respective phosphorus resonances.

Determination of Rate Constants. Rate constants were determined by simultaneous fit of the experimental data points to the values calculated using the differential equations (1)-(5). A computer program was written by employing the Simplex algorithm[14] to optimize the rate constants by nonlinear least squares minimization. Calculated data points were determined every 6 s for the first 10 min, every 30 s for the next 15 min, and every minute for the remaining time points. Constants k_1-k_4 were optimized by using weighting factors of 1, 1, 1, 0.3, and 0.3 for intermediates **2**, **3**, **4** + **5**, **6**, and **8**, respectively (see Scheme I).

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5-Amino-4-(diazoacetyl)-1-β-D-ribofuranosylimidazole, a New Antileukemic Agent

H. Jeanette Thomas, Lisa N. Avery, R. Wallace Brockman, and John A. Montgomery*

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35255. *Received June 2, 1986*

5-Amino-4-(diazoacetyl)-1-β-D-ribofuranosylimidazole (**15**), 5-amino-4-(chloroacetyl)-1-β-D-ribofuranosylimidazole (**16**), and a number of related imidazole ribonucleosides have been synthesized. Compounds **15** and **16** are cytotoxic to both H.Ep.-2 and L1210 leukemia cells in culture. The (diazoacetyl)imidazole **15** is also active against the P388 leukemia in mice.

Azaserine and 6-diazo-5-oxonorleucine (DON) inhibit a wide variety of experimental neoplasms and have shown activity in humans, presumably curing choriocarcinomas.[1] These compounds are glutamine antagonists interfering with a number of enzymes involved in the de novo pathways to purine and pyrimidine nucleotides. The enzyme most sensitive to their action appears to be phosphoribosylformylglycinamidine synthetase (E.C. 6.3.5.3), which they irreversibly inactivate through the reaction of the diazomethyl group with a thiol of the enzyme active site.[2] It is this inhibition that is thought to be responsible for the antineoplastic activity of these compounds. Their structures are the basis of the design of imidazoles such as 5-amino-4-(diazoacetyl)-1-β-D-ribofuranosylimidazole (**15**) as potential anticancer agents that might act, for example, by interfering with the enzymic reaction catalyzed by *N*-(5-amino-1-β-D-ribofuranosylimidazole-4-carbonyl)-L-aspartic acid 5′-phosphate synthetase (E.C. 6.3.2.6). Since 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide is phosphorylated by adenosine kinase,[3] which is ubiquitous, it seemed reasonable to assume that this closely related structure would also be phosphorylated in cells, a potential prerequisite for significant inhibition of this enzyme. For this reason, we undertook the synthesis of **15**.

5-Amino-1-β-D-ribofuranosylimidazole-4-carboxamide (**1**) was transformed by the method of Robins et al.[4] to 5-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylic acid (**3**), which was converted to the acid chloride **4** by treatment with thionyl chloride (Scheme I). Since reaction of **4** with potassium *tert*-butoxide and nitromethane gave only the *tert*-butyl ester **9**, it was converted to 5-amino-4-(imidazol-1-ylacetyl)-1-β-D-ribofuranosylimidazole (**10**), which reacted readily with ammonia to give **1**, but neither **10** nor its derivatives blocked with either a trifluoroacetyl or phthaloyl group on the 5-amino group would react as desired with potassium *tert*-butoxide and nitromethane.[5] Reaction of **4** with diazomethane was also unsuccessful, giving the methyl ester **8** with only a trace of the desired (diazoacetyl)imidazole **14**. Since interference by the amino group adjacent to the acid chloride of **4** seemed likely, it was blocked (in **3**) by formylation and by trifluoroacetylation, the latter procedure giving the mixed anhydride as well. Since neither of these intermediates, both still containing one proton of the amino group, could be converted to the desired (diazoacetyl)imidazole, phthaloylation of **3** was attempted. Because this attempt resulted in decarboxylation, the free acid (**3**) was converted by treatment with benzyl bromide and potassium carbonate in DMA to its benzyl ester (**7**), which could also be prepared by the reaction of **4** with benzyl alcohol. Reaction of **7** with [(ethoxycarbonyl)oxy]phthalimide gave the phthaloyl derivative **6**, which was debenzylated to the free acid **5**. Conversion of **5** to the acid chloride **11** followed by treatment with diazomethane gave the (diazoacetyl)imidazole **12**, which, on the basis of NMR and TLC data, is contaminated with a small amount of the chloromethyl ketone **13**. The phthaloyl and acetyl protective groups were removed from **12** by treatment with hydrazine, followed by ethanolic ammonia, to give the desired 5-amino-4-(diazoacetyl)-1-β-D-ribofuranosylimidazole (**15**). A slight excess of dry hydrogen chloride at room temperature converted **15** to the chloromethyl ketone **16**. Attempts to catalytically reduce the diazomethyl ketone **15** to the aminomethyl ketone **17** gave only the methyl ketone **18**.

Biologic Data

The diazomethyl ketone **15** inhibited the growth of both H.Ep.-2 and L1210 cells in culture with I_{50} values of 2 and 3 μM, respectively.[6] It also gave an 84% ILS of mice inoculated ip with 10^6 P388 leukemia cells when given qd 1-5 ip (100 mg/kg per dose).[6] It was less active given day 1 only (40% at 100 mg/kg) or q 3 h × 8 on days 1, 5, and 9 (61% ILS at 22.5 mg/kg per dose, 540 mg total dose). That a reactive group is essential to activity is attested to

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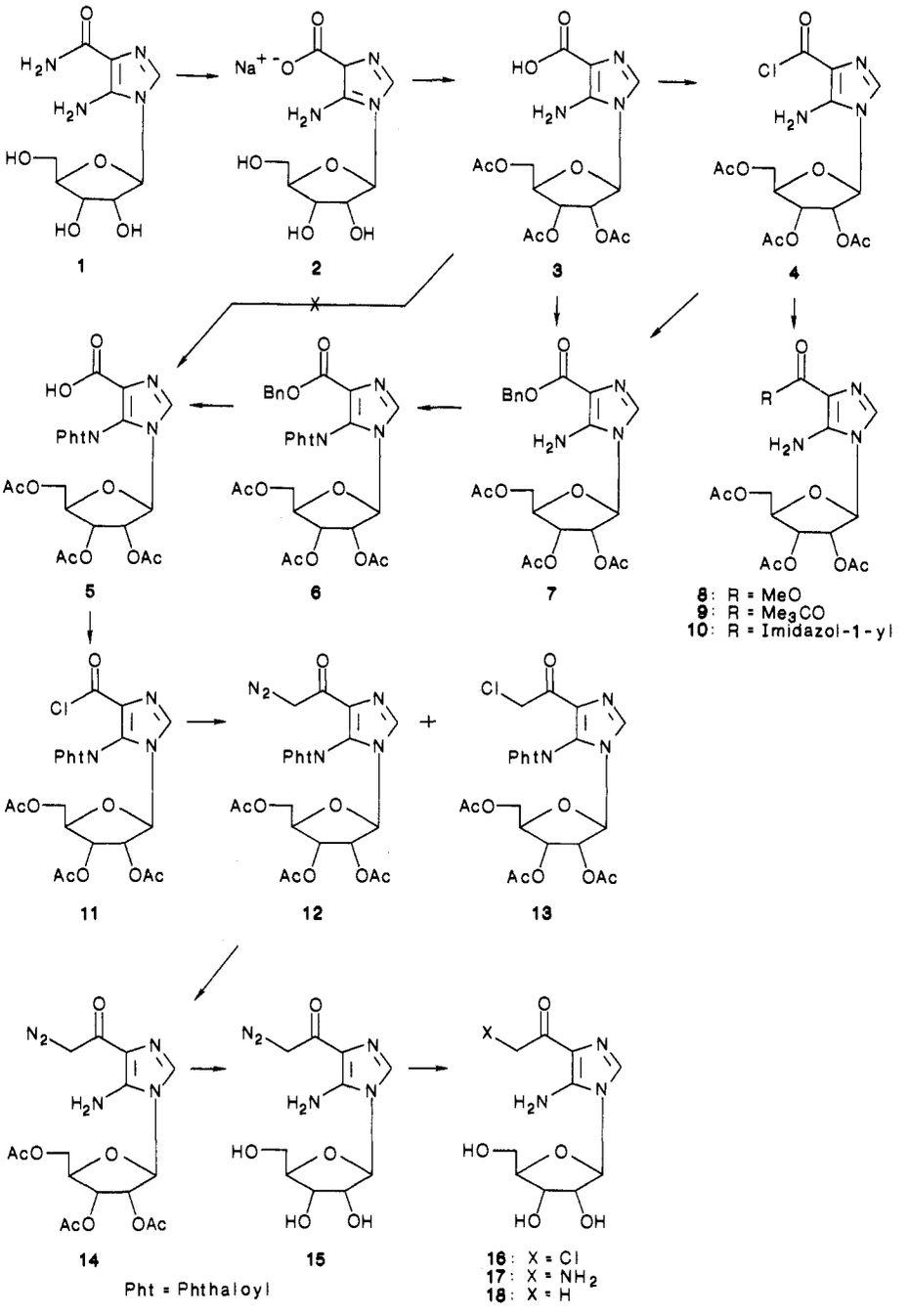
Scheme I



by the fact that the chloromethyl ketone **16** is also cytotoxic (I_{50} for L1210 cells 12 μM), whereas the methyl ketone **18** was completely inactive at 120 μM. The lower cytotoxicity of the chloromethyl ketone and its inactivity against the P388 leukemia in vivo may result from its relatively high chemical reactivity under physiologic conditions. The diazomethyl ketone **15** does not inhibit purine biosynthesis and studies of its effects on macromolecular synthesis in L1210 cells in culture showed that it does not interfere with DNA synthesis[7] unless cells are exposed for 24 h. A time study showed that effects of **15** on viability of L1210 cells in culture also requires a prolonged exposure (Figure 1). These results indicate that it is not acting as an antimetabolite or as a classical alkylating agent. Furthermore, it is equally toxic to the parent L1210 line and to a resistant strain lacking adenosine kinase, indicating that phosphorylation of **15** is not essential for biological activity. Studies are continuing on the mechanism of action of **15**.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. The UV absorption spectra were determined in the solvents specified with a Cary 17 spectrophotometer. ^{1}H NMR spectra were recorded on a Nicolet NMC 300NB spectrometer operating at 300.635 MHz, except where indicated. Chemical shifts are expressed in parts per million downfield from tetramethylsilane. When microanalytical data indicate a solvate, the solvents were seen in the proper amounts in the NMR spectra. Microanalyses were performed by the Molecular Spectroscopy Section, Southern Research Institute. TLC determinations were carried out on Analtech silica gel SGF (250 μm) plates using the solvents and detection methods given for each compound. Unless otherwise indicated, compounds were TLC homogeneous. The HPLC analysis was carried out with an ALC-242 liquid chromatograph (Waters Associates), using a μBondapak C_{18} column with UV monitoring. Mass spectra were recorded on a Varian

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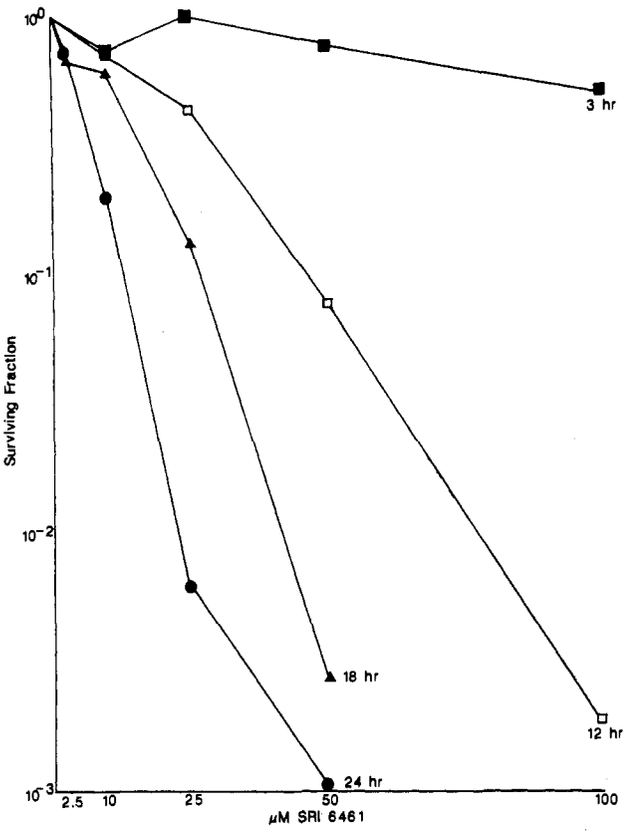

Figure 1. Effect of **15** on viability of L1210 cells determined by soft agar colony formation. Plot of surviving fraction vs. inhibitor concentration.

MAT 311A mass spectrometer in the fast atom bombardment (FAB) mode.

5-Phthalimido-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-imidazole-4-carboxylic Acid (5). A solution of 5-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylic acid benzyl ester (**7**; 14.4 g, 30.3 mmol) and *N*-carbethoxyphthalimide (13.3 g, 60.6 mmol) in dimethylformamide (250 mL) containing anhydrous potassium carbonate (8.36 g, 60.6 mmol) was stirred for 3 h at ambient temperature, then filtered, and evaporated to dryness in vacuo. An ethyl acetate solution of the residue was purified by flash chromatography on silica gel using ethyl acetate–cyclohexane (2:1) as the eluting solvent. A syrup (**6**) was obtained that crystallized on standing: yield 16.7 g (91%); mp indefinite; mass spectrum, m/z 259 $(sugar)^+$, 606 $(M + 1)^+$; TLC (EtOAc/cyclohexane (2:1), $(NH_4)_2SO_4$ char).

Crude **6** (16.7 g, 27.6 mmol) in ethyl acetate (600 mL) containing 30% palladium-on-carbon catalyst (9.6 g) was hydrogenated at ambient temperature and atmospheric pressure until the uptake of hydrogen ceased. It was then filtered and evaporated to dryness in vacuo. A white glass was obtained: yield 12.0 g (84%); mass spectrum, m/z 259 $(sugar)^+$, 516 $(M + 1)^+$; UV λ_{max} ($\epsilon \times 10^{-3}$) 220 nm at pH 1 (33) and 300 nm at pH 1 (1.68), 220 nm at pH 7 (40) and 300 nm at pH 7 (2.11), unstable at pH 13; ^1H NMR (Me_2SO-d_6) δ 1.19, 2.05, 4.02 (EtOAc), 1.94, 2.05 (s, 3 CH_3CO), 2.81, 8.02 (DMF), 4.31 (m, H-4′ and H-5′), 5.30 (dd, H-3′), 5.58 (ψt, H-2′), 5.98 (d, $J_{1',2'}$ = 5.7 Hz, H-1′), 8.35 (s, H-2), 17.22 (br s, CO_2H). Anal. Calcd for $C_{23}H_{21}N_3O_{11}$·0.5EtAc·0.3DMF: C, 53.51; H, 4.70; N, 7.95. Found: C, 53.13; H, 4.60; N, 7.90.

5-Amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-imidazole-4-carboxylic Acid Benzyl Ester (7). A solution of 5-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylic acid (**3**; 14.0 g, 36.3 mmol) in dimethylacetamide (100 mL) containing anhydrous potassium carbonate (5.01 g, 36.3 mmol) was stirred for 5 min at ambient temperature before the addition of benzyl bromide (6.21 g, 36.3 mmol). After stirring 16 h at ambient temperature, the mixture was filtered and evaporated to dryness in vacuo. The residue was partitioned between ethyl acetate and water. The ethyl acetate layer was dried over magnesium sulfate and evaporated to about 25 mL, whereupon the product was obtained as a crystalline solid: yield 11.2 g (65%); mp 129–130 °C; TLC (EtOAc, $(NH_4)_2SO_4$ char).

The analytical sample was obtained by recrystallization from ethanol. It was dried for 2 h at 78 °C (0.07 mm) over P_2O_5; mp 130–132 °C (lit.[4] mp 130–132 °C); UV λ_{max} ($\epsilon \times 10^{-3}$) 248 nm (sh) at pH 1 (11.7) and 268 nm at pH 1 (14.2), 270 nm at pH 7 (15.1), 271 nm at pH 13 (14.4); mass spectrum, m/z 259 $(sugar^+)$, 476 $(M + 1)^+$; ^1H NMR (Me_2SO-d_6) δ 2.04, 2.10 (2 s, 3 CH_3CO), 4.3 (m, H-4′ and H-5′), 5.23 (s, OCH_2 phenyl), 5.32 (dd, H-3′), 5.58 (ψt, H-2′), 5.95 (d, $J_{1',2'}$ = 6 Hz, H-1′), 6.30 (s, NH_2), 7.36 (m, phenyl), 7.46 (s, H-2). Anal. Calcd for $C_{22}H_{25}N_3O_9$: C, 55.57; H, 5.53; N, 8.84. Found: C, 55.64; H, 5.31; N, 8.77.

5-Amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-4-(imidazol-1-ylacetyl)imidazole (10). A cold (−20 °C) solution of 5-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylic acid (**3**; 385 mg, 1.00 mmol) in thionyl chloride (5 mL) was allowed to warm to ambient temperature where it was kept for 1 h, evaporated to dryness in vacuo, and treated with imidazole (680 mg, 10.0 mmol). A tetrahydrofuran (10 mL) solution of the mixture was kept at ambient temperature for 16 h, then filtered, and evaporated to dryness in vacuo. A chloroform solution of the residue was washed with water, dried over magnesium sulfate, and evaporated to dryness in vacuo. The residue crystallized from ethyl acetate solution as a white solid: yield 291 mg (67%); mp 143–145 °C; UV λ_{max} ($\epsilon \times 10^{-3}$) 324 nm at pH 1 (13.3), 314 nm at pH 7 (16.7), 268 nm at pH 13 (13.1); mass spectrum, m/z 436 $(M + 1)^+$; ^1H NMR (Me_2SO-d_6) δ 2.08, 2.12 (2 s, 3 CH_3CO), 4.30 (m, H-4′ and H-5′), 5.37 (m, H-3′), 5.65 (ψt, H-2′), 6.09 (d, $J_{1',2'}$ = 6 Hz, H-1′), 7.06 (m, H-4 of imidazole), 7.16 (s, NH_2), 7.72 (s, H-2), 8.15 (m, H-5 of imidazole), 8.92 (m, H-2 of imidazole); ^{13}C NMR (Me_2SO-d_6) δ 20.41, 20.45, 20.69 (CH_3), 62.13 ($C_{5'}$), 69.36 ($C_{3'}$), 72.93 ($C_{2'}$), 80.28 ($C_{4'}$), 86.93 ($C_{1'}$), 113.19 (C_4 of trisubstituted imidazole), 117.65 (C_5 of monosubstituted imidazole), 128.86 (C_2 of trisubstituted imidazole), 129.21 (C_2 of monosubstituted imidazole), 138.66 (C_4 of monosubstituted imidazole), 149.46 (C_5 of trisubstituted imidazole), 159.66 (CO of imidazole), 169.56, 170.04, 170.30 (CO of acetyls). Anal. Calcd for $C_{18}H_{21}N_5O_8$: C, 49.65; H, 4.86; N, 16.09. Found: C, 49.53; H, 4.97; N, 16.07.

4-(Diazoacetyl)-5-phthalimido-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole (12). To a solution of 5-phthalimido-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylic acid (**5**; 515 mg, 1.00 mmol) in anhydrous tetrahydrofuran (25 mL) was added *N,N*-dimethylchloroforminium chloride (256 mg, 2.00 mmol). A complete solution was obtained after about 5 min, and it was kept 1 h at ambient temperature before it was slowly added to a cold (−10 °C) solution of about 20 mmol of diazomethane in 100 mL of anhydrous ether. The solution was allowed to rise to ambient temperature where it was kept, loosely sealed, for 20 h before it was evaporated in vacuo to an orange syrup, suitable for conversion to **15** without further purification. **12**: TLC (EtOAc, NBP positive); mass spectrum, m/z 259 $(sugar)^+$, 540 $(M + 1)^+$; ^1H NMR (Me_2SO-d_6) δ 1.98, 2.02, 2.04 (3 s, 3 CH_3), 4.28 (m, 3 H, H-4′ and H-5′), 5.28 (dd, $J_{3',4'}$ = 4.4 Hz, H-3′), 5.58 (ψt, $J_{2',3'}$ = 6.4 Hz, H-2′), 5.99 (d, $J_{1',2'}$ = 5.8 Hz, H-1′), 6.59 (br s, CHN_2), 8.02 (m, 4 H, phenyl), 8.34 (s, H-2).

5-Amino-4-(diazoacetyl)-1-β-D-ribofuranosylimidazole (15). A solution of 4-(diazoacetyl)-5-phthalimido-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole (**12**; 3.20 g, 5.90 mmol) in anhydrous methylene chloride (100 mL) containing anhydrous hydrazine (189 mg, 5.9 mmol) was kept for 2 h at ambient temperature. Another 95 mg (2.96 mmol) of hydrazine was then added and the mixture kept 1 additional h, then filtered, and evaporated to dryness in vacuo, giving 5-amino-4-(diazoacetyl)-1-(2,3,5-tri-β-D-ribofuranosyl)imidazole (**14**) as a yellow syrup.

A solution of the syrup in 100 mL of ethanolic ammonia (saturated at 0 °C) was kept at 0–3 °C for 20 h before it was evaporated to dryness in vacuo. A solution of the residue in 25 mL of methanol was filtered to remove an insoluble solid and concentrated to about 10 mL, whereupon the product was obtained as a yellow crystalline solid: yield 1.21 g (72%); TLC ($CHCl_3$/MeOH (3:1), NBP positive).

The analytical sample was obtained from a previous reaction by recrystallization from methanol: mp 109–112 °C; UV λ_{max} ($\epsilon \times 10^{-3}$) 258 nm (sh) at pH 1 (3.63) and 294 nm at pH 1 (12.2), 277 nm at pH 7 (5.26) and 328 nm at pH 7 (22.6), 277 nm at pH 13 (5.47) and 329 nm at pH 13 (22.4); mass spectrum, m/z 284

(M + 1)$^+$; ^{1}H NMR (Me_2SO-d_6, XL-100) δ 3.31 (s, H_2O), 3.59 (m, 2 H-5'), 3.94 (m, H-4'), 4.08 (m, H-3'), 4.28 (m, H-2'), 5.15 (dd, $O_{3'}H$), 5.32 (m, H-4' and H-5'), 5.51 (d, H-1', $J_{1',2'}$ = 6 Hz), 6.12 (s, C*H*N_2), 6.58 (s, NH_2), 7.31 (s, H_2); ^{13}C NMR δ 50.29 (CHN_2), 61.02 ($C_{5'}$), 70.24 ($C_{3'}$), 72.69 ($C_{2'}$), 85.43 ($C_{4'}$), 87.40 ($C_{1'}$), 118.06 (C_4), 143.63 (C_5), 179.93 (CO). Anal. Calcd for $C_{10}H_{13}N_5O_5 \cdot 0.7H_2O$: C, 40.57; H, 4.91; N, 23.66. Found: C, 40.65; H, 5.28; N, 23.61.

5-Amino-4-(chloroacetyl)-1-β-D-ribofuranosylimidazole (16). A suspension of 5-amino-4-(diazoacetyl)-1-β-D-ribofuranosylimidazole (15; 190 mg, 0.67 mmol) in 15 mL of anhydrous acetonitrile was diluted with 20 mL of ethereal hydrogen chloride (saturated at 20 °C) and the mixture stirred at ambient temperature under anhydrous conditions until the bubbling ceased (about 10 min). The resulting solid was collected under nitrogen atmosphere and dried at ambient temperature and 0.07 nm over P_2O_5 for 1 h: yield 185 mg (95%); mp 155-157 °C dec; TLC ($CHCl_3$/MeOH (3:1), NBP positive).

The analytical sample was obtained from a similar reaction. It was recrystallized from ethanol: mp 154-156 °C dec; UV λ_{max} ($\epsilon \times 10^{-3}$) 300 nm at pH 1 (13.1), 310 nm at pH 7 (12.7), 312 nm at pH 13 (12.7); mass spectrum, *m/e* 291 (M$^+$; ^{1}H NMR (Me_2SO-d_6) δ 1.24 (s, EtOH), 3.32 (s, H_2O), 3.63 (ψt, 2 H-5'), 3.94 (dd, H-4'), 4.05 (dd, H-3'), 4.29 (dd, H-2'), 4.62 (s, $COCH_2Cl$), 5.20 (m, 3'-OH), 5.37 (ψt, 5'-OH), 5.43 (m, 2'-OH), 5.55 (d, H-1', $J_{1',2'}$ = 2 Hz), 7.05 (s, NH_2), 7.38 (s, H_2). Anal. Calcd for $C_{10}H_{14}ClN_3O_5 \cdot 0.5H_2O \cdot 0.3EtOH$: C, 40.48; H, 5.38; N, 13.27. Found: C, 40.39; H, 5.37; N, 13.27.

4-Acetyl-5-amino-1-β-D-ribofuranosylimidazole (18). A solution of 5-amino-4-(diazoacetyl)-1-β-D-ribofuranosylimidazole (15; 142 mg, 0.5 mmol) in methanol (10 mL) containing 117 mg of 30% palladium-on-carbon catalyst was hydrogenated at ambient temperature and atmospheric pressure, then filtered, and evaporated to dryness in vacuo. The syrup obtained was purified by preparative thin-layer chromatography on Brinkmann (2-mm) silica gel plates developed in $CHCl_3$/MeOH (3:1). The product was recrystallized from ethanol as a white solid: yield 35 mg (27%); mp 237-238 °C dec; TLC ($CHCl_3$/MeOH (3:1), $(NH_4)_2SO_4$ char); UV λ_{max} ($\epsilon \times 10^{-3}$); 256 nm (sh) at pH 1 (4.30) and 291 nm at pH 1 (13.3), 240 nm at pH 7 (3.07) and 302 nm at pH 7 (13.3), 240 nm (sh) at pH 13 (3.04) and 301 nm at pH 13 (13.4); mass spectrum (EI), *m/e* 257 (M$^+$); ^{1}H NMR (Me_2SO-d_6) δ 2.25 (s, CH_3), 3.60 (ψt, 2 H-5'), 3.93 (dd, H-4'), 4.05 (dd, H-3'), 4.29 (dd, H-2'), 5.19 (d, 3'-OH), 5.35 (ψt, 5'-OH), 5.41 (d, 2'-OH), 5.51 (d, H-1', $J_{1',2'}$ = 6 Hz), 6.79 (s, NH_2), 7.32 (s, H_2). Anal. Calcd for $C_{10}H_{15}N_3O_5$: C, 46.69; H, 5.88; N, 16.33. Found: C, 46.72; H, 6.02; N, 16.05.

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Prostaglandin-H Synthase Inhibition by Malonamides. Ring-Opened Analogues of Phenylbutazone

Jonathan L. Vennerstrom and Thomas J. Holmes, Jr.*

Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455.
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Recent reports of serious concern regarding the safe clinical use of phenylbutazone and its hydroxylated metabolite (oxyphenbutazone) as antiinflammatory agents have prompted the further investigation of ring-opened (malonamide) derivatives as potentially preferable therapeutic derivatives. Earlier reports have claimed reduced toxicity among similar derivatives. These studies reveal the relative degree of prostaglandin-H (PGH) synthase inhibitory activity among a series of malonamide derivatives. Contrary to observations in the pyrazolidinedione series, incorporation of a nonpolar butyl side chain in these malonamides was not beneficial but, rather, detrimental to enzyme-inhibitory activity. Although none of the reported nonbutylated malonamides was as potent an inhibitor of this enzyme as phenylbutazone, they all showed some inhibitory activity. PGH synthase inhibitory activity was especially pronounced in the bis(*p*-hydroxy anilide) derivatives, even extending to succinamide and adipamide derivatives. Of some interest is the observation that all of these *p*-hydroxy anilide derivatives were more potent inhibitors of this enzyme than acetaminophen.

The history of phenylbutazone (1) as a nonsteroidal antiinflammatory (NSAI) agent began in 1949 when the drug (which had been originally used as a solubilizing agent for aminopyrine) was introduced for the treatment of rheumatoid arthritis, acute gout, and allied disorders.[1] Clinically, phenylbutazone is 6 times more potent than the salicylates, but 5 times less potent than indomethacin. Phenylbutazone is an effective NSAI agent, but serious toxicity (especially aplastic anemia and agranulocytosis) limits its use in long-term therapy.[1,2]

A significant concern has been expressed about the toxicity of phenylbutazone (1) and oxyphenbutazone (2) in a number of recent reports[3,4] and letters to the editor.[5-8] Phenylbutazone and oxyphenbutazone have, in fact, been removed from the market in Bahrain, Jordan, and Norway.[8] Similar decisions are pending in Australia, the United States, and several other countries.[5,8] Meanwhile, the product labeling of phenylbutazone and oxyphenbutazone in the United States is being revised to reflect concern about the serious toxicity mentioned above.[4] A recent report in the *Drug and Therapeutics Bulletin*[3] acknowledged that while some physicians consider phenylbutazone to have special value in acute gout and an-

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