2-^{14}C-1-Allyl-3,5-diethyl-6-chlorouracil II: Isolation and Structures of the Major Sulfur-Free and Three Minor Sulfur-Containing Metabolites and Mechanism of Biotransformation

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Abstract □ The metabolites of 1-allyl-3,5-diethyl-6-chlorouracil in rabbit urine were isolated by preparative thick-layer, liquid-column, and gas chromatography. With the aid of mass and ^{1}H-NMR spectra, and by comparison with an authentic sample, the major metabolite, 1, was identified as 6,8-diethyl-2-hydroxymethyl-tetrahydrooxazolo-[3,2-*c*]-pyrimidine-5,7(4H,6H)-dione, Metabolite 2 as 1-allyl-3-ethyl-5-(1-hydroxyethyl)-6-methylthiouracil, Metabolite 3 as 1-allyl-3,5-diethyl-6-methylthiouracil, and Metabolite 4 as 6,8-diethyl-2-hydroxymethyl-tetrahydrothiazolo-[3,2-*c*]pyrimidine-5,7(4H,6H)-dione. The mechanism of the formation of sulfur-containing metabolites is discussed, and a new metabolic pathway for the formation of methylthio compounds is proposed.

Keyphrases □ 2-^{14}C-1-Allyl-3,5-diethyl-6-chlorouracil—isolation and structures of the sulfur-free and sulfur-containing metabolites, mechanism of biotransformation □ Metabolites—2-^{14}C-1-allyl-3,5-diethyl-6-chlorouracil, isolation and structures of the major sulfur-free and sulfur-containing, mechanism of biotransformation □ Biotransformation—2-^{14}C-1-allyl-6-chlorouracil, isolation and structures, sulfur-free and sulfur-containing metabolites, mechanism □ Sulfur—2-^{14}C-1-allyl-3,5-diethyl-6-chlorouracil metabolites, isolation and structures, mechanism of biotransformation

1-Allyl-3,5-diethyl-6-chlorouracil[1] (I) has shown promising therapeutic activity against herpes simplex and vaccinia virus (1, 2) and is used for the external treatment of herpes and other viral infections of the skin and mucous membranes. Its absorption in human skin and excretion, distribution, and metabolism in rats and rabbits using ^{14}C-I were reported previously (3). The isolation and structure determination of the major and three minor metabolites are reported in this study.

EXPERIMENTAL[2]

Application—For the isolation of the metabolites, four male rabbits, each 2–2.5 kg, were kept in metabolism cages. Each rabbit was given 5 mg of ^{14}C-I daily [specific activity 0.073 mCi/mmole (3)] mixed in the food over a period of 3 months. They were given dry food[3] and water and kept under normal laboratory conditions. The urine samples were collected daily and frozen to avoid any decomposition.

Radioactivity Measurements—The radioactive zones on TLC plates were located with a thin-layer scanner[4]. The radioassay in the solution was carried out in a two-canal scintillation counter[5], using a scintillation solution of 1000 ml of dioxane, 180 g of naphthalene, 8 g of 2,5-diphenyl-oxazole, and 0.1 g of 1,4-bis[2-(4-methyl-5-phenyl-oxazolyl)] benzene. The external standardization technique was employed. An aliquot (0.1 ml) of the sample solution was directly mixed into the scintillation solution and measured for radioactivity.

Chromatography—Analytical TLC was performed on 20 × 5-cm (0.25 mm) silica gel plates[6]. The solvent system was petroleum ether–ethyl acetate (1:1, v/v). Preparative TLC was performed on 20 × 20-cm (2 mm) silica gel plates with the described solvent system. Column chromatography was carried out on 0.05–0.2-mm silica gel with increasing proportions of ethyl acetate in petroleum ether.

Gas Chromatography—Analytical GLC determinations were performed on a gas chromatograph[7] equipped with a flame-ionization detector. The chromatographic column was glass tubing (1.5 m × 0.4-mm i.d.) packed with 3% SE-30 on 100–120 mesh Varaport 30. The operating conditions were: injection port temperature, 230°; oven temperature, 200°; detector temperature, 250°; and nitrogen (carrier gas) flow rate, 20 ml/min.

Preparative gas chromatography was performed on a gas chromatograph[8] with a flame-ionization detector. The chromatographic column was aluminum tubing (4 m × 6-mm i.d.) packed with 3% SE-30 on 100–120 mesh Varaport 30. The operating conditions were: detector/injector temperature, 225°; exit tip temperature, 200°; column oven temperature, 200°, isotherm; split 1:14; and nitrogen (carrier gas) flow rate, 350 ml/min.

Metabolite Isolation—The urine was adjusted to pH 7 and extracted in a liquid–liquid extractor with ether for 96 hr yielding ~90% of the radioactivity (Extract A), which on TLC showed Metabolites 1, 2, and 3 in the ratio 8.5:1.0:0.5 (3). GLC also showed Metabolite 4 (0.3%) but no unchanged I. For the isolation of the metabolites, Extract A was first purified to remove major impurities. It was applied on preparative TLC plates in a linear form and developed in the solvent system. The silica gel beyond the start line was removed, extracted with methanol in a soxhlet apparatus, solvent removed, and concentrated nearly to 5 ml. The process was repeated again. Purified Extract A (30 μCi or 100 mg of total metabolites) was concentrated to dryness and taken up in petroleum ether. It was loaded on a glass column (80 × 3 cm), filled with silica gel and eluted with increasing proportions of ethyl acetate in petroleum ether using an automatic fraction collector. Eight hundred 20-ml fractions were collected. A radioassay was carried out with each fraction. The loaded radioactivity was recovered fully. The following eluates were pooled together and concentrated after analysis in GLC:

100–150 (25% ethyl acetate in petroleum ether)—Metabolite 3
151–250 (30% ethyl acetate in petroleum ether)—Metabolite 2
450–650 (65% ethyl acetate in petroleum ether)—Metabolite 1
651–800 (70% ethyl acetate in petroleum ether)—Metabolite 4

The concentrated eluate of Metabolite 1 was brown-colored. It was loaded on a silica gel column and eluted with 60% ethyl acetate in petroleum ether. The solvent was removed and the residue was twice crystallized from methanol to yield 45 mg of Metabolite 1, mp 163–164°. Metabolites 2–4 could not be further purified by column chromatography. GLC showed the presence of a number of impurities. The substance was therefore purified by preparative GLC, to yield 20 mg of Metabolite 2 and 8 mg of Metabolite 3. Attempts to isolate and purify Metabolite 4 failed due to the minimal amount.

Synthesis—*Metabolite 1 (6,8-Diethyl-2-hydroxymethyl-tetrahydrooxazolo-[3,2-c]pyrimidine-5,7(4H,6H)-dione)* — 6,8-Diethyl-2-bromomethyl-tetrahydrooxazolo[3,2-*c*]pyrimidine-5,7(4H,6H)dione (1.5 g, 5 mmoles), prepared according to the literature method (4), was dissolved in 10 ml of dimethylformamide and heated under reflux for 10 hr with silver acetate (20 mmoles), which was obtained by heating 2.8 g of silver carbonate and 20 ml of acetic acid. After cooling, the insoluble

[1] Acluracil, Robugen GmbH, 7300 Esslingen/Neckar, West Germany.
[2] Melting points were taken on a Tottoli (Büchi, Switzerland) apparatus and are uncorrected. Mass spectra were measured at an ionizing potential of 70 eV with a CH-7 Varian MAT spectrometer using a direct evaporator inlet system or by combination with a gas chromatograph. ^{1}H-NMR were recorded on a Varian HA-100 or Bruker XL-90 spectrometer using deuterochloroform, deuterobenzene, or carbon tetrachloride as the solvent and trimethylsilane as the internal standard.
[3] Altromin, Altromin, Lage/Lippe, West Germany.
[4] Dr. Rudolf Berthold Co., Wildbad, West Germany.
[5] Tri-Carb model 3950, Packard Instrument Co., La Grange, Ill.
[6] Silica gel HF_{254}, E. Merck AG, Darmstadt, West Germany.
[7] Aerograph 1740, Varian.
[8] Aerograph 712, Varian.

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material was filtered off and the solvent evaporated. The residue was dissolved in chloroform and washed with a saturated solution of sodium bicarbonate followed by water. The organic layer was dried over sodium sulfate and filtered through active charcoal and the solvent was removed *in vacuo*. The residue was dissolved in methanol (10 ml). A solution of 0.6 g of sodium hydroxide in 3 ml of water was added and the reaction mixture kept for 16 hr at room temperature. The solvent was removed *in vacuo* and the residue dissolved in methanol–water (1:1) and loaded on an ion-exchange resin[9] (H^+) column and eluted with methanol–water (1:1). The eluates were pooled together and evaporated *in vacuo* and the residue crystallized from ethyl acetate–petroleum ether mixture to give Metabolite 1, mp 162.5–163.5°, in a 40% yield.

Anal.—Calc. for $C_{11}H_{16}N_2O_4$: C, 55.01; H, 6.71; N, 11.65. Found: C, 55.52; H, 6.28; N, 11.08.

Metabolite 3 (1-Allyl-3,5-diethyl-6-methylthiouracil)—Methyl mercaptan (10 g, 0.2 mole) in methanol was added to 50 ml of a 30% methanolic sodium methoxide solution. Compound I (36.4 g, 0.15 mole) was added with stirring. The reaction mixture was refluxed with stirring for 24 hr and poured into water. It was extracted with chloroform, the solvent evaporated, and the residue distilled *in vacuo* to give Metabolite 3, bp 109°/0.02 mm, in 36% yield.

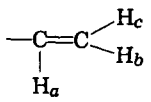
Anal.—Calc. for $C_{12}H_{18}N_2O_2S$: C, 56.67; H, 7.13; N, 11.01; S, 12.61. Found: C, 56.70; H, 7.35; N, 11.31; S, 12.52.

Metabolite 4 (6,8-Diethyl-2-hydroxymethyl-tetrahydrothiazolo-[3,2-c]pyrimidine-5,7(4H,6H)-dione) — 1-(2′,3′-Epoxypropyl)-3,5-diethyl-6-chlorouracil (2.6 g, 10 mmoles), prepared according to the described method (5), was dissolved in dry dimethyl sulfoxide (20 ml) and sodium hydrogen sulfide (1.1 g, 20 mmoles) was added. The reaction mixture was then concentrated *in vacuo* and the residue poured into water. After it was extracted twice with methylene chloride, the organic layer was dried over sodium sulfate. The solvent was removed and the milky viscous liquid was purified by column chromatography using silica gel (0.05–2 mm) with methylene chloride as elution solvent to give Metabolite 4, mp 83°, in 40% yield.

Anal.—Calc. for $C_{11}H_{16}N_2O_3S$: C, 51.54; H, 6.25; N, 10.93; S, 12.54. Found: C, 50.86; H, 6.55; N, 10.44; S, 12.12.

RESULTS AND DISCUSSION

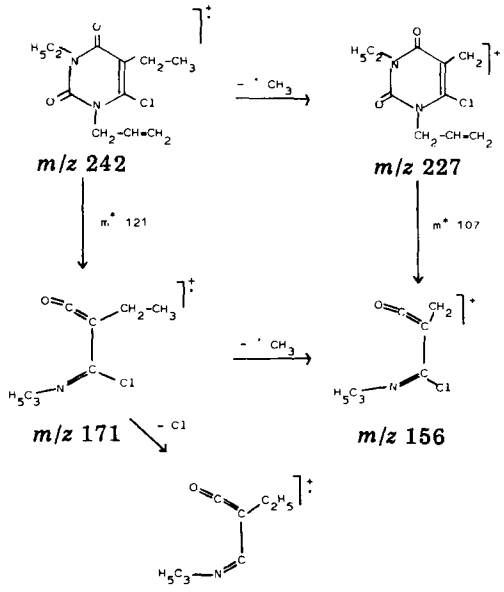
Mass and ^{1}H-NMR Spectrum of I—In the ^{1}H-NMR spectrum of I, both methyl groups appeared as triplets at δ 1.08 and 1.20 ppm. The methylene group at N-3 appeared as a quartet at δ 3.93, and the methylene group at N-1 appeared as a doublet at δ 4.65 ppm. In the allylic protons of



H_a appeared at δ 5.94 ppm as a multiplet, H_b at δ 5.22 ppm as a doublet, and H_c at δ 5.17 ppm as a doublet (J_{ab} = 12 Hz, J_{ac} = 17 Hz).

In the mass spectrum, the intensity ratio of the signals at *m/z* 242/244, 227/229, 201/203, 171/173, and 156/158 showed the presence of chlorine. The loss of ethyl isocyanate, C_2H_5CNO (242 → 171 or 227 → 156) supported by metastable peaks m* in the final sequence is characteristic of uracil and barbituric acid derivatives in a retro-Diels-Alder fragmentation (6–8). The fragmentation of I can be formulated according to Scheme I.

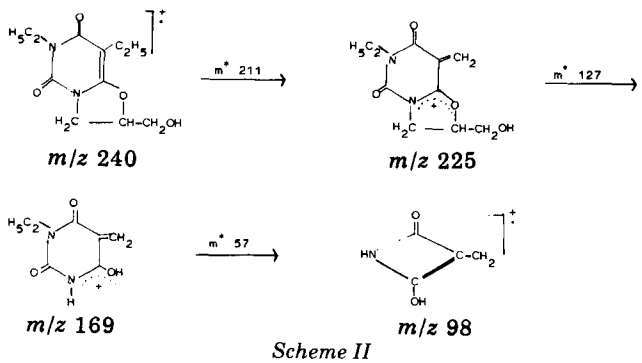
Structure of Metabolite 1—In the mass spectrum of the metabolite, the major fragmentation is formulated according to Scheme II, the formula of each fragment being established by high resolution mass spectrometry. Direct correlation between the fragments is demonstrated by the appearance of the metastable peaks m*. The mass spectrum of the metabolite showed the absence of chlorine in the molecule. First, the β-methyl group at C-8 was cleaved off the molecular ion leaving a highly stabilized cation. Next, the substituent at N-4 was removed: the expulsion of C_3H_4O showed one oxygen having been incorporated into the allylic side chain, while the other oxygen was bonded to the pyrimidine ring system. The loss of ethyl isocyanate (C_2H_5CNO) in the final sequence was similar to that of I. In the ^{1}H-NMR spectrum ($CDCl_3$), a signal appeared at δ 2.80 ppm (1 H) which, being exchangeable with deuterium oxide and also rather susceptible in position and line shape to both temperature and concentration, must be attributed to an hydroxide group. Since it was split into a triplet (J = 6.0 Hz), the metabolite must



Scheme I

contain a —CH_2OH moiety. The lone C-2 proton appeared at the lowest field, split into seven lines (not a heptuplet). The methylene proton at both C-3 and exocyclic carbon were nonequivalent, however, rendering the spectrum unsusceptible to first-order analysis. The theoretical spectra for Metabolite 1 and for closely related compounds prepared by independent synthesis were calculated and fitted to the experimental data within ±0.01 Hz. Table I lists the data thus obtained which confirm the heterobicyclic structure for the metabolite. In each case, the less shielded C-3 protons were assigned to H^A by virtue of the larger coupling to H^C (J *cis* > J *trans* for five-membered ring) (9). Spectral analysis of all compounds listed in Table I additionally was complicated by the N—CH_2 quartet being superimposed upon the partial spectra of H^A, H^B, and $H^{D,E}$. By changing the solvent from $CDCl_3$ to C_6D_6, however, all protons situated on the fused five-membered ring appeared better shielded by 0.8–1.3 ppm, while signals of the N-6 and C-8 substituent protons were shifted slightly to lower field. Apparently, the benzene molecules orient themselves in a manner known from other amide spectra (10): farthest away from the negative end of the N—C—O dipole, thus, placing substituents at N-4 and C-9 directly within the diamagnetic shielding cone due to arene ring current. If spectra accumulation was possible, the rather low solubility of these compounds in benzene (<5 × 10^{-2} mole/liter) was more than offset by the well separated partial spectra[10]. These data establish Metabolite 1 as 6,8-diethyl-2-hydroxymethyl-tetrahydrooxazolo-[3,2-c]pyrimidine-5,7(4H,6H)-dione. The metabolite structure was confirmed by its independent synthesis; the synthetic Metabolite 1 mass spectrum and ^{1}H-NMR, GLC, and TLC data were identical with those of the isolated product.

Structure of Metabolite 3—The mass spectrum of Metabolite 3 showed the absence of chlorine in the molecule. The methyl group was also cleaved off here (254 → 239) and in a retro-Diels-Alder reaction supported by metastable peaks, the loss of ethyl isocyanate (239 → 168) subsequently took place. In this respect, the fragmentation was similar



Scheme II

[9] Dowex 50 W × 4, Dow Chemical Co., Midland, Mich.

[10] In nitrobenzene-d_5 the effect is lost completely.

Table I—^{1}H-NMR Data of Tetrahydrooxazolo[3,2-*c*]pyrimidine-5,7(4H,6H)-dione Derivatives[a]

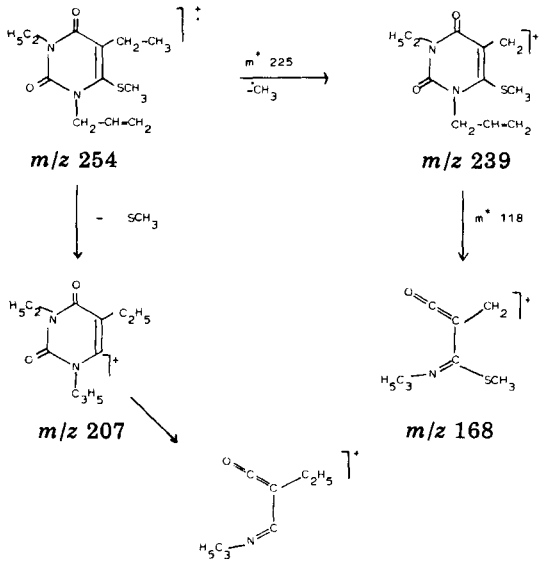
X	R^1	R^2	Solvent	H^{C}[b]	H^{A}	H^{B}	H^{D},H^{E}	J_{ac}	J_{bc}	$J_{dc,ec}$	J_{ab}	R^1		R^2	
Br	C_2H_5	C_2H_5	$CDCl_3$ (0.1 *M*)	5.124	4.277	4.061	3.639[c]	8.36	6.05	5.25	−10.90	CH_2	3.962	CH_2	2.354
												CH_3	1.213	CH_3	1.072
			C_6D_6 (0.01 *M*)	3.794	3.265	3.207	2.470	8.44	6.17	5.29	−10.75	CH_2	4.070	CH_2	~2.51
												CH_3	1.259	CH_3	1.23
Br	H	C_6H_5	$CDCl_3$ (0.003 *M*)	5.222	4.341	4.151	3.679[c]	8.55	6.12	4.84	−10.86	N—H	~7.88	C_6H_5	multi-plet
OCH_3	C_2H_5	C_2H_5	$CDCl_3$ (0.1 *M*)	4.981	4.156	4.016	3.687	8.64	6.69	3.70	−10.45	CH_2	3.959	CH_2	2.356
							3.645			4.49		CH_3	1.208	CH_3	1.064
			C_6D_6 (0.1 *M*)	3.890	3.276	3.264	2.781	8.72	6.63	3.84	−10.30	CH_2	4.074	CH_2	2.558
							2.665			4.37		CH_3	1.247	CH_3	1.247
OH^{Fd}	C_2H_5	C_2H_5	$CDCl_3$ (0.1 *M*)	4.982	4.173	4.100	4.030	8.59	6.94	3.35	−10.51	CH_2	3.951	CH_2	2.314
							3.831			3.71		CH_3	1.204	CH_3	1.051
			C_6D_6 (0.005 *M*)	3.678	3.165	3.337	2.956	8.71	6.82	3.51	−10.32	CH_2	4.092	CH_2	2.540
							2.714			4.02		CH_3	1.267	CH_3	1.255

[a] δ (ppm) relative to tetramethylsilane as internal standard; *J* (Hz); 30°. All spectra were measured at 90 MHz by Pulse-Fourier-Transform technique (8k interferogram, dwell time 560 or 840 μsec); number of scans varied between 1000 and 20,000, depending on concentration. [b] Values of δ and *J* given for H^A–H^F were obtained from theoretical spectra calculated with programs ITRCL1 and ITRCL2 (NICOLET users society) on a NICOLET BNC-12. [c] Spectra show some evidence of H^D,H^E-nonequivalence. [d] $\delta_{OH}F$ 2.797 ppm, J_{df} = 5.48 Hz, J_{ef} = 6.52 Hz.

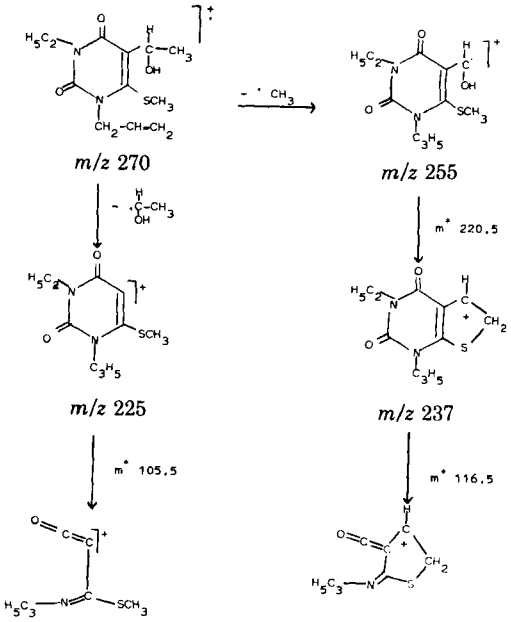
to that of I, as also the fragment at *m/z* 136 appeared in both the spectra. The intensity of ions at m + 2 with 5.1% relative intensity and the loss of 47 mass units showed the presence of an SCH_3 group. The fragmentation of Metabolite 3 can be formulated according to Scheme III. In the ^{1}H-NMR spectrum ($CDCl_3$), near the signals of unchanged allyl and alkyl groups, a singlet appeared at δ 2.44 (3 protons) showing the presence of an SCH_3 group. These data establish Metabolite 3 as 1-allyl-3,5-diethyl-6-methylthiouracil, which was further confirmed by an independent synthesis. The synthetic Metabolite 3 mass spectrum, ^{1}H-NMR, GLC, and TLC data were identical with those of the isolated product.

Structure of Metabolite 2—In the mass spectrum the intensity ratio of the ions at *m/z* 272/270 and 257/255 with 6.5% relative intensity showed the presence of sulfur. The fragmentation pattern of Metabolite 2 (Scheme IV) was identical with I and Metabolite 3. The behavior of certain ions showed the presence of a hydroxyethyl group. The methyl group at C-5 was knocked off (270 → 255) and then a water molecule was eliminated (255 → 237), and in a retro-Diels-Alder reaction, the ion at *m/z* 166 was formed. By loss of a hydroxyethyl group, the ion at *m/z* 225 was formed and subsequently by retro-Diels-Alder reaction the ion at *m/z* 154 also formed. The loss of an allyl group with 41 mass units was also similar to the fragmentation of I and Metabolite 3. ^{1}H-NMR showed the protons of allylic and *N*-ethyl groups. The singlet at δ 2.43 ppm (3 protons) was assigned to an SCH_3— group. A doublet at δ 1.55 ppm (3 protons) was assigned to an hydroxide group. Due to nonavailability of the synthetic product, a comparison could not be made but these data confirm the Metabolite 2 as 1-allyl-3-ethyl-5-(1-hydroxyethyl)-6-methylthiouracil.

Structure of Metabolite 4—As Metabolite 4 could not be isolated, its structure, 6,8-diethyl-2-hydroxymethyl-tetrahydrothiazolo-[3,2-*c*]-pyrimidine-5,7(4H,6H)-dione, was based on mass spectra obtained by



Scheme III

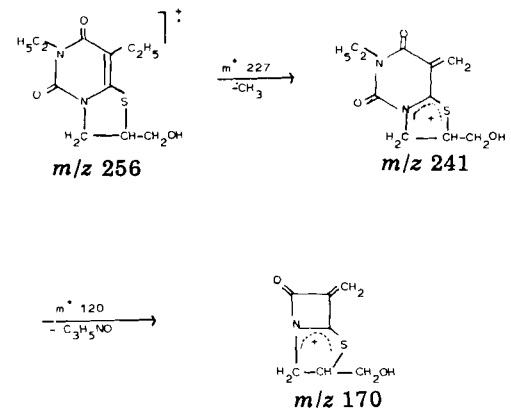


Scheme IV

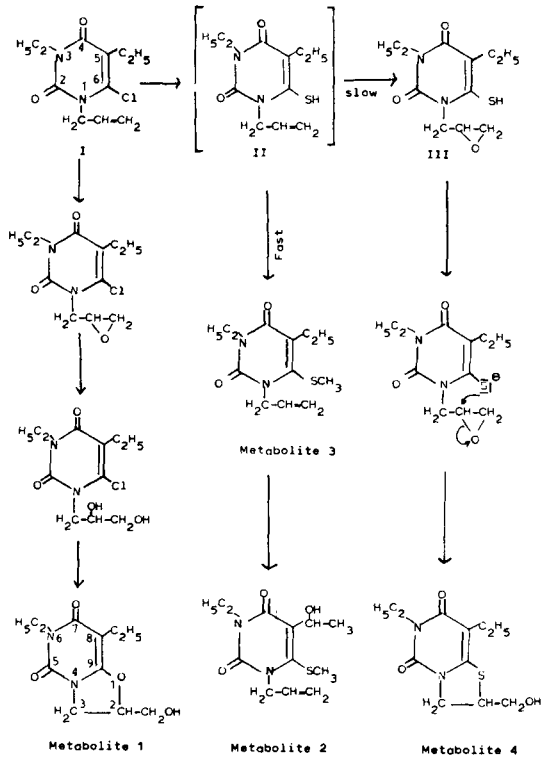
the combination of GLC–mass spectrometry. In the mass spectrum of the metabolite, the metastable peaks establish the following major fragmentation:

$$m/z\ 256 \xrightarrow{m^*\ 227} m/z\ 241 \xrightarrow{m^*\ 120} m/z\ 170$$

The intensity of the ions at m + 2 with 5.1% relative intensity showed the presence of sulfur. First, the β-methyl group at C-8 was cleaved off the molecular ion (256 → 241) leaving a highly stabilized cation. The loss of ethyl isocyanate (241 → 170) by a retro-Diels-Alder fragmentation sup-



Scheme V



Scheme VI

ported by a metastable peak in the final sequence is characteristic of uracil and barbituric acid derivatives. The structure of the metabolite was confirmed by its independent synthesis. The synthetic Metabolite 4 showed the fragmentation pattern as shown in Scheme V, the molecular formula of each fragment was established by high resolution mass spectrometry. The mass spectrum of Metabolite 4 was identical in all respects with the synthetic product, and thus established its structure as 6,8-diethyl - 2 - hydroxymethyl-tetrahydrothiazolo-[3,2-*c*]pyrimidine-5,7-(4H,6H)-dione.

Mechanism of Biotransformation—*Metabolite 1*—In the formation of Metabolite 1, the first step is epoxidation of the allylic double bond of I followed by hydrolysis to propane-2,3-diol as described for analogous compounds (11). Subsequently, the β-hydroxy group substitutes the heterocyclic chlorine, thus forming the fused bicyclic Metabolite I *via* an intramolecular S_N reaction.

Metabolites 2–4—Recently, the formation of sulfur-containing metabolites from sulfur-free drugs has developed increasing interest. The methylthio (—SCH_3) metabolites have been reported in a number of drugs (12–18). The origin and the mechanism of formation of these methylthio metabolites is not known so far. Two pathways have been proposed: direct attachment of a methylthio group and transformation of mercapturic acids or glutathione conjugates (19, 20). Metabolite 4, due to its unique structure, supports the mechanism of the formation of methylthio metabolites *via* the formation of an intermediate thiol (—SH). A bicyclic sulfur-containing barbituric acid derivative of this type as a product of biochemical degradation has not yet been reported in the literature. A new metabolic pathway for the formation of this sulfur-containing Metabolite 4 and for the methylthio metabolites, 2 and 3, is proposed according to Scheme VI. In the formation of Metabolite 4, the first step is the substitution of chlorine by a thiol group forming an intermediate compound (II). This reaction may be enzymatic. The epoxidation of the double bond takes place giving Compound III. Subsequently, the sulfur atom attacks the β-carbon atom of the epoxide ring thus forming the fused bicyclic Metabolite 4 *via* an intramolecular S_N-reaction. The formation of methylthio Metabolites 2 and 3 may take place through this intermediate (II), as the enzymatic methylation of the thiols is reported *in vitro* and *in vivo* (21). It seems that the methylation of the intermediate (II) is a fast preferential reaction, therefore, larger amounts of Metabolites 2 and 3 are formed. The epoxidation of II is a slow specific reaction. The epoxide (III) is unstable. It has a pronounced tendency to ring closure leading to the stable Metabolite 4.

REFERENCES

(1) K. K. Gauri and H. Kohlhage, *Chemotherapy*, **14,** 158 (1969).

(2) K. K. Gauri and B. Rohde, *Klin. Wochenschr.*, **47,** 375 (1969).

(3) R. Kaul, B. Hempel, and G. Kiefer, *J. Pharm. Sci.*, **71,** 893 (1982).

(4) E. E. Smissman and J. W. Ayres, *J. Org. Chem.*, **36,** 2407 (1971).

(5) G. Kiefer, R. Kaul, K. Keppeler, and B. Hempel, *Arch. Pharmaz.*, **315,** 444 (1982).

(6) T. Nishiwaki, *Tetrahedron*, **22,** 3117 (1966).

(7) J. M. Rice, G. O. Dudek, and M. Barber, *J. Am. Chem. Soc.*, **87,** 4569 (1965).

(8) R. F. Skinner, E. G. Gallaher, and D. B. Predmore, *Anal. Chem.*, **45,** 574 (1973).

(9) L. E. Erickson, *J. Am. Chem. Soc.*, **87,** 1867 (1965).

(10) J. V. Hatton and R. E. Richards, *Mol. Phys.*, **5,** 139 (1962).

(11) D. J. Harvey, L. Glazener, C. Stratton, D. B. Johnson, R. M. Hill, E. C. Horning, and M. G. Horning, *Res. Commun. Chem. Pathol. and Pharmacol.*, **4,** 247 (1972).

(12) K. Kamei, M. Matsuda, and A. Momose, *Chem. Pharm. Bull.*, **23,** 683 (1975).

(13) A. Focella, P. Hesling, and S. Teitel, *Can. J. Chem.*, **50,** 2025 (1972).

(14) D. H. Chatfield and W. H. Hunter, *Biochem. J.*, **134,** 879 (1973).

(15) T. Ou, K. Tatsumi, and H. Yoshimura, *Biochem. Biophys. Res. Commun.*, **75,** 401 (1977).

(16) B. Stock and G. Spiteller, *Arzneim.-Forsch./Drug Res.*, **27,** 982 (1977).

(17) W. G. Stillwell, *Pharmacologist*, **19,** 169 (1977).

(18) M. Tateishi and H. Shimizu, *Xenobiotica*, **6,** 431 (1976).

(19) Z. H. Israili, P. G. Dayton, and J. R. Kiechel, *Drug Metab. Dispos.*, **5,** 411 (1978).

(20) P. Jenner and B. Testa, *Xenobiotica*, **8,** 1 (1978).

(21) M. Tateishi, S. Suzuki, and H. Shimizu, *J. Biol. Chem.*, **253,** 8854 (1978).