866 © *1994 The Chemical Society of Japan* NOTES *Bull. Chem. Soc. Jpn., 67, 866—868(1994)* [Vol. 67, No. 3

Crystal and Molecular Structure of Asebotoxin IV

Mamoru SATO,* Yukiteru KATSUBE, Masaaki KATAI,† Jun'ichi KATAKAWA,†† and Tadahiro TETSUMI††
Institute for Protein Research, Osaka University, 3-2 Yamada-oka, Suita, Osaka 565
† Department of Applied Chemistry, Osaka Institute of Technology, Omiya 5-16-1, Asahi-ku, Osaka 535
†† Faculty of Pharmaceutical Sciences, Setsunan University, Hirakata, Osaka 573-01

(Received October 15, 1993)

Synopsis. The three-dimensional structure of asebotoxin IV has been determined by X-ray diffraction at room temperature. The crystals are triclinic, space group $P1$, with a=9.934(2), b=8.8746(9), c=7.0073(9) Å, α=71.05(1), β=80.04(1), γ=70.71(1)°, V=549.8(2) Å^3, Z=1, D_c=1.337 g cm^{-3}. The structure was solved by the direct method and refined by the full matrix least-squares method to a final R=0.038 for 1604 independent reflections with $F_o > 3\sigma$ (F_o). The molecule is based on a tetracyclic structure consisting of two five-membered, one six-membered and one seven-membered rings with various conformations.

Asebotoxin IV, (3β, 6β, 7α, 14R)- 6- propionyloxy-grayanotoxane-3,5,7,10,14,16-hexol, diterpenoids isolated from the flowers of *Pieris japonica* D. Don (Ericaceae), a poisonous tree in Japan.[1—3] The toxic activities of the diterpenoids are highly dependent of their ring conformations, thereby indicating particular importance of their detailed three-dimensional structure analyses. So, we here report on the X-ray crystal-structure determination of the title compound and describe the molecular architecture.

Experimental

Asebotoxin IV was isolated from the chloroform extract of leaves of *Pieris japonica* D. Don and purified by chromatography on silica gel. Colorless, plate-like crystals were grown by slow evaporation from ethyl acetate solution at room temperature. A crystal with approximate dimensions of 0.2×0.2×0.05 mm was selected for data collection on a Rigaku automated four-circle diffractometer equipped with a Rigaku RU200 rotating-anode X-ray generator operated at 40 kV, 200 mA. Unit-cell parameters were determined by the least-squares method from 20 intense reflections in the range 41.94°≦2θ≦45.66°.

Integrated intensities were measured by the θ–2θ scan technique using Ni-filtered Cu $K\alpha$ radiation (λ=1.5418 Å) and a monitor count technique. The scan width was $\Delta\theta$=(1.5+0.15 tan θ)° and scan speed 4° min^{-1}. Background intensities were measured for 5 s at both ends of a scan. A total of 1788 reflections; h k l range, $-10 \leqq h \leqq 11$, $-9 \leqq k \leqq 9$, $0 \leqq l \leqq 7$, were collected up to 2θ=120°. Three standard reflections (3 2 3, 2 4 2, and 2 $\bar{3}$ 0) were measured every 150 reflections to monitor crystal stability and orientation. No intensity decrease was observed during the data collection. Corrections were made for usual Lorentz and polarization effect but not for absorption nor extinction. The reflection

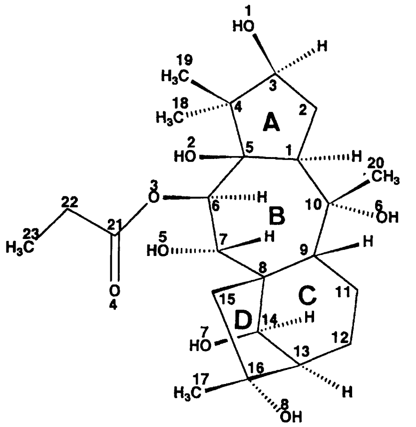

Fig. 1. A chemical structure of the molecule with the atom-numbering scheme.

Table 1. Final Atomic Coordinates and Their Equivalent Isotropic Thermal Parameters[a)]

Atom	x	y	z	B_{eq}/Å^2
O(1)	0.3357(0)	0.4331(0)	0.857(0)	3.8(2)
O(2)	0.2242(7)	0.7830(8)	0.894(1)	2.9(2)
O(3)	0.4281(7)	0.9525(7)	0.868(1)	2.8(2)
O(4)	0.6042(8)	1.055(1)	0.677(1)	5.5(3)
O(5)	0.2887(7)	1.2519(8)	0.638(1)	3.1(2)
O(6)	0.1230(8)	0.8533(8)	0.295(1)	3.5(2)
O(7)	0.3157(7)	1.2629(8)	0.253(1)	3.2(2)
O(8)	0.0876(8)	1.5604(8)	0.231(1)	4.0(2)
C(1)	0.285(1)	0.790(1)	0.536(1)	2.7(2)
C(2)	0.361(1)	0.613(1)	0.515(2)	3.4(3)
C(3)	0.432(1)	0.508(1)	0.710(2)	3.2(2)
C(4)	0.469(1)	0.635(1)	0.784(2)	3.0(2)
C(5)	0.3352(8)	0.793(1)	0.735(1)	2.5(2)
C(6)	0.366(1)	0.961(1)	0.689(2)	2.6(2)
C(7)	0.235(1)	1.114(1)	0.661(2)	2.6(2)
C(8)	0.1331(8)	1.162(1)	0.491(1)	2.5(2)
C(9)	0.0467(8)	1.036(1)	0.520(1)	2.8(2)
C(10)	0.126(1)	0.855(1)	0.502(1)	3.0(2)
C(11)	−0.077(1)	1.123(2)	0.376(2)	3.4(2)
C(12)	−0.032(1)	1.216(1)	0.161(2)	3.3(2)
C(13)	0.069(1)	1.318(1)	0.156(1)	2.8(2)
C(14)	0.1995(8)	1.197(1)	0.269(1)	2.5(2)
C(15)	0.022(1)	1.335(1)	0.498(2)	3.0(2)
C(16)	0.002(1)	1.445(1)	0.276(2)	3.2(2)
C(17)	−0.150(1)	1.550(2)	0.234(2)	4.6(3)
C(18)	0.605(1)	0.665(2)	0.658(2)	4.0(3)
C(19)	0.500(1)	0.565(1)	1.008(2)	3.6(3)
C(20)	0.037(2)	0.742(2)	0.639(2)	3.9(3)
C(21)	0.546(1)	1.007(1)	0.838(2)	3.5(3)
C(22)	0.590(1)	0.991(2)	1.040(2)	4.4(3)
C(23)	0.721(2)	1.048(2)	1.020(3)	8.1(6)

a) Equivalent isotropic B_{eq} defined as $B_{eq}=(4/3)\Sigma\Sigma B_{ij}\,a_i\cdot a_j$.

Table 2. Bond Lengths (l/Å) and Bond Angles (ϕ/°)

Bond lengths (l/Å)

O(1)–C(3)	1.434(9)	C(2)–C(3)	1.52(2)	C(9)–C(10)	1.57(2)
O(2)–C(5)	1.43(1)	C(3)–C(4)	1.55(2)	C(9)–C(11)	1.56(2)
O(3)–C(6)	1.46(2)	C(4)–C(5)	1.57(2)	C(10)–C(20)	1.54(2)
O(3)–C(21)	1.37(2)	C(4)–C(18)	1.53(2)	C(11)–C(12)	1.54(2)
O(4)–C(21)	1.19(2)	C(4)–C(19)	1.53(2)	C(12)–C(13)	1.55(2)
O(5)–C(7)	1.45(2)	C(5)–C(6)	1.54(2)	C(13)–C(14)	1.53(2)
O(6)–C(10)	1.47(2)	C(6)–C(7)	1.52(2)	C(13)–C(16)	1.53(2)
O(7)–C(14)	1.43(1)	C(7)–C(8)	1.56(2)	C(15)–C(16)	1.55(2)
O(8)–C(16)	1.46(2)	C(8)–C(9)	1.57(2)	C(16)–C(17)	1.51(2)
C(1)–C(2)	1.55(2)	C(8)–C(14)	1.56(2)	C(21)–C(22)	1.50(2)
C(1)–C(5)	1.57(2)	C(8)–C(15)	1.58(2)	C(22)–C(23)	1.51(2)
C(1)–C(10)	1.53(2)				

Bond angles (ϕ/°)

C(6)–O(3)–C(21)	117.7(7)	C(9)–C(8)–C(15)	107.7(7)
C(2)–C(1)–C(5)	106.0(7)	C(14)–C(8)–C(15)	103.2(7)
C(2)–C(1)–C(10)	114.9(7)	C(8)–C(9)–C(10)	120.4(7)
C(5)–C(1)–C(10)	119.2(7)	C(8)–C(9)–C(11)	108.1(7)
C(1)–C(2)–C(3)	107.3(8)	C(10)–C(9)–C(11)	110.3(7)
O(1)–C(3)–C(2)	111.0(7)	O(6)–C(10)–C(1)	103.1(7)
C(1)–C(3)–C(4)	110.1(7)	O(6)–C(10)–C(9)	109.2(7)
C(2)–C(3)–C(4)	104.0(7)	O(6)–C(10)–C(20)	106.7(7)
C(3)–C(4)–C(5)	103.2(7)	C(1)–C(10)–C(9)	116.0(7)
C(3)–C(4)–C(18)	106.3(7)	C(1)–C(10)–C(20)	114.5(8)
C(3)–C(4)–C(19)	111.3(7)	C(9)–C(10)–C(20)	106.8(7)
C(5)–C(4)–C(18)	112.6(7)	C(9)–C(11)–C(12)	113.9(8)
C(5)–C(4)–C(19)	114.6(7)	C(11)–C(12)–C(13)	113.3(8)
C(18)–C(4)–C(19)	108.5(7)	C(12)–C(13)–C(14)	107.2(7)
O(2)–C(5)–C(1)	110.2(7)	C(12)–C(13)–C(16)	112.2(7)
O(2)–C(5)–C(4)	111.2(7)	C(14)–C(13)–C(16)	104.6(7)
O(2)–C(5)–C(6)	108.7(7)	O(7)–C(14)–C(8)	109.3(7)
C(1)–C(5)–C(4)	104.3(7)	O(7)–C(14)–C(13)	115.7(7)
C(1)–C(5)–C(6)	107.2(7)	C(8)–C(14)–C(13)	100.8(7)
C(4)–C(5)–C(6)	115.1(7)	C(8)–C(15)–C(16)	107.5(7)
O(3)–C(6)–C(5)	106.1(7)	O(8)–C(16)–C(13)	109.3(7)
O(3)–C(6)–C(7)	105.4(7)	O(8)–C(16)–C(15)	107.9(7)
C(5)–C(6)–C(7)	115.7(7)	O(8)–C(16)–C(17)	107.0(7)
O(5)–C(7)–C(6)	105.6(7)	C(13)–C(16)–C(15)	102.4(7)
O(5)–C(7)–C(8)	109.2(7)	C(13)–C(16)–C(17)	115.4(8)
C(6)–C(7)–C(8)	121.8(7)	C(15)–C(16)–C(17)	114.5(8)
C(7)–C(8)–C(9)	114.5(7)	O(3)–C(21)–O(4)	124.7(9)
C(7)–C(8)–C(14)	117.1(7)	O(3)–C(21)–C(22)	108.8(8)
C(7)–C(8)–C(15)	106.3(7)	O(4)–C(21)–C(22)	126.9(9)
C(9)–C(8)–C(14)	107.1(7)	C(21)–C(22)–C(23)	112.0(9)

data thus collected were averaged for symmetry-related reflections to give 1632 independent reflections ($R_{\mathrm{int}}=0.008$).

Structure Analysis and Refinement

The structure was solved by direct methods with the SHELX86 program.[4] The atomic positions obtained from the direct method were automatically assessed and confirmed by alternatively applying Fourier syntheses and diagonal approximation least-squares that refine the atomic positions and their occupancies, instead of isotropic temperature factors.[5] The structure was then refined by the full-matrix least-squares procedure using the FMLS program:[6] the function minimized being $\Sigma w(F_\mathrm{o}-F_\mathrm{c})^2$ with $w=1.0$. Non-hydrogen atoms were refined anisotropically. The positions of hydrogen atoms were determined from difference Fourier maps and refined isotropically in the refinements. The final stage of the refinement that has 432 variables yields R (R_w)=0.038 (0.043), goodness-of-fit $S=0.237$, and ratio of maximum least-squares shift to error $(\Delta/\sigma)_{\mathrm{max}}=0.068$ ($(\Delta/\sigma)_{\mathrm{mean}}=0.008$) for 1604 non-zero independent reflections with $F_\mathrm{o}>3\sigma$ (F_o). Maximum positive and negative electron densities in the final difference Fourier map were +0.20 eÅ^{-3} and −0.10 eÅ^{-3}, respectively. The atomic scattering factors used for non-hydrogen atoms were taken from International Tables[7] and for hydrogen atoms from Stewart et al.[8] The absolute configuration of the molecule was assigned on the

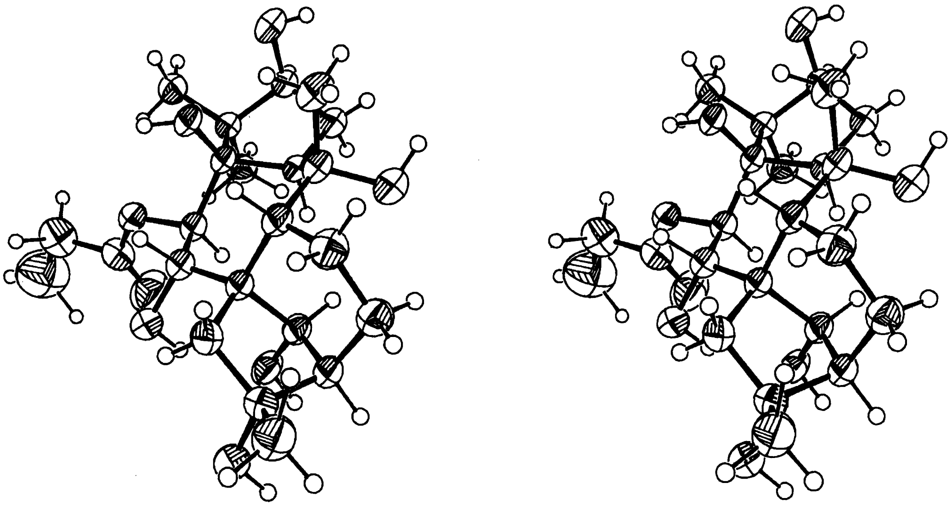

Fig. 2. A stereoscopic view of an ORTEP drawing of the molecule. The thermal ellipsoids correspond to 50% probability level.

Table 3. Summary of Intermolecular Hydrogen Bonds, X–H···Y

X	H	Y	X···Y (l/Å)	H···Y (l/Å)	X–H···Y (ϕ/°)
O(1)$^{a)}$	H(1)	O(5)	2.739(7)	2.0(1)	164(10)
O(6)$^{a)}$	H(4)	O(8)	2.915(10)	2.0(1)	160(8)
O(2)	H(2)	O(6)$^{b)}$	3.008(10)	2.3(1)	136(8)

a), b) Atoms with equivalent positions of (x, $1+y$, z) and (x, y, $1+z$), respectively.

basis of internal comparison with that of grayanotoxin III.[9] The final atomic parameters are listed in Table 1.# All computations were carried out on an NEC PC-9801 personal computer.

Results and Discussion

Figure 1 shows a chemical structure of the molecule with atom-numbering scheme. Figure 2 represents a stereoscopic view of an ORTEP-II[10] drawing of the molecule. Bond lengths and bond angles, along with their estimated standard deviations, are given in Table 2.

The molecule is based on a tetracyclic structure, composed of the four rings, designated as rings A, B, C, and D (Fig. 1), with various conformations. The two five-membered rings A and D have almost the same conformations of envelope forms, with C(4) and C(14) atoms deviating respectively from the mean planes formed by the remaining four atoms. The conformation of the seven-membered ring B corresponds approximately to a twist-chair form, the most stable form of cycloheptane, with a *trans*-junction to the ring A. The ring C takes a typical chair conformation and has a *cis*-junction to the ring B.

The C(23) atom has a relatively large temperature factor (Table 1), mainly due to the terminal atom of the propionyloxy group protruding from the ring B, thereby smearing the electron densities corresponding to the bound hydrogen atoms. The geometrical parameters of the intermolecular hydrogen bonds are given in Table 3. All the other intermolecular and intramolecular atomic distances for non-hydrogen atoms are usual van der Waals distances.

#List of observed and calculated structure factors, anisotropic thermal parameters and hydrogen atom parameters have been deposited as Document No. 67017 at the Office of the Editor of Bull. Chem. Soc. Jpn..

References

1) H. Hikino, K. Ito, and T. Takemoto, *Chem. Pharm. Bull.* (*Tokyo*), **17**, 854 (1969).

2) H. Hikino, K. Ito, T. Ohta, and T. Takemoto, *Chem. Pharm. Bull.* (*Tokyo*), **17**, 1078 (1969).

3) H. Hikino, M. Ogura, M. Fuzita, K. Ito, and T. Takemoto, *Chem. Pharm. Bull.* (*Tokyo*), **19**, 856 (1971).

4) G. M. Sheldrick, "Program for the Solution of Crystal Structures," University of Göttingen, Germany (1986).

5) N. Tanaka, K. Yamanouchi, Y. Katsube, and T. Ashida, *J. Crystallogr. Soc. Jpn.*, **31**, 27 (1989).

6) T. Ashida, "The Universal Crystallographic Computing System-Osaka," The Computing Center, Osaka University, Japan (1979), p. 60.

7) "International Tables for X-Ray Crystallography," The Kynoch Press, Birmingham (1974), Vol. IV.

8) R. F. Stewart, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, **42**, 3175 (1965).

9) P. W. Codding, *J. Am. Chem. Soc.*, **106**, 7905 (1984).

10) C. K. Johnson, "ORTEP-II, Report ORLN-5138," Oak Ridge National Laboratory, Tennessee, U.S.A. (1976).