THE CRYSTAL STRUCTURE AND ABSOLUTE CONFIGURATION OF FUSIDIC ACID METHYL ESTER 3-p-BROMOBENZOATE

A. COOPER* and D. C. HODGKIN

Chemical Crystallography Laboratory, South Parks Road, Oxford

Abstract—The crystal and molecular structure of the steroid antibiotic, fusidic acid, has been determined by X-ray diffraction methods. The structure was solved from the 3-dimensional Patterson and Fourier syntheses and was refined by block-diagonal least-squares to a final R factor of 0·15.

The steroid skeleton contains the unusual configurations 3α-OH, 4α-CH₃, 5α-H, 8α-CH₃, 9β-H, 10β-CH₃, 11α-OH, 13α-H, 14β-CH₃, 16β-OCOCH₃ and all ring junctions are *trans* fused with rings A and C *syn* to ring B. Rings A and C have chair conformations while ring B is a flattened boat. Comparison with other boat-shaped cyclohexane rings leads to the proposal that cyclohexane rings of this conformation may normally be flattened unless constrained to take up the more regular conformation usually assigned.

INTRODUCTION

FUSIDIC acid, first isolated in 1962[1] has been particularly successful in combatting staphylococcal infections, especially where the causative micro-organism has become resistant to other antibacterial agents. Preliminary chemical investigations suggested that the anitobiotic was a steroid of unusual stereochemistry, a first example of a non rearranged cyclization product of squalene, folded in the chair-boat-chair conformation. Details of the molecular structure proposed for fusidic acid (I) corresponded closely with features of the hypothetical carbonium ion (II)
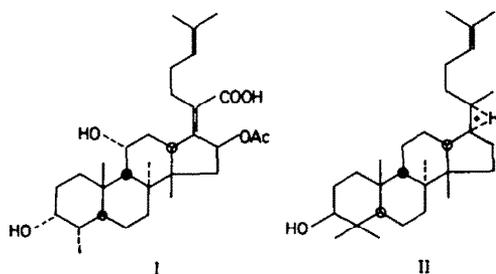
postulated in 1955 by Eschenmoser[4] to explain the cyclization of squalene to form cholesterol.

The structure of fusidic acid shown in I has now been fully confirmed by further chemical investigations and by a parallel X-ray crystallographic analysis. The chemi-

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* Present address: Centre for Crystallographic Research, Roswell Park Memorial Institute, 666 Elm Street, Buffalo, New York 14203.

cal researches have already been published[5] together with a preliminary crystallographic report.[6] The full X-ray analysis is reported below.
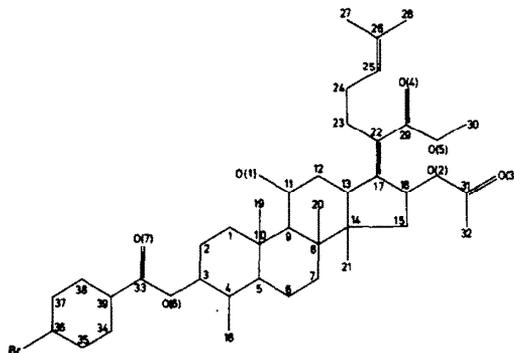

FIG. 1 Schematic diagram of fusidic acid giving the numbering system used to identify the atoms. Hydrogen atoms are given the same number as the carbon atom to which they are attached.

DISCUSSION

The principal stereochemical features of the molecule may be seen in Fig. 2. There are ten asymmetric centres in the nucleus and these were found to have the configurations 3α-(OH), 4α-CH$_3$, 5α-H, 8α-CH$_3$, 9β-H, 10β-CH$_3$, 11α-OH, 13α-H, 14β-CH$_3$, and 16β-OCOCH$_3$. All the ring junctions are *trans* fused and rings A and C are *syn* to ring B. The A/B ring junction is normal but the *trans* B/C ring junction has the opposite configuration to that normally found in natural steroids. As required by this configuration, ring B has the boat shaped conformation suggested by the mechanism proposed earlier for steroid biogenesis.[4] The C/D ring junction has normal
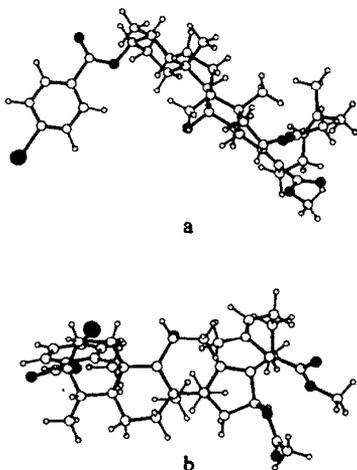

FIG. 2. Perspective views on the molecule:
(a) projection along the *a*-axis of the unit cell. Bromine ● ; Oxygen ● ; Carbon ○; Hydrogen ○.
(b) projection onto the least-squares plane through atoms C1 to C17.

stereochemistry but the configuration 13α-H, 14β-CH₃ is opposite to the normally found 13β and 14α functions.

Rings A, B and C were found to have chair, boat and chair conformations respectively and ring D has an envelope conformation. Examination of the dihedral angles for the steroid rings (Table 4) shows that rings C and D have normal geometry but rings A and B are severely flattened. In ring B, this flattening is due to the interaction between the 5α-H atom and the 8α-CH₃ group where the distance between C5 and C8 has increased from 2·57 Å, for an unstrained ring, to 2·78 Å. Chemical reactions suggesting the existence of strain in this system were observed by Arigoni.[4] Fig. 3 shows projections of ring B down both the C7—C6 and C10—C9 bonds. This figure clearly shows the type of strain present in the ring, which is a twisting about the C6—C7 bond producing a torsional angle C5—C6—C7—C8 of 30°, compared with the C5—C10—C9—C8 torsional angle of 4°. In view of the discontinuity introduced into the ring system of the steroid nucleus by ring B, the nonplanarity coefficient of 0·31 for the nucleus agrees surprisingly well with those values found for other steroids.[7]

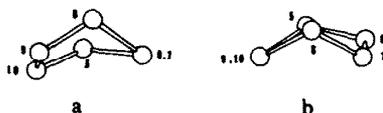FIG. 3 Projections of the B ring of the steroid nucleus showing the twisting of the ring about the C6—C7 bond:
    (a) projection along the C7—C6 bond.
    (b) projection along the C10—C9 bond.

The dihedral angles in the boat-shaped ring B are 132° and 145° (Table 4b) and it is interesting to compare them with those found in other boat-shaped rings, *viz.* 139° in cyclohexane-1,4-dione,[8] 114° in cyanocamphor,[9] 123° in *cis*-dinitroso-dichloro-cyclohexane[10] and 125° in 11,12-dibromo-3α,9-epoxy-cholanic acid methyl ester.[11] In all these examples except in cyclohexane-1,4-dione, the cyclohexane ring is held rigidly in the boat-shaped configuration by bridging between the "bow" and the "stern" and in these cases the dihedral angles are close to 120°. However in both fusidic acid and cyclohexane-1,4-dione no such constraint exists and the dihedral angles are in the region of 140° in both cases. It is possible then, that in fusidic acid, the flattening of the B ring may be as much due to the nature of the ring itself as to environmental factors.

The geometry of the side chains at ring D may be reduced to four essentially planar regions as shown in Fig. 4, and in each case the RMS deviation of the particular atoms from their respective planes is less than 0·03 Å. The major side chain attached at C17 is not elongated as is the case with saturated side chains, but curls back on itself as may be seen in Fig. 2. The configuration characteristic of this particular side chain is partially influenced by the formation of a weak interaction between one of the hydrogen atoms (H24B) attached to C24 and the carboxyl oxygen atom O4. Angular and distance considerations show that this interaction is not a normal hydrogen bond, but it may be regarded as a diffuse interaction of the hydrogen atom with the π electron distributions of O4, C29 and the unsaturated atom C22.
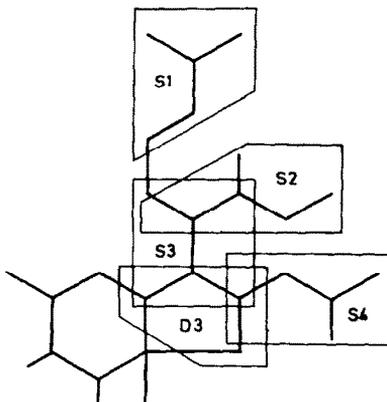
FIG. 4 Subdivisions of the ring D side chains into their major planar regions.

Similar interactions have been described by Mathieson[12] to explain certain preferred conformations of ester groups in relation to saturated ring systems, as for example, in the case of the bromobenzoate group attached to ring A of the steroid nucleus. The ester part of the bromobenzoate group (C3, O6, C33 and O7) is planar, and, in agreement with Mathieson's theory, H3 lies almost in this plane, 2·27 Å from the carboxyl oxygen atom O7, as shown in Fig. 6a. The analogy between this conformation and that found in the side chain as described above, may be seen in Fig. 6b, in which one of the atoms in the relevant portion of the side chain has been
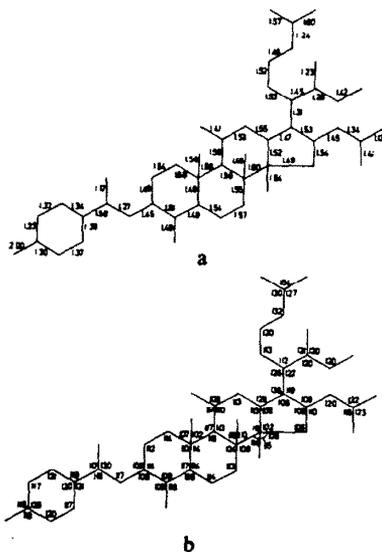

a

b

FIG. 5

(a) Interatomic distances expressed in Angstrom units (standard deviations 0·03 Å increasing to 0·06 Å at the ends of the side chains).

(b) Interatomic angles expressed in degrees (standard deviations 2° increasing to 4° at the ends of the side chains).

"eliminated" by projecting the atoms down the C22—C23 bond. The methylated acid group (C30, O5, C29, O4 and C22) is planar (side chain plane S2) and atom H24B lies 0·41 Å out of this plane, 2·32 Å from the carboxyl oxygen atom O4 (cf. Fig. 2b).
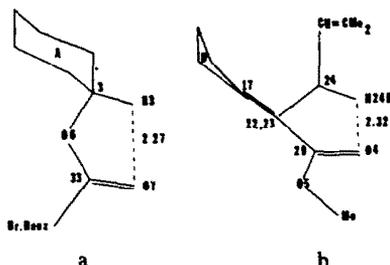

FIG. 6. Conformations of the two side chain ester groups:
(a) Projection down the *a*-axis of the unit cell, of ring A and portion of the bromobenzoate group;
(b) Projection along the C22—C23 bond, of ring D and of the major side chain. Both drawings are to the same scale but (b) is distorted by the projection.

At the other end of the molecule, the bromobenzoate group shows some minor distortions. The aromatic ring is planar to within 0·01 Å and the bromine atom lies 0·07 Å out of this plane. The ester part of the group (C33, O6, O7 and C3), as mentioned above, is planar, but due to distortions introduced by intermolecular packing forces involving the bromobenzoate group, this plane lies at 19° to the plane of the aromatic ring. The least-squares plane through the ten atoms of the bromobenzoate group makes an angle of 63° with the plane of the steroid nucleus and is perpendicular to the (010) face of the crystal, to within one standard deviation.

In the rigid portion of the steroid nucleus, the bond distances and angles have calculated standard deviations of 0·03 Å and 2° respectively. In the side chains the standard deviations increase gradually along the length of the side chains to 0·06 Å and 4° for the main chain and to 0·04 Å and 3° for the others. With these standard deviations, no bond length differs from the expected values except for C36—C37 (Fig. 5). The extreme shortening of this bond from the expected value of 1·39 Å cannot be explained on any chemical grounds but similar shortening of one of the bonds *ortho* to the heavy atom in halogen substituted aromatic systems has been noted in bromomiroestrol[13] and in suprasteryl II 4-iodo-5-nitrobenzoate[14] and this effect seems to be related to an anomolous diffraction effect due to the heavy atom.[15] The bond angles in the steroid nucleus are generally larger than the expected values for tetrahedral carbon atoms, but this enlargement is in accord with observations for other steroids.[7] There are two regions in which the bond angles depart considerably from the expected values. At C17, the point of attachment of the major side chain, the planarity imposed on the atoms C13, C16, C17, C22, C23 and C29 by the unsaturated bond C17═C22 brings the β hydrogen atom of C23 into the proximity of the two hydrogen atoms at C12. In order to overcome the steric hindrance between these three hydrogen atoms, the angle C13—C17—C22 has become enlarged. This steric hindrance may also contribute to the formation of the H24B—O4 interaction,

since the simultaneous enlargement of the angles C17—C22—C23 and C13—C17—C22 brings the β hydrogen atom at C24 and the carboxyl oxygen atom, O4, into the correct relationship to assist in this interaction. At the end of the main side chain the angles at C25 and C26 have standard deviations in the range 3–4°. Even so, these angles are significantly different from the expected ones and since this end of the side chain carries a number of the intermolecular packing contacts, it is likely that these have caused the observed distortions.

The packing of the molecules is shown in Figs. 7 and 8. In the steroid structures which have been determined by X-ray methods, angular side chains containing
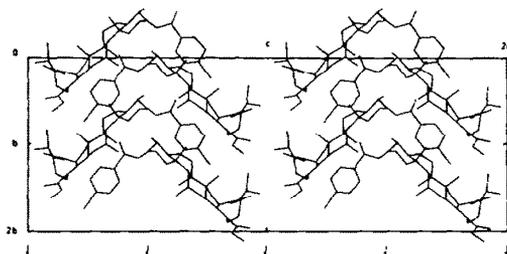
FIG. 7 Projection of four unit cells onto the plane (100).
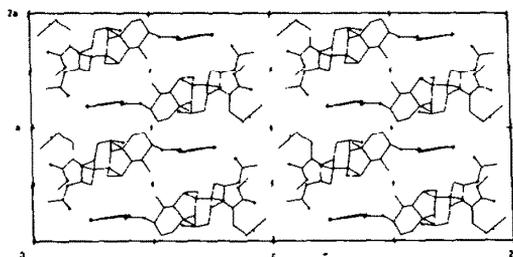
FIG. 8 Projection of four unit cells onto the plane (010).

oxygen atoms are usually found to be in comparatively close contact with hydrogen atoms of neighbouring molecules, allowing the steroid nuclei to pack closely, often with the assistance of intermolecular hydrogen bonding.[7] In fusidic acid, the number of axial groups on both the α and the β surfaces of the steroid nucleus prevents minimization of contacts between these groups by the simple staggering of molecules. There are no intermolecular distances less than 3·1 Å and of those less than 4·0 Å, only nine are less than 3·6 Å. These intermolecular contacts are summarized in Table 5. Weak van der Waals forces between the bromobenzoate groups and the remainder of adjacent molecules play the major part in the packing of the molecules (average distance 3·75 Å). The acetate groups carry the majority of the closer contacts (average 3·30 Å), although the benzoate, axial methyls, acid and main side chain groups all have some contacts in the range 3·1–3·6 Å. The bromine atoms

take little part in the packing, the closest contacts to them being with oxygen atoms (O3 and O7) at 3·65 and 3·81 Å.

TABLE 1. RÉSUMÉ OF THE OBSERVED AND CALCULATED STRUCTURE FACTORS AT THE END OF THE REFINEMENT

$|F_o|$ Range	Number of reflections	Average $|F_o|$	Average $|\Delta|$	R factor
0·0– 4·9	607	3·68	1·01	0·27
5·0– 9·9	759	7·87	1·79	0·23
10·0– 14·9	478	12·69	2·34	0·19
15·0– 19·9	265	17·72	2·41	0·14
20·0– 29·9	280	24·37	2·91	0·12
30·0– 39·9	103	35·26	3·17	0·09
40·0– 49·9	59	44·42	4·15	0·09
50·0– 74·9	69	60·12	5·99	0·10
75·0– 99·9	12	86·49	8·18	0·07
100·0–149·9	9	122·80	8·26	0·07
150·0–	1	158·60	2·18	0·01

TABLE 2. COMPARISON OF SELECTED INTENSITIES CALCULATED FOR TWO ENANTIOMORPHS, AS USED IN DETERMINING THE ABSOLUTE CONFIGURATION OF THE MOLECULES

h	k	l	$I_c(hkl)$	$I_c(h\bar{k}l)$	h	k	l	$I_c(hkl)$	$I_c(h\bar{k}l)$
0	1	1	530	929	0	3	10	190	223
0	2	7	158	201	0	4	3	677	635
0	2	8	893	780	5	3	1	349	325
0	2	10	158	121	5	3	$\bar{14}$	105	90
0	2	12	260	286	5	4	3	291	279

TABLE 3. ATOMIC PARAMETERS FOR THE ABSOLUTE CONFIGURATION OF THE MOLECULE. STANDARD DEVIATIONS FOR EACH PARAMETER ARE GIVEN IN PARENTHESES FOR THE LAST TWO DIGITS OF EACH NUMBER

Atom	x/a	y/b	z/c	B(iso)
Br	0·1861(3)	0·7500(6)	0·2368(1)	6·13(—)†
C1	0·0782(19)	0·2256(29)	0·5796(9)	4·01(37)
C2	0·0468(24)	0·0847(34)	0·5334(11)	5·15(49)
C3	0·1644(21)	0·0269(29)	0·5017(10)	4·24(40)
C4	0·2859(21)	−0·0142(27)	0·5464(9)	4·01(38)
C5	0·3038(19)	0·1318(26)	0·5884(9)	3·68(35)
C6	0·4269(24)	0·1006(32)	0·6281(11)	5·02(47)
C7	0·4338(24)	0·2069(33)	0·6879(11)	5·35(51)
C8	0·3626(20)	0·3777(27)	0·6802(9)	3·89(37)
C9	0·2174(19)	0·3270(25)	0·6714(9)	3·58(34)
C10	0·1883(19)	0·1853(24)	0·6229(9)	3·59(35)
C11	0·1219(23)	0·4848(31)	0·6718(10)	4·72(44)
C12	0·1442(22)	0·5884(29)	0·7295(10)	4·40(42)
C13	0·2884(19)	0·6316(25)	0·7426(9)	3·45(33)

TABLE 3—continued

Atom	x/a	y/b	z/c	B(iso)
C14	0·3759(20)	0·4771(26)	0·7432(9)	3·56(34)
C15	0·5063(26)	0·5538(37)	0·7557(12)	5·52(51)
C16	0·4826(21)	0·7027(28)	0·7985(10)	4·41(42)
C17	0·3368(18)	0·7358(30)	0·7937(8)	3·91(34)
C18	0·3963(27)	−0·0658(38)	0·5107(12)	5·94(57)
C19	0·1437(24)	0·0358(33)	0·6628(11)	5·07(47)
C20	0·4174(27)	0·4769(39)	0·6316(12)	5·87(55)
C21	0·3409(25)	0·3698(35)	0·7986(12)	5·34(50)
C22	0·2853(21)	0·8414(29)	0·8315(10)	4·11(39)
C23	0·1425(25)	0·8942(34)	0·8315(11)	5·18(49)
C24	0·0687(27)	0·8190(37)	0·8837(12)	5·84(55)
C25	0·0486(28)	0·6376(41)	0·8868(13)	6·37(60)
C26	0·1001(32)	0·5277(48)	0·9189(15)	7·59(75)
C27	0·0786(39)	0·3318(61)	0·9186(18)	9·42(99)
C28	0·2014(39)	0·5572(66)	0·9740(18)	9·46(01)
C29	0·3648(29)	0·9382(42)	0·8743(13)	6·50(62)
C30	0·5495(30)	1·0954(44)	0·8994(14)	6·76(65)
C31	0·6415(26)	0·6466(37)	0·8785(12)	5·60(51)
C32	0·6643(34)	0·5966(54)	0·9390(16)	8·35(84)
C33	0·2075(20)	0·1217(27)	0·4052(9)	3·96(38)
C34	0·2329(24)	0·4407(33)	0·3873(11)	5·04(47)
C35	0·2230(22)	0·5739(31)	0·3483(11)	4·65(44)
C36	0·2045(20)	0·5477(27)	0·2904(9)	3·80(37)
C37	0·1897(27)	0·4003(39)	0·2653(13)	6·04(57)
C38	0·1978(20)	0·2628(32)	0·3028(9)	4·27(38)
C39	0·2174(20)	0·2811(27)	0·3627(9)	4·14(41)
O1	0·1393(16)	0·5881(24)	0·6209(8)	5·48(35)
O2	0·5160(14)	0·6564(20)	0·8606(7)	4·46(28)
O3	0·7223(26)	0·6792(41)	0·8473(13)	10·34(72)
O4	0·3316(25)	0·9571(40)	0·9271(12)	9·57(64)
O5	0·4687(16)	1·0087(23)	0·8569(7)	5·30(33)
O6	0·2040(14)	0·1593(19)	0·4612(6)	4·27(27)
O7	0·2065(16)	−0·0106(22)	0·3815(7)	5·10(32)
H1A	−0·0073	0·2487	0·6055	
H1B	0·1028	0·3381	0·5549	
H2A	−0·0229	0·1318	0·5001	
H2B	0·0056	−0·0209	0·5569	
H3	0·1404	−0·0841	0·4754	
H4	0·2591	−0·1212	0·5736	
H5	0·3266	0·2377	0·5599	
H6A	0·5104	0·1313	0·6018	
H6B	0·4299	−0·0312	0·6402	
H7A	0·5343	0·2305	0·7001	
H7B	0·3892	0·1362	0·7236	
H9	0·1970	0·2647	0·7136	
H11	0·0232	0·4391	0·6706	
H12A	0·1086	0·5174	0·7673	
H12B	0·0904	0·7047	0·7249	
H13	0·3167	0·7007	0·7029	
H15A	0·5477	0·5974	0·7142	
H15B	0·5707	0·4631	0·7772	

TABLE 3—*continued*

Atom	x/a	y/b	z/c
H16	0·5367	0·8123	0·7849
H18A	0·4217	0·0348	0·4803
H18B	0·3709	−0·1769	0·4846
H18C	0·4780	−0·0936	0·5407
H19A	0·1213	−0·0722	0·6347
H19B	0·0588	0·0712	0·6875
H19C	0·2230	0·0068	0·6941
H20A	0·5187	0·5005	0·6420
H20B	0·3662	0·5953	0·6276
H20C	0·4084	0·4098	0·5891
H21A	0·2404	0·3316	0·7950
H21B	0·3568	0·4416	0·8397
H21C	0·4030	0·2602	0·7990
H23A	0·0965	0·8537	0·7894
H23B	0·1378	1·0298	0·8347
H24A	−0·0261	0·8766	0·8826
H24B	0·1204	0·8549	0·9249
H25	−0·0247	0·5907	0·8552
H27A	0·1000	0·2942	0·8729
H27B	−0·0238	0·3170	0·9257
H27C	0·1237	0·2356	0·9466
H28A	0·1518	0·6383	1·0051
H28B	0·2810	0·6250	0·9546
H28C	0·2464	0·4610	1·0021
H30A	0·5778	1·0054	0·9340
H30B	0·4909	1·1930	0·9191
H30C	0·6373	1·1551	0·8847
H32A	0·7176	0·6983	0·9606
H32B	0·7252	0·4861	0·9372
H32C	0·5868	0·5654	0·9689
H34	0·2521	0·4588	0·4352
H35	0·2304	0·7011	0·3655
H37	0·1718	0·3869	0·2171
H38	0·1882	0·1379	0·2838

† Equivalent B(iso). The anisotropic thermal parameters for the bromine atom, in the form $T = \exp - (10^5 \times \Sigma\, b_{ij}h_ih_j)$ are:

Atom	b_{11}	b_{22}	b_{33}	b_{12}	b_{13}	b_{23}
Br	1610(31)	2456(47)	277(6)	80(20)	−54(11)	299(16)

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TABLE 4a. LEAST-SQUARES PLANES THROUGH THE ATOMS†

Plane	Atoms	l	m	n	p
A1	C2, C3, C4	0·3116	0·9474	− 0·0724	0·167
A2	C1, C2, C4, C5	− 0·2675	− 0·5948	0·7581	− 8·492
A3B1	C1, C5, C6, C10	0·2867	0·9555	− 0·0696	− 0·921
B2	C6, C7, C9, C10	0·3102	0·7133	− 0·6285	6·999
B3C1	C7, C8, C9, C11	− 0·1151	− 0·0064	0·9933	− 14·544
C2	C8, C11, C12, C14	0·3334	0·7904	− 0·5140	4·265
C3D1	C12, C13, C14, C15	− 0·1605	− 0·1061	0·9813	− 15·133
D2	C13, C15, C16, C17	0·2446	0·6833	− 0·6880	7·267
D3	C14, C15, C16, C17	− 0·0513	− 0·5290	0·8471	− 11·646
A	C1, C2, C3, C4, C5, C10	0·3091	0·7796	− 0·5447	5·633
B	C5, C6, C7, C8, C9, C10	0·2986	0·6730	− 0·6767	7·594
C	C8, C9, C11, C12, C13, C14	− 0·2890	− 0·6001	0·7459	− 8·707
D	C13, C14, C15, C16, C17	− 0·2222	− 0·5436	0·8094	− 10·208
C1–C17		0·3201	0·6297	− 0·7078	7·848
S1	C24, C25, C26, C27, C28	0·7685	− 0·1071	− 0·6309	12·886
S2	C22, C29, C30, O4, O5	− 0·4887	0·8346	− 0·2543	0·263
S3	C13, C16, C17, C22, C23, C29	0·1777	0·7343	− 0·6552	6·638
S4	C16, C31, C32, O2, O3	− 0·0466	0·9564	0·2883	− 10·214

† The planes are in the form $lX + mY + nZ + p = 0$, where X, Y, Z and p are in Å units and are referred to an orthogonal coordinate system with $X \| a$, $Y \| cxa$ and $Z \| ax(cxa)$.

TABLE 4b. INTERPLANAR ANGLES

Plane 1	Plane 2	Angle
A1	A2	135°
A3B1	A2	134°
A3B1	B2	35°
B3C1	B2	132°
B3C1	C2	124°
C3D1	C2	130°
C3D1	D2	142°
C3D1	D3	26°
A	B	10°
B	C	174°
C	D	6°
C1–C17	(100)	71°
C1–C17	(010)	51°
C1–C17	(001)	135°
S1	D3	59°
S2	D3	129°
S4	D3	105°
S2	S4	42°

TABLE 4c. DEVIATIONS (IN Å UNITS) FROM THE LEAST-SQUARES PLANES

Atom	A	B	C	D	A2	A3B1	B2	B3C1	C2	C3D1	D2	D3	C1·C17
C1	0·20				0·02	0·03							0·07
C2	-0·20				-0·01								-0·01
C3	0·20				-0·60								0·59
C4	-0·21				0·01								0·08
C5	0·24	0·34			-0·02	-0·03	0·45						0·21
C6		-0·05				0·03	0·11						-0·17
C7		-0·36					-0·11	0·05					-0·56
C8		0·45	-0·27				0·74	-0·04	0·03				0·18
C9		-0·14	0·25				0·11	-0·06	-0·69				-0·41
C10	-0·22	-0·25			0·63	-0·03	-0·10						-0·45
C11			-0·21					0·05	-0·03				0·05
C12			0·19						0·03				-0·27
C13			-0·22	-0·23					0·65	0·00	0·04	-0·55	0·22
C14			0·26	0·24						0·01	-0·59	0·06	-0·28
C15				-0·16						0·00	-0·04	-0·10	0·34
C16				0·02						0·00	0·06	0·09	0·33
C17				0·13							-0·06	-0·06	0·09

TABLE 5. INTERMOLECULAR DISTANCES LESS THAN 4·0 Å

Atoms	Position	Distance	Atoms	Position	Distance
C1–C2	2/$\bar{1}$00†	3·84	C19–C37	2/$\bar{1}$00	3·96
C1–C3	2/$\bar{1}$00	3·77	C19–O1	1/0$\bar{1}$0	3·44
C1–C34	2/$\bar{1}\bar{1}$0	3·92	C19–C23	1/0$\bar{1}$0	3·87
C1–C35	2/$\bar{1}\bar{1}$0	3·70	C20–O7	2/000	3·90
C1–O7	2/$\bar{1}$00	3·65	C21–C29	1/0$\bar{1}$0	3·61
C2–C3	2/$\bar{1}$00	3·99	C21–C30	1/0$\bar{1}$0	3·66
C2–C34	2/$\bar{1}\bar{1}$0	3·58	C21–O5	1/0$\bar{1}$0	3·23
C2–C35	2/$\bar{1}\bar{1}$0	3·87	C23–C27	1/010	3·83
C2–O1	2/$\bar{1}\bar{1}$0	3·86	C23–C37	2/$\bar{1}$00	3·99
C4–O1	1/0$\bar{1}$0	3·71	C24–C27	1/010	3·87
C6–C34	2/0$\bar{1}$0	3·73	C24–O3	1/$\bar{1}$00	3·78
C6–C35	2/0$\bar{1}$0	3·64	C25–O3	1/$\bar{1}$00	3·47
C7–C35	2/0$\bar{1}$0	3·78	C27–O4	1/0$\bar{1}$0	3·81
C7–C36	2/0$\bar{1}$0	3·93	C28–C30	2/001	3·75
C11–C33	2/$\bar{1}$00	3·89	C30–C32	1/010	3·98
C11–C38	2/$\bar{1}$00	3·94	C32–O4	2/001	3·13
C11–O7	2/$\bar{1}$00	3·55	C33–O1	2/$\bar{1}$00	3·62
C12–C38	2/$\bar{1}$00	3·80	C35–O7	1/010	3·16
C12–C19	1/010	3·62	C36–O7	1/010	3·84
C13–C19	1/010	3·76	C37–O3	2/0$\bar{1}$0	3·13
C15–C38	2/000	3·69	C38–O3	2/0$\bar{1}$0	3·49
C15–Br	2/0$\bar{1}$0	3·89	C39–O1	2/$\bar{1}$00	3·97
C16–C37	2/000	3·98	O1–O6	2/$\bar{1}$00	3·97
C18–C20	2/0$\bar{1}$0	3·74	O1–O7	2/$\bar{1}$00	3·64
C19–C35	2/$\bar{1}$00	3·79	O3–Br	2/0$\bar{1}$0	3·81
C19–C36	2/$\bar{1}$00	3·76	O7–Br	1/0$\bar{1}$0	3·65

† Equivalent position nomenclature: C1–C2 2/$\bar{1}$00 is taken to mean atom C1 to (atom C2 at equivalent position 2, translated 1 unit cell in the $\bar{x}$ direction). Equivalent position 1 is xyz; equivalent position 2 is $\bar{x}, \frac{1}{2} + y, \bar{z}$.

EXPERIMENTAL

Crystals of fusidic acid were prepared by Godtfredsen in the form of the 3-*p*-bromobenzoate and these were thin monoclinic plates elongated along the *b*-axis, with the (001) face dominating. Oscillation and Weissenberg photographs showed that the *b*-axis was a symmetry axis and that the only systematically absent spectra were 0k0 when $k = 2n + 1$. The spacegroup is hence $P2_1$ or $P2_1/m$. Density measurements showed that there were two steroid molecules per unit cell and hence the non-centric spacegroup was chosen as the large number of asymmetric centres found in naturally occurring steroids precluded the additional molecular symmetry required by the centric spacegroup. The unit cell dimensions were measured on a Linear Diffractometer,[16] the averages being taken of the dimensions obtained from a number of crystals (the variation from crystal to crystal was approximately 0·1 %).

The X-ray diffraction data used in the structure analysis were obtained in three batches: with the *b*-axis as rotation axis, the intensities of spectra h0l–h4l were measured by a Linear Diffractometer using MoKα radiation; spectra h5l–h6l were recorded photographically on a Weissenberg goniometer using multiple film packs and CuKα radiation; spectra h7l–h, 10, l were recorded by a-axis Weissenberg photography using CuKα radiation. The observed intensities were corrected for Lorentz and polarization effects but no corrections were made for the effects of extinction or absorption. Additional a-axis spectra were recorded in order to place the three batches of data on a common intensity scale and this scaling was carried out by an iterative least-squares method.[17] A Wilson plot[18] then placed the intensities on an absolute scale and yielded an overall isotropic temperature factor of 4·9 Å². Two thousand six hundred and forty two intensities of observable magnitudes were employed in the analysis. The summarized crystal data are:

$C_{39}H_{53}O_7Br$, M = 713, monoclinic plates, $a = 10\cdot310 \pm 0\cdot011$, $b = 7\cdot935 \pm 0\cdot004$, $c = 22\cdot066 \pm 0\cdot026$ Å, $\beta = 91\cdot6 \pm 0\cdot1°$, $V_0 = 1804$ Å^3, $D_m = 1\cdot29 \pm 0\cdot03$ (by flotation), $Z = 2$, $D_c = 1\cdot313$ g cm^{-3}, F(000) = 756. Spacegroup $P2_1$ (C_2^2, No. 4). CuKα single crystal Weissenberg photography and MoKα single crystal diffractometry. (All errors are given as $\pm$ one standard deviation.)

Solution of the structure

A three dimensional Patterson synthesis was computed in which the Fourier coefficients were modified by the function exp $(5 \sin^2 \theta)/f_{Br}^2$ such that the Fourier series represented approximately, the diffraction of X-rays by point atoms at rest. The well resolved, heaviest peak on the Harker section $(x \frac{1}{2} z)$ gave the xz coordinates of the bromine atom in the unit cell, for which the y-coordinate was arbitrarily chosen as $0\cdot25$, and with the assistance of the a- and b-axis bromine phased Fourier projections, the full coordinates of the positions of the carbon atoms in the bromobenzoate group were obtained from the Patterson synthesis.

With the positions of the bromine atom and seven carbon atoms, a program written for the Mercury computer was used to scan automatically the derived Fourier synthesis for possible atomic sites.[19] As expected, this map contained pseudo mirror planes through the bromine atom positions at $y = 0\cdot25$ and $y = 0\cdot75$, but by giving preference to the heavier of the mirror related Fourier maxima and to those within reasonable bonding distances of other maxima, twelve new atomic sites were allocated from those maxima having electron densities greater than 2 eÅ^{-3}. With one half of the scattering matter of the molecule now represented in the Fourier synthesis phased on the twenty atoms so far located, the pseudo symmetry of the Fourier synthesis was completely destroyed and a further eleven positions were determined. A third round of structure factor and Fourier calculation brought forth six new atomic positions with electron densities greater than 2 eÅ^{-3} and by lowering the acceptance level to 1·5 eÅ^{-3}, five more chemically feasible atomic positions were obtained. A full three dimensional Fourier difference synthesis then brought up another five atoms and a further three dimensional Fourier difference synthesis showed no remaining unaccountable electron density.

Least-squares refinement of the atomic parameters was commenced using a large-block-diagonal approximation to the normal equations, having two $\frac{1}{2}(47)^2$, one $\frac{1}{2}(46)^2$ and one $\frac{1}{2}(48)^2$ normal equations blocks to include the $x_i x_j$, $z_i z_j$, $y_i y_j$ and GB_i† and $B_i B_j$ interactions respectively[19] (in order to fix the origin, the y-coordinate of the bromine atom was not refined). The terms of the normal equations were weighted according to Hughes' weighting scheme[20] with $F^* = 16$ electrons, and four cycles of iterative refinement were carried out. Fourier difference maps computed after cycles two and four showed that refinement was proceeding satisfactorily and that assignment of atomic types had been correct in all cases except for the terminal carboxyl oxygen and methyl carbon atoms of the acetate group, for which the identities had been reversed. The maps also indicated thermal anisotropy of the bromine atom.

The refinement was continued using a different computer program which allowed the simultaneous refinement of isotropic and anisotropic thermal parameters.[21] The normal equations matrices were divided into forty five $\frac{1}{2}(4)^2$ blocks for the individual positional and isotropic thermal parameters of the carbon and oxygen atoms and into one $\frac{1}{2}(13)^2$ block for the positional parameters of the bromine atom and atom C36 and for the anisotropic and isotropic thermal parameters of these two atoms respectively. A new weighting scheme was also derived such that the $w\Delta^2$ values remained invariant with the magnitude of $|F_o|$ $(1/w = 1 + 0\cdot0025 |F_o|^2)$. Three cycles of iterative least-squares refinement produced convergence of the minimization function with $\Sigma w\Delta^2/(m - n) = 6\cdot6$ and $R = 0\cdot15$. The relatively high R factor for this refinement is attributed to the large proportion of spectra with very low values of $|F_o|$ as may be seen in Table 1.

The absolute configuration of the molecules was determined by selecting from the photographic records of the $0kl$ and $5kl$ spectra, ten pairs of spectra for which the differences in the intensities $I(hkl)$ and $I(h\chi l)$ were great enough to be measured visually ($\Delta f_{Br}''$ for CuKα = $1\cdot46^{22}$). These intensities were compared with those calculated by vectorial combination of the bromine and light atom contributions to the structure factors for the two enantiomorphs (Table 2), and in all cases the intensities agreed with those calculated for the parameters given in Table 3 (the numbering in this Table refers to Fig. 1). These parameters thus give the absolute configuration of the molecules.‡

† G is the scalefactor which places $|F_c|$ on the same scale as $|F_o|$.

‡ A listing of the observed structure amplitudes and the values obtained from the refined coordinates may be had on application to the authors or from 'D.Phil. Thesis', A. Cooper, Oxford University, 1965.

Although no evidence for the positions of the hydrogen atoms could be obtained from the Fourier difference syntheses, the interest in the stereochemistry of this molecule made their location by theoretical considerations worthwhile. Consequently the positions of the hydrogen atoms were calculated such that the distance C—H was 1·08 Å and in each case the geometries of the hydrogen atoms about the respective carbon atoms were those to be expected from the configurations found in this analysis. Since the hydroxyl group at C11 is not oriented by hydrogen bonding, the hydrogen atom position at O1 could not be determined. The positions of the other hydrogen atoms are given in Table 3.

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