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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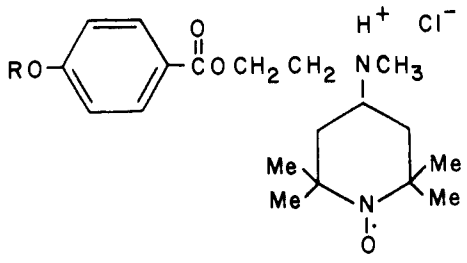
Various biological systems have been studied through the use of spin labels.[1-3] Our interest in the functional regions of neural membranes[4,5] has prompted us to investigate their interaction with the spin-labeled analogs of local anesthetics. The incorporation of nitroxide radicals into other drugs 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]

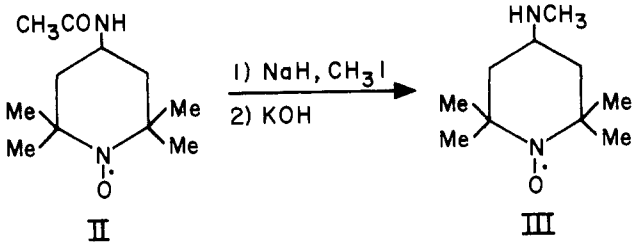
In order to ascertain that the nitroxide moiety is an acceptable perturbation in this series of drugs, they were tested for surface anesthesia in the guinea pig cornea. As seen in Table I the values for Ia-c parallel the data in the literature[9] for the non-spin-labeled analogs. The duration of activity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



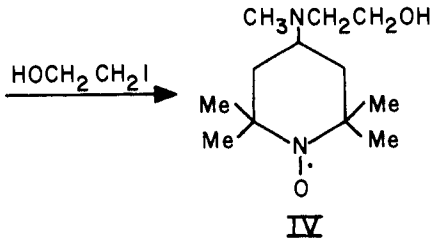
Ia, R = CH_2CH_3

b, R = $(CH_2)_5CH_3$

c, R = $(CH_2)_{11}CH_3$



II III



IV

the hexyloxy compound Ib is greater than for Ia. However, no activity was observed for the dodecyloxy homolog Ic even after doubling the concentration. Comparison of Ia and Ib with tetracaine shows that they are potent anesthetics.

Having demonstrated that the spin labels retain the anesthetic properties of the unmodified drugs, we are currently conducting experiments to discover their site of action in neural membranes and to study the effect of lengthening the alkyl group on the mobility of the probe in the membrane. It is hoped that such studies will lead to a better understanding of the mechanism of local anesthetic action.

Experimental Section

Melting points were determined on a Fischer-Johns hot stage apparatus and are uncorrected. Analytical data were furnished by the Micro Chemical Laboratory at the University of California at Berkeley and are within ±0.4% of theoretical values except where indicated. Mass spectra were measured on a Hitachi Perkin-Elmer RMU6E instrument.

4-Acetamido-2,2,6,6-tetramethylpiperidinooxy (II) was prepared from 4-amino-2,2,6,6-tetramethylpiperidine (Aldrich) by a method described in the literature.[11]

4-(*N*-Methylamino)-2,2,6,6-tetramethylpiperidinooxy (III). To a solution of II (6.5 g, 0.03 mol) in dry DMF (80 ml) was added 2.8 g (50% dispersion in mineral oil, 0.06 mol) of NaH. After stirring 2 hr at 60°, CH_3I (8.4 g, 0.06 mol) was added dropwise at ambient temperature and the mixture let stand overnight. The excess NaH was destroyed with water and the DMF evaporated. The residue was dissolved in CH_2Cl_2 and the NaI filtered. Removal of solvent gave 4-(*N*-methylacetamido)-2,2,6-6-tetramethylpiperidinooxy as an orange solid.

The crude amide was suspended in 10% aqueous KOH (85 ml) and refluxed for 48 hr. After saturating with K_2CO_3, the reaction mixture was extracted with ether and dried (K_2CO_3). Concentration and distillation of the residue gave 3.5 g of a red liquid (III): bp 71–74° (0.2 mm); 63% from II; M^+ −185; tlc (silica gel) showed only one spot.

4-[*N*-(2-Hydroxyethyl)-*N*-methylamino]-2,2,6,6-tetramethylpiperidinooxy (IV). A solution of III (2.5 g, 0.0135 mol), 2-iodoethanol[12] (2.3 g, 0.0135 mol), and diisopropylethylamine (1.7 g, 0.0135 mol) was stirred at ambient temperature for 48 hr. The mixture was diluted with EtOAc (30 ml) and filtered. The solvent was concentrated and the residue eluted from a basic Al_2O_3 column with EtO_2–CH_3OH (15:1). The product was contaminated with a small amount of III which was removed by distillation. The residue (IV) was a red viscous oil (1.7 g): 55% yield; M^+ −230; tlc (silica gel) showed only one spot. The methiodide salt precipitated from a solution of CH_3I and IV in CH_2Cl_2 and was filtered to give orange crystals, mp 220–222°. *Anal.* ($C_{13}H_{28}N_2O_2I$) C, H, N.

2-[*N*-Methyl-*N*-(2,2,6,6-tetramethylpiperidinooxy)]ethyl 4-Alkoxybenzoates·HCl (Ia–c). The following general procedure was used for the synthesis of I. Equimolar amounts of the acid chloride, IV, and diisopropylethylamine in $CHCl_3$ were stirred at ambient temperature for 24 hr. After washing with H_2O, the mixture was concentrated and eluted from a basic Al_2O_3 column with CH_2Cl_2 to yield the ester as a red viscous oil. The hydrochlorides were prepared by dissolving the ester in ice cold EtO_2–CH_3OH (1:1) and lowering the pH to 5–6 with dilute HCl. After drying ($CaSO_4$) and concentrating, the residual gum was triturated with ether to give analytically pure I (*ca.* 30%) as a pale yellow solid. Ia had mp 169–171°. *Anal.* ($C_{21}H_{34}N_2O_4Cl$) C, H, N. Ib had mp 148–150°. *Anal.* ($C_{25}H_{42}N_2O_4Cl$) C, H, N. Ic had mp 111–113°. *Anal.* ($C_{31}H_{54}N_2O_4Cl$) C, H, N.

Pharmacological Method. The duration of surface anesthesia in the guinea pig cornea was determined by a method reported in the literature,[13] except that the spin labels were allowed to remain in contact with the eye for 3 min.

Table I. Local Anesthetic Activities

Compd	Duration of anesthesia (min) in guinea pig cornea with 20 m*M* solution
Ia	20 ± 5
Ib	105 ± 10
Ic	0
Tetracaine	25 ± 5

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