Identification of Canine Urinary Metabolites of the Antiprotozoal Agent 3a,4,5,6,7,7a-Hexahydro-3-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,2-benzisoxazole

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Abstract □ Metabolite fractions from the urine of a dog dosed with 3a,4,5,6,7,7a-hexahydro-3-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,2-benzisoxazole (MK-0436) were obtained by the use of high-performance liquid chromatography. These fractions were of suitable purity for structural elucidation. Data obtained by mass spectrometry and NMR spectroscopy allowed the identification of seven major metabolites of this drug. Biotransformation in each case involved hydroxylation (mono or di) of the hexahydrobenzisoxazole ring.

Keyphrases □ 3a,4,5,6,7,7a-Hexahydro-3-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,2-benzisoxazole—identification of urinary metabolites, dogs □ Metabolites—of 3a,4,5,6,7,7a-hexahydro-3-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,2-benzisoxazole, identification, dog urine □ Antiprotozoal agents—3a,4,5,6,7,7a-hexahydro-3-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,2-benzisoxazole, identification of urinary metabolites by NMR spectroscopy, mass spectrometry, and capillary column GLC–mass spectrometry

3a,4,5,6,7,7a-Hexahydro-3-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,2-benzisoxazole (MK-0436, I) is an antiprotozoal agent (1) active against *Trypanosoma cruzi* (2) (Fig. 1). This nitroimidazole displayed antibacterial activity during mutagenicity testing[1] using the host-mediated mouse test and the Ames test with S9 liver microsomes (3) but not when the simple Ames spot test (4) was employed (5). These observations led to the presumption that metabolites of I might be antibacterial.

Several metabolite-containing fractions from the urine of a dog given I were found to possess antibacterial activity, and several canine metabolites (both unconjugated and the aglycones from conjugated metabolites) were partially characterized as mono- and dihydroxy species with substitution at positions 4, 5, 6, and/or 7 of the hexahydrobenzisoxazole ring (5). Further characterization of the metabolite mixture and unequivocal identification of several major metabolites of this drug are presented here.

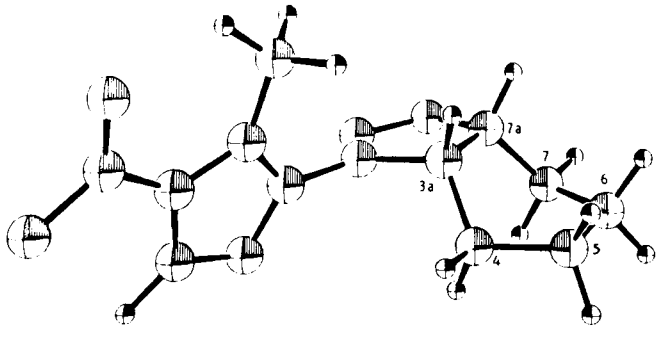

Figure 1—*Computer-generated ORTEP plot of I.*

[1] H. Skeggs, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486, personal communication.

EXPERIMENTAL

Two methanol solutions containing canine urinary metabolites of I (those originally present in the urine as unconjugated metabolites and the aglycones of those originally present as conjugates) were obtained as described previously (5). The two solutions were stored at 4°, and aliquots were taken for isolation and chromatographic purification of the metabolites.

Aliquots of the aglycone metabolite fraction (dissolved in methylene chloride) were subjected to high-performance liquid chromatography (HPLC) using a C_8 reversed-phase preparative column[2] with a step gradient (4.4 ml/min) of methanol–water (1:4) for 40 min, methanol–water (1:2) for 40 min, and methanol for 20 min. Fourteen fractions were collected in each of seven chromatographic runs. The initial column system conditions were regenerated following each run by passage of methanol–water (1:4) through the column for 15 min.

Five fractions (II–VI) yielded sufficient quantities of metabolites to pursue structural elucidation. Aliquots of the metabolite fraction from unhydrolyzed urine also were subjected to HPLC for metabolite isolation using a semipreparative C_{18} reversed-phase column[3]. The quantities of metabolites obtained were, except in one case, too small for successful characterization. Because the amount of bioactivity in the aglycone fraction was approximately twice that found in the nonconjugate fraction, efforts were concentrated on the former. Analytical HPLC was carried out on a C_{18} reversed-phase column[4] with methanol–water (1:4) at a flow rate of 1.5 ml/min. UV detection at 330 nm was used in all HPLC work.

GLC[5] and GLC–mass spectrometric[6] analyses were carried out on trimethylsilylated [bis(trimethylsilyl)trifluoroacetamide[7]–pyridine (3:1) for 3 hr at room temperature] metabolites using 10- or 15-m × 0.3-mm SE-30 glass capillary columns at 240° with helium as the carrier gas. The mass spectrometer[6] was operated with a 70-ev ionizing potential and a 0.8-mamp emission current. Direct-probe mass spectrometry[8] was carried out with a 70-ev ionizing potential, a 60-μamp trap current, and a 3.5-kv accelerating voltage. NMR spectra[9] were obtained from samples dissolved in deuterochloroform.

RESULTS AND DISCUSSION

HPLC (Fig. 2) and GLC (Fig. 3) (trimethylsilyl derivatives) analyses of the aglycone fraction resulting from enzyme hydrolysis of the urinary conjugates demonstrated that it was a multicomponent mixture. Although GLC–mass spectrometry indicated that most of the eluted components (trimethylsilyl derivatives) were drug related [earlier work showed the presence of at least six metabolites of I (5)], it was not possible to discern exact structures on the basis of the mass spectrometric data alone. NMR spectroscopic examination was needed to establish the metabolite structures unequivocally; but to utilize this technique, it was necessary to isolate and purify the metabolites. HPLC appeared to be the method of choice.

Preparative HPLC, followed in some cases by further purification on an analytical HPLC column, gave five metabolite fractions (II–VI in Fig.

[2] Lion Industries.
[3] E. S. Industries.
[4] Waters Associates.
[5] Hewlett-Packard model 5730A instrument with an SGE inlet splitter and a nitrogen-selective detector.
[6] Finnigan model 3200 instrument with a splitless injector.
[7] Supelco.
[8] LKB model 9000 instrument.
[9] Varian SC-300-MHz instrument equipped with a Fourier transform accessory.

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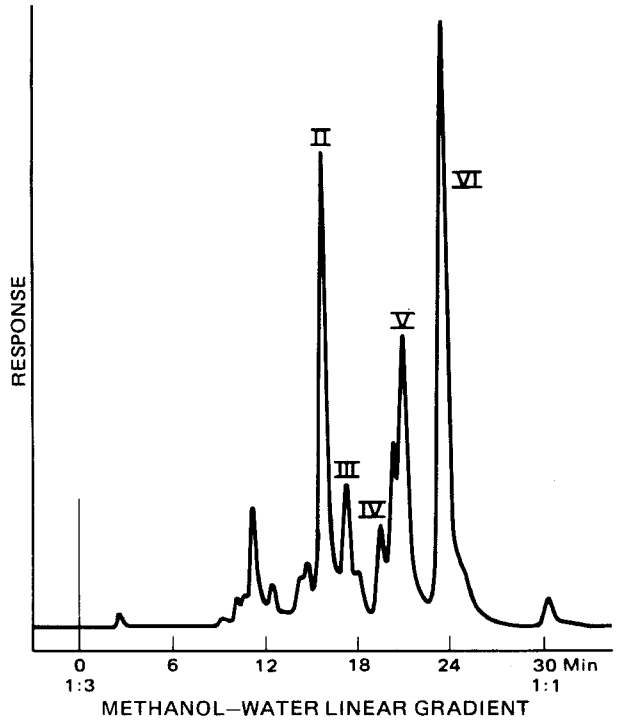


Figure 2—*High-performance liquid chromatogram (C_{18} reversed-phase column) of aglycone fraction (arising from enzyme hydrolysis of canine urinary conjugates).*

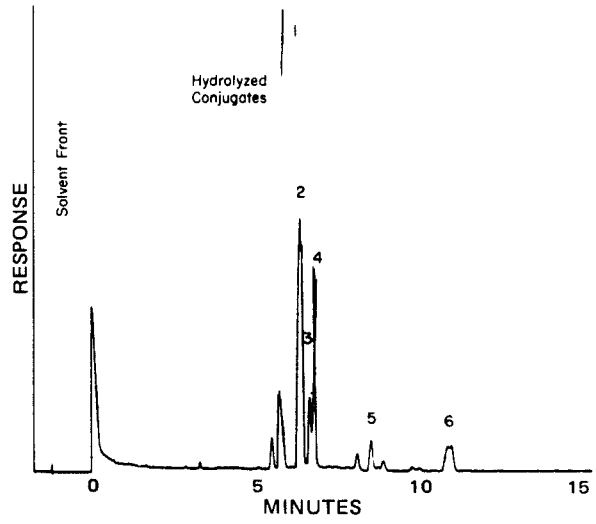


Figure 3—*Gas–liquid chromatogram produced from analysis of the trimethylsilylated aglycone fraction (arising from enzyme hydrolysis of canine urinary conjugates).*

2) of quality and quantity suitable for NMR examination. These fractions were examined first by direct-probe mass spectrometry. The resulting data (major, pertinent ions) are summarized in Table I. Capillary column GLC–mass spectrometric analysis of these samples (following trimethylsilylation) also showed that each sample contained mono- and/or dihydroxylated analogs of the parent drug [molecular ions for the derivatives of *m/e* 426 (dihydroxy metabolites) or *m/e* 338 (monohydroxy metabolites)].

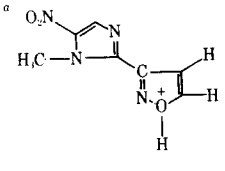
The NMR spectrum (Fig. 4) of I must be understood before structural assignments for the metabolites of this drug can be made. Since previous work demonstrated that the metabolic transformations occurred on carbons 4–7 (5), knowledge of the signal characteristics for the protons on the cyclohexane (hexahydrobenzisoxazole) ring was crucial. The parent drug exhibited proton absorption at δ 8.07 (imidazole H, not shown in Fig. 4), 4.43 (H_{7a}), 4.33 (NCH_3), 3.53 (H_{3a}), 2.29 (H_{7eq}), 2.14 (H_{4eq}), 1.82 (H_{7ax}), 1.67 (H_{6eq} and H_{5eq}), 1.53 (H_{6ax}), and 1.25 (H_{4ax} and H_{5ax}). The assignments, derived from inspection and double-irradiation experiments, provided the basis for the NMR characterization of the metabolites (Table II). The observed coupling constants in the sequence C_7H_2–$C_{7a}H$–$C_{3a}H$–C_4H_2 are consistent with a *cis*-ring fusion and a twist chair configuration for the six-membered ring. Spectrometric and chromatographic details for each isolated metabolite fraction will be given.

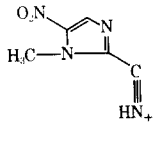
Metabolite Fraction II—GLC of the trimethylsilyl derivative of this fraction showed one main component (~80%) and two minor components (~10% each). GLC–mass spectrometry of the major component indicated a molecular ion of *m/e* 426, showing it to be a dihydroxylated metabolite of I. This also was true for the minor components. Direct-probe mass spectrometry of underivatized II gave spectra (Table I) with intense ions of *m/e* 282 (molecular ion of dihydroxy metabolites), and there was no evidence of monohydroxy metabolites (molecular ions of *m/e* 266). HPLC also indicated one major component and two minor components in the sample. The NMR spectrum of II showed two resonances that were not seen in the parent drug in the δ 3.5–4.0 region, indicating incorporation of two hydroxyl groups. Double-irradiation experiments established that the two carbinol methines were vicinal and that one was vicinal to H_{3a}. These findings allowed identification of the major metabolite as a 4,5-dihydroxy analog of I. Since $J_{4,5}$ and $J_{5,6ax}$ are large (>8 Hz), an axial–axial stereochemical relationship is indicated; thus, II must be the 4,5-di(equatorial)hydroxy derivative.

Metabolite Fraction III—Analytical HPLC of this fraction showed one major component and two minor components. However, capillary column GLC indicated the presence of only one minor component (~10%) and one major component (~90%). GLC–mass spectrometry of the trimethylsilylated sample showed that the more abundant component possessed a molecular ion of *m/e* 338, demonstrating that it is a monohydroxy analog of I. The minor component exhibited a molecular ion of *m/e* 426; thus, it is the bis(trimethylsilyl) derivative of a dihydroxy metabolite. The direct-probe mass spectrometric data for III (Table I) also demonstrate that this metabolite fraction is predominantly a

Table I—Major Ions Found in the Mass Spectra of I and Metabolites II–VIII

		Ion and Relative Intensity									
Compound	Substitution	*m/e* 282	*m/e* 266	*m/e* 265	*m/e* 250	*m/e* 249	*m/e* 247	*m/e* 237	*m/e* 233	*m/e* 195[a]	*m/e* 153[b]
I	—	—	—	—	M, 100	—	—	—	M-17, 42	36	70
II	4,5-Di-(eq)OH	M, 10	—	M − 17, 7	—	—	M − 35, 6	—	—	100	10
III	6-(ax)OH	(1)[c]	M, 9	—	—	M − 17, 9	—	—	—	100	76
IV	5-(eq)OH	(5)[c]	M, 37	—	—	M − 17, 27	—	M − 29, 32	—	100	66
V	7-(ax)OH	—	M, 19	—	—	M − 17, 13	M − 19, 19	M − 29, 6	—	72	100
VI	7-(eq)OH	—	M, 31	—	—	M − 17, 42	M − 19, 66	—	—	50	100
VII	6-(eq)OH	(6)[c]	M, 74	—	—	M − 17, 54	M − 19, 15	—	—	100	83
VIII	5-(eq), 7a-di-OH	M, 24	—	M − 17, 48	—	—	—	—	—	100	62

[a] 

[b] 

[c] M of minor dihydroxy metabolite component.

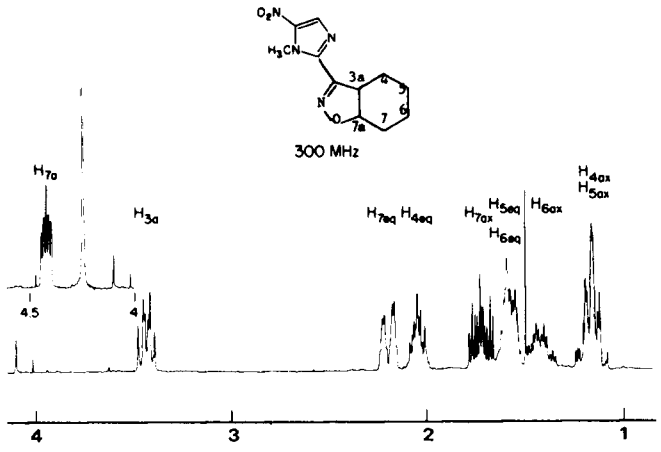


Figure 4—*NMR spectrum of I.*

monohydroxy metabolite contaminated with some dihydroxylated drug.

Metabolite III is assigned as the 6-(axial)hydroxyl derivative of I based on the absence of the characteristic 12.5-Hz coupling constant in the H_{7ax} resonance (Fig. 5). This large splitting is characteristic of an axial–axial relationship and has been identified as J_{H7ax}, J_{H6ax} in the parent drug. As expected, the multiplicities of the H_{3a} and H_{7a} signals are essentially unchanged from those of I, indicating that no substitution has occurred on the neighboring C-7 and C-4 atoms.

Metabolite Fraction IV—As with Fraction III, the direct-probe mass spectrometric data from Fraction IV (Table I) showed that the latter is composed mainly of monohydroxy metabolites plus a lesser amount of dihydroxy metabolites. Capillary column GLC of the trimethylsilyl derivative of IV showed a major component and three minor components, with the minor components each having an area of ~10% of that of the major peak. GLC–mass spectrometry of the major component gave a molecular ion of *m/e* 338, consistent with a monohydroxylated derivative of I. The multicomponent nature of the metabolite fraction (with one major component) also was indicated by analytical HPLC.

The major component of Fraction IV was characterized by NMR spectroscopy as having a single equatorial hydroxyl group at C-5. This conclusion was reached following elimination of other structural possibilities. The C-4 and C-7 sites were eliminated as possible positions of hydroxylation since the H_{3a} and H_{7a} signals retained the same multiplicity as in the parent drug. This required that the neighboring C-4 and C-7 positions be unsubstituted. The two C-6 possibilities were ruled out by Metabolites III and V, in which substitution at C-6 was firmly established by the reduced multiplicity of the recognizable axial H_7 signal. Finally, the linewidth of the carbinol CH group after deuterium oxide exchange ($W_{1/2}$ 20–24 Hz) necessitated at least one large vicinal constant, which is compelling evidence for an axial configuration.

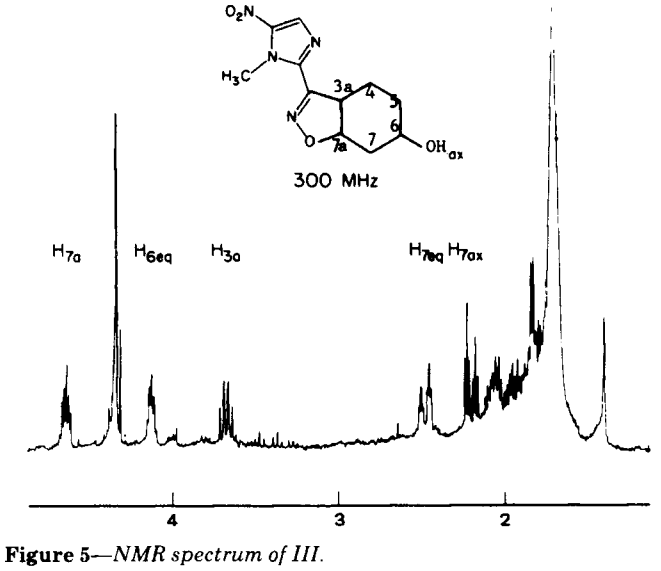


Figure 5—*NMR spectrum of III.*

Table II—NMR Data for I, Related Compounds, and Metabolites

Compound	3a	4 eq	4 ax	5 eq	5 ax	6 eq	6 ax	7 eq	7 ax	7a	NHC_3	HC=	OH
I	3.53 (10.0, 7.5, and 7.5)	2.14	~1.25	~1.67	~1.25	~1.67	~1.53	2.29	1.82 (15.0, 12.5, 4.5, and 4.5)	4.43 (7.5, 4.0, and 4.0)	4.33	8.07	—
Synthetic[a] 4-(eq)OH	3.22 t (8.3)	—	3.59 (11.5, 8.3, and 5.0)	~1.5[b]	1.28	2.10	~1.77	2.33	~1.77[b]	4.66	4.36	8.08	6.03
Synthetic[a] 6-(eq)OH, 7-(ax)OH	3.81 (8.8 and 6.8)	2.34	~1.65	~1.65	1.82	—	4.01	4.14	—	4.70 (8.6 and 4.3)	4.31	8.08	2.08 (3.7) C-6 3.53 (2.7) C-7
Synthetic[a] 5-(ax)OH, 6-(eq)OH	3.75 (11.0 and 7.7)	2.51 (14.5, 6.7, and 4.7)	~1.45	4.05	—	—	3.97	2.42 (14.5, 5.5, and 2.5)	2.20 (14.8, 10.7, and 5.0)	4.68	4.32	8.08	1.93 (6.0) C-6
II [4,5-di-(eq)OH]	3.33	—	3.45	—	3.59	~1.97	~1.65	2.43	~1.98	4.66	4.35	8.07	— —
III [6-(ax)OH]	3.64 q (8.0)	~1.93	~1.83	~1.7	~1.7	4.10 qn (~4.2)	—	2.38 (15.5 and 3.3)	2.10 (15.5, 4.0, and 4.0)	4.66	4.33	8.08	— —
IV [5-(eq)OH]	3.66	—	—	—	3.75	—	—	—	—	4.54	—	—	— —
V [7-(ax)OH]	3.76 q (8.0)	—	—	—	—	—	—	3.95	—	4.65 (8.0 and 3.7)	4.32	8.06	— —
VI [7-(eq)OH]	3.85 q (8.0)			2.0–2.8				—	4.06	4.47 (8.8 and 5.2)	4.29	8.07	— —
VII[c] [6-(eq)OH]	3.51	2.30	~1.32	—	—	—	—	2.63	1.78	4.71	—	—	— —
VIII [5-(eq)OH 7a-OH]	3.74 (12.0 and 7.5)	1.90[b]	1.70[b]	4.16	—	2.55[b]	1.30[b]	2.24	2.35	—	4.32	8.08	2.84

[a] Model compounds prepared by Dr. H. Mrozik, Synthetic Organic Department, Merck Sharp & Dohme Research Laboratories. [b] Tentative assignments. [c] Same as the minor component of metabolite Fraction V.

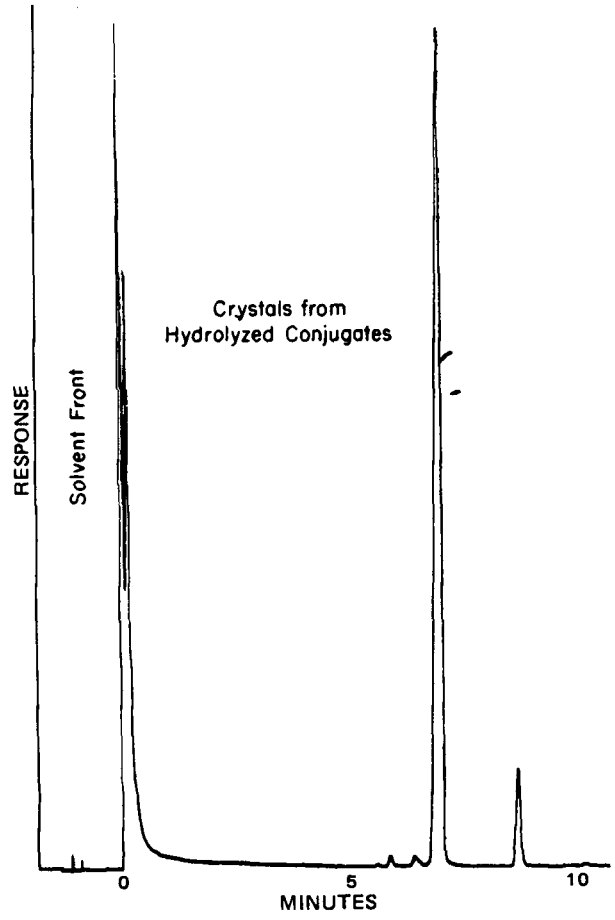


Figure 6—*Gas–liquid chromatogram produced by analysis of the trimethylsilylated metabolite Fraction VII.*

Metabolite Fractions V and VI—HPLC suggested that Fraction V contained one major and one minor component. GLC of the trimethylsilyl derivative showed three peaks with a relative area ratio of approximately 2:4:3. GLC–mass spectrometry of the three peaks gave molecular ions for each of *m/e* 338, as required for monohydroxylated metabolites; however, the fragmentation patterns in each case were somewhat different, suggesting isomeric species. Direct-probe mass spectrometry (Table I) of this metabolite fraction indicated the presence of monohydroxy metabolites only.

Analytical HPLC of Fraction VI and GLC of the trimethylsilyl product indicated a single component of >90% purity. GLC–mass spectrometry indicated a molecular ion at *m/e* 338, required for a monohydroxy compound. The direct-probe mass spectra from Fraction VI also showed that only monohydroxy (and no dihydroxy) metabolites were present (Table I).

The NMR spectra of Fractions V (major component) and VI showed an H_{7a} signal with reduced multiplicity, indicating substitution on a neighboring carbon atom. Since both spectra contained a normal H_{3a} resonance, it follows that the site of hydroxylation is C-7 and that the two metabolites are related as stereoisomers. Stereochemical assignments are not clearcut since the coupling constants between H_{7a} and the two C-7 protons are essentially the same in the parent molecule and differ by only 1 Hz in these two metabolites. However, there is reason for associating the larger J value with an axial H_7 [*i.e.*, a 7-(equatorial)hydroxyl group] since this would correspond to the findings for Metabolite III in which the assignments are unequivocal.

Inspection of the NMR features of the minor component in metabolite Fraction V eliminated C-4 and C-7 as possible sites for the hydroxylation since both the H_{3a} and H_{7a} signals retained a full complement of lines. Irradiation of the equatorial H_7 signal at δ 2.63 reduced the multiplicity of the axial H_7 to a doubled doublet (J = 11.5 and 4.5 Hz). Since the 4.5-Hz coupling constant represents $J_{7,7a}$ and J = 11.5 Hz arises from an axial–axial relationship, it follows that the axial H_7 has suffered a loss of spin interaction with its equatorial neighbor at C-6. Therefore, the minor component in Fraction V was assigned the C-6 equatorial hydroxy structure.

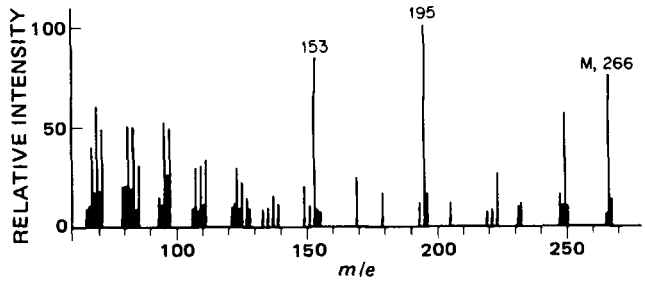


Figure 7—*Mass spectrum from metabolite Fraction VII.*

Metabolite Fraction VII—A small quantity (0.1 mg) of material crystallized in the bottom of the centrifuge tube (stored at 4°) containing the methanol solution of the aglycone metabolite fraction. GLC analysis of the crystals (Fraction VII) following their trimethylsilylation gave the chromatogram shown in Fig. 6. The major and minor components exhibited molecular ions of *m/e* 338 and 426, respectively, characterizing them as mono- and dihydroxy derivatives of I. The major component exhibited the same retention time as the shoulder (peak 3) on the leading edge of peak 4 in Fig. 3. Direct-probe mass spectrometry of underivatized VII gave the spectrum presented in Fig. 7. The molecular ion of *m/e* 266 confirmed that the spectrum arose from a monohydroxylated derivative of I, and the intense fragment ions of *m/e* 153 and 195 demonstrate that the hydroxyl group is substituted on C-4–C-7 of the hexahydrobenzisoxazole ring (5).

NMR examination of VII also indicated the presence of two components in the approximate ratio of 9:1. Signals for the major component matched those for the minor component of Fraction V. This finding indicates that the hydroxyl group in the major component is at C-6 and is in the equatorial configuration. The NMR data for the minor component in Fraction VII indicated it to be a dihydroxy metabolite of I, but no positional or conformational assignments could be made, except that substitution is on C-4–C-7 of the hexahydrobenzisoxazole ring.

Metabolite Fraction VIII—Both HPLC and GLC (following trimethylsilylation) of this nonconjugated metabolite fraction from unhydrolyzed dog urine indicated the presence of a major drug-related component (which appeared to be a dihydroxy metabolite) plus at least two related minor components. The dihydroxy nature of this metabolite also was suggested by direct-probe mass spectrometric data (Table I). Although only limited NMR data were obtained for Fraction VIII, analysis of the spectral features allowed a tentative structural assignment to the major component as 5-(axial),7a-dihydroxy-substituted drug. Key observations that led to this conclusion were: (*a*) the absence of a resonance in the δ 4.5–4.7 region diagnostic for H_{7a}; (*b*) a reduced multiplicity of the H_{3a} and both H_7 signals, indicative of hydroxylation on a neighboring carbon; and (*c*) the appearance of a new, weakly coupled, single proton peak at δ 4.18.

Observations *a* and *b*, taken together, constitute compelling evidence for hydroxylation at C_{7a}. The multiplicities of the H_{3a} and both H_7 signals require that C-4 and C-6 are unsubstituted, thus leaving C-5 as the site for the second hydroxyl group. The new carbinol CH group appears essentially as a broadened singlet with a width at half height of 9.5–10.0 Hz. This indicates that its couplings with the four neighboring protons at C-4 and C-6 all are small, strongly implying an equatorial configuration. Based on this tentative structural assignment, this is the first metabolite of I in which hydroxylation of the drug has occurred at a site other than C-4–C-7, but it is still on the hexahydrobenzisoxazole or cyclohexane ring portion of the drug.

When it was found that hydroxy metabolites of I are bioactive (5), several hydroxy analogs of the drug were synthesized[10]. Although NMR spectroscopy (and mass spectrometry) showed that these analogs were not identical to the metabolites, their NMR spectral data are presented in Table II for comparison.

This and other studies (5–8) have demonstrated that hydroxylation is a common metabolic route for cyclohexanes. Both mono- and dihydroxy metabolites are observed, and the latter probably arise from sequential hydroxylation steps. Thus, Elliott *et al.* (6) showed that cyclohexanol, a urinary metabolite of cyclohexane in the rabbit, is metabolized further to yield *trans*-cyclohexane-1,2-diol.

[10] H. Mrozik, P. Eskola, P. Kulsa, B. H. Arison, W. J. A. VandenHeuvel, J. A. Conroy, and B. M. Miller, Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065, unpublished data.

Agosin *et al.* (9) reported that the epimastigotes of *T. cruzi* possess an active drug-detoxifying (hydroxylating) system associated with the microsomal fraction, with properties like those of a monooxygenase system linked to cytochrome P-450. These authors speculated that the ability of *T. cruzi* to hydroxylate drugs to inactive metabolites may partially explain why no chemotherapeutic agents that cure Chagas' disease have been found; however, I, for which hydroxylation is a major route of metabolism in dogs (and mice) (5), is active against *T. cruzi* in mice (2, 5). Furthermore, since a synthetic dihydroxy derivative of this compound (the 6,7-*cis*-diol) exhibits severalfold greater activity than the parent drug (5), the possibility is raised that the enzyme system suggested (9) to detoxify other chemotherapeutic agents actually may enhance the bioactivity of I. Thus, the proposal of Agosin *et al.* (9) is of limited validity.

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Antimicrobial Activity of *N*-Chloramine Compounds

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Abstract □ Cellular mechanisms of action of two representative *N*-chloramines were studied. Both compounds, 3-chloro-4,4-dimethyl-2-oxazolidinone (I) and *N*-chlorosuccinimide (III), inhibited bacterial growth and exerted profound inhibition of bacterial DNA, RNA, and protein synthesis at a concentration of 10^{-5} *M*. Enzymes containing sulfhydryl groups generally were significantly inhibited by these chloramines at 10^{-4} *M*. Dihydrofolate reductase, which contains no sulfhydryl groups, also was inhibited but at much higher chloramine concentrations (10^{-2} *M*); ribonuclease, which also contains no sulfhydryl groups, was unaffected. All of these inhibitory effects of the chloramines could be prevented if sulfhydryl-containing reagents (mercaptoethanol or dithiothreitol) were added before or together with the chloramine. Once inhibition was produced by the chloramine, it was not reversible by later addition of the sulfhydryl reagents. These results suggest that these chloramines act at sulfhydryl sites as well as at other sites in both cells and purified enzymes.

Keyphrases □ *N*-Chloramines—antimicrobial activity, inhibition of bacterial DNA, RNA, and protein synthesis □ Antimicrobial activity—*N*-chloramine-induced inhibition of bacterial DNA, RNA, and protein synthesis □ Enzyme inhibition—*N*-chloramine-induced inhibition of sulfhydryl-containing enzymes in bacteria

Recent studies of the chemical (1–5) and antimicrobial (6–8) properties of *N*-chloramine compounds suggested that many of these compounds may be potentially useful antibacterial agents. Preliminary results indicated that these *N*-chloramines are effective antibacterial agents. It was hypothesized that the bactericidal actions of *N*-chloramines are derived from the direct transfer of positive chlorine ions from the *N*-chloramines to an appropriate cellular receptor (5).

The present study was an attempt to elucidate the cellular processes affected by these agents which might account for their observed antimicrobial properties. In seeking the mechanisms of action of such agents, the determination of the primary targets in terms of overall cellular function is vitally important. This paper reports the effects of two representative *N*-chloramines on overall DNA, RNA, and protein synthesis in bacteria. A more specific action in terms of direct inhibition of cellular enzymes also is discussed.

EXPERIMENTAL

Bacterial Cultures—Bacteria [*Escherichia coli* (ATCC 25922) and *Staphylococcus epidermidis* (ATCC 12228)] were grown and harvested as described previously (9). The bacterial cells then were resuspended in fresh nutrient medium[1] at a density of 3×10^8 colony-forming units/ml. Bacterial purity and the absence of contamination were established routinely by the Gram stain and by colony characteristics on blood agar plates.

DNA, RNA, and Protein Synthesis—All stock solutions of 3-chloro-4,4-dimethyl-2-oxazolidinone (I), 4,4-dimethyl-2-oxazolidinone (II), and *N*-chlorosuccinimide (III) were prepared in 0.1 *M* phosphate buffer (pH 7). The stock solutions were diluted with distilled, deionized water.

The bacterial suspensions were added to test tubes containing the respective agents diluted in water. All determinations were made in duplicate or triplicate along with controls containing an identical volume of bacteria without the agents. The bacteria then were incubated at 37° for 1 hr with various concentrations of the *N*-chloramines. Then 1–2 μCi of the following radioisotopes[2] were added: [*methyl*-^{3}H]thymidine, 90

[1] Bacto nutrient broth 003-03, Difco Laboratories.

[2] New England Nuclear Corp., Boston, Mass.

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