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SYNTHESIS AND CHARACTERIZATION OF OLIGOMERIC, ANIONIC THIOMALATO–SILVER(I) COMPLEXES WITH BIOLOGICAL ACTIVITIES

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Abstract—A water-soluble, thermally and light-stable, anionic Ag^I complex with a trianion of thiomalic acid, TMA^{3-} (H_3TMA = HOOCCH(SH)CH_2COOH) as a ligand was prepared and isolated as a typically yellow-coloured powder of the sodium salt, which showed excellent antimicrobial activity against some bacteria, yeast and moulds. All attempts at crystallization were unsuccessful. This compound was characterized by complete elemental analyses, TG/DTA, FT-IR, various NMR (^1H, ^{13}C and ^{109}Ag) spectroscopies, and molecular weight determination in aqueous solution by a combination of molmass measurement based on the cryoscopic method with degree of dissociation determination based on the [Na^+] measurement in the equilibrium state using an Na^+ ion-selective electrode. It was shown that this complex was not a monomeric species, but an oligomer with a formula of $\{NaH[Ag(TMA)] \cdot 0.5H_2O\}_n$ (n = 15–19; MW = 4320–5470). Analogues of this anionic Ag^I complex were also obtained as potassium, barium, ammonium, lithium and caesium salts, as well as a free-acid form.

In recent years, there has been considerable interest in anionic, water-soluble Ag^I complexes with biological activities. As an example of such complexes, bis(thiosulphato)argentate(I), $[Ag(S_2O_3)_2]^{3-}$ (usually called STS), has been known to possess the anti-ethylene property leading to delaying in senescence of ethylene-sensitive flowers such as carnation.[1] The sodium salt of the colourless STS complex is relatively light-sensitive in aqueous solution and much less stable or rapidly darkening when exposed to sunlight in the solid. We have anticipated the formation of other anionic, light-stable and water-soluble Ag^I complexes with biological activities, and sought out many organic ligands with SH, OH and/or COOH groups. In the aqueous system, we found that a thiomalic acid (H_3TMA = HOOCCH(SH)CH_2COOH) used in the racemic form (*R*, *S*-mixture) can form an anionic and light-stable Ag^I complex. This complexation was easily confirmed by observing no AgCl precipitation, even if an aqueous Cl^- solution was added to the pale yellow aqueous solution of mixed $AgNO_3$ and TMA with 1:1 molar ratio. In our preliminary experiments, the aqueous solution containing this TMA–Ag^I complex has evidently shown the life-prolongation effect for cut flowers of carnation, although its activity was lower than that of STS. In

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fact, the TMA–Ag^I complex was certainly transported within the xylem vessels in the cut flowers, because silver was quantitatively detected from their petals by analysis. Furthermore, the TMA–Ag^I complex showed excellent antimicrobial activity for some bacteria, yeast and moulds.

The TMA–Ag^I complexes reported previously[2–4] have been colourless or pale yellow, water-insoluble and polymeric species with TMA–Ag^I = 1 : 1 or 1 : 2. The 1 : 1 species, as a free acid form, has been thermally less stable. All of these compounds have been obtained by reactions of $AgNO_3$ and H_3TMA in aqueous or methanolic solutions with varied molar ratios under conditions lacking any alkaline hydroxides.

In the present work, we have first obtained an anionic TMA–Ag^I complex as the sodium salt, as well as potassium, barium, NH_4^+, lithium and caesium salts, using the corresponding hydroxides, all of which were water-soluble (except the barium salt), yellow-coloured, thermally and light-stable both in aqueous solution and in the solid. None of these salts would crystallize. Herein we report the synthesis and characterization of the sodium salt of the TMA–Ag^I complex by complete elemental analyses, TG/DTA measurement, FT-IR, molecular weight determination in aqueous solution based on the molmass measurement and degree of dissociation measurement, and ^1H, ^{13}C and ^{109}Ag NMR spectra. We also refer to the antimicrobial activity of the sodium salt.

EXPERIMENTAL

Chemicals

All chemicals were reagent grade and were used without further purification: thiomalic acid, $AgNO_3$, NaOH, KOH, $Ba(OH)_2$, aqueous NH_3, CH_3CN (Wako); $LiOH \cdot H_2O$, CsOH (Kanto); 99.8% D_2O (Merck).

Measurements and instrumentation

Elemental analyses were obtained from Mikroanalytisches Lab. Pascher (Remagen, Germany). IR spectra were obtained on a Nicolet 510 FT-IR spectrometer as KBr disks at room temperature. Thermogravimetric (TG) and differential thermal analyses (DTA) were done simultaneously using Seiko SSC 5000 TG/DTA 300, temperature programmed at 10.0°C per min between 20 and 500°C under air.

The ^{109}Ag NMR spectra (18.45 MHz) were recorded in 10 mm o.d. tubes on a Jeol JNM-EX 400 FT-NMR spectrometer equipped with a Jeol NM-40T10L low-frequency tunable probe. The ^{109}Ag NMR of the complex were measured in D_2O of 42.3% (w/v) concentration with reference to an external standard of saturated $AgNO_3$–D_2O solution by a substitution method. Spectral parameters for ^{109}Ag NMR include: pulse width 13.2 μs; acquisition time 0.39 s; repetition rate 0.7 s; sweep width $\pm$ 10,504.2 Hz. The ^1H NMR (399.65 MHz) and ^{13}C NMR (100.40 MHz) spectra were recorded in 5 mm o.d. tubes on a Jeol JNM-EX 400 FT-NMR spectrometer and Jeol EX-400 NMR data processing system. The ^1H and $^{13}C\{^1H\}$ NMR spectra of the complex were measured in D_2O solution of ~0.75% (w/v) concentration with reference to an internal DSS.

Molecular weight determination in aqueous solution

All molecular weight measurements in the solid state by mass spectroscopy (TOF-SIMS) were unsuccessful. Molmass measurement in aqueous solution was done by Mikroanalytisches Lab. Pascher (Remagen, Germany) based on the cryoscopic method for 7.95 mg of the complex in 0.40457 g of H_2O. In the control experiments, standard samples, NaCl (formula mass: 58) and disodium succinate (formula mass: 162), which are almost completely dissociative in aqueous solution, gave 29 and 59, respectively, as observed molmass values (within $\pm$5% of experimental errors). The number of species existing in the solution in equilibrium state multiplied by the observed molmass value results in the formula mass of the complex. For incompletely dissociative electrolytes, the number of species present in equilibrium state solution is a function of degree of dissociation α. In order to determine the degree of dissociation α, the pNa (pNa = $-\log [Na^+]$) or $[Na^+]$ values for varying concentrations (C_{Na} = 0.285–14.23 mM) of the complex in aqueous solution were measured at 25°C using an Na^+ ion-selective electrode (Horiba 1512A-10C) and a reference electrode (Horiba 2565A-10T) equipped with a pH/ion meter (Horiba F-23). The plot of observed $[Na^+]$ versus C_{Na} used for the measurement gave a straight line with a slope of the α value. The pHs were also measured at 25°C for varying concentrations (C_{Na} = 0.072–7.15 mM) of the complex.

Antimicrobial activity

Antimicrobial activities of some Ag^I compounds were estimated by MIC (minimum inhibitory concentration: $\mu g\ cm^{-3}$), as reported elsewhere.[5,6]

Bacteria and yeast were inoculated into 5 cm^3 of liquid medium (SCD medium for bacteria and GP medium for yeast), and cultured for 24 h at 35°C. The cultured fluids were diluted 100 times with distilled water and used for inoculation in the MIC test. As for the mould culture, the agar slant (GP agar medium) for 1 week's cultivation was washed with saline containing 0.05% Tween 80. The spore suspension obtained was adjusted to the concentration of 10^6 cm^{-3} and used for inoculation in the MIC test.

Test materials (Ag^I complexes) were dissolved or suspended in distilled water or dimethyl sulphoxide (DMSO) and then diluted twice with each culture medium. Each 1 cm^3 of culture medium containing various concentration of test materials was inoculated with 0.05 cm^3 of microorganism suspension prepared above.

Bacteria were cultured for 48 h at 35°C and yeast and mould for 1 week at 27°C, then the growth of microorganism was observed. When no growth of microorganism was observed in the medium containing the lowest concentration of test materials, the MIC of the test material was defined at this point of dilution. Compositions of the media were: SCD, casein peptone 17 g, soybean peptone 3 g, NaCl 5 g, K_2HPO_4 2.5 g, glucose 2.5 g, distilled water 1 dm^3, pH 7.1–7.5; GP, glucose 20 g, polypeptone 5 g, yeast extract 2 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, K_2HPO_4 1 g, distilled water 1 dm^3, pH 5.7.

Preparation and purification of the sodium salt of the TMA–Ag^I complex

The sodium salt of the TMA–Ag^I complex was prepared from an aqueous solution of the molar ratio of $H_3TMA–Ag^+–NaOH = 1:1:2$.

To thiomalic acid (H_3TMA; 7.51 g, 50 mmol) dissolved in 150 cm^3 water, an aqueous solution of NaOH (4.0 g, 100 mmol) in 150 cm^3 water was added. The resulting faint purple solution was added dropwise to a vigorously stirred $AgNO_3$ aqueous solution, which was prepared by dissolving $AgNO_3$ (8.49 g, 50 mmol) in 70 cm^3 water. The white precipitate initially formed was completely dissolved during stirring, resulting in a homogeneous, yellow solution. The clear, yellow filtrate obtained by filtering it through a fluted filter paper (Whatman no. 2) was rotavaporated until it became slightly viscous. This evaporated solution was kept in ice-cooled water bath to form an amorphous yellow powder. (This work-up is not a recrystallization but a reprecipitation, in spite of no addition of any other organic solvents.) At this temperature, the yellow supernatant mother liquor was removed using a pipette. Warming the remaining yellow powder to *ca* 50°C in a water bath, it dissolved and changed to a homogeneous yellow solution. From this yellow solution, a yellow powder was reprecipitated by keeping in an ice-cooled bath, followed by discarding the supernatant mother liquor. These procedures, consisting of reprecipitation by ice-cooling, next removing the supernatant mother liquor, and then formation of a yellow homogeneous solution by warming, were repeated three times. (These work-ups lead to a first purification step.) Finally, the yellow solution obtained by warming to *ca* 50°C was added to *ca* 150 cm^3 of CH_3CN with stirring. A yellow powder immediately precipitated. (This is a usual reprecipitation and also a second purification step.) The yellow precipitate was washed thoroughly with CH_3CN, then with ether and dried *in vacuo* overnight. Redissolving this yellow powder in small amounts of water, the purification by two types of reprecipitation was done once more. The final product, a light-stable yellow powder, soluble only in water and insoluble in organic solvents, was obtained in 2.38 g yield. The compound decomposes at ~180°C without melting. All attempts of crystallization of this compound were unsuccessful. Found: C, 17.1; H, 1.8; O, 24.4; S, 11.0; Ag, 38.3; Na, 7.3. Calc. for $\{NaH[Ag(OOCCH(S)CH_2COO)] \cdot 0.5H_2O\}_n$: C, 16.7; H, 1.8; O, 25.0; S, 11.1; Ag, 37.5; Na, 8.0%. Some prominent IR bands in the 1800–800 cm^{-1} region in the FT-IR spectrum measured as KBr disk were: 1700 (vs), 1600 (vs), 1410 (s), 1400 (vs), 1390 (s), 1220 (m), 1190 (m), 990 (w), 900 (vw), 860 (w) cm^{-1}. TG/DTA data: observed weight loss 3.16% below 150°C; an exothermic peak at 169.2°C; endothermic peaks at 197.4, 302.1 and 473.8°C. ^1H NMR data (Fig. 4) measured in D_2O with reference to an internal DSS at 23°C: 4.07 (1H, br, C*H*), 3.05 (1H, br, CH^aH^b), 2.98 ppm (1H, br, CH^aH^b); at 80°C: 4.05 (1H, m, C*H*), 3.03 (1H, m, CH^aH^b), 2.92 ppm (1H, m, CH^aH^b) with coupling constants (Hz): $^3J_{CH-H^a} = 9.28$, $^3J_{CH-H^b} = 5.37$ and $^2J_{H^a-H^b} = 16.1$. ^{13}C NMR data (Fig. 2) measured in D_2O at 26°C with an internal DSS: 183.7 (—*C*OO^-), 180.2 (—*C*OO^-), 48.8 (—*C*H_2—), 45.5 ppm (—*C*H—); at 70°C: 182.8 (—*C*OO^-), 179.3 (—*C*OO^-), 48.2 (—*C*H_2—), 45.1 ppm (—*C*H—). ^{109}Ag NMR data measured in D_2O at 22°C with an external $AgNO_3$ in D_2O: 868.7 ppm.

Preparation of other counter-cation salts of the TMA–Ag^I complex

Instead of NaOH used in the above preparation, equivalent amounts of some hydroxides such as KOH, $Ba(OH)_2$, aqueous ammonia, LiOH and CsOH were used for a preparation of the anionic

TMA–Ag^I complex with corresponding countercation. However, for the preparation of these salts, the order of addition of reagents, i.e. (1) or (2) below, was a significant factor; (1) to the aqueous $AgNO_3$ solution, aqueous thiomalic acid was initially added and finally aqueous metal hydroxide was added, or (2) to the aqueous $AgNO_3$ solution, an aqueous solution containing both thiomalic acid and the hydroxide was added. In fact, in the preparation of the lithium and caesium salts, the compounds obtained by (1) or (2) gave different IR spectra, although in the sodium salt preparation such a difference was not seen (the procedure based on (2) was described above). Thus, using the addition order based on (1) rather than (2) and the same purifications as in the sodium salt preparation, the water-soluble, thermally and light-stable Ag^I complexes were obtained as yellow powders for potassium, lithium, caesium and NH_4^+ salts, although only the barium salt was water-insoluble. None of these compounds would crystallize and all decomposed without melting.

Potassium salt: decomposition temperature ~120°C. Some prominent IR bands (KBr disk): 1700 (vs), 1580 (s), 1400 (vs), 1340 (s), 1290 (s), 1210 (m), 1190 (s), 990 (w), 900 (w), 860 cm^{-1} (m). ^1H NMR data measured in D_2O at 80°C: 4.11 (1H, m, C*H*), 3.09 (1H, m, C*H*^{a}H^b), 2.98 ppm (1H, m, CH^a*H*^{b}); ^{13}C NMR data measured at 25°C: 182.6 (—*C*OO$^-$), 179.0 (—*C*OO$^-$), 47.5 (—*C*H_2—), 44.8 ppm (—*C*H—).

Barium salt: decomposition temperature ~220°C. Some prominent IR bands (KBr disk): 1560 (vs), 1410 (vs), 1390 (vs), 1300 (w), 1260 (w), 1190 (w), 1000 (w), 950 (w), 880 cm^{-1} (m).

NH_4^+ salt: decomposition temperature ~130°C. Some prominent IR bands (KBr disk): 1700 (m), 1640 (m), 1580 (m), 1400 (vs), 1220 (w), 1190 (w), 1000 cm^{-1} (w). ^1H NMR data measured in D_2O at 28°C: 4.19 (1H, t, C*H*), 3.06 ppm (2H, d, C*H*$_2$); ^{13}C NMR data at 28°C: 182.8 (—*C*OO$^-$), 179.2 (—*C*OO$^-$), 47.6 (—*C*H_2—), 44.8 ppm (—*C*H—).

Lithium salt: decomposition temperature ~150°C. Main IR bands (KBr disk): 1693 (s), 1579 (vs), 1298 (m), 1235 (m), 1182 cm^{-1} (m). ^1H NMR data measured in D_2O at 24°C: 4.04 (1H, s, C*H*), 3.01 (1H, m C*H*^{a}H^b), 2.92 ppm (1H, m, CH^a*H*^{b}); ^{13}C NMR data measured at 24°C: 183.5 (—*C*OO$^-$), 179.9 (—*C*OO$^-$), 48.3 (—*C*H_2—), 45.3 ppm (—*C*H—).

Caesium salt: decomposition temperature ~180°C. Main IR bands (KBr disk): 1637 (vs), 1577 (vs), 1400 (s), 1228 (w), 1179 cm^{-1} (w). ^1H NMR data measured in D_2O at 24°C: 4.10 (1H, br, C*H*), 3.07 ppm (2H, br, C*H*$_2$); ^{13}C NMR data measured at 24°C: 183.0 (—*C*OO$^-$), 179.2 (—*C*OO$^-$), 47.9 (—*C*H_2—), 45.2 ppm (—*C*H—).

Preparation of the free-acid form of the TMA–Ag^I complex

The free-acid form has been obtained previously as a water-insoluble, but DMSO-soluble light yellow solid from methanol solution containing a H_3TMA–$AgNO_3$ 1 : 1 molar ratio without any alkaline hydroxide.[4]

This complex was isolated from some modifications of the literature. To $AgNO_3$ (1.70 g, 10 mmol) in 10 cm^3 water was added an aqueous solution of H_3TMA (1.50 g, 10 mmol) dissolved in 30 cm^3 water. Slow evaporation of the resulting clear yellow solution on a water bath at ~60°C gave a yellow–white precipitate, which was washed several times with ~100 cm^3 ethanol, then twice with acetone, finally twice with ether, and dried *in vacuo*. The yellow–white or pale yellow solid, the colour of which was less intense than that of the sodium salt, obtained in 1.06 g yield, was soluble in DMSO and DMF, but insoluble in water, ethanol, acetone, acetonitrile and hexane. The decomposition temperature was less than 60°C. Some main peaks of FT-IR (cm^{-1}) measured as KBr disk: 1700 (vs), 1548 (m), 1415 (s), 1312 (s), 1225 (s), 1177 (s), 937 (m), 844 (m).

RESULTS AND DISCUSSION

Characterization of the sodium salt of the TMA–Ag^I complex

The complete elemental analyses of C, H, S, O, Ag and Na for the sodium salt of the TMA–Ag^I complex showed that the composition or molar ratio in the monomeric complex is TMA^{3-}–Ag^I–Na^+ = 1 : 1 : 1 and half of a hydrated water molecule is present. If the thiomalic acid ligand is triply charged (TMA^{3-}), this complex should be monoprotonated. Thus, the composition of this complex is TMA^{3-}–Ag^I–Na^+–H^+–H_2O = 1 : 1 : 1 : 1 : 0.5 and its formula can be written as $\{NaH[Ag(TMA)] \cdot 0.5H_2O\}_n$ (formula weight: 288*n*). The presence of a half molecule of hydrated water is also supported by the TG/DTA measurement—the 3.16% weight loss observed under 150°C (calc. value 3.13%)—and also by a strong absorption band at ~3450 cm^{-1} observed in the solid FT-IR spectra. The composition of Ag^I–TMA = 1 : 1 in the complex is also compatible with the results of potentiometric titrations reported previously by Lenz and Martell;[7] Ag^I has no tendency to form the 1 : 2 complex in aqueous solution.

The pH values measured at 25°C in aqueous solution in the concentration range $C_{Na} = 0.072$–7.15 mM were within 5.8 ± 0.1, indicating that this complex was neutral in aqueous solution, or did not dissociate its protonated proton; thus, $\{NaH[Ag(TMA)] \cdot 0.5H_2O\}_n \rightarrow n Na^+ + \{[HAg(TMA)]^-\}_n$. Further, the observed value in molmass measurement by the cryoscopic method was 382 (experimental error: ±5%), indicating that this complex was not monomeric ($n \neq 1$), but oligomeric. Since the number of species present in solution becomes $(1 + n\alpha)$, where α is the degree of dissociation, the formula mass is shown as $288n = 382(1 + n\alpha)$. The plot of $[Na^+]$ versus C_{Na}, obtained from $[Na^+]$ or pNa measurement done at 25°C three times each for seven different concentrations ($C_{Na} = 0.285$–14.23 mM), gave a straight line with a correlation coefficient 0.997, in which the slope (α value) was determined to be 0.689–0.701. Thus, the degree of polymerization was derived as $n = 15$–19 and the molecular weight as 4320–5470. Control measurements using standard NaCl and disodium succinate aqueous solutions gave α values of 0.94 and 0.79, respectively, resulting in 56 (calc. 58) and 152 (calc. 162) as observed formula masses, respectively. It is not surprising that the present water-soluble TMA–Ag^I complex is a polynuclear compound rather than a mononuclear one, because many known complexes of Ag^I and Au^I with thiol ligands have been reported as polymeric and recently some phosphine ligands have been combined in order to limit such polymerizations.[6,8,9]

The solid FT-IR spectrum (Fig. 1e) of the sodium salt shows (1) one strong broad band at ~3450 cm^{-1} due to the hydrated water, (2) the disappearance by complex formation of S—H stretching band at ~2560 cm^{-1} due to the —SH group, suggesting Ag—S bond formation, and (3) the splitting to two strong bands at ~1700 and ~1600 cm^{-1} from one very strong carbonyl stretching band at ~1700 cm^{-1} in the carboxyl group of a free H_3TMA ligand (Fig. 1a). IR bands in the carbonyl stretching region of the free-acid form of the TMA–Ag^I complex (Fig. 1b) and the free H_3TMA ligand (Fig. 1a) are very close, whereas those of the sodium salts of the TMA ligand (Fig. 1c, d), which have been prepared by rotavaporating the aqueous solution containing H_3TMA and 2 or 3 equivalents of NaOH, are largely shifted to ~1600 cm^{-1} by salt formation such as $—COO^- \cdots Na^+$. These IR spectra strongly suggest that one carboxyl group of the sodium salt of the TMA–Ag^I complex remains as a free acid after the complexation and the other carboxyl group is attached to the Na^+ ion in the solid. The presence of one protonated carboxylic group in the sodium salt of the TMA–Ag^I complex is also reasonable from the analytical results. Thus, it is shown in the monomeric units of TMA–Ag^I complexes that the ligand donor atoms coordinating to the soft Ag^I atom are only soft sulphur atoms, but not carboxylic, hard oxygen atoms.

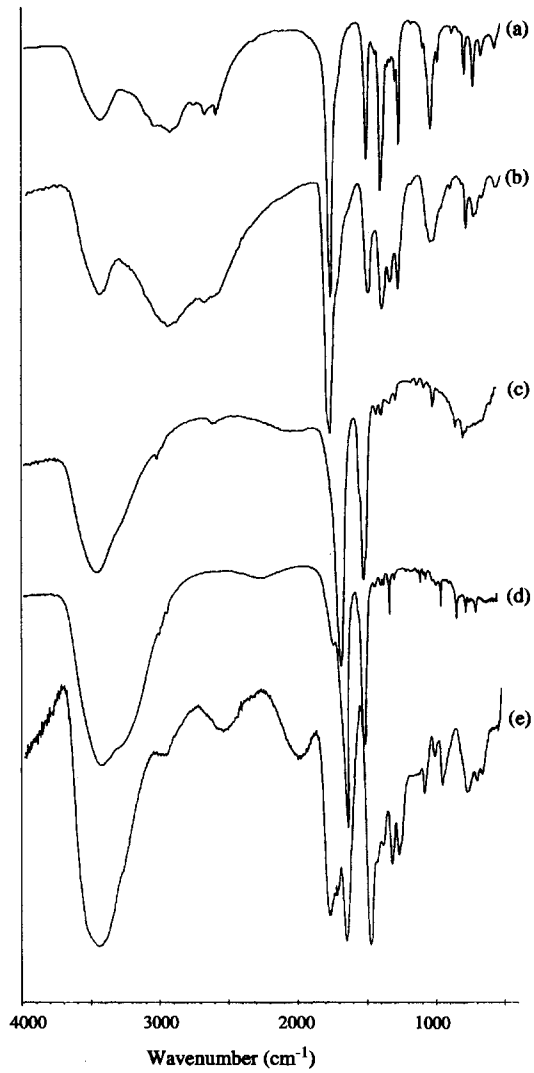

Fig. 1. Solid FT-IR spectra in the 4000–400 cm^{-1} region measured in KBr disks: (a) free H_3TMA; (b) the free acid form of the TMA–Ag^I complex; (c) the sodium salt of TMA, prepared by rotavaporating the aqueous solution containing H_3TMA and 2 equivs of NaOH; (d) the sodium salt of TMA, prepared by rotavaporating the aqueous solution containing H_3TMA and 3 equivs of NaOH; (e) the sodium salt of the TMA–Ag^I complex.

The Ag^I complex in aqueous solution is present as a single species, but not as an equilibrium species. There is only a dissociation of Na^+ ions as countercation in the complex, but there is no dissociation of Ag^I ions and protons. As a matter of fact, AgCl precipitate from free Ag^I ions was never detected at 25°C and even at 80°C by adding 40 cm^3 of satu-

rated NaCl aqueous solution to the 1.0 g of TMA–Ag^I complex dissolved in 10 cm^3 aqueous solution. Thus, the chemical equilibrium between free Ag^I ion and the TMA–Ag^I complex in aqueous solution is ruled out in consideration of all NMR measurements.

The ^{109}Ag NMR spectra observed as only one signal at 868.7 ppm, measured with reference to external $AgNO_3$ in D_2O, shows that all of Ag^I ions in this oligomeric complex are equivalent.

The ^{13}C NMR spectra (Fig. 2) give information of the coordinating TMA ligands in the TMA–Ag^I complex in aqueous solution. The ^{13}C NMR spectrum of free H_3TMA ligand in D_2O measured at 29°C shows four different carbon resonances due to two carboxyl carbons at δ 178.9 and 177.1 ppm, methylene carbon at δ 41.9 ppm and methyne carbon at δ 38.8 ppm. On the other hand, the ^{13}C NMR spectrum of the sodium salt of the complex, measured in D_2O at 26°C, also shows four different carbon signals due to two carboxyl carbons at δ 183.7 and 180.2 ppm, methylene carbon at δ 48.8 ppm and methyne carbon at δ 45.5 ppm. By this complexation, all carbon signals of the TMA ligand are shifted downfield. This simple ^{13}C NMR spectral pattern shows that all of the TMA ligands coordinating to Ag^I ions in the oligomeric complex are equivalent. These ^{13}C NMR spectra of the complex were essentially unchanged from those measured at an elevated temperature (~70°C). No significant difference of ^{13}C NMR chemical shifts at elevated temperature strongly indicates that there is no dissociation of TMA ligands from the TMA–Ag^I complex in aqueous solution. This property contrasts with that of the previously reported TMA–Au^I complex.[10] The simple four-line ^{13}C NMR spectrum of the complex further suggests that the TMA ligands are coordinated to Ag^I ions only through sulphur atoms, but not through carboxylic oxygens, because the coordination by one of two carboxylic groups in addition to the coordination by sulphur atoms should result in a much more complicated ^{13}C NMR spectrum. It has been generalized that the Ag^I ion shows a pronounced tendency to exhibit a linear two-fold coordination for nitrogen and sulphur donors in aqueous solution.[7,11] The results of ^{109}Ag and ^{13}C NMR spectral measurements, in addition to the FT-IR, clearly suggests that the oligomeric complex contains a repeat of a linear Ag^I–S_2 unit, where each Ag^I atom should be bonding to two bridging sulphur atoms. The suggested structure of the complex, shown in Fig. 3, also corresponds to that reported previously for the disodium salt of the Au^I–TMA^{3-} 1:1

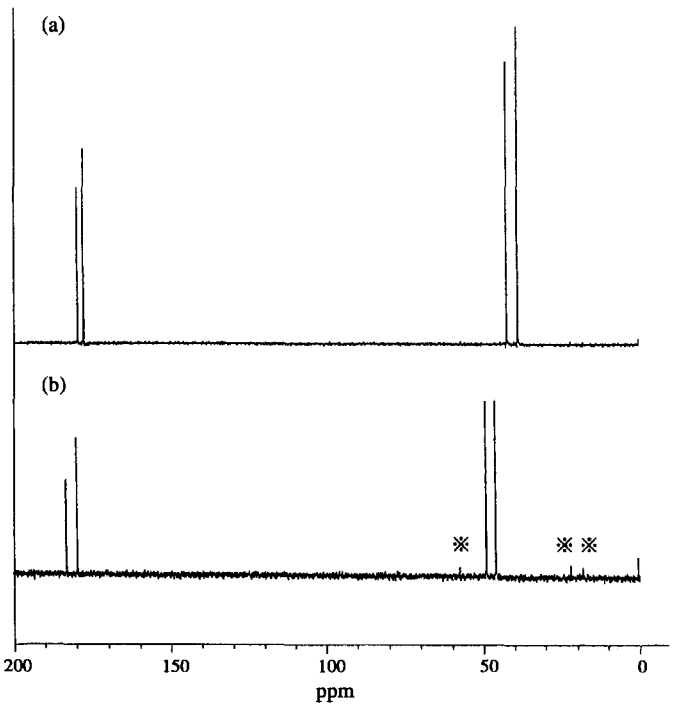

Fig. 2. $^{13}C\{^1H\}$ NMR measured in D_2O with reference to internal DSS: (a) free H_3TMA at 29°C; (b) the sodium salt of the TMA–Ag^I complex at 70°C, where carbon signals denoted by (※) are from methylene groups of the DSS.

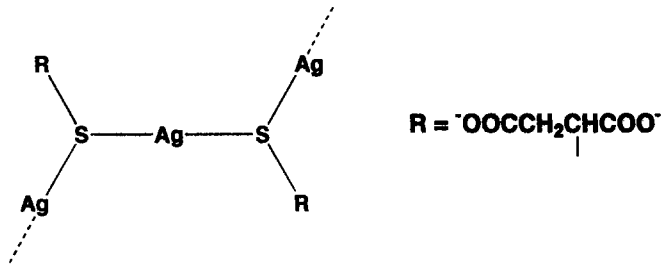

Fig. 3. Suggested structure of the sodium salt of the TMA–Ag^{I} complex.

complex (Myocrisin), which is an antiarthritic drug.[12,13]

The 400 MHz ^{1}H NMR spectra (Fig. 4) of the sodium salt of the TMA–Ag^{I} complex in D_2O were recorded at 23 and 80°C, and compared with that of free H_3TMA ligand recorded at 25°C. The ^{1}H NMR spectrum of the free H_3TMA ligand in D_2O shows an ABX system pattern consisting of a doub-

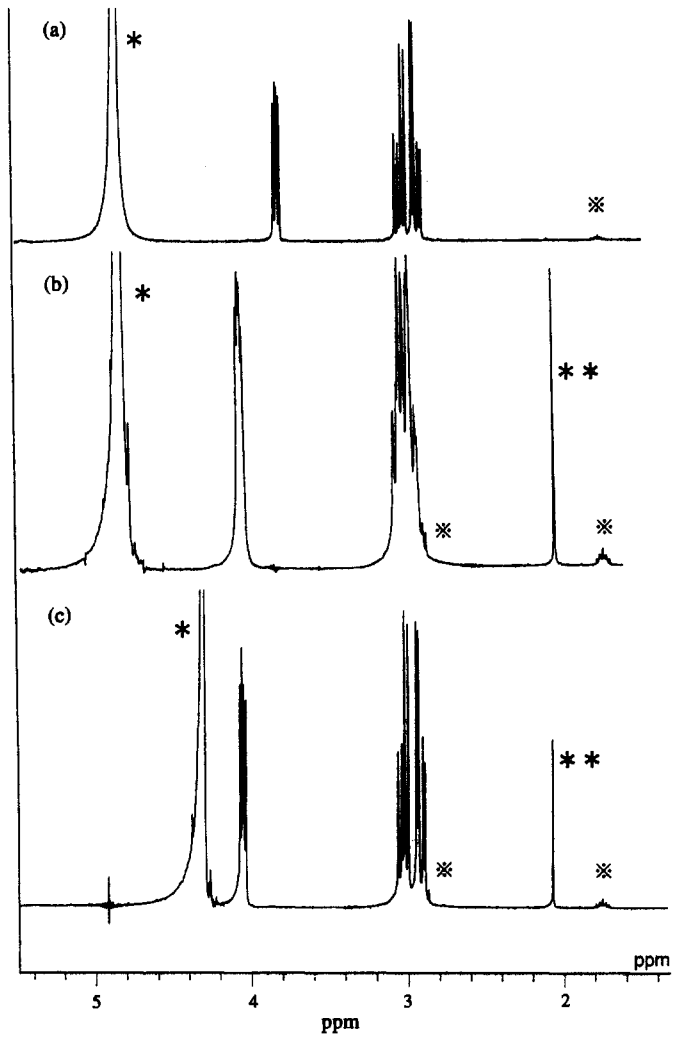

Fig. 4. ^{1}H NMR spectra measured in D_2O with reference to internal DSS: (a) free H_3TMA at 25°C; (b) the sodium salt of the TMA–Ag^{I} complex at 23°C; (c) spectrum at 80°C. Proton signals denoted by (*), (**) and (※) are from water (HOD), contaminated CH_3CN and methylene groups of the DSS, respectively.

Table 1. Antimicrobial activities of the TMA–Ag^I complex and related compounds evaluated by MIC[a]

Test organisms	Thiomalic acid (H_3TMA)	TMA–Ag^I complex	$AgNO_3$	Zeolite–Ag^+[b]	TCPN[c]	STS[d]
Escherichia coli	5000	31.3	6	390	>1000	39
Staphylococcus aureus		>2000	>1600	>50,000	15.6	
Bacillus subtilis	2500	>2000	100	195	15.6	1250
Pseudomonas aeruginosa		31.3	6	390	>1000	
Saccharomyces cerevisiae		3.9	1600	195	15.6	
Candida albicans		>2000	>1600	>50,000	31.3	
Aspergillus niger		>2000	>1600	3125	31.3	
Aureobasidium pullulans		31.3	>1600	1563	31.3	
Cladosporium sphaerospermum		125	>1600	1563	15.6	
Penicillium citrinum		>2000	>1600	3125	31.3	
Fusarium moniliforme		>2000	>1600	3125	15.6	
Rhizopus stolonifer		>2000	>1600	781	31.3	

[a] Minimum inhibitory concentration ($\mu g\ cm^{-3}$).
[b] Commercially available Zeolite-supported Ag^+.
[c] Tetrachloroisophthalonitrile.
[d] Aqueous solution of $Na_3[Ag(S_2O_3)_2]$.

let of doublets (four lines) for a vicinal proton of a methyne group and two double doublet (four lines × 2) signals of germinal protons (H^a and H^b) of the neighbouring methylene group. The coupling constants were determined as $^3J_{CH-H^a} = 8.79$ Hz, $^3J_{CH-H^b} = 5.86$ Hz and $^2J_{H^a-H^b} = 17.6$ Hz, and the chemical shift of the methyne proton was at δ 3.81 ppm, and those of the H^a and H^b protons at δ 3.02 and 2.94 ppm, respectively.

A similar spectral pattern was also observed in the ^1H NMR spectrum of the complex at 23°C. The only difference observed was broadening of all of the signals and the 0.21 ppm downfield shift of the methyne proton, whereas the chemical shifts of the methylene protons were almost unchanged (chemical shifts: methyne proton δ 4.07 ppm, H^a proton δ 3.05 ppm and H^b proton δ 2.98 ppm). The observed broadening of the signals may be interpreted as a higher molecular weight of the compound based on the oligomerization. The downfield shift of the methyne proton is attributed to TMA–Ag^I complex formation through S—Ag^I bonding. The ^1H NMR spectrum of the complex at elevated temperature (80°C) showed sharp signals, although the chemical shifts were essentially unchanged (chemical shifts: methyne proton δ 4.05 ppm, H^a proton δ 3.03 ppm and H^b proton δ 2.92 ppm). Such sharp signals can be accounted for by a decreased degree of oligomerization resulting from partial dissociation of S—Ag^I bonds at higher temperature. This lack of a significant difference of ^1H NMR chemical shifts at elevated temperature is also consistent with the observations in the ^{13}C NMR spectra, which suggests no dissociation of TMA ligand from the TMA–Ag^I complex in aqueous solution.

Antimicrobial activity

Antimicrobial activity exhibited by the sodium salt of the TMA–Ag^I complex is listed in Table 1, as estimated by the minimum inhibitory concentration (MIC; $\mu g\ cm^{-3}$), together with those of the aqueous $[Ag(S_2O_3)_2]^{3-}$ (STS) solution, Ag^+ ion as aqueous $AgNO_3$, free H_3TMA ligand, commercially available Zeolite-supported Ag^+ and an agricultural chemical, tetrachloroisophthalonitrile (TCPN). It should be noted that the TMA–Ag^I complex shows remarkable and superior activities against Gram-negative (*E. coli* and *P. aeruginosa*) bacteria, one yeast (*S. cerevisiae*) and two moulds (*A. pullulans* and *C. sphaerosperumum*). Although aqueous $AgNO_3$ itself has similar activities for bacteria, the complexation of Ag^I with the TMA ligand leads to appreciably enhanced activities for some moulds.

Properties of anionic, yellow TMA–Ag^I complexes

It is novel that all of the anionic TMA-Ag(I) complexes obtained here are characteristically yellow-coloured and light-stable both in the solid state and in aqueous solution. The TMA–Ag^I complexes do not have melting points. Thermal stability or decomposition temperature indicated by TG/DTA measurement is significantly influenced by the kind of counter-cation: barium salt (decomp. temp. 220°C) > sodium salt ~caesium salt (180°C) >

lithium salt (150°C) > NH_4^+ salt (130°C) > potassium salt (120°C) > > free acid (< 60°C). The compounds with the higher decomposition temperature were more light-stable. The sodium, lithium caesium, potassium and NH_4^+ salts are very soluble only in water, the barium salt insoluble in almost solvents and the free acid soluble only in DMSO and DMF. The water-soluble sodium, lithium, caesium, potassium and NH_4^+ salts cannot be recrystallized, but reprecipitated as yellow powder by cooling their aqueous solution at ice-water temperature. In concentrated solutions, such reprecipitation takes place even at room temperature. This aqueous system containing reprecipitated powder changes completely to a homogeneous, clear yellow solution on being warmed. These properties are utilized as purification work-up for the isolation of the TMA–Ag^I complexes.

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