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The Structure of the β Modification of Chloramphenicol Palmitate – a Redetermination

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Abstract

$C_{27}H_{42}Cl_2N_2O_6$, $M_r = 560 \cdot 6$, is orthorhombic, $P2_12_12_1$, with $a = 7 \cdot 805$ (3), $b = 52 \cdot 503$ (15), $c = 7 \cdot 414$ (2) Å, $U = 3038 \cdot 15$ Å^3, $Z = 4$, $D_x = 1 \cdot 226$ Mg m^{-3}. Final $R = 0 \cdot 064$ for 2378 reflections. All the H atoms were located. Thus, exact statements on the molecular geometry and especially on the hydrogen bonds are possible. There exists only one intermolecular O–H$\cdots$O hydrogen bond. The location of the H atoms shows that the intramolecular N–H$\cdots$O hydrogen bond [Eguchi & Iitaka (1974). *Acta Cryst.* B**30**, 2781–2783] does not exist. This result is in agreement with IR spectroscopic measurements.

Introduction

Several investigations on chloramphenicol palmitate (CAP) have been carried out to relate physicochemical properties with the therapeutic efficacy of this antibiotic (Tamura & Kuwano, 1961; Aguiar, Krc, Kinkel & Samyn, 1967; Aguiar & Zelmer, 1969; Miyamoto, Kiyotaki, Kisoh, Mitsunaga & Maeda, 1973; Andersgaard, Finholt, Gjermundsen & Hoyland, 1974; Burger, 1977; Szulzewsky, Kulpe, Schulz, Fichtner & Schinkowski, 1981). This contribution is concerned with a single-crystal X-ray analysis and IR spectroscopic measurements on the β modification of CAP. Crystallization proceeded in the following way: methods described by Burger (1977), Aguiar *et al.* (1967) and Tamura & Kuwano (1961) were used and crystals suitable for single-crystal diffractometry of the β modification and a crystalline powder of the α

modification were obtained. Crystals of the γ modification suitable for single-crystal diffractometry were obtained by heating a CAP–ethanol solution to ~350 K and then cooling to room temperature. Weissenberg and Guinier photographs of the γ modification indicated the space group $P2_1$ and lattice constants $a = 35 \cdot 528$ (20), $b = 16 \cdot 449$ (10), $c = 5 \cdot 185$ (6) Å, $\beta = 90 \cdot 15$ (4)° (Szulzewsky *et al.*, 1981). An attempt to reconcile the crystal structure of the β modification of CAP obtained by Eguchi & Iitaka (1974) with IR spectroscopic results failed. Neither the wavenumbers nor the shapes of all bands explain completely the proposed structure, especially the strong intramolecular hydrogen bond involving O(3) and N(1)–H. This prompted us to redetermine the structure of the β modification.

The intensities of the X-ray reflections were collected on a Hilger & Watts four-circle diffractometer operating in an ω-scan with Mo $K\alpha$ radiation ($\lambda = 0 \cdot 7107$ Å) monochromatized by a graphite crystal. 2395 reflections up to $\theta = 23°$ were measured. Intensities were corrected for Lorentz and polarization effects. The dimensions of the approximately cube-shaped crystal were $0 \cdot 35 \times 0 \cdot 25 \times 0 \cdot 40$ mm. For the structure determination the non-hydrogen parameters given by Eguchi & Iitaka (1974) were used to start with, and were refined by full-matrix least squares. The function minimized was $\sum w(|F_o| - |F_c|)^2$ with $w = 1$. Scattering factors for neutral Cl, O, N, C and H together with anomalous-dispersion terms for Cl were taken from *International Tables for X-ray Crystallography* (1974). The H atoms of tertiary CH groups, secondary CH$_2$ groups and aromatic CH groups were placed in their calculated positions with the CH$_3$, OH

Table 1. *Atomic positional parameters* ($\times 10^3$ *for H atoms and* $\times 10^4$ *for all others*) *and* U_{eq} *values* ($\text{Å}^2 \times 10^3$)

Equivalent isotropic thermal parameters for non-hydrogen atoms are calculated by $U_{eq} = \frac{1}{3} \sum_i \sum_j U_{ij} a_i^* a_j^* \mathbf{a}_i . \mathbf{a}_j$.

	x	y	z	U_{eq}
Cl(1)	11433 (2)	1000 (1)	2760 (3)	77
Cl(2)	8970 (3)	1188 (1)	179 (3)	100
C(1)	9973 (8)	912 (1)	1054 (8)	65
C(2)	8568 (8)	730 (1)	1657 (8)	56
C(3)	6918 (8)	550 (1)	4105 (7)	51
C(4)	5164 (8)	675 (1)	3894 (8)	62
C(5)	7401 (8)	476 (1)	6053 (8)	56
C(6)	8926 (8)	299 (1)	6088 (8)	54
C(7)	8699 (8)	43 (1)	5646 (8)	57
C(8)	10048 (8)	−123 (1)	5641 (8)	60
C(9)	11657 (8)	−28 (1)	6101 (9)	61
C(10)	11923 (8)	224 (1)	6542 (9)	65
C(11)	10564 (8)	388 (1)	6520 (8)	57
C(12)	4482 (8)	958 (1)	6361 (9)	64
C(13)	4826 (11)	1221 (1)	7008 (9)	74
C(14)	4136 (11)	1278 (1)	8864 (9)	79
C(15)	4658 (11)	1542 (1)	9516 (10)	81
C(16)	4021 (12)	1607 (1)	11388 (10)	86
C(17)	4617 (12)	1869 (1)	12028 (10)	92
C(18)	3946 (13)	1946 (1)	13867 (10)	93
C(19)	4608 (11)	2198 (1)	14547 (10)	87
C(20)	3914 (12)	2277 (1)	16372 (10)	94
C(21)	4630 (11)	2530 (1)	17060 (10)	88
C(22)	3920 (12)	2604 (1)	18881 (10)	94
C(23)	4645 (11)	2851 (1)	19618 (10)	91
C(24)	3887 (12)	2926 (1)	21437 (10)	98
C(25)	4615 (12)	3171 (1)	22218 (11)	96
C(26)	3805 (15)	3241 (2)	23980 (11)	115
C(27)	4498 (14)	3482 (2)	24779 (14)	144
O(1)	7787 (6)	612 (1)	483 (5)	69
O(2)	5173 (6)	928 (1)	4691 (6)	62
O(3)	7772 (6)	714 (1)	6949 (5)	63
O(4)	3733 (6)	794 (1)	7134 (5)	86
O(5)	12858 (6)	−431 (1)	5978 (5)	102
O(6)	14563 (6)	−115 (1)	6315 (5)	105
N(1)	8266 (6)	710 (1)	3400 (6)	51
N(2)	13144 (7)	−203 (1)	6149 (8)	74
H(30)	785	66	836	
H(N1)	915	80	397	
H(101)	1073	81	6	
H(103)	680	38	333	
H(104)	421	56	456	
H(204)	486	69	248	
H(105)	638	37	671	
H(107)	735	2	536	
H(108)	1021	−32	535	
H(110)	1327	24	683	
H(111)	1040	59	678	
H(113)	620	125	703	
H(213)	425	135	607	
H(114)	275	127	882	
H(214)	462	114	980	
H(115)	416	168	858	
H(215)	604	155	952	
H(116)	264	160	1138	
H(216)	449	147	1232	
H(117)	419	201	1106	
H(217)	600	187	1208	
H(118)	431	180	1482	
H(218)	257	196	1379	
H(119)	599	219	1464	

Table 1 (*cont.*)

	x	y	z
H(219)	426	234	1358
H(120)	254	229	1627
H(220)	423	213	1734
H(121)	431	268	1610
H(221)	601	251	1717
H(122)	255	262	1876
H(222)	421	245	1983
H(123)	601	283	1977
H(223)	438	300	1866
H(124)	413	277	2238
H(224)	252	295	2127
H(125)	440	332	2127
H(225)	597	315	2243
H(126)	244	326	2377
H(226)	402	309	2493
H(127)	386	352	2604
H(227)	428	364	2386
H(327)	586	346	2501

and NH groups oriented as found in a difference map. A fixed isotropic temperature factor ($U_{iso} = 0.06$ Å^2) was applied to all H atoms. 17 reflections clearly in error were omitted. The final $R = 0.064$ for 2378 reflections. Atomic parameters are listed in Table 1.* The final R of Eguchi & Iitaka (1974) calculated with 1249 reflections was 0·14.

Infrared spectra of the β modification of CAP were measured with a pressed disk of 2 mg CAP and 797 mg KBr and a DIGILAB FTS 20 spectrometer (200 scans with data-averaging for a resolution of 4 cm^{-1}).

Discussion

Bond distances and angles are shown in Fig. 1. The e.s.d.'s for the distances and angles, with the corresponding values obtained by Eguchi & Iitaka (1974) in parentheses, are: for the distances $\sigma = 0.006$ to 0·01 Å (0·03 Å), for the angles $\sigma = 0.5$ to 0·7° (2°). The proposed (Eguchi & Iitaka, 1974) packing of the molecules in the crystal is basically confirmed. However, many bond lengths and angles of our structure correspond better to the expected values than do those of Eguchi & Iitaka (1974). The mean C—C distance of the palmitoyl group is 1·515 Å with $\sigma = [\sum_N (X_N - \bar{X})^2 / (N - 1)]^{1/2} = 0.009$ Å, the mean C—C—C angle is 113·6° ($\sigma = 0.9°$). These values are in good agreement with the usual values for aliphatic long-chain molecules of 1·51 Å and 113·6° respectively (Sundell, 1977). The deviations from the ideal values of 1·54 Å and 112° (Kitaigorodsky, 1973) are probably due to thermal vibrations. Exact statements on the hydrogen bonds

and the configuration of functional groups may be derived from the positions of the H atoms. The intermolecular hydrogen bond O(3)–H(30)···O(1¹) may be seen in the stereoview in Fig. 2. The corresponding distances are given in Fig. 3. Further values of interest are O(3)···O(1¹) = 2·675 (11) Å and O(3)–H(30)···O(1¹) = 176·8°. H(30) lies close to the line connecting O(3) to O(1¹). This indicates a strong hydrogen bond. The intramolecular N(1)–H···O(3) contact, as well as the associated distances and angles, is shown in Fig. 4. Despite the relatively short distance N(1)···O(3) = 2·660 (11) Å there is no hydrogen

bond: this follows from the distance H(N1)···O(3) = 2·494 Å and the angle N(1)–H(N1)···O(3) = 89·7° (Schuster, Zundel & Sandorfy, 1976). N(1) and O(3) are each bonded to a tetrahedrally coordinated C(sp^3) atom in a staggered arrangement relative to one another, and with N(1) and O(3) in synclinal positions (Fig. 5). This explains the relatively short distance N(1)···O(3). If one of the two atoms concerned is chemically bonded to a H atom, this type of short N to O contact does not necessarily result in a hydrogen bond.
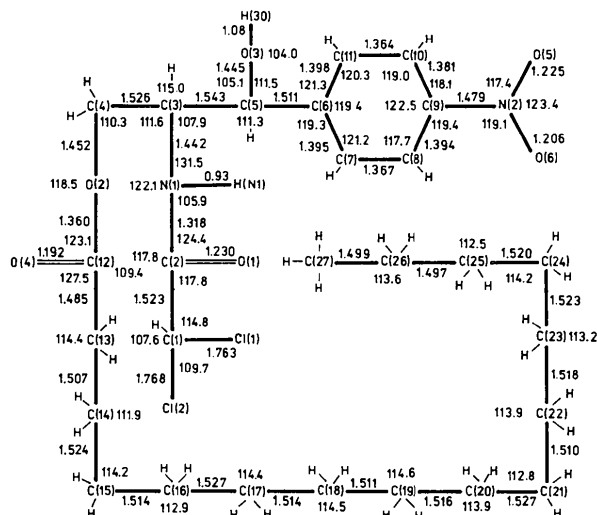
The configuration of the (group) is flat

and has a *trans* conformation. The conformation, as well as the distances and angles of this group, is in good agreement with those of peptide groups, as obtained from numerous X-ray analyses of amino acids and peptides (Dickerson & Geis, 1971).

Fig. 1. Bond lengths (Å) and angles (°) in the β modification of chloramphenicol palmitate.
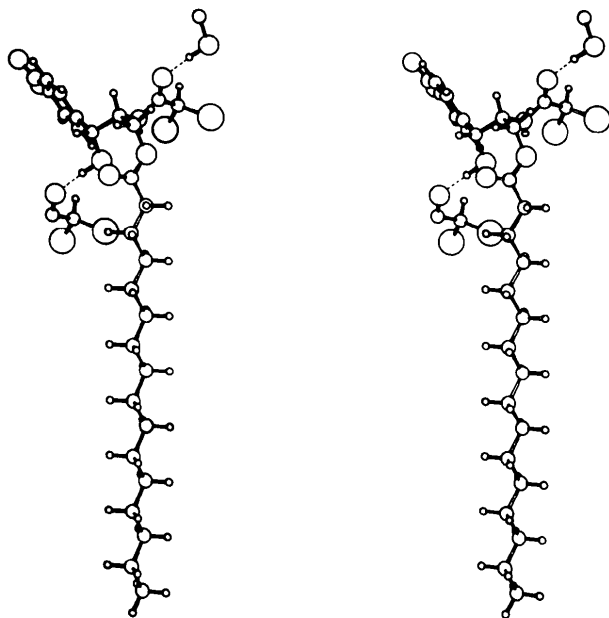
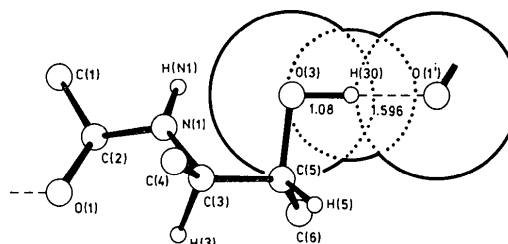Fig. 2. Stereoscopic view of the molecule showing hydrogen bonding.

Fig. 3. The intermolecular O(3)–H(30)···O(1¹) hydrogen bridge (distances in Å). Symmetry-equivalent atoms denoted by (i) correspond to $x, y, 1 + z$.
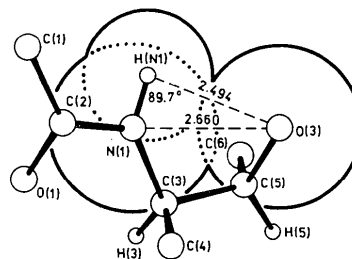

Fig. 4. The intramolecular N(1)···O(3) interaction (distances in Å). E.s.d.'s for distances not including H atoms are 0·011 Å.
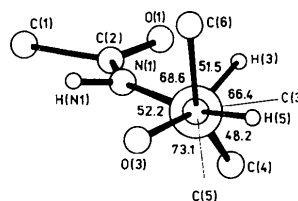

Fig. 5. A Newman projection along C(5)–C(3) showing torsion angles (°). E.s.d.'s for torsion angles not including H atoms are ≤0·7°.
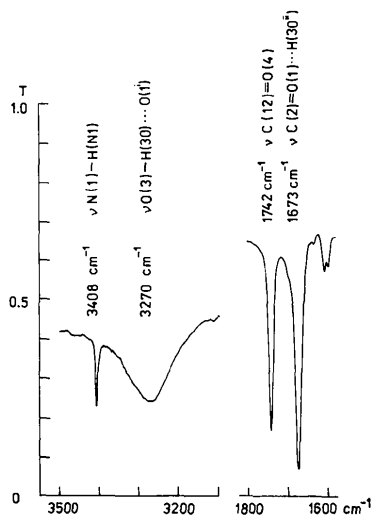
Fig. 6. Selected parts of the IR spectrum of the β modification with peak interpretation. Symmetry-equivalent atoms are denoted by (i) $x, y, 1 + z$, (ii) $x, y, z - 1$.

molecule, especially to the C atom moiety of the chloramphenicol part, are, in our opinion, of particular importance for the therapeutic efficacy of CAP. Certain observations suggest that in different modifications of CAP different conformers may be realized and that the difference in therapeutic efficacy may be due to this fact. Thus, the enzymatic hydrolysis separating the chloramphenicol part from the palmitoyl group, which is a precondition for the therapeutic effect and occurs with the α modification, might be inhibited in the β modification because of the different conformer present. Work to elucidate these questions is in progress.

Our results are in excellent agreement with the vibrational spectrum of the β modification of CAP. The relevant parts of the infrared spectrum are shown in Fig. 6. The intermolecular hydrogen bond linking the O(1) amide carbonyl with the O(3) secondary hydroxyl group gives rise to the absorption bands at 1673 and 3270 cm^{-1}. Both wavenumbers are considerably lower than the stretching vibrations of the undisturbed groups (1710 and 3595 cm^{-1}). The hydroxyl band is broadened and of a shape similar to bands of hydrogen bonds appearing in molecular crystals. The very small band at 3408 cm^{-1} is undoubtedly due to a hydrogen stretching vibration of a free imide group, namely N(1)—H. Furthermore, the O(4) of the ester carbonyl of the palmitoyl group does not interact with any electron acceptor: this may be concluded from the fact that the wavenumber of the carbonyl stretching vibration is at the expected position.

The dichloroacetamido group and its stereochemical arrangement relative to the other parts of the CAP

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