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Keyphrases

Thiazesim-^{14}C—excretion, distribution
Biotransformation, *in vivo*, *in vitro*—thiazesim-^{14}C
Column chromatography—separation
TLC—separation, identity
IR spectrophotometry—identity, purity
Electrophoresis—separation, radioactive metabolites

Metabolism of Thiazesim, 5-(2-Dimethylaminoethyl)-2,3-dihydro-2-phenyl-1,5-benzothiazepin-4(5*H*)-one, in the Rat *In Vivo* and *In Vitro*

By J. DREYFUSS, A. I. COHEN, and S. M. HESS

Three metabolites of thiazesim, 5-(2-dimethylaminoethyl)-2,3-dihydro-2-phenyl-1,5-benzothiazepin-4(5*H*)-one, were isolated from rat feces. Metabolite 1 contained a hydroxyl group in the 2-phenyl ring. Metabolite 2 was dihydroxylated at unknown positions of the 2-phenyl ring. Metabolite 3 was a derivative of Metabolite 1 and, in addition, appears to have had one carbon and two oxygens added to the side chain. Two metabolites of thiazesim (4 and 5) were isolated from rat liver homogenates incubated with NADPH and nicotinamide. Metabolite 4 has been identified as the sulfoxide derivative of thiazesim. Metabolite 5 has not yet been identified, but it was not similar to those isolated *in vivo*. Hydroxylation of either aromatic ring was not an observed metabolic reaction *in vitro*. Sulfoxidation was not an observed metabolic reaction *in vivo*.

A PAPER ELSEWHERE in this journal (1) has described aspects of the metabolism of the antidepressant agent, thiazesim-^{14}C, 5-(2-dimethylaminoethyl)-2,3-dihydro-2-phenyl-1,5-benzothiazepin-4(5*H*)-one, in the rat and dog that relate to its absorption, distribution, excretion, as well as the techniques developed for the separation of both conjugated and unconjugated metabolites. Also discussed were the results obtained by incubating thiazesim-^{14}C with liver preparations from various animal species.

This paper will describe the identification of the metabolic products of thiazesim-^{14}C formed by the rat *in vivo* or by rat liver preparations *in vitro;* these metabolites have not yet been tested for pharmacologic activity. The results showed that the metabolites formed by the rat *in vivo* were not the same as those produced by rat liver preparations *in vitro*.

EXPERIMENTAL

Isolation of Fecal Metabolites—Three metabolites of thiazesim were isolated from feces in an unconjugated form by the following procedure. Rats were repetitively dosed once a day for 4 days with 100 mg. of thiazesim per kg. and the feces samples combined. The feces were homogenized in 4 vol. of methanol that was 0.12 *N* with respect to HCl, and centrifuged. After centrifugation, the supernatant fraction was removed and saved, and the residue was extracted twice more with 4 vol.

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Dr. Josef Fried kindly performed the time-averaged NMR spectra. Dr. Walter McMurray performed the high-resolution mass spectra as well as the data processing required to construct the element maps. Dr. Phillip Funke obtained some of the low-resolution mass spectra.
The authors wish to thank Miss Barbara Keeler for determining the infrared spectra.

of unacidified methanol. The combined methanol extracts were adjusted to pH 1 to 2 with 1 *N* HCl and 2 vol. of *n*-heptane was added. After shaking, the lower layer was recovered, evaporated, and chromatographed on an alumina column for the isolation of Fraction 1, as described previously (1). The isolated Fraction 1 was evaporated and spotted on 1-mm. Silica Gel HF plates which were developed in benzene–ammonia–dioxane (10:10:80). The appropriate UV positive zones were scraped from the plate, pooled, and packed into columns, 4 cm. diam. × 2 cm. high, which were eluted with chloroform–methanol (5:1). When necessary, each eluted compound was rechromatographed on 1-mm. Silica Gel HF plates in benzene–ammonia–dioxane (60:5:35 or 10:10:80) in order to remove contaminating yellow material.

Preparation and Isolation of In Vitro Metabolites—For the preparation of metabolites *in vitro* in milligram quantities, the following procedure was used. Male Holtzman rats weighing 250–300 g. were killed by decapitation and the livers were quickly removed, chilled in ice-cold 1.15% KCl, blotted on filter paper, and weighed. The livers were homogenized in 2 vol. of 1.15% KCl with a Potter-Elvehjem tissue grinder with a Teflon pestle. The homogenate was centrifuged at 9,000 × *g* for 15 min. at 4°. The incubation mixture consisted of 7.5 mmoles of phosphate buffer, pH 7.4, 300 ml. of the supernatant fraction of rat liver (9,000×*g*), 368 μmoles of thiazesim, and the following amounts of cofactors: $MgCl_2$, 750 μmoles; glucose-6-phosphate, 840 μmoles; NADP, 57.5 μmoles; and nicotinamide, 9 mmoles. Quantities of cofactors equal to those cited above were added after incubation at 37° for 15 and again at 30 min. An additional 322 μmoles of thiazesim was added after 30 min. Incubation was for a total of 60 min. The reaction mixture was extracted with *n*-butanol as previously described (1).

In order to isolate metabolites uncontaminated with nicotinamide, the latter was first removed by chromatography of the methanol extract on a column of Whatman SG 31 silica gel. Sufficient dry silica gel was added to the methanol extract to form a paste which was then dried at 70°. The dry material was placed on top of a column of dry silica gel (5 × 10 cm.) and eluted with water until the effluent no longer absorbed at 260 mμ. At this time, the metabolites and unchanged thiazesim were eluted from the column with methanol–ammonia (95:5). The concentrated eluates were then chromatographed on thin-layer plates, as described above in the section on the isolation of fecal metabolites.

Spectroscopic Analysis—NMR spectra were obtained on either a Varian model A60 spectrometer or on a Varian model A60-A spectrometer equipped with a C1024 time averager.

Mass spectra were obtained on either an Associated Electrical Industries model MS9 spectrometer or on a Consolidated Electrodynamics Corporation model 103C spectrometer. Element maps were obtained by computer analysis from data recorded from the MS9 spectrometer (2).

Metabolite Designations—Three metabolites were isolated from rat feces (1). These metabolites had R_s values of 0.88, 0.64, and 0.52 in benzene–ammonia–dioxane (10:10:80) on Silica Gel HF chromatograms, and were designated as Metabolites 1, 2, and 3, respectively. Two metabolites were isolated after incubation of thiazesim-^{14}C with rat liver preparations. These metabolites had R_s values of 0.88 and 0.64 and were designated as Metabolites 4 and 5, respectively.

Infrared Spectra—Infrared spectra of all compounds were determined as micro-KBr pellets in order to assess the approximate purity of the samples. All samples gave well-defined spectra that were clearly derivatives of thiazesim.

Ultraviolet Spectra—The UV spectra taken at acid pH in methanol solution for the metabolites isolated *in vivo* resembled that found for thiazesim. In each case a prominent peak was seen at 276–278 mμ, as compared with 271–272 mμ for thiazesim. When the spectra for Metabolites 1, 2, and 3, and thiazesim were rerun at pH >10 there was a bathochromic shift to about 300 mμ for the three metabolites, but not for thiazesim. These data suggested the introduction of phenolic hydroxyl groups in the metabolites.

Metabolites 4 and 5, produced from rat liver preparations *in vitro*, exhibited UV spectra at acid pH in methanol solution that consisted of no well-defined peaks, but simply shoulders at 272, 265, 235, and 225 mμ for Metabolite 4 and 265 and 240 mμ for Metabolite 5; these spectral features did not change when the samples were rerun at pH >10. These data suggested that no phenolic hydroxyl groups were present in these metabolites.

Mass Spectra—The mass spectra of thiazesim and Metabolites 1 and 2 gave molecular ions at 326 ($C_{19}H_{22}N_2OS$), 342 and 358, respectively, as shown in Fig. 1. The increase in weight of 16 and 32 mass units of the molecular ions of Metabolites 1 and 2 strongly indicated that the compounds were the mono- and di-oxygenated derivatives of thiazesim. As confirmatory evidence, the mass of the parent ion of Metabolite 1, measured by high resolution mass spectrometry, was 342.1414 which corresponded to the formula $C_{19}H_{22}N_2O_2S$ (calcd. 342.1403).

The origins of the fragmentation peaks of Metabolites 1 and 2 and thiazesim are described below. As an aid in making the assignments, the element maps of Metabolite 1 and thiazesim were prepared. The base peak at *m/e* 58 and the next most intense peak, at *m/e* 71, present in thiazesim and Metabolites 1 and 2, corresponding to the $[CH_3N(CH_3)_2]^+$ and $[CH_2{=}CH{-}N(CH_3)_2]$ ions, respectively, showed the retention of the unaltered side chain in Metabolites 1 and 2, as shown in Scheme I. Furthermore, the presence of the ion at *m/e* 178 (C_9H_8NOS) in the spectra of thiazesim and Metabolites 1 and 2 suggested that the oxygen(s) was not substituted on the heterocyclic ring and must be substituted on the 2-phenyl group.

The assignment of the hydroxyl group(s) to the 2-phenyl ring was established from a comparison of selected peaks of thiazesim and those for Metabolites 1 and 2, which differed by 16 or 32 mass units (see Table I). The structures of the *m/e* 147, 120, and 107 peaks of Metabolite 1 are shown in Scheme II.

Prominent peaks common to all three compounds appeared at *m/e* 109, 136, 151, and 178, which corresponded to the following elemental compositions, as determined from the high resolution mass spectra

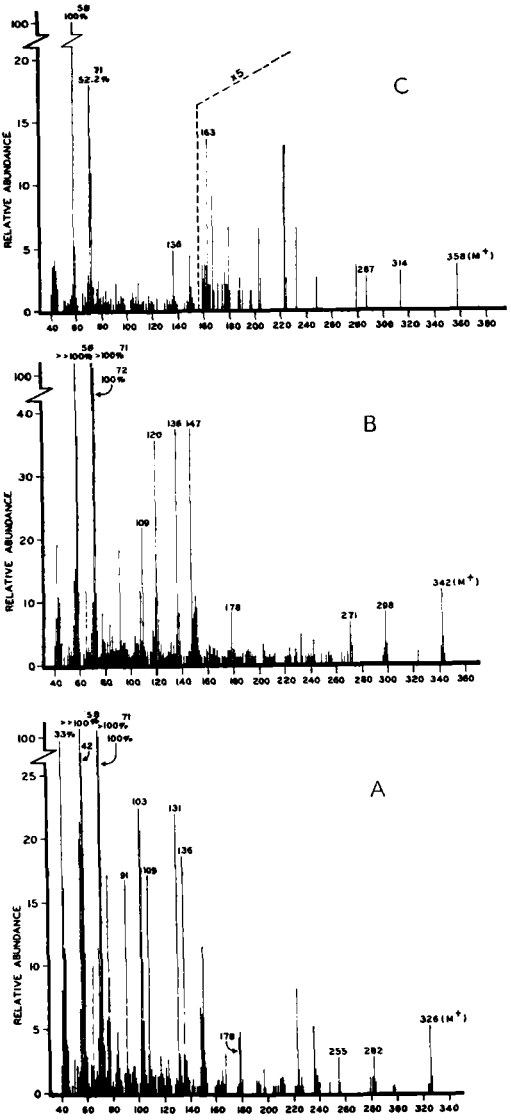
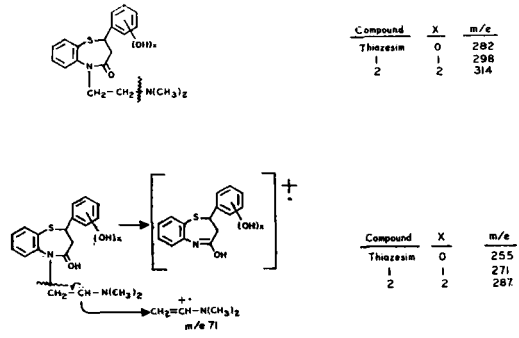
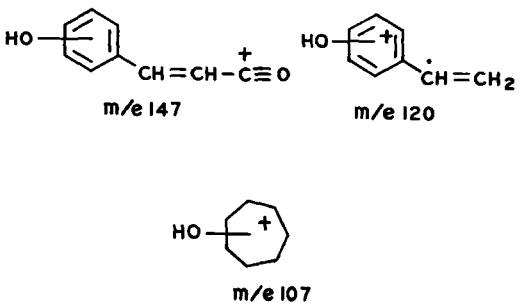


Fig. 1—Mass spectra of thiazesim (A), Metabolite 1 (B), and Metabolite 2 (C).

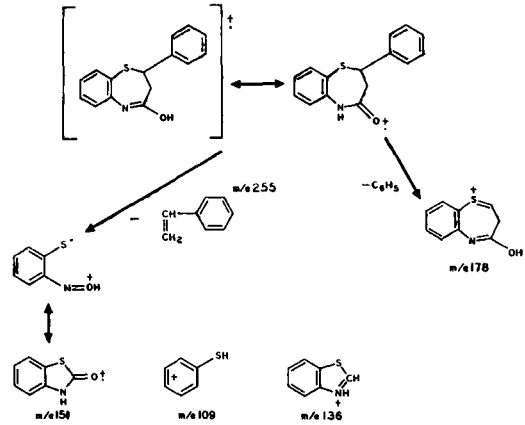


Scheme I—Fragmentation pattern of the side chain of thiazesim and Metabolites 1 and 2



Scheme II—Structures of selected fragments of Metabolite 1

of thiazesim and Metabolite 1, C_6H_5S, C_7H_6NS, C_7H_5NOS, and C_9H_8NOS, respectively. These peaks, corresponding to those aromatic fragments containing *both* nitrogen and sulfur, contained no hydroxyl group(s) either in the parent compound or in Metabolites 1 and 2. The probable structures of these fragments are depicted in Scheme III.



Scheme III—Structures of selected fragments of thiazesim

Metabolite 3 (see Fig. 2) with a molecular ion at *m/e* 386 ($C_{20}H_{22}N_2O_4S$) had fragments common to the nuclear portion of Metabolite 1 at *m/e* 91, 109, 120, 136 (C_7H_6NS), 147 ($C_9H_7O_2$), 242, and 271 ($C_{15}H_{13}NO_2S$). The absence of prominent peaks at *m/e* 58 and 71 demonstrated alterations(s) of the side chain. Further, peaks were present with the loss of oxygen at *m/e* 356 ($C_{19}H_{20}N_2O_3S$), 310 ($C_{18}H_{18}N_2OS$), 217 ($C_{12}H_{13}N_2S$), 203 ($C_{11}H_{11}N_2S$), and with the loss of sulfur at *m/e* 353 ($C_{20}H_{21}N_2O_4$), 323 ($C_{19}H_{20}N_2O_3$), and 277 ($C_{18}H_{17}N_2O$). It is to be noted that the low intensity peak, *m/e* 356, does not appear in the low resolution spectrum of Fig. 2. This peak was, however, detected and its exact mass determined by the use of the Nier peak-matching procedure.

The additional oxygen atoms present in Metabolite 3, compared with Metabolite 1, are not ketonic, aldehydic, acidic, or in an ester grouping, as determined from the infrared spectrum, therefore, the oxygens must be either alcoholic or ethereal. Moreover, the mass-measured formula of the mo-

TABLE I—MASS SPECTRA OF SELECTED FRAGMENTS OF THIAZESIM AND METABOLITES 1 AND 2

Thiazesim		Metabolite 1		Metabolite 2	
m/e	Elemental[a] Composition	m/e	Elemental[a] Composition	m/e	Elemental[b] Composition
282	$C_{17}H_{16}NOS$	298	$C_{17}H_{16}NO_2S$	314	$C_{17}H_{16}NO_3S$
255	$C_{15}H_{13}NOS$	271	$C_{15}H_{13}NO_2S$	287	$C_{15}H_{13}NO_3S$
131	C_9H_7O	147	$C_9H_7O_2$	163	$C_9H_7O_3$
104	C_8H_8	120	C_8H_8O	136	$C_8H_8O_2$
103	C_8H_7	119	C_8H_7O	135	$C_8H_7O_2$
91	C_7H_7	107	C_7H_7O	123	$C_7H_7O_2$

[a] Based on peak matching. [b] Based on low resolution data.

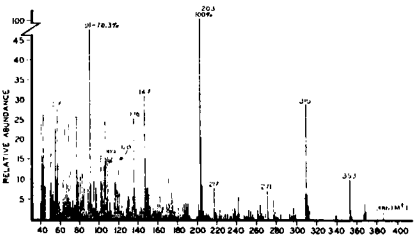
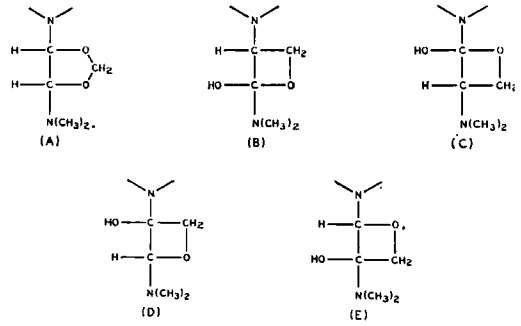


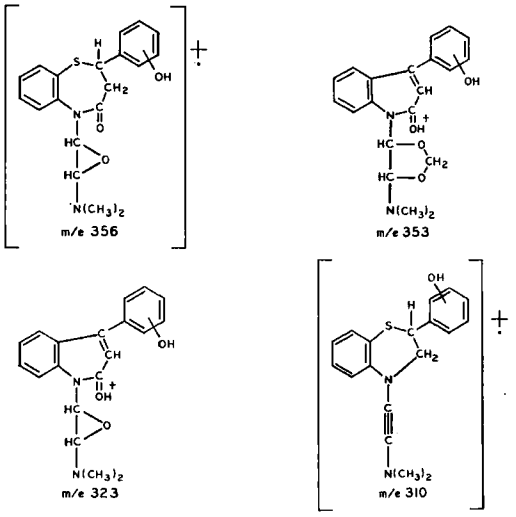
Fig. 2—Mass spectrum of Metabolite 3. This compound was thermally labile and gave variable spectra. Peaks of low intensity at m/e *323, 356, and 368 did not appear in this spectrum. In subsequent spectra, utilizing oscilloscope display of pertinent areas, these weak peaks were recognized and their exact masses determined. The elemental composition found for the mass measured peak at* m/e *368 did not give a reasonable structure based on the elemental composition of the parent ion and was considered to represent an impurity.*

lecular ion (*m/e* 386) corresponded to at least one more degree of unsaturation when compared with Metabolite 1. It can be concluded that at least one of the two incorporated oxygens must be a part of a cyclic ether. Reasonable structures for Metabolite 3 are the methylene dioxy group A or the cyclic ethers B, C, D, and E shown in Scheme IV.



Scheme IV—Possible structures for the side chain of Metabolite 3

It seems reasonable to eliminate from consideration substitution on the *N*-methyl carbon atoms, since atoms at this remote location would have had little effect on the fragmentation process of the remainder of the molecule. In those metabolites with a dimethylaminoethyl side chain, the fragmentation process appeared to be initiated at the side chain (*β*-cleavage). With changes in the side chain, the other fragmentation modes become apparent, particularly the loss of the sulfhydryl group. Since the fragmentation pattern of Metabolite 3 in the high mass region of the spectrum differed markedly from those obtained with thiazesim or Metabolites 1 or 2, it was suspected that modification of the side chain might alter the fragmentation mode of Metabolite 3. In support of this supposition an analog of thiazesim having a dimethylaminopropyl side chain was analyzed by the use of a mass spectrometer. This sample having a molecular ion at *m/e* 340 also gave a prominent *m/e* 307 peak (loss of SH). This result is similar to the loss of an SH group in the case of Metabolite 3. The side chain of thiazesim cleaves very readily since the carbon-carbon bond is *β* to two nitrogens. In a molecule which has an altered side chain, fragmentation modes, such as those originating from the nuclear portion of the molecule, may become more favored. The probable structure of the peaks of highest mass unique to Metabolite 3 are shown in Scheme V.



Scheme V—Structures of selected high-mass fragments of Metabolite 3

Of the five possible structures that accommodate the added elements of one carbon and three oxygens, compared with the parent drug (see Scheme IV), Structure A appears to be the most reasonable on the basis of NMR and mass spectral data. The aromatic proton resonances of Metabolites 1 and 3 were identical, therefore, Metabolite 3 was presumed also to have had the aryl substitution in the 2-phenyl ring. The NMR spectrum of Metabolite 3 had a prominent peak near 4.4 τ which was absent in the spectrum of Metabolite 1. The loca-

tion of this peak was reasonable for the assignment of the methylene dioxy protons. Although the proton count corresponded to only three protons, the region around 4.4 τ also may have included the other two protons on carbons bearing the two methylene dioxy oxygens. The relevant proton resonances of the protons of Structures B–E would have tended to have occurred at τ values higher than those found for the methylene dioxy protons, and certainly only one proton in Structures D and E would be expected to have appeared below 5 τ. In addition, Structures B, C, D, and E are 4-membered cyclic hemiacetals, and would not have been expected to exhibit the stability of the 5-membered cyclic methylene dioxy Structure A.

Although the data obtained with Metabolite 3 tend to support Structure A (Scheme IV), it should be stressed that this tentative proposal is made in the absence of the synthesized compound with which authentic spectra could be prepared.

The mass spectrum of Metabolite 4 gave a molecular ion at *m/e* 342, the same as that found for Metabolite 1, however, the fragmentation patterns for the two compounds were quite different (see Fig. 3). Metabolite 4 showed fragments of high intensity at *m/e* 325 (M-17, loss of OH), and at *m/e* 298 [M-44, loss of $-N(CH_3)_2$]. These results suggested that the additional oxygen incorporated into Metabolite 4 was not present on either of the substituted benzene rings. The strong peaks at *m/e* 58 and 71 indicated that no alteration had occurred to the side chain in Metabolite 4. Sulfoxidation seemed a likely possibility. Accordingly, the mass spectrum was run of the sulfoxide derivative of a thiazesim analog (SQ 10502) that contained an *N*-diethyl amino ethyl instead of an *N*-dimethyl amino ethyl side chain. The M^+ for this compound was found at *m/e* 370. Prominent peaks were found at *m/e* 353 (M-17) and *m/e* 298. The strong peaks found for Metabolite 4 at *m/e* 58 and *m/e* 71 were now displaced by 28 mass units to *m/e* 86 and *m/e* 99 due to the added C_2H_4 of the *N*-diethyl group. Other features of the mass spectra of Metabolite 4 and SQ 10502 were essentially identical. Similarly, the ultraviolet and infrared spectra of Metabolite 4 and SQ 10502 were identical.

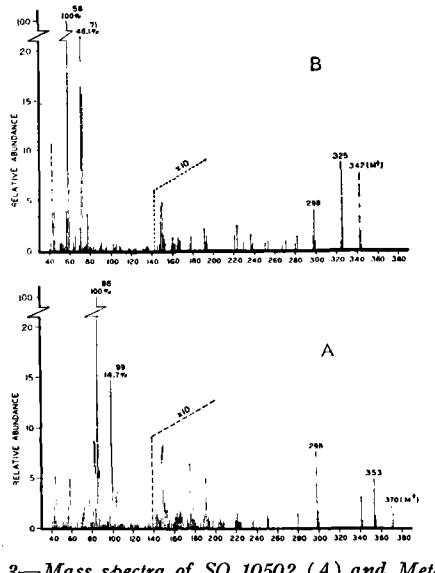


Fig. 3—Mass spectra of SQ 10502 (A) and Metabolite 4 (B). SQ 10502 is the sulfoxide derivative of thiazesim, which also contains an N*-dimethylaminoethyl side chain.*

The complete interpretation of the mass spectrum of Metabolite 5 has been complicated by the absence of a discernible peak for the parent ion. The presence of the peak at *m/e* 255 ($C_{15}H_{13}NOS$), as well as the peaks at *m/e* 136, 131, 109, 104, 103, and 91, demonstrated that the nuclear portion of thiazesim had remained intact. The presence of a stronger *m/e* 57 (C_3H_8N) peak rather than an *m/e* 58 peak, as well as the absence of both the *m/e* 71 peak and a strong *m/e* 44 peak, demonstrated a change in the fragmentation pattern from that observed for thiazesim. These data suggested that changes had occurred in the side chain of Metabolite 5.

Location of Hydroxyl Substituents—When strongly electron-donating groups, such as hydroxyl, are substituted in benzene the protons substituted *ortho* and *para* to the hydroxyl group are preferentially shielded (3). This is to be anticipated, because the increase in electron density will be greatest at the carbons *ortho* and *para* to the functional group and least at the *meta* position. The protons attached to the *ortho* and *para* carbons have an electron density that results in the observed chemical shifts of these protons occurring at a higher field than the *meta* proton.

The three isomeric cresols were used as model compounds for the determination of the position of substitution of the hydroxyl group in Metabolite 1. The three isomers exhibited unique aromatic proton resonances due to the presence of the hydroxyl group and the spin-spin coupling of the aromatic protons. *Para* cresol exhibited the typical A_2B_2 pattern of symmetrically disubstituted aromatic compounds with closely spaced proton resonances at 2.95 and 3.25 τ. The spectrum of the nonsymmetrically substituted *o*-cresol was less well defined, but resonances for the two protons *ortho* and *para* to the hydroxyl group fell in the region between 3.2 and 3.4 τ, while the *meta*-substituted protons resonated at lower fields between 2.8 and 3.17 τ as a complex multiplet. *Meta*-cresol, having three protons either *ortho* or *para* to the hydroxyl group, had these protons resonating near 3.3 τ as a complex multiplet, while the remaining *meta* protons resonated near 2.9 τ.

Figure 4 gives the proton resonances in the aromatic region of the NMR spectrum of the *ortho* and *para* methoxy derivatives on the 2-phenyl ring of thiazesim and of Metabolite 1. The time-averaged NMR spectrum of the aromatic portion of Metabolite 1 showed resonance for two protons rather than four, between 3.15 and 3.5 τ; these τ values agree with those found for *o*-cresol. The lack of symmetry in the aromatic portion of the spectra of *o*-cresol and Metabolite 1 added further evidence in favor of the *ortho* configuration for the hydroxyl group. The region under discussion provides evidence for only two protons; in addition, there is a noteworthy absence of the coupling pattern of an AB quartet which is present in the *p*-methoxy thiazesim spectrum (see Curve A, Fig. 4).

The *meta* substitution of the hydroxyl group may

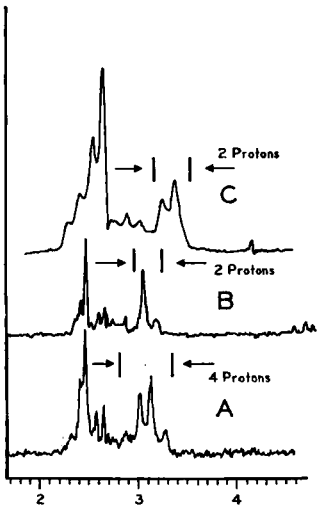


Fig. 4—Proton resonances of the aromatic region of the NMR spectra of p-*methoxy-thiazesim* (*A*), o-*methoxy-thiazesim* (*B*), *and hydroxy-thiazesim, Metabolite 1. The numbers on the abscissa represent τ-values.*

be eliminated *a priori* since the area under the curve between 3.15 and 3.5 τ accounted for only two of four protons. The lack of symmetry in the aromatic proton resonances ruled out the *para*-disubstituted isomer as the probable structure. These data support the view that the hydroxyl group of Metabolite 1 appears to be in the *ortho* position. This interpretation remains tentative, however, until the proposed structure will have been synthesized and the spectra analyzed.

The authors were unable to obtain a satisfactory time-averaged NMR spectrum of Metabolite 2, and, thus, have been unable to locate the hydroxyl groups on the 2-phenyl ring.

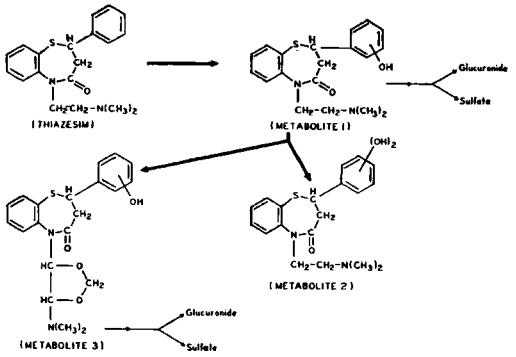
Metabolite 3 exhibited the same pattern for the proton resonances of the 2-phenyl ring as did Metabolite 1, and, therefore, appeared to be a derivative of the latter.

Scheme VI summarizes the probable metabolic events occurring during the biotransformation of thiazesim-^{14}C in the rat *in vivo*.

DISCUSSION

The inability to detect sulfoxidation *in vivo* may be attributed to two possibilities. First, sulfoxidation may actually have occurred, but the equilibrium for the reaction may strongly favor the sulfide form. Second, sulfoxidation could have been a favored reaction, but additional metabolic alterations may occur that result in a very low steady-state level of sulfoxide. A similar line of reasoning would apply if any sulfoxide formed were subsequently converted to the sulfone or sulfate derivatives. Since 30–40% of the dose, has not been identified, such secondary conversions might have been unrecognized.

It is more difficult to understand why hydroxylation, a predominant reaction *in vivo*, was not observed *in vitro*. One could argue that for some reason the conditions of incubation were not suitable for hydroxylation to have occurred. According to Gillette (4), hydroxylation reactions of aromatic



Scheme VI—Summary of biotransformation of thiazesim^{14}*C in the rat* in vivo

compounds require a liver microsomal enzyme system, NADPH, and oxygen. Likewise, Gillette and Kamm (5) have reported that the enzymatic formation of sulfoxides by guinea pig liver microsomes, requires NADPH and oxygen, the same requirements listed above for hydroxylation.

A common metabolic reaction during drug metabolism is *N*-dealkylation and a number of psychoactive drugs undergo this conversion (6). In the metabolism of thiazesim-^{14}C, either *in vivo* or *in vitro*, the authors were unable, so far, to detect the presence of any *N*-demethylated derivatives.

The elucidation of the biotransformation of drugs has been greatly simplified by the introduction of NMR and mass spectrometry. In addition to being able to work with microgram quantities of sample, the advantages of mass spectrometry as an analytical tool are in being able more readily to identify an unexpected structure. The more conventional and more restrictive approach to the identification of metabolites has been to synthesize "expected" metabolites and then to demonstrate their identity by chromatography in several different solvent systems, in comparison with an unknown compound. Schwartz and Bommer (7) have used high-resolution mass spectrometry to identify metabolites of diazepam in the rat.

Additional support for the unusual substitution to the side chain of Metabolite 3 comes from the fragmentation pattern of a known structure containing the methylene dioxy group (8). The compound, 1,3-dioxolane, gives a weak molecular ion (6.0%) at *m/e* 74. Prominent fragments are found at *m/e* 43 and *m/e* 44 which are easily rationalized by the loss of H_2CO, formaldehyde.

The methylene dioxy structure is found in several naturally occurring alkaloids (9). In these alkaloids, the 5-membered methylene dioxy ring is fused to an adjacent benzene ring. Presumably, the methylene dioxy group is formed by the oxidative cyclization of the *o*-methoxyphenol (10). Intermediates establishing the validity of the structure of Metabolite 3 by the latter pathway were not isolated. The transient presence of these compounds may have accounted for the authors' inability to find them; another possibility that existed, of course, is that the metabolic pathway was other than that considered here. To the authors' knowledge, there is no precedent for the natural occurrence of a methylene dioxy group attached to an alkyl side chain in either plants or

animals. In view of the unusual structure, however, this assignment should be considered tentative.

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Keyphrases

Thiazesim metabolism—*in vivo*, *in vitro*
Metabolites, thiazesim—isolated, identified
Liver, rats—*in vitro* metabolism
TLC—separation, identification
Column chromatography—separation
NMR spectroscopy—structure
Mass spectroscopy—structure
UV spectrophotometry—structure

Nonpolar Metabolites of Trifluoperazine in Rats

By C. L. HUANG and K. G. BHANSALI*

Urinary excretion of nonpolar metabolites of trifluoperazine was studied in six albino rats which were administered intraperitoneally with 5 mg. trifluoperazine dihydrochloride daily for a period of 5 days. The 24-hr. urine specimens were collected and analyzed for trifluoperazine sulfoxide and the unchanged drug by means of a spectrophotometric and thin-layer chromatographic techniques. The average urinary excretion of trifluoperazine sulfoxide was found to be 10.77% of the administered dose in the first 24-hr. period. Only a trace or a negligible amount of the unchanged drug was found in urine.

TRIFLUOPERAZINE (TOP) is one of the widely used neuroleptics today. During the past decade TOP has become established as an effective neuroleptic agent. Many clinical reports have appeared attesting to its efficacy in the treatment of certain types of psychotic symptomatology (1–12). TOP, a member of the phenothiazine family, is approximately 10 times more potent than chlorpromazine and is reported to have fewer side reactions. It differs from chlorpromazine by possessing a trifluoromethyl group on position 2 and a piperazinyl function on the propyl side chain. Comparative clinical studies (13–18) have indicated differences in the sedative effect, potency, rapidity of action, and incidence of neurological complications between TOP and other neuroleptics; however, factors responsible for these differences are as yet largely unknown. Despite a large number of clinical investigations reported in the past years, little is known as to its metabolic pathway. Because of the relatively small dosage level, metabolic study of this compound presents a formidable problem especially in man. It would be of interest to study the metabolic pathway of this relatively potent drug and compare it with that of chlorpromazine. Such comparative data might help to explain the question as to why this compound is more potent and has less side effects than chlorpromazine. A survey of the literature revealed that there is little information available regarding the metabolism of TOP. A rapid color test utilizing ferric chloride in mineral acids to detect urinary TOP metabolites was reported (19, 20).

The present investigation is focused on identification and quantitative determination of nonpolar metabolites of TOP in urine of rats. Thin-layer chromatography and paper partition chro-

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