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Synthesis and Therapeutic Testing of Mono- and Dialkyl Esters of Pentetic (Diethylenetriaminepentaacetic) Acid for Decorporation of Polymeric Plutonium

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Abstract □ The synthesis, characterization, and therapeutic evaluation of a series of partially esterified derivatives of pentetic (diethylenetriaminepentaacetic) acid are reported. These compounds were prepared in an attempt to promote increased decorporation of insoluble colloidal forms of plutonium, which are not removed by pentetic acid alone. The dimethyl, diethyl, dibutyl, dioctyl, and monoethyl esters were synthesized by reaction of the appropriate alcohol with the dianhydride of pentetic acid. These esters were injected intravenously into mice as their calcium chelates in saline. None of the esters was effective in removing plutonium from the liver. All esters removed approximately 20% of the plutonium in the skeleton. However, when the esters were given together with pentetic acid, only the dioctyl ester showed enhanced removal of plutonium compared to pentetic acid alone. The small increase in effectiveness and the increased acute toxicity make these esters of limited practical interest in plutonium decorporation therapy.

Keyphrases □ Pentetate esters, various—synthesized, evaluated for ability to decorporate polymeric plutonium in mice □ Plutonium, polymeric—decorporation in mice, various pentetate esters evaluated □ Structure-activity relationships—various pentetate esters evaluated for ability to decorporate polymeric plutonium in mice □ Chelating agents—various pentetate esters synthesized, evaluated for ability to decorporate polymeric plutonium in mice

The disposition kinetics of the easily hydrolyzable toxic radiometal plutonium following accidental exposure depend on the physicochemical form to which an individual is exposed as well as the route of exposure. Systemically deposited plutonium (*i.e.*, plutonium that has reached the circulation and is subsequently deposited in the tissues) is generally considered to be soluble ("monomeric") plutonium. However, aggregated insoluble forms of plutonium have been observed at later times in the liver and spleen (1, 2). As a model for insoluble systemically deposited plutonium, a colloidal ("polymeric") plutonium preparation is injected intravenously in animals. A large fraction of this plutonium is rapidly deposited in organs rich in reticuloendothelial elements, particularly the liver (3).

BACKGROUND

Pentetic acid (diethylenetriaminepentaacetic acid or DTPA), *N,N*-bis[2-[bis(carboxymethyl)amino]ethyl]glycine, administered intravenously as the calcium chelate trisodium salt (I), presently is considered the treatment of choice for accidental exposure to certain multivalent lanthanide and actinide radioelements such as plutonium. This drug has also been used for the treatment of lead poisoning, acute iron intoxication, and iron storage disease (4). Although I has been effective in removing soluble plutonium from the liver and, to a lesser extent, from the skeleton (5, 6), this chelating agent has not been successful in decorporating the more insoluble polymeric plutonium (7). Since hepatically deposited polymeric plutonium is primarily associated with lysosomes (8) and I is predominantly distributed in the extracellular space (9) and is rapidly excreted in the urine (10), it was reasoned that increased intracellular uptake of I might place a sufficient concentration of ligand at the site of plutonium deposition to promote increased plutonium decorporation.

Previously, two different approaches were attempted to increase the uptake of I into cells. Markley (11), using the pentaethyl ester of pentetic acid, found that this more lipid-soluble form removed additional plutonium from the mouse liver beyond that removed with I alone. When I and its pentaethyl ester were administered together, their effects in the liver were approximately additive, suggesting that each form was acting on a separate and distinct fraction of hepatic plutonium. However, the pentaethyl ester was much more toxic than I. In the second approach, I encapsulated in phospholipid liposomes was injected intravenously. The encapsulated I deposited intracellularly in the liver (12) to a large

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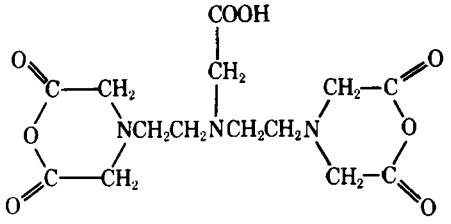
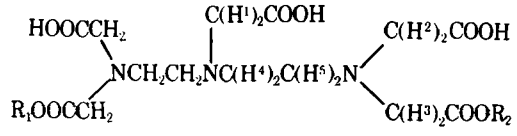


II



III–VII

extent and removed plutonium from the liver that could not be removed by I alone (13).

The present study was designed to test the possibility that partial esterification of pentetic acid with alcohols of varying chain length might provide compounds less toxic than the pentaethyl ester while retaining effective adjunct potency for removal of polymeric plutonium from the liver. A group of new mono- and diesters of pentetic acid was synthesized for experimental use in plutonium chelation therapy. Preparation of the esters was patterned after the synthesis of corresponding diesters of edetic acid (14, 15). The dianhydride (II) of pentetic acid was used as an intermediate; II reacts smoothly with primary alcohols to provide diesters III–VI (superscript numbers refer to NMR assignments) in good yield.

EXPERIMENTAL

Materials—Methanol and ethanol were purified by distillation from their respective magnesium alkoxides. Reagent grade pyridine and dimethylformamide were dried by storage over freshly activated molecular sieves[1]. Reagent grade pentetic acid[2], 1-butanol, and 1-octanol were used directly.

Methods—Melting points were determined on a hot-stage microscope[3] and are uncorrected. Samples to be characterized by NMR[4] spectroscopy were prepared in 99.5% deuterium oxide at a concentration of 5–10% (w/v); the pD was adjusted to 7.4 by the addition of 1 *M* KOD, Na_2CO_3, or DCl. Shifts are recorded in τ *versus* sodium 3-trimethylsilyl-2,2,3,3-tetradeuteropropionate. Elemental analyses were performed elsewhere[5].

Syntheses—*Dianhydride of Pentetic Acid (II)*—*N,N*-Bis[2-(2,6-dioxo-4-morpholinyl)]ethylglycine was prepared by previously published methods (15, 16).

Dimethyl Ester of Pentetic Acid (III)—A 100-mmole sample of II was refluxed with 250 ml of anhydrous methanol for 4 hr (drying tube), cooled, filtered, washed with 100 ml of methanol, and dried *in vacuo* at 75° overnight to provide 39.1 g (93%) of amorphous white powder, mp 194–195°; NMR: H^1 (6.13, s), H^2 (6.67, s), H^3 (6.37, s), H^4 (6.60, t), H^5 (6.91, t), and OCH_3 (6.17, s) ppm.

Anal.—Calc. for $C_{16}H_{27}N_3O_{10}$: C, 45.50; H, 6.46; N, 9.97. Found: C, 45.50; H, 6.47; N, 9.87.

Diethyl Ester of Pentetic Acid (IV)—A 105-mmole sample of II was refluxed (drying tube) in 250 ml of anhydrous ethanol for 1 hr. The cloudy solution was filtered hot through a medium frit glass filter with pressure. The product crystallized immediately upon cooling the filtrate and was recrystallized from 200 ml of anhydrous ethanol and dried *in vacuo* for 24 hr at 60°. A 39.2-g sample (83%) of white powder, mp 143–144°, was obtained; NMR: H^1 (6.13, s), H^2 (6.67, s), H^3 (6.39, s), H^4 (6.59, t), H^5 (6.90, t), OCH_2 (5.80, q), and CH_3 (8.72, t) ppm.

Anal.—Calc. for $C_{18}H_{31}N_3O_{10}$: C, 48.10; H, 6.95; N, 9.35. Found: C, 47.66; H, 7.11; N, 9.26.

Dibutyl (V) and Dioctyl (VI) Esters of Pentetic Acid—A mixture of 54 mmoles of II, 150 mmoles of the respective 1-alcohol, and 30–50 ml of anhydrous dimethylformamide was stirred at 80° for 4 hr. After precipitating slowly from the cooled clear amber solutions, the products were recrystallized from methanol and dried *in vacuo* to yield white powders: V, 60%, mp 154–156°; and VI, 68%, mp 153–156°; NMR (V): H^1 (6.14, s), H^2 (6.67, s), H^3 (6.39, s), H^4 (6.60, t), H^5 (6.91, t), OCH_2 (5.82); CH_2 (8.35 and 8.63), and CH_3 (9.08, q) ppm; NMR (VI): H^1 (6.13, s), H^2 (6.70, s), H^3 (6.41, s), H^4 (6.56, t), H^5 (6.97, t), OCH_2 (5.91), CH_2 (8.36 and 8.69), and CH_3 (9.12, q) ppm.

Anal.—Calc. for $C_{22}H_{39}N_3O_{10}$ (V): C, 52.26; H, 7.78; N, 8.31. Found: C, 52.16; H, 7.79; N, 8.33. Calc. for $C_{30}H_{55}N_3O_{10}$ (VI): C, 58.32; H, 8.97; N, 6.80. Found: C, 57.86; H, 8.88; N, 7.04.

Monoethyl Ester of Pentetic Acid (VII)—When a sample of IV was refluxed in 95% ethanol, a precipitate appeared. This material, mp 142–144°, was not recrystallized, but elemental analysis and the NMR spectrum showed it to be VII, contaminated with small amounts of pentetic acid; NMR: H^1 (6.15, s), H^3 (6.39, s), H^4 (6.59, t), H^5 (6.85, t), OCH_2 (5.74, q), and CH_3 (8.72, t) ppm. The two H^2 signals in VII were overlapped by corresponding signals from pentetic acid.

Anal.—Calc. for $C_{16}H_{27}N_3O_{10}$: C, 45.60; H, 6.46; N, 9.99. Found: C, 45.96; H, 6.44; N, 9.87.

Solutions for Injection—A quantity of each ester (III–VII) sufficient to give a final concentration of 100 mg/ml (except VI, which gave 50 mg/ml) was weighed into a volumetric flask and slurried with a stoichiometric amount of calcium carbonate and water until foaming subsided; 1.0 *M* Na_2CO_3 then was added dropwise to adjust the pH to 7.00 ± 0.05. The resulting solution was diluted to volume, filtered (fine-glass frit), and refrigerated at 5° until use. Similar solutions were prepared in deuterium oxide and examined by NMR spectroscopy. The results showed that negligible hydrolysis had occurred during preparation and that less than 10% of the esters were hydrolyzed after 60 days.

Biological Studies—Female $B6CF_1$/Anl mice, 80–100 days old, were injected with 1.0 μCi of polymeric plutonium/kg iv, which was 20% ultrafilterable through a cellophane membrane (17). Five days later, groups of five mice each were treated with I (0.25 mmole/kg) intraperitoneally, one of the esters intravenously (0.25 mmole/kg for all esters except VI, which was given at 0.12 mmole/kg), or both. Treatments with I, IV, or VII were continued twice weekly for 4 weeks, with III or V twice weekly for 3 weeks and with VI for 2 weeks. Toxicological considerations dictated the differences in treatment schedules. There were two plutonium control groups. In the first, the mice were treated with saline both intravenously and intraperitoneally. In the second, the mice received saline intravenously and I intraperitoneally.

The mice were sacrificed at 33 days after plutonium injection. Liver, spleen, lungs, and femurs (two per mouse) were removed, weighed, and assayed radiochemically for plutonium activity using published procedures (18). Preliminary toxicity testing of the new compounds was performed to determine a maximum injected dose that should not be acutely lethal to the test animals. This estimated dose was obtained by injecting small groups of animals (generally two to four) initially with a dose corresponding to the intravenous LD_{50} dose for I (*i.e.*, 4 mmoles/kg[6]). If all animals died, the dose was consecutively halved until a dose was reached where all animals survived. This dose was chosen as the initial therapeutic dose.

RESULTS AND DISCUSSION

The diesters III–VI and the monoester VII, reported here for the first time, were obtained as pure crystalline or amorphous compounds and were characterized by elemental analysis and NMR spectroscopy. The NMR spectra were virtually identical, differing only in features assigned to the different alkoxy groups. The monoester VII was prepared from IV. Refluxing IV in 95% ethanol slowly hydrolyzed the diester, resulting in the precipitation of the insoluble VII. The lower esters III, IV, and VII were moderately water soluble as the free acids; upon neutralization, all compounds were soluble to at least 0.1 *M*.

Attempts were made to activate the central carboxyl group of II to prepare symmetrical monoesters or triesters. The conditions employed included a larger excess of acetic anhydride, higher reaction temperatures, and trifluoroacetic anhydride instead of acetic anhydride at room temperature. Extensive decomposition to malodorous tars occurred in each case.

The limited toxicity testing of the synthesized compounds, based on a single intravenous injection, indicates that all of the esterified compounds were more acutely toxic than I. Compared to I, IV was about twice as toxic, III and VII were about three times, V was about six times, and

[1] Linde 4-A, Linde Division, Union Carbide Corp., New York, N.Y.
[2] G. Frederich Smith Chemical Co., Columbus, Ohio.
[3] E. Leitz, Inc., Rockleigh, NJ 07647.
[4] Model HR-220, Varian Associates, Palo Alto, Calif.
[5] Clark Microanalytical Laboratory, Urbana, Ill.

[6] Unpublished data.

Table I—Effect of Pentetic Acid Esters on Plutonium Content of Livers and Femurs of Mice

Compounds Administered[a]	Percent of Controls ± SD[b]		Significance for Femur Comparison[c]
	Liver	Femur	
Saline alone (control)[d]	24.5 ± 3.3	3.18 ± 0.36	
I alone (control)[d]	24.9 ± 1.1	2.29 ± 0.26	
III alone	115 ± 17	81.7 ± 10.8	$p < 0.02$
III + I	106 ± 7	90.5 ± 14.2	N.S.
IV alone	103 ± 4	79.3 ± 8.2	$p < 0.01$
IV + I	102 ± 3	106 ± 10	N.S.
V alone	105 ± 16	81.8 ± 10.9	$p < 0.02$
V + I	97.0 ± 6.7	84.0 ± 12.5	N.S.
VI alone	115 ± 16	82.8 ± 14.8	$p < 0.05$
VI + I	99.8 ± 12.5	84.1 ± 12.1	$p < 0.05$
VII alone	92.2 ± 4.5	79.7 ± 8.7	$p < 0.01$
VII + I	104 ± 5	106 ± 7	N.S.

[a] Compounds III–VII were injected intravenously, and I was given intraperitoneally; comparable saline injections were substituted for I in "alone" groups. [b] Percent figures were obtained by comparison of the ester "alone" groups with saline-treated controls and the ester + I groups with the I alone group. Standard deviations of the ratios were obtained by a propagation of error approximation technique as specified by Deming (20). [c] The test of independent means of treated and control groups (N.S. = no significant difference at $p < 0.05$). [d] Percent of injected dose ± *SD*.

VI was about 10 times as toxic. However, all esters were significantly less toxic than the pentaethyl ester of pentetic acid.

Table I summarizes the results obtained in trials designed to measure removal of polymeric plutonium from mouse liver and skeleton, using each of the esters, either alone or as an adjunct to I. Treatment efficacy is expressed as percent of the respective control level. With respect to the plutonium burden in the liver at 33 days, there was no effect of treatment with any of the esters, whether given alone or with I; nor did treatment with these esters have any significant effect on the plutonium content of the lung or spleen. In the skeleton, however, treatment with III, IV, or VII resulted in about a 20% reduction in the plutonium content of the femurs as compared to saline-treated controls. These reductions were statistically significant. When these esters were given together with I, there was no removal beyond that attributable to I alone. In contrast, treatment with V or VI resulted in a 15–20% reduction of the femur plutonium burden compared to the respective controls whether these esters were given alone or with I.

The objective of this study, removal of polymeric plutonium from the liver, was not achieved using any of the partially esterified compounds III–VII. Since Markley (11) previously demonstrated that the fully esterified pentaethyl ester of pentetic acid could remove a fraction of hepatic plutonium not removed by I, it seems reasonable to assume that the chemical modifications of I to the incompletely esterified forms were not sufficient to achieve the therapeutic action of the pentaethyl ester. This result may be due to the fact that esterification of one or two of the five acid groups does not sufficiently reduce the charge density of the molecule to enhance its cellular membrane transport. On the basis of present evidence, it is assumed that the effectiveness of the neutral pentaethyl ester is due to its increased intracellular uptake by plutonium-bearing liver cells followed by hydrolysis of the ester groups with "reactivation" of the ionic ligand within the vicinity of the target plutonium. This action is followed by chelation and subsequent excretion of the more soluble plutonium chelate.

An unexpected result was the demonstration of adjunct effectiveness of V and VI for removal of skeletal plutonium. The varying effectiveness of the esters tested suggests that partial esterification may have produced differences in their metabolism and/or distribution as compared to the unesterified ligand, I. Compounds III, IV, and VII, when given alone, removed skeletal plutonium; but when given together with I, they exerted no additional effect, suggesting that partial esterification with methyl or ethyl groups did little to alter the therapeutic effect of pentetic acid, at least as related to the removal of skeletal plutonium. Thus, the therapeutic effect noted when the esters were given alone was probably due to a chelating action similar to that of I. If III, IV, and VII behave like the ionic I, then adjunct potency would not be expected since the administered dose of I was shown previously to effect maximum removal of plutonium from the skeleton (19). In effect, the excess ligand, whether as acid or ester, was wasted.

Although the administration of either V or VI, given alone or with I, resulted in increased removal of skeletal plutonium, speculation on the mechanism of action of these esters seems inappropriate, particularly in view of the marginal effect produced. However, these esters are now being tested against other toxic metals, and one of the compounds (V) may be of interest for the decorporation of intracellularly deposited lead.

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