Clathrate-Type Complexation of Cephalosporins with β-Naphthol

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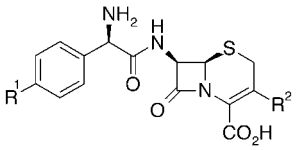
Abstract: The cephalosporin derivatives cephradine, cephalexin, cefaclor, and cefadroxil form complexes with β-naphthol, provided water is present. The crystal structures of these complexes have been determined by single-crystal X-ray diffraction. The complexes appear to be inclusion compounds of the clathrate type. In all cases, the cephalosporin molecules play the role of host, while β-naphthol is the guest molecule. Water molecules, which are accommodated in the crystal, play an essential role in the interaction between the guest and host molecules. Cephradine, cephalexin, and cefaclor form isomorphous complexes with β-naphthol, whereas cefadroxil crystallizes in a different morphology. The crystal structures are described in detail and discussed in terms of hydrogen-bonding, van der Waals and electrostatic interactions. The structure of the cefadroxil complex is basically different from that of the other three complexes, although there are notable structural similarities.

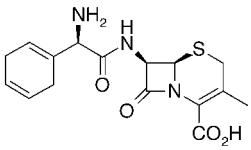
Keywords: cephalosporin · clathrates · hydrogen bonds · molecular recognition

Introduction

The cephalosporin derivatives cephalexin (**1**), cephradine (**2**), cefaclor (**3**), and cefadroxil (**4**) are important life-saving antibiotics, which have been in medical use for many years all over the world. Large-scale isolation and purification of the aforementioned cephalosporins from an aqueous solution is hampered by a number of problems. Owing to the β-lactam structure the molecules are very labile, especially under basic conditions. As a consequence the pH of the cephalosporin solution has to be monitored continuously during a reaction or purification procedure. It would, therefore, be highly convenient to isolate the cephalosporins by crystallization or co-crystallization with some additive, preferably immediately after their formation. It is known that selective cocrystallization can be achieved by adding β-naphthol to aqueous solutions of the cephalosporin derivatives **1**, **2**, **3**, and **4**.[1] These derivatives selectively form complexes with β-naphthol that precipitate from an aqueous solution. In the crystalline state, the cephalosporins are less susceptible to degradation. Moreover, complexation provides a method for the isolation of the product, since the precipitated complex can be separated from the reaction mixture by simple filtration. This facilitates down-stream processing in the large-scale production of these cephalosporins, which in turn indicates the industrial relevance of this method. After decomplexation, which can be achieved by acidification of an aqueous suspension followed by extraction with an organic solvent, the cephalosporins can be obtained in a pure state from the aqueous phase by neutralization and crystallization.



1 $R^1 = H$, $R^2 = CH_3$
3 $R^1 = H$, $R^2 = Cl$
4 $R^1 = OH$, $R^2 = CH_3$



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Despite the industrial relevance of efficient down-stream processing of the aforementioned cephalosporin derivatives, the molecular structures of their β-naphthol complexes have not been elucidated so far. Only the ratio of the cephalosporin and β-naphthol has been reported, as well as the water content.[1] Crystal structure analysis of complexes of β-naphthol with β-cyclodextrin[2] and with androsta-1,4-diene-3, 17-dione[3] revealed that in the former case β-naphthol is the guest molecule, whereas in the latter case no clear distinction can be made between host and guest molecules. Crystal-

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structure data of cephalosporins in either the pure or the complexed state are scarce. So far only the crystal structures of cefadroxil monohydrate and cefaclor dihydrate have been recorded.[4a, 4b, 5]

The aim of the present study is to elucidate the crystal structures of cephalosporin complexes with β-naphthol, in order to shed light on the nature of the interactions responsible for molecular recognition of the constituents in the solid state. An intriguing question is whether β-naphthol serves as the host or acts as a guest molecule. Single-crystal X-ray diffraction was used for this purpose.

Results and Discussion

Complexes of cephalosporins with β-naphthol are usually obtained as precipitates upon treatment of an aqueous solution of these antibiotics with β-naphthol. However, for the preparation of single crystals for the X-ray analyses methanol was used as a cosolvent. The presence of methanol enhances the solubility of β-naphthol and, moreover, allows cooling below 0 °C. From a 1% solution of cephalosporin in water or water/methanol no crystallization results, not even after cooling to 4 °C for 24 hours. However, after the addition of β-naphthol, crystallization starts instantaneously. β-Naphthol, which is only poorly soluble in water/methanol, undergoes a solid-to-solid transition from the pure state into the complexed state with cephalosporin. The crystal data of the respective cephalosporin/β-naphthol complexes are summarized in Table 1.

Remarkably, the crystal structures of the complexes of cephalexin, cephradine, and cefaclor with β-naphthol are isomorphous, as can be deduced from the powder diffraction patterns shown in Figure 1. Although the van der Waals radius of chlorine is comparable with that of a methyl group and partial saturation of a phenyl ring hardly results in structural changes, in general, such subtle molecular alterations can cause drastic structural deviations. The β-naphthol complexes of cephalosporins **1**, **2**, and **3** are of the clathrate type in which the cephalosporin serves as the host and β-naphthol as the guest molecule. In these clathrates, the host molecules form two-dimensional layers, which are held together by hydrogen-bonding and electrostatic interactions. The two-dimensional layers are packed in such a manner that a three-dimensional structure is formed. The remaining cavities are filled with β-naphthol and water molecules. In contrast, cefadroxil/β-naphthol complex is a clathrate with an entirely different three-dimensional structure that lacks the twofold symmetry

Table 1. Crystal data and data collection parameters for the β-naphthol complexes of cephalexin, cephradine, cefaclor and cefadroxil.

	Cephalexin/β-naphthol	Cephradine/β-naphthol	Cefaclor/β-naphthol	Cefadroxil/β-naphthol
crystal color	transparent orange-yellow	colorless transparent	light yellow-brown	pale yellow-brown
crystal shape	regular fragment	rather regular needle	regular rod	needle
crystal size [mm]	$0.38 \times 0.28 \times 0.20$	$0.60 \times 0.19 \times 0.05$	$0.28 \times 0.09 \times 0.06$	$0.31 \times 0.14 \times 0.13$
empirical formula	$C_{42}H_{55}N_6O_{14.50}S_2$	$C_{42}H_{56}N_6O_{14}S_2$	$C_{40}H_{47}Cl_2N_6O_{14.5}S_2$	$C_{42}H_{58}N_6O_{19}S_2$
M_w	940.04	933.05	978.86	1015.06
T [K]	293(2)	208(2)	293(2)	293(2)
crystal system	monoclinic	monoclinic	monoclinic	orthorhombic
space group	$C2$	$C2$	$C2$	$P2_12_12_1$
a [Å]	23.398(4)	23.4212(6)	23.447(3)	7.1117(2)
b [Å]	7.0623(18)	6.9715(2)	7.0262(8)	21.7170(8)
c [Å]	14.918(4)	15.0047(4)	14.8413(15)	30.9586(14)
α [°]	90	90	90	90
β [°]	109.80(3)	110.405(2)	110.550(10)	90
γ [°]	90	90	90	90
reflections	6	25	15	25
θ range [°]	22.329 – 40.260	40.234 – 46.787	17.322 – 22.038	19.907 – 26.398
V [Å^3]	2319.3(10)	2296.24(11)	2289.4(4)	4781.4(3)
Z	2	2	2	4
ρ_{calcd} [Mg m^{-3}]	1.346	1.349	1.420	1.410
absorption coefficient [mm^{-1}]	1.655	1.659	2.751	1.721
$F(000)$	994	988	1022	2144
θ range data collection[°]	4.02 – 69.91	3.14 – 70.00	3.18 – 70.01	2.85 – 69.30
index ranges	$-28 \le h \le 26$	$-26 \le h \le 28$	$-26 \le h \le 8$	$0 \le h \le 8$
	$-8 \le k \le 0$	$0 \le k \le 8$	$-8 \le k \le 0$	$0 \le k \le 26$
	$0 \le l \le 18$	$-18 \le l \le 0$	$-18 \le l \le 0$	$0 \le l \le 37$
reflections collected/unique	2486/2391	2475/ 2381	2452/ 2357	4870/ 4870
R(int)	0.0270	0.0322	0.0168	
observed reflections [$I_o > 2\sigma(I_o)$]	2130	2236	1675	3488
range of relative transmission factors	1.131/0.923	1.123/0.941	1.179/0.906	1.033/0.971
data/restraints/parameters	2391/249/346	2381/56/346	2391/222/345	4870/72/617
GOF on F^2	1.068	1.098	1.070	1.059
SHELXL-97 weight parameters	0.100400, 0.573100	0.072000, 1.253000	0.096200, 0.088500	0.103300, 7.821700
final R indices [$I > 2\sigma(I)$]	$R1 = 0.0488$	$R1 = 0.0413$	$R1 = 0.0568$	$R1 = 0.0740$
	$wR2 = 0.1372$	$wR2 = 0.1135$	$wR2 = 0.1507$	$wR2 = 0.1861$
R indices (all data)	$R1 = 0.0537$	$R1 = 0.0442$	$R1 = 0.0808$	$R1 = 0.1076$
	$wR2 = 0.1424$	$wR2 = 0.1162$	$wR2 = 0.1649$	$wR2 = 0.2112$
largest difference peak/hole [e Å^{-3}]	0.291/ – 0.255	0.330/ – 0.457	0.368/ – 0.232	0.642/ – 0.639

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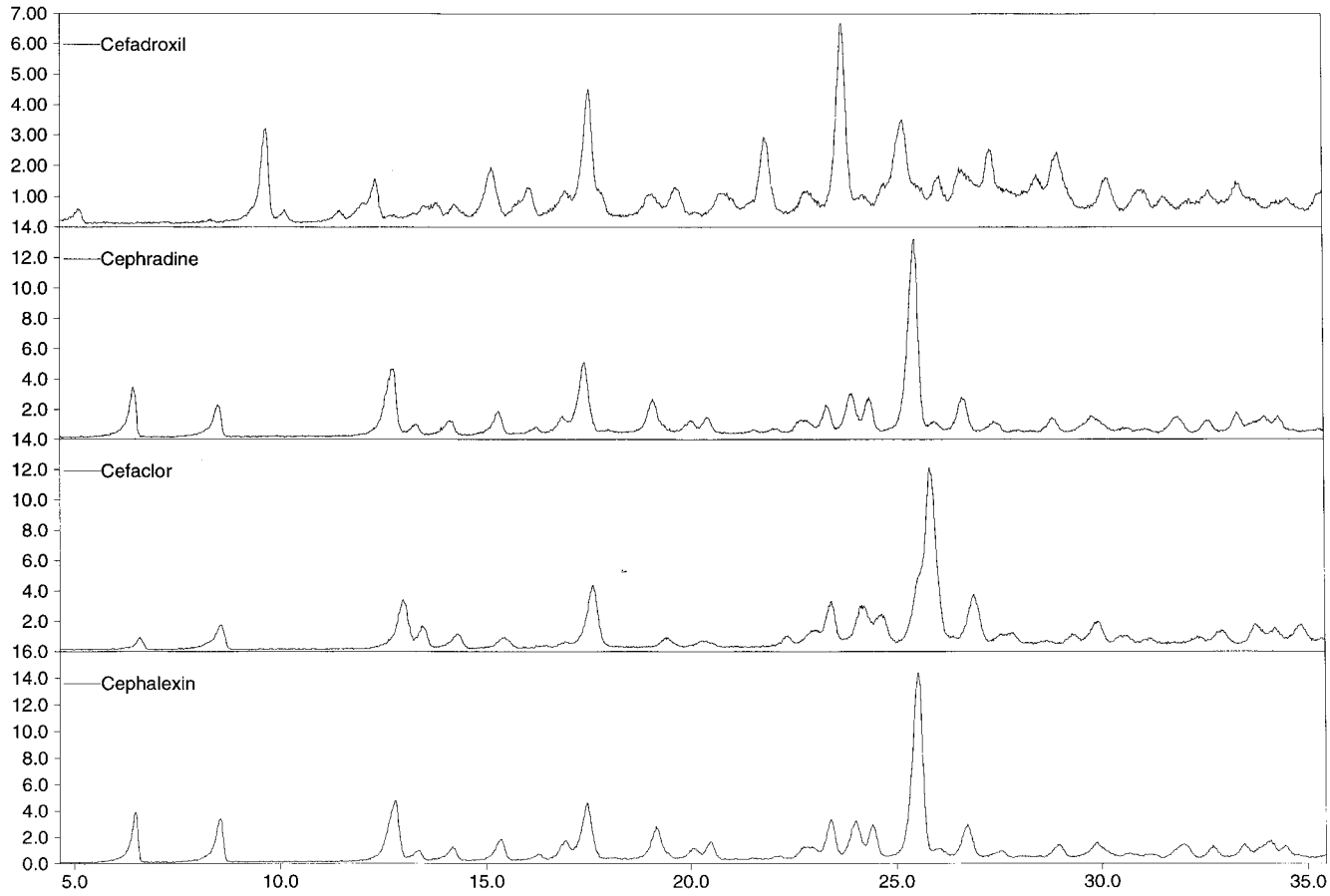


Figure 1. The powder diffraction patterns of the β-naphthol complexes of cephalexin, cephradine, cefaclor, and cefadroxil.

that is present in the complexes of **1**, **2**, and **3**. The large difference between the structure of the complex of cefadroxil and the complexes of cephalosporins **1**, **2**, and **3** is expressed in the powder diffraction patterns as is shown in Figure 1.

Figure 2 shows the crystal structure of the cephalexin/β-naphthol complex viewed in the direction of the *b* axis. The cephalosporin molecules are in the zwitterionic form with a negative charge on the carboxylate group and a positive charge on the ammonium group. Within the two-dimensional layers, the host molecules are assembled in a head-to-tail fashion. One carboxylate group has both hydrogen-bonding and electrostatic interactions with two ammonium groups and vice versa. This results in 4-point junctions at which the host molecules have strong noncovalent interactions with each other. In the crystal structures of the cephalexin, cephradine, and cefaclor complexes, the antibiotic molecules adopt an arrangement in which from a 4-point junction two molecules go up and two go down; this results in twofold symmetry along the *b* axis, as is visualized in Figure 3a. Building up these 4-point junctions leads to the formation of two-dimensional layers of cephalosporin molecules. In addition, this arrangement has the consequence that the two-dimensional layers contain holes as is shown in Figure 3b. This is the basis for the formation of channels when a three-dimensional cephalosporin framework is built up out of these two-dimensional layers.

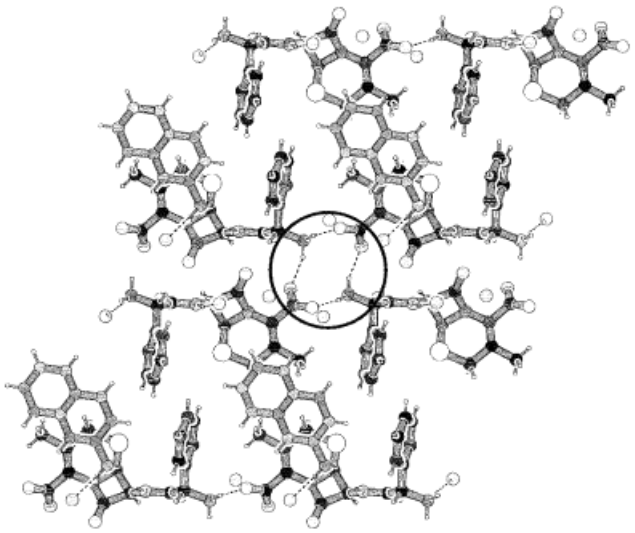
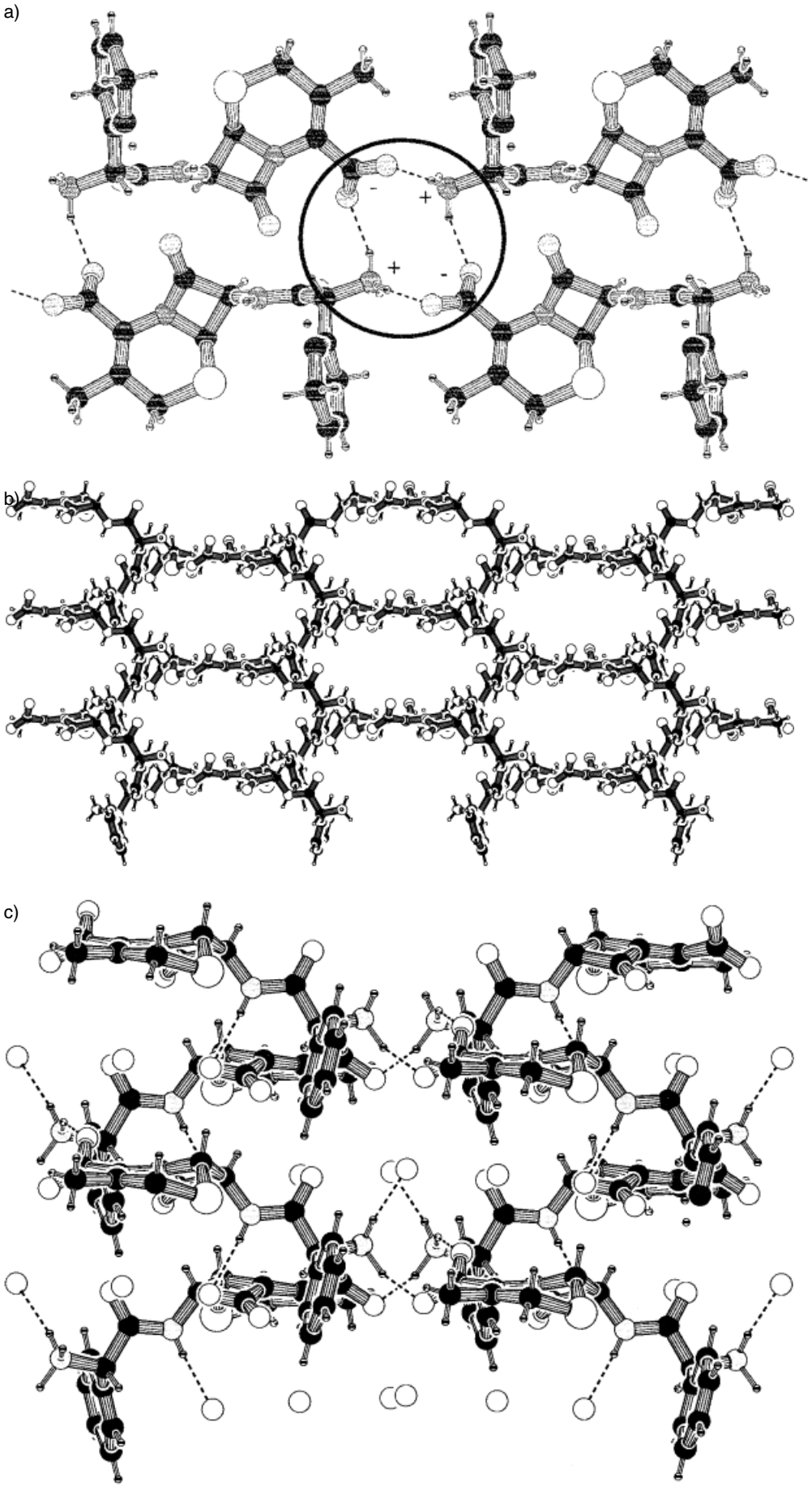


Figure 2. PLUTON[8] drawing of the structure of the cephalexin/β-naphthol complex viewed along the *b* axis.

The channels shown in Figure 3b, are filled with water and β-naphthol. The water molecules are positioned at the polar regions inside the channels, whereas β-naphthol is sandwiched between apolar parts of the antibiotic molecules. The water molecules are involved in multiple hydrogen bonding with both cephalosporin and other water molecules. The amino and amide hydrogen atoms point towards the oxygen atoms of the water molecules. In addition, the hydrogen – oxygen



distance is about 2 Å, which is the appropriate distance for effective hydrogen bonding (Figure 3c). Although the positions of the hydrogen atoms of the water molecules could not be determined by X-ray analysis, it is assumed that they also participate in the formation of the hydrogen bonding network. This assumption is justified as follows. The distance of the water molecules from the carbonyl and carboxylate groups is about 3 Å. Taking into account the length of the oxygen–hydrogen bond in water of 0.95 Å, it is reasonable to assume that a hydrogen bond is present. Except for the hydrogen-bonding and electrostatic interactions at the 4-point junctions, all other hydrogen-bonding interactions between host molecules involve water molecules. These interactions give the two-dimensional layers substantial additional strength. This suggests that the water molecules play the role of cement in the crystal. Recently it was shown that water incorporated in crystal structures often serves as a gluing agent.[6] The water molecules surround β-naphthol on two sides; this allows a hydrogen bond to be formed between the hydroxyl

Figure 3. a) A PLUTON[8] drawing of the 4-point junction formed by four cephradine molecules, each donating an ammonium or a carboxylate group. b) The two-dimensional layer viewed in the direction of the *c* axis. The holes that are present are a consequence of the arrangement of the cephalosporin molecules at a 4-point junction. c) The water molecules are positioned within the two-dimensional layers and form hydrogen bonds with the cephalosporin molecules. The hydrogen bonds between the hydrogens of water and acceptor atoms of the cephalosporins are not shown because the positions of the hydrogens could not be exactly determined.

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group of β-naphthol and a water molecule. This implies that the β-naphthol molecules are indirectly bound to the cephalosporin molecules through water.

The isomorphism found for the β-naphthol complexes of cephalexin, cephradine, and cefaclor, which was initially deduced from powder diffraction experiments, was confirmed by single-crystal X-ray diffraction, as is evident from the data in Table 1. Both the data in Table 1 and the powder diffraction patterns in Figure 1, clearly reveal that the crystal structure of the cefadroxil/β-naphthol complex, differs considerably from that of the other cephalosporin complexes. This difference must be attributed to the replacement of a hydrogen atom with a hydroxyl group on the phenyl ring. This replacement influences the molecular structure both sterically and electronically and has dramatic consequences for the recognition properties of the molecule.[7] This orthorhombic crystal structure contains cefadroxil, β-naphthol, and water in a ratio of 2:1:8. Thus, it contains three molecules of water per unit cell more than the cephalosporin complexes described above. The overall molecular geometry of the cefadroxil molecule in the β-naphthol complex is practically the same as its conformation in its uncomplexed monohydrate form[4a, 4b] and as the conformations of the other cephalosporin molecules in their complexes with β-naphthol. The cefadroxil/β-naphthol complex also forms a crystal structure of the clathrate type. As in the complexes of cephalexin, cephradine, and cefaclor with β-naphthol, cefadroxil is the host and β-naphthol is the guest. The β-naphthol molecule in this crystal structure is disordered, but could be refined in terms of two possible orientations. For the sake of clarity, only one orientation for β-naphthol is shown in Figure 4.

In the cefadroxil clathrate structure, 4-point junctions similar to those observed for cephalosporins **1**, **2**, and **3**, are present. However, the twofold symmetry is lacking in the case of cefadroxil. This deviating arrangement results in an essential difference between the cefadroxil complex and the complexes derived from the cephalosporins **1**, **2**, and **3**, namely, the dimensionality of the hydrogen-bonding network formed by the host molecules. While the cephalosporins **1**, **2**, and **3** form two-dimensional nets of hydrogen bonds, cefadroxil forms a three-dimensional network. Although the phenolic hydroxyl group is involved in hydrogen-bonding interactions, its function is not essential for the formation of the three-dimensional network. The three-dimensional network is constructed by interactions of only the ammonium and carboxylate groups of the cefadroxil molecules.

Conclusion

Cephalexin, cephradine, cefaclor, and cefadroxil form clathrates with β-naphthol in the presence of water. The essential feature of these clathrates is that the cephalosporins act as the host molecules and β-naphthol as the guest. The third constituent of the clathrates is water, which fulfils the role of cement in the crystal. The clathrates of cephalexin, cephradine, and cefaclor are isomorphous. Although these three cephalosporins have subtle structural differences, their complexation behavior with β-naphthol is essentially the same. The introduction of a hydroxyl group, as in cefadroxil, has a pronounced effect on the polarity and hydrophilicity of the molecule. In addition, it has a notable steric influence at that position of the molecule. As a consequence, the

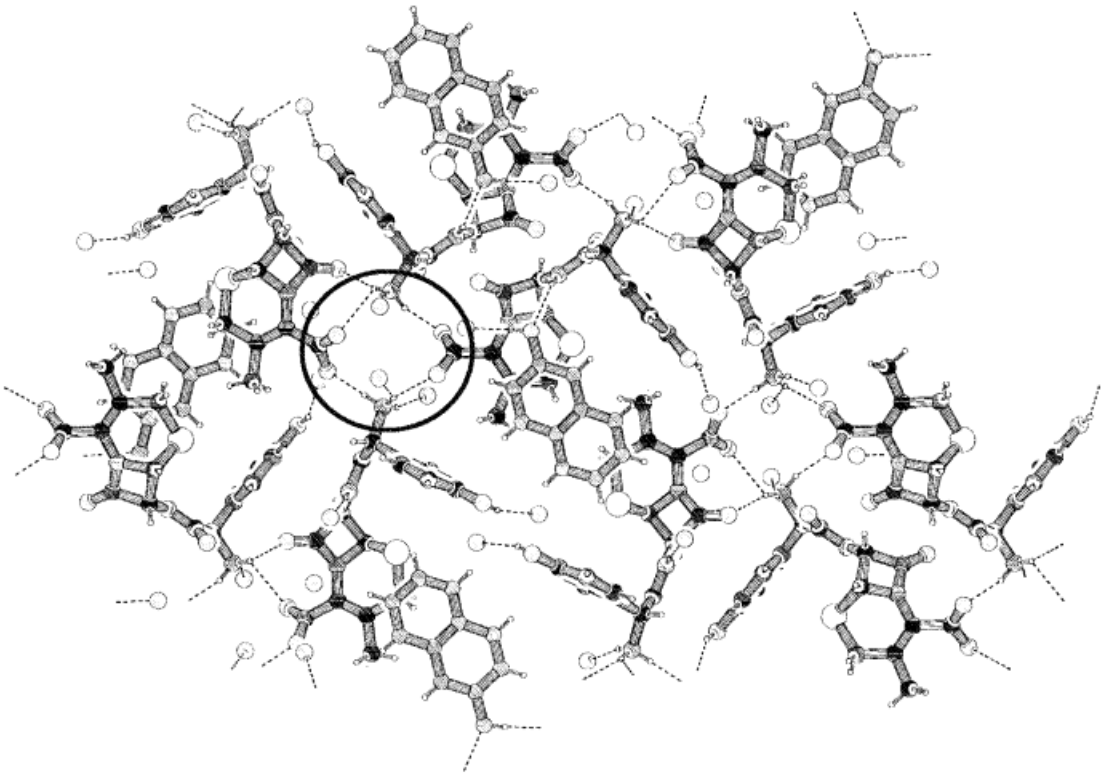

Figure 4. The crystal structure of the cefadroxil complex with β-naphthol. For sake of clarity only one orientation for β-naphthol is shown.

cefadroxil/β-naphthol complex is a clathrate with a different crystal structure. Remarkably, although the host framework formed by cefadroxil is very different, the remaining cavities are quite similar, because the same guest molecule can be accommodated. In both types of structures β-naphthol is hydrogen bonded to a water molecule. Apart from this hydrogen bond, β-naphthol has only van der Waals interactions with both cephalosporin and water molecules. The clathrate formation of cephalosporins with β-naphthol allows an effective withdrawal of cephalosporins from a diluted aqueous solution; this is highly relevant for industry.[1]

Experimental Section

The cephalosporins were obtained from DSM Life Sciences Group. β-Naphthol was purchased from ACROS.

Crystallization procedure: Cephalosporin monohydrate (500 mg) was dissolved in 20% aqueous methanol (approximately 50 mL). β-Naphthol (110 mg, 0.75 mmol) was dissolved in acetone (3 mL) and subsequently added to the cephalosporin solution. The solution was cooled at 4 °C overnight.

Crystal-structure solution of the β-naphthol complexes of 1, 2, 3, and 4: The crystals were measured on an ENRAF-Nonius CAD4 diffractometer. The radiation used was $Cu_{K\alpha}$ (graphite monochromated) with $\lambda = 1.54184$ Å. Intensity data were corrected for Lorentz and polarization effects. The structures were solved by the program system DIRDIF[10] by means of the program ORIENT and TRACOR[11] to orient and position a β-lactam fragment in the Patterson map and were refined anisotropically, by full-matrix least squares on F^2 (program SHELXL[12]). Crystal data and data collection parameters are given in Table 1.

Structure refinement for (cephalexin/β-naphthol), (cephradine/β-naphthol) and (cefaclor/β-naphthol): A crystallographic twofold axis passes through the center of the cavity in which the β-naphthol molecule is situated. As a consequence the β-naphthol molecule was disordered along this twofold axis. The two possible orientations of the β-naphthol molecule, which are related by twofold symmetry, were refined by use of a disorder model. The naphtalene skeletons belonging to the two possible orientations of the β-naphthol molecule did not overlap, but were shifted away from the twofold axis. The β-naphthol was (locally) surrounded by five water molecules: two at the side of the hydroxyl group, three at the other side of the cavity. The shift of the molecule with respect to the twofold axis may be the result of the replacement of a water molecule in the polar part of the cavity by the hydroxyl group of β-naphthol. The hydrogens attached to N18 were found in a difference Fourier map, but proved unstable during refinement. Therefore, these hydrogens and also the hydrogen atoms of the methyl group were refined as rigid rotors with idealized sp_3 hybridization and a C–H bond length of 0.97 Å to match maximum electron density in a difference fourier map. The hydrogens of the water molecules could not be localized and are therefore not included in the model. All other hydrogen atoms were placed at calculated positions and were refined riding on the parent atoms. The SQUEEZE procedure of the PLATON program[13] was used to correct for disordered solvent. For cephalexin/β-naphthol two voids at 0.500, 0.251, 0.000 and 0.000, 0.751, 0.000, with a volume of 33 and 34 Å^3, respectively, showed an electron count of 5 electrons indicating that in each void half a water molecule was present. For cefaclor/β-naphthol two voids at 0.500, 0.240, 0.000 and 0.000, 0.740, 0.000, with volumes of 29 Å^3, showed an electron count of 6 electrons indicating that in each void half a water molecule was present.

Structure refinement of cefadroxil/β-naphthol: The β-naphthol molecule showed severe disorder. Careful analysis showed that there were two possible ways in which the β-naphthol molecule could be positioned in the cavity. In both cases the hydroxyl group was bonded through a hydrogen bond to the same water molecule. The two possible orientations of the β-naphthol molecule are related by a 180° rotation of the naphthalene skeleton along the long axis of the molecule. The naphthalene skeletons belonging to the two possible orientations of the β-naphthol molecule do not overlap, but are slightly shifted with respect to each other, clearly, to optimize the fit in the cavity. The two possible orientations were refined by the use of a disorder model. The hydrogens attached to N18 were found in a difference Fourier map, but proved unstable during refinement. Therefore, these hydrogens and also the hydrogen atoms of the methyl group were refined as rigid rotors with idealized sp_3 hybridization and a C–H bond length of 0.97 Å to match maximum electron density in a difference Fourier map. The hydrogens of the water molecules could not be localized and are therefore not included in the model. All other hydrogen atoms were placed at calculated positions and were refined riding on the parent atoms. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC 109854, CCDC 109855, CCDC 109856, and CCDC 109857. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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