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Synthesis, characterization of strontium-bile acid salts and their bioactivity vs. the anti-osteoporosis drug strontium ranelate

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ABSTRACT

The strontium salts Sr(cholate)$_2$, (Compound **1**), Sr(dehydrocholate)$_2$, (Compound **2**) and Sr$_3$(3-dehydrocholanoyliden-L-tartrate)$_2$, (Compound **3**) have been prepared and characterized. The potential anti-osteoporotic activity of these compounds was tested on human primary osteoblasts (hOBs) and human primary osteoclasts (hOCs) in comparison with the bioactivity of strontium ranelate, previously registered as drug in the treatment of post-menopausal osteoporosis. Our results led to the hypothesis that the tested compounds, particularly Compound **2**, may have requirements for modulating skeletal tissue regeneration or at least down regulating the loss of bone mass. In fact, all tested compounds have been shown to induce maturation in human primary osteoblasts (hOBs) and apoptosis of human primary osteoclasts (hOCs) at the same time.

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1. Introduction

Evidence is growing that strontium (Sr) influences bone cells and bone metabolism in vitro and in vivo. Many studies have shown that Sr^{2+} can stimulate bone formation and inhibit bone resorption both in vitro and in vivo [1,2]. Recently strontium ranelate (SrR), a compound containing two ions of stable bivalent strontium combined with ranelic acid, which acts as a carrier, is registered as drug in the treatment of post-menopausal osteoporosis [3–5].

SrR is hypothesized to be a dual-acting agent with both anti-resorptive and anabolic skeletal benefits [6].

The precise molecular mechanism responsible for SrR effects is not clear and needs to be investigated. Functional analyses that have been performed suggest that different signaling pathways may be involved in the osteoblastic and osteoclastic responses to SrR [7–9]. Although, SrR is approved in several countries for the treatment of post-menopausal osteoporosis, it is a relatively new drug and its long-term safety still needs to be documented for the ideal management of osteopenic diseases.

Although, SrR is now being administered to women for osteoporosis, it could have potentially therapeutic value in different osteopenic disorders, including Paget's disease and cancer with bone metastases. To avoid possible adverse reactions in the greater population, it may be important to design analogous compounds that are better tolerated.

Since it is evident that the therapeutic action of SrR is due exclusively to Sr^{2+}, we reasoned that the performance of strontium-based drugs could be improved by modification of the carrier anion. Ranelate bears four carboxylic groups and allows the transport of a large amount of Sr^{2+} metal (two cations per tetra-charged anion, 34.1% in mass), but it showed a few drawbacks. First, it is known that a large proportion of Sr^{2+} administrated as ranelate is eliminated via gastrointestinal secretion [10,11]; therefore SrR needs to be administrated as a daily dose of 2 g, resulting in a low patient compliance. Second, synthesis of ranelic acid requires a long, multistep chemical process which contributes to the high price of SrR [12]. Therefore, it would be of first interest to couple the active Sr^{2+} cation to a readily available natural non-toxic anion that can increase the intestinal absorption of the metal.

Analysis of the characteristics of bile acids indicates that their anions may be promising alternative carriers for Sr^{2+}. Bile acids comprise a large group of natural or semi-synthetic molecules that are readily available at low cost; they have been used for pharmaceutical purposes for a long time, because of their known value as drug carriers [13]. Bile acid transport in the gastrointestinal tract is recognized as being an efficient high capacity system because of its involvement in reabsorption of bile salts following fat digestion. Bile acid anions are of therapeutic value because they are known to form complexes that increase the absorption of metal ions, like calcium and iron [14–16]. On this basis, we hypothesized that Sr^{2+} absorption also could be enhanced by the use of bile acids. To verify this hypothesis, and to ascertain if these observations can be

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exploited for the development of new strontium-based anti-osteoporosis drugs, it was essential to document that strontium bile salts are at least as active as SrR and are not cytotoxic. This was the aim of the present preliminary study.

2. Experimental

2.1. General

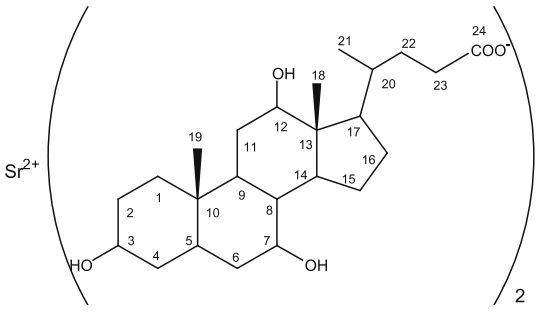
3-Dehydrocholanoyliden-L-tartaric acid was prepared as reported [17]. All the other chemicals and solvents were used as purchased (reagent grade). Elemental analysis (C, H, N) was performed using a Carlo Erba instrument, model EA1110.

The amount of strontium in each sample was determined by atomic absorption spectroscopy using 460.7 nm resonance line in an air-acetylene flame. Analysis was performed using a Perkin-Elmer atomic absorption spectrometer (Analyst 800); a strontium hollow cathode cave lamp was used (Slit width 0.2H, signal type AA time average, reading time 3 s, oxidant flow (air) 20 L/min, acetylene flow 2.5 L/min).

Samples and standards were added to 0.1% KCl solution, the 0.1% KCl solution being used as a calibration blank. Because considerable ionization of strontium occurs in an air-acetylene flame, it was controlled by the addition of an alkali salt. A calibration curve was made after reading four standard strontium solutions (1, 3, 5, 10 mg/L) prepared by diluting with deionized (18 MΩ cm) water (Milli-Q system, Waters Corp., Milford, MA). An atomic spectroscopy standard solution (1 μg/mL) of Strontium ($Sr(NO_3)_2$ in a HNO_3 2% matrix, Perkin-Elmer Pure) and a blank solution were also read. For each concentration level 3 readings were taken; three samples, marked with numbers **1**, **2** and **3** were read and their concentrations found to be in the calibration range. To check the accuracy of the method, a recovery test was performed adding these to those of known Sr concentration. Recoveries were found to be between 102% and 104%. The precision was tested taking triplicate readings, and the relative standard deviation (RSD%) was found to be less than 1%. FT-IR spectra were recorded on a Bruker Vertex 70 FT-IR instrument (4000–300 cm^{-1}). ^{1}HNMR spectra were recorded on a Bruker 200 AM. The splitting of proton resonances in the ^{1}H NMR spectra are defined as s = singlet, d = doublet, and m = multiplet. ^{13}C NMR spectra were recorded on a Varian 400 NMR spectrometer (^{13}C at 100.57). Peak positions shown are relative to tetramethylsilane.

2.2. Synthesis

2.2.1. Sr(cholate)$_2$ (1)



A solution of strontium hydroxide, $Sr(OH)_2 \cdot 8H_2O$ (0.5 g, 1.8×10^{-3} mol) in 75 mL of water was added to a suspension of cholic acid (1.5 g, 3.7×10^{-3} mol) in water (30 mL), which became immediately clear. The pH increased from 5.5 to 8.0. Occasional solid residues were eliminated by filtration. After stirring for 30 min at room temperature, the solution was evaporated to dryness giving a white residue that was washed with acetone and then dried at 60 °C for 4 h ($Sr(cholate)_2 \cdot 2H_2O$, 1.5 g, 1.6×10^{-3} mol, yield 89%). The solubility in water of **1** is 0.02 g/mL at 25 °C.

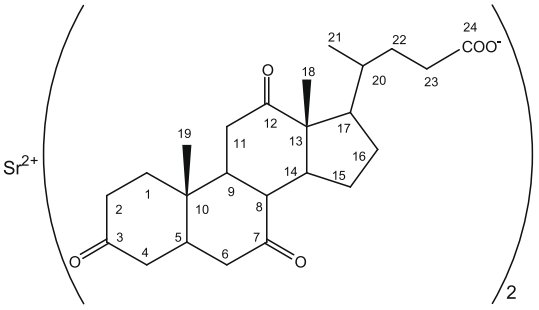
Elemental analyses: see Table 1.

IR (KBr, cm^{-1}) selected peaks: 3700–3100 (broad, OH), 1544 (COO^-), 1421.

^{1}H NMR (D_2O, ppm) 0.65 (3H, s, CH_3-19), 0.84 (3H, s, CH_3-18), 0.95 (3H, d, CH_3-21), 1.0–2.3 (24H, m, CH), 3.43 (1H, m, CH-3), 3.83 (1H, m, CH-7), 4.00 (1H, m, CH-12).

^{13}C NMR (D_2O, ppm,) selected peaks: 184.28 (C-24), 72.99 (C-12), 71.46 (C-3), 68.12 (C-7).

2.2.2. Sr(dehydrocholate)$_2$ (2)



The same procedure as above, using dehydrocholic acid suspended in water, gave $Sr(dehydrocholate)_2 \cdot H_2O$, (1.5 g, 1.65×10^{-3} mol, yield 91.7%).

The solubility in water of **2** is 0.01 g/mL at 25 °C.

Elemental analyses: see Table 1.

IR (KBr, cm^{-1}) selected peaks: 3800–3000 (broad, H_2O), 1714(ν C=O), 1557 (COO^-), 1430.

^{1}H NMR (D_2O, ppm): 0.71 (3H, d, CH_3-21), 1.08 (3H, s, CH_3-18), 1.26 (3H, m), 1.36 (3H, s, CH_3-19), 1.5–2.2 (12H, m, CH, CH_2), 2.37 (2H, m, CH_2-11), 2.97–3.29 (7H, m, CH_2-2, CH_2-4, CH_2-6, CH-8).

^{13}C NMR (D_2O, ppm,) selected peaks: 219.80 (C-12), 216.91 (C-7), 215.89 (C-3), 184.32 (C-24).

2.2.3. Sr$_3$(3-dehydrocholanoyliden-L-tartrate)$_2$ (3)

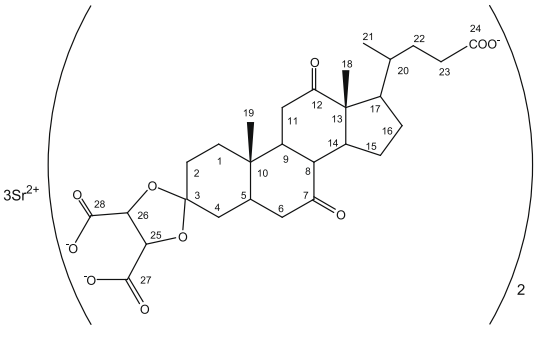


Table 1
Elemental analyses.

Compound	Formula	C%		H%		Sr%	
		Found	Calc.	Found	Calc.	Found	Calc.
Sr(cholate)$_2$ **(1)**	$C_{48}H_{78}O_{10}Sr \cdot 2H_2O$	61.28	61.45	8.55	8.74	9.50	9.30
Sr(dehydrocholate)$_2$ **(2)**	$C_{48}H_{66}O_{10}Sr \cdot H_2O$	63.22	63.54	7.05	7.50	10.07	9.70
Sr$_3$(3-dehydrocholanoyliden-L-tartrate)$_2$ **(3)**	$C_{56}H_{70}O_{20}Sr_3 \cdot 2H_2O$	49.15	49.40	5.35	5.43	19.63	19.30

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