in the freezer: it was repurified on silica gel preparative plates and crystallized from EtOH-Et_2O: mp 112-114 °C; UV (H_2O) λ_{max} 253.5 nm (ϵ 7550), 321 (2340). Anal. ($C_{10}H_{14}N_2O_4S$) H, S, N; C: calcd, 46.50; found, 45.97.

Biological Assay Procedures. HeLa Bu cells were infected with HSV-1 or HSV-2 virus at a multiplicity of 5-10 plaque-forming units per cell. After 1-h virus adsorption, the drugs were added at 0-h postinfection. Virus titer of 24-h postinfected cultures was examined according to the previously described procedure.[12]

Thymidine kinase from various sources (see Table II) was purified by previously published procedures.[14-16] No contamination of thymidine phosphorylase and nucleoside monophosphate phosphotransferase was present in our preparations. The apparent K_i was determined by the method of Cheng and Prusoff.[17]

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Synthesis of 2'-Deoxy-L-fucopyranosylcarminomycinone and -ϵ-pyrromycinone As Well As 2'-Deoxy-D-*erythro*-pentopyranosyldaunomycinone, -carminomycinone, and -ϵ-pyrromycinone

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Treatment of di-*O*-acetyl-2-deoxy-L-fucopyranosyl bromide with carminomycinone and ϵ-pyrromycinone in the presence of mercuric bromide and mercuric cyanide afforded 3',4'-di-*O*-acetyl-2'-deoxy-L-fucopyranosylcarminomycinone and -ϵ-pyrromycinone. Similarly, when di-*O*-acetyl-2-deoxy-D-*erytrho*-pentopyranosyl chloride was treated with daunomycinone, carminomycinone and ϵ-pyrromycinone, the di-*O*-acetyl derivatives of the anthracyclinone glycosides were obtained. Deacetylation of the previous acetates with sodium methoxide afforded 2'-deoxy-L-fucopyranosylcarminomycinone and -ϵ-pyrromycinone, as well as 2'-deoxy-D-*erythro*-pentopyranosyldaunomycinone, -carminomycinone, and -ϵ-pyrromycinone. 2'-Deoxy-L-fucopyranosylcarminomycinone was found to be more active than carminomycin at higher dosages on L1210.

The anthracyclines form a group of antibiotics that comprises a number of antineoplastic agents used clinically, such as doxorubicin (adriamycin), daunorubicin, and carminomycin.[1] These antibiotics possess a substituted tetrahydronaphthacenedione ring system linked by a glycosidic linkage to the amino sugar daunosamine.[2,3]

Although the anthracyclines have shown remarkable antitumor activity with a T/C on P388 of 200 or more, their success clinically has been limited because of their cardiotoxicity,[3-7] which necessitates the stoppage of treatment before complete remission is established to prevent irreversible damage to the heart tissues. To suppress this untoward effect, modifications of the anthracycline molecule have been made in both the aglycon and the sugar moiety.[8-28]

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Scheme I

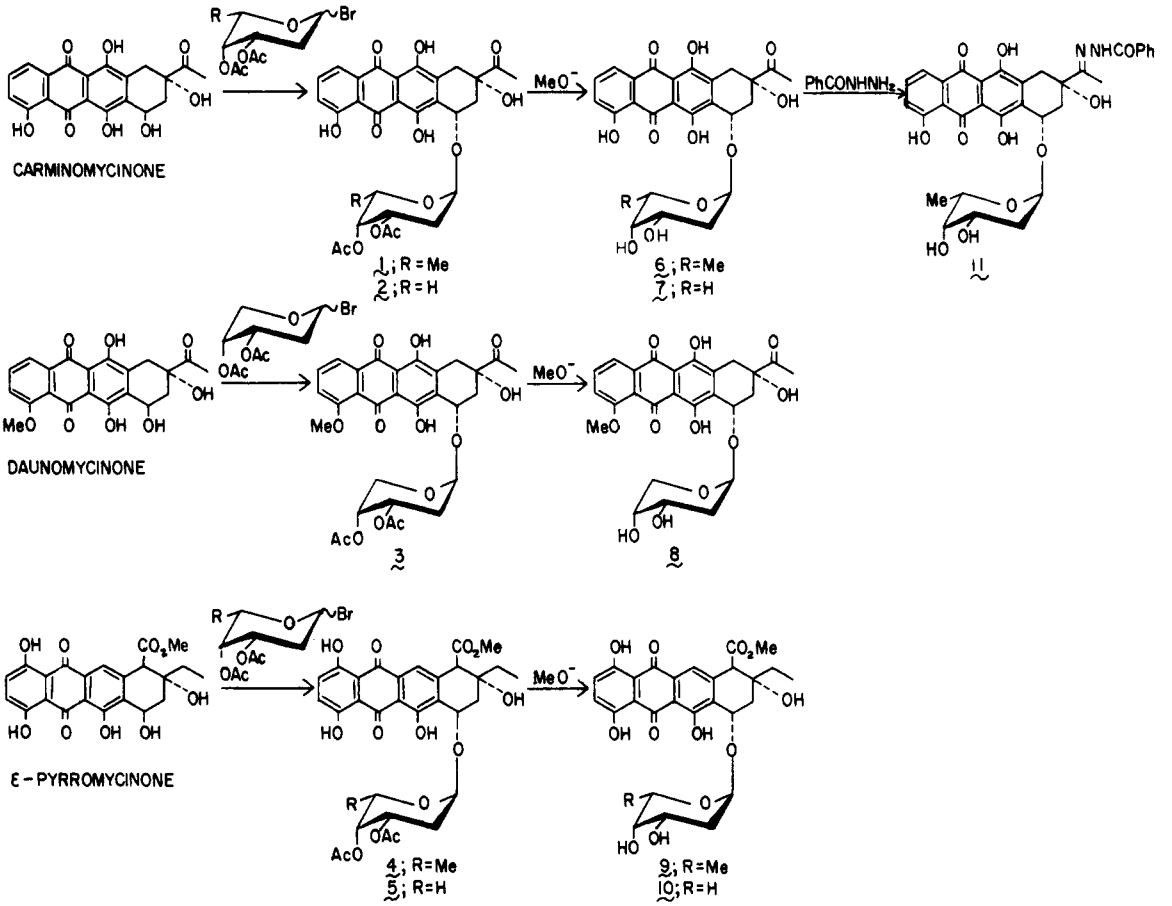

Table I. Antibacterial Screening[a]

no.	test organism	inhibition zone, mm dose, μg/mL: 50	25	12.5	6.25	3.1	1.6	0.8
6	BS-8[b]	21.5	21.5	21.3	19.5	17.2		
6	ILB[c]	2.3	1.3	1.2	1.1	0.8	0.7	0.8
8	BS-8[b]	18.8	15.8	12.5	10			
8	ILB[c]			0.1	3.3	3.3		
11	BS-8[b]	15	13.8	12.8	10.3			
11	ILB[c]	0.4	1.1	1.1	0.9	0.8		

[a] Compounds 1–5, 7, 9, and 10 showed no reaction in the above tests at 50 μg/mL. [b] When tested for antibiotic activity against *B. subtilis* ATTCC 6633 via a plate assay, carminomycin gave an inhibition zone of 20.2 mm at 12.5 μg/mL. [c] See K. E. Price, R. E. Buck, and J. Lein, *Appl. Microbiol.*, 12, 428 (1964). In this test, carminomycin gave an inhibition zone of 3.5 mm at 6.3 μg/mL.

The accepted mechanism of action for many anthracycline antibiotics assumes that the aromatic ring system is intercalated between the base pairs of DNA[29,30] and that the amino group of the sugar daunosamine forms a polar bond with the phosphate residue of the latter. By replacing the amino group with a hydroxyl group, this interaction with the phosphate residues could be weakened, leading to a less active product, but one which could have reduced toxicity and could thus show a better therapeutic index. Since mild activity was previously shown by the 2-deoxy-D-ribopyranosyl and 2-deoxy-L-fucopyranosyl derivatives of ε-rhodomycinone,[26] it was thought that linking these sugars to more powerful aglycons, such as carminomycinone, daunomycinone, and ε-pyrromycinone, could lead to active antineoplastic agents (Scheme I).

The protected glycosides were synthesized by reacting the desired anthracyclinone with either 3,4-di-*O*-acetyl-2,6-dideoxy-L-*lyxo*-hexopyranosyl bromide (di-*O*-acetyl-2-deoxy-L-fucosyl bromide)[31] or 2-deoxy-3,4-di-*O*-acetyl-D-*erythro*-pentopyranosyl chloride (di-*O*-acetyl-2-deoxy-D-ribopyranosyl chloride)[32] under Koenigs–Knorr condi-

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Table II. Antitumor Screening Results[a]

no.	treatment	tumor	dose, mg/kg	MST, days (% T/C)	av wt. change	survivors, day 5
6	once, day 1 (expt 1)	L1210	12.8	186	−0.4	6/6
			6.4	136	−1.1	6/6
			3.2	114	−0.9	6/6
			1.6	107	−0.8	6/6
			0.8	114	+1.2	6/6
			0.4	107	−0.8	6/6
6	once, day 1 (expt 2)	L1210	51.2	171	−1.9	5/6
			25.6	164	−1.2	6/6
			12.8	136	+0.3	6/6
			6.4	157	+0.7	6/6
			3.2	114	+0.2	6/6
			1.6	129	−0.6	6/6
6	QD 1-9	L1210	12.8	114	−1.6	5/6
			6.4	171	−1.7	6/6
			3.2	157	−0.3	5/6
			1.6	121	+0.6	6/6
			0.8	114	+2.3	6/6
			0.4	114	+0.6	6/6
8	once, day 1	L1210	12.8	107	+2.3	6/6
			6.4	93	+2.4	6/6
			3.2	100	+0.2	6/6
			1.6	86	+2.6	6/6
			0.8	100	+1.8	6/6
			0.4	100	+1.5	6/6
8	every 4th day	P388	50	110	+0.2	6/6
			25	110	−0.5	6/6
			12.5	112	−1.3	6/6
			6.25	100	−1.2	6/6
			3.13	100	−2.0	6/6
11	once, day 1	L1210	12.8	114	−0.3	6/6
			6.4	107	+1.6	6/6
			3.2	93	+1.9	6/6
			1.6	100	+2.0	6/6
			0.8	93	+1.5	6/6
			0.4	100	+2.3	6/6

[a] Tests done on L1210 were carried out at Bristol Laboratories according to the procedure of R. I. Geran, N. N. Greenberg, M. M. McDonald, A. M. Schumacher, and B. J. Abbott, Cancer Chemotherapy Report, part III, p. 9, 1972. Tests on P388 were carried out in the NCI according to the protocol described in Instruction 14, Screening Data Summary Interpretation and Outline of Current Screen, Drug Evaluation Branch, Drug Research and Development Program Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. Tumor inoculum: 10^6 ascites cells implanted ip into BDF_1 female mice. Evaluation: MST = median survival time in days; % T/C = MST treated/MST control × 100. Criteria: % T/C = 125 considered significant antitumor effect.

tions using mercuric bromide–mercuric cyanide catalysts.[33] Column chromatography was used to separate the unreacted sugars from the desired glycosides. The deacetylated glycosides were then prepared by saponification with sodium methoxide at room temperature. The yields of the blocked glycosides were above 80% and those of the deblocked ones nearly quantitative.

All compounds prepared gave correct elemental analysis, and their structures were confirmed by NMR spectroscopy. The latter showed the expected number of phenolic protons in the offset region of the spectra, i.e., two protons for the daunomycinone glycoside and three for the carminomycinone and ε-pyrromycinone glycosides, suggesting that the glycosidation took place either through the secondary or tertiary hydroxyl groups. The last possibility was excluded because this hydroxyl group is highly hindered and would not be expected to participiate in the reaction.

All the compounds prepared were screened at Bristol Laboratories, Syracuse, NY. They were first subjected to a preliminary screening for bacteriostatic activity on *Baccillus subtilis* at pH 8 (BS-8) and on ILB (see Table I). This was followed by in vivo testing on L1210 of those compounds found active in the first two screening systems, namely compounds **6**, **8**, and **11**. The rationale for not testing in vivo anthracyclines that did not show any bacteriostatic activity is that in Bristol Laboratories experience there has never been a case where an antitumor anthracycline did not show antibacterial activity. Compound **8** was also screened at the National Cancer Institute on the P388 tumor system. The activity observed for 2′-deoxy-L-fucopyranosylcarminomycinone (**6**) was consistently higher than that for the parent carminomycin, although at a higher dosage (see Table II). A similar result was obtained by Horton on 2-deoxy-L-fucopyranosyl adriamycinone.[24]

When 2′-deoxy-L-fucopyranosylcarminomycinone (**6**) was converted to the benzoyl hydrazone (**11**), i.e., the rubidazone analgoue, the antitumor activity was almost completely lost (see Table II).

Recently, several analogues of daunomycin were synthesized that were active antineoplastic agents but did not complex with DNA.[34] Instead they inhibited RNA synthesis. Since these compounds were more active than daunorubicin at higher doses, we thought that 2-deoxyfucopyranosylcarminomycinone (**6**) might act by the same mechanism. The lack of activity for the 2′-deoxy-D-ribopyranosyl derivative (**7**) is possibly due to the fact that it is not frozen into one conformation by the methyl

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Table III. Formulas and Physical Data of the Compounds Prepared

no.	mp, °C	anal.	formula
1	142-144	C, H	$C_{30}H_{30}O_{13}$
2	125-126	C, H	$C_{29}H_{28}O_{13}$
3	106-110	C, H	$C_{30}H_{30}O_{13}\cdot 0.5H_2O$
4	144-147	C, H	$C_{32}H_{32}O_{14}\cdot 0.5H_2O$
5	122-124	C, H	$C_{31}H_{32}O_{14}\cdot 0.5H_2O$
6	228-232	C, H	$C_{26}H_{25}O_{11}\cdot 0.5H_2O$
7	214-228	C, H	$C_{25}H_{24}O_{11}\cdot 0.5H_2O$
8	188-190	C, H	$C_{26}H_{26}O_{11}\cdot 0.5H_2O$
9	230-233	C, H	$C_{28}H_{30}O_{12}\cdot 0.5H_2O$
10	217-220	C, H	$C_{27}H_{28}O_{12}\cdot 0.5H_2O$

group at the 5′ position, so that the sugar can flip into a form that cannot bind with a receptor site. As for the rubidazone analogue, it might be too lipophilic to be active in vivo, due to solubility problems.

Experimental Section

Melting points were determined with a Kofler block and are uncorrected. Nuclear magnetic resonance spectra were run on Varian HA-100 or 360-A spectrometers. Microanalyses were run in the Department of Chemistry and Chemical Engineering microanalysis laboratory by Mrs. S. Brotherton on a Perkin-Elmer 240 elemental analyzer. Preparative chromatography columns were packed with Sargent-Welch SC14608 silica gel (60–200 mesh).

Synthesis of Blocked Glycosides. A suspension of the desired anthracyclinone (100 mg, 0.26 mmol), ground 3A molecular sieves (500 mg), mercuric bromide (110 mg, 128 mmol), and mercuric cyanide (10 mg, 0.40 mmol) in tetrahydrofuran (15 mL) was stirred at room temperature. The appropriate glycosyl halide (120 mg, 0.41 mmol) was added, and the mixture was refluxed for 0.5 h, after which another portion of the glycosyl halide (120 mg) was added, and the mixture was refluxed for another hour. The mixture was allowed to cool and was then filtered to remove the molecular sieves. The residue was washed well with chloroform, and the combined filtrates were evaporated to a syrup under reduced pressure. The syrup was dissolved in chloroform (500 mL) and washed with 2 M potassium iodide solution (4 × 50 mL). The chloroform solution was dried over sodium sulfate and evaporated to dryness under reduced pressure. The resulting product was dissolved in absolute ether (25 mL) and applied to a silica gel column (2 × 20 cm), which was washed with absolute ether (100 mL). The desired blocked glycoside was eluted with 2% methanol in chloroform crystallized from 95% ethanol. (See Table III).

Synthesis of Deblocked Glycosides. The blocked glycoside (50 mg) was dissolved in methanol (15 mL) containing an excess of freshly prepared sodium methoxide and stirred at room temperature for 20 min. The purple solution was then poured into a separately funnel containing a sodium hydrogen sulfate solution (0.5 M, 100 mL). The combined chloroform extracts were dried over sodium sulfate and evaporated to dryness under reduced pressure. The residue was dissolved in hot 95% ethanol (30 mL), filtered, reduced in volume to 10 mL, and allowed to evaporate slowly in an open beaker. The crystals were filtered and washed with ether. (See Table III.)

Synthesis of Rubidazone Analogue (11). 2′-Deoxy-L-fucopyranosylcarminomycinone (**6**; 10 mg, 19 μmol) and benzoyl hydrazine (2.7 mg, 20 μmol) were refluxed in absolute ethanol (5 mL) overnight. After the solvent was removed, the benzoylhydrazone (11) was crystallized from 95% ethanol, filtered, and washed with isopropyl ether, yield 8 mg (64%). Anal. ($C_{27}H_{22}N_2O_8$) C, H, N.

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2-(Aminomethyl)phenols, a New Class of Saluretic Agents. 2. Synthesis and Pharmacological Properties of the 5-Aza Isostere of 2-(Aminomethyl)-4-(1,1-dimethylethyl)-6-iodophenol

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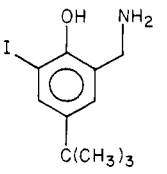
The synthesis and biological evaluation of 4-(aminomethyl)-6-(1,1-dimethylethyl)-2-iodo-3-pyridinol dihydrochloride (**7b**) are described. Compound **7b** proved to be highly active as a saluretic diuretic in both rats and dogs.

Recently, we reported[2] on a series of 2-(aminomethyl)-phenols which were shown to possess a high order of diuretic activity in rats and dogs. It was shown that the molecular features essential for activity are (1) a hydrogen, methyl, or methoxyl group in the 3 position; (2) a halo or C_3–C_4 α-branched alkyl substituent in the 4 position; (3) a hydrogen, lower alkyl, or lower alkoxy moiety in the 5 position; and (4) a chloro, bromo, or iodo group in the 6 position. The highest level of activity was achieved with 2-(aminomethyl)-4-(1,1-dimethylethyl)-6-iodophenol (**1**), which not only was an excellent diuretic but also displayed good antihypertensive activity in the spontaneously hypertensive rat.



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In this paper, we describe the synthesis and biological activity of 4-(aminomethyl)-6-(1,1-dimethylethyl)-2-iodo-3-pyridinol dihydrochloride (**7b**), the 5-aza isotere of **1**, as

(1) Deceased, May 31, 1977.
(2) For Part 1, see Stokker, G. E.; Deana, A. A.; deSolms, S. J.; Schultz, E. M.; Smith, R. L.; Cragoe, E. J., Jr.; Baer, J. E.; Ludden, C. T.; Russo, H. F.; Scriabine, A.; Sweet, C. S.; Watson, L. S. *J. Med. Chem.*, **1980**, *23*, 1414.

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