HUMAN METABOLISM OF CYPROHEPTADINE

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ABSTRACT

Gas-liquid chromatographic and mass, nuclear magnetic resonance, and infrared spectrometric techniques were utilized to identify some of the metabolites of cyproheptadine in the urine of human subjects who had ingested radiolabeled drug. Aromatic ring hydroxylation (followed by glucuronide conjugation), N-demethylation, and heterocyclic ring oxidation were shown to occur in man. The principal metabolite, however, was identified tentatively as a quaternary ammonium, glucuronide-like conjugate of cyproheptadine. No evidence was found for metabolic changes at the tricyclic ethylene bridge in this species.

Cyproheptadine, 1-methyl-4-(5*H*-dibenzo[*a,d*]-cycloheptenylidine)piperidine (fig. 1*a*), is a powerful antihistaminic and antiserotoninergic agent in animals and also has mild anticholinergic activity (1). Clinically, it is reported to be useful in treating a number of conditions, including the postgastrectomy dumping syndrome (2, 3) and pruritic dermatoses (4).

Little is known about the metabolism of cyproheptadine. N-Demethylation and 10,11-epoxidation (5-7), and N-oxidation (8) occur in animals. In the present work with man it is shown that although N-demethylation takes place to a minor extent, neither bridge hydroxylation nor N-oxidation seems to be an important metabolic transformation in this species. Aromatic ring hydroxylation, heterocyclic ring oxidation, and N-demethylation occur in man. The main metabolic product found in human urine, however, is a glucuronidase-sensitive conjugate involving only the nitrogen of cyproheptadine.

Materials and Methods

Equipment. The following thin-layer and column chromatography materials were used: Avicel (cellulose) and Silica Gel G plates (Uniplate, Analtech, Inc., Newark, Del.); Bio-Rex 63 (styrene-type phosphonic acid resin), Bio-Rex 5 (mixed tertiary and quaternary amine resin) and Cellex SE (sulfoethyl-cellulose), all from BioRad Laboratories (Richmond, Calif.); Amberlite XAD-2 resin (Rohm and Haas, Philadelphia, Pa.). The hydrolytic enzyme used was glucuronidase (Ketodase, Warner-Chilcott, Morris Plains, N.J.). Derivatizing agents used

were: bis(trimethylsilyl)acetamide (BSA)[1] (Supelco, Bellefonte, Pa.); acetic anhydride (Merck, Rahway, N.J.); trimethylanilinium hydroxide, 0.1 M methanolic solution (MethElute, Pierce Chemical Co., Rockford, Ill.); deuterated BSA (BSA-d_{18}), and acetic anhydride-d_6 (Merck Sharp & Dohme of Canada).

Mass spectra were obtained with an LKB model 9000 instrument, using 70 eV ionizing potential, 270°C source temperature, 50 μamp filament current and an accelerating potential of 3.5 kV. GLC was carried out with a Barber-Coleman model 5000 instrument equipped with a stream-splitter and with radioactivity and flame-ionization detectors. Six-foot columns packed with 3% OV-17 on acid-washed and silanized Gas-Chrom P were used. The column was temperature-programmed from 150° to 250°C, at a rate of 5°C/min except where noted.

NMR spectra were obtained with a Varian HA 100D spectrometer equipped with a Fourier transform accessory. With the sample in CD$_3$OD, 5000 pulses at a 1-sec acquisition time (total accumulation time 1.5 hr) permitted visualization of all features in the spectrum of a small amount (200 μg) of purified metabolite.

IR spectra were measured in KBr-pelleted samples with a Perkin-Elmer model 421 double-beam spectrometer equipped with a beam condensing unit. Sample acidification was achieved by treatment with alcoholic HCl and evaporation of solvent prior to pelleting.

Fractionation Procedure. Urine was collected from eight subjects for 48 hr after ingestion of ^{14}C-cyproheptadine (5,10,11-^{14}C, 4 mg, 16 μCi per subject) and passed through XAD-2 resin columns. The methanol eluate of the columns, containing 50 μCi of radioactivity, was concentrated under reduced pressure to a gum which was subjected to fractionation.

[1] The abbreviations used are: BSA, bis(trimethylsilyl)acetamide; DMC, desmethylcyproheptadine; TMSi, trimethylsilyl; GLC, gas-liquid chromatography; GLC-MS, coupled gas chromatography-mass spectrography; NMR, nuclear magnetic resonance; IR, infrared.

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a CYPROHEPTADINE *14C   b METABOLITE A3

c. METABOLITE A4   d

I   II

e.   f   g
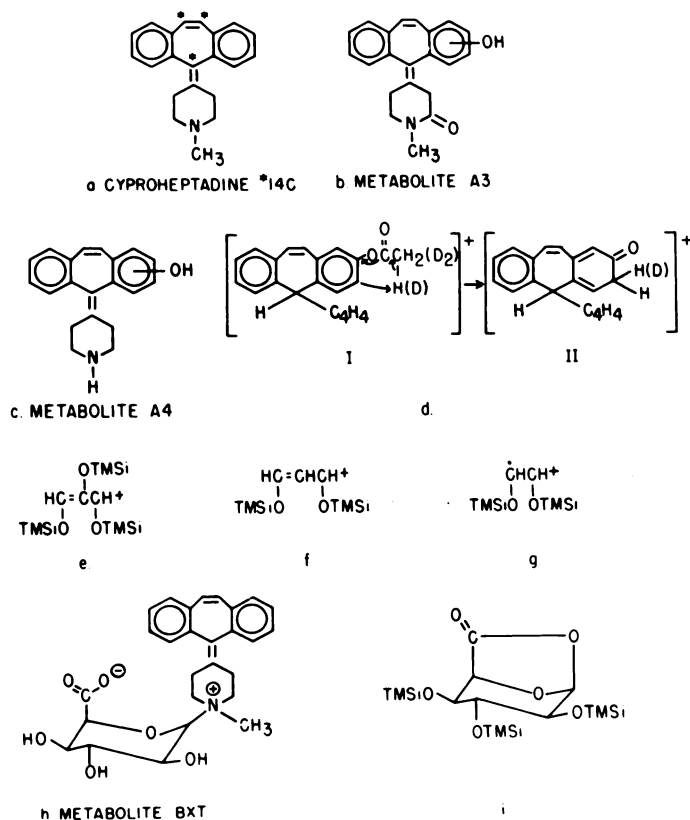
h METABOLITE BXT   i

FIG. 1. Structural formulas.

Fifty-tube countercurrent distribution of the concentrate between water and butanol/benzene (1:1, v/v) yielded three fractions: A, the aqueous end (25%); B, intermediate tubes (60%); and C, at the solvent end (15%). Cyproheptadine was identified as a component of the latter, but other metabolites present were in quantities too small to permit definitive examination.

Fraction A, after concentration under reduced pressure and solution in water, was applied to a column of Bio-Rex 5 resin (OH⁻) and washed through with water. The aqueous wash, which contained about one-half of the radioactivity in the fraction, was concentrated and spotted on 1000-μ Avicel plates, and the chromatograms were developed with butanol/benzene/water/methanol (2:1:1:1.25, v/v). The single radioactive band at R_f 0.69 was eluted with methanol (fraction A1).

The radioactivity retained by the Bio-Rex 5 resin was eluted with 0.5 N acetic acid. It was then adsorbed on a Bio-Rex 63 (H⁺) resin column, eluted with 1 N NH₄OH, concentrated, and chromatographed on 1000-μ Avicel plates with butanol/benzene/water/methanol, as above. The principal band of radioactivity, R_f 0.51, was eluted with methanol, concentrated, and treated with glucuronidase. The solution was made alkaline with Na₂CO₃ and then extracted with benzene. Removal of solvent and thin-layer chromatography of the residue on Silica Gel G plates (methanol/chloroform, 1:4, v/v) gave three major

radioactive zones (A2, A3, and A4) of R_f 0.43, 0.92, and 0.12, respectively. These were eluted from the adsorbant with methanol and each further purified using methanol/benzene (1:4, v/v) to give three major fractions, A2, A3, and A4 (R_f 0.35, 0.50, and 0.15, respectively).

After concentration under reduced pressure, fraction B was subjected to chromatography on 1000-μ Avicel plates with butanol/ethanol/water (17:3:20, v/v). A single peak of radioactivity was obtained (R_f 0.56). The radioactive bands were scraped off the plates and eluted with methanol, and the solvent was removed under reduced pressure. The residue was dissolved in water and applied to a Cellex SE (H⁺) column. The column was washed with water, and the fraction of effluent containing the radioactivity was concentrated to a small volume. A crystalline precipitate was obtained. The crystals were collected by centrifugation, dissolved in methanol, and separated from a little insoluble material by centrifugation. The methanolic solution was concentrated to a small volume, and about 10 volumes of water were added. After standing in the refrigerator overnight, the radioactive crystals (BXT) were collected, washed with a little cold water, and dried in vacuo over calcium chloride. Some of the crystalline product was subjected to enzyme hydrolysis and, after alkalinization, extracted with benzene. The benzene solution and the remaining aqueous solution were concentrated to dryness.

Trimethylsilylation and acetylation were carried out by dissolving the sample (~5 μg) in 20 μl of a 2:1 (v/v) mixture of derivatization agent and pyridine; the solution was allowed to stand at room temperature for 20 min prior to analysis. Methylation experiments were carried out by dissolving the sample (~5 μg) in 20 μl of MethElute just prior to analysis.

Results

Preliminary experiments showed that although cyproheptadine is extracted easily from aqueous solution (pH 9) with a variety of solvents such as benzene, dichloromethane, and ethyl acetate, little (4–6%) of the radioactivity was extracted by these solvents from the urine of subjects given a preparation of ^{14}C-cyproheptadine comparable to that used by Hucker et al. (8). Glucuronidase treatment of the urine increased the extractable radioactivity to 45–55%, the bulk of which proved to be cyproheptadine. Apparently cyproheptadine was excreted either as an enzyme-hydrolyzable conjugate or as a metabolite conjugate, the aglycone of which readily reverted to the parent drug. Fractionation of urine prior to glucuronidase treatment led to the isolation of a crystalline metabolite and of impure fractions which, after hydrolysis and further fractionation, yielded identifiable compounds.

Mass Spectra of Cyproheptadine and Oxygen-Containing Derivatives. The mass spectra of cyproheptadine and related compounds (fig. 2, a and c; table 1) show extensive fragmentation of the heterocyclic ring and relative stability of the tricyclic nucleus. Under the influence of electron impact, the exocyclic double bond migrates into the heterocyclic ring with an accompanying shift of a hydrogen atom to the carbocyclic system. The new C—C bond between the two rings then undergoes scission to yield the ions m/e 96 and M − 96 (cyproheptadine and 3-hydroxycyproheptadine) or m/e 82 and M − 82 (desmethylcyproheptadine; DMC). The heterocyclic ring undergoes fragmentation to yield M − 44, M − 58, and M − 72 ions (cyproheptadine and the 3-hydroxy compound). Analogous ions are found for DMC at M − 30, M − 44, and M − 58, respectively. The loss of 44 (cyproheptadine and 3-hydroxycyproheptadine) and 30 (DMC), formally M − (CH$_3$NCH$_3$) and M − (CH$_3$NH), respectively, results in a heterocyclic ring residue of C$_4$H$_4$ (fig. 1d). DMC yields an intense ion of M − 42; neither this nor the analogous ion 14 mass units higher is found in the spectra of cyproheptadine and 3-hydroxycyproheptadine.

Cyproheptadine 10,11-epoxide, like 3-hydrox-

ycyproheptadine, exhibits a molecular ion of m/e 303 (80% relative intensity); its fragmentation pattern is dominated by the ions M − 17 (70%) and M − 29 (100%) which are not observed in the mass spectrum of the latter compound. Another oxygenated cyproheptadine, 10(11)-keto-10,11-dihydrocyproheptadine, also exhibits the M − 17 ion (m/e 286; 15%). It forms mono-TMSi and monoacetyl derivatives of the enol tautomer, 10(11)-hydroxycyproheptadine. Likewise, 3-hydroxycyproheptadine is readily transformed to its TMSi and methyl ethers, and its acetyl ester; however, cyproheptadine 10,11-epoxide is unreactive under our conditions of derivative formation.

A1. GLC-mass spectrometry and direct-probe mass spectrometry of A1 and its TMSi derivative demonstrated that this fraction contained the major component of fraction B, *i.e.*, BXT (see below).

A2. The mass spectrum of the principal radioactive component of this metabolite fraction was found to be indistinguishable from that of 3-hydroxycyproheptadine (fig. 2, b and c), and distinct from that of 10(11)-hydroxycyproheptadine. The metabolite formed a mono-TMSi derivative (molecular ion, m/e 375), demonstrating that it was not the epoxide. The mass spectrum of the TMSi ether was identical to that of 3-hydroxycyproheptadine TMSi ether. In addition, the retention time of the metabolite was the same as that of 3-hydroxycyproheptadine (free and TMSi). A2 thus appears to be 3-hydroxycyproheptadine, although the possibility of a positional isomer is not excluded.

A3. The direct-probe MS of the underivatized fraction revealed the presence of a major drug-related component with a molecular ion of m/e 317, or 30 mass units greater than cyproheptadine. This component formed a mono-TMSi derivative, the mass spectrum of which is shown in fig. 2d. A comparison of the m/e values for the molecular and fragment ions of this derivative and of the TMSi-d$_9$ derivative of the metabolite with the ions of the corresponding derivatives of 3-hydroxycyproheptadine is presented in table 2. Based on the earlier discussion of fragmentation of the heterocyclic ring, the following conclusions can be drawn. The fragments of mass M − 96, M − 72, M − 58 and M − 44 from the TMSi and TMSi-d$_9$ derivatives of 3-hydroxycyproheptadine arise from fragmentation of the heterocyclic ring. With the metabolite, the fragments lost to yield ions M − 110 and M − 86 (m/e 279 and 303) are 14 mass units greater than those from 3-hydroxycyprohep-
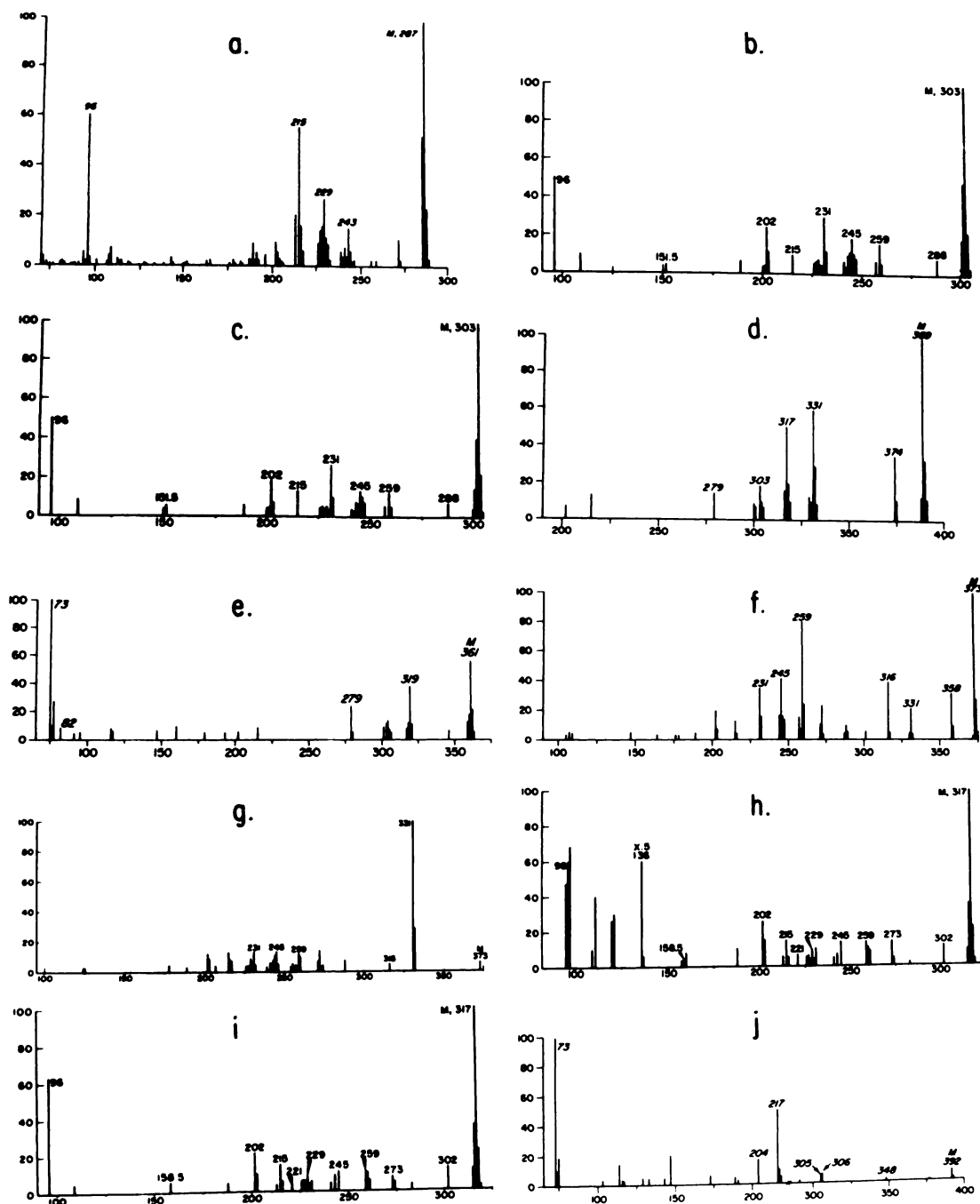
FIG. 2. Mass spectra (ordinates, relative intensity; abscissas, m/e).

a, cyproheptadine; b, metabolite A2; c, 3-hydroxycyproheptadine; d, TMSi derivative of metabolite A3; e, TMSi derivative of metabolite A4; f, acetylated metabolite A4; g, acetylated 10(11)-hydroxy-DMC; h, methylated metabolite A4; i, methylated 3-hydroxycyproheptadine; j, TMSi derivative of carbohydrate BX.

tadine. There is no loss of 44, but rather a loss of 44 plus 14 to give M − 58. Thus a likely metabolic change involves oxidation of the heterocyclic ring ($CH_2 \rightarrow CO$) as shown in fig. 1b. The additional 16

mass units necessary to account for the 30-mass unit increase in molecular weight can be ascribed to introduction of a derivatizable hydroxyl group, the position (bridgehead or aromatic ring) of which

TABLE 1

Mass-spectral ions from cyproheptadine and related compounds

m/e^a		
Cyproheptadine	3-Hydroxycypro-heptadine	Desmethylcypro-heptadine
96^b (60)	96^b (50)	82^b (33)
191, M − 96 (6)	207, M − 96 (5)	191, M − 82 (17)
215, M − 72 (56)	231, M − 72 (26)	215, M − 58 (68)
229, M − 58 (27)	245, M − 58 (13)	229, M − 44 (26)
243, M − 44 (15)	259, M − 44 (12)	231, M − 42 (53)
272, M − 15 (11)	288, M − 15 (7)	243, M − 30 (12)
287, M (100)	303, M (100)	258, M − 15 (51)
		273, M (100)

^{a} Relative intensities are shown in parentheses.
^{b} Heterocyclic fragment.

TABLE 2

Mass-spectral ions from TMSi derivatives of 3-hydroxycyproheptadine and metabolite A3

m/e			
3-Hydroxycyproheptadine		Metabolite A3	
TMSi	TMSi-d$_9$	TMSi	TMSi-d$_9$
96^a	96^a		
279, M − 96	288, M − 96	279, M − 110	288, M − 110
303, M − 72	312, M − 72	303, M − 86	312, M − 86
317, M − 58	326, M − 58	317, M − 72	326, M − 72
331, M − 44	340, M − 44	331, M − 58	340, M − 58
360, M − 15	366, M − 18	374, M − 15	380, M − 18
375, M	369, M − 15	389, M	383, M − 15
	384, M		398, M

^{a} Heterocyclic fragment.

is uncertain. Since the metabolite appeared in the conjugate fraction, we tentatively assign to it a phenolic structure.

A4. The fraction was derivatized with BSA, BSA-d$_{18}$, acetic anhydride, and acetic anhydride-d$_6$. The major radioactive component, as recognized by gas-liquid radiochromatography, gave molecular ions with m/e values of 361 (BSA, fig. 2e), 370 (BSA-d$_{18}$), 373 (Ac$_2$O, fig. 2f), and 379 (Ac$_2$O-d$_6$). Therefore, the metabolite must bear two functional groups, both of which were acetylated, but only one of which was trimethylsilylated under the conditions used. Its molecular weight is 289 [361 − 72 = 289; 373 − (2 × 42) = 289]. The TMSi-d$_9$ and acetyl-d$_3$ derivatives both displayed M − 15 signals, suggesting that the N-methyl group might still be present. The latter derivative of authentic DMC also displayed this ion, however, showing that the N-CH$_3$ group was not responsible for the signal.

The molecular weight of A4 is 2 mass units greater than that of cyproheptadine, and could result from loss of 14 mass units (CH$_2$) and addition of 16 (oxygen), i.e., metabolism to an

oxygenated DMC. Hintze and Fischer (6) have reported that 10,11-epoxy-DMC is a urinary metabolite of cyproheptadine in the rat. Since we have found that 10,11-epoxycyproheptadine is not trimethylsilylated or acetylated, it would appear that A4 is not the epoxide of DMC. A second possibility would be 10(11)-hydroxy-DMC, but the diacetyl derivative of this compound exhibited a spectrum different from that of diacetylated A4 (fig. 2, f and g). Also, the spectrum of the mono-TMSi derivative of A4 (fig. 2e) differed from that of the corresponding 10(11)-hydroxy-DMC derivative.

A structure compatible with the data, an aromatic ring-hydroxylated desmethylcyproheptadine, is shown in fig. 1c. The secondary amino group of this compound should react more slowly than the phenolic group with BSA, thus explaining the formation of the mono-TMSi derivative; however, both of the functional groups should undergo acetylation. The fragment ions of m/e 82, M − 82 [m/e 279 (TMSi), 288 (TMSi-d$_9$)], and M − 42 [m/e 319 (TMSi), 328 (TMSi-d$_9$)] from the TMSi derivative support the proposed structure. Additional supporting evidence was gained from the acetylation experiments. *Ion II* (fig. 1d) [m/e 259 (Ac$_2$O), 260 (Ac$_2$O-d$_6$)] was observed with the metabolite as well as with 3-hydroxycyproheptadine. It probably was formed by loss of ketene from the acetoxy precursor *ion I* [m/e 301 (Ac$_2$O), 304 (Ac$_2$O-d$_6$)] which was also observed.

The structure of this metabolite was substantiated further by use of MethElute. GLC of a solution of desmethylcyproheptadine in MethElute resulted in elution of cyproheptadine, i.e., N-methylation had occurred. The analogous experiment with 3-hydroxycyproheptadine resulted in O-methylation. Therefore, under similar conditions, the metabolite should yield a methoxycyproheptadine. A radioactive component with the same retention time as that of 3-methoxycyproheptadine was produced. Its mass spectrum is shown in fig. 2h. All of the ions from 3-methoxycyproheptadine (fig. 2i), including the molecular ion of m/e 317, are found in the spectrum of the dimethylated metabolite. Additional ions in the later spectrum probably are due to impurities, and it is reasonable to assign the structure shown in fig. 1d to metabolite A4, with the 3-position as a likely site for the hydroxy group. It should be noted that, according to Frigerio *et al.* (7), 10,11-epoxy-DMC forms a monomethyl derivative upon treatment with MethElute.

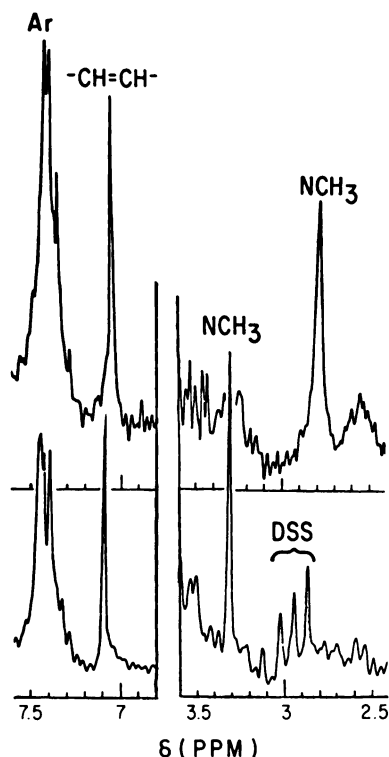*BXT.* The NMR spectrum of the crystalline material (fig. 3) showed a peak characteristic of

FIG. 3. *NMR spectra.*

Top, cyproheptadine and glucuronic acid, equimolar. *Bottom,* metabolite BXT (*DSS,* signals due to the internal standard, sodium 2,2-dimethyl-2-silapentane-5-sulfonate).

the 10,11-vinylic protons of cyproheptadine, indicating that the endocyclic double bond was unchanged. Absorption in the region characteristic of the —CH$_2$C= protons (2.5–3.0 δ) strongly suggests that the exocyclic double bond also was unaltered. The fact that the multiplet was shifted moderately downfield, 0.2–0.3 ppm in comparison to cyproheptadine, implies a structural change at a site more remote than an adjacent C-atom. Location of a substituent group on the piperidyl nitrogen can be inferred from a 0.5-ppm downfield displacement of the —NCH$_3$ signal relative to the parent compound cyproheptadine; a shift of similar magnitude was produced by N-methylation, *i.e.,* quaternization, of cyproheptadine. In addition to the peaks associated with the cyproheptadine nucleus, BXT showed signals between 3.5 and 4.0 δ (estimated relative area, 6–8 H) which suggest the presence of a carbohydrate moiety.

The IR spectrum of BXT, in addition to the expected aromatic bands at 800 and 750 cm^{-1}, showed broad 1100 cm^{-1} absorption (C—O) and a strong 1600 cm^{-1} band (COO$^-$) suggestive of a

glucuronide moiety. An absorption peak at 1715 cm^{-1} (COOH) was observed upon acidification of the sample.

The direct-probe mass spectrum of BXT was indistinguishable from that of cyproheptadine; however, the former required a significantly greater vaporization temperature than the latter. Trimethylsilylated BXT gave the mass spectrum of cyproheptadine, plus a number of signals (*e.g.,* m/e 204, 217, 305) but not all of those which are characteristic of TMSi derivatives of glucuronides and carbohydrates (9, 10). The apparent molecular ion of the trimethylsilylated species shifted from m/e 392 (TMSi) to 419 (TMSi-d$_9$); no signals of higher mass were observed.

GLC of BXT resulted in the elution of one radioactive component identical to cyproheptadine in retention time and mass spectrum. BXT subjected to trimethylsilylation prior to GLC yielded cyproheptadine and several nonradioactive substances, one of which seemed to be the TMSi derivative of a sugar-like compound (BX). Table 3 shows the m/e values of a number of ions found in the mass spectra of the TMSi (fig. 2*j*) and TMSi-d$_9$ derivatives of BX. The molecular ions are found at m/e 392 and 419; therefore, the molecular weight of the parent compound is 176 [392 − (3 × 72) = 176; 419 − (3 × 81) = 176]. Many of the fragment ions observed with the TMSi derivatives of carbohydrates (9) and glucuronides (10) are present in the spectra of the TMSi derivatives of BXT, and the deuterium shifts confirm the structures. Of particular interest are the following (table 3): C, M − 44, probably resulting from a loss of CO$_2$; E, M − 87, probably as shown in fig. 1*e* (this ion is common from TMSi derivatives of carbohydrates, and retains carbons 2, 3 and 4, principally); D, M

TABLE 3

Mass-spectral ions from TMSi derivatives of the carbohydrate portion (BX) of cyproheptadine metabolite BXT

Peak	m/e		Δ
	TMSi	TMSi-d$_9$	
A	392	419	27
B	377	401	24
C	348	375	27
D	306	333	27
E	305	332	27
F	291	315	24
G	217	235	18
H	204	222	18
I	191	209	18
J	189	204	15

– 86, probably E with a proton on the central carbon atom; G, fig. 1f; and H, fig. 1g. It should be noted that an ion of m/e 204, rather than 205, indicates a pyranose rather than a furanose ring structure (11).

Glucuronic acid, molecular weight 194, forms a penta-TMSi derivative. The molecular weight of BX is 176, 18 mass units less than that of glucuronic acid. Lactonization of a uronic acid reduces the molecular weight by 18 and the number of derivatizable groups to three. Glucofuranurono-(6 → 3)-lactone froms a tri-TMSi derivative with the same M (m/e 392) as the TMSi derivative of BX; these two compounds possess similar retention times and mass spectra. However, significant differences do exist between the spectra. M is more intense than M – 15 with tri-TMSi-BX, whereas the reverse is true with the TMSi derivative of the reference lactone. Tri-TMSi-glucofuranurono-(6 → 3)-lactone displayed an ion of M – 43 (M – 46, TMSi-d$_9$), probably M – (CH$_3$ + CO), but not M – 44. Further, the m/e 305 and 306 ions found with tri-TMSi-BX were absent from the spectrum of the reference compound. The latter yielded an intense signal at m/e 230, characteristic of a furanose system; this ion was absent in the spectrum of tri-TMSi-BX. Both spectra displayed intense m/e 217 ions.

Recently, Mrochek and Rainey (11) reported that trimethylsilylation of acyl glucuronides followed by GLC results in elution of the tri-TMSi ether of glucopyranurono-(6 → 1)-lactone (fig. 1i). The mass spectrum of this compound was presented by the authors, and closely matches (detailed comparison was not possible) that of the compound derived from trimethylsilylation of metabolite BXT. In our laboratory trimethylsilylation of an authentic acylglucuronide, followed by GLC, yielded a compound with identical GLC and MS properties to those of tri-TMSi-BX.

In 1973, Sinnott (12) reported that the β-D-galactopyranosylpyridinium cation undergoes β-galactosidase-catalyzed hydrolysis to yield galactose and pyridine. By analogy, the structure shown in fig. 1h can be suggested for metabolite BXT. Injection of such a compound into a GLC column at elevated temperatures should result in its thermal conversion to cyproheptadine and glucopyranurono-(6 → 1)-lactone which probably would not be eluted from the column. When injected into the column after trimethylsilylation, the lactone, an isomer of glucofuranurono-(6 → 3)-lactone, would form a tri-TMSi derivative (fig. 1i) and be eluted. Benzene extraction of hydrolyzed (glucuronidase)

BXT yielded a single radioactive compound which was found to be identical to cyproheptadine on the basis of TLC, GLC, NMR, IR, and mass spectrometry.

The total product resulting from glucuronidase treatment of BXT was examined by GLC-MS, following trimethylsilylation, in an attempt to obtain further information concerning the carbohydrate-like species associated with cyproheptadine. Several components of interest were observed, in addition to cyproheptadine. One of these possessed the mass spectrum of tri-TMSi-BX. Two others exhibited spectra nearly identical to those obtained when penta-TMSi-glucuronic acid, molecular weight 554, was analyzed similarly. Especially pertinent ions common to the derivatives of glucuronic acid and the hydrolysis product of BXT are m/e 539 (M – 15), 449 [M – (15 + 90)], 305, 292, 217, 204, and 191. A uronic acid is apparently released from BXT by treatment with glucuronidase.

An N-oxide of cyproheptadine has been identified as a minor metabolite in dogs and cats (8), and at least one N-oxide is known which reverts completely to the parent amine in the mass spectrometer (13). Therefore, it was important to establish that an N-oxide would not have been mistaken for the parent compound in the foregoing examination. The α- and β-N-oxides of cyproheptadine were examined; they exhibited GLC and MS behavior different from cyproheptadine. The chromatogram resulting from temperature-programmed GLC analysis showed the same three components (at 212°C, a shoulder on the 212°C peak, and at 223°C) to be eluted from both of the N-oxide isomers, whereas cyproheptadine gave a single peak at 212°. The mass spectrum of the shoulder of the 212° peak exhibited a molecular ion at m/e 303 (the molecular weight of the N-oxides), with the base peak at m/e 273, M – 30. The mass spectrum of the component which was eluted at 223° was dominated by a signal at m/e 285, 18 mass units lower than the molecular weight of the N-oxides, which may represent a dehydration product; only very low intensity signals were observed at m/e 303. The major component (212°C) was shown to be cyproheptadine.

Results from direct probe spectrometry of the N-oxides were quite similar to those obtained with GLC-MS, in that multiple components were observed. Early scans (least exposure to heat) gave spectra with the appropriate molecular ions, 303, but also with more intense 287 ions; later scans showed virtually no 303, but much elevated

(relative to 287) 285 signals. The final scans, *i.e.*, those with most exposure to heat, had m/e 285 as the base peak. Glucuronidase-incubated cyproheptadine-α-N-oxide gave the same MS results as nonincubated compound.

Discussion

The initial countercurrent fractionation procedure was not a particularly efficient one; however, it did lead to the isolation of the principal cyproheptadine metabolite in quantities sufficient for characterization. Strikingly, upon glucuronidase hydrolysis the compound yields cyproheptadine, which bears no obvious site for glucuronide conjugation. Apparently, in man cyproheptadine forms a quaternary, glucuronide-type conjugate.

All of the information available, particularly that gained from NMR spectroscopy, shows that the structure of cyproheptadine in the conjugate differs from that of the base compound only at the nitrogen atom. As quaternary N-alkoxy compounds have been prepared (14), the possibility was considered that the metabolite was a conjugate of N-hydroxycyproheptadine; however, glucuronidase treatment of the conjugate yielded only cyproheptadine as the aglycone. Cyproheptadine N-oxide, the only closely related synthetic compound available, was recovered unchanged after incubation with glucuronidase.

Mass spectral studies showed that cyproheptadine N-oxides underwent thermal degradation in the GLC column and on the mass spectrometer probe. Since unique products were the result, it is clear that the chance of confusing the N-oxides with cyproheptadine itself is remote.

It also seemed possible that the conjugate might be the glucuronide of a quaternary N-hydroxymethyl derivative of cyproheptadine. Although this type of metabolite seems not to have been described heretofore, the glucuronide conjugate of a hydroxymethyl tertiary amine was reported to be formed in rats (15), and a sugar conjugate of a similar type of compound has been found in plants (16). The base compound might undergo N-methylation, followed by methyl oxidation and then conjugation, or it might undergo methyl oxidation, followed by conjugation and then N-methylation. The first mentioned route seems improbable, since neither quaternization of amines nor methyl oxidation (demethylation) of quaternary compounds is recognized to occur appreciably in animals. The second route requires initial methyl oxidation which, as a step toward demethylation, is plausible. If the hydroxymethyl group formed a glucuro-

nide, this conjugate upon hydrolysis no doubt would yield desmethylcyproheptadine, a compound which was not found in any urinary fraction, although the phenolic derivative of the desmethyl compound was identified. Further, this route also would require N-methylation to yield a quaternary compound. Therefore, based on these considerations and the available data, characterization of the metabolite as a conjugate of cyproheptadine itself seems most reasonable at this time.

The other metabolic processes apparent from the data, namely, aromatic hydroxylation followed by glucuronide formation, N-demethylation, and heterocyclic ring oxidation (17, 18), are well recognized pathways and need no further discussion. Scission of the ring would be expected by analogy with other heterocyclic compounds (19). It is possible that compounds resulting from this type of metabolism were present in the unresolved urine fractions.

In confirmation of the recent report by Hintze *et al.* (20), no evidence was found in the present investigation that 10,11-epoxidation is a significant metabolic route for cyproheptadine in man. Although relative ion intensities in the mass spectrum of the epoxide reported by Frigerio *et al.* (7), by Hucker *et al.* (8), and in this paper are different, probably because of different instrumentation and ionizing potentials used in the three laboratories, the M − 29 ion was present consistently. This ion did not appear in the spectrum of any urine fraction.

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