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A Structure Analysis of Pieristoxin G by the X-Ray Diffraction Method

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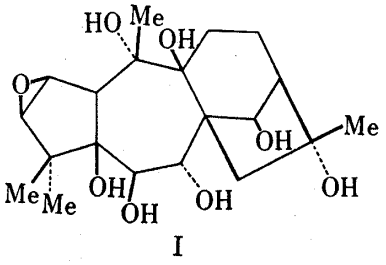
The stereochemistry of pieristoxin G, $C_{20}H_{32}O_8$, has been established by the X-ray diffraction method. The compound crystallized in the space group $P2_12_12_1$, with $a=13.668$, $b=6.567$, and $c=21.516$ Å. The structure was solved by the direct method and refined to $R=0.050$ for 1409 reflections. The conformations of the rings A, C, and D are envelope, distorted chair, and half-chair respectively. The junctions of the rings of A/B, B/C, and C/D are *trans*, *cis*, and *cis* respectively.

Extensive investigations have been done on the toxic constituents, such as grayanotoxins, asebotoxins, and leucothols, of the leaves of the Ericaceae species, famous poisonous trees in Japan.[2)] Recently Katai, *et al.* isolated new constituents from the leaves of the *Pieris japonica* D. Don, one of which was named pieristoxin G[3)] (abbreviated as P–G). The structure was presumed as I on the basis of chemical and spectroscopic evidence. The present X-ray structure analysis has been undertaken to determine the structure conclusively, and to obtain the details of the conformation of the molecule. The present paper describes the stereochemistry of P–G elucidated by the X-ray diffraction method.



I

Chart 1

Experimental and Structure Determination

P–G was crystallized from methanol–ethyl acetate solution in the form of colorless needles. Preliminary photographic investigation indicated orthorhombic symmetry. The systematic absences ($h00$ when h is odd, $0k0$ when k is odd, and $00l$ when l is odd) indicated the space group to be $P2_12_12_1$. The cell dimensions were obtained from the Bragg angles measured by a Toshiba four-circle diffractometer with Ni-filtered Cu Kα radiation. The crystal data are as follows; $C_{20}H_{32}O_8$, $M=400.5$, orthorhombic, space group $P2_12_12_1$, $a=13.668$ (4), $b=6.567$ (2), $c=21.516$ (6) Å, $V=1931.2$ Å^3, $Z=4$, $D_x=1.377$ g/cm^3, $\mu=8.9$ cm^{-1} (for Cu Kα).

A crystal with dimensions of *ca.* $0.1\times0.3\times0.1$ mm was mounted with its b axis parallel to the ϕ axis of the diffractometer. The stationary-crystal stationary-counter technique was applied, with a counting time of 30 sec. The intensities of 1412 independent reflections were measured up to $\sin\theta/\lambda=0.53$ with Ni-filtered Cu Kα radiation. The background for each reflection was taken from plots of the background as a function of 2θ. The intensities were corrected for the Lorentz and polarization factors, but not for absorption.

The structure factors were put on an absolute scale by Wilson statistics,[4)] and normalized structure amplitudes $|E_{hkl}|$ were derived. The structure was solved using 325 reflections with $|E|\geqq1.20$ by the multiple solution weighted tangent-formula procedure.[5)] Reflections 520, 011, and 1203 were used to define the origin,

1) Location: *a) Koyama, Tottori 680, Japan; b) Omiya 5-16-1, Asahi-ku, Osaka 535, Japan.*
2) *a)* H. Kakisawa, T. Kozima, M. Yanai, and K. Nakanishi, *Tetrahedron*, **21**, 3091 (1965); *b)* P. Narayanan, M. Röhrl, K. Zechmeister, and W. Hoppe, *Tetrahedron Letters*, **1970**, 3943; *c)* H. Hikino, M. Ogura, T. Ohta, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **18**, 1071 (1970); *d)* H. Hikino, M. Ogura, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **19**, 1980 (1971); *e)* A. Furusaki, N. Hamanaka, H. Miyakoshi T. Okuno, and T. Matsumoto, *Chemistry Letters*, **1972**, 783.
3) M. Katai, T. Matsushima, T. Terai, and H. Meguri, *Yakugaku Zasshi*, **95**, 778 (1975).
4) A.J.C. Wilson, *Acta Cryst.*, **2**, 378 (1949).
5) G. Germain, P. Main, and M.M. Woolfson, *Acta Cryst.*, **A27**, 368 (1971).

507 to define the enantimorph. Reflections 111, 8310, and 9310 were assigned initial phases of $\pm\pi/4$ and $\pm 3\pi/4$. The solution giving the highest figure of merit yielded an E map containing peaks corresponding to 27 of the expected 28 non-hydrogen atoms of P–G, and the remaining atom was found by a three-dimensional Fourier synthesis.

At the early stage of the refinement, the coordinates and isotropic temperature factors were refined by a block-diagonal least-squares method using the carbon scattering factor. The atomic species were assigned on the basis of the temperature factors as well as chemical informations. The refinement with anisotropic temperature factors for non-hydrogen atoms reduced the R value to 0.10. The intense reflections appeared to be affected by extinction, 011, 111, and 200, were omitted from the subsequent calculations. All of the thirty two hydrogen atoms were located in the difference Fourier syntheses. Including the positional parameters and isotropic temperature factors of these hydrogen atoms, the refinement reduced the R value to 0.050 for 1409 independent reflections. The weighting scheme used in the final cycles of refinement was: $w=0.4$ for $F_o=0$, $w=1.0$ for $0<F_o\leqq 18$, and $w=1.0/(1.0+0.2(F_o-18))$ for $18<F_o$. At the final stage of the refinement, the difference electron density map showed only featureless peaks having the maximum height of about the half heights of the hydrogen atoms. The final atomic parameters are given in Table I.[6]

The atomic scattering factors were taken from the International Tables for X-ray Crystallography.[7] The computations were executed on a TOSBAC 3400 computer at the Tottori University Computing Center, and on a NEAC 2200—N700 computer at the Computation Center of Osaka University.

Results and Discussion

The stereochemistry of P–G has been established by the present X-ray structure analysis. The structural formula is represented as I, which is identical with the formula given for the

Table I(a). Final Atomic Parameters and E.s.d.'s ($\times 10^4$) for Non-hydrogen Atoms

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C (1)	8140(3)	1398(8)	1468(2)	39(3)	201(13)	18(1)	6(11)	−4(3)	−6(7)
C (2)	8710(4)	−502(10)	1667(3)	48(3)	286(17)	27(1)	80(14)	−7(4)	8(9)
C (3)	7895(3)	2822(8)	2019(2)	36(3)	208(13)	17(1)	−3(11)	−1(3)	3(7)
C (4)	8700(4)	2844(10)	2495(2)	43(3)	320(17)	21(1)	−13(13)	−2(3)	−5(9)
C (5)	8301(4)	3200(9)	3120(2)	54(3)	284(16)	19(1)	−20(13)	−4(3)	−2(8)
C (6)	7204(4)	3542(8)	3063(2)	50(3)	218(14)	17(1)	−15(12)	4(3)	−4(7)
C (7)	6648(4)	2612(10)	3620(2)	67(3)	300(17)	17(1)	−39(15)	13(4)	20(8)
C (8)	7063(4)	5862(9)	3058(2)	65(4)	232(14)	20(1)	−18(14)	−2(4)	−16(8)
C (9)	6956(3)	2524(8)	2417(2)	41(3)	179(12)	16(1)	−1(11)	4(3)	9(7)
C (10)	6077(3)	3521(8)	2102(2)	38(3)	220(14)	16(1)	19(11)	8(3)	−10(7)
C (11)	5704(3)	2464(8)	1508(2)	32(2)	238(14)	14(1)	9(11)	−2(3)	10(7)
C (12)	6370(3)	2230(8)	927(2)	35(2)	172(12)	17(1)	1(11)	1(3)	−5(6)
C (13)	6770(3)	4176(8)	619(2)	37(3)	219(13)	18(1)	−9(11)	−4(3)	−2(7)
C (14)	6947(4)	3449(9)	−56(2)	42(3)	270(15)	16(1)	−17(12)	7(3)	12(7)
C (15)	7815(4)	2002(9)	−60(2)	42(3)	256(16)	19(1)	3(12)	0(3)	5(8)
C (16)	7689(4)	195(9)	376(2)	43(3)	265(15)	17(1)	17(12)	1(3)	−19(8)
C (17)	7263(3)	738(8)	1030(2)	36(2)	190(13)	17(1)	−8(11)	7(3)	−13(7)
C (18)	5702(3)	1335(8)	403(2)	40(3)	215(14)	18(1)	−26(11)	2(3)	−18(7)
C (19)	5975(3)	2420(9)	−214(2)	36(3)	228(14)	17(1)	44(12)	−8(3)	−10(8)
C (20)	6009(4)	957(10)	−769(2)	54(3)	303(18)	21(1)	33(14)	−10(3)	−43(8)
O (1)	8797(2)	2700(6)	1131(1)	35(2)	259(10)	20(1)	−14(8)	6(2)	−8(5)
O (2)	8614(3)	1165(7)	2946(2)	64(2)	318(11)	21(1)	53(10)	−11(2)	22(6)
O (3)	6725(2)	409(5)	2486(1)	48(2)	213(9)	19(1)	−10(8)	4(2)	13(5)
O (4)	5286(2)	3516(6)	2531(2)	41(2)	321(11)	19(1)	23(9)	5(2)	−19(6)
O (5)	4832(2)	3575(6)	1341(2)	36(2)	255(10)	21(1)	30(8)	1(2)	−2(5)
O (6)	6060(2)	5802(5)	632(1)	47(2)	196(9)	21(1)	6(8)	−8(2)	−11(5)
O (7)	6871(2)	−1155(5)	1277(2)	52(2)	178(9)	23(1)	−3(8)	3(2)	−3(5)
O (8)	5255(2)	3950(6)	−380(2)	52(2)	267(11)	20(1)	18(9)	−19(2)	−9(5)

The anisotropic temperature factors are of the form: $\exp(-\beta_{11}h^2-\beta_{22}k^2-\beta_{33}l^2-\beta_{12}hk-\beta_{13}hl-\beta_{23}kl)$.

6) A list of observed and calculated structure factors is available from the authors on request.
7) "International Tables for X-ray Crystallography," Vol. III, Kynoch Press, Birmingham, 1962, p. 202.

Table I(b). Final Positional Parameters ($\times 10^3$) and Isotropic Temperature Factors ($\times 10$) for Hydrogen Atoms

	x	y	z	B
H(O 1)	924(4)	188(9)	94(3)	29(14) Å^2
H(C 2)	930(5)	−57(11)	186(3)	51(17)
H(C 2)	879(4)	−145(10)	126(3)	40(15)
H(C 2)	831(4)	−132(9)	200(2)	24(12)
H(C 3)	790(4)	431(8)	187(2)	16(11)
H(C 4)	931(4)	326(10)	238(3)	35(15)
H(C 5)	860(4)	388(9)	350(2)	20(12)
H(C 7)	698(5)	346(10)	405(3)	50(16)
H(C 7)	679(4)	116(8)	363(2)	17(11)
H(C 7)	586(4)	277(10)	362(3)	32(13)
H(C 8)	738(4)	644(9)	348(2)	28(13)
H(C 8)	635(5)	621(10)	296(3)	39(14)
H(C 8)	746(4)	650(10)	271(3)	36(14)
H(O 3)	715(5)	−7(11)	271(3)	42(16)
H(C 10)	633(4)	508(8)	199(2)	15(11)
H(O 4)	471(5)	379(11)	238(3)	46(16)
H(C 11)	550(4)	107(9)	167(2)	25(12)
H(O 5)	521(5)	480(11)	118(3)	48(17)
H(C 13)	747(4)	456(9)	80(2)	23(12)
H(O 6)	629(5)	688(11)	98(3)	55(18)
H(C 14)	712(4)	473(9)	−36(3)	29(13)
H(C 15)	838(4)	291(9)	7(2)	20(12)
H(C 15)	800(4)	150(9)	−55(2)	30(13)
H(C 16)	838(3)	−50(8)	48(2)	14(11)
H(C 16)	721(4)	−76(10)	18(3)	29(13)
H(O 7)	681(5)	−58(13)	168(3)	57(18)
H(C 18)	502(4)	160(8)	54(2)	19(12)
H(C 18)	575(4)	−25(9)	39(2)	23(12)
H(O 8)	530(4)	494(10)	−6(3)	35(15)
H(C 20)	649(3)	−19(8)	−74(2)	15(11)
H(C 20)	611(5)	174(10)	−117(3)	48(16)
H(C 20)	528(4)	28(9)	−87(2)	27(13)

alkaline hydrolysis product of asebotoxin VII.[2d] A stereoscopic view of the molecule is shown in Fig. 1. The crystal structure and the numbering scheme of the atoms used in this paper are shown in Fig. 2. The absolute configuration of P–G drawn in these figures is based on the assumption that the configurations of the C and D rings in P–G are the same as those in leucothol A.[2e] The bond lengths and angles are listed in Table II. The conformation of P–G is represented by Fig. 3 and Table III. The five-membered ring A takes an envelope conformation; the C(9) atom deviates significantly (0.50 Å) from the plane through the C(3),C(4), C(5), and C(6) atoms. The dihedral angle between the above plane and the epoxide ring is 73.7°. The six-membered ring C takes distorted chair form; the torsion angles in the ring range from 43.6° to 70.9°. The C(13)—O(6) bond is equatorial. The five-membered ring D takes an half-chair conformation; the C(13) and C(14) atoms deviate by 0.40 and 0.37 Å respectively to opposite side of the plane through the C(12), C(18), and C(19) atoms. The junctions of the rings A/B, B/C, and C/D are *trans*, *cis*, and *cis* respectively.

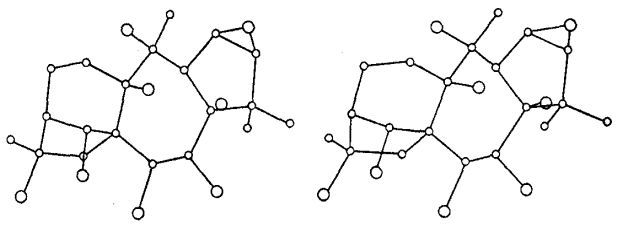


Fig. 1. Stereoscopic View of P–G
Hydrogen atoms are omitted for clarity.

The scheme of O–H·····O approachs of P–G in the crystal is summarized in Table IV. The molecule has five O–H·····O intramolecular approaches, of which the three are clearly

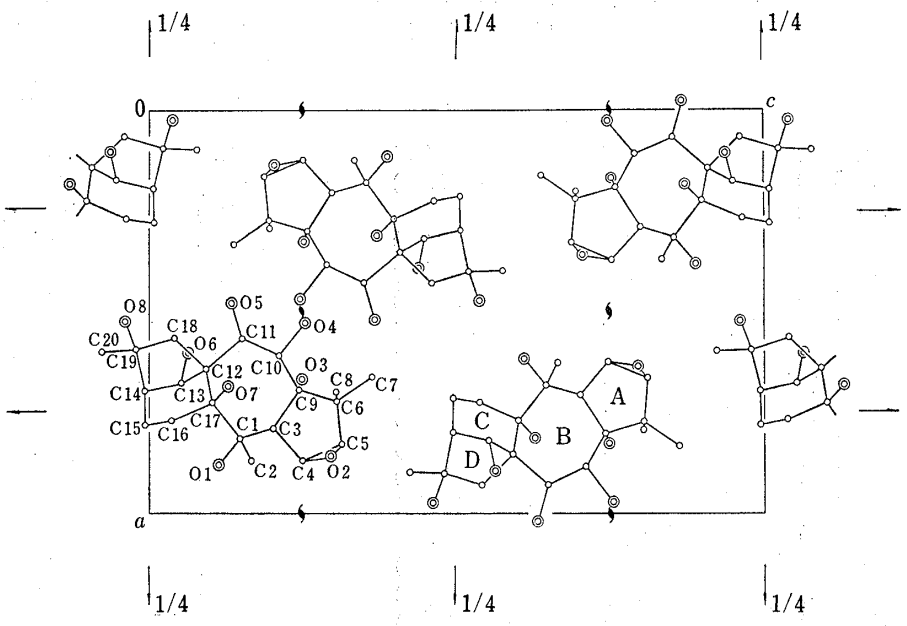


Fig. 2. Projection of the Crystal Structure along the *b* Axis and the Numbering of Atoms

TABLE II. Bond Lengths and Angles for Non-hydrogen Atoms

(a) Bond lengths (Å)

C (1)-C (2)	1.532(8)	C (1)-C (3)	1.548(7)
C (1)-C (17)	1.585(7)	C (1)-O (1)	1.436(6)
C (3)-C (4)	1.503(8)	C (3)-C (9)	1.555(7)
C (4)-C (5)	1.470(9)	C (4)-O (2)	1.474(8)
C (5)-C (6)	1.521(8)	C (5)-O (2)	1.452(7)
C (6)-C (7)	1.546(8)	C (6)-C (8)	1.535(8)
C (6)-C (9)	1.577(8)	C (9)-C (10)	1.528(8)
C (9)-O (3)	1.432(6)	C (10)-C (11)	1.542(8)
C (10)-O (4)	1.422(7)	C (11)-C (12)	1.553(7)
C (11)-O (5)	1.443(7)	C (12)-C (13)	1.540(7)
C (12)-C (17)	1.582(7)	C (12)-C (18)	1.565(7)
C (13)-C (14)	1.548(8)	C (13)-O (6)	1.444(6)
C (14)-C (15)	1.520(8)	C (14)-C (19)	1.528(8)
C (15)-C (16)	1.522(8)	C (16)-C (17)	1.563(8)
C (17)-O (7)	1.454(6)	C (18)-C (19)	1.551(8)
C (19)-C (20)	1.534(9)	C (19)-O (8)	1.451(7)

(b) Bond angles (°)

C (2)-C (1)-C (3)	112.8(4)	C (2)-C (1)-C (17)	109.2(4)
C (2)-C (1)-O (1)	107.9(4)	C (3)-C (1)-C (17)	117.2(4)
C (3)-C (1)-O (1)	99.3(4)	C (17)-C (1)-O (1)	109.6(4)
C (1)-C (3)-C (4)	111.7(4)	C (1)-C (3)-C (9)	121.7(4)
C (4)-C (3)-C (9)	103.3(4)	C (3)-C (4)-C (5)	110.7(5)
C (3)-C (4)-O (2)	112.6(5)	C (5)-C (4)-O (2)	59.1(4)
C (4)-C (5)-C (6)	108.4(5)	C (4)-C (5)-O (2)	60.6(4)
C (6)-C (5)-O (2)	114.0(5)	C (5)-C (6)-C (7)	111.3(5)
C (5)-C (6)-C (8)	105.7(5)	C (5)-C (6)-C (9)	102.7(4)
C (7)-C (6)-C (8)	109.6(5)	C (7)-C (6)-C (9)	114.2(5)
C (8)-C (6)-C (9)	112.9(5)	C (3)-C (9)-C (6)	104.7(4)
C (3)-C (9)-C (10)	110.5(4)	C (3)-C (9)-O (3)	111.1(4)
C (6)-C (9)-C (10)	112.3(4)	C (6)-C (9)-O (3)	111.6(4)
C (10)-C (9)-O (3)	106.8(4)	C (9)-C (10)-C (11)	115.8(5)
C (9)-C (10)-O (4)	108.0(4)	C (11)-C (10)-O (4)	106.6(4)
C (10)-C (11)-C (12)	121.2(5)	C (10)-C (11)-O (5)	104.6(4)
C (12)-C (11)-O (5)	109.5(4)	C (11)-C (12)-C (13)	118.2(4)

C (11)-C (12)-C (17)	113.7(4)	C (11)-C (12)-C (18)	106.0(4)
C (13)-C (12)-C (17)	107.4(4)	C (13)-C (12)-C (18)	102.1(4)
C (17)-C (12)-C (18)	108.5(4)	C (12)-C (13)-C (14)	101.7(4)
C (12)-C (13)-O (6)	111.5(4)	C (14)-C (13)-O (6)	110.5(4)
C (13)-C (14)-C (15)	108.6(5)	C (13)-C (14)-C (19)	102.1(4)
C (15)-C (14)-C (19)	113.6(5)	C (14)-C (15)-C (16)	113.3(5)
C (15)-C (16)-C (17)	114.8(5)	C (1)-C (17)-C (12)	119.8(4)
C (1)-C (17)-C (16)	108.4(4)	C (1)-C (17)-O (7)	107.2(4)
C (12)-C (17)-C (16)	107.6(4)	C (12)-C (17)-O (7)	107.2(4)
C (16)-C (17)-O (7)	105.7(4)	C (12)-C (18)-C (19)	107.7(4)
C (14)-C (19)-C (18)	102.9(4)	C (14)-C (19)-C (20)	115.1(5)
C (14)-C (19)-O (8)	109.7(5)	C (18)-C (19)-C (20)	112.7(5)
C (18)-C (19)-O (8)	111.5(4)	C (20)-C (19)-O (8)	105.2(5)
C (4)-O (2)-C (5)	60.3(4)		

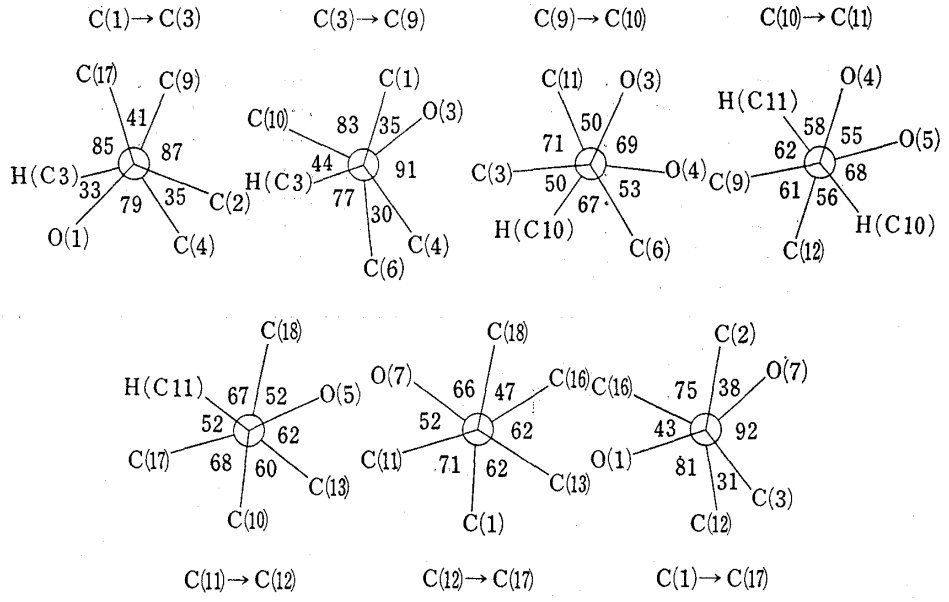


Fig. 3. Newman Projections

TABLE III. Torsion Angles (°)

C (1)-C (3)-C (4)-C (5)	−149.3	C (1)-C (3)-C (4)-O (2)	−85.3
C (9)-C (3)-C (4)-C (5)	−16.9	C (9)-C (3)-C (4)-O (2)	47.1
C (4)-C (5)-C (6)-C (7)	144.0	C (4)-C (5)-C (6)-C (8)	−97.1
C (4)-C (5)-C (6)-C (9)	21.4	O (2)-C (5)-C (6)-C (7)	78.7
O (2)-C (5)-C (6)-C (8)	−162.4	O (2)-C (5)-C (6)-C (9)	−43.9
C (5)-C (6)-C (9)-C (3)	−31.3	C (7)-C (6)-C (9)-C (3)	−152.0
C (8)-C (6)-C (9)-C (3)	82.1	C (5)-C (6)-C (9)-O (3)	89.0
C (7)-C (6)-C (9)-O (3)	−31.7	C (8)-C (6)-C (9)-O (3)	−157.6
C (5)-C (6)-C (9)-C (10)	−151.2	C (7)-C (6)-C (9)-C (10)	88.1
C (8)-C (6)-C (9)-C (10)	−37.8	C (11)-C (12)-C (13)-C (14)	−154.8
C (17)-C (12)-C (13)-C (14)	75.0	C (18)-C (12)-C (13)-C (14)	−39.1
C (11)-C (12)-C (13)-O (6)	−37.0	C (17)-C (12)-C (13)-O (6)	−167.2
C (18)-C (12)-C (13)-O (6)	78.7	C (17)-C (12)-C (18)-C (19)	−97.8
C (11)-C (12)-C (18)-C (19)	139.8	C (13)-C (12)-C (18)-C (19)	15.5
C (12)-C (13)-C (14)-C (15)	−70.9	O (6)-C (13)-C (14)-C (15)	170.6
C (12)-C (13)-C (14)-C (19)	49.4	O (6)-C (13)-C (14)-C (19)	−69.1
C (19)-C (14)-C (15)-C (16)	−56.5	C (13)-C (14)-C (15)-C (16)	56.4
C (14)-C (15)-C (16)-C (17)	−43.6	C (15)-C (16)-C (17)-C (12)	45.3
C (15)-C (16)-C (17)-O (7)	159.7	C (15)-C (16)-C (17)-C (1)	−85.7
C (12)-C (18)-C (19)-C (14)	14.4	C (12)-C (18)-C (19)-C (20)	138.9
C (12)-C (18)-C (19)-O (8)	−103.1	C (13)-C (14)-C (19)-C (18)	−38.6
C (15)-C (14)-C (19)-C (18)	78.1	C (13)-C (14)-C (19)-O (8)	80.2
C (15)-C (14)-C (19)-O (8)	−163.1	C (13)-C (14)-C (19)-C (20)	−161.5
C (15)-C (14)-C (19)-C (20)	−44.8		

Table IV. Scheme of O–H···O Approaches

O–H···O–C	O···O	O–H	H···O	∠O–H···O	∠H···O–C
O(1)–H(O1)···O(8)[a]–C(19)[a]	2.785Å	0.90Å	1.93Å	159°	117°
O(6)–H(O6)···O(7)[b]–C(17)[b]	2.674	1.08	1.64	158	135
O(3)–H(O3)···O(2)<C(4)[c], C(5)	2.809	0.82	2.22	130	101, 98
O(4)–H(O4)···O(5)–C(11)[c]	2.635	0.87	2.25	106	81
O(5)–H(O5)···O(6)–C(13)	2.698	1.01	1.79	148	100
O(7)–H(O7)···O(3)–C(9)	2.805	0.94	1.86	177	103
O(8)–H(O8)···O(6)–C(13)	2.725	0.95	1.90	144	98

a) $1/2+x, 1/2-y, -z$ b) $x, 1+y, z$ c) see text

Table V. Intermolecular Distances between Heavy-atoms (<3.6Å)

C(8)···O(3)[a]	3.263Å	C(13)···O(7)[a]	3.380Å
O(6)···O(7)[a]	2.674[e]	C(8)···O(5)[b]	3.399
O(4)···C(11)[b]	3.582	O(4)···O(3)[b]	3.017
O(4)···O(4)[b]	3.378	O(5)···C(7)[b]	3.336
O(5)···O(3)[b]	3.515	O(2)···C(20)[c]	3.138
C(15)···O(8)[d]	3.524	C(16)···O(8)[d]	3.553
O(1)···C(19)[d]	3.573	O(1)···C(20)[d]	3.244
O(1)···O(8)[d]	2.785[e]		

symmetry code:
a) $x, 1+y, z$ b) $1-x, 1/2+y, 1/2-z$ c) $3/2-x, -y, 1/2+z$
d) $1/2+x, 1/2-y, -z$ e) hydrogen bonds

recognized to be intramolecular hydrogen bonds. However the present analysis has not made it clear whether the other two approaches (c) in Table IV) depend on hydrogen bonds. The distance between the O(3) and O(4) atoms is fairly short (2.84 Å), and the final difference electron density map shows minor peaks around the O(4) atom, hence the existence of hydrogen bond between these atoms is uncertain. The molecules are linked by O(6)–H(06)·····O(7) and O(1)–H(01)·····O(8) intermolecular hydrogen bonds building infinite chains along **b** and along **a** respectively. The intermolecular distances less than 3.6 Å between heavy atoms are given in Table V. Except the two intermolecular hydrogen bonds described above, it may be explained with the aid of the final difference electron density map that the remaining contacts correspond to van der Waals contacts.

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