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Solid State Interaction of Steroids with Calixarenes. I. A Preliminary FTIR and DSC Study on 4-en-3-keto-Steroids

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Abstract. Both DSC and FTIR studies indicate that co-grinding and co-precipitation cause steroids to interact with calixarenes. This interaction leads to breaking of the crystal lattice of the steroids, dispersion of the steroidal carbonyls in a hydrophobic environment and formation of hydrogen bonds between steroidal and calixarene hydroxyls. This interaction seems to be specific, depending on the structure of the calixarene and of the steroid involved. It is reasonable to assume that inclusion complexes are formed.

Key words: Steroids, calixarenes, inclusion complexes, FTIR, DSC.

1. Introduction

The literature contains reports of various types of inclusion complexes of steroids. For example, inclusion complexes of steroids in cyclodextrins have been studied by Kralova *et al.* [1], through aqueous solubility curves; by Liu *et al.* [2], through elemental analysis, mass spectrometry, Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC); and by Djedaini *et al.* [3], through nuclear magnetic resonance. Marzona *et al.* [4] studied quantitative structure-stability relationships, correlating the physicochemical characteristics with the inclusion constants for steroids included in cyclodextrins published by Uekama *et al.* [5]. These complexes have been used for analysis [6–8] and biosynthetic transformations [9]. Steroid inclusion complexes in azoniacyclophanes have been studied by Kumar and Schneider [10] and in (m, n)-paracyclophanes by Carcanague and Diederich [11]. Ayoama *et al.* [12] reported complex formation of chiral steroidal polyols with an achiral resorcinol-aldehyde cyclotetramer studied by induced circular dichroism. Hydrogen-bond interaction between steroids and phenol derivatives in their inclusion crystals has been reported by Toda *et al.* [13].

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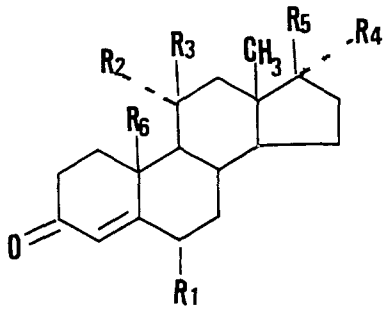
TABLE I. Steroids.



No.	Trivial name	R1	R2,R3	R4,R5	R6
I	Progesterone	H	H,H	H,$COCH_3$	CH_3
II	11α-Hydroxyprogesterone	H	OH,H	H,$COCH_3$	CH_3
III	11β-Hydroxyprogesterone	H	H,OH	H,$COCH_3$	CH_3
IV	17α-Hydroxyprogesterone	H	H,H	OH,$COCH_3$	CH_3
V	6β-Hydroxyprogesterone	OH	H,H	H,$COCH_3$	CH_3
VI	11-Ketoprogesterone	H	=O	H,$COCH_3$	CH_3
VII	Cortisone	H	=O	OH,$COCH_2OH$	CH_3
VIII	Hydrocortisone	H	H,OH	OH,$COCH_2OH$	CH_3
IX	19-Nortestosterone	H	H,H	H,OH	H
X	4-Androstene-3,17-dione	H	H,H	=O	CH_3

The study of complexes of steroids with calixarenes could increase our basic knowledge of the weak interactions responsible for molecular recognition between synthetic or biological receptors and steroids and make it possible to design even more selective synthetic receptors.

In this preliminary screening study, we have used FTIR and DSC to investigate the interactions of *p-tert*-butylcalix[n]arenes ($n = 4, 6, 8$) in the solid state with the steroids listed in Table I.

Nakai *et al.* [14–16] demonstrated the formation of some complexes of cyclodextrins in the solid state on co-precipitation and co-grinding. One method developed by Toda [17] to produce complexes was to react solid state material by grinding in an agate mortar and pestle. Using the method of Toda, we have prepared mixtures of steroids with *p-tert*-butylcalixarenes (1 : 1 molar ratio), ground for various times. Their FTIR spectra in KBr were compared with those of the physical mixture (time 0). The changes in the bands attributable to the ketones and hydroxyls were studied in relation to differences of the structures of the steroids. We also applied DSC to some of the mixtures to confirm the information obtained from the FTIR spectra.

2. Experimental

2.1. Materials

The steroids were obtained from Sigma, except for 6β-hydroxyprogesterone, which was from Steraloids. The *p-tert*-butylcalix[4]arene was obtained from Fluka, *p-tert*-butylcalix[6]arene and and *p-tert*-butylcalix[8]arene from Aldrich. The KBr, chloroform and tetrachloroethylene for spectrophotometry were from Merck; before use, the solvents were passed through aluminium oxide for chromatography to remove traces of water and of stabilizing agents. The FTIR spectra were recorded and analysed by a JASCO FTIR-5000. The DSC graphs were obtained on a Setaram DSC-92 instrument.

2.2. IR Spectra in Solution

Solutions containing 1–10 mg/mL of steroid in chloroform or in tetrachloroethylene were mixed with a solution of *p-tert*-butylcalix[6]arene (4 mg/mL) in the same solvent to obtain solutions with a molar ratio of 1 : 1. The spectra of solutions with the same final concentrations of steroid without calixarenes were also recorded. The spectra were recorded in NaCl cells of 0.1 mm pathlength immediately after preparation and one day later at room temperature.

2.3. IR Spectra in KBr

To prepare the co-ground samples, 1 mg of steroid alone or in a mixture with *p-tert*-butylcalix[n]arene ($n = 4, 6, 8$) (1 : 1 molar ratio) was ground for 0–15 minutes in an agate mortar and pestle before addition of 300 mg KBr. This was ground again for 1 minute and dried under vacuum at 35°C. To prepare the samples precipitated from chloroform, part of the solution prepared to record the IR spectra was evaporated slowly at room temperature and the residue dried under vacuum at 35°C. The samples were used to obtain the spectra in KBr discs.

2.4. Differential Scanning Calorimetry

DSC traces were obtained in aluminium capsules with non-hermetic seals into which the steroid samples or the steroid-calixarene mixtures were introduced, either unground, ground for 15 min or precipitated from chloroform. The weight of the sample, expressed as the steroids themselves or the content in the mixture, were 1.5–2.5 mg. The rate of heating was 3°C/min. The heat flow values are shown in the ordinate of the graph as mW/mg against the temperature in °C.

3. Results and Discussion

To study the interaction during co-grinding, the steroids were ground for different lengths of time with the different calixarenes. Differences have been found in the

IR spectra according to the structure of the steroid and to the calixarene, and also to the time of grinding. The changes are in the steroidal hydroxyl bands in the range 4000–3000 cm^{-1}. The major differences, however, are those in the range 1750–1600 cm^{-1}, related to the free carbonyl bond and those conjugated with C=C. Figures 1 and 2 show examples of the changes in these ranges with grinding time, with the structure of the steroid (11α- and 11β-hydroxyprogesterone) and with the calixarenes used ($n = 4, 6, 8$). Whereas, in the physical mixtures with *p-tert*-butylcalix[4]- and *p-tert*-butylcalix[6]arene, the steroid and calixarene hydroxyls are clearly distinguished, the grinding displaces the steroid -OH absorption to lower frequencies, so that the bands are superimposed. With $n = 8$, the calixarene hydroxyl band is much broader and obscures that of the steroid hydroxyls even in the physical mixture. Minor variations in the fingerprint region are difficult to interpret, due to the complexity of the vibrational spectra of two components.

In Table II are listed for all the steroids and all the calixarenes the maxima in cm^{-1} for the C=O bands of the standard steroid, the steroid ground for 15 minutes to see whether there is any polymorphic transformation and the mixture ground for 15 min. Except for hydrocortisone, all the changes in the standard steroids are negligible after grinding alone and for the unground mixture. In the mixtures ground for 15 min with *p-tert*-butylcalix[6]arene and *p-tert*-butylcalix[8]arene, the C=O bands are generally displaced toward higher frequencies, indicating a lower degree of hydrogen bonding. At the same time the steroid hydroxyl bands are displaced toward lower frequencies: we deduce that the hydrogen bonds of the steroid hydroxyls become stronger. In Table II we also report the C=O maxima of steroids in solution. When these steroids are dissolved in tetrachloroethylene, a solvent that cannot form hydrogen bonds, the C=O maxima are displaced in the same direction. Therefore, we deduce that there is interaction consequent to co-grinding of steroids with calixarenes due to crystal lattice breaking of the steroids, dispersion of the steroid carbonyls in a more hydrophobic environment and formation of hydrogen bonds between the steroidal and calixarene hydroxyls. This interaction is not aspecific but depends on the calixarene and on the steroid used. In fact grinding of many mixtures of steroids with *p-tert*-butylcalix[4]arene produces very little change compared to *p-tert*-butylcalix[6]arene and *p-tert*-butylcalix[8]arene. All the above data are in agreement with inclusion compound formation. The stability constants of steroid-cyclodextrin inclusion complexes determined by Uekama *et al.* were studied by Marzona *et al.* [4]. They concluded: "the concurrence of different effects on cyclodextrin inclusion complex stability: hydrophobicity, bulkiness, shape, charge distribution, hydrogen bond capability of the guest compound and cavity dimensions and solubility of the host cyclodextrin". The low interaction of *p-tert*-butylcalix[4]arene with all steroids may mainly be due to the small size of the host cavity, whereas it is more difficult to correlate the low affinity of cortisone for *p-tert*-butylcalix[6]arene with the steroid structure.

The results of co-precipitation after evaporation of a 1 : 1 mixture in chloroform were also compared with the results of precipitation without calixarene. The IR

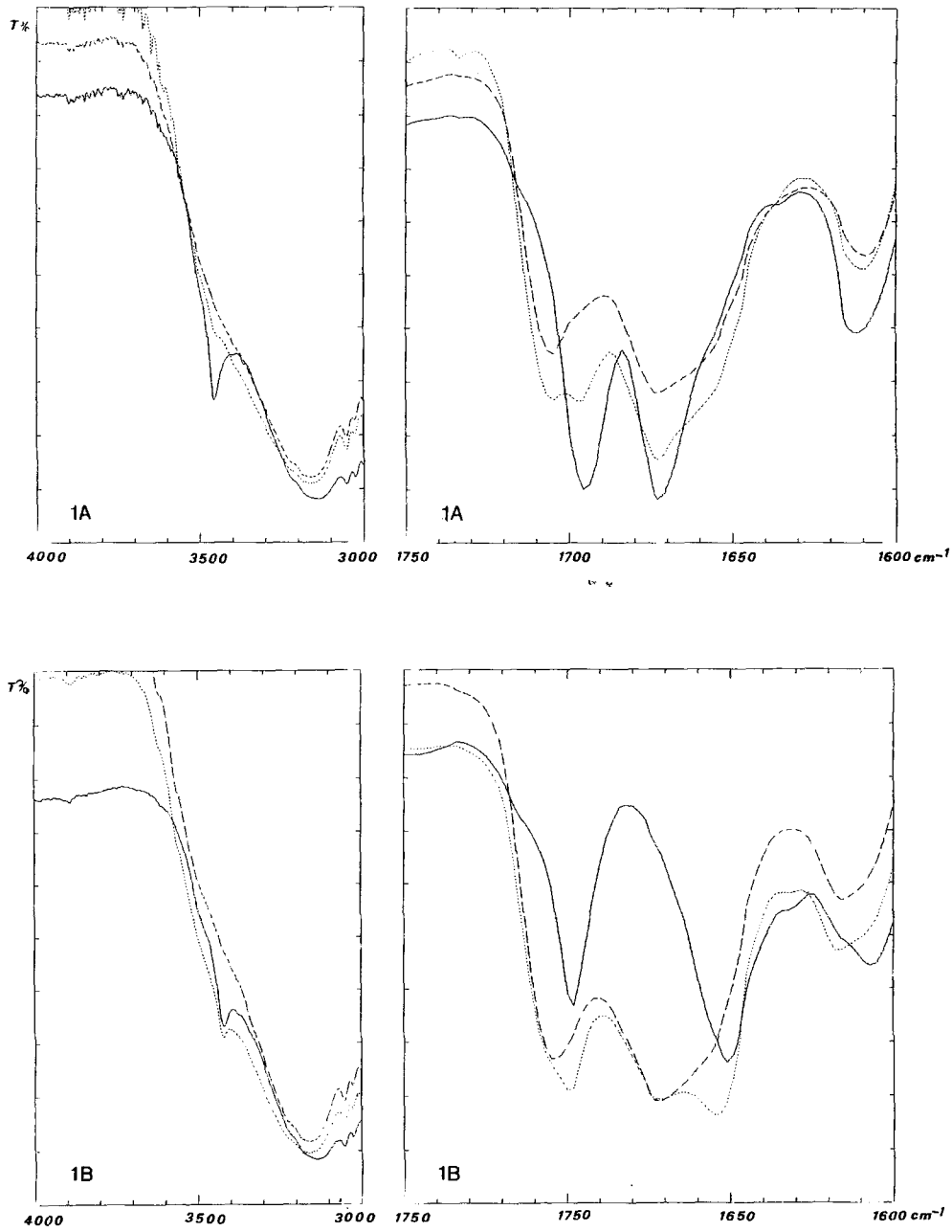


Fig. 1. Influence of steroid structure and of the time of grinding on IR spectra of ground steroid : *p-tert*-butylcalix[6]arene mixtures (1 : 1 molar ratio). 1A: 11α-hydroxyprogesterone (II); 1B: 11β-hydroxyprogesterone (III). —— time = 0; ..... time = 3 min; - - - - time = 15 min.

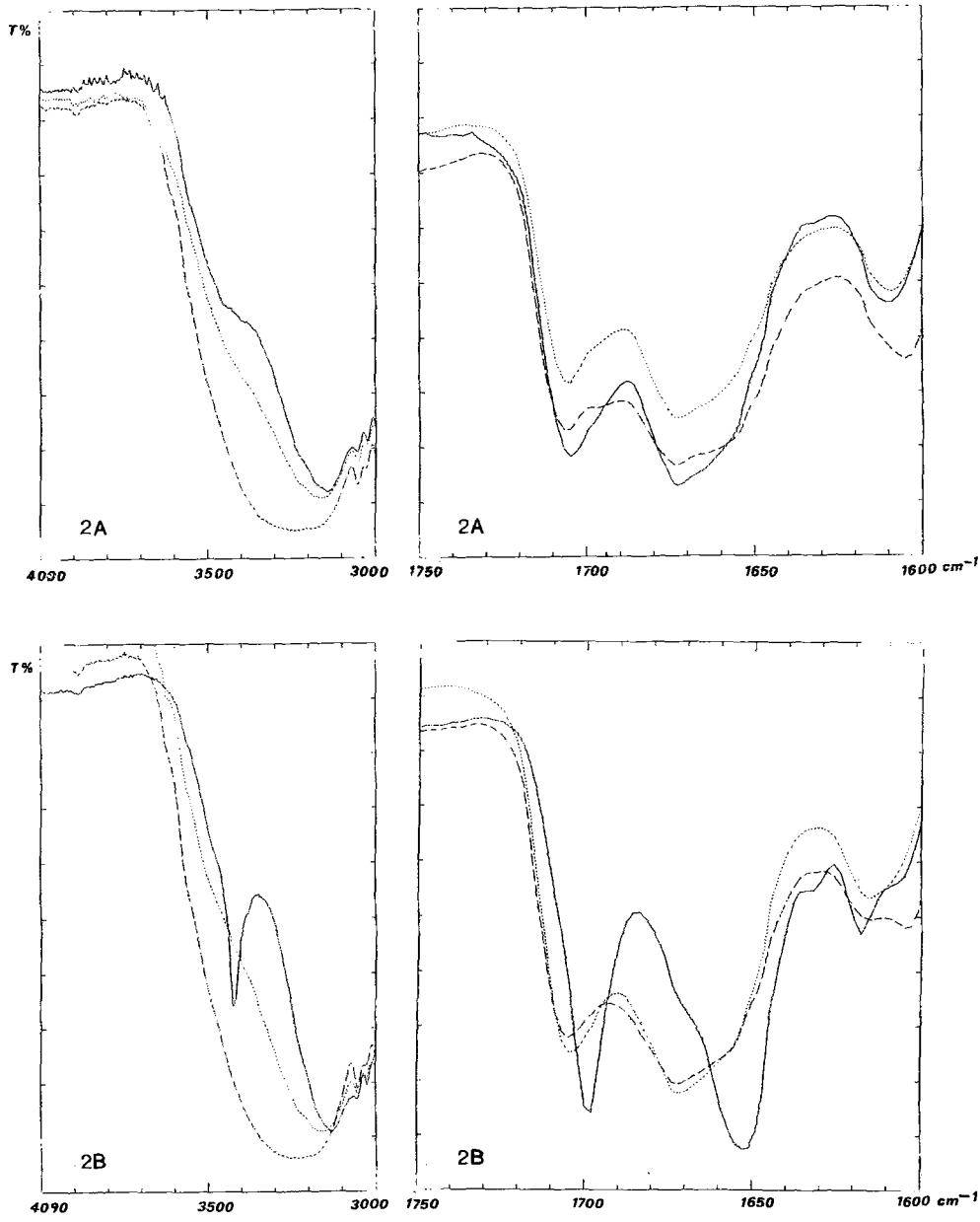


Fig. 2. Influence of steroid and calixarene structure on IR spectra of ground (time = 15 min) steroid : *p-tert*-butylcalix[n]arene mixtures (1 : 1 molar ratio). 2A: 11α-hydroxyprogesterone (II); 2B: 11β-hydroxyprogesterone (III). —— $n = 4$; ..... $n = 6$; - - - - $n = 8$.

TABLE II. IR frequencies (cm^{-1}) in chloroform (Clf) and in tetrachloroethylene (TCE) solutions, and in KBr pellets (KBr) of C=O bands (non-conjugated and conjugated) of steroids ($n = 0$) and of (1 : 1 molar ratio) steroid: *p-tert*-butylcalix[n]arene ($n = 4, 6, 8$) mixtures, not ground ($t = 0$), ground for 15 minutes ($t = 15$) and precipitated from chloroform (Prec).

Steroid	Clf $n=0$	TCE $n=0$	KBr $n=0$ $t=0$	KBr $n=0$ $t=15$	KBr $n=4$ $t=15$	KBr $n=6$ $t=15$	KBr $n=8$ $t=15$	KBr $n=0$ Prec	KBr $n=6$ Prec
I	1702	1709	1700	1700	1700	1705	1707	1700	1703
	1663	1682	1663	1663	1663	1678	1678	1663	1671
II	1702	1711	1696	1696	1705	1705	1707	1696	1707
	1663	1680	1673	1673	1673	1573	1673	1673	1671
III	1702	1709	1698	1698	1698	1705	1705	1698	1698
	1665	1676	1651	1651	1653	1671	1673	1651	1651
IV	1703	1713	1703	1703	1705	1709	1709	1696	1703
	1663	1682	1667	1667	1671	1678	1678	1673	1665
V	1702	1709	1700	1702	1705	1705	1707	1700	1700
	1678	1686	1678	1678	1682	1682	1684	1678	1680
			1671	1674				1671	
VI	1705	1713	1705	1705	1707	1709	1709	1705	1705
	1667	1684	1667	1667	1673	1676	1674	1667	1667
VII	1707	—	1705	1705	1707	1709	1709	1702	1702
	1669	—	1671	1671	1673	1672	1671	1651	1651
VIII	1709	—	1709	1711	1709	1709	1709	1711	1711
	1663	—	1644	1657	1655	1661	1661	1644	1644
IX	1663	1680	1655	1657	1659	1673	1674	1657	1669
									1663
X	1736	1746	1736	1736	1736	1742	1742	1736	1742
	1663	1682	1661	1663	1663	1678	1678	1661	1673

spectra were recorded and the results are presented in Table II. It can be seen that for steroids precipitated alone, changes are insignificant, but for the co-precipitates with calixarene, the spectra often change in the same way as in the co-grinding experiments.

There is no effect of calixarene on the C=O bands of steroid spectra recorded in chloroform or in tetrachloroethylene. Most likely this is because there is no interaction between steroid and calixarene in solution due to low stability of complexes in such solvents.

DSC traces were also recorded for some mixtures to see whether or not a different method would confirm the first results. Mixtures of six steroids with *p*-

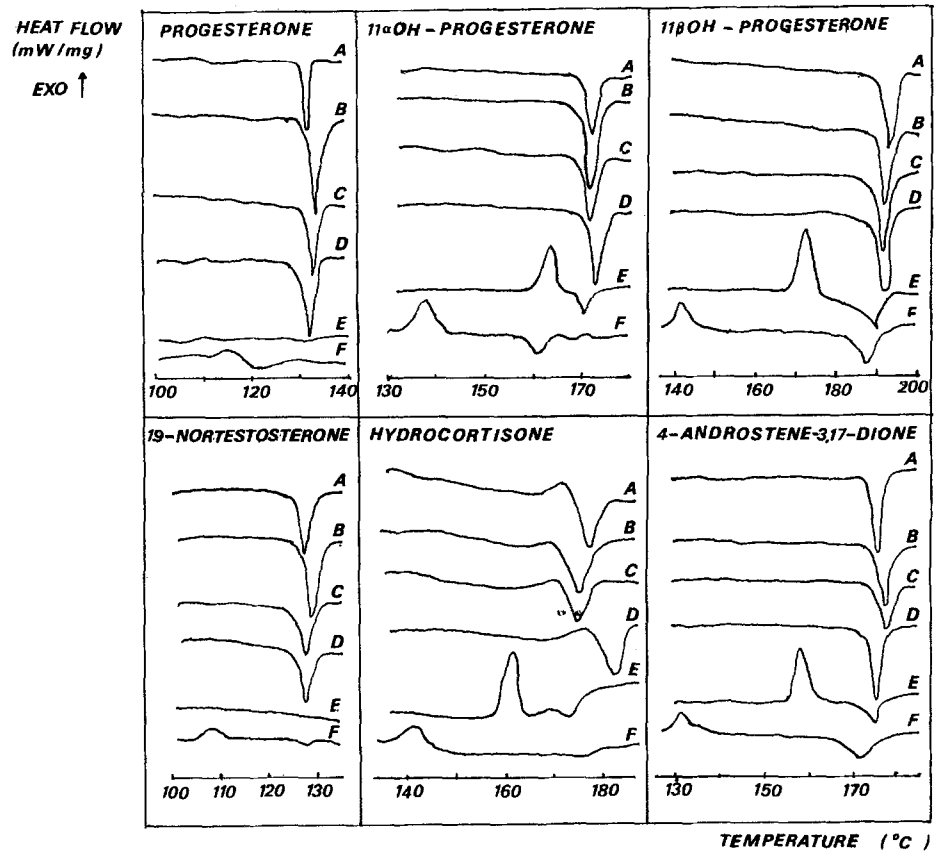


Fig. 3. DSC thermograms of steorids and of steroid : *p-tert*-butylcalix[6]arene mixtures (1 : 1 molar ratio). Temperature program: 3°C/min; sample weight, expressed as steroid content, 1.5–2.5 mg. A: standard steroid; B: steroid ground for 15 min; C: precipitated steroid; D: physical mixture; E: mixture co-ground for 15 min; F: co-precipitated mixture.

tert-butylcalix[6]arene were studied: the significant intervals from the DSC traces are shown in Figure 3. The DSC traces for the interval 100–250°C for *p-tert*-butylcalix[6]arene standard and after grinding for 15 min or precipitation from $CHCl_3$ are shown in Figure 4.

Examination of the traces reveals that the standard steroids when ground or precipitated without calixarene or simply mixed with calixarene have similar endothermic fusion peaks. After grinding with an equimolar amount of *p-tert*-butylcalix[6]arene, the fusion peaks generally decrease substantially or disappear, indicating a marked decrease or disappearance of free steroid. A similar behaviour has been observed in the literature [2] for steroids complexed with cyclodextrins.

The calixarene ground alone for 15 minutes shows an exothermic peak. In the mixture ground for 15 min one can also see the appearance of an exothermic peak

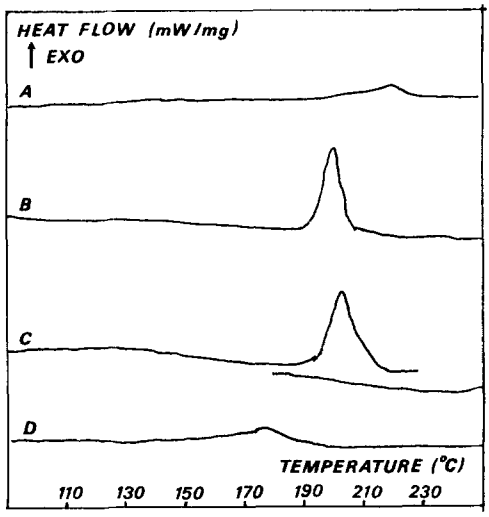


Fig. 4. DSC thermograms of *p-tert*-butylcalix[6]arene. A: standard; B, C: ground for 15 min; D: precipitated from chloroform. Temperature program: A, B, D: 5°C/min; C: heated from 50°C to 230°C at 5°C/min, cooled at 160°C, heated from 170°C to 250°C at 5°C/min.

at different temperatures, according to the steroid. The exothermic peaks for the progesterone : calixarene mixture and the 19-nortestosterone : calixarene mixture are not shown in Figure 3 because they appear at temperatures above the melting point. No such peak occurs for steroid ground alone for 15 min. Some of the complexes obtained by Nakai *et al.* [14–16] by co-grinding and by Giordano *et al.* [18] by co-kneading with cyclodextrins had an exothermic peak that disappeared when the sample heated to the end of the exothermic peak was cooled and heated again. This peak was attributed to transformation of an amorphous form into a crystalline one. Figure 4 shows that *p-tert*-butylcalix[6]arene ground for 15 min behaves in the same way. Also all the steroid : calixarene mixtures ground for 15 min behave in the same way, while any endothermic peak, if present, remains. Whereas the steroids precipitated alone retain the endothermic melting peak those co-precipitated with *p-tert*-butylcalix[6]arene from chloroform show a marked decrease or disappearance of the melting peak, indicating that in this case there is also disappearance or a marked reduction of the free steroid. A very much less intense exothermic peak, usually at a temperature lower than that for the co-ground mixture, suggests that the presence of chloroform leads to formation of a small quantity of amorphous precipitate. Variations of the IR and DSC for the different co-precipitates suggest that their composition is sensitive to differences in the experimental conditions.

The co-ground and the co-precipitated materials are not always identical: some differences in FTIR and DSC can be observed. Perhaps in co-precipitation there

is some influence of the chloroform which is known to form complexes with calixarenes [19].

4. Conclusions

In this paper evidence has been collected for the interaction between *p-tert*-butylcalix[6]arene and *p-tert*-butylcalix[8]arene and steroids in the solid state. DSC and FTIR studies agree in indicating that both co-grinding and co-precipitation can cause interaction between steroids and calixarenes. The IR spectra give information about the functional groups involved and also indicate that the interaction causes the breaking of the crystalline lattice of the steroids: the steroid carbonyl groups are located within a hydrophobic environment and hydrogen bonds between the steroid hydroxyl groups and the calixarene hydroxyl groups are formed. This interaction seems to be specific, depending on the nature of the calixarene and the steroid.

Due to its simplicity and reproducibility the co-grinding method is recommended for the preliminary screening of whether or not interaction between steroids and calixarenes takes place.

We are currently investigating steroid-calixarene complexes with the aim of obtaining more conclusive evidence on their structure.

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References

1. K. Kralova and L. Mitterhauszerova: *Pharmazie* **44**, 623 (1989).
2. F. Liu, D.O. Kildsig and A.K. Mitra: *Pharm. Res.* **7**, 869 (1990).
3. F. Djedaini and B. Perly: *J. Pharm. Sci.* **80**, 1157 (1991).
4. M. Marzona, R. Carpignano and P. Quagliotto: *Ann. Chimica* **82**, 517 (1992).
5. K. Uekama, T. Fujinaga, F. Hirayama, M. Otagiri and M. Yamasaki: *Int. J. Pharm.* **10**, 1 (1982).
6. D.W. Armstrong, W. DeMond, A. Alak, W.L. Hinze, T.E. Riel and K.H. Bui: *Anal. Chem.* **57**, 234 (1985).
7. B. Agnus, B. Sebille and M. Gosselet: *J. Chromatogr.* **552**, 583 (1991).
8. K. Shimada, T. Masue, K. Toyoda, M. Takani and T. Nambara: *J. Liq. Chromatogr.* **11**, 1475 (1988).
9. H.J. Woerdenbag, N. Pras, H.W. Frijklin, C.F. Lerk and T.M. Malingre': *Phytochem.* **29**, 1551 (1990).
10. S. Kumar and H.J. Schneider: *J. Chem. Soc., Perkin Trans.* **2**, 245 (1989).
11. D.R. Carcanague and F. Diederich: *Angew. Chem. Int. Ed. Engl.* **29**, 769 (1990).
12. Y. Kikuchi, K. Kobayashi and Y. Aoyama: *J. Am. Chem. Soc.* **114**, 1351 (1992).
13. F. Toda, K. Tanaka, H. Krupitsky and I. Goldberg: *Bull. Chem. Soc. Jpn.* **66**, 320 (1993).
14. Y. Nakai, A.E. Aboutaleb, K. Yamamoto, S.I. Saleh and M.O. Ahmed: *Chem. Pharm. Bull.* **38**, 728 (1990).

15. M.O. Ahmed, Y. Nakai, A.E. Aboutaleb, K. Yamamoto, A.A.Z.A. Rahman and S.I. Saleh: *Chem. Pharm. Bull.* **38**, 3423 (1990).
16. T. Hanawa, E. Yonemochi, T. Oguchi, Y. Nakai and K. Yamamoto: *J. Incl. Phenom.* **15**, 91 (1993).
17. F. Toda: *Bioorg. Chem.* **19**, 157 (1991).
18. F. Giordano, G. Bruni and G. Bettinetti: *J. Therm. Anal.* **38**, 2683 (1992).
19. C.D. Gutsche: *Calixarenes* (Monographs in Supramolecular Chemistry v. 1, Ed. J.F. Stoddart), Royal Society of Chemistry, Cambridge (1989).