ent cannabinoids used in this study supplied by Dr. Monique Braude, NIDA, Rockville, Md. We also acknowledge a generous sample of (+)-Δ^8-THC supplied by Dr. Gert Paschelke, Schering AG, Berlin, Germany.

References and Notes

(1) (a) J. L. Neumeyer and R. A. Shagoury, *J. Pharm. Sci.*, **60**, 1433 (1971); (b) "Marihuana", R. Mechoulam, Ed., Academic Press, New York and London, 1973.
(2) R. D. Sofia, S. D. Nalepa, J. J. Harakal, and H. B. Vassar, *J. Pharmacol. Exp. Ther.*, **186**, 646 (1973).
(3) G. B. Chesher, C. J. Dahl, M. Everingham, and D. M. Jackson, *Br. J. Pharmacol.*, **49**, 588 (1973).
(4) (a) R. D. Sofia and H. Barry, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **31**, 506 (1972); (b) D. M. Buxbaum, *Psychopharmacologia*, **25**, 275 (1972); (c) S. Kaymakcalan and G. A. Deneau, *Pharmacologist*, **13**, 247 (1971).
(5) S. Kaymakcalan and G. A. Deneau, *Acta Med. Turc., Suppl.*, 1, 1–27 (1972).
(6) S. Kaymakcalan, R. K. Turker, and M. N. Turker, *Psychopharmacologia*, **35**, 123 (1974).
(7) W. L. Dewey, L. S. Harris, and J. S. Kennedy, *Arch. Int. Pharmacodyn. Ther.*, **196**, 133 (1972).
(8) L. S. Harris, *Pharmacol. Rev.*, **23**, 285 (1971).
(9) (a) H. D. Christensen, R. I. Fruedenthal, J. T. Gidley, R. Rosenfeld, G. Boegli, L. Testino, D. R. Brine, C. G. Pitt, and M. E. Wall, *Science*, **172**, 165 (1971); (b) Z. Ben-Zvi, R. Mechoulam, and S. Burstein, *J. Am. Chem. Soc.*, **92**, 3468 (1970).
(10) M. Perez-Reyes, M. C. Timmons, M. A. Lipton, H. D. Christensen, K. H. Davis, and M. E. Wall, *Experentia*, **29**, 1009 (1973).
(11) (a) R. S. Wilson and E. L. May, *J. Med. Chem.*, **17**, 475 (1974); (b) B. R. Martin, L. S. Harris, W. L. Dewey, E. L. May, and R. S. Wilson, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **33**, 540 (1974).
(12) (a) *rac*-4 has been reported: K. E. Fahrenholtz, M. Lurie, and R. W. Kierstead, *J. Am. Chem. Soc.*, **89**, 5934 (1967). (b) The 1-methyl ether of (−)-**4** has been reported: J. W. Wildes, N. H. Martin, C. G. Pitt, and M. E. Wall, *J. Org. Chem.*, **36**, 721 (1971).
(13) R. Pappo, D. S. Allen, R. U. Lemieux, and W. S. Johnson, *J. Org. Chem.*, **21**, 478 (1956).
(14) R. A. Archer, D. B. Boyd, P. V. Demarco, I. J. Tyminski, and N. L. Allinger, *J. Am. Chem. Soc.*, **92**, 2500 (1970).
(15) J. C. Cradock, J. P. Davignon, C. L. Sitterst, and A. M. Guarino, *J. Pharm. Pharmacol.*, **25**, 345 (1973).
(16) (a) N. B. Eddy and D. Leimbach, *J. Pharmacol. Exp. Ther.*, **107**, 385 (1953); (b) A. E. Jacobson and E. L. May, *J. Med. Chem.*, **8**, 563 (1965).
(17) T. D. Perrine, L. Atwell, I. B. Tice, A. E. Jacobson, and E. L. May, *J. Pharm. Sci.*, **61**, 86 (1972).
(18) L. S. Harris, *Adv. Biochem. Psychopharmacol.*, **8**, 13 (1974).
(19) G. Jones, R. G. Pertwee, E. W. Gill, W. D. M. Paton, I. M. Nilsson, M. Widman, and S. Agurell, *Biochem. Pharmacol.*, **23**, 439 (1974).
(20) H. Edery, Y. Grunefeld, A. Ben-Zvi, and R. Mechoulam, *Ann. N.Y. Acad. Sci.*, **191**, 40 (1971).
(21) B. R. Martin, W. L. Dewey, L. S. Harris, J. Beckner, R. S. Wilson, and E. L. May, *Pharmacol. Biochem. Behav.*, in press.
(22) E. W. Gill, G. Jones, and D. K. Lawrence, *Biochem. Pharmacol.*, **22**, 175 (1973).
(23) E. W. Gill and G. Jones, *Biochem. Pharmacol.*, **21**, 2237 (1972).
(24) D. S. Kosersky, W. L. Dewey, and L. S. Harris, *Eur. J. Pharmacol.*, **24**, 1 (1973).
(25) S. H. Hill, R. Schwin, D. W. Goodwin, and B. Powell, *J. Pharmacol. Exp. Ther.*, **188**, 415 (1974).

Synthesis and Antitumor Properties of New Glycosides of Daunomycinone and Adriamycinone

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The synthesis of 4′-*epi*-daunorubicin and of 4′-*epi*-adriamycin was performed by condensation of 2,3,6-trideoxy-3-trifluoroacetamido-4-*O*-trifluoroacetyl-α-L-*arabino*-hexopyranosyl chloride with daunomycinone or the protected adriamycinone derivative **17**, respectively. Both the α and β anomers were obtained and characterized. All new compounds are biologically active in cultured cells and the α anomers display noticeable activity in experimental tumors in mice. Interestingly, 4′-*epi*-adriamycin (**4**) appears nontoxic to cultured heart cells up to a concentration of 5 μg/ml.

Our continuing concern with the glycosidic antitumor agents belonging to the anthracycline family of antibiotics[1,2] and with the structure–activity relationships of new derivatives of daunorubicin (**1**) and of adriamycin (**2**) with modifications in the amino sugar moiety[3] has prompted us to the synthesis and the biological evaluation of stereoisomers of **1** and of **2** in which the amino sugar residue is configurationally different with respect to the parent antibiotics. Synthesis of analogs of **1** with the sugar moiety substituted by D-glucose and D-glucosamine has been reported.[4] We now report the synthesis and biological activity of 4′-*epi*-daunorubicin (**3**), the corresponding β anomer **6**, 4′-*epi*-adriamycin (**4**), and its β anomer **7**. In the new compounds the natural amino sugar, daunosamine (3-amino-2,3,6-trideoxy-L-*lyxo*-hexose), is replaced by the corresponding 4-epi analog (3-amino-2,3,6-trideoxy-L-*arabino*-hexose).

Synthesis. Compounds **3** and **6** were obtained by coupling daunomycinone with 2,3,6-trideoxy-3-trifluoroacetamido-4-*O*-trifluoroacetyl-α-L-*arabino*-hexopyranosyl chloride (**16**) in the conditions of the Koenigs–Knorr reaction to give, after removal of the *O*-trifluoroacetyl group with methanol, the mixture of the *N*-trifluoroacetyl derivatives **5** and **8**. Removal of the protective group by mild alkaline treatment gave, after chromatographic separation, the glycosides **3** and **6**, which were isolated as the crystalline hydrochlorides. In order to obtain glycosides **4** and **7**, adriamycinone was converted to the dioxolane derivative

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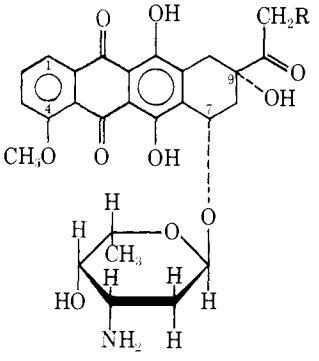
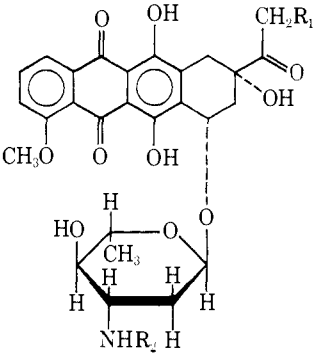
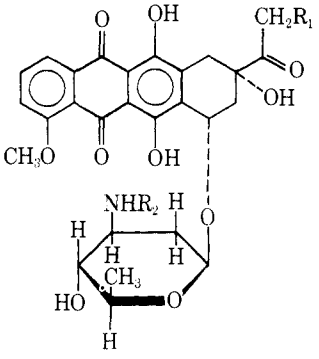
17 by reaction with dimethoxypropane in anhydrous acid conditions. Thus the primary hydroxy group of the side chain was blocked, which would otherwise interfere with the condensation step; moreover, the alkali-sensitive hydroxyacetyl side chain was protected against the basic condition during the hydrolysis of the *N*-trifluoroacetyl group. Compound **17** was coupled with **16** in the same conditions used for **3** and **6** to give, after removal of the *O*-trifluoroacetyl group, a mixture of **18** and **19**, which was separated by chromatography on a silica gel column. Subsequent de-



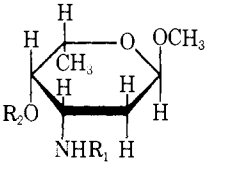
1, R = H
2, R = OH



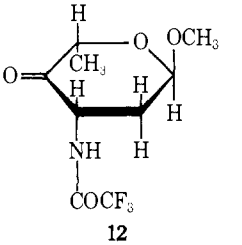
3, $R_1 = R_2 = H$
4, $R_1 = OH; R_2 = H$
5, $R_1 = H; R_2 = COCF_3$



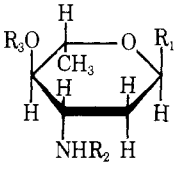
6, $R_1 = R_2 = H$
7, $R_1 = OH; R_2 = H$
8, $R_1 = H; R_2 = COCF_3$



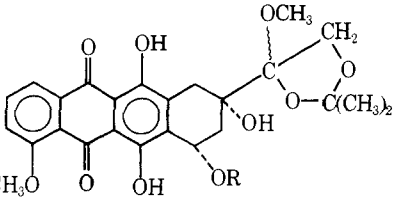
9, $R_1 = R_2 = H$
10, $R_1 = R_2 = COCF_3$
11, $R_1 = COCF_3; R_2 = H$



12



13, $R_1 = OCH_3; R_2 = COCF_3; R_3 = H$
14, $R_1 = OH; R_2 = COCF_3; R_3 = H$
15, $R_1 = OCOCF_3; R_2 = R_3 = COCF_3$
16, $R_1 = Cl; R_2 = R_3 = COCF_3$



17, R = H
18, R = 2,3,6-trideoxy-3-trifluoroacetamido-α-L-*arabino*-hexopyranosyl
19, R as in **18**, β-anomer

Table I. Colony-Forming Ability of Cultured HeLa Cells after Treatment with Compounds **1**, **2**, **3**, **4**, and **6**

		Time of exposure					
	Dose,	2 hr		8 hr		24 hr	
Compd	μg/ml	CN[a]	ID_{50}[b]	CN[a]	ID_{50}[b]	CN[a]	ID_{50}[b]
1	0.1	43		0		0	
	0.05	87	0.098	18	0.036	1	<0.025
	0.025	113		99		40	
2	0.5	40		14		0	
	0.25	55		35		6	
	0.1	65	0.33	62	0.19	18	<0.025
	0.05	78		72		36	
	0.025	102		83		42	
3	0.1	77		23		3	
	0.05	115	>0.1	56	0.056	23	<0.025
	0.025	113		88		48	
4	0.5	60		42		35	
	0.25	66	>0.5	54	0.35	46	0.3
	0.125	78		59		60	
6	1.0	98		17		6	
	0.5	101	>1.0	37	0.47	18	0.33
	0.25	108		86		70	

[a] Number of colonies expressed as percent of untreated controls. [b] 50% inhibiting dose (μg/ml).

blocking of the protective groups, first with alkali in order to remove the *N*-trifluoroacetyl group and then by an acid treatment in order to remove the dioxolane ring, gave glycosides **4** and **7**. The α and β anomers of 4′-epi derivatives are easily identified by ^1H NMR spectrometry. In fact, in the β anomers the signal of the axial anomeric proton is a pair of doublets ($J_{ax,eq} \sim 2$ Hz, $J_{ax,ax} \sim 9$ Hz), whereas in the α anomers it appears as a broad singlet ($W_H \sim 6$ Hz). Moreover, the C-1′ H signal in the β anomers shifts to higher field values (e.g., to δ 4.92 for **6** in DMSO-d_6) with respect to the corresponding α anomers, which display the C-1′ H signal at about δ 5.5. The reverse phenomenon is observed for the benzylic proton at C-7 whose signal is in the range δ 4.9–5.2 in the α series and in the range δ 5.3–5.5 (e.g., at δ 5.39 for **6**) in the β series. A similar behavior has been observed in the ^1H NMR spectra of the α and β anomers of the daunorubicin and adriamycin series.[5]

The protected chloro derivative **16** was prepared from methyl daunosaminide **9**,[6] which by treatment with $(CF_3CO)_2O$ gave the *N*,*O*-trifluoroacetyl derivative **10**. The oxidation with RuO_4 of methyl *N*-trifluoroacetyldaunosaminide (**11**), obtained by hydrolysis with MeOH of **10**, to the corresponding keto derivative **12**, followed by stereoselective reduction with $NaBH_4$, gave methyl 2,3,6-trideoxy-3-trifluoroacetamido-α-L-*arabino*-hexopyranoside (**13**), showing an axial orientation of the C-4 proton as indicated by the coupling constant values of C-4 H in the ^1H NMR spectrum. Acid hydrolysis of **13** gave **14**, which was treated with trifluoroacetic anhydride to give the 1,4-di-*O*-trifluoroacetyl-3-trifluoroacetamido derivative **15**. Finally, **16** was obtained by reaction of **15** with dry hydrogen chloride.

Biological Activity. The activity of the compounds on the colonies forming ability of HeLa cells is shown in Table I. It can be noted that the activity of 4′-*epi*-daunorubicin (**3**) and of 4′-*epi*-adriamycin (**4**) is lower than that of daunorubicin (**1**) and adriamycin (**2**), respectively. The β anomer of 4′-*epi*-daunorubicin (**6**) although still displaying a

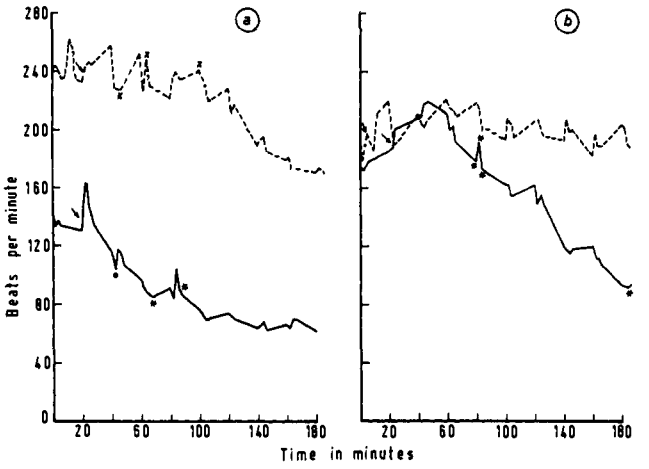


Figure 1. Effect of compounds 1–4 on the beating rate of cultured mouse cells. The substances were added at times shown by arrows. (a) Daunorubicin (1), 0.1 μg/ml (——); 4′-*epi*-daunorubicin (3), 0.5 μg/ml (– – –). (b)Adriamycin (2), 0.1 μg/ml (——); 4′-*epi*-adriamycin (4), 1 μg/ml (– – –). * indicates arrhythmias; x indicates asynchronous beating.

Table II. Activity of Compounds 1, 2, 3, 4, and 6 on MSV-M Foci Formation on MEF and on MEF Proliferation[a]

		MSV-M		MEF	
Compd	Dose, μg/ml	No. of foci[b]	ID_{50}[c]	No. of cells[b]	ID_{50}[c]
1	0.025	0		22	
	0.0125	17	0.006	45	0.008
	0.0062	51		54	
2	0.025	0		37	
	0.0125	24	0.005	42	0.01
	0.0062	46		61	
3	0.4	0		1	
	0.1	0	0.006	14	0.013
	0.025	0		23	
	0.0062	51		75	
4	0.025	0		40	
	0.0125	0	0.01	40	0.008
	0.0062	70		55	
	0.0031	90		62	
6	0.4	0		14	
	0.1	5		28	
	0.025	49	0.01	80	0.069
	0.062	48		87	

[a]Average data of two experiments. [b]As percent of untreated controls. [c]50% inhibiting dose (μg/ml).

Table III. Activity of New Glycosides on Sarcoma 180 Ascites in Mice

Compd	Dose[a]	AST[b]	LST[c]	Toxic deaths
1	0.2	106	1/10	
	1	140	3/10	
	5	140	0/10	2/10
2	0.2	126	2/10	
	1	190	4/30	
	1.5	333	4/10	
	2.25	290	4/10	
	5	210	4/20	
3	0.22	110	1/10	
	1.1	120	1/10	
	5.7	80	0/10	6/10
4	0.5	135	0/8	
	2	184	0/8	
	10	234	1/8	1/8
6	0.26	96	0/10	
	1.3	110	1/10	
	6.7	126	1/10	

[a]Treatment ip on day 1 (mg/kg of body weight). [b]Average survival time expressed as percent of untreated controls. [c]Long-term (60 days from tumor transplantation) survivors.

Table IV. Activity of New Glycosides in Comparison with Adriamycin on Transplanted Gross Leukemia in Mice

Compd	Dose[a]	AST[b]	Toxic deaths
2	2.25	171	
	2.5	185	
	3	185	
	3.5	200	
	3.75	200	
	4.5	164	8/18
3	1.5	114	
	2.25	150	
	3	178	
	3.75	142	2/10
	4.5	114	
4	1.5	135	
	2.5	157	
	3.5	178	
	4.5	171	
	6.0	128	5/10
6	1.5	100	
	2.25	100	
	3	114	

[a]Treatment iv on days 1–5 (mg/kg/day). [b]Average survival time expressed as percent of untreated controls.

noticeable inhibiting effect appears to be less active, on a weight basis, than the corresponding α anomer **3**.

Analysis of the behavior on cultured heart cells (Figure 1) shows that the beating rate decreased by about 50% with respect to the basal value after daunorubicin or adriamycin treatment and by 30% after treatment with 4′-*epi*-daunomycin, whereas it was not affected by 4′-*epi*-adriamycin at the concentration of 1 μg/ml. Also at the dose of 5 μg/ml, 4′-*epi*-adriamycin did not modify the beating rate of cultured heart cells.

The effect of the compounds under investigation on Murine Sarcoma virus-Moloney (MSV-M) foci formation and on mouse embryo fibroblasts (MEF) proliferation is shown in Table II. The activity of 4′-*epi*-daunorubicin (**3**) was of the same order of magnitude as that of **1**, but the β anomer **6** was somewhat less effective especially on cell proliferation, in agreement with the results on HeLa cell cultures (see above). 4′-*epi*-Adriamycin (**4**) showed an activity similar to that of adriamycin (**2**).

The results of the animal tests are reported in Tables III–V. On Sarcoma 180 ascites (Table III) 4′-*epi*-daunorubicin (**3**) and 4′-*epi*-adriamycin (**4**) were less active than respectively **1** and **2** and the β anomer **6** appeared endowed with even lower antitumor activity on a weight basis. Both **3** and **4**, and especially the latter, displayed noticeable activity on transplanted Gross leukemia (Table IV), whereas **6** exhibited no activity on this tumor at the doses tested. Compound **4** was compared with adriamycin (**2**) on solid Sarco-

Table V. Comparison of 4′-*epi*-Adriamycin with Adriamycin on Solid Sarcoma 180 in Mice

Compd	Dose[a]	Tumor growth[b]	Toxic deaths
Controls		100	0/10
2	2.5	36	2/10
	3.5	23	5/10
4	2.5	67	0/10
	5	29	2/10
	7.5		10/10

[a]Treatment iv on days 1–5 (mg/kg/day). [b]Tumor growth evaluated on day 11 after tumor implant.

ma 180 and it was found (Table V) that doses of comparable toxicity displayed comparable inhibition of tumor growth.

Discussion

The isolation of both α and β anomers as the products of the condensation of daunomycinone or of protected adriamycinone with 2,3,6-trideoxy-3-trifluoroacetamido-4-*O*-trifluoroacetyl-α-L-*arabino*-hexopyranosyl chloride is in agreement with the known course of the Koenigs–Knorr reaction when halo sugars without a participating group at C-2 are used.[7] The relative amounts of the two anomers are, however, dependent from the substituents in the sugar reagent and from the reaction conditions, and this should explain the different result of Acton et al.[8] who have obtained the α-glycoside with unexpected stereoselectivity with 4-*O*-*p*-nitrobenzoyl-3-*N*-trifluoroacetyldaunosaminyl bromide.

The biological activity displayed by the 4′-epi analogs (α anomers) is not unexpected since these compounds differ from the parent drugs in having the hydroxyl group at C-4′ equatorial instead of axial, whereas all chemical functions typical of the antitumor anthracyclines as well as other stereochemical features and conformation are retained. The different orientation of the C-4′ hydroxyl can however lead to important differences in the pharmacological behavior, as it is here exemplified by the absence of toxicity of 4′-*epi*-adriamycin in cultured heart cells for concentration values at which adriamycin is very effective. On the other hand, the same compound shows antitumor activity comparable to adriamycin in animal tests. These results suggest a remarkable potential therapeutic value of 4′-*epi*-adriamycin.

The low, albeit present, bioactivity of compound **6** suggests that β anomers should be also investigated in order to establish to what extent the inverted configuration at C-1′ modifies the pharmacological properties within this highly effective class of antitumor drugs.

Experimental Section

Chemical Synthesis. Melting points, taken at the Köfler hot stage, are uncorrected. Rotations were determined at 20° with a Perkin-Elmer 141 automatic polarimeter. Where analyses are indicated only by symbols of the elements, the analytical results obtained for those elements were within ±0.4% of theoretical values. ^{1}H NMR spectra were recorded with a Varian A-60A spectrometer, with Me_4Si as internal reference, in the indicated solvents. The mass spectra were recorded with a Perkin-Elmer 270 spectrometer (direct inlet technique) at an electron ionizing voltage of 70 V. The ir spectra were recorded with a Perkin-Elmer 457 spectrometer. TLC on silica gel HF (E. Merck) was used for identification purposes and the homogeneity test.

Methyl 2,3,6-Trideoxy-3-trifluoroacetamido-α-L-*lyxo*-hexopyranoside (11). To a suspension of **9**, as the hydrochloride (1 g, 5 mmol) in Et_2O (25 ml), $(CF_3CO)_2O$ (4 ml) was added at 0° with stirring. After 4 hr at room temperature the solvent was evaporated under vacuum and crude **10** (1.8 g), freed from acid by repeated evaporation of the Et_2O solution, was treated with MeOH (60 ml) at room temperature overnight and gave, after evaporation and crystallization from Et_2O–petroleum ether (bp 40–70°), 1.04 g of **11**: mp 108–109°; $[\alpha]_D$ −148° (c 0.5, $CHCl_3$); $[\alpha]_D$ −195° (c 0.5, MeOH). Anal. ($C_9H_{14}F_3NO_4$) H; C: calcd, 42.02; found, 41.55.

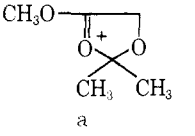
Methyl 2,3,6-Trideoxy-3-trifluoroacetamido-α-L-*threo*-hexopyranosid-4-ulose (12). Compound **11** (2 g, 7.6 mmol) in CH_2Cl_2 (40 ml) was added to a solution of KIO_4 (2.3 g), K_2CO_3 (0.25 g), and RuO_2 (0.12 g) in water (40 ml). The two-phase system was shaken overnight at room temperature. TLC [developing system $CHCl_3$–$(CH_3)_2CO$ (10:1 by volume); R_f for **11**, 0.1; R_f for **12**, 0.4] showed that about 60% of **12** was formed. Further addition of solid KIO_4 (2.3 g), K_2CO_3 (0.25 g), and RuO_2 (0.12 g) in 6 hr completed the oxidation. The organic layer was separated, filtered, washed, dried, and evaporated to give **12** (1.45 g, 74%): mp 77–80°; ir (KBr) 1735 (CO ketone), 1700 cm^{-1} (CO amide); mass spectrum *m/e* 255 (M^+); ^{1}H NMR ($CDCl_3$) δ 1.31 (d, J = 6.5 Hz, CH_3 C-5), 1.89 (m, C-2 H_{ax}), 2.88 (m, C-2 H_{eq}), 3.43 (s, CH_3O), 4.37 (d, J = 6.5 Hz, C-5 H), 4.83 (s, W_H = 4.5 Hz, C-1 H), 4.95 (two d, J = 12.5 Hz, J' = 6.0 Hz, C-3 H), and 7.00 (br s, NH). Anal. ($C_9H_{12}F_3NO_4$) C, H.

Methyl 2,3,6-Trideoxy-3-trifluoroacetamido-α-L-arabino-pyranoside (13). A solution of **12** (1 g, 3.9 mmol) in a mixture of dioxane (100 ml) and H_2O (10 ml) was treated with $NaBH_4$ (0.1 g, 2.7 mmol) at 5°. After 10 min the reduction was complete; then the solution was adjusted to pH 4 with Dowex W-X2 (H^+). The suspension was filtered and evaporation gave a crude product which was treated several times with MeOH in order to remove boric acid. **13** (0.65 g, 65%) was obtained: mp 195–197°; $[\alpha]_D$ −110° (c 0.2, MeOH), $[\alpha]_D$ −123° (c 0.5, $CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 1.07 (d, J = 6.0 Hz, CH_3 C-5), 1.5–1.9 (m, C-2 H_2), 3.0 [two d (after exchange with D_2O), J' = J'' = 9.5 Hz, C-4 H], 3.16 (s, CH_3O), 3.44 (two q, J' = 9.5 Hz, J'' = 6.0 Hz, C-5 H), 3.87 (m, C-3 H), 4.48 (s, W_H = 6.0 Hz, C-1 H), 4.84 (d, J = 6.5 Hz, COH), and 8.84 (br s, NH); TLC [$CHCl_3$–$(CH_3)_2CO$ (10:2 v/v)] single spot with R_f 0.33; **11** displayed R_f 0.37 (detection, H_2SO_4). Anal. ($C_9H_{14}F_3NO_4$) C, H.

2,3,6-Trideoxy-3-trifluoroacetamido-L-*arabino*-hexopyranose (14). Hydrolysis of **13** (1 g, 3.8 mmol) was performed in aqueous AcOH (20%) at 100° for 2 hr. Evaporation of the acid gave a solid substance which, by crystallization from MeOH–CH_2Cl_2 (1:3 by volume), gave **14** (0.6 g, 66%): mp 202° dec; $[\alpha]_D$ −51°; after 2 hr $[\alpha]_D$ −33.4° (c 0.5, dioxane). Anal. ($C_8H_{12}F_3NO_4$) C, H.

2,3,6-Trideoxy-3-trifluoroacetamido-4-*O*-trifluoroacetyl-α-L-*arabino*-hexopyranosyl Chloride (16). 2,3,6-Trideoxy-3-trifluoroacetamido-L-*arabino*-hexopyranose (**14**, 1 g, 3.8 mmol), suspended in 20 ml of anhydrous Et_2O, was treated at 0° with $(CF_3CO)_2O$ (8 ml) for 2 hr and, subsequently, for 1 hr at room temperature. Evaporation gave the crude 1,4-di-*O*-trifluoroacetyl-3-*N*-trifluoroacetyl derivative **15**, which was treated in anhydrous Et_2O at 0° with dry HCl. After standing at +5° overnight the solvent was removed in vacuo to give a quantitative yield of **16**: ^{1}H NMR ($CDCl_3$) δ 1.30 (d, J = 6.0 Hz, CH_3 C-5), 2.2–2.8 (m, C-2 H_2), 4.2–4.6 (m, C-5 H), 4.6–5.2 (m, C-3 H and C-4 H), 6.25 (m, W_H = 6.0 Hz, C-1 H), and 6.45 (br s, NH).

9-Deacetyl-9-(2′,2′-dimethyl-4′-methoxydioxolan-4′-yl)daunomycinone (17). Adriamycinone (1 g, 2.4 mmol) in dioxane (10 ml) and $CHCl_3$ (200 ml) was treated with dimethoxypropane (80 ml) and *p*-toluenesulfonic acid (0.17 g). The mixture, stirred at room temperature for 24 hr, was neutralized with solid $NaHCO_3$ (0.37 g) and washed with water to neutrality. Evaporation afforded a solid residue which was applied on a column of silicic acid and eluted with $CHCl_3$–$(CH_3)_2CO$ (10:1 by volume). The main reaction product (0.48 g, 36%), identified by TLC [developing solvent system $CHCl_3$–$(CH_3)_2CO$ (10:1), R_f 0.38], was **17**, a mixture (1:1) of epimers at C-13: ir no CO absorption at 1725 cm^{-1}; mass spectrum 486 (M^+), 454 (M − MeOH), 450 (M − $2H_2O$), *m/e* 131 (see structure a); ^{1}H NMR ($CDCl_3$) δ 1.47 and 1.63 (two s, gem CH_3), 3.46 (s, CH_3O C-13), 4.03 (s, CH_3O C-4), 4.0–4.6 (m, C-14 H_2), 5.22 (br s, C-7 H), 12.83 and 12.84 (two s, chelated OH), and 13.51 (s, chelated OH). Anal. ($C_{24}H_{24}O_{10}$) H; C: calcd, 61.01; found, 61.67.



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4′-*epi*-Daunomycin (3) and Its β Anomer (6). To a solution of

daunomycinone (0.5 g, 1.25 mmol) in anhydrous CH_2Cl_2 (120 ml), HgO (1 g, 4.6 mmol), $HgBr_2$ (0.25 g, 0.7 mmol), molecular sieve (Merck 3A, 10 g), and **16** (0.7 g, 1.95 mmol) were added with stirring. After 24 hr the mixture was filtered and evaporated under vacuum. The solid residue was dissolved in MeOH, refluxed 15 min, evaporated to dryness, and purified by chromatography on a silicic acid column using $CHCl_3$–MeOH (100:3 by volume) as the eluting system. The main product was a mixture (0.3 g, 38%) of **5** and **8** (70:30) obtained after crystallization from $CHCl_3$. The mixture was dissolved in aqueous 0.1 *N* NaOH (30 ml). After 30 min at room temperature, the pH of the solution was adjusted to 8.6 with 0.1 *N* HCl and the α- and β-glycosides as free bases were obtained by extraction with $CHCl_3$ and evaporation. The separation of the α and β anomers was performed by applying the mixture to a silica gel column using as eluent the solvent system $CHCl_3$–MeOH–H_2O (135:20:2 by volume). The hydrochlorides of 4′-*epi*-daunomycin (**3**) [0.16 g; mp 199–201° dec; $[\alpha]_D$ +314° (c 0.026, MeOH); R_f 0.65, solvent system $CHCl_3$–MeOH–H_2O (130:60:10 by volume)] and of its β anomer **6** [0.06 g; mp 187–189°; $[\alpha]_D$ +357° (c 0.02, MeOH); R_f 0.6] were obtained by dissolving the free bases in chloroform followed by the addition of the stoichiometric amount of methanolic HCl and ether. Anal. ($C_{27}H_{29}NO_{10}$·HCl) C, H.

4′-*epi*-Adriamycin (4) and Its β Anomer (7). A solution of **17** (0.7 g, 1.44 mmol) in CH_2Cl_2 (35 ml) was treated with HgO) 1.4 g), $HgBr_2$ (0.35 g), and 4 g of molecular sieve (Merck, 3A) as dehydrating agent. The mixture was stirred at room temperature and two additions of **16** (0.35 g, 1 mmol each) were performed after 1 and 3 hr. The stirring was continued overnight; then $CHCl_3$ (50 ml) was added. The solution obtained by filtration of the reaction mixture was evaporated under vacuum and the residue was dissolved in MeOH, refluxed 15 min, and evaporated to dryness. TLC of the crude product [solvent system $CHCl_3$–$(CH_3)_2CO$ (4:1 by volume)] revealed three spots at R_f 0.45, 0.26, and 0.22 corresponding to **17**, **18**, and **19**, respectively. The separation of the mixture was performed by chromatography on a silicic acid column using $CHCl_3$–C_6H_6–MeOH (100:20:4, by volume) as the eluting system. The fractions containing **18** and **19** were collected and evaporated to dryness separately. The residues (0.5 and 0.06 g, respectively) were treated as follows. NaOH (0.1 *N*, 50 ml) and acetone (10 ml) were added, after 30 min at room temperature the pH was adjusted to 8.4 with 0.1 *N* HCl, and the solution was repeatedly extracted with $CHCl_3$. The combined extracts were dried (Na_2SO_4) and evaporated under vacuum. The residue was taken up in 0.1 *N* aqueous HCl (50 ml) and left at room temperature for 36 hr. The acid solution was washed by extraction with $CHCl_3$ to eliminate traces of aglycone and then brought to pH 8.2 under stirring in the presence of $CHCl_3$ (50 ml) by slow addition of 0.1 *N* NaOH. The organic phase was then separated, dried (Na_2SO_4), and concentrated to 10 ml. Upon careful addition of the stoichiometric amount of methanolic HCl a red precipitate was obtained which was collected, washed with Et_2O, and dried under vacuum. **4** (0.4 g) had mp 185° dec; $[\alpha]_D$ +274° (c 0.01, MeOH); TLC on silica gel plate buffered at pH 7 (0.067 *M* phosphate), solvent system $CHCl_3$–MeOH–H_2O (130:60:10 by volume), R_f 0.55. Anal. ($C_{27}H_{29}NO_{11}$·HCl) C, H. **7** (0.05 g) had mp 190–193° dec; $[\alpha]_D$ +290° (c 0.01, MeOH); TLC (same conditions as **4**) R_f 0.43. Anal. ($C_{27}H_{29}NO_{11}$·HCl) H; C: calcd, 55.90; found, 56.46.

Biological Activity Evaluation. The compounds were evaluated for cytotoxic, antiviral, and cardiac toxicity in cultured cells and for antitumor activity in mice. In vitro tests were carried out on HeLa cells, on secondary mouse embryo fibroblasts (MEF) both infected and not infected with the Murine Sarcoma virus-Moloney (MSV-M), and on myocardial cells of newborn mice. HeLa cells were exposed to the drugs for 2, 8, and 24 hr; then the drugs were removed and cells were seeded on 60-mm Falcon plastic dishes (200 cells/plate). Colony number was evaluated microscopically 6 days later. MEF were plated on 35-mm Falcon plastic dishes, infected 24 hr later with MSV-M, and treated for 3 days with the compounds under study. The number of foci of transformed cells was evaluated microscopically 5 days after the infection. Uninfected MEF were similarly treated; at the end of the experiment, cells were counted in an haemocytometer.[9] Single cardiac cells were isolated by trypsinization from the heart of newborn mice. After 3 days at 37°, the cultures showing clusters of beating cells were treated with the compounds under study. The rate of beating and the rhythm were detected by microscopical examination.[10]

The antitumor activity of derivatives under study was tested on Sarcoma 180 (solid and ascites) and intravenously transplanted Gross leukemia. Tests on Sarcoma 180 and Gross leukemia were carried out in CD1 mice and C_3H mice, respectively, as previously described.[9] Each experimental group consisted of at least ten mice. Compounds were dissolved in Ringer solution and administered in a volume of 10 ml/kg of body weight, according to different schedules of treatment. Toxicity was evaluated by the macroscopic autopsy findings, mainly as reduction of spleen size.

Acknowledgments. Thanks are due to Professor S. Hanessian (Université de Montréal) for useful discussions, to Mrs. E. Gandini for the ^1H NMR spectra, and to Dr. B. Gioia for the mass spectra.

References and Notes

(1) A. Di Marco in "Antibiotics", Vol. I, D. Gottlieb and P. P. Show, Ed., Springer-Verlag, Berlin, 1966, p 190.
(2) F. Arcamone, G. Franceschi, A. Minghetti, S. Penco, S. Redaelli, A. Di Marco, A. M. Casazza, T. Dasdia, G. Di Fronzo, G. Giuliani, L. Lenaz, A. Necco, and C. Soranzo, *J. Med. Chem.*, **17**, 335 (1974).
(3) A. Di Marco and F. Arcamone, *Arzneim.-Forsch.*, **25**, 368 (1975).
(4) S. Penco, *Chim. Ind. (Milan)*, **50**, 908 (1968).
(5) F. Arcamone, S. Penco, and A. Vigevani, paper presented at "Adriamycin, New Drug Symposium", San Francisco, Calif., Jan 15 and 16, 1975 (to be published in *Cancer Chemother. Rep.*).
(6) F. Arcamone, G. Cassinelli, G. Franceschi, R. Mondelli, P. Orezzi, and S. Penco, *Gazz. Chim. Ital.*, **100**, 949 (1970).
(7) G. Wulff and G. Röhle, *Angew. Chem., Int. Ed. Engl.*, **13**, 157 (1974).
(8) E. M. Acton, A. N. Fujiwara, and D. W. Henry, *J. Med. Chem.*, **17**, 659 (1974).
(9) A. Di Marco, A. M. Casazza, T. Dasdia, F. Giuliani, L. Lenaz, A. Necco, and C. Soranzo, *Cancer Chemother. Rep.*, **57**, 269 (1973).
(10) A. Necco and T. Dasdia, *IRCS Libr. Compend.*, **2**, 1293 (1974).