In Vitro. Rat brains (6 mg) were homogenized in distilled water in a Sorvall blender at maximum speed for 2 min, filtered through four layers of cheese cloth, and centrifuged at 100,000g for 1 hr. The supernatant, containing 23% of the total activity (specific activity 600 nmol/mg protein/hr), was used for assaying the reactivators. Bovine erythrocyte AChE (Sigma) was prepared by dissolving the enzyme powder into histidine buffer (10 m*M*) adjusted to pH 7.4 to an activity of 0.1–0.2 μmol/min.

A standard assay was followed using 3.75 m*M* acetylthiocholine (Eastman recrystallized twice from ethanol) and 2.5 m*M* 5,5'-dithiobis(2-nitrobenzoic acid) in sodium phosphate buffer (0.1 *M*) at pH 8.[21] The enzyme was inhibited with 1 μ*M* paraoxon and excess inhibitor was removed by passing the enzyme mixture through a 1.2 by 12 cm Bio-gel column equilibrated in 10 m*M* histidine buffer (pH 7.4). The potential reactivator (1 m*M* in ethanol) or ethanol alone as control was then added to the inhibited enzyme and recovery monitored at room temperature.

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10,11-Dihydro-10,11-dihydroxy-5*H*-dibenz[*b,f*]azepine-5-carboxamide, a Metabolite of Carbamazepine Isolated from Human and Rat Urine

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The synthesis of 10,11-dihydro-10,11-dihydroxy-5*H*-dibenz[*b,f*]azepine-5-carboxamide from carbamazepine (Tegretol) is described and this is shown to be one of the metabolites of carbamazepine. The extraction procedure is described fully and further details about the metabolic pathway are discussed, in which carbamazepine epoxide is proposed as an intermediate. The synthesis of another possible metabolite, 10,11-dihydro-10-hydroxy-5*H*-dibenz[*b,f*]azepine-5-carboxamide, from carbamazepine epoxide is also described, although this has not been found as a metabolite in urine. The decomposition of 10,11-dihydroxy-10,11-dihydro-5*H*-dibenz[*b,f*]azepine-5-carboxaldehyde to acridine-9-carboxaldehyde under glc conditions is also discussed.

To date the metabolic fate of carbamazepine (Tegretol) (**1**) in humans and the experimental animal has not been completely determined. In previous reports we have described the pharmacokinetics of the drug in animals and humans[1–4] and the isolation and chemical and physical characterization of another metabolite, 10,11-dihydro-5*H*-dibenz[*b,f*]azepine-5-carboxamide 10,11-epoxide (**2**).[3–6] There was also evidence to suggest the presence of two hydroxylated derivatives in human urine following carbamazepine administration.[1–4] We report here the syntheses of 10,11-dihydro-10,11-dihydroxy-5*H*-dibenz[*b,f*]azepine-5-carboxamide (**3**) and 10,11-dihydro-10-hydroxy-5*H*-dibenz[*b,f*]azepine-5-carboxamide (**4**) and the isolation of one of these as a metabolite from rat and human urine. The mass spectrometric characterization of the dihydroxy compound **3** as well as the gas chromatographic behavior will be mentioned. The finding of metabolite **3** has also been very recently reported by other workers.[7,‡]

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Experimental Section

Melting points were determined with a Büchi capillary apparatus and are uncorrected. Ir spectra were obtained on a Perkin-Elmer 157 spectrophotometer as Nujol mulls and nmr spectra with a Varian A-60 spectrometer using tetramethylsilane as internal reference and DMSO-d_6 as solvent. Mass spectra were obtained on an LKB 9000 instrument operating under the previously described conditions.[3] Thin-layer chromatography was carried out on silica gel plates (Woelm precoated F256/366). Gas chromatography was carried out on a Carlo Erba Fractovap G1 chromatograph using a 2-m, 3% OV-17 or SE-30 column operating at 220–250° with an injection port temperature of 270°. Radiochromatograms were scanned on a Packard Radiochromato-Scanner Model 7201 and radioactive determinations were made on a Nuclear Chicago Isocap 300 liquid scintillation system. Samples were counted in 15 ml of a scintillation solution of butyl PBD (7 g) in toluene–ethylene glycol monomethyl ether (2:1) (1 l.). Counts were corrected by the external standard procedure with a counting efficiency of 80–89%. Cold and labeled **10,11-[$^{14}C_2$]-**carbamazepines were generously supplied by Ciba-Geigy, Basel.

10,11-Dihydro-10,11-dihydroxy-5*H*-dibenz[*b,f*]azepine-5-carboxamide (3). Carbamazepine (**1**)[8] (2.36 g, 10 mmol) in pyridine (1 ml) was added to a solution of OsO_4 (2.53 g, 10 mmol) in benzene (260 ml). The reaction mixture was stirred at room temperature for 2 days and the resulting dark precipitate collected and dried. Hydrolysis with a solution of *d*-mannitol (2.6 g) and NaOH (7 g) in water (50 ml) afforded a pale yellow solid which was washed with water and dried. Crystallization from EtOH gave **3** (1.5 g, 56%): mp 242–

245° dec (lit.[7] mp 191–193°); ν_{max} 3500–3300 (OH, NH_2), 1660 (C=O), 770 and 755 cm^{-1} (aromatic CH); nmr δ 4.98, 5.07 (2 s, 2, C_{10} and C_{11} protons), 5.34, 5.43 (2 s, 2, hydroxyl protons), 5.7 (broad, 2, amide protons), 7.32 (m, 8, aromatic protons).

10,11-Dihydro-10-hydroxy-5*H*-dibenz[*b,f*]azepine-5-carboxamide (4). A solution of 10,11-dihydro-5*H*-dibenz[*b,f*]azepine-5-carboxamide 10,11-epoxide (2)[6] (13 g, 52 mmol) in EtOH (1 l.) was reduced with hydrogen at room temperature and atmospheric pressure using 10% Pd on charcoal (4 g) as catalyst. After uptake of the theoretical amount of H_2, the catalyst was filtered off and the solvent was evaporated *in vacuo*. Crystallization from EtOAc–EtOH gave 4 (11.2 g, 72.5%): mp 188–192° (lit.[9] mp 195–196°); ν_{max} 3400 (OH, NH_2), 1650 (C=O), 775 and 760 cm^{-1} (aromatic CH); nmr δ 2.87 (q, 1, C_{11} axial proton, $J_{10(ax)-11(ax)}$ = 10 cps, J_{gem} = −15.5 cps), 3.35 (q, 1, C_{11} equatorial proton, $J_{10(ax)-11(eq)}$ = 5 cps), 5.05 (m, 1, C_{10} axial proton), 5.7 (1, hydroxyl proton), 5.7 (broad, 2, amide protons), 7.24 (m, 8, aromatic protons).

Animal Studies. Two male Sprague–Dawley rats (body wt 200 g) were injected intraperitoneally with a propylene glycol solution (0.5 ml) of 10,11-[$^{14}C_2$]carbamazepine diluted with cold carrier. The dose injected was 10 mg/kg with a specific activity of 0.56 μCi/mg. Animals were housed in plastic metabolic cages with water but no food. Urine was collected 24 and 48 hr after drug administration and the pooled urine was kept frozen until analyzed. Respiratory CO_2 was entrained in a stream of air passing through ethanolamine-ethylene glycol monomethyl ether (1:2) as previously described.[10] The activity in the total pooled urine accounted for 27% of the administered activity, while the activity found in the expired air accounted for only 0.5% of that administered.

A fraction of the pooled urine (3 ml) was extracted with ethylene dichloride (3 × 5 ml) (extract A) and then with ethyl acetate (3 × 5 ml) (extract B). The aqueous phase was incubated overnight with 2500 units of "ketodase" at pH 5 and then reextracted with ethyl acetate (3 × 5 ml) (extract C). The radioactivities recovered in each of the extracts were (A) 24%, (B) 5%, and (C) 11% of the activity present in the urine. Each extract was concentrated under N_2 at 60° to 0.1–0.2 ml and chromatographed on silica gel plates using a benzene–diethylamine–ethanol (8:1:1) system according to Christensen.§ The ethylene dichloride extract A gave a single radioactive peak, R_f 0.34, corresponding to that of authentic carbamazepine 10,11-epoxide (2) (Table I). The ethyl acetate extract B gave three radioactive peaks: an intense one of R_f 0.12 corresponding to authentic 10,11-dihydro-10,11-dihydroxy-5*H*-dibenz[*b,f*]azepine-5-carboxamide (3) and two smaller ones with R_f's 0.29 and 0.34 corresponding to 10,11-dihydro-10-hydroxy-5*H*-dibenz[*b,f*]azepine-5-carboxamide (4) and carbamazepine 10,11-epoxide (Table I). The extract C gave a single radioactive peak with R_f 0.12.

Human Studies. Carbamazepine (400 mg) was administered at 8 a.m. to two healthy volunteers fasted since the previous night, and urine was collected for the following 24 hr. Urine (5 ml) was extracted with ethylene dichloride (3 × 5 ml) (extract A') and subsequently with ethyl acetate (3 × 5 ml) (extract B'). The A' and B' extracts were concentrated and chromatographed as before. The ethylene dichloride extract (A') gave uv-quenching spots at R_f 0.43 and 0.34 corresponding to authentic carbamazepine and carbamazepine 10,11-epoxide. The ethyl acetate extract (B') gave spots with R_f's 0.34, 0.29, and 0.12.

The regions corresponding to the R_f's of the 10-hydroxy and 10,11-dihydroxy derivatives were scraped and the material was eluted from the silica gel with ethyl acetate. The eluates were evaporated to dryness under N_2 and subjected to mass spectrometry using the direct injection technique, the material coming off the probe at 100°.

Results and Discussion

Carbamazepine was administered to rats and to humans using the procedure described in the Experimental Section. The metabolite profile of the samples obtained from both sources appeared to be the same except that the rat urine contained no unchanged carbamazepine.

Treatment of the urine with ethylene dichloride extracted most of the epoxide metabolite 2. Extraction with ethyl acetate then provided a sample which contained both the epoxide and some more polar components on thin-layer chromatography. Incubation of the rat urine sample with β-glucuronidase and further extraction with ethyl acetate provided more of the polar material. Comparison of the chromatographic properties of the polar material with a synthetic sample of 10,11-dihydro-10,11-dihydroxy-5*H*-dibenz[*b,f*]azepine-5-carboxamide (3) showed that 3 was present. Chromatographic data from rat urine showed that 10,11-dihydro-10-hydroxy-5*H*-dibenz[*b,f*]azepine-5-carboxamide (4) may also have been present. The ethylene dichloride and ethyl acetate extracts from human urine were separated into their components by tlc, the concentration of the material in the rat urine being too low for adequate investigation. The extract A' (ethylene dichloride) contained unchanged carbamazepine and the previously identified epoxide.[3] The regions corresponding to the compounds 3 and 4 (Table I) of extract B' were scraped, and the material was washed from the silica gel. The washings were evaporated and subjected to mass spectrometry.

The material with the same R_f as 3, by direct injection into the mass spectrometer, gave a mass spectrum with a molecular ion at *m/e* 270, fragment ions at *m/e* 253, 252 (M − OH, M − H_2O), and a base peak at *m/e* 180 with the proposed structure 3. Comparison of the mass spectrum with that of authentic 10,11-dihydro-10,11-dihydroxy-5*H*-dibenz[*b,f*]azepine-5-carboxamide (3) showed that they were identical.# Synthesis of the 10,11-dihydroxycarbamazepine derivative 3 using the osmium tetroxide method would give the *cis*-dihydroxy derivative, and since the material isolated from the urine had the same R_f on tlc and the same mass spectrum, the metabolite probably also has the cis configuration for the hydroxyl groups. The compound 3 is unstable and decomposes at the melting point and the melting point of the synthetic material is higher than that of the material isolated from the urine[7] (242–245° *vs.* 191–193°).

Investigation of the spot with the same R_f as 10,11-dihydro-10-hydroxy-5*H*-dibenz[*b,f*]azepine-5-carboxamide (4) by mass spectrometry showed that none of the compound was present in the extract. A possible metabolic

Table I. Thin-Layer Chromatography of Processed Urine of Rats and Humans after Carbamazepine Administration

R_f values of radioactive or uv-quenching zones developed in benzene–ethanol–diethylamine (8:1:1)				
Rats			Humans	
Extract A	Extract B	Extract C	Extract A'	Extract B'
				0.80[e]
			0.43[a]	
0.34[b]	0.34[b]		0.34[b]	0.34[b]
	0.29[c]			0.29[c]
	0.12[d]	0.12[d]		0.12[d]
			0.08[e]	0.08[e]
				0.04[e]

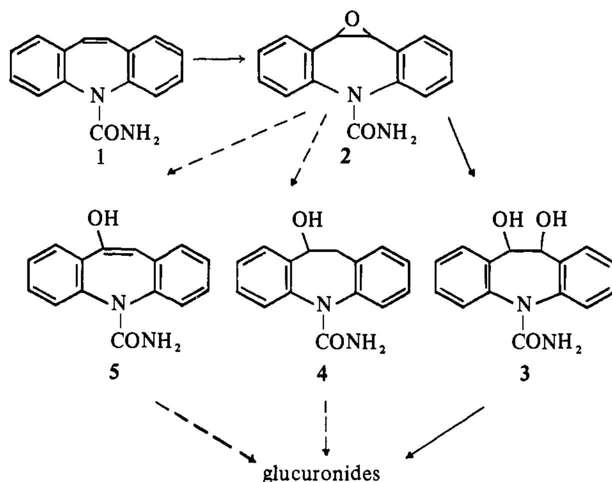
[a]Uv-quenching zone corresponding in R_f to carbamazepine (1). [b]Uv-quenching or radioactive zone corresponding in R_f to carbamazepine 10,11-epoxide (2). [c]Uv-quenching or radioactive zone corresponding in R_f to 10,11-dihydro-10-hydroxy-5*H*-dibenz[*b,f*]-azepine-5-carboxamide (4). [d]Uv-quenching or radioactive zone corresponding in R_f to 10,11-dihydro-10,11-dihydroxy-5*H*-dibenz-[*b,f*]azepine-5-carboxamide (3). [e]Uv-quenching zones also present in control urine; uv light of 254 nm.

§J. Christensen, personal communication.

#Supplementary material (the mass spectrum of 10,11-dihydro-10,11-dihydroxy-5*H*-dibenz[*b,f*]azepine-5-carboxamide) will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth Street, N.W., Washington D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JMED-73-703.

rationale for the formation of the 10,11-dihydroxy derivative **3** involving the epoxide **2** as an intermediate is shown in Scheme I. The material **5** is included in the scheme as another possible metabolite which could arise through the epoxide **2**.

Scheme I. Metabolic Pathway for Carbamazepine (1)[a]



[a]——→, known pathways; - - - →, possible pathways.

In attempting the gas-chromatographic analysis of the 10,11-dihydroxy metabolite **3** or authentic **3**, it was noticed that a relatively nonpolar material was eluted from the column. This occurred only on an OV-17 column and not on an SE-30 stationary phase. This material was collected by preparative glc and by direct injection mass spectrometry shown to be acridine-9-carboxaldehyde by comparison with authentic material.[6] Thus, the compound **3** had undergone a pinacol-type rearrangement under the acidic conditions of the column in a similar manner to the rearrangement of carbamazepine 10,11-epoxide (**2**) under the same conditions.[6] The present results confirm the previous hypotheses on the metabolism of carbamazepine and are in agreement with the intermediacy of the epoxide and the recent reports of other authors.

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Notes

Nitrohistidines and Nitrohistamines†

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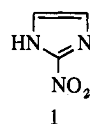
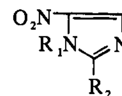
The efficacy of the antibiotic 2-nitroimidazole[1] (**1**) and the 5-nitroimidazole metronidazole (**2a**) in the treatment of trichomoniasis[2] and amebiasis[3] led to the development of dimetridazole[4] (**2b**) and ipronidazole[5] (**2c**), both potent histomonostats.[6] While interest in nitroimidazoles as antiprotozoal agents is currently evident,[7] it is noteworthy that the nitro derivatives of the essential amino acid histidine and its congener histamine, both containing the imidazole moiety, have thus far been overlooked. We therefore now report the synthesis and preliminary biological evaluation of a number of nitrohistidines and nitrohistamines.





2a, $R_1 = CH_2CH_2OH$; $R_2 = CH_3$
b, $R_1 = R_2 = CH_3$
c, $R_1 = CH_3$; $R_2 = CH(CH_3)_2$

The synthesis of 4(5)-nitro-L-histidine‡ (**6**) (Scheme I) was initially accomplished by converting L-histidine (**3**) into the *N*-acetyl intermediate **4** followed by nitration to yield the nitroamide **5** which was then hydrolyzed with acid. Subsequently, it was found that **3** could be directly nitrated to afford the desired nitro derivative **6** in 54% yield. As an extension of this simplification (Table I), D-histidine, as well as appropriately methyl-substituted L-histidines, was transformed by direct nitration to give **7**, the 1-methyl-4-nitro derivative **8**, the 1-methyl-5-nitro isomer **9**, and *N,N*-dimethyl-4(5)-nitro-L-histidine (**10**). In a similar manner 4(5)-nitrohistamine (**11**) and its *N,N*-dimethyl derivative **12** were also obtained while 1-methyl-5-

†A preliminary report of this work was presented by one of us (W. T.) at the Medicinal Chemistry Division of the 163rd National Meeting of the American Chemical Society, Boston, Mass., April 9-14, 1972.

‡The synthesis of racemic 4(5)-nitrohistidine was recently reported.[8]