

ATR-IR AND RAMAN SPECTROSCOPIC STUDY OF INTERACTION BETWEEN MAJOR CALIXARENE DERIVATIVES AND ORAL ANTICOAGULANTS

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Abstract

In this study we analyzed by ATR-IR and Raman spectroscopy the interaction between six major calixarene derivatives and two anticoagulants: warfarin and acenocoumarol. The six calixarene derivatives were: *p-tert-butyl-calix[4]arene* (C4), *p-tert-butyl-calix[6]arene* (C6) and *p-tert-butyl-calix[8]arene* (C8) and their ethyl-ester-derivatives: tetraester-*p-tert-butyl-calix[4]arene* (C4Es4), hexaester-*p-tert-butyl-calix[6]arene* (C6Es6), octaester-*p-tert-butyl-calix[8]arene* (C8Es8). The results showed the most intense interaction in terms of complex formation for the C6-acenocoumarol couple and for the C6-warfarin couple, respectively.

Rezumat

În acest studiu am analizat prin spectroscopie ATR-IR și Raman interacțiunea dintre șase derivați de calixarene majore și două anticoagulante: warfarina și acenocoumarolul. Cei șase derivați calixarenici analizați au fost: *p-terț-butil-calix[4]arena* (C4), *p-terț-butil-calix[6]arena* (C6) și *p-terț-butil-calix[8]arena* (C8) și etil-ester-derivații lor: tetraester-*p-terț-butil-calix[4]arena* (C4Es4), hexaester-*p-terț-butil-calix[6]arena* (C6Es6), octaester-*p-terț-butil-calix[8]arena* (C8Es8). Rezultatele au arătat cea mai puternică interacțiune din punct de vedere al formării complexelor pentru cuplul C6-acenocoumarol, respectiv pentru C6-warfarina.

Keywords: calix[n]arenes, anticoagulants, spectroscopy

Introduction

Calixarenes represent a versatile class of compounds utilized in complex formation research. Due to their macrocyclic structure, the

functional groups of the inner cavity enable non-covalent interactions with the guest molecules, from which hydrogen bonds are considered the most important (Figure 1). The literature reports their use as complexants of ions – metallic [17] or organic ions [8] and neutral molecules with different shapes and dimensions [18,22]. They have been used as stationary phases or mobile phase modifiers in separation techniques, even in enantioseparation [23,26], in detection of the complexed substances [2], also in recognition of enantiomers [3] and even as catalysts in different reactions [4,16]. Also, there have been reported some derivatives with pharmacological activity [6,19] and some calixarene derivatives have been used as carriers through liquid membranes [21]

Warfarin and its 5-nitro-derivative, acenocoumarol (Figure 2), are two widely used anticoagulants, showing a low therapeutic index, as well as frequent drug-drug interactions and many side effects have been reported. Moreover, a high variability in terms of absorption, metabolics and response to the administered dose has been observed [19].

IR or Raman spectroscopic techniques are useful tools for the study of complex formation between a “host” and a “guest”-molecule, such as calixarene derivatives and oral anticoagulants. IR and Raman spectroscopy are two complementary techniques which, apart from being non-invasive, have the advantage of giving short analysis times. Attenuated total reflectance infrared (ATR-IR) spectroscopy has the advantage of directly analyzing solid samples, readily applicable to small amounts of samples [5].

The aim of the present work was to detect and elucidate the interaction between different calixarene derivatives and the aforementioned anticoagulants and to observe the stability of the “host-guest” complexes formed. These complexes will be further used in separation techniques, for the enantiomer separation of the two anticoagulants. For this purpose, a characterization of the interaction between calixarene derivatives and the oral anticoagulants is very important.

Materials and Methods

Reagents

Six major calixarene derivatives were used as complexation ligands: *p-tert*-butyl-calix[4]arene (C4), *p-tert*-butyl-calix[6]arene (C6) and *p-tert*-butyl-calix[8]arene (C8) and their ethyl-ester-derivatives: tetraester-*p-tert*-butyl-calix[4]arene (C4Es4), hexaester-*p-tert*-butyl-calix[6]arene (C6Es6), octaester-*p-tert*-butyl-calix[8]arene (C8Es8) (Figure 1). The starting *p-tert*-butyl calix[n]arenes (C4, C6 and C8) were synthesized by slightly

modifying the procedure already described in the literature [11,12,20]. Ester derivatives of calix[n]arene (C4Es4, C6Es6 and C8Es8) were prepared by original procedures, by treating the parent calix[n]arene with ethyl bromoacetate in the presence of a base (K_2CO_3 or NaH) in organic media (THF/DMF or acetone) [24]. All the compounds were purified by recrystallization from toluene, chloroform or chloroform-ethanol mixture. The molecular structures of calixarene derivatives used in the study are presented in the Figure 1.

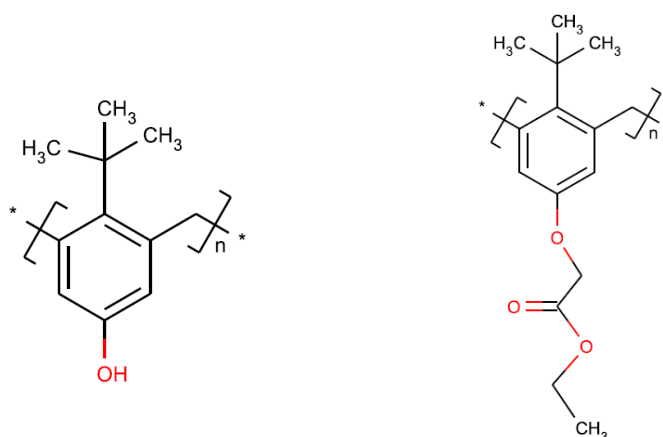


Figure 1

Structures of calixarenes utilized in the study; $n=4,6,8$

Warfarin (W) was purchased as analytical standard from Sigma-Aldrich (Germany). Acenocoumarol (A) was kindly donated by Labormed Pharmaceuticals S.A., Bucharest (Romania).

All solvents used in this study were of HPLC grade and were obtained from Sigma-Aldrich (Germany).

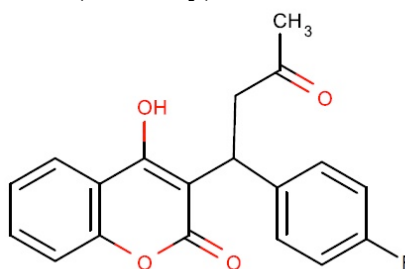


Figure 2

Structure of warfarin ($R=H$) and acenocoumarol ($R=NO_2$).

IR and Raman spectroscopy

Stock solutions of 20 mg/mL of calixarene derivatives and 8 mg/mL of anticoagulants in chloroform were prepared. Mixtures with stoichiometric ratios of 1:1 containing one of each host and one of the two anticoagulants were prepared. The obtained solutions were stirred with a magnetic stirrer for at least 10 minutes. Solvents were evaporated in a Heidolph LaboRota 4002 rotary evaporator and the obtained residues were dried under vacuum.

The obtained residues were analyzed by ATR-IR and Raman spectroscopy. For the ATR-IR spectroscopic studies, a Jasco FT-IR 4100 with a ZnSe crystal system was utilized. The spectra were recorded between 4000 and 500 cm^{-1} and interpreted using the Jasco Manager 2 software. For the Raman spectroscopic study a Perkin Elmer Raman station 400F was used. The resulted spectra were obtained as an average of 20 spectra recorded with an integration time of 1 second. Spectra were registered between 3620 and 90 cm^{-1} and processed using the Spectrum software.

Results and Discussion

Theoretical aspects

$^1\text{H-NMR}$ studies made on C4, C6 and C8 [1,7,9,10,15,25,27] show C4 tends to be the most rigid derivative of the three calixarenes and, as expected, it presents the smallest cavity, having an inner dimension of about 2.67 Å. The dimension of the inner cavity of C8, the biggest taken in study is about 2.74 Å. The conformational flexibility should increase with the increase of the cavity size, but NMR studies [10] have shown that C4 and C8 tend to act identically in non-polar solvents. However, C6 seems to be much more flexible, due to the flexibility of methylene groups linking the aromatic rings, which can be positioned inside and/or outside the plan formed by the phenolic groups.

Practical results and discussions

According to theoretical spectra of calixarenes[13], the phenyl IR bands were identified at 3134.72 cm^{-1} for C4, at 3120.74 cm^{-1} for C6 and for C8 at 3170.4 cm^{-1} . Also, warfarin and acenocoumarol spectra show a medium IR band at 3118 cm^{-1} and a weak IR band at 3079 cm^{-1} corresponding to phenyl group, a band between 1715 and 1680 cm^{-1} which is correlated with the aldehyde group and the NO_2 group in acenocoumarol gave two bands in the fingerprint region at 1560-1500 cm^{-1} and at 1390-1330 cm^{-1} [14].

Results showed different interactions between different calix[n]arenes and the same guest.

All band shifts observed in the IR spectra of acenocoumarol and calixarene derivatives correspond to aromatic nuclei, according to theoretical vibrational spectra [14], which lead to the conclusion that the non-covalent interactions formed between “host” and “guest”-molecules are probably of π - π type. However, formation of other type of non-covalent interactions, such as hydrogen bonds is not excluded.

The most intense interaction correlated with the highest IR vibrational band shift was observed in the case of C4-acenocoumarol couple (Figure 3).

In this case, the highest band shifting was from 3328 to 3294 cm^{-1} on the acenocoumarol spectrum and a band shifting from 2905 to 2927 cm^{-1}

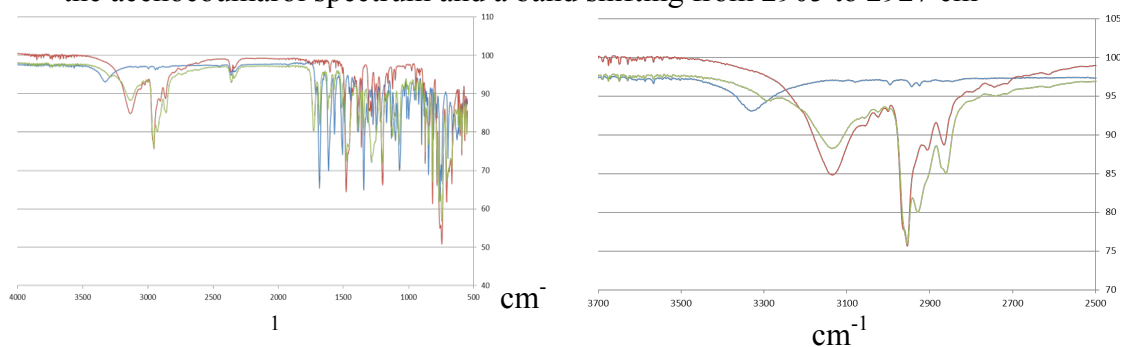


Figure 3

Band shifting in case of A-C4 complex: A (blue), C4(red), A-C4(green)

on the C4 spectrum. The other band shifts obtained are listed in the table I:

Table I

Band shifts observed in IR analysis of calixarene-anticoagulant complexes

Host-guest couple	Band shift in calix[n]arene spectra (cm^{-1})	Band shift in anticoagulant spectra (cm^{-1})
A-C4	2905→2927 (22) 2865→2860 (5)	3328→3294 (4) fingerprint
A-C6	2923→2927 (4) 2865→2861 (4)	3328→3291 (7)
A-C8		fingerprint
W-C4	3134→3137 (3) 2904→2928 (24) 2865→2863 (2)	
W-C6	2921 (band intensification)	2925→2928 (3)
W-C8		fingerprint

For C4Es4, C6Es6 and C8Es8 mixtures, we could observe band shifts only in the fingerprint region.

In the Raman spectroscopic study, the best evaluated interaction was observed in the A-C8 couple, due to the highest Raman band shifts observed, but especially due to a deformation of the bands appeared in the 1600 cm^{-1} region (Figure 4).

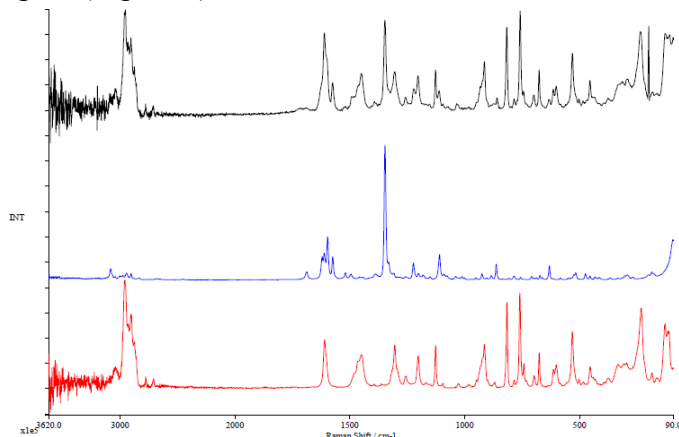


Figure 4

Comparison of spectra of C8(red), acenocoumarol (blue) and C8-A (black)

Polymorphism was also studied for the two guest substances in order to eliminate any chance of false interpretation. There was observed a modification of the spectrum of acenocoumarol before and after evaporation of the solvent, but this phenomenon did not seem to have any influence on the formation of complexes (Figures 5a and 5b).

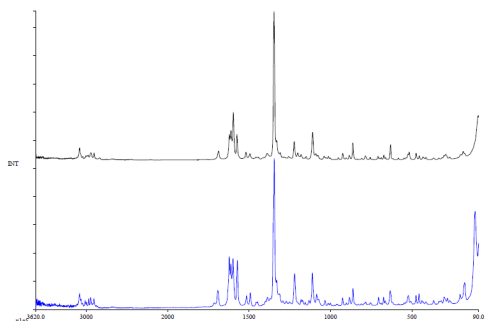


Figure 5a

Comparison of spectra of acenocoumarol before (black) and after (blue) recrystallization

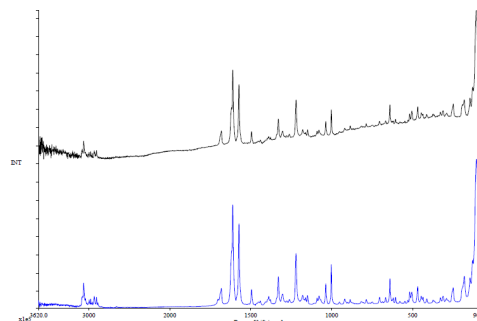


Figure 5b

Comparison of spectra of warfarin before (black) and after (blue) recrystallization

Results have shown that the non-derivatised calixarenes seem to have better complexation capacity towards acenocoumarol and warfarin than their ethyl-ester derivatives (Figure 6). The esteric functions seem to sterically block or prevent the formation of non-covalent bonds.

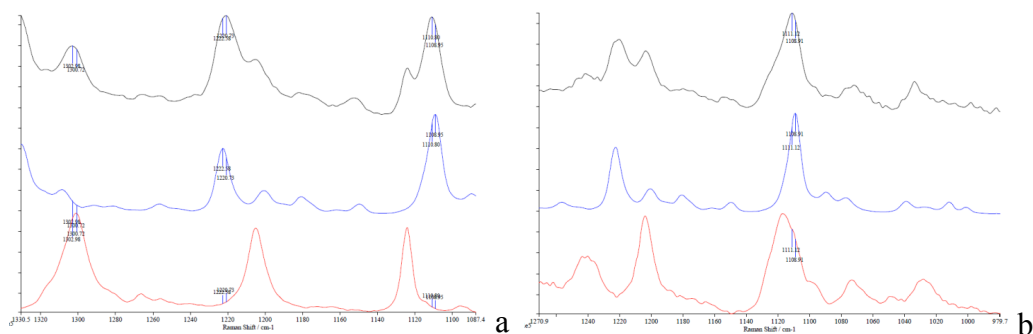


Figure 6

Comparison between spectra of A-C6 (a - black) and A-C6Es6 (b - black). Standard substances: A - blue; C6, C6Es6 – red

Even if in the ATR-IR study the strongest band shift obtained was in the A-C4 case, we suppose this could be due to the formation of an external complex, consequently to the lower flexibility of the molecule and because of the smaller inner cavity of the calixarene derivative compared to C6 or C8. Because only few band shifts in the IR (only in the fingerprint region) were observed and little difference between the Raman band shift of A-C6 and A-C8, we could predict the best interaction for the A-C6 couple, then for the W-C6 couple, where the dimension of the inner cavity of the calixarene could permit the guest to form an internal complex. These complexes are probably formed mainly by π - π interactions.

Conclusions

We prepared complexes between different calixarene derivatives and oral anticoagulants that were analyzed by ATR-IR and Raman spectroscopy.

In all cases, there was observed a band shift, indicating an interaction between the studied molecules. Results showed a higher band shift in case of C4 ligand in the IR study and a higher interaction in terms of band shift for C8 and C6, in the case of Raman study. Considering the smaller cavity of C4, where an external complex could be in this way formed, and the small differences between Raman band shifts in case of C6 and C8, our relative conclusion was that the most stable internal complex formed was between C6 and the two oral anticoagulants.

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