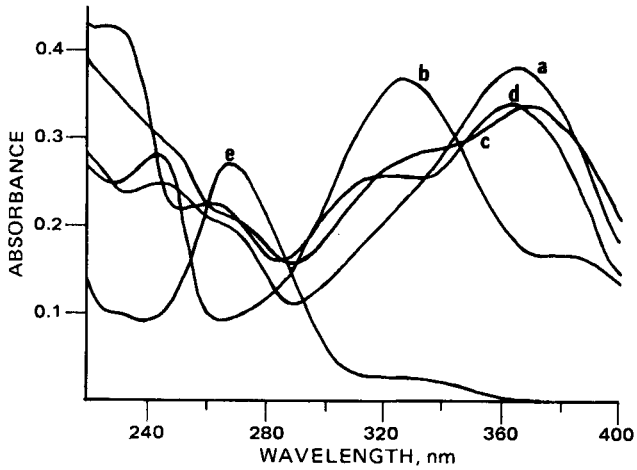
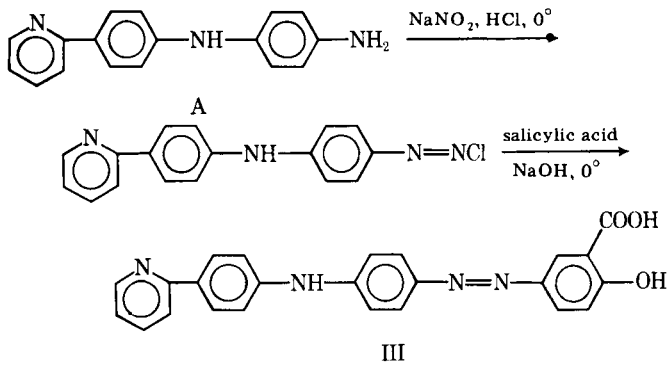


Figure 12—*UV spectra of sulfasalazine (a), I (b), II (c), III (d), and IV (e). Spectra were obtained in methanol (10^{-5} M).*

diazotized and coupled with salicylic acid, gave a product corresponding to impurity III. The sequence of synthetic steps is shown in Scheme VI. The synthesized material was found to be comparable with impurity III by TLC, mass spectrometry, and IR spectrophotometry (3400, 3100, 1660, 1595, 1485, 1429, 1308, 1249, 1168, 836, 758, and 683 cm^{-1}) (Fig. 10).

The mass spectrum of IV, isolated by TLC, is shown in Fig. 11 and was identified as sulfapyridine, *m/e* 249. The presence of sulfapyridine as an impurity in sulfasalazine was also confirmed by TLC. Impurity V, isolated from the TLC plate, was not fully characterized. The chemical-ionization mass spectrum showed a molecular ion, *m/e* 390.

Four lots of sulfasalazine from different manufacturers showed all impurities described; impurity III was the major one. The UV spectra of I–IV and sulfasalazine are shown in Fig. 12. Compounds I–III are the novel compounds, and their structures reveal some characteristic properties similar to sulfasalazine. Thus, the presence of these impurities in the drug will interfere in analytical methods such as UV spectroscopy,



Scheme VI

polarography, and nonaqueous titration. Identification of other impurities in sulfasalazine will be reported in the near future.

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Solid-State Dispersions Employing Urethan

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Abstract □ The dissolution rates of a number of drug–urethan solid-state dispersion systems were studied. A marked enhancement of the initial dissolution rates of several poorly water-soluble drugs was found when they were incorporated into a urethan matrix by heat fusion. These differences were considerable when pure substances such as griseofulvin, hydrocortisone, chloramphenicol, and acetaminophen were compared to the urethan–drug solid dispersion. Physical mixtures of the medicinal agents with urethan also gave a marked increase in the amount of drug in solution, with the value in most cases being over one-half that of the solid-state dispersion. Data are given, comparing ultrafiltration with samples filtered through cotton, regarding drug content remaining in solution.

Keyphrases □ Solid-state dispersions—various drug–urethan systems, dissolution rates compared to pure drug substances and physical mixtures □ Dissolution rates—various drug–urethan solid-state dispersions, compared to pure drug substances and physical mixtures □ Urethan—solid-state dispersions with various drugs, dissolution rates compared to pure drug substances and physical mixtures

The application of relatively soluble and nontoxic substances as vehicles for difficultly soluble medicinal agents in the form of solid-state dispersions is well documented (1–7). Dialkylamides and polyethylene glycols were shown to increase absorption of steroids and griseofulvin, respectively (8, 9).

Drugs may be poorly soluble in GI fluids, leading to difficult and erratic absorption when administered orally. Absorption of insoluble medicaments is sometimes a function of the dissolution rate of these substances in aqueous solutions (10–12). Solid solutions or dispersions may function to increase dissolution rates by breaking

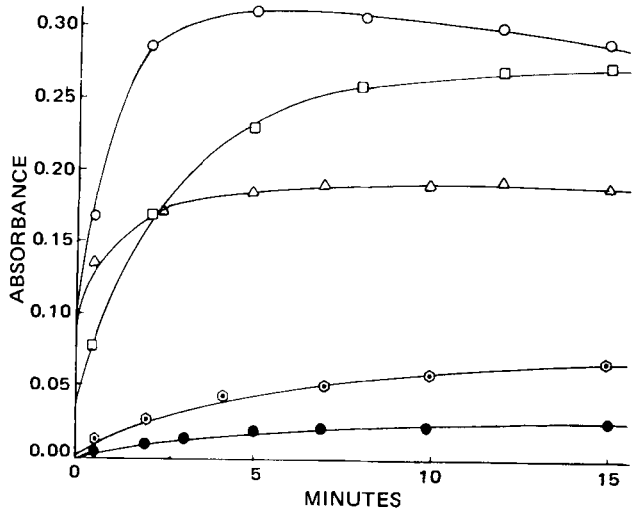

Figure 1—*Griseofulvin dissolution in pH 6.65 phosphate buffer (500 ml) at 37° and 60 rpm. Key: ●, wetted, nonmicronized griseofulvin powder, 25 mg; △, 5% griseofulvin solid-state dispersion in urethan, 500 mg total, 25 mg of griseofulvin; □, 5% griseofulvin solid-state dispersion in polyethylene glycol 4000, 500 mg total, 25 mg of griseofulvin; ⊙, 5% physical mixture of griseofulvin and urethan, 500 mg total, 25 mg of griseofulvin; and ○, 5% griseofulvin solid-state dispersion in urethan, 1 g total, 50 mg of griseofulvin.*

down crystal lattice energy, concomitantly decreasing the size of the medicament theoretically to the colloidal and even the molecular level. Thus, there is a subsequent increase in the rate of solution which is inversely proportional to particle size (3). If the dissolution process is the rate-limiting step in absorption, the greater the surface area a drug has, the faster and more complete should be the absorption. Levy (13) considered methods by which finely divided drugs may be presented to the GI tract.

The comparatively simple molecule, urethan, was utilized in these studies for several reasons. It is readily water soluble, has little tendency to form insoluble complexes with drugs, possesses a low melting point (important with heat-sensitive compounds), and is an efficient solvent for many organic molecules.

Urethan possesses the disadvantage of weak pharmacological activity, being used primarily in relatively large doses as a sedative. The compound also exhibits an ease of sublimation at relatively mild conditions.

EXPERIMENTAL

Chemicals—The following chemicals were used: urethan[1] (ethylcarbamate), mp 48–50°, reagent grade; hydrocortisone[2], micronized; griseofulvin[3], nonmicronized; chloramphenicol[4]; and polyethylene glycol 4000[5], powdered.

Dissolution Rates—A 60-rpm stirrer motor, fitted with a 2.54-cm propeller blade placed 4 cm from the bottom of an 800-ml beaker containing 500 ml of pH 6.65 phosphate buffer, was used in determining these rates. The phosphate buffer was prepared by adjusting the pH of 0.1 *M* KH_2PO_4 with sodium hydroxide solution. The drug, drug–urethan solid dispersion, or drug–urethan physical mixture was placed into a stirred solution from a height of about 1.5 cm. The temperature of the dissolution rate media was kept at 37 ± 0.1° by immersing the beaker in a constant-temperature bath.

Five-milliliter samples were periodically withdrawn for analyses by means of serological pipets with cotton filters attached[6] or by serological pipets followed by ultrafiltration utilizing 0.22-μm filters[7]. Spectrophotometric analyses[8] were carried out following dilution of the 5-ml samples. The compounds were read at the following wavelengths: chloramphenicol, 278 nm; griseofulvin, 295 nm; hydrocortisone, 274 nm; and acetaminophen, 250 nm.

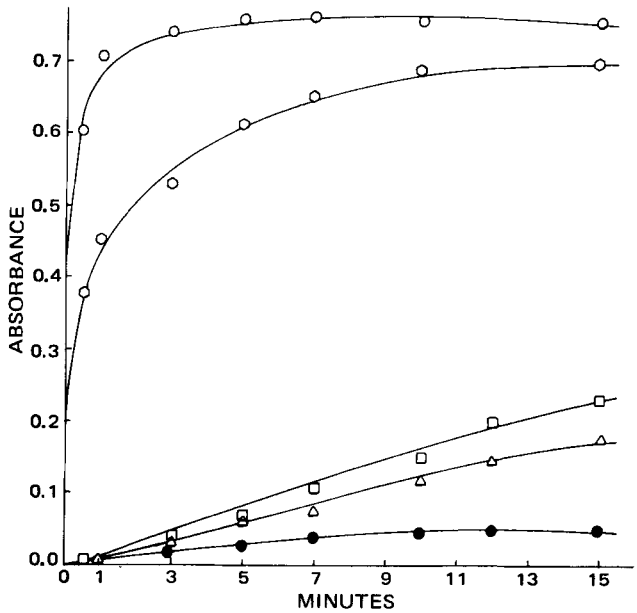

Figure 2—*Dissolution studies of hydrocortisone in pH 6.65 phosphate buffer (500 ml) at 37° and 60 rpm. Key: ●, micronized hydrocortisone, 50 mg; △, micronized hydrocortisone, 50 mg, added to dissolution media which previously had 950 mg of urethan dissolved in it; □, micronized hydrocortisone, 50 mg added to dissolution media which previously had 1.95 g of urethan dissolved in it; ○, 5% physical mixture of micronized hydrocortisone in urethan, 500 mg total, 50 mg of hydrocortisone; and ○, 5% solid-state dispersion of micronized hydrocortisone in urethan, 500 mg total, 50 mg of hydrocortisone.*

Preparation of Solid Dispersions—Solutions were produced by dissolution of 500 mg (5%) of the drug in 9.5 g of molten urethan, heated on a hot plate, and stirred magnetically. All substances exhibited miscibility under these conditions. The molten solution was poured onto a cold surface (metal pan), where it immediately congealed, and was then scraped up with a microspatula. This material was ground with mortar and pestle, if needed. A sample was used for dissolution rate studies with no further sizing. Higher percentages of actives were incorporated into urethan by the same procedure. Physical mixtures were prepared by carefully mixing the medicament with urethan in a mortar. Dissolution studies were run on an aliquot of this material.

Solubility Determinations—An excess of the substance under scrutiny was added to screw-capped vials (18-ml capacity) containing 15 ml of solvent, and varying amounts of urethan were added. The flasks were clamped on a motor-driven shaft and rotated vertically at 6 rpm in a constant-temperature bath at 37 ±0.1° until equilibrium was established. Samples were withdrawn, passed through filters[7] (0.22 μm), and analyzed spectrophotometrically for solubility.

RESULTS AND DISCUSSION

Several classes of drugs were incorporated into solid dispersion systems by dissolution in liquid urethan (mp 48–50°) followed by rapid chilling to solidify the solution. No difficulties were encountered with the procedure in concentrations up to 25% (w/w) drug–urethan. The resultant solid-state dispersion was examined for its ability to accelerate the dissolution rates of the relatively insoluble medicinal agents examined.

Properties of urethan such as a low melting point, solubility, and lack of assay interference favor its use as a carrier for drug molecules in spite of the fact that its low molecular weight does not fulfill the molecular size criterion for a host molecule of an interstitial molecular solution (9). Solid

[1] Fisher Scientific Co., Fair Lawn, N.J.
[2] Merck Sharp & Dohme, West Point, Pa.
[3] Schering Corp., Bloomfield, N.J.
[4] Mann Research Laboratories, New York, N.Y.
[5] Carbowax, Union Carbide, New York, N.Y.
[6] Kimble Products, Owens-Illinois, Toledo, Ohio.
[7] Millipore Corp., Bedford, Mass.
[8] Cary 14 recording spectrophotometer.

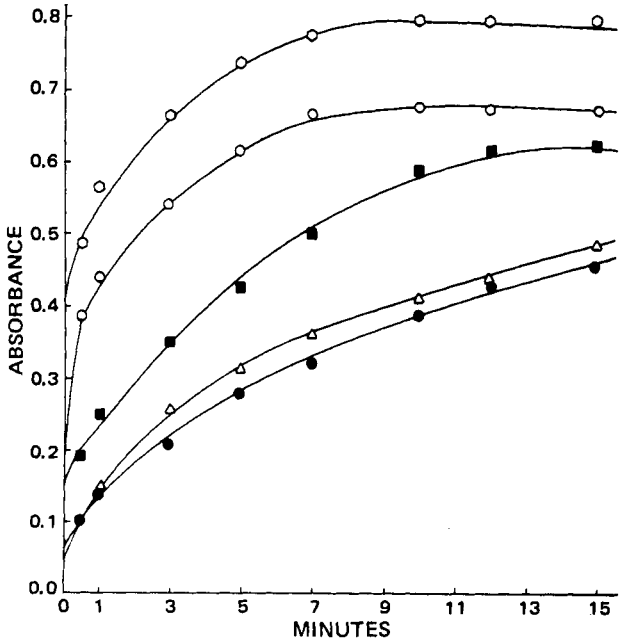

Figure 3—*Dissolution studies involving various percentage strengths solid-state dispersions of hydrocortisone in urethan (all equivalent to 50 mg of hydrocortisone). Key: ○, 5% hydrocortisone; ○, 10% hydrocortisone; ■, 15% hydrocortisone; △, 20% hydrocortisone; and ●, 25% hydrocortisone. Studies were carried out in pH 6.65 buffer at 37° and 60 rpm.*

dispersions prepared in matrixes of urethan are easy to work with because of their rather crystalline nature. For this reason, they are somewhat advantageous over polymeric matrixes, which are sometimes difficult to solidify.

Of the compounds studied, only a few are reported in this article for the sake of brevity. These compounds cover the spectrum, from the reasonably soluble acetaminophen to the relatively insoluble griseofulvin. Unfortunately, the data given are not as orderly as might be hoped when one compound is compared to another. The question may frequently arise as to whether one is visualizing a "dilution" effect or phenomena brought about by decreasing the particle size of the drug in the urethan matrix. The other matrixes examined, such as niacinamide, polyethylene glycols, urea, and urea–urethan mixtures, produced essentially the same qualitative results.

The solvent selected was pH 6.65 phosphate buffer, which was previously utilized in work with the ergot alkaloids (14). Few compounds studied ionized appreciably in this solvent system. Other conditions employed were those found acceptable in prior studies on differences in rates of solution (14).

No appreciable degradation of the drugs used was noted by TLC methods, as was reported (9) for griseofulvin. Such light-sensitive ergot alkaloids as dihydroergocristine showed no visible decomposition under the mild conditions of the process. Neither alterations nor shifts in UV spectra were noted.

Figure 1 illustrates a considerable enhancement of the amount of griseofulvin in solution upon incorporation of the antifungal into a 5% solid dispersion in urethan relative to the prewet nonmicronized griseofulvin itself. Polyethylene glycol 4000 containing 5% griseofulvin, prepared essentially after the method of Chiou and Riegelman (9), exhibited an even greater amount in solution than the 5% griseofulvin–urethan, although initially the latter showed higher levels in solution. This situation was characteristic of urethan as a carrier because exceptionally high levels of drug in solution were attained in the first 5–30 sec.

Figure 1 also indicates that a twofold increase in the quantity of solid dispersion used brings about an approximate doubling of the amount of griseofulvin in solution, as if it were a function of the quantity of dispersion added. The physical mixture was appreciably slower to dissolve than the solid dispersion, but this was not the situation with all drugs investigated.

Micronized hydrocortisone gave a great deal less in solution at times up to 15 min relative to the 5% solid dispersion (Fig. 2). However, the physical mixture, prepared by mixing in a mortar, gave values approaching those of the solid dispersion after 15 min. Some evidence of

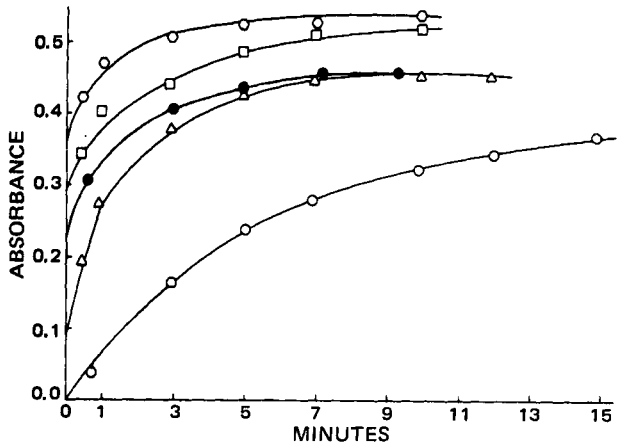

Figure 4—*Dissolution profile of chloramphenicol in pH 6.65 phosphate buffer (500 ml) at 37° and 60 rpm. Key: ○, chloramphenicol, 50 mg; △, 5% solid-state dispersion, chloramphenicol in polyethylene glycol 4000, 1 g; ●, 5% physical mixture, chloramphenicol in polyethylene glycol 4000, 1 g; ○, 5% solid-state dispersion, chloramphenicol in urethan, 1 g; and □, 5% physical mixture, chloramphenicol in urethan, 1 g.*

interaction between the steroid and urethan was indicated by the quantity of hydrocortisone in solution being doubled or tripled at elapsed times of 3 min or more when the drug was added to dissolution media containing previously dissolved urethan. This situation indicates that another variable is apparently operative in systems of this sort and complicates analysis.

The amounts of hydrocortisone in solution at a given time were inversely proportional to the percentage of steroid incorporated when equivalent amounts of hydrocortisone (50 mg of drug) were investigated (Fig. 3); this result is explainable from the standpoint of less isolation of colloidial particles by the soluble carrier. The more dilute the system, the larger is the amount of compound in solution. As the drug concentration is increased, one may think of the dissolution properties of the pure substance being approached, although this is probably not strictly correct.

The more soluble chloramphenicol exhibited an increase in the amount of compound in solution for the 5% solid dispersion, with all drug dissolved at 7 min (Fig. 4). The physical mixture of urethan and chloramphenicol showed more total drug in solution relative to the 5% physical mixture in polyethylene glycol 4000, which was greater than the solid solution (5%) of chloramphenicol in the polyethylene glycol. This finding illustrates the inconsistencies encountered from compound to compound, since the physical mixture was superior to the solid dispersion.

Acetaminophen was run in the same general manner as the other substances (150 mg of drug, 10% solid dispersion, and 1.5 g of total mixture), using distilled water (500 ml) as reported earlier (5). This compound, which possesses relatively good solubility properties comparable to griseofulvin, showed complete solution of both the 5% physical mixture and solid dispersion after 30 sec at 37°. At 27°, the solid dispersion was dissolved after 30 sec and the physical mixture was dissolved after 3 min, but the drug did not go into solution after 15 min.

Figure 5 demonstrates the differences in total drug found when samples were simply filtered through the cotton of serological pipets in contrast to aliquots being filtered serologically followed by ultrafiltration. An almost constant difference may be noted in the examples cited, possibly the consequence of entrapment of some compound on the ultrafilter[7] (0.22 μm) or other variables. This figure illustrates an almost direct relationship between milligrams of hydrocortisone added to the dissolution media and the elevation of total drug in solution at a given time. This occurrence was characteristic of many compounds examined.

Solubility studies were carried out with the more insoluble compounds and urethan, with no evidence of interaction up to 8 mg of urethan/ml.

The results represented in the figures were usually reproducible, ±15% or better, from day to day. The sequences presented in the figures were run on a given day.

Chloramphenicol palmitate[9] was run in 5% solid dispersions with

[9] Chloromycetin Palmitate, supplied by Dr. H. E. Machamer, Parke-Davis & Co.

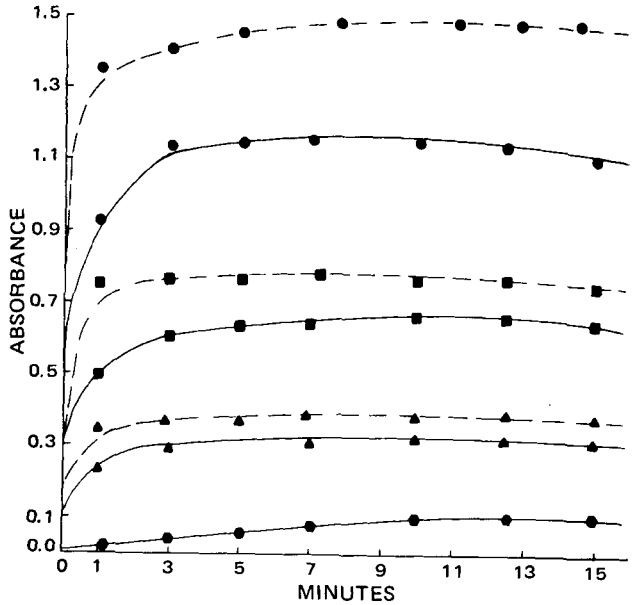

Figure 5—*Comparison of dissolution results of hydrocortisone filtered through serological pipets with cotton filters to samples filtered serologically followed by ultrafiltration with 0.22-μm filters. Key: - - -, serologically filtered; —, ultrafiltered; ▲, 5% solid-state dispersion in urethan, 500 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.

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

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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