

Potential Energy Calculations on the 2 : 1 Inclusion Compound Between Deoxycholic Acid and (+)-Camphor

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Abstract. The crystal structure of the 2 : 1 inclusion compound between deoxycholic acid and (+)-camphor has been solved by other authors. We have studied its crystal packing by potential energy calculations. Four arrangements of camphor, satisfactory from an energy point of view, were found and used to refine the crystal structure by a least-squares procedure. The results seem to indicate that more than one orientation is possible for the guest molecule and that the X-ray data do not allow us to establish which guest-molecule arrangements are actually present.

Key words: Choleic acid, guest location, potential energy calculations.

1. Introduction

The inclusion compounds between 3 α ,12 α -dihydroxy-5 β -cholan-24-oic acid (deoxycholic acid (DCA), see Figure 1) and a wide variety of guest molecules have been extensively studied in recent years [1–3]. They are termed ‘choleic acids’ and, in most cases, crystallize in the orthorhombic system giving rise to ‘channel-type’ inclusion compounds. The space group observed so far is $P2_12_12_1$, except for the (+)-camphor choleic acid compound (DCADCP) which belongs to $P2_12_12$ [4].

The DCA molecules give rise to pleated bilayers, extended parallel to the bc plane, which pack together leaving empty channels running along c . The ab projection of four choleic acids is shown as an example in Figure 2. The characteristic structural unit (the bilayer) is nearly identical in all the orthorhombic choleic acids investigated. Between adjacent bilayers there are either 2 $_1$ or pseudo-2 (or 2) axes parallel to c , giving rise to only two possible arrangements

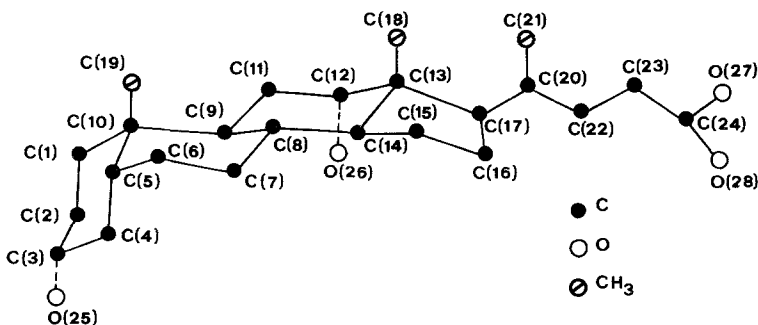


Fig. 1. Atomic numbering of DCA. Hydrogen atoms are omitted.

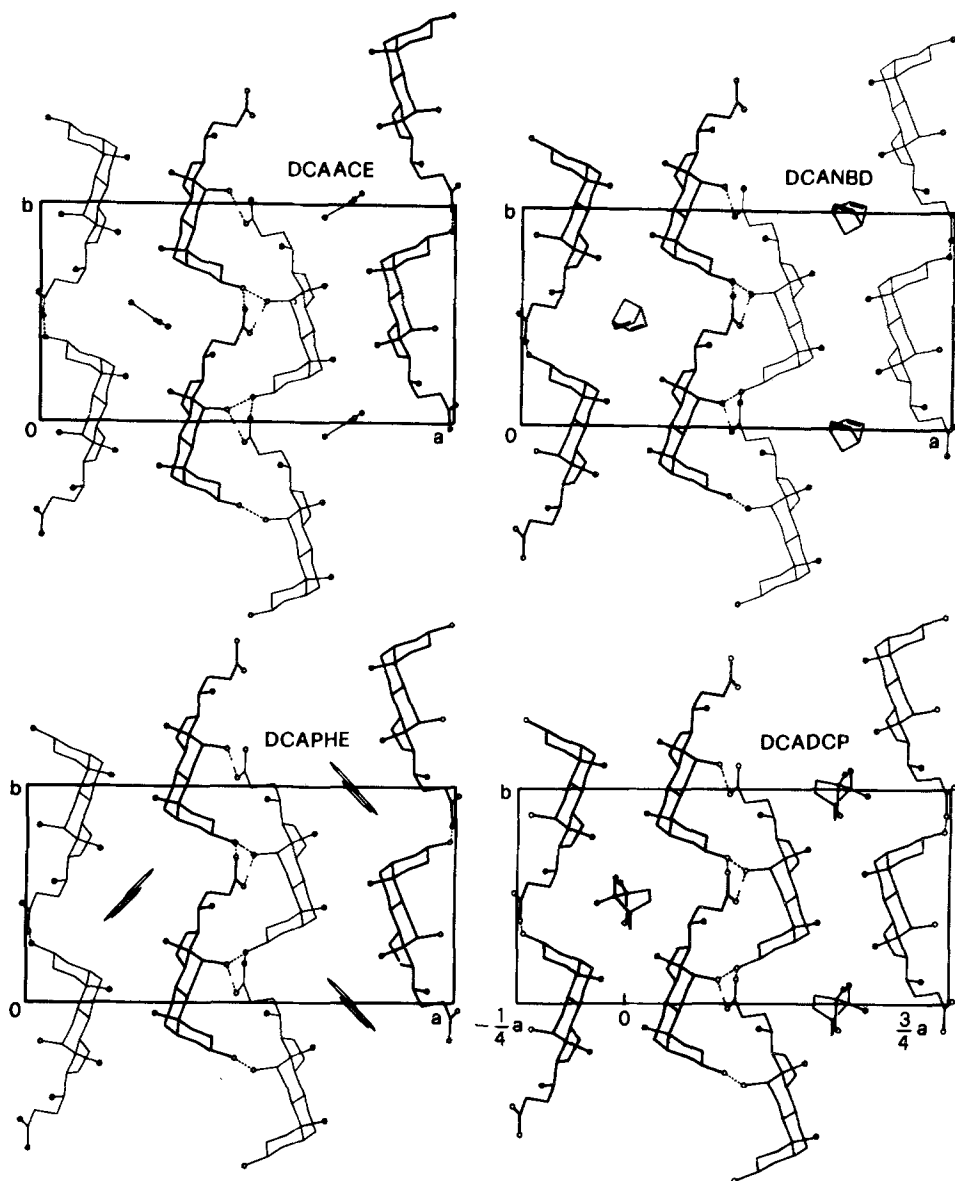


Fig. 2. Acetone (DCAACE), norbornadiene (DCANBD), phenanthrene (DCAPE) and (+)-camphor (DCADCP)-choleic acids viewed along c . The black and open circles are methyl groups and oxygen atoms respectively. The dotted lines represent hydrogen bonds.

along c [5]. The size of the guest molecule controls the separation between two adjacent antiparallel bilayers, which is equal to $a/2$. Moreover, the dimensions and shape of the channel cross-section can be changed by moving a bilayer along b (see Figure 2). For these reasons, van der Waals energy calculations were performed, in the $P2_12_12_1$ space group, for the two possible arrangements along c , neglecting the guest molecules, as a function of a and of the bilayer translation along b , with the aim of establishing if the energy minima correspond to

the observed DCA lattices in the choleic acids [5]. The calculations pointed out the occurrence of three minima, populated by the experimental crystal structures, corresponding to the DCAACE, DCAPHE, and DCANBD packings shown in Figure 2. The DCANBD packing is representative of one of the three minima and is very similar to that of DCADCP, since the molecules of the DCA bilayers in DCANBD are related by a pseudo-2 axis along c .

The results obtained for DCANBD [5], together with unpublished calculations accomplished in the space group $P2_12_12$, show no substantial differences between the energy curves of $P2_12_12_1$ and $P2_12_12$. The region of interest of the energy curves computed for DCANBD is reported in Figure 3, where the y value is the increment given to the y atomic coordinates of DCA found in the crystal structure of DCANBD. Both DCADCP and DCANBD have almost the same average value of the y coordinates, which differ by about 0.14 Å. Since the unit cell parameters of DCADCP ($a = 27.35$, $b = 13.81$, $c = 7.23$ Å) and DCANBD ($a = 27.13$, $b = 13.46$, $c = 14.21$ Å) are very similar in the a and b values, whereas the c value for DCADCP is similar to the approximate repeat along c of DCA in DCANBD (~ 7.1 Å), the shape and size of the cavity are approximately equal in both inclusion compounds, which present the same 2 : 1 host/guest molar ratio.

The guest molecule in DCANBD was located by van der Waals energy calculations [6]. This method is expected to be more powerful for locating (+)-camphor (DCP), which has a greater sterical hindrance than norbornadiene (NBD) and can engage in more specific interactions with DCA than can NBD owing to the methyl groups of both the host and guest molecules. Thus, DCP is probably more frozen than NBD in the interior of the DCA host lattice and should occur in a unique (or more than one) ordered arrangement as observed for NBD. In fact, the DCP-DCA contacts are generally shorter than those between NBD and DCA. However, the analysis of the DCADCP crystal structure [4] showed, for the atoms of

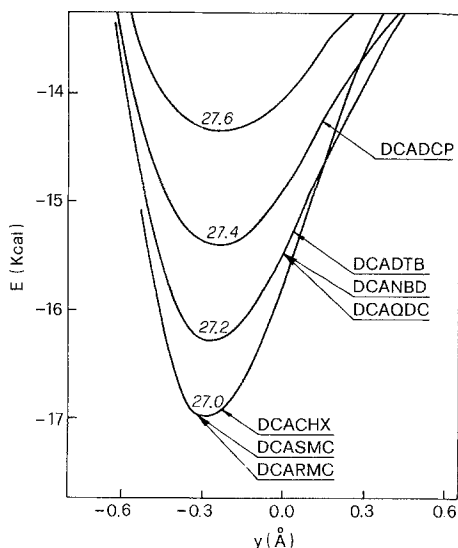


Fig. 3. Van der Waals energy vs. y for different values of a for DCANBD. The arrows show the positions of the experimental crystal structures. DCACHX, DCARMC, DCASMC, DCADTB and DCAQDC are abbreviations for inclusion compounds of DCA with cyclohexanone, *R*(3)-methyl cyclohexanone, *S*(3)-methyl cyclohexanone, di-*t*-butyl diperoxy carbonate and quadricyclane respectively.

DCP, uneven B_{eq} values, some of them very high, ranging from 9 to 45 Å². These values have to be compared with the lower and more uniform isotropic temperature factors of the atoms of the guest molecule in the DCACHX (15–23 Å²), DCASMC (13–21 Å²), DCADTB (17–30 Å²) and DCAQDC (18–25 Å²) crystal structures [2, 7], which are very similar to that of DCADCP and populate the same energy minimum of Figure 3. Moreover, difference Fourier syntheses, computed by us without including DCP, showed many unresolved peaks not satisfactorily interpretable just by means of the DCP atomic coordinates reported in [4], whose authors will be hereafter indicated as JSLL. These findings seem to suggest that more than one arrangement of DCP can better-fit the crystal data. We therefore decided to determine the possible arrangements of DCP in DCADCP by resorting to van der Waals energy calculations.

2. Van der Waals Energy Calculations

Semiempirical atom-atom potentials of the form

$$V(r) = a \exp(-br)/r^d - cr^{-6}$$

were used for the intermolecular interactions, with coefficients verified in known and unknown crystal structures [8, 9]. The methyl group was treated as one atom.

The DCA atoms were kept fixed, as obtained at the end of the refinement of the crystal structure by JSLL [4] and the DCP molecule was moved as a rigid body by varying three Eulerian angles ψ_1 , ψ_2 , and ψ_3 and three translations, t_x , t_y , and t_z along a , b , and c . The DCP was rotated counterclockwise around three axes OX , OY , and OZ parallel to a , b , and c respectively, where O is the point with coordinates which are the average of those of the DCP atoms, excluding the hydrogen atoms of the methyl groups. The angular and translational increments were progressively reduced from 10 to 2° and from 0.4 to 0.1 Å respectively. The DCP model (Figure 4) was that used by JSLL. The C–H bond lengths were 1.08 Å and the H–C–H bond angles were 109.5°. The hydrogen atoms had the same numbering as the carbon atoms to which they are attached.

The starting atomic coordinates in the calculations, corresponding to $\psi_1 = \psi_2 = \psi_3 = 0^\circ$ and $t_x = t_y = t_z = 0$ Å, were those published by JSLL [4] after a rigid body refinement of DCP. The DCP–DCA and DCP–DCP interactions were considered within a cut-off distance of 7 Å.

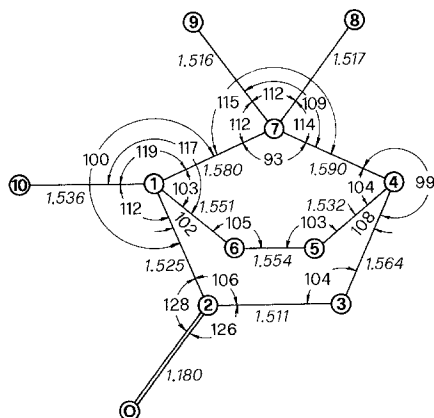


Fig. 4. Atomic numbering and bond lengths (Å) and angles (°) of DCP without hydrogen atoms.

Since DCP lies on a twofold rotation axis, each guest molecule can lie in one of two equivalent overlapping arrangements. Thus, when considering the DCP–DCP interactions, two separate calculations were carried out: one with adjacent DCP molecules related by a a translation (T) and the other by a c translation and a rotation of 180° about c (R). For both calculations four minima, indicated as A , B , C , and D , were found and their parameters are reported in Table I, where the energy values are given only for qualitative comparison. The very similar values of the parameters of each minimum for both calculations point out that the DCP–DCP interactions do not influence the DCP position. In fact, no short contacts are observed between adjacent guest molecules. For this reason we will only report the results obtained for the T minima.

Table I. Eulerian angles ($^\circ$), translations (\AA) and energy values (Kcal) corresponding to the van der Waals energy minima for DCP in DCADCP

Minimum		ψ_1	ψ_2	ψ_3	t_x	t_y	t_z	E
A	T	1	-10	1	-0.1	0.0	-0.3	-25.6
	R	-3	-2	1	-0.1	-0.1	-0.1	-25.2
B	T	-18	120	-90	-0.1	0.3	-0.4	-25.1
	R	-14	118	-84	-0.1	0.3	-0.4	-25.5
C	T	94	148	14	-0.2	0.0	-0.3	-24.0
	R	90	146	10	-0.2	0.0	-0.3	-23.8
D	T	-49	70	81	0.0	0.6	-0.5	-24.6
	R	-48	71	84	-0.1	0.6	-0.6	-24.6

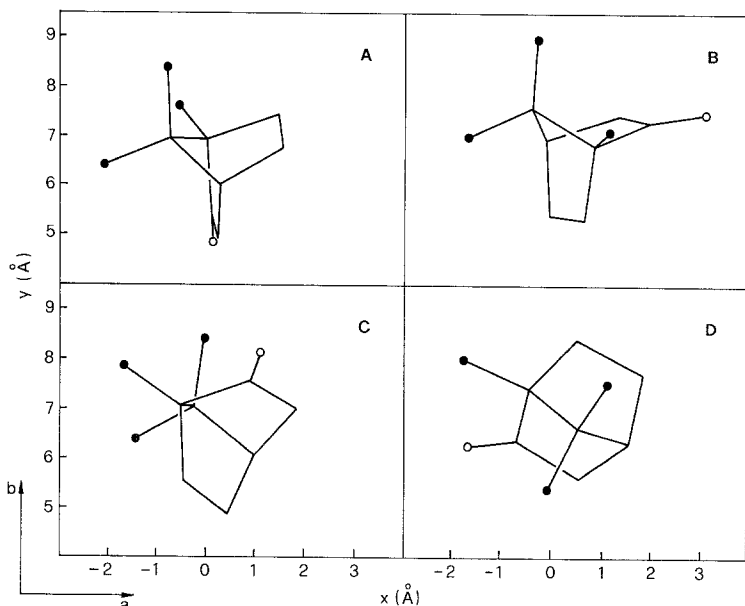


Fig. 5. Projection on the ab plane of DCP in the positions corresponding to the four energy minima. Black and open circles correspond to methyl groups and oxygen atoms respectively.

The four positions of DCP corresponding to the minima are shown in Figure 5. The DCP position of *A* is close to that reported by JSLL, but on the basis of the energy values it is not strongly favoured with respect to the other three positions in *B*, *C*, and *D*. In fact, *A* and *B* have nearly equal energy values which do not allow a discrimination between them. Moreover, the slightly higher energy values of *C* and *D* are due to the hardness of the potentials used by us and do not correspond to intermolecular contacts worse than those of *A* and *B*. The most relevant intermolecular interactions for *A*, *B*, *C* and *D*, together with those of the JSLL crystal structure, are reported in Table II.

Table II. Closest intermolecular distances (Å) in DCADCP for the positions of DCP corresponding to the energy minima and to the JSLL crystal structure. The first atom in the interactions DCA–DCP belongs to DCA. The H atoms have the same numbering of the C atoms to which they are bonded.

Interactions DCP–DCP (minimum B)									
C(3) ^b ...C(10)	3.5	H'(3) ^b ...C(10)	3.0	C(4) ^b ...C(10)	3.7	H(4) ^b ...C(10)	3.1		
Interactions DCA–DCP									
JSLL	<i>A</i>	<i>B</i>	<i>C</i>	<i>C</i>	<i>D</i>				
H(6) ^a ...H(3)	2.4	C(19) ^b ...H(3)	3.2	H(6) ^a ...H(5)	2.3	H(6) ^a ...H(5)	2.3	C(19) ^b ...C(8)	3.5
C(19) ^b ...H(3)	3.2	H'(1) ^b ...C(8)	3.2	H(6) ^c ...H(3)	2.4	H(5) ^a ...C(9)	3.2	H(5) ^f ...C(9)	3.0
H'(1) ^b ...C(8)	3.1	H(16) ^c ...H'(3)	2.3	C(19) ^f ...C(8)	3.8	H'(1) ^b ...H'(6)	2.3	H(16) ^e ...H(4)	2.2
C(16) ^c ...H'(3)	2.9	C(21) ^d ...H'(5)	3.1	H(22) ^c ...H'(3)	2.4	C(6) ^e ...C(8)	3.6	H(16) ^e ...C(3)	2.7
H(16) ^c ...H'(3)	2.2	H(22) ^g ...C(8)	3.1	H(20) ^d ...C(10)	3.2	H(6) ^e ...C(8)	3.1	H(16) ^e ...C(4)	2.8
H(22) ^c ...H(6)	2.2	H(16) ^g ...C(10)	3.1	H(16) ^g ...C(8)	3.1	H(16) ^c ...H(4)	2.1	H(22) ^c ...H(4)	2.4
C(21) ^d ...H'(5)	2.9	H(20) ^g ...C(10)	3.1	C(18) ^h ...C(8)	3.8	H(16) ^c ...H'(5)	2.2	C(18) ^d ...C(8)	3.7
H(16) ^g ...C(10)	2.9	C(18) ^h ...C(9)	3.7	C(21) ^h ...C(9)	3.9	H(16) ^c ...C(4)	2.6	H(16) ^g ...C(10)	2.9
C(18) ^h ...C(9)	3.6					H(16) ^c ...C(5)	2.7	C(16) ^g ...C(10)	3.7
						H(16) ^g ...C(8)	3.1	C(18) ^h ...C(10)	3.8
						C(22) ^g ...C(9)	3.7	C(21) ^h ...C(10)	3.7
						H(22) ^g ...C(9)	3.2		
						H(16) ^g ...C(10)	3.2		
						C(18) ^h ...C(10)	3.8		
						C(21) ^h ...C(10)	3.7		
						H(20) ^h ...C(10)	3.1		

Equivalent positions:

$$\begin{array}{llll}
 \text{a } x, y, z & \text{c } -x, -y, z & \text{e } -x, 1-y, z & \text{g } x, y+1, z \\
 \text{b } x, y, z+1 & \text{d } -x, -y, z+1 & \text{f } -x, 1-y, z+1 & \text{h } x, y+1, z+1
 \end{array}$$

In the positions corresponding to minima *B*, *C*, and *D*, the methyl groups and hydrogen atoms of DCP make good contacts with the DCA molecules belonging to all the four sides of the cavity, whereas for the minimum *A* no distances of DCP with one of the four sides of the cavity appear in Table II, indicating that the corresponding interactions are weaker than for the other minima. In agreement with this situation, the plot of the energy as a function of the translations t_x and t_y on the *ab* plane shows an elliptical shape of the minimum, which is more pronounced for the minimum *A* than for the other three (Figure 6). The long axis of the ellipse points approximately towards the *A* rings of DCA and, therefore, the motion of DCP is favoured along this axis. A plot of the energy as a function of t_z (Figure 7) around the minimum regions shows that the energy gradient along this direction is rather high and of the same order as on the *ab* plane.

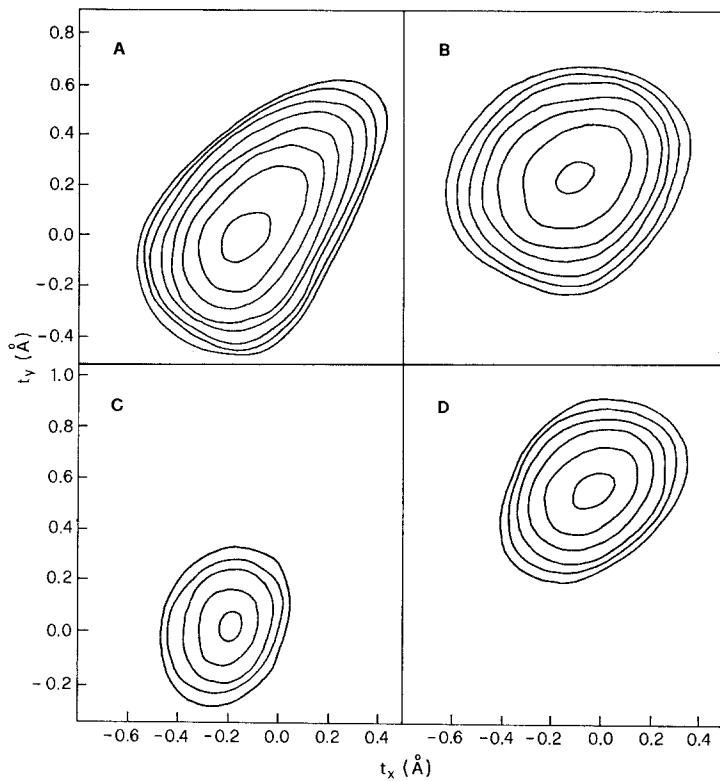


Fig. 6. Van der Waals energy of DCP as a function of t_x and t_y in the regions of the four minima. Contour lines are drawn at intervals of 0.5 Kcal starting from 22.0 Kcal.

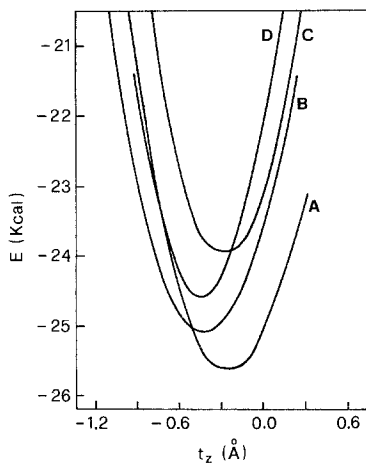


Fig. 7. Van der Waals energy of DCP as a function of t_z for the four minima.

3. Crystallographic Calculations

The presence, in the potential energy map, of four minima with similar energy values, all very sharp and corresponding to DCP arrangements suitable for good intermolecular interactions, do not preclude a unique solution. However, these findings supported our hypothesis that the thermal motion of DCP in the channels could not be very high and that such high and uneven values of the thermal parameters reported by JSLL came from an attempt to fit the electron density with only one model. To see if a better fit to the diffraction data could be achieved by considering the other DCP arrangements, after having obtained from *Acta Crystallographica* the structure factors list and the anisotropic thermal parameters of JSLL, we refined the structure using SHELX [10], starting for DCA from the coordinates and thermal parameters published and for DCP from the coordinates corresponding to *A*, *B*, *C*, and *D* in turn. The JSLL structure was also refined under the same conditions in order to make a comparison possible. The heavy atoms of DCA were refined anisotropically and the H atoms were riding on the atoms to which they are attached, with their temperature factors fixed to the average value of all the U_{eq} 's of the heavy atoms ($U_{\text{eq}} = \frac{1}{3}(U_{11} + U_{22} + U_{33})$). The DCP molecule was refined as a rigid body with isotropic temperature parameters U 's starting from 0.1 \AA^2 . We used the weighting scheme $w = k(a + b|F_0| + c|F_0|^2)^{-1}$ with $a = 1$, $b = 1$ and $c = 0.0116$. The k factor is redetermined after each refinement cycle.

The refinements gave agreement indices ranging within 0.08–0.11 with slightly better values for *A* and the JSLL structure. However, all the refinements were unsatisfactory, both because of very high thermal parameters of the DCP atoms and for the high shift/e.s.d. ratios which indicated that the refinements did not converge. The temperature factors after five least-squares cycles were for most atoms of DCP within the range 9–25 \AA^2 , but for a few atoms they were quite higher (up to 70 \AA^2) and tended to increase with further refinement cycles. An inspection of the DCAQDC crystal structure [2], nearly equal to that of DCADCP as far as the DCA host lattice is concerned, shows acceptable thermal parameters for quadricyclane within the range 18–25 \AA^2 and good shift/e.s.d. ratios, which are 5–10 times less than those of DCADCP, although the reflections of DCAQDC with $I > 2.5\sigma(I)$ are 2079 and those of DCADCP with $I > 3\sigma(I)$ are 2527. Hence, in order to get more information, it was decided to calculate two difference Fourier syntheses, the first after refining isotropically DCA only, starting from the atomic coordinates of JSLL, the R factor being 0.20, and the second with the DCA atomic coordinates and temperature factors of JSLL, without DCP. The two maps so obtained were practically equal in the region of DCP, at variance with the findings of JSLL, who were not able to locate DCP by means of a difference Fourier synthesis of the first type, which gave only 'one large, roughly spherical peak $\sim 7 \text{ \AA}$ in diameter on the two-fold rotation axis in the space between the deoxycholic acid layers' and could interpret the peaks of a difference Fourier synthesis of the second type computed after removing DCP treated as a giant atom. The fitting of the *A*, *B*, *C*, *D* and JSLL models of DCP with the electron density of the two maps was unsatisfactory for the misplacement of some atoms with respect to the regions of high electron density and for the presence of some regions of electron density not matched by atoms of DCP, even taking into account the two images related by the twofold axis. A slightly better fit was obtained with the JSLL and *A* models.

Some electron density peaks are populated by atoms belonging to different models and having low temperature factor values. This, together with the above-mentioned results, supports the occurrence of two or more models which would render the temperature more homogeneous once they are introduced together in the refinement. In fact, the introduction of more than one model in the refinement of the crystal structure gives rise to lower

temperature factors for the non-overlapping atoms of the different models, since their occupation factors decrease, and makes the values of the thermal parameters for all the DCP atoms less uneven and more realistic. To check this point, two refinements were carried out with both the JSLL and B models of DCP (which have the best energies) with an occupation factor of 0.375 and 0.125 respectively (*R1*) and with an equal occupation factor of 0.25 (*R2*). The occupation factor of the JSLL model was chosen higher than that of the *B* model in *R1*, owing to the slightly better results obtained in the calculations performed with the single JSLL model. The occupation factors were not refined because of their strong correlation with the thermal parameters. Both *R1* and *R2* gave better results than those obtained refining the JSLL structure (*R3*) in the same conditions. The agreement indices of *R1*, *R2* and *R3* were 0.070, 0.068 and 0.077, respectively. Refinement *R1* converged satisfactorily, whereas *R2* and *R3* did not but, *R2* has better shift/e.s.d. ratios than *R3*. Moreover, the e.s.d.'s of the atomic coordinates and thermal parameters are nearly equal for *R1* and *R2* and lower than those of *R3*. The temperature factors of the DCP atoms of the JSLL model were lower on the average and less uneven for *R1* and *R2* than for *R3*. Those of the DCP atoms of the *B* model were better for *R1* than for *R2*. The R^G values [11] are 0.104, 0.100 and 0.115 for *R1*, *R2* and *R3*, respectively and, as an indication only, the Hamilton test [11] would bring rejection at the 0.005 level of *R3* with respect to *R1* and *R2*. These findings support the presence of more than one model of DCP in the DCADCP crystal structure and favour *R1* with respect to *R2*, thus assigning a greater weight to the JSLL model.

4. Conclusions

In conclusion, potential energy calculations offer a convenient and reliable way of studying the location of the guest molecules in inclusion compounds when, as in the DCADCP case, the guest molecule can occupy more than one position and its contribution to the diffraction power is very low as compared with that of the host molecule [12]. Of course, the relative weights of the possible arrangements of the guest molecule can only be determined by refinement methods in those favourable cases in which the X-ray data are quantitatively adequate and very accurate.

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References

1. E. Giglio: *J. Mol. Struct.* **75**, 39 (1981).
2. V. M. Coiro, E. Giglio, F. Mazza, and N. V. Pavel: *J. Incl. Phenom.* **1**, 329 (1984).
3. E. Giglio: in *Inclusion Compounds*, vol. 2 (eds. J. L. Atwood, J. E. D. Davies, and D. D. MacNicol), pp. 207-229, Academic Press, London (1984).
4. J. G. Jones, S. Schwarzbaum, L. Lessinger, and B. W. Low: *Acta Crystallogr.* **B38**, 1207 (1982).
5. S. Candeloro De Sanctis and E. Giglio: *Acta Crystallogr.* **B35**, 2650 (1979).
6. A. D'Andrea, W. Fedeli, E. Giglio, F. Mazza, and N. V. Pavel: *Acta Crystallogr.* **B37**, 368 (1981).
7. C. P. Tang: PhD thesis, The Feinberg Graduate School, The Weizmann Institute of Science, Rehovot, Israel (1978).

8. N. V. Pavel, C. Quagliata, and N. Scarcelli: *Z. Kristallogr.* **144**, 64 (1976).
9. E. Gavuzzo, F. Mazza and E. Giglio: *Acta Crystallogr.* **B30**, 1351 (1974).
10. G. M. Sheldrick: SHELX 76. Program for crystal structure determination. University of Cambridge, England (1976).
11. W. C. Hamilton: *Acta Crystallogr.* **18**, 502 (1965).
12. S. Candeloro De Sanctis: *Acta Crystallogr.* **B39**, 366 (1983).