Bothner-By, A. A.; Dadok, S. *J. Am. Chem. Soc.* **1972**, *94*, 4015.
(3) Bothner-By, A. A.; Johner, P. E. *Biophys. J.* **1978**, *24*, 779.
(4) Williamson, M. P.; Williams, D. H. *J. Am. Chem. Soc.* **1981**, *103*, 6580.
(5) Roques, B. P.; Rao, R.; Marion, D. *Biochimie* **1980**, *62*, 753.
(6) Dubs, A.; Wagner, G.; Wüthrich, K. *Biochim. Biophys. Acta* **1979**, *577*, 177.

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