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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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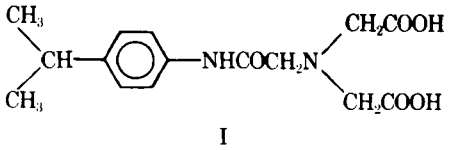
Abstract □ *N*-(3-Cyano-4,5-dimethyl-2-pyrrylcarbamoylmethyl)iminodiacetic acid (II*a*) and *N*-(3-cyano-4-methyl-5-benzyl-2-pyrrylcarbamoylmethyl)iminodiacetic acid (II*b*) were synthesized, labeled with technetium 99*m*, and compared with ^{99m}Tc-labeled *p*-isopropylacetanilidoiminodiacetic acid (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.

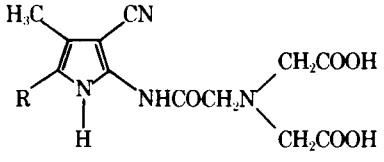
Keyphrases □ Iminodiacetic acid—^{99m}Tc-labeled, derivatives of 2-aminopyrroles, evaluation as hepatobiliary imaging agents, rats □ Lidocaine, 2-aminopyrrole analogs—^{99m}Tc-labeled iminodiacetic acid derivatives, evaluation as hepatobiliary imaging agents, rats □ Radiopharmaceuticals—^{99m}Tc-labeled 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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Table I—Parameters for the Blood Elimination Equation of Technetium 99*m* in Rats following Intravenous Injection of ^{99m}Tc-Labeled I, II*a*, and II*b*[a]

Parameter	I	II*a*	II*b*
A	0.76 (0.05)	0.73 (0.04)	0.81 (0.03)
α	0.40 (0.06)	0.52 (0.04)	0.69 (0.05)
B	0.24 (0.03)	0.27 (0.02)	0.19 (0.01)
β	0.030 (0.005)	0.023 (0.003)	0.027 (0.002)
R_2	91.8	98.2	97.8

[a] Standard errors are given in parentheses.

Labeling with Technetium 99*m*—Technetium 99*m* labeling of I was achieved by adding 7 mCi (1.0 ml) of sodium [^{99m}Tc]pertechnetate (III) eluate from a molybdenum 99–technetium 99*m* generator[2] to a vial containing a lyophilized preparation of 20 mg of I and 0.2 mg of stannous chloride[3].

Preparation of ^{99m}Tc-labeled II*a* and II*b* was accomplished by stannous chloride reduction of III in aqueous solution. Ten milligrams of pure compound was dissolved in 0.3 ml of 0.1 *N* NaOH. This solution was back-titrated with 0.05 *N* HCl to pH 7. Seven millicuries of III in 0.2 ml was added, and the resulting solution was purged with nitrogen for 5 min. After purging, 0.1 ml of freshly prepared stannous chloride (1 mg/ml in 0.05 *N* HCl) was added, and the mixture was kept at room temperature for 20 min. The final solution then was passed through a Millipore filter into a sterile evacuated vial.

The radiochemical purity of the ^{99m}Tc-labeled I was determined using instant TLC[4] with a chloroform–acetone (70:30) solvent system. The radiochemical purity of ^{99m}Tc-labeled II*a* and II*b* was determined using instant TLC with a methyl ethyl ketone solvent system. Examination of the chromatographic strips revealed the bound fraction to be 93% or greater for all of the radiopharmaceuticals.

***In Vivo* Studies**—Fasting, nonhydrated, male Sprague–Dawley[5] rats, 175–240 g, were used. The left jugular vein was exposed and cannulated for each animal after anesthesia was achieved with pentobarbital sodium (30 mg/kg). The animal then was positioned over a rectilinear scanner[6] modified to record the counts arising from the cardiac pool. Following the injection of 0.25 mCi of the ^{99m}Tc-labeled compound into the cannula, sequential counts were obtained for the duration of the study. These counts were used to model the disappearance of the radioactivity from the blood.

Each animal was killed 1 hr after injection of the radiopharmaceutical by overanesthesia with ether. Selected organs were removed from the animal and assayed for radioactivity. The results are reported as the percentage of the total injected radioactivity.

RESULTS

The disappearance of the radioactivity from the blood is described by a two-compartment open model. The computer-fitted equation[7] used to describe the data is:

$$C_B = A \exp(-\alpha t) + B \exp(-\beta t) \qquad \text{(Eq. 1)}$$

where C_B refers to the fraction of the dose retained in the blood (biological) and t is time (minutes). The parameters for Eq. 1 and the correlation coefficient (R^2) for each parameter are listed in Table I. Examination of these data reveals that there was no significant difference among the drugs for the β-phase of the elimination equation; the half-life for the β-phase ranged from 20 to 30 min.

Table II—Percent of Administered Radioactivity at 1 hr after Intravenous Injection of ^{99m}Tc-Labeled I, II*a*, and II*b* in Rat Organs[a]

Rat Organ	I	II*a*	II*b*
GI tract	78.7 (3.3)	38.0 (2.4)	81.5 (2.4)
Liver	2.6 (0.4)	8.0 (0.2)	6.7 (0.9)
Spleen	0.09 (0.03)	0.07 (0.05)	0.15 (0.08)
Kidneys	2.5 (0.2)	11.1 (3.4)	2.3 (0.3)
Lungs	0.17 (0.05)	0.30 (0.18)	0.20 (0.03)
Heart	0.02 (0.01)	0.02 (0.01)	0.07 (0.02)
Carcass	16.2 (3.0)	44.0 (2.8)	9.9 (0.9)

[a] Standard errors are given in parentheses.

Table II shows the distribution of technetium 99*m* in selected organs and tissues of the rat 1 hr after intravenous injection. The data are expressed as the percent of the injected dose and represent the average of three rats for each compound. The carcass values represent the remainder after the removal of the other organs and include the bladder, which accounted for much of the activity.

The organ data following injection of I or II*b* are indistinguishable except for the liver. Excretion into the GI tract accounted for almost 80% of the activity for both compounds. The kidneys retained about 2% of the injected dose, and the carcass contained about 10%. The content of technetium 99*m* in the liver at necropsy was significantly higher for the benzyl compound as compared to I.

The methyl derivative (II*a*) showed significantly less activity in the GI tract than either of the other compounds. Higher amounts of the injected activity were noted in the kidneys and remaining carcass.

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[2] CintiChem, Union Carbide Corp., Tuxedo, N.Y.
[3] Diagnostic Isotopes Inc., Bloomfield, N.J.
[4] ITLC SG, Gelman Instrument Co., Ann Arbor, Mich.
[5] GIBCO Animal Research Laboratories, Madison, Wis.
[6] Magnascanner 500, Picker Instruments, Cleveland, Ohio.
[7] NLIN, SAS 76, Raleigh, N.C.