Synthesis and Biological Studies of 3-(β-D-Ribofuranosyl)-2,3-dihydro-6*H*-1,3-oxazine-2,6-dione, a New Pyrimidine Nucleoside Analog Related to Uridine[1]

T. Ling Chwang, William F. Wood, J. Rodney Parkhurst, Stephen Nesnow, Peter V. Danenberg, and Charles Heidelberger*[2]

McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin 53706. *Received August 29, 1975*

Reaction of the trimethylsilyl derivative of 2,3-dihydro-6*H*-1,3-oxazine-2,6-dione (**2**, "uracil anhydride") with protected 1-*O*-acetylribofuranoses in the presence of stannic chloride gave the corresponding blocked nucleosides. 3-(2,3,-5-Tri-*O*-2',2',2'-trichloroethoxycarbonyl-β-D-ribofuranosyl)-2,3-dihydro-6*H*-1,3-oxazine-2,6-dione (**4c**) thus prepared from the protected sugar **3c**, 1-*O*-acetyl-2,3,5-tri-*O*-(2,2,2-trichloroethoxycarbonyl)ribofuranose, gave, on removal of the protecting groups with zinc dust, 3-(β-D-ribofuranosyl)-2,3-dihydro-6*H*-1,3-oxazine-2,6-dione (**1**). The structure of **1** was confirmed by uv, ir, NMR, and CD spectral data and was shown to be an N nucleoside. Uracil anhydride, **2**, and, to a lesser extent, its ribonucleoside **1** exert a moderate growth inhibition of mouse leukemia L5178Y, HeLa, and Novikoff hepatoma cells in culture. Both compounds produce weak inhibition of vaccinia viral replication in HeLa cells.

As part of our continuing effort to develop tumor inhibitory and antiviral drugs,[3,4] a number of nucleoside analogs with isosteric replacements of the nitrogen atoms in the pyrimidine ring, the 3-deazapyrimidines, were prepared.[5,6] This paper reports the preparation and biological evaluation of another pyrimidine nucleoside isosteric analog, 3-(β-D-ribofuranosyl)-2,3-dihydro-6*H*-1,3-oxazine-2,6-dione (**1**). The parent base, 2,3-dihydro-6*H*-1,3-oxazine-2,6-dione (**2**, "uracil anhydride"), is a pyrimidine analog with an oxygen isosteric replacement. We proposed in 1970[3] that due to their shape and size such nucleoside analogs would be expected to go to the active sites of certain enzymes concerned with the metabolism of pyrimidine nucleotides. Then because of the anhydride moiety they should react with nucleophilic centers at the active sites of the enzyme to form covalent bonds. We would then have a built-in "active site-directed irreversible inhibitor" as defined by Baker.[7]

Compound **2** was first synthesized in 1927 by Rinkes.[8] We found that **2**, prepared by either Rinkes' method or our route, vide infra, inhibited the growth of HeLa cells at a concentration of 10^{-4} M. While our work was in progress, new syntheses for compound **2** were reported by Washburne et al.,[9] Kuhar et al.,[10] and Skoda et al.[11] Compound **2** was reported to have moderate growth-inhibitory properties against *Escherichia coli*[10-12] and leukemia L1210 cells in vitro.[10] The synthesis of the deoxy ribonucleoside of **2** has been described, and it inhibits the growth of *Streptococcus faecium*.[13,14] More recently, the 5-fluoro derivative of **2** was synthesized, which inhibits the growth of *S. faecium* and L1210 cells.[14b]

Chemistry. Our initial preparation of **2** involved the Lossen rearrangement of a hydroxamic acid, analogous to the route used for the preparation of isatoic anhydride from phthalic anhydride.[15] Maleic anhydride was allowed to react with hydroxylamine hydrochloride to form a hydroxamic acid, which was then treated with *p*-nitrobenzoic acid, followed by sodium methoxide, and finally the mixture was heated in acetone. The yield of final product, **2**, isolated by column chromatography (silica gel with acetone as eluent) was, however, quite low.[16]

When Washburne's procedure of reacting maleic anhydride with trimethylsilyl azide was published and reported a better yield of **2**[9] (ca. 75%), gram quantities of **2** were synthesized by that method.

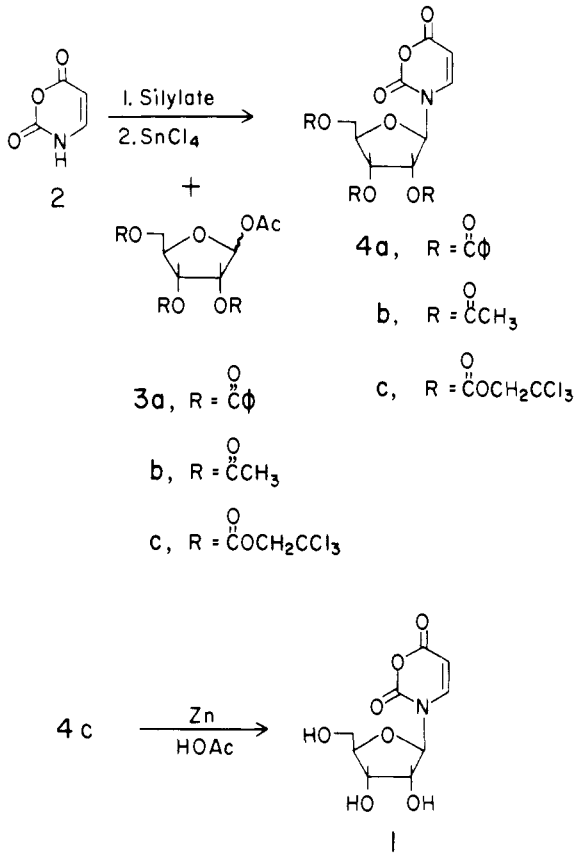
The synthesis of the desired ribonucleoside analog, **1**, was complicated by the lability to hydrolysis of **2**. "Uracil anhydride", **2**, decomposes slowly in ethanol even at room temperature. The decomposition pattern is quite complicated as evidenced by TLC of the reaction mixture at different time intervals. Kinetic studies by ultraviolet spectrophotometry on the hydrolysis of **2** at various pH values and incubation media have been reported.[11]

It has been shown that in place of 1-halo sugars, the more stable 1-acyloxy derivatives of protected furanoses can be used to condense with trimethylsilylated pyrimidine bases in the presence of a Friedel–Crafts catalyst to give the blocked nucleosides.[17-20] The conventional trimethylsilylation, which involves heating the pyrimidine base with hexamethyldisilazane in the presence of catalytic amounts of trimethylchlorosilane, invariably gave unidentified decomposition products of **2**. However, trimethylsilylation of **2** at room temperature with equimolar amounts of hexamethyldisilazane and trimethylchlorosilane in tetrahydrofuran was readily achieved in 15 min. After evaporation, the residue was condensed with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose or 1,2,3,4-tetra-*O*-acetyl-β-D-ribofuranose in the presence of stannic chloride and 1,2-dichloroethane to give, in good yields, 3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2,3-dihydro-6*H*-3-oxazine-2,6-dione (**4a**) and 3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2,3-dihydro-6*H*-1,3-oxazine-2,6-dione (**4b**), respectively. For the sake of experimental simplicity, rather than using the *N*-trimethylsilyl derivative of **2** obtained according to Washburne's method,[9] we preferred to start the reaction with compound **2** whose trimethylsilyl derivative can be readily prepared.

The two protecting groups, acetyl and benzoyl, initially used for the sugar hydroxyls, could not be removed without the decomposition of the labile oxazine ring. It was necessary, therefore, to select a protecting group that could be removed under nonhydrolytic conditions. Such a group is the 2,2,2-trichloroethoxycarbonyl, which can be removed by a β elimination using zinc dust in acetic acid[21,22] and which has not hitherto been used in nucleoside syntheses.

Methyl β-D-ribofuranoside[23] was acylated by a slight excess of 2,2,2-trichloroethoxycarbonyl chloride in DMF and anhydrous pyridine. Without extensive purification, the resulting product was treated with excess acetic acid and acetic anhydride in the presence of concentrated sulfuric acid[24] to afford the protected sugar, **3c**.

3-(2,3,5-Tri-*O*-2',2',2'-trichloroethoxycarbonyl-β-D-ribofuranosyl)-2,3-dihydro-6*H*-1,3-oxazine-2,6-dione (**4c**) was prepared from the protected sugar **3c** and the trimethylsilyl derivative of **2** in a manner analogous to that used for **4a** and **4b**.[25] The NMR spectrum of the blocked nucleoside **4c** shows the characteristic pair of doublets at δ 5.75 and 7.60 for the two protons in the oxazine ring with



a coupling constant of 8 Hz. The anomeric proton of **4c** is also a doublet centered at δ 5.96 with a coupling constant of 4.3 Hz. In dioxane, compound **4c** exhibits a uv absorption maximum at 260 nm, compared with 265 nm for compound **2** in water. The infrared stretching bands for the anhydride moiety are, however, obscured by the presence of the three 2,2,2-trichloroethoxycarbonyl groups. Removal of the protecting groups with zinc dust in acetic acid gave the final product, 1. Other reported methods[26,27] to remove the 2,2,2-trichloroethoxycarbonyl groups were tried without success.

It is generally recognized that condensation in the presence of a Lewis acid of silylated pyrimidines with sugars having a 2-acyloxy substituent leads almost exclusively to the β anomers of the nucleosides.[17-20] Aside from x-ray crystallography, which is impractical as a routine method, NMR and circular dichroism (CD) have been widely used to determine the anomeric configuration of the nucleosides. A value of about 3.5 Hz for the coupling constant ($J_{1',2'}$) of the anomeric proton is generally consistent with a β configuration for ribonucleosides. A value of 1 Hz or less is, however, highly desirable for unequivocal assignments of β configuration.[28] In addition, it is typical of pyrimidine β-ribonucleosides, with few exceptions, to show positive Cotton effects.[29] Thus, the β anomers of uridine,[29] 3-deazacytidine,[30] and 5-fluoro-3-deazauridine[5,6] all show positive molar ellipticities in their CD spectra.

The simplicity of the NMR spectra and the relatively small coupling constants of the doublets for the anomeric protons ($J_{1',2'}$ in the approximate region of 4 Hz) of **4a-c** and 1 suggest the β configuration. Moreover, the fact that both **4b** and 1 show a simple positive Cotton effect centered around 270 nm strongly indicates that they have the β configuration. Further stringent proof of the assigned β configuration of 1 was provided by a similar positive Cotton effect, centered around 270 nm, of the corresponding β-deoxyribosyl analog, 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-2,3-dihydro-1,3-6*H*-oxazine-2,6-dione. The anomeric configuration of this β-deoxy ribonucleoside was unequivocally assigned by its NMR spectrum which was characteristically different from that of its α anomer.[13] (We thank Dr. M. Bobek for providing us with a sample of the β-deoxy ribonucleoside.) The similarities of the ir (1785 and 1720 cm^{-1}, fluorolube) and uv (λ_{max} 265 nm, H_2O) spectra of **2** and 1 demonstrate that 1 is an N nucleoside.

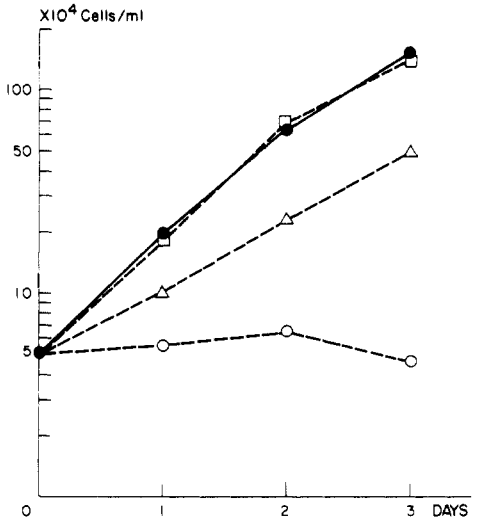


Figure 1. The effect of **2**, added on day 0, on the growth of L5178Y cells: •, control; ○, 10^{-4} M; △, 10^{-5} M; □, 10^{-6} M.

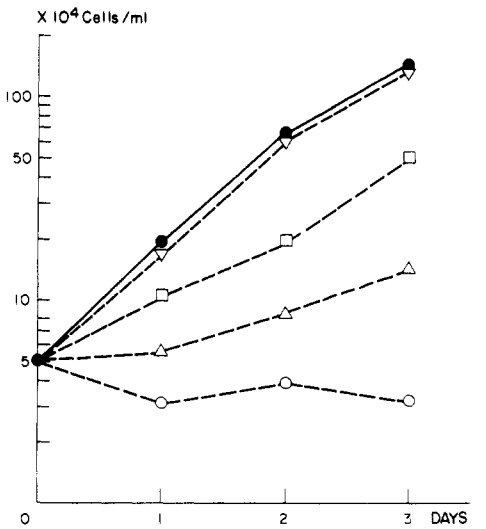


Figure 2. The effect of 1, added on day 0, on the growth of L5178Y cells: •, control; ○, 10^{-3} M; △, 10^{-4} M; □, 5×10^{-5} M; ▽, 10^{-5} M.

Biological. Kuhar et al.[10] reported that uracil anhydride, **2**, inhibits the growth of *E. coli* B and leukemia L1210 cells in vitro by 50% at a concentration of 4×10^{-7} and 4×10^{-5} M, respectively. Furthermore, this inhibition was reported to be competitively prevented by uracil and its nucleosides and to a lesser extent by cytosine and its nucleosides. The results of Skoda et al.[11,12] confirmed Kuhar's *E. coli* B observation[10] with "uracil anhydride", **2**, and extended it to show that DNA and RNA syntheses are rapidly inhibited by **2**. In addition, **2** was found to be a potent growth inhibitor of several gram-positive and gram-negative bacteria.[11]

Recently, the deoxy ribonucleoside of **2** was synthesized and was shown to inhibit the growth of *S. faecium* by 50%

Table I. Toxicity of 1 and 2 to Various Cell Lines. ID_{50}

Cells	1, M	2, M
L5178Y	7×10^{-5}	2.5×10^{-5}
HeLa	2×10^{-4}	5×10^{-5}
NS	2×10^{-4}	2×10^{-4}
NSF	1×10^{-4}	6×10^{-5}
E. coli B23	5×10^{-5}	5×10^{-5}

at a concentration of 5×10^{-8} M.[13] Thus, the deoxy ribonucleoside is approximately 1000-fold more antibacterial than the base.[13]

We have evaluated the effects of 1 and 2 on the growth of *E. coli* B23 in minimal medium. At a concentration of 10^{-4} M, both 1 and 2 showed complete growth inhibition, whereas at 10^{-5} M inhibition was only slight. *E. coli* B23 could be protected from the inhibitory effects of 2 (10^{-4} M) by simultaneous addition of an equal concentration of uridine; therefore, these data confirm the results reported by Skoda et al.[12]

Compounds 1 and 2 have also been tested for growth inhibitory activity against four tumor cell lines and vaccinia virus. The four cell lines used in these studies were leukemia L5178Y cells (L5178Y), HeLa cells, Novikoff hepatoma cells (NS), and Novikoff hepatoma cells lacking thymidine kinase (NSF). Figure 1 shows the inhibition of growth of L5178Y cells grown in the presence of 2 at concentrations between 10^{-4} and 10^{-6} M.[31] There is a pronounced growth inhibition at 10^{-4} M produced by the base and moderate inhibition at 10^{-5} M. In a similar manner, Figure 2 depicts the inhibition of L5178Y cells by 1 between 10^{-3} and 10^{-5} M. At 10^{-3} and 10^{-4} M, there is significant inhibition of L5178Y cells by 1 whereas at 5×10^{-5} M the inhibition is moderate. Our data indicate that in L5178Y cells, the base, "uracil anhydride", 2, is more growth inhibitory by at least twofold than its ribonucleoside analog, 1.

Attempts to reverse the toxic effects of 1 or 2 in L5178Y cells by simultaneous addition of pyrimidine analogs have not been successful. At 10^{-4} M 2 and 10^{-4} M orotic acid, cytidine, uracil, uridine, or thymidine, no stimulation of growth was observed in the experimental cultures when compared with cultures treated with 2 alone. Similar results were obtained with 1 at a concentration of 10^{-4} M in the presence of either 2×10^{-4} M uracil or uridine. There was slight reversal of inhibition of 1 (10^{-4} M), as judged by a stimulation of growth (5–10%), when 2×10^{-4} M cytidine, deoxycytidine, deoxyuridine, or thymidine was added at the same time as 1. Thus far, no compound tested has afforded complete protection against drug toxicity by 1 and 2 in L5178Y cells.

The inhibitory effects of compounds 1 and 2 on all cell lines and *E. coli* B23 are shown in Table I. The data are presented as the concentration of drug required to inhibit 50% of cellular growth (ID_{50}). The eukaryotic cells most sensitive to these compounds are L5178Y, followed by HeLa, NSF, and NS cells.

Compounds 1 and 2 have been tested for their ability to inhibit the formation of plaques produced from infection of HeLa cells monolayers by vaccinia virus. Compound 2 inhibited completely the formation of viral plaques at 10^{-3} M, slightly at 10^{-4} M, and not at all at 10^{-5} M, whereas 1 showed no inhibition at either 10^{-4} or 10^{-5} M. Thus, compounds 1 and 2 appear to have weak antiviral activity.

In view of the modest biological activities of compound 1, it does not warrant further study.

Experimental Section

General. Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. Ultraviolet spectra were recorded on a Gilford spectrophotometer Model 2400S or a Beckman DB-G. Proton magnetic resonance spectra were recorded on a Varian A-60 or Perkin-Elmer R-12 using tetramethylsilane as the internal standard. Circular dichroism spectra were obtained on a Cary 60 spectropolarimeter with a Model 6003 CD attachment. High-pressure liquid chromatography was performed on a Du Pont 830 liquid chromatograph with an ultraviolet photometer as detector. Elemental analyses were done by Galbraith Laboratories, Inc., Knoxville, Tenn., or by Spang Microanalytical Laboratory, Ann Arbor, Mich. Analytical TLC was run on Eastman chromagram sheets of silica gel with fluorescent indicator. Preparative TLC plates were prepared with silica gel PF-254 (EM Reagents). Detection was either by ultraviolet light or by a 5% sulfuric acid in ethanol spray followed by heating. Column chromatography was carried out with Baker silica gel 60–200 mesh or with Fisher Florisil 60–100 mesh. Chromatography solvent mixtures were by volume.

All silylation and stannic chloride reactions were carried out in an atmosphere of dry nitrogen. Solutions or solvents were usually transferred by hypodermic syringes.

Reagents and Solvents. The following materials were obtained from commercial sources and were used without further purification: sodium azide, D-(−)-ribose, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose, 1,2,3,4-tetra-*O*-acetyl-β-D-ribofuranose, zinc dust, maleic anhydride, 2,2,2-trichloroethoxycarbonyl chloride, minimal essential medium (MEM) (Gibco), and 0.45% tryptose phosphate broth (Difco).

Solvent distillation and drying were usually carried out under an atmosphere of dry nitrogen. Tetrahydrofuran (THF) was distilled from lithium aluminum hydride prior to use. Benzene was distilled from sodium. 1,2-Dichloroethane was distilled from calcium hydride. Fuming stannic chloride (Fisher) was used from a freshly opened bottle or was redistilled at normal pressure. Pyridine was distilled from sodium hydroxide and stored over Linde type 4A molecular sieve. *N,N*-Dimethylformamide (DMF) was distilled under reduced pressure or dried over molecular sieve.

2,3-Dihydro-6*H*-1,3-oxazine-2,6-dione (2). The procedure of Washburne et al.[9] was used. This procedure involves the reaction of trimethylsilyl azide[32] with maleic anhydride followed by hydrolysis with methanol. The uv spectrum (H_2O) shows a λ_{max} 264 (log ε 3.89). The solid has a melting point of 157–158° dec (lit. 158° dec[8] and 158–159° dec[9]).

3-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-2,3-dihydro-6*H*-1,3-oxazine-2,6-dione (4a). To 0.56 g (5 mmol) of 2 dissolved in 20 ml of THF was added an equimolar mixture of trimethylchlorosilane (1 g, 1.16 ml, 9 mmol) and hexamethyldisilazane (1.46 g, 1.9 ml, 9 mmol). After stirring at room temperature for 20 min, 2.02 g (4 mmol) of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (3a) in 20 ml of THF was added. After 5 min, the reaction mixture was evaporated under reduced pressure and anhydrous conditions. To the residue was then added 30 ml of 1,2-dichloroethane followed by 0.42 ml (3.6 mmol) of stannic chloride. After stirring at room temperature for 2.5 h under nitrogen, the yellow reaction mixture was filtered and washed with chloroform. The filtrate was concentrated in vacuo and the residue was taken up in chloroform, which was washed with dilute sodium carbonate and then with water. After drying over magnesium sulfate and evaporating, the white solid was recrystallized from methanol to yield 2.0 g (90% based on 3a) of 4a: mp 152–153° dec; NMR ($CDCl_3$) δ 8.30–7.95 (complex m, 5, phenyl), 7.70–7.35 (complex m, 11, phenyl and H-4), 6.28 (d, 1, $J_{1',2'}$ = 4.5 Hz, H-1'), 5.62 (d, 1, $J_{5,4}$ = 8 Hz, H-5); ir (KBr) ν 1780 (v s), 1740 (v s), 1640 (v s), 1600 cm^{-1} (m). Anal. ($C_{30}H_{23}O_{10}N$) C, H, N.

3-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-2,3-dihydro-6*H*-1,3-oxazine-2,6-dione (4b). Compound 2 was silylated as described above. The mixture from 0.6 g (5.3 mmol) of 2 was condensed with 1.4 g (4.4 mmol) of 1,2,3,4-tetra-*O*-acetyl-β-D-ribofuranose (3b) in the presence of stannic chloride (0.42 ml, 3.6 mmol) and 1,2-dichloroethane (30 ml) and then worked up as detailed above. Removal of the solvent yielded 1.28 g (91% based on 3b) of glassy semisolid, which was purified on a silica gel column eluted with benzene: ir (neat) ν 1810 (v s), 1720 (v s), 1640 cm^{-1} (v s); NMR ($CDCl_3$) δ 7.55 (d, 1, $J_{4,5}$ = 8 Hz, H-4), 5.9 (d, 1, $J_{1',2'}$ = 4.3 Hz, H-1'), 5.75 (d, 1, $J_{5,4}$ = 8 Hz, H-5), 2.15 (s, 9, OAc); uv

(dioxane) λ_{max} 262 nm (log ϵ 3.82); $[\theta]^{27}_{270}$ +5597. Anal. See ref 33.

1-*O*-Methyl-2,3,5-tri-*O*-(2,2,2-trichloroethoxycarbonyl)-ribofuranose. Methyl β-D-ribofuranoside was prepared from D-(−)-ribose and methanolic sulfuric acid.[23] A solution of 27.5 g (130 mmol) of 2,2,2-trichloroethoxycarbonyl chloride in 75 ml of DMF chilled with ice water was added rapidly to a stirred solution of methyl β-D-ribofuranoside (5.3 g, 32.5 mmol) in 50 ml of pyridine also chilled to 0°. The reaction was allowed to run at 0° for 2 h and then 25 ml of ice water was added. After filtration, the filtrate was concentrated under vacuum with the bath temperature kept around 50°. The gummy residue was taken up in chloroform, which was then washed with water. The organic phase was dried over magnesium sulfate and evaporated, yielding 13.25 g of syrup. The syrup (6 g) was dissolved in chloroform and chromatographed on 40 g of a silica gel column. The column was eluted with hexane, followed by 1:1 hexane–chloroform and then chloroform, to yield 4.3 g (mainly from chloroform cuts) of white syrup: NMR ($CDCl_3$) δ 5.22 (br s, 1, H-1'), 4.77 (s, 6, CH_2 of 2,2,2-trichloroethoxycarbonyl), 3.46 and 3.41 (2 s, total 3, OCH_3). The syrup could not be induced to crystallize and was used without further purification for subsequent reactions.

1-*O*-Acetyl-2,3,5-tri-*O*-(2,2,2-trichloroethoxycarbonyl)-ribofuranose (3c). Following Ryan's general procedure,[24] 0.9 ml of concentrated sulfuric acid was added to a chilled (ca. 10°) solution of 1 g (1.45 mmol) of 1-*O*-methyl-2,3,5-tri-*O*-(2,2,2-trichloroethoxycarbonyl)ribofuranose in 15 ml of glacial acetic acid and 3 ml of acetic anhydride. After 16 h at room temperature, the reaction was treated with 100 ml of a mixture of ice and water. The aqueous phase was then extracted with chloroform, which was then washed with saturated sodium bicarbonate solution and water. The organic phase was dried over magnesium sulfate and evaporated to give 1.4 g of syrup. The syrup was purified by chromatography on Florisil eluted with hexane and then benzene, followed by methylene chloride. All fractions (mainly from benzene cuts) containing **3c** (monitored by acid spray and charring TLC on silica gel employing the solvent system of 1% acetone in benzene) were combined and evaporated to yield 0.6 g (58%) of foam: NMR ($CDCl_3$) δ 6.31 (s, 1, H-1'), 4.81 (s, 6, CH_2 of 2,2,2-trichloroethoxycarbonyl), 2.13 (s, 3, OAc). An analytical sample of **3c** was obtained from preparative TLC on silica gel employing a solvent of 1% acetone in benzene. Anal. ($C_{16}H_{15}O_{12}Cl_9$) C, H, N.

3-(2,3,5-Tri-*O*-2,2,2-trichloroethoxycarbonyl-β-D-ribofuranosyl)-2,3-dihydro-6*H*-1,3-oxazine (4c). To 0.48 g (4.12 mmol) of **2** dissolved in 20 ml of dry THF was added an equimolar mixture of trimethylchlorosilane (0.87 ml, 6.7 mmol) and hexamethyldisilazane (1.43 ml, 6.7 mmol). After 10 min of stirring at room temperature, 1.3 g (1.8 mmol) of **3c** in 15 ml of THF was introduced. Ten minutes later, the reaction mixture was evaporated under reduced pressure and in an inert atmosphere. To the residue was added 25 ml of 1,2-dichloroethane followed by 1 ml (8.2 mmol) of stannic chloride. After 24 h of stirring at room temperature, and work-up as described previously for **4a**, 1.62 g of yellow syrup was obtained. The syrup was passed through a silica gel column, which was eluted with hexane with a progressively increasing amount of chloroform. All fractions (mainly from 2:1 hexane–chloroform) containing **4c** (monitored by TLC on silica gel employing the solvent system of 1:1 cyclohexane–acetone) were combined and evaporated to yield 1.2 g (87% based on **3c**) of yellowish solid: mp 110° dec; NMR ($CDCl_3$) δ 7.60 (d, 1, $J_{4,5}$ = 8 Hz, H-4), 5.96 (d, 1, $J_{1',2'}$ = 4.3 Hz, H-1'), 5.75 (d, 1, $J_{5,4}$ = 8 Hz, H-5), 4.81 (br s, 6, CH_2 of 2,2,2-trichloroethoxycarbonyl); uv (dioxane) λ_{max} 260 nm (log ϵ 3.78). Anal. See ref 33.

3-(β-D-Ribofuranosyl)-2,3-dihydro-6*H*-1,3-oxazine-2,6-dione (1). To 1 g (1.3 mmol) of **4c** in 50 ml of glacial acetic acid was added 3 g of zinc dust. After stirring at room temperature for 2.5 h, the solid was filtered and washed with acetic acid. The filtrate was concentrated and taken up in 100 ml of water. Gaseous hydrogen sulfide was then bubbled into the mixture for 45 min. The solid was removed by filtration and the filtrate was washed with chloroform. The aqueous phase was concentrated and eluted with water through an Amberlite IRC-50 column (H^+ form). The eluent was evaporated under reduced pressure to dryness. The resulting gummy residue was then repeatedly (three times) purified by preparative TLC employing a solvent system of 2:1:1 ethyl acetate–cyclohexane–acetone. The extracted product was again passed through an Amberlite IRC-50 column (H^+ form) using water as the eluent. Evaporation of the aqueous phase yielded 63 mg (20%) of **1** (hydroscopic solid). An analytical sample was obtained by high-pressure liquid chromatography purification (25 cm × 2.1 mm i.d. "Zorbax"-SIL column at ambient temperature and 2000 psi of pressure) utilizing a solvent mixture of 86.14:12.5:1.26:0.1 methylene chloride–methanol–water–formic acid: ir (fluorolube) ν 1785 (v s), 1720 cm^{-1} (v s); NMR (Me_2SO-d_6) δ 8.35 (d, 1, $J_{4,5}$ = 8 Hz, H-4), 5.92 (d, 1, $J_{5,4}$ = 8 Hz, H-5), 5.72 (d, 1, $J_{1',2'}$ = 4 Hz, H-1'); uv (H_2O) λ_{max} 265 nm (log ϵ 3.86); $[\theta]^{27}_{270}$ +5279. Anal. ($C_9H_{11}O_7N$) C, H, N.

Inhibition of Leukemia L5178Y, HeLa, NS, and NSF Cells. The cytotoxicity of the compounds in these cell culture systems was determined according to the published procedures.[31]

Inhibition of Vaccinia Virus Replication in HeLa Cells. The procedure used is a slight modification of the published method.[34] Confluent monolayers of HeLa S_3 cells in 30-mm dishes were washed once with 2 ml of phosphate-buffered saline and infected with 0.20 ml of a diluent medium containing vaccinia virus in the presence or absence of the test compound. The virus diluent medium was composed of minimal essential medium (MEM), 5.5% bovine serum albumin fraction V, penicillin (50 units/ml) and streptomycin (50 μg/ml). The virus inoculated dishes were incubated for 1 h in a humidified 5% CO_2 incubator at 37° with occasional rocking. A minimum of three plates were used for each experimental culture. One hour postinfection the dishes received an overlay medium (1.8 ml) consisting of 86% MEM, 0.45% tryptose phosphate broth, 0.15% sodium bicarbonate, penicillin (50 units/ml), streptomycin (50 μg/ml), 8% starch (starch hydrolyzed for gel electrophoresis, Lot 245-1, Connaught Medical Research Laboratories, Toronto, Canada), and drug at the appropriate concentration. The dishes were incubated for 48 h followed by addition of a second overlay containing 1.0% neutral red; 24 h later the plaques were counted.

Inhibition of *E. coli* B23. The method of Skoda et al.[12] was followed in determining the inhibition of *E. coli* B23 growth by monitoring the change in absorbance at 575 nm.

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References and Notes

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(33) Although all available spectroscopic data are fully consistent with the assigned structures, no satisfactory analytical results could be obtained under the conditions used for the elemental analysis. However, purification by preparative TLC on silica gel with 10% acetic acid added to the developing solvent mixtures (for **4b**, 1:1 acetone–cyclohexane with 10% acetic acid; for **4c**, 2:1 carbon tetrachloride–ethyl ether with 10% acetic acid) gave satisfactory analysis with the inclusion of 2 mol of acetic acid for **4b** and 1.5 mol of acetic acid for **4c**. The inclusion of acetic acid in the samples purified by TLC is substantiated by the appearance of additional NMR resonances around δ 2.1, characteristic for the methyl group in acetic acid.
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Metabolic Formation and Synthesis of 1-(3-Chlorophenyl)-2-(4-hydroxyphenyl)-1-methyl-2-(2-pyridine)ethanol. A Potential Hypocholesteremic Agent

J. E. Sinsheimer,* E. Van den Eeckhout, L. E. Hewitt, Y. Kido, D. R. Wade, D. W. Hansen Jr., J. C. Drach, and J. H. Burckhalter

College of Pharmacy, University of Michigan, Ann Arbor, Michigan 48109. *Received July 23, 1975*

1-(3-Chlorophenyl)-2-(4-hydroxyphenyl)-1-methyl-2-(2-pyridine)ethanol (**8a**) has been synthesized and found to be the major urinary metabolite following intraperitoneal administration of 1-(3-chlorophenyl)-1-methyl-2-phenyl-2-(2-pyridine)ethanol (1) to rats. This metabolite has a hypocholesteremic effect in rats similar to that of the parent drug.

The higher melting of two enantiomeric pairs of compound 1 was selected from a series of 133 2-(2-pyridine)-1,2-diarylalkanols for study of its hypocholesteremic effect in humans.[1] Although the compound was nontoxic and was found to lower serum cholesterol in rats, it had no hypocholesteremic effect in monkeys or man. This species difference in activity prompted the present investigation of the metabolism of compound 1 on the premise that a metabolite was responsible for hypocholesteremic activity. Compound 1 has been prepared with tritium uniformly distributed in the unsubstituted benzene ring.[2] This report describes characterization of the principal metabolites following ip injection into male Sprague–Dawley rats, synthesis of the metabolites, and preliminary evaluation of their hypocholestermic activity.

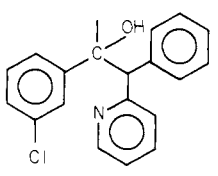

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Figure 1 shows the daily rate and cumulative urinary excretion for 13 days of three rats which had been administered 40 mg/kg (1.71 μCi) of compound 1. The first day's urine contained 5% of the original ^{3}H activity which decreased to less than 1% in 7 days. Excreted ^{3}H in feces followed a pattern similar to that of the urine with about 7% of the total dose excreted in the first day falling in 13 days to less than 1% per day. Total activity excreted in the feces averaged about 25% of the dose over this period. There was no appreciable ^{3}H detected in any of the 11 tissues examined after sacrifice. Total activity retained in body tissues was estimated to be less than 1.4%. The