A Study of the Relationship Between Chemical Structure and Bone Localization of Tc-99m Diphosphonic Acids: Concise Communication

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This study was undertaken to evaluate the effect on bone localization of substitutions at the bridge carbon atom of methylenediphosphonic acid. Two monosubstituted compounds (ethylidenediphosphonic acid and benzylmethylenediphosphonic acid), and one disubstituted compound (isopropylidenediphosphonic acid), were synthesized. Their biological behavior was compared with that of MDP and dichloro-MDP in the rat, using Tc-99m as a label. Ethylidenediphosphonic acid showed the highest femur-to-liver and femur-to-muscle ratios. MDP exhibited a slightly faster blood clearance than ethylidenediphosphonic acid.

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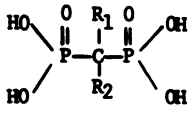
Technetium-99m-labeled methylenediphosphonic acid (MDP) has been reported to be a superior agent for skeletal imaging (*1*). A monosubstitution of the methylene hydrogen of MDP with alkylamino, arylamino, or hetrocyclic groups has been studied in the hope of improving the property of bone localization (*2*). The objective of the present study was to evaluate the effect on bone localization of substitutions, such as monomethyl, monobenzyl, dimethyl, and dichloro groups, at the bridge carbon atom of MDP. We therefore synthesized ethylidenediphosphonic acid (compound A), benzylmethylenediphosphonic acid (compound B), and isopropylidenediphosphonic acid (compound C). These were compared with MDP and dichloromethylenediphos-



	R_1	R_2
MDP	H	H
Compound A	H	CH_3
Compound B	H	CH_2-C_6H_5
Compound C	CH_3	CH_3
Compound D	Cl	Cl

FIG. 1. Chemical structures of diphosphonic acids.

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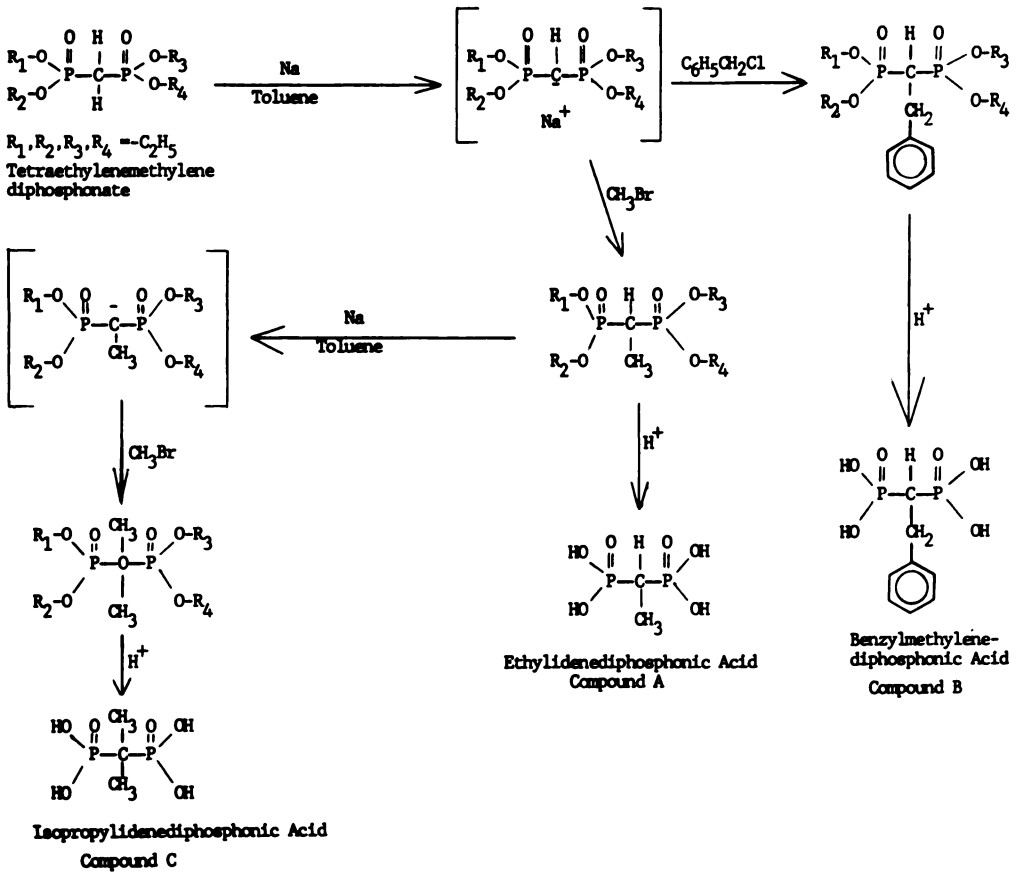

FIG. 2. Synthetic schemes of compounds A, B, and C.

phonic acid (compound D) for bone-seeking properties. Their structures are shown in Fig. 1.

METHODS

Syntheses. (Fig. 2) (*3,4*) Tetraethylethylidene diphosphonate, sodium (11.5 g, 0.5 mole) was dispersed in boiling toluene (300 ml) with high-speed stirring, then cooled to room temperature. To this dispersion tetraethylmethylene diphosphonate* (144.1 g, 0.5 mole) was added dropwise. External cooling in an acetone-dry-ice bath maintained temperature at 25-30°C. When the reaction was complete, bromomethane(52.3 g, 0.55 mole) in 100 ml toluene was added slowly to the solution while the temperature was held below 30°C. (This reaction is highly exothermic and requires considerable cooling.) When the addition was complete, the reaction mixture was heated to 80°C for 4 hr and then cooled to room temperature. The precipitate (NaBr) was filtered out and the filtrate concentrated in vacuo. The residue was diluted with water and extracted three times with ethyl acetate. The extracts were combined and concentrated in vacuo. The oily residue was distilled under vacuum. The product was collected at 145-149°C/0.5 mm. The yield was 74.3 g (49.2%). Analyses for carbon, hydrogen, and phosphorus are shown in Table 1.

Ethylidenediphosphonic acid (A). A mixture of tetraethylethylidene diphosphonate (6.0 g, 20 millimoles) and concentrated hydrochloric acid (40 ml) was refluxed for 3 hr. The reaction mixture was cooled to room temperature and the acid evaporated in vacuo. The residue was washed with acetone, then with petroleum ether (30-60°C). The product was crystallized and recrystallized from ethanol:ether (1:1); mp 179-181°C. The yield was 3.1 g (83%). Analyses and NMR spectra are shown in Table 1.

Tetraethylbenzylmethylene diphosphonate was prepared by a method similar to that used for tetraethylethylidene diphosphonate, except that benzyl chloride (69.7 g, 0.55 mole) was used instead of bromomethane. The product was distilled at 178-180°C/0.5 mm. The yield was 98.4 g (51.5%). Analyses for carbon, hydrogen, and phosphorus are shown in Table 1.

Benzylmethylenediphosphonic acid (B) was prepared using the same procedure as for the preparation of ethylidenediphosphonic acid. The yield was 4.5 g (84.3%). Mp 210-213°C. Analyses for carbon, hydrogen, and phosphorus are shown in Table 1.

Tetraethylisopropylidene diphosphonate was prepared by a method similar to that used for synthesis of tetraethylethylidene diphosphonate, reacting equal moles of sodium, tetraethylethylidene diphosphonate, and

TABLE 1. DATA FROM ANALYSES, NMR SPECTRA, AND LABELING EFFICIENCIES, OF TETRAETHYL ESTERS AND DIPHOSPHONIC ACIDS*

Compound	Formula	% Carbon		% Hydrogen		% Phosphorus		NMR (P-31)†	Tc-99m labeling efficiency
		Calculated	Found	Calculated	Found	Calculated	Found		
Tetraethylethylidene diphosphonate	$C_{10}H_{24}O_6P_2$	39.74	39.53	8.00	7.96	20.50	20.48		
Tetraethylbenzylmethylene diphosphonate	$C_{16}H_{28}O_6P_2$	50.26	50.55	7.38	7.41	16.20	16.13		
Tetraethylisopropylidene diphosphonate	$C_{11}H_{26}O_6P_2$	41.77	41.60	8.27	8.49	19.76	19.84		
Ethylidenediphosphonic acid (Compound A)	$C_2H_8O_6P_2$	12.64	12.66	4.24	4.21	32.60	32.20	24.38 ppm from $O{=}P(OCH_3)_3$	95%
Benzylmethylenediphosphonic acid (Compound B)	$C_8H_{12}O_6P_2$	36.11	36.32	4.55	4.40	23.28	23.50		95%
Isopropylidenediphosphonic acid (Compound C)	$C_3H_{10}O_6P_2$	17.66	17.49	5.29	5.10	30.36	30.30	27.97 ppm from $O{=}P(OCH_3)_3$	95%

* Microanalyses by Galbraith Laboratories Inc., Knoxville, TN.
† Joel-PS-100 FT 40 MHz for P-31. Proton was tritiated.

bromomethane. The product was isolated at 163–166°C/0.5 mm. The yield was 97.7 g (61.8%). Analyses for carbon, hydrogen, and phosphorus are shown in Table 1.

Isopropylidenediphosphonic acid (C) was prepared in a fashion similar to that used for preparing ethylidenediphosphonic acid. The product was crystallized and recrystallized from acetone:ether (40:60). The yield was 2.9 g (71.3%). Mp 227–229°C. Analyses and NMR spectra are shown in Table 1.

Labeling. Compounds A, B, C, D (dichloromethylenediphosphonic acid†), and MDP were labeled with Tc-99m in the presence of tin (II) as follows: 10 mg of each acid were dissolved in 5 ml of normal saline. To this solution was added 0.1 ml of 0.1% $SnCl_2$ in normal HCl solution. Sodium [^{99m}Tc]pertechnetate, 5–6 mCi, was then added and the pH adjusted to 6.5–7.0 with 5% sodium bicarbonate solution. The product was then passed through a 0.22-μm sterile Swimmex filter. Labeling efficiencies (Table 1) were determined by instant thin layer chromatography in acetone and saline systems (*5*).

Animal studies. Tissue distribution studies were performed in at least six rats (Sprague-Dawley) for each compound. The rats were killed 2 hr after i.v. injection of 0.3–0.4 ml (150–250 μCi, 10–15 μg of acid) of the labeled radiopharmaceutical. The organs of interest were removed, weighed, and radioactivity content was measured in a well scintillation counter. The data are summarized in Table 2. Venous blood samples were obtained for clearance studies in at least three rats at 15, 30, 60, and 120 min after injection (Fig. 3).

RESULTS AND DISCUSSION

Analytical data of the synthesized compounds, la-

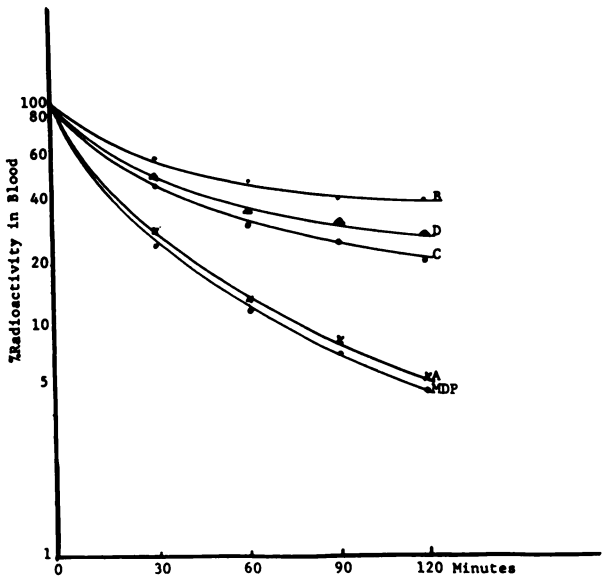

FIG. 3. Blood clearance of several Tc-99m diphosphonic acids in rats.

TABLE 2. TISSUE DISTRIBUTION OF Tc-99m DIPHOSPHONIC ACIDS IN RATS* AT 2 HR AFTER I.v. INJECTION

Organ	MDP†	% injected dose per gram tissue			
		A	B	C	D
Blood	0.025 ± 0.009	0.029 ± 0.008	0.214 ± 0.071	0.053 ± 0.008	0.054 ± 0.010
Liver	0.021 ± 0.012	0.016 ± 0.005	0.062 ± 0.010	0.029 ± 0.010	0.025 ± 0.010
Spleen	0.023 ± 0.007	0.018 ± 0.004	0.048 ± 0.011	0.028 ± 0.004	0.040 ± 0.014
Kidney	0.827 ± 0.210	0.882 ± 0.325	1.361 ± 0.373	0.668 ± 0.173	0.720 ± 0.102
Heart	0.027 ± 0.009	0.024 ± 0.006	0.055 ± 0.008	0.021 ± 0.005	0.064 ± 0.011
Lung	0.032 ± 0.012	0.025 ± 0.007	0.086 ± 0.014	0.040 ± 0.017	0.064 ± 0.011
Muscle	0.009 ± 0.004	0.006 ± 0.002	0.053 ± 0.004	0.011 ± 0.003	0.012 ± 0.007
Femur	1.027 ± 0.176	1.261 ± 0.123	0.771 ± 0.078	0.759 ± 0.098	0.691 ± 0.079
		Target-to-nontarget ratios (g/g)			
Femur/blood	40.45	42.98	3.60	14.30	12.80
Femur/liver	48.70	75.91	12.31	26.18	26.80
Femur/muscle	107.04	203.24	14.51	65.46	56.67

* Average of six rats. Mean ±s.d.
† For MDP, A, B, C, D, see text.

beling efficiencies, and blood clearance data are summarized in Table 1 and Fig. 3. The distribution studies revealed that increasing bone localization occurred in the order B < D < C < MDP ≃ A. Ethylidenediphosphonic acid (A) showed the highest femur-to-liver and femur-to-muscle ratios. Its blood clearance, however, was slightly slower than that of MDP. Compounds C and D were also found to bind with Tc-99m, but exhibited only half the amount of radioactivity in bone and twice the amount in the blood compared with MDP or ethylidenediphosphonic acid at 2 hr after injection.

A single-crystal structure of MDP has been studied

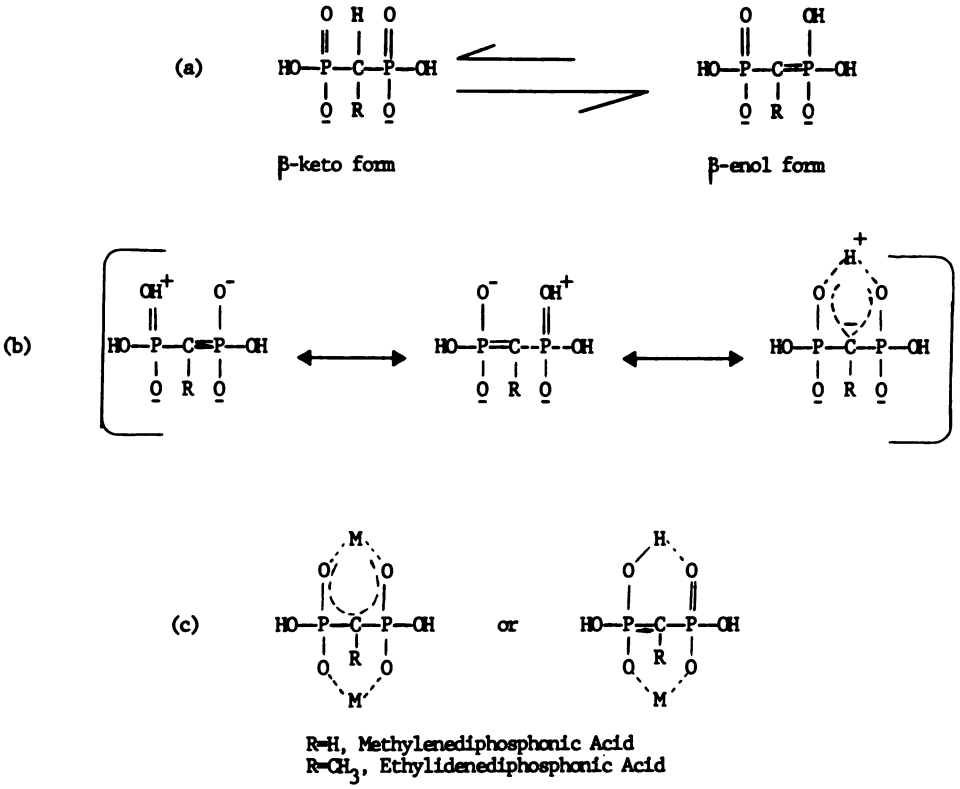

FIG. 4. Tautomeric, resonance, and chelating structures of MDP and ethylidenediphosphonic acid.

by DeLaMatter and coworkers, who suggested that it might have a staggered configuration (*6*). However, ethylidenediphosphonic acid (A) has been studied by Uchtman and coworkers, who suggested it might have an eclipsed configuration (*7*). Formation of Tc-99m diphosphonate chelate appears first to use the phosphoryl oxygen from each phosphonate group. The second binding site, meanwhile, may be created in the case of MDP and ethylidenediphosphonic acid by "keto-enol" type of tautomeric structure. There are at least three factors that could contribute to stabilization of the postulated enolization (Fig. 4): (a) conjugation of the carbon-phosphorus double bond with the second phosphorus group, (b) resonance structures, and (c) chelation of the enol form by either a metal or an intramolecular hydrogen bond between the enolic hydroxy and the second phosphonyl oxygen.

Our studies indicate strong similarities between MDP and ethylidenediphosphonic acid. Both MDP, with its suggested staggered configuration, and ethylidenediphosphonic acid, with its suggested eclipsed configuration (*6,7*), undergo excellent binding with Tc-99m. The possibility exists that "keto-enol" tautomerism in MDP and ethylidenediphosphonic acid may also play an active role in the metal binding and the resultant bone localization, since compounds that lack such tautomerism,—i.e., benzylmethylenediphosphonic acid (B), isopropylidenediphosphonic acid (C), and dichloromethylenediphosphonic acid (D)—exhibited poor localization in bone. It is hoped that these data relating chemical structure to bone-seeking potential may lead to the design of superior bone-imaging agents.

FOOTNOTES

* Orgamet Inc., East Hemptead, NH.
† Proctor & Gamble Co., Cincinnati, OH.
‡ P-L. Biochemical Laboratories, Inc., Milwaukee, WI.

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