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PHENOLIC METABOLITES OF CHLORPROTHIXENE IN MAN AND DOG

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ABSTRACT:

From urine and feces of dogs and urine of patients given chlorprothixene (CPT) *per os*, metabolites were extracted without or with enzymatic deconjugation and separated by repeated TLC. Purified compounds were characterized by UV, NMR, and mass spectrometry, by color reactions, and by chemical interconversions. Both species excreted 6- and 7-hydroxy-CPT besides the sulfoxidic and demethylated analogues. In urine, the phenols were largely present as conjugates. The major metabolites in dog feces were 5-hydroxy-CPT and its demethylated derivative, whereas 5-hydroxylation was not detected in man. Dog excreta also contained 6-hydroxy-7-methoxy (or 7-hydroxy-6-methoxy)-CPT; further, a 5-hydroxy compound was detected in which the exocyclic double bond was hydrated. In the other metabolites, the *Z*-configuration of CPT had been retained, but small quantities of *E*-isomers were formed during isolation. According to preliminary quantitative data, phenols accounted for a small part of extractable metabolites in human urine, whereas they predominated in dog feces.

Chlorprothixene [*Z*-2-chloro-9-(3'-dimethylaminopropylidene)thioxanthene] is a major psychoactive drug with neuroleptic as well as antidepressant properties (1). Structurally, it differs from chlorpromazine only by a substitution of a carbon atom carrying an exocyclic double bond for a nitrogen in the central ring. Nonetheless, literature reports indicated an important difference in the *in vivo* metabolism of the two compounds in mammals. Sulfoxide formation, *N*-demethylation, and *N*-oxidation are common to both (*cf.* ref. 2); however, the introduction of one or two hydroxy groups into the aromatic rings was described as a major route of metabolism for chlorpromazine (*cf.* ref. 3) and other phenothiazines (4, 5), while phenols could not be identified as biotransformation products of chlorprothixene (6–8) or of other thioxanthenes (9–11).

Phenolic metabolites of tricyclic psychoactive drugs attract special attention, since several of them including 7-hydroxychlorpromazine were shown to exert biological actions similar to those of the parent compounds (*cf.* ref. 12). It was therefore a challenging question whether aromatic hydroxylation can also occur in CPT.[1] An indication of such a possibility was the observation that in urine of dogs given CPT β-glucuronidase treatment liberated two compounds which gave color reactions suggestive of a phenolic character (8). Positive identification was hampered by the lack of synthetic reference compounds. Meanwhile, some hydroxy derivatives of CPT became available by synthesis (13). These could serve for a comparison with metabolites isolated from human and dog urine and dog feces that, on the basis of spectroscopic measurements, had been described as isomeric phenols in a preliminary communication (14). In some cases, the comparison revealed that the assignment of the hydroxy group position had been erroneous. Besides, an incorrect nomenclature had been used for the positions in the Cl-free aromatic ring.

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[1] Abbreviations used: CPT, chlorprothixene; DM_1-, monodesmethyl; DM_2-didesmethyl; NOE, nuclear Overhauser effect; EI, electron impact; TMS, trimethylsilyl.

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Materials and Methods

Drugs and Metabolites. *Z*-CPT hydrochloride, *Z*-DM_1-CPT acetate, and *Z*-CPT-SO hydrochloride were kindly supplied by Troponwerke (Köln, F. R. G.); *E*-CPT was from Dr. A. Jorgensen (Lundbeck, Copenhagen, Denmark). The following hydroxy derivatives were available from syntheses (13): 3-OH-CPT, *Z*/*E*-mixture, 4-OH-CPT, *Z*- and *E*-isomer, *E*-6-OH-CPT, and *E*-7-OH-CPT.

Drug Administration and Sample Collection. *Dog.* One male and two female beagles weighing 11–14.5 kg received on three successive days 16.5 mg/kg CPT HCl (corresponding to 15 mg/kg free base) *per os* in a gelatine capsule. After drug administration, they were placed into a metabolic cage for 6 hr and then urine was obtained by catheterization. They spent the rest of the time in ordinary boxes from which feces were collected.

Man. Urine was collected from 7 a.m. to noon by three male patients under continuous oral treatment with CPT (Truxal, 120–400 mg/day); one of them received haloperidol (40 mg/day) in addition. A further patient repeatedly collected morning urine specimens following ingestion of 25–50 mg of CPT HCl at bedtime. The samples were stored at −20°C.

Extraction of Biological Samples. *Procedure for Selective Isolation of Phenolic Metabolites.* For each dog, urine samples from the 3 days were combined yielding 250–450 ml. Human urine samples comprising more than 600 ml were concentrated to about 30% of their original volume by repeated freezing and thawing with eventual removal of the colorless upper layer (modified from ref. 15). Acetic acid was added to pH 4.5 followed by β-glucuronidase/arylsulfatase (5.2 + 2.6 units/ml, Boehringer Mannheim, F. R. G.) at a quantity of 0.5 ml/100 ml of urine. After incubation at 37°C for 24 hr, the urine was brought to pH 9 with 5 N NaOH and twice extracted with an equal volume of diethyl ether. From the combined ether layers, weakly acidic compounds were extracted into four to five successive portions of 1 N NaOH (20 ml/100 ml of ether), until the aqueous layer remained colorless. After pH adjustment to 8.5 with acetic acid, it was twice extracted with an equal volume of chloroform and the extract was evaporated under reduced pressure.

TABLE 1

R_F values in TLC and spectroscopic data of CPT, its metabolites, and the E-isomers of some of the compounds

Solvent A: chloroform/isopropanol/water/25% ammonia (15:10:0.36:0.24, v/v); B: chloroform/isopropanol (20:2, v/v), atmosphere saturated with ammonia vapor; C: diisopropyl ether/isopropanol (20:2, v/v), atmosphere saturated with ammonia vapor; D: acetone/isopropranol/2 N ammonia (10:10:1.2, v/v); E: isopropanol/25% ammonia (20:2, v/v).

Structure Assigned	R_F in Solvent					UV Maxima	
	A	B	C	D	E	pH 1	pH 9
						nm	
Z-CPT	0.89	1.00	0.86	0.57	0.80	230, 268, 325	230, 268, 325
E-CPT		1.00	0.82	0.44	0.80	230, 268, 325	230, 268, 325
Z-DM_1-CPT	0.61	0.91	0.57	0.28	0.67	230, 268, 325	230, 268, 325
Z-CPT-SO	0.82	0.95	0.61	0.44	0.79	220, 260	220, 260
E-CPT-SO	0.74		0.55	0.33	0.73	220, 260	220, 260
Z-DM_1-CPT-SO	0.41	0.81		0.17	0.56	220, 260	220, 260
E-DM_1-CPT-SO	0.30	0.71		0.13	0.45	220, 259	220, 259
Z-5-OH-CPT	0.84	0.61	0.34	0.64	0.72	229, 269, 324	221, 303
E-5-OH-CPT	0.67	0.54		0.50		229, 270, 324	221, 303
Z-5-OH-DM_1-CPT	0.48	0.46		0.31	0.57	230, 269, 324	221, 303
5,9-$(OH)_2$-9,1′-H_2-CPT	0.55	0.60		0.55	0.66	270, 288	280, 311
Z-6-OH-CPT	0.79	0.60	0.39	0.62	0.74	236, 272, 325	248
E-6-OH-CPT	0.58	0.48	0.23	0.46	0.62	236, 272, 320	247
Z-6-OH-DM_1-CPT	0.41	0.43		0.28		235, 272, 320	247
Z-6-OH-DM_2-CPT	0.61	0.31		0.73		235, 271, 325	247
Z-6-OH-CPT-SO	0.69	0.25	0.07	0.47	0.53	225	227
E-6-OH-CPT-SO	0.44	0.18		0.32	0.36	225	230
Z-6-OH-DM_1-CPT-SO	0.23	0.15		0.18	0.30	225	228
Z-7-OH-CPT	0.68	0.55	0.36	0.64	0.72	213, 266, 336	222, 269, 350
E-7-OH-CPT	0.51	0.53	0.24	0.45	0.57	212, 267, 335	222, 268, 350
Z-7-OH-DM_1-CPT	0.30	0.30		0.28	0.44	213, 264, 335	221, 269, 345
Z-7-OH-DM_2-CPT	0.44	0.28		0.66		213, 267, 335	221, 270, 340
Z-7-OH-CPT-SO	0.31	0.10		0.28	0.33	224, 261, 300	224, 298, 335
Z-6,7-(OH,OCH_3)-CPT	0.73	0.52	0.09	0.36	0.57	217, 277, 330	220, 247, 300, 340
E-6,7-(OH,OCH_3)-CPT	0.42				0.40	217, 277, 330	220, 249, 298, 335
Z-6,7-(OH,OCH_3)-DM_1-CPT	0.35	0.48			0.34	217, 276, 331	220, 247, 300, 340

From dog feces, only nonconjugated phenols were extracted by mixing 10 g of feces with 20 ml of 2 N aqueous ammonia and shaking with two 25-ml portions of ether. The filtered ether layers were processed as described above.

Procedure for Isolation of Total Basic Metabolites. Urine specimens were twice extracted at pH 9 with an equal volume of chloroform to yield nonconjugated metabolites. Subsequently, incubation with β-glucuronidase/arylsulfatase at pH 4.5 and extraction with ether at pH 9 were performed as described above. Liberated metabolites were extracted from the ether phase into three 20-ml portions of 1 N sulfuric acid. The combined acid extracts were alkalinized with 25% ammonia and extracted twice with 20 ml of chloroform.

Dog feces were extracted with ether as described. The combined ether phases from 160 g of feces were shaken four times with 20 ml of 1 N sulfuric acid, the aqueous extract being handled in the same way as in urine analyses.

Thin Layer Chromatography. From the extracts, single compounds were isolated by two or three successive preparative chromatographic steps. In some of the qualitative experiments, 20 × 20 cm plates were manually coated with 0.25 mm of silica gel with fluorescence indicator (Kieselgel GF_{254}, type 60, Merck, Darmstadt, F.R.G.). In other experiments and particularly in quantitative analyses, precoated sheets with silica gel (Alugram Sil G/UV_{254}, Macherey-Nagel, Düren, F. R. G.) were used. The total urine metabolite fraction or an extract from 80 g of dog feces was applied as a band to one plate along with reference compounds. Chloroform/isopropanol (20:2, v/v) was allowed to run to the upper edge; this measure reduced interference from lipids and impurities in the ensuing separation. After drying for 3 min, the chromatogram was developed by running solvent A (table 1) to a height of 12 cm. Five to seven bands visualized under UV light were removed with a spatula after moistening the gel by spraying with water. The gel was suspended in 1–2 ml of 2 N ammonia and substances were extracted with two 2-ml portions of chloroform. The dried extracts mostly were purified by rechromatography in solvent B and, if substances then isolated proved to be inhomogeneous upon chromatography in solvent D or E, a third run in one of these solvents followed. When the separated mixtures also contained nonphenolic compounds, rechromatography of the bands with R_F 0.75 or greater in solvent A was performed in solvent C. Substance losses in one chromatographic step amounted to 2–10%.

Prior to NMR and mass spectrometry, the homogeneous compounds were run in solvent B or D on manually coated plates that had been prewashed with heptane and subsequently with methanol by running to the upper edge. The purified substances were sealed under nitrogen for mailing.

For purposes of identification, a solvent system composed of 1,2-dichloroethane/ethyl acetate/ethanol/acetic acid/water (4:9:2.7:2.7:1.6, v/v) was used in addition to those given in table 1. Color reactions on chromatograms were carried out by spraying with 1% $Ce(SO_4)_2$ in a 1:1 mixture of ethanol and 85% phosphoric acid or with ninhydrin and heating to 100°C for 3 min.

UV Spectroscopy. Aliquots of purified substances were dissolved in 3 ml of 0.1 N H_2SO_4 and the absorption was measured from 210 to 380 nm in a Zeiss PMQ II or a Uvikon LC 720 spectrophotometer. The measurement was repeated after addition of 0.1 ml of 25% ammonia to sample and reference cuvettes. Quantitation was based on molar absorptions measured on weighed samples of synthetic compounds or purified metabolites. *E*- and *Z*-isomers always exhibited identical spectra (table 1), and for *E*- and *Z*-CPT, molar absorptions were found to be the same. Therefore, it was assumed that for the metabolites absorptions of *E*- and *Z*-isomers were also identical. The same assumption was made with regard to *N*-demethylated derivatives.

Mass Spectrometry. The EI mass spectra (ionization energy, 70 eV)

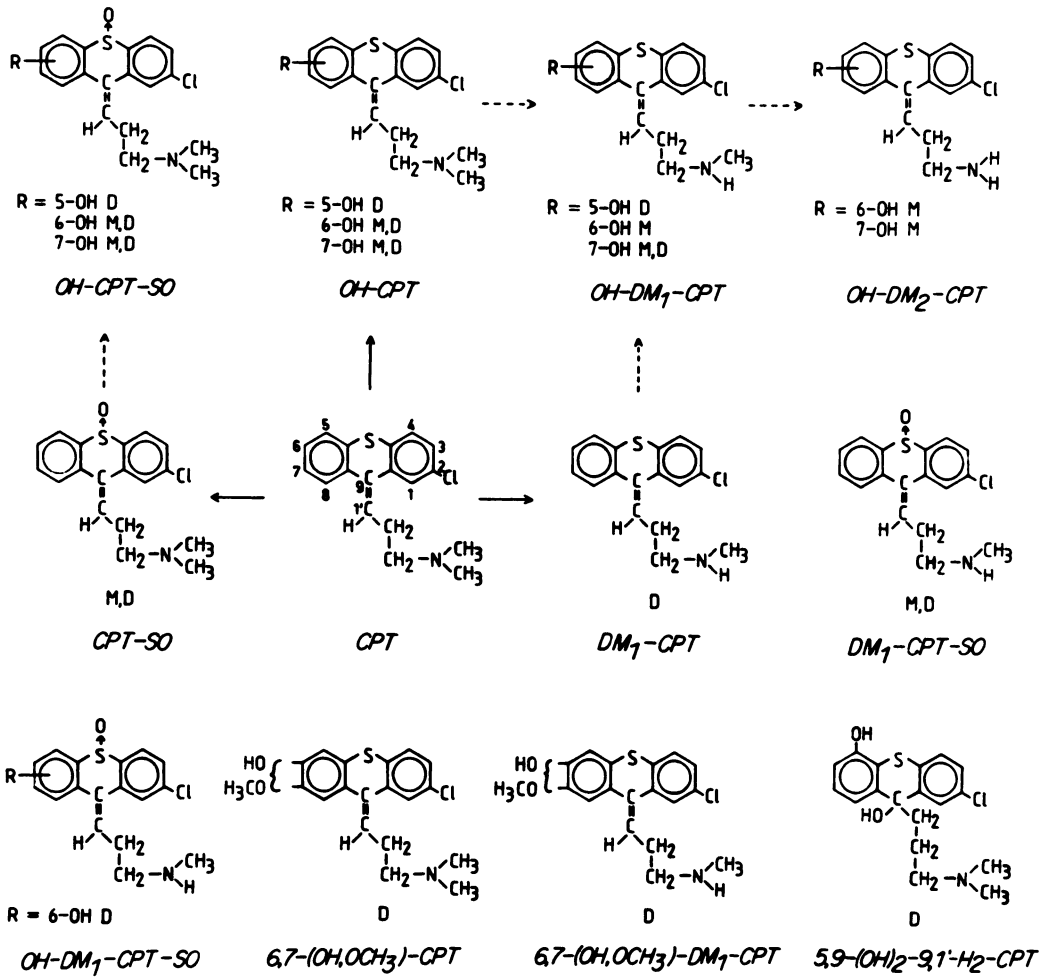

FIG. 1. *Structural formulas of CPT metabolites produced in man (M) and dog (D).*

were recorded with a Kratos AEI MS 902 linked to an Incos data system (Finnigan). The samples were introduced *via* a direct inlet system and evaporated by heating the source to 120–150°C. High resolution mass spectra were measured with a resolving power of 10,000 (10% valley definition). Mostly three to four single high resolution scans were merged to improve mass refinements. All mass measurements were within 10 ppm of the theoretical values.

NMR Spectroscopy. The ^1H NMR spectra were recorded in pulse Fourier transform mode using 80, 300, and 400 MHz spectrometers of Bruker (Karlsruhe, F. R. G.). The samples were dissolved in deuterated chloroform, dimethyl sulfoxide, or methanol or mixtures thereof (table 2). The internal standard was TMS with $\delta = 0$. For measurements of NOE, samples were degassed by careful warming of the tubes cooled in liquid nitrogen under vacuum and pouring with a stream of dried nitrogen gas. This procedure was repeated up to three times.

Chemical Interconversions. Sulfoxides were reduced to sulfides with $TiCl_3$ (16). Oxidation of sulfides to sulfoxides occurred upon heating them in 1 N acetic acid containing 3% H_2O_2 for 60 min to 60°C. In both cases, the reaction mixtures were alkalinized with ammonia and extracted with chloroform. *N*-Methylation of small quantities of primary and secondary amines was carried out by dissolving them in 0.5 ml of ethanol, adding 10 μl of CH_3I and leaving the solution for 4 hr at room temperature. The methylation of secondary amines was alternatively achieved with formaldehyde and sodium cyanoborohydride (17).

Results

***Z/E*-Isomerism.** Complete resolution of metabolite mixtures and structural elucidation of individual compounds was complicated by interconversion of *Z/E*-isomers. During sample processing, it occurred as a consequence of exposure to UV light, for instance when TLC sheets were visualized at 254 nm. When chromatographic bands containing about 260 nmol of *Z*-7-OH-CPT were exposed to this light, the fraction converted to the *E*-isomer increased linearly from 0.5 to 3.9% upon increasing the exposure time from 0.5 to 10 min. Irradiation of a solution of *E*-7-OH-CPT in methanol (1 mg/ml) led to the formation of about 40% of the *Z*-isomer within 11 hr, the identity of the product being confirmed by NMR spectrometry. In most cases, the quantity of *E*-isomers isolated from extracts of biological samples corresponded to 4–8% of the respective *Z*-isomers; they are regarded as *in vitro* artifacts (metabonates), since comparable quantities were produced in model experiments from freshly purified *Z*-compounds. Chromatographic data of *E*-configurated substances are included in table 1, because the TLC procedure had to be designed for their separation from the original metabolites.

Structural Elucidation. A survey of compounds identified in dog and human excreta is given in table 1 and fig. 1. Evidence from which the assigned structures were deduced is presented in the following.

Nonphenolic Metabolites. *Z*-CPT, *E*-CPT, *Z*-CPT-SO and *Z*-DM_1-CPT isolated from biological material were identical with synthetic reference compounds with regard to UV spectra, color reactions, and R_F values in TLC. *E*-CPT-SO and *Z*-DM_1-CPT-SO produced from the authentic sulfides matched the properties

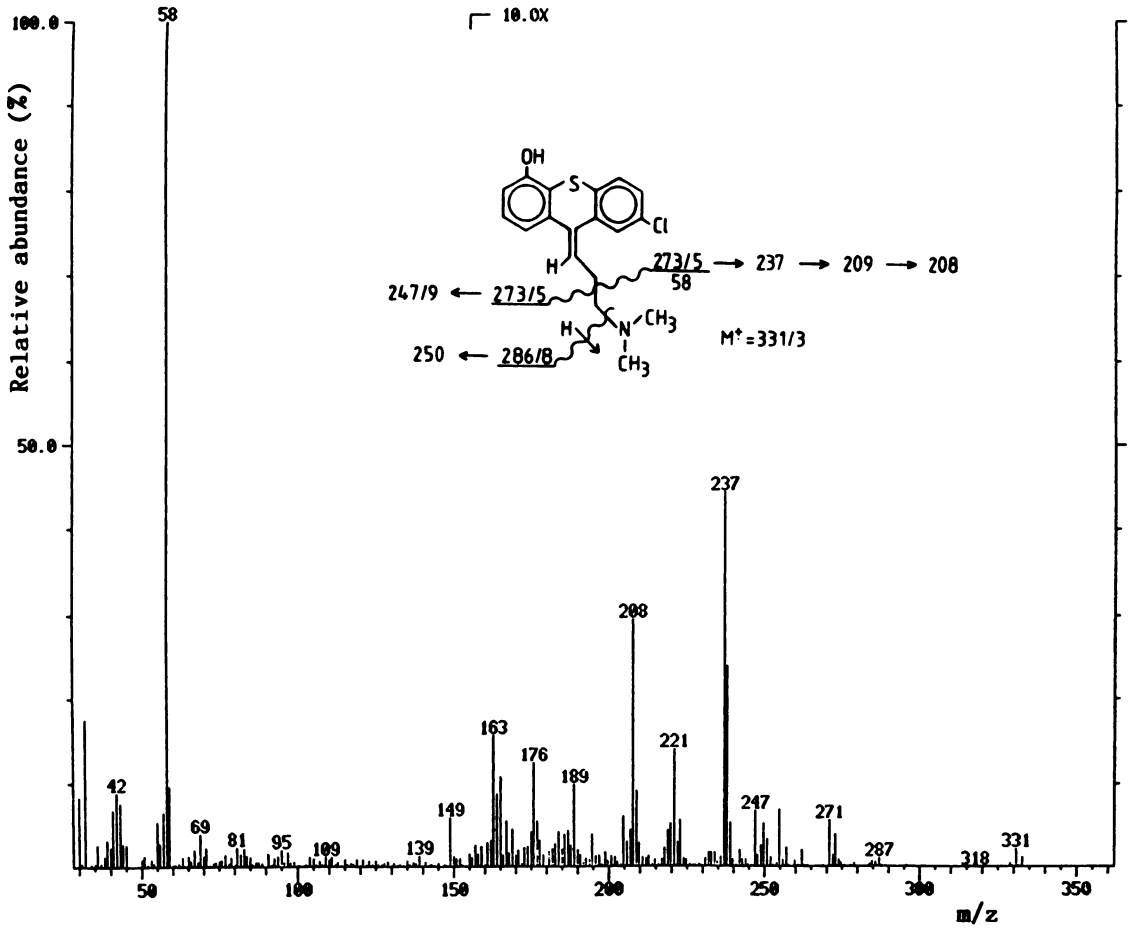

FIG. 2. *Mass spectrum and fragmentation pattern of Z-5-OH-CPT.*

of the respective substances obtained from urine and feces. *E*-DM_1-CPT-SO was converted to the *Z*-isomer upon storage in solution and could be methylated to *E*-CPT-SO.

5-OH-CPT. The major metabolite in dog feces did not cochromatograph nor did its UV spectrum show concordance with any of the reference compounds. Marked differences between the spectra at pH 1 and 9 and the violet color produced with $Ce(SO_4)_2$ pointed to the presence of a phenolic group. The structure of a tertiary amine sulfide was indicated by the failure to react with $TiCl_3$ or methyl iodide or to strain with ninhydrin. Confirmation came from the mass spectrum (fig. 2) that gave molecular ions *m/z* 331/3. High resolution revealed the composition $C_{18}H_{18}{}^{35}ClNOS$ according to a mass 331.0778. The base peak *m/z* 58 (C_3H_8N) indicated the presence of the dimethylamino side chain. The pattern of the fragment ions representing the aromatic tricyclic part of the molecule was similar to that of CPT shifted by 16 amu.

In the aromatic region of the ^1H NMR spectrum, the resonances of H-1, H-3, and H-4 could be easily assigned (table 2, fig. 3). The coupling patterns of the remaining three multiplets showed that each proton of the second aromatic ring had at least one more proton in *ortho* position. Consequently the OH substituent was in the 5- or 8-position. Differentiation was achieved by NOE: the irradiation of H-1′ caused an increase of the intensity of the doublet of doublets at $\delta = 7.02$. On the basis of chemical shift and coupling constants, these signals had to be assigned to H-5 or H-8; the NOE indicated that the multiplet belonged to a proton in close neighborhood to H-1′, and thus to H-8. Consequently, the OH group had to be in the 5-position. In addition, the NOE confirmed the *Z*-configuration of the side chain, since in an *E*-configured compound the intensity of the H-1 signal at $\delta = 7.37$ would have been increased.

Z-5-OH-DM_1-CPT. UV spectra and color reaction with Ce(IV) were identical with those of 5-OH-CPT; methylation converted the metabolite to 5-OH-CPT. The structure of a phenolic secondary amine was deduced from the mass spectrum in fig. 4. The base peak *m/z* 44 was characteristic for a monodesmethyl side chain. The molecular ions *m/z* 317/9 could be clearly identified; high resolution revealed the mass 317.0647 corresponding to $C_{17}H_{16}{}^{35}ClNOS$. Compared with the tertiary amine, the ion fragments of the aromatic moiety showed a somewhat different pattern. The ^1H NMR spectrum (table 2) and NOE confirmed the 5-position of the hydroxyl group and the *Z*-configuration.

Z-5-OH-CPT-SO. This metabolite was reduced to *Z*-5-OH-CPT and was produced from the latter upon sulfoxidation. The identities of the products were demonstrated by TLC in three solvent systems and by UV spectroscopy.

5,9-Dihydroxy-9,1′-dihydrochlorprothixene. This metabolite from dog urine and feces stained violet with $Ce(SO_4)_2$ already in the cold. It was stable at room temperature, but heating in 0.1 N sulfuric acid to 100°C for 1 hr led to more than 70% decomposition with the formation of *E*- and *Z*-5-OH-CPT as main products as revealed by TLC in three solvents and by UV spectra. Conversely, *Z*-5-OH-CPT under the same conditions produced 7% of the compound with the hydrated exocyclic double bond.

Of the weak molecular ions *m/z* 349/51, the more abundant one had an exact mass of 349.0928 that confirmed the compo-

TABLE 2

^{1}H NMR signals of phenolic CPT metabolites

Chemical shifts δ; coupling constants J (Hz) are in parentheses; dd, doublet of doublets.

	Z-5-OH-CPT[a]	Z-5-OH-DM$_1$-CPT[a]	5,9-(OH)$_2$-9,1′-H$_2$-CPT[b]	E-6-OH-CPT[b]	Z-6-OH-CPT-SO[c]	Z-7-OH-CPT[b]	E-7-OH-CPT[b]	6,7-(OH,OCH$_3$)-CPT[b]
Solvent	$CDCl_3/CD_3OD$	$CDCl_3/CD_3OD$	CD_3OD	$CDCl_3$	$CDCl_3/CD_3OD$	$CDCl_3$	$CDCl_3$	CD_3OD
H-1	7.37 d (2.3)	7.38 d (2.5)	7.81 d (2)	7.43 d (2)	7.4–7.7 m	7.32 d (2)	7.40 d (2)	7.44 d (2)
H-3	7.24 dd (2.3, 8.6)	7.22 dd (2.5, 8)	7.24 dd (2, 8)	7.15 dd (2, 8)		7.19 dd (2, 8)	7.16 dd (2, 8)	7.26 dd (2, 8.4)
H-4	7.45 d (8.6)	7.42 d (8)	7.39 d (8)	7.26 d (8)	7.95 d (8)	7.35 d (8)	7.28 d (8)	7.41 d (8.4)
H-5				6.72 d (3)	7.35 d (3)	7.02 d (8)	7.14 d (8)	6.77 s[d]
H-6	6.79 dd (1.3, 7.9)	6.79 dd (1.4, 8)	6.76 dd (1, 8)			6.49 dd (3, 8)	6.47 dd (2, 8)	
H-7	7.14 t (7.9)	7.14 t (8)	7.15 t (8)	6.49 dd (3, 8)	7.05 dd (3, 8)			
H-8	7.02 dd (1.3, 7.9)	7.02 dd (1.4, 8)	7.33 dd (1, 8)	7.13 d (8)	7.4–7.7 m	6.83 d (3)	6.78 d (2)	7.09 s[d]
H-1′	5.83 t (6.9)	5.82 t (7)	1.25–2.30	5.71 t (7)	6.25 t	6.02 t (7)	5.78 t (7)	5.88 t (7)
H-2′	2.6–2.8	2.66 q (7)		2.75 m	2.63	2.64	2.74 q (7)	2.55–2.70
H-3′		2.73 t (7)		2.57 m			2.58 t (7)	
N-CH_3	2.48 s	2.34 s	2.20 s	2.34 s	2.30 s	2.28 s	2.35 s	2.29 s

[a] 300 MHz spectrum.
[b] 400 MHz spectrum.
[c] 80 MHz spectrum.
[d] Assignment to H-5 and H-8 is arbitrary.

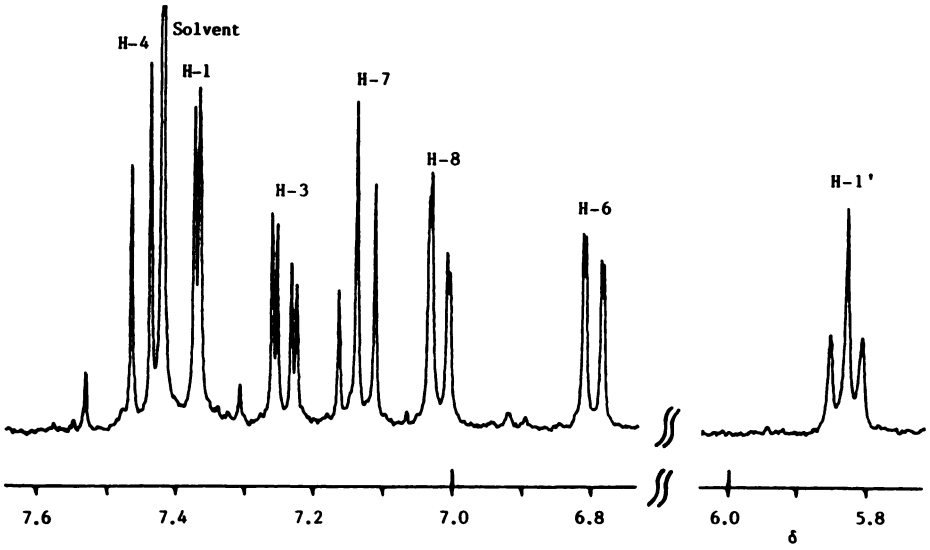

FIG. 3. *Aromatic proton part of the 300 MHz ^{1}H NMR spectrum of Z-5-OH-CPT.*

sition $C_{18}H_{20}{}^{35}ClNO_2S$. The characteristic loss of 86 amu ($C_5H_{12}N$) from M^+ leading to *m/z* 263/5 ($C_{13}H_8ClO_2S$) in the mass spectrum in fig. 5 could only be explained by the formal addition of water to the olefinic double bond. This assumption was supported by the mass spectrum of the TMS derivative which showed weak molecular ions at *m/z* 493/5, indicating the attachment of two TMS residues. Again, a pronounced loss of 86 amu from the molecular ion could be observed. It should be mentioned, however, that the metabolite was slightly contaminated by a compound containing one less oxygen.

In the ^{1}H NMR spectrum (table 2, fig. 6), the Cl-free ring showed the pattern of a 2,3-disubstituted phenol as in 5-OH-CPT. Furthermore the characteristic triplet of the olefinic proton H-1′ was missing. The methylene groups of the side chain showed a complex pattern at δ = 1.25–2.30, whereas the two N-CH_3 groups appeared as a singlet at δ = 2.20.

6-OH-CPT. The metabolite present in human and dog excreta exhibited the same UV spectra as synthetic 6-OH-CPT and produced the same light red color upon heating with Ce(IV). However, the TLC data of the metabolite did not match those of the freshly dissolved synthetic compound, but those of a minor component the quantity of which increased upon storage of the solution and handling in daylight. The synthetic 6-OH-CPT had been ascribed the *Z*-configuration on the basis of the IR spec-

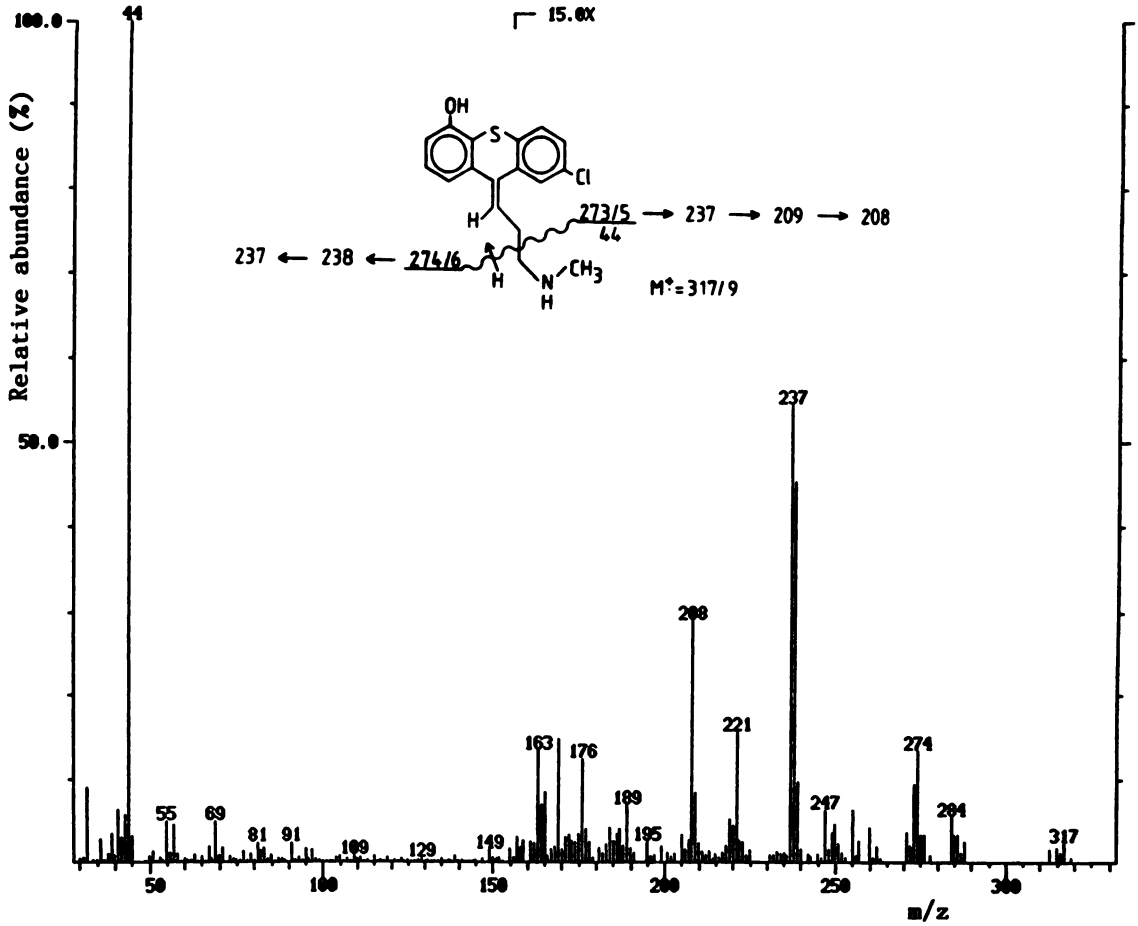

FIG. 4. *Mass spectrum and fragmentation pattern of Z-5-OH-DM*$_1$*-CPT.*

trum, though the precursor was an *E*-isomer (13). Since for the metabolite the *Z*-configuration had to be assumed, the synthetic compound was subjected to NMR spectroscopic investigation (table 2). In NOE, the degassed probe was irradiated at $\delta = 5.7$. This caused an enhancement of 17% of the signal of H-1 at $\delta = 7.43$, indicating the neighborhood of the protons H-1 and H-1′. Thus, the synthetic sample must be *E*-configured, whereas the metabolite should have *Z*-configuration.

In mass spectrometry, the metabolite showed the expected molecular ions at *m/z* 331/3, and a base peak resulting from the side chain at *m/z* 58. In principle, the breakdown products were the same as those of CPT shifted by 16 amu.

Z-6-OH-DM$_1$*-CPT.* The metabolite occurring in human urine only had the same UV spectra and produced the same Ce(IV) reaction as Z-6-OH-CPT to which it was converted with methyl iodide. Confirmation of the structure could only be achieved by high resolution mass spectrometry which showed molecular ions at *m/z* 317/9 with an exact mass 317.0624 according to $C_{17}H_{16}{}^{35}ClNOS$. As expected, *m/z* 44 (C_2H_6N) was the base peak (fig. 7). Although there were distinct similarities with the mass spectrum of Z-5-OH-DM$_1$-CPT (fig. 4) as far as the elimination of 43 (C_2H_5N) and 44 amu and consecutive processes are concerned, *m/z* 302/4 (M − CH_3) and *m/z* 288/90 (M − C_2H_5) were more abundant. The composition of these fragment ions was confirmed by high resolution mass measurements. They are suggested to be produced by rearrangement *via* a *para*-quinoid intermediate that does not occur in the fragmentation of 5- or 7-hydroxy compounds.

Z-6-OH-DM$_2$*-CPT.* This metabolite from human urine exhibited the same chromophore as the above tertiary and secondary amines and it was converted to them by CH_3I. Ninhydrin gave a color in the cold. The lighter of the molecular ions *m/z* 303/5 was found to correspond to a composition of $C_{16}H_{14}{}^{35}ClNOS$, achieving an exact mass of 303.0477. The breakdown was analogous to that of Z-6-OH-DM$_1$-CPT.

6-OH-CPT-SO. The major metabolite failed to stain with the Ce(IV) reagent, but $TiCl_3$ reduced it to a compound staining light red. In UV spectroscopy and TLC, the reduction product could not be distinguished from Z-6-OH-CPT. Moreover, H_2O_2 treatment of the latter produced a sulfoxide the properties of which agreed with those of the metabolite.

The mass spectrum (fig. 8) showed a base peak at *m/z* 58 and very weak molecular ions at *m/z* 347/9 which immediately eliminated water to form *m/z* 329/31. Loss of oxygen to *m/z* 331/3, however, occurred to a minor extent only. The spectrum was complicated by the superposition of the fragmentation pathways commencing with the loss of oxygen or water, respectively. The former pathway resulted in fragments also occurring in the spectrum of Z-6-OH-CPT as could be established by high resolution. The initial loss of water led, for instance, to *m/z* 285/7 → 250, *m/z* 286/8 → 260/2, and *m/z* 271/3.

The ^1H NMR spectrum (table 2) substantially differed from that of sulfidic compounds. The signals of H-1, H-3, and H-8 formed a multiplet at $\delta = 7.4$–7.7.

A minor compound isolated from dog excreta exhibited the same UV spectra as Z-6-OH-CPT-SO, but $TiCl_3$ treatment led

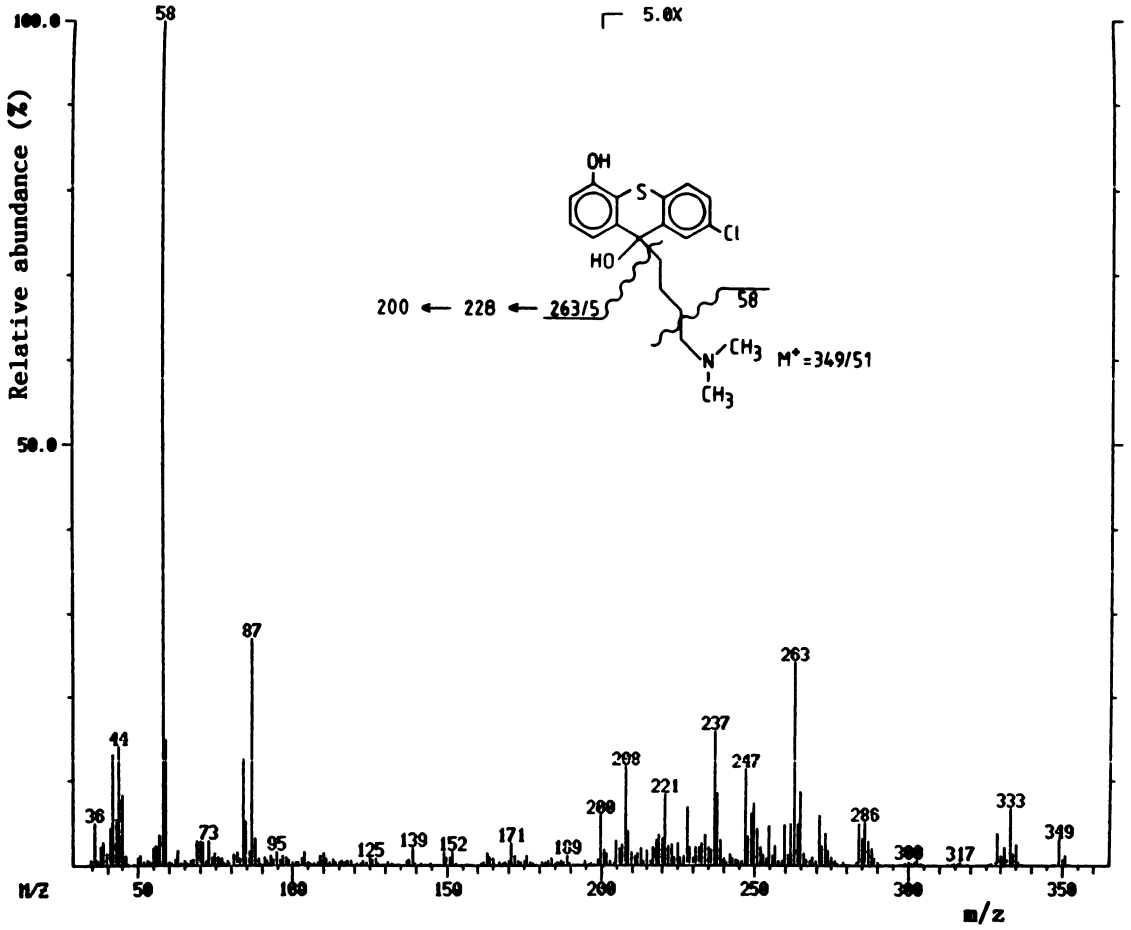

FIG. 5. *Mass spectrum and fragmentation pattern of 5,9-(OH)$_2$-9,1'-H$_2$-CPT.*

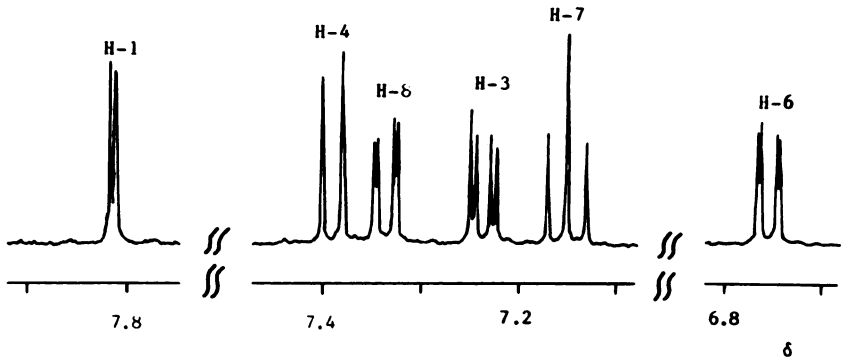

FIG. 6. *Aromatic proton part of the 400 MHz ^{1}H NMR spectrum of 5,9-(OH)$_2$-9,1'-H$_2$-CPT.*

to a sulfide that had the chromatographic properties of *E*-6-OH-CPT. The two sulfoxides showed mutual interconversion upon storage.

Z-6-OH-DM$_1$-CPT-SO. The structure of the metabolite occurring in dog urine and feces was derived from the UV spectra, from the ninhydrin reaction, and from its methylation to *Z*-6-OH-CPT-SO.

7-OH-CPT. The metabolite from human and dog excreta agreed with synthetic *E*-7-OH-CPT with respect to the UV spectra and to the dark red color reaction with $Ce(SO_4)_2$. Concerning behavior in TLC, the same discrepancies were observed as with 6-OH-CPT (table 1). The mass spectrum confirmed the suggested phenolic metabolite, showing molecular ions at *m/z* 331/3 and the base peak at *m/z* 58. The fragmentation pattern was very similar to that of *Z*-5-OH-CPT (fig. 2).

The ^{1}H NMR spectrum was in accordance with the assumed structure and distinctly different from that of the synthetic *E*-isomer (table 2). The configurations were confirmed by NOE of the degassed samples. In the metabolite's spectrum irradiation at δ = 6.01 (triplet of H-1') enhanced the doublet at δ = 6.83 (H-8) by nearly 15%, whereas irradiation at δ = 2.65 (center of the multiplet of H-2' and H-3') led to an increase of about 20% of the doublet at δ = 7.32 (H-1). Therefore, the configuration of the metabolite must be *Z*. On the other hand, irradiation of the synthetic compound at δ = 5.78 (H-1') enhanced the doublet at δ = 7.40 (H-1), whereas the irradiation of the quartet at δ = 2.73 (H-2') caused an enhancement of 22% of the doublet at δ = 6.78

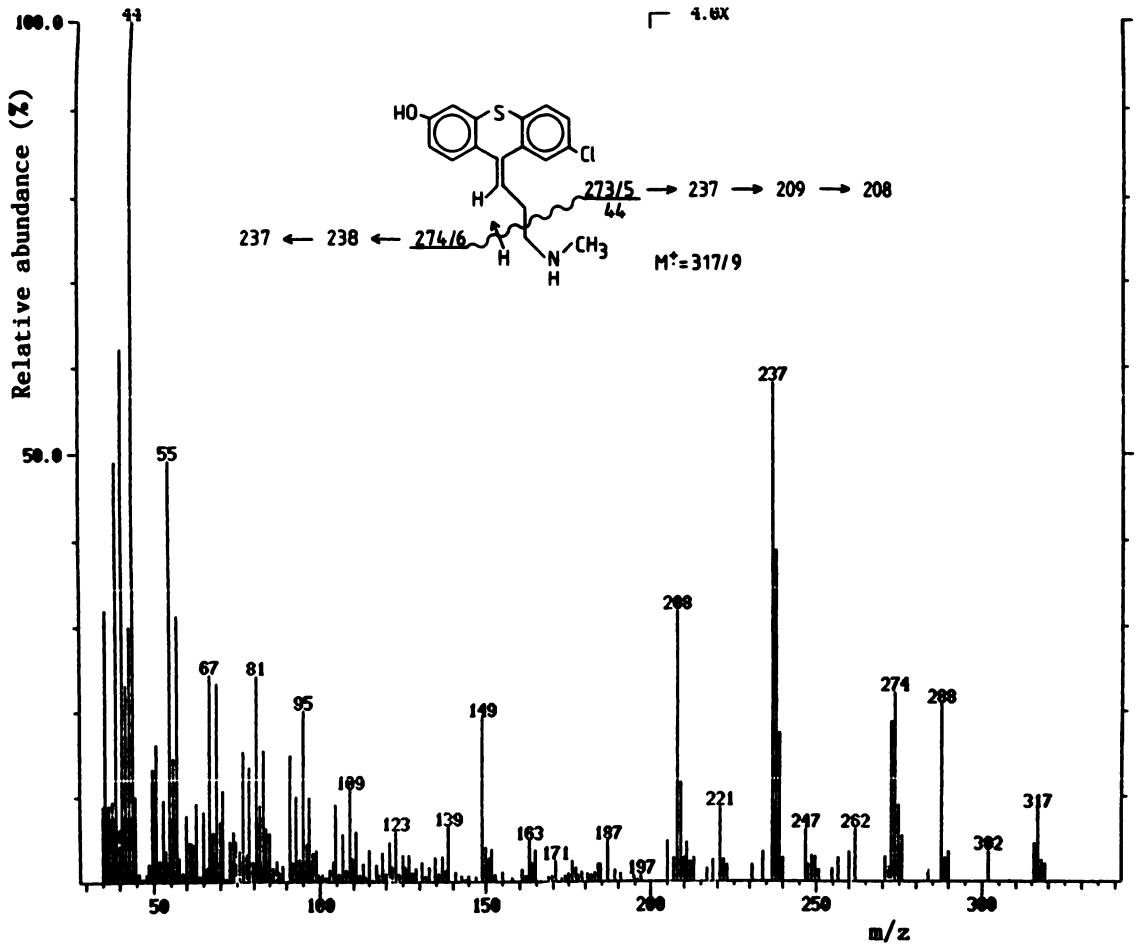

FIG. 7. *Mass spectrum and fragmentation pattern of 6-OH-DM$_1$-CPT.*

(H-8). These results confirmed the postulated *E*-configuration of the synthetic 7-OH-CPT.

Z-7-OH-DM$_1$-CPT. This structure was suggested on the basis of the UV spectral properties, the color resulting with Ce(IV) and the conversion to *Z*-7-OH-CPT with CH_3I. Confirmation came from the mass spectrum which showed molecular ions at *m/z* 317/9 and the base peak at *m/z* 44. The fragmentation corresponded to that of *Z*-5-OH-DM$_1$-CPT (fig. 4) and showed very low abundances for M − 15 and M − 29.

Z-7-OH-DM$_2$-CPT. The evidence leading to the proposition of the structure was analogous to the one described for the 6-hydroxy isomer. The suggested composition $C_{16}H_{14}{}^{35}ClNOS$ according to a mass 303.0486 for the lighter of the molecular ions *m/z* 303/5 was established by high resolution. The absence of intense ions at *m/z* 58 and 44 agreed with the postulated primary amine structure. The fragmentation paralleled that of *Z*-6-OH-DM$_2$-CPT, albeit the corresponding ions differed in their intensities.

Z-7-OH-CPT-SO. The assignment of this structure was based on findings analogous to those with the 6-OH isomer

6-Hydroxy-7-methoxy (or 7-Hydroxy-6-methoxy)-CPT. Compounds ascribed to the structure of hydroxy-methoxy derivatives were easily decomposed on chromatograms as evidenced by a brownish red discoloration, particularly upon exposure to UV light. They stained bright yellow with $Ce(SO_4)_2$. *Z/E*-Isomerization seemed to proceed at a higher rate than with simple phenols. The structure was derived from the ^1H NMR spectrum (table 2, fig. 9) in which the protons belonging to the chlorine-substituted ring could be easily identified. The singlets at $\delta = 6.77$ and 7.09 suggested the attachment of OH and OCH_3 to C-6 and C-7, but the exact position of the two substituents remained open.

The major component was assumed to possess the *Z*-configuration in analogy to the other phenolic metabolites; moreover, it had higher R_F values in solvents A and E than the isomer, a feature consistent with its nature as *Z*-configured compound.

6,7-(OH,OCH$_3$)-DM$_1$-CPT. The metabolite apparently possessed the same chromophore as the above tertiary amine and was converted to it upon methylation. The proposed structure was confirmed by the mass spectrum showing the molecular ions at *m/z* 347/9 and the base peak at *m/z* 44. In the high mass region, the spectrum resembled that of *Z*-6-OH-DM$_1$-CPT shifted by 30 amu.

Metabolite Patterns in Excreta. No appreciable differences existed among the three dogs with regard to the occurrence of phenolic CPT metabolites in urine and feces. Quantitation of nonphenolic compounds in addition to the phenols was done in one dog only (table 3). Nonphenolic metabolites were primarily recovered from the nonconjugated fraction of urinary metabolites and phenols from the conjugated fraction, but both classes were present in either fraction. While the ratio of phenolic to nonphenolic substances was 0.4 in urine, it amounted to 5 in feces.

Humans excreted phenolic sulfides and sulfoxides with the OH group in the 6- or 7-position. While phenolic tertiary and secondary amines were detected in all samples, the corresponding primary amines could only be identified under treatment with

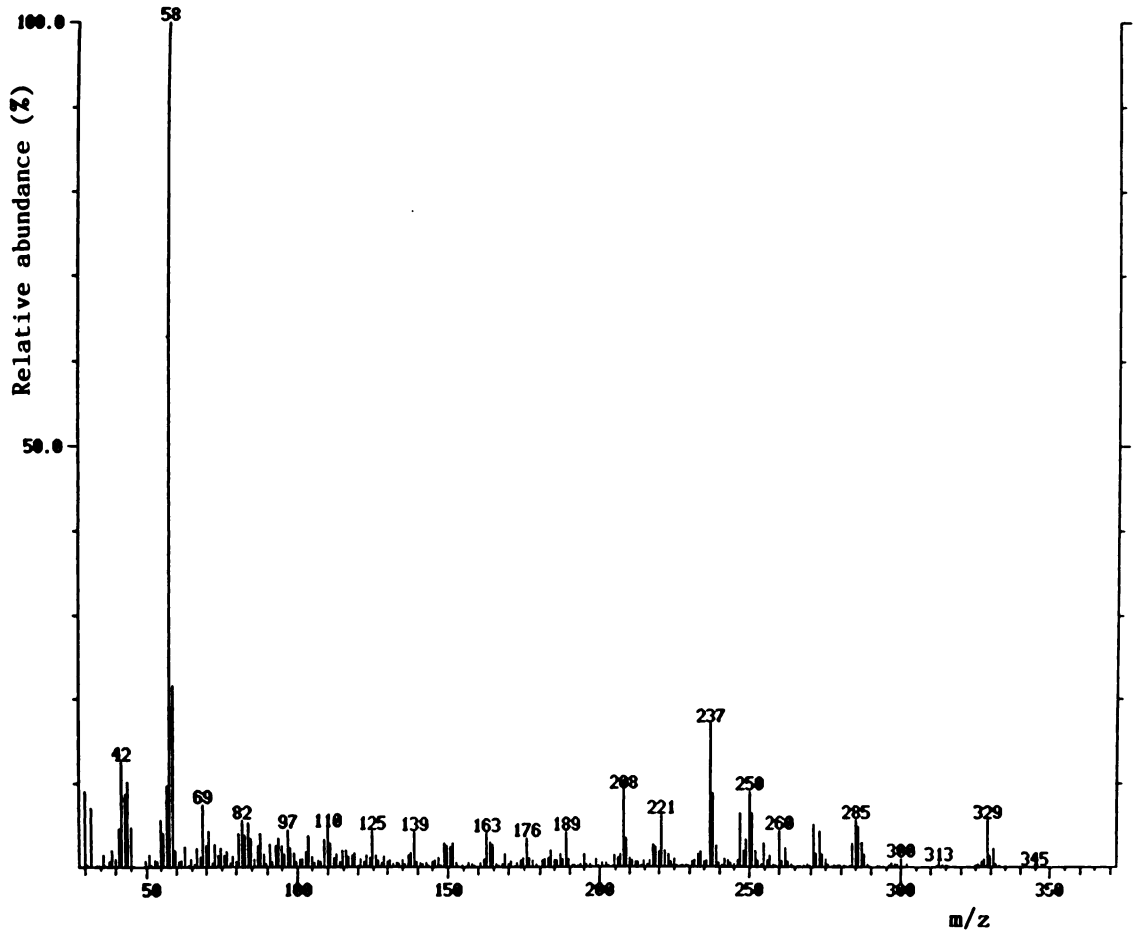

FIG. 8. *Mass spectrum of 6-OH-CPT-SO.*

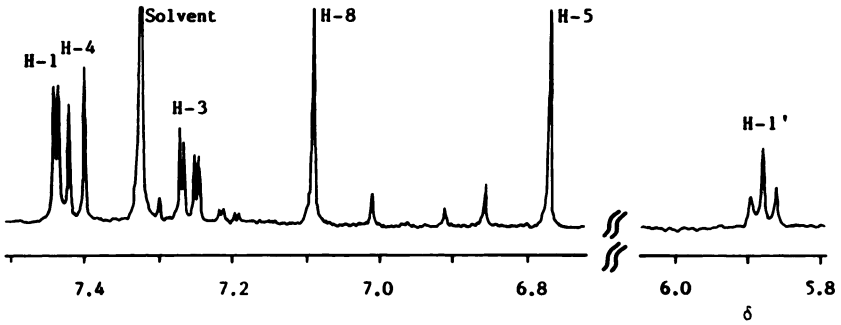

FIG. 9. *Aromatic proton part of the 400 MHz ^1H NMR spectrum of 6,7-(OH,OCH_3)-CPT.*

doses exceeding 100 mg/day. Apart from that, the composition of the phenol fraction was similar in all samples. In one of them, also nonphenolic metabolites were quantitated and a ratio of phenols to nonphenols of 0.05 was found (table 3). The only phenol detected in nonconjugated form was 7-OH-CPT-SO.

Discussion

With regard to *Z/E*-isomerization by UV light, CPT metabolites exhibited the same behavior as the parent drug (18, 19).

The present results show that the thioxanthene ring system of CPT can be attacked in man and dog by aromatic hydroxylation. Dog differed from man by the preferential formation of 5-hydroxy compounds, while these were absent from human urine. In both species, 6- and 7-hydroxylation was regularly observed. These reactions correspond to hydroxylations in positions 7 and 8, respectively, of 2-substituted phenothiazine drugs. Carbon atom 7 is the preferred site for hydroxylation of chlorpromazine (20, 21) and fluphenazine (22), while 8-hydroxy derivatives play a minor role (23). This is probably due to the directing influence of the ring nitrogen atom that is absent in CPT. The occurrence of phenolic sulfoxides that has been described with chlorpromazine (20, 24) was also observed with CPT in man and dog.

Though few quantitative data were obtained, it can be stated that in man aromatic hydroxylation makes a very small contribution to urinary metabolites of CPT. In contrast, phenolic glucuronides were reported to constitute the major fraction of chlorpromazine metabolites in patient urine (25–28). CPT metabolites with presumed phenolic character had been detected in dog urine by Huus and Khan (8). In accordance with this, a more prominent part was played by phenols in dog than in

TABLE 3

Results of quantitative analyses of chlorprothixene metabolites in dog and human excreta

Urine was obtained from a female dog on three successive days within 6 hr after oral administration of 15 mg/kg CPT and pooled. Feces (320 g) were collected on the third day of the experiment. Three morning urine specimens were collected by a patient after ingestion of 30–40 mg of CPT at bedtime and pooled. Values are sums of *Z*- and *E*-isomers and are not corrected for recovery.

Substance	Quantity Isolated from		
	Dog Urine	Dog Feces	Human Urine
		μmol	
CPT	0.27	1.60	ND[a]
DM_1-CPT	0.09	0.21	ND
CPT-SO	10.5	2.6	8.4
DM_1-CPT-SO	1.95	2.1	3.5
5-OH-CPT	0.18	10.8	ND
5-OH-DM_1-CPT	ND	6.1	ND
5-OH-CPT-SO	ND	2.5	ND
5,9-$(OH)_2$-9,1′-H_2-CPT	1.15	2.03	ND
6-OH-CPT	0.18	0.49	0.03
6-OH-DM_1-CPT	ND	ND	0.04
6-OH-CPT-SO	0.97	1.50	0.04
6-OH-DM_1-CPT-SO	0.22	0.27	ND
7-OH-CPT	1.35	5.3	0.21
7-OH-DM_1-CPT	0.36	2.15	0.07
7-OH-CPT-SO	0.33	0.16	0.22
6,7-(OH,OCH_3)-CPT	ND	0.83	ND
6,7-(OH,OCH_3)-DM_1-CPT	ND	0.44	ND

[a] ND, not detected.

human urine. In dog feces, the quantity of phenols exceeded that of nonphenols severalfold. It must, however, be emphasized that the fraction of the daily dose represented by the measured metabolites was only about 6% in feces collected during one day.

An unusual metabolite present in dog excreta was a 5-hydroxy compound in which the exocyclic double bond of CPT was hydrated. No such reaction has to our knowledge been described in drugs with thioxanthene or dibenzocycloheptadiene ring systems. It can, however, not be excluded that this reaction was due to bacterial attack at 5-OH-CPT secreted into dog intestine *via* the bile. The urinary excretion of the metabolite may have been secondary to reabsorption of the transformed compound.

Some of the phenolic CPT derivatives obtained by synthesis were slightly sedative, but none was cataleptogenic (13). Since they were not identical with any of the metabolites, a pharmacological activity of the latter cannot be excluded. In view of the minor quantities produced in man, a contribution to neuroleptic effects by phenols is very improbable. An oxidative attack at an aromatic nucleus of a drug may, however, open a possibility for covalent binding to endogenous macromolecules and thus for immunization towards the drug. Allergic reactions to CPT are infrequent, but skin reactions apparently caused by the drug have been observed in some cases (29, 30).

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