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Physico-chemical characterization of meglumine antimoniate

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The leishmanicidal drug, meglumine antimoniate (MA), has been synthesized by the reaction of antimony oxyhydrated and N-methyl glucamine. Infrared and solid state NMR ^{13}C analysis of MA and the ligand strongly suggests that antimony binds to N-methyl glucamine through the oxygen of C-3 carbon. Potentiometric titration indicated that, between pH 4.5 and 7.5, MA exists in the zwitterionic form.

Keywords: meglumine antimoniate, structure, leishmaniasis

Introduction

The use of antimonials to treat leishmaniasis is nearly historical, and they are still in use today (Marsden 1985). Tartar emetic (antimony III potassium tartrate) was the first antimony derivative to show a leishmanicidal effect. Other trivalent antimonials (i.e. stibophen, anthiomaline) were tested and found to be as effective and less toxic. They have been widely used until the much less toxic pentavalent antimonials were discovered and introduced in the forties. Meglumine antimoniate (antimony(V) N-methyl-glucamine) and sodium stibogluconate (sodium antimony(V) gluconate) are now in common use for the treatment of leishmaniasis. Surprisingly, the exact structure of these compounds, and their mechanism of action and toxicity, have not been defined until now. As a result, rational design of new more potent and less toxic antimonials was not achieved.

In the present work, we determined some structural and physicochemical features of meglumine antimoniate (MA). I.R. and solid state NMR analysis brought informations about the site of attachment of antimony in the ligand. On the other hand, we evaluated the pH-dependence of MA ionization state.

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Materials and Methods

General experimental techniques

N-methyl glucamine and Sb_2O_5 (Aldrich) were used as received. All chemicals used were of the reagent grade. IR spectra of the compounds have been recorded on a Galaxy Series FTIR 3000 spectrophotometer using KBr pellets. ^{13}C NMR spectra have been recorded at 400.13 MHz on a Bruker DRX 400 AVANCE spectrometer, with a MAS 400 SB-BLA multinuclear sound and a 7 mm KEL-F rotor. Conductivity data were obtained with a YSI Conductivity Bridge, model 31, with a conductivity cell ($C=8.8x10^{-2}cm^{-1}$). The TG was obtained on a Shimadzu TGA-50 under nitrogen atmosphere. C, H and N analysis were carried out using a Perkin-Elmer 240 Elemental Analyzer. Antimony content was determined by atomic absorption using a Hitachi Z8200 spectrophotometer.

Preparation of meglumine antimoniate

To a solution of N-methyl glucamine (5 g) in warm water (20 ml), freshly precipitated and still wet hydrated antimony pentoxide is added until the solution remains milky. The solution thus obtained is filtered and after cooling precipitated by addition of alcohol.

The solid obtained was analyzed by elemental analysis (C,H,N) and atomic absorption (Sb). Thermogravimetric data indicated the presence of three molecules of water. Calculated for $C_7H_{24}O_{11}Sb$: C, 20.07; H, 5.70; N, 3.58; Sb, 28.03. Found: C, 20.45; H, 5.57; N, 3.56; Sb, 28.9.

Potentiometric titration

Potentiometric titrations of MA were performed with a Metrohm automatic system equipped with an electrode pair of glass and calomel. The MA concentration was in the range of 0.01–0.04 mol/L. The solutions were clear throughout the whole pH range studied. Values for the protonation constants were calculated using the computer program SUPERQUAD (Gans *et al.* 1985). Species distribution diagram was calculated using the computer program SCECS (Duarte *et al.* 1994). Measurements were performed at 25°C in aqueous solution ($NaClO_4$, I = 0.1 mol/L) under nitrogen atmosphere.

Results and discussion

Following reaction between antimony oxyhydrated and N-methyl glucamine, we obtained an amorphic solid which, analyzed by elemental and thermal analysis and atomic absorption, showed a ligand: Sb molar stochiœmetry of 1:1. The resulting product was further analyzed by I.R. and ^{13}C NMR spectroscopies. Fig. 1 displays I.R. spectra of the MA synthesized in this study. Our MA showed the same I.R. spectrum than 2168-RP, the commercially available meglumine antimoniate (data not shown). This data strongly suggests that these are the same product. I.R. spectrum of MA, when compared to that of N-methyl glucamine, showed changes in the absorption bands. The strong absorption around 3000 cm^{-1}, with multiple bands extending to 2300 cm^{-1}, can be attributed to stretching vibrations in the

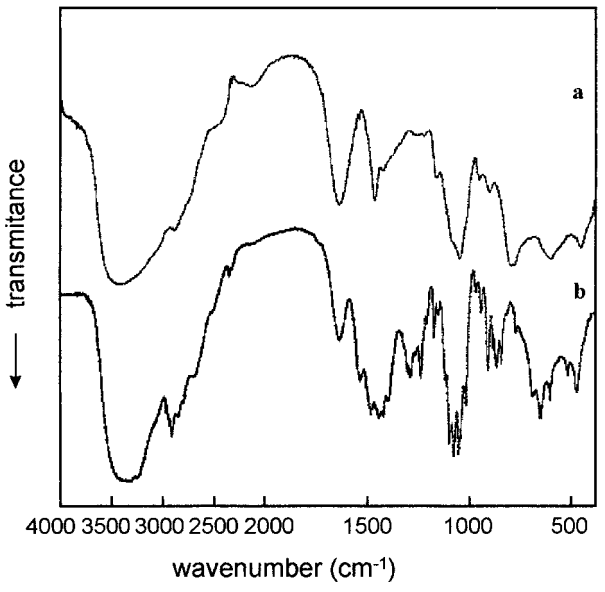
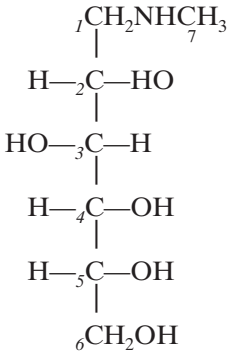


Figure 1. Infrared spectra of (a) N-methyl glucamine and (b) meglumine antimoniate

$-NH_2^+-$ group, and is therefore due to protonation of secondary amine of the complex. Bands due to the angular deformation in the plane of O-H groups (1500–1200 cm^{-1}) and C-O stretching vibrations (1100–1000 cm^{-1}) were found to be wider in the case of the complex than in the case of the ligand. MA also displayed a new band around 800 cm^{-1} due to the rocking vibration of the $-NH_2^+-$ (Bellamy 1978). Finally, it was not possible to localize the Sb-O stretching vibration in MA spectrum expected in the 500–650 cm^{-1} region (Holmes *et al.* 1987), because of the presence of a large band in this region due to the ligand.

The ^{13}C NMR spectra of N-methyl glucamine in D_2O solution displayed only one signal at 71.8 δ for C-2, C-3, C-4 and C-5.



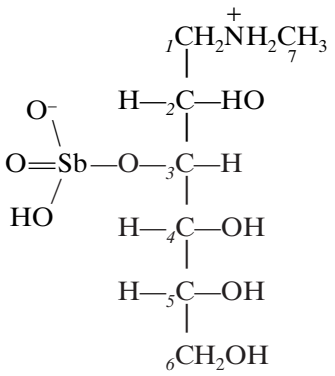
N-methyl glucamine

However, when this compound was in the solid state, the ^{13}C NMR spectrum showed the same chemical shift only for C-2 and C-3. Considering the empirical effect of the substituent groups, it was then possible to assign the ^{13}C NMR chemical shift of all carbons. The result of this assignment is displayed in Table 1. In the case of MA in the solid state, the ^{13}C NMR spectrum showed a strongly overlapped band in the region of 50 to 80 δ. This spectrum was then deconvoluted computationally with the fitting

Table 1. Solid state ^{13}C NMR of N-methyl glucamine (ligand) and meglumine antimoniate (MA)

carbon	Chemical shift (δ)	
	Ligand	MA
C-1	55.9	54.6
C-2	71.1	70.1
C-3	71.1	79.7
C-4	68.0	65.4
C-5	73.8	74.9
C-6	62.9	61.5
C-7	37.8	35.8

program BANDFIT (Lopes *et al.* 1992–1993). Fig. 2 displays the experimental ^{13}C NMR spectrum and the resulting calculated bands of MA in region of 50 to 80 δ. Thus, six components were detected. In order to evaluate the accuracy of the band envelope, the root mean square of residual (DIS) was used. The optimized parameters obtained with a DIS value of 0.005 transmittance units demonstrated the good agreement between experimental and theoretical data (Maddams 1980). Comparison of ^{13}C NMR data between the complex and the ligand (Table 1) showed a signal shift from 71.1 δ to 79.7 δ. This shift can be attributed to the binding of antimony either to C-2 or C-3 oxygens. As the C-4 signal is more affected by the complexation than the C-1 one, we proposed that C-3 oxygen is the binding site of antimony. From these data, the following molecular structure for MA can be proposed:



Meglumine antimoniate (MA)

Another point we wished to address in this study is the pH-dependence of the ionization state of MA. It is well established that *Leishmania* parasites live and replicate within an acidified vacuole of the mammalian macrophage (Alexander 1992). This localization also implies that the drug, in order to reach the parasite, have to cross distinct compartments of different pH. Determination of MA ionization state is therefore important to evaluate the possible influence of drug ionization on its passage through biological membrane and its retention inside the acidic vacuole. Protonation constant values obtained for MA were 10.26±0.02 and 12.36±0.02. Therefore, MA contains two dissociable protons which can be attributed to the amino group (pKa_2=10.26) and to the antimonic acid group (pKa_1=2.10). Fig. 3 displays the diagram of species distribution as a function of pH. It shows that, between pH 4.5 and 7.5, the complex exists as 100% in the zwitterionic form. That was confirmed by conductivity measurements (data not shown). This data therefore indicates that MA ionization state does not depend on pH, in the range of physiological pH (between 5 and 7.5).

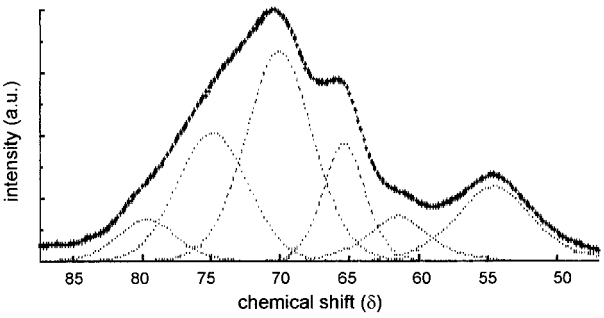


Figure 2. Experimental and calculated ^{13}C NMR spectrum (400 MHz; MAS) and the resulting deconvoluted bands of meglumine antimoniate in the solid state.

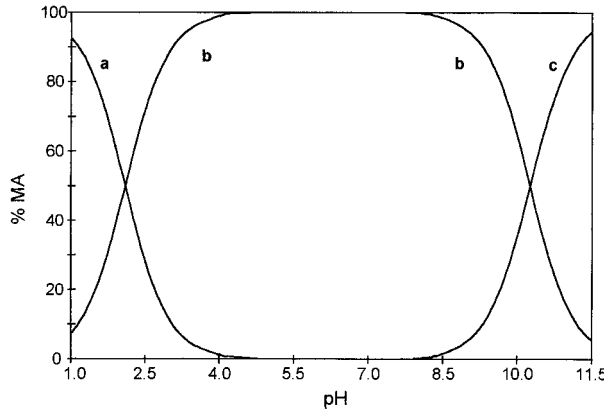


Figure 3. Species distribution curve for meglumine antimoniate as a function of pH, calculated for MA concentration of 0.04 mol/L. (a) protonated complex, (b) zwitterionic complex, (c) deprotonated complex.

Acknowledgments

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