Novel Precursor for the Synthesis of C-Nucleoside Analogues. Synthesis of the C-Nucleoside Analogues of Ribavirin, Bredinin, and Related Compounds

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Received May 29, 1979

Synthesis of β-D-ribofuranosyl-1-carboximidic acid methyl ester, a novel and versatile precursor for the synthesis of C-nucleoside analogues, has been described. The utility of this precursor has been illustrated by the preparation of several C-nucleoside analogues of 1,2,4-triazole and imidazole nucleosides, including the C-nucleoside analogues or ribavirin and bredinin. The synthesis and structural determination of an N-methyl derivative of the C-nucleoside analogue of ribavirin have also been described.

Since a majority of the biologically active C-nucleosides possess the β-stereochemistry at C-1 of the sugar moiety,[2] several methods have been described in the recent literature for synthesis of β-oriented C-nucleosides.[3] A key step in developing syntheses for such C-nucleoside analogues is the establishment of a functionalized C–C bond with the β configuration at C-1 of the carbohydrate precursor. Hanessian and Pernet[4] first synthesized carbohydrate derivatives with a functionalized carbon fragment at the C-1 position by condensation of tetra-O-acetyl-α-D-glucopyranosyl bromide with diethyl or dibenzyl sodiomalonate. Ohrui and Fox[5a] synthesized a ribosylmalonate in a similar manner and cyclized it with urea to give a pyrimidine C-nucleoside. Since then, several reports on the syntheses of a variety of C-nucleosides from functionalized carbon fragments containing glycosyl intermediates have appeared.[5b] The β-oriented 1-cyanoribosyl derivative synthesized by Bobek and Farkas[6] has previously been utilized for the synthesis of various C-nucleoside analogues.[7,8] In our approach the 1-cyanoribosyl derivative of Bobek and Farkas has been transformed to β-D-ribofuranosyl-1-carboximidic acid methyl ester which has proven to be a versatile precursor for the synthesis of a variety of C-nucleoside analogues.

Results and Discussion

Synthesis of β-D-Ribofuranosyl-1-carboximidic Acid Methyl Ester (2) and Its Use in the Synthesis of C-Nucleoside Analogues of Ribavirin,[9] Bredinin,[10] and Related Nucleosides. Compound **2** was easily prepared by reacting 2,3,5-tri-O-benzoyl-β-D-ribofuranosyl 1-cyanide[6] with a catalytic amount of sodium methoxide in methanol. The crystallized compound is quite stable for extended periods under anhydrous conditions. The unavailability of the other anomer of the imidic ester made the assignment of its anomeric configuration on the basis of its ^{1}H NMR $J_{1',2'}$ (=2 Hz) difficult. However, since it was derived from a β-oriented glycosyl cyanide[11] and the C-nucleoside analogues synthesized from it were confirmed to be β anomers, its stereochemistry was therefore assumed to be β.

On the basis of the literature report[12] that N-acyl derivatives of acetamidrazones readily cyclized to 1,2,4-triazole derivatives under the action of heat, intermediate **3** (Scheme I) was considered to be an appropriate precursor for the synthesis of the C-nucleoside analogue of ribavirin. Compound **3** was obtained in 70% yield by reaction of the imidic ester **2** with oxamidohydrazide. On fusion (135 °C), intermediate **3** was converted to the C-nucleoside analogue of ribavirin **8** in almost quantitative yield. Similar to the preparation of **3**, intermediates **4**, **5**, and **6** were prepared by reaction of the corresponding hydrazides with imidate **2**. However, on fusion only intermediate **4** cyclized to the corresponding C-triazole nucleoside **9**; compounds **5** and **6** were recovered unchanged. In addition to C-triazole nucleoside **9**, the fusion of intermediate **4** produced about 5% of C-oxadiazole nucleoside **20**.

Analogous to the nucleophilic reactions of oxamidohydrazide, aminoguanidine and thiosemicarbazide reacted with **2** to yield the open-chain intermediates **7** and **13**, respectively. On fusion, compound **7** produced the C-nucleoside analogue of 5-amino-1,2,4-triazole **12**, and intermediate **13** yielded 3-(β-D-ribofuranosyl)-1,2,4-triazole-5-(1*H*,4*H*)-thione (**14**). A synthesis of the C-nucleoside analogue **12** by a multistep procedure has been reported by Just and co-workers.[13]

The C-nucleoside analogue of the immunosuppressive and antiviral agent bredinin[10] was prepared from methyl imidate **2** in a two-step procedure (Scheme I). In the first step, the reaction of aminomalonamide with **2** in dimethyl

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Scheme I

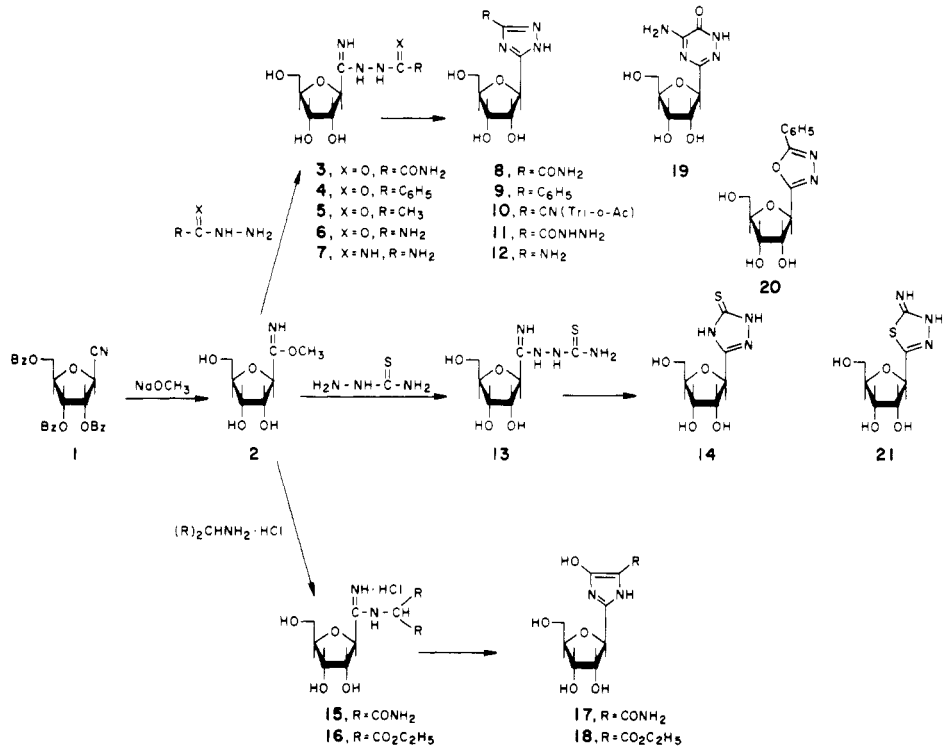

Table I. Pertinent Proton NMR[a] Data for *C*-Nucleoside Analogues and Their 2′,3′-*O*-Isopropylidene Derivatives

compd	H_1', δ	$J_{1',2'}$, Hz	CH_3, δ	δ Δ CH_3	H_4', δ (multiplicity)
8	4.73	5			
22	4.94	4	1.32, 1.50	0.18	4.05 (m)
9	4.81	4.5			
12	4.48	4			
10 (tri-*O*-Ac)	5.24	4.5			
11	4.75	5.0			
14	4.49	6.5			
14 (2′,3′-*O*-isop)	4.73	5.5	1.3, 1.47	0.17	4.02 (m)
17	4.58	6.0			
17 (2′,3′-*O*-isop)	4.75	*b*	1.27, 1.48	0.21	4.02 (m)
18	4.58	5.0			

[a] Spectra were recorded in Me_2SO-d_6 with tetramethylsilane as internal standard. The chemical shift values (δ) are in parts per million downfield from Me_4Si. [b] Not measurable.

sulfoxide at room temperature yielded the intermediate **15**. Subsequent heating of this intermediate in water led to the cyclized product **17** in high yield (74%). The carboxy ester derivative **18** was obtained in a somewhat similar manner by the reaction of diethyl aminomalonate with **2** and subsequent conversion of the open-chain precursor **16** to **18**. The yield of the final product **18**, however, was only 35%.

Structure Determination. (a) Anomeric Configuration of the *C*-Nucleoside Analogues. Due to the uncertainties of using proton NMR $J_{1',2'}$ values for the assignment of anomeric configuration of *C*-nucleosides, the data obtained from the 2′,3′-*O*-isopropylidene derivatives of **8**, **14**, and **17** were used as a basis for these determinations. As listed in Table I, the proton NMR chemical shift differential (δΔ) of the two methyl groups in the isopropylidene derivatives of **8**, **14**, and **17** (0.18, 0.17, and 0.21 ppm, respectively) is indicative of β stereochemistry in accordance with Imbach's rules.[14] In addition, the H4′ resonance in each of the above isopropylidene derivatives gave a multiplet which also is supportive of the β configuration, according to a recent publication.[15] Since the other *C*-nucleoside analogues in each series were obtained by similar reactions and their $J_{1',2'}$ values (Table I) are in the same range as the above examples, they are also assigned the β configuration.

(b) Structure of the Heterocyclic Moiety in Compounds 8 and 14. Except for these two compounds, the structure of the heterocyclic moiety in the other *C*-nucleosides discussed above was derived from elemental analysis and proton NMR. In the case of compound **8**, the alternate structure of triazinedione **19** was considered because precursor **3** could cyclize to either of these compounds depending upon which of the two carbonyls is the target of nucleophilic attack by the imine nitrogen. NMR and IR data were inconclusive in discriminating between these two alternatives. However, conversion of the product to the cyanotriazole derivative **10** ruled out the triazinedione structure **19**. Finally, **8** was established to be the correct structure for this *C*-triazole nucleoside by X-ray crystallographic analysis.[18]

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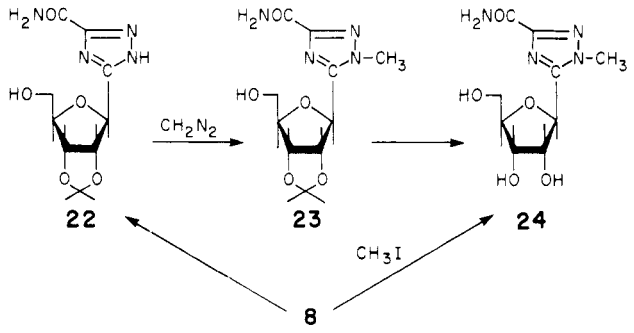
Scheme II



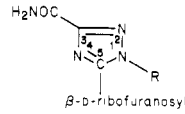
For compound 14 the alternate structure 21 was considered. NMR spectroscopy was unable to distinguish between the two alternatives. However, when the detailed UV spectra of this material are compared [0.1 N HCl, λ_{max} 208 nm (ϵ 5800), 248 (16 800); pH 7, λ_{max} 215 nm (ϵ 8400), 240 (12 700); 0.1 N KOH, λ_{max} 229 nm (ϵ 11 800)] with commercially available 1*H*-1,2,4-triazole-3-thiol [0.1 N HCl, λ_{max} 208 nm (ϵ 6200), 245 (15 100); pH 7, λ_{max} 215 nm (ϵ 6950), 243 (13 200); 0.1 N KOH, λ_{max} 234 nm (ϵ 12 500)], the similarity of chromophore in the two compounds was obvious. These data strongly supported structure 14 over the alternative thiadiazole isomer 21. Measurements of pK_a also were consistent with the above indication [compound 14, pK_{a_1} = 6.05 ± 0.1, pK_{a_2} = 12.9 ± 0.1; 1,2,4-triazole-3-thiol, pK_{a_1} = 6.87 ± 0.1, pK_{a_2} = 13.2 ± 0.1]. Finally the structure 14 was established to be correct by X-ray analysis.[18]

Methylation of the *C*-Nucleoside Analogue of Ribavirin. N-Methylation of the *C*-nucleoside analogue of ribavirin was carried out by two methods (Scheme II), and the isolated *N*-methyl products from both methods were found to be identical. The diazomethane method involved initial preparation of the 2′,3′-*O*-isopropylidene derivative **22** which was then subjected to diazomethane treatment followed by removal of the isopropylidene group. On the other hand, direct methylation could be carried out with CH_3I by using unprotected *C*-ribavirin. In the latter procedure formation of two isomers was indicated in the crude reaction mixture (proton NMR: two *N*-methyl resonances at δ 3.94 and 4.11, two H1′ doublets at δ 4.64 and 4.90). Only one of these isomers could be eluted from the silica gel column. The structure of the *N*-methyl derivative obtained by either method was established to be the *N*-methyl derivative **24** on the basis of ^{13}C NMR spectroscopy. The ^{13}C resonances of carbons 3 and 5 in the *N*-methyl compound were compared with the corresponding resonances in the *C*-nucleoside analogue of ribavirin 8. As shown in Table II, ^{13}C resonances of carbon 5 and carbon 3 in compound **24** undergo an upfield shift of 12.4 ppm and a downfield shift of 5.3 ppm, respectively, as compared to the corresponding resonances in compound 8. According to the observations made on N-substituted triazoles,[16] these data are compatible with structure **24**. The other two possible *N*-methyl isomers (N-2 and N-4) would require either an upfield shift in the ^{13}C resonance of carbon 3 (α carbon, N-2 isomer) or an upfield shift in the ^{13}C resonance of both carbons 3 and 5 (both α carbons, N-4 isomer).

Experimental Section

General Methods. Melting points were determined in a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton and ^{13}C magnetic resonance spectra were obtained in Me_2SO-d_6 on a Varian XL-100 or a Bruker HFX-10 spectrometer, respectively, with tetramethylsilane as internal reference. Solvents were dried by storage over molecular sieves (4A) with the exception of methanol which was stored over 3A molecular sieves. Reactions and products were monitored by TLC using 0.25-mm silica gel plates (60F-254) purchased from Brinkmann Instruments.

Table II. ^{13}C NMR[a] Results for the Triazole Moiety of the *C*-Nucleoside Analogue of Ribavirin, 8, and Its *N*-Methyl Derivative 24



carbon	R = H	R = CH_3	shift
C-3	153.2	158.5	downfield shift of 5.3 ppm
C-5	159.0	146.6	upfield shift of 12.4 ppm

[a] Spectra were recorded in Me_2SO-d_2 with tetramethylsilane as internal standard. The chemical shift values of the ^{13}C spectra are in parts per million downfield from Me_4Si.

β-D-Ribofuranosyl-1-carboximidic Acid Methyl Ester, 2. To a suspension of 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl cyanide[6] (1; 124.1 g, 263.23 mmol) in 675 mL of dry methanol was added sodium methoxide (3.15 g, 58.4 mmol), and the solution, which became homogeneous in 5 min, was stirred for 2.5 h at room temperature. For neutralization of the base, cation-exchange resin (AG 50W-X8, H^+, dried at 100 °C (0.05 mm Hg), 16 h, 13 g, 5.1 molar equiv/g) was added to the stirred solution. The resin was filtered, and the solvent was distilled at about 40 °C. The residue was isolated and washed with methanol. The methanol washings were concentrated to obtain second and third crops of **2**. The three crops were combined and recrystallized from dry methanol: yield 35.4 g (70%); mp 140–142 °C; NMR δ 3.59 (s, 3, OCH_3), 3.50–3.90 (m, 5, 2′-, 3′-, and 4′-CH and 5′-CH_2), 4.06 (d, 1, 1′-CH, $J_{1',2'}$ = 2 Hz), 4.93 (br s, 3, 2′-, 3′-, 5′-OH), 8.25 (s, 1, C=NH).

Anal. Calcd for $C_7H_{13}NO_5$: C, 43.97; H, 6.85; N, 7.33. Found: C, 44.05; H, 7.01; N, 7.22.

2-[(Aminocarbonyl)carbonyl]-1-(β-D-ribofuranosyliminomethyl)hydrazine, 3. Methyl imidate **2** (3.49 g, 33.87 mmol) and oxamidohydrazide (6.475 g, 33.87 mmol) were dissolved in 350 mL of dimethyl sulfoxide. After the reaction solution was stirred for 20 h at room temperature, the solvent was distilled off at 55 °C in vacuo. The residual solid was suspended in methanol, and the soluble portion was collected by filtration (the insoluble solid was found to be unreacted hydrazide) and concentrated to about 25 mL. By addition of this solution dropwise into acetonitrile (500 mL) a precipitate was obtained: yield 6.33 g (71.3%); NMR δ 3.6 (m, 2, 2′- and 3′-CH), 3.8 (m, 1, 4′-CH), 3.95 (m, 2, 5′-CH_2), 4.15 (d, 1, 1′-CH, $J_{1',2'} \approx 1$ Hz), 5.2 (br s, 2, CONHNHC), 10.05 (br s, 1, C=NH).

Anal. Calcd for $C_8H_{14}N_4O_6$: C, 36.64; H, 5.38; N, 21.37. Found: C, 36.41; H, 5.60; N, 20.09. (Due to the unstable and hygroscopic nature of this compound good elemental analysis could not be obtained.)

1-(β-D-Ribofuranosyliminomethyl)-2-benzoylhydrazine, 4. A solution of methyl imidate **2** (1.912 g, 10 mmol) and a stoichiometric amount of freshly recrystallized benzoylhydrazine in methanol (100 mL) was kept at room temperature with stirring for 17 h. The precipitated solid was isolated. A second crop of the product was obtained on concentrating the filtrate and allowing the oil to stand for approximately 1 h. Thus, 2.52 g (85.3%) of TLC-pure material, mp 187 °C, was obtained: NMR δ 3.60 (m, 2, 5′-CH_2), 3.78 (m, 1, 4′-CH), 4.00 (m, 2, 2′-, 3′-CH), 4.21 (br s, 1, 1′-H, $J_{1',2'} \approx 0$ Hz), 5.0 (br, 3, 3 OH), 6.41 (br s, 2, -NHNH-), 7.3–7.9 (2 m, 5, aromatic protons), 9.78 (br s, 1, C=NH).

Anal. Calcd for $C_{13}H_{17}N_3O_5$: C, 52.82; H, 5.80; N, 14.22. Found: C, 52.76; H, 5.62; N, 14.05.

1-(β-D-Ribofuranosyliminomethyl)-2-acetylhydrazine, 5. A solution of imidate **2** (191.2 mg, 1 mmol) in methanol (10 mL) was treated with an equimolar amount of acethydrazide for 18 h at room temperature. The reaction mixture was concentrated,

(18) J. F. Blount, E. F. Nowoswiat, and M. S. Poonian, unpublished results.

and the residue was recrystallized from methanol. The yield of the product was 85%, and NMR showed it to be a mixture of two *N*-acetyl rotomers: NMR δ 1.79 and 1.95 (2 s, 3, $NCOCH_3$, the two singlets coalesced into a single resonance at 85 °C), 3.54 (m, 2, 5′-CH_2), 3.72 (m, 1, 4′-CH), 3.94 (m, 3, 1′-, 2′-, and 3′-CH), 4.95 (br, 3, 3 OH), 6.04 (br s, 2, NH_2), 9.3 (s, 1, NH) (the ratio of the two rotomers was 55:45 as judged by the integration of the two *N*-acetyl signals); IR (KBr) 1555, 1655 cm^{-1}.

Anal. Calcd for $C_8H_{16}N_3O_5$: C, 41.20; H, 6.69; N, 18.02. Found: C, 40.84; H, 6.69; N, 17.76.

2-(Aminocarbonyl)-1-(β-D-ribofuranosyliminomethyl)-hydrazine, 6. A solution of the imidate **2** (1.912 g, 10 mmol) and a stoichiometric amount of semicarbazide hydrochloride in dimethyl sulfoxide was stirred for 18 h at room temperature. The solvent was removed in vacuo (0.1 mm) at 60 °C and the residual oil dissolved in methanol (~15 mL). The product was precipitated by adding the methanol solution to acetonitrile (200 mL). The precipitate was isolated in a dry atmosphere (under argon) and dried at 56 °C (0.1 mm) for 17 h. To convert the hydrochloride salt to the free base, an aqueous solution of the product was passed through a DE-23 (diethylamino)ethylcellulose column (3.5 × 35 cm). The product was collected in a 500-mL fraction which was concentrated to an oil, coevaporated once with ethanol, and dissolved in the minimum amount of methanol by heating on a steam bath. When the methanol solution was allowed to stand at room temperature, a solid precipitated. Three more crops of **6** were obtained by concentrating the mother liquors: yield 567 mg (24%); mp 179–180 °C; NMR δ 3.5 (m, 2, 5′-CH_2), 3.68 (m, 1, 4′-CH), 3.85 (m, 3, 2′- and 3′-CH and OH), 4.7 (d, 1, 1′-CH, $J_{1'2'}$ = 4.5 Hz), 4.94 (m, 2, 2 OH), 5.85 and 5.95 (d, 4, $NHNHCONH_2$), 8.26 (s, 1, C=NH); IR (KBr) 3600–3200, 1695, 1677, 1100 cm^{-1}.

Anal. Calcd for $C_7H_{14}N_4O_5$: C, 35.90; H, 6.03; N, 23.92. Found: C, 35.98; H, 6.25; N, 24.22.

(β-D-Ribofuranosyliminomethyl)guanidinoamine Dihydrochloride, 7. To a stirred solution of aminoguanidine dihydrochloride (1.47 g, 10 mmol) in 100 mL of anhydrous methanol was added methyl imidate **2** (1.91 g, 10 mmol). After 18 h at room temperature the solvent was distilled off and the white residue recrystallized from methanol to obtain 1.48 g (48%) of a colorless powder: mp 181–183 °C; NMR δ 3.63 (m, 2, 5′-CH_2), 3.93 (m, 1, 4′-CH), 3.96 and 4.12 (2 m, 1 each, 2′- and 3′-CH), 4.55 (d, 1, 1′-CH, $J_{1'2'}$ = 4.5 Hz), 4.7–6.8 (br, 3 OH signals), 8.04 (s, 5, =NH, NH_2·2HCl), 9.63 (br s, 2, –NHNH–), 10.35 (br, 1, C=NH).

Anal. Calcd for $C_7H_{15}N_5O_4$·2HCl: C, 27.46; H, 5.60; N, 22.88; Cl^-, 23.16. Found: C, 27.96; H, 5.67; N, 22.78; Cl^-, 22.39.

5-(Aminocarbonyl)-3-(β-D-ribofuranosyl)-1,2,4-triazole, 8. A sample of compound **3** (6.33 g, 24.2 mmol) was heated under vacuum (0.1 mm) at 135 °C for 15 min. After the flask was cooled, the glassy material was treated with methanol and heated on a steam bath. During this process a solid started to precipitate. After about 2 h, the solid was isolated, and a second crop was obtained on concentrating the filtrate. The total yield of the product was 3.68 g (62.2%), mp 195–196 °C. This material was recrystallized from ethanol–H_2O (5:1) for single-crystal X-ray analysis: NMR δ 3.53 (m, 2, 5′-CH_2), 3.82 (m, 1, 4′-CH), 3.45 and 4.17 (m, 1 each, 2′- and 3′-CH), 4.73 (d, 1, 1′-CH, $J_{1'2'}$ = 5 Hz), 7.64 and 7.84 (br s, 1 each, $CONH_2$), extremely broad hydroxyl and NH protons between δ 5 and 7. However, the tri-*O*-acetate derivative of **8** gave three acetyl signals (δ 1.98, 2.06, and 2.07) and an NH signal at δ 14.9. For the 2′,3′-*O*-isopropylidene derivative **22**: mp 143–145 °C; NMR δ 1.32 and 1.50 [2 s, 3 each, $C(CH_3)_2$], 3.40 (d, 2, 5′-CH_2), 3.45 (br, 1, 5′-OH), 4.05 (m, 1, 4′-CH), 4.73 (m, 1, 3′-CH), 4.94 (d, 1, 1′-CH, $J_{1'2'}$ = 4 Hz), 5.05 (m, 1, 2′-CH), 7.69 and 7.91 (2 br s, 1 each, $CONH_2$), 1 NH proton signal buried under $CONH_2$ signals.

Anal. Calcd for $C_8H_{12}N_4O_5$ (**8**): C, 39.35; H, 4.95; N, 22.94. Found: C, 39.19; H, 5.17; N, 22.74.

5-Phenyl-3-(β-D-ribofuranosyl)-4*H*-1,2,4-triazole, 9. The open-chain precursor **4** (1.88 g, 6.39 mmol) was heated at 185 °C under vacuum (0.05 mm) for 15 min. After cooling, the residue was dissolved in a mixture of chloroform and methanol (9:1), and the solution was allowed to stand. The solid material obtained was isolated, recrystallized from acetone, and dried. A 31% yield (0.55 g) of the product, mp 150–153 °C, was obtained. The filtrate obtained above was concentrated to an oil and chromatographed on a prepacked silica column (3.7 × 44 cm, eluent chloroform–ethanol, 9:1). This afforded 5% of another product which was identified to be **20** on the basis of mass spectral and NMR analysis. For **9**: NMR δ 3.52–3.63 (m, 2, 5′-CH_2), 3.88 (m, 1, 4′-CH), 4.02–4.21 (2 t, 1 each, 2′- and 3′-CH), 4.81 (d, 1, 1′-CH, $J_{1'2'}$ = 4.5 Hz), three hydroxyl protons and one NH proton between 4.2 and 5.2 (br), 7.32 and 7.98 (2 m, 5, phenyl protons); IR (KBr) 1690 cm^{-1} (aromatic); mass spectrum *m/e* 277, and compatible fragmentation pattern; UV (H_2O) 244 nm (ε 14 650). For **20**: NMR δ 3.54 (m, 2, 5′-CH_2), 3.92, 4.08 and 4.39 (each m, 1 each, 4′-, 3′- and 2′-CH), 4.72, 5.06, and 5.36 (t, d, d, 1 each, 3 OH), 4.90 (d, 1, 1′-CH, $J_{1'2'}$ = 4.5 Hz), 7.6 and 8.0 (2 m, 5, phenyl protons); mass spectrum *m/e* 278, the fragmentation pattern was compatible and was analogous to the fragmentation pattern of **9** except that significant peaks were up by one mass unit as expected.

Anal. Calcd for $C_{13}H_{15}N_3O_4$ (**9**): C, 56.31; H, 5.45; N, 15.15. Found: C, 56.09; H, 5.75; N, 14.91.

5-Cyano-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole, 10. A solution of compound **8** (1.221 g, 5 mmol) in 50 mL of anhydrous pyridine was treated with 10 mL of acetic anhydride for 16 h at room temperature. The solvents were removed by evaporation under vacuum. TLC analysis of the residual oil showed quantitative acetylation. The oil was redissolved in pyridine (50 mL), and freshly distilled phosphorous oxychloride (10 mL, 106 mmol) was carefully added. The solution was heated at reflux for 1 h, cooled to room temperature, and slowly poured over crushed ice. The reaction mixture was concentrated to an oil and partitioned between water and ethyl acetate. The organic phase was washed with water (3 × 25 mL), dried over anhydrous sodium sulfate, and concentrated to an oil. The crude oil was fractionated on a silica gel column (2.8 × 40 cm, eluent ethyl ether). The fractions containing the product were concentrated, decolorized with charcoal, and filtered through a bed of Celite. The filtrate was concentrated to an oil which was dried at 79 °C (0.05 mm) for 17 h: yield of the oily product 1.03 g (58%); NMR δ 1.94 (s, 3, $OCOCH_3$), 2.07 (s, 6, 2 $OCOCH_3$), 3.9–4.4 (m, 3, 4′-CH and 5′-CH_2), 5.24 (d, 1, 1′-CH, $J_{1'2'}$ = 5 Hz), 5.31 and 5.56 (2 t, 1 each, 2′- and 3′-CH); IR ($CHCl_3$) 2260 cm^{-1}.

Anal. Calcd for $C_{19}H_{16}N_4O_7$: C, 47.73; H, 4.58; N, 15.90. Found: C, 47.87; H, 4.82; N, 15.92.

3-(β-D-Ribofuranosyl)-5-(hydrazinocarbonyl)-1,2,4-triazole, 11. Compound **8** (2.0 g, 8.19 mmol) in 20 mL of 85% hydrazine hydrate was stirred at ambient temperature for 16 h. Unreacted hydrazine hydrate was removed at 50 °C (0.05 mm) and the residue coevaporated once with ethanol. The oily residue was dissolved in a minimal amount of the solvent mixture (CH_3OH–H_2O, 75:25) and allowed to stand overnight. The crystals were isolated, washed with methanol, and dried at 76 °C (0.05 mm) for 20 h. In the first crop, 490 mg (mp 202–204 °C) of product was isolated. On further concentration of the filtrate, 441 mg of second crop crystals (mp 196–200 °C) was obtained: yield 43.8%; NMR δ 3.53 (m, 2, 5′-CH_2), 3.82 (m, 1, 4′-CH), 3.95, 4.17 (2 m, 1 each, 2′- and 3′-CH), 4.74 (d, 1, 1′-CH, $J_{1'2'}$ = 5.0 Hz), 4.3–5.4 (broad hydroxyl signals, 3 OH), 9.74 (broad ring NH and CONHN signals).

Anal. Calcd for $C_8H_{13}N_5O_5$: C, 37.06; H, 5.05; N, 27.02. Found: C, 37.08; H, 5.23; N, 26.73.

5-Amino-3-(β-D-ribofuranosyl)-4*H*-1,2,4-triazole, 12. Compound **7** (1.02 g, 3.33 mmol) was heated under vacuum (50 μm) at 183 °C for 12 min. The flask was cooled down to room temperature, and the glassy residue was taken up in methanol. The methanol solution was concentrated, and the residual material was passed through a DEAE-cellulose column in water to remove the hydrochloric acid. The effluent from the column was concentrated to obtain a highly hygroscopic white solid: yield ~60%; NMR δ 3.42–3.56 (m, 2, 5′-CH_2), 3.77 (m, 1, 4′-CH), 4.02 and 4.06 (2 m, 1 each, 2′- and 3′-CH), 4.48 (d, 1, 1′-CH, $J_{1'2'}$ = 5 Hz), 5.4–6.6 (br, 3 OH, NH, and NH_2); mass spectrum *m/e* 216, compatible fragmentation pattern. The best elemental analysis was consistent with 0.5 mol of water.

Anal. Calcd for $C_7H_{12}N_4O_4 \cdot {}^1/_2H_2O$: C, 37.3; H, 5.7; N, 24.4. Found: C, 37.58; H, 5.42; N, 24.84.

3-(β-D-Ribofuranosyl)-1,2,4-triazole-5(1*H*,4*H*)-thione, 14. Methyl imidate **2** (1.912 g, 10 mmol) was dissolved in dimethyl sulfoxide (25 mL) and treated with thiosemicarbazide (0.915 g, 10 mmol). The reaction solution was stirred at room temperature for 19 h. The solvent was distilled off under vacuum and the

residual oil treated with ethanol (60 mL). When this solution was allowed to stand, unreacted thiosemicarbazide precipitated. Most of the unreacted thiosemicarbazide was thus removed by successive concentration and precipitation. The filtrate was concentrated to an oil, dissolved in methanol (10 mL), and gradually dropped into ethyl acetate (150 mL). Due to the extreme hygroscopic nature of the precipitated material it was isolated under argon and dried at room temperature (0.05 mm, 20 h). NMR spectral analysis of this material supported structure **13**: δ 3.53 (m, 2, 5′-CH_2), 3.74 (m, 1, 4′-CH), 4.00 (m, 3, 1′-, 2′-, and 3′-CH), 4.49 (br, 3, 3 OH), 6.39 (br s, 2, C(S)NH_2), 7.10 and 7.63 (2 br s, 2, -NHNH-), 9.61 (br s, 1, C=NH). The presence of one atom of sulfur was shown by elemental analysis. Without further purification, this material was heated (130 °C, 0.05 mm, 15 min). Methanol was added to the cooled glassy material, and on stirring and slight warming, the precipitation of compound **14** resulted: mp 237–239 °C; yield 0.763 g (32.7%). A sample of this material was recrystallized from water for X-ray crystallographic analysis:[18] NMR δ 3.50 (m, 2, 5′-CH_2), 3.8–4.2 (m, 4, one NH or OH proton, 2′-, 3′-, and 4′-CH), 4.49 (d, 1, 1′-CH, $J_{1',2'}$ = 6.5 Hz), 4.8–5.6 (br exchangeables, 3 OH), 12.50–14.00 (br exchangeable, 1, NH); IR (KBr) 1603, 1510 cm^{-1}; mass spectrum m/e 233; UV (H_2O) λ_{max} 247 nm (ϵ 15 800), 212 (sh, 6580). NMR of the 2′,3′-*O*-isopropylidene derivative of **14**: $\delta\Delta CH_3$ = 0.17 and $H_{4'}$ a multiplet.

Anal. Calcd for $C_7H_{11}N_3O_4S$ (**14**): C, 36.04; H, 4.75; N, 18.01; S, 13.75. Found: C, 35.67; H, 4.87; N, 18.15; S, 13.49.

4-(Aminocarbonyl)-5-hydroxy-2-(β-D-ribofuranosyl)-imidazole, *C*-Nucleoside Analogue of Bredinin, 17. To a solution of imidic ester **2** (5.74 g, 30 mmol) in 200 mL of dry dimethyl sulfoxide was added aminomalonamide hydrochloride (4.67 g, 30 mmol), and the resultant solution was stirred for 17 h at room temperature. The solvent was distilled off (0.01 mm, ~40 °C) and the oily residue coevaporated once with methanol. A solution of the oil in methanol (50 mL) was then added dropwise to acetonitrile (800 mL). The precipitate obtained was isolated, washed with ethanol, and dried at room temperature (0.05 mm). The recovered material weighed 7.5 g. A TLC profile of this material showed two UV spots, but the analysis was consistent with the molecular formula of **15**.

Anal. Calcd for $C_9H_{17}N_4O_6Cl$: C, 34.56; H, 5.48; N, 17.92; Cl^-, 11.34. Found: C, 34.97; H, 5.69; N, 17.92; Cl^-, 11.22.

The NMR spectrum of the above mixture of rotomers showed ~30% of the minor component; the data for the major component is as follows: δ 3.3–4.2 (m, 5, 2′-, 3′-, and 4′-CH and 5′-CH_2), 4.67 (d, 1, 1′-CH, $J_{1',2'}$ = 4.5 Hz, the anomeric proton of the other component appeared at δ 4.54), 5.14 (s, 1, $NCH(CO)_2$), 5–6 (br, 3 OH), 7.72 and 8.0 (2 br s, 2 each, both $CONH_2$), 8.7–10.0 (br, =NHNH·HCl). A sample of the above heterogeneous material (2.0 g, 6.4 mmol) was dissolved in water (5 mL) and heated in a 100 °C oil bath for 5 min. A clear solution was obtained for a fraction of a minute, after which a solid product started to precipitate. The solid material was filtered, washed with ethanol, and dried at 76 °C (0.05 mm, 16 h). Two crops of the product gave a total of 1.23 g (74% yield). No clear melting point was observed; the compound chars above 240 °C. The compound was recrystallized from water: NMR δ 3.52 (m, 2, 5′-CH_2), 3.78 (m, 1, 4′-CH), 3.97 (m, 2, 2′- and 3′-CH), 4.55 (d, 1, 1′-CH, $J_{1',2'}$ = 6 Hz), 5.0–8.0 (v br, 4 OH's and ring NH), 6.8 (br s, 2, $CONH_2$); UV λ_{max} (H_2O) 244 nm (ϵ 6750), 283 (15 800); IR (KBr) 1650 cm^{-1}.

Anal. Calcd for $C_9H_{13}N_3O_6$: C, 41.70; H, 5.05; N, 16.21. Found: C, 41.64; H, 5.23; N, 16.21.

4-(Carboethoxy)-5-hydroxy-2-(β-D-ribofuranosyl)-imidazole, 18. Methyl imidate **2** (1.912 g, 10 mmol) was dissolved in 100 mL of methanol, and diethyl aminomalonate hydrochloride (2.116 g, 10 mmol) was added. The contents were stirred for 17 h at room temperature. The solvent was removed and the oil treated with 20 mL of methanolic ammonia. After several minutes the solution was concentrated to an oil and redissolved in methanol (20 mL). This solution was then added dropwise to acetonitrile (500 mL). The precipitate formed was isolated and redissolved in methanol by heating on a steam bath. The methanolic solution was allowed to stand at room temperature. The crystallized product was filtered, washed with methanol, and dried (76 °C, 0.05 mm, 16 h). A total of 1.021 g (35% yield) was obtained from three crops of **18**. The compound carbonized before melting around 200 °C: NMR δ 1.23 (t, 3, CH_3 of OEt), 3.55 (m, 2, 5′-CH_2), 3.79 (m, 1, 4′-CH), 3.9–4.3 (m, 3, 2′-, 3′-CH, and one OH), 4.15 (q, 2, CH_2 of OEt), 4.58 (d, 1, 1′-CH, $J_{1',2'}$ = 4.5 Hz), 4.93, 5.18 (2 br, 2, 2 OH), 6.7–8.6 (v br, for ring NH), 12.40 (br s, 1, hydrogen-bonded vinylic OH); IR (KBr) 1700, 1670 cm^{-1}; UV (H_2O) λ_{max} 242 nm (ϵ 7775), 282 (16 685), negative Cotton effect.

Anal. Calcd for $C_{11}H_{16}N_2O_7$: C, 45.84; H, 5.60; N, 9.72. Found: C, 45.89; H, 5.86; N, 9.68.

3-(Aminocarbonyl)-5-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)-1-methyl-1*H*-1,2,4-triazole, 23. A mixture of compound **8** (2.015 g, 8.25 mmol), dimethoxypropane (12.5 mL), and *p*-toluenesulfonic acid (5.1 g, 26.9 mmol) in 100 mL of anhydrous acetone was stirred at room temperature overnight. The reaction mixture was filtered through a bed of Celite, the filtrate concentrated to an oil, and the oil dissolved in methanol (50 mL). A quantitative reaction was indicated by silica gel TLC. An excess of diazomethane (generated from 52.4 mmol of *N*-methyl-*N*-nitrosoguanidine[17]) in ether was added to the above methanolic solution of the isopropylidene derivative **22**. The solution was allowed to stand for 3 h at room temperature and then evaporated on a steam bath. The remaining oil was taken into acetone–toluene (80:20) and fractionated on an E & M, size C, prepacked silica column (3.8 × 43 cm). Part of the chromatographic peak containing the product still had some contaminants. The contaminated fractions were pooled separately, concentrated, and rechromatographed on a size A prepacked column (1 × 20 cm). The combined purified product (oil) was dried (100 °C, 0.05 mm) for 16 h. The amount of the product obtained was 0.430 g (21.3% yield): NMR δ 1.31 and 1.49 (2 s, 3 each, 2 CH_3), 3.42 (m, 2, 5′-CH_2), 3.99 (m, 1, 4′-CH), 4.09 (s, 3, NCH_3), 4.71 (m, 1, 3′-CH), 5.05 (m, 1, 2′-CH), 7.90 and 8.08 (2 br s, 1 each, $CONH_2$).

Anal. Calcd for $C_{12}H_{18}N_4O_5$: C, 48.32; H, 6.08; N, 18.78. Found: C, 48.32; H, 6.02; N, 18.60.

3-(Aminocarbonyl)-5-(β-D-ribofuranosyl)-1-methyl-1*H*-1,2,4-triazole, 24. Compound **23** (0.334 g, 1.15 mmol) was treated with 1 mL of 90% trifluoroacetic acid in water at room temperature for 15 min. The acid was evaporated, and the residue was washed with ethanol. After the residue was dried, only 11.4% of the product was obtained: NMR δ 3.49 (m, 2, 5′-CH_2), 3.79 (m, 1, 4′-CH), 3.96 (m, 1, 3′-CH), 4.08 (s, 3, NCH_3), 4.21 (m, 1, 2′-CH), 4.61 (d, 1, 1′-CH, $J_{1',2'}$ = 6 Hz), 3 OH resonances buried under 1′-CH proton, 7.88 and 8.03 (2 br s, 1 each, $CONH_2$); IR (KBr) 1690 cm^{-1}; mass spectrum m/e 259; UV (H_2O) 220 nm (sh, ϵ 7400), no maxima observed above 200 nm, probably masked by $CONH_2$ absorption. ^{13}C NMR data were used to assign the site of N-methylation. See the Results and Discussion section and Table II.

Preparation of 3-(Aminocarbonyl)-5-(β-D-ribofuranosyl)-1-methyl-1*H*-1,2,4-triazole, 24, by the Methyl Iodide Method. The *C*-nucleoside analogue of ribavirin (**8**; 4.24 g, 17.36 mmol) was reacted with methyl iodide (5 mL, 80 mmol) in the presence of potassium carbonate (3.93 g, 28.46 mmol) in Me_2SO (65 mL) at room temperature for 2 h. After the solvent was distilled off, the mixture was chromatographed on a silica column (100 × 5 cm) which was eluted with a mixture of $CHCl_3$ and methanol (4:1). The fractions of the desired material were pooled and concentrated. The oil was dissolved in a minimum amount of $CHCl_3$–CH_3OH (4:1) mixture and the solution allowed to stand overnight. The crystalline material obtained was filtered, washed, and dried. A yield of 7.6% (0.339 g, mp 139–142 °C) was obtained. This product was found to be identical with the one obtained by the diazomethane method (TLC and spectroscopic criteria).

Anal. Calcd for $C_9H_{14}N_4O_5$: C, 41.86; H, 5.46; N, 21.70. Found: C, 41.78; H, 5.60; N, 21.83.

Acknowledgment. We express our gratitude to Dr. J. Blount, who determined structures of several compounds by X-ray analysis. Also we are thankful to Dr. T. Williams (NMR), Dr. V. Toome (UV), Dr. F. Scheidl (microanalysis), and Mr. S. Traiman (IR). Continued support of this project by Dr. A Cook is deeply appreciated.

Registry No. **1**, 23316-67-8; **2**, 62404-62-0; **3**, 62404-63-1; **4**, 72161-01-4; **5**, 72161-02-5; **6**, 72161-03-6; **7**·2HCl, 72161-04-7; **8**, 62404-64-2; **8** tri-*O*-Ac, 72161-05-8; **9**, 72161-06-9; **10**, 62404-66-4; **11**, 72161-07-0; **12**, 56159-34-3; **13**, 72161-08-1; **14**, 72161-09-2; **14** 2′,3′-

O-isop, 72161-10-5; **15**, 72161-11-6; **17**, 72161-12-7; **17** 2',3'-O-isop, 72161-13-8; **18**, 72161-14-9; **20**, 72161-15-0; **22**, 62404-67-5; **23**, 72161-16-1; **24**, 72161-17-2; oxamidohydrazide, 515-96-8; benzoylhydrazine, 613-94-5; acetylhydrazide, 1068-57-1; semicarbazide hydrochloride, 563-41-7; aminoguanidine dihydrochloride, 55457-88-0; hydrazine, 302-01-2; thiosemicarbazide, 79-19-6; aminomalonamide hydrochloride, 57471-66-6; diethyl aminomalonate hydrochloride, 13433-00-6.

The Structure of Thalibrunine, a Reinvestigation and Revision[1]

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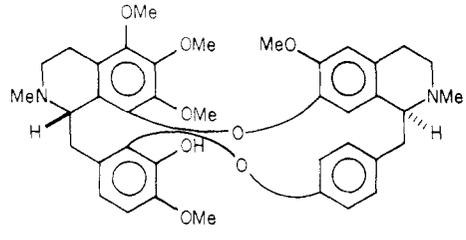
Received August 31, 1979

Evidence is presented that thalibrunine has structure **2** and is the 2'-hydroxy derivative of hernandezine. Ceric ammonium nitrate oxidation of thalibrunine acetate (**3**) gave 2-methoxy-4-acetoxy-4',5-diformyldiphenyl ether (**6**), which was also prepared synthetically from 2-methoxy-4-(benzyloxy)phenol and 4-bromobenzaldehyde in four steps, thereby firmly establishing the tail-to-tail diphenyl ether unit. Anomalous products, *o*-cresol **14** and methyl ether **15**, obtained on $NaBH_4$ reduction of the neutral fraction from the ceric ammonium nitrate oxidation of thalibrunine acetate (**3**), were characterized from studies on model compounds. The cryptophenolic nature of thalibrunine (**2**) is due to the strong hydrogen bond between the phenolic group and the unshared electron pair of the tertiary nitrogen. The hydrogen-bonded structure persists in the Na/NH_3 cleavage products (e.g., **17**), lacking the head-to-head diphenyl ether group. The H bond in these products can be broken by protonation, a feature not observed for thalibrunine. CD spectral data reflecting those changes and supporting the *S,S* configuration are presented. Thalibrunimine should have its structure revised to **18**.

Thalibrunine, a bis(benzyltetrahydroisoquinoline) alkaloid from *Thalictrum rochebrunianum* Franc. and Sav. (family Ranunculaceae) was first reported[2] in 1966, and structure **1** was proposed[3] for it in 1974. The head-to-head



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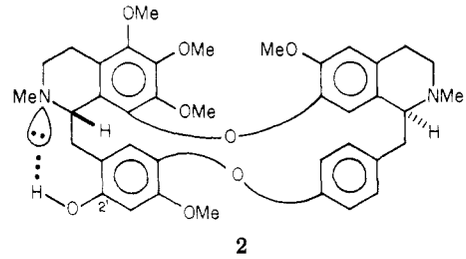

2

or bis(tetrahydroisoquinoline) ether-linked portion was firmly established by direct comparison of the reduced photooxidized product from thalibrunine with synthetically prepared material. The tail-to-tail, or ether-linked bis(benzyl), portion, on the other hand, rests only on biogenetic consideration and the Gibbs test for para-unsubstituted phenols.[4] Availability of additional plant material gave a supply of thalibrunine that made possible a further study of this alkaloid and also provided a mixture of crude bases from which four new thalibrunine-related alkaloids were isolated. These are reported in the following paper.[5]

The first piece of information placing structure **1** in doubt was the ^1H NMR spectrum taken in acetone-d_6 under pulsed-signal Fourier transform conditions.[6] The aromatic region which was resolved more clearly and contained virtually no background noise did not show the outer less intense peaks of a typical AB quartet expected for the ortho protons of the trioxygenated benzylic ring. Also, an ABXY pattern for the monooxygenated benzylic ring was observed as a double set of AB quartets with additional splitting. The remaining peaks were four distinct one-proton singlets. This would require para protons in the trioxygenated benzylic ring, for which six structures can be theoretically considered. Two structures each are possible for three different ring systems, 17-, 18-, and 19-membered. The latter possibility is biogenetically least likely, since the diphenyl ether would involve the para position of each benzylic ring, and none of the six structures would possess an unsubstituted position para to the phenolic hydroxyl. Rechecking the Gibbs test on a scrupulously purified sample of thalibrunine produced a negative result,[7] invalidating the earlier evidence that led to proposal of structure **1**.

The ^1H NMR spectrum of thalibrunine taken in $CDCl_3$ or acetone-d_6 shows a broad one-proton signal considerably downfield (δ 12.4 and 11.9, respectively) that is characteristic of hydrogen-bonded phenolic hydroxyls.[8] These signals do not readily exchange with D_2O, as was observed.

(1) Alkaloids of Thalictrum. 28. For part 27, see W.-T. Liao, J. L. Beal, W.-N. Wu, and R. W. Doskotch, *Lloydia*, **41**, 271 (1978).
(2) H. H. S. Fong, J. L. Beal, and M. P. Cava, *Lloydia*, **29**, 94 (1966).
(3) M. P. Cava, J. M. Saá, M. V. Lakshmikantham, M. J. Mitchell, J. L. Beal, R. W. Doskotch, A. Ray, D. C. DeJongh, and S. R. Shrader, *Tetrahedron Lett.*, 4259 (1974).
(4) The reliability of the test can be improved by the procedure of F. E. King, T. J. King, and L. C. Manning [*J. Chem. Soc.*, 563 (1957)] over that of H. D. Gibbs [*J. Biol. Chem.*, **72**, 653 (1927)]. A referee has requested that a statement be included suggesting strongly that the Gibbs test in any form be abandoned. Our experience would suggest that positive results should not be accepted without other corroborating evidence.
(5) J. Wu, J. L. Beal and R. W. Doskotch, *J. Org. Chem.* following paper in this issue.
(6) In acetone-d_6 or methanol-d_4—but not in $CDCl_3$—the aromatic region is composed of almost total first-order patterns, and because it is more spread out, it is readily analyzable. Double-resonance experiments substantiated the pattern relationships.
(7) The earlier positive test must have been due to a very minor contaminant whose presence was not indicated by spectral or TLC examination.
(8) L. M. Jackman and S. Sternhell, "Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", 2nd ed., Pergamon Press, New York, 1969, Chapters 3-7.

0022-3263/80/1945-0208$01.00/0