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

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Received March 20, 1981, from the *College of Pharmacy, University of South Carolina, Columbia SC 29208.* Accepted for publication July 28, 1981.

Abstract □ The synthesis and biodistribution properties of ^{99m}Tc-labeled 5-substituted *N*-(3-cyano-4-methyl-2-pyrrylcarbamoylmethyl)iminodiacetic acids and a similar series of N^1-methyl analogs are described. These compounds were compared with ^{99m}Tc-labeled *N*-(2,6-dimethylphenylcarbamoylmethyl)iminodiacetic acid for hepatobiliary activity in the rat. The effects of structural modifications on biological activity are also reported.

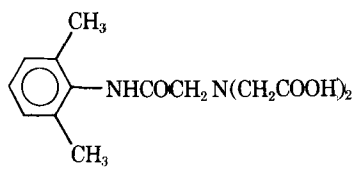
Keyphrases □ Imaging agents—^{99m}Tc-labeled iminodiacetic acid derivatives of substituted 2-aminopyrroles, rats □ Iminodiacetic acid—^{99m}Tc labeled derivatives of 2-substituted aminopyrroles, as hepatobiliary imaging agents in rats □ 2-Aminopyrroles—substituted, ^{99m}Tc-labeled derivatives, as hepatobiliary imaging agents in rats

Diseases of the gall bladder and biliary tract are a major health problem. Visualization of the hepatobiliary system in humans can be used in the differential diagnosis of these diseases. The use of radiopharmaceuticals as diagnostic tools for the dynamic evaluation of the hepatobiliary system was initiated by Taplin *et al.* (1) when they introduced the rose bengal sodium iodine 131 test.

BACKGROUND

Rose bengal sodium iodine 131 has many drawbacks because of the physical characteristics of the iodine 131 label. Recently, many technetium 99*m* complexes have been produced and studied as possible replacements for rose bengal sodium iodine 131. These technetium 99*m* complexes include penicillamine (2), dihydrothioctic acid (3), tetracycline (4), mercaptoisobutyric acid (5), and pyridoxalamine acids (6). Among the most promising of the agents are the iminodiacetic acid derivatives (7), such as *N*-(2,6-dimethylphenylcarbamoylmethyl)iminodiacetic acid (I). Many analogs of I have been synthesized by altering the lipophilic substituent on the benzene ring and many of these analogs are being investigated clinically in humans. To date, however, no ideal hepatobiliary agent has been introduced because all of these complexes are adversely affected by elevated serum bilirubin levels.

To make a more effective hepatobiliary agent more needs to be known about the structure–activity relationship. It was reported previously (8) that a chelate must have a molecular weight between 300 and 1000, exist as an organic anion, contain at least two aromatic rings, and bind to albumin to be effective as a hepatobiliary scintigraphic agent. Another report (9) proposed a bichelate structure for ^{99m}Tc–I with a molecular weight of the lidocaine derivative that favors biliary excretion. One of the first attempts at indicating a structure–activity relationship of the lidocaine analogs (10) reported a linear relationship between the biliary excretion in mice and the natural logarithm of the absolute value of the molecular weight of the technetium 99*m* chelate divided by the net charge on the chelate.



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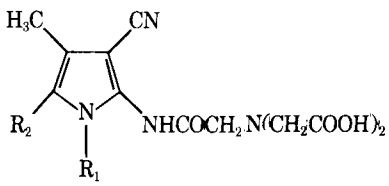

II: R_1 = H
III: R_1 = CH_3

Recently, the synthesis and the biodistribution properties of ^{99m}Tc-labeled 2-aminopyrrole analogs of I were reported (11). The activity exhibited by these compounds prompted the synthesis and evaluation of other 5-substituted *N*-(3-cyano-4-methyl-2-pyrrylcarbamoylmethyl)-iminodiacetic acids (II) and a similar series of N^1-methyl analogs (III). All of the derivatives were labeled with technetium 99*m* and compared with ^{99m}Tc–I for hepatobiliary activity in rats. The effects of N^1-methylation and the effects of C-5 substitution on biological activity are reported (Table I).

EXPERIMENTAL[1]

Chemistry—*N-(3-Cyano-1,4,5-trimethyl-2-pyrrylcarbamoylmethyl)-iminodiacetic Acid (IIIa)*—The procedure for the synthesis of IIIa is given as a general method for II*a–e* and III*b–f*. A modified procedure of Callery *et al.* (12) was used. A solution of 2-chloroacetamido-3-cyano-1,4,5-trimethylpyrrole (6.77 g, 0.03 mole) (13), disodium iminodiacetic acid monohydrate (5.85 g, 0.03 mole), and sodium hydroxide (1.2 g, 0.03 mole) in 100 ml methanol–water (3:1) was refluxed with stirring for 3 hr. The solution was stirred at ambient temperature overnight, diluted with water (100 ml), and the methanol removed *in vacuo*. The resulting suspension was diluted with additional water (50 ml), warmed gently, and filtered. After acidification of the filtrate to pH 3 by dropwise addition of concentrated hydrochloric acid, the precipitate was collected and dried. The crude product (7.25 g, 75.0%) was recrystallized from water to yield a light lavender powder (homogeneous on TLC, methanol, R_f 0.57), mp 184–185° dec., IR (KBr): 3300, 3020, 2860, 2210, 1680 (broad), 1560, 1410, 1350, and 970 cm^{-1}; NMR (dimethyl sulfoxide-d_6): δ 2.00 (s, 3H, CH_3 at C_4), 2.07 (s, 3H, CH_3 at C_5), 3.27 (s, 3H, CH_3 at N_1), 3.53 (s, 6H, —CH_2—alpha to amino group), 7.80–9.20 (broad s, 3H, amide NH and COOH) ppm.

N-(2,6-Dimethylphenylcarbamoylmethyl)iminodiacetic acid (I)—A solution of 1-chloroacetamido-2,6-dimethylbenzene (5.93 g, 0.03 mole), disodium iminodiacetic acid monohydrate (5.85 g, 0.03 mole), and sodium hydroxide (1.2 g 0.03, mole) in 100 ml of methanol–water (3:1) was refluxed with stirring for 4.5 hr. The methanol was removed *in vacuo* and the crude product (5.5 g, 69%) was isolated according to the procedure described for III*a*. The product was recrystallized from water to yield white crystals, mp 216–217° [lit. mp 215–216° (4)]; IR (KBr): 3180, 1750, 1610, 1530, 1215, 1155, and 770 cm^{-1}.

Anal.—Calc. for $C_{14}H_{18}N_2O_5$: C, 57.13; H, 6.16; N, 9.52. Found: C, 57.16; H, 6.18; N, 9.51.

Labeling with Technetium 99*m*—The iminodiacetic acid derivatives (I, II*a–e*, III*a–f*) were labeled by a stannous chloride reduction of sodium (^{99m}Tc) pertechnetate (IV) eluate from a molybdenum 99–technetium

[1] IR spectral data 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 Chromatogram sheets, type 6060 (silica gel), and the sheets were developed in an iodine chamber. Carbon, hydrogen, and nitrogen values were obtained from analysis performed by Atlantic Microlabs, Inc., Atlanta, Ga.

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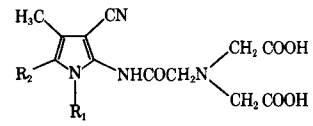

Table I—Data for Various *N*-(3-Cyano-4-methyl-2-pyrrylcarbamoylmethyl)iminodiacetic Acids

Compound	R_1	R_2	Yield, %	Recrystallization Solvent	Melting Point	R_f[a]	Formula	Analysis, % Calc.	Found
IIa[b]	—H	—CH_3							
IIb	—H	—iso-C_4H_9	63.5	water	163–166.5° dec.	0.37	$C_{16}H_{22}N_4O_5$	C 54.84 H 6.33 N 15.99	54.87 6.33 15.98
IIc	—H	—$CH_2CH_2SCH_3$	57.0	dimethyl sulfoxide–acetone–ether (1:4:1)	184–186° dec.	0.41	$C_{15}H_{20}N_4O_5S$	C 48.90 H 5.47 N 15.21 8.70	48.71 5.54 15.14 8.66
IId[b]	—H	—$CH_2C_6H_5$							
IIe	—H	—$CH_2C_6H_4$-p-OH	50.0	water	111.5–115°	0.28	$C_{19}H_{20}N_4O_6$ · 0.5 H_2O	C 55.74 H 5.17 N 13.69	55.63 5.29 13.82
IIIa	—CH_3	—CH_3	75.0	water	184–186° dec.	0.57	$C_{14}H_{18}N_4O_5$	C 52.17 H 5.63 N 17.38	52.03 5.63 17.33
IIIb	—CH_3	—iso-C_4H_9	73.5	water	170–171° dec.	0.57	$C_{17}H_{24}N_4O_5$	C 56.03 H 6.64 N 15.38	56.04 6.65 15.37
IIIc	—CH_3	—$CH_2CH_2SCH_3$	60.9	water	144–145°	0.57	$C_{16}H_{22}N_4O_5S$	C 50.25 H 5.80 N 14.65 S 8.39	50.42 5.86 14.75 8.41
IIId	—CH_3	—C_6H_5	60.7	water	162–165° dec.	0.62	$C_{19}H_{20}N_4O_5$	C 59.37 H 5.24 N 14.58	59.30 5.27 14.57
IIIe	—CH_3	—$CH_2C_6H_5$	73.6	water	168–169° dec.	0.55	$C_{20}H_{22}N_4O_5$	C 60.29 H 5.57 N 14.06	60.06 5.65 13.99
IIIf	—CH_3	—CH_2—C_6H_4-p-OCH_3	73.9	water	171–172° dec.	0.62	$C_{21}H_{24}N_4O_6$	C 58.87 H 5.65 N 13.07	58.62 5.72 13.02

[a] Methanol. [b] Data previously reported in ref. 11.

Table II—Parameters for the Blood Elimination Equation of ^{99m}Tc in the Rat following Intravenous Injection of ^{99m}Tc-Labeled Iminodiacetic Acid Derivatives[a]

	A	α	*B*	β	R^2
I	0.63 (0.02)	0.23 (0.02)	0.36 (0.02)	0.013 (0.00013)	99.8
IIa[b]	0.73 (0.04)	0.52 (0.04)	0.27 (0.02)	0.023 (0.003)	98.2
IIb	0.73 (0.03)	0.50 (0.03)	0.27 (0.01)	0.019 (0.001)	99.7
IIc	0.67 (0.02)	0.32 (0.01)	0.33 (0.01)	0.0104 (0.0011)	99.7
IId[b]	0.81 (0.03)	0.69 (0.05)	0.19 (0.01)	0.027 (0.002)	97.8
IIe	0.79 (0.03)	0.58 (0.03)	0.21 (0.01)	0.023 (0.002)	99.5
IIIa	0.54 (0.03)	0.43 (0.04)	0.44 (0.01)	0.015 (0.001)	99.7
IIIb	0.57 (0.03)	0.37 (0.03)	0.43 (0.01)	0.013 (0.001)	99.7
IIIc	0.56 (0.03)	0.25 (0.02)	0.43 (0.02)	0.012 (0.001)	99.7
IIId	0.67 (0.02)	0.26 (0.02)	0.33 (0.01)	0.016 (0.001)	99.8
IIIe	0.58 (0.03)	0.28 (0.03)	0.42 (0.02)	0.011 (0.001)	99.7
IIIf	0.56 (0.02)	0.26 (0.02)	0.44 (0.01)	0.008 (0.001)	99.9

[a] Standard error in parentheses. [b] Data previously reported in ref. 11.

99*m* generator[2] in an aqueous solution. Ten milligrams of each compound were dissolved in minimal amounts of sodium hydroxide (1 *N*). This solution was back titrated with hydrochloric acid (0.05 *N*) to pH 7. Seven millicuries of IV 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 solution[3] (1 mg/ml) was added and the solution was kept at room temperature for 20 min. The molar ratio of ligand to tin varied from 52:1 to 77:1. The final solution was passed through a millipore filter into a sterile evacuated vial.

The radiochemical purities of technetium 99*m* labeled IIc, IIe, IIIa, and IIIc compounds were determined using instant TLC[4] with a chloroform–acetone (70:30) solvent system. The radiochemical purities of 99*m* Tc-labeled I, IIa, IIb, IId, IIId, IIIe, and IIIf compounds were determined using instant TLC with methyl–ethyl ketone, 85% methanol, and saline solvent systems. Examination of the chromatographic strips revealed the bound fraction to be 95% or greater for all of the radiopharmaceuticals.

***In Vivo* Studies**—Fasting, nonhydrated, male Sprague-Dawley[5] rats, 175–250 g, were used. The left jugular vein was exposed and cannulated for each animal after anesthetizing with pentobarbital sodium (30 mg/kg). The animal was then 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 sacrificed 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 (min). The parameters for Eq. 1 and the correlation

[2] CintiChem, Union Carbide Corp., Tuxedo, N.Y.
[3] The stannous chloride solution was prepared daily by dissolving 15 mg of $SnCl_2 \cdot 2H_2O$ in 15 ml of hydrochloric acid (0.05 *N*).
[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.

Table III—Percent of Administered Radioactivity at 1 hr after Intravenous Injection of ^{99m}Tc-Labeled Iminodiacetic Acid Derivatives in Selected Organs of the Rat[a]

	GI Tract	Liver	Spleen	Kidneys	Lung	Heart	Carcass
I	64.7	3.89	0.06	4.11	0.19	0.07	27.0
	(5.83)	(0.55)	(0.02)	(0.59)	(0.07)	(0.04)	(4.70)
IIa[b]	38.0	8.00	0.07	11.1	0.30	0.02	44.0
	(2.40)	(0.21)	(0.05)	(3.41)	(0.18)	(0.01)	(2.80)
IIb	72.9	6.06	0.08	5.92	0.21	0.05	14.8
	(2.71)	(1.43)	(0.01)	(0.60)	(0.05)	(0.01)	(1.26)
IIc	75.2	5.50	0.04	6.80	0.16	0.05	12.0
	(2.24)	(1.43)	(0.01)	(0.07)	(0.03)	(0.02)	(1.56)
IId[b]	81.5	6.72	0.15	2.34	0.20	0.07	9.90
	(2.48)	(0.91)	(0.08)	(0.35)	(0.03)	(0.02)	(0.92)
IIe	76.1	4.67	0.07	3.04	0.20	0.07	15.8
	(2.29)	(1.02)	(0.01)	(0.21)	(0.02)	(0.01)	(1.20)
IIIa	21.9	11.3	0.14	6.8	0.38	0.16	59.3
	(3.60)	(4.70)	(0.09)	(0.6)	(0.08)	(0.09)	(3.61)
IIIb	53.3	10.8	0.04	4.03	0.24	0.06	31.4
	(11.8)	(2.41)	(0.01)	(1.22)	(0.03)	(0.03)	(9.57)
IIIc	58.1	6.40	0.06	2.81	0.26	0.09	32.0
	(3.69)	(1.59)	(0.01)	(0.27)	(0.02)	(0.02)	(3.73)
IIId	78.6	4.00	0.03	1.63	0.17	0.03	15.3
	(1.94)	(0.40)	(0.01)	(0.02)	(0.04)	(0.01)	(1.62)
IIIe	69.1	6.62	0.07	2.37	0.49	0.14	20.6
	(0.47)	(0.72)	(0.01)	(0.24)	(0.08)	(0.01)	(0.52)
IIIf	59.6	11.8	0.12	2.23	0.54	0.19	24.9
	(2.59)	(2.41)	(0.01)	(0.37)	(0.04)	(0.09)	(2.20)

[a] Standard error in parentheses. [b] Data previously reported in reference 11.

coefficient (R^2) for each parameter are listed in Table II. The data represents the combined data for three rats for each compound.

Table III 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 a percentage 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.

This experiment was a completely randomized block design which permitted the calculation of differences between the ^{99m}Tc-labeled pyrrole derivatives IIa–*e*, IIIa–*f*, and ^{99m}Tc–I. Differences were tested by two-sided Student *t* tests at a 90% confidence level. Student *t* statistics between ^{99m}Tc–I and the ^{99m}Tc-labeled iminodiacetic acid derivatives of 5-substituted 2-aminopyrroles were computed for the β phases of the blood curves, the percentage of injected radioactivity in the GI tracts, carcasses, livers, and kidneys.

The results show that ^{99m}Tc–IIa, ^{99m}Tc–II*d*, and ^{99m}Tc–IIIa are different from ^{99m}Tc–I. When ^{99m}Tc–IIa and ^{99m}Tc–IIIa were compared with ^{99m}Tc–I, these complexes showed less of the radioactivity accumulating in the GI tract and more remaining in the carcass at 1 hr after injection. A comparison between ^{99m}Tc–II*d* and ^{99m}Tc–I showed that ^{99m}Tc–II*d* had less radioactivity remaining in the carcass and more accumulating in the GI tract.

The experimental design allowed testing of the effects of N^1-methylation and C-5 substitution on the pyrrole ring by a two-way analysis of variance. The analysis of variance was performed on the β phase rate constants and on the percentages of radioactivity in the carcass, GI tract, liver, and kidneys.

The results show that N^1-methylation significantly decreased the rate constant, increased the accumulation of radioactivity in the carcass and kidneys, and decreased the accumulation of technetium 99*m* in the GI tract. One possible explanation for these results is that these chelates are in the structure proposed by Loberg and Fields (9) and that the technetium 99*m* labeled compounds IIa–*e* can undergo intramolecular hydrogen bonding between the N^1 hydrogen and the carbonyl group at position 2. The N^1-methyl series (IIIa–*f*) cannot undergo this intramolecular hydrogen bonding. Therefore, the free carbonyl group of compounds IIIa–*f* can interact with water to a greater extent through intermolecular hydrogen bonding which reduces protein binding and biliary excretion.

The two-way analysis of variance also revealed that the C-5 substitution effect was significant for the rate constant and the percentages of radioactivity in the carcass, GI tract, and kidneys.

Since the C-5 substitution was significant for these measured parameters, correlation analysis between the molecular weight of the C-5 substituent and the amount of radioactivity found in the GI tract was performed.

The correlation analysis revealed that when the C-5 substituent of either the N^1-H series (II) or the N^1-methyl series was aliphatic, increasing the molecular weight of the technetium 99*m* complex increased biliary excretion. The correlation coefficient for the N^1-H aliphatic series is 0.86; for the N^1-methyl series it is 0.77. When the C-5 substituent was aromatic, an increase in molecular weight of the technetium 99*m* complex decreased biliary excretion. For the N^1-H series, the correlation coefficient is −0.90; for the N^1-methyl series it is −0.62.

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ACKNOWLEDGMENTS

Abstracted in part from a thesis submitted by C. M. DeJuliis to the Graduate School of the University of South Carolina in partial fulfillment of Master of Science degree requirements.