Synthesis of 5-(β-D-Ribofuranosyl)-1,2,4-oxadiazole-3-carboxamide

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The title compound was synthesized in three steps from ethoxycarbonylformamide oxime (**5**) and 3,4,6-tri-*O*-acetyl-2,5-anhydroallonyl chloride (**4b**) in 62% overall yield. An acid catalyzed de-esterification was required to prevent a facile base catalyzed elimination reaction.

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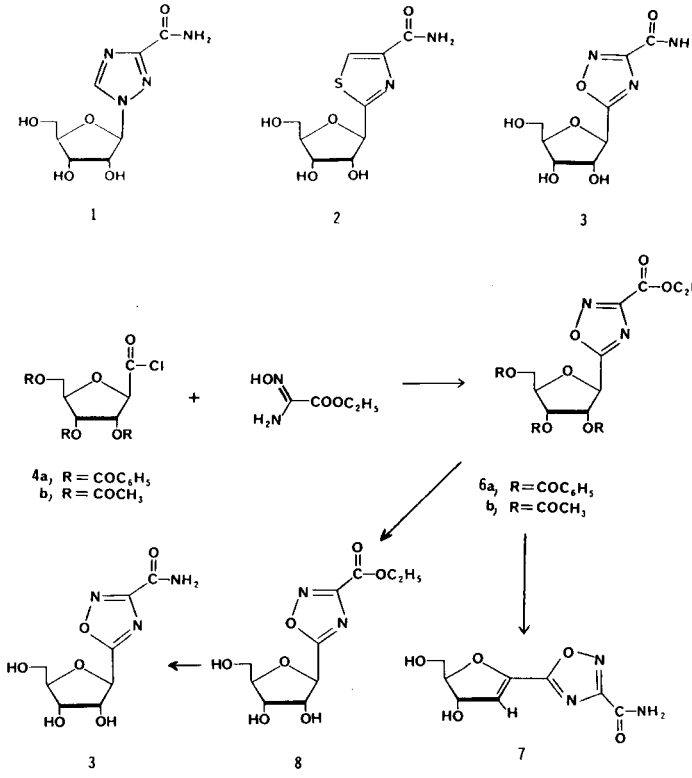
The development of new classes of antiviral and antineoplastic agents have led in our laboratory to the preparation of 1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide [1] (ribavirin [2]) **1** and 2-(β-D-ribofuranosyl)thiazole-4-carboxamide [3] (tiazofurin [4]) **2**. Animal studies have demonstrated the effectiveness of ribavirin **1** as an antiviral agent [5] and tiazofurin **2** as an antineoplastic agent [6]. In devising new potentially active compounds we studied the structure-activity relationships of **1** and **2** and their analogs [3,7]. The features necessary for biological activity appear to be the presence of a ring nitrogen adjacent to the carboxamide function, and the β-D-ribofuranosyl moiety as the glycosyl component. We have now synthesized 5-(β-D-ribofuranosyl)-1,2,4-oxadiazole-3-carboxamide (**3**) as a hybrid of **1** and **2** with an oxygen atom in place of the sulfur atom.

The functionalized oxadiazole ring was first formed by the condensation of the acid chloride **4a** (3,4,5-tri-*O*-benzoyl-2,5-anhydroallonyl chloride [7]) with the amide oxime **5** (ethoxycarbonylformamide oxime [8]) in anhydrous pyridine to give the blocked nucleoside ethyl 5-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-oxadiazole-3-carboxylate (**6a**) in 29% yield after chromatography. Deblocking of **6a** under standard conditions with methanolic ammonia gave the elimination product 5-(4′*R*,5′*S*,4′-hydroxy-5′-hydroxymethylene-4′,5′-dihydrofuran-2′-yl)-1,2,4-oxadiazole-3-carboxamide (**7**) as the sole product. None of the desired **3** was obtained from **6a** under a variety of experimental conditions using basic deblocking agents.

Ethyl 5-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-oxadiazole-3-carboxylate (**6b**) was obtained in 66% yield from the condensation of **4b** (3,4,6-tri-*O*-acetyl-2,5-anhydroallonyl chloride [9]) and **5** in pyridine. The elimination reaction leading to **7** was then circumvented by treatment of **6b** with 0.5 *N* hydrogen chloride in absolute ethanol at 4° to give ethyl 5-(β-D-ribofuranosyl-1,2,4-oxadiazole-3-carboxylate (**8**) (*ca* 100% yield). Ammonolysis of **8** in methanolic ammonia provided **3** in 94% yield.

Compound **3** was shown to give 46% inhibition of leukemia L1210 and 43% inhibition of leukemia P388 at 1×10^{-4} *M* in cell culture. Compound **3** also showed a virus rating (V.R.) of 0.38 and 0.51 against vaccinia and HSV-2 viral lines respectively with very little cellular toxicity.



EXPERIMENTAL

General Methods.

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H-nmr) spectra were determined at 90 MHz with JEOL FX-90Q spectrometer. Carbon nuclear magnetic resonance (^{13}C-nmr) were determined at 22.5 MHz on the same instrument. The Chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. Mass spectra (ms) were obtained on a Hitachi Perkin-Elmer RMU-6E at an ionizing voltage of 70 eV. Optical rotations were recorded on a Perkin-Elmer 241 automatic polarimeter. Elemental analyses were performed by Robertson Laboratory, Florham Park, NJ. Thin-layer chromatography (tlc) was run on aluminum backed silica gel 60 F-254 (EM Reagents) plates. Preparative scale chromatography was conducted using flash chromatography techniques. J. T. Baker silica gel (~40 μm) or Kiesel gel 60 EM Reagents (40-63 μm) was used for flash chromatography. Detection of components on tlc was by uv light and with 10% sulfuric acid in methanol spray followed by heating. Evaporations were carried out under reduced pressure with the bath temperature below 35°.

Ethyl 5-(2′,3′,5′-Tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-oxadiazole-3-carboxylate (**6a**).

A solution of 5.0 g (10 mmoles) of **4a** (3,4,6-tri-*O*-benzoyl-2,5-anhydroallonyl chloride [7] and 1.48 (11 mmoles) of **5** (ethoxycarbonylformamide oxime [8]) in 50 ml of anhydrous pyridine was heated at 80° for 1 hour then allowed to stand at room temperature for 18 hours. The reaction mixture was evaporated *in vacuo* and the residue coevaporated with toluene (2 × 50 ml). The residue was dissolved in dichloromethane and added to the top of a 4 cm × 38 cm silica gel column and the product was eluted with hexane:ethyl acetate (8:2 → 7:3). The product fractions were pooled and evaporated to dryness to yield 1.7 g (29%) of **4a** as a viscous syrup; ^{1}H-nmr (deuteriochloroform): δ 1.41 (t, J = 7.2 Hz, 3H), 4.48 (q, J = 7.2 Hz, 2H), 4.66-4.90 (m, 1H), 4.75 (d, J = 2.7 Hz, 2H), 5.61 (d, J = 4.5 Hz, 1H, H-1'), 5.90-6.18 (m, 2H), 7.24-7.65 (m, 9H), 7.83-8.16 (m, 6H); tlc: (hexane:ethyl acetate, 6:4) R_f 0.30.

Anal. Calcd. for $C_{31}H_{26}N_2O_{10}$: C, 63.48; H, 4.47; N, 4.78. Found: C, 63.22; H, 4.57; N, 4.51.

Ethyl 5-(2',3',5'-Tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-oxadiazole-3-carboxylate (**6b**).

A mixture of 4.4 g (33 mmoles) of ethoxycarbonylformamide oxime (**5**) and 20 g (66 mmoles) of 3,4,6-tri-*O*-acetyl-2,5-anhydroallonyl chloride (**4b**) was dissolved with stirring in 100 ml of pyridine. After the initial exothermic reaction subsided the mixture was stirred at room temperature for 18 hours. The pyridine was evaporated *in vacuo* and the residue coevaporated with toluene (2 × 150 ml). The residue was dissolved in methanol (75 ml), 35 g of 60-200 mesh silica gel was added and the methanol evaporated *in vacuo* followed by coevaporation of the residue with toluene (150 ml). The dry powder was added to the top of a 4.5 cm × 36 cm column of silica gel. The column was developed with 2.5 ℓ of 4:1 hexane:-ethyl acetate. The product fractions were pooled and evaporated *in vacuo* to give 8.75 g (66%) of **6b** as a clear colorless syrup; ^{1}H-nmr (deuteriochloroform): δ 1.44 (t, J = 7.1 Hz, 3H), 2.10 (s, 3H), 2.12 (s, 3H), 2.14 (s, 3H), 4.04-4.31 (m, 1H), 4.35-4.52 (m, 2H), 4.51 (q, J = 7.1 Hz, 2H), 5.30 (d, J = 5.4 Hz, 1H, H-1'), 5.40-5.57 (m, 1H), 5.58-5.77 (m, 1H); ^{13}C-nmr (deuteriochloroform): δ 13.84, 20.16, 20.29, 20.46, 62.51, 62.91, 71.45, 73.77, 74.51, 81.08, 156.92, 161.90, 169.03, 169.26, 170.17, 176.96; tlc: (hexane:ethyl acetate, 6:4) R_f 0.28.

Anal. Calcd. for $C_{16}H_{20}N_2O_{10}$: C, 48.00; H, 5.04; N, 7.00. Found: C, 48.24; H, 4.99; N, 7.02.

5-(4'*R*,5'*S*,4'-Hydroxy-5'-hydroxymethylene-4',5'-dihydrofuran-2'-yl)-1,2,4-oxadiazole-3-carboxamide (**7**).

Ethyl 5-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-oxadiazole-3-carboxylate (**6a**) 3.11 g (5.3 mmoles) was dissolved in 100 ml of methanolic ammonia (saturated at 0°) and stirred in a sealed low pressure reaction vessel. After 24 hours the solvent was evaporated to yield a solid 1.2 g (100%), mp 136-138°. The solid was recrystallized from 2-propanol (40 ml) to yield 1.05 g (87%) of **7**, mp 153-154°; ^{1}H-nmr (DMSO-d_6): δ 3.53 (dd, J = 4.7 and 4.1 Hz, 2H), 4.48 (dt, J = 4.1 and 4.7 Hz, 1H), 4.85 (ddd, J = 2.9, 4.1 and 6.3 Hz, 1H), 5.04 (t, J = 4.1 Hz, DCl exchanged, 1H), 5.63 (d, J = 6.3 Hz, DCl exchanged, 1H), 6.24 (d, J = 2.9 Hz, 1H), 8.16 (bd, DCl exchanged, 1H), 8.39 (bd, DCl exchanged, 1H); ^{13}C-nmr (DMSO-d_6): δ 61.07, 73.86, 90.95, 113.25, 143.25, 157.17, 164.02, 168.83; tlc: (chloroform:methanol, 8:2) R_f 0.18.

Anal. Calcd. for $C_8H_9N_3O_5$: C, 42.30; H, 3.99; N, 18.50. Found: C, 42.49; H, 4.08; N, 18.23.

Ethyl 5-(β-D-Ribofuranosyl)-1,2,4-oxadiazole-3-carboxylate (**8**).

Ethyl (2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-oxadiazole-3-carboxylate (**6b**) (4.0 g, 10 mmoles) was dissolved in 100 ml of ice-cold 0.5 *N* ethanolic hydrogen chloride and stored at 4°. After 40 hours the solution was evaporated *in vacuo* and the residue coevaporated with ethanol (2 × 100 ml). The residue was chromatographed on a silica gel column (4 cm × 19 cm) using 5% ethanol in chloroform as the eluting solvent. After evaporation of the solvent from the product fractions 2.6 g (98%) of **8** was obtained as an analytically pure syrup. A sample was crystallized from ether-dichloromethane, mp 65°; ^{1}H-nmr (DMSO-d_6): δ 1.35 (t, J = 7.0 Hz, 3H), 3.44-3.63 (m, 2H), 3.88-4.15 (m, 2H), 4.28-4.43 (m, 1H), 4.41 (q, J = 7.0 Hz, 2H), 4.86 (t, J = 4.9 Hz, 1H, DCl exchanged), 5.02 (d, J = 5.0 Hz, 1H, H-1'), 5.24 (d, J = 4.9, 1H, DCl exchanged), 5.58 (d, J = 7.2 Hz, 1H, DCl exchanged); ^{13}C-nmr (DMSO-d_6): δ 13.97, 61.74, 62.82, 71.48, 75.10, 76.12, 86.31, 157.17, 161.59, 179.59; $[\alpha]_D^{23}$ −27.9° (c1, methanol); tlc: (chloroform:methanol, 9:1) R_f 0.19.

Anal. Calcd. for $C_{10}H_{14}N_2O_7$: C, 43.80; H, 5.15; N, 10.22. Found: C, 44.01; H, 5.30; N, 10.04.

5-(β-d-Ribofuranosyl)-1,2,4-oxadiazole-3-carboxamide (**3**).

One g (3.65 mmoles) of ethyl 5-(β-D-ribofuranosyl)-1,2,4-oxadiazole-3-carboxylate (**6b**) was dissolved in 100 ml of methanolic ammonia (previously saturated at 0°). After 18 hours at room temperature the reaction mixture was evaporated to dryness to yield 0.84 g (94%) of compound **3** as a chromatographically clean amorphous solid. A sample was crystallized from acetone-hexane, mp 78°; ^{1}H-nmr (DMSO-d_6): δ 3.40-3.62 (m, 2H), 3.80-4.12 (m, 2H), 4.16-4.22 (m, 1H), 4.78 (t, J = 5.4 Hz, 1H), 4.93 (d, J = 5.5 Hz, 1H, H-1'), 5.18 (d, J = 5.4 Hz, 1H), 5.30 (d, J = 6.3 Hz, 1H), 8.09 (bd s, 1H), 8.31 (bd s, 1H); ^{13}C-nmr (DMSO-d_6): δ 61.80, 71.48, 75.05, 76.06, 86.25, 157.80, 163.80, 178.91; $[\alpha]_D^{23}$ −38.0° (c 0.5, methanol); ms: m/e 246 (M + 1), 228, 210, 192, 178; tlc: (chloroform:methanol, 2:1) R_f 0.48.

Anal. Calcd. for $C_8H_{11}N_3O_6$: C, 39.19; H, 4.52; N, 17.14. Found: C, 39.04; H, 4.71; N, 16.91.

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