The Crystal and Molecular Structure of Bicyclomycin, a New Antibiotic

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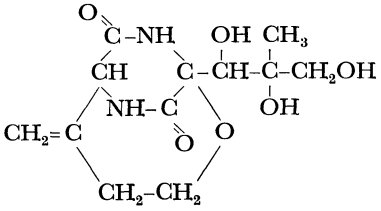
The molecular structure of a new antibiotic, Bicyclomycin ($C_{12}H_{18}O_7N_2$), has been elucidated by X-ray diffraction analysis. Bicyclomycin crystallizes from aqueous solution in two forms, an orthorhombic and a monoclinic systems. The crystal data for the former are $a=11.72$, $b=12.72$, $c=8.80$ Å and the space group $P2_12_12_1$, while those for the latter are $a=10.27$, $b=10.91$, $c=6.67$ Å, $\beta=101.4°$, and the space group $P2_1$. The crystal structure of the former was solved by the direct method and refined by least-squares method to a final R factor of 0.07 for 1623 reflections. The established structure is



The diketopiperazine ring is folded into a boat form. The conformations of the side chain are all *gauche* or nearly so. All the oxygen and nitrogen atoms participate in two intramolecular and four intermolecular hydrogen bonds.

A new antibiotic Bicyclomycin was obtained from the culture filtrate of *Streptomyces sapporonensis* which was isolated from a soil sample collected at Sapporo City. Bicyclomycin has an antibacterial activity against Gram-negative bacteria and exhibits no cross resistance to major antibiotics for human use.[1] Chemical and spectroscopic investigations[2] suggested several functional groups in the molecule, but the precise stereochemistry of the structure remained to be unknown. X-Ray diffraction study was thus undertaken to establish the whole structure of the molecule.

Experimental

Bicyclomycin crystallizes from aqueous solution in two forms, colorless prisms and plates. Oscillation and Weissenberg photographs uniquely identified the space groups as $P2_12_12_1$ for the former and $P2_1$ for the latter. The unit cell dimensions were determined by the least-squares calculation using the high angle spots of Cu$K\alpha_1$ and Cu$K\alpha_2$ on zero-layer Weissenberg photographs superimposed by the Al wire patterns for calibration. The densities were measured by a floatation method in a chloroform–carbon tetrachloride mixture for the orthorhombic crystal and a benzene–carbon tetrachloride mixture for the monoclinic one, respectively. Crystal data were summarized in Table 1. The intensity data for the orthorhombic modification were collected from the multiple-film equi-inclination Weissenberg photographs for the layer lines 0 to 8 on the a axis and 0 to 6 on the c axis, respectively using the Ni-filtered Cu$K\alpha$ radiation. The intensities were estimated visually by comparison with a calibrated film strip. Corrections were made for Lorentz and polarization factors and spot sizes. A total of 1623 intensities was recorded, but 74 of these were too weak to be measured. Structure factor magnitudes $|F|$ and normalized structure factor magnitudes $|E|$ were derived by the Wilson's method. The values of the statistical average, $\langle|E|\rangle$ and $\langle|E|^2-1\rangle$, were 0.867 and 0.778, respectively, as compared with 0.886 and 0.736, the theoretical values for noncentrosymmetric space groups.

Table 1. Crystal data

Crystal system	orthorhombic	monoclinic
Molecular formula	$C_{12}H_{18}O_7N_2$	$C_{12}H_{18}O_7N_2\cdot H_2O$
Unit cell constants	$a=11.72(1)$Å	$a=10.27(1)$Å
	$b=12.72(1)$	$b=10.91(1)$
	$c=8.80(1)$	$c=6.67(1)$
		$\beta=101.4(1)°$
V	1312 Å^3	732 Å^3
D_m	1.54	1.46
D_c	1.53 g/cm^3	1.45 g/cm^3
Z	4	2
Absent spectra	$h00$ when h is odd,	$0k0$ when k is odd.
	$0k0$ when k is odd,	
	$00l$ when l is odd.	
Space group	$P2_12_12_1$	$P2_1$

Structure Determination

The structure of Bicyclomycin was solved using the symbolic addition technique applied to noncentrosymmetric structures.[3] The Eq. (1) was used to define 30 phases ($|E|\geq1.5$) in terms of initial set of phases listed in Table 2. From multiple indications, it soon became obvious that $2b\simeq0$, $2c\simeq\pi$, and $2p\simeq\pi$. As for the value a, there were no confirmative indications.

$$\varphi_{\boldsymbol{h}} \simeq \langle\varphi_{\boldsymbol{k}}+\varphi_{\boldsymbol{h}-\boldsymbol{k}}\rangle_{\boldsymbol{k}r} \qquad (1)$$

Thus, various combinations of symbolic phases for

Table 2. Initial set of phases

Reflection	Phase	$\|E\|$
0 3 7	$\pi/2$	2.56
0 9 4	$\pi/2$	2.73
5 0 8	0	3.29
4 2 10	a	2.67
5 10 4	b	2.69
10 4 1	c	2.78
4 5 8	p	2.57

the use of the tangent formula were assigned as follows:

$$a = \pi/6, 3\pi/6, 5\pi/6, -\pi/6, -3\pi/6, -5\pi/6$$
$$b = 0, \pi$$
$$c = \pi/2, -\pi/2$$
$$p = \pi/2, -\pi/2.$$

For each of the 48 sets with 185 phases ($|E| \geq 1.50$), six cycles of tangent refinement were carried out. Only four sets of them converged characteristically at lowest R' index,[3] defined by

$$R' = \frac{\sum_{kr} ||E_h|_{obs} - |E_h|_{calc}|}{\sum_{kr} |E_h|_{obs}} \quad . \quad (2)$$

Further eight cycles of tangent refinement for each of these four sets of 391 phases ($|E| \geq 1.20$) converged at the same R' index of 0.26.

Three dimensional E-maps with 391 terms were then calculated for these sets. The 21 largest peaks appeared on the respective four E-maps; each of them was found to give the same molecular structure. There was only one unique set, however, to which the other three were related by a mirror symmetry or by a translation. Assuming all the atoms to be nitrogen atom, structure factor calculation based on these 21 atoms gave an R factor of 0.25 for all reflections, and the corresponding Fourier synthesis showed no spurious peaks. All the 21 non-hydrogen atoms were assigned to 12 carbon, 7 oxygen and 2 nitrogen atoms, respectively on the basis of this Fourier map and the partial structure known from chemical and spectroscopic investigations.[2]

The atomic coordinates and isotropic thermal parameters for the 21 atoms were refined by block-diagonal least-squares method for four cycles. The function minimized was $\sum w(|F_o| - |F_c|)^2$. Then, anisotropic temperature factors for all non-hydrogen atoms were introduced, and two more cycles of refinement reduced the R factor to 0.09. A difference map calculated at this stage revealed all the 18 hydrogen atoms. Setting the isotropic temperature factor of each hydrogen atom

Table 3. The fractional atomic coordinates and their estimated standard deviations in parentheses

Atom	x	y	z
C(1)	0.1686(3)	0.2872(3)	0.2093(4)
C(2)	0.2539(3)	0.2885(3)	0.3436(4)
C(4)	0.3899(3)	0.3824(3)	0.1740(4)
C(6)	0.2563(3)	0.5317(3)	0.2167(5)
C(7)	0.1437(4)	0.4891(4)	0.1549(5)
C(8)	0.0988(3)	0.3887(4)	0.2235(4)
C(10)	0.3371(3)	0.3203(3)	0.0399(4)
C(13)	0.5216(3)	0.3802(3)	0.1637(4)
C(15)	0.5869(3)	0.4369(3)	0.2948(4)
C(17)	0.7150(4)	0.4338(4)	0.2601(6)
C(18)	0.5495(4)	0.5514(3)	0.3199(5)
C(20)	0.0006(4)	0.3870(5)	0.3001(6)
N(3)	0.3527(3)	0.3412(3)	0.3188(3)
N(9)	0.2323(3)	0.2819(3)	0.0654(3)
O(5)	0.3575(2)	0.4898(2)	0.1452(3)
O(11)	0.1035(3)	0.1967(3)	0.2230(4)
O(12)	0.2311(3)	0.2516(3)	0.4675(3)
O(14)	0.5587(2)	0.2740(2)	0.1537(3)
O(16)	0.5610(3)	0.3868(3)	0.4359(3)
O(19)	0.5455(4)	0.6134(3)	0.1831(4)
O(21)	0.3845(3)	0.3150(3)	−0.0840(3)

Table 4. The anisotropic temperature factors[a] and their estimated standard deviations in parentheses (multiplied by 10^4)

Atom	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
C(1)	24(2)	36(3)	15(4)	−24(4)	−10(6)	− 5(6)
C(2)	21(2)	34(2)	19(4)	− 5(4)	10(6)	12(5)
C(4)	16(2)	20(2)	7(3)	3(4)	3(5)	2(5)
C(6)	24(2)	26(2)	33(4)	23(4)	−12(6)	− 6(5)
C(7)	27(3)	40(3)	31(4)	22(5)	−30(7)	6(6)
C(8)	22(2)	42(3)	18(4)	3(4)	−14(6)	−17(6)
C(10)	22(2)	22(2)	8(4)	16(4)	− 3(6)	7(5)
C(13)	15(2)	16(2)	19(4)	1(3)	14(6)	− 5(5)
C(15)	16(2)	26(2)	24(4)	− 1(4)	3(6)	− 7(5)
C(17)	17(2)	52(3)	79(6)	−12(5)	3(7)	−15(8)
C(18)	40(3)	24(2)	58(5)	− 3(5)	−13(8)	−18(6)
C(20)	31(3)	71(4)	92(7)	−10(6)	34(9)	−58(10)
N(3)	17(2)	26(2)	8(3)	− 3(3)	− 6(5)	5(4)
N(9)	28(2)	33(2)	5(3)	− 7(4)	−22(5)	−24(5)
O(5)	22(2)	21(2)	34(3)	13(3)	8(5)	13(4)
O(11)	28(2)	44(2)	71(4)	−34(4)	−13(5)	11(5)
O(12)	41(2)	62(2)	18(3)	−42(4)	0(5)	35(5)
O(14)	27(2)	17(1)	44(3)	11(3)	23(5)	− 8(4)
O(16)	25(2)	40(2)	28(3)	8(3)	−16(5)	17(4)
O(19)	60(3)	22(2)	84(5)	−14(4)	27(7)	12(5)
O(21)	31(2)	49(2)	10(3)	10(4)	8(5)	−12(4)

a) The aniostropic temperature factor is in the form: $\exp\{-(B_{11}h^2+B_{22}k^2+B_{33}l^2+B_{12}hk+B_{13}hl+B_{23}kl)\}$.

Table 5. The fractional atomic coordinates for hydrogen atoms and their X–H bond lengths (X=C, N, or O)

Atom	Bonded to	x	y	z	X–H (Å)
H(1)	N(3)	0.405	0.353	0.395	0.92
H(2)	C(6)	0.258	0.605	0.192	0.96
H(3)	C(6)	0.255	0.520	0.327	0.98
H(4)	C(7)	0.156	0.472	0.049	0.96
H(5)	C(7)	0.081	0.550	0.171	1.08
H(6)	N(9)	0.191	0.266	−0.009	0.84
H(7)	O(11)	0.034	0.209	0.157	1.01
H(8)	C(13)	0.536	0.412	0.071	0.93
H(9)	O(14)	0.522	0.238	0.214	0.82
H(10)	O(16)	0.612	0.343	0.467	0.86
H(11)	C(17)	0.753	0.484	0.341	1.06
H(12)	C(17)	0.733	0.344	0.243	1.17
H(13)	C(17)	0.730	0.464	0.166	0.93
H(14)	C(18)	0.614	0.587	0.392	1.09
H(15)	C(18)	0.473	0.554	0.384	1.06
H(16)	O(19)	0.469	0.587	0.136	1.05
H(17)	C(20)	−0.043	0.459	0.314	1.05
H(18)	C(20)	−0.028	0.326	0.338	0.91

The mean standard deviation in atomic coordinates is 0.08 Å, and that of the distance X–H is 0.09 Å.

equal to 3.0 Å^2, four more cycles of least-squares refinement brought the R factor down to 0.07, the positions of all the hydrogen atoms being converged satisfactorily. The maximum shift of the non-hydrogen atom coordinates became less than the estimated standard deviations; their mean value for C, N, and O was 0.004 Å (0.003~0.007 Å). The weighting scheme adopted in the final least-squares calculation was:

$$w = 0.4 \qquad \text{for } |F_0| < 2.0$$

$$w = 1.0 \qquad \text{for } 2.0 \leq |F_0| < 10.0$$

$$w = 10.0/|F_0| \quad \text{for } |F_0| \geq 10.0.$$

The scattering factors for H, C, N, and O were taken from International Tables for X-ray Crystallography.[4] The final atomic coordinates and thermal parameters for non-hydrogen atoms are given in Tables 3 and 4, and those for hydrogen atoms in Table 5. The observed and calculated structure factors are given in Table 6.

Most of the computer programs used were in the UNICS.[5] Tangent refinement was performed with the program TANG, written by T. Ashida. All the programs were modified by the authors to allow calculations on middle size of computer, HITAC 8400 at Nippon Business Consultant and Fujisawa Pharmaceutical Co., Ltds.

TABLE 6. THE OBSERVED AND CALCULATED STRUCTURE FACTORS

L FO FC	L FO FC	L FO FC	L FO FC	L FO FC	L FO FC	L FO FC	L FO FC	L FO FC
H,K= 0 0	3 245 272	10 126 125	9 43 40	0 31 46	7 145 134	5 274 264	1 64 68	H,K= 5 5
4 50 35	4 136 128	H,K= 5 1	10 42 48	1 200 185	8 101 100	6 84 76	2 251 266	0 19 1
6 326 339	5 185 192	0 880 957	11 88 95	2 263 287	9 42 52	7 299 332	3 100 102	1 280 263
8 474 487	6 139 141	1 310 299	H,K= 1 2	3 189 221	10 189 164	8 201 222	4 181 190	2 118 110
10 157 170	H,K= 10 0	2 302 276	0 507 559	4 154 154	H,K= 5 3	9 34 43	5 111 115	3 286 280
H,K= 1 0	0 304 316	3 241 212	1 504 551	5 185 189	0 253 252	10 143 145	6 138 137	4 131 140
1 630 738	1 23 12	4 257 247	2 702 756	6 82 76	1 174 150	H,K= 1 4	H,K= 10 4	5 135 120
2 402 410	2 96 105	5 185 180	3 191 187	H,K= 10 2	2 123 122	0 622 639	0 287 286	6 104 92
3 336 343	3 0 16	6 276 301	4 317 330	0 23 16	3 298 279	1 280 289	1 401 414	7 280 303
4 243 237	4 227 224	7 109 89	5 170 180	1 88 100	4 221 217	2 254 229	2 135 155	8 149 145
5 455 479	5 0 20	8 185 171	6 99 99	2 127 138	5 255 256	3 413 416	3 132 146	9 83 77
6 266 281	6 226 253	9 31 36	7 206 193	3 191 222	6 107 91	4 251 259	4 218 201	10 22 29
7 411 428	H,K= 11 0	10 143 131	8 227 232	4 118 121	7 134 134	5 194 183	5 84 82	H,K= 6 5
8 198 210	1 336 318	H,K= 6 1	9 107 119	5 70 77	8 192 195	6 100 82	6 138 130	0 62 53
9 0 24	2 88 87	0 535 558	10 119 104	6 53 55	9 49 43	7 287 278	H,K= 11 4	1 107 102
10 139 128	3 192 205	1 476 454	11 73 72	H,K= 11 2	10 121 117	8 115 114	0 62 57	2 150 151
11 125 132	4 198 214	2 341 346	H,K= 2 2	0 49 33	H,K= 6 3	9 106 105	1 71 71	3 202 197
H,K= 2 0	5 96 92	3 203 190	0 364 385	1 41 37	0 167 151	10 41 55	2 106 94	4 238 254
0 99 94	6 68 64	4 200 185	1 75 75	2 113 127	1 245 218	H,K= 2 4	3 77 75	5 206 215
1 372 390	H,K= 12 0	5 119 108	2 509 513	3 153 164	2 123 115	1 266 253	4 125 133	6 94 93
2 484 477	0 78 91	6 88 78	3 588 607	4 178 189	3 127 122	2 370 347	5 190 189	7 113 110
3 541 530	1 0 20	7 181 179	4 119 109	5 102 108	4 202 189	3 107 104	6 159 149	8 152 132
4 348 341	2 44 56	8 162 177	5 290 271	6 89 69	5 131 133	4 108 95	H,K= 12 4	9 169 145
5 391 399	3 88 86	9 0 14	6 127 141	H,K= 12 2	6 134 132	5 219 208	0 63 64	H,K= 7 5
6 158 169	4 124 123	10 97 79	7 0 13	0 77 88	7 180 210	6 78 65	1 80 84	0 232 216
7 45 17	5 72 65	H,K= 7 1	8 209 220	1 132 150	8 99 92	7 96 91	2 86 77	1 119 113
8 119 120	6 0 5	0 400 395	9 53 58	2 0 12	9 42 51	8 202 208	3 32 36	2 224 220
9 106 115	H,K= 13 0	1 278 274	10 25 33	3 114 116	10 95 92	9 100 114	4 62 60	3 252 270
10 95 101	1 31 42	2 170 154	11 30 38	4 145 139	H,K= 7 3	10 175 156	5 128 104	4 197 191
11 32 42	2 25 25	3 87 66	H,K= 3 2	5 70 62	0 142 138	H,K= 3 4	6 28 28	5 157 165
H,K= 3 0	3 0 18	4 137 117	0 497 609	6 71 75	1 185 173	0 50 46	H,K= 13 4	6 56 73
1 263 252	4 101 95	5 42 36	1 454 477	H,K= 13 2	2 280 292	1 166 160	0 82 89	7 59 60
2 185 196	5 36 39	6 96 105	2 497 510	0 0 7	3 226 211	2 267 249	1 66 71	8 180 154
3 187 175	H,K= 14 0	7 115 113	3 148 136	1 27 27	4 281 271	3 227 212	2 0 19	9 95 82
4 202 172	0 23 25	8 169 130	4 47 52	2 108 121	5 140 122	4 158 151	3 95 82	H,K= 8 5
5 73 62	1 44 46	9 178 142	5 104 89	3 0 1	6 162 153	5 252 248	4 73 65	0 147 154
6 201 207	2 34 36	H,K= 8 1	6 164 169	4 59 48	7 113 115	6 80 83	H,K= 14 4	1 149 145
7 0 10	3 80 75	0 347 330	7 214 203	5 103 104	8 47 50	7 186 187	0 36 39	2 167 156
8 138 132	4 54 53	1 75 70	8 110 107	H,K= 14 2	9 85 68	8 182 193	1 75 73	3 262 297
9 85 93	H,K= 0 1	2 159 148	9 25 7	0 168 158	H,K= 8 3	9 69 72	2 16 16	4 44 52
10 132 134	1 342 325	3 129 118	10 213 220	1 103 98	0 101 89	10 45 49	3 43 48	5 112 118
11 58 69	2 324 299	4 238 240	H,K= 4 2	2 77 70	1 240 229	H,K= 4 4	H,K= 0 5	6 49 43
H,K= 4 0	3 230 220	5 211 227	0 154 145	3 47 49	2 390 433	0 168 152	1 71 67	7 179 172
1 10 5	4 311 330	6 60 64	1 219 205	H,K= 0 3	3 197 204	1 423 424	2 133 106	8 0 18
2 194 167	5 152 155	7 205 206	2 402 398	1 312 309	4 239 243	2 208 201	3 354 356	H,K= 9 5
3 142 118	6 132 161	8 80 72	3 192 178	2 429 414	5 132 125	3 144 131	4 285 295	0 358 375
4 158 152	7 163 159	9 26 24	4 284 279	3 266 267	6 53 58	4 264 270	5 76 71	1 127 128
5 110 113	8 223 228	H,K= 9 1	5 163 147	4 15 17	7 147 126	5 139 116	6 36 48	2 212 228
6 52 45	9 35 33	0 307 296	6 270 301	5 80 69	8 45 44	6 112 103	7 95 92	3 66 66
7 233 232	10 54 78	1 133 136	7 196 187	6 135 147	9 140 121	7 81 80	8 81 76	4 253 240
8 0 4	11 52 53	2 130 132	8 67 75	7 439 422	H,K= 9 3	8 136 129	9 135 142	5 74 75
9 51 50	H,K= 1 1	3 138 137	9 93 86	8 79 70	0 106 106	9 125 116	10 97 103	6 141 136
10 111 106	1 625 666	4 119 112	10 245 231	9 81 86	1 36 43	10 32 32	H,K= 1 5	H,K= 10 5
H,K= 5 0	2 108 99	5 52 52	H,K= 5 2	10 40 46	2 235 248	H,K= 5 4	0 792 846	0 65 61
1 131 129	3 256 257	6 71 67	0 420 439	11 39 34	3 108 108	0 355 350	1 389 354	1 131 132
2 412 359	4 276 274	H,K= 10 1	1 42 53	H,K= 1 3	4 44 36	1 120 119	2 328 320	2 192 212
3 203 180	5 154 146	0 16 12	2 66 54	0 293 294	5 0 16	2 346 342	3 458 465	3 262 275
4 343 340	6 277 286	1 88 88	3 69 61	1 529 546	6 72 68	3 76 81	4 149 149	4 56 50
5 150 137	7 412 377	2 161 165	4 172 161	2 230 225	H,K= 10 3	4 68 66	5 221 219	5 163 174
6 186 189	8 325 326	3 100 105	5 11 19	3 354 356	0 28 30	5 73 66	6 35 29	6 133 109
7 236 246	9 81 86	4 115 124	6 233 250	4 137 123	1 47 54	6 193 191	7 128 128	H,K= 11 5
8 414 429	10 43 51	5 66 67	7 182 171	5 419 447	2 194 209	7 30 14	8 114 118	0 212 228
9 93 92	11 52 52	6 0 4	8 184 182	6 102 83	3 133 129	8 197 184	9 102 99	1 0 17
10 144 142	H,K= 2 1	H,K= 11 1	9 76 59	7 79 76	4 152 161	9 101 88	10 125 112	2 169 166
H,K= 6 0	0 262 270	0 86 104	10 180 196	8 191 186	5 34 42	10 34 40	H,K= 2 5	3 0 22
0 530 540	1 394 390	1 50 49	H,K= 6 2	9 79 87	6 102 85	H,K= 6 4	0 529 549	4 152 132
1 219 204	2 439 427	2 113 122	0 217 231	10 93 83	H,K= 11 3	0 157 151	1 407 389	5 47 51
2 91 65	3 275 267	3 93 97	1 178 179	11 73 74	0 23 17	1 139 133	2 203 182	6 108 95
3 288 276	4 355 323	4 56 52	2 249 226	H,K= 2 3	1 77 86	2 50 51	3 208 204	H,K= 12 5
4 186 174	5 268 244	5 36 29	3 125 131	0 241 229	2 60 66	3 121 112	4 65 69	0 139 138
5 194 193	6 188 203	6 67 55	4 285 280	1 320 338	3 75 77	4 46 48	5 211 213	1 58 64
6 211 212	7 297 298	H,K= 12 1	5 113 108	2 347 340	4 87 85	5 237 264	6 160 159	2 86 79
7 172 150	8 161 162	0 54 52	6 112 112	3 371 366	5 96 97	6 109 113	7 0 25	3 93 96
8 61 64	9 106 114	1 27 36	7 110 109	4 517 495	6 113 103	7 64 60	8 123 135	4 48 42
9 149 124	10 49 52	2 207 205	8 144 143	5 102 91	H,K= 12 3	8 79 70	9 89 98	5 77 66
10 0 13	11 0 15	3 115 110	9 76 66	6 286 295	0 85 98	9 99 89	10 29 38	H,K= 13 5
H,K= 7 0	H,K= 3 1	4 106 105	10 196 208	7 236 230	1 0 12	H,K= 7 4	H,K= 3 5	0 48 50
1 265 256	0 328 339	5 72 68	H,K= 7 2	8 127 119	2 36 27	0 218 211	0 160 155	1 21 21
2 125 99	1 50 42	6 92 81	0 0 0	9 78 73	3 64 52	1 32 30	1 250 213	2 30 35
3 178 166	2 415 417	H,K= 13 1	1 123 110	10 0 16	4 150 135	2 179 173	2 348 334	3 0 11
4 426 456	3 879 932	0 70 73	2 127 108	H,K= 3 3	5 137 128	3 218 208	3 106 102	4 35 36
5 0 10	4 444 410	1 137 150	3 129 128	0 299 284	6 82 87	4 295 289	4 187 190	H,K= 14 5
6 98 92	5 236 230	2 35 32	4 86 76	1 657 687	H,K= 13 3	5 293 324	5 53 52	0 103 101
7 96 77	6 52 42	3 63 61	5 247 260	2 116 113	0 103 101	6 134 127	6 66 53	1 95 107
8 116 91	7 145 137	4 152 149	6 199 185	3 534 537	1 153 166	7 33 29	7 177 185	H,K= 0 6
9 142 123	8 232 236	5 62 71	7 114 112	4 122 114	2 72 65	8 164 134	8 83 92	0 152 114
H,K= 8 0	9 36 37	H,K= 14 1	8 48 51	5 246 232	3 119 125	9 26 31	9 36 40	1 133 126
0 346 343	10 108 104	0 118 109	9 89 65	6 124 118	4 70 81	H,K= 8 4	10 78 69	2 50 29
1 117 107	11 126 141	1 66 74	H,K= 8 2	7 216 195	5 122 144	0 110 117	H,K= 4 5	3 115 115
2 113 104	H,K= 4 1	2 63 60	0 31 25	8 72 74	H,K= 14 3	1 126 120	0 248 237	4 221 230
3 238 242	0 210 222	3 82 79	1 114 112	9 72 80	0 68 70	2 191 189	1 300 269	5 173 170
4 80 45	1 599 594	4 80 87	2 179 182	10 212 194	1 46 46	3 213 235	2 116 101	6 53 25
5 186 188	2 196 172	H,K= 0 2	3 171 165	H,K= 4 3	2 194 191	4 158 167	3 114 110	7 45 40
6 53 50	3 185 159	2 652 704	4 266 280	0 20 20	3 108 116	5 28 35	4 240 224	8 35 41
7 0 15	4 189 168	3 16 6	5 237 244	1 149 134	H,K= 0 4	6 115 103	5 201 200	9 151 157
8 0 14	5 204 192	4 168 161	6 117 114	2 561 596	0 123 120	7 69 56	6 229 258	10 0 22
9 59 49	6 135 129	5 77 51	7 179 178	3 260 246	1 33 38	8 51 50	7 135 126	H,K= 1 6
H,K= 9 0	7 233 219	6 143 124	8 108 92	4 133 120	2 262 247	9 137 145	8 301 330	0 214 213
1 318 298	8 170 173	7 293 291	9 0 3	5 32 15	3 170 162	H,K= 9 4	9 136 136	1 394 373
2 97 91	9 129 129	8 122 131	H,K= 9 2	6 224 232	4 113 117	0 22 32	10 29 41	2 303 288

Table 6. Continued.

L	FO	FC	L	FO	FC	L	FO	FC	L	FO	FC	L	FO	FC	L	FO	FC	L	FO	FC	L	FO	FC	L	FO	FC
H,K= 10 6			2	111	95	H,K= 1 8			0	27	22	0	145	156	1	144	135	2	36	45	0	62	45	H,K= 10 12			1	96	95
4	67	65	3	129	137	0	38	19	1	0	21	1	168	142	2	53	36	3	146	149	1	51	49	0	59	44	2	94	85
5	160	149	4	160	174	1	241	218	2	114	115	2	102	97	3	73	70	4	29	34	2	57	66	1	44	55	3	62	56
6	105	83	5	56	62	2	121	115	3	68	61	3	56	53	4	119	119	5	113	131	3	53	60	H,K= 0 13			4	111	115
H,K= 11 6			6	117	121	3	161	164	4	69	67	4	120	130	5	136	153	6	22	25	H,K= 11 11			1	8	11	5	25	27
0	133	137	7	136	117	4	88	95	5	89	84	5	148	166	6	81	90	7	0	4	0	37	39	2	16	33	H,K= 5 14		
1	46	52	8	48	57	5	154	157	6	83	86	6	93	97	7	100	92	8	135	141	H,K= 0 12			3	0	0	0	11	23
2	90	83	9	68	67	6	209	228	H,K= 10 8			7	0	17	8	0	2	H,K= 1 11			0	221	233	4	161	172	1	62	56
3	73	70	H,K= 6 7			7	259	276	0	170	118	8	99	98	H,K= 3 10			0	75	63	1	132	138	5	80	76	2	0	10
4	118	109	0	120	108	8	0	18	1	21	22	H,K= 6 9			0	267	246	1	188	175	2	186	197	6	113	116	3	37	37
5	98	81	1	31	34	9	38	48	2	0	14	0	84	86	1	118	112	2	40	36	3	93	92	H,K= 1 13			4	107	113
6	67	65	2	41	42	H,K= 2 8			3	42	35	1	149	142	2	44	42	3	80	91	4	0	7	0	11	11	5	52	53
H,K= 12 6			3	138	128	0	202	196	4	127	121	2	65	66	3	104	99	4	134	139	5	21	28	1	31	31	H,K= 4 14		
0	0	0	4	200	215	1	87	84	5	88	88	3	166	178	4	96	101	5	62	65	6	24	19	2	53	52	0	11	15
1	41	44	5	135	134	2	155	150	6	70	66	4	63	60	5	125	135	6	91	95	7	69	55	3	123	110	1	110	94
2	132	132	6	179	207	3	69	61	H,K= 11 8			5	68	81	6	49	45	7	110	109	H,K= 1 12			4	69	70	2	92	88
3	83	79	7	64	58	4	61	62	0	30	22	6	137	153	7	0	13	8	91	87	0	0	11	5	115	118	3	31	26
4	41	35	8	149	141	5	97	100	1	49	52	7	101	82	8	65	57	H,K= 2 11			1	129	127	6	66	64	4	0	13
5	49	49	9	89	88	6	55	54	2	73	60	8	73	71	H,K= 4 10			0	75	80	2	132	144	H,K= 2 13			H,K= 5 14		
H,K= 13 6			H,K= 7 7			7	81	83	3	31	51	H,K= 7 9			0	172	184	1	167	172	3	87	89	0	198	163	0	46	36
0	0	9	0	55	64	8	67	60	4	60	49	0	271	279	1	101	103	2	44	51	4	0	7	1	11	16	1	77	66
1	113	110	1	232	226	9	42	48	5	42	49	1	58	61	2	38	30	3	82	85	5	99	95	2	41	39	2	74	61
2	68	69	2	186	200	H,K= 3 8			H,K= 12 8			2	54	62	3	165	180	4	60	67	6	176	183	3	116	110	3	9	17
3	59	70	3	137	128	0	317	289	0	43	46	3	138	137	4	150	140	5	0	21	7	162	139	4	25	21	4	63	70
H,K= 0 7			4	172	178	1	67	64	1	121	116	4	44	45	5	148	116	6	98	97	H,K= 2 12			5	0	4	H,K= 6 14		
1	433	423	5	90	88	2	70	66	2	19	20	5	74	71	6	125	128	7	125	127	0	144	150	6	57	44	0	9	10
2	184	171	6	57	52	3	28	26	3	79	76	6	149	142	7	120	110	8	132	129	1	70	66	H,K= 3 13			1	55	44
3	222	227	7	178	164	4	297	339	H,K= 13 8			7	104	71	8	84	79	H,K= 3 11			2	119	124	0	49	50	2	45	48
4	294	293	8	86	74	5	99	89	0	73	78	H,K= 8 9			H,K= 5 10			0	267	276	3	46	44	1	112	110	3	30	31
5	93	91	H,K= 8 7			6	57	54	1	112	139	0	31	29	0	66	66	1	185	197	4	72	68	2	111	102	H,K= 7 14		
6	38	43	0	134	138	7	117	106	H,K= 0 9			1	181	199	1	47	57	2	51	48	5	94	98	3	0	12	0	27	27
7	94	90	1	11	25	8	80	79	1	140	149	2	58	59	2	203	204	3	176	185	6	111	99	4	40	42	1	0	11
8	66	59	2	186	206	9	116	110	2	535	540	3	70	64	3	276	317	4	113	105	7	25	32	5	78	73	2	24	27
9	75	75	3	74	70	H,K= 4 8			3	190	188	4	183	189	4	313	345	5	0	10	H,K= 3 12			6	50	56	H,K= 0 15		
10	51	53	4	109	117	0	206	186	4	427	489	5	81	77	5	143	151	6	54	59	0	103	113	H,K= 4 13			1	10	22
H,K= 1 7			5	88	94	1	277	263	5	55	67	6	25	18	6	28	30	7	22	28	1	233	240	0	54	52	2	36	44
0	238	190	6	146	150	2	104	110	6	58	58	7	143	171	7	165	151	H,K= 4 11			2	43	37	1	122	124	3	32	30
1	80	75	7	74	57	3	228	235	7	255	253	H,K= 9 9			8	66	72	0	11	23	3	124	125	2	26	29	4	39	44
2	342	323	8	113	107	4	145	143	8	35	35	0	34	31	H,K= 6 10			1	117	113	4	64	64	3	30	28	H,K= 1 15		
3	118	112	H,K= 9 7			5	130	136	9	93	84	1	0	11	0	28	21	2	91	93	5	13	21	4	40	35	0	47	55
4	140	145	0	46	48	6	121	138	H,K= 1 9			2	113	122	1	116	119	3	298	325	6	0	18	5	97	100	1	50	46
5	180	178	1	128	123	7	96	97	0	236	213	3	104	107	2	151	137	4	0	21	H,K= 4 12			6	59	51	2	63	61
6	117	121	2	210	236	8	133	121	1	123	107	4	143	129	3	191	202	5	214	234	0	180	178	H,K= 5 13			3	54	53
7	60	60	3	73	65	9	35	33	2	219	218	5	46	45	4	50	61	6	96	90	1	68	73	0	12	9	4	105	121
8	74	75	4	75	78	H,K= 5 8			3	472	494	6	91	88	5	76	64	7	81	59	2	73	69	1	98	86	H,K= 2 15		
9	72	67	5	119	127	0	141	138	4	108	101	H,K= 10 9			6	80	82	H,K= 5 11			3	72	75	2	123	118	0	72	69
10	76	73	6	193	193	1	166	161	5	247	253	0	56	52	7	77	69	0	72	61	4	136	134	3	62	60	1	0	12
H,K= 2 7			H,K= 10 7			2	268	276	6	40	39	1	31	31	H,K= 7 10			1	32	27	5	76	32	4	55	53	2	0	19
0	346	321	0	15	19	3	167	165	7	152	154	2	84	84	0	33	33	2	104	110	6	114	111	5	32	36	3	21	21
1	352	318	1	103	106	4	268	273	8	42	40	3	60	57	1	30	30	3	48	56	7	45	48	H,K= 6 13			4	30	38
2	255	247	2	41	45	5	126	128	9	124	117	4	131	122	2	52	49	4	0	4	H,K= 5 12			0	11	19	H,K= 3 15		
3	154	155	3	85	86	6	31	30	H,K= 2 9			5	72	75	3	59	60	5	28	22	0	0	2	1	70	74	0	34	35
4	82	78	4	41	44	7	133	124	0	97	80	H,K= 11 9			4	75	79	6	53	51	1	49	50	2	47	48	1	96	82
5	135	136	5	120	104	8	141	128	1	153	150	0	71	63	5	14	19	7	20	12	2	0	10	3	86	80	2	23	28
6	186	196	6	147	133	H,K= 6 8			2	303	290	1	38	41	6	72	62	H,K= 6 11			3	40	44	4	92	89	3	78	77
7	201	221	H,K= 11 7			0	72	69	3	48	45	2	0	13	7	109	104	0	11	24	4	151	163	5	38	47	H,K= 4 15		
8	125	117	0	14	13	1	220	198	4	267	283	3	83	86	H,K= 8 10			1	84	98	5	24	15	H,K= 7 13			0	37	22
9	94	90	1	64	71	2	214	225	5	115	125	4	109	112	0	86	100	2	126	142	6	70	67	0	184	155	1	97	89
10	68	77	2	43	39	3	261	335	6	77	81	H,K= 12 9			1	82	86	3	127	127	H,K= 6 12			1	97	86	2	98	91
H,K= 3 7			3	139	142	4	112	107	7	105	107	0	36	24	2	145	100	4	125	130	0	166	165	2	114	98	3	66	71
0	537	521	4	112	98	5	186	194	8	162	143	1	56	61	3	56	32	5	50	48	1	149	146	3	96	86	H,K= 5 15		
1	121	125	5	76	70	6	78	78	9	17	17	2	77	92	4	97	89	6	11	22	2	117	107	H,K= 8 13			0	16	6
2	224	206	H,K= 12 7			7	152	141	H,K= 3 9			H,K= 0 10			5	86	77	H,K= 7 11			3	50	61	0	24	25	1	100	95
3	73	66	0	108	106	8	57	48	0	247	241	0	517	509	6	59	55	0	145	142	4	39	38	1	160	153	2	81	90
4	119	120	1	132	134	H,K= 7 8			1	144	140	1	243	239	H,K= 9 10			1	119	128	5	67	58	2	70	60	H,K= 0 16		
5	113	121	2	59	53	0	216	210	2	135	137	2	83	72	0	103	103	2	147	141	6	25	23	3	96	110	0	106	92
6	173	192	3	88	74	1	126	116	3	125	126	3	207	231	1	50	54	3	0	13	H,K= 7 12			H,K= 9 13			1	20	0
7	80	91	4	0	4	2	218	235	4	133	95	4	104	104	2	25	32	4	12	24	0	95	90	0	105	118	2	0	4
8	95	101	H,K= 13 7			3	99	94	5	121	131	5	168	186	3	34	22	5	46	44	1	104	102	H,K= 0 14			H,K= 1 16		
9	134	118	0	157	148	4	120	125	6	50	54	6	13	20	4	22	24	6	95	97	2	111	101	0	56	71	0	0	1
H,K= 4 7			1	97	99	5	43	35	7	54	56	7	35	44	5	124	133	H,K= 8 11			3	31	30	1	86	94	1	67	55
0	37	33	2	70	76	6	83	77	8	131	117	8	0	17	H,K= 10 11			0	112	111	4	10	16	2	61	55	2	68	83
1	111	98	3	29	32	7	51	57	9	72	76	H,K= 1 10			0	21	21	1	125	129	5	69	61	3	59	36	H,K= 2 16		
2	142	138	H,K= 0 8			8	99	85	H,K= 4 9			0	54	34	1	42	49	2	47	39	H,K= 8 12			4	77	76	0	127	118
3	75	58	0	249	231	H,K= 8 8			0	30	37	1	151	153	2	80	67	3	35	33	0	28	21	5	82	84	1	113	115
4	145	132	1	223	216	0	64	63	1	83	85	2	137	143	3	108	98	4	32	75	1	49	49	H,K= 1 14					
5	238	246	2	18	20	1	97	97	2	174	180	3	192	179	4	94	82	5	29	27	2	91	81	0	7	10			
6	91	83	3	0	30	2	86	85	3	75	71	4	166	171	H,K= 11 10			H,K= 9 11			3	69	69	1	17	11			
7	54	51	4	188	212	3	55	55	4	248	264	5	53	59	0	23	24	0	49	42	4	62	49	2	38	33			
8	0	15	5	95	91	4	12	14	5	75	78	6	135	144	1	55	43	1	107	99	H,K= 9 12			3	34	32			
9	42	38	6	248	289	5	97	91	6	26	22	7	35	34	2	42	45	2	68	59	0	23	27	4	53	52			
H,K= 5 7			7	0	18	6	155	161	7	77	76	8	94	85	3	45	45	3	139	129	1	23	26	5	60	62			
0	195	178	8	22	30	7	120	108	8	25	23	H,K= 12 10			H,K= 0 11			4	101	97	2	85	83	H,K= 2 14					
1	197	175	9	62	51	H,K= 9 8			H,K= 5 9			0	109	104	1	124	125	H,K= 10 11			3	86	86	0	120	111			

Results and Discussion

The configuration of the Bicyclomycin molecule can be seen in Fig. 1. The bond distances and angles of the non-hydrogen atoms are shown in Fig. 2. The mean estimated standard deviations are 0.006 Å and 0.3° for bond distances and angles, respectively. The bond lengths involving the hydrogen atoms are listed in Table 5.

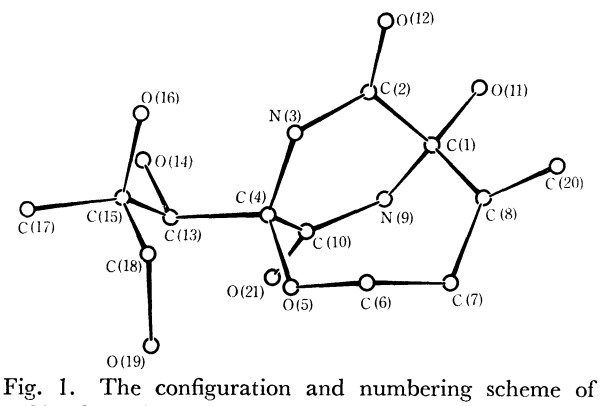


Fig. 1. The configuration and numbering scheme of Bicyclomycin molecule.

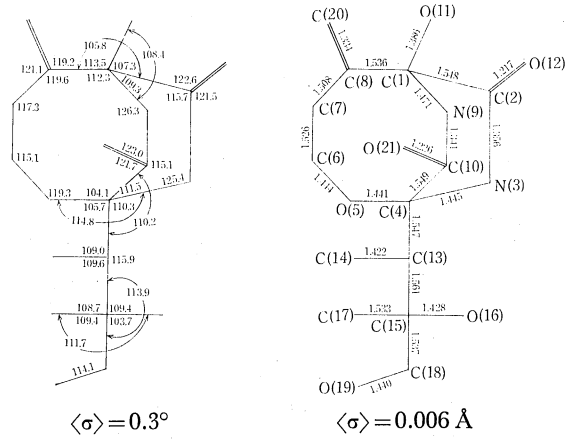


Fig. 2. Bond distances and angles.

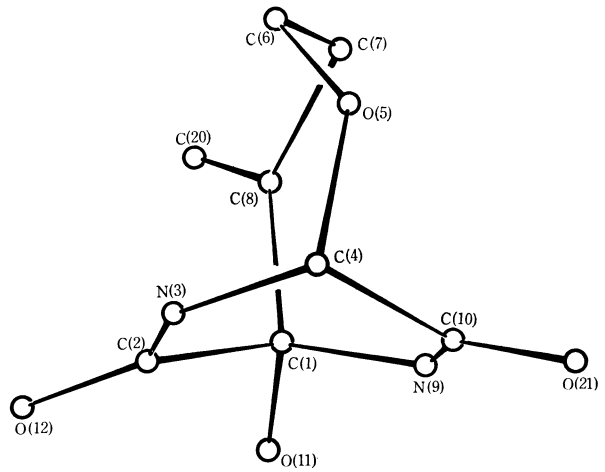


Fig. 3. A part of the molecule showing the cage type of ring system.

The diketopiperazine ring is folded into a boat conformation. Bridging the axial bonds of C(1) and C(4) in the ring, four atoms, O(5), C(6), C(7), and C(8), constitute the two eight-membered rings together with >C–CO–NH–C< moieties of the six-membered ring and thus establish the cage type of ring system. These features are shown in Fig. 3. There exists an approximate two fold axis going through the center of the diketopiperazine ring and the middle point of the C(6)–C(7) bond.

Atoms C(1), C(2), N(3), and O(12) are coplanar within 0.03 Å (amide plane I) and those C(1), C(4), N(9), C(10), and O(21) are also coplanar within 0.03 Å (amide plane II). The dihedral angle between amide plane I and II is 144°. The moiety, C(1), C(8), C(7), and C(20) involving the methylene group, displays the good planarity (methylene plane). The equations of the best planes and the deviations of respective atoms from the planes are listed in Table 7.

The distances, C(2)–N(3) (1.36 Å) and C(10)–N(9) (1.34 Å) significantly shorter than that of C–N single

Table 7. Some of the least-squares planes and the deviation of atoms from the planes (Å)

1) Amide plane I			
$0.4088X-0.8604Y-0.3045Z=-2.888$			
C(1)	−0.008	O(12)	0.011
C(2)	0.026	C(4)*	0.105
N(3)	−0.010	H(1)*	−0.09
2) Amide plane II			
$0.4286X-0.8493Y+0.3084Z=-1.690$			
C(1)	0.002	C(10)	0.031
C(4)	−0.010	O(21)	−0.010
N(9)	−0.011	H(6)*	−0.25
3) Methylene plane			
$0.4823X+0.2134Y+0.8496Z=3.295$			
C(1)	−0.003	C(20)	−0.004
C(7)	−0.003	H(17)*	−0.06
C(8)	0.010	H(18)*	0.04

In the above equations, X, Y, and Z are the rectangular coordinates in Å unit.
Asterisks indicate atoms not included in the calculation of the plane.

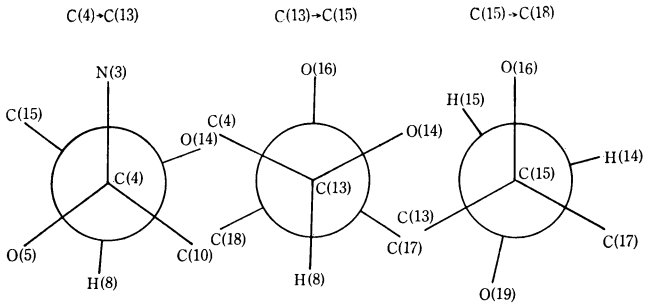


Fig. 4. Newman projections about C(4)–C(13), C(13)–C(15), and C(15)–C(18) bonds.

bond length (1.47 Å), indicate some double bond character; similar shortening of the bond length has been reported in diketopiperazine[6] and in many peptides.[7] The distance C(1)–O(11) (1.39 Å) seems to be shorter than that of the corresponding single bond length.

The conformation of the side chain is presented by the Newman projections down the C(4)–C(13), C(13)–C(15), and C(15)–C(18) bonds. As shown in Fig. 4,

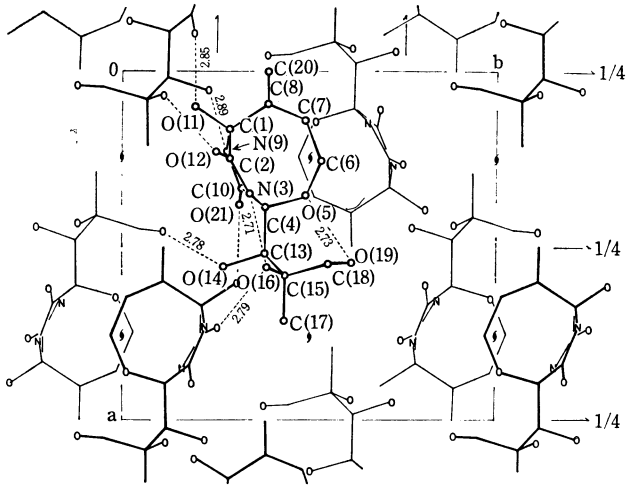


Fig. 5. The crystal structure viewed along the c axis. The hydrogen bonds are indicated by dashed lines.

Table 8. Inter- and intramolecular hydrogen bonds (D–H···A)

Donor	H	Acceptor	D···A (Å)	H···A (Å)	D–H···A (°)
I) Intermolecular					
N(9)	H(6)	$O(14)^{I}$	2.89	2.07	166
O(11)	H(7)	$O(21)^{I}$	2.85	1.89	156
O(14)	H(9)	$O(19)^{II}$	2.78	1.99	161
O(16)	H(10)	$O(12)^{III}$	2.79	1.93	178
II) Intramolecular					
N(3)	H(1)	O(16)	2.71	1.91	144
O(19)	H(16)	O(5)	2.73	1.80	146

Roman numerals as superscripts denote the following transformations relative to the reference molecule at x, y, z:

I	$-1/2+x$,	$1/2-y$,	$-z$,
II	$1-x$,	$-1/2+y$,	$1/2-z$,
III	$1/2+x$,	$1/2-y$,	$1-z$,

all the conformations are *gauche* or nearly so.

All the oxygen and nitrogen atoms in the molecule participate in two intramolecular and four intermolecular hydrogen bonds. Two oxygen atoms, O(16) and O(19), in the side chain participate in intramolecular hydrogen bonds, N(3)–H(1)···O(16) and O(19)–H(16)···O(5). These hydrogen bonds are of particular contribution to retain the molecular frameworks.

The crystal structure viewed along the *c* axis is shown in Fig. 5. The hydrogen bonds are indicated by dashed lines and detailed features of these hydrogen bonds are listed in Table 8.

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