Structural Factors in the *In Vivo* Chelate Mobilization of Aged Cadmium Deposits

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The role of structural factors in determining the relative efficacy of dithiocarbamates as chelating agents for the *in vivo* mobilization of aged cadmium deposits is examined for 23 newly synthesized compounds of this type. The critical feature in determining the efficacy of the compounds in mobilizing intracellular cadmium is the balance between hydrophobic and hydrophilic groups. This balance also governs the other properties of these compounds such as the organ specificity of action and the relative propensity to carry cadmium to the brain. The transport of cadmium to the brain by dithiocarbamate can be greatly reduced by the incorporation of appropriate hydrophilic groups that prevent the formation of lipid-soluble cadmium complexes that pass readily into the brain. If the chelating agents carry an additional ionic charge, their ability to pass through cellular membranes and react with intracellular deposits of cadmium is significantly reduced, with other structural factors being equal. The structural features that optimize mobilization of cadmium from the kidney do not appear to be identical with those that optimize its mobilization from the liver. The correlation of cadmium-mobilizing properties of these chelating agents with the sum of the Hansch π constants for the parts of the molecular structures other than the dithiocarbamate grouping ($\Sigma\pi$) is reasonably good for the removal of renal cadmium by derivatives of D-glucamine and D-xylamine. Another aspect of the molecular structure that appears to play a role is the presence of uncharged polar groups having the ability to form hydrogen bonds. The relevance of these factors in designing chelating agents to enhance the excretion of other toxic metals from their intracellular sites is discussed.

Introduction

Human ingestion of toxic metals from the environment is a matter of continuing concern. For many such metals, treatments based on using chelating agents are available for use in clinics. In general, the success of the treatment depends upon the time interval between exposure or ingestion of the toxic metal and the initiation of treatment. Early treatment allows that metal which is ingested but not absorbed, for example, from the gastrointestinal tract, to be more effectively removed. As this time interval increases, the amount of metal absorbed increases, and a greater proportion of the absorbed metal can reach intracellular sites where its removal is often difficult. For many toxic metals there are very few chelating agents that have been used successfully in the treatment of heavy metal toxicity when such treatment is delayed for an extended period of time. As a result, the structural requirements needed to insure efficacy in such compounds are not clearly understood. We have, accordingly, undertaken the study of an extensive series of dithiocarbamates in the mobilization of cadmium from its aged deposits in mice in order to sort out the structural factors that are favorable to the efficacy of such compounds when treatment is delayed. The results obtained may serve as a guide in designing more effective chelating agents for the delayed treatment of toxicity because of other environmentally important toxic metals.

The ability of sodium diethyl dithiocarbamate (DDTC) to act as an antagonist for acute cadmium chloride intoxication was first reported by Gale and his co-workers (*1*). Subsequent studies have confirmed and extended these results and showed that dithiocarbamates are capable of reacting with and mobilizing intracellular deposits of cadmium (*2–10*), a significant environmental poison. The ability of the cadmium complex, that is formed with DDTC, to pass through the blood-brain barrier results in an increase in the brain levels of cadmium in such a situation (*4*). It was shown that the use of dithiocarbamates bearing more polar substituents on the nitrogen prevented this passage of the dithiocarbamate-cadmium complex through the blood-brain barrier (*5,8*).

Subsequent to these findings a large number of dithiocarbamates have been developed, which are capable of removing aged cadmium from its intracellular deposits in the liver, the kidney, and some other organs, without simultaneously transferring any of the cadmium to the brain. For most of the compounds providing data, they

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are found to enhance the biliary excretion of cadmium (*11*) and cause an appreciable increase in the fecal excretion of cadmium (*3,7,12–15*). These results are of general interest because the majority of therapeutic chelating agents used to remove toxic heavy metals from the mammalian body are unable to gain access to such intracellular deposits.

While the typical approach to developing more efficacious therapeutic chelating agents for toxic metals is via a systematic study of the factors that affect the stability constants of the metal complexes that are formed (*16–19*), this approach has not been taken in the present study because of the solubility problems that arise in determining the stability constants of dithiocarbamate-cadmium complexes (*20*). Instead, we have taken an approach where the effect of structural variations on biological properties, associated with a specific functional group in a molecule, have been used to indicate the direction in which future synthetic effort should be directed. This has been useful because, in the present case at least, the pharmacological properties of the chelating agent are of key significance in determining whether that compound is able to gain access to, and to mobilize intracellular deposits of cadmium *in vivo*. Following ingestion, cadmium moves rapidly to intracellular sites (*21*) where typical water-soluble chelating agents (even those with very high values for their stability constants with cadmium) are unable to remove it (*22–26*). Thus, an approach based solely on structural variations designed to optimize the stability constant of the complex of the cadmium ion with the chelating agent was shown not to be a fruitful approach in early studies (*22–25*). The only type of chelating group, other than dithiocarbamate that has been shown capable of mobilizing intracellular cadmium, is the vicinal dithiol group, which has been examined in some detail by Cherian and his co-workers (*27,28*). The mobilization of intracellular cadmium is also a problem in which the rates of the various processes involved are critical in determining the extent of cadmium mobilization that is achieved (*29*). The present study started from the demonstrated ability of the dithiocarbamate moiety to remove cadmium from its aged deposits *in vivo*.

It is customary in most structure-activity studies to determine the concentration at which a given biological effect is obtained with different compounds. Studies in which a toxic metal is removed from animals would require an enormous number of animals, many experiments, and an extended period of time for each compound for the collection of such data. It is much more convenient, as well as customary, to use a standard set of conditions in which all the animals are loaded the same way with the toxic metal and then each of the groups is subjected to a standard mobilization procedure with the administration of exactly the same molar dosage of chelating agent. The results of such experiments allow the chelating agents to be ranked on the basis of relative efficacy (for each of the important organs if necessary), and then this relative efficacy can be examined to determine the correlation of the trend of mobilizing ability with various structural changes.

Another problem that arises with such correlations involving many chelating agents is the presence of ionic groups. Such groups are essential for proper functioning of the chelating agents and can rarely be eliminated from the structures. Since it is generally considered that the assignment of π contributions for such polar groups is difficult, the values for such groupings are usually not found in standard compilations. While estimates for the values of these substituent constants can be obtained from the literature (*15,30*), the manner in which they are to be incorporated into standard compilations is somewhat arbitrary. An additional complication that arises with several of the compounds prepared here is the variation in the values for aromatic-aliphatic combinations of the sort used here (*31*); thus the π value of the benzyl group is quite dependent on the group attached to it, and these values have not been estimated for the dithiocarbamates. The net result is that structure-activity studies can be carried out on these systems, but not in quite the same manner as is customary with a series of electrically neutral organic compounds.

Experimental Section

Preparation of N-Substituted Xylamines (7a–m), and Glucamines (10f–h, j–m).

All the substituted xylamines and glucamines were prepared by following a reported procedure (*47*) with some variation (*34*). In general, for Schiff base formation, D-(+)xylose (6) or α-D-(+)-glucose (9) is allowed to react with a slight excess (ca. 1–2%) of amine (5a–m) until gel formation or a viscous oily reaction mixture is obtained to ensure completion of the reaction. Hydrogenation was carried out as usual (*34*) in the presence of platinum oxide catalyst. In cases where solid product could not be isolated, the highly viscous oil was used as such in the next step (Tables 1 and 2).

Preparation of Dithiocarbamates (2, 4a,b, 8a–m, 11f–h, j–m)

The reported (*33,34*) procedure was successfully employed to obtain dithiocarbamates as white solids. For the preparation of 2 and 4a,b, 2 mole of NaOH was used, whereas for the rest (8a–m, and 11f–h, j–m) only 1 mole was used. The amines were dissolved preferably in 1,4-dioxane, but occasionally methanol was used due to solubility reasons. The products were characterized by IR, ^{1}H NMR(300 MHz) and elemental analyses (Tables 3–5).

Animal Studies

Male ICR mice (Harlan Industries, Indianapolis, IN) weighing 31 $\pm$ 2 g were loaded with cadmium via a series of IP injections by administering one 1-mg and three 3-mg

Table 1. Substituted xylamines 7a–m, $RNHCH_2(CHOH)_3CH_2OH$.

Compound number	R	Yield, %	Melting point, °C
7a	$C_6H_5CH_2$	81.3	44–46
7b	$3\text{-}CF_3C_6H_4CH_2$	75.5	82–85
7c	$4\text{-}CH_3C_6H_4CH_2$	81.6	91–94
7d	$4\text{-}CH_3OC_6H_4CH_2$	71.9	75–77
7e	$4\text{-}ClC_6H_4CH_2$	72.6	54–57
7f	$C_6H_5(CH_2)_3$	95.0[a]	(Highly viscous oil)[b]
7g	$C_6H_5(CH_2)_4$	96.0[a]	(Highly viscous oil)[b]
7h	$CH_3(CH_2)_6$	63.9	70–72
7i	$CH_3(CH_2)_6$	73.9	71–73
7j	$CH_3(CH_2)7$	83.0	75–77
7k	$CH_3(CH_2)_9$	68.7	82–84
7l	$c\text{-}C_6H_{11}$(cyclohexyl)	50.8	108–110
7m	$O(CH_2CH_2)_2N(CH_2)_3$	95.0[a]	(Viscous oil)[b]

[a]Yield refers to crude product which was used as such in the next step.
[b]Obtained as viscous oil and could not be crystallized.

Table 2. Substituted glucamines 10 f–h,j–m, $RNHCH_2(CHOH)_4CH_2OH$.

Compound number	R	Yield, %	Melting point, °C
10f	$C_6H_5(CH_2)_3$	40.9	147–149
10g	$C_6H_5(CH_2)_4$	49.4	123–124
10h	$CH_3(CH_2)_5$	61.0	120–122
10j	$CH_3(CH_2)_7$	70.7	121–123
10k	$CH_3(CH_2)_9$	35.8	122–124
10l	$c\text{-}C_6H_{11}$(cyclohexyl)	50.8	138–140
10m	$O(CH_2CH_2)_2N(CH_2)_3$	73.1	113–115

Table 3. Dithiocarbamates of amino acids 2, 4a,b.

Compound number	R	Formula, anal.	Yield, %	Melting point, °C	^1H NMR (D_2O/DSS), δ, ppm
$2 \cdot 0.5\ H_2O$	—	$C_{10}H_9NNa_2O_2S_2 \cdot 0.5\ H_2O$ (C,H,N,S[a])	65.0	>250	7.32–7.14 (m, 5H), 5.28 (s, 2H), 4.43 (s, 2H)
$4a \cdot 0.0\ H_2O$	H	$C_6H_7NNa_2O_2S_2 \cdot 0.0\ H_2O$ (C,H,N,S)	95.0	>300	4.84–4.81 (m, 1H), 3.96–3.75 (m, 2H), 2.38–2.26 (m, 1H), 2.09–1.95 (m, 3H)
$4b \cdot 1.2\ H_2O$	OH	$C_6H_7NNa_2O_3S_2 \cdot 1.2\ H_2O$ (C[b],H,N,S[c])	90.8	191–195	4.83 (t, J=7.7 Hz, 1H), 4.55–4.49 (m, 1H), 4.17–4.13 (2t, J=13.2, 1.4 Hz, 1H), 3.97–3.93 (2d, J=13.2, 4.9 Hz, 1H), 2.44–2.35 (m, 1H), 2.27–2.18 (m, 1H)

[a]S, calculated 21.79 (found 19.96). Analysis not within ± 0.40%.
[b]C, calculated 26.41 (found 27.10). Analysis not within ± 0.40 %.
[c]S, calculated 23.50 (found 23.07). Analysis not within ± 0.40%.

Table 4. Substituted xylamine dithiocarbamates 8a–m.

Compound number	R	Formula, anal.	Yield, %	Melting point, °C	^{1}H NMR (D_2O/DSS), δ, ppm
8a • 0.8 H_2O	$C_6H_5CH_2$	$C_{13}H_{18}NNaO_4S_2$ • 0.8 H_2O (C,H,N,S[a])	71.0	185–186	7.44–7.21 (m, 5H), 5.75 (d, J=15.8 Hz, 1H), 5.25 (d, J=15.8 Hz, 1H), 4.44–4.39 (dd, J=13.7, 4.9 Hz, 1H), 4.28–4.23 (m, 1H), 3.98–3.94 (dd, J=14.1, 8.3 Hz, 1H) 3.84–3.79 (m, 1H), 3.67–3.63 (dd, J=11.8, 3.9 Hz, 1H), 3.58–3.50 (m, 2H).
8b • 2.3 H_2O	3-$CF_3C_6H_4CH_2$	$C_{14}H_{17}F_3NNaO_4S_2$ • 2.3 H_2O (C,H,N,S)	43.3	160–162	7.66–7.49 (m, 4H), 5.81 (d, J=16.1 Hz, 1H), 5.32 (d, J=16.1 Hz, 1H), 4.48–4.43 (dd, J=13.8, 4.8 Hz, 1H), 4.31–4.26 (m, 1H), 3.99–3.95 (dd, J=13.7, 8.1 Hz, 1H), 3.86–3.80 (m, 1H), 3.71–3.52 (m, 3H).
8c • 0.7 H_2O	4-$CH_3C_6H_4CH_2$	$C_{14}H_{20}NNaO_4S_2$ • 0.7 H_2O (C,H,N,S)	69.9	215–217	7.25 (d, J=8.1 Hz, 2H), 7.19 (d, J=8.1 Hz, 2H), 5.71 (d, 15.6 Hz, 1H), 5.19 (d, J = 15.6 Hz, 1H), 4.41–4.36 (dd, J=13.8, 4.9 Hz, 1H), 4.25–4.20 (m, 1H), 3.96–3.92 (dd, J=13.7, 7.9 Hz, 1H), 3.84–3.78 (m, 1H), 3.66–3.62 (dd, J=11.7, 3.8 Hz, 1H), 3.57–3.48 (m, 2H), 2.32 (s, 3H).
8d • 0.0 H_2O	4-$CH_3OC_6H_4CH_2$	$C_{14}H_{20}NNaO_5S_2$ • 0.0 H_2O (C,H,N,S)	83.2	197–198	7.26 (d, J=8.6 Hz, 2H), 7.00 (d, J=8.6 Hz, 2H), 5.69 (d, J=15.4 Hz, 1H), 5.18 (d, J=15.4 Hz, 1H), 4.40–4.35 (dd, J=13.7, 4.9 Hz, 1H), 4.25–4.19 (m, 1H), 3.96–3.92 (dd, J=13.7, 7.9 Hz, 1H), 3.82 (s, 3H), 3.81–3.77 (m, 1H), 3.66–3.62 (dd, J=11.8, 4.0 Hz, 1H), 3.58–3.48 (m, 2H).
8e • 1.0 H_2O	4-$ClC_6H_4CH_2$	$C_{13}H_{17}ClNNaO_4S_2$ • 1.0 H_2O (C,H,N,S)	50.7	186–187	7.39 (d, J=8.4 Hz, 2H), 7.23 (d, J=8.4 Hz, 2H), 5.71 (d, J=15.9 Hz, 1H), 5.21 (d, J=15.9 Hz, 1H), 4.43–4.39 (dd, J=13.8, 4.8 Hz, 1H), 4.29–4.23 (m, 1H), 3.97–3.92 (dd, J=13.4, 8.1 Hz, 1H), 3.86–3.80 (m, 1H), 3.69–3.65 (dd, J=11.8, 3.9 Hz, 1H), 3.60–3.51 (m, 2H).
8f • 1.2 H_2O	$C_6H_5(CH_2)_3$	$C_{15}H_{22}NNaO_4S_2$ • 1.2 H_2O (C,H,N,S[b])	47.3	65–70	7.42–7.20 (m, 5H), 4.42–4.18 (m, 3H), 4.04–3.79 (m, 3H), 3.79–3.46 (m, 3H), 2.67 (t, 2H), 2.15–2.00 (m, 2H).
8g • 1.2 H_2O	$C_6H_5(CH_2)_4$	$C_{16}H_{24}NAO_4S_2$ • 1.2 H_2O (C,H[c] N,S[d])	69.0	182–184	7.38–7.22 (m, 5H), 4.39–4.34 (dd, J=13.6, 5.2 Hz, 1H), 4.29–4.22 (m, 2H), 3.98–3.56 (m, 6H), 3.53–3.51 (m, 1H), 2.66 (t, J=7.4 Hz, 2H), 1.77–1.59 (m, 4H).
8h • 1.0 H_2O	$CH_3(CH_2)_5$	$C_{12}H_{24}NNaO_4S_2$ • 1.0 H_2O (C,H,N,S)	75.0	146–148	4.43–4.38 (dd, J=13.6, 5.0 Hz, 1H), 4.30–4.20 (m, 2H), 4.01–3.97 (dd, J=13.5, 7.8 Hz, 1H), 3.89–3.80 (m, 2H), 3.65–3.54 (m, 3H), 1.74–1.70 (m, 2H), 1.30 (br, s, 6H), 0.86 (t, J=7.0 Hz, 3H).
8i • 0.8 H_2O	$CH_3(CH_2)_6$	$C_{13}H_{26}NNaO_4S_2$ • 0.8 H_2O (C,H,N,S)	88.6	155–157	4.48–4.43 (dd, J=13.6, 5.2 Hz, 1H), 4.27–4.24 (m, 2H), 4.05–3.96 (m, 1H), 3.90–3.82 (m, 2H), 3.74–3.54 (m, 3H, 1.74–1.70(m, 2H), 1.30 (br, m, 8H), 0.86 (t, J=6.5 Hz, 3H)
8j • 0.8 H_2O	$CH_3(CH_2)_7$	$C_{14}H_{28}NNaO_4S_2$ • 0.8 H_2O (C,H,N,S)	82.8	154–156	4.44–4.40 (dd, 1H), 4.27–4.21 (m, 2H), 4.01–3.97 (dd, 1H), 3.86–3.80 (m, 2H), 3.75–3.56 (m, 3H), 1.75–1.70 (m, 2H), 1.30 (m, br, 8H), 0.86 (t, 3H).
8k • 0.5 H_2O	$CH_3(CH_2)_9$	$C_{16}H_{32}NNaO_4S_2$ • 0.5 H_2O (C,H,N,S)	89.8	153–155	4.53–4.48 (dd, 1H), 4.31–4.21 (m, 2H), 3.99–3.93 (m, 2H), 3.79–3.73 (m, 2H), 3.67–3.56 (m, 2H), 1.80–1.68 (m, br, 2H), 1.29 (m, 14H), 0.88 (t, J=6.5 Hz, 3H).
8l • 0.4 H_2O	c-C_6H_{11}	$C_{12}H_{22}NNaO_4S_2$ • 0.4 H_2O (C,H,N,S[e])	67.7	165–167	5.48 (t, 1H), 4.34–4.12 (m, 3H), 3.87–3.55 (m, 4H), 1.84–1.81 (m, 4H), 1.64 (d, 1H), 1.52–1.28 (septet, 4H), 1.17 (t, 1H).
8m • 0.0 H_2O	$O(CH_2CH_2)_2N(CH_2)_3$	$CC_{13}H_{25}N_2NaO_5S_2$ • 0.0 H_2O (C,H,N,S)	89.5	189–191	4.43–4.39 (dd, 1H), 4.36–4.22 (m, 2H), 4.02–3.81 (m, 3H), 3.77–3.51 (m, 7H), 2.56 (br, s, 4H), 2.42 (t, 2H), 2.04–1.90 (m, 2H).

[a]S, calculated 18.12 (found 18.72). Analysis not within ± 0.40%.
[b]S, calculated 16.48 (found 17.77). Analysis not within ± 0.40%.
[c]H, calculated 6.60 (found 6.14). Analysis not within ± 0.40%.
[c]S, calculated 15.91 (found 14.15). Analysis not within ± 0.40%.
[e]S, calculated 18.94 (found 18.11). Analysis not within ± 0.40%.

Table 5. Substituted glucamine dithiocarbamates 11f–h, j–m.

Compound number	R	Formula, anal.	Yield, %	Melting point, °C	^{1}H NMR (D_2O/DSS), δ, ppm[a]
11f	$C_6H_5(CH_2)_3$	$C_{16}H_{24}NNaO_5S_2 \cdot 1.25\ H_2O$ (C,H,N,S)	75.6	179–181.5	7.39–7.24 (m, 5H), 4.42–4.26 (m, 3H), 3.93–3.60 (m, 7H), 2.66 (t, 2H), 2.11–2.01 (m, 2H).
11g	$C_6H_5(CH_2)_4$	$C_{17}H_{26}NNaO_5S_2 \cdot 1.0\ H_2O$ (C,H,N,S)	86.1	177–179.5	7.39–7.22 (m, 5H), 4.42–4.27 (m, 3H), 3.92–3.60 (m, 7H), 2.67 (t, 2H), 1.79–1.57 (m, 4H).)
11h	$CH_3(CH_2)_5$	$C_{13}H_{26}NNaO_5S_2 \cdot 0.9\ H_2O$ (C,H,N,S)	65.0	153–155	4.76 (dd, 1H), 4.35–4.20 (m, 2H), 3.93-3.62 (m, 7H), 1.72 (br, s, 2H), 1.30 (br, s, 6H), 0.86 (br, t, 3H).
11j	$CH_3(CH_2)_7$	$C_{15}H_{30}NNaO_5S_2 \cdot 0.0\ H_2O$ (C,[b]H,N,S)	85.7	146–150	*4.67–4.62 (dd, 1H), 4.43–4.32 (m, 1H), 4.26–4.17 (m, 1H), 3.87–3.70 (m, 6H), 3.65–3.60 (dd, 1H), 1.86–1.67 (m, 2H), 1.39–1.22 (m, br, 10H), 0.90 (t, 3H).
11k	$CH_3(CH_2)_9$	$C_{17}H_{34}NNaO_5S_2 \cdot 0.5\ H_2O$ (C,H,N,S)	72.6	157–160	*4.66–4.63 (dd, 1H), 4.43–4.33 (m, 1H), 4.25–4.18 (m, 1H), 3.87–3.59 (m, 7H), 1.86–1.70 (m, 2H), 1.34 (br, s, 14H), 0.91 (t, 3H).
11l	c-C_6H_{11}	$C_{13}H_{24}NNaO_5S_2 \cdot 1.0\ H_2O$ (C,H,N,S)	76.3	169–170	5.48 (t, 1H), 4.24–4.10 (m, 3H), 3.82–3.76 (m, 4H), 3.66–3.60 (m, 1H), 1.83–1.75 (m, 4H), 1.63 (d, 1H), 152–1.27 (septet, 4H), 1.19–1.11 (m, 1H).
11m	$O(CH_2CH_2)_2N(CH_2)_3$	$C_{14}H_{27}N_2NaO_6S_2 \cdot 0.0\ H_2O$ (C,H,N,S)	88.1	154–156	4.48–4.43 (dd, 1H), 4.37–4.29 (m, 2H), 2.95–3.86 (m, 2H), 3.83–3.73 (m, 8H), 3.67–3.61 (m, 1H), 2.56 (t, 4H), 2.42 (t, 2H), 2.02–1.92 (m, 2H).

[a]In d_4-MeOH. Analysis not within ± 0.40%.
[b]C, calculated 46.02 (found 46.56).

injections $CdCl_2 \cdot 2.5\ H_2O$/kg in 0.5 mL of 0.9% saline on 4 consecutive days. After a 3-day interval the animals were randomly divided into groups of five each. One group that had not been given cadmium served as a normal control and was given 0.9% saline for all injections. One group in each experimental set served as thecadmium control group and was given 0.9% saline injections instead of chelating agent. Each other group was given an IP injection of one of the chelating agents at a level of 1.0 mmole/kg each day for 5 consecutive days. Each injection contained the appropriate amount of chelating agent in 0.5 mL of 0.9% saline. Two days after the last injection of chelating agent, all the animals were sacrificed by cervical dislocation and dissected. Weighed amounts of each animal's liver, kidney, and brain were digested in pure nitric acid on a heating block (80°C), taken to dryness, redissolved in 1% nitric acid, and analyzed for cadmium using a Perkin Elmer model 403 atomic absorption spectrophotometer. Each separate shipment of mice had its own control groups. The SD found for average organ analyses covered the range of 5 to 20% and were generally larger for lower cadmium levels. To bring all of these to a common basis, the relative renal and hepatic cadmium levels for each compound were obtained by dividing the observed cadmium organ levels values by the corresponding levels in the cadmium control groups. The use of none of the compounds reported here resulted in any elevation of cadmium levels in the brains of the animals (data not shown). These levels were of the order of 40 to 70 parts per billion.

Table 6. Relative cadmium mobilizations (Cd treated/ Cd control).[a]

Compound number	Liver	Kidney	$-\Sigma\pi$
2	0.97	0.86	—
4a	1.12	0.67	—
4b	0.94	0.66	—
8a	1.03	0.90	1.09
8b	1.19	0.76	1.97
8c	0.89	0.85	1.65
8d	0.66	0.52	1.07
8e	0.80	0.79	1.80
8f	0.81	0.92	1.83
8g	0.99	0.99	2.25
8h	0.73	0.83	3.21
8i	0.83	1.20	3.75
8j	1.00	1.03	4.29
8k	—[b]	—[b]	2.51
8l	1.04	0.51	2.76
8m	0.74	0.56	0.62
11f	0.61	0.75	1.83
11g	0.48	1.04	2.25
11h	0.94	0.83	3.21
11j	1.17	0.96	4.29
11k	1.12	1.06	5.37
11l	0.80	0.35	2.51
11m	0.77	0.53	0.62

[a]All the compounds were injected at a dosage of 1.0 mmole/kg, IP each day on 5 consecutive days except for 11j (0.4 mmole/kg) and 11k (0.1 mmole/kg) because of their toxicity. The data on these compounds were not used in the correlations.
[b]All animals in this group died after one injection at 1.0 mmole/kg.

All animals were kept in an AALAC-approved animal care facility during the course of the experiments and were provided with free access to food and water. The statistical analysis of the data was carried out using standard analysis of variance and least squares curve-fitting methods.

Results and Discussion

The properties of the newly prepared amines (xylamine and glucamines) are given in Tables 1 and 2. The novel dithiocarbamates derived from them and their characteristics are given in Table 3 (amino acid derivatives), Table 4 (xylamine dithiocarbamates), and Table 5 (glucamine dithiocarbamates). The reactions used in the preparation of these compounds are shown in Figures 1, 2, and 3, respectively.

The search for parameters that correlate with the *in vivo* findings is, most obviously, centered on those related to the presumption that the ability of the dithiocarbamate to pass through the cellular membrane is crucial to the success of the overall decorporation process. This suggests the π parameter of Hansch and Leo (*32*), though a number of related parameters have served in the same fashion (*33,34*). The correlation of relative cadmium levels in the liver and kidney following a standard chelate treatment has been examined. In all cases the summations of the parameters ($\Sigma\pi$) used to characterize the compounds have involved all of those parts of the molecular other than the dithiocarbamate grouping [$-N(CS_2Na)-$]. We have used standard procedures (*32*) to obtain an estimate of the contribution of the morpholine group (8m, 11m) to our $\Sigma\pi$ parameters.

Table 6 contains data on the relative efficacies of these compounds in removing cadmium from the liver and kidney of mice previously loaded with 10 mg $CdCl_2 \cdot 2.5\ H_2O$/kg, IP. For purposes of comparison, sodium *N*-benzyl-D-glucamine dithiocarbamate monohydrate, under comparable conditions, reduced liver levels to 0.78, which are those of the cadmium control animals, and renal levels to 0.51, which are those of the cadmium controls (*34*).

The correlation of the cadmium mobilizing efficacy with the structures of the various dithiocarbamates was examined using the π parameters of Hansch and Leo (*32*), supplemented by values from Martin (*35*) and Tute (*31*) where necessary. Table 6 shows the relative reductions observed in cadmium burdens for both the liver and kidney. Each series of closely related compounds was examined separately. For the glucamine dithiocarbamates, the correlation observed for the reductions in renal cadmium levels with the compounds listed (11f–11m), when combined with data on four compounds published previously (*34*) it was found to be described by the equation:

$$\{Cd_{treated}/Cd_{control}\} = 1.918 + 1.055(\Sigma\pi) + 0.174(\Sigma\pi)^2$$
$$n = 10;\ r = 0.937;\ s = 0.51;\ 0.0001 < p \leq 0.005$$

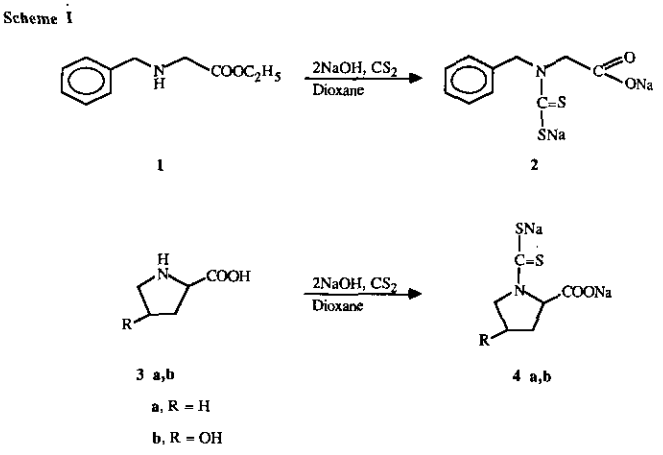

FIGURE 1. Reaction scheme showing the preparation of the compounds, 2, 3a,b, and 4a,b.

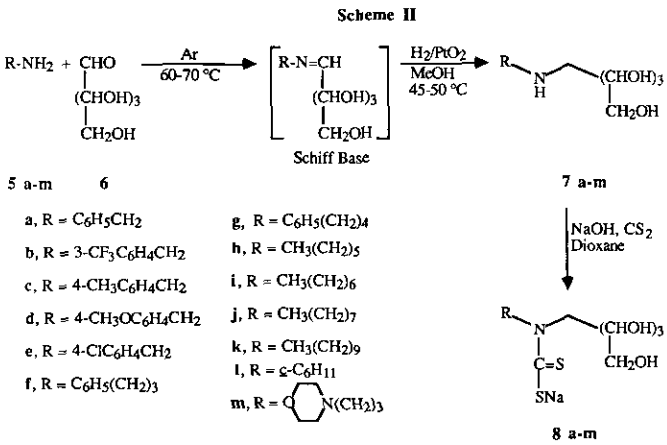

FIGURE 2. Reaction scheme showing the preparation of the xylamines, 7a–m, and the corresponding dithiocarbamates, 8a–m.

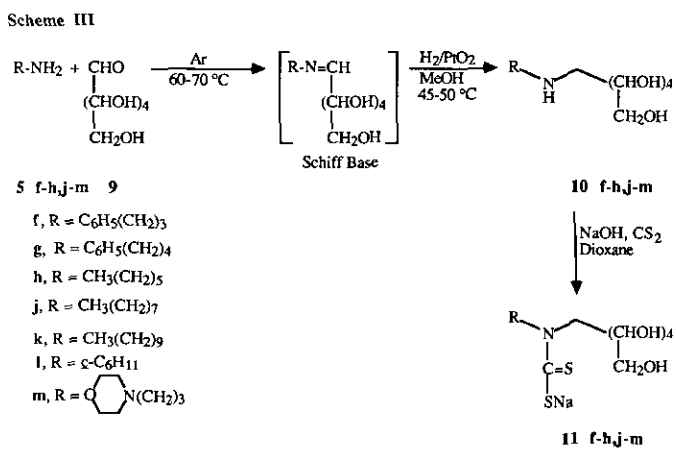

FIGURE 3. Reaction scheme showing the preparation of glucamines 10f–h and 10j–m, and the corresponding dithiocarbamates, 11f–h, and 11j–m.

This relationship has a minimum at $\Sigma\pi = -3.02$, which indicates that the optimally effective compounds (i.e., those which give the lowest residual cadmium levels) are expected to have values of $\Sigma\pi$ which are close to -3.00. For the hepatic data obtained with the glucamine derivative, no reliable correlation with $\Sigma\pi$ was found, though the relative reductions correlated to an extent with the molecular weight (MW) of the dithiocarbamate via the equation:

$$\{Cd_{treated}/Cd_{control}\} = -0.005(MW) + 2.758$$
$$n = 10;\ r = 0.74;\ s = 0.45;\ 0.01 < p \leq 0.025$$

This relationship is consistent with the fact that these compounds cause predominantly biliary excretion, which is favored by higher molecular weights.

The xylamine dithiocarbamates are almost uniformly less effective than the corresponding glucamines. The correlation of $\Sigma\pi$ with renal cadmium level reductions for the xylamines is given by the equation:

$$\{Cd_{treated}/Cd_{control}\} = 1.07 + 0.295(\Sigma\pi) + 0.04(\Sigma\pi)^2$$
$$n = 12;\ r = 0.735;\ s = 0.50;\ 0.025 < p \leq 0.05$$

The lower value of r indicates that this correlation is not so satisfactory as that obtained for renal cadmium levels with the glucamine dithiocarbamates. Only three of the xylamine derivatives were quite effective in reducing renal cadmium levels (8d, 8l, and 8m). Table 6 shows that some compounds that are very effective in reducing hepatic cadmium levels are not effective in reducing renal cadmium levels (e.g., 11g). In general, the xylamine dithiocarbamates are less effective than the glucamine dithiocarbamates, sometimes by very appreciable amounts. Of the newly prepared compounds reported here that are quite effective (8d, 8m, 11l, and 11m), three (8d, 8m, and 11m) are capable of forming hydrogen bonds via oxygen atoms in the substituent group. The fourth compound, 11l, the cyclohexyl derivative, is more effective than its xylamine analog, especially in removing cadmium from the liver. Compounds 2, 4a, and 4b, all of which have ionized carboxylic acid groups at physiological pH, have relatively little effect on hepatic cadmium levels, though they are more effective in reducing kidney cadmium levels. Several of the compounds in Table 6 (i.e., 8d, 8m, 11l, and 11m) are approximately as effective as the parent benzyl-D-glucamine dithiocarbamate. Compound 11l is expected to be very similar structurally to the parent benzyl compound. Dithiocarbamate treatment may cause cadmium in other organs to migrate towards the liver (*36,37*), so a temporary increase in liver cadmium may be found for some dithiocarbamates and dosing schedules.

It is of some interest to examine those compounds that are both better and worse than anticipated. One such compound is the 4-phenylbutyl derivative (11g) of D-glucamine, which is very ineffective in removing renal cadmium and very effective in removing hepatic cadmium. The cyclohexyl derivatives of both xylamine- (8l) and D-glucamine dithiocarbamates (11l) are very effective in removing renal cadmium, but the D-glucamine derivative is superior here, as elsewhere, in removing cadmium from the liver. The dithiocarbamates that carry alkyl chains were quite disappointing, as they were both relatively more toxic and much less effective than anticipated. The fact that none of the alkyl derivatives is among the most effective compounds indicates that, in addition to any requirements based on π (polarity), there are structural or geometric requirements for optimum efficacy that cannot be satisfied easily by an unsubstituted alkyl group. There is a considerable increase in the toxicity and decrease in the efficacy with an increase in the chain length of the purely alkyl xylamine (8h–8k) and D-glucamine (11h–11k) dithiocarbamates.

Because the kidney is a much smaller organ than the liver, a compound that is very effective in mobilizing only hepatic cadmium may produce a much more pronounced decrease in the whole body cadmium-burden than one that is equally effective in mobilizing only renal cadmium. The removal of renal cadmium may prove more important, as renal damage is the first and also the most serious consequence of chronic cadmium intoxication (*38*).

The structural features required for substantially mobilizing hepatic cadmium are not generally coincident with those for renal cadmium. This may be related to the requirements for biliary excretion of the resulting cadmium complexes, where the presence of nonionic polar groups is quite important (*11*). With very few exceptions, of which 11g is the most notable, the enhancement of renal cadmium mobilization is a prerequirement for the enhancement of hepatic cadmium mobilization.

A comparison of the compounds described here with those reported earlier on the mobilization of cadmium by dithiocarbamates is very useful in allowing certain general characteristics of effective and ineffective compounds to be seen more clearly. In an examination of a series of compounds closely related to sodium diethyldithiocarbamate (*6,8*), $R_1R_2NCS_2Na$, it was shown that if $R_1 = R_2 = C_2H_5$ or CH_3, cadmium mobilization was accompanied by the transport of cadmium into the brain; if either R_1 or $R_2 = CH_2COOH$, the compounds were rather ineffective at mobilizing intracellular cadmium. Finally, if either R_1 or R_2 (or both) $= CH_2CH_2OH$, while the other was a methyl, ethyl, or β-hydroxyethyl group, cadmium mobilization occurred with a significant reduction in the cadmium transport to the brain. Of the compounds examined at that time, $(HOCH_2CH_2)_2NCS_2Na$ was the least toxic and carried the least cadmium to the brain (*5,6*). This compound, unfortunately, did not possess satisfactory stability at room temperature. The search for a compound of superior stability lead to $HOCH_2(CHOH)_4N(CH_3)CS_2Na$ which proved to be a compound of low toxicity, excellent stability, and as effective as a cadmium mobilizing agent when given at 2.2 mmole/kg (*9*). Two piperidine derivatives:; $HOCH\text{-}(CH_2CH_2)_2NCS_2Na$ and $H_2NCOCH(CH_2CH_2)_2NCS_2Na$ were also found to be very effective when given at this level (*7,39*).

A series of compounds prepared from the primary amines, R_1 = H and R_2 = CH_3, C_2H_5, n-C_3H_7, n-C_4H_9 proved to have the ability to mobilize cadmium, but had poor chemical stability and were too toxic (*40*).

A very significant step was Kojima and his co-workers finding that replacement of the methyl group in $HOCH_2(CHOH)_4CH_2N(CH_3)CS_2Na$ by a benzyl group to give $HOCH_2(CHOH)_4CH_2N(CH_2C_6H_5)CS_2Na$ resulted in a compound that was both significantly more effective and equally nontoxic (*41*). The corresponding 4-methylbenzyl and 4-isopropylbenzyl compounds were also quite effective (*33*). The application of the Topliss procedure to this series of compounds, with a planned range of substitutions on the benzene ring, showed that of the 4-Cl, 4-CH_3O, 4-CH_3, 3-CF_3 derivatives and the parent compound, the 4-CH_3O was significantly superior to all of the others on the basis of reductions it effected in both renal and hepatic cadmium levels (*34*). Studies previously published (*15,33,34,41–43*) showed clearly the advantages of what may be called an amphipathic chelating agent, i.e., one containing both a polar and a nonpolar group on the nitrogen atom. The preparation and examination of the cyclohexyl derivatives (c-C_6H_{11})NR_1-(CS_2Na), where R_1 = $CH_2CH_2SO_3Na$, $CH_2CH_2CH_2SO_3Na$ and $CH_2CH(OH)CH_2SO_3Na$ showed that more than one category of amphipathic carbodithioates is capable of mobilizing cadmium in an impressive fashion (*15*); the higher dosage required for these latter three compounds shows that the presence of a charged group (SO_3) introduces too great a polarity to be adequately balanced by a cyclohexyl group for optimum efficacy. The related compounds 8l and 11l, which bear uncharged polar groups are effective at significantly lower dosage levels.

Of the compounds newly reported here, three (8m, 11l, and 11m) are equal or superior in decreasing hepatic and renal cadmium to sodium *N*-benzyl-D-glucamine dithiocarbamate, reported by Kojima and his co-workers (*41*), which is the most effective of such compounds previously reported from other laboratories.

Conclusion

Cadmium can be mobilized from its aged intracellular deposits in rodents by a variety of dithiocarbamates. The most effective of these are amphipathic chelating agents in which the nitrogen bears both a polar and a nonpolar substituent. The efficacy of a particular compound is dependent upon the balance achieved between these two groups. If the compound bears an ionizable polar group, efficacy decreases, presumably because of the greater difficulty in passing through cellular membranes. If the compounds are too nonpolar, they have an enhanced toxicity and generally cause an increase in the cadmium content of the brain. The most effective compounds include those structures that bear a D-glucamine residue and a substituted benzyl or a 3-(4-morpho-linyl)/propyl residue that is capable of forming hydrogen bonds. Analogous structures bearing a cyclohexyl group are quite effective in removing renal cadmium, but they are less effective in removing cadmium from hepatic sites.

The trend shown in the correlation equations given, where greater efficacies are found for glucamine dithiocarbamates at an optimum $\Sigma\pi$ value around -3.00, as well as the known lower efficacies of compounds bearing charged groups such as SO_3^- or COO^-, which have large negative $\Sigma\Pi$ values, indicates the existence of a region of maximum efficacy as $\Sigma\pi$ changes. Such a trend has been found in many other studies involving these parameters (*31,35*).

The dosage levels at which the most effective of these compounds have demonstrated their efficacy in animal tests are still too high (by a factor of about 5) to be given serious consideration for clinical use. On the basis of the compounds examined so far, it is reasonable to suspect that such a compound can be prepared by the appropriate exploitation of the structural information currently on hand.

The general trends found here may prove useful in the design of chelating agents for the decorporation of other toxic metals that are tightly bound to intracellular sites *in vivo*. It should be noted that the fraction of intracellular cadmium mobilized with the most effective of these chelating agents is quite high. An analogous problem of the mobilization of intracellular deposits of toxic metal is also important in cases of plutonium intoxication (*44,45*), iron overload from repeated transfusions (*16–18*), and lead intoxication (*46*).

Our results suggest that the presence of large numbers of ionized substitutents on a chelate structure can constitute a major deterrent to accessing that chelating agent to intracellular deposits of toxic metals. At the present time the chelating agents used for plutonium and lead and most of those used for iron possess such ionic groups. Thus it is not uncommon to test chelating agents for plutonium mobilization by injecting them into the animals a few minutes after the administration of the plutonium (*44*). It would appear that related chelate structures, without such a preponderance of ionized groups, might prove significantly superior to those compounds currently used or under investigation for these two elements. It must also be realized, however, that the presence of a suitable number of polar (but not necessarily ionic) groups will usually also result in a significant decrease in the toxicity of the basic structure. Our results also suggest that variations of the chelating agent structure need to consider the optimization of the balance of polar and nonpolar groups to achieve membrane permeability, a factor that may prove useful in improving the removal of intracellular deposits of toxic metals other than cadmium.

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REFERENCES

1. Gale, G. R., Smith, A. B., and Walker, E. M., Jr. Diethyldithiocarbamate in the treatment of acute cadmium poisoning. Ann. Clin. Lab. Sci. 11:476–483 (1981).
2. Gale, G. R., Atkins, L. M., and Walker, E. M., Jr. Effects of diethyldithiocarbamate on organ distribution and excretion of cadmium. Ann. Clin. Lab. Sci. 12: 463–470 (1982).
3. Gale, G. R., Atkins, L. M., Walker, E. M., and Smith, A. B. Comparative effects of diethyldithiocarbamate, dimercaptosuccinate, and diethylenetriaminepentaacetate on organ distribution and excretion of cadmium. Ann. Clin. Lab. Sci. 13: 33–44 (1983).
4. Jones, S. G., Basinger, M. A., Jones, M. M., and Gibbs, S. J. A comparison of diethyldithiocarbamate and EDTA as antidotes for acute cadmium intoxication. Res. Commun. Chem. Pathol. Pharmacol. 38: 271–278 (1982).
5. Jones, S. G., Jones, M. M., Basinger, M. A., Burka, L. T., and Shinobu, L. A. Polarity and antidotal efficacy of dithiocarbamates in acute cadmium intoxication. Res. Commun. Chem. Pathol. Pharmacol. 40: 155–163 (1983).
6. Shinobu, L. A., Jones, S. G., and Jones, M. M. Mobilization of aged cadmium deposits by dithiocarbamates. Arch. Toxicol. 54: 235–242 (1983).
7. Gale, G. R., Atkins, L. M., Smith, A. B., Walker, E. M., Jr., Jones, M. M., and Hodge, R. P. Heterocylcic *N*-dithiocarboxylates as cadmium antagonists: 4-hydroxypiperidine- and 4-carboxamidopiperidine-N-dithiocarboxylate. Res. Commun. Chem. Pathol. Pharmacol. 43: 281–298 (1984).
8. Jones, S. G., and Jones, M. M. Structure-activity relationships among dithiocarbamate antidotes for acute cadmium chloride intoxication. Environ. Health Perspect. 54: 285–290 (1984).
9. Shinobu, L. A., Jones, S. G., and Jones, M. M. Sodium N-methyl-D-glucamine dithiocarbamate and cadmium intoxication. Acta Pharmacol. Toxicol. 54: 189–194 (1984).
10. Gale, G. R., Smith, A. B., Atkins, L. M., and Jones, M. M. Effects of diethyldithiocarbamate and N-methyl-N-dithiocarboxyglucamine on murine hepatic cadmium-metallothionnein *in vitro*. Res. Commun. Chem. Pathol. Pharmacol. 49: 423–434 (1985).
11. Cikrt, M., Basinger, M. A., Jones, S. G., and Jones, M. M. Structural effects in the dithiocarbamate-enhanced biliary excretion of cadmium. J. Toxicol. Environ. Health 17: 429–439 (1986).
12. Gale, G. R., Shinobu, L. A., Jones, M. M., Atkins, L. M., and Smith, A. B. Effects of sodium N-methyl-N-dithiocarbaoxyglucamine on cadmium distribution and excretion. Life Sci. 35: 2571–2578 (1984).
13. Cantilena, L. R., Jr., and Klaassen, C. D. The effects of repeated administration of several chelators on the distribution and excretion of cadmium. Toxicol. Appl. Pharmacol. 66: 361–367 (1982).
14. Cantilena, L. R., Jr., Klaassen, C. D. Comparison of the effectiveness of several chelators after single administration on the toxicity, excretion and distribution of cadmium. Toxicol. Appl. Pharmacol. 58: 452–460 (1981).
15. Gale, G. R., Atkins, L. R., Smith, A. G., Jones, S. G., and Jones, M. M. Amphipathic dithiocarbamates as cadmium antagonists: N-cyclohexyl-N-sulfonatoalkyl derivatives. Res. Commun. Chem. Pathol. Pharmacol. 58: 371–391 (1987).
16. Pitt, C. G., Gupta, G., Estes, W. E., Rosenkrantz, H. T., Metterville, J. J., Crumbliss, A. L., Palmer, R. A., Nordquest, K. W., Hardy, K. A. S., Whitcomb, D. R., Byers, B. R., Arcenaux, J. E. L., Gaines, G. G., and Sciortino, C. V. The selection and evaluation of new chelating agents for the treatment of iron overload. J. Pharmacol. Exp. Ther. 208: 12–18 (1979).
17. Pecoraro, V. L., Weitl, F. L., and Raymond, K. N. Ferric-ion specific sequestering agents 7. Synthesis, iron-exchange kinetics and stability constants of N-substituted, sulfonated catechoylamide analogs of enterobactin. J. Am. Chem. Soc. 103: 5133–5140 (1981).
18. Martell, A. E., Motekaitis, R. J., Murasee, I., Sala, L. F., Stoldt, R., Ng, C. Y., Rosenkrantz, H., and Metterville, J. J. Development of iron chelators for Cooley's anemia. Inorg. Chim. Acta 138: 215–230 (1987).
19. Jones, D. C., Smith, G. L., May, P. M., and Williams, D. R. Assessment of pharmaceutical agents for removing cadmium from humans using chemical speciation models. Inorg. Chim. Acta 93: 93–100 (1984).
20. Scharfe, R. R., Sastri, V. S. and Chakrabarti, C. L. Stability of metal dithiocarbamate complexes. Anal. Chem. 45: 413–415 (1973).
21. Shaikh, Z. A., and Lucis, O. J. Biological differences in cadmium and zinc turnover. Arch. Environ. Toxicol. 24: 410–418 (1972).
22. Voight, G. E., and Skold, G. Uber den schutzeffekt der diathylentriaminpenta-essigsaure (DTPA) bei der akuten cadmiumvergiftung. Z. Gesamte Expt. Med. 136: 326–330 (1963).
23. Eybl. V., Sykora, J. and Mertl, F. Wirkung von CaADTA and CaDTPA bei der kadmiumvergiftung. Acta Biol. Med. Ger. 17: 178–185 (1966).
24. Niemeier, B. Der einfluss von chelatbildern auf verteilung and toxicitat van cadmium. Int. Arch. Gewerbepathol. Gewerbehyg. 24: 160–168 (1967).
25. Cantilena, L. R., Jr. and Klaassen, C. D. Decreased effectiveness of chelation therapy with time after acute cadmium poisoning. Toxicol. Appl. Pharmacol. 63: 173–180 (1982).
26. Waalkes, M. P., Watkins, J. B., and Klaassen, C. D. Minimal role of metallothionein in decreased chelator efficacy for cadmium. Toxicol. Appl. Pharmacol. 68: 392–398 (1983).
27. Cherian, M. G. Biliary excretion of cadmium in the rat IV. Mobilization of cadmium from metallothionein by 2,3-dimercaptopropanol. J. Toxicol. Environ. Health 6: 379–391 (1980).
28. Cherian, M. G., Onosaka, S., Carson, G. K., and Dean, P. A. W. Biliary excretion of cadmium in the rat V. Effects of structurally related mercaptans on chelation of cadmium metallothionein. J. Toxicol. Environ. Health 9: 389–399 (1982).
29. Jones, M. M., Wilson, D. J., Topping, R. J., and Laurie, S. H. The role of rate-determining steps in the decorporation of toxic metal ions. Inorg. Chim. Acta 152: 159–170 (1988).
30. Schmidt, V.S. Some problems of the development of the physicochemical principles of modern extraction technology [English translation]. Russ. Chem. Rev. 56: 792–809 (1987).
31. Tute, M. S. Principles and practice of Hansch analysis: a guide to structure-activity correlation for the medicinal chemist. Adv. Drug Res. 6: 1–77 (1971).
32. Hansch, C., and Leo, A. Correlation Constants for Correlation Analysis in Chemistry and Biology. John Wiley and Sons, New York, 1979, p. 49.
33. Kojima, S., Kiyozumi, M., Honda, T., Senba, Y., Kaminaka, K., and Ohnishi, M. Studies on the poisonous metals XVIII. Effects of several dithiocarbamates on tissue distribution and excretion of cadmium in rats. Chem. Pharm. Bull. 36: 3838–3844 (1987).
34. Jones, S. G., Singh, P. K., and Jones, M. M. Use of the Topliss scheme for the design of more effective chelating agents for cadmium decorporation. Chem. Res. Toxicol. 1: 234–237 (1988).
35. Martin, Y. C. Quantitative Drug Design, A Critical Introduction. Marcel Dekker, New York, 1978, p. 377.
36. Gale, G. R., Atkins, L. M., Walker, E. M., Jr., Smith, A. B., and Jones, M. M. Dithiocarbamates and cadmium metabolism: further correlations of cadmium chelate partition coefficients with pharmacologic activity. Ann. Clin. Lab. Sci. 14: 137–145 (1984).
37. Gale, G. R., Atkins, L. M., Smith, A. B., and Jones, M. M. Effects of diethyldithiocarbamate and selected analogs on cadmium metabolism following chronic cadmium ingestion. Res. Commun. Chem. Pathol. Pharmacol. 47: 107–114 (1985).
38. Friberg, L., Elinder, C. G., Kjellstrom, T., and Nordberg, G. Cadmium and Health: A Toxicological and Epidemiological Appraisal, Vol 1. CRC Press, Boca Raton, FL, 1985, pp. 1–5.
39. Cikrt, M., Lepsi, P., Horakova, L., Blaha, K., Jr., Bittnerova, D., and Jones, M. M. Effects of dithiocarbamates on cadmium distribution and excretion in chronically exposed rats. J. Toxicol. Environ. Health. 17: 419–427 (1986).
40. Jones, M. M., Gale, G. R., Atkins, L. M., and Smith, A. B. Effects of monoalkyl dithiocarbamates on mobilization of cadmium in the mouse. J. Toxicol. Environ. Health 17: 81–89 (1986).
41. Kojima, S., Kaminaka, K., Kiyozumi, M., and Honda, T. Comparative effects of three chelating agents on distribution and excretion of cadmium in rats. Toxicol. Appl. Pharmacol. 83: 516–524 (1986).

42. Kojima, S., Kaminaka, K. Kiyozumi, M., and Honda, T. Effects of chelating agents on biliary and urinary excretion and tissue distribution of cadmium in rats. Toxicol. Lett. 34: 41–46 (1986).
43. Kojima, S., Kiyozumi, J., Honda, T., Kaminaka, K., Oda, Y., and Senba, Y. Effect of N-benzyl-D-glucamine dithiocarbamate on distribution and excretion of cadmium in rats. Toxicology 45: 93–102 (1987).
44. Mays, C. W., Lloyd, R. D., Jones, C. W., Bruenger, F. W., Taylor, G. F., Durbin, P. W., and Raymond, K. N. Decorporation of Pu and Am from beagles with delayed daily injections of 3,4,3-LICAM(C) or Zn-DTPA. Health Phys. 50: 530–534 (1986).
45. Durbin, P. W., Jueng, N., Jones, E. S., Weitl, F. L., and Raymond, K. N. Specific sequestering agents for the actinides. 10. Enhancement of ^{238}Pu elimination from mice by poly(catechoylamide) ligands. Radiat. Res. 99: 85–105 (1984).
46. Haust, H. L., Poon, H. C., Inwood, M., O'Hea, E. K., Haines, D. S. M., Forret, C. J., and Keys, D. S. Occupational lead exposure: studies in two brothers showing differential susceptibility to lead. Clin. Biochem. 18: 102–108 (1985).
47. Kagan, F., Rebenstorf, M. A., Heinzelman, R. V. The preparation of glycamines. J. Am. Chem. Soc. 79:3541-3544 (1957).