In view of existing evidence, it appears that a methyl group alpha to the alkylamino function influences anticholinergic activity. Furthermore, the postulated ability of this group to alter the orientation between the molecule and the receptor should further substantiate the premise that the binding of an important moiety of an antagonist may be affected considerably by modifying other parts of the same molecule.

REFERENCES

(1) R. B. Barlow, "Introduction to Chemical Pharmacology," Methuen, London, England, 1964, p. 198.

(2) H. R. Ing, "The Alkaloids," vol. 5, R. H. F. Manske and H. L. Holmes, Eds., Academic, New York, N.Y., 1955, p. 243.

(3) W. R. Buckett and C. G. Haining, *Br. J. Pharmacol.*, **24**, 138 (1965).

(4) A. Bebbington and R. W. Brimblecombe, *Adv. Drug Res.*, **2**, 154 (1965).

(5) E. J. Ariens, in "Advances in Drug Research," vol. 3, N. J. Harper and A. B. Simmonds, Eds., Academic, London, England, 1966, p. 260.

(6) B. W. J. Ellenbroek, R. J. F. Nivard, J. M. Van Rossum, and E. J. Ariens, *J. Pharm. Pharmacol.*, **17**, 393 (1965).

(7) M. May, H. F. Ridley, and D. J. Triggle, *J. Med. Chem.*, **12**, 320 (1969).

(8) R. W. Brimblecombe and T. D. Inch, *J. Pharm. Pharmacol.*, **22**, 881 (1970).

(9) A. M. Lands, E. Ananenko, G. Jones, J. O. Hoppe, and T. J. Becker, *J. Pharmacol. Exp. Ther.*, **96**, 1 (1949).

(10) M. M. Kochhar, R. G. Brown, and J. N. Delgado, *J. Pharm. Sci.*, **54**, 393 (1965).

(11) S. K. Gomer, E. I. Isaacson, R. G. Brown, and J. N. Delgado, *ibid.*, **57**, 1586 (1968).

(12) W. G. Haney, Jr., Ph.D. dissertation, University of Texas, Austin, Tex., 1970. W. G. Haney, R. G. Brown, J. N. Delgado, and E. I. Isaacson, *J. Pharm. Sci.*, in press.

(13) R. B. Barlow, *J. Pharm. Pharmacol.*, **23**, 90 (1971).

(14) R. B. Barlow, F. M. Frank, and J. D. M. Pearson, *J. Med. Chem.*, **16**, 439 (1973).

(15) B. B. Wylie, E. I. Isaacson, and J. N. Delgado, *J. Pharm. Sci.*, **54**, 1373 (1965).

(16) A. Liberles, "Introduction to Theoretical Organic Chemistry," Macmillan, New York, N.Y., 1968, p. 591.

(17) E. S. Gould, "Mechanism and Structure in Organic Chemistry," Holt, Rinehart and Winston, New York, N.Y., 1959, p. 570, and references cited therein.

(18) E. L. Eliel, "Stereochemistry of Carbon Compounds," McGraw-Hill, New York, N.Y., 1962, p. 47, and references cited therein.

(19) R. Bentley, "Molecular Asymmetry in Biology," vol. 1, Academic, New York, N.Y., 1969, p. 69, and references cited therein.

(20) F. A. Bovey, "Nuclear Magnetic Resonance Spectroscopy," Academic, New York, N.Y., 1969, pp. 161, 164, and references cited therein.

(21) C. C. Pfeiffer, *Science*, **124**, 29 (1956).

(22) F. B. Abramson, R. B. Barlow, M. G. Mustafa, and R. P. Stephenson, *Br. J. Pharmacol.*, **37**, 207 (1969).

(23) R. W. Brimblecombe, D. M. Green, T. D. Inch, and P. B. J. Thompson, *J. Pharm. Pharmacol.*, **23**, 745 (1971).

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Chelates of Dicumarol I: Preparation and Structure Identification of Magnesium Chelate

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Abstract □ A magnesium chelate of dicumarol was prepared by reacting a suspension of dicumarol and magnesium oxide in 50% water–methanol. GLC, thermogravimetric, and elemental analyses showed that this compound has a 2:1 ligand–metal stoichiometry with 2 moles of water associated with the complex. Although the chelate does not melt, two endothermic peaks at 205 and 274° were observed in the thermogram, in contrast to a single endothermic peak corresponding to a melting point of 288° for dicumarol. IR spectroscopy indicated that the magnesium is bonded between the carbonyl at C-2 and the oxygen at C-4′ (or vice versa).

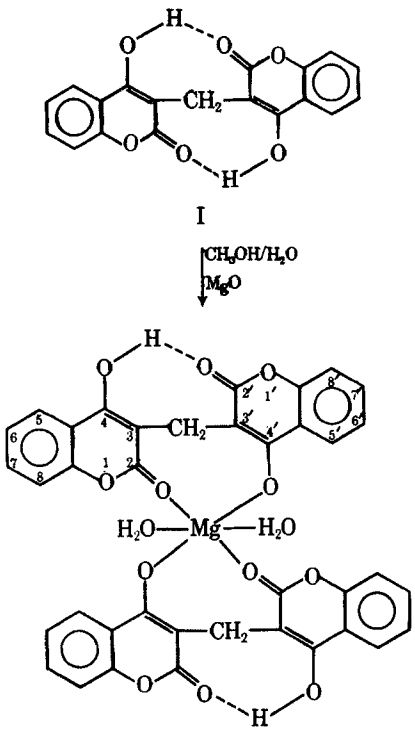
Keyphrases □ Dicumarol—chelate with magnesium prepared, structure elucidated □ Chelates—dicumarol–magnesium, prepared, structure elucidated □ Magnesium—chelate with dicumarol prepared, structure elucidated □ Metals—magnesium, chelate with dicumarol prepared, structure elucidated □ Anticoagulants—dicumarol, chelate with magnesium prepared, structure elucidated

Dicumarol [3,3′-methylenebis(4-hydroxycoumarin)] (I) is an oral anticoagulant utilized for the prevention and therapy of thromboembolic vascular disease. However, many problems are associated with its use: concomitant administration with certain drugs can inhibit or potentiate its anticoagulant effect (1), it is slowly and erratically absorbed from tablet dosage forms (2), and its bioavailability from tablets depends on the type and amount of excipients in the formulation (3).

Reported bioavailability differences from various tablet formulations provided the stimulus for a study of potential solid–solid interactions between I and various metal-containing excipients (4). Chemisorption of I occurred with several excipients, but the interaction with the magnesium-containing excipients was postulated to be chelation.

In subsequent studies (5, 6) in dogs and humans, concomitant administration of I with magnesium-containing adjuvants resulted in faster and more complete drug absorption. These investigators postulated that the enhanced bioavailability may be due to chelate formation within the GI tract.

This investigation was undertaken to prepare, isolate, and study the physicochemical properties and bioavailability of the magnesium chelate of dicumarol. This report details the preparation, stoichiometry, and structure



Scheme I

identification of the dicumarol–magnesium chelate (II). The physicochemical properties and bioavailability will be the subject of a later report.

EXPERIMENTAL

Materials—Dicumarol[1], methanol[2], ethylenediaminetetraacetic acid disodium salt[2], eriochrome black T[2], 2-propanol[2], heavy magnesium oxide[3], dimethylformamide[4], methanolic tetrabutylammonium hydroxide solution[4] (25%), atomic absorption magnesium standard[5], zinc metal[5], dimethyl sulfoxide[5], benzoic acid[5], pyridine[6], 4A molecular sieves[6], ammonium chloride[7], hydrochloric acid[8], tetrabutylammonium chloride[9], ammonium hydroxide[10] (58%), benzene[10], sodium hydroxide[10], and ethanol[11] were used without further purification, except that I was recrystallized from benzene. The melting point of I was verified using a differential thermal analyzer.

Ammonium chloride–ammonium hydroxide buffer (pH 10) was prepared by dissolving 14 g of ammonium chloride in 114 ml of ammonium hydroxide and diluting to 200 ml with distilled water (7).

A 0.5% (w/v) eriochrome black T solution was prepared by dissolving 0.125 g in sufficient ethanol to make 25 ml.

Ethylenediaminetetraacetic acid disodium salt solution (0.025 *M*) was prepared by dissolving 9.306 g in enough water to make 1000 ml. Standardization was accomplished by titrating with a solution of zinc chloride, prepared by dissolving an accurately weighed amount of zinc metal in dilute hydrochloric acid USP (7).

Tetrabutylammonium hydroxide solution was standardized by potentiometrically titrating a 0.0106 *M* methanolic benzoic acid solution using a modified procedure described by Connors (8).

Preparation of II—Three grams (0.0089 mole) of I, recrystallized from benzene, and 0.324 g (0.008 mole) of heavy magnesium oxide were suspended in 600 ml of methanol (Scheme I). When the reactants were wetted, 600 ml of distilled water was added, and the mixture was stirred in a covered, light-protected beaker for 24 hr. Undissolved material was removed by filtration; the filtrate was collected in a 1000-ml round-bottom flask, covered, and placed in a refrigerator (5°) until crystallization was complete. The crystals were collected on a Büchner funnel, washed thoroughly with distilled water, and dried *in vacuo* at room temperature for 24 hr. Approximately 1.5 g of white crystalline material was obtained.

Atomic Absorption—An atomic absorption spectrophotometer[12], equipped with a single-slot burner head (10-cm pathlength) and a magnesium hollow cathode lamp, was used to determine the magnesium content of II. Instrumental parameters were: wavelength, 285.2 nm; hollow cathode lamp current, 5 mamp; fuel, acetylene (flowmeter 4, 10 psig); oxidizer, air (flowmeter 20, 35 psig); monochromator entrance slit width, 50 μm; monochromator exit slit width, 75 μm; gain, 1; and pathlength, 10 cm.

Commercially available magnesium reference standard (10,000 ppm) was diluted with dimethylformamide to prepare standard solutions equivalent to 0.1–1.0 ppm of magnesium. After optimizing the wavelength and zeroing the instrument while aspirating dimethylformamide, a standard curve was prepared from the standard solutions by plotting absorbance *versus* concentration. Standard solutions of magnesium spiked with I showed no enhancement or depression of absorption.

Solutions of II were prepared by dissolving approximately 25 mg in 100 ml of dimethylformamide and diluting an aliquot 20-fold with dimethylformamide to give a magnesium concentration of approximately 0.4 ppm. The actual magnesium content of II was obtained by reference to the standard curve.

Complexometric Titration—The magnesium content of II also was determined by a modified complexometric titration procedure (9). To release the magnesium, the chelate was reacted with base prior to titration by dissolving accurately weighed samples of II (approximately 125 mg) in 10 ml of 1 *N* sodium hydroxide, adding 10 ml of distilled water, and stirring for 15 min. The magnesium was solubilized by adding 10 ml of dilute hydrochloric acid USP and stirring the solution an additional 15 min.

The acidic solution was filtered to remove the precipitated I, and the precipitate was washed with distilled water until the washings were neutral to litmus paper. After neutralizing the filtrate with 1 *N* sodium hydroxide, 5 ml of ammonium chloride–ammonium hydroxide buffer and 0.5 ml of 0.5% (w/v) ethanolic eriochrome black T indicator solution were added. Then the filtrate was titrated with 0.025 *M* ethylenediaminetetraacetic acid disodium salt solution until the wine-red color changed to blue.

GLC—A gas chromatograph[13], equipped with a thermal conductivity detector and two commercially prepared 3-mm × 1.83-m (6-ft) stainless steel columns packed with 10% Carbowax 20M on 80–100-mesh Chromosorb W AW[14], was used to confirm the presence of water in II. Operating conditions were: injection port, 240°; column, 180°; detector, 260°; carrier gas (helium), 40 ml/min (50 psig); attenuation, 2; and bridge current, 175 mamp.

Dimethyl sulfoxide, dried over 4A molecular sieves, was spiked with known amounts of water, and 5-μl samples containing 5–20 μg of water were injected[15]. The retention times, from injection to peak maximum, were 0.4 min for water and 2.2 min for dimethyl sulfoxide. Confirmation of water in II was obtained by dissolving samples, which had been dried at 85°, in dried dimethyl sulfoxide, injecting 5 μl, and comparing retention times to those of the water-spiked dimethyl sulfoxide samples.

Thermogravimetric Analysis—The water content of II also was determined by mass loss on a thermobalance[16]. A heating rate of 20°/min, a *y*-axis sensitivity of 0.4 mg/2.54 cm, and a dynamic nitrogen atmosphere (40 ml/min, 5 psig) were used for all analyses. Sample sizes ranged from 14 to 17 mg.

Differential Thermal Analysis—Endothermic or exothermic transitions were determined by thermal analysis[17] using 2–3-mg samples. A heating rate of 20°/min and a dynamic nitrogen atmosphere (flowmeter 3, 5 psig) were used for all analyses. The differential temperature sensitivities used were 1°/2.54 cm for I and 0.2°/2.54 cm for II. Temperatures were corrected for the nonlinear response of the chromel–alumel thermocouple.

[1] Sigma Chemical Co.
[2] Reagent, J. T. Baker.
[3] Technical, Matheson, Coleman & Bell.
[4] Reagent, Matheson, Coleman & Bell.
[5] Certified, Fisher Scientific Co.
[6] Technical, Fisher Scientific Co.
[7] Laboratory, Fisher Scientific Co.
[8] Reagent, Fisher Scientific Co.
[9] Eastman Organic Chemicals.
[10] Reagent, Mallinckrodt Chemical Works.
[11] U.S. Industrial Chemicals Co.
[12] Model 82-270, Jarrel-Ash.
[13] Model 5750B, Hewlett-Packard.
[14] Supelco.
[15] 701SN, Hamilton Co.
[16] Model 950, DuPont.
[17] Model 900, DuPont.

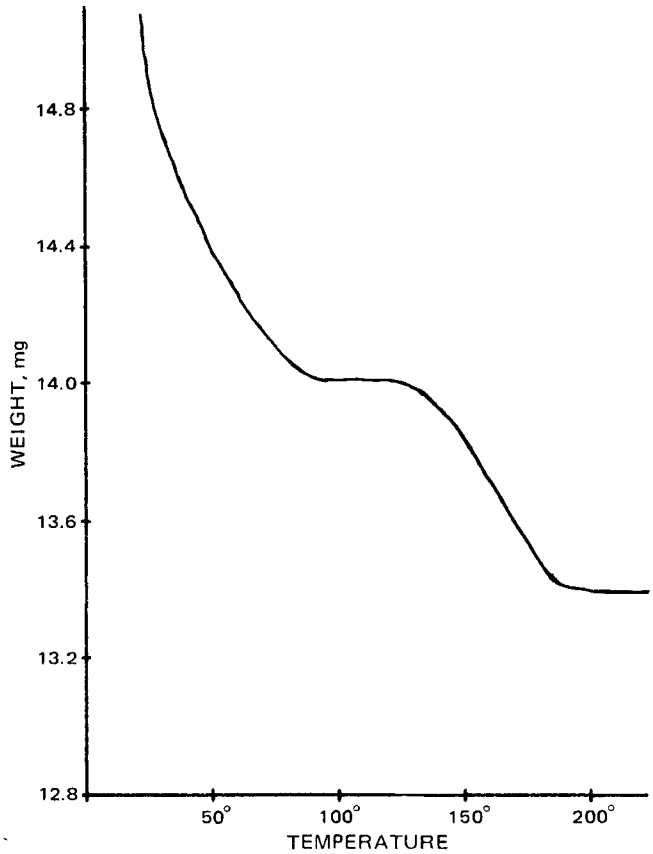


Figure 1—*Thermogravimetric analysis of dicumarol–magnesium chelate.*

IR Spectroscopy—IR spectra were obtained[18] from approximately a 0.33% dispersion in potassium bromide using a 5-min scan period.

NMR Spectroscopy—Proton spectra were obtained[19] in deuterated chloroform. Chemical shifts were reported in parts per million relative to tetramethylsilane as an internal standard.

Nonaqueous Titration—A nonaqueous titration was performed on II to quantitate the enolic protons. An accurately weighed sample (40–80 mg), dissolved in 50 ml of pyridine, was titrated with 1.027 *M* methanolic tetrabutylammonium hydroxide solution delivered from a micrometer syringe[20]. Potential measurements were recorded using a digital pH meter[21] equipped with a combination electrode. End-point drift was minimized by using a saturated solution of tetrabutylammonium chloride in 2-propanol as the internal electrolyte.

Carbon and Hydrogen Analyses—Carbon and hydrogen analyses were performed[22] on II, and calculated percentages were based on a molecular formula of $C_{38}H_{26}MgO_{14}$.

RESULTS AND DISCUSSION

Initial attempts to prepare the magnesium chelate of I centered around the preparation of the disodium salt of I, which could subsequently be reacted with a water-soluble magnesium salt. Preparation of the disodium salt was attempted by four different procedures: (*a*) reacting I suspended in 2-propanol with sodium hydroxide, (*b*) reacting I suspended in ether with sodium hydride, (*c*) adding I to a pH 8.9 phosphate buffer, and (*d*) dissolving I in an equivalent amount of 1.0 *N* sodium hydroxide and adjusting to a known volume with distilled water (10). None of these methods was successful; the isolated product was yellow or became yellow upon standing, suggesting that degradation had occurred.

Subsequently, direct preparation of II was accomplished by reacting a suspension of I and magnesium oxide in a 50% water–methanol mixture (Scheme I). As the reaction progressed, the solution became less turbid.

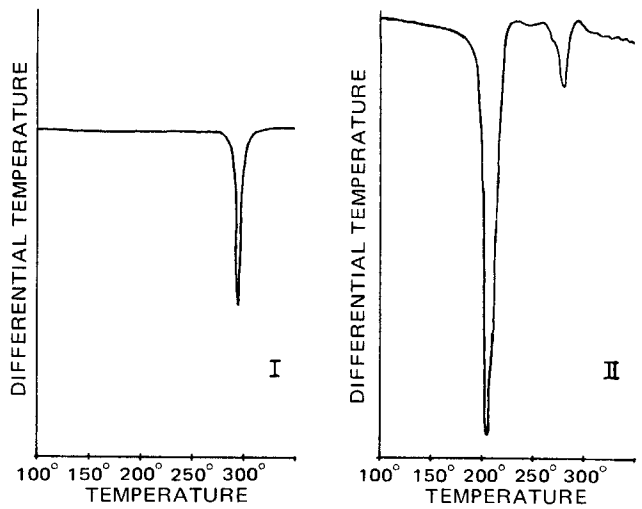


Figure 2—*Differential thermal analysis of I and II.*

The isolated reaction product, later identified as II, was a white, crystalline material. Methanol was used in the reaction medium because water alone would not wet I; however, the volume was chosen empirically. Subsequently, the solubility of II was found to be greater in methanol–water mixtures, preventing precipitation of II and aiding its separation from unreacted materials after preparation.

The optimum reaction time was determined by spectrophotometrically analyzing samples of the supernate for total ligand (I). After 24 hr, no further increase in absorbance was observed. Although water-soluble magnesium salts were tried in an attempt to prepare the chelate by this procedure, only magnesium oxide was useful. The pH increase in the microenvironment produced by the magnesium hydroxide may be sufficiently high to remove an enolic proton from the ligand, enabling the chelate to form.

Before a reliable estimate of the stoichiometry of II could be obtained, it was necessary to determine the number of water molecules associated with the complex. The presumption of the presence of water subsequently was confirmed by thermogravimetric analysis and GLC. Thermograms of II (Fig. 1) showed a loss of loosely bound water between 30 and 100°, followed by constant weight to 140°. Between 140 and 190°, sigmoidal curves representing a mass loss of 4.81–4.98% were obtained. The theoretical percentage of water in a 2:1 chelate, based on two water molecules, is 4.93%. Identical treatment of I resulted in no mass loss; consequently, two molecules of water were associated with each chelate molecule.

However, since thermogravimetric analysis represents only mass loss, GLC was used qualitatively to confirm that the weight loss was due to water. Chromatograms of samples of II, dried *in vacuo* at 85° to remove surface water, showed a peak with the same retention time as water-spiked dimethyl sulfoxide samples. Under the chromatographic conditions used, II was not eluted from the column. These results substantiated that the mass loss observed between 140 and 190° was attributable to water associated with the chelate.

After quantitation of the water of coordination or, possibly, water of crystallization occupying lattice sites (11) and after carbon, hydrogen, and magnesium analyses, the analytical data clearly indicate a 2:1 ligand–metal stoichiometry.

Anal.—Calc.: C, 62.45; H, 3.59; Mg, 3.33; water, 4.93. Found (standard error of the mean of three determinations): C, 62.66; H, 3.67; Mg, 3.29 (0.012) (complexometric titration) and 3.33 (0.026) (atomic absorption); water, 4.91 (0.050) (thermogravimetry).

Differential thermal analysis of I and II showed substantial differences (Fig. 2). Thermograms of I exhibited a single, sharp endothermic peak at a corrected temperature of 288°, which was within the reported melting point range of 287–293° (12). The thermogram of II showed two broad endothermic peaks: a large peak at 205° and a small peak at 274°. Visual observation in a melting-point apparatus suggested that both peaks may have been due to degradation. A color change from white to yellow was observed at approximately 200° and a brown carmelized mass resulted at 280°, but no melt was observed at any temperature.

NMR spectra obtained in deuterated chloroform showed identical chemical shifts for I and II. The spectra had a single signal of 3.7 ppm arising from the methylene protons, a complex multiplet between 7 and 8 ppm corresponding to the aromatic protons, and a single signal at 11.3

[18] Model 267, Perkin-Elmer.
[19] Bruker Hx 90 equipped with an sxp RF pulse amplifier and an R-NC 12 data system.
[20] Burroughs Wellcome & Co.
[21] Model 76008, Beckman.
[22] Galbraith Laboratories, Knoxville, Tenn.

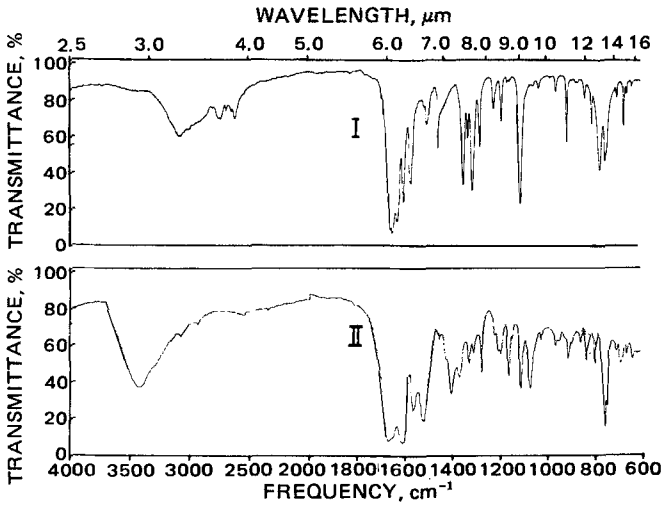


Figure 3—*IR spectra of I and II.*

ppm due to the hydrogen-bonded (enolic) protons at C-4 and/or C-4′ (13).

The poor solubility of II in deuterated chloroform necessitated the use of an NMR spectrometer equipped with a Fourier transform data system. The sample was analyzed overnight; all peaks were identifiable and appeared at their expected chemical shifts, but the spectrum could not be quantitated. Therefore, nonaqueous titration was used to quantitate the enolic protons. As expected, titration curves of II yielded two sharp breaks corresponding to two protons. One proton is believed to be at C-4 or C-4′ on each ligand molecule.

The IR spectra of I and II are shown in Fig. 3. Due to intramolecular hydrogen bonding, the carbonyl stretching frequency of I appeared at 1660 cm^{-1} (13). When I was *O*-methylated and no intramolecular hydrogen bonding could occur, the carbonyl peak was shifted to 1725 cm^{-1} (13). Since there was no shift to longer wave numbers in the carbonyl stretching frequency of II, both carbonyl groups in each ligand probably were bonded. Based on the presence of two titratable protons on II, it seems reasonable that one carbonyl group in each ligand was still intramolecularly hydrogen bonded and that the other carbonyl was bonded with magnesium.

Intramolecular hydrogen bonding also affected the position of the hydroxyl peak in the IR region. This peak appeared at 3100 cm^{-1} for I; however, II exhibited a large, broad water band between 3700 and 2800 cm^{-1}. Since II is believed to retain one intramolecular hydrogen bond on each ligand, the hydroxyl band at 3100 cm^{-1} was probably concealed beneath the water band.

In summary, elemental analyses conclusively established a 2:1 ligand–metal stoichiometry for II and the IR and other analytical data are consistent with Structure II in Scheme I. X-ray studies on the chelate are currently underway to substantiate further the structure.

REFERENCES

(1) D. Bernstein, *Drug Intel. Clin. Pharm.*, **5**, 276 (1971).

(2) R. A. O'Reilly, P. M. Aggeler, and L. S. Leong, *Thromb. Diath. Haemorrh.*, **11**, 1 (1964).

(3) E. Losinski, *Can. Med. Assoc. J.*, **83**, 177 (1960).

(4) J. L. Lach and L. D. Bighley, *J. Pharm. Sci.*, **59**, 1261 (1970).

(5) M. J. Akers, J. L. Lach, and L. J. Fischer, *ibid.*, **62**, 391 (1973).

(6) J. J. Ambre and L. J. Fischer, *Clin. Pharmacol. Ther.*, **14**, 231 (1973).

(7) K. A. Connors, "A Textbook of Pharmaceutical Analysis," Wiley, New York, N.Y., 1967, pp. 68, 69.

(8) K. A. Connors, "A Textbook of Pharmaceutical Analysis," Wiley–Interscience, New York, N.Y., 1974, p. 61.

(9) J. P. Leyda, in "Pharmaceutical Chemistry," vol. 1, L. G. Chatten, Ed.; Dekker, New York, N.Y., 1966, p. 277.

(10) J. D. McCallister, T. F. Chin, and J. L. Lach, *J. Pharm. Sci.*, **59**, 1286 (1970).

(11) R. H. Gore and W. W. Wendlandt, *Anal. Chem. Acta*, **52**, 83 (1970).

(12) "The Merck Index," 7th ed., Merck & Co., Rahway, N.J., 1960, p. 153.

(13) D. W. Hutchinson and J. A. Tomlinson, *Tetrahedron*, **25**, 2531 (1969).

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X-Ray Analysis of Sulfur-Containing Colchicine Derivatives

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Abstract □ The crystal and molecular structures of deacetylthiocolchicine hydrochloride dihydrate and thiocolchicine hexahydrate were determined by X-ray diffraction. The replacement of oxygen by sulfur on the C ring methoxy group causes greater puckering of the troponoid ring. The conformation of one A ring methoxy group differs from that of colchicine derivatives that do not contain sulfur.

Keyphrases □ Colchicine derivatives—X-ray diffraction determination of crystal and molecular structures □ Thiocolchicines, various—X-ray diffraction determination of crystal and molecular structures □ X-ray diffraction—determination of crystal and molecular structures of various thiocolchicines □ Crystal and molecular structures—various thiocolchicines, X-ray diffraction determination

Colchicine (I), an ancient drug, is used primarily in the treatment of gout. Attempts to use this powerful mitotic inhibitor in the treatment of human malignancies have been largely unsuccessful. Other mitotic poisons, however, are being used successfully with human tumors. In particular, vinblastine and vincristine are used for the treat-