directed toward identification of such a residue and examination of the binding of 3 to β-lactamases in an effort to rationalize its biological activity.

Experimental Section

Crystal Structure Determination. Tazobactam was crystallized from a 70:30 ethanol/water mixture as colorless stout hexagonal prisms. Crystals exhibit orthorhombic symmetry, space group $P2_12_12_1$. Unit cell parameters (Table I) were refined by least-squares analysis of setting angles of 25 reflections obtained on an Enraf-Nonius CAD4 diffractometer. A crystal 0.83 mm long × 0.33 mm wide × 0.40 mm thick was selected for collection of intensity data. Although a Ψ scan exhibited intensity variation of less than ±6% from the mean, an empirical absorption correction was made with DIFABS.[20] By the ω–2θ scan technique a total of 2464 observed reflections with $I \geq 3\sigma$ were collected in the range $2° \leq \theta \leq 25°$ for graphite-monochromated Mo Kα radiation.

The structure was solved by direct methods using the RANTAN procedure within MULTAN.[21] Two independent molecules of 3 are present. After missing non-hydrogen atoms were located in an electron density map, least-squares refinement was carried out with SHELX.[22] All hydrogen atoms except those in carboxyl groups were introduced in calculated positions determined by the molecular geometry, methyl groups were treated as rotatable rigid bodies, and other H atoms were assumed to ride on their attached atoms. Carboxyl hydrogen atom H(12) was located unambiguously in a difference electron density map. Two plausible alternatives were found for H(12'), but one was selected because it gave the molecule a heat of formation from MOPAC[14] 20 kcal mol^{-1} lower than the other, its intramolecular geometry was better, and only it engaged in reasonable hydrogen bonding. Coordinates and isotropic temperature factors were refined for H(12) and H(12') in the final cycles, along with isotropic temperature factors for other H atoms, coordinates and anisotropic thermal parameters for non-hydrogen atoms, and an empirical extinction correction. The observed reflections were weighted by $1/[\sigma^2(F) + 0.000519F^2]$. At termination no parameter shifted by more than 0.03 ESD, the final discrepancy index was $R = 0.033$, and no peak in a difference electron density map exceeded 0.30 eÅ^{-3}. Fractional coordinates and thermal parameters have been deposited as supplementary material.

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Registry No. 3, 89786-04-9.

Supplementary Material Available: Tables listing fractional coordinates and thermal parameters, bond distances, bond angles, and torsion angles for tazobactam (7 pages). Ordering information is given on any current masthead page.

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Additional Nucleotide Derivatives of Mitosenes. Synthesis and Activity against Parental and Multidrug Resistant L1210 Leukemia

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Cytidine 5'-monophosphate and 5'-*ara*-CMP conjugates of 2,7-diaminomitosene, with the phosphate groups linked to C-1, were prepared by treating mitomycin C with the appropriate nucleotides. 5'-UMP conjugates were prepared from mitomycin A, 7 (M-83), and 8 (BMY-25282) by similar procedures. A conjugate could not be prepared from mitomycin C and 6-MPRP, but a sulfur-linked derivative was made with 6-MP ribonucleoside. The corresponding 1-hydroxy-2-aminomitosenes were prepared from the parent mitomycin analogues for structure–activity comparisons. All compounds were tested against L1210 murine leukemia in the MTT tetrazolium dye assay. In general, the conjugates were less potent than the parent mitomycins; however 5'-*ara*-CMP conjugate **14** derived from mitomycin C was more potent than the parent compound or any mitomycin tested except mitomycin A. It also was more potent than *ara*-C. This result establishes the value of this approach to prodrugs, at least in cell culture. Against a multidrug-resistant L1210 cell line, all of the conjugates derived from mitomycin C were more potent than the parent compound. 6-Mercaptopurine ribonucleoside conjugate **15** was more active against the resistant cells than it was against the parental cell line.

In a previous article on nucleotide conjugates of 2,7-diaminomitosene, we reported that treatment of mitomycin C (1) with 5'-UMP under acidic conditions gave the cis isomer of a product (2) in which C-1 of the mitosene was substituted by the phosphate group of the uridylate[1] (Scheme I). Catalytic reduction of 2 in $H_2{}^{18}O$ resulted in release of the nucleotide and formation of 2,7-diamino-1-hydroxymitosene (3) with 60% of the ^{18}O incorporated at C-1. This process was thought to occur by way of intermediate 5. Reduction of the conjugate by sodium dithionite in the presence of 2'-deoxyguanosine gave bifunctional alkylation involving C-1 and C-10 of the resulting decarbamoylmitosene and the 2-amino groups of two 2'-deoxyguanosines. This is the same product obtained from mitomycin C under the same conditions.[1] Catalytic reduction of 2 in the presence of calf thymus DNA resulted in covalent binding to an extent slightly less than that obtained with mitomycin C. A product of monoalkylation at C-1 (4) is expected under these conditions.[2]

5'-UMP derivative 2 was readily taken up by L1210 leukemia cells in culture and it was highly cytotoxic to these cells: about $^1/_5$ as potent as mitomycin C on 1-h exposure, but equally potent on 8–9-h exposure. It also prolonged the life span of mice bearing P388 lymphocytic

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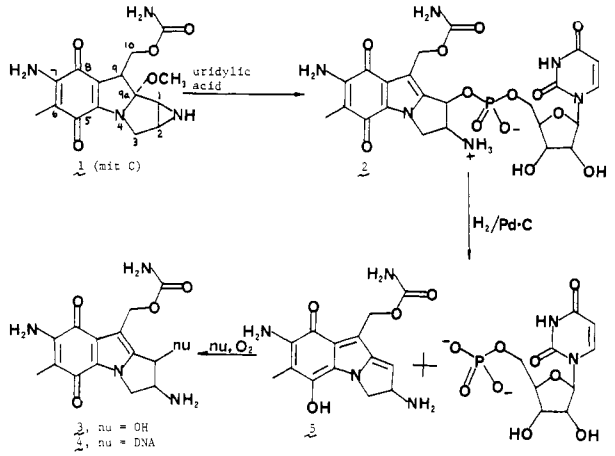
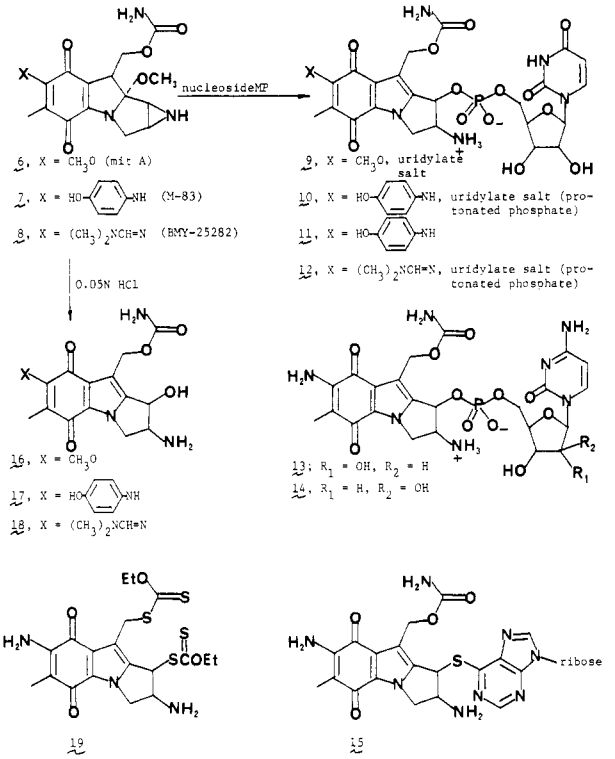
0022-2623/91/1834-1947$02.50/0

Scheme I



Scheme II



leukemia to about the same extent as mitomycin C (40% increased lifespan). The corresponding 5-fluoro-2′-deoxy-5′-UMP derivative also was active against L1210 leukemia cells in culture, but it was slightly less potent than **2**.[1]

The previous results described above encouraged us to extend this study to additional nucleotide conjugates of mitosenes. One set of these conjugates was based on the 5′-UMP derivatives of highly active mitomycin C analogues including mitomycin A (**6**), N^7-(4-hydroxyphenyl)mitomycin C (M-83, **7**),[3] and the dimethylamidino analogue of mitomycin C (BMY-25282, **8**).[4] A second set of conjugates was to include highly active nucleotides such as 5′-*ara*-CMP and 6-mercaptopurine 5′-monophosphate linked to 2,7-diaminomitosene. Thus, both the mitosene and the nucleotide moieties would be varied.

Chemistry

The 5′-UMP derivative **9** was prepared by treating mitomycin A (**6**) with uridine 5′-monophosphate in dry *p*-dioxane (Scheme II). It was obtained as the uridylic acid salt after a workup that included chromatography on a C-18 column with acetonitrile–water as solvent. Similar treatment of M-83 (**7**), but with a workup based on sequential washing of the crude product with acetone and methanol, gave 5′-UMP derivative **10** as the uridylic acid salt. The corresponding neutral form **11** was obtained when the workup was changed to chromatography on silica gel with 5% 1 N NH_4OH in 2-propanol as solvent. Treatment of BMY-25282 (**8**) with 5′-UMP, followed by the workup procedure involving washing with acetone and methanol gave 5′-UMP conjugate **12** as the uridylic acid salt. The yields of all of these products were low (17–32%) and they were highly solvated.

Heating mitomycin C (**1**) with 5′-*ara*-CMP in *N*,*N*-dimethylformamide at steam bath temperature gave the 2,7-diaminomitosene derivative **14** in low yield. The corresponding 5′-CMP derivative **13** was prepared in the same way. It served as an analogue of **14** in which the liberated nucleotide would not be cytotoxic, thus affording a measure of the contribution of the 5′-*ara*-CMP moiety of **14** to the cytotoxicity. We were unable to prepare a conjugate from mitomycin C and 6-mercaptopurine ribonucleoside 5′-monophosphate (6-MPRP) by this procedure. Very small amounts of a number of products were found. This result was disappointing because we wanted to know whether the phosphate group or the sulfur atom of 6-MPRP would react preferentially at C-1 of the mitomycin. It was possible to prepare a conjugate (**15**) from mitomycin C and 6-MP ribonucleoside. As discussed below, this structure had C-1 of 2,7-diaminomitosene substituted by the sulfur atom.

Reduction of the conjugate **9**–**12** is expected to result in liberation of 5′-UMP and formation of 1-hydroxymitosenes **16**–**18**, in addition to alkylation of DNA and perhaps other biological nucleophiles. It seemed important, therefore, to determine if compounds **16**–**18** would have antitumor activity of their own. Although **16** had been prepared previously,[5] **17** and **18** were unknown. Each of these compounds was readily prepared by treating mitomycin analogues **6**–**8** with 0.05 N hydrochloric acid. Yields were in the range of 18–43%.

Structures of the above compounds were verified by microanalysis and various spectroscopic measurements (Experimental Section). There was considerable overlap of peaks in the ^1H NMR spectra, but it was possible in every case to discern the hydrogens in the 7-substituent of the mitosene part of the structures. In the nucleotide moieties, the anomeric protons and protons on H-5 of uracil and cytosine were found. The ultraviolet spectra of mitosenes are very characteristic and they agreed with the proposed structures. In some cases, ^{13}C NMR and mass spectra provided additional confirmations of these structures. The structural assignment of most concern was that of compound **15**, in which it had to be determined whether the sulfur atom or one of the oxygen atoms of 6-MP ribonucleoside was attached to C-1 of 2,7-diaminomitosene. That the sulfur was attached was evident in the ^{13}C NMR spectrum, which showed C-1 at δ 43.74. This chemical shift is close to that of C-1 in ethyl xanthate derivative **19** (δ 49.1),[5] but far from the value of C-1 when substituted by oxygen, as found in **3** (δ 65.1).[6] There was

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Table I. Cytotoxicity of Mitomycin and 6-MP Analogues in Parental and Multidrug-Resistant L1210 Murine Leukemia Cells in Vitro[a]

compd	IC_{50}, μM parental (P) L1210	multidrug-resistant (R) L1210[b]	resistance ratio R/P
1 (mit C)	0.09	1.50	16.7
2	0.32	0.32	1.0
3	28.1	6.87	0.24
6	0.0003	0.0023	7.67
7	0.070	0.54	8.34
8	0.051	0.64	12.5
9	0.22	7.39	33.3
10	1.92	33.6	17.5
11	3.76	9.74	2.59
12	24.8	31.8	1.28
13	0.35	0.42	1.2
14	0.011	0.115	10.2
ara-C	0.036	0.036	1.0
ara-CMP	0.43	0.41	0.95
15	0.55	0.19	0.35
6-MP ribonucleoside	0.010	0.068	6.8
6-MP	0.020	0.024	1.18
16	0.60	7.46	12.5
17	2.42	27.9	11.5
18	0.746	20.8	27.9

[a] Six-day MTT assay, continuous exposure (method of Scudiero, D. A.; Shoemaker, R. H.; Paull, K. D.; Monks, A.; Tierney, S.; Nofziger, T. H.; Currens, M. I.; Seniff, D.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 4827. [b] Multidrug-resistant (MDR) L1210 cells were developed by continuous exposure to increasing concentrations of mitomycin C. These cells exhibit collateral resistance to other natural product anticancer agents such as doxorubicin and vincristine and they express high levels of the MW 180 000 membrane P-glycoprotein. (Dorr, R. T.; Liddil, J. D.; Trent, J. M.; Dalton, W. S. *Biochem. Pharmacol.* **1987**, *36*, 3115.)

only one isomer of **15** (TLC evidence) and it was 1,2-cis, based on the chemical shift at C-1. The corresponding trans isomer would be expected to have a more deshielded C-1 at about δ 54, based on values for the cis and trans isomers of 1-hydroxy-2,7-diaminomitosene.[6,7]

Biology

Table I gives the IC_{50} values for all of the mitomycins, their 2-amino-1-hydroxymitosene transformation products, and the nucleotide and nucleoside conjugates against parental (sensitive) and multidrug-resistant L1210 leukemia cells as determined by the microculture tetrazolium dye assay. The purine and pyrimidine compounds with known antitumor activity that are expected to be released inside L1210 cells on bioreductive activation of the conjugates are included for comparison. Among the mitomycins, mitomycin A (**6**) is much more potent against the parental L1210 cells than mitomycin C (**1**), M-83 (**7**), and BMY-25282 (**8**), whose potencies differ by less than a factor of 2. The 2-amino-1-hydroxymitosenes **3**, **16**, **17**, and **18** are considerably less active than the present mitomycins, with the relative decreases in potency for **3** and **16** being greater than those of the other two compounds.

Antitumor potencies of the nucleotide and nucleoside derivatives against the parental L1210 leukemia cells show some interesting and important differences. Among the 5′-UMP derivatives, the mitomycin C and mitomycin A derived conjugates **2** and **9** are more active than the M-83 and BMY-25282 derived conjugates **10**, **11**, and **12**, with **12** having surprisingly low potency. When the mitomycin C derived conjugates with variation in the nucleotide are compared, it is seen that *ara*-CMP conjugate **14** is significantly more potent than the others. It also is more potent than mitomycin C, *ara*-C, and *ara*-CMP. This result demonstrates that it is possible to achieve the enhancement in activity desired in a mitomycin–nucleotide conjugate prodrug. The 6-MP ribonucleoside conjugate **15** derived from mitomycin C does not show such an enhancement in potency, being much less active than mitomycin C, 6-MP, or 6-MP ribonucleoside.

All of the mitomycins were less potent against the multidrug-resistant L1210 cells than the parental cells. The decreases ranged from 8-fold for mitomycin A to 17-fold for mitomycin C. Similar decreases in potency were found for the 2-amino-1-hydroxymitosenes, with the notable exception of mitomycin C transformation product **3**, which was 4-fold more potent against the resistant cells. The mitomycin C conjugates maintained good potencies against the resistant cells. 5′-CMP conjugate **13** and 5′-UMP conjugate **2** showed little or no decrease in potency, whereas 6-MP ribonucleoside conjugate **15** showed about a 3-fold increase in potency. A 10-fold decrease in potency was observed for 5′-*ara*-CMP conjugate **14**, but it still was more active than any compound except mitomycin A against the multidrug-resistant cells. The 5′-UMP conjugates **9**, **10**, and **11** derived from mitomycin A and M-83 lost potency against the resistant cells. The loss for **10** was much greater than for **11**. The reason for this substantial difference is not apparent. A significant change in potency did not occur for the conjugate **12** derived from BMY-25282, although **12** was not very active against the parental cells. Among the purine and pyrimidine standards, there was no loss of potency against the resistant cells for *ara*-C, *ara*-CMP, or 6-MP. Howeverr, 6-MP ribonucleoside showed a loss of potency.

Decreased potencies of some of the above compounds against the multidrug-resistant cells are presumed to be caused by their affinity for P-glycoprotein, which aids in their transport out of these cells. There does not appear to be a simple relationship between chemical structure and potency decrease. However, the mitomycin C derivatives should be more hydrophilic than those from the other mitomycins. This property suggests that compounds such as **3**, **13**, and **15** might not bind tightly to P-glycoprotein. It does not account for the decreased potencies of mitomycin C and **14**, which also should be hydrophilic.

Catalytic reduction of **15** in water was conducted in order to determine if it would liberate 6-MP ribonucleoside and form 2,7-diamino-1-hydroxymitosene (**3**) upon deconjugation. Trace amounts of both products were detected by TLC. The main two spots, however, differed from these compounds and from 2,7-diaminomitosene (which might be formed by capture of a proton by intermediate **5** in Scheme I).[8] Their small amounts precluded further analysis. In a similar manner, trace amounts of **3** and *ara*-CMP were detected after reduction of **14** in water. The main spot differed from these compounds and from 2,7-diaminomitosene. Considerable decomposition occurred.

Conclusions

The preparation of nucleotide conjugates was extended to a variety of nucleotides and mitomycin C analogues. The conjugate obtained from mitomycin C and 5′-*ara*-CMP was more active than either parent compound against a sensitive line of L1210 leukemia cells, demon-

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strating that this type of prodrug is successful, at least in cell culture. All of the mitomycin C derived conjugates were more potent than mitomycin C against a multidrug-resistant line of these cells, although they were less potent than mitomycin A. These results suggest that additional conjugates should be prepared and that the phenomenon of increased potency against resistant cells should be investigated further.

Experimental Section

Melting points were determined with a Mel-Temp apparatus and are uncorrected. IR spectra were recorded on a Beckman IR-33 spectrophotometer and UV–visible spectra were recorded on a Perkin-Elmer Lambda 3A spectrophotometer. NMR spectra were taken on Brucker WM250 and AM500 spectrometers or a JEOL FX90Q spectrometer, with Me_4Si as the internal reference standard. Mass spectra were measured on a Varian-MAT 311 spectrometer. Elemental analyses were performed by Desert Analytics, Inc., Tucson, AZ. The results obtained were within ±0.4% of the theoretical values unless indicated otherwise.

2-Amino-7-methoxymitosene 1-Uridylate Uridylic Acid Salt (9). A mixture of mitomycin A (50 mg, 0.143 mmol), uridine 5′-monophosphate (93 mg, 0.29 mmol), and 15 mL of freshly distilled *p*-dioxane was stirred for 3 h at room temperature. The resulting orange precipitate was collected, washed with ethyl acetate (2 × 10 mL), dissolved in 1 mL of water, and purified by chromatography on C-18 silica with acetonitrile–water (20:80 v/v) as solvent. Evaporation of the orange fraction under reduced pressure gave 35 mg (25% yield) of **9** as orange crystals with mp 171–180 °C dec: ^{1}H NMR (D_2O) δ 3.90 (s, 3, 7″-OCH_3), 5.20 (d, 2, 10″-CH_2), 5.60 (br s, 1,1″-H), 5.80 (m, 2, 1′-Hs), 5.96 (d, 2, 5-H, J = 7.9 Hz), 7.97 (d, 2, 6-H, J = 7.9 Hz); IR (KBr) 3100–3500, 1610–1710, 1260–1270, 1050–1100 cm^{-1}; UV (H_2O) λ_{max} 235.3, 338–358, 438–440 nm. Anal. ($C_{24}H_{28}N_5O_{14}P{\cdot}C_9H_{13}N_2O_9P{\cdot}0.75H_2O$) C, N; H: calcd, 4.38; found, 4.96.

2-Amino-7-(4-hydroxyanilino)mitosene 1-Uridylate Uridylic Acid Salt (10). A mixture of **7** (50 mg, 0.117 mmol),[3] uridine 5′-monophosphate (76 mg, 0.234 mmol), and 15 mL of freshly distilled *p*-dioxane was stirred 3 h at room temperature and then evaporated under reduced pressure. The blue residue was suspended in ethyl acetate (10 mL) and filtered. The resulting solids were washed successively with acetone (2 × 5 mL) and methanol (2 × 5 mL), suspended in 5 mL of methanol, filtered, and dried to give 25 mg (21% yield) of **10** as the uridylic acid salt, a dark blue hygroscopic solid with mp > 250 °C dec: ^{1}H NMR (Me_2SO_4-d_6 + D_2O) δ 5.15 (s, 2, 10″-CH_2), 5.6–6.0 (br s, 4, 1′-Hs and 5-Hs), 6.6–7.1 (br s, 4, phenyl), 7.7–8.1 (br s, 2, 6-Hs); ^{13}C NMR (Me_2SO-d_6) δ 11.76 (6″-CH_3), 52.94 (C-3″), 54.67 (C-2″), 59.65 (C-10″), 64.65 (C-5′), 65.61 (br s, C-1″), 69.95 (C-3′), 72.87 (C-2′), 82.95 (C-4′), 87.61 (C-1′), 101.8 (C-5), 109.5 (C-6″), 114.0 (C-9″), 114.8 (C-2 of phenyl), 124.66 (C-3 of phenyl), 127.8 (C-9a″), 131.8 (C-1 of phenyl), 140.48 (C-6), 143.8 (C-7″), 150.56 (C-4), 152.0 (C-5a″), 153.5 (C-4 of phenyl), 156.5 (C-10″a), 162.91 (C-2), 174.0 (C-8″), 179.0 (C-5″); IR (KBr) 3200–3480, 1700, 1600–1690, 1600, 1495, 1220, 1050–1110 cm^{-1}; UV (H_2O) λ_{max} 264, 296–300, 330–335, 550–590 nm; decoupled ^{31}P NMR [Me_2SO_4-d_6: external reference UMP] δ 68.08 (br s). Anal. ($C_{29}H_{31}N_6O_{14}P{\cdot}C_9H_{13}N_2O_9P{\cdot}6H_2O$) C, H, N.

2-Amino-7-(4-hydroxyanilino)mitosene 1-Uridylate (11). A mixture of **7** (17.5 mg, 0.04 mmol),[3] uridine 5′-monophosphate (15 mg, 0.046 mg), and 20 mL of *p*-dioxane was stirred 3 h at room temperature and then evaporated under reduced pressure. The blue residue was chromatographed on silica gel with 5% of 1 N NH_4OH in 2-propanol as solvent. Evaporation of the blue band under reduced pressure gave 5 mg (17% yield) of **11** as the neutral form, a blue solid with mp 170–180 °C dec: ^{1}H NMR (Me_2SO-d_6) δ 2.6 (s, 3, 6a″-CH_3), 5.05 (s, 2, 10″-CH_2), 5.65 (d, 1, 5-H, J = 7.9 Hz), 5.85 (d, 1, 1′-H, J = 4.4 Hz), 6.5 (br s, 7″-NH + 10a″-$CONH_2$), 6.8 (2 d, 4, aromatic protons, J = 7.0 Hz), 8.15 (d, 1, 6-H, J = 7.9 Hz), 11.35 (s, 1, phenolic OH); IR (KBr) 3200–3400, 1700, 1670, 1050–1100, 1020 cm^{-1}; MS (negative FAB in glycerol) M − H = 717.

This compound gave an identical R_f on TLC (silica gel, 1 N NH_4OH–2-propanol, 2:8 v/v) with that of a neutralized sample of **10**.

2-Amino-7-(dimethylamidino)mitosene 1-Uridylate Uridylic Acid Salt (12). A mixture of **8** (30 mg, 0.077 mmol),[4] uridine 5′-monophosphate (50 mg, 0.15 mmol), and 10 mL of freshly distilled *p*-dioxane was stirred 16 h at room temperature and then evaporated under reduced pressure. The brown solid residue was suspended in 10 mL of ethyl acetate and filtered. The solids were washed with acetone (2 × 5 mL), dried, triturated with 5 mL of methanol, filtered, and redried to give 25 mg (32% yield) of **12** as the uridylic acid salt. Crystallization from acetone–water gave solid with mp 170–180 °C dec: ^{1}H NMR (D_2O) δ 1.88 (s, 3, 6″-CH_3), 3.10 (s, 6, $N(CH_3)_2$), 5.15 (s, 2, 10″-CH_2), 5.92 (d, 2, 5-Hs, J = 7.9 Hz), 5.96 (br s, 2 1′-Hs), 7.91 (s, 1, NCH=N), 7.95 (d, 2, 6-Hs, J = 7.9 Hz); IR (KBr) 3100–3500, 1700, 1690, 1680, 1600, 1080, 1040 cm^{-1}; UV (H_2O) λ_{max} 259–260, 302, 350–354, 440–450 nm. Anal. ($C_{26}H_{32}N_7O_{13}P{\cdot}C_9H_{13}N_2O_9P{\cdot}8.25H_2O$) H; C: calcd, 36.42; found, 35.96; N, calcd, 10.92; found, 11.41.

2,7-Diaminomitosene 1-Cytidylate (13). A mixture of mitomycin C (50 mg, 0.15 mmol), cytidine 5′-monophosphate (100 mg, 0.31 mmol), and dry *N*,*N*-dimethylformamide (8 mL) was heated on a steam bath for 30 min. The resulting purple solution showed no remaining starting material by TLC (silica gel; CH_3OH–$CHCl_3$, 2:8 v/v). It was cooled and evaporated and the solid residue was extracted with ethyl acetate and filtered. Washing the solids successively with ethyl acetate (2 × 5 mL), acetone (2 × 5 mL), and methanol (2 × 5 mL), followed by drying in air, gave 25 mg (27% yield) of **13** as purple solid with mp 210–215 °C dec: ^{1}H NMR (Me_2SO-d_6 + D_2O) δ 1.75 (s, 3, 6″-CH_3), 5.06 (s, 2, 10″-CH_2), 5.80 (d, 1, 5-H, J = 7.9 Hz), 6.00 (d, 1, 1′-H), 7.88 (d, 1, 6-H, J = 7.9 Hz); IR (KBr) 3300–3500, 1700, 1670, 1600, 1090, 1040 cm^{-1}; UV (MeOH) λ_{max} 228, 247.5, 307.5, 351.2, 513–524 nm. The analytical sample was recrystallized from CH_3OH–toluene. Anal. ($C_{23}H_{28}N_7O_{12}P{\cdot}0.85C_7H_8$) C, H, N.

On a small-scale experiment, it was found that the addition of perchloric acid to this reaction produced additional decomposition.

2,7-Diaminomitosene 1-(β-D-Arabinofuranosylcytosine 5′-Monophosphate) (14). A mixture of mitomycin C (25 mg, 0.075 mmol), cytosine β-D-arabinofuranoside 5′-monophosphate (50 mg, 0.15 mmol, Sigma), and 5 mL of dry *N*,*N*-dimethylformamide was heated 1 h on a steam bath, cooled, and filtered. The filtrate was evaporated to dryness under reduced pressure and the purple residue was purified by chromatography on silica gel with 5% 1 N NH_4OH in 2-propanol as solvent. Evaporation of eluate from the slow-moving purple band gave 10 mg (21% yield) of **14** as a purple solid with mp 130–136 °C dec: ^{1}H NMR (Me_2SO-d_6) δ 1.75 (s, 3, 6″-CH_3), 5.05 (s, 2, 10″-CH_2), 5.85 (d, 1, 5-H, J = 7.9 Hz), 5.9 (br s, 1, 1′-H), 6.5 (br s, 4, 7″-NH_2 + $CONH_2$), 7.5 (br s, 2, 4-NH_2), 7.85 (d, 1, 6-H, J = 7.9 Hz); IR (KBr) 3300–3500, 1700, 1670, 1600, 1090, 1040 cm^{-1}; UV (MeOH) λ_{max} 225, 247.5, 307.5, 351–352, 508–525 mm; MS (positive FAB, 0.2 M TSA/glycerol) MH+ = 626. Anal. ($C_{23}H_{28}N_7O_{12}P{\cdot}4.5H_2O$) C, H, N.

2,7-Diaminomitosene 1-(9-β-D-Ribofuranosyl-6-mercaptopurine) (15). A mixture of mitomycin C (80 mg, 0.24 mmol), 6-mercaptopurine ribonucleoside (70 mg, 0.246 mmol, Aldrich), and 10 mL of water was warmed to 40 °C to give a clear solution, treated with 20 mg of 10% palladium-on-carbon catalyst, and hydrogenated for 30 min, while the mixture was kept warm and well stirred. The resulting purple mixture was evacuated, flushed with nitrogen, and stirred in air for 15 min. After filtration and concentration under reduced pressure, the residue was extracted with 20 mL of ethyl acetate and filtered. The solids were washed successively with acetone (2 × 5 mL) and methanol (2 × 5 mL) and dried to give 60 mg (43% yield) of **15** as a purple solid that showed a single spot on TLC (silica gel, 2-propanol–1 N aqueous NH_4OH) and had mp 240–250 °C: ^{1}H NMR (Me_2SO-d_6) δ 1.75 (s, 3, 6″-CH_3), 5.04 (s, 2, 10″-CH_2), 5.9 (d, 1, 1′-H, J = 4.4 Hz), 6.5 (m, 4, 7″-NH_2 + $CONH_2$), 8.22 (s, 1, 2-H or 8-H), 8.63 (s, 1, 2-H or 8-H); ^{13}C NMR (Me_2SO-d_6) δ 8.28 (6″-CH_3), 43.74 (C-1″), 46.0 (C-3″), 51.65 (C-2″), 56.96 (C-10″), 62.02 (C-5″), 71.02 (C-3′), 74.92 (C-2′), 86.62 (C-4′), 89.22 (C-1′), 105.80 (C-6″), 131.69 (C-5), 144.26 (C-2 or C-8), 145.99 (C-2 or C-8), 152.39 (C-4), 160.95 (C-6), 175 (C-5″), 177.5 (C-8″); IR (KBr) 3200–3480, 1709, 1600, 1201, 1078, 1060 cm^{-1}; UV (MeOH) λ_{max} 214, 253, 284, 291 (sh), 310, 350–355, 520–530 nm; MS (positive FAB, 0.2 M TSA/glycerol), MH^+ = 587. The analytical sample was recrystallized from MeOH.

Anal. ($C_{24}H_{26}N_8O_8S\cdot3.5CH_3OH$) C, N, S; H: calcd, 5.77; found, 4.60.

2-Amino-1-hydroxy-7-(4-hydroxyanilino)mitosene (17). A mixture of 7 (30 mg, 0.07 mmol)[3] and 5 mL of 0.05 N HCl was stirred at room temperature for 2 h. The resulting purple solution was cooled in an ice bath and brought to pH 8.0 by adding 5% $NaHCO_3$ solution. The mixture was extracted with ethyl acetate (4 × 25 mL) and the combined extract was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by preparative TLC on silica gel (20 × 20 × 0.2 cm) with MeOH–$CHCl_3$ (2:8 v/v) as solvent. The major purple band was scraped off the plate, extracted with MeOH–CH_2Cl_2, filtered, and concentrated to give 10 mg (35% yield) of **17** as a purple solid with mp >250 °C dec; ^1H NMR (Me_2SO-d_6) δ 1.70 (s, 3, 6-CH_3), 3.6–4.2 (m, 3, H-3 + H-2), 4.5 (br s, 1, H-1), 5.2 (s, 2, 10-CH_2), 6.5 (s, 2, $CONH_2$), 6.7–6.9 (br s, 4, phenyl), 8.15 (s, 1 phenolic OH): IR (KBr) 3480, 3400–3420, 3320–3340, 3200, 1700, 1590, 1500 cm^{-1}; UV (MeOH) λ_{max} 254–260, 286–290, 325, 368, 530–550 nm. Anal. ($C_{20}H_{20}N_4O_6\cdot CH_3OH$) C, H, N.

2-Amino-7-(dimethylamidino)-1-hydroxymitosene (18). This compound was prepared by the same procedure as that described for **17**. From 30 mg of **8**[4] was obtained 9 mg (18% yield) of **18** as purple solid with mp >250 °C dec: ^1H NMR (D_2O) δ 1.75 (s, 3, 6-CH_3), 3.08 (s, 6, $N(CH_3)_2$), 3.6–4.2 (m, 3, H-3 + H-2), 4.5 (br s, 1, H-1), 5.06 (s, 2, 10-CH_2), 7.45 (s, 1, NCH=N); ^{13}C NMR (Me_2SO-d_6) δ 11.9 (6-CH_3), 31.48 (NCH_3), 35.82 (NCH_3), 63.01 (C-1), 151.5 (NCH=N); MS (EI, high resolution) calcd for $C_{17}H_{21}N_5O_5$ m/e 375.1543, found m/e 375.1545.

Reduction of 15 by Sodium Dithionite. A solution of 1 mg of **15** in 1 mL of water was deaerated by bubbling N_2 through it for 15 min. A solution of 2 mg of sodium dithionite in 1 mL of water was added and the resulting solution was stirred for 11 min. It turned pale yellow during this time. The nitrogen stream was replaced by oxygen for 5 min, which restored the purple color of the quinone. The reaction mixture was analyzed by TLC on silica gel with two systems, MeOH–chloroform (2:8 v/v) or 2-propanol–1 N NH_4OH (2:8 v/v). Faint spots corresponding to 2,7-diamino-1-hydroxymitosene (**3**) and 6-mercaptopurine ribonucleoside were observed (comparison with authentic samples in parallel spots). There were two more intense spots, neither of which corresponded to 2,7-diaminomitosene. They were not examined further.

Reduction of 14 by Sodium Dithionite. This experiment was carried out in the same way as described for **15**, but on a 5-mg scale. Analysis of the product by TLC using MeOH–chloroform as solvent showed traces of 2,7-diamino-1-hydroxymitosene (**3**) and *ara*-CMP, together with a faster moving unknown spot and a few faint spots near the baseline.

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Synthetic Approaches to the Guanosine and Xanthosine Analogues 5-Amino-3-β-D-ribofuranosylpyrazolo[3,4-*e*][1,3]oxazin-7-one and 3-β-D-Ribofuranosylpyrazolo[3,4-*e*][1,3]oxazine-5,7-dione and Studies of Their Antitumor Potential

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5-Amino-3-β-D-ribofuranosylpyrazolo[3,4-*e*][1,3]oxazin-7-one has been synthesized via cyclization of the appropriately protected pyrazofurin derivatives and subsequent transformations of the heterocyclic moiety. This guanosine analogue was marginally cytotoxic to L1210 cells in vitro. The xanthosine analogue 3-β-D-ribofuranosylpyrazolo[3,4-*e*]-[1,3]oxazine-5,7-dione was also synthesized, and was found to be highly cytotoxic. It appeared to act as a prodrug of pyrazofurin.

Introduction

Certain purines, purine nucleosides, and purine-like nucleoside antibiotics [e.g., tubercidin (**1**), sangivamycin (**2**), toyocamycin (**3**), formycin (**4**), formycin B (**5**), etc.] have shown significant chemotherapeutic and biological activities.[1-10] Considerable research effort has been devoted to the improvement of the chemotherapeutic and biological activity of these nucleosides by selective chemical

(1) Symposium on Bioorganic Chemistry and Drug Design, Academy of Sciences Latvian SSR, May and June, 1982, Riga, Latvia.

(2) Conference on *Structure–Activity Relationships of Anti-tumor Agents*, Presented lecture on topic entitled, "Antimetabolites" Leewenhorst Conference Centre, 11–13 March 1982, The Netherlands.

(3) New York Academy of Sciences conference on *The Chemistry, Biology and Clinical Uses of Nucleoside Analogs*, 4–6 September 1974, New York, NY.

(4) *Symposium of the Chemistry, Biochemistry and Clinical Aspects of Nucleosides*; Fourteenth National American Chemical Society Medicinal Chemistry Symposium, Durham, NH, June 1974, American Chemical Society: Washington, DC, 1974.

(5) *First International Round Table on Nucleosides and Their Biological Activities*; 28–30 October 1974, Montpellier, France. *Second International Round Table on the Chemistry and Biology of Nucleosides and Nucleotides*; 172nd National Meeting of the American Chemical Society, Carbohydrate Division, 29 August–3 September 1976, San Francisco, CA. *Third International Round Table on the Chemistry and Biology of Nucleosides and Nucleotides*; Montpellier, France, 4–6 October 1978 (published by INSERM as a monograph in 1979). *Fourth International Round Table on Nucleosides and Their Biological Activities*; Antwerp, Belgium, 3-8 February 1981.

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