New Compounds: Fatty Acid and Long Chain Hydrocarbon Derivatives Containing a Strong Chelating Agent

WILLIAM C. ECKELMAN *‡x, STEPHEN M. KARESH *, and RICHARD C. REBA *‡

Abstract □ Fatty acid and long chain hydrocarbon analogs that contain a strong chelating group were synthesized as part of an effort to determine if the biological function of the fatty acid can be used to transport metallic isotopes to the myocardium.

Keyphrases □ Fatty acid analogs containing chelating groups—synthesis, potential radiopharmaceutical imaging agents □ Chelating agents—synthesis of fatty acid and long chain hydrocarbon analogs

Radiolabeled fatty acid analogs have been suggested as myocardial imaging agents for the detection of myocardial infarcts. Preliminary results with radioiodinated fatty acids indicate that infarcts can be detected by external counting of the emitted γ-rays (1–3). However, a combination of poor physical properties and expense has prevented the widespread use of any of the various isotopes of iodine in the compounds.

A technetium compound (^{99m}Tc, $t_{1/2}$ 6 hr, 140 kev gamma) would overcome these problems, because technetium possesses ideal physical properties and ready availability (4). However, the direct labeling of molecules with technetium usually does not create strong chemical bonds (5, 6). (Carboxymethyl)iminobis(ethylenenitrilo)tetraacetic acid (I) forms the most stable ^{99m}Tc chelate[1] known at this time (7), and even this chelate appears to dissociate to a small extent *in vivo* (8). Therefore, any chelating group that is less stable than the I group will create a weak chelate which would dissociate and result in complicated *in vivo* distribution. However, if I could be attached to a biological substrate such as a fatty acid in such a way as not to diminish the biological activity, then this new biological compound would possess both a functional group to bind technetium and a functional group to bring about localization in the myocardium.

It is not the intent to produce a strict analog of a fatty acid, but rather a molecule that is taken up in the myocardium as a fatty acid but is not completely metabolized as such. This type of molecule apparently could produce even higher concentrations of the radioisotope in the myocardium than the concentration reached by the rapidly metabolized fatty acid (9, 10). This hypothesis appears to be true for radioiodinated cholesterol localization, which is used for external visualization of the adrenal glands (11).

Previous work has shown that alkylating agents can successfully be delivered to hormone responsive tissue by binding the alkylating agent to steroid hormones (12–14).

The group of compounds in this study contain either I or an (ethylenedinitrilo)tetraacetic acid[2] (II)-like structure linked to a fatty acid or long chain hydrocarbon moiety. Ester derivatives of II have been prepared by the reaction of excess alcohol with an acid catalyst (15) and by the reaction of the dianhydride of II and an alcohol (16). No similar derivatives of I have been prepared. The preparation of monoester derivatives of II and I has not been reported. These monoderivatives would possess the optimal chelating strength.

Substitution on the nitrogen atom of I-like structures can be obtained by reacting ethylenediamine or diethylenetriamine with the appropriate alkyl bromide before carboxymethylation. The synthesis of (*N*-*n*-octyl)iminobis(ethylenenitrilo)tetraacetic acid has been reported (17).

Four classes of compounds have been synthesized (V–VII; IX and X; XI and XIII; and XIV), and all have been shown[3] to chelate strongly carrier-free radioisotopes such as ^{57}Co, ^{99m}Tc, and ^{113m}In.

EXPERIMENTAL

The physical properties[4] of the compounds are given in Table I; elemental analyses[5] are given in Table II.

***N*-*n*-Hexadecyldiethylenetriamine Trihydrochloride (III)**—A solution of diethylenetriamine (50 ml, 0.5 mole) and *n*-bromohexadecane (30.6 g, 0.10 mole) in ethanol (50 ml) was heated to reflux. A solution of sodium hydroxide (4.5 g) in water (6 g) was added dropwise over 5 min, and then the mixture was refluxed for 3 hr. The product was extracted into the organic layer after addition of *n*-butanol (40 ml), benzene (40 ml), and water (200 ml). The organic layer was washed twice with water, dried, and distilled at 180° at 0.05 mm pressure. The product was dissolved in ether (200 ml) and converted to the trihydrochloride with dry hydrogen chloride gas. The precipitated product was washed with ether.

***N*-*n*-Octadecyldiethylenetriamine Trihydrochloride (IV)**—A solution of diethylenetriamine (50 ml, 0.50 mole) and *n*-bromooctadecane (33.3 g, 0.10 mole) in ethanol (50 ml) was reacted according to the procedure for III.

(*n*-Dodecyl)iminobis(ethylenenitrilo)tetraacetic Acid Monohydrate (V)—A solution of sodium chloroacetate (15.4 g, 0.132 mole) and 4-*n*-dodecyldiethylenetriamine (10 g, 0.037 mole) was heated at 50° for 6 hr at pH 11.0 ± 0.5, maintained by the addition of sodium hydroxide (18). A 1.0-ml aliquot of the reaction mixture was titrated at pH 12 with 0.100 *N* calcium chloride solution to the permanent turbidity end-point, using sodium oxalate as the indicator. The reaction mixture was then adjusted to the chelation concentration that titrates 80 mg of calcium carbonate/ml of solution. Sulfuric acid (1:4) was added to the solution at 80° to a final pH of 2.6, and the solution was seeded with a crystal of I and cooled overnight at 5°. The precipitate was washed with water.

(*n*-Hexadecyl)iminobis(ethylenenitrilo)tetraacetic Acid

[1] ^{99m}Tc-Diethylenetriaminepentaacetic acid (DTPA).

[2] Ethylenediaminetetraacetic acid (EDTA).

[3] Unpublished data.

[4] Determined with the following instruments: Thomas-Hoover capillary melting-point apparatus (melting points are uncorrected), Perkin-Elmer model 337 grating infrared spectrophotometer, and Varian model A-60A spectrometer. Potentiometric titrations were performed using a Corning glass electrode, a Corning model 8 pH meter, and a Heathkit chart recorder.

[5] Performed by F. Kasler, University of Maryland.

Table I—Physical Properties of New Compounds

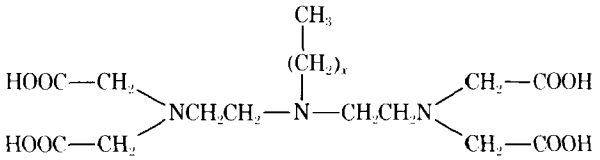
Compound	Yield, %	Recrystallization Solvent	Melting Point	IR Data, cm^{-1}
III	50	Water–isopropanol (4:1)	257–258°	1605, 2850, 2890
IV	37	Water–isopropanol (4:1)	251–254°	1605, 2850, 2890
V	55	Water–isopropanol (4:1)	159–160°	1640, 1730, 2850
VI	64	Water–isopropanol (4:1)	—	1650, 1730, 2848
VII	62	Water–isopropanol (4:1)	—	1650, 1730, 2848
VIII	95	Insoluble	193–195° dec.	1760, 1810, 1130
IX	49	Benzene	103–104°	1740, 1750, 2850
X	50	Benzene	105–106°	1740, 1750, 2850
XI	80	Methanol	149–151°	1600, 1730, 2850
XII	67	Ethyl acetate	94–95°	1685, 2850, 2910
XIII	72	Methanol	148–151°	1620, 1720, 2850
XIV	6	Water–isopropanol (4:1)	160–161°	1655, 1740, 2850
XV	84	Insoluble	182° dec.	1780, 1830, 2960

Table II—Elemental Analysis of New Compounds

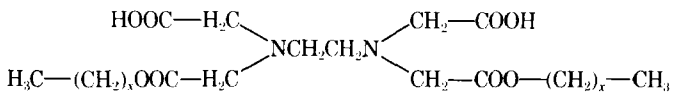
Compound	Molecular Formula	Calculated, %			Found, %		
		C	H	N	C	H	N
III	$C_{20}H_{48}Cl_3N_3$	54.97	11.07	9.61	55.25	11.22	9.30
IV	$C_{22}H_{52}Cl_3N_3$	56.82	11.27	9.04	56.97	11.24	8.78
V	$C_{24}H_{47}N_3O_9$	55.26	9.08	8.06	55.75	9.11	8.09
VI	$C_{28}H_{57}N_3O_{10}$	56.45	9.64	7.05	56.91	9.47	6.85
VII	$C_{30}H_{61}N_3O_{10}$	57.76	9.85	6.74	58.25	9.70	6.70
VIII	$C_{10}H_{12}N_2O_6$	46.87	4.73	10.93	46.68	4.88	10.82
IX	$C_{42}H_{80}N_2O_8$	67.38	10.74	3.93	67.41	11.02	3.80
X	$C_{46}H_{88}N_2O_8$	68.71	11.01	3.64	68.95	11.30	3.50
XI	$C_{40}H_{73}N_2O_{12.5}$	61.43	9.40	3.58	61.23	9.80	3.64
XII	$C_{16}H_{32}O_3$	70.52	11.84	—	70.66	12.05	—
XIII	$C_{42}H_{76}N_2O_{12}$	62.97	9.56	3.50	62.87	9.74	3.25
XIV	$C_{28}H_{51}N_3O_{11}$	55.52	8.49	6.94	55.47	8.58	7.18
XV	$C_{14}H_{19}N_3O_8$	47.06	5.36	11.74	46.25	5.37	11.46

Dihydrate (VI)—Compound III (23.1 g, 0.053 mole), sodium chloroacetate (37.5 g, 0.32 mole), and $Na_2CO_3 \cdot 10H_2O$ (30.5 g, 0.110 mole) were dissolved in water (100 ml) and heated at 95° for 24 hr (19). The product was isolated as for V.

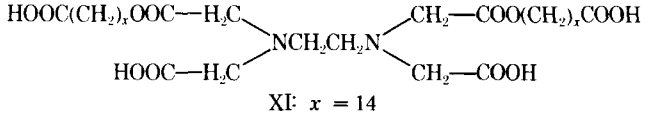
(*n*-Octadecyl)iminobis(ethylenenitrilo)tetraacetic Acid Dihydrate (VII)—A solution of IV (24.6 g, 0.053 mole), sodium chloroacetate (37.5 g, 0.320 mole), and $Na_2CO_3 \cdot 10H_2O$ (30.5 g, 0.110 mole) was dissolved in water (100 ml) and then heated at 95° for 24 hr. The product was isolated as for V.



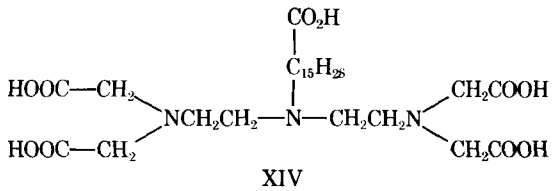
V: $x = 11$
VI: $x = 15$
VII: $x = 17$



IX: $x = 15$
X: $x = 17$



XI: $x = 14$
XIII: $x = 15$



XIV

Dianhydride of II (VIII)—Compound II (182 g, 0.71 mole) was suspended in pyridine (300 g), acetic anhydride (260 g, 2.54 moles) was added, and the mixture was stirred at 65° for 24 hr. The product was filtered, washed with acetic anhydride and ether, and dried.

***N,N'*-Dihexadecyl Ester of II (IX)**—A mixture of the dianhydride of II (32 g, 0.125 mole) and *n*-hexadecanol (60.5 g, 0.250 mole) in benzene (315 ml) was refluxed for 24 hr and then filtered hot. The collected precipitate was washed with boiling benzene, and the total filtrate was cooled to produce the crystalline product.

***N,N'*-Dioctadecyl Ester of II (X)**—A mixture of the dianhydride of II (32 g, 0.125 mole) and *n*-octadecanol (67.5 g, 0.250 mole) in benzene (315 ml) was refluxed for 24 hr, and the product was isolated according to the procedure for IX.

***N,N'*-Di(14-carboxytetradecyl) Ester of II (Hemihydrate) (XI)**—A mixture of 15-hydroxypentadecanoic acid (2.50 g, 0.010 mole) and the dianhydride of II (1.00 g, 0.004 mole) in benzene (15 ml) was refluxed for 18 hr. The precipitate was filtered, washed with boiling benzene, and dried.

16-Hydroxypalmitic Acid (XII)—A solution of juniperic acid lactone (9.1 g, 0.036 mole) in ethanol (10 g) was mixed with sodium hydroxide (5 *N*, 25 ml) and water (25 ml) and refluxed for 4 hr. The reaction mixture was washed with ethyl acetate and then acidified with 6 *N* HCl to pH 1.00. The product was then extracted into ethyl acetate.

***N,N'*-Di(15-carboxypentadecyl) Ester of II (XIII)**—A solution of XII (1.506 g, 0.006 mole) in benzene (15 ml) was mixed with the dianhydride of II (0.707 g, 0.003 mole) and refluxed for 16 hr. The resulting precipitate was filtered, washed with boiling benzene, and dried.

***N* - (15 - Carboxy - 9 - pentadecenyl)iminobis(ethylenenitrilo)-tetraacetic Acid Monohydrate (XIV)**—A solution of 16-bromo-9-hexadecenoic acid (10 g, 0.030 mole) and diethylenetriamine (16.5 ml, 0.150 mole) was mixed in ethanol (15 ml) and heated to reflux. A solution of sodium hydroxide (1.5 g) in water (1.8 g) was added dropwise over 5 min, and reflux was continued for 4 hr. The

product was isolated as the trihydrochloride according to the procedure for III. It was then carboxymethylated according to the procedure for V.

(Carboxymethyl)iminobis(ethylenenitrilo)tetraacetic Acid Dianhydride[6] (XV)—Compound I (39.3 g, 0.10 mole) was suspended in pyridine (50 g), and acetic anhydride (40.8 g, 0.40 mole) was added. The mixture was heated at 65° for 24 hr. The product was filtered, washed with acetic anhydride and ether, and dried.

REFERENCES

(1) J. R. Evans, R. W. Gunton, and R. G. Baker, *Circ. Res.*, **16**, 1(1965).

(2) R. W. Gunton, J. R. Evans, and R. G. Baker, *Amer. J. Cardiol.*, **16**, 482(1965).

(3) F. J. Bonte, K. D. Graham, and J. G. Moore, *Radiology*, **108**, 195(1973).

(4) R. Richards, "Radioactive Pharmaceuticals," CONF-651111, Atomic Energy Commission, 1966, pp. 323–334.

(5) W. C. Eckelman and P. Richards, *J. Nucl. Med.*, **13**, 202(1972).

(6) P. E. Valk, C. A. Dilts, and J. McRae, *ibid.*, **14**, 235(1973).

(7) W. C. Eckelman and P. Richards, *ibid.*, **11**, 761(1970).

(8) J. F. Klopper, W. Hauser, and H. L. Atkins, *ibid.*, **13**, 107(1972).

(9) J. H. Bragdon and R. Gordon, Jr., *J. Clin. Invest.*, **37**, 574(1958).

(10) C. L. Malmendier, *ibid.*, **41**, 185(1962).

(11) R. J. Blair, W. H. Beierwaltes, and L. M. Lieberman, *J. Nucl. Med.*, **12**, 176(1971).

(12) L. F. Larinov, S. A. Degteva, and N. A. Lesnaia, *Vop. Onkol.*, **8**, 12(1962).

(13) S. A. Degteva, *ibid.*, **10**, 52(1964).

(14) M. E. Wall, G. S. Abernethy, and F. I. Carroll, *J. Med. Chem.*, **12**, 180(1969).

(15) A. Badinand, A. Boucheule, and C. Charbonnier, *Soc. Chim. Fr., Bull., 5 Ser.*, **1960**, 382.

(16) W. S. Lennon, U.S. pat. 3,497,535 (1970); through *Chem. Abstr.*, **72**, P120264X(1970).

(17) V. F. Vasil'eva, O. Y. Lavrova, and N. M. Dyatlova, *Zh. Obshch. Khim.*, **36**, 724(1966).

(18) A. Vanyolos, *Rev. Chim. (Bucharest)*, **6**, 378(1955); through *Chem. Abstr.*, **50**, 16671f(1956).

(19) F. Münz, U.S. pat. 2,130,505 (1938).

[6] DTPA dianhydride.

ACKNOWLEDGMENTS AND ADDRESSES

Received July 18, 1974, from the *Department of Nuclear Medicine, Washington Hospital Center, Washington, DC 20010, and the ‡Department of Radiology, George Washington University, Washington, D.C.

Accepted for publication October 8, 1974.

Adapted from a dissertation to be submitted by S. M. Karesh to the Graduate School, University of Maryland, in partial fulfillment of the Doctor of Philosophy degree requirements.

Supported in part by the Research Foundation of the Washington Hospital Center, the Women's Auxillary of the Washington Hospital Center, and National Institutes of Health Grant GM 16262.

The authors thank Dr. W. J. Rzeszotarski and Dr. A. Mauger for many helpful discussions. This work was performed at the George Hyman Memorial Research Building.

[x] To whom inquiries should be directed (at the Washington Hospital Center).

COMMUNICATIONS

Nonclassical Phase Transfer Behavior of Phenylbutazone

Keyphrases □ Phenylbutazone—nonclassical phase transfer behavior, pH and buffer effects, dissolution and ionization rates □ Dissolution—phenylbutazone, nonclassical phase transfer behavior □ Ionization—phenylbutazone, nonclassical phase transfer behavior

To the Editor:

Lovering and Black (1, 2) recently alluded to the nonclassical behavior of phenylbutazone in its transfer through a dimethylsiloxane membrane and through an everted rat intestine as a function of pH.

I measured the ionization rates of phenylbutazone in aqueous buffered solution (μ = 0.1) at 25 ± 0.2° using a stopped-flow spectrophotometer[1] and found the protonation of the phenylbutazone anion and the deprotonation of phenylbutazone to be *noninstantaneous*[2]. As expected, both protonation and deprotonation were highly dependent on the pH of the solution as well as buffer concentration.

As an example, the half-life for the deprotonation of phenylbutazone (taken to zero buffer concentration) at pH 5.5 was 55 msec and it was 67 msec at pH 7.0. The half-life for protonation at pH 3.5 was 10.3 msec, while at pH 4.0 it was 22.6 msec. Phenylbutazone is a carbon acid[3] of pKa 4.50–4.70 (3–5), and the ionization rates of carbon acids are slow relative to the approximately diffusion-controlled ionization rates of other acids (6–10).

Apart from the phase transfer anomalies noted here, the chemical properties of carbon acids often show anomalies when compared to other acids. For example, carbon acids show large negative deviations

[1] Durrum stopped-flow spectrophotometer with a thermostated cell and syringes maintained at 25 ± 0.2°.

[2] The word noninstantaneous is used to describe phenomena taking place at rates considerably slower than the diffusion-controlled limit of $\sim 2 \times 10^{10}$ M^{-1} sec^{-1}.

[3] Carbon acids are acids in which the dissociating proton is bound to a carbon atom instead of a heteroatom such as oxygen or nitrogen.