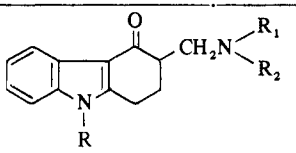
Table II. 3-Disubstituted-2,3-dihydro-4(1*H*)-carbazolones



R	NR_1R_2	Reaction solvent	Yield, %	Recrystn solvent	Mp, °C	Formula	Analyses
H	$N(CH_3)_2$	THF	27	Acetone–hexane	194–196	$C_{15}H_{18}N_2O$	C, H, N
H	Δ^3-Pyrrolino	THF	11	Acetone–hexane	180–183	$C_{17}H_{18}N_2O$	H, N; C[a]
H	Pyrrolidino	THF	9	Acetone–hexane	198–200	$C_{17}H_{20}N_2O$	C, H, N
H	Morpholino	EtOH	23	MeOH	227–229	$C_{17}H_{20}N_2O_2$	C, H, N
H	$N(C_2H_5)_2$	THF	13	Acetone–hexane	143–145	$C_{17}H_{22}N_2O$	C, H, N
H	Piperidino	THF	36	Acetone–hexane	182–184	$C_{18}H_{22}N_2O$	C, H, N
CH_3	Morpholino	EtOH	33	Acetone–hexane	171–174	$C_{18}H_{22}N_2O_2$	C, H, N
CH_3	Piperidino	EtOH	40	Acetone–hexane	136–137	$C_{19}H_{24}N_2O$	C, H, N
H	3-Methylpiperidino	EtOH	16	Acetone–hexane	173–176	$C_{19}H_{24}N_2O$	C, H, N
H	4-Methylpiperidino	EtOH	46	Acetone–hexane	178–181	$C_{19}H_{24}N_2O$	C, H, N
CH_3	4-Methyl-1-piperazinyl	EtOH	35	Acetone–hexane	128–130	$C_{19}H_{25}N_3O$	C, H, N
CH_3	3-Methylpiperidino	EtOH	13	Acetone–hexane	118–121	$C_{20}H_{26}N_2O$	C, H, N
CH_3	4-Methylpiperidino	EtOH	49	Acetone–hexane	146–148	$C_{20}H_{26}N_2O$	C, H, N

[a] C: calcd, 76.66; found, 76.17.

their ability to induce ataxia, to decrease locomotor activity, and to afford protection against electroshock- and strychnine-induced convulsions in mice. In general, the compounds described herein failed to afford protection at an acceptable dose (≤50 mg/kg) against convulsions induced by either method. However, many compounds induced ataxia, as judged by impairment of ability to traverse a suspended rod, and decreased motor activity. The data for the more interesting compounds are summarized in Table I; comparable data for Mannich base **1** are included.

Experimental Section

Melting points were determined in open capillary tubes on a Mel-Temp apparatus and are uncorrected. All products upon which yields are based were homogeneous as judged by tlc using Eastman Chromatogram No. 6060 sheets (silica gel with fluorescent indicator) developed by $CHCl_3$–hexane–EtOH (1:1:1) or acetone–HOAc–MeOH–benzene (5:5:20:100). Where analyses are indicated only by symbols of the elements, analytical results were within ±0.4% of the calculated values.

Mannich Reactions. The following preparation of 3-(dimethylaminomethyl)-2,3-dihydro-4(1*H*)-carbazolone illustrates the general procedure. A soln of 1.00 g (5.5 mmoles) of 2,3-dihydro-4(1*H*)-carbazolone (**3**), 500 mg (6.0 mmoles) of dimethylamine hydrochloride, and 210 mg (6.3 mmoles) of paraformaldehyde in 60 ml of tetrahydrofuran† containing 3 ml of 10% ethanolic HCl was heated at reflux temperature for 16 hr. The solvents were removed under reduced pressure, and the residue was treated with 20% HOAc. Extraction with EtOAc removed the starting ketone. The acid layer was rendered alkaline with NH_4OH to give 350 mg of crystals, mp 195–197° dec. The characterization of this substance and those prepared in a similar manner is given in Table II.

Acknowledgment. Microanalyses were furnished by Mr. L. Brancone and his staff, and the pharmacological tests were done with the technical assistance of Mrs. J. Kurowski and Mrs. F. Milano.

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†Alternatively, EtOH was used in certain preparations, as indicated in Table II.

Synthesis of Some Spin-Labeled Analogs of Drug Molecules

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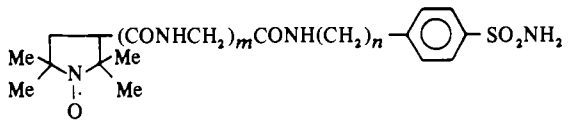
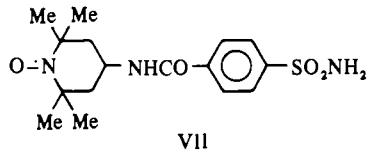
Spin labels are stable free radicals that can be used as reporter groups to study the interaction of drugs and other ligands with biologically important macromolecules such as enzymes, nucleic acids, and membranes.[1-3] The most commonly employed spin label is the nitroxide free radical, since this group is very stable in aqueous systems at physiological pH values. Furthermore, the electron spin resonance of the nitroxide group is exquisitely sensitive to changes in its microenvironment.[1-3] Spin-labeled drugs have recently become important in studies of drug mechanisms at a molecular level. For example, drug analogs containing the nitroxide moiety have been used to study the topography of specific binding sites in receptor macromolecules.[4,5] Spin-labeled drugs have also been used, in conjunction with immunoassay techniques, to detect and assay low concentrations of drugs and their metabolites in biological fluids such as urine, plasma, and saliva.[6,7]

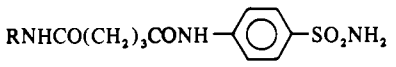
In this report, we describe the synthesis of spin-labeled analogs of three classes of drugs, the sulfonamides, the barbiturates, and the choline esters. The spin-labeled sulfon-

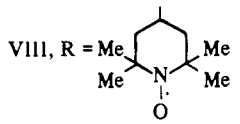


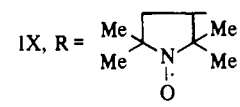
IV, $m = 0; n = 0$
V, $m = 0; n = 1$
VI, $m = 1; n = 0$

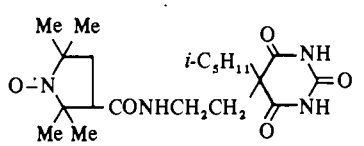


VII

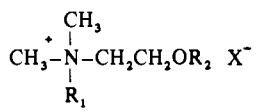


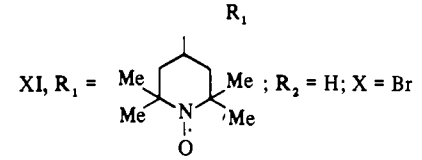


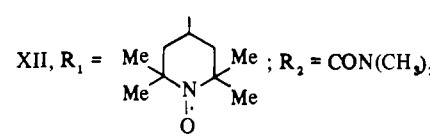




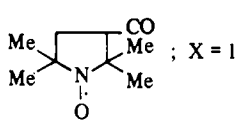
X







XIII, $R_1 = CH_3$; R_2 =



; X = I

amides (IV-IX) have been used to probe the active site of bovine erythrocyte carbonic anhydrase B and human erythrocyte carbonic anhydrase B and C.[4,†] The choline esters (XI-XIII) were synthesized as probes for the active site of acetylcholinesterase. Such analogs may also be of use in the detection of proteins capable of binding acetylcholine during the isolation of the acetylcholine receptor. Finally, the spin-labeled barbituric acid (X) derivative was synthesized to study its interaction with membrane systems in an attempt to ascertain the mode of action of this class of drugs.

The syntheses described below have been selected to show how simple chemical procedures may be used to incorporate the nitroxide group into a drug molecule. The amide bond has been used extensively in these syntheses since this linkage is easily formed and yet is very stable in aqueous systems at physiological pH values.

†C. F. Chignell, D. K. Starkweather, and R. H. Erlich, unpublished work.

Experimental Section

Melting points were obtained with a Kofler micro hot stage apparatus and are uncorrected. All elemental analytical results for the compounds were within ±0.4% of the theoretical values, except where indicated.

3-Carboxy-2,2,5,5-tetramethyl-1-pyrrolidinyloxyl (I) was prepared by refluxing 3-carbamoyl-2,2,5,5-tetramethyl-1-pyrrolidinyloxyl (Frinton Laboratories) with aqueous $Ba(OH)_2$ according to the procedure of Rozantsev and Krinitskaya.[8] 3-Amino-2,2,5,5-tetramethyl-1-pyrrolidinyloxyl (II) was prepared from the same intermediate by the method of Krinitskaya, *et al.*[9] 4-Amino-2,2,6,6-tetramethylpiperidinooxyl (III) was synthesized from 4-acetylamino-2,2,6,6-tetramethylpiperidine (Aldrich) by the procedure of Rozantsev and Kokhanov.[10] The spin-labeled sulfonamides and the barbituric acid derivative were synthesized from one of these intermediates by a condensation reaction that employed either *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (Aldrich) or ethyl (or isobutyl) chloroformate (Eastman). Typical reactions are given below together with details of the somewhat different routes used to synthesize the choline esters.

2,2,5,5-Tetramethyl-3-[(*p*-sulfamoylbenzyl)carbamoyl]-1-pyrrolidinyloxyl (V) (Method 1). Freshly distilled ethyl chloroformate (0.86 ml, 0.009 mole) was added dropwise to a cooled (ice-salt bath) solution of I (1.67 g, 0.009 mole) in dry THF (20 ml). After stirring for 1 hr, triethylamine (2.54 ml, 0.018 mole) was added, followed by a suspension of *p*-aminomethylbenzenesulfonamide hydrochloride (2.0 g; 0.009 mole) in THF (20 ml). The reaction was allowed to warm to room temperature and then stirred for a further 4 hr. After the reaction mixture had been evaporated to dryness, the residue was dissolved in water (10 ml) and then acidified with HCl and extracted with EtOAc (3 × 10 ml). The EtOAc was dried (Na_2SO_4) and evaporated to dryness. The residue (3.0 g) was recrystallized from MeOH to give V as yellow prismatic crystals, mp 202–203°. *Anal.* ($C_{16}H_{24}N_3O_4S$) C, H, N.

2,2,6,6-Tetramethyl-4-(*p*-sulfamoylbenzamido)piperidinooxyl (VII) (Method 2). A solution of *p*-sulfamylbenzoic acid (1.0 g, 0.005 mole), III (0.86 g, 0.005 mole), *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (1.24 g, 0.005 mole) in THF–EtOH (1:1, 70 ml) was stirred at 35° for 18 hr. The solvent was then removed under reduced pressure, and the residue was placed on a silica gel column and eluted with $CHCl_3$ containing increasing proportions of MeOH. Recrystallization from $CHCl_3$–MeOH (1:1) yielded orange needle crystals (1.1 g) of VII, mp 223–225° dec. *Anal.* ($C_{16}H_{24}N_3O_4S$) C, H, N.

2,2,5,5-Tetramethyl-3-[(*p*-sulfamoylphenyl)carbamoyl]-1-pyrrolidinyloxyl (IV) was prepared from 4-aminobenzenesulfonamide (0.86 g) and I (0.93 g) by method 1 (ethyl chloroformate) and recrystallized from Me_2CO–hexane to give needle crystals (1.2 g), mp 226–227° dec. *Anal.* ($C_{15}H_{22}N_3O_4S$) C, H, N.

2,2,5,5-Tetramethyl-3-[(carbethoxymethyl)carbamoyl]-1-pyrrolidinyloxyl was prepared from glycine ethyl ester (0.72 g) and I (0.68 g) by method 2 and recrystallized from EtOAc–hexane to give yellow prismatic crystals (1.0 g), mp 117–118°. *Anal.* ($C_{13}H_{23}N_2O_4$) C, H, N.

2,2,5,5-Tetramethyl-3-{[(*p*-sulfamoylphenyl)carbamoyl]-methyl}carbamoyl)-1-pyrrolidinyloxyl (VI). *N*-3-(1-Oxyl-2,2,5,5-tetramethyl)pyrrolidinecarboxyglycine ethyl ester (1.2 g) was dissolved in 10 ml of water, and an equivalent amount of solid NaOH was added. After stirring for 1 hr at 25°, the solution was adjusted to pH 3.0 with dilute HCl, then extracted with EtOAc (3 × 10 ml). The EtOAc solution was dried (Na_2SO_4), then evaporated to dryness to give crude *N*-3-(1-oxyl-2,2,5,5-tetramethyl)pyrrolidinecarboxyglycine (0.87 g). The latter compound was condensed with 4-aminobenzenesulfonamide (0.63 g) using method 1 (ethyl chloroformate) to give VI as pale yellow prismatic crystals (0.6 g) from MeOH, mp 224–225°. *Anal.* ($C_{17}H_{25}N_4O_5S$) C, H, N.

4-Glutarimidobenzenesulfonamide. Sulfanilamide (2.0 g) and glutaric anhydride (1.32 g) were dissolved in Me_2CO (10 ml), and the mixture was allowed to stand overnight. The precipitate (2.8 g) was recrystallized from THF to give 4-glutarimidobenzenesulfonamide as colorless needle crystals, mp 188–189°. *Anal.* ($C_{11}H_{14}N_2O_5S$) C, H, N.

2,2,6,6-Tetramethyl-4-{4-[(*p*-sulfamoylphenyl)carbamoyl]butyramido}piperidinooxyl (VIII). 4-Glutarimidobenzenesulfonamide

(1.15 g) was condensed with III (0.69 g) using method 1 (isobutyl chloroformate) to give VIII (1.5 g) as pale orange needles from MeOH, mp 204–205°. *Anal.* ($C_{20}H_{31}N_4O_5S$) C, H, N.

2,2,5,5-Tetramethyl-3{4-[(*p*-sulfamoylphenyl)carbamoyl]butyramido}-1-pyrrolidinyloxyl (IX). 4-Glutarimidobenzenesulfonamide (0.8 g) was condensed with II (0.44 g) using method 1 (isobutyl chloroformate) to give IX (0.85 g) as pale yellow prismatic crystals, mp 217–218°. *Anal.* ($C_{19}H_{29}N_4O_5S$) C, H, N.

3{[2-(Hexahydro-5-isopentyl-2,4,6-trioxo-5-pyrimidinyl)ethyl]carbamoyl}-1-pyrrolidinyloxyl (X). I (1.0 g) was condensed with 5-(2-aminoethyl)-5-isopentylbarbituric acid (1.30 g) (Aldrich) by method 1 (ethyl chloroformate) to give X (1.0 g) as pale yellow prismatic crystals, mp 227–228° dec. *Anal.* ($C_{20}H_{33}N_4O_5$) C, H, N.

4-[(2-Hydroxyethyl)dimethylammonio]-2,2,6,6-tetramethylpiperidinooxyl Bromide (XI). 4-*N*,*N*-Dimethylamino-2,2,6,6-tetramethylpiperidine (15.2 g), prepared by the method of Hubbell, *et al.*,[11] was refluxed for 5 hr with 2-bromoethanol (15.2 g) and absolute EtOH (30 ml). The reaction mixture was then poured into dry ether (250 ml) and the precipitated *N*,*N*-dimethyl-*N*'-(2',2',6',6'-tetramethyl-4'-piperidyl)-2-hydroxyethylammonium bromide (XIV) (34.7 g) filtered off, washed exhaustively with dry ether, and dried. The preparation of XI and XIV was achieved by a modification of the method of Kornberg and McConnell.[12] XIV (34.7 g) was mixed with disodium ethylenediaminetetraacetate (9.63 g), sodium hydroxide (54.2 g), $Na_2WO_4 \cdot 2H_2O$ (11.26 g), 30% H_2O_2 solution (34.0 ml), and water (450 ml). After the reaction mixture had been allowed to stand at room temperature for 3 days, a further 42 ml of 30% H_2O_2 solution was added and the pH of the mixture adjusted to 10 by the addition of solid NaOH. After 2 more days, the mixture was stirred vigorously with a magnetic stirrer to destroy unreacted H_2O_2 and then freeze-dried. The residue was taken up in a minimum amount of hot EtOH and filtered, and the filtrate evaporated to dryness. The residue was then dissolved in a minimum volume of water and the solution passed through a column (30 cm × 2 cm) of AG1 X-8 anion-exchange resin in the Br^- form. The pale orange eluate was collected, freeze-dried, and then crystallized from EtOH to give XI as orange needle crystals (26.9 g), mp 209–210°. *Anal.* ($C_{13}H_{28}BrN_2O_2$) C, H, N.

4-[(2-Hydroxyethyl)dimethylammonio]-2,2,6,6-tetramethylpiperidinooxyl Bromide Dimethylcarbamate (XII). To a suspension of XI (2.0 g) and K_2CO_3 (1.93 g) in dry Me_2CO (20 ml) was added dimethylcarbamyl chloride (1.37 g), and the mixture was refluxed for 16 hr. The reaction mixture was then evaporated to dryness and the residue treated with hot EtOH (20 ml). The EtOAc extract was filtered and the filtrate evaporated to dryness. The residue was dissolved in a minimum amount of water and passed through a column (30 cm × 2 cm) of AG1 X-8 anion-exchange resin in the Br^- form. The pale orange eluate was freeze-dried and the residue recrystallized from EtOH–ether to give XII (2.5 g) as deep orange prismatic crystals, mp 194–195°. *Anal.* ($C_{16}H_{33}BrN_3O_3$) C, H, N.

3-Carboxy-2,2,5,5-tetramethyl-1-pyrrolidinyloxyl Ester with Choline Iodide (XIII). To an ice-cooled suspension of IV (0.55 g) in dry pyridine (0.3 ml) was added SO_2Cl (0.28 ml) in one lot. The mixture was allowed to warm to room temperature, then stirred for 1 hr, and centrifuged. The supernatant was carefully removed and mixed with dimethylaminoethanol (2.0 g) and then refluxed gently for 2 hr. The solvent was then removed under reduced pressure and the pale yellow oily residue dissolved in dry MeOH (5 ml) and treated with MeI (2 g). After refluxing for 3 hr, the solvent was removed under reduced pressure and the residue (0.5 g) crystallized from MeOH–ether to give pale yellow prismatic crystals of XIII, mp 175–176°. *Anal.* ($C_{14}H_{28}IN_2O_3$) C, H, N.

Acknowledgment. The authors wish to thank Dr. William C. Alford and Miss Paula Parisius of the Microanalytical Laboratory, National Institute of Arthritis and Metabolic Diseases, for performing the microanalyses.

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Book Reviews

Pharmacognosy. By G. E. Trease and W. C. Evans. 10th ed. The Williams and Wilkins Company, Baltimore, Md. 1971. viii + 795 pp. 14 × 22 cm. $23.00.

This tenth edition of "Pharmacognosy" is essentially similar to the previous edition and fulfills only part of the requirements for an introductory textbook. It is readable and understandable, and gives an excellent description of the botanical aspects of pharmacognosy. On the other hand, there is hardly any discussion of topics such as antibiotics, hormones, vitamins, and enzymes which are of particular interest to the pharmacognosist.

The text is organized in 40 chapters and divided into nine parts. In the first six parts (Introduction, Plants and Their Structure, Phytochemistry, Biosynthetic Pathways, Genetics and Comparative Phytochemistry, and From Plants to Crude Drugs) the authors attempted to place slightly more emphasis on phytochemical aspects, including metabolic pathways and such topics as genetics and comparative phytochemistry. By and large, these first six parts are essentially similar to those in the previous edition with some modification and reorganization of the subject matter. Part seven, which makes up one third of the book, deals with the drugs of botanical origin which are arranged according to the latest Engler system, the monocotyledons following the dicotyledons. Under each family an outline is given not only of the salient botanical features but also of the interesting chemical compounds found in the family. A good portion of this part has been rewritten and rearranged and should provide a useful service to investigators in the field. Part eight on drugs of animal origin and part nine on microscopical technique and commercial fibres are essentially similar to those in the previous edition. Of particular interest to researchers is the enlarged phytochemical appendix, which lists selected abstracts from January 1966 (volume 64 of *Chemical Abstracts*) to the end of December 1970 (volume 73 of *Chemical Abstracts*).

In view of the many changes in emphasis on various aspects of pharmacognosy in the last 20 years, which changes have been primarily in the area of phytochemistry and biosynthesis, it is the opinion of the reviewer that this textbook would be of little value to the undergraduate student in pharmacognosy in the United States. However, the book may be recommended to investigators in the field and those who wish to acquire the appropriate background.

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