Structural Investigation of Bistrifluron Using X-Ray Crystallography, NMR Spectroscopy, and Molecular Modeling

Joon Kwan Moon, Jeong-Han Kim,[*] Sangkee Rhee, Gangbeom Kim,[†] Hoseop Yun,[†] Bong-Jin Chung,[‡] Sangsan Lee,[§] and Yoongho Lim[#,*]

School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea
[†]Department of Molecular Science and Technology, Ajou University, Suwon 442-749, Korea
[‡]Dongbu Research Institute, Dongbuhannong Chemical Inc., Whasung 445-960, Korea
[§]Supercomputing Center, Korea Institute of Science and Technology Information, Daejon 305-350, Korea
[#]Department of Applied Biology and Chemistry, Bio/Molecular Informatics Center, Konkuk University, Seoul 143-701, Korea
Received February 21, 2002

A new insecticide, bistrifluron acts as an inhibitor of insect development and interferes with the cuticle formation of insects. Since it shows low acute oral and dermal toxicities, it can be one of potent insecticides. Based on X-ray crystallography, NMR spectroscopy and molecular modeling, the structural studies of bistrifluron have been carried out.

Key Words : Bistrifluron, X-Ray crystallography, NMR spectroscopy, Molecular modeling

Introduction

Bistrifluron, *N*-({[2-chloro-3,5-bis(trifluoromethyl)phenyl]-amino}carbonyl)-2,6-difluorobenzamide, is a new benzoyl-phenylurea insecticide developed by Dongbu-hannong Chemical Co., Korea (Figure 1). It is found to be an inhibitor of insect development and acts by interference with the formation of the insects cuticle, probably by inactivating of the chitin synthetase as the other benzoylphenylureas do.[1] It is active against lepidopteran pest, whitefly as an insect growth regulator. In addition, it can be widely used on apple, *Brassica leafy* vegetables, tomato, persimmon and other fruits. The moulting process is thus inhibited, leading to death.[2] The water solubility is approximately 0.03 mg/L and its vapor pressure, 2.05×10^{-8} torr. It has low acute oral and dermal toxicity (rat) of >5,000 mg/kg and >2,000 mg/kg, respectively.

Since the information about its structure may help the modification for the development of more potent pesticides, authors report the results of its three dimensional structural studies here.

Materials and Methods

Chemicals. Bistrifluron (purity 97.3%) was obtained from Dongbuhannong chemical Co. All solvents used for preparing crystal were reagent grade or better (Duksan, Korea).

X-Ray crystallography. A crystal sample was prepared in a mixture of dichloromethane and hexane. Data collection was performed with MoKα_1 radiation (λ = 0.71073 Å) on a MXC3 Diffractometer (Mac Science, Japan) equipped with an incident beam graphite monochromator. The unit cell parameters and the orientation matrix for data collection were obtained from the least-squares refinement using the setting angles of 15 reflections in the range $20^o < 2\theta(\text{MoK}\alpha_1) < 28^o$. Intensity data were collected with the ω-2θ scan techniques. The intensities of two standard reflections showed no significant deviations during the data collection.

The initial positions for all non hydrogen atoms were obtained by using direct methods of the SHELXS-86 program.[3] The structure was refined by full matrix least-squares technique with the use of the SHELXL-93 program.[4] Anisotropic thermal motion for non-hydrogen atoms and isotropic extinction parameters were included. The final cycle of refinement performed on Fo^2 with all 3081 unique reflections afforded residuals wR2 = 0.1538 and the conventional R index based on the reflections having $Fo>2\sigma(Fo^2)$ was 0.0640.

NMR spectroscopy. The ^{1}H NMR spectrum was obtained on a Bruker DRX 600 (14.1 T, Karlsruhe, Germany) and other NMR spectra were obtained on a Bruker Avance 400 (9.4 T, Karlsruhe, Germany) instrument in $CDCl_3$ and DMSO-d_6. For the ^{1}H-NMR experiment, 32 transients were acquired with 1 sec relaxation delay using 32 K data points,

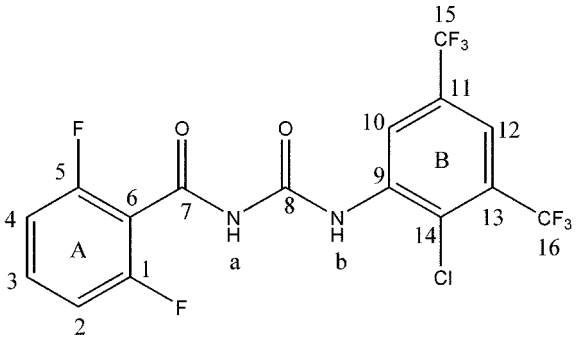

Figure 1. The structure and nomenclature of bistrifluron.

[*]Coresponding authors. Yoongho Lim(Phone: +82-2-450-3760, Fax: +82-2-453-3761, e-mail: yoongho@konkuk.ac.kr), Jeong-Han Kim(Phone: +82-31-290-2404, Fax: +82-31-293-8608, e-mail: kjh2404@snu.ac.kr)

and the 90° pulse was 12.8 μsec, with spectral width of 6,000 Hz. For the ^{13}C-NMR and DEPT[5] experiments, 3000 transients were acquired with a 2 sec relaxation delay using 64K data points, and the 90° pulse was 9.8 μsec with spectral width of 22,000 Hz. Two-dimensional spectra were acquired with 2048 data points in t2 and 256 in t1 increments. The COSY,[6] HMQC,[7] and HMBC[8] spectra were collected with the magnitude method, and the NOESY[9] spectrum, with the phase sensitive mode.

Molecular modeling. All computational calculations were performed using Accelrys software (San Diego, CA) on a Silicon Graphics O2 R12000 workstation, where the consistent-valence forcefield (cvff) and the extensible-systematic forcefield (esff) were used for 500 psec.

Results and Discussion

X-Ray crystallography. ORTEP diagram and numbering scheme for bistrifluron is shown in Figure 2. Atomic coordinates and equivalent isotropic displacement parameters for bistrifluron are listed in Table 1. Crystal size was approximately 0.30 × 0.20 × 0.14 mm and unit cell was triclinic. The triclinic cell parameters and calculated volumes are a = 7.794(3) Å, b = 15.272(6) Å, c = 15.288(6) Å, α = 89.00(3)°, β = 77.64(3)°, γ = 79.12(3)°.

The systematic extinction was indicative of the space group $P_{\bar{1}}$. Bond length of carbon-carbon, carbon-fluorine, and carbonchlorine is about 1.495, 1.346 and 1.731 Å, respectively. Double bond length of carbon-oxygen is 1.218 Å. Bond length of difluorobenzoyl ring carbons is 1.378 Å and phenyl ring carbons is 1.386 Å.

Bond angle of C-F sp^3 hybrid is 106.2° and sp^2 hybrid of benzene ring is 120°. Torsion angle of C5-C6-C7-N1 is 56.6 and C10-C9-N2-C8 is 11.9. Ring A and ring B are not in the same plane. Figure 3 shows the packing mode of molecules in the unit cell. Two molecules of bistrifluron are packed at the opposite direction.

NMR spectroscopy. In order to investigate the three dimensional structure using NMR, nOe has been used. Unfortunately, bistrifluron does not contain sufficient protons

Table 1. Atomic coordinates (× 10^{-4}) and equivalent isotropic displacement parameters (Å^2 × 10^{-3}) for bistrifluron (Esd in parentheses)

	x	y	z	U (eq)
C(1)	4676(9)	4244(4)	1013(4)	69(2)
C(2)	4958(10)	5066(4)	1241(4)	84(2)
C(3)	3523(13)	5762(5)	1365(5)	97(2)
C(4)	1866(11)	5651(4)	1272(5)	93(2)
C(5)	1648(10)	4803(4)	1058(4)	71(2)
C(6)	3052(9)	4084(3)	919(3)	60(2)
C(7)	2869(8)	3182(3)	625(3)	59(1)
C(8)	1233(7)	1940(3)	1119(3)	49(1)
C(9)	2307(6)	545(3)	204(3)	44(1)
C(10)	1551(6)	-25(3)	821(3)	48(1)
C(11)	1707(6)	-908(3)	600(3)	47(1)
C(12)	2567(6)	-1237(3)	-252(3)	52(1)
C(13)	3324(6)	-680(3)	-879(3)	51(1)
C(14)	3213(6)	207(3)	-646(3)	47(1)
C(15)	984(8)	-1529(4)	1283(4)	59(1)
C(16)	4239(8)	-1048(4)	-1797(4)	66(2)
Cl(1)	4220(2)	916(1)	-1395(1)	63(1)
F(1)	6083(5)	3561(2)	899(3)	97(1)
F(2)	39(5)	4685(2)	950(2)	93(1)
F(3)	-405(4)	-1119(2)	1894(2)	75(1)
F(4)	424(5)	-2186(2)	927(2)	90(1)
F(5)	2196(5)	-1913(2)	1739(2)	88(1)
F(6)	5950(5)	-969(3)	-2008(2)	92(1)
F(7)	4227(6)	-1914(2)	-1874(2)	104(1)
F(8)	3473(5)	-650(2)	-2427(2)	91(1)
N(1)	1563(6)	2808(2)	1162(3)	54(1)
N(2)	2241(5)	1448(2)	399(2)	51(1)
O(1)	3887(6)	2803(2)	-39(3)	82(1)
O(2)	143(5)	1686(2)	1708(2)	57(1)

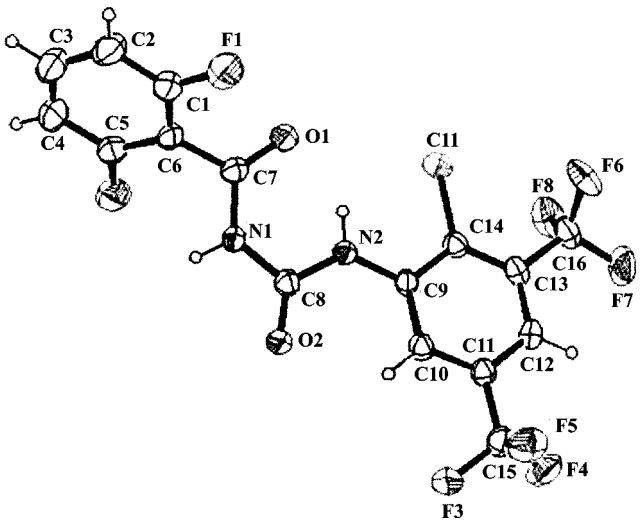

Figure 2. ORTEP diagram and numbering scheme for bistrifluron.

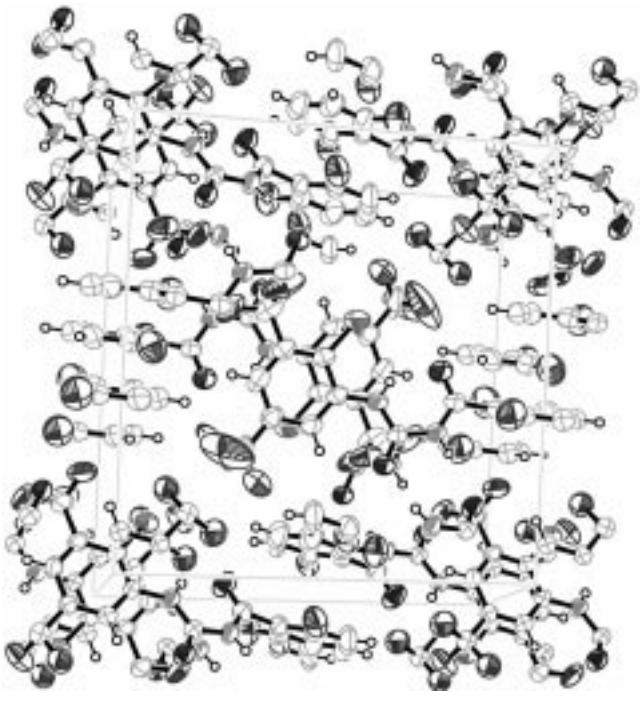

Figure 3. Packing mode in the unit cell for bistrifluron.

Table 2. Complete assignments of the ^{1}H and ^{13}C NMR data

δ ^{13}C/ppm	CH$_n$[a]	δ ^{1}H/ppm (J/Hz)[b]	assignments
111.6	d	7.08 (m)	C2, 4
118.1	d	7.75 (d, 1.7)	C12
120.8	d	8.95 (d, 1.7)	C10
121.4	s	–	C9
123.2	s	–	C13
124.1	s	–	C11
128.7	s	–	C15
129.1	s	–	C16
133.4	d	7.55 (m)	C3
137.7	s	–	C14
149.6	s	–	C8
157.7	s	–	C1, 5
160.3	s	–	C6
162.5	s	–	C7
-NH	–	10.9 (s)	a, b

[a]data determined by DEPT. [b]data determined by HMQC.

showing nOes because it has only seven protons. As a result, structural studies on bistrifluron using NMR could be focused on its complete assignments of the ^{1}H and ^{13}C NMR data. There are fourteen ^{13}C signals in the ^{13}C NMR spectrum. Their multiplicities were determined by DEPT. C2/C4 and C1/C5 were determined based on the interpretation of DEPT. Since the correlation between H2/H4 and H3 was observed in COSY, H3 was determined. C6-C16 were assigned by HMBC. Because bistrifluron includes several quaternary carbons in order to observe long ranged coupling between the carbon and proton signals, HMBC applied by a delay time for a long ranged coupling of 70 ms was carried out. Complete assignments of the ^{1}H and ^{13}C NMR data are listed in Table 2.

Molecular modeling. The minimum energy conformation of bistrifluron is shown in Figure 4. The bond lengths, bond angles, and torsion angles determined by molecular modeling calculations are compared with X-ray crystallographic data.

The results reveal that the conformer with two ketone groups placed at the same direction has higher total energy of 10 kcal/mol than the conformer placed at the opposite direction. While the torsion angles N1-C8-N2-C9, C6-C7-N1-C8, and C5-C6-C7-N1 determined by X-ray crystallography were 175.2°, -171.3°, and -56.6°, those calculated using molecular modeling were 168.2°, 170.34°, and -174.04°, respectively. The values of former two torsion angles obtained by molecular modeling agree with those by X-ray crystallography. It can be considered that two torsion angles N1-C8-N2-C9 and C6-C7-N1-C8 are close to the plane because they have double bond character. But in case of X-ray crystallography two rings are twisted by only 52.6°, and in case of molecular modeling they are more twisted by 15.5°. While when the compound is placed at solid state two rings are twisted, when it is placed in vacuo two rings are positioned almost at the same plane.

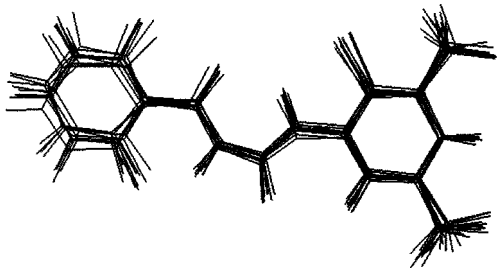

Figure 4. The superimposed structures of bistrifluron obtained by molecular modeling.

Acknowledgment. This work was supported by BK21 project in part. Authors thank Dongbu-hannong Co. for financial support. All NMR measurements by a Bruker Avance 400 spectrometer system were partially supported by Shared Research Equipment Assistance Program 2001 of Korea Basic Science Institute. The molecular modeling experiment was partially supported by ministry of information and communication through national grid infrastructure implementation project. Dr. Rhee thanks for the grant by Korea Research Foundation (KRF-2000-015DP0286).

References

1. Nakagawa, Y.; Izumi, K.; Oikawa, N.; Kurozumi, A.; Iwamura, H.; Fujita, T. *Pestic. Biochem. Physiol*. **1991**, *40*, 12.
2. Song, C.; Shin, W. K.; Cho, K. Y. *Korean J. Pestic. Sci*. **2000**, *4*, 63.
3. Sheldrick, G. M. *Acta Crystallogr*. **1990**, *A46*, 467.
4. Sheldrick, G. M. In *SHELXL93, Program for the refinement of crystal Structures*; University of Göttingen: Germany, 1993.
5. Dodrell, D.; Pegg, D.; Bendall, M. *J. Magn. Reson.* **1982**, *48*, 323.
6. Bax, A.; Freeman, R. *J. Magn. Reson*. **1981**, *43*, 333.
7. Bax, A.; Griffey, R.; Hawkins, B. *J. Magn. Reson*. **1983**, *55*, 301.
8. Bax, A.; Summers, M. *J. Am. Chem. Soc*. **1986**, *108*, 2093.
9. Turner, D. L. *J. Magn. Reson*. **1985**, *61*, 28.