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Allosteric effects in norbadione A. A clue for the accumulation process of ^{137}Cs in mushrooms?†

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A fruitful combination of potentiometry, absorption spectrophotometry, ESMS and ^{1}H NMR enabled the characterisation of two caesium complexes with norbadione A and the determination of the respective stability constants of a mononuclear and a dinuclear caesium complex at pH ~ 6; a preliminary study allowed the assignment of five protonation sites of this pigment; a positively cooperative binding of the second Cs$^+$ cation was observed.

Uptake of potentially highly toxic metals from polluted environments by various organisms such as plants or fungi, and their subsequent transfer to man *via* the food chain, has become a great matter of concern in terms of health hazards.[1–3] Fungi, in particular, often exhibit a remarkable ability to accumulate a large variety of elements, ranging from the heaviest of the transition metals such as lead or mercury, to the alkali metals, including radioisotopes like ^{137}Cs.[1–11] However, while evaluation and quantification of the transfer of toxic elements from soils to fungi have been extensively studied, comparatively little research has been conducted on the molecular aspect leading to the uptake, translocation and finally sequestration of metal cations in mushrooms, and more precisely concerning the nature and the properties of the organic chelators involved in these processes. In particular, the high concentrations of ^{137}Cs from atmospheric radioactive fallouts regularly reported in the fruiting bodies of the bay boletus (*Xerocomus badius* (Fr.:Fr.)Gilbert), an edible mushroom, could be ascribed to the complexation of this radionuclide essentially by the two major cap pigments, badione A and norbadione A, normally present as potassium salts.[5–9] Norbadione A is a naphthalenoid pulvinic derivative (Fig. 1) which possesses seven protonation sites (two enols, two carboxyls and three phenols).

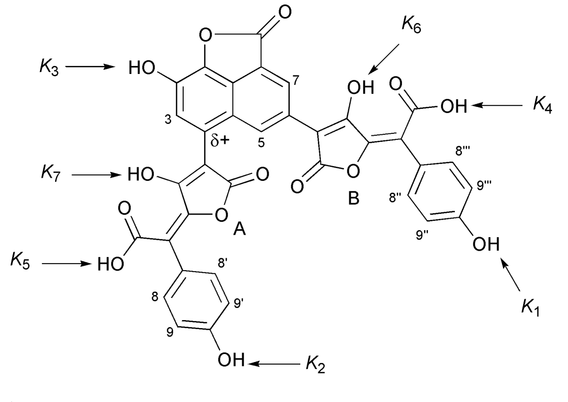

Fig. 1 Chemical structure and atom-numbering scheme of norbadione A (noted $\mathbf{LH}_7$).

A fruitful combination of potentiometry, absorption spectrophotometry, ESMS and ^{1}H NMR allowed us to calculate and assign five protonation constants in methanol : water (80 : 20 by weight) and to determine the binding constant of one and two Cs$^+$ cations in the same solvent. Dipotassium salt of norbadione A was obtained from Dr M. Gill (University of Melbourne, Australia). Acidification of the salt[9] led to the fully protonated form of norbadione A, which was further purified using multiple chromatography on Sephadex LH-20 with methanol as eluent.[10]

Five protonation constants were determined using both UV-visible absorption spectrophotometric and potentiometric methods in a span of pH between 1.0 and 11.0. The potentiometric data were processed by a non-linear least-squares method (Hyperquad[12]). Norbadione A pigment strongly absorbs in the UV-visible region, which enables us to monitor the absorbance changes upon pH variation. The spectrophotometric data were refined using the Specfit software.[13] The successive protonation constants K_3, K_4, K_5, K_6 and K_7, respectively related to the formation of $\mathbf{LH}_3$, $\mathbf{LH}_4$, $\mathbf{LH}_5$, $\mathbf{LH}_6$ and $\mathbf{LH}_7$ are given in Table 1, as well as the corresponding absorption maxima of the various protonated species of norbadione A (Table 1).

Constants K_1 and K_2, which were found to be higher than 11.5, were easily attributed to the phenolic moieties in agreement with data available in literature.[14] K_3 could be associated with the protonation of the β-naphthol group and is in excellent agreement with literature data.[15] Qualitative and structural information on the different protonated species of norbadione A were obtained from ^{1}H NMR experiments in the same mixed solvent. It is noteworthy to observe that unsymmetrical electronic effects (Fig. 1) lead to more shielded protons for arm B as compared with the corresponding ones borne by arm A. When pD varies from 1.0 to 3.4, notable variations in chemical shifts concern protons H^3, H^5 and H^7, as well as protons at positions 8–8‴, suggesting deprotonation of either the neighbouring enol or carboxylic functions. At pD values of 3.4 and 6.4, the major norbadione A form remains the doubly deprotonated species $\mathbf{LH}_5$, as shown by the similarity of the ^{1}H NMR spectra. Between pD values of 6.4 and 10.0, the signals of the isochronous aromatic protons pairs H^8, H$^{8'}$ and H$^{8''}$, H$^{8'''}$ of the phenolic units are by far the most affected. This observation is clearly consistent with the deprotonation of the vicinal carboxylic acids and consequently shows that acidity constants K_6 and K_7 correspond to the two enol functions. Furthermore these conclusions are in agreement with ESMS data showing that decarboxylated fragments still coordinate one or two caesium cations (Fig. 2). The relatively high values observed for the protonation constants K_4 and K_5 of the carboxylic groups are likely to be due to hydrogen bonds formed with the neighbouring enolates. Moreover, spectral evolution *versus* time was observed from pD > 9.0 showing a

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† Electronic supplementary information (ESI) available: ^{1}H NMR, absorption spectra, spectrophotometric and potentiometric titration data. See http://www.rsc.org/suppdata/cc/b2/b201929a/

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Table 1 Norbadione A: protonation constants,[a] stability constants of the Cs$^+$ complexes[b] and spectrophotometric data

Equilibrium	log K (σ)	λ_{max} ($\varepsilon \times 10^{-4}$) nm (M^{-1} cm^{-1})
$\mathbf{L} + H \overset{K_1}{\rightleftarrows} \mathbf{L}H$	> 11.5[c]	—
$\mathbf{L}H + H \overset{K_2}{\rightleftarrows} \mathbf{L}H_2$	> 11.5[c]	316 (4.9), 370 (1.4), 496 (1.1)
$\mathbf{L}H_2 + H \overset{K_3}{\rightleftarrows} \mathbf{L}H_3$	10.17(3)	320 (3.9), 374 (sh), 478 (1.1)
$\mathbf{L}H_3 + H \overset{K_4}{\rightleftarrows} \mathbf{L}H_4$	9.07(6)	274 (3.8), 318 (2.6), 368 (2.3)
$\mathbf{L}H_4 + H \overset{K_5}{\rightleftarrows} \mathbf{L}H_5$	7.74(7)	266 (4.6), 368 (2.5), 425 (sh)
$\mathbf{L}H_5 + H \overset{K_6}{\rightleftarrows} \mathbf{L}H_6$	1.94(5)[d]	268 (4.7), 368 (2.3) 425 (sh)
$\mathbf{L}H_6 + H \overset{K_7}{\rightleftarrows} \mathbf{L}H_7$	0.7(1)[d]	258 (3.6), 369 (3.2), 400 (3.1)
$\mathbf{L}H_5 + Cs \overset{K_{LCs}}{\rightleftarrows} (\mathbf{L}H_5)Cs$	5.4(1)	265 (5.0), 370 (2.3), 415 (sh)
$(\mathbf{L}H_5)Cs + Cs \overset{K_{LCs2}}{\rightleftarrows} (\mathbf{L}H_5)Cs_2$	5.9(2)	267 (4.4), 364 (2.3), 415 (sh)

Solvent: methanol : water (80 : 20 by weight); T = 25.0(2) °C.[a] I = 0.1 M (NEt$_4$ClO$_4$). [b] pH = 6.1 (0.1 M succinic acid–TMAOH buffer). [c] Ref. 14. [d] The ionic strength varied within the errors interval. For the sake of simplicity charges have been omitted. sh = shoulder.

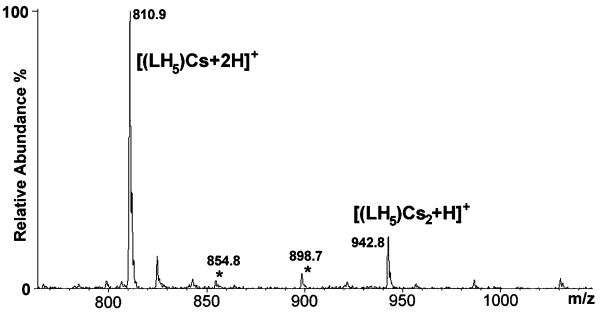

Fig. 2 Electrospray mass spectrum. Solvent: methanol : water (80 : 20 by weight); [norbadione A]$_{tot}$ = [Cs$^+$]$_{tot}$ = 4.10 × 10^{-4} M; V_c = 192 V; positive mode; * decarboxylated fragments.

slow transformation of norbadione A. This reaction corresponds to the base mediated opening of the lactone group with the subsequent oxidation of the catechol unit,[16] and leads to the formation of pisoquinone, a pigment[17] present in the fungus *Pisolithus arhizus* (Scop.:Pers.)Rauschert. Combination of UV-visible spectrophotometry and ESMS led to the characterisation of two norbadione A caesium complexes (($\mathbf{L}H_5$)Cs, ($\mathbf{L}H_5$)Cs$_2$) in methanol : water (80 : 20 by weight) at pH 6.1 (Fig. 2).

The corresponding successive stability constants were also determined (Table 1). At pH = 6.1, the ratio K_{LCs2}/K_{LCs} = 3(2), larger than 0.25 which is the value expected for a statistical model of two identical binding sites, clearly indicates that the stability of ($\mathbf{L}H_5$)Cs$_2$ is higher than that of its mononuclear analogue ($\mathbf{L}H_5$)Cs due to allosteric effects in norbadione A (Fig. 3). Changes in solvation and/or in structural features induced by the coordination of the first Cs$^+$ cation could explain this result.

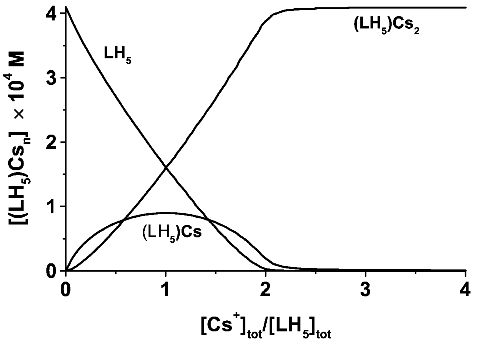

Fig. 3 Distribution curves of the caesium complexes with norbadione A. [$\mathbf{L}H_5$]$_{tot}$ = 4.1 × 10^{-4} M; pH = 6.1.

In conclusion, our results represent the first extensive physico-chemical study of norbadione A, including its acido-basic properties and its ability to coordinate caesium. ^{1}H NMR studies combined with potentiometry allowed us to attribute five protonation constants and to show the crucial role played by the enolates in the coordination process, along with an eventual participation of the protonated carboxylic groups. Positively cooperative interactions between the two caesium cations in the dinuclear complex ($\mathbf{L}H_5$)Cs$_2$ are in line with the ability of norbadione A to store radioactive and toxic ^{137}Cs in bay boletus.[5–9] Further studies on the coordination of alkali cations and toxic divalent metals Cd(II) and Ni(II) by norbadione A are on hand. An exhaustive examination of the coordination properties of norbadione A finds potential applications in pollution problems and in new processes of phytoremediation of contaminated soils. Preliminary studies showed strong interferences of sodium and potassium with caesium norbadione A complexes, which could eventually prevent the efficient use of this pigment as a novel chelator of ^{137}Cs in human therapy.

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