Table II—Recovery of Indicine-*N*-oxide from Plasma Samples

Amount Added, μg	Amount Found[a], μg	Recovery, %
1	0.78 ± 0.04	78
3	3.07 ± 0.04	102
6	5.33 ± 0.07	89
9	7.99 ± 0.04	89
13	11.7 ± 0.1	90
15	12.9 ± 0.1	86
17	15.2 ± 0.1	90
20	16.1	81
Average		88 ± 7

[a] Average of duplicate samples.

(10:9:1) as the developing solvent. Components were visualized with Dragendorff's spray reagent (9).

RESULTS AND DISCUSSION

Solutions of indicine-*N*-oxide in pH 4.6 buffer exhibited a differential pulse polarographic maximum at −0.72 ± 0.03 v (saturated calomel electrode) (Fig. 1). The potential for this maximum was pH dependent, becoming more negative at higher pH values (Table I). Since similar reduction peak potentials and pH dependencies have been observed for other *N*-oxides (10–12), these data are consistent with reduction of an *N*-oxide moiety. In support of this reduction, no wave was detected for indicine (NSC 136052), the reduced form of indicine-*N*-oxide, under similar polarographic conditions. The pKa of indicine-*N*-oxide was 4.25 ± 0.02 at 23°. This value is consistent with values of other *N*-oxides, which are typically in the 4.3–5.4 range (10–12). The average purity of the drug was 95.9 ± 3% based on the number of millimoles of hydrogen ion added at the equivalence point to the millimoles of *N*-oxide added (based on the weight).

A pH of 4.63 was chosen for the analysis medium because the effect of pH on the peak potential in this region was minimal and the intensity of response was the greatest (Table I). A linear polarographic response was observed over a range of 0.5–10 μg/ml of pH 4.6 buffer. The standard curve parameters determined by a least-squares fit of the data indicated a response of 0.122 ± 0.002 μamp/μg/ml and a correlation coefficient of 0.9997.

Extraction of indicine-*N*-oxide from plasma samples was possible only after lyophilization. Both normal aluminum oxide and reversed-phase (C_{18} bonded silica gel) chromatographic columns were needed to remove other chemical species that gave polarographic responses in the same region as the drug. Qualitative confirmation of the drug in the methanolic extract of the lyophilized residue, the methanolic eluate from the aluminum oxide column, and the methanol–water eluate from the reversed-phase silica gel column was determined by TLC (R_f 0.41). Indicine-*N*-oxide was not retained to any significant extent by either column, as indicated by the recovery data.

Analysis of plasma spiked with known concentrations of indicine-*N*-oxide gave an overall recovery of 88 ± 7% (*SD*) (Table II) over a concentration range of 1–20 μg/ml of plasma. The plasma assay presented here is a reliable, sensitive method that is applicable for clinical use. The method is suitable for the analysis of the parent *N*-oxide in the presence of the known metabolic reduction product, indicine (2).

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Synthesis of Phenylurethans of 1,2-Dialkyl-4-pyrazolidinols as Anticonvulsant Agents

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Received December 10, 1979, from the *College of Pharmacy, University of Kentucky, Lexington, KY 40506.* Accepted for publication January 18, 1980.

Abstract □ Twelve phenylurethans of 1,2-dialkyl-4-pyrazolidinols were synthesized and tested for anticonvulsant activity. Several compounds exhibited activity.

Keyphrases □ Phenylurethans—synthesized from 1,2-dialkyl-4-pyrazolidinols, evaluation as anticonvulsant agents □ Anticonvulsant activity—phenylurethans of 1,2-dialkyl-4-pyrazolidinols, synthesis and evaluation for activity □ 1,2-Dialkyl-4-pyrazolidinols—phenylurethan derivatives, synthesis and testing for anticonvulsant activity

4-Pyrazolidinyl benzoates, ester derivatives of 1,2-dialkyl-4-pyrazolidinols (I), reportedly possess local anesthetic activity (1). Since lidocaine (2) and many urethans (3) show anticonvulsant properties, synthesis and testing of a series of phenylurethans (III*a*–III*l*) of 1,2-dialkyl-4-pyrazolidinols as potential anticonvulsant agents were desired.

DISCUSSION

The necessary 1,2-dialkyl-4-pyrazolidinols (I) were obtained from the reaction of epichlorohydrin and 1,2-dialkylhydrazines according to a reported procedure (4). Hydrazinoalcohols possessing methyl (I*a*), ethyl (I*b*), and *n*-propyl (I*c*) substituents were prepared. The 1,2-dialkyl-4-

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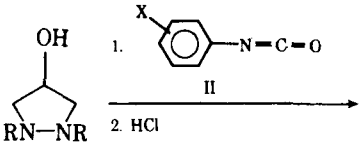
Table I—Physical Properties of Hydrochlorides of Phenylurethans of 1,2-Dialkyl-4-pyrazolidinols

Compound	X	R	Melting Point	Yield, %	Formula	Analysis, % Calc.	Found
IIIa	o-CH_3	CH_3	208–209°	72	$C_{13}H_{19}N_3O_2$·HCl	C 54.64	54.67
						H 7.05	6.96
						N 14.70	14.80
IIIb	p-C_2H_5O	CH_3	176–179°	75	$C_{14}H_{21}N_3O_3$·HCl	C 53.25	53.29
						H 7.02	7.02
						N 13.31	13.28
IIIc	2-Cl,6-CH_3	CH_3	226–227°	75	$C_{13}H_{18}ClN_3O_2$·HCl	C 48.76	49.25
						H 5.98	6.33
						N 13.12	13.24
IIId	2,6-$(CH_3)_2$	CH_3	234–235° dec.	77	$C_{14}H_{21}N_3O_2$·HCl	C 56.09	56.23
						H 7.40	7.43
						N 14.02	14.07
IIIe	H	C_2H_5	135–136°	64	$C_{14}H_{21}N_3O_2$·HCl	C 56.09	56.18
						H 7.40	7.48
						N 14.02	14.05
IIIf	p-CH_3	C_2H_5	135–137°	32	$C_{15}H_{23}N_3O_2$·HCl	C 57.41	57.59
						H 7.71	7.59
						N 13.39	13.48
IIIg	2-Cl,6-CH_3	C_2H_5	212–213°	66	$C_{15}H_{22}ClN_3O_2$·HCl	C 51.73	52.22
						H 6.66	6.88
						N 12.07	12.19
IIIh	2,6-$(CH_3)_2$	C_2H_5	219–220°	69	$C_{16}H_{25}N_3O_2$·HCl	C 58.62	58.66
						H 7.99	7.88
						N 12.82	12.81
IIIi	m-Cl	$CH_3(CH_2)_2$	157–159°	68	$C_{16}H_{24}ClN_3O_2$·HCl	C 53.04	53.40
						H 6.96	7.36
						N 11.60	11.60
IIIj	p-CH_3O	$CH_3(CH_2)_2$	168–171°	74	$C_{17}H_{27}N_3O_3$·HCl	C 57.05	57.01
						H 7.89	7.77
						N 11.74	11.64
IIIk	p-CH_3	$CH_3(CH_2)_2$	157.5–160°	74	$C_{17}H_{27}N_3O_2$·HCl	C 59.72	59.77
						H 8.26	8.34
						N 12.29	12.26
IIIl	m-CF_3	$CH_3(CH_2)_2$	143–146°	30	$C_{17}H_{24}F_3N_3O_2$·HCl	C 51.58	51.41
						H 6.37	6.88
						N 10.61	10.54

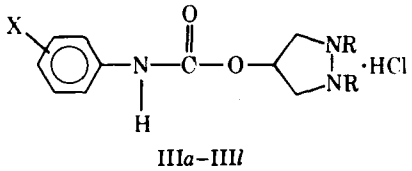
Table II—Anticonvulsant and Toxic Effects

Compound	MES Activity[a] 0.5 hr	MES Activity[a] 4 hr	sc Met Activity[a] 0.5 hr	sc Met Activity[a] 4 hr	Toxicity[a] 0.5 hr	Toxicity[a] 4 hr
IIIa	–	–	–	–	–	–
IIIb	–	–	+	–	+	–
IIIc	+	–	+	–	+	–
IIId	+	–	+	–	+	–
IIIe	–	–	–	–	–	–
IIIf	–	[b]	–	–	–	–
IIIg	–	–	–	–	+	–
IIIh	+	–	++	–	+	–
IIIi	–	–	+	+	+	–
IIIj	–	–	–	–	+	–
IIIk	+	–	–	++[c]	+	–
IIIl	+	–	+	–	+	–

[a] Activity and toxicity at 30, 100, and 300 mg/kg are represented by +++, ++, and +, respectively; – denotes no activity or toxicity observed at 300 mg/kg. [b] No activity observed at 100 mg/kg. [c] Rescreening gave inconsistent results; activity was observed at 30 and 100 but not at 300 mg/kg; no toxicity at all three doses.



Ia: R = CH_3
Ib: R = CH_3CH_2
Ic: R = $CH_3(CH_2)_2$



IIIa–IIIl

Scheme I

pyrazolidinols added readily to aryl substituted isocyanates (II) to give the adducts IIIa–IIIl (Scheme I). The latter compounds were isolated uniformly as their hydrochloride salts, and their physical properties are given in Table I.

Compounds IIIa–IIIl were examined in the maximal electroshock seizure (MES) and subcutaneous pentylenetetrazol seizure threshold (sc Met) tests for anticonvulsant activity and neurotoxicity in male mice[1] by reported procedures (5). Seven of the 12 compounds tested showed some degree of anticonvulsant activity (Table II). The most promising compound, IIIh, was evaluated further. The values for MES ED_{50}, sc Met ED_{50}, and TD_{50} (median toxic dose in the rotorod test) for IIIh were 193 (163–218), 336 (234–495), and 414 (353–499) mg/kg, respectively. Because of the moderate activity in this series of compounds, further work appears to be unwarranted.

EXPERIMENTAL[2]

A typical reaction for preparing the hydrochlorides of phenylurethans of 1,2-dialkyl-4-pyrazolidinols (IIIa–IIIl) is described using IIIk as the example. A mixture of 3.48 g (0.03 mole) of Ic (4), 3.99 g (0.03 mole) of p-tolyl isocyanate, and 45 ml of dry toluene was refluxed for 22 hr. The mixture was cooled and extracted twice with 50-ml portions of 2 *N* HCl. The combined acidic extract was washed with 50 ml of benzene, filtered through a sintered-glass funnel, and made basic with solid sodium carbonate. The separated oil was extracted into methylene chloride and dried over magnesium sulfate. Evaporation of the solution under reduced pressure gave an oily residue.

The oily free base was dissolved in absolute ethanol and acidified to pH ~2–3 by dropwise addition of concentrated hydrochloric acid. Upon cooling, a white solid precipitated, which was filtered and washed with cold absolute ethanol. Drying afforded 7.59 g (74% yield) of the hydrochloride salt, mp 156–159°. Recrystallization from absolute ethanol–ether gave analytically pure material, mp 157.5–160°; IR (KBr): 1720 (C=O) cm^{-1}.

[1] No. 1, Carworth Farms.

[2] Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. IR spectra were taken on a Perkin-Elmer 700 spectrophotometer as either liquid films or as potassium bromide pellets. Elemental analyses were performed by Dr. Kurt Eder, Geneva, Switzerland.

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Evaluation of ^{99m}Tc-Labeled Iminodiacetic Acid Derivatives of Substituted 2-Aminopyrroles as Hepatobiliary Imaging Agents I

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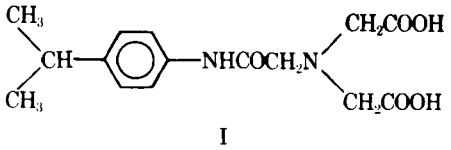
Received November 5, 1979, from the *College of Pharmacy, University of South Carolina, Columbia, SC 29208.* Accepted for publication January 14, 1980.

Abstract □ *N*-(3-Cyano-4,5-dimethyl-2-pyrrylcarbamoylmethyl)iminodiacetic acid (II*a*) and *N*-(3-cyano-4-methyl-5-benzyl-2-pyrrylcarbamoylmethyl)iminodiacetic acid (II*b*) were synthesized, labeled with technetium 99*m*, and compared with ^{99m}Tc-labeled *p*-isopropylacetanilidoiminodiacetic acid (I) for hepatobiliary activity in rats. All three compounds showed similar clearance of radioactivity from the blood. Comparison of the amount of radioactivity in various organs 1 hr after injection showed no significant difference between I and II*b*. Compound II*a* showed significantly less radioactivity in the GI tract and a higher amount in the kidneys and bladder.

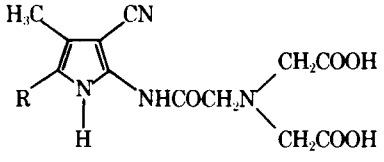
Keyphrases □ Iminodiacetic acid—^{99m}Tc-labeled, derivatives of 2-aminopyrroles, evaluation as hepatobiliary imaging agents, rats □ Lidocaine, 2-aminopyrrole analogs—^{99m}Tc-labeled iminodiacetic acid derivatives, evaluation as hepatobiliary imaging agents, rats □ Radiopharmaceuticals—^{99m}Tc-labeled iminodiacetic acid derivatives of 2-aminopyrroles, evaluation as hepatobiliary imaging agents, rats

Efforts have been made in recent years to formulate ^{99m}Tc-labeled radiopharmaceuticals for use in hepatobiliary scintigraphic imaging. Many lidocaine analogs with an iminodiacetic acid functional group capable of forming a stable complex with technetium 99*m* have been evaluated (1). Most lidocaine derivatives were synthesized by altering the lipophilic substituents on the benzene ring. One compound, *p*-isopropylacetanilidoiminodiacetic acid (I), has shown desirable characteristics and now is available commercially.

Johnson *et al.* (2) recently synthesized a series of 2-diethylaminoacetamido-3-cyano-4-methyl-5-substituted pyrrole analogs of lidocaine. These compounds compared favorably with lidocaine in local anesthetic and antiarrhythmic activities. The iminodiacetic acid derivatives of the methyl- (II*a*) and benzyl- (II*b*) substituted compounds were prepared. These two compounds were labeled with technetium 99*m* and compared with ^{99m}Tc-labeled I for hepatobiliary activity in rats.



I



II

II*a*: R = CH_3
II*b*: R = $CH_2C_6H_5$

EXPERIMENTAL

Chemistry[1]—*N-(3-Cyano-4,5-dimethyl-2-pyrrylcarbamoylmethyl)-iminodiacetic Acid (II*a*)*—A modified procedure of Callery *et al.* (3) was used. A mixture of 2-chloroacetamido-3-cyano-4,5-dimethylpyrrole (5.72 g, 0.027 mole) (2), disodium iminodiacetic acid monohydrate (5.44 g, 0.027 mole), and sodium hydroxide (1.10 g, 0.027 mole) in 100 ml of methanol–water (3:1) was refluxed for 24 hr. The methanol was removed *in vacuo*, 100 ml of water was added to the residue, the suspension was filtered, and the pH of the filtrate was adjusted to 3 by the dropwise addition of concentrated hydrochloric acid. The precipitate was collected and air dried.

The crude product (6.2 g, 76.8% yield) was washed several times with boiling water to yield a brown powder (homogeneous on TLC in methanol, R_f 0.55), mp 203–204.5° dec.; IR (KBr): 3610, 3330, 2210, 1675, 1640, 1430, 1400, 1220, and 725 cm^{-1}; NMR (dimethyl sulfoxide-d_6): δ 1.92 (s, 3H, CH_3 at C-4), 2.03 (s, 3H, CH_3 at C-5), 3.47 (s, 6H, CH_2), 10.75 (s, 1H, N_1H), and 10.3–12.8 (broad s, 3H, NHCO, COOH, and $^+$NH) ppm.

Anal.—Calc. for $C_{13}H_{16}N_4O_5$: C, 50.64; H, 5.23; N, 18.17. Found: C, 50.53; H, 5.23; N, 18.16.

*N-(3-Cyano-4-methyl-5-benzyl -2- pyrrylcarbamoylmethyl)iminodiacetic Acid (II*b*)*—A mixture of 2-chloroacetamido-3-cyano-4-methyl-5-benzylpyrrole (7.77 g, 0.027 mole) (2), disodium iminodiacetic acid monohydrate (5.44 g, 0.027 mole), and sodium hydroxide (1.10 g, 0.027 mole) was condensed according to the procedure described for II*a*. A brown powder was obtained (6.5 g, 62.6% yield) (homogeneous on TLC in methanol, R_f 0.51), mp 190–192° dec.; IR (KBr): 3400, 3330, 2210, 1675, 1620, 1250, 1215, 950, and 710 cm^{-1}; NMR (dimethyl sulfoxide-d_6): δ 1.96 (s, 3H, CH_3 at C-4), 3.79 (s, 2H, CH_2 at C-5), 4.2 (broad s, 6H, CH_2), 7.07 (s, 5H, C_6H_5), 11.25 (broad s, 3H, NHCO, COOH, and $^+$NH), and 11.5 (broad s, 1H, N_1H) ppm.

Anal.—Calc. for $C_{19}H_{20}N_4O_5$: C, 59.37; H, 5.24; N, 14.58. Found: C, 59.23; H, 5.26; N, 14.53.

[1] IR spectra were determined on a Beckman Acculab 4 spectrophotometer using the potassium bromide technique. NMR spectra were determined on a Hitachi Perkin-Elmer R24 high-resolution spectrometer with tetramethylsilane as the internal reference. Melting points were obtained using a Thomas-Hoover capillary apparatus and are uncorrected. TLC was performed using Eastman type 6060 chromatogram sheets (silica gel), and the sheets were developed in an iodine chamber. Carbon, hydrogen, and nitrogen values were obtained from analyses performed by Atlantic Microlabs., Atlanta, Ga.

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