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Nucleoside Conjugates as Potential Antitumor Agents. 2. Synthesis and Biological Activity of 1-(β-D-Arabinofuranosyl)cytosine Conjugates of Prednisolone and Prednisone[1]

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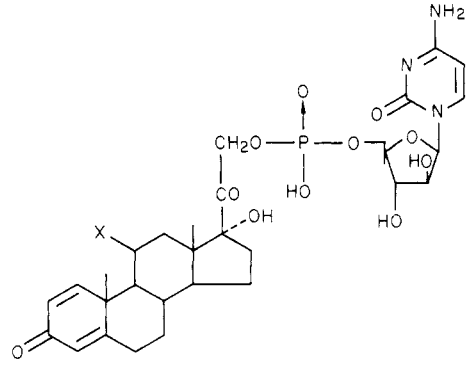
Two of the new anticancer drugs recently synthesized in our laboratory from conjugation of *ara*-C[2] and several corticosteroids linked through a phosphodiester bond include prednisolone- (I) and prednisone-p-*ara*-C (II). They were demonstrated to be enzymatically hydrolyzed to the corresponding steroid and *ara*-CMP and the latter was further shown to be hydrolyzed to *ara*-C by phosphodiesterase I, snake venom, 5'-nucleotidase, and acid phosphatase. However, the conjugates were shown to be resistant to hydrolysis by alkaline phosphatase. The activity of conjugates I and II against L1210 lymphoid leukemia in female mice ($C_3D_2F_1/J$) was significantly greater than that of *ara*-C alone or in combination with the steroid. In fact, when the optimum dosage of 75 (μmol/kg)/day × 5 was used, the administration of *ara*-C alone was followed by an increased life span (ILS) of 45%. This result is similar to that previously reported.[3] With the same equimolar doses of mixtures of *ara*-C and either prednisolone or prednisone, the ILS values were 40 and 44%, respectively. However, when the conjugates were used, the ILS values were 89 and 100%, respectively. These findings seem promising and have provided the bases for continued study of these new compounds.

Because 1-(β-D-arabinofuranosyl)cytosine (*ara*-C) has a specific mode of action at the S phase of the cell cycle[4] and is rapidly deactivated by cytidine deaminase,[5] it requires a very complex and precise dosage schedule to be efficacious. As a result of its rapid degradation, the plasma half-life in patients is generally too short for effective and convenient administration.[6] In an attempt to overcome some of these difficulties, others[3,7-10] have synthesized lipophilic derivatives. For example, *ara*-C 5'-phosphate L-1,2-dipalmitin has recently been developed and reported to show strong therapeutic potential.[10] Furthermore, studies[11] on the use of steroids as covalent carriers of cytotoxic groups, including nucleoside bases,[12] have been presented. The present study was designed to (1) synthesize conjugates of *ara*-C and steroids and (2) to determine the extent to which the favorable response of *ara*-C might be enhanced by the chemical linkage of this compound to steroids. A description of the synthesis and biological activity of conjugates of *ara*-C and two steroids, prednisolone- (I) and prednisone-p-*ara*-C (II), is provided in the present report.

Chemistry. Three chemical reactions were used sequentially to prepare *ara*-C conjugates of prednisolone and of prednisone. The first was the direct phosphorylation of *ara*-C with $POCl_3$ and $(EtO)_3PO$ by a modified method of Yoshigawa et al.[13] and Hong et al.[14] By this method, the yield for *ara*-CMP was 80%. In the next step, the latter compound was acetylated with Ac_2O in the presence of pyridine. The Ac_3-*ara*-CMP prepared demonstrated one spot on TLC, and the mobilities in various solvents were identical with that of the compound prepared by phosphorylation of Ac_3-*ara*-C.[15] The observed UV maxima for the pyridine-free compound were 243 and 290 nm and were near that for Ac_3-*ara*-C (245 and 295 nm). Finally, Ac_3-*ara*-CMP was condensed with 2 molar equiv of either prednisolone or prednisone. This was analogous to the preparation of prednisolone 21-phosphate by condensation of prednisolone with 2-cyanoethyl phosphate in the presence of DCC and pyridine.[16] After these procedures were completed and the protective groups removed, conjugates I and II were separated on a DE-52 column using a HOAc gradient (0–1.5 N). The conjugates were actually eluted out in 1–1.5 N HOAc fractions and the yield for each was 30%. Attempts at condensation of prednisolone 21-phosphate or prednisone 21-phosphate with Ac_3-*ara*-C in the presence of DCC and pyridine at room temperature and at reflux were not successful.



I, X = OH
II, X = =O

Confirmation of the molecular structures of the conjugates was provided by elemental analysis; UV, IR, and NMR spectra; and especially by chemical and enzymatic hydrolyses of the phosphodiester bonds. Hydrolyses of

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Table I. Effects of Compounds on the Viability of L1210 Cells in Culture

compound	concn (μM) for 50% loss of viability at 72 h (ED_{50})
prednisolone-p-*ara*-C (I)	0.05
prednisone-p-*ara*-C (II)	0.5
ara-C	0.1
ara-CMP	0.05
prednisolone	100
prednisone	>100
ara-CMP + prednisolone (5 μM)	0.01
ara-CMP + prednisolone (100 μM)	0.005

prednisolone- and prednisone-*p*-*ara*-C by 0.1 N $Ba(OH)_2$ yielded prednisolone 21-phosphate and *ara*-C and prednisone 21-phosphate and *ara*-C, respectively. These steroid phosphates were further hydrolyzed to the corresponding steroid. When enzymatic hydrolysis was used, the products were the steroid and *ara*-CMP. The latter compound was further hydrolyzed to *ara*-C by 5'-nucleotidase at pH 9.0.

Biological Results. Antiproliferative Activity in Vitro. This was determined for *ara*-C, *ara*-CMP, the steroids, the steroids plus *ara*-CMP, and the conjugates by the measurement of growth inhibition of L1210 lymphoid leukemic cells in culture.[17] The concentration of each compound which resulted in 50% inhibition (ED_{50}) at 72 h was estimated from response curves of three separate experiments[18] as shown in Table I. *ara*-CMP was found to be twice as active as *ara*-C and prednisolone was marginally so, while prednisone was clearly inactive. However, the simple combination of prednisolone and *ara*-CMP was strongly synergistic at concentrations of 100 to 5 μM and 0.01 to 0.005 μM, respectively. Conjugate I was equally as active as *ara*-CMP but conjugate II was less so. These findings have provided the bases for continued study of the effects of these compounds in vivo.

Antitumor Activity in Vivo. In this part of our study, *ara*-C, *ara*-CMP, prednisolone, prednisone, prednisolone plus *ara*-C, prednisone plus *ara*-C, and the conjugates of these steroids and *ara*-C linked by a phosphodiester bond were each tested against L1210 lymphoid leukemia in mice ($C_3D_2F_1/J$) according to the NCI protocol[19] with slight modifications of size of inoculum (1×10^6 cells) and injection schedule (qd, days 1–5). After the abdominal cavity of each mouse was inoculated with 1×10^6 L1210 lymphoid leukemic cells, all nontreated or control animals died between 8 and 10 days. As shown in Table II, the most effective dose for prednisolone- (I) and prednisone-p-*ara*-C (II) was 75 (μmol/kg)/day. Neither prednisolone nor prednisone alone at the same molar dose was active against the tumor cells in mice. When *ara*-C and *ara*-CMP at the same concentrations were used, the mice were found to have an ILS of 45 and 65%, respectively. In simple combination with either steroid, the ILS values found for *ara*-C were nearly the same as when this agent was used alone. However, when conjugates I and II were administered, the ILS values (89 and 100%) were increased by near twofold or more.

Aside from the highly significant activity demonstrated for conjugates I and II against L1210 lymphoid leukemia in mice, average loss of weight of the animals provided preliminary indications that toxicity at the optimum dosage was less than that for *ara*-C or *ara*-CMP. In fact, on the 8th day after the start of administration of optimum dosages of conjugates I and II, average reduction in weight of the mice was about 0.3 g. However, when *ara*-C and *ara*-CMP were given in the same dosage, the decreases in weight found at this same time were more than tenfold greater. Based on our findings for ILS values and for weight loss, it is clear that the conjugates I and II were superior to all other compounds shown in Table II. Further studies are needed to assess more extensively responses and toxicities in other in vivo experimental systems.

Enzymatic Hydrolysis. Enzymatic hydrolysis of prednisolone- (I) and prednisone-p-*ara*-C (II) with purified phosphodiesterase I (EC 3.1.4.1), snake venom, and 5'-nucleotidase (EC 3.1.3.5) from *Crotalus adamanteus* showed that the conjugates were first slowly hydrolyzed to the corresponding steroid and *ara*-CMP. The latter was further hydrolyzed to *ara*-C during a 24-h incubation

Table II. Effects of the Conjugates on Survival Time and Changes in Weights of Mice with Intraperitoneally Implanted L1210 Lymphoid Leukemia[a]

compd	dose,[b] [mg(μmol)/kg]/day × 5	wt change (g/mouse) on day 8	survival time, days		% ILS[d]
			range	median (T/C)[c]	
prednisolone-p-*ara*-C (I)	25 (37.5)	−0.07	11–18	15.5/9.0	72.7
	50 (75.0)	−0.28	15–36	17.0/9.0	88.9
	75 (112.7)	−0.71	14–21	16.0/9.0	77.8
	100 (150.0)	−1.50	9–15	10.5/9.0	16.7
prednisone-p-*ara*-C (II)	25 (37.7)	−0.14	12–17	14.5/9.0	61.1
	50 (75.4)	−0.30	14–20	18.0/9.0	100.0
	75 (113.0	−0.75	14–16	14.0/9.0	55.6
	100 (150.7)	−1.50	9–15	10.0/9.0	11.1
ara-C and prednisolone	18.2 and 27.0 (75.0 each)	−2.46	8–15	14.0/10.0	40.0
ara-C and prednisone	18.3 and 27.0 (75.4 each)	−1.25	12–15	13.0/9.0	44.0
ara-C	18.2 (75.0)	−3.64	10–19	14.5/10.0	45.0
	25 (102.8)	−3.80	9–16	9.5/8.0	18.8
	50 (205.6)	−4.20	8–11	10.0/9.0	11.1
ara-CMP	24.2 (75.0)	−3.52	12–18	16.5/10.0	65.0
	50 (154.7)	−4.32	7–13	9.0/9.0	0
prednisolone	27 (75.0)	−0.85	8–10	9.5/10.0	−5.0
prednisone	27 (75.4)	−1.01	7–12	9.5/10.0	−5.0

[a] Female $C_3D_2F_1/J$ mice in groups of eight (average weight 20 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 40 mice used for the control study was 9.0 ± 1.0 (SD) days and changes in weight 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 were determined as follows: (T/C − 1) × 100.

period at 37 °C and pH 9.0, except when purified phosphodiesterase I was used; only 20% of the *ara*-CMP was hydrolyzed to *ara*-C. Acid phosphatase (EC 3.1.3.2) hydrolyzed 87% of the conjugates during a 24-h incubation period at 25 °C and pH 4.8, and the resulting products were the conjugate (8%), *ara*-CMP (5%), *ara*-C (42%), and the corresponding steroid (45%). Bacteria alkaline phosphatase (EC 3.1.3.1) did not hydrolyze the conjugates during a 24-h incubation period. However, 39% of conjugate I and 15% of conjugate II were hydrolyzed at 37 °C during a 24-h incubation period in plasma of a baboon.

Discussion

Under the conditions of our experimental procedures, conjugates I and II were found to exhibit a substantial increase in the life span (ILS 89 and 100%) in L1210 bearing mice at a dosage of [50 mg (75 μmol)/kg]/day × 5, exceeding the life expectancy produced by *ara*-C, *ara*-CMP, the steroids, and combinations of the steroids and *ara*-C at the same molar dosage (Table II).

Previous work by Evans et al.[20] shows that when *ara*-C·HCl was given to mice at the dosage of (71.7 μmol/kg)/day × 7 the ILS value found was 187.5%. In contrast, when we administered *ara*-C to mice at a dosage of (75 μmol/kg)/day × 5, the ILS value was 45%. This difference cannot be completely explained without additional data. It is noteworthy that we used $C_3D_2F_1/J$ female mice inoculated with 1×10^6 L1210 lymphoid leukemic cells and the mice were treated for only 5 days. Evans et al.[20] reported the use of BDF_1 mice and treated for 7 days. However, the size of inoculum was not presented. It is of further interest that under similar conditions, as in our work, Neil et al.[3] found nearly the same results as we detected. Therefore, it is apparent that differences between the results of Evans et al. and our work is attributable to differences in methodology and biological materials.

The conjugates were found to be resistant to enzymatic hydrolysis by alkaline phosphatase. In serum, alkaline phosphatase is more prevalent in concentration compared to acid phosphatase levels. On the other hand, they were relatively sensitive to phosphodiesterase I, 5′-nucleotidase, and acid phosphatase enzymatic hydrolysis. However, after incubation with baboon plasma at 37 °C for 24 h, 60–85% of the conjugates remained intact. Thus, conjugates of this type might serve as a sustained release form of the parent nucleoside and, therefore, may be rendered more effective antineoplastic agents.

The availability of the conjugate would provide a means to investigate whether compounds of this type improve the therapeutic index of the parent nucleoside clinically and to test the hypothesis that the compounds will be taken up more rapidly by cells and subsequently by hydrolyzed enzymatically intracellularly in a similar manner as shown by the in vitro experiments. Further studies on this point and on conjugates of other nucleoside antitumor agents and steroids are in progress in our laboratory.[21]

Experimental Section

Synthesis. Melting points were determined in capillary tubes using a Mel-Temp apparatus and are uncorrected. UV spectra were recorded with a Beckman Acta V spectrophotometer, and IR absorptions in KBr pellets were determined with a Perkin-Elmer 297 infrared spectrophotometer. NMR spectra were obtained with a Varian XL-100 spectrometer using Me_4Si as an internal standard. AG1-X8 (formate; Bio-Rad), diethylaminoethylcellulose (DE-52, Whatman), and cellulose powder (CC31, Whatman) were used for column chromatography. Evaporations were performed in vacuo at 30 °C. TLC was carried out 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 performed on Whatman No. 3MM paper using the following solvent systems: (A) *i*-PrOH–H_2O–concentrated NH_4OH (7:2:1); (B) EtOAc–*n*-PrOH–H_2O (4:1:2); and (C) EtOH–0.5 M NH_4OAc, pH 7.5 (5:2). Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Where analyses are indicated only by symbols of the elements, the analytical results for those elements were within ±0.4% of the theoretical values unless designated otherwise.

1-(β-D-Arabinofuranosyl)cytosine 5′-Monophosphate. This compound was prepared by previously published procedures[13,14] with some modification. To a cooled mixture (0 °C) of 2 mL (ca. 20 mmol) of $POCl_3$ and 50 mL of $(EtO)_3PO$ was added 2.43 g (10 mmol) of *ara*-C. The mixture was stirred at 0 °C for 18 h and then poured into 500 mL of Et_2O–petroleum ether (bp 37–60 °C) (1:1). The suspension was then extracted with a total 500 mL of H_2O, and the aqueous layer was neutralized to pH 7.0 with concentrated NH_4OH. The aqueous solution was applied to an AG1-X8 (formate) column (100 g, 3 × 45 cm) prepacked in H_2O. The column was eluted with H_2O (1 L) and then with 0.5 N HCO_2H (1 L). The 0.5 N HCO_2H eluate (1 L) was evaporated to dryness and the residue was treated with Me_2CO. The resulting white solid was filtered and washed with Me_2CO: yield 2.56 g (79.2%). The chromatographic mobilities in various solvent systems and the IR spectra are identical with those of the authentic *ara*-CMP. Enzymatic hydrolysis with 5′-nucleotidase (EC 3.1.3.5) gave *ara*-C.

N^4,2′,3′-Triacetyl-1-(β-D-arabinofuranosyl)cytosine 5′-Monophosphate. A mixture of 1.62 g (5 mmol) of dried *ara*-CMP, 30 mL of Ac_2O, and 60 mL of anhydrous pyridine was stirred at room temperature for 18 h and then 20 mL of H_2O was added to the ice-cooled mixture. After stirring the mixture at room temperature for 2 h, it was evaporated to dryness and the syrup was evaporated with pyridine three times to remove the residual H_2O. TLC monitoring of the syrup in solvents A and C showed one spot, and the mobilities were identical with those of the compound prepared by phosphorylation of Ac_3-*ara*-C[15] with $POCl_3$ and $(EtO)_3PO$: TLC R_f (A) 0.19, R_f (C) 0.56. The UV of the pyridine-free compound taken in 50% EtOH showed maxima at 243 and 290 nm. The syrupy compound was employed without further purification.

5′-(Prednisolone-21-phosphoryl)-1-(β-D-arabinofuranosyl)cytosine (I). Ac_3-*ara*-CMP, prepared by acetylation of 1.62 g (5 mmol) of *ara*-CMP with AC_2O (30 mL) and pyridine (60 mL) described as above, was stirred with 3.60 g (10 mmol) of prednisolone 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) and extracted with 100 mL of 50% EtOH. The insoluble urea was filtered and washed with 50% EtOH (20 mL). The combined filtrate was evaporated to dryness, and 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 dissolved in 50 mL of 50% EtOH, and the solution was applied to a DE-52 (acetate) column (200 g, 5 × 25 cm) prepacked in 50% EtOH. The column was then eluted by a linear gradient with 0–1.5 N AcOH in 50% EtOH (1 L each). The eluate between 1250 and 2000 mL was evaporated to dryness and the residue was treated with Me_2CO. The resulting white solid was filtered and washed with Me_2CO: yield 1.03 g (31%). The analytical sample (as the NH_4 salt) was prepared by passing the product (200 mg) through a cellulose column (30 g, 2.5 × 23 cm) with solvent A as described previously:[22] mp 210–220 °C (slowly dec); TLC R_f (A) 0.60, R_f (B) 0.02, R_f (C) 0.72; UV λ_{max} (H_2O) 242 nm (ε 20 300), 260 sh (18 100); UV λ_{max} (0.1 N HCl) 250 nm sh (ε 16 700), 268 (17 900); UV λ_{max} (0.1 N NaOH) 240 nm (ε 19 400), 260 sh (17 100); IR (KBr) 3400 (NH_2), 1740 (C=O), 1657, 1610 (C=O, C=C, C=N), 1220 (P=O), 1080, and 1055 cm^{-1} (POC); NMR (Me_2SO-d_6) δ 0.80 (s, 3, CH_3), 1.40 (s, 3, CH_3), 0.94–2.40 (br m, 13 H), 3.96–5.40 (br m, 13 H), 5.94 (s, 1, C_4 H), 6.07 (d, 1, J = 4 Hz, $C_{1'}$ H), 6.09 (d, 1, J = 8 Hz, cytosine C_5 H), 6.18 (d, 1, J = 10 Hz, C_2 H), 7.37 (d, 1, J = 10 Hz, C_1 H), 8.07 (d, 1, J = 8 Hz, cytosine C_6 H), 9.48 (br s, 2, NH_2). Anal. ($C_{30}H_{39}N_3O_{12}P \cdot NH_4 \cdot 3H_2O$) C, H, N, P.

5'-(Prednisone-21-phosphoryl)-1-(β-D-arabinofuranosyl)cytosine (II). The compound was prepared by condensation of Ac_3-*ara*-CMP, prepared from *ara*-CMP (1.62 g, 5 mmol), with 3.58 g (10 mmol) of prednisone in the presence of 4.12 g (20 mmol) of DCC in 250 mL of anhydrous pyridine and by subsequent removal of the protective groups in 2 N NH_3–MeOH (200 mL) as described above. The product was separated by a DE-52 column (acetate) with a linear gradient of AcOH (0 to 1.5 N) in 50% EtOH (1 L each) as described above. The eluate between 1300 and 2000 mL was evaporated to dryness, and the residue was treated with Me_2CO. The white solid was filtered and washed with Me_2CO: yield 0.95 g (28.6%). The analytical sample (as the NH_4 salt) was prepared as described previously:[22] mp 210–220 °C (slowly dec), TLC R_f (A) 0.62, R_f (B) 0.02, R_f (C) 0.70; UV λ_{max} (H_2O) 238 nm (ε 21 800), 260 sh (17 700); UV λ_{max} (0.1 N HCl) 244 nm (ε 16 600), 268 (16 600); UV λ_{max} (0.1 N NaOH) 236 nm (ε 21 400), 260 sh (16 700); IR (KBr) 3300 (NH_2), 1720, 1705 (C=O), 1655, 1610 (C=O, C=C, C=N), 1220 (P=O), 1080 and 1040 cm^{-1} (POC); NMR (Me_2SO-d_6) δ 0.52 (s, 3, CH_3), 1.38 (s, 3, CH_3), 0.62–2.30 (br m, 13 H), 3.95–5.36 (br m, 11 H), 5.91 (d, 1, J = 8 Hz, cytosine C_5 H), 6.04 (s, 1, C_4 H), 6.06 (d, 1 J = 4 Hz, $C_{1'}$ H), 6.12 (d, 1, J = 10 Hz, C_2 H), 7.64 (d, 1, J = 10 Hz, C_1 H), 7.88 (d, 1, J = 8 Hz, cytosine C_6 H), 8.48 (br s, 2, NH_2). Anal. ($C_{30}H_{37}N_3O_{12}P \cdot NH_4 \cdot 2H_2O$) C, H, N, P.

Hydrolysis of the Conjugates with $Ba(OH)_2$. The conjugate (0.01 mmol) and $Ba(OH)_2$ (0.1 mmol) in H_2O (1 mL) were heated at 90–95 °C in a stoppered vial. The clear solution which resulted on warming turned cloudy as the reaction proceeded. Aliquots (0.1 mL) were removed at the designated time, treated with Dowex 50 (H^+) resin, and examined chromatographically in solvents A and C. Hydrolysis of the conjugate was completed in 5 min and the products were the steroid 21-phosphate and *ara*-C. After standing at room temperature overnight, the phosphate in the hydrolysate was further hydrolyzed to the corresponding steroid.

Biochemical Studies. Growth Inhibitory Assays in Cultured Cells. Cultured cells derived from mouse lymphoid leukemia L1210 were used for the determination of growth inhibitory properties of the compounds shown in Table I. The compounds were dissolved in Me_2SO and allowed to remain at 37 °C overnight to achieve sterilization. Sterile growth medium (RPMI no. 1640 + 10% fetal calf serum) was then added to bring the final concentration of Me_2SO to 0.5% and those of the compounds to 100 μM or lower. Cells of stock cultures were inoculated into sterile test tubes containing the growth medium and the compounds in a density previously determined to initiate logarithmic growth (2–3 × 10^5 cells/mL). At 24-h intervals, 0.2-mL samples were removed and mixed with Trypan blue to a final concentration of 0.05%, and the number of viable cells, as determined by exclusion of the dye, were counted in a hemocytometer. After 24, 48, and 72 h, viable cell counts were performed on cultures incubated with each compound or mixture as shown in Table I. Viable cell density in the presence of the compound was divided by viable cell density in the absence of the compound, and the concentration of the compound required to produce 50% inhibiting of growth (ED_{50}) after 72 h was determined by interpolation[18] (Table I).

Antitumor Activity of Mice. 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) was employed. The assay was performed according to NCI protocol[19] with slight modifications. Intraperitoneal implantation of 1 × 10^6 cells in 0.5 mL of suspension to the control and the treated groups (each eight $C_3D_2F_1$/J female mice, average wt 20 g) was carried out using donor mice (DBA/2Ha, supplied by Roswell Park Memorial Institute) bearing 3–5 day old tumor cells. Compounds were dissolved in 0.9% NaCl, and a dose (0.5 mL) was administered ip daily. Treatment began 24 h after implantation for 5 consecutive days. Control animals received a 0.5-mL injection of saline. Testing was followed as described in the protocol. 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 (%), as shown in Table II.

Enzymatic Hydrolysis. Enzymatic cleavage of the phosphodiester bond of the conjugates I and II was studied by incubating the compounds with the enzymes in a manner described as follows: 0.2-mL of the 24-h incubation mixtures (0.5 mL) was streaked on Whatman 3MM paper (23 × 57 cm) with authentic markers and this was developed in solvent A. Each band was eluted with H_2O and quantitated by UV.

(a) Phosphodiesterase I (EC 3.1.4.1). To 5 μmol of the conjugate in a total volume of 0.4 mL, consisting of 0.05 mL of 1 M Tris-HCl buffer (pH 8.8) and 0.35 mL of H_2O, was added 1.5 mg of phosphodiesterase I from *Crotalus adamanteus* (Sigma Chemical Co.) in 0.1 mL of H_2O. This mixture was incubated at 37 °C for 24 h. Both conjugates were hydrolyzed completely, the products being *ara*-CMP (40%), *ara*-C (10%), and prednisolone or prednisone (50%).

(b) Snake Venom. To 5 μmol of the conjugate in a total volume of 0.4 mL, consisting of 0.05 mL of 1 M Tris-HCl buffer (pH 8.8) and 0.35 mL of H_2O, was added 2.5 mg of crude *Crotalus adamanteus* venom (Sigma Chemical Co.) in 0.1 mL of H_2O, and the mixture was incubated at 37 °C for 24 h. Both conjugates were hydrolyzed completely, the products being *ara*-C (50%) and the steroid (50%).

(c) 5'-Nucleotidase (EC 3.1.3.5). To 5 μmol of the conjugate in a total of 0.4 mL, consisting of 0.05 mL of 1 M Tris-HCl (pH 9.0), and 0.05 M $MgSO_4$ and 0.35 mL of H_2O, was added 2 mg of purified 5'-nucleotidase from *Crotalus adamanteus* (Sigma Chemical Co.) in 0.1 mL of H_2O, and the mixture was incubated for 24 h. Both conjugates were hydrolyzed completely, the products being *ara*-C (50%) and the steroid (50%).

(d) Acid Phosphatase (EC 3.1.3.2). To 5 μmol of the conjugate in a total volume of 0.4 mL, consisting of 0.05 mL of 0.15 M NaOAc (pH 4.8) and 0.35 mL of H_2O, was added 3 mg of crude wheat germ acid phosphatase (Sigma Chemical Co.) in 0.1 mL of H_2O, and the mixture was incubated at 25 °C for 24 h. Prednisolone-p-*ara*-C (I) was 86% hydrolyzed, the final mixture being conjugate I (8%), *ara*-CMP (5%), *ara*-C (42%), and prednisolone (45%). Prednisone-p-*ara*-C (II) was 88% hydrolyzed to conjugate II (7%), *ara*-CMP (4%), *ara*-C (43%), and prednisone (46%).

(e) Alkaline Phosphatase (EC 3.1.3.1). To 5 μmol of the conjugate in a total volume of 0.4 mL, consisting of 0.05 mL of 1 M Tris-HCl (pH 8.0) and 0.35 mL of H_2O, was added 0.1 mL of suspension of bacterial alkaline phosphatase (Worthington Biochemical Corp), and the mixture was incubated at 25 °C for 24 h. Neither of the conjugates was hydrolyzed.

(f) Plasma. The conjugate (5 μmol) was incubated with fresh baboon plasma (0.5 mL) at 37 °C for 24 h. Prednisolone-p-*ara*-C (I) was 39% hydrolyzed, the final mixture being conjugate I (44%), *ara*-CMP (7%), *ara*-U (21%), and prednisolone (28%). However, prednisone-p-*ara*-C (II) was 15% hydrolyzed, the final mixture being conjugate II (74%), *ara*-CMP (13%), and prednisone (13%).

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References and Notes

(1) This work has been presented in part at the 176th National Meeting of the American Chemical Society, Miami Beach, Fla., Sept 1978, Abstract MEDI 39. Presented in part in *Proc. Am. Assoc. Cancer Res.*, abstract 378 (1979).
(2) Abbreviations used are: *ara*-C, 1-(β-D-arabinofuranosyl)-cytosine; *ara*-CMP, 1-(β-D-arabinofuranosyl)cytosine 5'-monophosphate; Ac_3-*ara*-C, N^4,2',3'-triacetyl-1-(β-D-arabinofuranosyl)cytosine; Ac_3-*ara*-CMP, N^4,2',3'-triacetyl-1-(β-D-arabinofuranosyl)cytosine 5'-monophosphate; DCC, *N*,*N'*-dicyclohexylcarbodiimide; prednisolone-p-*ara*-C, 5'-(prednisolone-21-phosphoryl)-1-(β-D-arabinofuranosyl)cytosine; prednisone-p-*ara*-C, 5'-(prednisone-21-phosphoryl)-1-(β-D-arabinofuranosyl)cytosine; *ara*-U, 1-(β-D-arabinofuranosyl)uracil; ip, intraperitoneally.
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Book Reviews

Applications of High Performance Liquid Chromatography. By A. Pryde and M. T. Gilbert. Halsted Press (Wiley), New York. 1979. xii + 255 pp. 16 × 24 cm. $29.95.

As the field of high-performance LC has broadened and diversified in its applicability to research problems, it has evolved from a subdiscipline populated by analytical chemists to a powerful research tool applicable to a variety of problems. Of particular significance is that its use profile has changed dramatically in the last 3 or 4 years. Formerly, LC was promoted as a method complimentary to GC and TLC, one that would allow the investigation of nonvolatile or thermally labile substances not suitable for the more commonly used methods. Recently, LC analysis has become a viable and highly competitive alternative to the other methods that also accomplish the analysis satisfactorily. Needless to say, this change is of remarkable consequence to the practicing scientist, and its importance is in part reflected by the recent increase in the number of instrument manufacturers offering LC hardware. Also reflecting this "coming of age" is the increase in the number of texts and monographs devoted to the subject of high-performance LC.

The present work seems to represent an extension of an earlier book, "Introduction to High Performance Liquid Chromatography", by Hamilton and Sewell, reviewed in the July, 1979, issue of this journal. Both the former work and the one considered here are published in Europe by Chapman and Hall (London), and both seem to be products of a consistent editorial direction. The current book has an obviously more applied orientation, as reflected in the title.

The book is divided into five major parts, four of which are concerned exclusively with applications. The first part, entitled "Theory and Practice of HPLC", uses most of its 53 pages to describe the equipment and modes used in chromatography, as well as several practical tips on how to accomplish an analysis. Only 7 pages are devoted to theory per se, and those consist mostly of definitions of parameters. The remaining sections are devoted to "Pharmaceutical Analysis", "Biochemical Analysis", "Environmental Analysis", and "Miscellaneous Applications", in that order. In the section on "Pharmaceutical Analysis" (54 pages), coverage is devoted to several major classes of drugs and chemotherapeutic agents, and when possible separate discussions occur for the analysis of dosage forms and the analysis of metabolites and biologic fluid. The "Biochemical Analysis" section (59 pages) essentially covers analysis of endogenous materials, with chapters on lipids, steroids, carbohydrates, biogenic amines and proteins, nucleotides and related substances, vitamins, and several other categories. Obviously, to cover such a wide range of applications in the space provided, detail is somewhat limited, but this is not a deficiency due to the copious use of references. The "Environmental Analysis" section (20 pages) consists of one chapter devoted to pesticides, carcinogens, and industrial pollutants, with most of the examples and discussion focusing on the first two. In the "Miscellaneous Applications" section (12 pages), topics such as plant products, food products, inorganic analysis, and optical resolution receive very brief coverage.

As mentioned above, the book makes extensive use of references, with some 876 included through Spring, 1977. Considering the monumental task of compiling and organizing the material, the authors are to be commended for the creation of a work that will be useful to both neophytes and established researchers. Inexperienced users will find guidance both on how to begin and on the types of separations reported by others, while experienced users will find the work a useful literature review for reference. The only shortcoming of the book is that it is a victim of the very characteristic that has made the field so impressive—the rapidity of its development. In this regard, it is not up to date, but it is probably impossible to produce a hard-cover, fully typeset, and illustrated book such as this one in much shorter time. With this fact in mind, it is a book worthy of inclusion in the libraries of those who are working in, or hope to be working in, the area of high-performance LC.

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Biological/Biomedical Applications of Liquid Chromatography. Edited by Gerald L. Hawk. Marcel Dekker, New York. 1979. xv + 736 pp. 15.5 × 23.5 cm. Swiss francs 106.00.

This book contains the contributions of 36 groups who presented papers at the First Liquid Chromatography Symposium, held in Oct 1977 under the auspices of the Waters Co. With such a wide group of contributions, it is not unexpected that the quality of the various papers varied from excellent to mediocre. Topics covered included applications of high-performance LC to prostaglandin analysis, the analysis of proteins and peptides, nucleotides, pteridines, cancer chemotherapy, and antibiotics. The drawbacks of this book are (1) it was to some extent, although not exclusively, restricted to the use of Waters instrumentation; (2) readers will seek in vain for advances in the state of the art. The papers were presented essentially from the viewpoint of individuals using state-of-the-art instrumentation of the 1974 to