Photoreversion. The adduct of interest was dissolved in 50% methanol/water at a concentration of 10 μg/mL. This solution was irradiated at 254 nm with a low-intensity mercury hand lamp for 1–5 min. The solution was then analyzed by HPLC on the Ultrasphere ODS column. Identification of products was achieved by coinjection with authentic standards.

Registry No. 8b, 86290-69-9; 8c, 86290-65-5; 8d, 14256-46-3; 9a, 2009-24-7; 9b, 88589-76-8; 9c, 88589-77-9; 9d, 88589-78-0; 10a, 298-81-7; 10b, 5263-08-1; 10c, 5307-52-8; 10d, 5307-51-7; 11a, 82460-28-4; 11b, 88589-79-1; 11c, 88589-80-4; 11d, 88589-81-5; poly(dA-dT)·poly(dA-dT), 26966-61-0.

(±)-3-(4-Amino-1H-pyrrolo[2,3-d]pyrimidin-1-yl)-5-(hydroxymethyl)-(1α,2α,3β,5β)-1,2-cyclopentanediol, the Carbocyclic Analogue of Tubercidin

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(±)-3-(4-Amino-1H-pyrrolo[2,3-d]pyrimidin-1-yl)-5-(hydroxymethyl)-(1α,2α,3β,5β)-1,2-cyclopentanediol (9), the carbocyclic analogue of tubercidin, prepared from (±)-3-amino-5-(hydroxymethyl)-(1α,2α,3β,5β)-1,2-cyclopentanediol (6), is cytotoxic to cells containing adenosine kinase but not to cells that do not, indicating that its activity depends on phosphorylation. Although inactive against P388 leukemia in mice and against herpes and influenza viruses in vitro, it showed marginal activity against respiratory syncytial, vesicular stomatitis, and rhino viruses in vitro.

Carbocyclic analogues of nucleosides have proven to possess interesting biological activities.[1] Carbocyclic adenosine (CAdo, 1)[2] is cytotoxic to both H.Ep.-2 and

1

2: X = O
3: X = CH₂

4
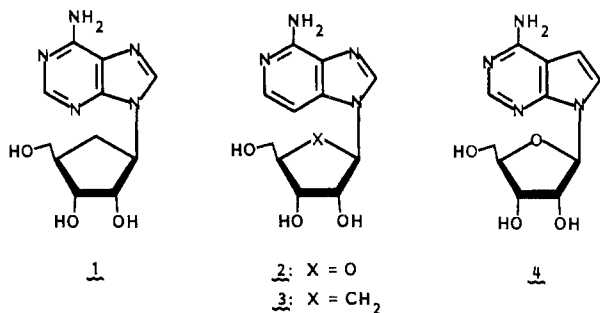
L-1210 cells in culture, although it is not effective against L-1210 leukemia in vivo at the maximum tolerated dose, indicating a lack of selectivity for neoplastic cells. It was inactive against herpes simplex type 1, vaccinia, rhino type 1A, and influenza (Ao/PR-8/34) viruses. CAdo is deaminated by adenosine deaminase and phosphorylated by adenosine kinase.[3] Even so it is cytotoxic to cells deficient in adenosine kinase, indicating that the nucleoside analogue itself can kill cells.[3] CAdo is an extremely potent inhibitor of S-adenosyl-L-homocysteinase,[4] the inhibition of which causes a perturbation in biological methylations by effecting a buildup of S-adenosyl-L-homocysteine, which is a potent feedback inhibitor of methyltransferases utilizing S-adenosylmethionine.[5] These inhibitions may explain the toxicity of CAdo itself to cells that cannot phosphorylate it. 3-Deazaadenosine (3-deaza-Ado, 2),[6] which is not detectably deaminated[7] and only very poorly

Scheme I
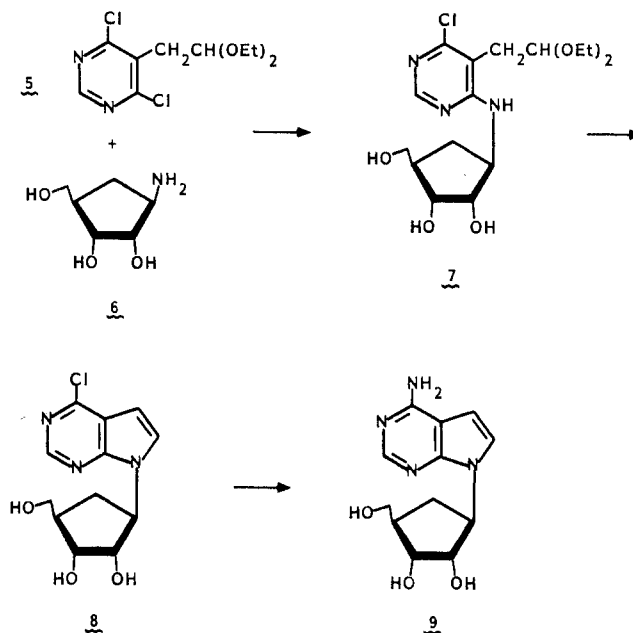
phosphorylated,[8] is also an excellent competitive inhibitor of S-adenosyl-L-homocysteinase (although less potent than CAdo)[4] and exhibits some antiviral activity.[9] Carbocyclic 3-deazaadenosine (3-deaza-CAdo, 3), which is neither phosphorylated nor deaminated, is an equally good inhibitor of S-adenosyl-L-homocysteinase[10] and exhibits potent activity against vaccinia and a number of RNA viruses in vitro and activity against VSV and vaccinia in vivo.[9] The activity of 3-deaza-CAdo (3) and of tubercidin (7-deazaadenosine, 4)[11] suggested the synthesis and evaluation of carbocyclic tubercidin [7-deaza-CAdo, (±)-3-(4-amino-1H-pyrrolo[2,3-d]pyrimidin-1-yl)-5-(hydroxy-

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Table I. Cytotoxicity of Carbocyclic 7-Deazaadenosine and Some Related Compounds

compd	H.Ep.-2	H.Ep.-2 (AK$^-$)	L-1210	L-1210 (AK$^-$)[b]
3-deazaadenine	7			
3-deazaadenosine (2)	20			
carbocyclic 3-deaza- adenosine (3)	0.6		4	1
7-deazaadenine	>70			
7-deazaadenosine (4)	0.002	>3		
carbocyclic 7-deaza- adenosine (9)	1.4		4	>40
carbocyclic adenosine (1)	0.7	20		

Column header: IC$_{50}$,[a] μM

[a] The concentration required to inhibit the growth of treated cells to 50% of untreated controls. [b] Cells selected for resistance to 6-(methylthio)purine ribonucleoside.

methyl)-(1α,2α,3β,5β)-1,2-cyclopentanediol, **9**] as another potential chemotherapeutic agent. Tubercidin (**4**), which is phosphorylated to the triphosphate but not deaminated,[11] has exhibited some anticancer[12] and antiviral activity.[11] Recently, several carbocyclic analogues of 6-methyltubercidin have been prepared, but these showed neither cytotoxicity nor antiviral activity (herpes simplex type 1).[13]

Preparation of 7-deaza-CAdo (**9**) was carried out by the sequence shown in Scheme I, which has been used for the preparation of other tubercidin analogues.[14] Treatment of (±)-3-amino-5-(hydroxymethyl)-(1α,2α,3β,5β)-1,2-cyclopentanediol (**6**) with a slight excess of 4,6-dichloro-5-(2,2-diethoxyethyl)pyrimidine (**5**) produced the pyrimidine **7**. Liberation of the aldehyde, accomplished by treating **7** with dilute HCl in dioxane, was followed by spontaneous cyclization to **8**. Displacement of the chlorine of **8** to produce 7-deaza-CAdo (**9**) was carried out with ethanolic ammonia at 140 °C.

Biological Evaluation. 7-Deaza-CAdo (**9**) inhibited *S*-adenosyl-L-homocysteinase from L-1210 cells with a K_i of 44 μM compared to 3-deaza-CAdo (**3**) with a K_i of 1.0 μM. In the same experiment, tubercidin (**4**) itself was not inhibitory. The cytotoxicity of 7-deaza-CAdo (**9**), given in Table I with that of some related compounds, is similar to the toxicity of 3-deaza-CAdo (**3**) and CAdo (**1**) itself to H.Ep.-2 and L-1210 cells. L-1210 cells deficient in adenosine kinase (L-1210 AK$^-$), however, appear to be highly resistant to **9**, indicating that its activity is dependent on phosphorylation, as is the case with tubercidin itself.[15] CAdo, on the other hand, as mentioned above, is still toxic to the AK$^-$ cells, although the IC$_{50}$ is significantly higher than for AK$^+$ cells. Carbocyclic 3-deazaadenosine (**3**), however, is at least as cytotoxic to AK$^-$ as it is to AK$^+$ cells, which indicates that phosphorylation does not play a role in its activity. Carbocyclic 7-deazaadenosine is not deaminated by adenosine deaminase, even when exposed to a large excess of enzyme overnight. The compound was toxic to mice at a 100 mg/kg dose on a qd 1–5 schedule and showed no activity at 50 mg/kg against P388 leukemia. Carbocyclic 7-deazaadenosine showed marginal activity against respiratory syncytial, vesicular stomatitis, and rhino

type 1A viruses in vitro. It was inactive against herpes type 1 (S-148) and influenza type A$_2$/Aichi/2/68 and type A$_0$/PR/8/34.[16] No antileishmanial activity was seen either in vitro or in vivo.[18]

Experimental Section

Melting points were determined on a Mel-Temp apparatus, and are uncorrected. NMR spectra were recorded with a Varian XL-100-15 spectrometer operating at 100.1 MHz for ^{1}H NMR and 25.16 MHz for ^{13}C NMR. Chemical shifts are expressed in parts per million downfield from internal tetramethylsilane. The ultraviolet absorption spectrum was determined with a Cary 17 spectrophotometer by dissolving the compound in ethanol and diluting tenfold with 0.1 N HCl, pH 7 buffer, and 0.1 N NaOH. The numbers in parentheses are extinction coefficients ($\epsilon \times 10^{-3}$). Microanalyses were performed by Atlantic Microlabs, Inc., Atlanta, GA, or in the Molecular Spectroscopy Section of Southern Research Institute. Thin-layer chromatography was carried out on Analtech precoated silica gel GF plates (0.25-mm thickness), and preparative layer chromatography was carried out on Brinkman precoated silica gel plates (2.0-mm thickness). Triethylamine was dried over Linde 4Å molecular sieves.

(±)-4-Chloro-5-(2,2-diethoxyethyl)-6-[[(1α,2β,3β,4α)-2,3-dihydroxy-4-(hydroxymethyl)cyclopentyl]amino]pyrimidine (7). To a solution of 4.41 g (16.6 mmol) of 4,6-dichloro-5-(2,2-diethoxyethyl)pyrimidine (**5**)[14] and 2.3 g (15.6 mmol) of **6**[2] in 114 mL of absolute ethanol was added 9 mL of triethylamine. After the solution had heated at reflux for 18 h, it was examined by TLC (9:1 CHCl$_3$–CH$_3$OH). If no **6** was present, processing was begun. If some **6** was still present, an additional 3 mL of triethylamine was added and the solution was again heated at reflux until all **6** had disappeared (<24 h). The solution was evaporated to dryness, the residue was dissolved in water, and the excess **5** was removed by extraction with petroleum ether. The product was then extracted into CHCl$_3$ (3 × 50 mL), and the organic extracts were dried (MgSO$_4$) and evaporated to dryness. The residual syrup, 5.8 g (98% crude yield), was used without further purification. For the purposes of obtaining a useful ^{1}H NMR spectrum, a small amount of crude **7** was purified by preparative TLC (9:1 CHCl$_3$–CH$_3$OH): ^{1}H NMR (CDCl$_3$) δ 1.20 and 1.22 (2 t, 6, 2 CH$_3$), 1.4 and 2.3 (2 m, 3, H-4', cyclopentane ring CH$_2$), 2.92 (d, 2, J = 5 Hz, pyrimidine CH$_2$), 3.3–4.4 (m, 12, 2 CH$_3$CH$_2$, 5'-CH$_2$, H-1', -2', -3', 3 OH's), 4.59 (t, 1, pyrimidine CH$_2$CH), 6.49 (d, 1, J = 5 Hz, NH), 8.24 (s, 1, H-2).

(±)-3-(4-Chloro-1*H*-pyrrolo[2,3-*d*]pyrimidin-1-yl)-5-(hydroxymethyl)-(1α,2α,3β,5β)-1,2-cyclopentanediol (8). To the syrup **7** (5.8 g, 15.4 mmol) in 90 mL of dioxane was added 23.2 mL of 1 N HCl, and the solution was stirred for 2 days at room temperature, at which time the reaction was judged complete by TLC (9:1 CHCl$_3$–CH$_3$OH). After cooling to 0–5 °C, the solution was neutralized with concentrated ammonium hydroxide and evaporated to dryness. Traces of water were removed from the residue azeotropically with ethanol, and then inorganic salts were removed by trituration with ethanol, followed by filtration. The filtrate was evaporated to dryness, and the residue (crude weight 75–100% of theory) was used in the amination step without further purification.

In one run, a small sample was purified by preparative TLC (CH$_3$OH), followed by recrystallization from ethanol: mp 135–136 °C; ^{1}H NMR (Me$_2$SO-d_6) δ 1.65 and 2.2 (2 m, 3, H-4', cyclopentane ring CH$_2$), 3.50 (m, 2, 2 H-5'), 3.86 (m, 1, H-3'), 4.16–4.50 (m, 2, H-2', OH), 4.75 (br s, 2, 2 OH), 5.06 (m, 1, H-1'), 6.69 (d, 1, $J_{5,6}$ = 3.5 Hz, H-5), 7.90 (d, 1, H-6), 8.62 (s, 1, H-2); after addition of D$_2$O, δ 3.87 (dd, 1, $J_{2',3'}$ = 5 Hz, $J_{3',4'}$ = 2 Hz, H-3'), 4.26 (dd, $J_{1',2'}$ = 9 Hz, H-2'); ^{13}C NMR (Me$_2$SO-d_6) δ 29.34 (cyclopentane ring CH$_2$), 44.94 (C-4'), 59.41 (C-1'), 62.72 (C-5'), 71.61, 75.16 (C-2', C-3'), 98.90 (C-5), 117.08 (C-4a), 129.08 (C-6), 149.89 (C-2), 150.60, 150.98 (C-4, C-7a). Anal. (C$_{12}$H$_{14}$ClN$_3$O$_3$·0.1H$_2$O) C, H, N.

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(16) Antiviral data were gathered by using standard cpe-inhibition assays in mammalian cell cultures as described in ref 17.

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(18) Antileishmanial screening was conducted through the Walter Reed Army Institute of Research.

(±)-3-(4-Amino-1*H*-pyrrolo[2,3-*d*]pyrimidin-1-yl)-5-(hydroxymethyl)-(1α,2α,3β,5β)-cyclopentanediol (9). Crude 8 from the above reaction was dissolved in 50 mL of ethanol saturated at 5 °C with anhydrous ammonia and heated in a glass-lined stainless-steel vessel at 140 °C for 3 days. After cooling, the reaction solution was evaporated to dryness, the residue was dissolved in water, and the pH was adjusted to 8 with ammonium hydroxide. Cooling to 0–5 °C caused the precipitation of a gray solid, which was collected and allowed to recrystallize from water after decolorization. The white solid was filtered, washed with cold water, and dried under reduced pressure at 100 °C: yield 1.14 g (overall yield from 7, 28%); mp 243–245 °C dec; UV λ_{max} (pH 1) 275 nm (10.0); UV λ_{max} (pH 7) 273 nm (10.0); UV λ_{max} (pH 13) 272 nm (10.0); ^{1}H NMR (Me$_2$SO-d_6) δ 1.58 and 2.09 (2 m, 3, H-4′ and cyclopentane ring CH$_2$), 3.49 (m, 2, 2 H-5′), 3.84 (m, 1, H-3′), 4.18 (m, 1, H-2′), 4.45–5.05 (m, 4, H-1′, 3 OH), 6.55 (d, 1, $J_{5,6}$ = 3.5 Hz, H-5), 6.89 (s, 2, NH$_2$), 7.24 (d, 1, H-6), 8.04 (s, 1,

H-2); after the addition of D$_2$O, δ 3.83 (dd, 1, $J_{2',3'}$ = 5 Hz, $J_{3',4'}$ = 3 Hz, H-3′), 4.17 (dd, 1, $J_{1',2'}$ = 8.5 Hz, H-2′); ^{13}C NMR (Me$_2$SO-d_6) δ 29.81 (cyclopentane ring CH$_2$), 45.00 (C-4′), 58.67 (C-1′), 62.72 (C-5′), 71.75, 75.18 (C-2′, C-3′), 98.58 (C-5), 102.58 (C-4a), 121.91 (C-6), 149.86 (C-7a), 151.06 (C-2), 157.14 (C-4). Anal. (C$_{12}$H$_{16}$N$_4$O$_3$) C, H, N.

Racemic and Optically Active 2,9-Dimethyl-5-(*m*-hydroxyphenyl)morphans and Pharmacological Comparison with the 9-Demethyl Homologues

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2,9α-Dimethyl-5-(*m*-hydroxyphenyl)morphan (3a) has been synthesized from 5-(*m*-methoxyphenyl)-2-methyl-9-oxomorphan (4) and resolved into its enantiomers (+)-3a and (−)-3a. The assigned α-orientation of the 9-methyl group was derived from studies of induced NMR shifts using Eu(fod)$_3$-d_{27}. Compound (+)-3a has inappreciable agonist (antinociceptive) activity in mice, and (−)-3a shows codeine-like potency in the hot-plate and writhing tests only. The 9-demethyl homologues, (+)-1 and (−)-1, are strong agonists, about as potent as morphine in these tests as well as in the tail-flick assay. The racemic compound 3a and (+)-3a, but not (−)-3a, exhibit low-potency, narcotic-antagonist activity in mice (tail-flick test, vs. morphine). All three, however, precipitate abstinence in nonwithdrawn, morphine-dependent rhesus monkeys. Monkey studies with the 9-demethyl homologues confirmed earlier results showing that (+)-1, suppressing abstinence in withdrawn animals, has high physical dependence capacity, while (−)-1 has none. Instead, (−)-1 precipitates abstinence in nonwithdrawn animals. Studies in rats and isolated organs (guinea pig ileum and mouse vas deferens) and receptor-binding assays confirm the quite different opioid-action profiles of (+)-1 and (−)-1, which thus might interact with different opioid receptors. Catalytic hydrogenation of the methiodide (7) of 5 gave, instead of the expected epimer of 3a, ring-opened compound 8.

5-(*m*-Hydroxyphenyl)-2-methylmorphan[1] (1, Chart I) and its enantinomers[2–4] [(+)-1 and (−)-1] have been shown to possess antinociceptive potency comparable to that of morphine in the mouse hot-plate test. The (+) isomer completely supports morphine dependence in rhesus monkeys, in contrast to the (−) isomer, which, instead, precipitates withdrawal symptoms. A few years ago, Zimmerman et al.[5] demonstrated that 4β-(*m*-hydroxyphenyl)-1,3β,4α-trimethylpiperidine (2) is a "pure" antagonist (apparently by virtue of the 3-methyl substituent), unique at that time, for *N*-methyl compounds of the 4-phenylpiperidine series. These observations prompted us to introduce a methyl group into 1 in a position corresponding to the 3-position of 2. Of the alternatives, i.e., 4- or 9-methyl derivative of 1, we chose the latter because of the availability of 5-(*m*-methoxyphenyl)-2-methyl-9-oxomorphan[1,2] (4, Scheme I) as a suitable intermediate. We now describe the synthesis of 2,9α-dimethyl-5-(*m*-hydroxyphenyl)morphan (3a; obtained under the employed
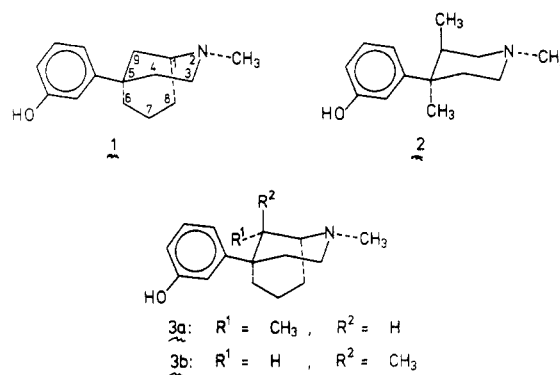
experimental conditions) and its resolution into its enantiomers, (+)-3a and (−)-3a, and present the results of pharmacological studies of these compounds and appropriate reference substances. Also included are additional pharmacological studies and receptor-binding data for (+)-1 and (−)-1 of known stereochemistry.[4]

Chart I

3a: R^1 = CH$_3$, R^2 = H
3b: R^1 = H , R^2 = CH$_3$

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