BUSING, W. R., MARTIN, K. O. & LEVY, H. A. (1962). *ORFLS*. Report ORNL-TM-305. Oak Ridge National Laboratory, Tennessee.

CANTRELL, J. S., STALZER, R. A. & BECKER, T. L. (1974). *Acta Cryst.* B30, 154–161.

CRUICKSHANK, D. W. J. (1961). *Computing Methods and the Phase Problem in X-ray Crystal Analysis*, p. 45. Oxford: Pergamon Press.

DESTRO, R. (1979). *Acta Cryst.* B35, 1714–1717.

JAMES, M. N. G. & WILLIAMS, G. J. B. (1973). *Acta Cryst.* B29, 1172–1174.

International Tables for X-ray Crystallography (1962). Tome III, pp. 202–203. Birmingham: Kynoch Press.

MARSH, R. E., RAMAKUMAR, S. & VENKATESAN, K. (1976). *Acta Cryst.* B32, 66–70.

SMITH, J. L., CHWANG, A. & SUNDARALINGAM, M. (1980). *Acta Cryst.* B36, 833–837.

STEWART, R. F., DAVIDSON, E. R. & SIMPSON, W. T. (1965). *J. Chem. Phys.* 42, 3175–3187.

WRIGHT, R. W. & MARCHESSAULT, R. M. (1968). *Can. J. Chem.* 46, 2567–2575.

Benzyloxycarbonylprolylproline and N-Benzyloxycarbonylprolyl-L-azetidine-2-carboxylic Acid

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Abstract

Both Z-Pro-Pro-OH, $C_{18}H_{22}N_2O_5$, $M_r = 346 \cdot 39$, and Z-Pro-Aze-OH, $C_{17}H_{20}N_2O_5$, $M_r = 332 \cdot 36$, crystallize in the monoclinic space group $P2_1$ with $Z = 2$. Their respective lattice parameters are $a = 10 \cdot 446$ (4), $b = 14 \cdot 527$ (6), $c = 6 \cdot 107$ (3) Å, $\beta = 114 \cdot 79$ (5)°, with $V = 841$ (1) Å^3, $d_x = 1 \cdot 367$ (2) g cm^{-3} and μ(Mo $K\alpha$) = $1 \cdot 08$ cm^{-1}; and $a = 10 \cdot 161$ (7), $b = 14 \cdot 743$ (13), $c = 5 \cdot 640$ (5) Å, $\beta = 109 \cdot 63$ (4)°, with $V = 796$ (2) Å^3, $d_m = 1 \cdot 38$ (1), $d_x = 1 \cdot 387$ (3) g cm^{-3} and μ(Mo $K\alpha$) = $1 \cdot 11$ cm^{-1}. For both crystals counter data were measured to $(\sin \theta)/\lambda = 0 \cdot 70$ Å^{-1}. The structures were solved by direct methods and refined by full-matrix least-squares fit to the data with $F_o > 2\sigma(F_o)$. The final values of $R = \sum |F_o - F_c|/\sum F_o$ were $0 \cdot 064$ (1425 data) and $0 \cdot 048$ (1702 data) respectively. The two crystals are isostructural. In each molecule the peptide bond is *trans*, but the urethane bond is *cis*, so that the benzyloxycarbonyl group is folded back on the dipeptide. The dipeptide conformations are semi-extended. The conformation angles (φ, ψ) for Z-Pro-Pro-OH are -65 and $155°$ for the first residue and -53 and $142°$ for the second; the corresponding values for Z-Pro-Aze-OH are -60 and $152°$, and -52 and $139°$.

Introduction

Azetidine-2-carboxylic acid (Aze, 1) and proline (Pro, 2) differ in chemical structure by a single methylene group. The crystal structure of L-Aze has been analyzed by Berman, McGandy, Burgner & Van Etten (1969). They cited reports of toxic effects of Aze reversible by simultaneous administration of Pro, and they concluded that the conformations of Aze and Pro differ enough that replacement of Pro by Aze in proteins would cause structural changes large enough to account for the toxic effects. Whereas Berman *et al.* (1969) were able to compare Aze and Pro as the free, zwitterionic, imino acid structures, (1) and (2), the two molecules named in the title (hereafter Z-Pro-Pro-OH and Z-Pro-Aze-OH respectively) allow a comparison of Aze and Pro as corresponding peptide residues.

(1)   (2)

Experimental

Crystals suitable for the X-ray diffraction measurements were kindly provided by Professor Robert L. Van Etten of Purdue University. Crystal quality, diffraction symmetry, and approximate lattice dimensions were determined from Buerger precession photographs. The density of Z-Pro-Aze-OH was measured by flotation in a mixture of chlorobenzene and bromobenzene.

Quantitative diffraction data were measured on a Picker four-circle diffractometer equipped with the Picker–Furnas monochromator. Mo $K\alpha$ X-rays (λ =

Table 1. *Coordinates* ($\times 10^4$) *and equivalent* (*Hamilton*, 1959) *isotropic temperature factors* ($\mathring{A}^2 \times 10^2$)

Z-Pro-Pro-OH

	x	y	z	B_{iso}
C(0)	3155 (6)	4033 (5)	7288 (10)	346 (18)
C(1)	4924 (5)	5490 (5)	6111 (9)	322 (16)
C(1A)	3600 (6)	5000	4359 (9)	340 (17)
C(1B)	2615 (7)	5695 (6)	2461 (11)	493 (22)
C(1D)	1372 (6)	5134 (5)	4742 (11)	442 (20)
C(1G)	1166 (7)	5469 (6)	2239 (13)	611 (24)
C(2)	7119 (6)	6936 (5)	7817 (10)	344 (18)
C(2A)	7349 (5)	5964 (5)	7182 (9)	328 (17)
C(2B)	8226 (7)	5958 (6)	5665 (13)	557 (26)
C(2D)	6129 (6)	5100 (6)	3413 (10)	434 (20)
C(2G)	7668 (7)	5159 (7)	4014 (12)	571 (26)
C(4)	5036 (6)	2977 (5)	9378 (10)	379 (18)
C(5)	6612 (5)	2979 (5)	10295 (9)	320 (16)
C(6)	7256 (6)	3274 (6)	8810 (11)	429 (21)
C(7)	8706 (7)	3277 (6)	9685 (14)	540 (26)
C(8)	9533 (6)	2989 (6)	12014 (13)	501 (23)
C(9)	8909 (6)	2693 (6)	13486 (11)	491 (21)
C(10)	7433 (6)	2689 (5)	12575 (11)	420 (20)
N(1)	2753 (4)	4673 (5)	5578 (8)	353 (15)
N(2)	6026 (4)	5512 (5)	5523 (7)	306 (13)
O(0)	2447 (4)	3740 (4)	8277 (8)	492 (16)
O(1)	4965 (4)	5861 (4)	7959 (6)	380 (12)
O(3)	4493 (4)	3762 (4)	7820 (6)	347 (11)
O(2")	7926 (4)	7121 (4)	10087 (7)	412 (13)
O(2')	6335 (5)	7474 (5)	6393 (7)	529 (16)
H(1A)	3880	4445	3463	338
H(1BA)	2678	5606	753	492
H(1BB)	2898	6394	3069	492
H(1DA)	549	4661	4644	443
H(1DB)	1398	5702	5906	443
H(1GA)	689	4934	915	608
H(1GB)	497	6070	1728	608
H(2A)	7876	5554	8795	327
H(2BA)	9332	5875	6802	557
H(2BB)	8068	6589	4649	557
H(2DA)	5770	4390	3175	434
H(2DB)	5512	5478	1740	434
H(2GA)	8110	4530	4891	576
H(2GB)	7831	5254	2392	576
H(4A)	4743	3015	10871	377
H(4B)	4613	2348	8384	377
H(6)	6624	3496	7000	434
H(7)	9110	3496	8553	532
H(8)	10664	2989	12678	495
H(9)	9546	2468	15303	486
H(10)	6941	2450	13703	418
H(2")	7914	7728	10726	408

Z-Pro-Aze-OH

	x	y	z	B_{iso}
C(0)	3240 (3)	3979 (3)	6909 (6)	294 (9)
C(1)	5228 (3)	5432 (3)	6346 (5)	271 (8)
C(1A)	4060 (3)	5000	4234 (6)	285 (9)
C(1B)	3251 (4)	5724 (3)	2305 (6)	350 (9)
C(1D)	1653 (3)	5088 (3)	4136 (7)	350 (10)
C(1G)	1710 (3)	5461 (3)	1667 (6)	402 (10)
C(2)	7533 (3)	6856 (3)	8254 (6)	303 (9)
C(2A)	7751 (3)	5871 (3)	7767 (6)	307 (9)
C(2B)	8439 (4)	5673 (3)	5735 (7)	403 (11)
C(2G)	7065 (4)	5218 (3)	4101 (6)	367 (10)
C(4)	4984 (4)	2958 (3)	9358 (6)	356 (10)
C(5)	6556 (3)	2968 (3)	10457 (6)	302 (8)
C(6)	7386 (4)	3327 (3)	9193 (7)	407 (11)
C(7)	8840 (4)	3318 (4)	10296 (9)	509 (14)
C(8)	9453 (4)	2946 (4)	12647 (8)	482 (12)
C(9)	8628 (5)	2585 (4)	13878 (8)	515 (13)
C(10)	7189 (4)	2584 (4)	12810 (7)	442 (12)
N(1)	2992 (3)	4622 (3)	5160 (5)	309 (7)
N(2)	6490 (3)	5442 (3)	6106 (5)	316 (7)
O(0)	2374 (2)	3695 (3)	7780 (5)	382 (7)
O(1)	5041 (2)	5774 (3)	8189 (4)	359 (7)
O(3)	4573 (2)	3696 (3)	7582 (4)	344 (6)
O(2')	6863 (3)	7371 (3)	6679 (5)	473 (8)
O(2")	8175 (2)	7080 (3)	10627 (4)	388 (7)
H(1A)	4472	4487	3310	282
H(1BA)	3555	5702	641	348
H(1BB)	3447	6394	3109	348
H(1DA)	787	4629	3839	348
H(1DB)	1562	5631	5371	348
H(1GA)	1410	4952	206	405
H(1GB)	1043	6046	1060	405
H(2A)	8246	5496	9487	304
H(2BA)	9327	5218	6338	407
H(2BB)	8727	6269	4908	407
H(2GA)	7143	4505	3763	365
H(2GB)	6548	5554	2317	365
H(4A)	4536	3046	10832	359
H(4B)	4625	2319	8419	359
H(6)	6910	3614	7344	403
H(7)	9490	3599	9322	499
H(8)	10574	2930	13493	484
H(9)	9117	2295	15720	513
H(10)	6562	2284	13802	446
H(2")	8118	7695	11182	382

0·71069 Å) and a graphite monochromator crystal were used. The specimen crystal of Z-Pro-Pro-OH measured 0·17 × 0·27 × 0·4 mm along a, b, and c, respectively, and it was mounted with c* approximately parallel to the diffractometer φ axis. The Z-Pro-Aze-OH specimen was larger; it measured 0·22 × 0·17 × 0·77 mm along a, b, and c, and it was mounted approximately parallel to d*($10\bar{1}$).

Lattice dimensions were determined by least-squares fit to manually optimized setting angles for reflections with $2\theta < 27°$. Intensity data were measured for $2\theta \leq 60°$, i.e. $(\sin \theta)/\lambda \leq 0·70 \mathring{A}^{-1}$. Reflections were

integrated as $\theta/2\theta$ scans of width $(1·5 + 0·692 \tan \theta)°$ (2θ). The scan rate was 2° (2θ) min^{-1}, and background was counted for 10 s at each end of each scan. The total numbers of reflections measured were 2952 for Z-Pro-Pro-OH and 2790 for Z-Pro-Aze-OH. Variations in the scaling factors derived from periodically measured reference reflection intensities were less than ±3%.

Structure-factor magnitudes were evaluated from net intensities according to: $F = (I/Lp)^{1/2}$ and $\sigma(F) = (F/2)\sigma(I)/I$ if $I \geq 2\sigma(I)$; $F = \sigma(F) = [0·5\sigma(I)/Lp]^{1/2}$ if $I < 2\sigma(I)$. The e.s.d. $\sigma(I)$ was based on counting

Table 2. *Refinement indices*

	Z-Pro-Pro-OH	Z-Pro-Aze-OH
$R = \sum |F_o - F_c|/\sum F_o$		
All data	0·114 (2543 data)	0·075 (2393 data)
Data with $w \neq 0$	0·064 (1425 data)	0·048 (1702 data)
$R_w = [\sum w(F_o - F_c)^2/\sum wF_o^2]^{1/2}$	0·064	0·051
$S = [\sum w(F_o - F_c)^2/(N_o - N_p)]^{1/2}$	1·51	1·30
N_o observations	1425	1702
N_p parameters	225	216

statistics only. Absorption effects were ignored, as transmission factors were calculated to vary only from 0·92 to 0·98. Extinction effects were also ignored, and subsequent agreement between observed and calculated structure factors confirmed that extinction was not significant in either crystal. After the data were reduced and replicate and equivalent measurements were averaged, there were 2543 data of which 1425 had $F > 2\sigma(F)$ in the Z-Pro-Pro-OH data set, and 2393 with 1702 having $F > 2\sigma(F)$ in the Z-Pro-Aze-OH set.

Both crystal structures were solved using the direct-methods program *QTAN* (Langs & DeTitta, 1975). The structures were refined by full-matrix least-squares iterations minimizing $\sum w(F_o - F_c/K)^2$, where $w = [\sigma^2(F_o) + q^2 F_o^2]^{-1}$ if $F_o \geq 2\sigma(F_o)$ and $w = 0$ if $F_o < 2\sigma(F_o)$. For both refinements a value $q = 0·025$ was assumed for the proportionality factor for instrumental uncertainties. Positional and anisotropic thermal vibration parameters for the non-H atoms were refined. Parameters for the H atoms were not refined, but were included in the structure-factor calculations. H-atom positional parameters were calculated assuming standard molecular geometry, and each H atom was assigned an isotropic thermal parameter equivalent (Hamilton, 1959) to the anisotropic parameters of the atom to which the H atom was bound. The calculated H-atom parameters were updated after every two or three cycles of refinement. Final parameters are listed in Table 1, and refinement indices are given in Table 2.* In the final cycles of each refinement, all parameter shifts were smaller than 0·15 e.s.d.'s. Final electron-density difference maps for each structure showed only random fluctuations, everywhere smaller in absolute value than 0·3 e Å^{-3}.

Results and discussion

Fig. 1 shows that the two crystals are isostructural. In each structure neighboring molecules related to each other by the twofold screw axis are linked through hydrogen bonds from the carboxylic hydroxyl group of one molecule to the urethane carbonyl O atom of the

* Tables of anisotropic thermal parameters and observed and calculated structure factors have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 36482 (26 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

next. The O···O distance in this hydrogen bond is 2·648 (9) Å in Z-Pro-Pro-OH and 2·669 (6) Å in Z-Pro-Aze-OH. Within experimental error, these distances are equal. There are no other hydrogen bonds in
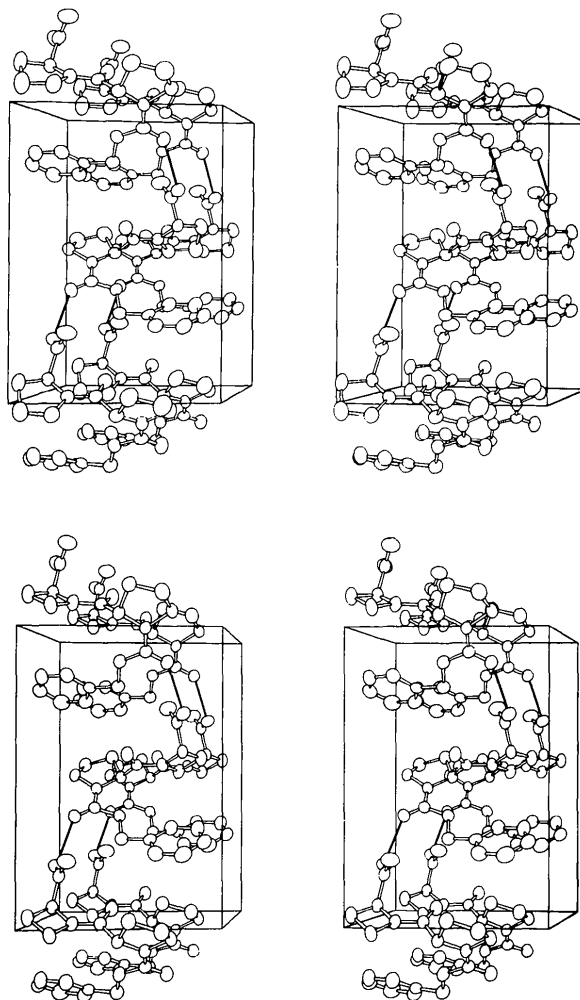

Fig. 1. Stereoscopic diagrams (Johnson, 1965) of the crystal structures of Z-Pro-Pro-OH (top) and Z-Pro-Aze-OH (bottom). In both diagrams the scale is the same, and the crystallographic a axis is horizontal, directed from left to right, and the b axis is vertical, directed from bottom to top.
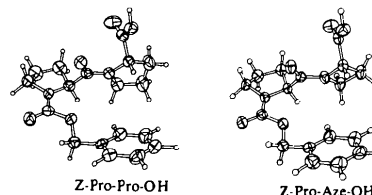

Z-Pro-Pro-OH   Z-Pro-Aze-OH

Fig. 2. Perspective drawings (Johnson, 1965) of the molecular structures. In both diagrams the scale is the same, and the orientation is as in Fig. 1. Non-H atoms are represented as 50% probability thermal vibration ellipsoids, and H atoms as spheres of radius 0·1 Å.
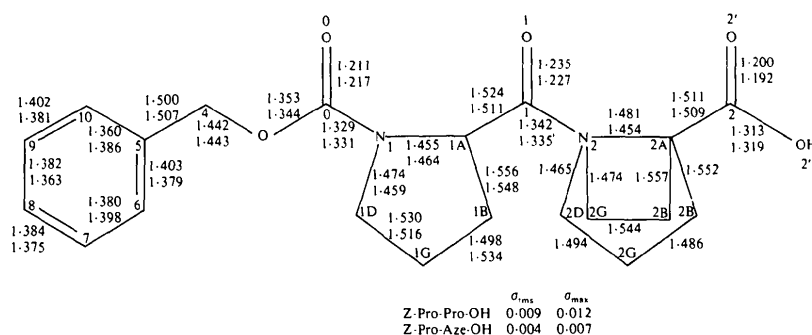
Fig. 3. Bond lengths (Å). The upper value in each pair of values is for Z-Pro-Pro-OH and the lower for Z-Pro-Aze-OH.
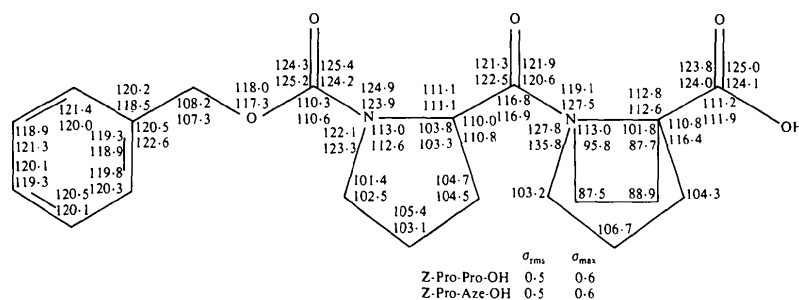

Fig. 4. Bond angles (°). The upper value in each pair of values is for Z-Pro-Pro-OH and the lower for Z-Pro-Aze-OH.

the structures, because the carboxyl group is the only group capable of being a hydrogen-bond donor.

The molecular structures and thermal vibration amplitudes are shown in Fig. 2. In each molecule the peptide bond is *trans*, but the urethane bond is *cis*, so that the benzyloxycarbonyl group is folded back on the dipeptide. The vibration amplitudes in the benzyl-oxycarbonyl group are about the same in both crystals, but the amplitudes in the Pro-Aze dipeptide are somewhat smaller than those in the Pro-Pro dipeptide. This is consistent with the facts that the Z-Pro-Aze-OH crystals are slightly denser and gave a larger number of data with $F > 2\sigma(F)$ (Table 2) than the Z-Pro-Pro-OH crystals, and with our finding that there are more short intermolecular contacts ($d < 3.5$ Å) involving Pro-Aze atoms than Pro-Pro atoms.

Numerical comparisons of intramolecular dimensions are presented in Figs. 3, 4 and 5. Fig. 5 also illustrates the result of a least-squares superposition of the two molecular structures that minimized the separations between the atoms of the Z-Pro-N(2)—C(2)α—COO portions of the molecules. The r.m.s. separation between the fitted atoms was 0·107 Å, and the maximum separation was 0·200 Å between the N(2) atoms. On the whole, chemically equivalent bond lengths and bond angles (Figs. 3 and 4) in the two molecules are equal within experimental error. The only bond lengths that differ by more than three e.s.d.'s of their difference are the lengths of the N(2)—C(2)α

bonds. Of course, the bond angles at the N(2) and C(2)α atoms differ significantly because the Pro(2) and Aze(2) rings are of different size.
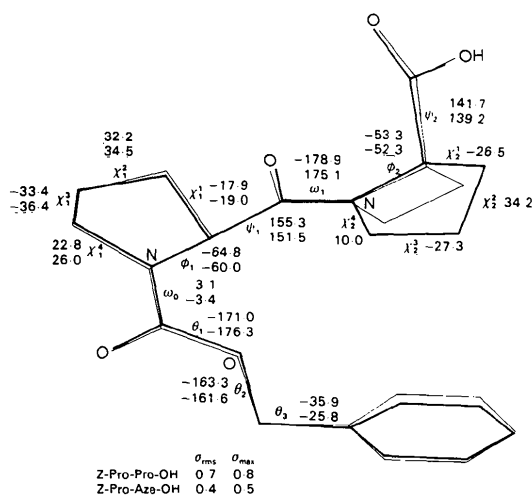

Fig. 5. Conformation angles (°) according to IUPAC–IUB Commission on Biochemical Nomenclature (1971). The angle ψ_2 is defined as involving the hydroxylic O atom. The upper value in each pair of values is for Z-Pro-Pro-OH, which is diagrammed in the heavier line, and the lower value is for Z-Pro-Aze-OH, diagrammed in the lighter line. The diagram represents a least-squares superposition of the molecular structures (see text). The orientation corresponds to Figs. 1 and 2.

All three Pro residues in these structures have C^γ exo with respect to the peptide chain (Fig. 2). In both Pro(1) residues the pyrrolidine rings have an 'envelope' conformation that approximates idealized mirror symmetry with the mirror passing through $C(1)\gamma$ and between $N(1)$ and $C(1)\alpha$. In the Pro(2) residue the ring has a 'half chair' conformation that approximates twofold axial symmetry with the axis passing through $N(2)$ and between $C(2)\beta$ and $C(2)\gamma$. In the Aze(2) residue the four-membered azetidine ring is flat (r.m.s. out-of-plane deviation is 0·004 Å).

The overall conformations of the two molecules (Figs. 2 and 5) hardly differ at all. The φ and ψ conformation angles at the Aze(2) residue do not differ from those at the Pro(2) residue by any more than the corresponding differences between the two chemically equivalent Pro(1) residues. Thus, substitution of Aze for Pro in a protein should be expected to affect the protein structure mainly through side-group steric effects and not directly through effects on the conformation of the main polypeptide chain.

References

BERMAN, H. M., McGANDY, E. L., BURGNER, J. W. II. & VAN ETTEN, R. L. (1969). *J. Am. Chem. Soc.* **91**, 6177–6182.
HAMILTON, W. C. (1959). *Acta Cryst.* **12**, 609–610.
IUPAC–IUB COMMISSION ON BIOCHEMICAL NOMENCLATURE (1971). *Biochem. J.* **121**, 577–585.
JOHNSON, C. K. (1965). *ORTEP.* Report ORNL-3794, revised. Oak Ridge National Laboratory, Tennessee.
LANGS, D. A. & DeTITTA, G. T. (1975). *Acta Cryst.* A**31**, S16.

The Structure of the 2:1 Complex between the Bile Acid Deoxycholic Acid and (+)-Camphor

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Abstract

The 2:1 deoxycholic acid–camphor complex is orthorhombic, $P2_12_12$, with $a = 27\cdot353$ (3), $b = 13\cdot814$ (2), $c = 7\cdot233$ (1) Å, $Z = 4(C_{24}H_{40}O_4\cdot\frac{1}{2}C_{10}H_{16}O)$/unit cell. The crystal structure was solved by direct methods and refined by least squares to $R = 0\cdot07$ for all 2933 measured X-ray reflections. As in other previously determined orthorhombic crystal complexes of this bile acid, the deoxycholic acid molecules form hydrogen-bonded bilayers stacked in an array which leaves hydrophobic channels between them. Filling the channels, which in this particular case are centered on crystallographic twofold axes and nearly cylindrical, are columns of camphor molecules distributed between two possible orientations related by the twofold axis. Comparison of this structure with other orthorhombic deoxycholic acid complexes shows that while the steroid bilayers are very similar in all cases, they are shifted parallel to each other in either or both of two directions, thus forming channels of differing sizes, shapes, and orientations, to accommodate guest molecules with optimal van der Waals guest–host interactions. Variations in geometric and/or electronic structure of guest molecules yield different complexes. The relations among the types so far observed are

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