The Crystal Structure of Hydrated Beauvericin

BY A. J. GEDDES AND IN PART BY D. AKRIGG

Astbury Department of Biophysics, University of Leeds, Leeds LS2 9JT, England

The structure of the ionophorous cyclic depsipeptide antibiotic beauvericin [-L-N-methyl-phenylalanyl-D-α-hydroxyisovaleryl-]₃, $C_{45}H_{60}N_6O_6$, has been determined by direct methods from counter intensities and refined to an R of 0·096. The hydrated material crystallizes in the monoclinic space group $P2_1$ with $a = 15·936$ (4), $b = 17·165$ (2), $c = 8·337$ (12) Å, $\beta = 92·14$ (6)°, $Z = 2$. The conformation is similar to that found in a Ba picrate complex and also to the conformation which has been proposed to exist in polar solvents from spectroscopic studies and conformational analyses.

Introduction

Beauvericin (Bv) is a cyclic hexadepsipeptide of alternating L-N-methyl-phenylalanyl and D-α-hydroxyisovaleryl residues (Hamill, Higgins, Boaz & Gorman, 1969). It is a member of the enniatin group of antibiotics known to be active against gram positive and mycobacteria. The enniatins have the general formula (L-MeX-D-Hylv)₃ where X is Ile, Val, Leu or Phe in enniatins A, B and C, and Bv respectively. The antimicrobial activity of Bv closely resembles that of enniatin A (Ovchinnikov, Ivanov & Mikhaleva, 1971) and its antibiotic action results from its ability to affect the transport of metal ions across biological membranes (Dorschner & Lardy, 1968). It was proposed earlier that the enniatins and other macrocyclic antibiotics could affect translocation by forming stable complexes with the metal cation (Mueller & Rudin, 1967) and it is now known that such complexes are lipid soluble and these antibiotics act as mobile carriers which ferry cations across cell membranes.

In contrast to valinomycin, which is perhaps the most widely known of these antibiotics, Bv is effective with alkaline earth as well as alkali metal ions (Roeske, Isaac, King & Steinrauf, 1974; Prince, Crofts & Steinrauf, 1974). While not having the very considerable cation specificity exhibited by valinomycin, Bv is unusual in showing an anion-dependent cation specificity (Estrada-O, Gomez-Louero & Montal, 1972). Ivanov *et al.* (1973) have reported enniatin complexes with 2:1 and 3:2 macrocycle:cation ratios.

Spectroscopic studies carried out at the Shemyakin Institute indicate two spatial forms of the enniatins, an asymmetric 'N' form in non-polar solvents and a symmetric 'P' form in polar solvents, and a recent crystallographic study of a Bv Ba picrate complex (Hamilton, Steinrauf & Braden, 1975) shows (Bv . Ba . Pic₃ . Ba . Bv)⁺Pic⁻ as the unit of structure.

Structure determination and refinement*

The Bv was synthesized by Dr Roeske (Roeske *et al.*, 1974) and crystals were grown in the laboratory of Dr

---

* With technical assistance from D. Akrigg.

Steinrauf by cooling overnight from a hot solution of *n*-heptane.

Intensities were collected on an Enraf–Nonius CAD-4 diffractometer. 3678 reflexions were measured by the moving-crystal moving-counter technique in the range $3° \leq \theta \leq 68°$ with graphite-monochromatized Cu $K\alpha$ radiation, $\lambda = 1·54051$ Å. A control reflexion measured every 50 reflexions showed a gradual decline in intensity to about 93% of its initial value, but no correction was made for this nor for absorption.

The space group was determined as $P2_1$ with $a = 15·936$ (4), $b = 17·165$ (2), $c = 8·337$ (12) Å and $\beta = 92·14$ (6)°, the standard deviations being calculated from 15 observations. Another crystal form frequently crystallized with the above conditions and this had the space group $P222$ with $a = 15·13$, $b = 15·65$ and $c = 19·04$ Å. These crystals were always split and intensities have not yet been collected.

The structure was solved with *MULTAN* (Germain, Main & Woolfson, 1971). We used a recent version of *MULTAN* written by Drs J. P. Declercq, G. Germain, P. Main, L. Lessinger and Professor M. M. Woolfson. The normalized structure factors (E's) were analysed as follows:

	Experimental	Theoretical Non-centrosymmetric	Theoretical Centrosymmetric
$\langle |E^2 - 1| \rangle$	0·7114	0·7360	0·9680
$\langle |E| \rangle$	0·8948	0·8860	0·7980.

There were 276 reflexions with $E \geq 1·60$ and these were used for phase generation. The reflexions used in the starting set are given in Table 1.

Table 1. *Starting set of reflexions*

h	k	l	E	Phase	
2	0	$\bar{2}$	2·32	180	A $\sum_1$ reflexion; probability that phase is correct is 0·927
1	3	$\bar{1}$	3·07	±45	Origin-fixing reflexions
9	2	$\bar{4}$	2·98	45 Fixes enantiomorph	
3	1	2	2·54	360	
6	0	2	2·76	360, 180	
4	5	7	2·47		Multiple assignment
12	4	1	2·40	45, 135, 225, 315	
13	11	0	2·33		

256 sets of phases were generated. Nine of these had combined figures of merit (CFOM) of 2·6 to 2·7 while none of the remaining sets had a CFOM greater than 2·2. The peak-searching routine was applied to the electron density distributions calculated from the nine different sets of phases. In each case the projected positions of the 87 highest peaks were output graphically on the line-printer. For one of these nine sets an almost completed ring structure was apparent and it was considered likely that this corresponded to the backbone of the cyclic depsipeptide. 33 atoms were selected on the basis of the known chemical structure of Bv. A Fourier synthesis was computed with phases specified by these 33 atoms and from the resultant electron density map a further 19 atoms were selected and three of the original atoms discarded. One further synthesis revealed the positions of the remaining eight non-H atoms in the Bv molecule. One of the original atoms retained throughout was only 80th in the list of 87 peaks in order of decreasing intensity.

The structure was refined by full-matrix least squares with X-RAY 72 (Stewart, Kruger, Ammon, Dickinson & Hall, 1972) initially with isotropic and subsequently with anisotropic temperature factors to an R of 0·157. At this stage a difference synthesis revealed one strong peak about 3 Å from two of the amide carbonyl O atoms and this was interpreted as the O atom of a molecule of water. Further refinement, including this atom, was stopped when R reached 0·129, and another difference map was computed. Two additional peaks were present. One of these was quite distinct, although weak, and about 3 Å from the three ester carbonyl O atoms. This was interpreted as the O atom of a water molecule with a population parameter much less than unity (this parameter eventually refined to 0·26). The other peak was weak and diffuse and was interpreted

Table 2. *Fractional atomic coordinates* ($\times 10^5$)

Standard deviations are in parentheses.

	x	y	z
N(1)	33375 (37)	12660 (0)	112835 (70)
C(2)	31405 (57)	10237 (72)	129075 (100)
C(3)	41440 (42)	10468 (45)	106440 (79)
C(4)	48651 (46)	10893 (52)	119216 (93)
C(5)	57188 (53)	9331 (53)	112954 (103)
C(6)	60848 (52)	14037 (58)	101878 (105)
C(7)	68652 (59)	12348 (82)	95969 (128)
C(8)	72775 (65)	5928 (94)	101271 (140)
C(9)	69542 (65)	945 (71)	112202 (137)
C(10)	61657 (59)	2600 (62)	118273 (105)
C(11)	40811 (45)	2383 (47)	99232 (92)
O(12)	35810 (40)	−2468 (40)	102256 (90)
O(13)	46715 (34)	1438 (33)	88481 (61)
C(14)	47115 (53)	−6393 (51)	80799 (96)
C(15)	56150 (52)	−7951 (55)	77239 (111)
C(16)	59887 (64)	−2226 (75)	65717 (145)
C(17)	57141 (70)	−16453 (70)	71707 (165)
C(18)	41200 (55)	−5813 (49)	65739 (103)
O(19)	41597 (45)	−363 (35)	56755 (80)
N(20)	35948 (41)	−11861 (38)	62938 (77)
C(21)	34575 (58)	−18527 (56)	73851 (116)
C(22)	30389 (48)	−11360 (47)	48425 (93)

Table 2 (*cont.*)

	x	y	z
C(23)	30460 (53)	−18857 (51)	38094 (110)
C(24)	39263 (52)	−21438 (50)	34796 (90)
C(25)	44560 (68)	−16802 (61)	26617 (126)
C(26)	52728 (76)	−18961 (85)	23712 (143)
C(27)	55712 (72)	−26240 (84)	29452 (157)
C(28)	50335 (72)	−30994 (67)	37219 (130)
C(29)	42087 (60)	−28791 (59)	40248 (107)
C(30)	21572 (53)	−9203 (49)	52940 (110)
O(31)	18759 (40)	−9356 (48)	66159 (72)
O(32)	16979 (34)	−7152 (37)	39698 (61)
C(33)	8421 (45)	−4927 (47)	42697 (89)
C(34)	4152 (54)	−3871 (56)	25929 (107)
C(35)	−4545 (61)	−461 (84)	27124 (128)
C(36)	3883 (84)	−11820 (72)	17115 (132)
C(37)	8897 (46)	2868 (51)	51677 (92)
O(38)	12929 (37)	8178 (35)	46202 (67)
N(39)	4374 (39)	3690 (39)	64866 (79)
C(40)	22 (58)	−2987 (64)	72252 (112)
C(41)	4948 (49)	11064 (47)	73717 (92)
C(42)	−3764 (48)	14356 (61)	77901 (107)
C(43)	−9268 (47)	15275 (58)	62941 (106)
C(44)	−16184 (51)	10420 (75)	60768 (139)
C(45)	−20866 (63)	11254 (93)	46183 (186)
C(46)	−18838 (91)	16540 (103)	34969 (172)
C(47)	−12056 (92)	21329 (90)	37143 (149)
C(48)	−7206 (59)	20673 (63)	51454 (118)
C(49)	10769 (46)	10071 (52)	88415 (94)
O(50)	13408 (42)	3989 (37)	93771 (77)
O(51)	12805 (31)	17151 (32)	94430 (61)
C(52)	18686 (42)	17032 (49)	108225 (93)
C(53)	18505 (54)	25161 (53)	115513 (98)
C(54)	9686 (68)	26650 (74)	122185 (137)
C(55)	21289 (84)	31460 (63)	103954 (144)
C(56)	27299 (50)	15003 (46)	101973 (94)
O(57)	28811 (35)	15568 (46)	87770 (66)
O(H₂O)	21259 (57)	22027 (55)	59429 (95)
O(H₂O′)	20269	−13607	586
O(H₂O″)	78995	−12565	45910

Fractional atomic coordinates of hydrogen atoms ($\times 10^4$)

	x	y	z		x	y	z
HC(2)	3587	852	13705	HC(29)	3822	−3241	4624
H′C(2)	2738	579	12783	HC(33)	565	−905	4952
H″C(2)	2852	1477	13401	HC(34)	770	−21	1936
HC(3)	4300	1436	9783	HC(35)	−731	22	1627
HC(4)	4753	688	12787	H′C(35)	−411	473	3255
H′C(4)	4868	1634	12410	H″C(35)	−803	−405	3357
HC(6)	5774	1890	9790	HC(36)	112	−1114	626
HC(7)	7124	1591	8773	H′C(36)	62	−1565	2340
HC(8)	7854	474	9695	H″C(36)	973	−1379	1599
HC(9)	7280	−389	11588	HC(40)	−333	−238	8203
HC(10)	5914	−102	12649	H′C(40)	447	−693	7501
HC(14)	4509	−1061	8834	H″C(40)	−392	−514	6375
HC(15)	5983	−703	8732	HC(41)	726	1529	6653
HC(16)	5951	311	7043	HC(42)	−299	1966	8329
H′C(16)	5649	−243	5541	H′C(42)	−657	1062	8553
H″C(16)	6589	−326	6335	HC(44)	−1779	647	6923
HC(17)	6314	−1749	6934	HC(45)	−2595	775	4403
H′C(17)	5354	−1735	6182	HC(46)	−2246	1695	2467
H″C(17)	5537	−2005	8036	HC(47)	−1055	2528	2860
HC(21)	3068	−2301	7177	HC(48)	−212	2421	5340
H′C(21)	3270	−1613	8401	HC(52)	1691	1293	11625
H″C(21)	4024	−2093	7574	HC(53)	2296	2556	12458
HC(22)	3262	−721	4096	HC(54)	957	3199	12697
HC(23)	2731	−1779	2746	H′C(54)	852	2269	13058
H′C(23)	2751	−2321	4401	H″C(54)	532	2626	11330
HC(25)	4242	−1154	2248	HC(55)	2117	3680	10874
HC(26)	5653	−1536	1753	H′C(55)	1745	3137	9421
HC(27)	6176	−2791	2782	H″C(55)	2715	3029	10084
HC(28)	5240	−3634	4095				

as a water molecule within an intermolecular cavity and having considerable freedom of movement. Although the solvent may have been contaminated with water it seems more likely that water molecules were absorbed by the crystal during the period it was left standing in the laboratory prior to examination.

Further refinement including the O atoms of these water molecules (the first having been assigned a low population parameter but a normal isotropic temperature factor, and the second given a population parameter of unity but an isotropic temperature factor three to four times the average) reduced R to 0·111. A final differ-

Table 3. *Anisotropic thermal parameters* ($\times 10^4$)

Standard deviations are in parentheses. The temperature factor is of the form:

$$\exp\left[-2\pi^2(U_{11}a^{*2}h^2 + U_{22}b^{*2}k^2 + U_{33}c^{*2}l^2 + 2U_{12}a^*b^*hk + 2U_{13}a^*c^*hl + 2U_{23}b^*c^*kl)\right].$$

	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
N(1)	360 (33)	471 (40)	332 (33)	25 (31)	−16 (26)	46 (30)
C(2)	552 (52)	1069 (84)	366 (46)	210 (58)	76 (38)	137 (54)
C(3)	322 (36)	400 (41)	287 (35)	68 (33)	−39 (28)	81 (33)
C(4)	349 (40)	566 (53)	455 (46)	47 (39)	−71 (33)	−114 (42)
C(5)	510 (49)	509 (51)	519 (52)	−56 (43)	−243 (40)	−92 (44)
C(6)	427 (46)	607 (59)	551 (52)	3 (44)	−156 (40)	38 (48)
C(7)	417 (51)	1140 (100)	723 (72)	4 (61)	28 (48)	121 (71)
C(8)	471 (58)	1379 (128)	643 (73)	−12 (73)	−59 (52)	−267 (78)
C(9)	562 (61)	801 (81)	809 (73)	259 (58)	−276 (54)	282 (65)
C(10)	596 (58)	686 (66)	480 (50)	−57 (53)	−210 (43)	−38 (51)
C(11)	291 (36)	411 (43)	454 (43)	−16 (34)	−9 (32)	124 (37)
O(12)	569 (40)	534 (40)	986 (54)	−131 (35)	236 (37)	−104 (38)
O(13)	476 (32)	420 (33)	429 (30)	−141 (27)	46 (25)	−119 (26)
C(14)	533 (49)	452 (48)	411 (44)	94 (41)	−182 (37)	−101 (39)
C(15)	396 (45)	556 (53)	630 (56)	62 (41)	−114 (40)	−169 (46)
C(16)	557 (63)	867 (83)	907 (84)	−58 (61)	163 (58)	27 (70)
C(17)	659 (71)	694 (76)	1227 (109)	224 (61)	−188 (71)	−309 (77)
C(18)	567 (52)	342 (43)	510 (50)	30 (39)	−104 (40)	−139 (41)
O(19)	944 (51)	312 (32)	701 (43)	−192 (33)	−382 (38)	143 (32)
N(20)	466 (37)	325 (34)	435 (37)	19 (29)	−135 (29)	−50 (29)
C(21)	592 (55)	480 (55)	715 (62)	−236 (46)	−151 (48)	142 (49)
C(22)	375 (40)	404 (43)	443 (44)	101 (34)	−65 (33)	−48 (37)
C(23)	470 (48)	400 (47)	690 (58)	164 (40)	−108 (42)	−279 (44)
C(24)	512 (47)	462 (48)	357 (42)	152 (41)	−157 (36)	−104 (37)
C(25)	752 (69)	560 (59)	684 (65)	115 (53)	78 (54)	−12 (53)
C(26)	766 (80)	918 (94)	825 (79)	−249 (72)	244 (63)	−165 (73)
C(27)	580 (67)	949 (103)	909 (87)	−17 (67)	68 (61)	−376 (78)
C(28)	798 (73)	734 (76)	680 (66)	356 (63)	−265 (57)	−385 (60)
C(29)	628 (58)	586 (56)	488 (52)	100 (48)	−129 (44)	−136 (45)
C(30)	491 (49)	346 (45)	609 (57)	66 (38)	−44 (43)	−144 (42)
O(31)	600 (39)	991 (57)	425 (35)	248 (39)	−25 (30)	39 (37)
O(32)	492 (32)	642 (38)	313 (27)	180 (30)	−136 (24)	−15 (27)
C(33)	310 (37)	452 (47)	388 (42)	132 (34)	−12 (31)	−65 (36)
C(34)	458 (49)	549 (55)	555 (54)	14 (43)	−17 (40)	−104 (45)
C(35)	464 (56)	1344 (118)	678 (68)	246 (66)	−224 (48)	−113 (74)
C(36)	1244 (102)	752 (79)	618 (67)	208 (75)	−418 (68)	−175 (61)
C(37)	306 (36)	501 (48)	408 (41)	89 (37)	−27 (32)	−100 (40)
O(38)	598 (36)	454 (35)	461 (32)	−137 (30)	212 (27)	−19 (27)
N(39)	369 (33)	386 (36)	510 (39)	−36 (30)	−8 (29)	−24 (32)
C(40)	553 (55)	707 (65)	544 (56)	−176 (51)	189 (44)	−69 (51)
C(41)	486 (46)	381 (44)	396 (42)	−4 (37)	−44 (35)	−4 (36)
C(42)	316 (41)	763 (67)	593 (55)	163 (44)	60 (38)	−181 (52)
C(43)	301 (39)	654 (61)	578 (53)	128 (40)	−25 (36)	−143 (48)
C(44)	260 (42)	1042 (91)	1066 (84)	124 (53)	−73 (47)	−361 (76)
C(45)	372 (54)	1126 (116)	1330 (116)	60 (67)	−274 (67)	−509 (99)
C(46)	812 (91)	1172 (130)	996 (102)	499 (93)	−362 (81)	−413 (94)
C(47)	1042 (100)	1114 (110)	774 (83)	581 (91)	−270 (75)	−30 (80)
C(48)	565 (57)	699 (66)	624 (61)	110 (51)	19 (47)	−45 (53)
C(49)	320 (39)	492 (48)	463 (45)	80 (37)	100 (33)	3 (41)
O(50)	794 (46)	419 (36)	649 (41)	79 (33)	−197 (34)	72 (32)
O(51)	362 (27)	424 (30)	421 (30)	54 (25)	−54 (23)	6 (25)
C(52)	217 (33)	491 (46)	454 (44)	59 (33)	−61 (30)	4 (38)
C(53)	509 (50)	517 (53)	417 (45)	92 (43)	−101 (38)	−145 (40)
C(54)	698 (68)	819 (80)	848 (79)	152 (64)	−11 (58)	−188 (68)
C(55)	1245 (101)	420 (58)	893 (83)	−121 (63)	−189 (74)	135 (60)
C(56)	473 (46)	347 (42)	441 (45)	−73 (35)	−31 (36)	51 (36)
O(57)	441 (32)	1089 (58)	361 (32)	76 (36)	21 (25)	239 (36)
O(H₂O)	1154 (70)	1071 (67)	741 (52)	−349 (57)	−104 (48)	270 (502)
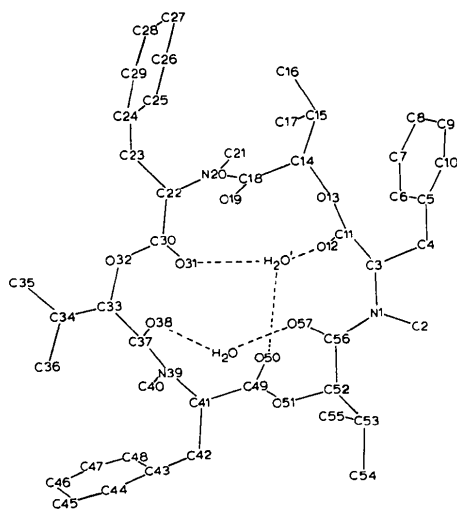
(a)
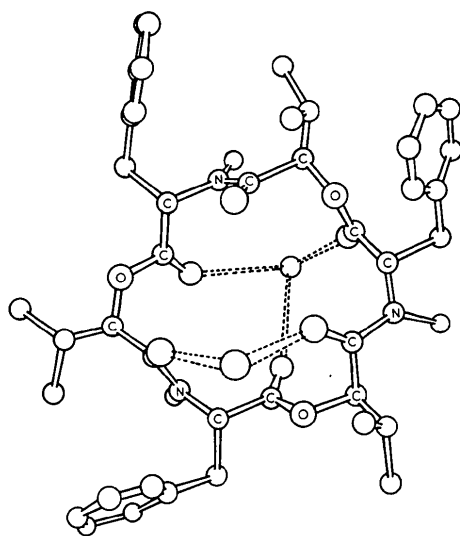
(b)
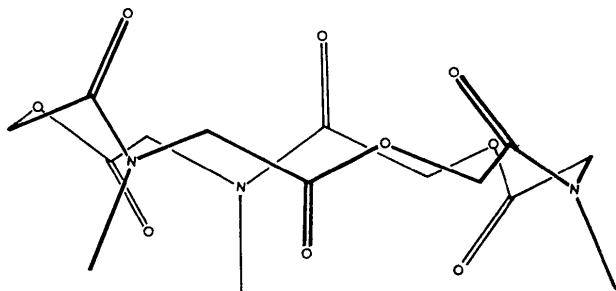
(c)

Fig. 1. (a) Beauvericin. The atom-numbering scheme. (b) A perspective view of the beauvericin molecule looking along the normal to the plane of best fit through the backbone atoms. (c) A schematic diagram of the beauvericin molecule viewed parallel to the plane of best fit through the backbone atoms. The side groups have been omitted since they obscure the backbone in this projection.

ence map revealed all 57 H atoms on the Bv molecule. These were included in a final cycle of refinement but their positions were not refined. The final value of R was 0·096.*

Description of the structure

The atomic coordinates, thermal parameters, bond lengths, bond angles, and torsion angles are shown in Tables 2–6. The numbering of the atoms is shown in Fig. 1(a) and a perspective view normal to the least-squares plane through the backbone atoms is shown in Fig. 1(b).

Table 4. Bond lengths (Å)

Standard deviations are in parentheses.

N(1)—C(2)	1·466 (11)	O(32)——C(33)	1·448 (9)
N(1)—C(3)	1·460 (9)	C(33)——C(34)	1·545 (11)
C(3)—C(4)	1·540 (10)	C(33)——C(37)	1·534 (12)
C(3)—C(11)	1·514 (11)	C(34)——C(35)	1·512 (14)
C(4)—C(5)	1·501 (11)	C(34)——C(36)	1·550 (15)
C(5)—C(6)	1·375 (13)	C(37)——O(38)	1·215 (10)
C(5)—C(10)	1·419 (13)	C(37)——N(39)	1·348 (10)
C(6)—C(7)	1·387 (13)	N(39)——C(40)	1·486 (12)
C(7)—C(8)	1·349 (19)	N(39)——C(41)	1·467 (10)
C(8)—C(9)	1·367 (18)	C(41)——C(42)	1·551 (12)
C(9)—C(10)	1·403 (14)	C(41)——C(49)	1·520 (11)
C(11)—O(12)	1·186 (10)	C(42)——C(43)	1·508 (12)
C(11)—O(13)	1·336 (9)	C(43)——C(44)	1·388 (13)
O(13)—C(14)	1·492 (10)	C(43)——C(48)	1·383 (14)
C(14)—C(15)	1·506 (12)	C(44)——C(45)	1·412 (18)
C(14)—C(18)	1·546 (12)	C(45)——C(46)	1·353 (22)
C(15)—C(16)	1·514 (15)	C(46)——C(47)	1·364 (22)
C(15)—C(17)	1·540 (15)	C(47)——C(48)	1·403 (16)
C(18)—O(19)	1·203 (11)	C(49)——O(50)	1·205 (11)
C(18)—N(20)	1·348 (11)	C(49)——O(51)	1·349 (10)
N(20)-C(21)	1·485 (12)	O(51)——C(52)	1·458 (9)
N(20)-C(22)	1·477 (10)	C(52)——C(53)	1·523 (12)
C(22)-C(23)	1·550 (12)	C(52)——C(56)	1·528 (11)
C(22)-C(30)	1·515 (12)	C(53)——C(54)	1·553 (14)
C(23)-C(24)	1·507 (12)	C(53)——C(55)	1·527 (15)
C(24)-C(25)	1·363 (14)	C(56)——O(57)	1·224 (10)
C(24)-C(29)	1·409 (13)	C(56)——N(1)	1·362 (10)
C(25)-C(26)	1·384 (17)	O(H₂O)—-O(38)	2·918 (11)
C(26)-C(27)	1·414 (20)	O(H₂O)—-O(57)	2·841 (10)
C(27)-C(28)	1·366 (17)	O(H₂O′) -O(12)	3·13
C(28)-C(29)	1·400 (15)	O(H₂O′) -O(31)	2·97
C(30)-O(31)	1·209 (11)	O(H₂O′) -O(50)	3·25
C(30)-O(32)	1·350 (10)	O(H₂O)—-O(H₂O″)	2·68

The molecule is disc-shaped with the side groups projecting in pseudo-equatorial fashion. The amide carbonyl groups project to one side of the disc and the ester carbonyl atoms and amide N-methyl groups to the other (Fig. 1c).

Torsion angles

The molecule departs from exact threefold symmetry, slightly in backbone conformation and considerably

---

in side-chain orientation. Thus two of the amino acid residues are almost identical in backbone conformation with φ, ψ angles of -103, 167 and -101, 166° [the convention used for torsion angles is that described by the IUPAC–IUB Commission on Biochemical Nomenclature (1970)]. The third is about 24° smaller in φ and 10° smaller in ψ. For the three hydroxy acid residues there are similar departures of threefold symmetry, the φ, ψ angles being 90, -135 and 70, -133 and 73, $-164°$. Two of the MePh side chains have very similar conformations with C^β–C^γ gauche to N–C^α and C^γ–C^δ gauche to C^α–C^β. The third differs in that C^β–C^γ is trans to N–C^α. This difference would seem to result from intermolecular packing forces. The three HyIv side chains are all different, with C^β–$H(C^\beta)$ trans to each of C^α–C', C^α–$H(C^\alpha)$, and C^α–O(ether). There are no significant departures from planarity of either the amide groups C^α–CO–N–C^α ($\omega = 177{\cdot}6$, $-179{\cdot}7$, $-177{\cdot}4$) or the ester groups C^α–CO–O–C ($\omega = -179{\cdot}1$,

$178{\cdot}6$, $177{\cdot}7°$). The H atoms of all the HyIv methyl groups are staggered relative to the arrangement on the C^β atoms. The H atoms of the N-methyl groups, while not so clearly defined on the electron density maps, seem to be arranged so that one C–H bond in each N-methyl group is trans to the N–C' bond, leaving the other two H atoms of each group bracketing the hydroxy acid $H(C^\alpha)$ atoms.

Bond lengths and angles

Most of the bond lengths and angles are close to normal but there are a few exceptions. Apparently, ring-closure has been achieved by the slight compression of 6 of the 18 ring angles, viz the three C'–C^α–O angles, which are 3 or 4° less than the normal tetrahedral value, and the three C^α–N–C' angles, which are all 4 or 5° less than the average values found in linear peptides (Dickerson & Geis, 1969). Since Bv is composed of three chemically identical pairs of residues,

Table 5. Bond angles (°)

Standard deviations are in parentheses.

C(2)—N(1)—C(3)	119·3 (0·6)	O(31)–C(30)—O(32)	123·2 (0·8)
C(2)—N(1)—C(56)	121·9 (0·7)	C(30)–O(32)—C(33)	114·3 (0·6)
C(3)—N(1)—C(56)	116·4 (0·6)	O(32)–C(33)—C(34)	105·0 (0·6)
N(1)—C(3)—C(4)	112·2 (0·6)	O(32)–C(33)—C(37)	106·6 (0·6)
N(1)—C(3)—C(11)	109·6 (0·5)	C(34)–C(33)—C(37)	110·7 (0·7)
C(4)—C(3)—C(11)	110·8 (0·6)	C(33)–C(34)—C(35)	111·0 (0·7)
C(3)—C(4)—C(5)	114·3 (0·6)	C(33)–C(34)—C(36)	109·4 (0·8)
C(4)—C(5)—C(6)	123·0 (0·8)	C(35)–C(34)—C(36)	111·3 (0·9)
C(4)—C(5)—C(10)	119·1 (0·8)	C(33)–C(37)—O(38)	119·1 (0·7)
C(6)—C(5)—C(10)	117·9 (0·8)	C(33)–C(37)—N(39)	118·3 (0·7)
C(5)—C(6)—C(7)	121·8 (0·9)	C(38)–C(37)—N(39)	122·5 (0·8)
C(6)—C(7)—C(8)	119·1 (1·1)	C(37)–N(39)—C(40)	122·2 (0·7)
C(7)—C(8)—C(9)	122·6 (1·0)	C(37)–N(39)—C(41)	118·6 (0·7)
C(8)—C(9)—C(10)	118·8 (1·1)	C(40)–N(39)—C(41)	118·4 (0·7)
C(9)—C(10)–C(5)	119·8 (0·9)	N(39)–C(41)—C(42)	112·9 (0·7)
C(3)—C(11)–O(12)	126·3 (0·7)	N(39)–C(41)—C(49)	109·6 (0·7)
C(3)—C(11)–O(13)	110·0 (0·6)	C(42)–C(41)—C(49)	112·4 (0·7)
O(12)–C(11)–O(13)	123·7 (0·8)	C(41)–C(42)—C(43)	110·4 (0·7)
C(11)–O(13)–C(14)	116·2 (0·6)	C(42)–C(43)—C(44)	118·6 (0·9)
O(13)–C(14)–C(15)	107·6 (0·7)	C(42)–C(43)—C(48)	120·0 (0·8)
O(13)–C(14)–C(18)	105·0 (0·6)	C(44)–C(43)—C(48)	121·3 (0·9)
C(15)–C(14)–C(18)	114·1 (0·7)	C(43)–C(44)—C(45)	116·3 (1·1)
C(14)–C(15)–C(16)	114·4 (0·8)	C(44)–C(45)—C(46)	122·3 (1·2)
C(14)–C(15)–C(17)	109·7 (0·8)	C(45)–C(46)—C(47)	121·4 (1·3)
C(16)–C(15)–C(17)	112·1 (0·9)	C(46)–C(47)—C(48)	118·2 (1·3)
C(14)–C(18)–O(19)	120·9 (0·8)	C(47)–C(48)—C(43)	120·5 (1·0)
C(14)–C(18)–N(20)	116·5 (0·7)	C(41)–C(49)—O(50)	126·2 (0·8)
O(19)–C(18)–N(20)	122·5 (0·8)	C(41)–C(49)—O(51)	109·3 (0·7)
C(18)–N(20)–C(21)	126·3 (0·7)	O(50)–C(49)—O(51)	124·5 (0·7)
C(18)–N(20)–C(22)	116·5 (0·7)	C(49)–O(51)—C(52)	114·8 (0·6)
C(21)–N(20)–C(22)	116·9 (0·6)	O(51)–C(52)—C(53)	106·4 (0·6)
N(20)–C(22)–C(23)	113·1 (0·6)	O(51)–C(52)—C(56)	106·9 (0·6)
N(20)–C(22)–C(30)	110·0 (0·7)	C(53)–C(52)—C(56)	112·1 (0·6)
C(23)–C(22)–C(30)	111·6 (0·7)	C(52)–C(53)—C(54)	109·0 (0·7)
C(22)–C(23)–C(24)	111·9 (0·7)	C(52)–C(53)—C(55)	112·6 (0·7)
C(23)–C(24)–C(25)	121·3 (0·8)	C(54)–C(53)—C(55)	113·6 (0·9)
C(23)–C(24)–C(29)	119·5 (0·8)	C(52)–C(56)—O(57)	121·8 (0·7)
C(25)–C(24)–C(29)	119·2 (0·8)	C(52)–C(56)—N(1)	117·6 (0·7)
C(24)–C(25)–C(26)	122·5 (1·0)	O(57)–C(56)—N(1)	120·5 (0·7)
C(25)–C(26)–C(27)	118·9 (1·1)	O(38)–O(H₂O)–O(57)	99·8 (0·3)
C(26)–C(27)–C(28)	118·7 (1·1)	O(57)–O(H₂O)–O(H₂O″)	122
C(27)–C(28)–C(29)	122·4 (1·1)	O(12)–O(H₂O′)–O(31)	86
C(28)–C(29)–C(24)	118·3 (0·9)	O(12)–O(H₂O′)–O(50)	73
C(22)–C(30)–O(31)	127·1 (0·8)	O(31)–O(H₂O′)–O(50)	66
C(22)–C(30)–O(32)	109·7 (0·7)		

and since all the atoms have been refined independently, we can attach more significance to small departures from normal values of bond lengths if these are repeated in each of the three pairs of residues. Thus, the angular distortion at the HyIv C^α atom would seem to be associated with a slight lengthening of the C^α–C' and C^α–O bonds of about 0·02 Å, and a shortening of the C^α–C^β and one of the C^β–C^γ bonds by the same amount.

The planarity of the amide group in peptides is usually ascribed to electron delocalization such that the carbonyl C=O bond lengthens from about 1·20 to 1·24 Å and the C'–N bond shortens from about 1·44 to 1·33 Å. In the present case all the C'–N and C'–O(ether) bonds are shorter than normal single-bond values (by about 0·10 and 0·05 Å respectively) and this partial double-bond character is sufficient to sustain the amide and ester groups in a planar conformation. However, there is no corresponding observed lengthening of the C=O double bonds. Perhaps these bonds may be a little longer than the values quoted in the tables since the carbonyl O atoms have a larger thermal vibration than the average for the backbone atoms and no correction has been made for the apparent contraction. However, the N-methyl C atoms would have to be treated similarly and the observed N–C(Me) lengths do not depart significantly from normal. The partial double-bond character of the C'–N bonds may be associated with the angular distortion at the N atom and there could be a contribution from the neighbouring C^α–C' bonds which are longer than normal. The partial double-bond character of the C'–O(ether) bond may result from a contribution from the lone pair of electrons on the ether O atom; the C'–O–C^α angles are a little large, but not abnormally so.

Non-bonded contacts

There are no inter- or intramolecular non-bonded contacts at distances closer than normal. An examination of the complete list of non-bonded contact distances shows a high proportion of interatomic separations at or around values corresponding to the van der Waals potential-energy minimum so that the observed structure will have considerable stability. Moreover, the atoms are so well locked together that any departure from the observed conformation of the backbone would involve a complex pattern of linked synchronous rotations about several torsion angles.

Comparison with other structural studies

Several papers have appeared from the Shemyakin Institute describing spectroscopic experiments on the enniatin antibiotics, including Bv. These have been summarized by Ovchinnikov et al. (1974) where references to the earlier publications can be found.

ORD spectra clearly show a conformational change on passing from non-polar solvents such as n-heptane to polar solvents such as trifluoroethanol. The conformation of the macrocycle when complexed with the cation in a variety of solvents seems to be the same as the conformation of the uncomplexed form in polar solvents.

NMR studies of enniatin B over a wide temperature range indicated that the conformation in non-polar solvents is devoid of symmetry but that in polar solvents the molecule is (threefold) symmetrical. In both forms of all the enniatins the side chains are pseudo-equatorial. In Bv the $^3J_{C\alpha H–C\beta H}$ constants of the HyIv residues indicate mixed *gauche* and *trans* rotamers. The $^3J_{C\alpha C–H\beta H1,2}$ constants of the MePh residues

Table 6. *Torsion angles* (°)

C(56)–N(1)—C(3)—C(11)	−77·8	C(22)–C(30)–O(32)–C(33)	−179·7	C(52)–C(56)–N(1)—C(3)	177·7
C(56)–N(1)—C(3)—C(4)	158·7	O(31)–C(30)–O(32)–C(33)	1·6	C(52)–C(56)–N(1)—C(2)	15·2
C(2)—N(1)—C(3)—C(11)	85·2	C(30)–O(32)–C(33)–C(37)	69·5	O(57)–C(56)–N(1)—C(3)	−3·5
C(2)—N(1)—C(3)—C(4)	−38·4	C(30)–O(32)–C(33)–C(34)	−173·1	O(57)–C(56)–N(1)—C(2)	−166·0
N(1)—C(3)—C(11)–O(13)	157·2	O(32)–C(33)–C(37)–N(39)	−132·6	N(1)—C(3)—C(4)—C(5)	−175·2
N(1)—C(3)—C(11)–O(12)	−21·8	O(32)–C(33)–C(37)–O(38)	51·4	C(11)–C(3)—C(4)—C(5)	61·8
C(4)—C(3)—C(11)–O(13)	−78·5	C(34)–C(33)–C(37)–N(39)	113·8	C(3)—C(4)—C(5)—C(6)	63·7
C(4)—C(3)—C(11)–O(12)	102·6	C(34)–C(33)–C(37)–O(38)	−62·2	C(3)—C(4)—C(5)—C(10)	−114·5
C(3)—C(11)–O(13)–C(14)	177·6	C(33)–C(37)–N(39)–C(41)	178·6	N(20)–C(22)–C(23)–C(24)	−50·2
O(12)–C(11)–O(13)–C(14)	−3·4	C(33)–C(37)–N(39)–C(40)	0·2	C(30)–C(22)–C(23)–C(24)	−174·9
C(11)–O(13)–C(14)–C(18)	90·2	O(38)–C(37)–N(39)–C(41)	−5·5	C(22)–C(23)–C(24)–C(25)	−61·3
C(11)–O(13)–C(14)–C(15)	−148·0	O(38)–C(37)–N(39)–C(40)	−174·9	C(22)–C(23)–C(24)–C(29)	118·7
O(13)–C(14)–C(18)–N(20)	−135·5	C(37)–N(39)–C(41)–C(49)	−101·2	N(39)–C(41)–C(42)–C(43)	−55·7
O(13)–C(14)–C(18)–O(19)	47·6	C(37)–N(39)–C(41)–C(42)	132·7	C(49)–C(41)–C(42)–C(43)	179·7
C(15)–C(14)–C(18)–N(20)	107·0	C(40)–N(39)–C(41)–C(49)	68·6	C(41)–C(42)–C(43)–C(44)	111·3
C(15)–C(14)–C(18)–O(19)	−69·9	C(40)–N(39)–C(41)–C(42)	−57·5	C(41)–C(42)–C(43)–C(48)	−66·4
C(14)–C(18)–N(20)–C(22)	−179·1	N(39)–C(41)–C(49)–O(51)	166·1	O(13)–C(14)–C(15)–C(16)	−61·8
C(14)–C(18)–N(20)–C(21)	7·9	N(39)–C(41)–C(49)–O(50)	−12·0	O(13)–C(14)–C(15)–C(17)	171·3
O(19)–C(18)–N(20)–C(22)	−2·2	C(42)–C(41)–C(49)–O(51)	−67·5	C(18)–C(14)–C(15)–C(16)	54·2
O(19)–C(18)–N(20)–C(21)	−175·2	C(42)–C(41)–C(49)–O(50)	114·4	C(18)–C(14)–C(15)–C(17)	−72·8
C(18)–N(20)–C(22)–C(30)	−103·2	C(41)–C(49)–O(51)–C(52)	−177·4	O(32)–C(33)–C(34)–C(35)	−172·2
C(18)–N(20)–C(22)–C(23)	131·2	O(50)–C(49)–O(51)–C(52)	0·7	O(32)–C(33)–C(34)–C(36)	−62·4
C(21)–N(20)–C(22)–C(30)	70·5	C(49)–O(51)–C(52)–C(56)	73·4	C(37)–C(33)–C(34)–C(35)	−57·6
C(21)–N(20)–C(22)–C(23)	−55·1	C(49)–O(51)–C(52)–C(53)	−166·6	C(37)–C(33)–C(34)–C(36)	52·3
N(20)–C(22)–C(30)–O(32)	167·2	O(51)–C(52)–C(56)–N(1)	−164·1	O(51)–C(52)–C(53)–C(54)	65·7
N(20)–C(22)–C(30)–O(31)	−14·2	O(51)–C(52)–C(56)–O(57)	17·1	O(51)–C(52)–C(53)–C(55)	−61·3
C(23)–C(22)–C(30)–O(32)	−66·4	C(53)–C(52)–C(56)–N(1)	79·7	C(56)–C(52)–C(53)–C(54)	−177·7
C(23)–C(22)–C(30)–O(31)	112·2	C(53)–C(52)–C(56)–O(57)	−99·1	C(56)–C(52)–C(53)–C(55)	55·3

indicate a *gauche–trans* conformation [$\chi \simeq -60$ or $180°$ where χ is defined as $H(C^\alpha)–C^\alpha–C^\beta–H(C^\beta)$].

Apart from minor details the structure of the uncomplexed form of Bv has all the features required to account for the spectroscopic data from Bv in *polar* solvents. In the crystal the departure of the backbone from exact threefold symmetry is small, and only one of the MePh side chains has χ at about $+60$ instead of $-60°$. It is not expected that the small differences in backbone conformation between the crystallographic and spectroscopic models would account for the large differences in ORD spectra observed between Bv in polar and non-polar solvents. Moreover, the conformation (P) described by Ovchinnikov *et al.* (1974) as the most likely to occur in polar solvents, *viz* φ, ψ (MePh) = -103, $171°$; φ, ψ(HyIv) = 74, $-136°$, is almost identical with the observed crystal structure.

The exact values of the torsion angles were derived by Popov, Pletnev, Evstratov, Ivanov & Ovchinnikov (1970) from semi-empirical potential-energy calculations. A similar minimum-energy structure was deduced independently in the author's laboratory (McCaldon, unpublished) and it is interesting to compare the two procedures briefly. Whereas Popov *et al.* used Kitaigorodsky's (1965) function, included electrostatic interactions, used a 'resilient spring' function for ring closure, and treated ω and τ as variables (as well as φ and ψ), we used a 6–exp function, took no account of electrostatic interactions, varied φ and ψ only, and used an arbitrarily chosen 'end-to-end' distance criterion to decide whether the ring would close without a significant increase in energy. Although ours was a fairly crude calculation, the fact that both procedures gave a similar minimum-energy structure implies that this is indeed a stable conformation.

Our calculations also indicate an alternative low-energy conformation involving *cis* MePh residues at about φ, ψ(MePh) = 60, $60°$; φ, ψ(HyIv) = 160, $200°$, although the width of the potential well is not as great as in the structure discussed above.

The structure of the Bv Ba picrate complex (Hamilton *et al.*, 1975) is $(Bv.Ba.Pic_3.Ba.Bv)^+Pic^-$. The complex anion involves two identical Bv molecules each coordinated to a Ba ion through the three HyIv carbonyl O atoms. The Ba ions are *not* in the central cavity of the Bv molecule but are displaced to that side of the disc opposite to which the *N*-methyl groups project. The two Ba ions are held 4.13 Å apart by coordination with six O atoms contributed by three picrate ions. The backbone conformation of each Bv molecule in this Ba picrate complex is similar to the form (P) proposed by the Russian group from their spectroscopic studies and conformational analysis, and to the conformation of the uncomplexed Bv presented in this paper. There is a non-crystallographic threefold symmetry in side-group orientations which is not observed in the Bv molecules in another crystal form of the Ba picrate complex currently under investigation.

A more detailed comparison of these two complexes will be presented later.

Also under investigation are crystals of the Rb picrate complex. The space group is $R3$ and six Bv molecules and six Rb ions are stacked along the three-fold axis giving a cell dimension of 48 Å along this unique axis. The positions of the peaks on the Patterson map indicate that there is some similarity between this structure and the Ba picrate complexes. However, the unit difference in charge between Rb^+ and Ba^{2+} means that only two $(Bv.Rb.Pic_3.Rb.Bv)$ groups would be allowed. The other two Rb ions must bear a different relation to the Bv molecules. Preliminary electron density maps do not show any Rb ions bound within the central cavity of Bv molecules but such an arrangement cannot be completely ruled out at this stage.

Discussion

A general feature of the naturally occurring ionophorous antibiotics is that they undergo a conformational change on binding the cation. In valinomycin, for example, there is a stepwise replacement by carbonyl O atoms of the water molecules coordinated to the ion, and this involves the breakage and formation of hydrogen bonds. In Bv (where there is no possibility of intramolecular hydrogen-bond formation) the conformations of the native molecule and the Ba picrate complex are apparently identical, apart from possible differences in rotation in the side chains. The complex is formed, not by adjusting the conformation to fit the ion, but by bringing two antibiotic molecules together to surround a cation–anion complex. Obviously the nature of the anion will affect the stability of the complex and this could explain the results of Estrada-O *et al.* (1972) who demonstrated an anion-dependent cation specificity of Bv in mitochondria. Hamilton *et al.* (1975) point out that their structure could also account for the observed second-order concentration dependence on Bv in lipid bilayer membranes (Yafuso, Freeman, Steinrauf & Roeske, 1974), and for the apparent charge of $+1$ for Ca in the Bv mediated membrane transport of bacterial chromatophores (Prince *et al.*, 1974).

There is as yet no convincing crystallographic evidence that the enniatin antibiotics enclose a metal ion within the central cavity. As Hamilton *et al.* suggest, it is possible that the sixfold coordination provided by the carbonyl O atoms is inadequate for Ba and probably Ca ions as well. A further argument against ions larger than K^+ being contained within the central cavity is the close approach ($2.8–3.0$ Å) of carbonyl C atoms in the backbone to the centre of the molecule. Ions smaller than K^+ ($K^+–O \simeq 2.60$ Å minimum) could enter the central cavity without necessitating a conformational change in the antibiotic. However, the average distance from the centroid of the six carbonyl O atoms to these atoms is about 2.8 Å so that the molecule would have to close in upon small

ions to provide reasonable coordination distances. In view of the close-packed nature of the observed structure this may not be an energetically favoured process, in which case the ion would probably prefer to lie closer to either the triad of MePh carbonyl O atoms or to the triad of HyIv carbonyl O atoms and there would then be no good reason for choosing the inside rather than the outside of the cavity. At the moment there does not seem to be any experimental evidence for a significant covalent contribution to the $M^+\cdots O$ bond which might cause the ion to prefer to be outside the cavity.

Finally, we note that the crystal structure conformation of uncomplexed Bv crystallized from n-heptane is similar to that proposed by spectroscopic studies for Bv in *polar* solvents. The predominant environment of the molecule in the crystal is non-polar, with only 1 to 2 molecules of water for each Bv molecule. The structure described would seem to be a likely candidate for the uncomplexed molecule within biological membranes.

References

DICKERSON, R. E. & GEIS, I. (1969). In *The Structure and Action of Proteins*. New York: Harper & Row.

DORSCHNER, E. & LARDY, H. (1968). *Antimicrobial Agents Ann.* pp. 11–19.

ESTRADA-O, S., GOMEZ-LOUERO, C. & MONTAL, M. (1972). *Bioenergetics*, **3**, 417–428.

GERMAIN, G., MAIN, P. & WOOLFSON, M. M. (1971). *Acta Cryst.* A**27**, 368–376.

HAMILL, R. L., HIGGINS, C. E., BOAZ, H. E. & GORMAN, M. (1969). *Tetrahedron Lett.* pp. 4255–4258.

HAMILTON, J. A., STEINRAUF, L. K. & BRADEN, B. (1975). *Biochem. Biophys. Res. Commun.* **64**, 151–156.

IUPAC–IUB COMMISSION ON BIOCHEMICAL NOMENCLATURE (1970). *J. Mol. Biol.* **52**, 1–17.

IVANOV, V. T., EVSTRATOV, A. V., SUMSKAYA, L. V., MELNIK, E. I., CHUMBURIDZE, T. S., PORTNOVA, S. L., BALASHOVA, T. A. & OVCHINNIKOV, YU. A. (1973). *FEBS Lett.* **36**, 65–71.

KITAIGORODSKY, A. I. (1965). *Acta Cryst.* **18**, 585–590.

MUELLER, P. & RUDIN, D. O. (1967). *Biochem. Biophys. Res. Commun.* **26**, 398–404.

OVCHINNIKOV, YU. A., IVANOV, V. T., EVSTRATOV, A. V., MIKHALEVA, I. I., BYSTROV, V. F., PORTNOVA, S. L., BALASHOVA, T. A., MESHCHERYAKOVA, E. N. & TULCHINSKY, V. M. (1974). *Int. J. Peptide Protein Res.* **6**, 465–498.

OVCHINNIKOV, YU. A., IVANOV, V. T. & MIKHALEVA, I. I. (1971). *Tetrahedron Lett.* 159–162.

POPOV, E. M., PLETNEV, B. Z., EVSTRATOV, A. V., IVANOV, V. T. & OVCHINNIKOV, YU. A. (1970). *Khim. Prir. Soedin.* **5**, 616–622.

PRINCE, R. C., CROFTS, A. R. & STEINRAUF, L. K. (1974). *Biochem. Biophys. Res. Commun.* **59**, 697–703.

ROESKE, R. W., ISAAC, S., KING, T. E. & STEINRAUF, L. K. (1974). *Biochem. Biophys. Res. Commun.* **57**, 554–561.

STEWART, J. M., KRUGER, G. J., AMMON, H. L., DICKINSON, C. & HALL, S. R. (1972). The X-RAY system – version of June 1972, Tech. Rep. TR-192. Computer Science Center, Univ. of Maryland, College Park, Maryland.

YAFUSO, M., FREEMAN, A. R., STEINRAUF, L. K. & ROESKE, R. W. (1974). *Fed. Proc. Abstr.* **33**, 1258.

The Crystal and Molecular Structure of 2,4-Dinitrophenol

BY TATSUIKI KAGAWA, RYOICHI KAWAI, SETSUO KASHINO AND MASAO HAISA

Department of Chemistry, Faculty of Science, Okayama University, Tsushima, Okayama 700, Japan

The crystal structure of 2,4-dinitrophenol has been determined from visually estimated Cu $K\alpha$ X-ray data. The crystals are orthorhombic, space group $P2_12_12_1$, with $a = 6\cdot106$ (6), $b = 23\cdot24$ (2), $c = 5\cdot168$ (5) Å and $Z = 4$. The structure was solved by the Patterson method and refined by block-diagonal least-squares calculations to $R = 0\cdot076$ for 768 non-zero reflexions. The molecules related by an a translation are stacked to form a column. The columns are held together by an interaction between the phenolic and nitro groups [O$\cdots$H $2\cdot23$ Å] of the molecules related by a twofold screw axis along the c axis, to form a sheet parallel to (010). The sheets are stacked along the b axis by van der Waals interactions. The molecule is nearly planar and the o-quinonoid nature of the benzene ring is remarkable. The phenolic C–O bond length $1\cdot331$ (7) Å is appreciably shortened. The geometry of the intramolecular hydrogen bonding between the phenolic and o-nitro groups is similar to that of the other o-nitrophenols [O$\cdots$O $2\cdot593$ (7) Å, O–H$\cdots$O 130 (6)°, H$\cdots$O $1\cdot89$ (7) Å].

Introduction

As part of a programme of studies on the crystal and molecular structures of substituted phenols (Kawai,

Kashino & Haisa, 1976), the structure of 2,4-dinitrophenol, the prototype of 'uncouplers' of oxidative phosphorylation (White, Handler & Smith, 1973), has been studied by X-ray analysis. The main object of