Unusually Strong Binding of Ca^{2+} Ions by the Novel Antibiotic Squalestatin-1

Wojciech Bal,[a,b] Alex F. Drake,[a] Malgorzata Jezowska-Bojczuk,[b] Henryk Kozlowski,[b] Leslie D. Pettit[c] and Peter J. Sadler*[a]

[a] *Department of Chemistry, Birkbeck College, University of London, Gordon House and Christopher Ingold Laboratories, 29 Gordon Square, London, UK WC1H 0PP*
[b] *Institute of Chemistry, University of Wroclaw, ul. F. Joliot-Curie 14, 50-383 Wroclaw, Poland*
[c] *School of Chemistry, University of Leeds, Leeds, UK LS2 9JT*

The squalestatins, inhibitors of squalene synthase and potent antimicrobial agents, have potential both as cholesterol-lowering drugs and as antibiotics; using potentiometry and circular dichroism, we show that squalestatin-1 has a much higher affinity for Ca^{2+} than citrate, and readily inserts into SDS micelles (a model membrane), processes which may be relevant to its mechanism of action.

Squalestatins are extracted from a natural source, the fermentation broths of a newly isolated species of *Phoma*, a soil fungus.[1] They are a novel class of inhibitors of the enzyme squalene synthase and have potential as cholesterol-lowering agents in man.[2] They also exhibit a potent broad spectrum of antifungal activity *in vitro*, including activity against important pathogens. In this work we have used potentiometry and circular dichroism (CD) to demonstrate that squalestatin-1 (SQ1) has an unusually high affinity for Ca^{2+} ions and readily inserts into negatively-charged sodium dodecyl sulfate (SDS) micelles, a model for a membrane system.[3] These features may play a role in the biological activity of squalestatins.

SQ1 contains the bicyclic core[4] [1*S*-(1α, 3α, 4β, 5α, 6α, 7β)]-4,6,-7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-3,4,5-tricarboxylic acid in which the citric acid like moiety has potential as a strong metal-binding agent. An initial study of the protonation of SQ1‡ in aqueous solution using CD spectroscopy revealed an irreversible spectral change below pH 3. Subsequent potentiometric studies were therefore limited to the range pH 3.5 to 10.

The stability constants of H^+ and Ca^{2+} complexes of SQ1 in the presence of 0.1 mol dm^{-3} KCl were determined at 25 °C using pH-metric titrations (pH changes monitored using a combined glass–calomel electrode calibrated in hydrogen concentrations using $HClO_4$ with the Molspin automatic titrator, 5 cm^3 solution, 0.2–0.4 mmol dm^{-3} SQ1, mol ratio SQ1 : Ca^{2+} 1 : 1 to 10 : 1). Addition of Ca^{2+} to SQ1 at a 1 : 1 mol ratio at pH 5.5 led to precipitation with SQ1 concentrations > 0.5 mmol dm^{-3}, and therefore potentiometry was carried out within a lower concentration range. The data were analysed using the program SUPERQUAD,[5] and the results are shown in Table 1 where they are compared with similar data[6] for citrate, $CH_2(CO_2^-)C(OH)(CO_2^-)CH_2CO_2^-$.

The two protonation constants can be associated with two of the three SQ1 carboxy groups. It seems likely that the third pK_a value is <3 and that this could not be measured because of irreversible reactions that occurred in highly acidic solutions, perhaps involving aggregation *via* the long hydrophobic tails and ring opening.

The increased stability of the calcium SQ1 complex compared to calcium citrate by more than an order of magnitude (Table 1) is notable, particularly in view of the significantly lower affinity of SQ1 for protons when compared to citrate. This can be attributed to the presence of the SQ1 ring system which constrains the spatial orientation of the

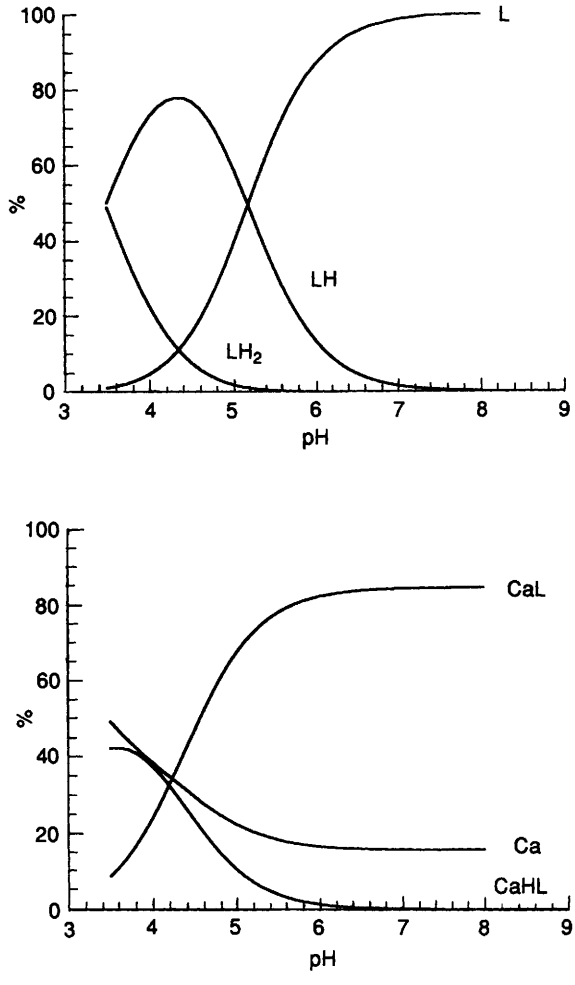
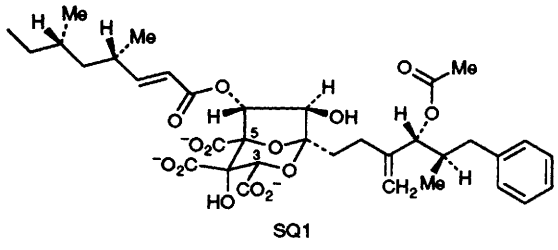


Fig. 1 Species distribution diagrams for protonation of SQ1 (*a*) and Ca^{2+} binding to SQ1 (1 : 1, 0.4 mmol dm^{-3}) (*b*). L is the trianion of SQ1 with all three carboxylate groups deprotoanted and Ca refers to unbound (aquated) Ca^{2+} ions.

Table 1 Potentiometric data (errors ± 0.01 log unit) at 25 °C and I = 0.10 mol dm^{-3} (KCl).[a]

Protonation			Complexation		
	log K			log β	
Species	SQ1	Citrate	Species	SQ1	Citrate
LH	5.19	5.83	CaLH	9.13	7.86
LH_2	3.49	4.34	CaL	4.94	3.63
LH_3	—	2.89			

[a] The data for citrate are taken from ref. 6.



SQ1

carboxy and hydroxy groups. This, in turn, would limit stabilization of protonated species by hydrogen bonding but favour chelation to the larger Ca^{2+} ion, probably *via* the carboxylate groups at C(3) and C(4) together with the C(4) hydroxy group. Such tridentate coordination is found in 1:1 Mg^{2+} citrate[7] and Mn^{2+} citrate[8] complexes.

We found that white precipitates of CaSQ1 are readily soluble in SDS solutions. The CD spectrum of SQ1 (Fig. 2) contains contributions from the three carboxylate and two ester chromophores. However, the correlation of the relatively long wavelength 220 nm CD band with the strong UV absorption maximum at *ca.* 215 nm (ε *ca.* 20000 mol^{-1} dm^3 cm^{-1}) permits assignment of the latter to the α,β-unsaturated ester chromphore at C(6). The observed change in the CD spectrum of SQ1 in micellar SDS solutions[9] can be related to this group which responds to the insertion of the hydrophobic tails of SQ1 into the micelles. No further change in the CD spectrum was observed on addition of Ca^{2+} ions to SQ1 (1:1, pH 7) in SDS micelles, which ought to be the case for Ca^{2+} ions binding to exposed carboxylate groups with little effect on the conformation of the hydrophobic tails which remain buried. It is clear, as noted previously,[10] that CD spectroscopy provides an elegant method for monitoring ion–ionophore–micelle (liposome) interactions.

We have shown here that SQ1 has potent Ca^{2+} binding properties and that membrane insertion *via* the two hydrophobic tails is facile. Ca^{2+} plays an important role in membrane stabilization, in activating membrane bound enzymes, and in triggering intracellular events.[11] SQ1 has a Ca^{2+} affinity intermediate between that of citrate, which is present in extracellular fluids, and intracellular Ca-binding proteins such as calmodulin, and this suggests a possible role for SQ1 as a novel membrane transport agent for Ca^{2+}. It is likely that SQ1 will also have a high affinity for a variety of other metal ions, including redox-active transition metal ions, and these will be the subject of further studies.

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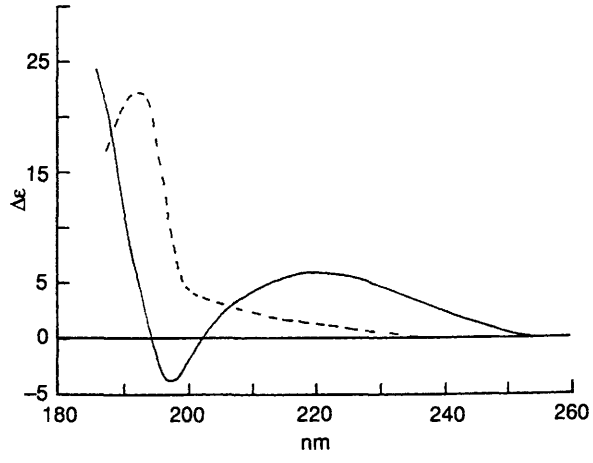

Fig. 2 CD spectrum of SQ1 (0.2 mmol dm^{-3}): (---) water, pH 7; (- - -) SDS (0.1 mol dm^{-3}), pH 7. Ca^{2+} addition (1:1) had little effect on the CD spectrum either in the presence or absence of SDS.

Footnotes

† The tripotassium salt of SQ1 (L) was the gift of Glaxo (Greenford, UK). The free acid is designated as LH_3 in the stability constant studies.

‡ CD Spectra were recorded on a JASCO J600 spectropolarimeter in 0.5 mm pathlength cells at ambient temperature.

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