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Some physical-chemical properties of dantrolene and two of its analogues

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Summary

Some physical-chemical properties of dantrolene (1-[[5-(*p*-nitrophenyl)furfurylidene]amino]hydantoin), *p*-chlorodantrolene ((des-*p*-nitro)-*p*-chlorodantrolene) and *o*-nitrodantrolene ((des-*p*-nitro)-*o*-nitrodantrolene) have been studied. The solubility was measured in the range 20–37 ° C, at pH 5.4, 7.4 and 9.4. The solubility was found to increase as a function of pH and temperature. The thermodynamic solubility parameters were determined. Distribution experiments in the system octanol-buffer (20 ° C) yielded a value for the logarithm of the partition coefficient of the neutral compound of 1.70 ± 0.05 and a pK value of 7.5. In the presence of cyclodextrins the solubility increased, indicating complex formation. The corresponding complex formation constants were highest for β-cyclodextrin, with no detectable pH dependence. The inclusion of *p*-Cl-dantrolene was also studied using modelling experiments and molecular mechanics calculations. Inclusion of the chlorophenyl group proved to be possible in all cyclodextrins studied; inclusion of the hydantoin part was only feasible with β- and γ-cyclodextrin. The calculated energy of interaction indicated that Cl-dantrolene has complete rotational freedom in γ-cyclodextrin, but limited freedom in α- and β-cyclodextrin.

Introduction

We are investigating the influence of cyclodextrins (CDs) on the absorption of dantrolene (1-[[5-(*p*-nitrophenyl)furfurylidene]amino]hydantoin) by the rat (Jansen et al., 1988; Poelma et al., 1989). As a part of this investigation, it was felt necessary to gain more insight into the physical-chemical properties of dantrolene. We therefore began a study in which besides dantrolene two related compounds were investigated: *p*-Cl-dantrolene ((des-*p*-nitro-)-*p*-Cl-dantrolene) and *o*-nitrodantrolene ((des-*p*-nitro)-*o*-nitrodantrolene) (see Fig. 1 for the structural formulae of these compounds).

Several physical-chemical properties might be important for the absorption process. The following items have been studied: solubility; lipophilicity in terms of partitioning behaviour; complexation by α-, β- and γ-CD; molecular modelling and in computro studies of the complexation by CDs. As the pK of dantrolene is around 7.5 (Clarke, 1986) the studies were performed at at

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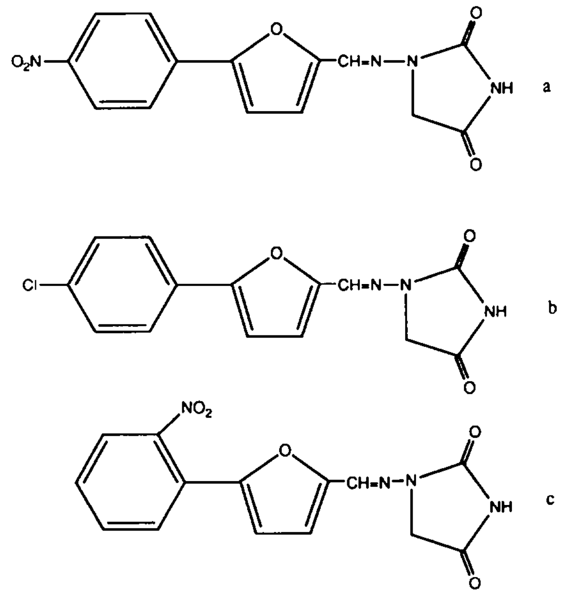

Fig. 1. The structural formulae of the dantrolene derivatives used in this study: (a) dantrolene; (b) *p*-Cl-dantrolene; (c) *o*-nitrodantrolene.

least three pH values, viz. 5.4, 7.4 and 9.4. In some instances, the influence of temperature was also investigated.

Several methods are available for studying the complex formation by CDs. The direct spectrophotometric method appeared not to be useful because of the very small changes in the spectra upon inclusion. Because of the low solubility of the compounds and our intention to perform the measurements as a function of pH and temperature, the competitive methods were also of no use. We therefore decided to make use of the solubility method (Szejtli, 1982).

Materials and Methods

Dantrolene, *p*-Cl-dantrolene and *o*-nitrodantrolene were a gift from Norwich Eaton, Norwich, USA. The CDs were a gift from AVEBE, Foxhol, The Netherlands. All compounds were used as obtained. A correction was made for the amount of water in the CDs.

The solubility measurements were performed in three-fold, with 3 × 6 tubes on one disc rotating in a thermostated bath. The concentrations were measured spectrophotometrically, at a maximum at about 300 nm, depending on the kind of dantrolene, pH and CD used. The solutions and the cuvet were thermostated at 20, 25, 30 and $37 \pm 0.1\,^\circ\mathrm{C}$. The concentrations of the CDs were varied from 10^{-3} to 10^{-2} M. As the concentrations of the dantrolene complexes reached maximally 5% of that of the CDs, the binding constants were calculated from the following equation: $K = [\text{complex}]/([\text{dantrolene}_{\text{free}}] \times [\text{CD}_{\text{total}}]) = \text{slope}/(\text{intercept} \times (1 - \text{slope}))$.

For the solubility experiments isotonic phosphate buffer solutions were prepared (Pharmaceutical Handbook, 1980). From the measured solubility, the (apparent) standard free energy of solution ΔG° is obtained. From the temperature dependence of ΔG°, ΔH° is determined using the van 't Hoff equation. Then ΔS° is obtained by calculation from $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$.

The distribution coefficient D (defined as the total concentration of substance in the organic phase divided by the total concentration of the substance in the aqueous phase) of dantrolene is measured in the system *n*-octanol/(0.001 M citrate/Tris)buffer (shake flask method (Van der Giesen and Janssen, 1982)) at 20 °C and at the pH values 4, 5, 6, 7, 8 and 9. The concentration in both layers was measured by HPLC (Poelma et al., 1989).

The modelling was carried out using Chem-X (Chemical Design, April 1989 release). In order to obtain cyclodextrin structures that gave a good fit with dantrolene compounds, the α- and β-CD molecules were constructed using a special procedure (Kostense et al., 1991; Van Helden et al., 1991). The structure of γ-CD, for which no such procedure is available, was taken from the Cambridge Structural Database. The dantrolene molecule was built using Chem-X substructures and default values (extended force field).

The position and orientation of the guest compound that result in the lowest interaction energy were found using the 'calculate conformations' facility in Chem-X: the guest was placed at various positions in the cavity and at each position the interaction energy between guest and cyclodextrin was calculated (Van Helden et al., 1991). The complex with the lowest interaction energy was then completely optimised using Chem-X.

Results and Discussion

Solubility as a function of pH

Results of the solubility experiments on dantrolene are reported in Table 1, which shows the dependence of the solubility on pH and temperature. It is seen that the solubilities fall within the micromolar range. No literature values are known to us.

The solubility of dantrolene is observed to increase from pH 5.4 to 9.4. This pH dependence is given by $S(\mathrm{pH}) = S_0(1-\alpha)^{-1}$ or

$$\log S(\mathrm{pH}) = \log S_0 - \log(1-\alpha) \qquad (1)$$

(Martin et al., 1983) in which S(pH) represents the solubility at a given pH, S_0 the solubility of the undissociated dantrolene and α the degree of dissociation at the given pH.

The pK of dantrolene has been reported to be 7.5 (Clarke, 1986). This means that at pH 5.4, we are dealing with the solubility of only the undissociated acid, which equals S_0 in Eqn 1. From the measured solubility data at pH 7.4, 25 °C, a pK value of 8.3 ± 0.1 is found; similarly, the data at pH 9.4 are consistent with a pK of 8.55 ± 0.01. Using the data obtained at other temperatures, comparable values are obtained. The conclusion is that a discrepancy is observed between the pK value reported in the literature and that estimated from the solubility experiments.

TABLE 2

The thermodynamic solubility parameters of dantrolenes at 25 °C

	$\Delta G°$ (kJ/mol)	$\Delta H°$ (kJ/mol)	$\Delta S°$ (J/K per mol)
pH 5.4			
dantrolene	29.2 ± 0.02	38.2 ± 1.3	29.9 ± 4.3
Cl-dantrolene	29.7 ± 0.00	14.4 ± 1.5	−51.1 ± 4.9
o-nitrodantrolene	25.2 ± 0.02	32.7 ± 1.1	25.1 ± 3.6
pH 7.4			
dantrolene	28.9 ± 0.01	48.4 ± 2.0	65.4 ± 6.4
Cl-dantrolene	28.9 ± 0.06	35.4 ± 2.8	22.2 ± 9.1
o-nitrodantrolene	23.6 ± 0.06	39.4 ± 0.8	53.2 ± 2.2
pH 9.4			
dantrolene	24.1 ± 0.08	47.9 ± 2.3	79.7 ± 7.7
Cl-dantrolene	23.4 ± 0.04	43.3 ± 2.1	66.7 ± 6.8
o-nitrodantrolene	20.2 ± 0.01	41.5 ± 2.4	71.4 ± 8.0

In Table 1 the solubility data of Cl-dantrolene are also listed. It can be observed that the solubilities of dantrolene and Cl-dantrolene are very

TABLE 1

Solubility of dantrolenes (in μM) at various temperatures and pH values

		pH 5.4	pH 7.4	pH 9.4
20 °C	dantrolene	5.99 ± 0.05	6.47 ± 0.06	4.04 ± 0.21
	Cl-dantrolene	5.96 ± 0.33	7.43 ± 0.11	5.91 ± 0.10
	o-nitrodantrolene	30.8 ± 1.0	57.3 ± 0.5	228 ± 1
25 °C	dantrolene	7.38 ± 0.07	8.34 ± 0.05	5.97 ± 0.19
	Cl-dantrolene	6.32 ± 0.01	8.72 ± 0.22	7.67 ± 0.12
	o-nitrodantrolene	37.2 ± 0.3	72.5 ± 1.7	283 ± 1
30 °C	dantrolene	9.59 ± 0.09	11.51 ± 0.12	7.58 ± 0.36
	Cl-dantrolene	6.93 ± 0.22	10.82 ± 0.29	9.81 ± 0.03
	o-nitrodantrolene	46.1 ± 0.5	96.0 ± 4.1	363 ± 11
37 °C	dantrolene	13.70 ± 0.09	18.22 ± 0.10	12.10 ± 0.71
	Cl-dantrolene	8.13 ± 0.06	15.68 ± 0.06	15.20 ± 0.20
	o-nitrodantrolene	63.0 ± 1.6	137 ± 10	553 ± 13

Each value is the mean ± S.D. of three determinations.

similar. Using the aforementioned method, pK values can be estimated for Cl-dantrolene. From the data at pH 7.4, 25 ° C and pH 9.4, 25 ° C, pK values of 7.9 ± 0.1 and 8.37 ± 0.02 are found. The agreement between these two values is less satisfactory than in the case of dantrolene. Comparing the pK values of dantrolene and Cl-dantrolene hardly any influence of the Cl substituent on the pK might be expected, because of the large distance between the substituent and the centre of ionization.

From the solubility data for *o*-nitrodantrolene (Table 1) at pH 7.4, 25 ° C and pH 9.4, 25 ° C, pK values of 7.42 ± 0.04 and 8.58 ± 0.01 are found. Similar remarks to those given above for Cl-dantrolene can be given here. Thus far, we are unable to provide an explanation for the discrepancies described.

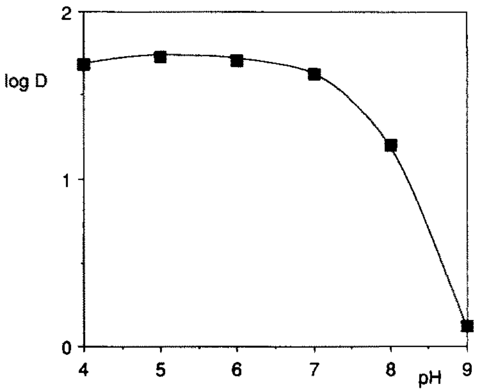

Fig. 2. Log D vs pH of dantrolene in the system octanol-water, at 20 ° C.

Thermodynamic solubility parameters

In Table 2 the thermodynamic parameters describing the solubility process are given. The data at pH 5.4 relate to the undissociated acid. The influence of pH on ΔH follows from the temperature dependence of the solubility (as expressed in Eqn 1) and can be written as:

$$\Delta H(\text{pH}) = \Delta H^{\circ} + \alpha \Delta H(\text{ion}) \qquad (2)$$

In this equation ΔH(pH) represents the observed ΔH, ΔH° the heat of solution of the undissociated compound, ΔH(ion) the heat of ionisation of the compound and α the degree of dissociation at the given pH. The increase in ΔH(pH) on going from pH 5.4 to pH 9.4 is caused by the term $\alpha \Delta H$(ion), which is expected to be positive. Using the data for dantrolene at pH 9.4 and at 25 ° C, the term $\alpha \Delta H$(ion) is found to be 23 ± 3 kJ mol^{-1}.

From Eqns 1 and 2 the following expression for the entropy term can be derived:

$$\Delta S(\text{pH}) = \Delta S^{\circ} + \alpha \Delta H(\text{ion}) T^{-1} - R \ln(1 - \alpha) \qquad (3)$$

This equation clearly demonstrates that the entropy is determined by several factors. From the

TABLE 3

Binding constants (M^{-1}) of dantrolenes and CDs at various temperatures and pH values

	pH 5.4			pH 7.4			pH 9.4		
CD:	α	β	γ	α	β	γ	α	β	γ
25 ° C									
dantrolene	89	281	28	134	315	42	136	324	83
Cl-dantrolene	177	310	33	210	474	45	241	531	88
o-nitrodantrolene	13	120	91	17	133	106	[a]	[a]	[a]
37 ° C									
dantrolene	67	240	33	96	284	45	106	300	104
Cl-dantrolene	143	401	47	168	498	63	180	581	113
o-nitrodantrolene	14	101	81	15	45	35	[a]	[a]	[a]

[a] No value, because of precipitation of the complex.

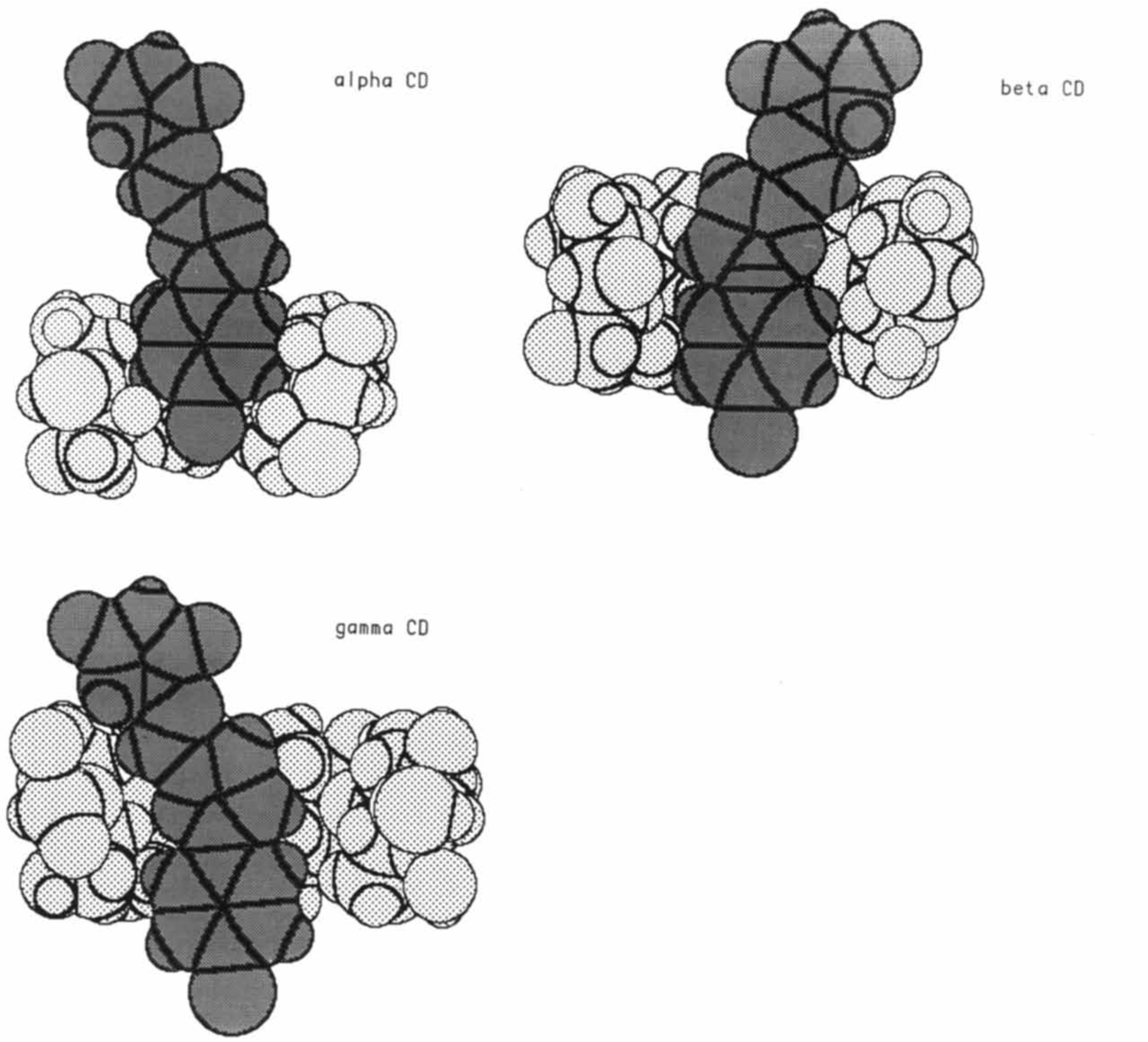

Fig. 3. Inclusion of the chlorophenyl part of Cl-dantrolene by α-, β- and γ-CD.

data given above we find for dantrolene at pH 9.4:

$$\alpha \Delta H(\text{ion}) T^{-1}$$

$$= 79 \pm 9 \text{ J K}^{-1} \text{ mol}^{-1} \text{ and } -R \ln(1-\alpha)$$

$$= 17 \pm 3 \text{ J K}^{-1} \text{ mol}^{-1}.$$

In view of the fact that the pH dependence of the solubility cannot be explained satisfactorily with a single pK value, it cannot be expected that consistent data are obtained for all compounds at all pH values. The numbers given should be considered as an illustration.

Distribution coefficient of dantrolene

Results of partitioning experiments on dantrolene are given in Fig. 2, where log D is plotted vs pH. The pH dependence follows the behaviour to be expected for the partitioning of an acid compound. The log D value at low pH is equal to the logarithm of the partition coefficient (log P) of the neutral compound and is found to be 1.70 ± 0.05. No literature values are available; also, a comparison with calculated values is not possible because of missing fragmental values (Hansch and Leo, 1979).

The second important parameter which follows from this curve is the pK value. A value around 7.5 follows from these experiments, in accord with the reported value in the literature (Clarke, 1986). This result warrants a further study of the pH dependence of the solubility of dantrolene and its derivatives.

Low solubility is often attributed to the hydrophobic character (expressed as log P measured in the system octanol/water) and/or to a high melting point (m.p.) of a compound (Valvani and Yalkowsky, 1980) via the equation: log molar solubility $= -\log P - 0.01 \times \text{m.p.} + 1.05$. As the m.p. of dantrolene is 280 °C (Clarke, 1986) and log $P = 1.7 \pm 0.05$, the solubility of dantrolene at 25 °C calculated via this equation should be 3.5×10^{-4} M. This disagrees completely with the measured value of 7.38×10^{-6} M. In fact, the difference is 1.7 log units. In the experimental equations (Valvani and Yalkowsky, 1980), corrections of 1 log unit are found for rigid polycyclic aromatic hydrocarbons and for steroid hormones. Therefore, the complicated and rigid structure of the molecule is possibly the reason for the discrepancy found.

Interaction of dantrolene and derivatives with α-, β- and γ-CD

Solubility experiments in the presence of CD indicated an interaction between these two compounds. In Table 3 the results are expressed in terms of binding constants. The interaction is strongest for β-CD and weakest for γ-CD.

Not shown are the results of the temperature dependence of the binding constants, since reliable ΔH values could not be determined from this solubility method, or from microcalorimetric experiments, due to low solubility. The trend observed was that ΔH increased, for both dantrolene and Cl-dantrolene, going from α- to β- to γ-CD, at all pH values. A detailed analysis, however is not warranted.

The data in Table 3 indicate that there is no detectable pH dependence. This means that no major pK shifts occur, and it excludes the possibility that either the neutral or the anion is exclusively bound. These observations might be important regarding the question of which part of the molecule is included in the CD. For investigating

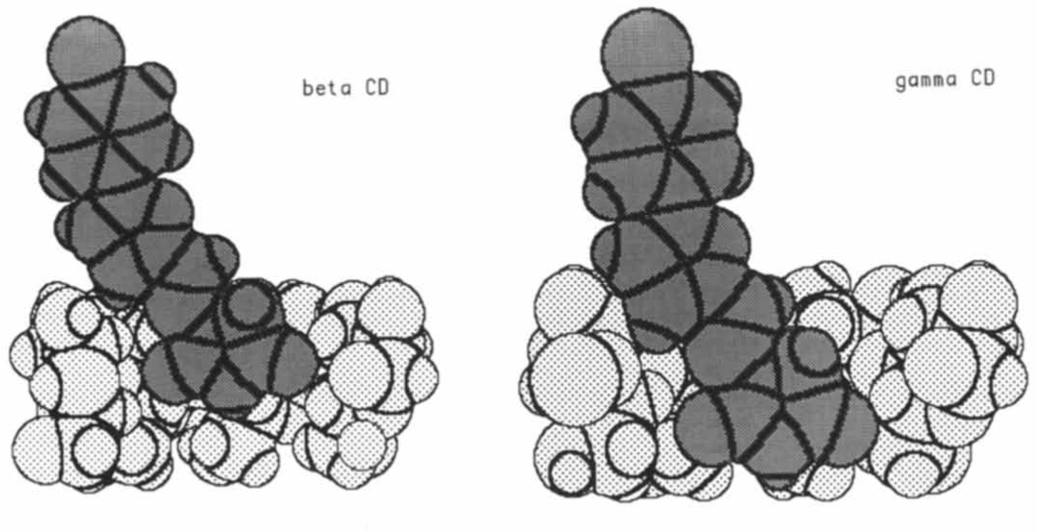

Fig. 4. Inclusion of the hydantoin part of Cl-dantrolene by α-, β- and γ-CD.

this problem, modelling experiments and calculations in computro on interaction energies were performed.

Interaction of dantrolene and derivatives studied in computro

In Fig. 3 the inclusion of the chlorophenyl part of Cl-dantrolene by α-, β- and γ-CD is shown. Inclusion appears to be possible; in β- and γ-CD the chlorophenyl group even protrudes. To check the possibility of reversed inclusion, viz. the inclusion of the hydantoin part, similar modelling experiments were performed. This reversed inclusion is indeed feasible with β- and γ-CD (see Fig. 4). However, this type of inclusion might lead to a measurable influence on the pK value, analogous to that described for the inclusion of p-nitrophenol (Eftink and Harrison, 1981), hence, we suggest that the inclusion as shown in Fig. 3 is more likely.

The interaction energy, defined and calculated as described in Materials and Methods, has been calculated for Cl-dantrolene, as a function of the rotation along an axis perpendicular to the plane of the O(4)-atoms in α-, β- and γ-CD. The results are shown in Fig. 5, where the most favourable minimum value has been scaled to the same zero value in all the three cases studied. In α-CD there is a strong dependence of the interaction energy on the angle of rotation, in contrast to γ-CD, where free rotation seems to exist. Applying the Boltzmann distribution law to these data it can be estimated that 99% of the molecules will have an interaction energy between 0 and 8 kJ/mol. This implies that these complexes have a rotational freedom of ±10 ° in α- and ±45 ° in β-CD. This clearly indicates the different degree of rotational freedom depending on the size of the CD.

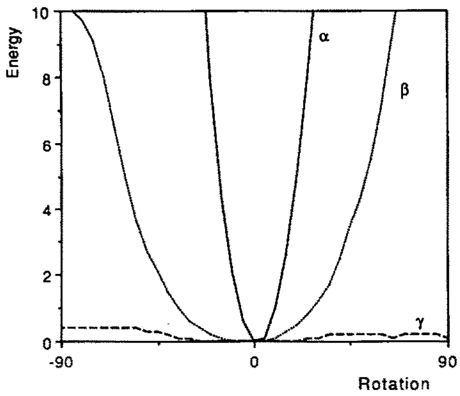

Fig. 5. Interaction energy E (kcal/mol) of Cl-dantrolene with α-, β- and γ-CD.

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