Study on the Inhibition of Adenosine Deaminase

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4(*R*)-(1-Hydroxyethyl)-5-methyl-1-β-D-ribofuranosylimidazole (**10**), which contains only the asymmetric alcohol center of the diazepinol ring of the adenosine deaminase inhibitor coformycin (**12**), is a much less potent inhibitor of the enzyme but still binds to the enzyme about as tightly as the normal substrate.

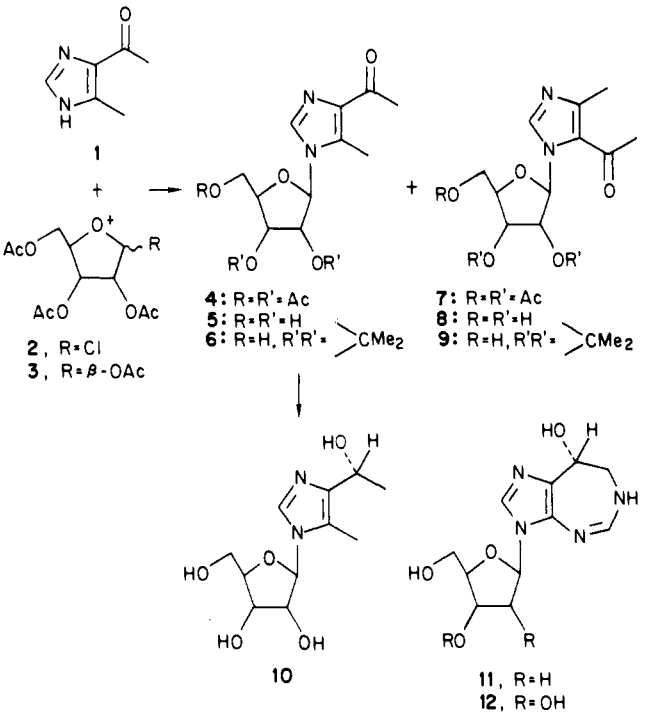
2-Deoxycoformycin (**11**) and coformycin (**12**), its ribo analogue, are both exceedingly tight binding inhibitors of adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4),[1] presumably because they resemble the transition state of the enzymatic reaction.[2] These inhibitors have the *R* configuration at C-8, and the *S* diastereomer of 2′-deoxycoformycin has less than 0.1% of the inhibitory activity of the *R* isomer,[3] establishing the importance of the stereochemistry at that asymmetric center to the enzyme inhibition. The contribution of the seven-membered ring, other than providing stability, is not clear.

In an attempt to learn more about the contribution of the seven-membered ring relative to that of the asymmetric center to tight binding of the coformycins **11** and **12**, we undertook the synthesis of 4-(1-hydroxyethyl)-5-methyl-1-β-D-ribofuranosylimidazole (**10**), a compound resembling the imidazole ribonucleoside portion of coformycin but having essentially only the asymmetric carbon of the diazepinol. This synthesis was accomplished by reaction of 4-acetyl-5-methylimidazole (**1**)[4] with a D-ribofuranose derivative followed by removal of the blocking groups and reduction of the carbonyl group. The reaction of **1** with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride in nitromethane in the presence of mercuric cyanide gave a 4:1 mixture of two nucleosides. The identity of these nucleosides (**4** and **7**) was deduced from the ^1H and ^{13}C NMR spectra of their deacetylation products to be the desired **5** (four parts) and its 3-isomer **8** (one part). The β-configuration of **5** and **8** was established from the NMR spectra of their 2′,3′-*O*-isopropylidine derivatives,[5] prepared by our standard method. Definitive proof of the structure of **10**, and therefore of **5**, was obtained by X-ray crystallography, which also established the absolute configuration of the carbon attached to C-4. This in turn proved the structure of **8** because it had already been shown to be a β-ribonucleoside. The fusion reaction of **1** with tetra-*O*-acetyl-D-ribofuranose gave, along with **4** and **7**, a third nucleoside identified by the NMR spectra of its deacetylated product as the α-anomer of **4**. The ratio of the three products was 10:1:1.

Reduction of the carbonyl group of **5** was carried out with both sodium borohydride and Raney Nickel with essentially the same results. The product, obtained in approximately 50% yield, was a crystalline solid that was identified by X-ray crystallography as the *R* diastereomer **10**. The *S* isomer was not detected.

Crystallographic Analysis of Compound 10. Crystals of compound **10** were grown by slow evaporation of a methanol/butyl ether solution at room temperature. These crystals are orthorhombic, space group $P2_12_12_1$, with $a = 7.156$ (4), $b = 8.379$ (3), and $c = 19.98$ (2) Å. The structure was solved by direct methods, using 1148 observed reflections ($F_o^2 > \sigma(F_o^2)$, in the range $2\theta \geq 2° \leq 128°$, measured with a Picker FACS-1 diffractometer, using Ni-filtered copper radiation and a θ–2θ scan technique.

Scheme I



Structure factors were assigned standard deviations based upon counting statistics, plus an instrumental uncertainty term. The structure was refined by full-matrix least squares, by minimizing $\sum w(|F_o| - |F_c|)^2$, where $w = 1/\sigma^2(F_o)$. All hydrogen atoms were located at difference Fourier maps calculated during the latter stages of refinement. The configuration of the molecule was established by assigning the D-ribose configuration to the sugar moiety. Final cycles of refinement included a scale factor, positional and anisotropic thermal parameters for all non-hydrogen atoms, positional and anisotropic thermal parameters for all hydrogen atoms except H(05′), which assumed unreasonable parameters during initial refinement efforts, and an isotropic secondary extinction parameter. The final value for the standard crystallographic *R* index is 0.051; the weighted *R* index is 0.064; and the goodness-of-fit is 3.13. A final difference Fourier map showed no peaks or troughs with magnitudes exceeding 0.45 e/Å^3. A drawing of the molecular structure is shown in Figure 1.

Enzyme Assay. Compounds **5** and **10** were assayed[6] for their ability to inhibit the deamination of adenosine[6]

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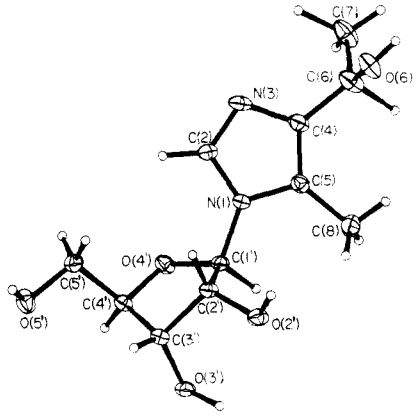

Figure 1. Crystal structure of compound **10**. Non-hydrogen atoms are represented by thermal ellipsoids scaled to include 50% probability. Hydrogen atoms are represented by spheres of arbitrary radius.

by calf intestine adenosine deaminase.[7] Compound **10** was found to have a K_i of 61 μM with a K_m for adenosine of 39 μM, indicating little difference in its binding and that of the normal substrate, although the structural resemblance is somewhat remote. Compound **5**, which lacks the asymmetric carbon of **10**, did not inhibit. Since the K_i of **10** is approximately 3×10^6 that of coformycin, it is clear that the seven-membered ring makes a very important contribution to the binding of that inhibitor to adenosine deaminase. Perhaps one of its functions is to hold the asymmetric center in the proper orientation relative to the transition state. This would be in keeping with the proposed conformational change in the enzyme produced by the coformycins.[8]

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. NMR spectra were recorded on a Varian XL-100-15 spectrometer operating at 100.1 MHz for ^{1}H NMR and 25.2 MHz for ^{13}C NMR. Chemical shifts are expressed in parts per million downfield for tetramethylsilane: Chemical shifts (δ) quoted for multiplets are measured from the approximate center. Ultraviolet absorption spectra were determined on a Cary 17 spectrophotometer by dissolving each compound in ethanol and diluting 10-fold with 0.1 N HCl, pH 7 buffer, and 0.1 N NaOH. Values are in nanometers, and the numbers in parentheses are extinction coefficients ($\epsilon \times 10^{-3}$). The microanalysis was performed by Atlantic Microlab, Inc., Atlanta, GA. Thin-layer and preparative-layer chromatography were carried out on Brinkman precoated silica gel plates (0.25- or 2.0-mm thickness). HPLC was carried out with an ALC-242 liquid chromatograph (Waters Associates), using a μBondapak C_{18} column and monitoring with a UV detector at 254 nm. Mass spectra were recorded on a Varian MAT 311A mass spectrometer in the electron-impact (EI) or fast atom bombardment (FAB) mode.

4-Acetyl-5-methyl-1-β-D-ribofuranosylimidazole (5) and 5-Acetyl-4-methyl-1-β-D-ribofuranosylimidazole (8). Method A. To an azeotropically dried solution of methyl 5(4)-methylimidazole-4(5)-yl ketone (1; 300 mg, 2.42 mmol) in nitromethane containing mercuric cyanide (758 mg, 3 mmol) was added a solution of 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride (2; 3 mmol) in azeotropically dried nitromethane (30 mL). After refluxing for 3 h with stirring, the solution was filtered and evaporated to dryness in vacuo. The syrup obtained, dissolved in anhydrous methanol (50 mL), was treated with 4 mL of 1 N methanolic sodium methoxide. After 1 h at ambient temperature, the solution was neutralized with glacial acetic acid and evaporated to dryness in vacuo. The residue was purified by preparative thin-layer chromatography using butanol/water (86:14) as the developing solvent. The bands containing product were purified further by preparative thin-layer chromatography using chloroform/methanol (3:1) as the developing solvent. Product **5** crystallized from methanol as a white solid, yield 275 mg (44%). The analytical sample was obtained from a previous reaction by recrystallization from methanol/ethyl acetate: mp 145 °C; UV λ_{max} at pH 1, 243 (11.8); at pH 7, 262 (10.8); at pH 13, 265 (11.6); ^{1}H NMR (Me_2SO-d_6) δ 2.42 and 2.53 (2 s, 2 CH_3), 3.62 (m, CH_2), 3.95 (q, H-4'), 4.10 (t, H-3'), 4.21 (t, H-2'), 5.57 (d, H-1', $J_{1'2'}$ = 5 Hz), 7.98 (s, H-2); ^{13}C NMR (Me_2SO-d_6) δ 9.63 (CH_3), 27.14 (CH_3CO), 60.94 (C-5'), 69.95 and 75.22 (C-2' and C-3'), 85.56 (C-4'), 87.94 (C-1'), 133.44 and 136.58 (C-4 and C-5), 134.20 (C-2), 194.98 (CO). Anal. Calcd for $C_{11}H_{16}N_2O_5 \cdot 0.4MeOH \cdot 0.4H_2O$: C, 49.56; H, 6.71, N, 10.14. Found: C, 49.73; H, 6.41; N, 10.03.

Product II was obtained in 11% yield. The analytical sample was obtained from a previous reaction by recrystallization from ethanol: mp 152–154 °C; UV λ_{max} at pH 1, 244 (11.0); at pH 7, 264 (13.0); at pH 13, 265 (12.7); ^{1}H NMR (Me_2SO-d_6) δ 2.44 and 2.48 (2 s, 2 CH_3), 3.7 and 3.9 (2 m, H-2', H-3', H-4', H-5'), 6.2 (d, H-1', $J_{1'2'}$ = 3 Hz), 8.38 (H-2); ^{13}C NMR (Me_2SO-d_6) δ 16.92 (CH_3), 30.12 (CH_3CO), 59.73 (CH_2), 68.07 and 75.69 (C-2' and C-3'), 83.58 (C-4'), 80.36 (C-1'), 126.93 and 147.53 (C-4 and C-5), 138.74 (C-2), 188.28 (CO). Anal. Calcd for $C_{11}H_{16}N_2O_5 \cdot 0.44H_2O$: C, 50.01; H, 6.44; N, 10.60. Found: C, 50.04; H, 6.38; N, 10.44.

Method B. A mixture of methyl 5(4)-methylimidazole-4(5)-yl ketone (1; 544 mg, 4.39 mmol), 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**3**; 2.79 g, 8.78 mmol), and *p*-toluenesulfonic acid (38 mg) was stirred and heated at 140 °C until a clear melt was obtained. After addition of 38 mg more of *p*-toluenesulfonic acid to the mixture, it was stirred and heated at 175–180 °C for 2 h. The process was repeated with 19 mg of μ-toluenesulfonic acid and heated for 1 h. A methylene chloride solution of the dark reaction mixture was absorbed on silica gel (60 PF-254). Elution of the silica gel with methylene chloride and methanol gave a syrup that was purified by preparative thin-layer chromatograhy using methylene chloride. The blocked nucleoside was obtained as a syrup, yield 1.25 g.

Deblocking was carried out in methanolic sodium methoxide. Purification by preparative thick plates of silica gel using initially butanol/water (86:14) followed by chloroform/methanol/ammonium hydroxide (3:1:0.2) gave **5**, (203 mg, 24%), **8** (22 mg, 2.6%), and the α-anomer of **5** (17 mg, 2%). ^{1}H NMR (Me_2SO-d_6): δ 2.38 and 2.46 (2 s, 2 CH_3), 3.46 (m, CH_2), 4.15 (m, H-2', H-3', and H-4'), 5.88 (d, H-1', $J_{1'2'}$ = 5 Hz), 7.86 (H-2).

4-Acetyl-5-methyl-1-(2',3'-*O*-isopropylidene-β-D-ribofuranosyl)imidazole (6). A solution of 66 μL of 2,2-dimethoxypropane and 90 μL of 70% perchloric acid in 20 mL of anhydrous acetone was kept 5 min before adding 4-acetyl-5-methyl-1-β-D-ribofuranosylimidazole (**5**; 51 mg, 0.2 mmol). The resulting solution was stirred at ambient temperature for 1 h, neutralized with 100 μL of pyridine, and evaporated to dryness in vacuo. The residue was partitioned between chloroform and water. The chloroform layer was dried with magnesium sulfate and evaporated to dryness in vacuo. The residue was dissolved in acetone/cyclohexane and evaporated to dryness. It crystallized on standing; yield 60 mg (98%).

The analytical sample was obtained by recrystallization from ethanol/ether: mp 134–135 °C; UV λ_{max} at pH 1, 244 (12.7); at pH 7, 264 (11.8); at pH 13, 264 (11.7); ^{1}H NMR ($CDCl_3$) δ 1.37 and 1.6 (2 s, CH_3 of IP), 1.43 (cyclohexane), 2.52 and 2.6 (2 s, 4 CH_3, CH_3CO), 3.9 (m, CH_2), 4.46 (m, H-4'), 4.8 (q, H-2'), 4.98 (m, H-3'), 5.2 (s, OH), 5.76 (d, H-1', $J_{1'2'}$ = 3 Hz), 8.0 (s, H-2); ^{13}C NMR (Me_2SO-d_6) δ 10.16 (4-CH_3), 25.31 and 27.34 (CH_3 of IP), 26.96 (cyclohexane), 27.60 (CH_3CO), 62.30 (CH_2), 81.61, 86.07, 86.43 (C-2', C-3', C-4'), 92.09 (C-1'), 114.31 (C of IP), 133.54 and 137.16 (C-4 and C-5), 133.98 (C-2), 195.53 (CO); mass spectrum (FAB) *m/e* 173 (sugar)$^+$, 297 (M + 1)$^+$. Anal. Calcd for $C_{14}H_{20}N_2O_5 \cdot 0.12C_6H_{12}$: C, 57.70; H, 7.05; N, 9.14. Found C, 57.79; H, 7.09; N, 9.38.

5-Acetyl-4-methyl-1-(2',3'-*O*-isopropylidene-β-D-ribofuranosyl)imidazole (9). A solution of 66 μL of 2,2-dimethoxypropane and 90 μL of 70% perchloric acid in 20 mL of anhydrous acetone was kept 5 min before adding 5-acetyl-4-methyl-1-β-D-ribofuranosylimidazole (**8**; 51 mg, 0.2 mmol). The resulting solution was stirred at ambient temperature for 1 h, neutralized with 100 μL of pyridine, and evaporated to dryness

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in vacuo. The residue was partitioned between chloroform and water. After drying with magnesium sulfate, the chloroform layer was evaporated to dryness in vacuo, giving the product as a syrup. Trituration with ether and cyclohexane gave a glass: yield 43 mg (67%); UV λ_{max} at pH 1, 245 (9.07); at pH 7 and pH 13, 264 (11.2); ^{1}H NMR ($CDCl_3$) δ 1.38 and 1.62 (2 s, CH_3 of IP), 1.44 (cyclohexane), 2.52 and 2.58 (2 s, 4-CH_3 and CH_3CO), 3.9 (m, CH_2), 4.35 (m, H-4'), 4.74 (m, H-2'), 4.88 (s, OH), 4.96 (m, H-3'), 6.48 (d, H-1', $J_{1'2'}$ = 2 Hz), 8.33 (H-2); ^{13}C NMR ($CDCl_3$) δ 16.91 (4-CH_3), 25.55 and 27.34 (CH_3 of IP), 26.96 (cyclohexane), 30.12 (CH_3CO), 61.71 (CH_2), 79.76 (C-3'), 86.89 and 87.21 (C-2' and C-4'), 93.14 (C-1'), 114.15 (C of IP), 127.50 and 147.60 (C-4 and C-5), 139.44 (C-2), 188.62 (CO); mass spectrum (FAB) m/e 173 (sugar)$^+$, 297 (M + 1)$^+$. Anal. Calcd for $C_{14}H_{20}N_2O_5 \cdot 0.12C_6H_{12} \cdot 0.47H_2O$: C, 56.15; H, 7.16; N, 8.90. Found: C, 56.13; H, 7.02; N, 8.80.

4(*R*)-(1-Hydroxyethyl)-5-methyl-1-β-D-ribofuranosylimidazole (10). Method A. To a cold (ice bath) solution of 4-acetyl-5-methyl-1-β-D-ribofuranosylimidazole (**5**; 100 mg, 0.39 mmol) in 15 mL of anhydrous ethanol was added a solution of sodium borohydride (22.3 mg, 0.59 mmol) in 2.23 mL of anhydrous ethanol. After 4 h at ambient temperature, the solution was acidified (pH 2) with 1 N hydrochloric acid and evaporated to dryness. The residue was purified by preparative thin-layer chromatography (3:1 chloroform/methanol) on silica gel. The product was obtained by methanol extraction. Evaporation of the solvent gave a syrup that crystallized on standing; yield 52 mg (51%).

The analytical sample was obtained by absorbing an aqueous solution of the material on rexyn 101 (H) ion-exchange resin and eluting with 50% aqueous ammonium hydroxide. The solid thus obtained was recrystallized from ethanol: mp 170–172 °C; mass spectrum (EI) m/e 258 (M$^+$); ^{1}H NMR (Me_2SO-d_6) δ 1.32 (d, CH_3CHOH, J = 7 Hz), 2.19 (s, 4-CH_3), 3.55 (m, CH_2), 3.85 (m, H-4'), 4.02 (m, H-3'), 4.2 (m, H-2'), 4.5-5.5 (OH), 4.64 (m, CH_3CHOH), 5.4 (d, H-1', $J_{1'2'}$ = 6 Hz), 7.7 (s, H-2); assignments of CH_3CHOH and H-2' verified by spin-decoupling. Anal. Calcd for $C_{11}H_{18}N_2O_5 \cdot 0.15H_2O$: C, 50.63; H, 7.07; N, 10.73. Found: C, 50.63; H, 7.24; N, 10.72.

Method B. A solution of 4-acetyl-5-methyl-1-β-D-ribofuranosylimidazole (**5**; 100 mg, 0.39 mmol) in ethanol (25 mL) containing Raney Nickel catalyst (25 mg) was hydrogenated at ambient temperature and 30 psi for 48 h, then filtered, and evaporated to dryness in vacuo. The residue was purified by flash chromatography on Merck silica gel 60 (70–230 mesh, 20 g) using chloroform/methanol (3:1) as the eluting solvent to give 82 mg of a glass. A crystalline solid was obtained from ethanol; yield 51 mg (51%). A solution of 20 mg of this material in ethanol (5 mL) was allowed to evaporate slowly at ambient temperature. A crystalline residue identical with that obtained by the borohydride reduction was obtained.

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Registry No. **1**, 23328-91-8; **2**, 40554-98-1; **3**, 13035-61-5; **5** (β anomer), 98483-23-9; **5** (α anomer), 98483-24-0; **6**, 98483-25-1; **8**, 31281-18-2; **9**, 31281-20-6; **10**, 98483-26-2; adenosine deaminase, 9026-93-1.

Book Reviews

Biologically Active Principles of Natural Products. Edited by W. Voelter and G. D. Daves, Jr. Georg Thieme Verlag, Stuttgart. 1984. 310 pp. 16 × 23.5 cm. $26.00

Nothing on the title page of this volume reveals that it is a Festschrift honoring Karl Folkers and that it was assembled by the editors following a symposium at Lehigh University toward the end of 1981, which celebrated Karl Folker's 75th birthday.

In their preface the editors state that Dr. Folkers's life-long research interests have dealt with "chemical regulation of life processes", which might not have been a bad title for a book, that deals with the chemistry, biochemistry, and some clinical aspects of vitamins. hormones, and antibiotics. In his own contribution the Festschrift—which would have made an excellent opening chapter—Dr. Folkers reflects on some of his teachers and their influence on his research. Among those guidelines is one that characterizes this book well, namely the attempt to bring chemistry and medicine closer together. The remarkable aspect of Folkers's research career and hence of the Festschrift is the fact that "medicine" is not equivalent to biochemistry, molecular biology, or physiology, but to clinical medicine.

The 27 contributions cover a wide spectrum of biomedical topics, among them coenzyme Q_{10}, vitamins B_6 and B_{12}, biosynthesis, structure–activity relationships, and even organic synthesis and analysis. Slightly over half of the contributions are mini-reviews by single authors, as one would expect for a symposium celebrating a scientist and his career. Chapters with post-1981 references are the exception rather than the rule, despite the 3-year interval between symposium and publication dates, which according to the editors was needed to update the symposium lectures.

Festschriften are no substitutes for the periodical literature or for monographs. However, they are valuable markers in the history of science and often are fun to read. In this case, the breadth of the topics provides added stimulation for scientists and students of many disciplines in the biomedical research community, particularly for those who would, with Karl Folkers, like to forge a closer link between chemistry and medicine.

The book, which is produced by offset printing, includes author and subject indices and some good-quality black and white photographs. Its modest price should make it an attractive addition to many personal libraries—this despite the inexcusable typo in the preface, where Folkers becomes Folker—not once but twice!

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Minimum Steric Difference. The MTD Method for QSAR Studies. Vol. 7 in the Chemometrics Series. By Z. Simon, A. Chiriac, S. Holban, D. Ciubotaru and G. I. Mihalas. Ed. by D. Bawden. Wiley, New York. 1984. ix + 173 pp. 15 × 23.5 cm. ISBN 0471-90438-4. $47.95.

The ultimate goal of a QSAR study is to get a picture of the interaction between the receptor and the effector substance. In Volume 1 of *The Chemometrics Series* the distance geometry method of mapping receptor–effector interactions was presented. In Volume 7 the editor returns to this important topic. The five authors present their approach to the receptor mapping problem—the MSD and MTD methods. The aim of the two methods is to reduce the large number of structure descriptors necessary for describing the receptor–effector interaction to a single number, which is then used as a variable in traditional multiple-regression QSARs. Such a tremendous reduction of the description of the molecular structure seems somewhat hazardous. The authors could have avoided this problem by the use of modern methods of multivariate analysis.