Chemical and ^{1}H NMR Spectroscopic Investigations of Stereoisomeric Tc(V) DMSA Complexes

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Abstract

Stereoisomeric Tc(V) complexes derived from *meso* and *racemic* 2,3-dimercaptosuccinic acid dimethylester have been identified by ^{1}H NMR spectroscopy and some of them have been isolated. In methanolic amine or potassium hydroxide solution, complexes of the *meso* ligand racemize to give the complex compounds of the *racemic* 2,3-dimercaptosuccinic acid dimethylester. In methanolic potassium hydroxide solution, Tc(V) complexes of *racemic* 2,3-dimercaptosuccinic acid are formed by ester hydrolysis.

Introduction

The different behaviour of $^{99m/99}$Tc(V) complexes of diastereoisomeric 2,3-dimercaptosuccinic acids (DMSA) in distribution studies in animals is a well known fact [1, 2]. *meso* DMSA gives Tc complexes with osteotropic properties, while the corresponding product derived from the *racemic* ligand is eliminated without any accumulation in the organism. From earlier work [3] it became evident that DMSA and its ester as 1,2-dithiole ligands form Tc(V) complexes of the general formula $[TcOL_2]^-$. Hence, the different *in vivo* behaviour of these products should be assigned to isomers derived from stereoisomeric ligand structures which differ in the steric arrangement of carboxylic groups.

Therefore, it is important to get more information on the stereochemistry of this type of compound. In the present paper, chemical and ^{1}H NMR spectroscopic investigations are presented.

Experimental

^{1}H NMR spectra were recorded on a FT-NMR-spectrometer WH 90 DS (Bruker Spectrospin). Thin layer chromatography was performed on Silufol^R plates with water as eluant.

Preparation of Compounds

The ligands *meso* and *racemic* 2,3-dimercaptosuccinic acid (DMSA) and *meso* and *racemic* 2,3-dimercaptosuccinic acid dimethylester (DMSA-ester) were obtained according to ref. 4.

$Et_4N[TcO(meso\text{-}DMSAester)_2]$ (**1**) was prepared by the ligand exchange reaction of Tc(V) gluconate with *meso* dimercaptosuccinic acid dimethylester as previously described [3]. This product shows two spots in TLC with R_f values of 0.4 and 0.7 in an intensity ratio of about 1:1. The yield of crude product was 74%. Repeated crystallization of **1** from acetone/methanol leads to the stereoisomer uniform compounds: **1a**, m.p.* 170–171 °C, R_f 0.7, yield 34%; **1b**, m.p. 138 °C, R_f 0.4, yield 20% and **1c**, m.p. about 165 °C (contaminated with **1b**), yield 3%.

n-Bu$_4$N[TcO(meso-DMS)$_2$] (2)

25 μmol ammonium pertechnetate were dissolved in 1 ml of dry methanol. HCl gas was bubbled through the solution for 0.5 h. The green solution was evaporated to dryness under vacuum and the residue was dissolved in 0.5 ml methanol. Into this solution 50 μmol of *meso* dimercaptosuccinic acid (as disodium salt) in 0.5 ml of methanol were dropped; then 10 mg tetrabutylammonium bromide in 1 ml of water was added. After standing overnight yellow-–brown crystals were separated and washed with dioxane/ether. Yield: 6.2 mg (35% based on Tc). *Anal.* Calc. for $C_{24}H_{44}NO_9S_4Tc$: C, 40.2; H, 6.2; N, 2.0; S, 17.9; Found: C, 40.0; H, 6.2; N, 2.1; S, 17.5%.

Et$_4$N[TcO(racemic-DMSA-ester)$_2$] (3)

The procedure was the same as that described for **1** with *racemic* DMSA-ester as the ligand. Fractional crystallization of **3** from methanol gives **3b**, m.p. 140 °C, yield 9% based on Tc.

Et$_4$N[TcO(racemic-DMSA)$_2$] (4)

(a) The compound was previously obtained by the Tc gluconate method [3]. (b) By hydrolysis of

*m.p. = melting point.

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1a. 16.5 mg (25 μmol) **1a** were dissolved in 1 ml of 2% methanolic KOH by shaking. After two hours, the solvent was evaporated under a nitrogen stream and the red–brown residue was dissolved in some drops of water. The complex was separated from KOH by Dowex 50 (column 0.5 × 5 cm, water as eluant). The eluate was dropped into an aqueous solution of 100 mg of tetraethylammonium bromide. The product did not precipitate.

Evaporation of the solvent and recrystallization of the residue from nitromethane/methanol gave brown crystals, m.p.: 202–204 °C. Yield: 8.0 mg (53%).

n-Bu$_4$N[TcO(racemic-DMSA)$_2$]

By hydrolysis of **1a**, tetrabutylammonium bromide was used instead of tetraethylammonium bromide. A yellow precipitate formed immediately which was separated, washed with water and dried under vacuum, m.p.: 194–196 °C. Yield 52% based on Tc. *Anal.* Calc. for $C_{24}H_{44}NO_9S_4Tc$: C, 40.2; H, 6.2; N, 2.0; S, 17.9. Found: C, 40.0; H, 6.2; N, 2.0; S, 17.5%.

Results and Discussion

Stereochemical Considerations

Ligands

The dithiole ligands 2,3-dimercaptosuccinic acid and its dimethylester have been prepared and separated into dissymmetric *racemic* and internally compensated *meso* diastereoisomers [4] (Fig. 1).

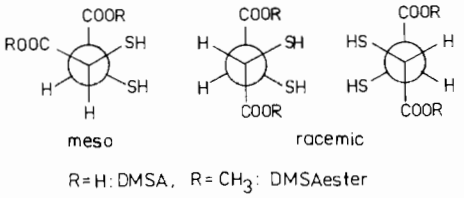

Fig. 1. Stereoisomers of 2,3-dimercaptosuccinic acid and 2,3-dimercaptosuccinic acid dimethylester.

Complexes

Generally, simple dithiole ligands form square-pyramidal bis(dithiolato)oxotechnetate(V) complexes [5, 6]. 1,2-disubstituted ligands result in forming different stereoisomeric complexes. The *meso* diastereoisomer of each ligand may be linked to the Tc=O group to form one *anti*- and two chemically different *syn*-isomers with the substituents *exo* or *endo* to the axial Tc=O group, as shown in Fig. 2.

Derived from the *racemic* ligand, three stereoisomeric complexes should be considered, two of them being enantiomers and one *meso* form (Fig. 2).

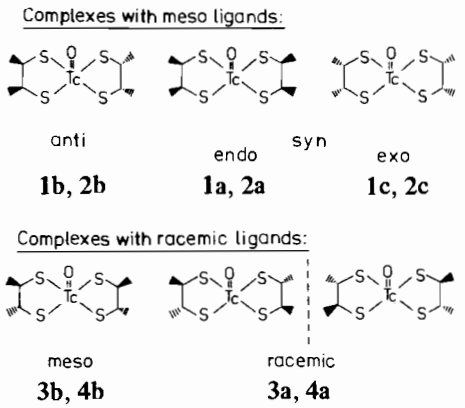

Fig. 2. Representation of stereochemistry of square-pyramidal complexes $[TcOL_2]^-$ with *meso* and *racemic* DMSA and DMSA-ester ligands (◀, ◀ represent the orientation of the carb(meth)oxy groups).

Preparation of Complexes

In principle, the complexes of all four ligands can be obtained by the reaction of the ligand with aqueous Tc(V) gluconate solution [3]. However, this method is not the favourable one for the isolation of complexes of dimercaptosuccinic acids because of the good solubility of the resulting compounds in water. Therefore, tetrachlorooxotechnetate(V) [7] in methanolic solution has also been used as starting material. If not otherwise noted, the complexes were separated as tetraethylammonium salts. The *meso* DMSA-ester with Tc(V) gluconate resulted in a product without a sharp melting point and which shows two spots in TLC. Uniform compounds (**1a–c**) were obtained by repeated recrystallization of this product.

The reaction of *meso* DMSA with tetrachlorooxotechnetate(V) yielded a mixture of complexes (**2a–c**, tetrabutylammonium salts). Attempts to separate stereochemically uniform compounds failed. The original Tc(V) complexes of the *racemic* DMSA-ester obtained by the Tc(V) gluconate method consists of at least two different forms (**3a, 3b**). One of the compounds (**3b**) was separated from the mixture by recrystallization.

Tc chelates of *racemic* DMSA (**4a, 4b**) were obtained by the Tc(V) gluconate method with a low yield [3].

*Racemization of **1a** in Alkaline Solution*

A further attempt to obtain a stereochemically uniform Tc(V) complex of *meso* DMSA by treating the pure compound **1a** with methanolic potassium hydroxide did not lead to the desired product. The ^{1}H NMR pattern in the SC–H region (*vide infra*) indicates that the compounds formed belong to the *racemic* series.

If the reaction (**1a** in 2% methanolic KOH at room temperature) was stopped after some minutes by addition of acetic acid, a nonhydrolyzed product could be isolated which is identical with the Tc(V) complex of *racemic* DMSA-ester (**3a, 3b**). At longer reaction times (about 2 h for quantitative reaction), Tc chelates of *racemic* DMSA (**4a, 4b**) were formed and isolated as both the tetraethylammonium and the tetrabutylammonium salt:

$$\mathbf{1a} \xrightarrow[\text{fast}]{OH^-} \mathbf{3a, 3b} \xrightarrow[\text{slow}]{OH^-/H_2O} \mathbf{4a, 4b}$$

Obviously, the complex-bounded *meso* ligand was racemized under strongly basic conditions, followed by hydrolysis of the ester groups. The racemization reaction also takes place at lower rates with ammonia and amines. This process can be thought to proceed by a very simple mechanism involving the formation of a carbanion intermediate by α-proton abstraction in basic media and subsequent readdition of the proton from the opposite site. Because the stability of the carbanion intermediate depends on the electron-withdrawing and resonance-stabilizing capacities of the substitutents attached to the carbanion, only the Tc complex of *meso* 2,3-dimercaptosuccinic acid dimethylester is racemized, whereas the Tc complex of *meso* 2,3-dimercaptosuccinic acid does not racemize under the same conditions due to the formation of the stable carboxylate ion.

Discussion of ^1H NMR Spectra

A suitable tool to detect and identify different stereoisomers in this system is ^1H NMR spectroscopy. The chelate ring protons SC–H of isomers have chemical shifts sufficiently different to analyse mixtures.

The spectrum of the product derived from *meso* DMSA-ester (**1a–c**) in the region of the chelate ring protons consists of an intensive signal, confirming the formation of different complex forms. The two central signals have an intensity ratio of 1:1 in all mixtures (Fig. 3).

The structure of compound **1a** has been determined earlier by X-ray analysis [8]. It has a square-pyramidal $TcOS_4$ core similar to that of some other investigated oxotechnetium(V) dithiolato complexes [5, 6]. With regard to the carbmethoxy groups, it has been shown that all four substituents are arranged in the *endo* position with respect to the Tc=O group. On the basis of these results, the intensive peak at 4.37 ppm in the spectrum (Table I) is assigned to the four equivalent protons in **1a**. The signal at 4.53 ppm consequently arises from the *syn–exo* compound (**1c**). Finally, the chemical shifts 4.39 and 4.49 ppm of equal signal intensity both are due to the *anti*-configurated complex (**1b**), e.g., to the *exo*- and *endo*-oriented side, respectively.

The original Tc(V) complexes of *racemic* 2,3-dimercaptosuccinic acid dimethylester (**3**) are characterized by an eight-line pattern (see Fig. 3), representing a superposition of two AB spectra. Different intensities of both spectra confirm that this product contains at least two different forms (**3a, 3b**). The separable compound (**3b**) shows a pure AB spectrum.

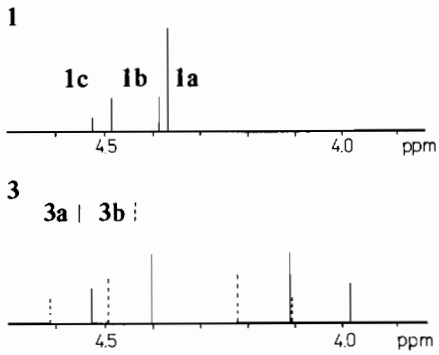

Fig. 3. ^1H NMR spectra (region of chelate ring protons) of Tc(V) complexes of *meso* DMSA-ester (1) and *racemic* DMSA-ester (3).

TABLE I. ^1H NMR Spectral Parameters of Identified Complexes[a]

Ligand	Complex	δ(CH–S) (ppm) position of H		$^3J_{HH}$ (Hz)	δ(OCH_3) (ppm)
		exo	*endo*		
meso-DMSA-ester	**1a** *syn–endo*	4.37			
	1b *anti*	4.39	4.49		3.57
	1c *syn-exo*		4.53		
meso-DMSA	**2a**[b] *syn–endo*	4.35			
	2b[b] *anti*	4.43	4.46		
	2c[b] *syn–exo*		4.52		
racemic-DMSA-ester	**3a**[b] *racemic*	4.06	4.45	11.7	3.73
	3b *meso*	4.17	4.55	10.4	3.717/3.714
racemic-DMSA	**4a**[b] *racemic*	4.04	4.45	11.8	
	4b[b] *meso*	4.15	4.55	10.8	

[a]Solvent: acetone-d_6. [b]in mixture.

The assignment of these compounds to stereoisomeric forms can not be made with certainty. For the complexes derived from the *meso* ligands, it can be assumed that the high field absorption of the B-part in the AB spectra of the complexes of *racemic* ligands should belong to proton in the *exo* position. Unlike the complexes with *meso* ligands, the complexes with *racemic* ligands show substantially greater differences in the chemical shifts between *exo* and *endo* protons, which reflects a significant distortion of symmetry due to the change of substituent arrangement. The steric hindrance between substituents, which leads to a decrease of the trans dihedral angle, should be smaller in the enantiomeric compounds **3a** than that in the *meso* complex **3b**. Therefore, we assume that the compound with the smaller value of $^3J_{HH}$ (**3b**) can be assigned to the *meso* complex. The CH_3 group peaks occur at different chemical shifts for the *meso* and *racemic* complexes. The methyl groups of the *meso* complex have a slight difference of about 0.003 ppm; e.g., this complex seems to be more deformed compared to the *racemic* complexes. A similar behaviour was observed in complexes with *racemic* DMSA. The spectral characteristics and the intensity ratio of the different AB parts in the eight-line spectrum indicate that both the stereochemical relations and the distribution of products should be comparable to that obtained with the *racemic* DMSA-ester complexes.

Conclusion

The formation of stereoisomeric Tc(V) complexes of 2,3-dimercaptosuccinic acids and 2,3-dimercaptosuccinic acid dimethylester has been proved and some of them have been isolated and identified by ^1H NMR spectroscopy. The main complex obtained by *meso* DMSA-ester is the isomer with the carbmethoxy groups in *syn* position and on the same side as the Tc=O group. The alternative complexes were also isolated. Tc complexes derived from the *racemic* ligands are characterized by AB spectra. The observed eight-line pattern is interpeted as a superposition of the AB spectra of the *meso* complex **3b** and of the *racemic* complexes **3a**. Tc complexes of *meso* DMSA-ester racemize under basic conditions. Therefore, the preparation of a stereochemically pure complex of *meso* DMSA was not successful. This reactions yields Tc compounds of *racemic* DMSA-ester or, at longer reaction time, to the corresponding *racemic* DMSA complexes.

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