The Protonation and Deprotonation Equilibria of Hypericin Revisited

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Summary. The protonation and deprotonation behaviour and the assignment of $pK_a$ values of hypericin are reviewed and discussed. Three experiments (electrospray MS, $^1$H NMR, acid–base indicator equilibria) provided additional evidences for the assignment of $pK_a$ values of $-5$ and $-6$ to mono- and diprotonation at the carbonyl groups of hypericin, of $pK_a = 2$ to monodeprotonation at the bay-region, and of $pK_a = 11$ to dideprotonation at the bay- and peri-regions.

Keywords. Hypericin; Indicator; ES-MS; $^1$H NMR; Acid–base equilibria.

Introduction

Although it is presently clear that hypericin (1) is involved in a series of protonation and deprotonation equilibria [1], there is some uncertainty and even disagreement in the literature which species belongs to which characteristic absorption spectrum, and which species prevails under certain experimental conditions, e.g. at different pH values or in solutions of certain solvents [2–21]. The aim of the present paper is to summarize the experimental and theoretical arguments accumulated so far and to present additional key results to clarify this issue.

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Results and Discussion

Assignments advanced so far

Before describing new experiments to ultimately settle the question of protonation and deprotonation of hypericin it seems to be appropriate to review experiments and assignments obtained so far, and to critically discuss their pros and cons.

The very first indication that hypericin (1) is a rather strong acid has been reported by Song more than twenty years ago. He observed that 1 liberated CO$_2$ from a sodium bicarbonate solution [2], a routine reaction to discriminate phenols from carboxylic acids having $pK_a$ values well below 7 [22] ($pK_a$(NaHCO$_3$) = 6.37 [23]). This has been later on substantiated by Mazur by the preparation of the sodium salt of 1 by directly reacting 1 with an equivalent of aqueous sodium bicarbonate; within this paper, the preparation of the lysine salt of 1 has been also reported [5]. More than ten years later it has been found in our group that the ‘soluble form’ of 1 occurring in Hypericum species consists mainly of its potassium salt [3].

In 1992, $pK_a$ values of 1 and a series of analogous model compounds have been determined by Falk’s group yielding values in the range of −6 and in the range of 11 in 80% aqueous DMSO. The first value has been assigned to monoprotonation, the second one to monodeprotonation of 1 [4]. A deprotonation step at $pK_a$ = 11.7 has been observed for 1 incorporated into aqueous Triton micelles and was also interpreted as monodeprotonation [7]. The 2,5,9,12-tetrabromohypericin derivative has been shown to display titrable groups characterized by $pK_a$ values of 1.2 and 8.8, which were attributed to protonation and deprotonation equilibria [8].

A stable pyridinium salt of 1 ($1^− \cdot py \cdot H^+$) has been prepared in 1993; its structure was determined by X-ray crystallography [6]. Thereby, the deprotonation site has been unequivocally assigned to the bay region (positions 3/4). Moreover, because the $pK_a$ of pyridine amounts to 5.4 [23], a stable salt would only be obtainable if the $pK_a$ of the corresponding acid were equal to or lower than this value. This result has been confirmed and refined later on with a different crystal form of this salt ($1^− \cdot py \cdot H^+ \cdot py \cdot H_2O$) [9]. In addition, $pK_a$ values for the mono- and dideprotonation steps using 80% ethanol have been measured to amount to 1.7 and 12.5 [9].

Taken together, the results achieved up to this time indicated that in addition to an acidic group characterized by a $pK_a$ value of about 11 there ought to be another much more acidic group present in the molecule. For the latter, one of the bay-hydroxyl groups is a proper candidate. This has been complemented by spectrophotometrically titrating fringelike D (2), which displays two pairs of bay-hydroxyl groups. A two-step titrable system has been observed in this case [9, 11], as well as in the case of stentorin and isostentorin [11], instead of the one-step system in the same region of 1 ($pK_a \approx 2$) [10]. Conductivity measurements also revealed that 2 is dissociated in pure DMSO depending on its concentration and that this dissociation is characterized by a mean $pK_a$ value of 3.1, nicely corroborating the spectrophotometric titration experiment. Accordingly, the step in the range of $pK_a = 2$ had to be assigned to monodeprotonation, whereas the step in the region of $pK_a = 11$ had to be reassigned to dideprotonation.