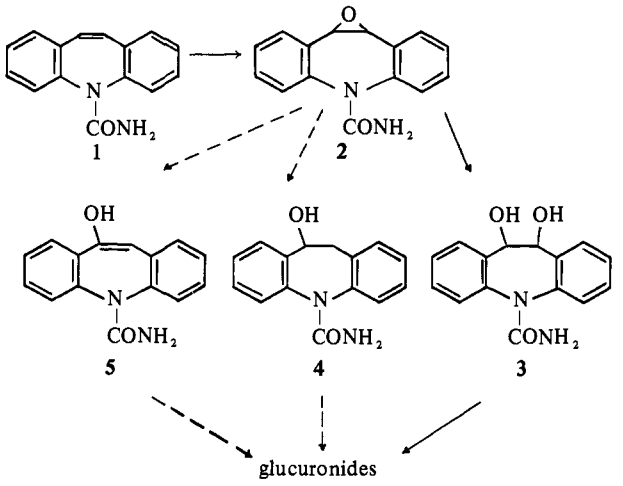
rationale for the formation of the 10,11-dihydroxy derivative **3** involving the epoxide **2** as an intermediate is shown in Scheme I. The material **5** is included in the scheme as another possible metabolite which could arise through the epoxide **2**.

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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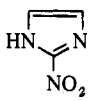
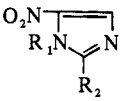
The efficacy of the antibiotic 2-nitroimidazole[1] (**1**) and the 5-nitroimidazole metronidazole (**2a**) in the treatment of trichomoniasis[2] and amebiasis[3] led to the development of dimetridazole[4] (**2b**) and ipronidazole[5] (**2c**), both potent histomonostats.[6] While interest in nitroimidazoles 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.



1



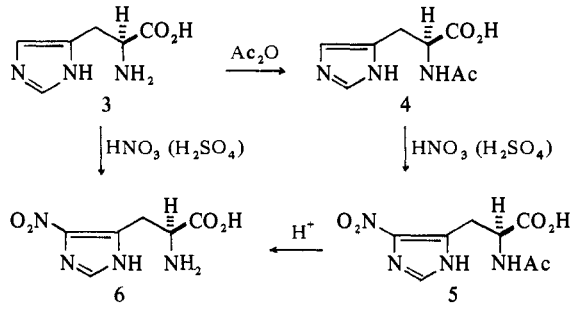
2a, $R_1 = CH_2CH_2OH$; $R_2 = CH_3$
b, $R_1 = R_2 = CH_3$
c, $R_1 = CH_3$; $R_2 = CH(CH_3)_2$

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-

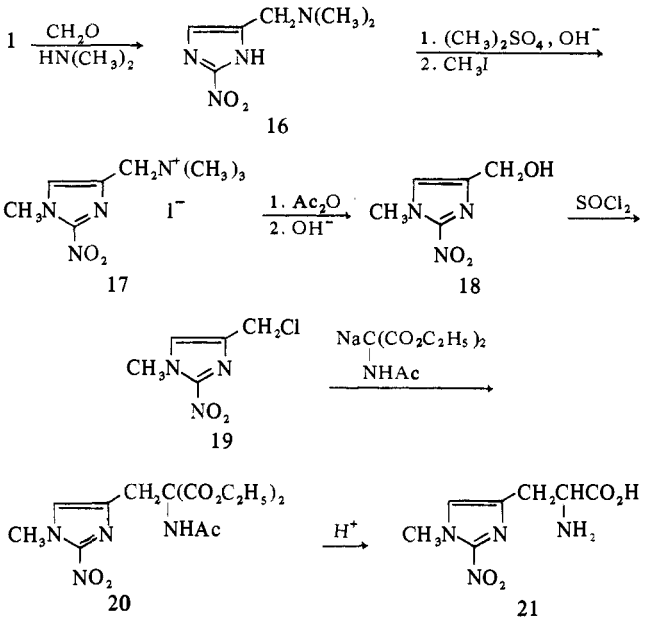
†A preliminary report of this work was presented by one of us (W. T.) at the Medicinal Chemistry Division of the 163rd National Meeting of the American Chemical Society, Boston, Mass., April 9–14, 1972.

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

Scheme I



Scheme II



nitrohistamine (**15**) was prepared by treatment of the dichloroacetamide **13**, obtained from **11** and dichloroacetic anhydride, with Me_2SO_4 in toluene followed by acid hydrolysis.

In contrast, the 2-nitrohistidine **21** was synthesized by the following sequences (Scheme II) since imidazoles cannot be directly nitrated in the 2 position except under very special conditions.[9] Treatment of 2-nitroimidazole (**1**) with CH_2O and Me_2NH_2 gave the Mannich base **16** which was successively treated with Me_2SO_4 and MeI to give the quaternary salt **17** followed by acetylation and alkaline hydrolysis to provide the hydroxymethyl derivative **18**. Reaction of **18** with $SOCl_2$ and treatment of the resulting chloride **19** with the sodium salt of ethyl acetamidomalonate yielded **20** which was then hydrolyzed and decarboxylated to afford the (±)-2-nitrohistidine **21**.

Biological Results. The 5-nitroimidazole, metronidazole **2a**, has been generally accepted as a highly active trichomonacide and amebicide in man.[2,3] High activity against these protozoal infections in experimental animals has also been reported for the antibiotic azomycin which is 2-nitroimidazole.[1] Therefore, it was of interest to determine the *in vivo* antiprotozoal activity of the nitro-substituted histidines **6–9** and of 5-nitrohistamine **11**. In addition, the *in vivo* antibacterial activity of the compounds was investigated. The techniques employed have been described in detail by Grunberg, *et al.*[10,11]

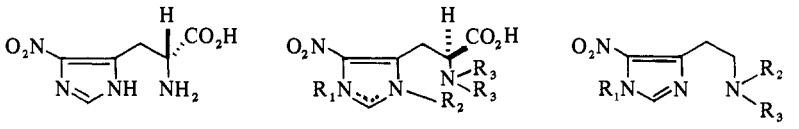
The compounds were administered to mice infected subcutaneously with *Trichomonas vaginalis* or intraperitoneally with *Trichomonas foetus* and to rats infected intracecally with *Entamoeba histolytica*. The four compounds were inactive at a dose of 1000 mg/ml when infiltrated subcutaneously into the site of the *T. vaginalis* infection. When administered orally, compounds **6–9** at a dose of 100 mg/kg and compound **11** at doses of 250 and 200 mg/kg were without activity against *T. vaginalis* and *T. foetus*, respectively. Compounds **6** and **7** were also inactive against *E. histolytica* at doses of 75 and 120 mg/kg, respectively.

Compound **6** was without activity against systemic infections in mice with *Staphylococcus aureus*, *Streptococcus pyogenes*, *Proteus vulgaris*, *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa* when administered orally at a dose of 100 mg/kg. Compounds **7–9** were inactive against *S. aureus* and *P. vulgaris* when administered subcutaneously at a dose of 200 mg/kg. In addition, none of the histamine derivatives **11–15** exhibited any noteworthy blood pressure lowering effects in dogs at a dose of 10 mg/kg iv.

Experimental Section

L-1-Acetamido-2-[4(5)-nitro-5(4)-imidazolyl]propionic Acid (5). To a mixture of 17.8 g (0.083 mol) of *N*-acetyl-L-histidine[12] in 37 ml of concentrated H_2SO_4 was added 20 ml (0.286 mol) of 90% HNO_3 at a rate to maintain the temperature at 40–45°. The resulting solution was stirred at 40° for 2 hr and poured onto 250 g of ice

Table I. 4- and 5-Nitrohistidines and 5-Nitrohistamines



7 8–10 11–15

No.	R_1	R_2	R_3	Yield, %	Crystn solvent[a]	Mp, °C	$[\alpha]D$,[b] deg	Formula[c]
7				25	A	198–199	−25.6	$C_6H_8N_4O_4 \cdot H_2O$[d]
8[e]		CH_3	H	28	B-A	254–255	+40.5	$C_7H_{10}N_4O_4$
9[e]	CH_3		H	35	B-A	205–206	+28.8	$C_7H_{10}N_4O_4$
10[f]	H	H	CH_3	28	B-A	174–175	+115.6	$C_6H_{12}N_4O_4$
11	H	H	H	77	B-A	271–272		$C_5H_8N_4O_2 \cdot HCl$
12[g]	H	CH_3	CH_3	90	B-A	207–209		$C_7H_{12}N_4O_2 \cdot HCl$[h]
13	H	H	$COCHCl_2$	71	B	204–206		$C_7H_8Cl_2N_4O_3 \cdot HCl$[h]
14	CH_3	H	$COCHCl_2$	68	C	120–122		$C_8H_{10}Cl_2N_4O_4$[h]
15	CH_3	H	H	88	D	196–198		$C_6H_{10}N_4O_2 \cdot 2HCl$[h]

[a] Crystallization solvents: A = water; B = ethanol; C = ethyl acetate; D = methanol. [b] Determined as a 1% solution in 5 *N* HCl at 25°. [c] All compounds were analyzed for C, H, and N. [d] Water was determined by the Karl Fisher reagent. [e] 1-Methyl-L-histidine and 3-methyl-L-histidine were purchased from Pfaltz and Bauer, Inc. [f] For the preparation of α-*N*,*N*-dimethyl-L-histidine hydrochloride, see Y. N. Rheinhold, Y. Ishikawa, and D. B. Melville, *J. Med. Chem.*, 11, 258 (1968). [g] For the preparation of 4(5)-(2-dimethylaminoethyl)imidazole, see B. Garforth and F. L. Pyman, *J. Chem. Soc.*, 489 (1935). [h] Also analyzed for Cl.

and the mixture adjusted to pH 2 with Na_2CO_3. The solids were collected and crystallized from H_2O to give 10.8 g (54%) of **5**, dec 236–237°, $[\alpha]^{25}D$ −35.9° (*c* 1, 5 *N* HCl). *Anal.* ($C_8H_{10}N_4O_5$) C, H, N.

L-α-Amino-β-[4(5)-nitro-4(5)-imidazolyl]propionic Acid Monohydrate (6). A mixture of 21.5 g (0.089 mol) of **5** in 200 ml of 2 *N* HCl was refluxed for 2 hr, cooled, and adjusted to pH 5 with dilute NaOH. The resulting crystals were collected to give 17.5 g (90%) of **6**, dec 197–198°, $[\alpha]^{25}D$ +26.8° (*c* 1, 5 *N* HCl). *Anal.* ($C_6H_5N_4O_4 \cdot H_2O$) C, H, N. Alternatively, 10 g (0.032 mol) of L-histidine (**3**) was nitrated by the procedure given for the preparation of 5 to afford 36% of **6**.

4(5)-(2-Dichloroacetamidoethyl)-5(4)-nitroimidazole (13). To a mixture of 19.3 g (0.1 mol) of 11 in 200 ml of dry C_5H_5N at 5°, a solution of 36.0 g (0.15 mol) of $(Cl_2CHCO)_2O$ in 25 ml of C_6H_6 was added dropwise over a period of 0.5 hr. After stirring at 5° for 0.5 hr and storage at room temperature for 5 hr, the mixture was evaporated under reduced pressure. To the residual oil 200 ml of H_2O was added; the mixture was adjusted to pH 1 and refrigerated. The resulting solids were collected and crystallized from EtOH to give 19.0 g (71%) of **13**, mp 204–206°. *Anal.* ($C_7H_8Cl_2N_4O_3$) C, H, N, Cl.

1-Methyl-4-(2-dichloroacetamidoethyl)-5-nitroimidazole (14). To a suspension of 37.3 g (0.14 mol) of **13** in 400 ml of refluxing toluene, 18 ml (0.19 mol) of $(CH_3)_2SO_4$ was added dropwise over a period of 0.5 hr. After refluxing an additional 2 hr, the reaction mixture was evaporated under reduced pressure. The residual oil was dissolved in 200 ml of H_2O, cooled, and adjusted to pH 9.5. After refrigeration, the solids were collected and crystallized from EtOAc to give 26.5 g (68%) of **14**, mp 120–122°. *Anal.* ($C_8H_{10}Cl_2N_4O_3$) C, H, N, Cl.

1-Methyl-4-(2-aminoethyl)-5-nitroimidazole Dihydrochloride (15). A mixture of 19.2 g (0.072 mol) of **14** and 150 ml of 2 *N* HCl was refluxed for 2 hr and then evaporated under reduced pressure. The residual solids were slurried with acetone and filtered to give 15.4 g of **15**, mp 196–198°. *Anal.* ($C_6H_{10}N_4O_2 \cdot 2HCl$) C, H, N, Cl.

2-Nitro-4(5)-dimethylaminomethylimidazole (16). A mixture of 61.2 g (0.54 mol) of 2-nitroimidazole[13] (**1**), 59 ml of 37% CH_2O, and 104 ml of 25% $(CH_3)_2NH$ in 3.5 l. of EtOH was refluxed for 24 hr and evaporated under reduced pressure. The residual oil was dissolved in 100 ml of H_2O and acidified to pH 2, the insolubles were filtered, the filtrate was adjusted to pH 7.4, and the resulting crystals were collected to give 35.3 g (38%) of **16**, dec 187–189°. *Anal.* ($C_6H_{10}N_4O_2$) C, H, N.

1-Methyl-2-nitro-4-trimethylaminoethylimidazole Iodide (17). To a solution of 70.2 g (0.412 mol) of **16** in 455 ml of 1 *N* NaOH was added 43 ml (0.455 mol) of $(CH_3)_2SO_4$. The reaction mixture was stored at room temperature for 2 hr and then evaporated under reduced pressure. The residual oil was dissolved in 400 ml of MeOH, 75 ml of MeI added, and the mixture refrigerated overnight and filtered. The crystals were recrystallized from MeOH to give 72.1 g of **17**, dec 200–201°. *Anal.* ($C_8H_{15}IN_4O_2$) C, H, N.

4-Hydroxymethyl-1-methyl-2-nitroimidazole (18). A mixture of 72.1 g (0.221 mol) of **17**, 650 ml of Ac_2O, and 72 ml of AcOH was refluxed for 6 hr and evaporated under reduced pressure. To the residual oil was added 100 ml of EtOH, the mixture stored at 4° overnight, and 33.8 g of recovered **17** collected. The filtrate was evaporated; the residue was dissolved in 175 ml of 1 *N* NaOH and extracted with give 1-l. portions of EtOAc. The combined extracts were evaporated and the residue was triturated with Et_2O to give 4 g of solids which were crystallized from 40 ml of EtOH to yield 3.3 g (9.3%) of **18**, mp 150–151°. *Anal.* ($C_5H_7N_3O_3$) C, H, N.

4-Chloromethyl-1-methyl-2-nitroimidazole (19). To a solution of 2.73 g (0.017 mol) of **18** in 1.5 ml of C_5H_5N at 5° was slowly added 1.6 ml (0.023 mol) of $SOCl_2$. After storage at 5° for 30 min and at 45–55° for 2 hr, a mixture of 100 ml of EtOAc and 25 ml of 1 *N* HCl was added. The organic layer was separated, washed with aqueous Na_2CO_3, and evaporated, and the residue was crystallized from Et_2O to give 2.32 g (76%) of **19**, mp 61–63°. *Anal.* ($C_5H_6ClN_3O_2$) C, H, N, Cl.

Ethyl α-Acetamido-α-carbethoxy-β-(1-methyl-2-nitro-4-imidazolyl)propionate (20). A mixture of 2.32 g (0.013 mol) of **19** and 3.74 g (0.017 mol) of diethyl acetamidomalonate in 50 ml of 0.32 *N* NaOEt in EtOH was refluxed for 1 hr and evaporated. The solids were distributed between 100 ml of EtOAc and 40 ml of 0.1 *N* NaOH. The organic layer was separated, washed with 0.1 *N* NaOH, and evaporated. The residue was crystallized from EtOH to give 2.6 g (55%) of **20**, mp 163–165°. *Anal.* ($C_{14}H_{20}N_4O_7$) C, H, N.

α-Amino-β-(1-methyl-2-nitro-4-imidazolyl)propionic Acid Monohydrate (21). A solution of 8.2 g (0.023 mol) of **20** in 37 ml of 2 *N* H_2SO_4 was refluxed for 13 hr and cooled, 7.1 g (0.0375 mol) of $Ba(OH)_2 \cdot H_2O$ was added, and the solution was filtered. The reaction product in the filtrate was isolated by ion-exchange chromatography and crystallized from EtOH–H_2O to give 2.53 g (47%) of **21**, dec 150°. *Anal.* ($C_7H_{10}N_4O_4 \cdot H_2O$) C, H, N.

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Spin-Labeled Analogs of Local Anesthetics

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Various biological systems have been studied through the use of spin labels.[1-3] Our interest in the functional regions of neural membranes[4,5] has prompted us to investigate their interaction with the spin-labeled analogs of local anesthetics. The incorporation of nitroxide radicals into other drugs such as sulfonamides, barbiturates, choline esters,[6] and morphine[7] has been recently reported.

In this report we describe the synthesis and pharmacology of a series of 2-[*N*-methyl-*N*-(2,2,6,6-tetramethylpiperidinooxyl)]ethyl 4-alkoxybenzoates (I). These compounds are the spin-labeled analogs of the 2-(*N*,*N*-diethylamino)ethyl 4-alkoxybenzoates whose physical[8] and biological properties[9] have been extensively studied. An interesting feature of this system is that the activity increases as the alkoxy chain is lengthened to six carbons but abruptly diminishes in the higher homologs.

The spin-labeled drugs Ia-c were prepared by acylation of 4-[*N*-(2-hydroxyethyl)-*N*-methylamino]-2,2,6,6-tetramethylpiperidinooxy (IV) with the appropriate 4-alkoxybenzoyl chloride. Synthesis of IV was accomplished by conversion of the primary amide II to the secondary amine III, followed by alkylation with 2-iodoethanol. The esters possess the structural characteristics associated with local anesthetics: a hydrophobic part and hydrophilic part connected by an intermediate chain.[10]

In order to ascertain that the nitroxide moiety is an acceptable perturbation in this series of drugs, they were tested for surface anesthesia in the guinea pig cornea. As seen in Table I the values for Ia-c parallel the data in the literature[9] for the non-spin-labeled analogs. The duration of activity for