G-25 (BPW system). Several trace impurities revealed by TLC (cellulose) were successfully removed by countercurrent distribution (BAW, 10 ml each phase, 900 transfers): TLC (cellulose) R_f 0.52 (BAW), 0.53 (BAWP), 0.72 (BEAW), 0.24 (BPW); amino acid analysis Arg 1.03, Val 0.93, Tyr 0.90, Ile 0.96, His 0.95, Pro 1.05, Thr 1.08; $[\alpha]^{24}D$ −67.0° (c 0.5); ^1H NMR δ 0.87 (m, Val and Ile-CH_3), 1.25 (d, Thr-CH_3), 6.73 and 7.03 (d, Tyr-arom), 7.38 and 8.76 (s, His-arom).

[Sar^1,Ser(Me)^8]angiotensin II. After cleavage of the peptide from the resin support with HBr–TFA, the monotosyl octapeptide was obtained in a homogeneous state by chromatography on Sephadex G-25 (0.1 M HOAc). The tosyl group was removed with HF, and the free peptide was chromatographed on AG-1-X2 in the usual manner. The product obtained was homogeneous in three TLC systems without further purification: TLC (cellulose) R_f 0.28 (BPW), 0.51 (BAWP), 0.4 (BAW); amino acid analysis Sar 0.96, Arg 1.00, Val 1.06, Tyr 0.99, Ile 0.85, His 1.07, Pro 1.03 (*O*-methylserine was partially degraded during hydrolysis and was not calculated); $[\alpha]^{21}D$ −71.4° (c 0.5); ^1H NMR δ 0.8–1.0 (m, Val and Ile-CH_3), 2.83 (s, Sar-NCH_3), 3.37 [s, Ser(Me)-OCH_3], 4.06 (s, Sar-CH_2), 6.73 and 7.03 (d, Tyr-arom), 7.43 and 8.78 (s, His-arom).

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References and Notes

(1) Abbreviated designation of amino acid derivatives and peptides is according to the recommendation of the IUPAC–IUB commission (IUPAC information bulletin no. 26). In addition, the following abbreviations have been used: Prd = pyridine, GdnAc = guanidineacetic acid, Ser(Me) = *O*-methylserine, Thr(Me) = *O*-methylthreonine, HOBt = 1-hydroxybenzotriazole.
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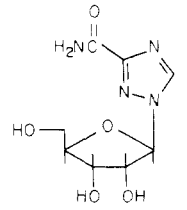
Synthesis and Antiviral Activity of Certain Thiazole *C*-Nucleosides

Prem C. Srivastava,* Michael V. Pickering, Lois B. Allen, David G. Streeter, Marie T. Campbell, Joseph T. Witkowski, Robert W. Sidwell, and Roland K. Robins

ICN Pharmaceuticals, Inc., Nucleic Acid Research Institute, Irvine, California 92715. *Received May 10, 1976*

A general reaction of glycosyl cyanides with liquid hydrogen sulfide in the presence of 4-dimethylaminopyridine to provide the corresponding glycosylthiocarboxamides is described. These glycosylthiocarboxamides were utilized as the precursors for the synthesis of 2-D-ribofuranosylthiazole-4-carboxamide and 2-β-D-ribofuranosylthiazole-5-carboxamide (**23**). The structural modification of 2-β-D-ribofuranosylthiazole-4-carboxamide (**12**) into 2-(2,3,-5-tri-*O*-acetyl-β-D-ribofuranosyl)thiazole-4-carboxamide (**15**), 2-β-D-ribofuranosylthiazole-4-thiocarboxamide (**17**), and 2-(5-deoxy-β-D-ribofuranosyl)thiazole-4-carboxamide (**19**) is also described. These thiazole nucleosides were tested for in vitro activity against type 1 herpes virus, type 3 parainfluenza virus, and type 13 rhinovirus and an in vivo experiment was run against parainfluenza virus. They were also evaluated as potential inhibitors of purine nucleotide biosynthesis. It was shown that the compounds (**12** and **15**) which possessed the most significant antiviral activity were also active inhibitors (40–70%) of guanine nucleotide biosynthesis.

Certain naturally occurring *C*-glycosyl nucleosides possess a variety of biological properties that are of potential medicinal importance.[1-5] In recent years, considerable work has been directed toward the synthesis of such compounds. In search of a potent antiviral drug several *N*-glycosyl nucleosides[6,7] including ribavirin[8] (**1**) have been prepared and studied in this Laboratory. Studies with ribavirin[9] and certain imidazole nucleosides[6] (**2**) have shown that those compounds which exhibited significant antiviral activity were also active inhibitors of guanine nucleotide biosynthesis. This suggested that the selective regulation of this important pathway to nucleic acid biosynthesis may have specific chemotherapeutic application. The present report describes the synthesis and antiviral evaluation of certain *C*-glycosylthiazoles structurally related to ribavirin.



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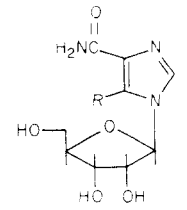
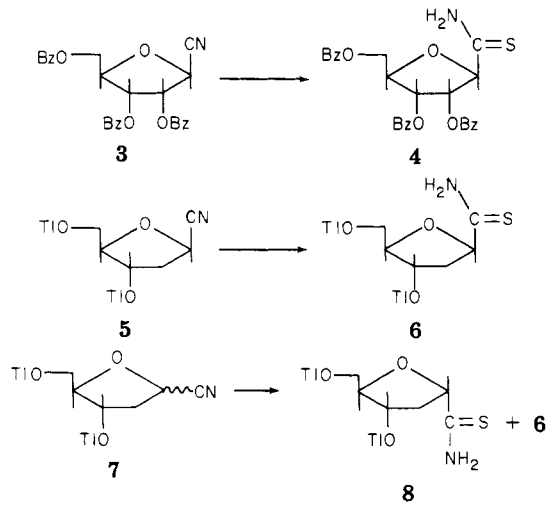


2, R = H or halogen

Various approaches for the synthesis of *C*-nucleosides have been summarized in the literature.[10] Of these, the conversion of β-D-aldofuranosyl cyanides into suitably functionalized anhydroalditols, in which further elabo-

Scheme I



ration of the aglycon provides C-glycosyl nucleosides, appeared to be the most interesting.[11-15]

Recently this Laboratory has succeeded[16] in the transformation of 2,3,5-tri-O-benzoyl-β-D-ribofuranosyl cyanide[11] (3) into 2,5-anhydro-3,4,6-tri-O-benzoyl-D-allonthioamide (4) via the treatment of the former compound with liquid hydrogen sulfide in the presence of a catalytic amount of 4-dimethylaminopyridine. This method provided high yields (>80%) of the product 4 with no benzoate elimination and in this regard is superior to literature procedures.[17] This method was found to be rather general and 2,5-anhydro-3-deoxy-4,6-di-O-*p*-toluoyl-D-*ribo*-hexonthioamide (6) and 2,5-anhydro-3-deoxy-4,6-di-O-*p*-toluoyl-D-*arabino*-hexonthioamide (8) were similarly prepared in 80–85% yields, respectively, from 2-deoxy-3,5-di-O-*p*-toluoyl-D-*erythro*-pentofuranosyl cyanide.[18] It is important to mention here that difficulties were encountered in separating chromatographically pure 2-deoxy-3,5-di-O-*p*-toluoyl-α-D-*erythro*-pentofuranosyl cyanide from its anomeric mixture 7. Therefore, 7 was treated with liquid hydrogen sulfide and 4-dimethylaminopyridine to provide the anomeric mixture of thioamides from which 6 and 8 were then conveniently separated by silica gel column chromatography (Scheme I).

The conversion of thioamides into ethyl thiazole-4-carboxylates has been effected via the treatment of the former compounds with ethyl bromopyruvate.[19] Compound 4 reacted readily with ethyl bromopyruvate in acetonitrile to provide a mixture of one minor and two major products, which were separated in pure form by silica gel column chromatography. The structure of the minor product (6.9%) was determined to be ethyl 2-(5-benzoyloxymethylfuran-2-yl)thiazole-4-carboxylate (11) on the basis of its ^{1}H NMR spectrum, which did not exhibit a signal for the anomeric proton. Instead, it showed the presence of two doublets at δ 6.66 and 7.18 ($J_{3,4}$ = 3.5 Hz) for the 3- and 4-furanyl protons. The optical rotation of such a planar molecule would be expected to be zero, which indeed was observed, thus further supporting the structure of 11. The formation of this compound could arise from benzoate elimination of 4 during the cyclization reaction. Similar benzoate elimination has been reported earlier.[20] The structure of one of the major products (42%), which was fast moving on TLC, was determined to be ethyl 2-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)thiazole-4-carboxylate (9), whereas the second slower moving product (22%) was ethyl 2-(2,3,5-tri-O-benzoyl-α-D-ribofuranosyl)thiazole-4-carboxylate (10). This structural assignment was based on ^{1}H NMR spectral studies of 2-β-D-ribofuranosylthiazole-4-carboxamide (12) and 2-α-D-ribofuranosylthiazole-4-carboxamide (13), which were obtained via the treatment of 9 and 10, respectively, with methanolic ammonia. The observed upfield shift of the anomeric proton of 12 relative to that of 13 is consistent with the β and α configurations, respectively. A similar observation was reported for the relative chemical shifts of $C_{1'}$H of known anomeric pairs of *N*- and *C*-glycosyl nucleosides.[21-24] Compound 11 was also treated with methanolic ammonia to provide 2-(5-hydroxymethylfuran-2-yl)thiazole-4-carboxamide (14).

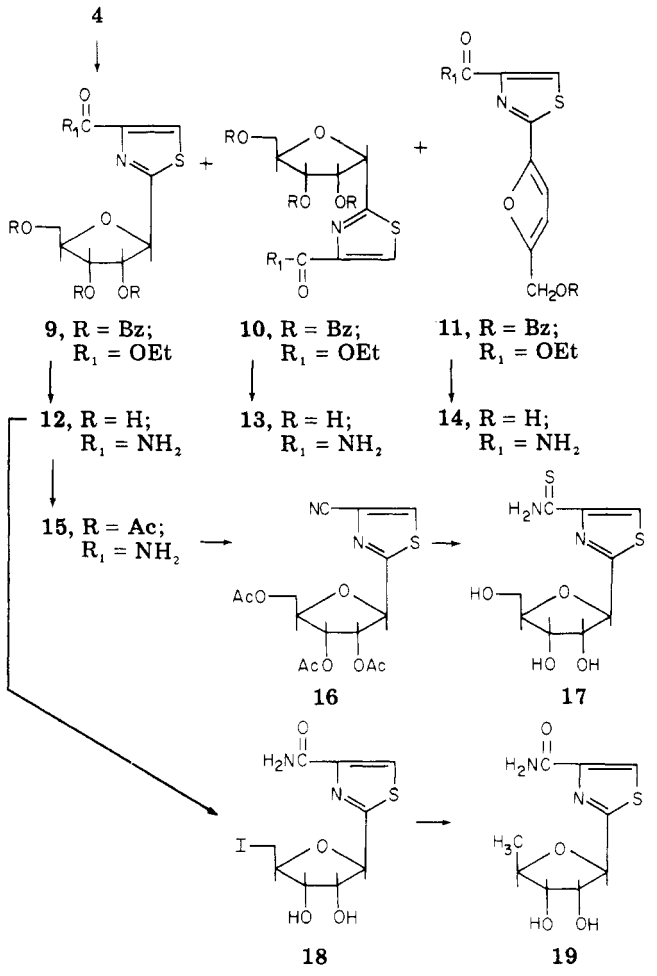
Since compound 12 was found effective against a variety of viruses in vitro, its structural modification in the base as well as in the sugar moiety was considered important from a structure–activity point of view. The simplest of these modifications was the synthesis of 2-β-D-ribofuranosylthiazole-4-thiocarboxamide (17), which has similar steric requirements but different hydrogen-bonding properties compared to those of 12. A similar argument was given for the synthesis of 1-β-D-ribofuranosyl-1,2,4-triazole-3-thiocarboxamide, a structural analogue of ribavirin (1), in which this modification resulted in loss of broad-spectrum antiviral activity but retention of activity against herpes simplex virus.[25]

Compound 12 was acetylated with acetic anhydride in the presence of pyridine to provide 2-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)thiazole-4-carboxamide (15) in 90% yield. The dehydration of 15 was achieved in chloroform with phosphorus oxychloride and triethylamine to furnish a 90% yield of 2-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-thiazole-4-carbonitrile (16). When compound 16 was treated with potassium hydrosulfide in boiling ethanol, the desired product, 2-β-D-ribofuranosylthiazole-4-thiocarboxamide (17), was obtained in 70% yield as light yellow needles.

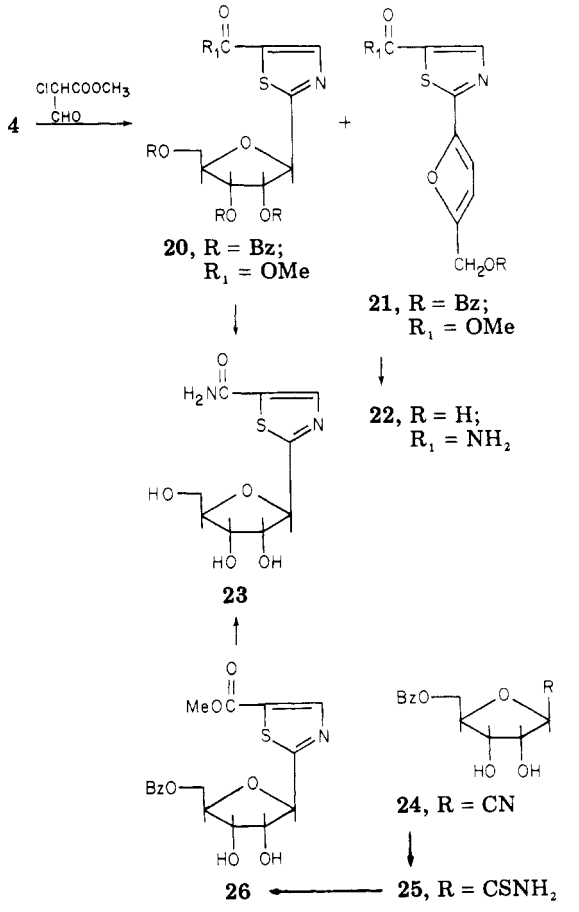
Following the rationale discussed in an earlier report,[26] the 5′-deoxy analogue of 12 was synthesized as follows. Selective iodination of 12 using methyltriphenoxyphosphonium iodide[27] (Rydon reagent) in DMF gave crystalline 2-(5-deoxy-5-iodo-β-D-ribofuranosyl)thiazole-4-carboxamide (18) in 58% yield. Hydrogenation of 18 using Pd/C catalyst provided 2-(5-deoxy-β-D-ribofuranosyl)thiazole-4-carboxamide (19) in 58% yield (Scheme II).

The most important compound to be synthesized for antiviral evaluation in this series was 2-β-D-ribofuranosylthiazole-5-carboxamide (23), a positional isomer of 12. A procedure described in the literature[19] for the synthesis of ethyl 2-alkylthiazole-4-carboxylates utilizes the condensation of alkylthioamides with ethyl formylchloroacetate. In a similar approach methyl formylchloroacetate[28] was condensed with the thioamide 4. As expected, the cyclized product, methyl 2-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)thiazole-5-carboxylate (20), was indeed obtained, but extensive benzoate elimination occurred, providing methyl 2-(5-benzoyloxymethylfuran-2-yl)thiazole-5-carboxylate (21) as the major product. The structure of 21 was assigned in the same way as that of 11. The product 21 was converted into 2-(5-hydroxymethylfuran-2-yl)thiazole-5-carboxamide (22) via treatment of 21 with methanolic ammonia. The desired product 23 was obtained when the benzoyl derivative 20 was treated with methanolic ammonia. An alternate route for the synthesis of 20 was adopted in order to avoid the facile benzoate elimination during cyclization. 2,5-Anhydro-6-O-benzoyl-D-allonthioamide (25) was chosen as the starting material. Compound 25 was readily obtained in crystalline form when 5-benzoyl-β-D-ribofuranosyl cyanide[20] (24) was

Scheme II



Scheme III



treated with liquid hydrogen sulfide and 4-dimethylaminopyridine as described above. The thioamide **25** was then condensed with methyl formylchloroacetate in DMF to provide methyl 2-(5-*O*-benzoyl-β-D-ribofuranosyl)-thiazole-5-carboxylate (**26**) as the major product, which was separated from some slow-moving impurities by silica gel column chromatography. Ammonolysis of **26** with methanolic ammonia yielded (60%) crystalline 2-β-D-ribofuranosylthiazole-5-carboxamide (**23**), which was found identical (TLC mobility and UV and ^{1}H NMR) with **23** obtained from **20**. The position of $H_{1'}$ of **23** in the ^{1}H NMR spectrum was similar to that of **12**. The 0.33-ppm upfield shift of the anomeric proton of **23** compared to the α anomer of the isomeric nucleoside **13** supports the β configuration of **23** (Scheme III).

Biological Activity. These studies have given some insight into structure–activity relationships which have shown specifically that in a five-membered heterocyclic nucleoside the carboxamide group situated β to the site of ribosylation and adjacent to a nitrogen appears necessary for antiviral activity. These features are found in ribavirin (**1**), certain imidazole nucleosides (**2**), and the *C*-glycosylthiazole (**12**). It is interesting to note that compound **23**, which lacks a nitrogen adjacent to the carboxamide, did not show any antiviral activity. The biochemical and antiviral evaluation of these compounds, which further establishes a correlation between inhibition of purine nucleotide biosynthesis and antiviral activity, is described below.

Biochemical Evaluation. Some of the compounds in this series possessed a spectrum of antiviral activity similar to that of ribavirin. Because of this fact and the structural similarities between these compounds and ribavirin, the compounds were tested as potential inhibitors of purine nucleotide biosynthesis in Ehrlich ascites tumor cell suspensions.[29] Ribavirin is a rather specific inhibitor of the biosynthesis of guanine nucleotides in this assay,[6,30] and this inhibition has been linked to the mode of antiviral action of this compound.[9] Table I shows the effects of six compounds on the incorporation of [^{14}C]hypoxanthine into adenine and guanine nucleotides as determined by thin-layer chromatographic separation of the various nucleotides. Two of the compounds tested, **12** and **15**, show a very specific inhibition of guanine nucleotide biosynthesis ranging from 42 to 70%. This activity seems to correlate well with the in vitro antiviral evaluation of the series since these same two compounds possess the

Table I. Inhibition of Purine Nucleotide Biosynthesis in Ehrlich Ascites Tumor Cells[a]

Compd	% inhibition: Adenine nucleotides (AMP + ADP + ATP)	% inhibition: Guanine nucleotides (GMP + GDP + GTP)
12	0	69
14	0	0
15	0	42
17	0	12
19	0	0
23	1.0	6.8

[a] Approximately 6 × 10^6 cells/ml were incubated at 37 °C, 20 min, with and without 1 mM of the test compounds. [^{14}C]Hypoxanthine (55 μCi/μmol) was then added to a final concentration of 0.1 mM and the incubation continued for 60 min. Adenine and guanine nucleotides were separated on PEI-cellulose as previously described.[29]

Table II. Comparative in Vitro Antiviral Activity (Virus Rating)[a]

Compd	Type I herpes simplex virus	Type 3 parainfluenza virus	Type 13 rhinovirus
12	0.6	0.6	0.5
14	0.0	0.0	0.0
15	0.6	0.2	0.3
17	0.1	0.0	0.0
19	0.0	0.0	0.0
23	0.0	0.0	0.0

[a] See ref 31.

most significant antiviral activity of the series.

Antiviral Evaluation. The compounds synthesized were tested for inhibition of type 1 herpes simplex virus (DNA virus), type 3 parainfluenza virus and type 13 rhinovirus (RNA viruses) in concentrations ranging from 1000 to 1 μg/ml using human carcinoma of the nasopharynx (KB) cells.[31] The antiviral activity was determined in terms of virus rating[31] (VR) by observing inhibition of viral cytopathic effect after a 72-h incubation at 37 °C. Using this system, any VR of less than 0.5 suggested only slight or no antiviral activity; a VR of 0.5 or greater was indicative of moderate to definite antiviral activity. Of the compounds tested 12 showed significant in vitro antiviral activity against both RNA and DNA viruses while its triacetate 15 was found inhibitory only against herpes simplex virus. The data presented in Table II are the average of one to five tests.

Since compound 12 was effective in vitro against parainfluenza viruses, this nucleoside and its triacetate 15 were tested in parallel with ribavirin in mice infected with type 1 parainfluenza (Sendai) virus. Female mice (18–20 g) were infected intranasally with an LD_{90} of virus. Drugs were administered orally thrice daily for 8 days starting 1 h postvirus inoculation. All three compounds were effective in increasing the mean day of death, but only ribavirin significantly increased survivor numbers (Table III). None of the compounds tested caused toxicity control deaths, although reduction of weight gain was observed in those control animals receiving the highest dose of compound, suggesting each compound was used at a concentration approaching the maximum tolerated dose for mice. Although this series of compounds had a spectrum of activity similar to that of ribavirin, their lesser potency did not suggest their further development as antiviral compounds.

Experimental Section

The physical properties were determined with the following instruments: melting point, Thomas-Hoover apparatus (uncorrected); IR, Perkin-Elmer Model 257 spectrophotometer (KBr); UV spectra, Cary 15 UV spectrophotometer (pH 1 and pH 11); specific rotation, Perkin-Elmer Model 141 polarimeter; and ^1H NMR, Hitachi Perkin-Elmer Model R-20A spectrometer (DSS). The presence of exchangeable protons was confirmed by ^1H NMR spectroscopy in absolute Me_2SO-d_6 by exchange with D_2O followed by reintegration. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Where analyses are indicated by only symbols of the elements, the analytical results for those elements were within ±0.4% of the theoretical value. Woelm silica gel (0.063–0.2 mm) purchased from ICN Life Science Group, Cleveland, Ohio, was used for column chromatography and Woelm TLC plates (silica gel F_{254}) were used to check the purity of the compounds. The spray test[32] was used to detect the presence of the thioamide group.

2,5-Anhydro-3,4,6-tri-*O*-benzoyl-D-allonthioamide (4). A mixture of 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl cyanide[11] (3, 25.0 g, 0.53 mol), 4-dimethylaminopyridine (0.5 g), and liquid hydrogen sulfide (125 ml) was stirred in a sealed bomb at room temperature for 17 h. The hydrogen sulfide was allowed to evaporate and the residue (foam) was dissolved in methylene chloride (250 ml), washed with saline water (2 × 100 ml), and dried ($MgSO_4$). Evaporation of the solvent in vacuo yielded 26 g of 2 in the form of light yellow foam which was of sufficient purity for further reactions. The product was purified by chromatography on a silica gel column (5 × 70 cm) packed in chloroform. Elution with 5% (v/v) ethyl acetate in chloroform provided 22 g (83%) of analytically pure product as a light yellow foam: $[\alpha]^{25}D$ +2.84° (c 1, $CHCl_3$); ^1H NMR (Me_2SO-d_6) δ 5.08 (d, 1, J = 5 Hz, H_1'), 7.2–8.3 (m, 15, benzoyls), 9.4 and 10.25 [pair of s (br), 2, $CSNH_2$]. Anal. ($C_{27}H_{23}NO_7S$) C, H, N, S.

2,5-Anhydro-3-deoxy-4,6-di-*O*-*p*-toluoyl-D-*ribo*-hexonthioamide (6). A mixture of 2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl cyanide[18] (5, 7.58 g, 0.020 mol) and 4-dimethylaminopyridine (150 mg) was allowed to react with liquid hydrogen sulfide (ca. 50 ml) as described above for 20 h. Hydrogen sulfide was allowed to evaporate and the residue (light yellow foam) was dissolved in ether (200 ml). The ether portion was washed with saline water (100 ml × 2) and dried ($MgSO_4$). The solvent was evaporated in vacuo to provide a residue which, with the aid of seed crystals, was crystallized from ethanol as shining white needles. Seed crystals were obtained by chromatography of the crude product on a silica gel column using 5% (v/v) ethyl acetate in chloroform as eluent. Homogenous fractions were evaporated and the product was crystallized from ethanol: yield 7.0 g (85%); mp 154–155 °C; $[\alpha]^{25}D$ −12.9° (c 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.42 (s, 6, methyls), 8.35 [s (br), 2, $CSNH_2$]. Anal. ($C_{22}H_{23}NO_5S$) C, H, N, S.

2,5-Anhydro-3-deoxy-4,6-di-*O*-*p*-toluoyl-D-*arabino*-hexonthioamide (8). An anomeric mixture[18] (7) was used for the synthesis of 8 as it was found much more convenient to separate epimers of the thiocarboxamide than separating the α and β anomers of 7 by chromatography.

The mixture 7 (5.0 g, 0.013 mmol), 4-dimethylaminopyridine (100 mg), and freshly condensed hydrogen sulfide (35 ml) were stirred in a sealed bomb at room temperature for 20 h. Hydrogen

Table III. Effect of Equimolar Doses of Ribavirin (1), 12, and 15 on Parainfluenza Virus Induced Deaths in Mice

Compd	Dose, mg/kg/day	Toxicity control, surv/total	Infected-treated, surv/total	Survivor increase, p[a]	Infected-treated, mean day of death	Mean day of death increase, p[b]
Placebo (CMC)[c]			2/20		8.9	
1	99	5/5	6/10	0.006	10.0	<0.02
	49.5		3/10	0.16	12.0	<0.001
12	104	5/5	0/10		12.3	<0.001
	52		1/10		11.0	<0.001
	26		1/10		10.9	<0.001
15	155	5/5	0/10		8.6	
	77.5	5/5	0/10		11.9	<0.001
	38.8		0/10		11.0	<0.001

[a] Probability (Fisher's exact test). [b] Probability (t test). [c] All compounds prepared in carboxymethylcellulose (0.125%, CMC).

sulfide was allowed to evaporate; the light yellow foam was dissolved in ethyl ether (200 ml), washed with saline water, and dried ($MgSO_4$). The evaporation of ether in vacuo gave a light yellow foam. Chromatography on a column (2.5 × 6 cm) of silica gel packed in chloroform using 5% (v/v) ethyl acetate in chloroform as eluent separated the two products (**6** and **8**) to provide 2.0 g of **6** as the faster migrating component and 1.0 g of **8** which was crystallized from ethanol: mp 152 °C; $[\alpha]^{25}D$ −39.0° (*c* 1, $CHCl_3$); ^1H NMR (Me_2SO-d_6) δ 2.42 (s, 6, methyls), 8.5 and 9.4 [pair of s (br), 2, $CSNH_2$]. Anal. ($C_{22}H_{23}NO_5S$) C, H, N, S.

Reaction of 2,5-Anhydro-3,4,6-tri-*O*-benzoyl-D-allonthioamide with Ethyl Bromopyruvate. A cold (0 °C) solution of ethyl bromopyruvate (18 g) in acetonitrile (60 ml) was added to an ice-cold solution of **4** (15 g, 29.7 mmol) in acetonitrile (180 ml). After 5 min of stirring the ice bath was removed and the reaction solution was stirred at room temperature for 1 h. The reaction solution turned deep orange, the solvent was evaporated in vacuo (20 °C), and the residue (syrup) was triturated with a saturated solution of sodium bicarbonate (150 ml). This was extracted with ether (250 ml × 2); the ether portion was washed with water and dried ($MgSO_4$). The ether was evaporated in vacuo to provide a syrup which was passed through a column of silica gel (600 g) packed in chloroform. Elution of the column with 10% (v/v) ethyl acetate in chloroform provided one minor (**11**) and two major (**9** and **10**) products described below.

Ethyl 2-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)thiazole-4-carboxylate (9). Fractions containing the major, fast-moving product were collected and evaporated in vacuo to provide 7.5 g (42%) of the pure product **9** as a syrup: ^1H NMR ($CDCl_3$) δ 1.35 (t, 3, *J* = 7 Hz, CH_3 of ethyl carboxylate), 4.40 (q, 2, *J* = 7 Hz, CH_2 of ethyl carboxylate), 4.85 (m, 3, $H_{1'}$ and $H_{5'}$), 5.90 (m, 3, $H_{2'}$, $H_{4'}$), 7.2–8.13 (m, 15, tri-*O*-benzoyl), 8.17 (s, 1, H_5). Anal. ($C_{32}H_{27}NO_9S$) C, H, N, S.

Ethyl 2-(2,3,5-Tri-*O*-benzoyl-α-D-ribofuranosyl)thiazole-4-carboxylate (10). Fractions containing the slower moving product (on TLC, very much like **4**) were collected and the solvent was evaporated in vacuo to provide 4.0 g (22%) of **10** in the form of a homogeneous syrup. The syrup was crystallized on keeping and scratching. The crystalline product was recrystallized from ethanol: mp 85–86 °C; ^1H NMR ($CDCl_3$) δ 1.33 (t, 3, *J* = 7.5 Hz, CH_3 of ethyl carboxylate), 4.37 (q, 2, CH_2 of ethyl carboxylate), 4.6–5.0 (m, 3, $H_{1'}$, $H_{5'}$), 5.9–6.35 (m, 3, $H_{2'}$, $H_{3'}$, $H_{4'}$), 7.2–8.2 (m, 15, tri-*O*-benzoyls), 8.23 (s, 1, H_5). Anal. ($C_{32}H_{27}NO_9S$) C, H, N, S.

Ethyl 2-(5-Benzoyloxymethylfuran-2-yl)thiazole-4-carboxylate (11). Fractions containing one minor product (moving slower but very close to **9**) were evaporated in vacuo to provide a syrup which was crystallized from methanol to provide 734 mg (7%) of **11**: mp 99–100 °C; $[\alpha]^{25}D$ 0.0° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.38 (s, 2, CH_2 of benzoyloxymethyl), 6.66 and 7.17 (dd, 2, H_3 and H_4). Anal. ($C_{18}H_{15}NO_5S$) C, H, N, S.

2-β-D-Ribofuranosylthiazole-4-carboxamide (12). A concentrated solution of **9** (5.0 g, 8.31 mmol) in methanol (15 ml) was stirred with methanolic ammonia (saturated at 0 °C, 100 ml) in a pressure bottle at room temperature for 2 days. The solvent was evaporated and the residue was chromatographed through a column (2.5 × 35 cm) of silica gel (100 g) packed in ethyl acetate. Elution of the column with a solvent system (ethyl acetate–1-propanol–water, 4:1:2; v/v; top layer) removed the fast-moving methyl benzoate and benzamide. The slower moving, major, UV and sugar-positive fractions were collected and the solvent was evaporated in vacuo. The residue (syrup), thus obtained, was readily crystallized from ethanol–ethyl acetate to provide 1.6 g (74%) of pure product **12**: mp 144–145 °C; $[\alpha]^{25}D$ −14.3° (*c* 1, DMF); UV λ_{max}^{pH1} 237 nm (ε 8640); UV λ_{max}^{pH11} 238 nm (ε 8100); ^1H NMR (Me_2SO-d_6) δ 7.5–7.8 [s (br), 2, $CONH_2$]; ^1H NMR (Me_2SO-d_6–D_2O) δ 4.99 (d, 1, *J* = 5 Hz, $H_{1'}$), 8.25 (s, 1, H_5). Anal. ($C_9H_{12}N_2O_5S$) C, H, N, S.

2-α-D-Ribofuranosylthiazole-4-carboxamide (13). The synthesis of **13** was achieved from **10** following the same procedure as described for the synthesis of **12**. In this case the product obtained after column chromatography was crystallized from ethanol–water to provide a 75% yield of pure product: mp 214–215 °C; $[\alpha]^{25}D$ −51.7° (*c* 1, DMF); UV λ_{max}^{pH1} 237 nm (ε 8420); UV λ_{max}^{pH11} 238 nm (ε 8290); ^1H NMR (Me_2SO-d_6) δ 7.4–7.7 [s (br), 2, $CONH_2$]; ^1H NMR (Me_2SO-d_6–D_2O) δ 5.3 (d, 1, *J* = 3.5 Hz, $H_{1'}$), 8.27 (s, 1, H_5). Anal. ($C_9H_{12}N_2O_5S$) C, H, N, S.

2-(5-Hydroxymethylfuran-2-yl)thiazole-4-carboxamide (14). A solution of **11** (714 mg, 2.0 mmol) was stirred with methanolic ammonia (saturated at 0 °C, 50 ml) in a pressure bottle at room temperature for 4 days. The solvent was evaporated. The residue was triturated with water, and the separated solid was filtered and crystallized from water to provide 225 mg (50%) of **14** as needles: mp 192–193 °C; UV λ_{max}^{pH1} 227 nm (ε 12715) and 309 (16371); UV λ_{max}^{pH11} 230 nm (ε 11737) and 308 (16628); ^1H NMR (Me_2SO-d_6) δ 4.55 [s (br), 2, CH_2], 5.45 [s (br), 1, OH], 6.58 and 7.15 (dd, 2, *J* = 4 Hz, H_3 and H_4 furan), 7.7 [s (br), 2, $CONH_2$], 8.3 (s, 1, H_5 of thiazole).

2-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)thiazole-4-carboxamide (15). Acetic anhydride (2.0 ml) was added to an ice-cold solution of **12** (1.04 g, 4 mmol) in anhydrous pyridine (16 ml) and the reaction solution was stirred at room temperature for 17 h. The solvent was evaporated in vacuo, the residue was dissolved in ethyl acetate, and the solution was washed with water and dried ($MgSO_4$). The ethyl acetate portion was evaporated in vacuo and the residue thus obtained was crystallized from water to provide 1.4 g (90%) of **15** as white needles: mp 103 °C; ^1H NMR ($CDCl_3$) δ 2.1 (3 s, 9, tri-*O*-acetyl), 6.2 and 7.15 [pair of s (br), 2, $CONH_2$], 8.2 (s, 1, H_5). Anal. ($C_{15}H_{18}N_2O_8S$) C, H, N, S.

2-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)thiazole-4-carbonitrile (16). A solution of **15** (1.16 g, 3 mmol) in chloroform (24 ml) was cooled to 0 °C and triethylamine (6.0 ml) was added to it followed by the addition of phosphorus oxychloride (0.75 ml). The reaction solution was stirred at room temperature for 2 h. The solvent was evaporated in vacuo and the residue was dissolved in chloroform (75 ml). It was washed with a saturated solution of $NaHCO_3$ (50 ml). The organic layer was separated, washed with water, and dried ($MgSO_4$). The solvent was evaporated in vacuo to provide a syrup (1.10 g, 100%) which was of sufficient purity for further reaction. The crude product was passed through a column of silica gel (80 g, packed in chloroform) and eluted with 20% (v/v) ethyl acetate in chloroform. The major, UV and sugar-positive fractions were collected and evaporated to provide 990 mg (90%) of analytically pure product **16** as a syrup: IR (neat in NaCl plates) ν_{max} 2228 cm^{-1} (CN). Anal. ($C_{15}H_{16}N_2O_7S$) C, H, N, S.

2-β-D-Ribofuranosylthiazole-4-thiocarboxamide (17). A magnetically stirred mixture of potassium hydroxide (225 mg, 4 mmol) in ethanol (10 ml) was saturated with hydrogen sulfide at room temperature. The clear solution was evaporated in vacuo and the residue was coevaporated in vacuo first with absolute ethanol and then with benzene. The dried residue (potassium hydrosulfide) was dissolved in absolute ethanol (10 ml). To this was added a solution of **16** (368 mg, 1 mmol) in ethanol (2 ml) and the clear solution was refluxed on steam bath for 1 h. The solvent was evaporated in vacuo, the residue was dissolved in water, and the solution was adjusted to pH 7 by adding diluted acetic acid. The solvent was again evaporated and the residue dried in vacuo. The residue was stirred in absolute ethanol (ca. 20 ml). The ethanol portion was filtered and evaporated in vacuo to provide a residue which was crystallized from ethanol, ethyl acetate, and ligroine to yield 165 mg (60%) of pure **17**: mp 134–135 °C; UV $\lambda_{max}^{pH1or11}$ 255 nm (ε 12032) and 306 (8210). Anal. ($C_9H_{12}N_2O_4S_2$) C, H, N, S.

2-(5-Deoxy-5-iodo-β-D-ribofuranosyl)thiazole-4-carboxamide (18). Rydon reagent[27] (3.2 g, 7.0 mmol) was added to a solution of **12** (1.0 g, 3.8 mmol) in dry DMF (15 ml) under nitrogen atmosphere and the resultant solution was stirred for 1 h at room temperature with exclusion of moisture. Methanol (1.0 ml) and sodium thiosulfate (2.0 g) were added and the reaction mixture was stirred for additional 5 min. The insoluble material was discarded by filtration and the filtrate was evaporated in vacuo. The residue was passed through a column packed with silica gel (2.5 × 36 cm, slurry in chloroform) which was eluted with 25% (v/v) chloroform in ethyl acetate. The homogeneous UV and sugar-positive fractions were collected and evaporated in vacuo to provide a crystalline compound which was recrystallized from ethyl acetate to provide 750 mg (58%) of **18** as fine needles: mp 156–157 °C; ^1H NMR (Me_2SO-d_6) δ 5.02 (d, 1, *J* = 4.5 Hz, $H_{1'}$), 8.27 (s, 1, H_5). Anal. ($C_9H_{11}N_2O_4SI$) C, H, N, I.

2-(5-Deoxy-β-D-ribofuranosyl)thiazole-4-carboxamide (19). Sodium acetate (200 mg) and Pd/C catalyst (10%, 200 mg) were

added to a solution of 18 (370 mg, 1.0 mmol) in ethanol (25 ml). The mixture was hydrogenated at room temperature in a Parr apparatus at 45 psi for 2 h. The reaction mixture was filtered through a Celite pad and the pad was washed with hot ethanol. The washings and filtrate were combined and evaporated in vacuo. The residue was dissolved in water (2 ml) and adjusted to pH 8 by adding $NaHCO_3$. The solvent was evaporated again and the residue was chromatographed through a column of silica gel (2.5 × 40 cm, slurry in ethyl acetate). Elution of the column with ethyl acetate provided the pure product **19** which was crystallized from ethyl acetate: yield 140 mg (56%); mp 154 °C; ^{1}H NMR (Me_2SO-d_6–D_2O) δ 1.33 (d, 3, J = 6 Hz, $H_{5'}$), 4.99 (d, 1, J = 3.5 Hz, $H_{1'}$), 8.25 (s, 1, H_5). Anal. ($C_9H_{12}N_2O_4S$) C, H, N, S.

Methyl 2-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)thiazole-5-carboxylate (20) and Methyl 2-(5-Benzoyloxymethylfuran-2-yl)thiazole-5-carboxylate (21). To a solution of **4** (5.00 g, 9.9 mmol) in benzene (5 ml) was added methyl formylchloroacetate (2.0 g) and the reaction solution was heated on a steam bath for 1 h. To this was added anhydrous sodium carbonate (525 mg, 5 mmol). Methyl formylchloroacetate (3.0 g) was further added in portions (1.0 g × 3) at intervals of 1 h and the refluxing was continued for an additional 1 h. The solvent was evaporated and the residue was triturated with a saturated solution of $NaHCO_3$ (150 ml). This was extracted with chloroform (150 ml) and then with ethyl acetate (150 ml). The chloroform and ethyl acetate portions were separately washed with water and then mixed together and dried ($MgSO_4$). The solvent was evaporated and the residue (syrup, 3.7 g) was chromatographed through a column of silica gel (3.5 × 5.5 cm, slurry in chloroform). Elution of the column with 5% (v/v) ethyl acetate in chloroform provided **20** and **21** as follows.

Methyl 2-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)thiazole-5-carboxylate (20). Slow-migrating homogeneous fractions which did not show fluorescence under UV light were collected and evaporated in vacuo to provide 300 mg (5.3%) of **20** as a syrup. This was characterized and analyzed after converting into **23**.

Methyl 2-(5-Benzoyloxymethylfuran-2-yl)thiazole-5-carboxylate (21). The fast-migrating homogeneous fractions, showing fluorescence under UV light were collected and evaporated to give a crystalline residue which was recrystallized from chloroform–ligroine to provide 1.0 g (29.4%) of the pure product **21**: mp 81–82 °C; ^{1}H NMR ($CDCl_3$) δ 3.92 (s, 3, CH_3), 5.38 (s, 2, CH_2 of 5-benzoyloxymethyl), 6.66 and 7.13 [s (pair), 2, H_3 and H_4 furan), 7.4–8.2 (m, 5, benzoyl), 8.4 (s, 1, H_4 of thiazole). Anal. ($C_{17}H_{13}NO_5S$) C, H, N, S.

2-(5-Hydroxymethylfuran-2-yl)thiazole-5-carboxamide (22). A solution of **21** (1.0 g, 2.91 mmol) in methanol (15 ml) was treated with methanolic ammonia (saturated at 0 °C, 50 ml) and the product isolated exactly as described for the synthesis of **14** to provide 260 mg (40%) of **22** as pale yellow needles: mp 225 °C dec; $[\alpha]^{25}_D$ +0.70° (*c* 1, DMF); UV $\lambda_{max}^{pH1or11}$ 331 nm (ε 23669); ^{1}H NMR (Me_2SO-d_6) δ 4.55 (d, 2, J = 6 Hz, CH_2 of hydroxymethyl), 5.5 (t, 1, J = 6.5 Hz, OH of hydroxymethyl), 6.58 and 7.18 (pair of s, 2, J = 3.0 and 3.5 Hz, respectively, H_3 and H_4 of furan), 7.75 and 8.15 [pair of s (br), 2, $CONH_2$], 8.45 (s, 1, H_4 of thiazole). Anal. ($C_9H_8N_2O_3S$) C, H, N, S.

2-β-D-Ribofuranosylthiazole-5-carboxamide (23). The samples of nucleoside **23** obtained separately from **20** or **26** were found identical (melting point, ^{1}H NMR, and TLC). The general procedure of synthesis is described. A mixture of **20** or **26** (0.25 mmol) and methanolic ammonia (saturated at 0 °C, 20 ml) was stirred in a sealed bottle at room temperature for 2 days. The solvent was evaporated and the residue was chromatographed through a column of silica gel (2 × 30 cm slurry in ethyl acetate). It was eluted with a solvent system (ethyl acetate–1-propanol–H_2O, 4:1:2 v/v; top layer) and the main sugar-positive and UV-absorbing fractions were collected. The solvent was evaporated and the residue was crystallized from ethanol–ethyl acetate: yield 52 mg (80%); mp 96–97 °C; ^{1}H NMR (Me_2SO-d_6) δ 7.55 and 8.00 [pair of s (br), 2, $CONH_2$]; ^{1}H NMR (Me_2SO-d_6–D_2O) δ 4.97 (d, 1, J = 5 Hz, $H_{1'}$), 8.32 (s, 1, H_4). Anal. ($C_9H_{12}N_2O_5S$) C, H, N.

2,5-Anhydro-6-*O*-benzoyl-D-allonthioamide (25). A mixture of 5-*O*-benzoyl-β-D-ribofuranosyl cyanide[20] (**24**, 10.0 g, 38.13 mmol), 4-dimethylaminopyridine (200 mg), and liquid hydrogen sulfide (50.0 ml) was stirred in a sealed bomb at room temperature for 2 days. Hydrogen sulfide was evaporated at atmospheric pressure at room temperature. The residue was dissolved in ethyl acetate, washed with water, and dried ($MgSO_4$). Evaporation of ethyl acetate in vacuo gave a crystalline residue which was recrystallized from ethyl acetate–ligroine to provide 9.5 g (84%) of **25** as a crystalline product: mp 140 °C; ^{1}H NMR (Me_2SO-d_6) δ 3.0–4.65 (6, sugar protons), 5.18 and 5.41 (dd, 2, J = 6 and 4.5 Hz, respectively, C_2OH and C_3OH), 7.6 and 8.05 [m (pair), 5, benzoyl], 8.92 and 10.05 [pair of s (br), 2, $CSNH_2$]. Anal. ($C_{13}H_{14}NO_5S$) C, H, N, S.

Methyl 2-(5-*O*-Benzoyl-β-D-ribofuranosyl)thiazole-5-carboxylate (26). A solution of **25** (600 mg, 2.20 mmol) in DMF (2 ml) was cooled to ca. 5 °C and methyl formylchloroacetate (0.50 g, 3.66 mmol) was added. The reaction solution was stirred at room temperature for 2 days. The solvent was evaporated and the residue was triturated with a saturated solution of sodium bicarbonate (20 ml). The mixture was extracted with ethyl acetate (2 × 50 ml) and the ethyl acetate solution was dried ($MgSO_4$) and evaporated in vacuo. The residue was chromatographed through a column of silica gel (50 g) packed in chloroform. The slow-moving compound was separated from the two faster moving products (the fastest was a fluorescent compound, possibly the benzoate elimination product, and the other was found to be the unchanged starting material **24**). The fractions containing the slow-moving product were collected and evaporated in vacuo. The residue was crystallized from chloroform–ligroine to provide 185 mg (25%) of **26** as a crystalline product: mp 114 °C (shrinks at 111–112 °C); ^{1}H NMR (Me_2SO-d_6) δ 3.81 (s, 3, CH_3), 4.20 and 4.55 [m (pair), 5, $H_{2'}$, $H_{3'}$, $H_{4'}$, and $H_{5'}$], 5.10 (d, 1, J = 3.5 Hz, $H_{1'}$), 7.62 and 8.00 [m (pair), 5, benzoyl], 8.39 (s, 1, H_4 of thiazole). Anal. ($C_{17}H_{17}NO_7S$·H_2O) C, H, N, S.

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A Study of the Mechanism of Halothane-Induced Liver Necrosis. Role of Covalent Binding of Halothane Metabolites to Liver Proteins in the Rat[1]

G. Subba Rao

Pharmacology Laboratory, Division of Biochemistry, Research Institute, American Dental Association Health Foundation, Chicago, Illinois 60611. *Received July 19, 1976*

Various anesthetic and nonanesthetic doses of [1-^{14}C]halothane were administered separately to normal and phenobarbital-pretreated (PBP) rats by ip route. The rats were sacrificed at 0.5–24 h after dosing, and livers were removed and examined histopathogically for tissue necrosis. Only PBP rats that received anesthetic doses of halothane (11.5 or 23 mmol/kg) and sacrificed 24 h after dosing exhibited liver toxicity. Determination of the radioactivity distribution among various liver macromolecules revealed that the protein fraction contained the highest activity at all time points in all animals. The lipid fraction showed some radioactivity during the initial 1–6-h period which disappeared after 6–8 h, while the DNA fraction was devoid of radioactivity in all animals injected with [^{14}C] halothane. All the PBP rats that exhibited liver necrosis consistently attained higher covalent binding of halothane metabolites to liver proteins (2.13–2.20 nmol/mg of protein) when compared with the protein binding (1.12–1.41 nmol/mg of protein) observed among the rats that did not exhibit liver toxicity during the same time period. These results suggest a correlation between covalent binding of halothane metabolites to liver proteins and halothane-induced liver necrosis.

Halothane ($CF_3CHBrCl$), a widely used general anesthetic agent, is implicated in occasional fatal hepatic necrosis observed among exposed humans.[2-4] The toxic hazards appear to increase with repeated anesthetic exposures to halothane.[4] Although there were numerous studies in recent years,[5-20] the mechanism of halothane-induced liver necrosis remains poorly understood. The formation of chemically reactive metabolites of halothane by the liver microsomal cytochrome P-450 enzyme system and covalent binding of these reactive metabolites to hepatic lipids and proteins were reported by several investigators.[7-20] However, the covalent binding of halothane metabolites to liver macromolecules has not been correlated with liver toxicity. Recently, good correlation between tissue toxicity and covalent binding of reactive metabolites to target tissue macromolecules was observed with several halogenated hydrocarbons, e.g., chloroform, carbon tetrachloride, and bromobenzene.[21-23]

The present studies were initiated to evaluate possible relationship between covalent binding of halothane metabolites to liver macromolecules and halothane-induced liver necrosis. Rats were used in these experiments since earlier studies[24,25] showed that halothane toxicity can be induced in this species following phenobarbital pretreatment (PBP).

Experimental Section

Animals. Male Sprague–Dawley rats, weighing 100–120 g, were purchased from Camm Research Institute, Wayne, N.J., and maintained on Purina rat chow. The animals were fasted overnight prior to halothane administration and were fed thereafter. Drinking water was available ad libitum. The rats were divided into two major groups, normal and phenobarbital pretreated.

Phenobarbital Pretreatment (PBP). Rats were pretreated with phenobarbital sodium USP (80 mg/kg, in distilled water, ip) for three consecutive days and were used on the fourth day.[26,27]

Labeled Halothane Solutions. [1-^{14}C]Halothane (New England Nuclear, Boston, Mass.; specific activity 1.35 mCi/mmol) was dissolved in olive oil and made up to the requisite concentrations with unlabeled halothane (Ayerst Laboratories, Inc., New York, N.Y.). The halothane doses used were 4.6, 11.5, and 23.0 mmol/kg. Each animal received 50 μCi of ^{14}C/kg.

Halothane Administration. The protocol employed for in vivo studies with [^{14}C]halothane in normal and PBP rats is described in Table I. The requisite doses of halothane were administered by ip route. Six rats were used at each time point