Inclusion Complexes with Podophyllotoxin, Structural Characterization and Chiral Recognition

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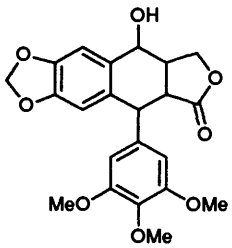
The biologically active compound podophyllotoxin can form host–guest complexes which contain two types of guests, *i.e.* water and a smaller organic molecule. Inclusion compounds which contain two podophyllotoxin and two water molecules per organic guest have been prepared with 15 different guests. They were found to crystallize in the orthorhombic space group $P2_12_12_1$ with unit-cell dimensions *a* 10.3–10.6 Å, *b* 17.3–17.8 Å and *c* 25.8–26.1 Å and one small organic molecule per asymmetric unit. The crystal structures have been determined from low-temperature X-ray diffraction data for the three complexes formed with bromobenzene, nitrobenzene, and racemic butan-2-ol, respectively. The crystal structures display a regular pattern of hydrogen bonds which connects the two crystallographically independent podophyllotoxin and water molecules. Water acts as a donor towards the oxo group from one podophyllotoxin and the hydroxy group from another podophyllotoxin molecule related by translational symmetry along the *a*-axis, and it accepts a hydrogen from the hydroxy group of a podophyllotoxin molecule which is crystallographically inequivalent. The arrangement leads to voids in the structure bound by the more hydrophobic part of six podophyllotoxin molecules (three of each type). These voids accommodate the smaller organic guests. Both the bromobenzene and the butan-2-ol complexes are disordered. Bromobenzene is found in a plane which contains the molecule in two orientations. The more populated (80%) has bromine in a volume, which corresponds to that containing the nitro group in the nitrobenzene complex. Partial resolution of butan-2-ol has been achieved in the inclusion complex. It contains 70% of the *R*-form and the less populated *S*-form (30%) is oriented in such a way that it shares the carbon chain with the *R*-form. The crystallographically independent podophyllotoxin molecules differ only in the relative orientation of their 3,4,5-trimethoxyphenyl groups.

Since early times podophyllin resins obtained by extraction from plants of the genus *Podophyllum* have been used in medicine as cathartics and cholagogues. However, during the period 1940–1955 they were removed from several pharmacopoeias for such purposes,[1] but almost at the same time novel interesting pharmacological properties of the podophyllins were observed, *i.e.*, their ability to cure venereal warts (condylomata acuminata)[2] and their cytostatic properties.[3] This led to an increasing scientific interest in the active compounds found in podophyllins, especially in the major component podophyllotoxin, shown in Fig. 1. Whereas solutions or ointments



Podophyllotoxin

containing podophyllotoxin as the active principle are now the choice drug against condylomata, no clinical use of podophyllotoxin or any of its congeners has emerged, due to toxic side-effects.[4] However, as the result of strong efforts to develop podophyllotoxin derivatives as drugs, two important new anticancer agents, VP 16-213 or etoposide and V26 or teniposide, already approved for use in several countries, have emerged.[5]

Podophyllotoxin and its congeners or semisynthetic derivatives appear to exert their cytostatic activity by at least two different mechanisms. In the first one, *e.g.* podophyllotoxin serves to arrest cell division in the metaphase, a process which is connected with inhibition of microtubule assembly.[6] In the second one, *e.g.* VP 16-213 effects cell division in the late S or G_2 phase of the cell cycle. This is connected with DNA cleavage.[7,8] Furthermore, both podophyllotoxin and many of its derivatives, including the otherwise biologically inactive picropodophyllotoxin, act competitively in various other ways in cells, *e.g.* by inhibiting nucleoside transport.[9] These actions require higher concentrations than those required for arresting mitosis.

The use of podophyllotoxin in the clinic[2] as well as its use as a starting material in the synthesis of etoposide and teniposide and their clinical use, *etc.*, have led to increased research activity both around the chemistry and the biology of such compounds, and we have recently examined the thermal stability of podophyllotoxin,[10] and synthesized a series of new derivatives which were subjected to biological testing.[11]

The crystallization of podophyllotoxin is by no means trivial. Earlier it had been reported that podophyllotoxin could be obtained in several crystal forms. The postulated modifications were examined by X-ray diffraction methods, which unambiguously showed that pure podophyllotoxin crystallized in one orthorhombic form with m.p. 182–183 °C and not in two as postulated earlier.[12] Podophyllotoxin can also be isolated as a trigonal hydrate with m.p. 162–164 °C and as an orthorhombic solvate of water and benzene with m.p. 114–118 °C (foaming). Chemical analysis of the latter modification showed that it contained two podophyllotoxin and two water molecules per benzene molecule. These results indicate that podophyllotoxin,

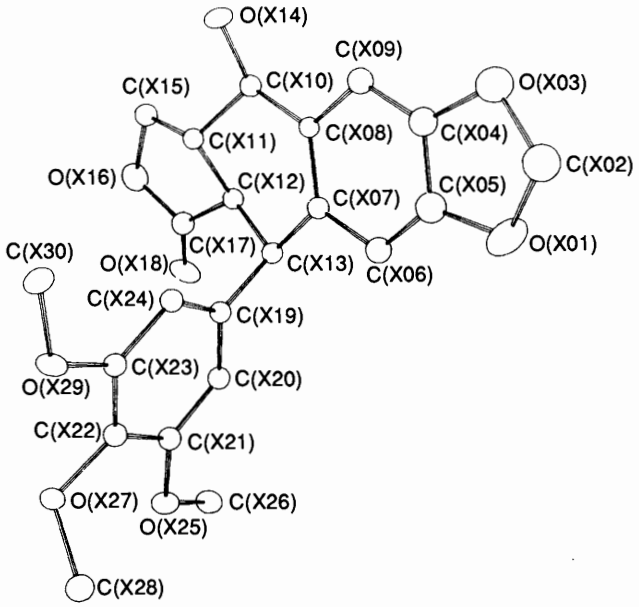


Fig. 1. An ORTEP drawing illustrating the numbering of podophyllotoxin. The two crystallographically independent molecules correspond to X = 1 and 2.

besides its interesting biological properties, also has the ability to act as a host for both water and organic guest molecules.

Inclusion compounds or guest–host complexes have attracted considerable attention recently.[13] The simplest example of lattice inclusion complexes are solvates. Apparently, podophyllotoxin can act as a host for two different types of guest, *i.e.* hydrophilic water and hydrophobic organic molecules. The drawing in Fig. 1 shows that podophyllotoxin has a hydrophilic part which contains the hydroxy and the oxo groups. The remaining part of the molecule has a more hydrophobic character. Pure podophyllotoxin crystallizes without included water, so it is likely that the ability of podophyllotoxin to form inclusion complexes is connected with the presence of water. Apparently, hydrogen bonds between one of the guest molecules (water) and podophyllotoxin are essential for the formation of a molecular arrangement which can accommodate larger and more hydrophobic guest molecules.

It appears that it is both the topological features and the presence of functional groups in podophyllotoxin that are important for the formation of the inclusion complexes. In this aspect it displays similarities with the co-ordinate clathrate host 1,1′-binaphthyl-2,2′-carboxylic acid extensively studied by Weber *et al.*,[14] but the functional groups in podophyllotoxin do not provide the possibility of forming similar hydrogen-bond arrangements. In addition, the host lattice formed by the chiral podophyllotoxin could be expected to provide chiral recognition of enantiomeric guest molecules.

This paper describes the structural characterization of the inclusion complexes formed by podophyllotoxin and their ability to perform chiral recognition based on the crystal-structure determinations for three compounds with bromobenzene, nitrobenzene, and butan-2-ol as guest molecules in the podophyllotoxin–water lattice.

Experimental

Materials.—Podophyllotoxin {>98%, HPLC; m.p. 181–182 °C; $[\alpha]_D^{20}$ −132.0° (*c* 1, $CHCl_3$)} was provided by pHarma-medica A/S, Vesterlundsvej 19, Herlev, Denmark. All other chemicals for syntheses were of best commercial grade. Liquids were distilled before use.

Preparations. The compounds listed in Table 1 were prepared by methods 1–4 described below. CHN-analyses were performed by the microanalytical laboratory at the H. C. Ørsted Institute.

Formation of Complexes.—Method 1. Podophyllotoxin (1.0 g) and the guest compound (1 cm^3) were dissolved in 70% ethanol (7.0 cm^3) and the solutions were heated to 60–70 °C. Subsequently the solutions were left at room temperature for 0.5–5 h, during which time the inclusion compounds crystallized out, except that of nitrobenzene, but by addition of more water and scratching on the side of the reaction vessel with a glass spatula this compound also crystallized out. The complexes were collected by suction and washed several times with ice-cold 70% ethanol. They were recrystallized from 70% ethanol and finally dried *in vacuo* over Siccapent for 2 days.

Method 2. The inclusion compounds were prepared as described in Method 1, but after being washed with ice-cold 70% ethanol they were washed with diethyl ether and dried in air for 2 days.

Method 3. Essentially by using the same procedure as in Method 1, compound **7** was precipitated from ethanol (40 cm^3). Similarly compounds **5**, **8**, **9**, **10** and **12** were precipitated from 80% ethanol (5.6 cm^3) and washed with ice-cold 80% ethanol. Finally they were all washed with ice-cold 96% ethanol and dried over Siccapent for 2 days.

Method 4. Compound **12** was prepared from podophyllotoxin (0.82 g, 2.00 mmol) and phenol (0.100 g, 1.06 mmol) dissolved in a mixture of ethanol (50 cm^3) and water (1.2 cm^3) at 60–70 °C. After cooling, the crystals were collected by suction, washed with ice-cold 96% ethanol and dried at 50 °C for 1 h. Complexes **13** and **15** were prepared as described above but from benzyl alcohol (0.113 g, 1.06 mmol) and butan-2-ol (0.1572 g, 2.12 mmol) instead of phenol. To obtain crystallization of the latter complex **15** it was necessary to evaporate some of the solvent and add a small amount of water. The crystals were isolated and washed and dried as above.

X-Ray Crystallography.—The inclusion compounds which gave the best quality of crystals suitable for diffraction work were characterized by X-ray crystallographic methods. All complexes crystallized in the orthorhombic space group $P2_12_12_1$. Table 1 lists their unit-cell dimensions at room temperature as determined from Weissenberg photographs. The crystal structures of the complexes with bromobenzene, nitrobenzene, and butan-2-ol were determined using low-temperature X-ray diffraction data collected with a CAD-4 diffractometer. For the three data collections, intensities of three control reflections were measured after every 10 000 s, and the orientation of the crystals was checked after every 300 reflections. Table 2 lists the crystal data and summarizes results from data reduction and structure refinements. Further details are given in the following sections.

Podophyllotoxin–Bromobenzene–Water Complex.—The unit-cell dimensions were determined from the setting angles of 22 reflections with 15.9° < θ < 19.9°. Analysis of the intensity control reflections showed a decrease with exposure time, *t*, to a total of 9%. The intensities of the standard reflections were analysed and the following expression were derived to correct for the degradation $I = I(t)\exp(1.48 \times 10^{-1}\, t^2 + 3.22 \cdot 10^{-5}\, t)$. The data were corrected for Lorentz, polarization, and absorption effects and averaged according to the crystal-class symmetry. The structure was solved using the heavy-atom method. The position of the bromine atom was derived from the Patterson function. Subsequent Fourier analyses gave the

Table 1. Inclusion compounds formed with podophyllotoxin. The numbers given for the guests molecules are calculated from the chemical analysis relative to one molecule of podophyllotoxin.

Guest 1	Composition		Molar proportions	Cell dimensions (room temperature)		
	Guest 1	Guest 2 (water)	host:guest 1:guest 2	*a*/Å	*b*/Å	*c*/Å
1 Toluene [1]	0.466	1.14	2:1:2	10.43	17.67	26.09
2 *o*-Xylene [1]	0.497	1.17	2:1:2			
3 *m*-Xylene [1]	0.479	1.25	2:1:2			
4 *p*-Xylene [1]	0.494	1.12	2:1:2			
5 Chlorobenzene [3]	0.487	0.869	2:1:2			
6 Bromobenzene [2]	0.471	1.23	2:1:2	10.50	17.63	25.73
7 Nitrobenzene [2,3]	0.452, 0.462	1.16, 0.85	2:1:2	10.60	17.81	25.81
8 Aniline [3]	0.529	0.813	2:1:2			
9 *N*,*N*-Dimethylaniline [1,3]	0.449, 0.529	1.046, 0.823	2:1:2			
10 Pyridine [3]	0.454	0.950	2:1:2			
11 Quinoline [1]	0.465	1.27	2:1:2			
12 Phenol [4]	0.584	1.76	2:1:4	10.43	17.62	26.01
13 Benzyl alcohol [4]	0.574	1.35	2:1:3	10.34	17.46	26.05
14 Anisole [1]	0.501	1.16	2:1:2			
15 Butan-2-ol [4]	0.480	1.53	2:1:3	10.50	17.29	25.95

1, 2, 3 and 4 refer to the different modes in the preparation of the inclusion compounds listed in the Experimental section.

Table 2. Crystal data and a summary of refinement results for the podophyllotoxin inclusion complexes.

	Organic guest		
	Bromobenzene	Nitrobenzene	Butan-2-ol
Formula	$C_{50}H_{61}BrO_{18}$	$C_{50}H_{61}NO_{20}$	$C_{48}H_{66}O_{19}$
Formula weight/g mol^{-1}	1029.94	965.80	947.06
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Crystal size/mm	0.23 × 0.31 × 0.45	0.20 × 0.40 × 0.45	0.07 × 0.13 × 0.33
T/°C	−163	−163	−163
a/Å	10.442(5)	10.459(2)	10.24(2)
b/Å	17.549(6)	17.668(3)	17.294(8)
c/Å	25.384(10)	25.248(7)	25.80(1)
V/Å^3	4652(5)	4666(3)	4566(13)
Z	4	4	4
λ/Å	0.710 73	0.710 73	1.541 78
μ/cm^{-1}	11.07	1.012	8.453
Scan type	ω	ω	ω − 2θ
Scan width Δω/°	1.0 × 0.35 . tan θ	1.0 + 0.35 . tan θ	0.8 + 0.15 . tan θ
Octants measured	*h*, *k* ±*l*	*h*, *k* ±*l*	*h*, *k* ±*l*
Max scan time/s	120	120	180
Number of observations	11 191	5726	5264
Number of contributing reflections	7200	4274	3534
Weights (w^{-1})	$\sigma_{cs}^2(F) + 0.0006\|F\|^2$	$\sigma_{cs}^2(F) + 0.0004\|F\|^2$	$\sigma_{cs}^2(F) + 0.0009\|F\|$
Variables	655	640	608
R	0.046	0.041	0.062
R_w	0.053	0.044	0.084

positions of the other non-hydrogen atoms in the structure. The least-squares method minimizing $\Sigma w(|F_o| - |F_c|)^2$ was used for the refinement. A refinement with anisotropic thermal parameters for Br and O led to physically unrealistic thermal parameters for some of the atoms in the bromobenzene molecule. The difference Fourier map showed considerable residual density close to the bromobenzene molecule, which could be interpreted as a partly populated bromobenzene molecule oriented with its bromine atom close to the 4-carbon atom. The population parameters of the two bromobenzene molecules were included in the refinements. The two partly populated bromobenzene molecules are oriented in such a way that only C(1), C(3) and C(5) do not coincide with atoms from the other molecule, as shown in Fig. 2. The population of the molecules was determined from the average of the parameters for C(1), C(3) and C(5) which gave 0.80(1) for the more populated and 0.20(2) for the less populated molecule, and these values were used in the final refinements. Anisotropic thermal parameters were used for all the non-hydrogen atoms in the structure except for the carbon atoms in the less populated bromobenzene molecule.

A difference Fourier map showed the positions of the hydrogen atoms. They were introduced in idealized positions with a common thermal parameter, $U = 0.015$ Å^2. The absolute configuration of the molecules were determined as described by Rogers.[15] The X-RAY system[16] was used for the crystallographic calculations using the scattering factors from Cromer and Wabers[17] except for hydrogen, where the values by Stewart *et al.*[18] were employed. The scattering factors for bromine and oxygen were corrected for the effect of anomalous scattering. The maximum shift in the last cycle was 0.8 σ and the maximum peak in difference density was 0.7 e Å^{-3}.

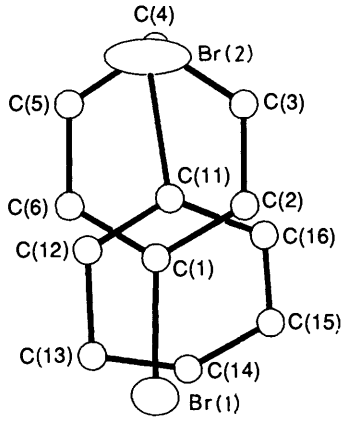


Fig. 2. An illustration of the relative orientation of the two partly populated bromobenzene guest molecules; atoms labelled Br(1)–C(1) · · · C(6) correspond to the more (0.80) and Br(2)–C(11) · · · C(16) to the less (0.20) populated.

Podophyllotoxin–Nitrobenzene–Water Complex.—22 Reflections with $18.1° < \theta < 19.9°$ were used in the determination of unit-cell parameters. The data were corrected for Lorentz and polarization effects. An analysis of the standard reflections showed a decay of the crystal up to 10%. The data were corrected for the degradation by the function $I = I(t)\exp(2.91 \times 10^{-7} t^2 + 2.53 \times 10^{-5} t)$ and symmetry reflections including Friedel pairs were averaged. The structure was solved by direct methods. A standard run using the program system MULTAN[19] provided positional parameters for all the non-hydrogen atoms in one podophyllotoxin molecule and a large fraction (25 atoms) of the other molecule. The structure refinement was analogous to that described previously for the bromobenzene complex. The chirality of the molecules were made identical with that of podophyllotoxin in the bromobenzene complex. The maximum shift in the last cycle was 0.03 σ and the largest peak in the difference density was 0.4 e Å^{-3}.

Podophyllotoxin–Butan-2-ol–Water Complex.—To compensate for the poor diffraction power of this compound Cu-Kα radiation was used for the data collection. Reflections with $19.3° < \theta < 40.2°$ were used for the determination of unit-cell parameters. The data were corrected for Lorentz and polarization effects, and symmetry-related reflections including Friedel pairs were averaged. Starting positions for the podophyllotoxin and the water molecules were taken from the refined structure of the podophyllotoxin–nitrobenzene complex. The co-ordinates for the guest molecules were found in a difference Fourier map.

The large thermal vibrations of the guest molecule made it difficult to distinguish between the methyl and the hydroxy group in butan-2-ol. However, an examination of interatomic distances showed that one of the atoms, which had the shortest bond distance, also had a short distance of 3.01 Å to the ether oxygen O(201), which is in accord with the expected dimension of the weak hydrogen bond between an ether oxygen and a hydroxy group, observed in the crystal structure of pure podophyllotoxin.[20] Placing a methyl group in this position would lead to energetically unfavourable contact distances. In addition a refinement with the hydroxy and the methyl groups interchanged resulted in significantly higher *R*-value. Based on those arguments, the peak with the short distance to O(201) was assigned as an oxygen atom.

A difference Fourier showed the positions of the hydrogen atoms of the podophyllotoxin molecules. They were introduced in idealized positions with a common thermal parameter $B = 3.0$ Å^2.

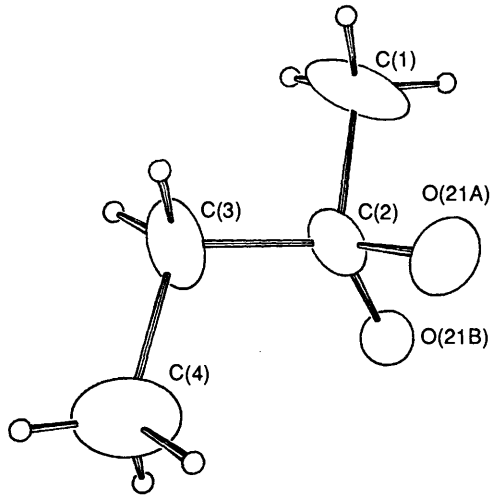


Fig. 3. The guest molecule butan-2-ol, O(21A), corresponds to the more (0.70) populated *R*-isomer and O(21B) to the less (0.30) populated *S*-isomer.

This refinement resulted in large displacement parameters for the hydroxy group of the guest molecule. Furthermore, the difference Fourier map displayed one peak of 0.9 e Å^{-3} which was significantly over the noise level (±0.5 e Å^{-3}). Both the distance from the peak to C(2) of 1.42 Å as well as the stereochemistry around C(2) were consistent with the interpretation that this peak represents the hydroxy group of the other enantiomer of butan-2-ol, oriented such that the enantiomers have a common C–C–C–C backbone as shown in Fig. 3. In the subsequent refinement cycles this atom, O(21B), was introduced, and the population parameters of the oxygen corresponding to the hydroxy groups of the two enantiomers were included in the refinements. The population parameters were scaled to give the sum 1.0 and were not included in the final cycles. The maximum shift in the final cycle is 0.07 σ and the largest peak in the difference Fourier map is 0.4 e Å^{-3}. The SDP-system[21] supplied by ENRAF-Nonius was used for the crystallographic computations; the atomic scattering factors were from the same sources as above.

Similar care was taken in the three crystal-structure determinations, but the quality of the crystals varied so much that the accuracy of the structure determinations differ significantly. Table 3 lists the positional parameters for the most accurate structure determination of the nitrobenzene complex. The positional parameters of the more populated guest molecules in the two other structures, bromobenzene and butan-2-ol, are presented in Table 4. Full lists of positional parameters have been deposited.

Table 3. Positional parameters for the podophyllotoxin–water–nitrobenzene complex.

Atom	*x*	*y*	*z*
O(101)	0.610 2(2)	0.327 89(13)	0.172 46(9)
C(102)	0.736 2(3)	0.358 5(2)	0.179 68(14)
O(103)	0.769 2(2)	0.349 11(14)	0.234 61(9)
C(104)	0.651 9(3)	0.343 0(2)	0.259 62(12)
C(105)	0.557 5(4)	0.329 7(2)	0.222 64(12)
C(106)	0.432 9(3)	0.319 2(2)	0.236 96(12)
C(107)	0.402 0(3)	0.322 5(2)	0.291 37(12)
C(108)	0.498 3(3)	0.334 2(2)	0.328 60(12)
C(109)	0.626 3(3)	0.345 1(2)	0.312 64(13)
C(110)	0.471 8(3)	0.339 3(2)	0.388 54(12)
C(111)	0.338 6(3)	0.311 1(2)	0.400 25(12)
C(112)	0.245 2(3)	0.346 4(2)	0.361 37(12)
C(113)	0.260 7(3)	0.313 3(2)	0.305 79(12)

Table 3 (*continued*)

Atom	*x*	*y*	*z*
O(114)	0.567 2(3)	0.303 32(13)	0.419 90(9)
C(115)	0.280 1(3)	0.332 7(2)	0.453 60(13)
O(116)	0.142 0(2)	0.340 27(13)	0.443 10(8)
C(117)	0.120 2(3)	0.347 4(2)	0.390 49(13)
O(118)	0.012 8(2)	0.355 14(13)	0.373 90(9)
C(119)	0.213 2(3)	0.232 0(2)	0.300 51(12)
C(120)	0.081 6(3)	0.218 6(2)	0.300 27(12)
C(121)	0.035 8(3)	0.144 7(2)	0.296 64(12)
C(122)	0.120 0(3)	0.084 3(2)	0.289 73(12)
C(123)	0.251 1(3)	0.097 7(2)	0.289 61(12)
C(124)	0.298 6(3)	0.171 3(2)	0.296 07(12)
O(125)	−0.091 0(2)	0.125 41(13)	0.300 12(9)
C(126)	−0.179 6(3)	0.186 6(2)	0.308 13(13)
O(127)	0.073 0(2)	0.011 66(12)	0.284 92(9)
C(128)	0.050 3(4)	0.009 8(2)	0.231 08(14)
O(129)	0.326 1(2)	0.035 14(13)	0.283 63(9)
C(130)	0.462 0(3)	0.046 3(2)	0.279 36(14)
O(201)	0.256 3(3)	−0.272 61(14)	0.456 35(10)
C(202)	0.387 9(4)	−0.278 1(2)	0.440 3(2)
O(203)	0.446 7(2)	−0.207 07(13)	0.452 80(10)
C(204)	0.345 7(3)	−0.156 3(2)	0.450 10(13)
C(205)	0.231 7(4)	−0.195 8(2)	0.453 06(13)
C(206)	0.116 7(3)	−0.160 2(2)	0.453 40(13)
C(207)	0.116 9(3)	−0.080 5(2)	0.449 10(12)
C(208)	0.232 1(3)	−0.040 3(2)	0.444 81(12)
C(209)	0.349 7(3)	−0.079 5(2)	0.445 71(13)
C(210)	0.236 3(3)	0.045 9(2)	0.435 90(12)
C(211)	0.111 9(3)	0.080 5(2)	0.453 82(12)
C(212)	0.003 2(3)	0.036 8(2)	0.428 45(12)
C(213)	−0.011 6(3)	−0.041 2(2)	0.453 55(12)
O(214)	0.346 0(2)	0.080 25(13)	0.459 89(9)
C(215)	0.083 0(3)	0.161 3(2)	0.438 03(13)
O(216)	−0.057 7(2)	0.163 84(13)	0.433 71(9)
C(217)	−0.105 3(4)	0.092 9(2)	0.429 52(12)
O(218)	−0.219 0(2)	0.081 24(13)	0.425 93(9)
C(219)	−0.057 5(3)	−0.039 3(2)	0.511 08(12)
C(220)	−0.187 5(3)	−0.035 8(2)	0.521 98(13)
C(221)	−0.230 3(3)	−0.033 6(2)	0.574 39(13)
C(222)	−0.143 3(3)	−0.038 2(2)	0.616 00(12)
C(223)	−0.013 1(3)	−0.044 3(2)	0.605 31(13)
C(224)	0.029 4(3)	−0.043 3(2)	0.553 02(12)
O(225)	−0.355 6(2)	−0.026 95(14)	0.589 20(8)
C(226)	−0.450 1(4)	−0.031 3(2)	0.548 6(2)
O(227)	−0.184 6(2)	−0.033 63(14)	0.667 98(9)
C(228)	−0.235 0(4)	−0.103 3(2)	0.687 5(2)
O(229)	0.063 9(2)	−0.050 6(2)	0.648 56(9)
C(230)	0.196 9(4)	−0.065 5(3)	0.639 1(2)
Organic guest			
N(1)	0.052 8(4)	0.234 2(2)	0.143 2(2)
O(3)	0.075 7(3)	0.183 4(2)	0.111 7(2)
O(4)	−0.034 0(3)	0.232 8(2)	0.175 53(13)
C(1)	0.133 6(4)	0.302 3(2)	0.141 2(2)
C(2)	0.236 5(4)	0.303 7(2)	0.106 7(2)
C(3)	0.311 5(4)	0.368 9(3)	0.105 4(2)
C(4)	0.284 6(4)	0.429 4(2)	0.137 6(2)
C(5)	0.180 1(4)	0.427 3(2)	0.171 1(2)
C(6)	0.101 7(4)	0.362 2(2)	0.173 35(14)
Water molecules			
O(1)	0.544 5(2)	0.151 21(13)	0.404 12(9)
O(2)	0.293 3(2)	0.114 0(2)	0.561 93(10)

Results and Discussion

Elemental analyses showed that most of the inclusion complexes have identical compositions, *i.e.* the proportions organic guest–water–podophyllotoxin being 1:2:2. The complexes which deviate from this pattern all contain alcohol as the organic guest molecule. However, X-ray diffraction photographs of these compounds showed that they are isostructural with the other complexes, which indicates that they either have water bound to the surface or may contain water, which is hydrogen bonded to the organic guest molecule. A guest–host complex has also been found with 2-bromopodophyllotoxin as a host.[22] This compound has one guest molecule, ethyl acetate, and two 2-bromopodophyllotoxin molecules per asymmetric unit. In contrast with the complexes formed with podophyllotoxin this compound does not contain any water of hydration; instead hydrogen bonds between the hydroxy and oxo groups connect the molecules in the crystal.

Table 4. Positional parameters[a] for the guest molecules in bromobenzene and butan-2-ol complexes.

	PP[b]	*x*	*y*	*z*
Bromobenzene				
Br(1)	0.80	0.030 41(6)	0.202 09(3)	0.140 59(3)
C(1)	0.80	0.130 5(5)	0.291 2(3)	0.138 7(2)
C(2)	0.80	0.233 1(6)	0.297 1(4)	0.103 6(3)
C(3)	0.80	0.304 4(5)	0.362 4(3)	0.101 9(2)
C(4)	0.80	0.279(2)	0.424 6(11)	0.134 5(6)
C(5)	0.80	0.173 8(5)	0.417 6(3)	0.169 5(2)
C(6)	0.80	0.101 9(5)	0.352 7(4)	0.171 0(2)
Br(2)	0.20	0.270 9(8)	0.423 6(6)	0.141 4(4)
C(11)	0.20	0.177(2)	0.322 9(13)	0.132 0(9)
C(12)	0.20	0.094(2)	0.314(2)	0.172 6(11)
C(13)	0.20	0.037(2)	0.245 9(12)	0.168 7(8)
C(14)	0.20	0.069(3)	0.191(2)	0.118 6(11)
C(15)	0.20	0.145(2)	0.216 9(10)	0.085 8(7)
C(16)	0.20	0.206(2)	0.282 2(13)	0.089 9(9)
Butan-2-ol				
C(1)	1.0	0.062(1)	0.213 8(5)	0.129 8(7)
C(2)	1.0	0.169(1)	0.277 4(5)	0.119 3(4)
C(3)	1.0	0.125(1)	0.348 1(5)	0.151 5(3)
C(4)	1.0	0.237(1)	0.415 0(7)	0.149 8(5)
O(21A)	0.70	0.171(1)	0.287 4(9)	0.068 1(4)
O(21B)	0.30	0.214(2)	0.753(1)	0.662 6(7)

[a] The positional parameters given correspond to the appropriate cell dimensions listed in Table 2. [b] Population parameter.

Packing in the Crystals.—Hydrogen bonding plays an important role for the packing in the podophyllotoxin inclusion complexes, and all the possible donor atoms are hydrogen bonded. These bonds are depicted as thin lines on the stereo pairs in Figs. 4–6 which illustrate the packing arrangements in the crystals. The geometry of the hydrogen bonds in the three structures is presented in Table 5.

The pattern of hydrogen bonds which involve the two crystallographically independent podophyllotoxin molecules A and B and the water molecules displays remarkable symmetry. Each of the water molecules acts as a donor towards a hydroxy and an oxo group, respectively, from two podophyllotoxin molecules related by translational symmetry along the *a*-axis, e.g. water molecule O(1) connects the podophyllotoxin molecules B (X = 2) and water O(2) connects molecules of the type A (X = 1). The hydroxy groups in podophyllotoxin act as donors towards the water molecules in such a way that the water molecules O(1), which donate protons to molecules of type B as described previously, accept protons from podophyllotoxin molecule of type A. Analogously molecules O(2) act as acceptors of protons from type-B molecules.

This system of hydrogen bonds between hydroxy groups and water molecules forms chains of crystallographically independent molecules, and molecules of the same type related by the

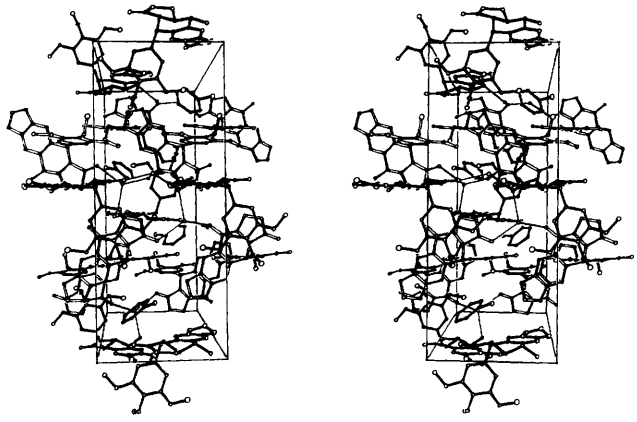


Fig. 4. A stereo pair illustrating the packing in the bromobenzene–water–podophyllotoxin complex viewed along the crystallographic *b*-axis. Bromobenzene is shown in the more populated orientation. Hydrogen bonds are indicated as thin lines. To differentiate between the two crystallographically independent molecules, type A molecules (X = 1) have open and those of type B (X = 2) full bonds.

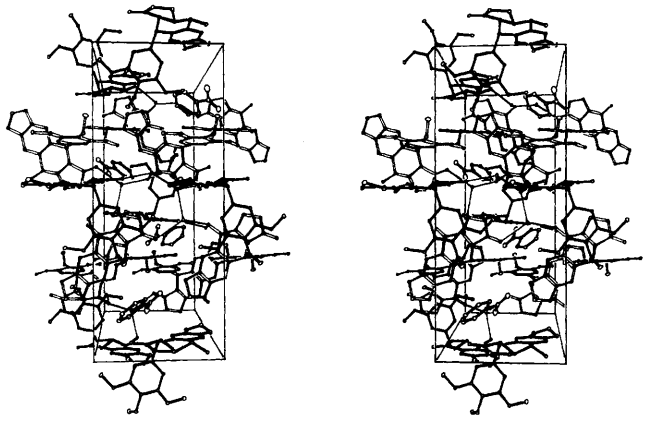


Fig. 5. The packing in the nitrobenzene–water–podophyllotoxin complex drawn as in Fig. 4.

twofold screw axis parallel to the *a*-axis. A schematic illustration of the hydrogen-bond system is given in Fig. 7. This extensive hydrogen-bond system gives rise to significant intermolecular interactions in the direction of the *a*-axis, which are consistent with the observation that the crystals are elongated in the direction of the *a*-axis. The relative orientation of the podophyllotoxin molecules in the crystal is another interesting feature of the packing. The podophyllotoxin molecule consists of a system of four fused rings with the 3-, 4-, 5-trimethoxyphenyl group almost perpendicular to the ring system. In the crystal the molecule are oriented in a way which makes the phenyl group from molecule A almost parallel to the fused ring system of molecule B. Similarly, phenyl groups from molecule B are almost coplanar with the ring system of molecule A. The molecules are packed in the crystal such that the planar phenyl group of molecule A is almost perpendicular (86°) to the *c*-axis, and the phenyl group of molecule B is virtually parallel (1°) to the *a*–*c* plane. As is apparent also from an inspection of Figs. 4–6, the packing pattern in the crystals displays significant regularities.

Only a fraction of podophyllotoxin is involved in the hydrogen-bond system, and it is the orientation of the remaining and more hydrophobic part of the molecules which is important for the formation of guest complexes.

Packing and Geometry of the Guest Molecules.—The crystal-

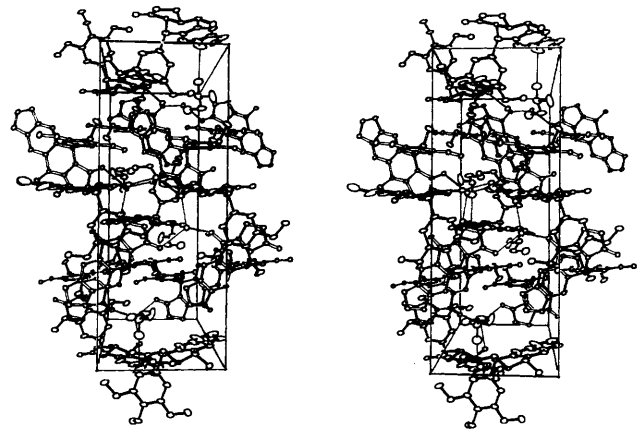


Fig. 6. The packing in the butan-2-ol–water–podophyllotoxin complex drawn as in Fig. 4.

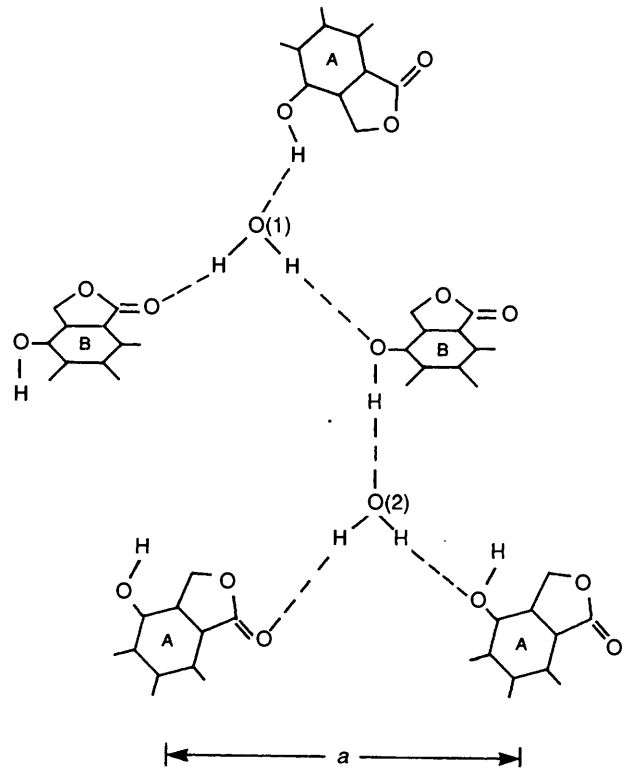


Fig. 7. A schematic representation of the hydrogen-bond structure in the podophyllotoxin inclusion complexes.

structure determinations showed that the three different guest molecules occupy similar positions in the unit cell. They are enclosed in a hydrophobic pocket formed by six podophyllotoxin molecules, three of each crystallographically independent type. The environment for the three guest molecules is shown in Figs. 8–10. As seen from these illustrations the trimethoxyphenyl group and the five-membered ring, O(X01), C(X02), O(X03), C(X04), C(X05) are close to the guest molecule. The intermolecular distances which are shorter than the sum of van der Waals radii[23] plus an additional 0.2 Å are listed in Table 6.

The aromatic guest molecules bromobenzene and nitrobenzene are enclosed in an almost identical way, with the bromine from the more populated bromobenzene molecule placed similarly to the nitro group. The plane of the phenyl groups is tilted relative to the planar groups of the surrounding guest molecules. Table 6 shows that most of the interatomic

distances involve the nitro group. The oxygen atoms are involved in interactions with the five-membered ring from molecule A, a trimethoxyphenyl group from another molecule related by translational symmetry along the *a*-axis, and methoxy groups from molecule B. The other part of the guest molecule has close contacts to the ring system of both molecule A [C(106)] and B [C(203)] as well as a methoxy group of molecule B. The interatomic distances seem to be slightly larger or of the same magnitude as the sum of the atomic van der Waals radii.[23]

From the drawing of the two partly populated bromobenzene molecules in Fig. 2, it can be seen that they occupy almost identical areas of space; as a consequence they have very similar contacts. The more (0.80) populated molecule is shown in Fig. 8. It is apparent from Table 6 that the contacts between bromobenzene and the surrounding podophyllotoxin molecules are analogous to those described previously for nitrobenzene. It should be noted that in this case, one contact between C(3) and O(203), 3.112 Å, is significantly shorter than the sum of the van der Waals radii.

The most interesting guest molecule is butan-2-ol, which is chiral and aliphatic. The structure analysis showed that though the void formed by the host molecules tends to favour aromatic molecules, it can also accommodate an aliphatic molecule. The electron density of the guest molecule in the hydrophobic void is interpreted as originating from the two enantiomers of butan-2-ol which are oriented so they have a common carbon chain as shown in Fig. 3. The terminal methyl group C(4) is found in the same area as the 4-carbon atoms of the benzene rings in the previously described guest–host systems.

Based on the refinement results it emerges that the crystal contains 70% of the *R*-form and 30% of the *S*-form, which shows that the podophyllotoxin water host lattice performs chiral recognition. The enantiomer excess is similar to that observed in the inclusion complex of cyclodextrin with racemic fenoprofen.[24]

The hydrogen group, O(21B), of the less populated *S*-form of butan-2-ol is pointing out of the hydrophobic pocket such that it has short contact distances to other podophyllotoxin molecules than is the case with bromobenzene and nitrobenzene. One of the distances [O(21B)–C(201)] is so short that one could expect it to be energetically repulsive, which could explain why the crystal contains only 30% of the *S*-form.

Hydrogen bonding between the hydroxy group and ether oxygen atoms plays a role for both enantiomers of butan-2-ol. The hydrogen bonds tends to fix the guest molecule in the cavity. The favouring of the *R*-isomer seems to be caused by repulsive interactions with the surrounding podophyllotoxin molecules for the *S*-isomer.

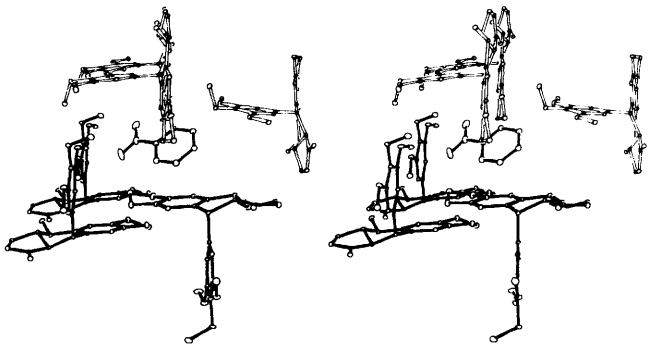


Figure 9. The surroundings of the guest molecule nitrobenzene drawn as described in Fig. 8.

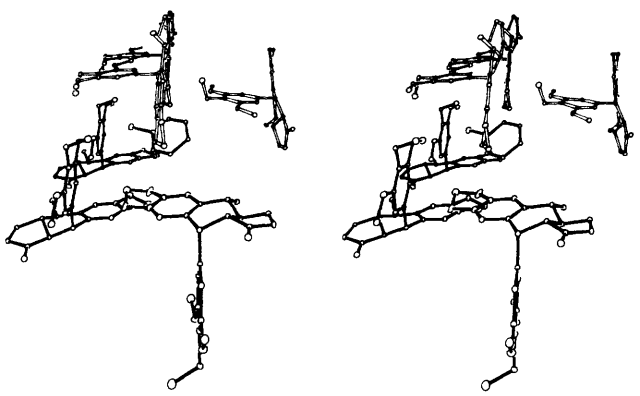


Fig. 8. The surroundings of the more populated orientation of the bromobenzene guest molecule illustrated by a stereo pair viewed along the *a*-axis. The podophyllotoxin molecules are drawn as described in Fig. 4.

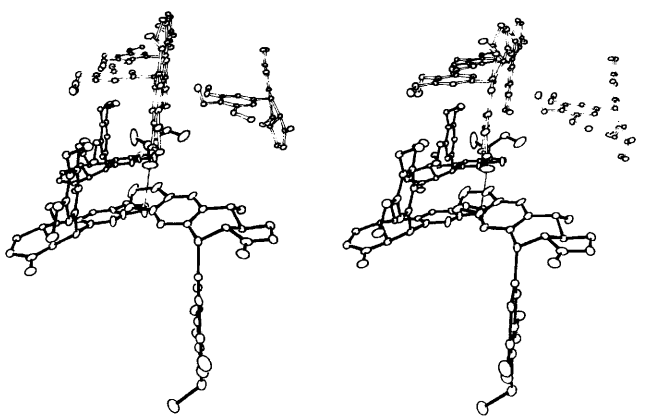


Figure 10. The surroundings of the more populated *R*-isomer of butan-2-ol drawn as in Figs. 8 and 9.

Table 5. Geometry of the hydrogen bonds in the three complexes.

	Organic guest				
	Bromobenzene		Nitrobenzene	Butan-2-ol	
D–H · · · A	D–A (Å)	D–H–A	D–A (Å)	D–H–A	D–A (Å)
O(1)–H(1) · · · O(214)	2.786(4)	169°	2.805(3)	174°	2.685(5)
O(1)–H(2) · · · O(218)[a]	2.802(4)	167°	2.820(3)	170°	2.785(6)
O(2)–H(3) · · · O(114)[b]	2.821(4)	158°	2.818(3)	157°	2.738(5)
O(2)–H(4) · · · O(118)[c]	2.881(4)	138°	2.862(3)	147°	2.808(5)
O(114)–H(114) · · · O(1)	2.699(4)	148°	2.727(3)	172°	2.710(5)
O(214)–H(214) · · · O(2)	2.709(4)	157°	2.701(3)	171°	2.691(5)

[a] $(1 + x, y, z)$. [b] $(x - \frac{1}{2}, \frac{1}{2} - y, 1 - z)$. [c] $(\frac{1}{2} + x, \frac{1}{2} - y, 1 - z)$.

Table 6. Short* intermolecular contact distances (Å) between the organic guest molecules and podophyllotoxin.

Bromobenzene complex			
C(4)–C(106)	3.61(2)	Br(1)–C(230)[c]	3.793(5)
C(13)–C(120)	3.43(2)	Br(1)–O(225)[d]	3.669(3)
C(13)–C(102)[a]	3.57(2)	C(3)–O(203)[e]	3.112(6)
C(5)–O(127)[b]	3.217(6)		
Nitrobenzene complex			
C(4)–C(106)	3.534(4)	O(3)–C(230)[c]	3.237(6)
O(4)–C(120)	3.383(5)	O(3)–C(226)[d]	3.389(5)
O(4)–C(102)[a]	3.274(5)	O(4)–C(228)[d]	3.341(5)
O(4)–O(103)[a]	3.269(4)	C(3)–O(203)[e]	3.218(5)
C(5)–O(127)[b]	3.235(5)		
C(6)–O(127)[b]	3.380(4)		
Butan-2-ol complex			
C(4)–C(106)	3.552(10)	C(1)–C(230)[c]	3.662(10)
C(3)–O(127)[b]	3.528(9)	C(4)–O(218)[b]	3.340(9)
C(3)–C(128)[b]	3.666(10)	O(21A)–O(201)[d]	3.086(9)
		O(21A)–C(203)[e]	3.329(11)
O(21B)–C(202)[f]	2.96(2)		
		O(21A)–O(213)[b]	3.514(13)
O(21B)–O(20)[f]	2.90(2)		
O(21B)–C(230)[g]	3.46(2)		
O(21B)–C(106)[d]	3.47(2)		

[a] $(x-1, y, z)$. [b] $(-x, \frac{1}{2}+y, \frac{1}{2}-z)$. [c] $(\frac{1}{2}-x, -y, z-\frac{1}{2})$. [d] $(-x-\frac{1}{2}, -y, z-\frac{1}{2})$. [e] $(1-x, \frac{1}{2}-y, \frac{1}{2}-z)$. [f] $(x-\frac{1}{2}, \frac{1}{2}-y, 1-z)$. [g] $(x, 1+y, z)$.

Characteristics of Podophyllotoxin Inclusion Complexes.— The crystal-structure determinations showed that the presence of water is essential if podophyllotoxin is to act as a host for smaller organic molecules. Podophyllotoxin is linked to the water molecules by a very regular system of hydrogen bonds as described previously. The geometry of the hydrogen bonds seems unaffected by the identity of the organic host. The preparations have all been performed in solutions of either methanol or ethanol and one could have expected that these alcohols could also have served as the hydrophilic guests since they readily form hydrogen bonds. However, the structure determinations showed clearly that the two protons of water are essential for the formation of the water–podophyllotoxin lattice.

This arrangement of the irregular (T-shaped) podophyllotoxin molecules leads to hydrophobic voids and requires the presence of smaller organic molecules in order to stabilize the structure. The cell dimensions of the inclusion complexes vary with the size of the organic guest. Considering that the system of hydrogen bonds is along the direction of the *a*-axis, it is surprising that the *a*-axis varies as much as the *c*-axis, since hydrophobic interactions seem to be more important in the direction of the *c*-axis.

The inclusion complexes listed in Table 1 represent most of the successful preparations. Attempts to make similar complexes with other organic guest molecules by the same experimental procedure have been performed, but the suitability of guest molecules has not been conveyed in a systematic way. Preliminary experiments seem to indicate that cyclohexanol, 3,3-dimethylbutan-2-ol, and 1-phenylethanol are not suitable guests, which shows that the hydrophobic void in the podophyllotoxin–water complex is of a size which allows only molecules of a limited size and shape as guests.

Using co-ordinative and topological interactions to differentiate between the different types of clathrates, Weber has suggested a new system for classification of these compounds.[25] According to this system, the inclusion complexes of podophyllotoxin display a co-ordinative host–guest interaction with the guest molecule water. The guest–host interactions with

Table 7. Bond lengths (Å) and angles (°) averaged over the two crystallographically independent podophyllotoxin molecules in the nitrobenzene complex.

O(X01)–C(X02)	1.437(5)	C(X12)–C(X17)	1.503(5)
O(X01)–C(X05)	1.383(4)	C(X13)–C(X19)	1.527(4)
C(X02)–O(X03)	1.435(4)	C(X15)–O(X16)	1.475(4)
O(X03)–C(X04)	1.385(4)	O(X16)–C(X17)	1.353(4)
C(X04)–C(X05)	1.382(5)	C(X17)–O(X18)	1.208(4)
C(X04)–C(X09)	1.364(5)	C(X19)–C(X20)	1.393(5)
C(X05)–C(X06)	1.361(5)	C(X19)–C(X24)	1.399(5)
C(X06)–C(X07)	1.412(5)	C(X20)–C(X21)	1.395(5)
C(X07)–C(X08)	1.398(5)	C(X21)–C(X22)	1.393(5)
C(X07)–C(X13)	1.524(5)	C(X21)–O(X25)	1.369(4)
C(X08)–C(X09)	1.412(5)	C(X22)–C(X23)	1.392(5)
C(X08)–C(X10)	1.540(5)	C(X22)–O(X27)	1.382(4)
C(X10)–C(X11)	1.508(5)	C(X23)–C(X24)	1.397(5)
C(X10)–O(X14)	1.428(4)	C(X23)–O(X29)	1.362(4)
C(X11)–C(X12)	1.518(5)	O(X25)–C(X26)	1.432(4)
C(X11)–C(X15)	1.520(5)	O(X27)–C(X28)	1.429(4)
C(X12)–C(X13)	1.526(4)	O(X29)–C(X30)	1.437(4)
C(X02)–O(X01)–C(X05)	103.6(3)		
O(X01)–C(X02)–O(X03)	107.1(3)		
C(X02)–O(X03)–C(X04)	103.5(3)		
O(X03)–C(X04)–C(X05)	109.4(3)		
O(X03)–C(X04)–C(X09)	128.5(3)		
C(X05)–C(X04)–C(X09)	122.1(3)		
O(X01)–C(X05)–C(X04)	107.8(3)		
O(X01)–C(X05)–C(X06)	128.3(3)		
C(X04)–C(X05)–C(X06)	121.9(3)		
C(X05)–C(X06)–C(X07)	117.7(3)		
C(X06)–C(X07)–C(X08)	120.3(3)		
C(X06)–C(X07)–C(X13)	116.6(3)		
C(X08)–C(X07)–C(X13)	123.0(3)		
C(X07)–C(X08)–C(X09)	120.4(3)		
C(X07)–C(X08)–C(X10)	122.6(3)		
C(X04)–C(X09)–C(X08)	117.6(3)		
C(X08)–C(X10)–C(X11)	109.6(3)		
C(X08)–C(X10)–C(X14)	112.8(3)		
C(X11)–C(X10)–C(X14)	113.0(3)		
C(X10)–C(X11)–C(X12)	108.8(3)		
C(X10)–C(X11)–C(X15)	117.9(3)		
C(X12)–C(X11)–C(X15)	112.4(3)		
C(X11)–C(X12)–C(X13)	111.3(3)		
C(X11)–C(X12)–C(X17)	103.6(3)		
C(X13)–C(X12)–C(X17)	121.9(3)		
C(X07)–C(X13)–C(X12)	106.6(3)		
C(X07)–C(X13)–C(X19)	112.1(3)		
C(X12)–C(X13)–C(X19)	114.0(3)		
C(X11)–C(X15)–O(X16)	104.6(2)		
C(X15)–O(X16)–C(X17)	110.3(2)		
C(X12)–C(X17)–O(X16)	109.5(3)		
C(X12)–C(X17)–O(X18)	129.4(3)		
O(X16)–C(X17)–O(X18)	121.1(3)		
C(X13)–C(X19)–C(X20)	119.3(3)		
C(X13)–C(X19)–C(X24)	121.1(3)		
C(X20)–C(X19)–C(X24)	119.6(3)		
C(X19)–C(X20)–C(X21)	120.0(3)		
C(X20)–C(X21)–C(X22)	120.4(3)		
C(X20)–C(X21)–O(X25)	124.4(3)		
C(X21)–C(X22)–O(X27)	120.2(3)		
C(X22)–C(X23)–O(X29)	115.5(3)		
C(X09)–C(X08)–C(X10)	116.9(3)		
C(X22)–C(X21)–O(X25)	115.2(3)		
C(X21)–C(X22)–C(X23)	119.7(3)		
C(X23)–C(X22)–O(X27)	120.1(3)		
C(X22)–C(X23)–C(X24)	120.0(3)		
C(X22)–C(X23)–O(X29)	115.4(3)		
C(X24)–C(X23)–O(X29)	124.5(3)		
C(X19)–C(X24)–C(X23)	120.2(3)		
C(X21)–O(X25)–C(X26)	117.0(3)		
C(X22)–O(X27)–C(X28)	113.0(3)		
C(X23)–O(X29)–C(X30)	117.4(3)		

Table 8. Torsion angles (°) involving the 3,4,5-trimethoxyphenyl moiety.

	Guest molecule		
	Bromo-benzene	Nitro-benzene	Butan-2-ol
C(120)–C(121)–O(125)–C(126)	−2.6(4)	−0.1(3)	−1.5(7)
C(220)–C(221)–O(225)–C(226)	2.7(5)	7.6(5)	−1.0(8)
C(121)–C(122)–O(127)–C(128)	86.6(4)	91.7(4)	80.1(6)
C(221)–C(222)–O(227)–C(228)	88.0(4)	80.5(4)	85.1(7)
C(122)–C(123)–O(129)–C(130)	177.2(3)	175.6(3)	177.3(5)
C(222)–C(223)–O(229)–C(230)	173.4(4)	173.2(4)	−178.8(6)

the other guest molecules are of topological origin forming an aediculate.

Their potential to separate enantiomers in a racemic mixture is one of the fascinating aspects of inclusion compounds. The structure determination of the butan-2-ol complex shows that partial resolution has been achieved. The hydrophobic voids contain an excessive amount of the *R*-form of butan-2-ol (70% of the *R*- and 30% of the *S*-form). There are only a very few examples of complete resolution of racemates by guest–host complexes.[26] Owing to the size of the hydrophobic pocket the guest–host complexes of podophyllotoxin can only be used to separate small chiral molecules; as an example 1-phenylethanol is too large for the void.

There are other examples of butan-2-ol as a guest in chiral host lattices.[27] However, the quoted enatiomeric excesses found in the butan-2-ol complexes with these host molecules are much smaller than that found in the podophyllotoxin–water complex, which indicates that the podophyllotoxin clathrates may perform suitable resolution of other small chiral molecules.

Comparison of the Different Podophyllotoxin Molecules in the Crystal Lattices.—This investigation of the inclusion complexes of podophyllotoxin provides the basis for six independent observations of the molecular geometry of podophyllotoxin.

A comparison of the bond lengths and angles found in the structures reveals no systematic differences or variations. The two types of crystallographically independent molecules agree well and there are no variations between the three inclusion complexes. Table 7 lists bond lengths and angles averaged over the two crystallographically independent podophyllotoxin molecules in the nitrobenzene complex.

The two crystallographically independent molecules differ slightly in the orientation of the 3,4,5-trimethoxyphenyl group as illustrated by the torsion angle C(X07)–C(X13)–C(X19)–C(X20) which is found in the range 13–15° in molecule A and 33–36° in molecule B in the three structures. It seems likely that this difference in conformation between molecules A and B is caused by the packing around the hydrophobic pocket. The only significant difference between the podophyllotoxin molecules is found in the orientation of the methoxy groups. The methoxy groups in the 3 and 5 positions are virtually coplanar with the phenyl group and 4 methoxy group is almost perpendicular to the plane. However, deviations of up to *ca.* 10° from this general picture are observed in the three structures as illustrated by the torsion angles given in Table 8.

Acknowledgements

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