Solubility Behavior of Phenolic Compounds in Hexane–Ethyl Acetate, Hexane–Ethyl Myristate, and Hexane–Ethyl Pivalate Cosolvent Systems

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Received February 1, 1980, from the *School of Pharmacy, Kobe-Gakuin University, Tarumi-ku, Kobe, Japan,* ‡*Takeda Chemical Industries, Ltd., Tokyo, Japan,* and the §*College of Dentistry and the* ¶*College of Pharmacy, University of Kentucky, Lexington, KY 40506.* Accepted for publication March 19, 1981.

Abstract □ Interactions of phenolic compounds 4-hexylresorcinol and 3,4-dimethylphenol with esters were studied using hexane–ester cosolvent systems by both phase solubility and partitioning methods. The data obtained by the phase solubility method were variable and could not be analyzed by any mathematical model. The data obtained by the partitioning method, however, strongly suggest that 4-hexylresorcinol forms 1:1 and 1:2 complexes with the esters in hexane, while 3,4-dimethylphenol forms only 1:1 complexes with the same esters.

Keyphrases □ Phenolic compounds—interaction with esters using hexane–ester cosolvent systems, phase solubility and partitioning methods □ Phase solubility—interaction of phenolic compounds with esters □ 4-Hexylresorcinol—formation of 1:1 and 1:2 complexes with esters in hexane □ 3,4-Dimethylphenol—formation of 1:1 complexes with esters

In a previous investigation (1), the permeability coefficient of 4-hexylresorcinol through an ethylene–vinyl acetate copolymer membrane varied in a nonlinear fashion as a function of the vinyl acetate content of the copolymers. In the same study, the partition coefficient of the drug in the membrane varied nonlinearly as a function of the vinyl acetate content. The results were rationalized on the basis that the dihydroxy compound forms 1:1 and 1:2 complexes with the vinyl acetate portion of the copolymers.

A study was undertaken to determine the solubility behavior of 4-hexylresorcinol in hexane–ethyl acetate cosolvent systems; hexane and ethyl acetate represent the polyethylene and the vinyl acetate portions of the copolymer, respectively. Additional data in other cosolvent systems, hexane–ethyl myristate and hexane–ethyl pivalate, also were studied. To obtain further insight on the mechanism of interaction of 4-hexylresorcinol in the described cosolvent systems, the solubility of a monohydroxy compound, 3,4-dimethylphenol, also was studied.

EXPERIMENTAL

Materials—4-Hexylresorcinol[1] and 3,4-dimethylphenol[2] were crystallized from hot benzene. Ethyl myristate, ethyl acetate, and ethyl pivalate, reagent grades, were purified by distillation.

Phase Solubility Study—To each 1.0 g of the phenols placed in respective 10-ml volumetric flasks, a 10-ml solution containing 1.5–30 × 10^{-2} *M* of the esters in hexane was added. The flasks were shaken for 48 hr in a water bath shaker at 25°. The solutions were centrifuged, exactly 1 ml of supernate was pipetted into a volumetric flask, and the volume was completed to the mark with hexane. Further dilutions were carried out when necessary to obtain an absorbance range within the Beer's law region. The absorbance of the solutions was determined spectrophotometrically[3] at 277.5 nm. The solubilities of the phenols were calculated using a standard curve constructed for them in hexane. This procedure was previously reported (2).

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Partitioning Study—Two hundred milligrams of the phenol was dissolved in 1 liter of phosphate buffer (0.1 *M*, pH 7.5), and exactly 5 ml of the aqueous solution was pipetted into a 15-ml test tube with a stopper. Exact volumes (between 2 and 5 ml) of the solutions containing 1.5–30 × 10^{-2} *M* of the esters in hexane were added to the test tubes, which then were shaken for 10 min. After the test tubes were centrifuged, the aqueous layer was taken and the absorbance of the solution was determined spectrophotometrically without dilution at 277.5 nm. The partition coefficients (*PC*) were calculated from the change in UV absorbance at 277.5 nm in the aqueous layer before and after the partition:

$$PC = \frac{(\text{volume of aqueous layer})(A_0 - A)}{(\text{volume of organic layer})(A)} \qquad \text{(Eq. 1)}$$

where $A_0 - A$ is the absorbance change in the aqueous layer and A is the absorbance in the aqueous layer.

RESULTS AND DISCUSSION

Tables I, II, and III show the total solubility of 4-hexylresorcinol as a function of the ethyl acetate, ethyl myristate, and ethyl pivalate concentrations in hexane, respectively. Figure 1 shows the solubility diagrams of the drug in the three cosolvent systems and demonstrates that the solubility increased in a nonlinear fashion as a function of the added esters and that the solubility behavior was different in the three cosolvent systems.

To characterize these systems mathematically, it was first assumed that 4-hexylresorcinol forms 1:1 and 1:2 complexes with esters according

Table I—Solubility of 4-Hexylresorcinol in Ethyl Acetate–Hexane

Concentration of Total Ethyl Acetate in Hexane, × 10^2 *M*	Total Solubility of 4-Hexylresorcinol, × 10^3 *M*
0	4.5
2.27	4.5
4.54	—
6.80	5.7
9.08	4.9
11.4	5.2
22.7	410
34.1	779
45.4	820

Table II—Solubility of 4-Hexylresorcinol in Ethyl Myristate–Hexane

Concentration of Total Ethyl Acetate in Hexane, × 10^2 *M*	Total Solubility of 4-Hexylresorcinol, × 10^3 *M*
0	4.5
0.61	5.7
1.22	6.5
1.84	7.7
2.45	6.9
3.01	12.3
6.14	40.2
9.21	48.7
12.28	82.8
30.7	362

0022-3549/81/1100-1225$01.00/0
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Table III—Solubility of 4-Hexylresorcinol in Ethyl Pivalate–Hexane

Concentration of Total Ethyl Pivalate in Hexane, $\times 10^2$ M	Total Solubility of 4-Hexylresorcinol, $\times 10^3$ M
1.53	4.7
3.06	4.7
4.59	5.2
6.12	5.2
7.7	6.3
15.3	153
23.0	—
30.6	291

to Eqs. 2 and 3 (3, 4):

$$HR_0 + E_0 = HR - E \quad \text{(Eq. 2)}$$

$$HR_0 + 2E_0 = HR - E_2 \quad \text{(Eq. 3)}$$

$$K_{1:1} = \frac{[HR - E]}{[HR_0][E_0]} \quad \text{(Eq. 4)}$$

$$K_{1:2} = \frac{[HR - E_2]}{[HR_0][E_0]^2} \quad \text{(Eq. 5)}$$

where $[HR_0]$, $[E_0]$, $[HR - E]$, and $[HR - E_2]$ represent the concentrations of free 4-hexylresorcinol, free ester, the 1:1 complex, and the 1:2 complex, respectively.

The total concentration of 4-hexylresorcinol, $[HR_T]$, and the total concentration of the ester, $[E_T]$, can be written according to:

$$[HR_T] = [HR_0](1 + K_{1:1}[E_0] + K_{1:2}[E_0]^2) \quad \text{(Eq. 6)}$$

$$[E_T] = [E_0] + K_{1:1}[E_0][HR_0] + 2K_{1:2}[E_0]^2[HR_0] \quad \text{(Eq. 7)}$$

Elimination of $[E_0]^2$ from Eq. 7 by using Eq. 6 gives:

$$[E_0] = \frac{1}{1 - K_{1:1}[HR_0]}\{[E_T] - 2([HR_T] - [HR_0])\} \quad \text{(Eq. 8)}$$

The combination of Eqs. 6 and 8 to eliminate $[E_0]$ gives:

$$[HR_T] = [HR_0] + \frac{K_{1:1}[HR_0]}{1 - K_{1:1}[HR_0]}\{[E_T] - 2([HR_T] - [HR_0])\} + \frac{K_{1:2}[HR_0]}{(1 - K_{1:1}[HR_0])^2}\{[E_T] - 2([HR_T] - [HR_0])\}^2 \quad \text{(Eq. 9)}$$

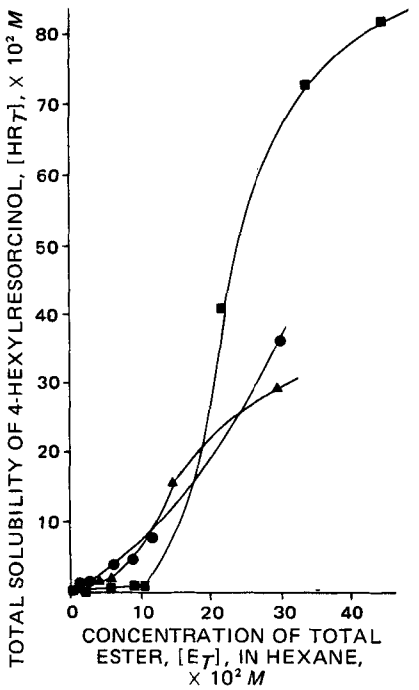

Figure 1—*Plot of the total solubility of 4-hexylresorinol, $[HR_T]$, as a function of the total ester concentration $[E_T]$, in hexane at 25°. Key: ■, ethyl acetate; ●, ethyl myristate; and ▲, ethyl pivalate.*

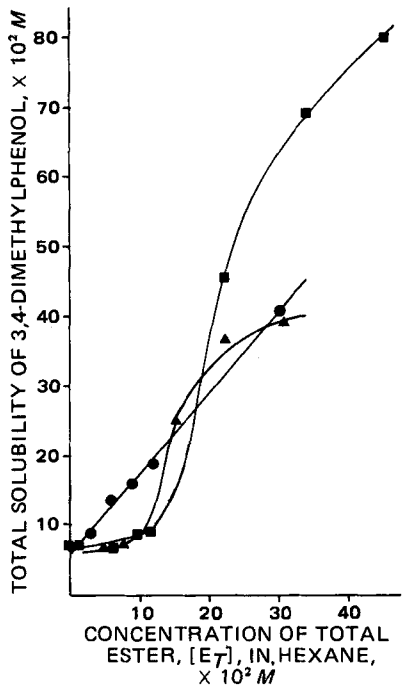

Figure 2—*Plot of the total solubility of 3,4-dimethylphenol as a function of the total ester concentration, $[E_T]$, in hexane at 25°. Key: ■, ethyl acetate; ●, ethyl myristate; and ▲, ethyl pivalate.*

Bringing $[HR_0]$ to the left side of Eq. 9 and dividing both sides of Eq. 9 by $[E_T] - 2([HR_T] - [HR_0])$ gives:

$$\frac{[HR_T] - [HR_0]}{[E_T] - 2([HR_T] - [HR_0])} = \frac{K_{1:1}[HR_0]}{1 - K_{1:1}[HR_0]} + \frac{K_{1:2}[HR_0]}{(1 - K_{1:1}[HR_0])^2}\{[E_T] - 2([HR_T] - [HR_0])\} \quad \text{(Eq. 10)}$$

A plot of the left-hand side of Eq. 10 *versus* $[E_T] - 2([HR_T] - [HR_0])$ should give a straight line with a positive intercept. However, the phase solubility data plotted according to Eq. 10 gave a negative intercept value, and the concentration of the free ester, $[E_0]$, calculated from Eq. 8 also gave a negative value. This result was evident from the data in Tables I–III where at 30×10^{-2} M esters in hexane, the total solubility of 4-hexylresorcinol was equivalent or slightly greater than that of the ester added.

Other assumptions based on the formation of dimer or higher order complexes failed to explain the solubility behavior of 4-hexylresorcinol in these systems. Similarly, the phase solubility diagrams of 3,4-di-

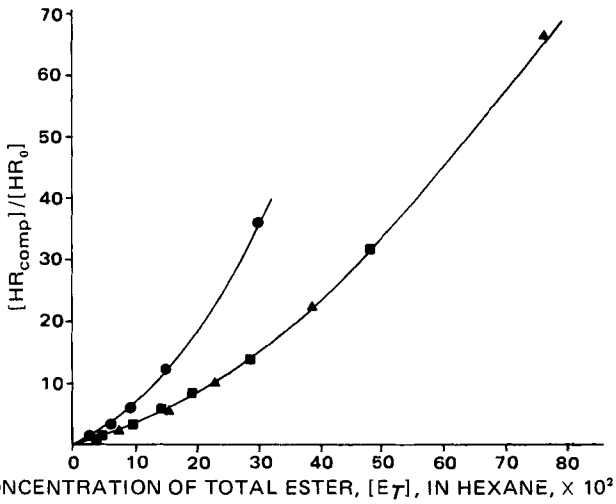

Figure 3—*Plot of $[HR_{comp}]/[HR_0]$ as a function of the total ester concentration, $[E_T]$, in hexane ($[HR_{comp}] = [HR_T] - [HR_0]$). Key: ■, ethyl acetate; ●, ethyl myristate; and ▲, ethyl pivalate.*

Table IV—Extent of Complex Formation between 4-Hexylresorcinol and Ethyl Acetate in Hexane Determined by Partitioning Study

Concentration of Total Ethyl Acetate, $\times 10^2$ M	Partition Coefficient	$\frac{[HR_{comp}]}{[HR_0]}$
0	0.754	0
4.80	1.97	1.48
9.61	3.46	3.36
14.4	5.57	6.03
19.2	7.55	8.52
28.5	12.1	14.2
48.1	25.7	31.5

Table V—Extent of Complex Formation between 4-Hexylresorcinol and Ethyl Myristate in Hexane Determined by Partitioning Study

Concentration of Total Ethyl Myristate, $\times 10^2$ M	Partition Coefficient	$\frac{[HR_{comp}]}{[HR_0]}$
0	0.754	0
1.53	1.33	0.674
3.07	1.90	1.40
6.14	3.45	3.35
9.21	5.48	5.91
15.0	10.7	12.4
30.1	29.5	36.2

Table VI—Extent of Complex Formation between 4-Hexylresorcinol and Ethyl Pivalate in Hexane Determined by Partitioning Study

Concentration of Total Ethyl Pivalate, $\times 10^2$ M	Partition Coefficient	$\frac{[HR_{comp}]}{[HR_0]}$
0	0.754	0
3.84	1.56	0.962
7.68	2.66	2.361
15.4	5.26	5.63
23.0	9.23	10.6
38.4	18.6	22.5
76.4	54.3	66.6

methylphenol were different in the three cosolvent systems (Fig. 2). It is known that the monohydroxy compound forms a 1:1 complex with esters. If that is the case, then one should observe a linear increase in the total solubility of the phenol as a function of added esters in hexane. In Fig. 2, the increase in the solubility of 3,4-dimethylphenol as a function of added esters in hexane is nonlinear for ethyl pivalate and ethyl acetate and appears to be linear for ethyl myristate.

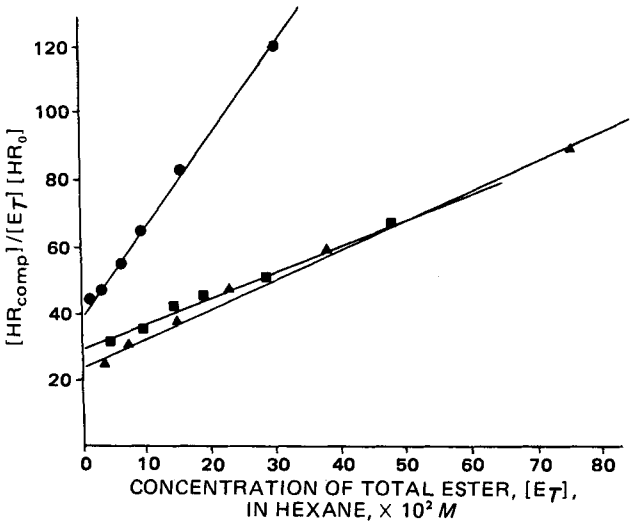

Figure 4—*Plot of $[HR_{comp}]/[HR_0][E_T]$ as a function of the total ester concentration, $[E_T]$, in hexane according to Eq. 16 ($[HR_{comp}] = [HR_T] - [HR_0]$). Key: ■, ethyl acetate; ●, ethyl myristate; and ▲, ethyl pivalate.*

Further examination of the equilibrated systems showed that, in addition to the supernatant and crystalline phases, an oily third phase existed. It was assumed that the abnormalities in the phase solubility diagrams of 4-hexylresorcinol and 3,4-dimethylphenol were due to the formation of a eutectic phase between the low-melting-point phenols and the esters. It is known that low-melting-point phenols form a eutectic mixture upon their interaction with a low-melting-point proton acceptor.

The irreproducibility of the phase solubility data of 4-hexylresorcinol led to a study to characterize the nature of the complexes formed using partitioning methods. The data obtained for 4-hexylresorcinol in the three cosolvent systems are shown in Tables IV–VI. The concentration of free 4-hexylresorcinol, $[HR_0]$, in hexane was obtained as follows:

$$[HR_0] = (PC \text{ at } 0\% \text{ ester})\,[HR_{H_2O}] \qquad \text{(Eq. 11)}$$

where PC at 0% ester is the partition coefficient of 4-hexylresorcinol at zero ester concentration, $[HR_{H_2O}]$ is the aqueous phase concentration, and the concentration of 4-hexylresorcinol in the complex is equal to $[HR_T] - [HR_0]$. A plot of $([HR_T] - [HR_0])/[HR_0]$ *versus* added ester is shown in Fig. 3 for ethyl acetate, ethyl myristate, and ethyl pivalate. As seen in Fig. 3, the increase in the total drug concentration as a function of added esters showed a positive curvature. The ratio of the free form, $[HR_0]$, and the complex form of 4-hexylresorcinol, $[HR_{comp}]$, in the organic layer is:

$$\frac{[HR_{comp}]}{[HR_0]} = \frac{[HR_T] - [HR_0]}{[HR_0]} \qquad \text{(Eq. 12)}$$

$[HR_T] - [HR_0]$ is given from Eq. 6; *i.e.*,

$$[HR_T] - [HR_0] = K_{1:1}[E_0][HR_0] + K_{1:2}[HR_0][E_0]^2 \qquad \text{(Eq. 13)}$$

Substituting the $[HR_T] - [HR_0]$ value from Eq. 12 in Eq. 13 gives:

$$\frac{[HR_{comp}]}{[HR_0]} = K_{1:1}[E_0] + K_{1:2}[E_0]^2 \qquad \text{(Eq. 14)}$$

Dividing both sides of Eq. 14 by $[E_0]$ gives:

$$\frac{[HR_{comp}]}{[HR_0][E_0]} = K_{1:1} + K_{1:2}[E_0] \qquad \text{(Eq. 15)}$$

Since the partitioning study used very dilute solutions of 4-hexylresorcinol, the concentrations of the complexes also are fairly small. Even if 100% of 4-hexylresorcinol complexes with esters, it is reasonable to replace the concentration of the free ester, $[E_0]$, by the concentration of the total ester concentration, $[E_T]$:

$$\frac{[HR_{comp}]}{[HR_0][E_T]} = K_{1:1} + K_{1:2}[E_T] \qquad \text{(Eq. 16)}$$

A plot of $[HR_{comp}]/[HR_0][E_T]$ *versus* $[E_T]$ provides a straight line with a slope of $K_{1:2}$ and an intercept of $K_{1:1}$. The experiments were repeated

Table VII—Stability Constants of 4-Hexylresorcinol and 3,4-Dimethylphenol in Hexane–Ester Systems

Ester	4-Hexylresorcinol $K_{1:1}$[a], M^{-1}	4-Hexylresorcinol $K_{1:2}$[b], M^{-2}	3,4-Dimethylphenol, $K_{1:1}$, M^{-1}
Ethyl acetate	28	80	9.24
Ethyl pivalate	21	100	9.18
Ethyl myristate	40	266	15.5

[a] Stability constant of 1:1 complex. [b] Stability constant of 1:2 complex.

Table VIII—Extent of Complex Formation between 3,4-Dimethylphenol and Ethyl Acetate Determined by Partitioning Study

Total Concentration of 3,4-Dimethylphenol, $\times 10^2$ M	Partition Coefficient	$\frac{[DMP_{comp}]}{[DMP_0]}$
0	1.37	—
4.80	1.96	0.44
9.16	2.53	0.85
19.23	3.86	1.83
28.48	5.00	2.67
48.07	7.60	4.57

Table IX—Extent of Complex Formation between 3,4-Dimethylphenol and Ethyl Myristate Determined by Partitioning Study

Total Concentration of 3,4-Dimethylphenol, $\times 10^2 M$	Partition Coefficient	$\frac{[DMP_{comp}]}{[DMP_0]}$
0	1.37	—
1.53	1.73	0.27
3.07	1.96	0.43
6.14	2.61	0.91
9.21	3.31	1.43
15.03	4.57	2.35
30.00	7.94	4.83

for various cosolvent systems, and each showed a straight-line relationship between the two parameters (Fig. 4). The stability constants, $K_{1:1}$ and $K_{1:2}$, for each ester were calculated from Fig. 4 and are shown in Table VII. It is evident from these results that 4-hexylresorcinol forms not only 1:1 but also 1:2 complexes with esters in hexane. The stability constant values obtained for ethyl myristate were somewhat higher than those for the other esters. This result is probably due to the fact that ethyl myristate has a larger hydrocarbon chain, which results in a better interaction with the hydrophobic portion of phenols.

To ascertain whether the formation of 1:2 complexes is due to the involvement of the two hydroxy groups of 4-hexylresorcinol, the partitioning study was repeated with 3,4-dimethylphenol. The data obtained from this study are shown in Tables VIII–X. A plot of $[DMP_{comp}]/[DMP_0][E_T]$ *versus* the total ester concentration is shown in Fig. 5; $[DMP_{comp}]$ is the concentration of 3,4-dimethylphenol in the complex form, and $[DMP_0]$ is the concentration of the free form.

Table X—Extent of Complex Formation between 3,4-Dimethylphenol and Ethyl Pivalate Determined by Partitioning Study

Total Concentration of 3,4-Dimethylphenol, $\times 10^2 M$	Partition Coefficient	$\frac{[DMP_{comp}]}{[DMP_0]}$
0	1.37	—
3.82	1.78	0.30
7.68	2.33	0.70
15.36	3.33	1.44
23.04	4.32	2.17
38.40	6.63	3.86

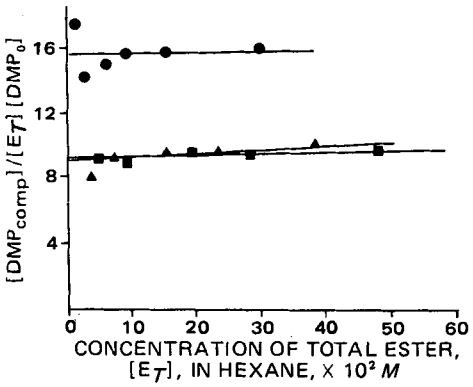

Figure 5—*Plot of $[DMP_{comp}]/[DMP_{free}][E_T]$ as a function of the total ester concentration, $[E_T]$, in hexane; $[DMP_{comp}]$ and $[DMP_{free}]$ represent the concentrations of complexed and free forms of 3,4-dimethylphenol, respectively. ($[DMP_{comp}] = [DMP_T] - [DMP_0]$). Key: ■, ethyl acetate; ●, ethyl myristate; and ▲, ethyl pivalate.*

As seen in Fig. 5, the monohydroxy compound forms only a 1:1 complex with the esters. The stability constants calculated from Fig. 5 are given in Table VII. The results of this study substantiate the conclusion that the diffusion of 4-hexylresorcinol through ethylene–vinyl acetate copolymers involved the formation of 1:1 and 1:2 complexes between the drug and the vinyl acetate portion of the copolymers.

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High-Performance Liquid Chromatographic Analysis of Chemical Stability of 5-Aza-2′-deoxycytidine

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Received December 17, 1979, from the *Centre de Recherche Pédiatrique, Hôpital Sainte-Justine, and the ‡Département de Pharmacologie, Université de Montréal, Montréal, Québec, Canada H3T 1C5. Accepted for publication March 31, 1981.

Abstract □ The chemical stability of 5-aza-2′-deoxycytidine (I) in acidic, neutral, and alkaline solutions was analyzed by high-performance liquid chromatography. In alkaline solution, I underwent rapid reversible decomposition to *N*-(formylamidino)-*N*′-β-D-2-deoxyribofuranosylurea (II), which decomposed irreversibly to form 1-β-D-2′-deoxyribofuranosyl-3-guanylurea (III). The pseudo-first-order rate constants for this reaction were determined. The decomposition of I in alkaline solution was identical to that reported previously for the related analog, 5-azacytidine. However, in neutral solution (or water), there was a marked difference in the decomposition of I and 5-azacytidine. The same decomposition products were formed from 5-azacytidine in neutral solution as in alkaline solution. However, in neutral solution, I decomposed to II and three unknown compounds that were chromophoric at 254 nm. Compound I was most stable when stored in neutral solution at low temperature.

Keyphrases □ 5-Aza-2′-deoxycytidine—analysis of chemical stability using high-performance liquid chromatography □ Antileukemic agents—5-aza-2′-deoxycytidine, analysis of chemical stability using high-performance liquid chromatography □ High-performance liquid chromatography—analysis of chemical stability of 5-aza-2′-deoxycytidine

5-Aza-2′-deoxycytidine (I), a nucleoside antimetabolite, is a very active antileukemic agent in mice (1, 2) and a potent cytotoxic agent against neoplastic cells *in vitro* (2, 3). This antimetabolite is related to 5-azacytidine, an agent currently used in the clinical treatment of acute leukemia (4).

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