of anhydrous benzene was added 14.43 g (0.069 mol) of phosphorus pentachloride and the suspension stirred at ambient temperature for 4 h. The clear solution was concentrated in vacuo to provide the diacid chloride as a light tan solid which was dissolved in 140 ml of anhydrous methylene chloride; 17.9 g (0.068 mol) of stannic chloride in 20 ml of methylene chloride was slowly added and the solution was stirred at ambient temperature for an additional 24 h. The reaction was made basic and filtered; the organic layer was separated and after extraction with ether the aqueous phase was acidified with concentrated hydrochloric acid to provide a brown solid which was washed with water and dried. Recrystallization from acetonitrile provided 3.71 g of a light brown solid, mp 177–178 °C.

4,10-Dihydro-10-oxothieno[3,2-*c*][1]benzoxepin-8-acetic Acid (2). This compound was prepared by modification of a method used by Stach and Spingler.[5] To 3.5 ml of absolute ethanol was slowly added 5.80 g (0.04 mol) of phosphorus pentoxide, the temperature being kept below 80 °C; the white viscous mixture was then heated at 110 °C for 1 h. After adding 25 ml of sulfolane, the reaction temperature was adjusted to 81–83 °C and 2.70 g (0.01 mol) of 4-(2-carboxy-3-thienylmethoxy)-phenylacetic acid was added. After 3 h, the mixture was decanted into water, made basic, and extracted with toluene. Acidification of the ice-cooled aqueous phase with concentrated hydrochloric acid provided a brown solid which was extracted with chloroform, dried (Na_2SO_4), filtered, and concentrated in vacuo to a yellow solid. Trituration with ether provided 1.0 g of light yellow crystals, mp 162–164 °C.

4-(2-Carboxy-3-furylmethoxy)phenylacetic Acid (3). A mixture of 20.0 g (0.09 mol) of methyl 3-bromomethyl-2-furoate, 15.12 g (0.09 mol) of methyl 3-(4-hydroxyphenyl)acetate, 52.0 g (0.4 mol) of potassium carbonate, 360 ml of 2-butanone, and 1.0 g of sodium iodide was refluxed for 17 h. The reaction was cooled and filtered, and the filtrate was concentrated in vacuo to a dark brown oil. The oil was dissolved in ether, washed with water, 5% sodium hydroxide, and water, and dried (Na_2SO_4). Filtration and concentration in vacuo gave an oil which was refluxed with 51.0 g (0.91 mol) of potassium hydroxide in 255 ml of ethanol and 26 ml of water for 17 h. The reaction was concentrated in vacuo to a brown solid which was dissolved in water and extracted with ether; the aqueous phase was acidified with concentrated hydrochloric acid and the resulting brown precipitate was filtered and dried. Recrystallization from acetonitrile afforded 3.78 g (33%) of off-white crystals, mp 204–205 °C. Anal. ($C_{14}H_{12}O_6$) C, H.

4-(2-Carboxy-3-thienylmethoxy)phenylacetic Acid (4). This compound was prepared according to the method used for compound **3**. Recrystallization from 2-propanol followed by washing with ether provided 16.5 g (62%) of beige crystals, mp 222 °C. Anal. ($C_{14}H_{12}O_5S$) C, H, S.

3-Methyl-2-thiophenecarboxylic Acid (8). This compound was prepared by a procedure developed by Campaigne et al.[4] To 8.40 g (0.21 mol) of sodium hydroxide in 72 ml of water was added 18.0 g (0.11 mol) of silver nitrate and the suspension cooled to 5 °C; 6.30 g (0.05 mol) of 3-methylthiophene-2-carboxaldehyde was then added portionwise. The mixture was stirred at ambient temperature for 1.5 h and filtered, and the precipitate was washed with 70 ml of water. Acidification of the ice-cooled filtrate provided a solid which was collected and dried to yield 6.10 g (86%) of colorless crystals, mp 141–143 °C (lit.[3] 147–148 °C, H_2O) (IR, NMR).

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Synthesis and Antitumor Activity of 1,2-Dihydro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-2-oxo-5-methylpyrazine 4-Oxide, a Structural Analogue of Thymidine

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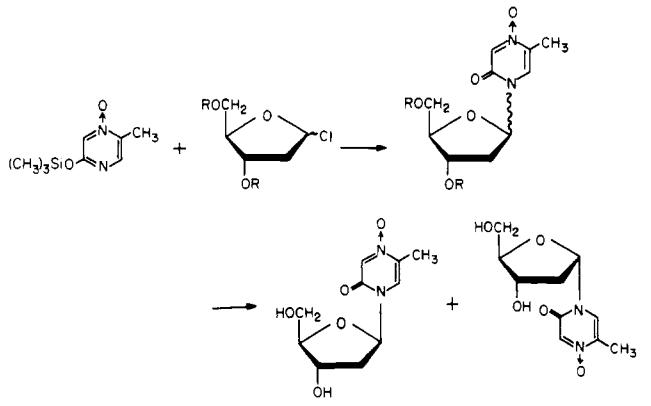
1,2-Dihydro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-2-oxo-5-methylpyrazine 4-oxide was synthesized by condensation of the silylated pyrazine base with the blocked chloro sugar, followed by removal of the protecting groups. The compound inhibited the growth of leukemia L1210 cells in vitro by 50% at 9×10^{-7} M. At 400 mg/kg/day × 6 it increased the life-span of leukemia L1210 bearing mice by approximately 55%, without apparent toxicity to the host.

We have previously reported on the synthesis and biological activity of pyrazine analogues of various natural pyrimidine and purine nucleosides[1-3] and showed that some of these compounds have pronounced antibacterial activity, while being essentially inactive against leukemia L1210 cells. We have now prepared the pyrazine analogue

Scheme I



of thymidine, 1,2-dihydro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-2-oxo-5-methylpyrazine 4-oxide (**5**) (Scheme I), which demonstrated a reverse selectivity, being weakly active against bacterial cells but significantly active against leukemia L1210 cells in vitro and in vivo.

Protected **5** was synthesized by condensation of the trimethylsilyl derivative of 1,2-dihydro-2-oxo-5-methylpyrazine 4-oxide with blocked 2-deoxy-D-*erythro*-pentofuranosyl chloride. Because the yield and anomeric composition of the condensation product is influenced by the reaction conditions,[4] two synthetic approaches were investigated. In one approach condensation of 2-deoxy-3,5-di-*O*-*p*-chlorobenzoyl-α-D-*erythro*-pentofuranosyl chloride with the silylated base was carried out in the presence of molecular sieves, since it has been shown[5] that molecular sieves enhance formation of the β anomer in the condensation of 3,5-di-*O*-*p*-toluoyl-2-deoxy-D-ribofuranosyl chloride with silylated 6-methylcytosine. Under the conditions used, the yield of crude anomeric mixture was 54%, from which the pure, blocked β anomer **1** was obtained in 10% and the α anomer **2** in 25% yield. Thus, the presence of molecular sieves does not in itself assure the predominant formation of the β anomer.

The second approach involved addition of $HgO–HgBr_2$ in place of the molecular sieves to the reaction mixture containing *p*-toluoyl blocked sugar chloride and the silylated pyrazine base. Mercuric oxide reacts rapidly with trimethylsilyl chloride to give hexamethyldisiloxane and mercuric chloride[7] and rapid removal of trimethylsilyl chloride, a by-product of the condensation reaction, had been suggested[6] to favor formation of the β anomer. This method gave a 71% yield of blocked anomeric mixture. The anomers were separated by fractional crystallization and silica gel chromatography, and the pure, blocked β nucleoside was obtained in 44% overall yield. The overall yield of the α anomer was 24%. Removal of the *p*-chlorobenzoyl groups from **1** and **2** with sodium methoxide in methanol for 1.5 h gave approximately 66% yield of the free nucleosides **5** and **6**. Treatment of the *p*-toluoyl blocked nucleosides **3** and **4** with sodium methoxide in methanol–methylene chloride for 8 h yielded 50% of the mixture of **5** and **6**.

The structures of **5** and **6** were established on the basis of their UV and NMR spectra. The UV spectra are consistent with the spectra of various N-1 substituted pyrazine *N*-oxides and differ considerably from the spectra of O-substituted pyrazine *N*-oxides.[1,2] The NMR spectrum of **5** gave a triplet (peak width of 13 Hz) for the anomeric proton, while that of **6** gave a quartet with a peak width of 8 Hz, supporting the β configuration for **5** and the α configuration for **6**. Whereas the free nucleosides gave the expected splitting patterns for the anomeric protons (a "pseudo triplet" for β and a "quartet" for the α-anomeric proton), the anomeric protons of both anomers of the protected nucleosides gave rise to apparent quartets, a pattern which in the past[8,9] had been observed for other blocked nucleosides.

Since previously prepared pyrazine analogues of various natural pyrimidines were inhibitors of bacterial growth in vitro, but were only marginally active against tumor cells,[1,2] it was of interest to find that compound **5** inhibited the growth of leukemia L1210 cells in vitro by 50% at 9×10^{-7} M. On the other hand, the compound had marginal activity against bacteria, inhibiting *Streptococcus faecium* growth by 50% at 8×10^{-5} M and *Escherichia coli* and K_{12} growth by 20 and 30%, respectively, at 1×10^{-3} M. The pyrazine analogue of thymine was essentially inactive against L1210 cells.[1] Because of the in vitro inhibition of leukemia L1210 cell proliferation, **5** was also evaluated for its effect on the growth of this tumor in DBA/2 Ha mice. When administered intraperitoneally at 100, 200, and 400 mg/kg/day × 6, the life-span of the tumor bearing mice was increased by 18, 30, and 55%, respectively. At these doses, no overt signs of host toxicity were discernable.

To gain information on the possible site of action of **5**, the effect of various natural pyrimidines on the inhibition of leukemia L1210 cell growth in vitro was evaluated. At 1×10^{-5} M thymidine, the ratio of inhibitor concentration to substrate concentration ([I]/[S]) at which 50% growth occurs was found to be approximately 50. With deoxyuridine and deoxycytidine as substrates, the ratios were approximately 2 and 0.2, respectively. With uridine and cytidine as substrates, ratios of 0.04 and 0.02, respectively, were obtained.

These results suggest that compound **5** may act along the metabolic path leading to DNA synthesis and function. Experimental evaluation of this suggestion is currently in progress.

Experimental Section

Melting points were determined on a Fisher-Johns melting point apparatus and were not corrected. UV spectra were recorded on a Cary Model 14 spectrometer and IR on a Perkin-Elmer 457 infrared spectrometer. NMR spectra were recorded using Varian XL-100 and JOEL MH-100 instruments. Satisfactory analyses (C, H, and N within ±0.4% of the theoretical values) were obtained from Robertson Laboratory, Florham Park, N.J. Evaporations were carried out under reduced pressure in a rotary evaporator.

1,2-Dihydro-1-(2-deoxy-3,5-di-*O*-*p*-chlorobenzoyl-β-D-*erythro*-pentofuranosyl)-2-oxo-5-methylpyrazine 4-Oxide (1) and Its α Anomer 2. A mixture of 1,2-dihydro-2-oxo-5-methylpyrazine 4-oxide[1] (0.781 g, 6.20 mmol), hexamethyldisilazane (8 ml), and trimethylchlorosilane (0.1 ml) was stirred at 95–100 °C for 1 h, protected from atmospheric moisture. The resulting solution was diluted with dry toluene (50 ml), and the solvent was removed by evaporation. Dry toluene (50 ml) was added to the residue and the solvent was again evaporated. The residue was dissolved in dry benzene (150 ml), and 19 g of 4 Å molecular sieves followed by 2-deoxy-3,5-di-*O*-*p*-chlorobenzoyl-α-D-*erythro*-pentofuranosyl chloride (5.33 g, 12.4 mmol)[6] was added to this solution. The reaction mixture was stirred at room temperature for 3 days with exclusion of atmospheric moisture and was then filtered into methanol (40 ml). The filtrate was evaporated in vacuo to a syrupy residue which was suspended in methylene chloride (20 ml) and filtered. The filtered solids were washed with ether to give 0.214 g of 1,2-dihydro-2-oxo-5-methylpyrazine 4-oxide. The combined filtrate and washings were concentrated to a syrupy residue (2.20 g) which was purified by dry-column chromatography on silica gel using chloroform–ethyl acetate (3:2) as the solvent. Evaporation of the solvent gave 1.79 g (54%) of the anomeric mixture of the 2′-deoxyribonucleosides of 2-oxo-5-methylpyrazine 4-oxide. The anomeric ratio was shown by NMR (using the ratio of peak heights corresponding

to the 5-methyl protons of the α and β anomers at δ 2.15 and 2.05, respectively) to be α:β = 2:1. Recrystallization of this mixture from toluene gave 0.947 g of crude α anomer, which was purified by recrystallization from toluene (three times) to give 0.789 g (24.5%) of 1,2-dihydro-1-(2-deoxy-3,5-di-*O*-*p*-chlorobenzoyl-α-D-*erythro*-pentofuranosyl)-2-oxo-5-methylpyrazine 4-oxide: mp 215 °C dec; $[\alpha]^{25}D$ −114° (*c* 0.5, $CHCl_3$); IR (KBr) 1735 (C=O, ester), 1680 (C=O, amide), 1233, 855 cm^{-1} (N → O); NMR ($CDCl_3$) δ 6.40 (q, 1, $J_{1',2',2''}$ = 2 and 6 Hz, peak width = 8 Hz, C_1, H), 2.15 (s, 3, CH_3); UV_{max} (EtOH) 228 nm (ε 43800), 283 (9620), 345 (6260). Anal. C, H, N.

The combined filtrates remaining after (re)crystallization of the α anomer were evaporated to give 0.79 g of crude β anomer which was purified by several recrystallizations from toluene–petroleum ether and finally from chloroform–petroleum ether. The yield of the pure β anomer was 0.301 g (9.7%): mp 186 °C dec; $[\alpha]^{25}D$ +108° (*c* 0.5, $CHCl_3$); IR (KBr) 1715 (C=O, ester), 1670 (C=O, amide), 1240, 845 cm^{-1} (N → O); NMR ($CDCl_3$) δ 2.05 (s, 3, CH_3), 6.40 (q, 1, $J_{1',2',2''}$ = 5.4 and 8 Hz, peak width = 13 Hz, C_1, H); UV_{max} (EtOH) 232 nm (ε 43900), 283 (10700), 345 (5680). Anal. C, H, N.

1,2-Dihydro-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-2-oxo-5-methylpyrazine 4-Oxide (3) and Its α Anomer 4. 2-Deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride[10] (15.0 g) was added to a cold (5 °C) mixture of dry HgO (6.5 g), $HgBr_2$ (10.5 g), and the trimethylsilyl derivative of 1,2-dihydro-2-oxo-5-methylpyrazine 4-oxide (prepared from 3.6 g of the pyrazine base). The mixture was stirred at 5 °C for 0.5 h and at room temperature for 0.5 h. It was then filtered, and the solids were washed with chloroform (200 ml). The combined filtrates were washed with a 30% KI solution (2 × 100 ml) and H_2O (2 × 100 ml) and dried (Na_2SO_4). The solution was evaporated to approximately 30 ml, when crystallization occurred. Ethanol (100 ml) was added to the mixture and the crystals were filtered and washed successively with ethanol and ether to give 8.8 g of a mixture of blocked α and β anomers. The crystalline fractions and the filtrate fraction were then worked up as follows.

The combined filtrate and washings were evaporated, and the syrupy residue was purified by silica gel chromatography. The column (2.5 × 60 cm) was eluted first with 500 ml of ethyl acetate–benzene (1:3) and then ethyl acetate–benzene (1:1). The appropriate fractions were collected and evaporated to dryness. The crystalline residue was recrystallized from 100 ml of ethanol to give 0.85 g of pure α anomer. The total yield of α anomer was 23.8% (3.26 g): mp 170–171.5 °C; NMR ($CDCl_3$) δ 2.15 (s, 3, CH_3), 2.41, 2.42 (2 s, 6, toluoyl CH_3), 6.40 (q, 1, $J_{1',2',2''}$ = 1 and 6 Hz, peak width = 10 Hz, C_1, H). Anal. C, H, N.

The original crystalline anomeric mixture (8.8 g) was dissolved in chloroform and the solution was evaporated to a syrup. Crystallization of this syrup from chloroform–acetone (7:10, 60 ml) gave 5.50 g of the pure β anomer. The filtrate remaining after separation of crystals was evaporated to a syrup from which α anomer (2.35 g) crystallized upon addition of ethanol (150 ml). Repeated work-up of the filtrate gave 0.38 g of the β anomer. The filtrate was evaporated to a syrupy mixture (240 mg) of **3** and **4** which was separated by silica gel chromatography and fractional crystallization to give an additional 0.1 g of **3**. The β anomer was obtained in 43.7% (5.98 g) yield: mp 200.5–201.5 °C dec; NMR ($CDCl_3$) δ 1.95 (s, 3, CH_3), 2.44, 2.45 (2 s, 6, toluoyl CH_3), 6.27 (q, 1, $J_{1',2',2''}$ = 5 and 7.5 Hz, peak width = 15 Hz, C_1, H). Anal. C, H, N.

1,2-Dihydro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-2-oxo-5-methylpyrazine 4-Oxide (5). A suspension of the blocked β anomer **1** (0.260 g) in 26 ml of methanol containing a catalytic amount of sodium methoxide was stirred at room temperature for 1.5 h. The solution was neutralized with Dowex 50 [H^+] ion-exchange resin and filtered, and the filtrate was evaporated to a syrupy residue. The syrup was extracted with benzene (20 ml) to remove methyl *p*-chlorobenzoate and was then dissolved in 2 ml of 1:1 EtOH–ethyl acetate. After several hours at room temperature, the crystalline product was collected by filtration and washed with a small amount of acetone: yield 0.08 g (66%). An analytical sample was obtained by recrystallization from ethanol: mp 141.5–143 °C; $[\alpha]^{25}D$ +149° (*c* 0.1, MeOH); IR (KBr) 3420, 3330 (OH), 1655 (C=O), 1220, 850 cm^{-1} (N → O); NMR (Me_2SO-d_6) δ 2.11 (s, 3, CH_3), 6.22 (t, 1, $J_{1',2'}$ = 6.5 Hz, peak width = 13 Hz, C_1, H), 7.63 (s, 1, C_6H), 8.04 (s, 1, C_3H); UV_{max} (95% EtOH) 224 nm (ε 21400), 283 (10500), 345 (5700). Anal. C, H, N.

1,2-Dihydro-1-(2-deoxy-α-D-*erythro*-pentofuranosyl)-2-oxo-5-methylpyrazine 4-Oxide (6). Treatment of the blocked α anomer in the manner described for the β anomer gave a syrup which was purified by dry column chromatography on silica gel, using ethyl acetate–methanol (5:1) as the solvent. The free syrupy α anomer **6** was obtained by evaporating the solvent: $[\alpha]^{25}D$ −146° (*c* 0.1, MeOH); NMR (MeOH-d_4) δ 6.22 (q, 1, $J_{1',2',2''}$ = 2 and 6 Hz, peak width = 8 Hz, C_1, H); UV_{max} (95% EtOH) 224 nm (ε 20800), 283 (8700), 345 (6100). Anal. C, H, N.

The procedure used for assaying the effect of the compound on the in vitro growth of the bacterial strains used has been published previously.[11]

Determination of the growth-inhibiting potency of the compounds against leukmia L1210 cells in vitro was carried out by our micro technique, whereby 0.5-ml aliquots of medium (RPMI 1640 + 10% fetal calf serum) containing the analogue are introduced into 16 × 125 nm screw cap culture tubes, followed by 0.5-ml portions of the medium containing 3 × 10^5 L1210 cells. The cultures are incubated at 37 °C for 40 h, after which time the viable cells are counted by trypan blue exclusion. During this time, the cell number in the controls increases ca. eight- to ninefold with an average cell viability of 99%.

The effect of the compounds on leukemia L1210 cell growth in vivo was evaluated by inoculating female DBA/2J CR mice (5–7 weeks old, 17–19 g) ip with 1 × 10^6 leukemia L1210 cells. After 24 h the compound, dissolved in saline, was administered ip once daily for 6 consecutive days.

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