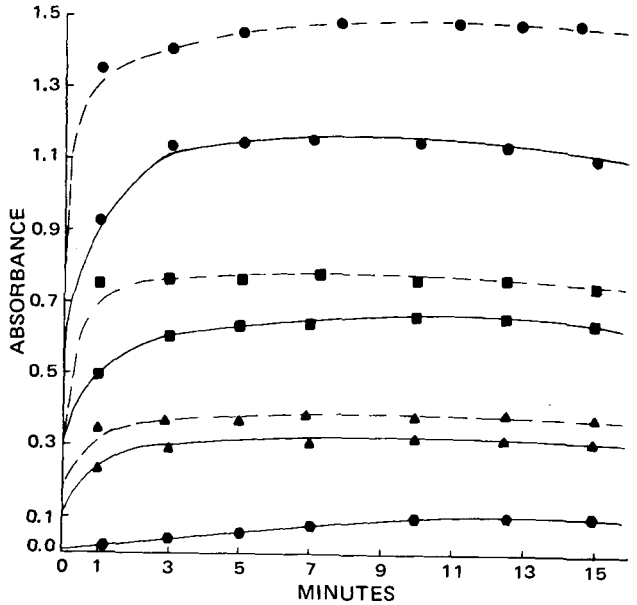


Figure 5—*Comparison of dissolution results of hydrocortisone filtered through serological pipets with cotton filters to samples filtered serologically followed by ultrafiltration with 0.22-μm filters. Key: - - -, serologically filtered; —, ultrafiltered; ▲, 5% solid-state dispersion in urethan, 500 mg, 25 mg of steroid; ■, 5% solid-state dispersion in urethan, 1 g, 50 mg of steroid; ●, 5% solid-state dispersion in urethan, 2 g, 100 mg of steroid; and ●, micronized hydrocortisone, 50 mg. Studies were carried out in pH 6.65 phosphate buffer (500 ml) at 37° and 60 rpm.*

urethan and polyethylene glycol 4000. A 5% physical mixture in urethan was also utilized. None of these systems gave an appreciable elevation in the dissolution rate over the pure drug. The amount solubilized was extremely low in all cases, indicating almost the absence of an appreciable effect.

SUMMARY

Many variables are present in these dissolution studies, rendering analysis a complicated matter, and typical results should not be inferred as general from one substance to another. As with any system of this nature, animal testing is imperative before reconciliation of the *in vitro* data can be completed. This testing was done by Chiou and Riegelman (9) with griseofulvin and polyethylene glycols.

When various medicinal agents are prepared in 5% solid-state dispersions in urethan, a large augmentation of the amount in solution at time periods up to 15 min is observed relative to the pure drug. This result is likely a partial consequence of diminution of particle sizes to the colloidal level in the freely water-soluble urethan.

The probability of some change in the crystalline state of the medicament in the solid-state dispersion cannot be discounted. This change could lead to supersaturated solutions along with the concomitant decrease in particle size.

Simple admixture of the drug with urethan and other diluents leads to an increase in the drug in solution. For this reason, care must be taken in ascribing observed effects entirely to a particle-size decrease rather than partially to simple dilution.

REFERENCES

(1) S. Feldman and M. Gibaldi, *J. Pharm. Sci.*, **56**, 370 (1967).

(2) M. Mayersohn and M. Gibaldi, *ibid.*, **55**, 1323 (1966).

(3) A. H. Goldberg, M. Gibaldi, and J. L. Kanig, *ibid.*, **54**, 1145 (1965).

(4) A. H. Goldberg, M. Gibaldi, J. L. Kanig, and M. Mayersohn, *ibid.*, **55**, 581 (1966).

(5) A. H. Goldberg, M. Gibaldi, and J. L. Kanig, *ibid.*, **55**, 482 (1966).

(6) *Ibid.*, **55**, 487 (1966).

(7) W. L. Chiou and S. Riegelman, *J. Pharm. Sci.*, **58**, 1505 (1969).

(8) W. L. Hayton, D. E. Guttman, and G. Levy, *ibid.*, **59**, 575 (1970).

(9) W. L. Chiou and S. Riegelman, *ibid.*, **59**, 937 (1970).

(10) L. J. Edwards, *Trans. Faraday Soc.*, **47**, 1191 (1951).

(11) E. Nelson, *J. Am. Pharm. Assoc., Sci. Ed.*, **48**, 96 (1959).

(12) E. Nelson and I. Schaldemose, *ibid.*, **48**, 489 (1959).

(13) G. Levy, *Am. J. Pharm.*, **135**, 78 (1963).

(14) H. V. Maulding and M. A. Zoglio, *J. Pharm. Sci.*, **59**, 384 (1970).

Synthesis and Evaluation of *N*-Deacetyl-*N*-glycosylalkylthiocolchicines as Antileukemic Agents

GEORGE T. SHIAU, KALYAN K. DE, and ROBERT E. HARMON ˣ

Received February 17, 1977, from the *Department of Chemistry, Western Michigan University, Kalamazoo, MI 49008.* Accepted for publication June 10, 1977.

Abstract □ A series of *N*-deacetyl-*N*-glycosylalkylthiocolchicines (glucosyl, galactosyl, mannosyl, ribosyl, and arabinosyl) was prepared by heating *N*-deacetylalkylthiocolchicines with the appropriate monosaccharides in methanol. Some compounds (glucosyl-, mannosyl-, and ribosylalkylthiocolchicines) were per-*O*-acetylated in dry pyridine with acetic anhydride. The compounds were tested against leukemia L-1210 and P-388 systems. Preliminary results showed that the antileukemic activity of the glycosyl compounds *in vitro* is similar to that of the *N*-deacetylalkylthiocolchicines used for their preparation. However, the presence of a glycosyl moiety in the molecule gives the advantage of greater solubility in water. Of the results obtained to date in lymphoid leukemia screening *in vivo*, five glycosyl compounds showed promising activity levels and have now reached confirmed active status.

Keyphrases □ *Thiocolchicines, various substituted*—synthesized, antileukemic and cytotoxic activity evaluated □ Antileukemic activity—various substituted thiocolchicines evaluated □ Cytotoxic activity—various substituted thiocolchicines evaluated □ Structure-activity relationships—various substituted thiocolchicines evaluated for antileukemic and cytotoxic activity

Thiocolchicoside (β-D-glucopyranosylmethylthiocolchicine) (Ic), prepared from acetyldemethylcolchicine by the action of methyl mercaptan followed by ether cleavage and condensation with acetobromoglucose (1), has shown muscle relaxant activity (2) and found clinical application (3). In a program to prepare new derivatives of colchicine

Table I—Physical Constants of *N*-Deacetyl-*N*-glycosylalkylthiocolchicines and Their Per-*O*-acetyl Derivatives

Compound	Yield, %	Recrystallization Solvent	Melting Point		Specific Rotation		Formula	Analysis, %			
			Present	Reported[a]	Present[b]	Reported[a]		C	H	N	S
IIa	87	Ethanol–water	161–162°	190°	−220°	−505° (0.5% H_2O)	$C_{26}H_{33}NO_9S\cdot C_2H_5OH$	57.81 58.08	6.75 6.86	2.41 2.34	5.51[c] 5.59[d]
IIb	84	Ethanol–water	152–153°	184°	−204°	−445° (0.2% H_2O)	$C_{26}H_{33}NO_9S\cdot C_2H_5OH$	57.81 57.70	6.75 6.54	2.41 2.30	5.51 5.32
IIc	85	Methanol	153–155°	—	−244°	—	$C_{26}H_{33}NO_9S\cdot H_2O$	56.41 56.67	6.37 6.28	2.53 2.58	5.79 5.51
IId	77	Methanol	151–153°	175°	−233°	−345° (0.2% H_2O)	$C_{25}H_{31}NO_8S\cdot CH_3OH$	58.09 58.61	6.56 6.53	2.61 2.36	5.97 6.44
IIe	87	Ethanol	123–125°	159°	−207°	−220° (0.2% CH_3OH)	$C_{25}H_{31}NO_8S\cdot C_2H_5OH$	58.79 58.59	6.76 6.67	2.54 2.68	5.81 5.77
IIf	70	—[e]	—	—	—	—	—	—	—	—	—
IIIa	90	Methanol	230–231°	—	−183°	—	$C_{34}H_{41}NO_{13}S$	58.03 58.16	5.87 5.98	1.99 1.95	
IIIb	85	Methanol–water	140–142°	—	—	—	—	—	—	—	—
IIIc	70	Water	135°[e]	—	—	—	—	—	—	—	—
IIId	82	Ethanol	161–163°	—	−189°	—	$C_{35}H_{43}NO_{13}S$	58.57 58.72	6.04 6.14	1.95 1.90	

[a] Reference 4. [b] Sample of 0.10 g in 100 ml of 95% ethanol solution. [c] Calculated. [d] Found. [e] An amorphous powder.

as potential anticancer agents, a series of compounds of this type was prepared for evaluation of intracranial antileukemic activity.

RESULTS AND DISCUSSION

The preparation of *N*-deacetyl-*N*-glycosylalkylthiocolchicines by heating *N*-deacetylalkylthiocolchicine with monosaccharide in methanol was reported (4). The same procedure was followed, for example, for the preparation of *N*-deacetyl-*N*-(D-glucosyl)methylthiocolchicine (IIa). The product crystallized from methanol. Although it had the same melting point, ~190°, as reported (4), it turned out to be a mixture of the starting material and the product expected as based on TLC, IR, and NMR evidence. However, the required Compound IIa, mp 161–162°, was separated from the filtrate and recrystallized from aqueous 85% ethanol.

Other glycosyl analogs were prepared in good yield (Table I). Some compounds were per-*O*-acetylated with excess acetic anhydride in dry pyridine at 0° (5). The products were recrystallized from either methanol or ethanol. Yields were in excess of 80% (Table I).

All compounds were examined by TLC (Table II) and characterized by IR, NMR, and mass spectrometry, specific rotation, melting point, and elemental analysis (Table I). Compounds IIa–IIe in water had identical UV spectra to the spectrum of Ia in water, *i.e.*, λ_{max} 380 (ε 18,200), λ_{min} 315, λ_{max} 285 (ε 11,000), and λ_{max} 260 (ε 21,700) nm.

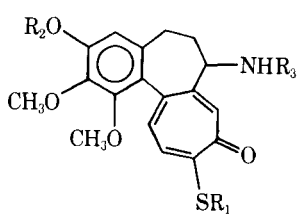
N-Deacetyl-*N*-glycosylalkylthiocolchicines were not sufficiently volatile for mass spectrometry; but, as expected (6), the trimethylsilyl derivatives had much greater volatility. Peaks corresponding to the molecular ions of trimethylsilyl derivatives were weak, but their per-*O*-acetylated analogs (IIIa–IIId) gave very intense peaks of molecular ions. This result strongly indicates that the coupling of compounds (Ia and Ib) with monosaccharide has taken place. Compounds IIa–IIf had very strong and wide IR absorptions at 1750 cm^{-1} due to OH and NH stretching, respectively. On the other hand, the per-*O*-acetylated analogs, IIIa–IIId, gave weak absorptions at 3350 cm^{-1} and very strong absorptions at 1750 cm^{-1}, as expected, due to NH stretching and acetyl C=O stretching, respectively.

In the IR spectra of IIa–IIf and IIIa–IIId, there was no absorption at the 1620–1700-cm^{-1} region due to the Schiff base C=N stretching (7). Therefore, the suspected structure of a Schiff base was eliminated. The 100-MHz spectrum of IIIa showed that the coupling constants were $J_{1,2} = J_{2,3} = 9$ Hz, suggesting that H-1, H-2, and H-3 of the glucosyl moiety were *ax*, indicating the β-configuration. The specific rotation data also supported this configuration.

The compounds were tested[1] in an *in vitro* assay against L-1210 (mouse leukemia) and were also examined by the National Cancer Institute's screening service against L-1210 and P-388 leukemia cell cultures (Tables III–V).

These preliminary results show that the antileukemia activity of IIa–IIf, *in vitro*, is similar to that of compounds used for their preparation. However, the presence of a glycosyl moiety in the molecule gives the advantage of greater solubility in water (Table VI).

Compound IIa was active against KB cells with an ED_{50} of 2.6×10^{-3} μg/ml (4.8×10^{-9} *M*). Thus, its activity was confirmed in cell culture. In the lymphoid leukemia screen *in vivo*, all compounds tested, except IIb,



Ia: $R_1 = R_2 = CH_3$, $R_3 = H$
Ib: $R_1 = C_2H_5$, $R_2 = CH_3$, $R_3 = H$
Ic: $R_1 = CH_3$, R_2 = D-glucosyl, $R_3 = COCH_3$
IIa: $R_1 = R_2 = CH_3$, R_3 = D-glucosyl
IIb: $R_1 = R_2 = CH_3$, R_3 = D-galactosyl
IIc: $R_1 = R_2 = CH_3$, R_3 = D-mannosyl
IId: $R_1 = R_2 = CH_3$, R_3 = D-ribosyl
IIe: $R_1 = R_2 = CH_3$, R_3 = L-arabinosyl
IIf: $R_1 = C_2H_5$, $R_2 = CH_3$, R_3 = D-glucosyl
IIIa: $R_1 = R_2 = CH_3$, R_3 = per-*O*-acetyl-D-glucosyl
IIIb: $R_1 = R_2 = CH_3$, R_3 = per-*O*-acetyl-D-mannosyl
IIIc: $R_1 = R_2 = CH_3$, R_3 = per-*O*-acetyl-D-ribosyl
IIId: $R_1 = C_2H_5$, $R_2 = CH_3$, R_3 = per-*O*-acetyl-D-glucosyl

Table II—R_f Value of *N*-Deacetyl-*N*-glycosylmethylthiocolchicines

Solvent	Compound					
	Ia	IIa	IIb	IIc	IId	IIe
Ethanol–ethyl acetate, 1:4 (v/v)	0.41	0.32	0.31	0.37	0.50	0.45
Acetone	0.73	0.22	0.19	0.28	0.49	0.25

Table III—Antileukemic Activities of *N*-Deacetyl-*N*-glycosylalkylthiocolchicines and Their Per-*O*-acetyl Analogs

Compound	L-1210 Tube Dilution *In Vitro* Assay, μg/ml	
	ID_{50}	ID_{90}
Ia	0.0070	0.011[a]
Ib	0.069	0.098[a]
IIa	0.016	0.023
IIb	0.017	0.023
IIc	0.017	0.023
IId	0.0092	0.013
IIe	0.017	0.024
IIf	0.069	0.098
IIIa	0.1	0.1
IIIb	0.1	0.1
IIIc	0.1	0.1
IIId	0.1	0.1

[a] Reference 8.

[1] At The Upjohn Co., Kalamazoo, Mich.

Table IV—Evaluation of *N*-Deacetylalkylthiocolchicines and *N*-Deacetyl-*N*-glycosylalkylthiocolchicines against Lymphoid Leukemia L-1210 *In Vivo* [a]

Compound		Dose[b], mg/kg	Toxicity on Day 5[c]	Control Body Weight Change[d]	Weight Difference (T − C)[e]	Tumor Evaluation[f]		Percent T/C[g]
Number	NSC Number					Test	Control	
I*a*	9170	400	5/6	−2.2	0.8	8.8	9.3	94
		200	6/6	−2.2	0.7	9.7	9.3	104
		100	6/6	−2.2	0.7	9.5	9.3	102
I*b*	186306	50.0	3/3	−0.5	0.5	10.0	10.2	98
		25.0	3/3	−0.5	0.0	9.7	10.2	95
		12.5	3/3	−0.5	1.0	11.0	10.2	107
II*b*	225332	50.0	4/4	−1.8	1.0	11.5	10.2	112
		25.0	4/4	−1.8	−1.5	10.3	10.2	100
		12.5	4/4	−1.8	1.5	10.3	10.2	100
II*c*	221659	200	6/6	0.3	−0.1	6.8	9.9	
		100	6/6	0.3	−0.5	13.5	9.9	136
		50.0	6/6	0.3	0.1	13.0	9.9	131
		150	4/6	−1.8	−2.0	12.3	10.2	120
		100	6/6	−1.8	0.2	13.2	10.2	129
		50.0	6/6	−1.8	0	13.0	10.2	127
		25.0	6/6	−1.8	0	10.5	10.2	102
II*d*	221660	200	0/4	0.0	0	0.0	9.9	—
		100	1/4	0.0	−3.0	6.0	9.9	—
		50.0	3/4	0.0	−5.7	6.0	9.9	—
		25.0	4/4	−1.8	0.3	12.5	10.2	122
		12.5	3/4	−1.8	−0.1	13.3	10.2	130
		6.25	4/4	−1.8	0.1	11.0	10.2	107
		3.12	3/4	−1.8	1.1	11.3	10.2	110
II*f*	186309	400	6/6	2.1	−2.1	14.0	9.0	155
		200	6/6	2.1	−1.1	12.0	9.0	133
		100	6/6	2.1	−1.1	10.4	9.0	115
		50.0	6/6	2.1	0.2	10.0	9.0	111
II*e*	248896	96.0	6/6	1.4	−3.6	11.5	9.0	127
		48.0	6/6	1.4	−2.4	12.2	9.0	135
		24.0	6/6	1.4	−1.9	11.3	9.0	125
		12.0	6/6	1.4	−0.9	10.8	9.0	120
		6.0	6/6	1.4	−0.1	9.2	9.0	102
II*a*	186308	200	0/6	−0.3	0.3	0.0	9.5	—
		100	5/6	−0.3	−5.0	6.2	9.5	—
		50.0	6/6	−0.3	−2.0	14.0	9.5	147
		75.0	5/6	1.1	−4.3	9.4	9.8	95
		50.0	6/6	1.1	−2.2	14.7	9.8	150
		35.0	6/6	1.1	−3.0	13.7	9.8	139
		100	2/6	0.1	−3.0	8.0	8.5	—
		50.0	6/6	0.1	−3.3	11.5	8.5	135
		25.0	6/6	0.1	−1.1	12.0	8.5	141
		12.5	6/6	1.0	−0.8	12.0	8.5	141
		6.25	6/6	0.1	−0.7	10.7	8.5	125
		3.13	6/6	0.1	−0.6	10.0	8.5	117

[a] Mice were implanted intraperitoneally with 1×10^5 L-1210 cells. [b] Dose per injection; nine doses. [c] Number of survivors/total number of mice on Day 5 in survival systems as a measure of drug toxicity. [d] Average weight change of control animals in grams (toxicity minus initial day). [e] Average weight of test group minus average weight of control animals in grams. [f] Survival time in days. [g] Ratio of survival time of treated animals to control animals expressed as a percentage; a compound is considered active if it has a T/C of ≥125%.

Table V—Evaluation of *N*-Deacetyl-*N*-glycosylalkylthiocolchicines against P-388 Lymphocytic Leukemia *In Vivo* [a]

Compound	Dose[b], mg/kg	Toxicity on Day 5[c]	Control Body Weight Change[d]	Weight Difference (T − C)[e]	Tumor Evaluation[f]		Percent T/C[g]
					Test	Control	
II*a*	200	0/6	0.3	−0.3	4.7	11.1	—
	100	3/6	0.3	−4.3	6.0	11.1	—
	50.0	6/6	0.3	−1.0	17.3	11.1	155
II*e*	48.0	5/6	1.2	−2.6	17.1	11.0	155
	36.0	6/6	1.2	−2.7	16.3	11.0	148
	24.0	6/6	1.2	−0.2	16.8	11.0	152
	12.0	6/6	1.2	−1.3	16.0	11.0	145
II*f*	400	6/6	2.1	−2.1	14.0	9.0	155
	200	6/6	2.1	−1.1	12.0	9.0	133
	100	6/6	2.1	−1.1	10.4	9.0	115
	50.0	6/6	2.1	0.2	10.0	9.0	111
III*a* (NSC 251700)	192	6/6	2.0	−3.1	16.4	10.9	150
	96.0	6/6	2.0	−3.4	14.0	10.9	128
	48.0	6/6	2.0	−2.5	14.1	10.9	129
	24.0	6/6	2.0	−1.3	13.1	10.9	120
	12.0	6/6	2.0	−1.0	12.8	10.9	117
	6.0	6/6	2.0	−0.2	11.7	10.9	107

[a] Mice were implanted intraperitoneally with 1×10^6 P-388 cells. [b] Dose per injection; nine injections. The first injection was given 1 day after tumor was implanted. [c–g] See Table IV.

Table VI—Solubility of *N*-Deacetyl-*N*-glycosylmethylthiocolchicines in Water at 25°

Compound	Solubility, *M*
I*a*	2.9×10^{-4}
II*a*	1.7×10^{-2}
II*b*	$>3.0 \times 10^{-1}$
II*c*	4.3×10^{-2}
II*d*	5.2×10^{-3}
II*e*	3.2×10^{-2}

showed much better activity than the compounds (I*a* and I*b*) used for their preparation and have now reached confirmed active status.

EXPERIMENTAL[2]

***N*-Deacetyl-*N*-(D-glucosyl)methylthiocolchicine (II*a*)**—To a solution of 11.2 g of *N*-deacetylmethylthiocolchicine (8) in 200 ml of methanol was added 5.4 g of D-(+)-glucose. The mixture was heated at 70° for 2 hr and then concentrated to about 100 ml. On standing overnight at room temperature, some solid deposited. The mixture was filtered, and the filtrate was further concentrated to about 70 ml and stored at 10°.

Over several days, it gave a pale-yellow solid, which was washed with cold methanol to yield 14.8 g (87%) of II*a*, mp 157–159°. Recrystallization from aqueous 85% ethanol gave pale-yellow rectangular prisms, mp 161–162°. This compound was characterized as containing one molecule of ethanol by elemental analyses and NMR spectroscopy.

***N*-Deacetyl-*N*-(D-galactosyl)methylthiocolchicine (II*b*)**—A mixture of 2.00 g of I*a* and 1.00 g of D-(+)-galactose in 60 ml of methanol was refluxed for 2 hr. The clear solution was concentrated to 10 ml and then stored at 10°. The crystals were filtered and washed with ethanol, yielding 2.48 g (84%). For further purification, 0.70 g of the product was dissolved in 50 ml of ethanol by heating. On cooling slowly to room temperature, the solution gave 0.56 g of II*b* as pale-yellow rectangular prisms, mp 152–153°.

A similar procedure was used to obtain IIc–II*f*.

***N*-Deacetyl-*N*-(tetra-*O*-acetyl-D-glucosyl)methylthiocolchicine (III*a*)**—To a stirred ice-cold solution of acetic anhydride (10 ml) in anhydrous pyridine (20 ml) was slowly added II*a* (5.8 g, 10 mmoles). The solution was left overnight at room temperature and then slowly poured into ice water (200 ml) with stirring. The precipitate was filtered, washed with water, and dried by pressing on a porous plate. Recrystallization from methanol gave 6.3 g (90%) of yellow fine needles of III*a*, mp 230–231°.

A similar procedure was used to obtain III*b*–III*d*.

Solubility Measurement—To 100 mg of each sample in small test tubes was added distilled water (0.5 ml). The test tubes were kept in a constant-temperature bath at 37° for 3 hr and then at 25° overnight so that some dissolved material crystallized out. The mixture was then filtered. The concentrations of the filtrates were measured at 380 nm. An average extinction coefficient of 18,200 at 380 nm was used in the calculation. The experimental error was within ±5%. The results are listed in Table VI.

[2] Melting points were measured with a Thomas-Hoover Uni-melt apparatus and are uncorrected. IR spectra were obtained on Beckman IR-8 and Perkin-Elmer 457 spectrophotometers. UV spectra were recorded with a Beckman model 24 spectrophotometer. NMR spectra were measured on Varian A-60 and Varian XL-100 spectrometers using tetramethylsilane as the internal standard and dimethyl sulfoxide-d_6 or deuterochloroform as the solvent. Mass spectra were recorded at 70 ev on an LKB 9000 SN mass spectrometer. Elemental analyses were performed by Midwest Microlab, Inc., Indianapolis, Ind. Eastman chromagram sheet 6061 silica gel was used for TLC, and the chromatograms were examined with UV light.

REFERENCES

(1) L. Vellez and G. Muller, *Bull. Soc. Chim. Fr.*, **1954**, 755; **1955**, 194.

(2) G. Plotka and R. Jequir, *Arch. Int. Pharmacodyn. Ther.*, **109**, 386 (1957).

(3) R. Pluvinage, D. Lemoine, and H. Gros, *Sem. Hop. Paris*, **33**, 3602 (1957).

(4) L. Velluz and G. Muller, French pat. 1, 175. 407 (1955). L. Velluz and G. Muller, British pat. 733, 729 (1957).

(5) M. L. Wolfrom and A. Thompson, in "Methods in Carbohydrate Chemistry," R. L. Whistler and M. L. Wolfrom, Eds., Academic, New York, N.Y., 1963, p. 211.

(6) T. Radford and D. C. Dejongh, in "Biochemical Applications of Mass Spectrometry," G. R. Waller, Ed., Wiley–Interscience, New York, N.Y., 1972, p. 318.

(7) N. B. Colthup, L. H. Daly, and S. E. Wiberly, "Introduction to Infrared and Raman Spectroscopy," Academic, New York, N.Y., 1964, p. 282.

(8) G. T. Shiau, K. K. De, and R. E. Harmon, *J. Pharm. Sci.*, **64**, 646 (1975).

ACKNOWLEDGMENTS

Presented in part at the 168th American Chemical Society National Meeting, Atlantic City, N.J., September 1974.

Supported by Grant CA 13119-02A1 from the National Institutes of Health.

The authors thank Mr. V. K. Sood and Miss S. L. Kuentzel, The Upjohn Co., Kalamazoo, Mich., for mass spectral measurements and antileukemic activity determinations, respectively.