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Review Article

Inclusion Compounds of Psychotropic Agents and Cyclodextrins

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Abstract. Literature data on inclusion compounds of psychotropic agents (hypno-sedative, anticonvulsive and antiepileptic drugs, neuroleptics and anxiolytics) with cyclodextrins and their derivatives are presented. Inclusion compounds of two novel psychotropic agents, gidazepam and cinazepam, with β-cyclodextrin (1 : 1 and 1 : 2) were obtained. The structure of these complexes has been established by one- and two-dimensional ^{1}H-NMR (for solutions in DMSO) and IR (for solid state) spectroscopy.

Key words: psychotropic agents, cyclodextrins, phenazepam, cinazepam, inclusion compounds, structure, ^{1}H-NMR spectroscopy, NOESY spectra, d-contacts, Nuclear Overhauser effect.

1. Introduction

Inclusion compounds of psychotropic agents with cyclodextrins (CDs) may be used for modeling the interaction of psychopharmacologically active endogenic compounds and xenobiotics with the central nervous system receptors and for the elucidation of some problems in pharmaceutical chemistry (bioavailability, solubility, stability of drugs etc).

The inclusion complexes of different psychotropic agents with CD and their derivatives have been reported: e.g. inclusion complexes of hypno-sedative drugs e.g. barbiturates [1], and the central nervous system stimulants, ephedrine and caffeine [2,3]. Such complexation can significantly improve the bioavailability of the drug: the inclusion complex of the anticonvulsive and antiepileptic drug, carbamazepine, with 2-hydroxypropyl-β-CD gives a peak plasma concentration of carbamazepine in blood plasma of 25.4 $\pm$ 4.9 μg/mL approximately 2 h after administration, whereas with the pure drug it appears in blood plasma at a level of 10.7 $\pm$ 0.21 μg/mL approximately 4 h after administration [4].

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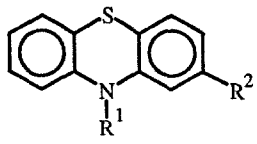
2. Complexes of the Phenothiazines

The interaction of the neuroleptic drugs, the phenothiazines (**I**), with α- and β-CD in aqueous solutions was investigated by Otagiri and co-authors [5] using circular dichroism, ultraviolet absorption and proton magnetic resonance spectroscopies.



I

1. Promazine, $R^1 = (CH_2)_3N(CH_3)_2$, $R^2 = H$;
2. Chloropromazine, $R^1 = (CH_2)_3N(CH_3)_2$, $R^2 = Cl$;
3. Methopromazine, $R^1 = (CH_2)_3N(CH_3)_2$, $R^2 = OCH_3$;
4. Levomepromazine, $R^1 = CH_2CHCH_2N(CH_3)_2$, $R^2 = OCH_3$;
   CH_3
5. Prochloperazine, $R^1 = (CH_2)_3$—N⟨ ⟩N—CH_3, $R^2 = Cl$.

α-CD showed no appreciable complex formation with any of the compounds studied, suggesting that the cavity of α-CD is not large enough to include the bulky phenothiazine moiety.

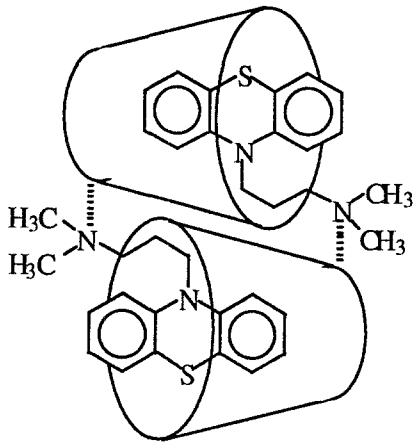
Phenothiazines form 1 :1 complexes with β-CD. In the β-CD–drug system protons located in the β-CD cavity were found to be subjected to anisotropic shielding, while protons of the phenyl and N-substituted groups in the drug shifted to low field with remarkable broadening. The formation constants of the inclusion complexes were correlated with the partition coefficients of compounds **1–5**.

Taking into account these data, the authors [5] have suggested that the aromatic part of the drug was included into the hydrophobic cavity of β-CD, while the N-substituents of the drug interacted with the external groups of the β-CD cavity (**II**).

3. Complexes of the Benzodiazepines

Benzodiazepine anxiolytics (**III**) are used more than any other psychotropic agents. Their main characteristic is the ability to cut off negative emotions: anxiety, nervousness, fear and panic states. These drugs, as a rule, also have hypnotic, sedative, anticonvulsive and myorelaxive properties. The combination of these properties in each benzodiazepine agent forms its psychopharmacologic spectrum. More than 30 benzodiazepine anxiolytics are used in medical practice. As a rule, compounds of a given series have low solubility in water, which limits their bio-availability.

Numerous chemical investigations, particularly on the pharmaceutical chemistry of inclusion compounds of benzodiazepine anxiolytics, have therefore been carried out during the last 10–15 years. The purposes of such investigations are to increase the bioavailability of agents and to change the spectrum of pharmaceutical activity.



II

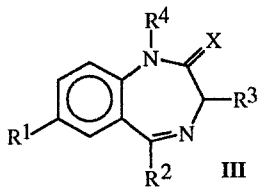
Our joint investigations with the Moscow Institutes of Pharmacy and Pharmacology have shown that formation of phenazepam clathrate complexes with polyvinylpyrrolidone gives stable injection solutions of a given agent and also essentially increases the anxioselectivity. On the basis of the data obtained a novel medicinal form of phenazepam was obtained: a 0.1% injection solution [6].

Biopharmaceutical investigations on benzodiazepines (**III**) have shown that a rapid plasma appearance of benzodiazepines is therapeutically essential, particularly in the treatment of acute convulsive attacks. Accordingly, a rapidly dissolving form of benzodiazepines with high aqueous solubility is preferable for rapid absorption in oral benzodiazepine therapy.

Inclusion complexes of benzodiazepines (**III**) with α-, β- and γ-CD in aqueous solution and in the solid phase were studied by solubility methods, spectroscopy (UV, CD and IR), thermal analysis and X-ray diffractometry [7,8].

The solid complexes of benzodiazepines (BD) with γ-CD were prepared by mixing appropriate amounts of CD and BD in water. The amounts were calculated from the descending curvature of the phase solubility diagram. The complex, which precipitated as a microcrystalline powder, was filtered and dried under vacuum. The powder corresponded to a 2 : 3 or 1 : 2 benzodiazepine/γ-CD complex. Neither α- nor β-CD yielded any solid complexes.

The magnitudes of stability constants (K) usually increase in the order of $\beta > \gamma > \alpha$-CD, suggesting that in aqueous media the size of the cavity of β-CD most favourably accommodates the benzodiazepine molecules.



III

		R^1	R^2	R^3	R^4	X
6	Diazepam	Cl	C_6H_5	H	CH_3	O
7	Medazepam	Cl	C_6H_5	H	CH_3	H_2
8	Fludiazepam	Cl	o-FC_6H_4	H	CH_3	O
9	Nitrazepam	NO_2	C_6H_5	H	H	O
10	Nimetazepam	NO_2	C_6H_5	H	CH_3	O
11	Flunitrazepam	NO_2	o-FC_6H_4	H	CH_3	O
12	Clonazepam	NO_2	o-ClC_6H_4	H	H	O
13	Flurazepam	Cl	o-FC_6H_4	H	$(CH_2)_2N(C_2H_5)_2$	O
14	Lorazepam	Cl	o-ClC_6H_4	OH	H	O
15	Oxazepam	Cl	C_6H_5	H	H	O
16	Bromazepam	Br	2'-C_5H_4N	H	H	O
17	Phenazepan	Br	o-ClC_6H_4			

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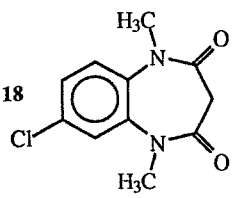

CLOBAZAM

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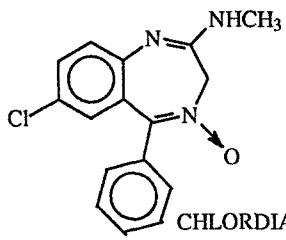


CHLORDIAZEPOXIDE

A good correlation was also found in the case of the phenothiazine neuroleptics (**I**) between the stability constant of the complex and the partition coefficient of the drug molecule, indicating that the more hydrophobic guest molecule exhibited the stronger binding to CD. These findings have confirmed that the hydrophobic nature of the guest molecule and steric factors between the host and guest molecules were responsible for these interactions.

In the IR spectrum of the diazepam (**6**) -γ-CD complex the 1685 cm^{-1} band (C=O) shifted to 1665 cm^{-1}, suggesting the formation of intermolecular hydrogen bonding between diazepam and γ-CD.

It was shown in this work that the γ-CD complex of benzodiazepines dissolved much more rapidly than the drug alone. The enhanced dissolution rate may be due to the increase in solubility and the decrease in crystallinity of the drug by inclusion complexation.

Experiments on rabbits showed that the serum levels of the drug administered as the complex were much higher during the initial 30 min period than on administrating the drug alone. In the case of diazepam, the maximum serum levels (C_{max}) of 0.59 ± 0.08 μg/mL was observed at 30 ± 8 min. In contrast, the γ-CD complex resulted in the rapid appearance of diazepam in the serum, showing a C_{max} value of 1.05 ± 0.16 μg/mL at 24 ± 9 min. The authors concluded that the γ-CD complex of diazepam could be used to improve the oral bio-availability of diazepam.

The formation of inclusion complexes of nitrazepam (**9**) with α-, β-, γ-CD, heptakis-(2,6-di-*O*-methyl)-β-CD (DM-β-CD) and heptakis-(2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD) was confirmed by a group of Japanese and Egyptian scientists [9]. The highest stability constant and the solubilized amount of nitrazepam was obtained with DM-β-CD in the order:

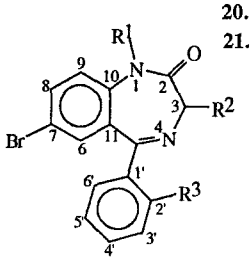
$$\text{DM-}\beta\text{-CD} > \beta\text{-CD} > \text{TM-}\beta\text{-CD} > \gamma\text{-CD} > \alpha\text{-CD}$$

A crystalline complex of nitrazepam with DM-β-CD in a 1 : 2 molar ratio was obtained by the coprecipitation method. IR spectra showed that the frequency shifts for the carbonyl stretching band of nitrazepam in the complexes with DM- and TM-β-CD, were similar to those of nitrazepam in $CHCl_3$ solution, arising from the monomolecular dispersion of nitrazepam in a hydrophobic environment.

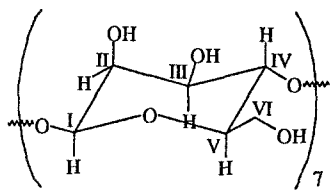
4. Complexes of Gidazepam and Cinazepam

We have investigated inclusion compounds of two novel psychotropic agents of the 1,4-benzodiazepine series (**III**): gidazepam (**20**) and cinazepam (**21**) with β-CD. Gidazepam is a selective anxiolytic [10]. Cinazepam has strong anxiolytic and hypnotic effects. In contrast to other hypnotics of the benzodiazepine series cinazepam has a significant advantage: the structure of sleep is not changed by it. Sleep induced by cinazepam does not differ from the normal, physiologic sleep [11].

20. Gidazepam: $R^1 = C^{1"}H_2C^{2"}ONHNH_2$; $R^2=R^3=H$
21. Cinazepam: $R^1=H$; $R^2=O^{1"}C^{2"}OC^{3"}H_2C^{4"}H_2C^{5"}OOH$; $R^3=Cl$



III



IV

Inclusion compounds of gidazepam and cinazepam were obtained by the coprecipitation method: a hot methanol solution of the appropriate benzodiazepine was added to a hot aqueous solution of cyclodextrin in ratios of 1 : 1 and 2 : 1 of cyclodextrin: benzodiazepine. The solutions were mixed when hot, cooled and the precipitate was separated and dried. The elemental analysis of the precipitate corresponded to inclusion compounds of composition 1 : 1 and 2 : 1. The formation of inclusion compounds was confirmed using thermogravimetry, IR and ^{1}H-NMR spectroscopy.

The structure of the inclusion compounds were studied using one- and two-dimensional ^{1}H-NMR spectroscopy, using the reported assignments of the signals in the ^{1}H-NMR-spectrum of gidazepam [12]. ^{13}C-^{1}H Correlation spectroscopy at the transition of magnetization from protons on nuclei of carbon following the DEPT method was used to assign the signals in the NMR spectrum of cinazepam. The β-CD signals were assigned on the basis of literature data [13,14].

The spectra of the inclusion compounds are the superposition of 'host' and 'guest' spectra with selectively shifted signals. The absence of signals of 'guest' and 'host' molecules in unbonded forms demonstrates that all 'guest' and 'host' molecules in the complexes of both compositions participate in clathrate formation.

Analysis of the shifts ($\Delta\delta$) of signals of gidazepam protons on formation of the 1 : 1 and 2 : 1 complexes has allowed us to conclude that the oxygen atom of the hydrazide fragment forms a hydrogen bond with the hydrogen atom of one of the hydroxyls of β-CD, and that the protons of the NH_2 group are hydrogen-bonded with other β-CD hydroxyl groups.

At the transition of the gidazepam complex with β-CD of 1 : 2 composition to the complex with 1 : 1 composition the high-field shift of the water signal by 0.05 ppm is observed to take place. This can be explained by the displacement of water molecules from the cavity of the second β-CD molecule. Therefore, it can be concluded that the complex with 1 : 1 composition actually has a 2 : 2 composition.

The signal of the carboxyl group of the protons in spectra of inclusion compounds of cinazepam with β-CD is shifted to higher field, which may be due to its desolvatation on clathrate formation. The higher shielding is observed in the proton of the amide fragment. It may be considered that this is the result of H-bond formation of the oxygen atom of the same fragment with one of the β-CD hydroxyl groups.

Significantly more extensive information on the structure of the inclusion compounds of gidazepam and cinazepam with β-CD has been obtained using cross-relaxation two-dimensional spectroscopy (NOESY). NOESY spectra, besides trivial intramolecular *d*-contacts of the C^6H—$C^{2'}H$, C^8H—C^9H, $C^3H'_2$—$C^3H''_2$, C^1H_2—NH_2 type etc., have numerous *d*-contacts between 'host' and 'guest' molecules.

From the NOESY spectra it is seen that the phenyl ring of gidazepam and the *o*-chlorophenyl ring of cinazepam are in the β-CD hydrophobic cavity. This fact is attested by the ($C^{2'}H$—$C^{4'}H$)—C*II*H (C*IV*H), C*V*H (C*III*H, C*VI*H_2), C*VI*OH contacts in the spectra of inclusion compounds of gidazepam with β-CD and the

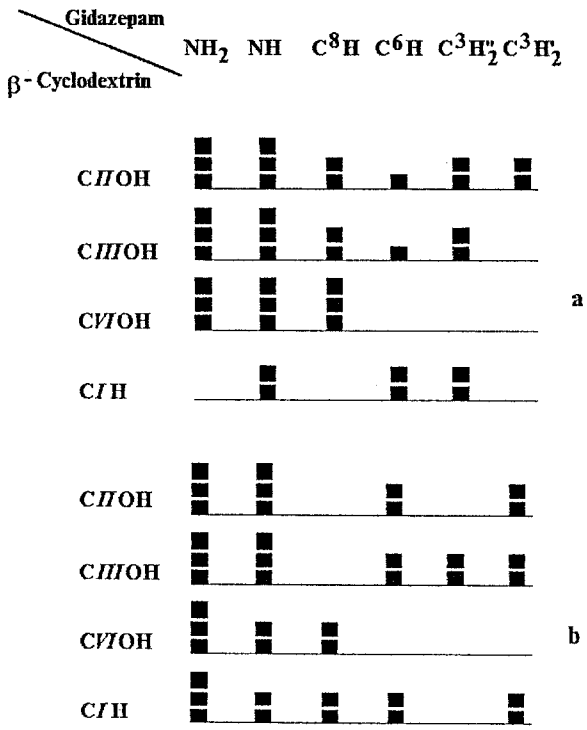


Figure 1. Diagram of the *d*-contacts for gidazepam and β-CD in the inclusion compounds of (a) 2 : 2 (b) 1 : 2 compositions.

($C^{3'}H$—$C^{5'}H$)—C*I*H, C*V*H, C*VI*OH contacts in the spectra of the cinazepam complex with β-CD.

Diagrams of some of the *d*-contacts between 'host' and 'guest' molecules of the inclusion complexes with the indication of cross-peak intensity are shown in Figures 1 and 2. The presence of C^6H—C*I*H and C^6H—C*II*OH, C*III*OH *d*-contacts in the spectra of the gidazepam complexes with β-CD indicates the disposition of the C^6 atom close to the middle line of the β-CD bracelet (at the level of the C*I* atoms).

Intense *d*-contacts of C^8H with C*VI*OH and medium-intense contacts with C*II*OH and C*III*OH (for the 1 : 1 complex, Figure 1a) indicate the simultaneous disposition of C^8H near the wide and narrow bases of the bracelet. This is possible with the 2 : 2 complex, but not with the 1 : 1 complex as two β-CD molecules are close to each other by the wide and narrow bases (Figure 3). A similar association is simultaneously confirmed by intensive cross-peaks NH, NH_2—C*II*OH, C*III*OH and C*VI*OH. These cross-peaks indicate that the hydrazide fragment of the gidazepam molecule is close to the hydroxyl groups of their 'host' and C*VI*OH of the associated clathrate.

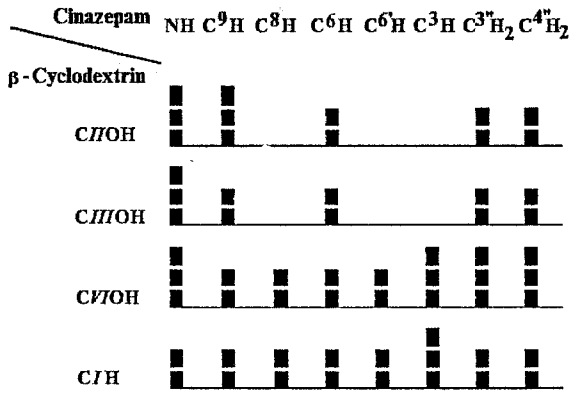


Figure 2. Diagram of the *d*-contacts for cinazepam and β-CD in the 1 : 2 inclusion compound.

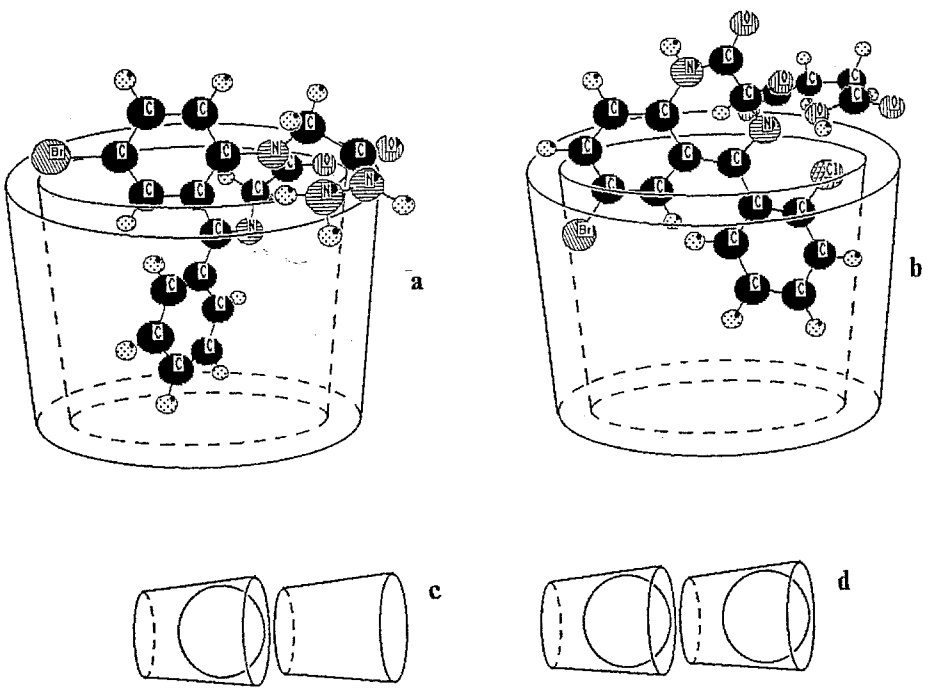


Figure 3. Models of the inclusion of the molecules of gidazepam (a) and cinazepam (b) in the β-CD cavity; schemes of constitution of inclusion complexes of gidazepam and cinazepam 1 : 2 (c) and 2 : 2 (d).

The absence of cross-peaks between the C^9H and $C^{1''}H_2$ signals and signals of β-CD protons is noteworthy. This indicates that the distance between corresponding 'guest' and 'host' protons exceeds 0.5 nm. The mentioned fragments of the gidazepam molecule are close to the main axis of the dimeric associate, beyond the limits of the β-CD cavity. At the same time, the *d*-contacts of average intensity

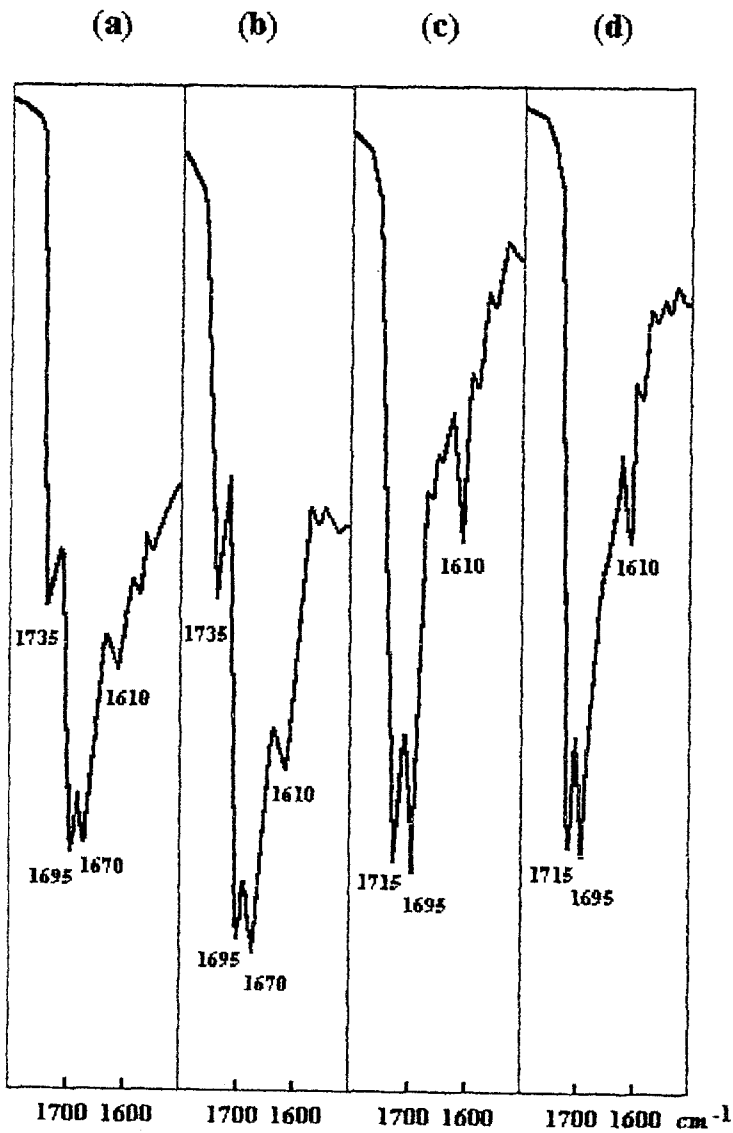


Figure 4. Solid state IR spectra (1500–1800 cm^{-1}) of (a) cinazepam, (b) mechanical mixture of cinazepam with β-CD, (c) inclusion complex of cinazepam with β-CD (1 : 1); (d) inclusion complex of cinazepam with β-CD (1:2).

between C^3H_2 and *CIIOH*, *CIIIOH* protons as well as *CIH* testify to the disposition of the methylene fragments of benzodiazepine within the hydrophobic cavity near the wide base of β-CD.

Comparison of diagrams of *d*-contacts for complexes with 2 : 2 and 2 : 1 composition (Figure 1) confirms that the spatial orientation of the β-CD molecules in these complexes is the same. The difference lies in the fact that the hydrazide frag-

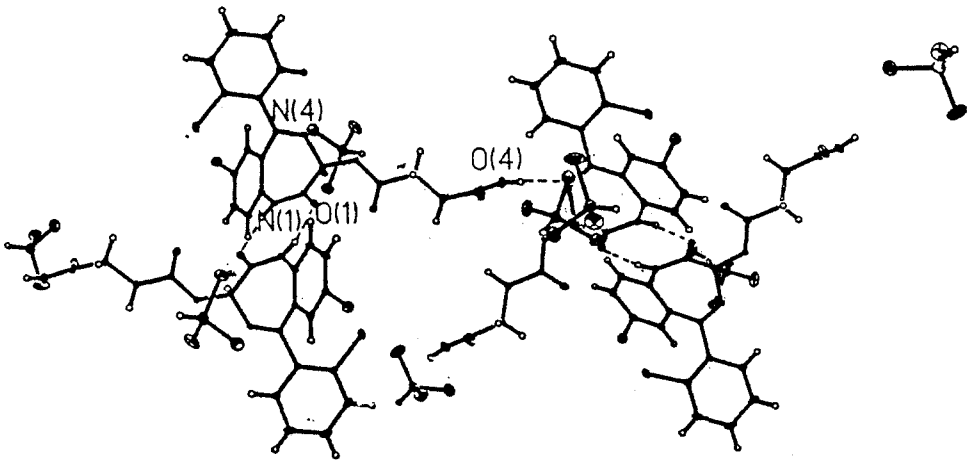


Figure 5. Crystal structure of cinazepam.

ment of gidazepam has been absorbed in the cavity of the empty β-CD molecule of the 2 : 1 complex (contact NH_2—C*I*H). The absence of C^8H—C*III*OH, C*III*OH *d*-contacts, but the appearance of the C^8H—C*I*H *d*-contact in the 2 : 1 complex testifies to the attempt of the benzo group of the gidazepam molecule to penetrate into the hydrophobic cavity of the second β-CD molecule from the side of the narrow base.

The presence of C^6H, C^8H, C^9H—C*I*H, C*II*OH, C*III*OH *d*-contacts in the cinazepam spectra (Figure 2) indicates the disposition of the C^6, C^8 and C^9 atoms near the wide base of the bracelet. At the same time C^6H, C^8H, C^9H—C*VI*OH *d*-contacts could be only explained by the localization of these atoms near the narrow base of the bracelet. The simultaneous disposition of C^6, C^8 and C^9 (as for gidazepam) near the wide as well as the narrow bases is possible only in the case of the approach of two β-CD molecules to each other by wide and narrow bases. A similar association is confirmed by intensive NH, $C^{3''}H_2$, $C^{4''}H_2$—C*VI*OH cross-peaks.

The presence in the inclusion compound of cinazepam with β-CD of $C^{3''}H_2$, $C^{4''}H_2$—C*I*H, C*II*OH and C*III*OH cross-peaks of average intensity in the NOESY spectra indicates that the hemi-succinyl fragment is inside the β-CD bracelet near its wide base.

Measurements of the cross-peak intensities in NOESY spectra at the time of mixing τ_m = 0.2, 0.4 and 0.6 s have revealed a linear dependence of the intensities on τ_m in the abovementioned diapazones. The distances between the closed protons from values of the nuclear Overhauser effect (NOE) were calculated on this basis.

As the relationship between the NOE value and the interproton distances d is determined by the known correlation $(NOE)^{-1} \sim d^6$, distances between any pairs of protons interacting by the dipole–dipole mechanism can be calculated by

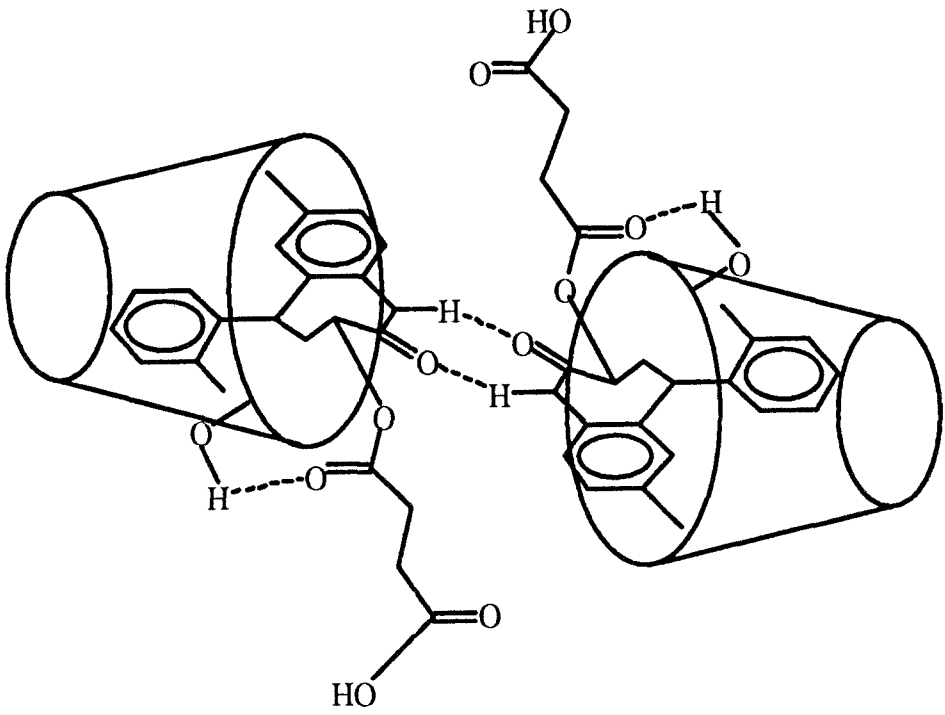


Figure 6. Possible structure of the 2 : 2 inclusion complex of cinazepam and β-CD in the solid state.

comparison with the NOE and distance values of 'reference' groups. The NOE values and distances between the two heminal protons C^3H', C^3H'' of gidazepam (0.18 nm), and between the C^8H—C^9H protons of cinazepam (0.25 nm by MM2 calculation) were used as the basis for the calculations.

The spatial structure in DMSO solutions on the basis of established values of interatomic distances in the inclusion complexes of gidazepam and cinazepam has been determined. As seen from Figure 3, the essential difference is the size of the angle between the axis of symmetry of β-CD and the axis which lies through the centres of the aromatic rings. This angle is 0° for the gidazepam complex (Figure 3a), and 60° for the cinazepam complex (Figure 3b). This difference, evidently, is caused by the position of the substituents participating in the formation of hydrogen bonds with hydroxyl groups near the wide base of the β-CD bracelet: the hydrazinocarbonylmethyl moiety of gidazepam near the N^1 atom, and the hemisuccinylhydroxy-group of cinazepam near C^3.

IR spectra of crystalline samples of inclusion compounds of cinazepam with β-CD indicate that the structure of the complexes in the solid state can be different from those described above. The IR spectra of cinazepam, a mechanical mixture of cinazepam and β-CD and the cinazepam inclusion compounds with β-CD of composition 1 : 1 and 1 : 2 in the region 1500–1800 cm^{-1} are given in Figure 4.

The spectrum of cinazepam, where there are clear bands of the carbonyl group of the ester fragment (1695 cm^{-1}) and of the carboxyl group (1670 cm^{-1}) is shown in Figure 4a. The spectrum of a mechanical mixture of cinazepam and β-CD is practically the same as that of cinazepam (Figure 4b).

Significant changes in the spectra occur after formation of the cinazepam/β-CD inclusion compounds of ratio 1 : 1 and 1 : 2. The bands of the ester carbonyl group are shifted by 20 cm^{-1} to higher wave number and this indicates participation of this group in intermolecular relationships of cinazepam and β-CD (evidently, with one of its hydroxyl groups). The position of the absorption band of the C=O bond of the carboxyl group is shifted by 25 cm^{-1} to higher wave number. This can be explained by the breakage of the intermolecular hydrogen bond between the carboxyl group and the nitrogen atom of the azomethin group of the neighbouring cinazepam molecule. The position of the absorption bands of the C=O bond of the lactam fragment (1610 cm^{-1}) does not change. This testifies that intermolecular dimer associates remain in inclusion compounds due to hydrogen bonds between the amide fragments of neighbouring molecules. The afore-mentioned hydrogen bonds between amide groups, as well as between the carboxyl group and the azomethin nitrogen atom, have been detected using spectral methods and X-ray diffraction analysis of cinazepam which was carried out by Ya. Lipkovsky. These bonds are shown in Figure 5. On the basis of these spectral data, the X-ray diffraction results and data on the size of the β-CD cavity the proposed structure of the 2 : 2 cinazepam/β-CD inclusion compound is shown in Figure 6.

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