concd *in vacuo*. The residue was dild with H_2O, and the mixt was extd with CH_2Cl_2. The exts were washed with H_2O, dried, and concd to leave 55.2 g (96%) of a colorless liquid, bp 169–174° (0.4 mm). *Anal.* ($C_{15}H_{19}NO_2$) H, N; C: calcd, 73.44; found, 72.45.

1,1-Diphenyl-4-(2-ketopiperidinyl)butanol (10). To a soln of PhMgBr, prepd from 23.5 g (0.15 mole) of PhBr and 2.6 g (0.15 g-atom) of Mg in 150 ml of THF, at 0° was added dropwise a soln of 24.5 g (0.1 mole) of 4-(2-ketopiperidinyl)butyrophenone in 150 ml of THF. After being stirred at 0° for 5 hr, the mixt was concd *in vacuo*. The residue was dild with excess NH_4Cl–H_2O. The resulting mixt was extd with CH_2Cl_2. The exts were dried and concd to leave 23.9 g of colorless crystals, mp 173–174° (EtOH). *Anal.* ($C_{21}H_{25}NO_2$) C, H, N.

A sample of 1-[^{3}H]Ph-**10** was prepd from [*G*-^{3}H]PhBr (sp activity 50 mCi/mg) in the same way.

***N*-(4,4-Diphenyl-4-hydroxybutyl)-δ-aminovaleric Acid (11).** A mixt of 8 g (0.025 mole) of **10** in 275 ml of EtOH and 37 g (0.12 mole) of $Ba(OH)_2 \cdot 8H_2O$ in 165 ml of H_2O was stirred and refluxed for 48 hr. After distn of EtOH, the mixt was cooled to 0° and 150 ml of 2 *N* H_2SO_4 was added dropwise. The mixt was stirred for 15 min, then it was made alk (2 *N* KOH), heated to boiling, and filtered through Super-Cel. The filtrate was cooled to 0° and brought to pH 7 with AcOH. The cryst ppt was filtered and washed (H_2O, EtOH, and Et_2O) to give 6.6 g (78%) of colorless crystals, mp 207–209° dec. *Anal.* ($C_{21}H_{27}NO_3$) C, H, N.

A sample of 4-[^{3}H]Ph-**11** was prepd from **1**-[^{3}H]Ph-**10** by the same procedure. Radiochemical purity was 99% as determined by segmentation[21] of the tlc (system 4) and liquid scintillation counting.

Lactamization of 11. A suspension of 0.5 g (1.5 mmoles) of **11** in 35 ml of xylene was stirred and refluxed for 1 hr. The resulting soln was concd *in vacuo* and the residue was crystd from EtOH to give 0.45 g (97%) of colorless crystals, mp and mmp with **10**, 173–174°.

Acknowledgment. We are indebted to Mr. George C. Heil for the pharmacological testing results.

References

(1) J. E. Swagzdis, T. L. Flanagan, C. Kaiser, C. L. Zirkle, and H. Green, *Pharmacologist*, 9, 215 (1967).
(2) C. A. Leonard, T. Fujita, D. H. Tedeschi, C. L. Zirkle, and E. J. Fellows, *J. Pharmacol. Exp. Ther.*, **154**, 339 (1966).
(3) R. T. Williams, "Detoxication Mechanisms," Chapman and Hall, Ltd., London, 1959.
(4) D. V. Parke, "The Biochemistry of Foreign Compounds," Pergamon Press, Oxford, 1968.
(5) H. House and J. W. Blaker, *J. Org. Chem.*, **23**, 334 (1958).
(6) F. Feigl, "Spot Tests in Organic Analysis," 6th ed, Elsevier Publishing Co., Amsterdam, 1960, p 273.
(7) J. L. Lambert and V. E. Cates, *Anal. Chem.*, **29**, 508 (1957).
(8) G. A. Lugg, *ibid.*, **35**, 899 (1963).
(9) L. Katz and L. S. Karger, *J. Amer. Chem. Soc.*, **74**, 4085 (1952).
(10) C. Kaiser, A. M. Pavloff, E. Garvey, P. J. Fowler, D. H. Tedeschi, C. L. Zirkle, E. A. Nodiff, and A. J. Saggiomo, *J. Med. Chem.*, **15**, 665 (1972).
(11) H. Steudel, *Hoppe-Seyler's Z. Physiol. Chem.*, **213**, 11 (1932).
(12) H. McKennis, Jr., L. B. Turnbull, and E. R. Bowman, *J. Amer. Chem. Soc.*, 79, 6342 (1957).
(13) H. McKennis, Jr., L. B. Turnbull, and E. R. Bowman, *ibid.*, **80**, 6597 (1958).
(14) H. B. Hucker, J. R. Gillette, and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, **129**, 94 (1960).
(15) A. K. Cho, A. S. Fairhurst, W. L. Haslett, and D. J. Jenden, *Proc. Int. Pharmacol. Meet. 2nd*, **1963**, 75 (1964).
(16) W. Hammer, B. Holmstedt, B. Karlén, F. Sjöqvist, and J. Vessman, *Biochem. Pharmacol.*, **17**, 1931 (1968).
(17) G. Chen and C. R. Ensor, *J. Pharmacol. Exp. Ther.*, 98, 245 (1950).
(18) I. M. Hais and K. Macek, "Paper Chromatography, A Comprehensive Treatise," Publishing House Czechoslovakia Academy of Science, Prague, 1963.
(19) A. Marxer, *Helv. Chim. Acta*, **24**, 209E (1941).
(20) Valette, *Bull. Soc. Chim. Fr.*, **47**, 289 (1930); *Chem. Abstr.*, 24, 3005 (1930).
(21) F. Snyder and H. Kimble, *Anal. Biochem.*, 11, 510 (1965).

Design, Synthesis, and Broad Spectrum Antiviral Activity of 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide† and Related Nucleosides[1,2]

J. T. Witkowski,* Roland K. Robins, Robert W. Sidwell, and Lionel N. Simon

ICN Nucleic Acid Reserach Institute, Irvine, California 92664. *Received May 12, 1972*

The first synthetic broad-spectrum, noninterferon-inducing, antiviral agent 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (**1**) has been prepared and tested against a variety of both RNA and DNA viruses in tissue culture. The syntheses of **1** and related nucleosides by the silylation–glycosylation procedure and the acid-catalyzed fusion procedure are described. The concepts underlying the development of this agent are discussed. Comparison of antiviral activity is made with the structurally related nucleoside antibiotics pyrazomycin, formycin, and showdomycin. Reproducible broad-spectrum antiviral activity both *in vitro* and *in vivo* at nontoxic dosage levels is shown by **1**. These findings demonstrate the feasibility of practical antiviral chemotherapy despite the problem that viral infection is intimately linked to the biochemistry of the host cell. This nucleoside (**1**) should prove a most useful probe in the study of the molecular biology of virus replication.

Although broad-spectrum antibacterial agents and antibiotics have been developed to wide clinical usefulness over the period of the past 40 years, the comparable screening effort in the direction of the development of antiviral agents has yielded results far short of the same degree of success.[3-6] This situation prevails despite the fact that respiratory diseases, principally of viral origin, are responsible for more than 50% of all acute human illnesses.[7-9]

In searching for a broad-spectrum antiviral agent an effort was made to concentrate on the synthesis of compounds which have the potential to affect enzymatic processes which are common to all known viruses such as viral-induced nucleic acid and protein synthesis. These processes are carried out by enzymes specifically coded for in the viral genome. Another common feature of all viruses is their lack of protein-synthesizing capability. It is conceivable that initiation of virus-specific protein synthesis and/or RNA synthesis may utilize unique viral enzymes which could be specifically inhibited.

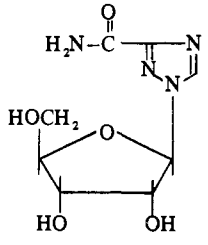
Several nucleosides such as 5-iodo-2′-deoxyuridine and 1-β-D-arabinofuranosylcytosine have been used with limited success against herpes virus infection in man.[10-12] One of the more potentially clinically useful agents, 9-β-D-arabino-

†1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide is identified by the International Chemical and Nuclear Corporation by the name Virazole.

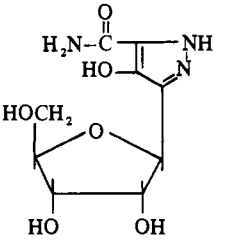
furanosyladenine, possesses marked antiviral activity *in vitro* and *in vivo* against herpes and vaccinia viruses.[5,13-19] It should be noted, however, that these nucleosides are active only against certain DNA viruses and, therefore, possess a very limited antiviral spectrum.

More recently the ribofuranosyl nucleoside antibiotic pyrazomycin, 3-β-D-ribofuranosyl-4-hydroxypyrazole-5-carboxamide (**2**), has been shown to have antiviral activity against rhinovirus, measles, herpes simplex, and vaccinia viruses in tissue culture.[20-22] Apparently inhibition of viral replication *in vivo* and toxicity to the host could be only partially separated[23] in studies of **2**. Another ribonucleoside antibiotic, formycin,[24] has also demonstrated *in vitro* antiviral activity.[25,26] These considerations led us to concentrate on the synthesis of a substantial number of β-D-ribofuranosyl derivatives as potential wide-spectrum antiviral agents.

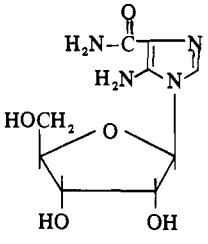
The ribonucleoside antibiotic, showdomycin[27] (**4**), is an example of a biologically active nucleoside with the β-D-ribofuranose moiety attached to a 5-membered heterocyclic ring. Further studies with other 5-membered heterocycles have resulted in the synthesis of β-D-ribofuranosyl derivatives of various imidazoles[28-30] and 1,2,4-triazoles.[31]



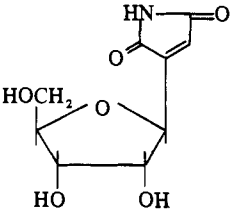
1



2



3



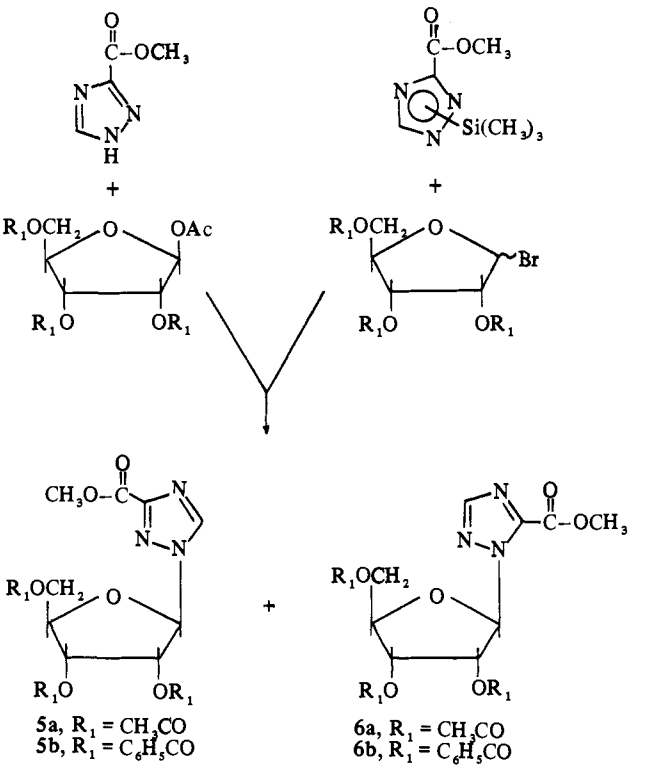
4

In our present work we have investigated the synthesis of 1,2,4-triazole nucleosides with carboxamide and related substituents. The nucleoside 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (**1**) is structurally related to 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (**3**). The structural similarity of **1** and pyrazomycin (**2**) is also apparent. The synthesis of **1** was approached as follows.

Treatment of the trimethylsilyl derivative of methyl 1,2,4-triazole-3-carboxylate with an acyl-blocked ribofuranosyl bromide in MeCN at room temperature provided a 1:1 mixture of the two nucleosides **5** and **6** in greater than 90% yield. These isomers were readily separated by chromatography of the blocked nucleosides over silica gel.

Alternatively, the acid-catalyzed fusion procedure[32] with methyl 1,2,4-triazole-3-carboxylate and 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose or 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose provided an 85% yield of the same nucleosides **5** and **6** in a 10:1 ratio.

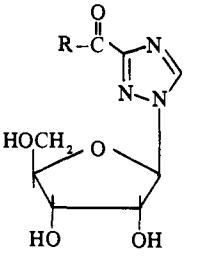
Treatment of the blocked methyl ester nucleosides **5** and **6** with methanolic NH_3 provided 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (**1**) and 1-β-D-ribofuranosyl-1,2,4-triazole-5-carboxamide (**7**), respectively.



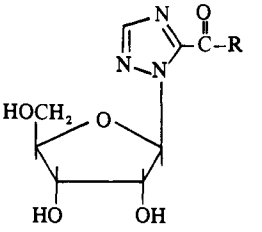
5a, $R_1 = CH_3CO$
5b, $R_1 = C_6H_5CO$

6a, $R_1 = CH_3CO$
6b, $R_1 = C_6H_5CO$

Deacylation of **5** and **6** with NaOMe in MeOH afforded the corresponding deblocked methyl ester nucleosides **8** and **9** which were convenient intermediates for preparing other nucleosides. The carboxhydrazides (**10** and **11**) of each isomer were readily obtained on treatment of **8** and **9** with N_2H_4 at room temperature. Similarly the carbohydroxamic acid **12** was prepared from methyl ester **8** and NH_2OH.



1, R = NH_2
8, R = OCH_3
10, R = $NHNH_2$
12, R = NHOH
13, R = OH



7, R = NH_2
9, R = OCH_3
11, R = $NHNH_2$
14, R = OH

The structures of these nucleosides have been assigned[1,33] on the basis of their pmr and ^{13}C nmr spectra and by chemical degradation. Hydrolysis of both methyl esters **8** and **9** with aqueous base and subsequent decarboxylation of the carboxylic acids **13** and **14** provided in each case 1-β-D-ribofuranosyl-1,2,4-triazole (**15**) which eliminated the possibility of glycosylation at N-4 of the triazole. This degradation also served to establish the β configuration of these nucleosides since the synthesis and assignment of structure of **15** have previously been reported.[31] While 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxylic acid (**13**) required heating in DMSO at 180° for decarboxylation, the isomeric nucleoside **14** decarboxylated at room temperature on acidification of the Na salt with Dowex 50 (H).

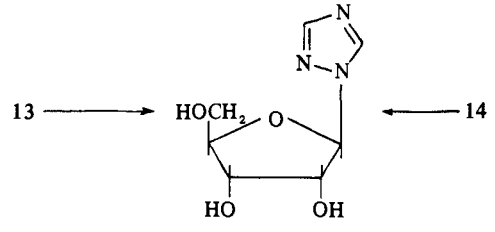
1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (**1**) has been found in our laboratory to possess highly significant antiviral activity in tissue culture against adenovirus, herpes

Table I. *In Vitro* Antiviral Activity of 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (1)

Virus	Parameter(s)[a] for evaluation	Activity[b]
DNA viruses		
Type 1 herpes simplex	CPE, VTR	+
Type 2 herpes simplex	CPE	+
Pseudorabies	CPE	±[c]
Murine cytomegalo	CPE	+
Vaccinia	CPE, VTR	+
Myxoma	CPE	+
Type 3 adeno	CPE	+
RNA viruses		
Type 3 parainfluenza	CPE, VTR	+
Type 1 parainfluenza	CPE, HAR, VTR	+
Influenza A_2	CPE, HAR	+
Influenza B	HAR	+
Type 1A rhino	CPE	+
Type 13 rhino	CPE	+
Type 56 rhino	CPE	+
Coxsackie	CPE, VTR	±
Type 2 polio	CPE	±[c]
Vesicular stomatitis	CPE, PR, VTR	+

[a] CPE = Inhibition of virus-induced cytopathogenic effects as determined by microscopic examination of cells treated with 1 72 hr after exposure to virus. VTR = Virus titer reduction in supernate and washed cells of cultures treated with 1 72 hr after exposure to virus. HAR = Hemagglutinin reduction in supernates of cells treated with 1 72 hr after exposure to virus. PR = Plaque reduction in cells treated with 1 72 hr after exposure to virus. [b] + = Significant CPE inhibition, VTR, HAR, or PR. Whenever feasible, activity was considered "+" if essentially equivalent to that seen using "known positive" drugs (*i.e.*, drugs reported by others to be active against the particular virus). The known positive drugs used included 5-iodo-2'-deoxyuridine, 9-β-D-arabinofuranosylcytosine (all DNA viruses except adenovirus), 1-adamantanamine·HCl (influenza A_2, influenza B), 2-methyl-4-[(5-methyl-5*H*-*as*-triazino[5,6-*b*]indol-3-yl)amino]-2-butanol (rhino viruses), pyrazomycin (rhino viruses, vesicular stomatitis virus), and guanidine·HCl (polio virus). ± = Moderate CPE inhibition, VTR, HAR, or PR, indicated when definite, reproducible activity which was less extensive than that exhibited by known positive compounds was demonstrated. – = Essentially no CPE inhibition, VTR, HAR, or PR, at any concentration of the compound. [c] Moderate antiviral activity seen against these viruses only if the cells were preexposed to 1.

virus type 1 and type 2, vaccinia virus, myxoma virus, parainfluenza virus, rhinovirus, coxsackie virus, influenza A_2 virus, and influenza B virus[1,2,34] (see Table I). In these



15

experiments, concentrations of 1 ranging in one-half log levels from 1 to 1000 μg/ml were used against a standard dose (100 cell culture 50% infectious doses) of virus. Antiviral activity was seen using concentrations of 1 ranging as low as 32 to 1 μg/ml, depending on the virus utilized.

The broad-spectrum antiviral activity of 1 has been confirmed with both DNA and RNA viruses *in vivo*[2,34] against established infections (see Table II). However, 1 has proved negative *in vivo* against herpes simplex encephalitis, vaccinia, and other types of encephalitis presumably due to the inability of 1 to cross the blood-brain barrier.

A comparison of the *in vitro* antiviral activity of 1, pyrazomycin,‡ formycin,[24] and showdomycin[27] is presented in Table III. The data indicate the general superiority of 1 in the systems tested. Showdomycin was essentially negative against the viruses chosen for our study.

The single-dose LD_{50} of 1 in mice is 1.3 g/kg ip and in the rat, 5.3 g/kg orally. This nucleoside (1) has been shown *not* to induce interferon in mice. The mechanism of action of 1 appears to involve an effect on macromolecular processes of viral replication. The precise site and mode of action of 1 is presently under active investigation. The practical utility of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (1) remains a subject of great interest and further study.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Evaporations were accomplished with a Buchler rotating evaporater under reduced pressure with a bath temp of <35°. The pmr spectra were recorded at 60 MHz on a Perkin-Elmer Hitachi R20A spectrometer and chemical shifts are reported in parts per million (δ) with DSS as an internal reference. Specific rotations were determined with a Perkin-Elmer Model 141 polarimeter. Analytical results were within ±0.4% of the theoretical values.

1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylic Acid Methyl Ester (5a) and 1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-5-carboxylic Acid Methyl Ester (6a). Method 1. A mixture of methyl 1,2,4-triazole-3-carboxylate[42] (12.7 g, 0.10 mole) and 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (31.8 g, 0.10 mole) was heated in an oil bath maintained at 160–165° until the sugar had melted. Bis(*p*-nitrophenyl)phosphate (250 mg) was added and heating at 160–165° was continued under diminished pressure for 15–20 min. The residue was crystd from MeOH to give **5a** (30.0 g, 78%) with mp 107–109°. *Anal.* ($C_{15}H_{19}N_3O_9$) C, H, N.

Chromatography of the filtrate from crystn of **5a** over silica gel with $CHCl_3$–Me_2CO (20:1) provided 2.8 g (7.3%) of **6a** as a syrup. *Anal.* ($C_{15}H_{19}N_3O_9$) C, H, N.

Method 2. A soln of the trimethylsilyl derivative of methyl 1,2,4-triazole-3-carboxylate (prepd by treatment of 4.19 g, 33.0 mmoles of the ester with excess hexamethyldisilazane at reflux) and 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide (from 9.54 g, 30.0 mmoles of the 1-*O*-acetyl deriv) in dry MeCN (200 ml) was kept at 25° for 3 days. The soln was then evapd to dryness, and the residue was dissolved in $CHCl_3$ and applied to a silica gel (265 g) column. Elution with $CHCl_3$–Me_2CO (20:1) provided **6a** (5.28 g, 46%) as the first product and **5a** (5.85 g, 51%) in subsequent fractions.

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl-1,2,4-triazole-3-carboxylic acid methyl ester (5b) [mp 137–139°, *Anal.* ($C_{30}H_{25}N_3O_9$) C, H, N] and **1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazole-5-carboxylic acid methyl ester (6b)** [mp 123–124°, *Anal.* ($C_{30}H_{25}N_3O_9$) C, H, N] were prepd by the same procedures (methods 1 and 2) used in the prepn of **5a** and **6a** above in essentially the same yields.

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (1). Method 1. A soln of **5a** (10.0 g, 26.0 mmoles) in MeOH (70 ml) satd at 0° with NH_3 was kept in a sealed pressure flask at 25° for 18 hr. The solvent was removed, and the product was crystd from EtOH to give **1** (5.70 g, 90%) with mp 174–176°. Recrystn of the product from aqueous EtOH provided a second cryst form of the nucleoside with mp 166–168°: $[\alpha]^{25}D$ –36.5° (*c* 1.0, H_2O); nmr (DMSO-d_6) δ 5.85 (d, 1, $J_{1',2'}$ = 3.8 Hz, 1'-H), 8.91 (s, 1, 5-H). *Anal.* ($C_8H_{12}N_4O_5$) C, H, N.

Method 2. A soln of **5b** (16.0 g, 28.0 mmoles) in MeOH (250 ml) satd at 0° with NH_3 was kept in a sealed pressure flask at 25° for 3 days. The solvent was removed, and the product was crystd from EtOH to give **1** (6.70 g, 98%) with mp 174–176°.

1-β-D-Ribofuranosyl-1,2,4-triazole-5-carboxamide (7). Method 1. Treatment of the methyl ester **6a** (2.09 g, 5.20 mmoles) with MeOH (50 ml) satd at 0° with NH_3 for 16 hr at 25° provided **7** (1.0 g, 79%) with mp 148–150° (from EtOAc–MeOH); $[\alpha]^{25}D$ –48.0° (*c* 1.0, H_2O); nmr (DMSO-d_6) δ 6.77 (d, 1, $J_{1',2'}$ = 3.0 Hz, 1'-H), 8.16 (s, 1, 3-H). *Anal.* ($C_8H_{12}N_4O_5$) C, H, N.

Method 2. A soln of **6b** (6.0 g, 10.5 mmoles) and MeOH (60 ml) satd at 0° with NH_3 was kept in a sealed pressure flask at 25° for 3 days to provide **7** (2.20 g, 86%) with mp 148–150°.

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxylic Acid Methyl Ester (8). A soln of **5a** (42.0 g) and NaOMe (400 mg) in MeOH

‡ The authors wish to thank Dr. Koert Gerzon of the Research Laboratories of the Eli Lilly Company for a sample of pyrazomycin.

Table II. *In Vivo* Antiviral Activity of 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (1)

Virus	System	Reference to description of system	Treatment route[a]	Activity[b]
DNA viruses				
Herpes simplex	Rabbit keratitis	35	Eye	+
Herpes simplex	Hamster keratitis–encephalitis	36	Eye	±
Herpes simplex	Mouse encephalitis	37	Ip	–
Herpes simplex	Mouse tail lesion	38	Top	+
Vaccinia	Mouse tail lesion	38	Top	+
Vaccinia	Mouse encephalitis	37	Ip	–
Vaccinia	Rabbit skin lesion	39	Po	±
Vaccinia	Rabbit skin lesion	39	Top	±
RNA viruses				
Friend leukemia	Mouse splenomeg–hepatomeg	4	Ip	+
Influenza A_0	Mouse pneumonia	5, 40	Ip, po	+
Influenza A_2	Mouse pneumonia	5, 40	Ip, po	+
Influenza B	Mouse pneumonia	5, 40	Ip, po	+
Parainfluenza 1	Mouse pneumonia	40	Ip, po, in	+
Semliki forest	Mouse encephalitis	37	Ip, iv, ic	–
Vesicular stomatitis	Mouse tail lesion	38	Top	±
Western equine encephalitis	Mouse encephalitis	37	Ip, iv, ic	–

[a] Eye = eye irrigation (drops or ointment); ip = intraperitoneal; top = topical (ointment or cotton swab); po = *per os* (oral gavage); in = intranasal; iv = intravenous; ic = intracerebral. [b] + = Treatment with 1 resulted in statistically highly significant (a) decreases in symptoms of the disease, in the case of keratitis, tail lesion, pneumonia, and splenomegaly–hepatomegaly experiments, or (b) increases in survivor number or mean survival time in the encephalitis and pneumonia experiments. ± = Treatment with 1 resulted in statistically moderately significant alterations in the infection. – = Treatment with 1 did not appreciably alter the infection (statistically insignificant).

(200 ml) was kept at room temp for 4 hr. The soln was neutralized with Bio-Rad AG50W-X2(H) and filtered, and the filtrate was concd to a syrup. Crystn of the product from EtOAc–MeOH provided **8** (26.4 g, 93%) with mp 117–119°: $[\alpha]^{25}D$ −36.3° (*c* 1.0, H_2O); nmr (DMSO-d_6) δ 5.90 (d, 1, $J_{1',2'}$ = 3.7 Hz, 1'-H), 8.99 (s, 1, 5-H). *Anal.* ($C_9H_{13}N_3O_6$) C, H, N.

1-β-D-Ribofuranosyl-1,2,4-triazole-5-carboxylic Acid Methyl Ester (9). Treatment of 6a by the same procedure for preparing **8** provided the deblocked ester **9** in 98% yield with mp 129–131° (from *i*-PrOH): $[\alpha]^{25}D$ −50.2° (*c* 1.0, H_2O); nmr (DMSO-d_6) δ 6.54 (d, 1, $J_{1',2'}$ = 2.9 Hz, 1'-H), 8.29 (s, 1, 3-H). *Anal.* ($C_9H_{13}N_3O_6$) C, H, N.

Table III. Comparative Antiviral Activity of 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (1), Pyrazomycin, Formycin, and Showdomycin

Virus	1	Pyrazomycin	Formycin	Showdomycin
	In vitro antiviral activity (virus rating)[a]			
Type 3 adeno	0.7	0.1	0.0	0.0
Type 1 herpes simplex	1.2	0.2	0.1	0.0
Type 2 herpes simplex	1.1	0.0	0.3	0.1
Vaccinia	0.9	0.1	0.1	0.2
Type 3 parainfluenza	0.8	0.3	0.0	0.1
Type 1 parainfluenza	0.9	0.2	0.1	0.0
Type 1A rhino	0.6	0.5	0.4	0.0
Type 13 rhino	0.8	0.8	0.0	0.0
Coxsackie	0.4	0.3	0.0	0.0
Vesicular stomatitis	0.7	0.7	0.3	0.0
	In vitro antiviral activity (therapeutic index)[b]			
Influenza A_2	3.2	1.0	<1.0	<1.0
Influenza B	10	3.2	<1.0	<1.0

[a] Virus rating (VR) = a numerical expression of antiviral activity in which the degree of inhibition of viral cytopathogenic effect, toxicity of the compound to the cell monolayer, and concentration of the virus used are considered.§ A VR of 0.1–0.4 suggests only slight antiviral activity, whereas 0.5–0.9 indicates moderate activity, and >1.0 signifies marked antiviral activity. [b] Therapeutic index = highest nontoxic concentration of chemical divided by the lowest effective concentration of the chemical. In these studies, toxicity was evidenced by granulation, vacuolization, rounding, and disintegration of the cells as determined by microscopic examination. An "effective" concentration was one which reduced the average hemagglutinin titer at least one twofold dilution below the average virus control titer.

§ Virus rating (VR) originally described by Ehrlich, *et al.*,[41a] has been modified by Sidwell and Huffman.[41b]

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxhydrazide (10). Hydrazine (95%, 0.7 ml) was added to a soln of **8** (2.59 g, 10.0 mmoles) in MeOH (30 ml) with stirring at room temp. Stirring was continued for 12 hr and the resulting ppt was collected and crystd from aqueous EtOH to give 2.30 g (89%) of **10** with mp 145–147° (softens): $[\alpha]^{25}D$ −29.5° (*c* 1.0, H_2O). *Anal.* ($C_8H_{13}N_5O_5$) C, H, N.

1-β-D-Ribofuranosyl-1,2,4-triazole-5-carboxhydrazide (11) was obtained in 70% yield from **9** by the same procedure used for the prepn of **10**: mp 151–153°; $[\alpha]^{25}D$ −5.60° (*c* 1.0, H_2O). *Anal.* ($C_8H_{13}N_5O_5$) C, H, N.

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carbohydroxamic Acid (12). A soln of **8** (1.00 g, 3.86 mmoles), NH_2OH (660 mg), and EtOH was refluxed for 1 hr and then cooled, and the resulting ppt was collected. Crystn from aqueous EtOH provided **12** (800 mg, 80%) with mp 196–197° dec: $[\alpha]^{25}D$ −34.7° (*c* 1.0, H_2O). *Anal.* ($C_8H_{12}N_4O_6$) C, H, N.

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxylic Acid (13). A soln of the methyl ester (**8**) (5.18 g, 20.0 mmoles) and NaOH (2.0 g) in H_2O (50 ml) was kept at room temperature for 24 hr. The soln was neutralized with Bio-Rad AG50W-X2 (H) and concd to a small volume. The product was crystd from H_2O to give 4.6 g (94%) of **13** with mp 188–190° dec: $[\alpha]^{25}D$ −34.0° (*c* 1.0, H_2O). *Anal.* ($C_8H_{11}N_3O_6$) C, H, N.

1-β-D-Ribofuranosyl-1,2,4-triazole (15). Method 1. The carboxylic acid **13** (200 mg) and DMSO (1 ml) were heated in an oil bath at 180° for 5 min. The solvent was removed, and the product (**15**) was obtained after chromatography over silica gel (12 g) with EtOAc.

Method 2. A soln of **9** (1.30 g, 5.0 mmoles) and NaOH (0.60 g) in H_2O (10 ml) was kept at room temp for 24 hr to give the Na salt of the acid **14**. The soln was applied to a Bio-Rad AG50W-X2 (H) (100 ml) column, and evolution of a gas was noted. Elution with H_2O (300 ml) followed by 1 *N* NH_4OH provided 0.90 g (90%) of **15**. All properties of **15** prepd by methods 1 and 2 were identical with those of an authentic sample.[31]

Acknowledgment. The authors wish to thank Jon Miller and M. P. Schweizer for their helpful suggestions, G. P. Khare and John H. Huffman for assistance in obtaining the antiviral data, and O. P. Crews, Jr., and his staff for the large-scale preparation of chemical intermediates.

References

(1) J. T. Witkowski, R. K. Robins, and R. W. Sidwell, Abstracts, 163rd National Meeting of the American Chemical Society, Boston, Mass., April 1972, No. MEDI 19.
(2) J. H. Huffman, J. T. Witkowski, R. K. Robins, R. W. Sidwell, G. P. Khare, W. B. Jolley, L. N. Simon, L. P. Gebhardt, and D. G. Streeter, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **31**, 2035, 2036, 2037, 2038 (1972).

(3) C. R. Stuart-Harris, *Brit. Med. J.*, **1**, 387 (1971).
(4) R. W. Sidwell, G. J. Dixon, S. M. Sellers, and F. M. Schabel, Jr., *Cancer Chemother. Rep.*, **50**, 299 (1966).
(5) R. W. Sidwell, G. J. Dixon, S. M. Sellers, and F. M. Schabel, Jr., *Appl. Microbiol.*, **16**, 370 (1968).
(6) R. W. Sidwell, *Progr. Antimicrob. Anticancer Chemother.*, 2, 803 (1971).
(7) C. G. Loosli, *Amer. Rev. Resp. Dis.*, **88**, i (1963).
(8) D. A. Stevens and T. C. Merigan, *Rational Drug Ther.*, **5**, 1 (1971).
(9) F. L. Horsfall, *Pub. Health Rep.*, **72**, 905 (1957).
(10) H. E. Kaufman, *Proc. Soc. Exp. Biol. Med.*, **109**, 251 (1962).
(11) B. E. Juel-Jensen, *Brit. Med. J.*, **2**, 154 (1970).
(12) J. Foerster and W. Hryniuk, *Lancet*, 712 (Sept 25, 1971).
(13) F. M. Schabel, Jr., *Chemotherapy*, **13**, 321 (1968).
(14) R. W. Sidwell, G. J. Dixon, F. M. Schabel, Jr., and D. H. Kamp, *Antimicrob. Ag. Chemother.*, 148 (1968).
(15) F. A. Miller, G. J. Dixon, J. Ehrlich, B. J. Sloan, and I. W. McLean, Jr., *ibid.*, 136 (1968).
(16) J. L. Schardein and R. W. Sidwell, *ibid.*, 155 (1968).
(17) B. J. Sloan, F. A. Miller, J. Ehrlich, I. W. McLean, Jr., and H. E. Machamer, *ibid.*, 141 (1968).
(18) G. J. Dixon, R. W. Sidwell, F. A. Miller, and B. J. Sloan, *ibid.*, 172 (1968).
(19) R. W. Sidwell, G. Arnett, and F. M. Schabel, Jr., *Progr. Antimicrob. Anticancer Chemother., Proc. Int. Congr. Chemother., 6th*, 1969, A14-44-48 (1970).
(20) R. H. Williams, K. Gerzon, M. Hoehn, M. Gorman, and D. C. DeLong, Abstracts, 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969, No. MICR 38.
(21) D. C. DeLong, L. A. Baker, K. Gerzon, G. E. Gutowski, R. H. Williams, and R. L. Hamill, *Int. Congr. Chemother., Proc., 7th*, 1970, **1**, A-5/35 (1971).
(22) *Chem. Eng. News*, 43 (Sept 15, 1969).
(23) F. Streightoff, J. D. Nelson, J. C. Cline, K. Gerzon, R. H. Williams, and D. C. DeLong, Abstracts, Ninth Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, D. C., Oct 1969.
(24) R. K. Robins, L. B. Townsend, F. Cassidy, J. F. Gerster, R. F. Lewis, and R. L. Miller, *J. Heterocycl. Chem.*, **3**, 110 (1966).
(25) N. Ishida, M. Homna, K. Kumegai, M. Shimizu, and A. Igawa, *J. Antibiot.*, **20**, 49 (1967).
(26) C. Nishimura and H. Tsukeda, *Progr. Antimicrob. Anticancer Chemother., Proc. Int. Congr. Chemother., 6th*, 1969, **2**, 20 (1970).
(27) K. R. Darnall, L. B. Townsend, and R. K. Robins, *Proc. Nat. Acad. Sci. U. S.*, **57**, 548 (1967).
(28) R. J. Rousseau, L. B. Townsend, and R. K. Robins, *Chem. Commun.*, 265 (1966).
(29) R. J. Rousseau, R. K. Robins, and L. B. Townsend, *J. Heterocycl. Chem.*, **4**, 311 (1967).
(30) R. J. Rousseau, R. K. Robins, and L. B. Townsend, *J. Amer. Chem. Soc.*, **90**, 2661 (1968).
(31) J. T. Witkowski and R. K. Robins, *J. Org. Chem.*, **35**, 2635 (1970).
(32) T. Sato, T. Shimidate, and Y. Ishido, *Nippon Kagaku Zasshi*, 81, 1440 (1960).
(33) G. P. Kreishman, J. T. Witkowski, R. K. Robins, and M. P. Schweizer, *J. Amer. Chem. Soc.*, **94**, 5894 (1972).
(34) R. W. Sidwell, J. H. Huffman, G. P. Khare, L. B. Allen, J. T. Witkowski, and R. K. Robins, *Science*, **177**, 705 (1972).
(35) M. E. Corwin, V. Coleman, S. Riegelman, M. Okumoto, E. Jawetz, and P. Thygeson, *Invest. Ophthalmol.*, **2**, 578 (1963).
(36) R. W. Sidwell, S. M. Sellers, and G. J. Dixon, *Antimicrob. Ag. Chemother.*, 1966, 483 (1967).
(37) R. W. Sidwell, G. J. Dixon, S. M. Sellers, and F. M. Schabel, Jr., *Appl. Microbiol.*, **13**, 579 (1965).
(38) S. Yoshimura, R. T. Christian, G. D. Mayer, and R. F. Krueger, *Progr. Antimicrob. Anticancer Chemother., Proc. Int. Congr. Chemother., 6th*, 1969, **1**, 481 (1970).
(39) C. C. Mascoli and R. G. Burrell, "Experimental Virology," Burgess Publishing Co., Minneapolis, Minn., 1965, p 4.
(40) G. Middlebrook, *Proc. Soc. Exp. Biol. Med.*, **80**, 105 (1952).
(41) (a) J. Ehrlich, B. J. Sloan, F. A. Miller, and H. E. Machamer, *Ann. N. Y. Acad. Sci.*, **130**, 5 (1965); (b) R. W. Sidwell and J. H. Huffman, *Appl. Microbiol.*, 22, 797 (1971).
(42) G. I. Chipen and V. Ya. Grinshtein, *Chem. Heterocycl. Compounds (USSR)*, 1, 420 (1965).

Potential Antitumor Agents. 6. Possible Irreversible Inhibitors of Ribonucleoside Diphosphate Reductase†

Krishna C. Agrawal,* Barbara A. Booth, E. Colleen Moore, and Alan C. Sartorelli

Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510, and Department of Biochemistry, The University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, Houston, Texas 77025. *Received May 30, 1972*

1-Formylisoquinoline thiosemicarbazone, a potent antineoplastic agent, blocks DNA synthesis by inhibiting the enzyme ribonucleoside diphosphate reductase. Several groups potentially capable of alkylation of the enzyme were introduced at the 5 position of the isoquinoline nucleus to design possible irreversible inhibitors of ribonucleoside diphosphate reductase. Two series of compounds were made using either 5-amino or 5-hydroxy derivatives to yield corresponding amides [$-NHSO_2CH_3$, $-NHCOC_6H_4$ (*m*- or *p*-SO_2F)] or esters [$-OSO_2CH_3$, $-OCO_2C_2H_5$, $-OCO_2C_6H_5$, $-OCOC_6H_4$ (*m*- or *p*-SO_2F), $-OSO_2C_6H_4$ (*o*-, *m*-, or *p*-SO_2F)]. In addition, a bis(β-chloroethyl)amino group was introduced at the 5 position by nucleophilic substitution of 5-chloro-1-formylisoquinoline. Although these agents were potent inhibitors of the enzyme *in vitro*, requiring in the range of 10^{-6}–10^{-8} *M* concentration for 50% inhibition of the enzyme, only two derivatives, those containing a $-OCO_2C_2H_5$ or a $-OCO_2C_6H_5$ moiety, were shown to have potent tumor-inhibitory activity in mice bearing Sarcoma 180 ascites cells.

A number of α-(N)-heterocyclic carboxaldehyde thiosemicarbazones, which exhibit growth-inhibitory activity against a relatively wide spectrum of transplanted rodent tumors,[1-9] spontaneous lymphomas of dogs,[10] and DNA viruses of the Herpes group,[11] have been shown to be potent inhibitors of the activity of the mammalian form of the enzyme ribonucleoside diphosphate reductase.[11-14] The most active compounds of this series are 80 to 5000 times more potent than hydroxyurea, guanazole, and *meso*-α,β-diphenylsuccinate, the other known inhibitors of this enzyme. Interference with the activity of ribonucleoside diphosphate reductase prevents the conversion of ribonucleotides to deoxyribonucleotides and subsequently results in inhibition of the synthesis of DNA. From studies on the mechanism by which α-(N)-heterocyclic carboxaldehyde thiosemicarbazones inhibit the activity of ribonucleoside diphosphate reductase, it has been postulated that inhibition is due to either the coordination of iron by these compounds in the metal-bound enzyme or that an iron chelate

†Presented in part before the Division of Medicinal Chemistry at the 160th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1970; this work was supported by Grant CA-02817 from the National Cancer Institute, U. S. Public Health Service.