PREPARATION, CHARACTERIZATION AND ORGAN DISTRIBUTION OF THE OXOCHLORO-BIS-(1,10-PHENANTHROLINE)TECHNETIUM (V) CATION

K. Schwochau*, K.H. Linse, P. Pleger, U. Pleger, C. Kremer
Institut für Nuklearchemie

and A.A. de Graaf
Institut für Biotechnologie (IBT-1)
Forschungszentrum Jülich GmbH (KFA), D-52425 Jülich, Germany

*Author for correspondence. Present address:
Institut für Chemie und Dynamik der Geosphäre,
Institut 4, Erdöl und Organische Geochemie,
Forschungszentrum Jülich GmbH (KFA),
D-52425 Jülich, Germany

SUMMARY

$[TcOCl(phen)_2]Cl_2$ was prepared by controlled potential cathodic reduction or dithionite reduction of TcO_4^- in aqueous alcoholic solution containing 1,10-phenanthroline(phen) monohydrate. The compound was also obtained by ligand exchange reaction of $[TcOCl_4]^-$ with phen-H_2O in methanol. The strong IR absorption at 895 cm^{-1} was attributed to the Tc=O stretching vibration. The ^1H NMR spectrum displayed 16 distinct resonances which were assigned by HH-COSY-experiments to the non-equivalent aromatic protons of the two phen ligands. The maximum uptake of $[^{99m}TcOCl(phen)_2]^{2+}$ of 4.2 % dose/g heart in mice, 1 min p.i., was low, probably reflecting the dipositive charge and the high hydrophilicity of the complex.

Key words: *technetium (V), phenanthroline complex, preparation, characterization, biodistribution.*

1. INTRODUCTION

Various mixed ligand technetium complexes containing 1,10-phenanthroline (phen) were synthesized and characterized [1 - 12]. In addition recently the pure phen chelates $[Tc(phen)_3]^+$ [13, 14], $[Tc(phen)_3]^{2+}$ [15 - 17] and $[Tc(phen)_3]^{3+}$ [18] have been prepared and identified. Following the objective to synthesize new cationic ^{99m}Tc radiopharmaceuticals, we obtained and characterized $[Tc(phen)_3]Cl_2$ and evaluated its

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organ distribution in mice [17]. Potential controlled reduction of $[Tc(phen)_3]Cl_2$ in 0.15 M NaCl yielded $[Tc(phen)_3]Cl$ [14], which, however, is readily reoxidized to the starting compound. In the course of preparing new technetium 1,10-phenanthroline complexes, we frequently observed the formation of rather stable, red-violet solutions in water and organic solvents. The synthesis, characterization, and biodistribution of the underlying compound is the subject of this investigation.

2. MATERIALS AND METHODS

2.1 GENERAL

1,10-Phenanthroline monohydrate was obtained from Fluka Chemika Company, Germany, and ammonium pertechnetate from Oak Ridge National Laboratory, U.S.A. ^{99m}Tc generators of DuPont Pharma GmbH, Germany, were used to elute $Na^{99m}TcO_4$. The IR spectra were recorded with a Shimadzu IR-460 spectrophotometer over the range 4000 - 500 cm^{-1} using KBr pellets and the VIS/UV spectra with a Beckman Acta III C spectrophotometer over the range 15000 - 45000 cm^{-1}. The ^1H NMR spectra were measured with a AMX-400 WB Bruker spectrometer employing a standard 5 mm inverse probe head. A HPLC system of Knauer, equipped with a variable wavelength monitor and a Hamilton polystyrene cation exchange column PRP-X 200 (8 x 250 mm) was used for the separation of the $^{99}Tc/^{99m}Tc$ phenanthroline complex. For the ^{99m}Tc detection a Berthold γ-monitor was applied in series with a mass detector.

2.2 SYNTHESIS OF $[^{99}TcOCl(phen)_2]Cl_2$

Oxochloro-bis(1,10-phenanthroline)technetium(V)chloride can be obtained by several routes of preparation:

a) Controlled potential cathodic reduction of TcO_4 in 0.15 M $NaCl/C_2H_5OH$ solution containing phenanthroline:

$TcO_4^- + 2$ phen + 6 HCl + 2 $e^- \rightarrow [TcOCl(phen)_2]Cl_2 + 3\ H_2O + 3\ Cl^-$

75 mg of 1,10 phenanthroline was dissolved in 3 ml ethanol and the solution mixed with 7 ml 0.15 M saline containing 8.8 mg of NH_4TcO_4. The pH 3 of the solution was adjusted by adding hydrochloric acid and the mixture electrolyzed under an argon atmosphere for 2 hr in a three-compartment cell between platinum electrodes at the potential of 1.0 volt vs the saturated Ag/AgCl electrode. A dark red-violet solution of the complex was obtained in the cathodic compartment, simultaneously with the formation of TcO_2-hydrate which precipitated at the platinum cathode.

b) Chemical reduction of TcO_4^- with $S_2O_4^{2-}$ in isopropanol/water containing phenanthroline:

$TcO_4^- + S_2O_4^{2-} + 3Cl^- + 2H^+ + 2$ phen $\rightarrow [TcOCl(phen)_2]Cl_2 + 2\ SO_3^{2-} + H_2O$

10 mg of NH_4TcO_4 was dissolved in 1 ml H_2O and 150 mg of 1,10-phenanthroline in.8 ml isopropanol. To the mixture of both solutions 58 mg of $Na_2S_2O_4$ dissolved in 6 ml of H_2O was added and the pH adjusted to 8. The reaction was accomplished at 50°C within 15 min under an argon atmosphere.

c) Reaction of $[TcOCl_4]$- with phenanthroline in methanol:

$[TcOCl_4]^- + 2$ phen $\rightarrow [TcOCl(phen)_2]Cl_2 + Cl^-$

A solution of 648 mg 1,10-phenanthroline in 10 ml methanol was added dropwise to a solution of 93 mg [n-Bu_4N][$TcOCl_4$] in 5 ml methanol. The colour of the mixture immediately turned to red-violet and the reaction was accomplished by stirring the mixture for 2 hr at room temperature.

The solutions of the complex were purified by removal of anionic impurities using the macroporous anion exchanger AG-MP-1 of Biorad, by extraction of excess phenanthroline with dichloromethane, and removal of salt impurities by evaporation of the complex solutions to dryness and extraction of $[TcOCl(phen)_2]Cl_2$ with acetonitrile. Finally, the complex was separated by HPLC using a mixture 80/20 (v/v) of methanol/0.05 M NaCl as the mobile phase.

2.3 SYNTHESIS OF $[^{99m}TcOCl(phen)_2]Cl_2$ FOR BIODISTRIBUTION MEASUREMENTS

A solution of 3.1 GBq $Na^{99m}TcO_4$ in 5 ml 0.15 M NaCl was reacted with a mixture of 5.5 mg 1,10-phenanthroline in 5 ml isopropanol and 6 mg $Na_2S_2O_4$ in 5 ml $Na_2CO_3/NaHCO_3$ buffer solution of pH 9.2. The reaction was completed within 15 min at 50°C under an argon atmosphere. The solution was cooled to room temperature, passed through the anion exchanger AG-MP-1 and the eluate through the cation exchanger AG-MP-50. The cation exchanger was eluted with 1 M NaCl in 50/50 (v/v) of methanol/water and the eluate evaporated to dryness. The residue was dissolved in ammoniacal water and excess phenanthroline extracted with dichloromethane. The aqueous phase was brought to dryness and the complex extracted with acetonitrile. After HPLC separation of $[^{99m}TcOCl(phen)_2]Cl_2$, a radiochemical purity of more than 90% was achieved. Finally, the complex was dissolved in isotonic saline and injected into groups of 3 - 5 NMRI mice. The activity injected per mouse was around 110 kBq. The animals were killed at different intervals of time. The organs of interest were isolated, weighed, and the γ-activity was assayed under standard geometry.

3. RESULTS AND DISCUSSION

$[TcOCl(phen)_2]Cl_2$ is readily soluble in water, less soluble in methanol or acetonitrile and is rather stable in solution. In spite of repeated HPLC purifications, the compound could not be obtained in a pure crystalline state. It precipitated from aqueous solutions by adding large anions, such as $[PF_6]^-$, however, efforts to purify the complex by precipitation were unsuccessful. Thus, its composition could be confirmed by elemental analysis only approximately. The calculated values are given in parentheses: Tc, 16.94 (17.00); N, 9.13 (9.63); C, 51.67 (49.55) wt %. In particular, the complete separation of free phenanthroline appears to be difficult.

The complex is also formed by reaction of freshly prepared TcO_2-hydrate at 80°C with a solution of phenanthroline in a water/isopropanol mixture which was acidified to pH 4.5 by adding hydrochloric acid. During the reaction air was bubbled through the reaction mixture. It should be emphasized that the complex formation was only observed in the presence of both, chloride ions and oxygen.

The red-violet solution of $[TcOCl(phen)_2]Cl_2$ in acetonitrile absorbs in the VIS at 714, 573, and 513 (sh) nm, in the UV at 350 (sh), 334 (sh), 297 (sh), 273, 233, and 212 (sh) nm. The latter three strong bands may correspond to $\pi \rightarrow \pi^*$ transitions of 1,10-phenanthroline [19].

The IR spectrum is dominated by the spectrum of phenanthroline. The Tc-N stretching vibrations are to be expected at wave-numbers much lower than 500 cm^{-1} [19]. A strong band at 895 cm^{-1} is assigned to the Tc-O stretch, the frequency of which is relatively low compared to the Tc-O stretching vibrations found for [TcOCl(1,2-ethanediolate)(phen)] [4] and $[TcO_3Cl(phen)]$ [2] at 952 and 977 cm^{-1}, respectively. However, both compounds contain only one phenanthroline ligand. Another strong band at 1471 cm-1 and its satellites may be attributed to in-plane antisymmetric ring deformations involving C-C and C-N stretching modes of phenanthroline [20].

The ^1H NMR spectrum of $[TcOCl(phen)_2]Cl_2$, dissolved in deuterated methanol, displays 16 aromatic proton resonances of the two phenanthroline ligands: δ 6.92 (dd,F_1), 7.07 (dd,F_3), 7.48 (dd,E3), 7.69 (dd,D3), 7.82 (dd,E1), 7.93 (dd,C3), 7.95 (d,B2), 8.07 (d,A2), 8.29 (d,B1), 8.33 (dd,F2), 8.37 (d,A1), 8.40 (dd,D2), 8.77 (dd,E2), 8.93 (dd,C2), 9.15 (dd,D1), 9.51 (dd,C1). For the assignment of the resonances see Fig. 1.

The structure of the complex cation, calculated with the Hyperchem Molecular Modelling Programm, is shown in Fig. 1.

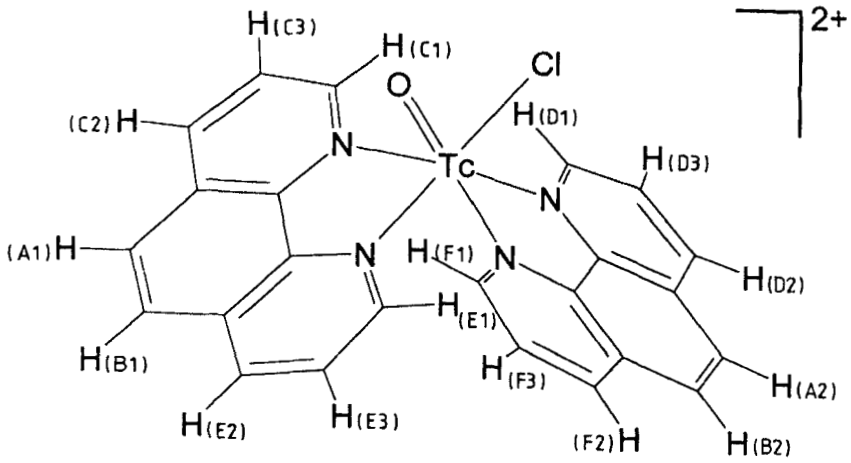

Fig. 1 Hyperchem Molecular Modelling drawing of $[TcOCl(phen)_2]^{2+}$

The assignment of the 16 resonances to the 16 non-equivalent aromatic protons of the two phenanthroline molecules was achieved by using HH-COSY-experiments. The occurrence of 16 distinct proton signals suggests that the oxygen and the chlorine atom assume *cis* configuration yielding a completely asymmetrical environment [4]. In agreement with the proposed complex structure (Fig. 1), the C1 proton (δ = 9.51) is deshielded by the anisotropic effect of the Tc=O bond, whereas the F1 proton (δ = 6.92) is strongly shielded by the ring current field of the condensed pyridine ring located in the other phenanthroline ligand. Compared with the ^{1}H NMR spectrum of free 1,10-phenanthroline, the spectrum of $[TcOCl(phen)_2]Cl_2$ shows no contact shift or line broadening, indicating the diamagnetism of the complex.

We studied the biodistribution of the dipositive cation $[TcOCl(phen)_2]^{2+}$ in mice, in spite of the known, but not always valid hypothesis that unipositive cationic complexes tend to accumulate in the myocardium [21]. The results are summarized in Table 1.

The uptake of the compound in the myocardium of 4.2, 2.0 and 1.2 % i.d/g organ at 1,10 and 100 min p.i., respectively, is rather low and the degree of washout from the heart considerable. The relatively high hydrophilicity of the complex salt may explain

Table 1. Organ distribution of [^{99m}TcOCl(phen)$_2$]Cl$_2$ in mice

	% Dose/g organ		
Post-injection (p.i.) time	1 min	10 min	100 min
No. of mice:	5	4	3
Heart	4.2 ± 0.3	2.0 ± 0.2	1.2 ± 0.2
Blood	8.6 ± 0.6	3.7 ± 0.2	1.8 ± 0.1
Liver	16.6 ± 2.2	16.6 ± 1.4	16.3 ± 1.1
Lungs	13.8 ± 2.7	6.2 ± 1.8	2.7 ± 0.7
Kidneys	28.6 ± 2.2	17.8 ± 2.1	10.3 ± 0.8
Brain	0.4 ± 0.08	0.2 ± 0.02	0.1 ± 0.01
Activity-ratio			
Heart/blood	0.49	0.55	0.68
Heart/liver	0.25	0.12	0.07
Heart/lungs	0.31	0.32	0.45

the low heart activity, the acceptable rate of clearance from the blood and the predominant excretion of activity by the kidneys. In spite of the low lipophilicity, the activity retention in the lungs and particularly in the liver is unfavourable, reflecting poor heart/lungs and heart/liver ratios. The heart/blood ratio is only 0.68 at 100 min p.i. Consequently, [^{99m}TcOCl(phen)$_2$]$^{2+}$ appears not to be appropriate for myocardial imaging. The almost constant activity of greater than 16% dose/g liver for a period of 100 min might stimulate interest in testing this complex for liver imaging.

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REFERENCES

[1] Hieber W., Lux F., Herget C. - Z. Naturforschg. 20b: 1159 (1965)

[2] Davison A., Jones A.G., Abrams M.J. - Inorg. Chem. 20: 4300 (1981)

[3] Pearlstein R.M., Davison A. - Polyhedron 7: 1981 (1988)

[4] Pearlstein R.M. Lock C.J.L., Faggiani R., Costello C.E., Zeng C.-H., Jones A.G., Davison A. - Inorg. Chem. 27: 2409 (1988)

[5] Wilcox B.E., Ho D.M., Deutsch E. - Inorg. Chem. 28: 1743 (1989)

[6] Wilcox B.E., Ho D.M., Deutsch E. - Inorg. Chem. 28: 3917 (1989)

[7] O'Connell L.A., Dewan J., Jones A.G., Davison A. - Inorg. Chem. 29: 3539 (1990)

[8] Breikss A.J., Nicholson T., Jones A.G., Davison A. - Inorg. Chem. 29: 640 (1990)

[9] Lu J., Clarke M.J. - J. Chem. Soc. Dalton Trans 1992: 1243

[10] Clarke M.J., Lu J. - Inorg. Chem. 31: 2476 (1992)

[11] Du Preez J.G.H., Gerber T.I.A., Kemp H.J. - J. Coord. Chem. 26: 177 (1992)

[12] Lu J., Hiller C.D., Clarke M.J. - Inorg. Chem. 32: 1417 (1993)

[13] Breikss A.J., Davison A., Jones A.G. - Technetium and Rhenium in Chemistry and Nuclear Medicine 3 (Nicolini M., Bandoli G., Mazzi U., Eds.) Cortina International, Verona, p. 85 (1990)

[14] Schwochau K., Linse K.H., Cerda M.F., Kremer C. - Technetium and Rhenium in Chemistry and Nuclear Medicine 4 (Nicolini M., Bandoli G., Mazzi U., Eds.) SGE Ditoriali, Padova, p. 247 (1995)

[15] Archer C.M., Dilworth J.R. - J. Nucl. Med. 30: 939 (1989)

[16] Archer C.M., Dilworth J.R., Thompson R.M., McPartlin M., Povey D.C., Kelly J.D., - Technetium and Rhenium in Chemistry and Nuclear Medicine 3 (Nicolini M., Bandoli G., Mazzi U., Eds.) Cortina International, Verona, p. 201 (1990)

[17] Schwochau K., Linse K.H., Su Z.F. - Appl. Radiat. Isot. 43: 1079 (1992)

[18] Kremer, C., Kremer E. - J. Radioanal. Nucl. Chem., Letters 175: 445 (1993)

[19] McWhinnie W.R., Miller J.D. - Advan. Inorg. Chem. Radiochem. 12: 135 (1969)

[20] Singh S.S. - Z. Naturforsch. 24a: 2015 (1969)

[21] Deutsch E., Bushong W., Glavan K.A., Elder R.C. - Science 24: 85 (1981)