

The Structure Determination of a Herbicidal Compound, 3D5

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The structure of herbicidal compound, 3D5, isolated from the culture broth of *Streptomyces* sp. 3D5, was elucidated as a 16-membered diene macrolide by the spectroscopic method. It was identical with bafilomycin D which has been known to be an insecticidal compound and an inhibitor of K⁺-dependent ATPase. However, this is the first report which shows that bafilomycin D has a herbicidal activity.

In the preceding paper (6), the taxonomy, fermentation, isolation, physico-chemical properties, and biological activity of the 3D5 compound were presented.

Herein we described the full account of structure determination of 3D5.

MATERIALS AND METHODS

Electron impact (EI) mass spectrum and secondary ion (SI) mass spectrum were obtained with a Hitachi M-80 mass spectrometer.

Proton and carbon NMR spectra were recorded on a Jeol JNM GX-400 spectrometer. The chemical shifts were reported in ppm relative to internal tetramethylsilane, and the coupling constants were expressed in Hz.

RESULTS AND DISCUSSION

The molecular formula of 3D5 compound was established as C₃₅H₅₆O₈ on the basis of EI-MS (M⁺=m/z 604, M-18=m/z 586, M-C₃H₇-2H₂O=m/z 525), SIMS (M⁺+K⁺=m/z 643) and ¹³C NMR spectral data.

The decoupled ¹³C NMR and ¹H NMR spectral data including the distortionless enhancement by polarization transfer (DEPT) experiment revealed 35 carbons of the following functional groups (accounting for 53 protons): 9X CH₃, 1X CH₂, 6X CH, 2X OCH₃, 5X O-CH, 7X

$=\text{CH}-$, $3\text{X}=\overset{\text{O}}{\text{C}}-$, $1\text{X}=\overset{\text{O}}{\text{C}}-\text{O}$, $1\text{X}=\overset{\text{O}}{\text{C}}-\text{R}'$. These groups accounted for all the hydrogens in 3D5 compound except for 3 exchangeable ones which were assumed to be hydroxyl functions on δ_{C} 72.77, 79.91, and 81.37 ppm based on deuterium induced upfield shifts observed in the ¹³C NMR spectrum (2, 5).

The 400 MHz proton NMR spectrum of 3D5 compound in CDCl₃ was complex and an unambiguous assignment by the conventional one dimensional NMR experiment was not possible. Homonuclear shift correlation spectroscopy (2D ¹H-¹H cosy) was therefore used to establish the connectivity between all pairs of mutually coupled protons while heteronuclear shift correlation spectroscopy (2D ¹H-¹³C cosy) was used to correlate the chemical shifts of all directly bonded proton and carbon pairs in the molecules.

Unequivocal assignments of all the proton and carbon resonances were obtained based on 2D NMR experiments such as ¹H-¹H cosy (Fig. 1) and ¹H-¹³C cosy (Fig. 2), with the exception of those for 9 methyl groups, 2 methoxyl groups and 3 hydroxyl groups which were unambiguously assigned by the aid of heteronuclear multiple bond correlation (HMBC), conventional proton spin decoupling, and nuclear overhauser effect (NOE) experiment.

2D ¹H-¹H cosy and 2D ¹H-¹³C cosy spectra of 3D5 compound in CDCl₃ and in benzene-d₆ revealed two

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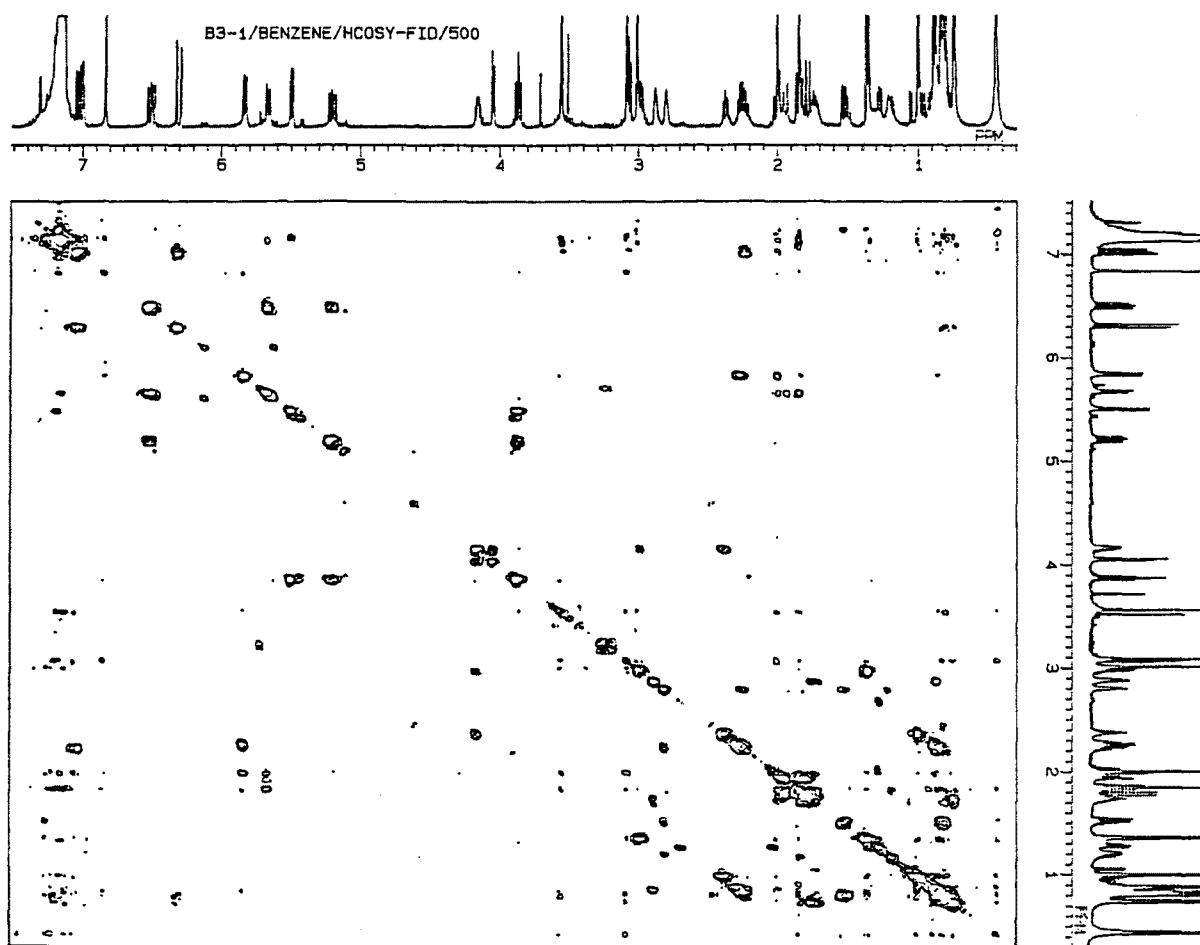


Fig. 1. ¹H-¹H cosy spectrum of 3D5 compound (400 MHz, benzene-d₆).

partial structures, A (C-1~C-15) and B (C-16~C-25) as shown in Fig. 3. The location of methoxyl groups at C-2 and C-14 was determined by HMBC. Methoxyl protons at δ_{H} 3.68 ppm (CH₃O-C-2) and δ_{H} 3.22 ppm (CH₃O-C-14) were long range coupled with δ_{C} 141.57 ppm (C-2) and δ_{C} 83.48 ppm (C-14), respectively, and also NOE enhancements were observed at CH₃ (C-4) and H-14 when methoxyls at δ 3.68 and δ 3.22 were irradiated, respectively.

The presence of hydroxyl functions at C-7 (δ_{C} 81.37 ppm), C-17 (δ_{C} 72.77 ppm) and C-23 (δ_{C} 79.91 ppm) was established by the couplings from hydroxy protons, δ_{H} 1.51 ppm, δ_{H} 1.57 ppm, and δ_{H} 3.63 ppm to H-7 (δ_{H} 3.31 ppm), H-23 (δ_{H} 3.18 ppm), and H-17 (δ_{H} 3.76 ppm), respectively in the proton cosy spectrum in CDCl₃, and by the deuterium induced upfield shift of the C-7, C-17, and C-23 signals. The H-15 (δ_{H} 5.06) proton, which resonated in the lowest field among the methine protons bearing an oxygen function, should be esterified with

the C-1 carbonyl, and also methine proton of H-15 showed long range coupling to C-1 (δ_{C} 166.49), C-12 (δ_{C} 132.79 ppm), C-13 (δ_{C} 127.24 ppm), C-16 (δ_{C} 38.67 ppm) and C-17 (δ_{C} 72.77 ppm). The lower field resonance of olefinic signal C-21 (δ_{C} 148.65), H-21 (δ_{H} 6.92), in the NMR spectra suggested that this proton is attached to the β -carbon on the double bond to carbonyl group. ¹H-¹³C long range correlations from an allylic methyl (δ_{C} 14.09, CH₃-26) to three olefinic carbons (C₃, C₄ and C₅), and from H-3 (δ_{H} 6.65) to an ester carbonyl (δ_{C} 166.49, C-1) suggested that these units formed a conjugated diene-carbonyl system causing the characteristic UV absorption of 3D5 compound.

In the highest field of methyl signal region at 0.94 ppm of the ¹H NMR spectrum, there was a complicated overlapped signal containing 4 methyl groups. Two of them corresponded to δ_{C} 16.90 and δ_{C} 19.77 ppm respectively, in the ¹³C NMR spectrum, both correlate with the identical methine proton at δ_{H} 1.72 ppm. Obviously,

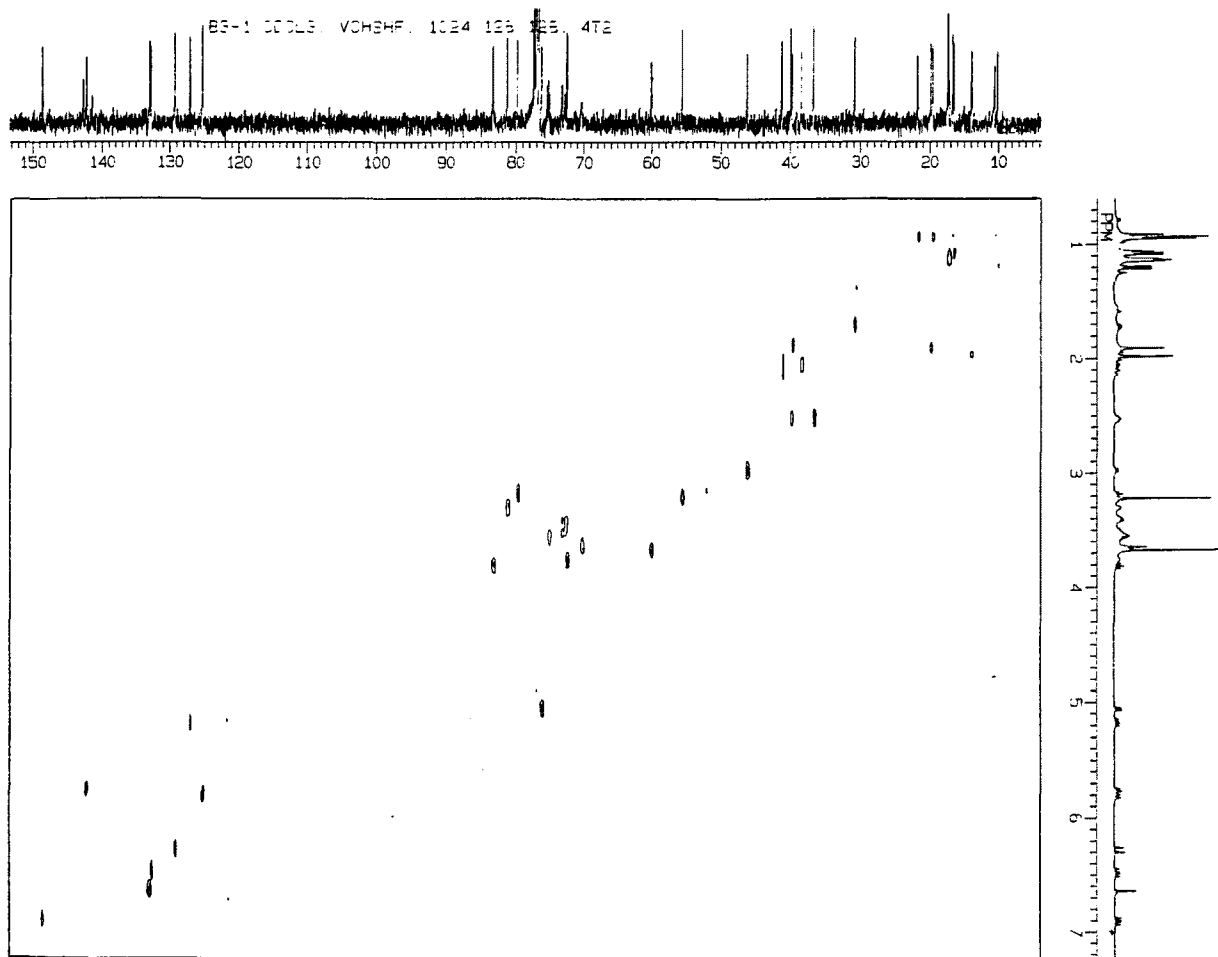


Fig. 2. ^1H - ^{13}C cosy spectrum of 3D5 compound (400 MHz, CDCl_3).

these two methyl signals were due to methyls of the CH_3 -25 and CH_3 -33 and this methine proton was H-24. The δ_{H} 0.94 ppm signal also corresponded to δ_{C} 10.82 and δ_{C} 21.99 in the ^{13}C NMR spectrum, correlated with δ_{H} 2.06 ppm and δ_{H} 2.13 ppm respectively in the ^1H - ^{13}C cosy spectrum, representing two methyl signals due to CH_3 -30 and CH_3 -28. Also upon irradiation of δ_{H} 0.94 ppm signal containing 4 methyl groups, H-16 (δ_{H} 2.06 ppm) collapsed to doublet of quartet, H-24 (δ_{H} 1.72 ppm) to simple, and H-8 (δ_{H} 1.91 ppm) to doublet in the ^1H spin decoupling experiment. A broad methyl signal at δ_{H} 1.07 ppm coupled with two methine protons at δ_{H} 2.57 which in turn coupled with H-21 (δ_{H} 6.92 ppm) and H-5 (δ_{H} 5.78 ppm), suggesting that δ_{H} 1.07 ppm signal contained two methyl groups, CH_3 -27 and CH_3 -32. Methyl singlet at δ_{H} 1.98 ppm was long range coupled with C-4 (δ_{C} 132.85 ppm) and C-5 (δ_{C} 142.33 ppm) while methyl singlet at δ_{H} 1.91 ppm was long range coupled with C-11 (δ_{C} 125.42 ppm)

and C-10 (δ_{C} 142.86 ppm). These two methyl singlets were assigned to CH_3 -26 and CH_3 -29, respectively.

High order couplings and inadequate separation of H-8 and one of H-9 methylene protons in the proton NMR spectrum taken in CDCl_3 prevented the establishment of the relationship between these protons. In the proton NMR spectrum measured in benzene- d_6 , however, H-8 and H-9 were well separated and could be analyzed easily. Almost complete overlapping at δ_{H} 2.09 ppm was also well separated in benzene- d_6 and could be assigned to be H-15 and another H-9 methylene proton.

The proton H-5 showed allylic coupling with CH_3 -26 and H-3. By irradiation at H-5 (δ_{H} 5.78 ppm), the long range coupling of CH_3 -26 and H-3 disappeared. By irradiation at H-3 in a NOE experiment, only the resonance of H-5 was seen in the difference spectrum. This was in accordance with an *E* configuration of diene structure C_2 - C_3 - C_4 - C_5 , methoxy substitution at C_2 and methyl sub-

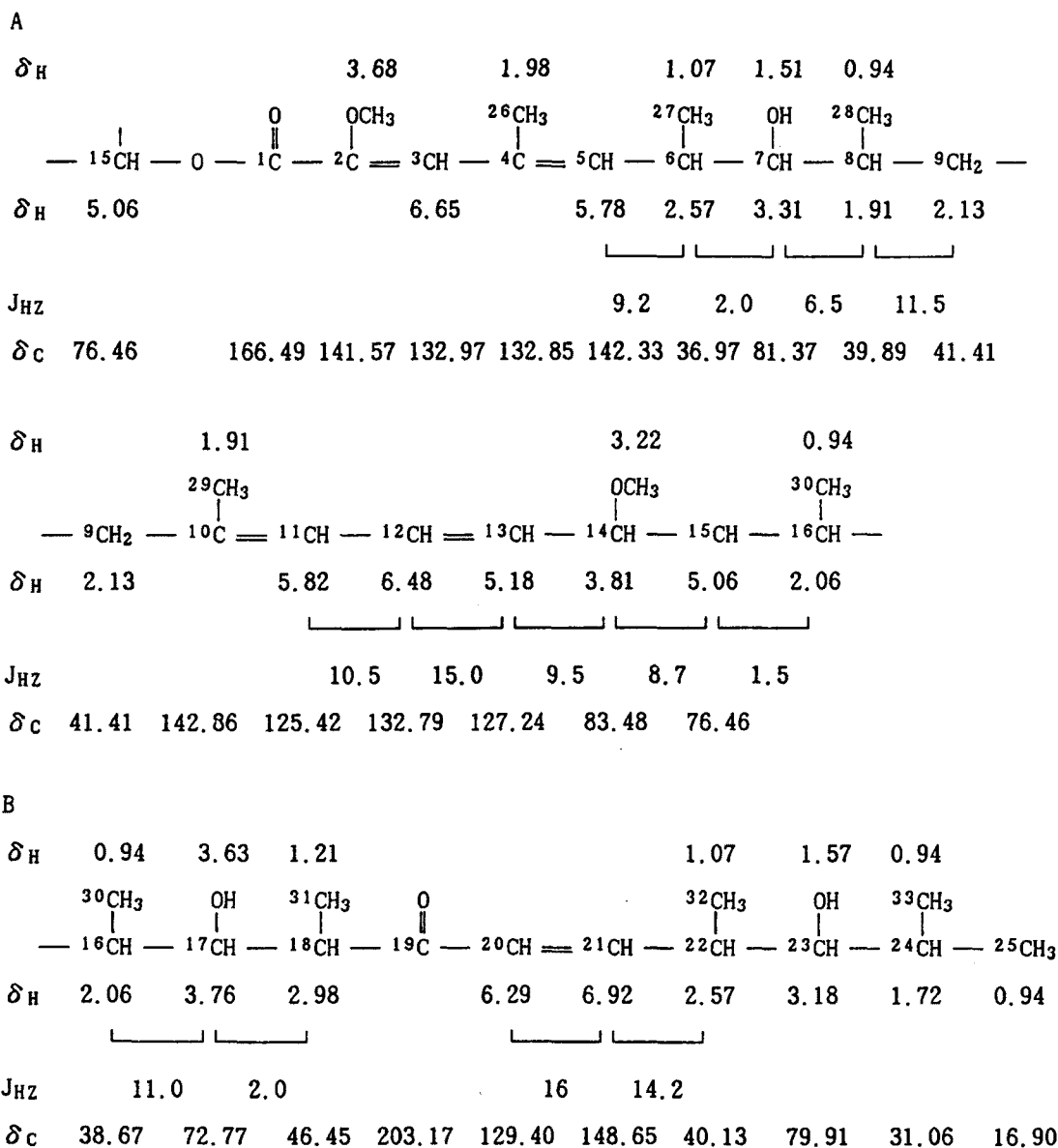


Fig. 3. Partial structures A and B for 3D5 compound.

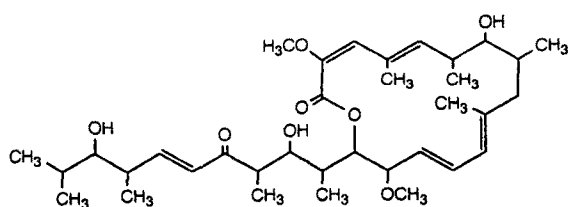


Fig. 4. The structure of 3D5 compound.

stitution at C_4 . The coupling constant ($J=15$ Hz) between two olefinic protons H-12 and H-13 indicated an *E*-geometrical configuration of C_{10} - C_{11} - C_{12} - C_{13} also (4).

The complete assignments of proton and carbon signals of 3D5 compound were established as shown in Table 1 and 2.

Based on these analyses, the structure of 3D5 compound was determined as shown in Fig. 4, a 16-membered diene macrolide which was identical with bafilomycin D (3).

Bafilomycin D has been reported to be an insecticidal

Table 1. Proton NMR assignments of 3D5 compound

Chemical shift (ppm)	assignments, integration, coupling constant (J in Hz), multiplicity			Coupling signal (ppm)	
6.92	=CH-21	(1H)	$J=16, 14.2$	d of d	6.29, 2.57
6.65	=CH-3	(1H)		s	
6.48	=CH-12	(1H)	$J=15, 10.5$	d of d	5.82, 5.18
6.29	=CH-20	(1H)	$J=16$	d	6.92
5.82	=CH-11	(1H)	$J=10.5$	d	6.48
5.78	=CH-5	(1H)	$J=9.2$	d	2.57
5.18	=CH-13	(1H)	$J=15.0, 9.5$	d of d	6.48, 3.81
5.06	OCH-15	(1H)	$J=8.7, 1.2$	d of d	3.81, 2.06
3.81	OCH-14	(1H)	$J=9.5, 8.7$	d of d	5.18, 5.06
3.76	OCH-17	(1H)	$J=11.0, 4.1, 2.0$	d of d of d	3.63, 2.98, 2.06
3.68	OCH ₃	(3H)		s	
3.63	OH-17	(1H)		d	3.76
3.31	CH-7	(1H)	$J=2.0, 6.5$	m	2.57, 1.91, 1.51
3.22	OCH ₃	(3H)		s	
3.18	OCH-23	(1H)		m	2.57, 1.72, 1.57
2.98	CH-18	(1H)	$J=2.0$	m	3.76, 1.21
2.57	CH-22	(1H)	$J=14.2$	m	6.92, 3.18, 1.07
	CH-6	(1H)	$J=2.0, 9.2$	d of q of d	5.78, 1.07
2.13	CH ₂ -9	(2H)	$J=14, 11.5$	d	1.91
2.06	CH-16	(1H)	$J=11.0, 6.7, 1.2$	d of q of d	5.06, 3.76, 0.94
1.98	CH ₃ -26	(3H)			
1.91	CH-8	(1H)	$J=11.5, 6.5$	d of q	3.31, 0.94
	CH ₃ -29				
1.72	CH-24	(1H)	$J=12.0, 8.0$	m	3.18, 0.94
1.57	OH-23	(1H)		m	3.18
1.51	OH-7	(1H)		m	2.98
1.21	CH ₃ -31	(3H)		d	2.57
1.07	CH ₃ -32	(3H)		m	
	CH ₃ -27	(3H)			
0.94	CH ₃ -28	(3H)			1.72, 2.06
	CH ₃ -30	(3H)			1.91
	CH ₃ -25	(3H)			
	CH ₃ -33	(3H)			

Table 2. ^{13}C NMR data of 3D5 compound

Chemical shift δ_{C} (ppm)	aggnments	position (carbon number)	Chemical shift δ_{C} (ppm)	aggnments	position (carbon number)
10.351	CH ₃	31	81.370	O-CH	7
10.819	CH ₃	30	83.476	O-CH	14
14.095	CH ₃	26	125.423	=CH-	11
16.728	CH ₃	32	127.236	=CH-	13
16.903	CH ₃	25	129.401	=CH-	20
17.489	CH ₃	27			
19.770	CH ₃	33	132.792	=CH-	12
20.004	CH ₃	29			
21.993	CH ₃	28	132.850	=C-	4
31.059	CH	24	132.970	=CH-	3
36.968	CH	6			
38.665	CH	16	141.570	=C-	2
39.893	CH	8			
40.127	CH	22	142.330	=CH-	5
41.414	CH ₂	9			
46.446	CH	18	142.857	=C-	10
55.747	O-CH ₃		148.648	=CH-	21
60.252	O-CH ₃				
72.771	O-CH	17			
76.456	O-CH	15	166.492	$\begin{array}{c} \text{O} \\ \\ -\text{C}=\text{O} \end{array}$	1
79.908	O-CH	23			
			203.173	$\begin{array}{c} \\ -\text{C}=\text{O} \end{array}$	19

compound and it has been known as an inhibitor of K^+ -dependent ATPase (1). However, this is the first report which shows that bafilomycin D has a herbicidal activity.

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