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The Use of Electrospray Mass Spectrometry (ES/MS) for the Differential Detection of Some Steroids as Calix-[n]-arene Sulphonate Complexes

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Abstract

Non-covalent inclusion complexes formed between three *p*-sulphonato-calix-[n]-arenes (**1** n = 4, **2** n = 6 and **3** n = 8) and three steroids (progesterone, testosterone and oestradiol) have been studied by electrospray mass spectrometry (ES/MS). Mass spectrometric titration experiments have demonstrated differences with regard to selectivity of each *p*-sulphonato-calix-[n]-arene against the steroids. *p*-sulphonato-calix-[8]-arene interacts more strongly with oestradiol and *p*-sulphonato-calix-[6]-arene with progesterone. Studies in which different orifice voltages were applied show that all oestradiol complexes are reduced in signal intensity at 50 V as compared to 20 V, whereas the intensities observed for the testosterone and progesterone complexes do not vary with voltage. Competition experiments confirm the selectivity of the complexation.

Introduction

The differential detection of non-derivatised steroids at very low concentrations represents a major interest in analytical biochemistry [1–5]. In general such methods involve complex extraction and chromatographic processes [6–8]. The development of modern mass-spectrometry techniques, such as, Electrospray Mass Spectrometry (ES/MS) [9] and MALDI has allowed, under relatively mild conditions, the investigation of non-covalent complexation, such as protein/protein interactions [10], enzyme/ inhibitor complexes [11, 12], assemblies of DNA with proteins [13–15], oligonucleotides and drugs [16], supramolecular metal complexes [17] and ternary complexes with aminoacids of amino acids with Cu^{2+} and phenanthroline [18].

The use of electrospray mass spectrometry as a detection method for steroids, has received considerable attention [19–20]. One possible method to avoid extraction and chromatographic separation lies in the use of selective complexation agents for the steroids, which will show clear differential behaviour and thus allow facile determination of both the presence and nature of the steroids. Supramolecular complexation agents [21, 22] have received attention in this regard include native cyclodextrins [23], modified cyclodextrins [24], including chromopheric and fluoropheric derivatives [25], cyclodextrin-calixarene systems [26], calixresorcinarenes in both solution [27] and on surfaces [28, 29]. Recent works, report the HPLC study in solution of the complexation of *p*-sulphonato-calix-[n]-arenes with testosterone [30]. Solid-state interactions of steroids with calixarenes have also been reported [31]. While such molecules can selectively complex various steroids, obtaining differential behaviour, i.e., obtaining a set of variable responses to the presence of different steroids, is still a non-trivial problem.

In this paper, we present a study by ES/MS of the complexation properties of *p*-sulphonato-calix-[4]-arene (**1**), *p*-sulphonato-calix-[6]-arene (**2**) and *p*-sulphonato-calix-[8]-arene (**3**) towards three steroids: progesterone (Pr), testosterone (Ts) and oestradiol (Od). The behaviour of the complexation curves and the differing response to changes in the orifice voltage allow clear differentiation between the steroids. The selectivity of the complexation process is also demonstrated, in complexation competition experiments.

Experimental

Reagents

1, **2** and **3** were synthetised by the published method [32] and the differents steroids used have been provided by Dr C.-Y. Cuilleron. Acetonitrile was purchased from Carlo Erba. Solvents and chemicals were used without further purification.

Apparatus

ES/MS experiments

The ES/MS experiments were performed on a single quadrupole *Perkin Elmer* API 165 Sciex mass spectrometer. A

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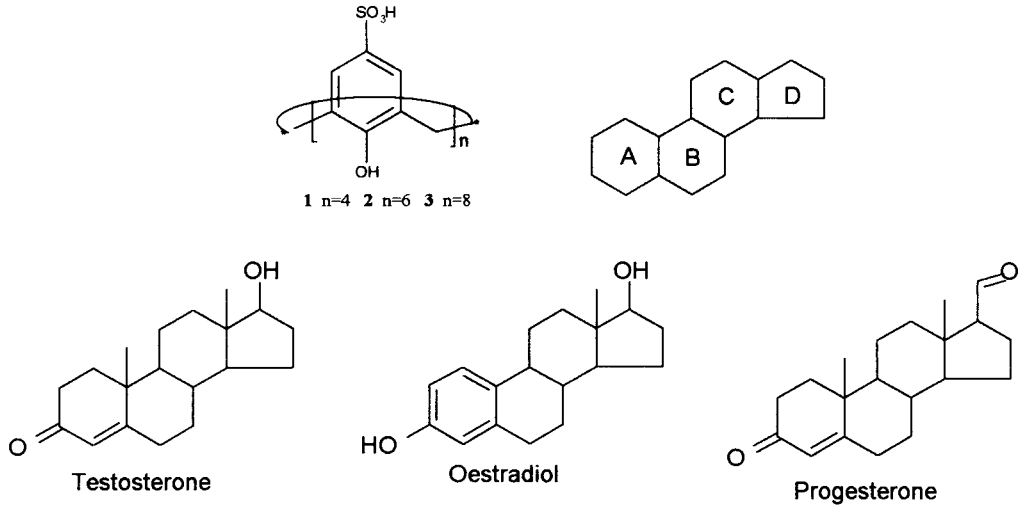

Figure 1. Structure of **1**, **2**, **3** and steroids: progesterone, testosterone and oestradiol.

solution of the steroid was prepared in the mixture acetonitrile/water/formic acid 50:50:0.1% (concentration of steroid 200 pmol/μL). To this a solution of *p*-sulphonato-calix-[n]-arene is added to obtain of steroid:calix molar ratios of 1:1, 1:5, 1:10, 1:20 and 1:30. To improve the signal-to-noise ratio, 10 scans were accumulated; ion spray and orifice potential were 20 or 50V respectively.

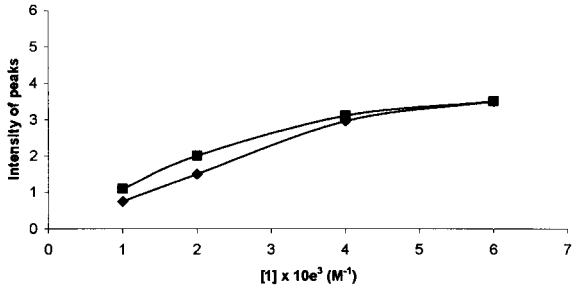

Figure 2a. Intensity of peaks versus concentration of **1** at orifice voltage 20 (♦) and 50 V (■).

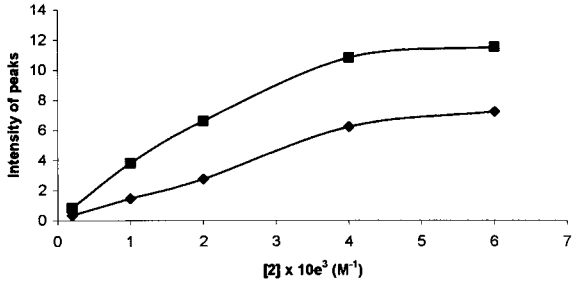

Figure 2b. Intensity of peaks versus concentration of **2** at orifice voltage 20 (♦) and 50 V (■).

Results and discussion

The structures of **1**, **2**, **3** and of the three steroids; progesterone (Pr), testosterone (Ts) and oestradiol (Od) are given in Figure 1. These three steroids have been chosen for the differences of their structures in rings A and D. In order to ensure that the intensity of the observed peaks is a correct measurement of the relative concentrations of the molecules present, the ES/MS peak intensities of **1**, **2** and **3** were measured as a function of their concentrations and the results are presented in Figures 2a, 2b and 2c. It can be seen that in the concentration range 0–4 mM for orifice voltages of 20 and 50 V there is a linear response curve, at concentrations above this the intensity reaches a plateau. The ES/MS titration curves for the complexation of **1**, **2** and **3** with progesterone, testosterone and oestradiol at orifice voltages of 20 and 50 V are presented in Figures 3a, 3b and 3c. In all cases, progesterone appears to interact more strongly with **1**, **2** and **3**, giving higher ES/MS peak intensities. For **1**, a maximum point on the curve is observed, such behaviour has been previously observed in ES/MS titration curves of Cu/PrP [33] and may be explained by the formation of a 1:2 complex. A peak at 1059 (m/z) is observed, corresponding to this system, however the intensity is only marginally above the noise level. For **2**, the signal intensity for all 1:1 complexes increases in a near linear manner with increasing calix-[*n*]-arene concentration. In the case of the complexes with **3**, there is only a low intensity for the complexes up to **3**: steroid ratios of 20:1 above this ratio the intensity of the complex signal increases rapidly. On changing the orifice voltages between 20 and 50 V, the intensities of the signals

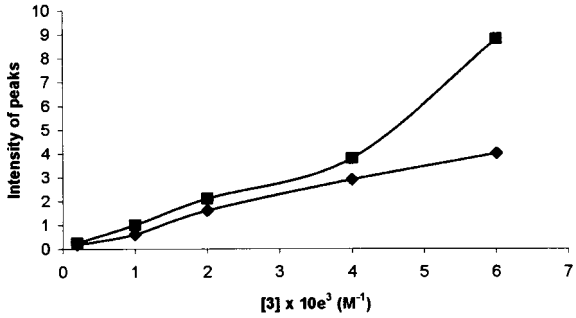

Figure 2c. Intensity of peaks versus concentration of **3** at orifice voltage 20 V (♦) and 50 V (■).

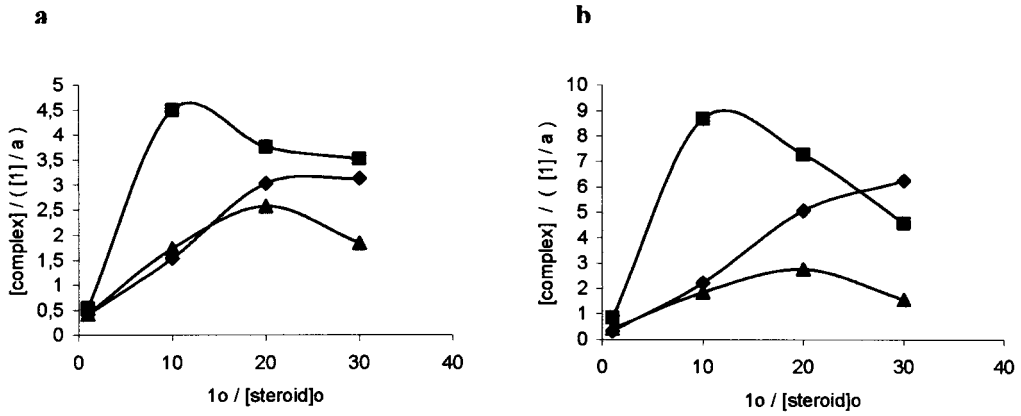

Figure 3a. Plot of [complex]/ ([**1**]/a) vs [**1**]o/[steroid]o where [**1**]o is the initial concentration of calix, [steroid]o is the initial concentration of steroid and a is the ratio of [1]o/[steroid]o at orifice voltage 20V (a) and 50V (b), with testosterone (♦), progesterone (■) and oestradiol (▲).

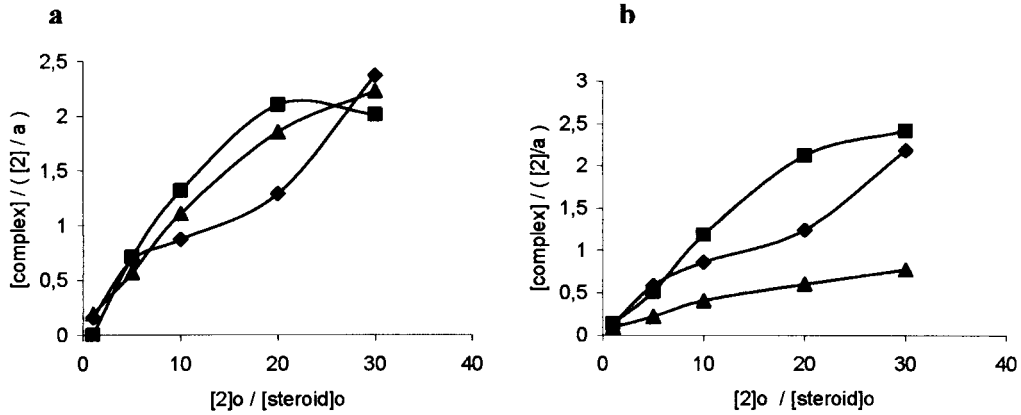

Figure 3b. Plot of [complex]/ ([**2**]/a) vs [**2**]o/[steroid]o where [**2**]o is the initial concentration of calix, [steroid]o is the initial concentration of steroid and a is the ratio of [**2**]o/[steroid]o at orifice voltage 20V (a) and 50 V (b), with testosterone (♦), progesterone (■) and oestradiol (▲).

for the complexes with progesterone and testosterone remain constant implying complexes between water-soluble calixarenes and steroids are stable. In all cases for the oestradiol complex changing orifice voltage from 20 V to 50 V leads to a decrease in the intensity of the peak corresponding to the complex. This implies a lower stability of this complex.

Complexation selectivity

The selectivity of the complexation of the three steroids with **1**, **2**, **3** was investigated by ES/MS experiments on equimolar mixtures in the presence of excess calix, for **2** molar ratios of 1:20 and 1:30 were used whereas for **3** a single molar ratio of 1:30 was studied. The results are given in Figures 4a and 4b and the corresponding spectra for **2** with the steroids mixture are given in Figures 5a and 5b. Under the experimental conditions, a lack of selectivity should lead to 33%, 33%, 33% signal ratio for the three complexes. As can be clearly seen this is not the case and selectivity is observed. For **2**, a clear selectivity is observed, especially at an orifice voltage of 20V and about 60% at the higher orifice voltage. At a 1:30 molar ratio, progesterone is slightly favoured at orifice voltage of 20V , but a higher value the selectivity for progesterone increases to 50%.

With respect to **3**, at low orifice voltage oestradiol is selectively observed, (50%) over progesterone but this effect is, as expected, inversed at the higher orifice voltage. The competition selectivity experiments, thus, accord well with the titration experiments.

Summarising the results with respect to the behaviour of the steroids in the ES/MS experiments:

1. Testosterone is complexed in a invariant manner with respect to the applied orifice voltage, and with little discrimination between the calix-arenes studied.
2. Progesterone is again complexed in an invariant manner with respect to the orifice voltage. It is selectively favoured in competition experiments involving *p*-sulphonato-calix-[6]-arene.
3. Oestradiol shows strong orifice voltage dependent behaviour; at low orifice voltages it is favoured in complexation experiments with *p*-sulphonato-calix-[8]-arene.

Conclusion

Electrospray mass spectrometry is a valid tool for study the weak interaction between steroids and calixarenes; it requires a minimal sample and the information is more easily obtained than with more classical analytical techniques. It is been show that the steroids interact selectively with water-soluble *p*-sulphonato-calixarenes. The combination of an

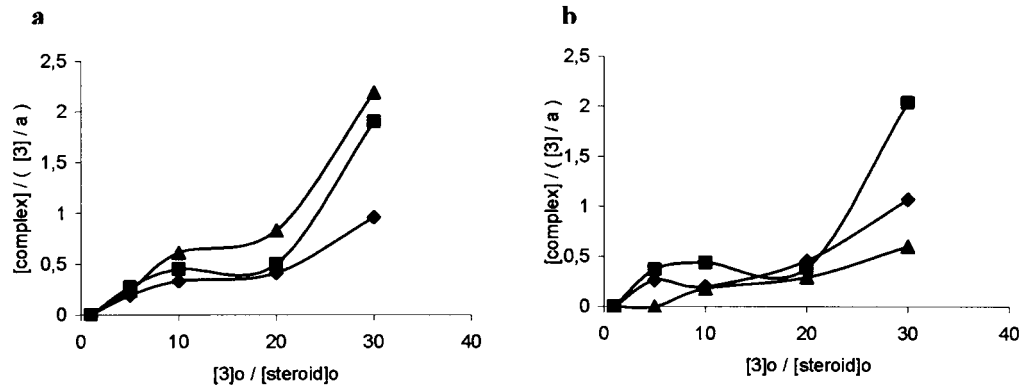

Figure 3c. Plot of [complex]/ ([**3**]/a) vs [**3**]o/[steroid]o where [**3**]o is the initial concentration of calix, [steroid]o is the initial concentration of steroid and a is the ratio of [**3**]o/[steroid]o at orifice voltage 20V (a) and 50 V (b), with testosterone (♦), progesterone (■) and oestradiol (▲).

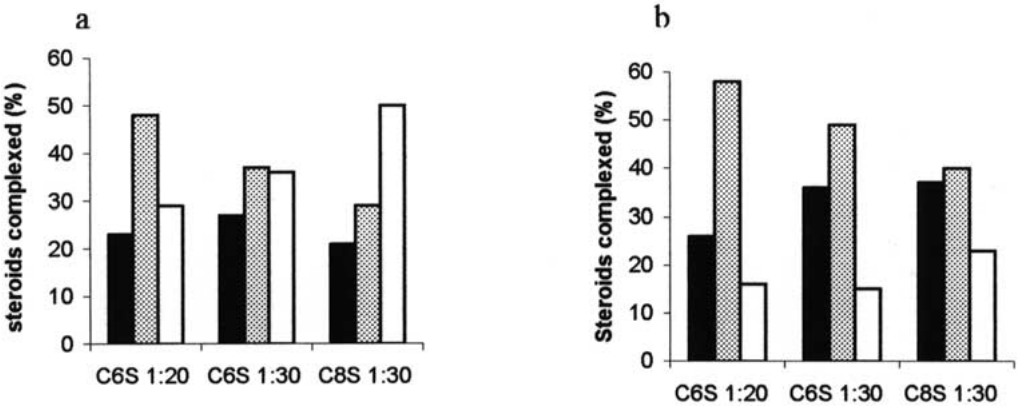

Figure 4. Percentage of steroid complexed for steroid:calixarene molar ratio of 1:20 and 1:30 at orifice voltages 20V (a); and 50V (b) with testosterone (black), progesterone (grey) and oestradiol (white).

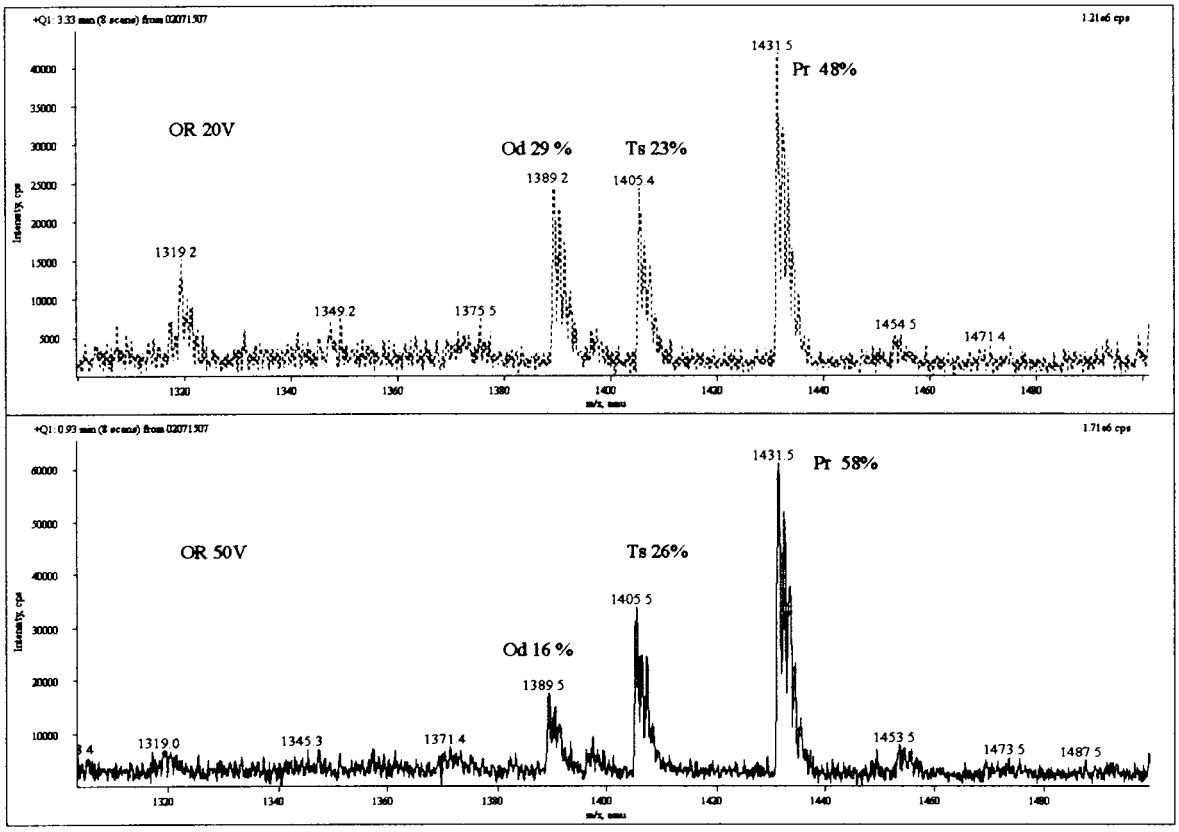

Figure 5. ES/MS spectra of complex formed between **2**, progesterone, testosterone and oestradiol at molar ratio 20:1:1:1 at orifice voltage 20V and 50V.

array of calixarenes coupled with variable ES/MS conditions may prove a powerful tool in steroid analysis.

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