bound agents per nucleotide on DNA. Log $([\eta]/[\eta]_0)$ is first plotted as a function of log $(1 + 2r)$. If monointercalation occurs, the slope of the curve is expected to be between 2.3 and 3. When a slope higher than 4 is obtained, log $([\eta]/[\eta]_0)$ is plotted vs. log $(1 + 4r)$. If bisintercalation is the dominant process, the slope value is between 2.3 and 3.

Biological Testing. L1210 was used to evaluate the antitumor activity of the dimers in the same way as described in the preceding paper.[1]

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Nucleoside Conjugates as Potential Antitumor Agents. 3. Synthesis and Antitumor Activity of 1-(β-D-Arabinofuranosyl)cytosine Conjugates of Corticosteroids[1]

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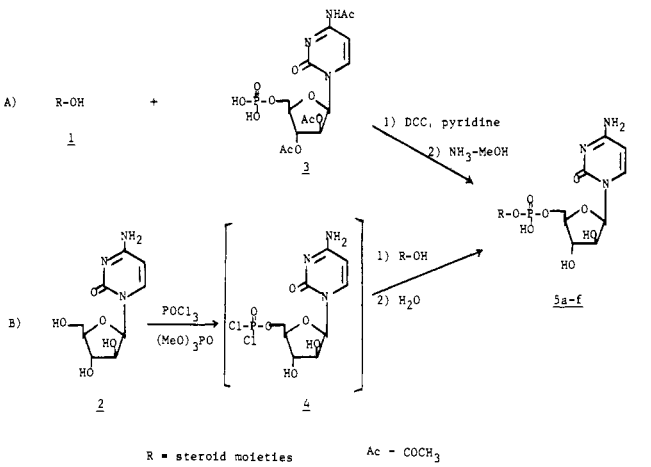
Six 5′-(steroid-21-phosphoryl)-1-(β-D-arabinofuranosyl)cytosines have been prepared and evaluated against L1210 lymphoid leukemia in culture and in mice ($C_3D_2F_1/J$). These include the *ara*-C conjugates of 11-deoxycorticosterone (**5a**), corticosterone (**5b**), cortexolone (**5c**), fludrocortisone (**5d**), 6α-methylprednisolone (**5e**), and dexamethasone (**5f**). When the optimum dosage of *ara*-C [38 (μmol/kg)/day × 5] was given to mice bearing L1210, the ILS value found was 89%. A simple mixture of each steroid and *ara*-C gave ILS values that were on the whole significantly less than that of the parent nucleoside. However, of six conjugates, all but two (**5d** and **5f**) were more active than *ara*-C at their optimal doses. Both corticosterone- (**5b**) and cortexolone-*p*-*ara*-C (**5c**) were especially effective at the respective optimal doses of 76.7 and 115 (μmol/kg)/day × 5. These gave ILS values of 200% each. All of the conjugates were demonstrated to be enzymatically hydrolyzed to the corresponding steroid and *ara*-CMP, and the latter was further shown to be hydrolyzed to *ara*-C by phosphodiesterase I, 5′-nucleotidase, and acid phosphatase. However, they were shown to be resistant to hydrolysis by alkaline phosphatase.

As a result of our continuing efforts to develop more effective nucleoside derivatives for the treatment of malignant tumors, we have recently reported the synthesis and antitumor activity of 1-(β-D-arabinofuranosyl)cytosine (*ara*-C)[2] conjugates of cortisol and cortisone[3] and of prednisolone and prednisone.[4] The greater antitumor activities demonstrated for these conjugates as compared to those of *ara*-C alone or in combination with the steroid against L1210 lymphoid leukemia in mice have prompted us to synthesize and test conjugates of other available corticosteroids. The present report describes methods of syntheses and antitumor properties of six new *ara*-C conjugates of corticosteroids. Three of these steroids (11-deoxycorticosterone, corticosterone, and cortexolone) are naturally occurring and the other three (fludrocortisone, 6α-methylprednisolone, and dexamethasone) are synthetic corticoids.

Scheme I



Chemistry. The *ara*-C conjugates (**5a–f**) were prepeared by two methods (Scheme I). The first method (A) was the condensation of Ac_3-*ara*-CMP (**3**) with 2 molar equiv of steroid (1) in the presence of DCC and pyridine at room temperature for 2 days.[3,4] After the acetyl groups were removed in 2 N NH_3–MeOH, the conjugates were separated on a DE-52 (acetate) column using a HOAc gradient (0–2.0 N). The yields varied according to the steroid used, ranging from a high with the use of 11-deoxycorticosterone to lower relatively similar values for the others (Table I). In an attempt to improve the yield, the following conditions were varied: molar ratio of Ac_3-*ara*-CMP, steroid, and DCC, reaction temperature, time, and solvents. None of these increased the yield and

(1) This work has been previously presented. See Hong, C. I.; Nechaev, A.; West, C. R. In "Abstracts of Papers", 178th National Meeting of the American Chemical Society, Washington, D.C., Sept. 1979; American Chemical Society: Washington, D.C., 1979; Abstract MEDI 43.

(2) Abbreviations used are: *ara*-C, 1-(β-D-arabinofuranosyl)cytosine; *ara*-CMP, 1-(β-D-arabinofuranosyl)cytosine 5′-monophosphate; Ac_3-*ara*-CMP, N^4,2′,3′-triacetyl-1-(β-D-arabinofuranosyl)cytosine 5′-monophosphate; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DOC-*p*-*ara*-C, 5′-(11-deoxycorticosterone-21-phosphoryl)-1-(β-D-arabinofuranosyl)cytosine; corticosterone-*p*-*ara*-C, 5′-(corticosterone-21-phosphoryl)-1-(β-D-arabinofuranosyl)cytosine; cortexolone-*p*-*ara*-C, 5′-(cortexolone-21-phosphoryl)-1-(β-D-arabinofuranosyl)cytosine; fludrocortisone-*p*-*ara*-C, 5′-(fludrocortisone-21-phosphoryl)-1-(β-D-arabinofuranosyl)cytosine; MePred-*p*-*ara*-C, 5′-(6α-methylprednisolone-21-phosphoryl)-1-(β-D-arabinofuranosyl)cytosine; DXM-*p*-*ara*-C, 5′-(dexamethasone-21-phosphoryl)-1-(β-D-arabinofuranosyl)cytosine; ip, intraperitoneally.

(3) Hong, C. I.; Nechaev, A.; West, C. R. *Biochem. Biophys. Res. Commun.* **1979**, *88*, 1223.

(4) Hong, C. I.; Nechaev, A.; West, C. R. *J. Med. Chem.* **1979**, *22*, 1428.

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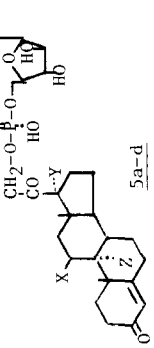
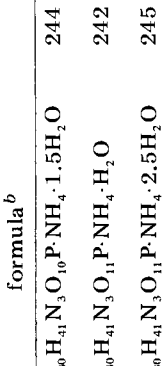
Table I. Physical Data of the Conjugates



5a–d



5e,f

no.	compound	X	Y	Z	method	mp,[a] °C	yield, %	formula[b]	UV max, nm ($\epsilon \times 10^{-3}$)[c] H_2O	0.1 N HCl	0.1 N NaOH
5a	DOC-*p*-*ara*-C	H	H	H	A	215–225 dec	36.3	$C_{30}H_{41}N_3O_{10}P\cdot NH_4\cdot 1.5H_2O$	244 (20.5)	248 (16.7)	244 (18.7)
					B	215–225 dec	21.0			272 sh (13.6)	
5b	corticosterone-*p*-*ara*-C	OH	H	H	A	215–225 dec	10.0	$C_{30}H_{41}N_3O_{11}P\cdot NH_4\cdot H_2O$	242 (20.3)	247 (17.5)	243 (20.5)
					B	200–213 dec	5.4			272 sh (13.0)	
5c	cortexolone-*p*-*ara*-C	H	OH	H	A	200–210 dec	15.3	$C_{30}H_{41}N_3O_{11}P\cdot NH_4\cdot 2.5H_2O$	245 (19.7)	250 (16.6)	243 (19.4)
										272 sh (13.7)	
5d	fludrocortisone-*p*-*ara*-C	OH	OH	F	A	216–228 dec	13.6	$C_{30}H_{40}FN_3O_{12}P\cdot NH_4\cdot 2H_2O$	238 (20.9)	242 (16.0)	238 (20.9)
									265 sh (10.7)	275 (12.9)	265 sh (11.3)
5e	MePred-*p*-*ara*-C	H	H	CH_3	A	210–215 dec	13.1	$C_{31}H_{41}N_3O_{12}P\cdot NH_4\cdot 2.75H_2O$	240 (20.3)	252 sh (17.7)	240 (20.8)
									258 (18.7)	268 (19.2)	258 sh (18.8)
5f	DXM-*p*-*ara*-C	F	CH_3	H	A	215–220 dec	10.0	$C_{31}H_{40}FN_3O_{12}P\cdot NH_4\cdot 2H_2O$	234 (21.2)	239 (16.8)	234 (22.0)
									260 sh (18.1)	270 (17.3)	260 sh (16.0)

[a] No distinct mp. Slowly decomposed. [b] All compounds were analyzed for C, H, N, and P. Analytical results were within ±0.4% of the theoretical values. [c] sh = shoulder.

Table II. Effects of Compounds on the Viability of L1210 Lymphoid Leukemia Cells in Culture

no.	compound	length of pre-incubation, h	concn, μM, for 50% loss of viability at 72 h (ED_{50})
5a	DOC-*p*-*ara* C	0	0.05
5b	corticosterone-*p*-*ara*-C	0	0.09
		24	0.09
		48	0.09
5c	cortexolone-*p*-*ara*-C	0	0.08
5d	fludrocortisone-*p*-*ara*-C	0	0.08
5e	MePred-*p*-*ara*-C	0	0.45
5f	DXM-*p*-*ara*-C	0	0.08
	ara-C	0	0.1
	11-deoxycorticosterone (DOC)	0	21.0
	ara-CMP + corticosterone	0	0.08

neither did altering the order or rate of addition of the reactants. With the use of column chromatographic separation, *ara*-CMP was recovered at 40%. This product was utilized for further preparation of the conjugates.

The second method (B) was a direct route in which *ara*-C (**2**) and steroid (**1**) were linked through a phosphodiester bond from a reaction with $POCl_3$ in $(MeO)_3PO$.[5] Conjugates **5a** and **5b** were prepared by this method and the yields were 21 and 5.4%, respectively. Even though these yields were relatively small, this method eliminated several steps, including the blocking of the nucleoside and use of the condensing agent. The low yield might be attributable to the low reactivity of the 21-OH group of steroids with the 5-*O*-dichlorophosphate ester (**4**) of *ara*-C. The conjugates prepared by this method had chromatographic mobilities in various solvents and IR and UV spectra that were identical with those of the same compound prepared by the condensation method.

The observed UV maxima in H_2O for the conjugates were between 234–245 ($\epsilon \sim 20000$) and 260 nm (shoulder). The latter was more prominent for the conjugates **5e** and **5f**, both of which have a quinone A ring in the steroid.

Biological Results. Antiproliferative Activity in Vitro. Growth inhibition of L1210 lymphoid leukemia cells in culture by compounds listed in Table II was measured by Trypan blue exclusion from the viable cells.[6] The concentration of each drug which resulted in 50% inhibition (ED_{50}) of growth at 72 h is shown in Table II. Only 11-deoxycorticosterone among the steroids used alone demonstrated an ED_{50} that was less than 100 μM and, therefore, values above this for the other corticoids are not included here. Activity against L1210 cells was demonstrated for all conjugates of *ara*-C in vitro, as shown in Table II. DOC-*p*-*ara*-C (**5a**) was the most effective and was twice as active as *ara*-C. Corticosterone- (**5b**), cortexolone- (**5c**), fludrocortisone- (**5d**), and DXM-*p*-*ara*-C (**5f**) were either as active as *ara*-C or nearly so. Although MePred-*p*-*ara*-C (**5e**) clearly demonstrated activity, it was only about 10% as effective as DOC-*p*-*ara*-C.

In order to determine the effects of preincubation in the medium on activity, the most active of the conjugates in vivo (**5b**) was incubated at 37 °C for 24 and 48 h before

(5) Kim, S. H.; Rosowsky, A. *J. Carbohydr., Nucleosides, Nucleotides* **1979**, *6*, 229.
(6) Tritsch, G. L.; Nechaev, A.; Mittelman, A. *Cancer Biochem. Biophys.* **1977**, *2*, 87.

the in vitro assay. As seen in Table II, conjugate **5b** showed the same activity (ED_{50} = 0.09 μM) regardless of the preincubation period shown. Furthermore, the combination of *ara*-CMP and corticosterone showed a similar activity (ED_{50} = 0.08 μM). Therefore, these results failed to demonstrate evidence for cleavage of the conjugate to the parent drug (*ara*-C or *ara*-CMP) and the corresponding steroid. In support of this finding, analysis of incubation mixtures (24 and 48 h) by TLC, and quantitated by UV, showed that neither *ara*-C nor *ara*-CMP was detected and the conjugate remained intact.

Antitumor Activity in Vivo. This is determined for *ara*-C alone, *ara*-C plus each steroid, and conjugates against intraperitoneal implants of L1210 lymphoid leukemia in mice ($C_3D_2F_1$/J) by comparison of median survival times of control vs. treated animals. The procedure was in accord with NCI protocol,[7] with the exception that the inoculum in our studies contained 1×10^6 cells instead of 1×10^5. Moreover, our injection schedule was 5 days rather than the 9, as previously reported.[7]

Under these conditions, it can be seen in Table III that the optimum dose found for *ara*-C against L1210 lymphoid leukemia in mice in vivo was 38 (μmol/kg)/day. This dose given daily for 5 successive days was followed by a significant increase in life span of 89%. However, when *ara*-C was combined in a simple mixture with each of the steroids [either 38 or 75 (μmol/kg)/day, shown in Table III], the results obtained were clearly less than that of the nucleoside used alone. In contrast, when *ara*-C linked to each steroid through a phosphodiester bond was administered as a conjugate, DOC- (**5a**), corticosterone- (**5b**), cortexolone- (**5c**), and MePred-*p*-*ara*-C (**5e**) were highly effective, providing for ILS values (138, 200, 200, and 156%, respectively) that exceeded that of the parent compound by 55 to 125%. Only the two conjugates (**5d** and **5f**) which contained the synthetic fluorocorticoid moieties (fludrocortisone and dexamethasone) gave ILS values that were clearly less than that of *ara*-C or any of the other conjugates shown in Table III. However, they are still more effective than combination of *ara*-C and the corresponding steroid at the same molar doses.

Toxicity of *ara*-C and the four superior conjugates at the optimal dosages, as reflected by weight loss (Table III), was either minimum or not detectable by this method. Thus, the results obtained on activity and toxicity suggest that these highly active conjugates merit further studies in the direction toward clinical trials.

Enzymatic Hydrolysis. Enzymatic hydrolysis of the conjugates with purified phosphodiesterase I (EC 3.1.4.1), 5′-nucleotidase (EC 3.1.3.5) from *Crotalus adamanteus*, and acid phosphatase (EC 3.1.3.2) from wheat germ showed that the products were the steroid and *ara*-CMP. The latter was further hydrolyzed to *ara*-C during a 24-h incubation period at the appropriate temperature and pH.[3,4] For example, when corticosterone-*p*-*ara*-C (**5b**) was incubated with phosphodiesterase I, 50% of the conjugate was hydrolyzed within 20 min and the hydrolysis was completed within 6 h of incubation. However, only 30% of the conjugate was hydrolyzed after a 6-h incubation with both 5′-nucleotidase and acid phosphatase, and 20% of the conjugate remained intact at the end of a 24-h incubation. All conjugates were found to be resistant to enzymatic hydrolysis by bacterial alkaline phosphatase (EC 3.1.3.1). When the conjugates were incubated with normal mouse plasma at 37 °C, 80 and 60% of each conjugate remained intact after a 24- and 48-h incubation, respectively. However, when the conjugates were incubated with normal human plasma at 37 °C for 24 h, approximately 50% of each conjugate remained intact.

Discussion

The present study on the syntheses and antitumor activity of the *ara*-C conjugates of three naturally occurring steroids (11-deoxycorticosterone, corticosterone, and cortexolone) and three synthetic corticoids (fludrocortisone, 6α-methylprednisolone, and dexamethasone) has demonstrated four additional derivatives which are more active than the parent antineoplastic agent. Thus, we have now found eight steroid derivatives of *ara*-C which are far more active than this agent alone against L1210 lymphoid leukemia in mice in vivo. From previous studies, these included the *ara*-C conjugates of cortisol and cortisone[3] and prednisolone and prednisone;[4] in the present work, these included 11-deoxycorticosterone, corticosterone, cortexolone, and 6α-methylprednisolone. Only those of fludrocortisone and dexamethasone were less active than *ara*-C alone.

The optimum dose detected for *ara*-C alone in the present study was 9 [mg (38 μmol)/kg]/day × 5, which increased the life span of the mice bearing L1210 lymphoid leukemia by 89%. A simple mixture of *ara*-C and each steroid at the same molar doses of the optimal doses of both *ara*-C and the conjugates shown in Table III were clearly less effective than that of this nucleoside used alone. However, it was of interest to note that all conjugates of the three natural steroids shown in Table III elicited exceptional responses, increasing the life span of the mice by 138–200%. Furthermore, toxicity was either minimum or not existent. Only the conjugate of 6α-methylprednisolone (**5e**) among the synthetic groups offered an advantage (ILS, 156%) above that of *ara*-C alone.

The conjugates with more potent antiiflammatory steroids (dexamethasone and fludrocortisone) showed disappointing results. Previous work by Jaffe et al.[8] shows that these steroids exerted marked cytolytic action upon the leukemic cells in vivo. However, they also were comparably more toxic in leukemic mice, indicating that the poor therapeutic indices of these compounds limited the full expression of their capacity to destroy leukemic cells. This might be the case for the conjugates with dexamethasone (**5f**) and fludrocortisone (**5d**). However, toxcity from neither of these drugs was reflected in animal weight loss.

In a previous investigation by Evans et al.,[9] *ara*-C·HCl in a dose of 71.7 (μmol/kg)/day × 7 given to mice bearing L1210 lymphoid leukemia was followed by an ILS of 187.5%. At a similar dose for 5 days against implants of 1×10^6 cells in $C_3D_2F_1$/J mice in our previous studies,[4] the ILS value found was 45%. The present study demonstrates results that are consistent with our previous work[4] but continues to differ from that of the previous investigations,[9] namely, an optimum dose for *ara*-C of 38 (μmol/kg)/day × 5 and an ILS value of 89%. Again, it is apparent that the differences are attributable to methods and biological materials.

In order to understand the molecular mode of action of these conjugates, a series of studies are needed. One of the first trials in these studies is that of preincubation of the conjugate in the medium for 24 and 48 h prior to

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(8) Jaffee, J. J.; Fischer, G. A.; Welch, A. D. *Biochem. Pharmacol.* **1963**, *12*, 1081.

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Table III. Effects of the Conjugates, *ara*-C Alone and in Combination with Steroids, on the Survival Time and Weight Changes of Mice with Intraperitoneally Implanted L1210 Lymphoid Leukemia[a]

no.	compound	dose,[b] [mg(μmol)/kg]/ day × 5	wt change, g/mouse, on day 8	survival time, days		% ILS[d]	30-day survivors
				range	median (T/C)[c]		
	ara-C	5.0 (20.6)	+2.00	9-22	13.5/9.0	50	0/7
		9.2 (38.0)	−0.63	14-42	17.0/9.0	89	1/7
		18.2 (75.0)	−3.64	10-19	14.5/10.0	45	0/8
		25.0 (102.8)	−3.80	9-16	9.5/8.0	19	0/8
		Naturally Occurring Steroids					
5a	DOC-*p*-*ara*-C	25.0 (39.3)	−0.54	8-21	16.0/8.5	88	0/8
		50.0 (78.8)	−0.94	17-24	19.0/8.0	138	0/8
		75.0 (117.9)	−0.09	14 to >45	18.5/9.5	95	1/8
	ara-C and 11-deoxy-corticosterone	9.2 and 12.6 (38.0 each)	−3.58	13-23	16.5/11.0	50	0/6
		19.1 and 26.0 (78.8 each)	−4.25	9-12	10.5/10.0	5	0/8
5b	corticosterone-*p*-*ara*-C	25.0 (38.4)	+0.06	14-18	16.5/8.5	94	0/8
		50.0 (76.7)	−1.24	15-42	24.0/8.0	200	2/8
		75.0 (115.1)	−0.59	12-21	17.0/8.5	100	0/8
	ara-C and corticosterone	9.2 and 13.2 (38.0 each)	−3.37	15-28	16.0/11.0	46	0/6
		18.7 and 26.6 (76.7 each)	−1.48	8-24	12.0/9.0	33	0/8
5c	cortexolone-*p*-*ara*-C	25.0 (38.4)	+1.55	14-27	18.0/9.5	90	0/8
		50.0 (76.7)	−2.50	14-23	20.5/8.5	141	0/8
		75.0 (115.1)	+0.46	18-37	28.5/9.5	200	3/8
		100.0 (153.4)	−3.82	10-16	16.0/11.0	46	0/6
	ara-C and cortexolone	9.2 and 13.2 (38.0 each)	−4.76	13-24	17.5/11.0	59	0/6
		18.7 and 26.6 (76.7 each)	−1.36	12-22	15.0/8.5	77	0/8
		Synthetic Steroids					
5d	fludrocortisone-*p*-*ara*-C	25.0 (36.5)	−1.09	12-18	14.0/8.5	65	0/8
		50.0 (72.9)	+0.39	14-17	16.0/9.5	68	0/8
		75.0 (109.4)	+0.52	14-18	17.5/9.5	84	0/8
		100.0 (145.8)		7-8	7.0/11.0	−36	0/6
	ara-C and fludrocortisone	9.2 and 14.5 (38.0 each)	−4.47	12-16	12.0/11.0	9	0/6
		17.7 and 27.7 (72.9 each)	−3.48	7-17	10.5/9.5	11	0/8
5e	MePred-*p*-*ara*-C	25.0 (36.8)	+2.00	13 to >45	17.5/9.5	84	1/8
		50.0 (73.6)	−1.08	14-24	20.5/8.0	156	0/8
		75.0 (110.3)	−1.07	15-37	18.0/9.5	90	1/8
	ara-C and 6α-methyl-prednisolone	9.2 and 13.2 (38.0 each)	−2.38	11-28	15.0/11.0	36	0/6
		18.0 and 27.7 (73.6 each)	−1.38	9-17	13.0/9.0	44	0/8
5f	DXM-*p*-*ara*-C	25.0 (36.0)	−1.25	7-15	11.5/9.0	28	0/8
		50.0 (71.7)	−1.05	10-17	13.5/9.0	50	0/8
		100.0 (143.4)	−2.02	8-13	9.5/9.0	6	0/8
	ara-C and dexamethasone	9.2 and 14.9 (38.0 each)	−4.23	8-10	9.5/11.0	−14	0/6
		34.8 and 56.1 (143.4 each)	−2.72	8-9	8.5/9.0	-6	0/8

[a] $C_3D_2F_1$/J mice in groups of six to eight (av wt 25 g) were inoculated ip with 1×10^6 cells of L1210 lymphoid leukemia. [b] Doses were started 24 h after tumor implantation. [c] Median survival time of 48 mice used for the control study was 9.33 ± 1.08 (SD) days and weight changes on day 8 averaged +1.81 ± 0.79 (SD) g/mouse. [d] Percentage increase in life span of treated animals as compared with control tumor bearers was determined as follows: (T/C − 1) × 100.

beginning the in vitro assay. It seems clear that the medium played no role in splitting the conjugate to the parent drug and steroid. In fact, when the times of preincubation of the conjugate were 0, 24, and 48 h, the activities found were identical. Furthermore, when the conjugate was incubated in the medium and the products were quantitated by UV after separation, no other product was detected except the intact conjugate. In addition, because conjugate **5b** remained 80 to 60% intact during the 24- to 48-h incubation period in mouse plasma, it seems likely the conjugate serves as a prodrug form of *ara*-C or *ara*-CMP against enzymes present in plasma of these mice. Furthermore, it suggests that the parent drug is released possibly mainly at the cellular level rather than the plasma. Further studies will be needed to verify this point.

Studies on responses, toxicities, pharmacokinetics, and metabolism of these more active conjugates in animals and others to evaluate additional advantages of these conjugates, particularly with regard to targeting to specific tissues, are in progress in our laboratory.

Experimental Section

Synthesis. Melting points were determined in capillary tubes using a Mel-Temp apparatus and are uncorrected. UV absorption spectra were obtained on a Beckman Acta V spectrophotometer. IR spectra were recorded on a Perkin-Elmer 297 infrared spectrophotometer. AG1-X8 (formate; Bio-Rad), diethylaminoethylcellulose (DE-52, Whatman) and cellulose powder (CC 31, Whatman) were used for column chromatography. Evaporations

were performed in vacuo at 30 °C. TLC was performed on glass plates coated with a 0.25-mm layer of silica gel PF-254 (Brinkman) and on polygrams silG UV 254 plates (Brinkman), and descending paper chromatography was carried out on Whatman No. 3MM paper using the following solvent systems: (A) *i*-PrOH–H_2O–concentrated NH_4OH (7:2:1) and (B) EtOH–0.5 M NH_4OAc, pH 7.5 (5:2). Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Where analyses are reported only by the element symbols, results were within ±0.4% of the theoretical values.

5′-(11-Deoxycorticosterone-21-phosphoryl)-1-(β-D-arabinofuranosyl)cytosine (5a). Method A. By Condensation. Ac_3-*ara*-CMP (**3**), prepared by acetylation of 1.62 g (5 mmol) of *ara*-CMP with Ac_2O (30 mL) and pyridine (60 mL) as reported previously,[3,4] was stirred with 3.31 g (10 mmol) of 11-deoxycorticosterone and 4.12 g (20 mmol) of DCC in 250 mL of anhydrous pyridine at room temperature for 2 days. Water (10 mL) was then added and the suspension was stirred at room temperature overnight. After evaporating to dryness, the residue was coevaporated with toluene (10 mL) to remove the residual pyridine and treated with 100 mL of 50% EtOH. The insoluble urea was removed by filtration and the filtrate was evaporated to dryness. The residue was stirred in 200 mL of 2 N NH_3–MeOH at room temperature overnight, followed by evaporating to dryness. The residue was then dissolved in 50 mL of 50% EtOH, and the solution was applied to a DE-52 (acetate) column (300 g, 5.5 × 30 cm) prepacked in 50% EtOH. The column was then eluted by a linear gradient of HOAc in 50% EtOH (0 to 2.0 N, 2 L each). The product was eluted out between 1500 and 2200 mL, and the combined eluate was evaporated to dryness. The residue was then treated with Me_2CO. The resulting white solid was filtered and washed with Me_2CO, yielding 1.153 g (36.3%). The analytical sample (as the NH_4 salt) was prepared by passing the product (100 mg) through a cellulose column (30 g, 2.5 × 23 cm) with solvent A as described previously:[10] mp 215–225 °C (slowly dec); TLC R_f (A) 0.58, R_f (B) 0.80; IR (KBr) 3360 (NH_2) 3200, 2930, 1710 (C=O), 1640, 1610 (C=O, C=C, C=N), 1490, 1220 (P=O), 1055 cm^{-1} (POC). *ara*-CMP was recovered (40%) in the subsequent fractions. Table I lists the conjugates prepared in an analogous manner.

Method B. By Direct Route. To a cooled mixture (–10 °C) of 0.973 g (4 mmol) of dried *ara*-C and 25 mL of redistilled $(MeO)_3PO$ was added 0.7 mL (ca. 7.42 mmol) of $POCl_3$. The mixture was stirred at 0–5 °C for 3 h, and then 2.644 g (8 mmol) of dried 11-deoxycorticosterone was added to the clear reaction mixture. The mixture was stirred at 0–5 °C for 5 days, and the clear solution was poured slowly into ice–water (100 mL) containing $NaHCO_3$ (1 g). The resulting suspension was mixed with 95% EtOH to get a 50% EtOH solution, and the insoluble solid was removed by filtration. The filtrate was then neutralized to pH 7.0 with 1 N NaOH and applied to a DE-52 (acetate) column (300 g, 5.5 × 30 cm) prepacked in 50% EtOH. The product was separated out with a linear gradient of AcOH in 50% EtOH (0 to 2.0 N, 2 L each) as described above, yielding 533 mg (21%). The analytical sample (as the NH_4 salt) was prepared as described previously:[10] mp 215–225 °C (slowly dec); TLC R_f (A) 0.58, R_f (B) 0.80; IR (KBr) 3380 (NH_2), 3200, 2940, 1720 (C=O), 1650, 1610 (C=O, C=C, C=N), 1490, 1210 (P=O), 1070 cm^{-1} (POC). Table I lists the conjugate prepared in an analogous manner.

Biochemical Studies. Growth-Inhibiting Assays in Cultured Cells. Compounds shown in Table II were screened for in vitro growth-inhibiting activity against L1210 lymphoid leukemia in culture using the methodology described previously.[3,4,6] In some instances, the conjugate was preincubated in the medium at 37 °C for various lengths of time, and the assays were performed as described previously.

Antitumor Activity in Vivo. Compounds shown in Table III were screened for in vivo antitumor activity against the ascites cell form of L1210 lymphoid leukemia grown in $C_3D_2F_1/J$ female mice (C_3H/HEJ female × DBA/2J male mice, supplied by Jackson Labs) using the methodology described previously.[3,4,7] The mice in groups of six to eight (average weight 25 g) were inoculated ip with 1×10^6 cells of L1210 from donor mice (DBA/2Ha, supplied by Roswell Park Memorial Institute) bearing 3–5 day old tumor cells. Compounds were dissolved first in 0.154 N NaOH and then the solutions were neutralized with 0.154 N HCl (final 0.9% NaCl solution). The dose (0.5 mL) was administered ip daily for 5 consecutive days beginning 24 h after tumor implantation. Antitumor activity was evaluated by the comparison of the median survival time of the treated animals (T) to that of the control animals (C), i.e., the percentage increase in life span (ILS), (T/C – 1) × 100 (%).

Enzymatic Hydrolysis. Enzymatic cleavage of the phosphodiester bond of the conjugates was studied by incubating the compounds (5 μmol) with phosphodiesterase I (EC 3.1.4.1), 5′-nucleotidase (EC 3.1.3.5), acid phosphatase (EC 3.1.3.2), alkaline phosphatase (EC 3.1.3.1), and mouse and human plasmas in appropriate buffer (final volume 1.0 mL) as described previously.[4] Aliquots (0.1 mL) of the incubation mixtures at various lengths of time were streaked on Whatman No. 3 MM paper (23 × 57 cm) or on TLC plates (0.05 × 10 × 20 cm) with authentic markers, followed by developing in solvent A. Each band was eluted with 50% EtOH and quantitated by UV.

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N,N′-Dialkylbis(dichlorophenyl)ethylenediamines and -imidazolidines: Relationship between Structure and Estradiol Receptor Affinity

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Diastereomeric *N,N′*-dialkylbis(dichlorophenyl)ethylenediamines (**2**) and the corresponding imidazolidines (**3**) with chlorine in the 2,4, 2,6, 3,4, and 3,5 positions were synthesized. Only the stereoisomers of the 2,6-dichloro-substituted compounds exhibit for N-CH_3 (**2e**, **3e**), N-C_2H_5 (**2f**, **3f**), and N-C_3H_7 (**2g**, **3g**) an affinity to the estradiol receptor (K_a values ranging from 9.1×10^4 to 9.1×10^6), because the nitrogen atoms are shielded by the ortho-located chlorine atoms; therefore, a binding interaction with hydrophobic receptor areas is possible. These substances show weak uterotrophic activity and no significant effect on the growth of the DMBA-induced hormone-dependent mammary adenocarcinoma of the rat.

Structural modification of the synthetic estrogen hexestrol (**1**) by variation of the hydroxy position[1,2] and the length (C_4–C_{10})[3] and ramification of the alkyl chain[4] yields antiestrogens, which show a marked inhibition on the

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