Communication to the Editor

RESPIRANTIN, A NOVEL INSECTICIDAL CYCLODEPSIPEPTIDE FROM *Streptomyces*

Sir:

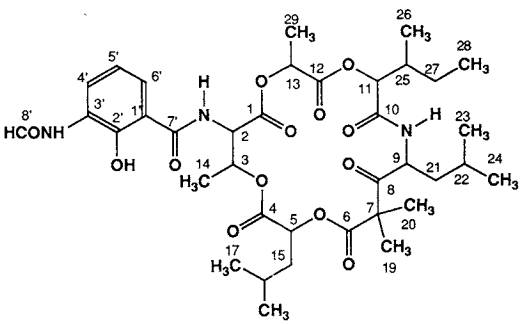
In the course of our screening search for insect growth regulators from microorganism, we have found a new insecticidal antibiotic termed respirantin (**1**) from the mycelial extract of a *Streptomyces* sp. No. 4403, which was isolated from a soil sample collected at Eiheiji-cho, Fukui, Japan. In this communication, we report the fermentation, isolation, structure elucidation and biological activities of **1**.

Bioassay for insect growth regulators was carried out according to the method previously reported[1,2]. A cultured broth was suspended with an artificial diet for *Pseudalitia* (*Leucania*) *separata*[3] in a flask, which was then autoclaved at 110°C for 15 minutes. Assay larvae of *P. separata* had been previously reared under aseptic conditions and three larvae in the third larval molting were transferred to an assay flask. These assay flasks were kept at 25°C and the effect of each sample was examined.

The fermentation was carried out in 500-ml Erlenmeyer flasks containing: 100 ml of medium with a following composition: Glucose 0.4%, yeast extract 0.4% and malt extract 1%. The pH of the medium was adjusted to 7.3 before sterilization. The seed culture was inoculated to the flask, and the fermentation was carried out at 26.5°C for 5 days on a rotary shaker.

The acetone extract of the mycelial cake was concentrated under reduced pressure and the resulting aqueous solution was extracted with ethyl acetate. After concentration of the ethyl acetate layer, the residue was chromatographed on silica gel and developed with hexane - ethyl acetate (2 : 1). The active fraction was concentrated under reduced pressure and the resulting residue was applied to preparative HPLC with a reversed phase column. The conditions were as follows: Column, Senshu Pak ODS-H-5252 (2 × 25 cm); solvent, 50% 2-propanol; flow rate, 4 ml/minute; detection, UV absorption at 254 nm. The active fraction was concentrated to dryness under reduced pressure to give **1** as an amorphous powder.

Fig. 1. The structure of respirantin (**1**).



The physico-chemical properties of **1** are summarized in Table 1. The molecular formula of **1** was determined as $C_{37}H_{53}N_3O_{13}$ by HRFAB-MS. The UV spectrum showed characteristic absorption maxima at 228 and 320 nm. The FT-IR spectrum showed absorption bands at 1744, 1711, 1690, 1680 and 1641 cm^{-1} assignable to several carbonyl groups.

The ^1H and ^{13}C NMR spectral data for **1** are shown in Table 2. The ^1H NMR spectrum showed the presence of a 1,2,3-trisubstituted phenyl ring (δ_H 8.54, 6.94 and 7.34) with an H-bonded hydroxy group (δ_H 12.51), consistent with the positive reaction of **1** with alcoholic ferric chloride. The ^{13}C NMR spectrum of **1** indicated eight carbonyl carbons (δ_C 208.0, 173.3, 171.8, 170.4, 169.9, 169.5, 167.5 and 159.0) and one aliphatic quaternary carbon (δ_C 53.0).

The ^1H-^1H COSY and ^{13}C-^1H COSY spectra of **1** showed the presence of following partial structures: –NH–CH–CH(CH_3)–O–, –O–CH–CH_2–CH(CH_3)–CH_3, –NH–CH–CH_2–CH(CH_3)–CH_3, –O–CH–CH(CH_3)–CH_2–CH_3, –O–CH–CH_3, –NH–CHO.

The connectivities of the partial structures and the other fragments were determined by HMBC experiments. As shown in Fig. 2, the HMBC

Table 1. Physico-chemical properties of respirantin.

Appearance	Amorphous powder
$[\alpha]_D^{25}$	−10° (*c* 0.23, MeOH)
Molecular formula	$C_{37}H_{53}N_3O_{13}$
FAB-MS (*m/z*)	748 $(M+H)^+$
HRFAB-MS Calcd:	748.3656
Found:	748.3701 $(M+H)^+$
UV λ_{max}^{MeOH} nm (ε)	228 (27,900), 320 (5,300)
FT-IR ν_{max} cm^{-1}	3324, 2961, 1744, 1711, 1690, 1680, 1641, 1530

Table 2. ^{1}H and ^{13}C NMR spectral data for respirantin in $CDCl_3$.

No.	δ_C	δ_H
1	167.5	
2	55.6	5.19 (dd, 8.7, 2.8)[a]
3	72.3	5.99 (dq, 6.4, 2.8)
4	171.8	
5	72.0	4.66 (dd, 10.1, 4.1)
6	173.3	
7	53.0	
8	208.0	
9	56.5	4.88 (ddd, 10.5, 9.6, 3.7)
10	169.9	
11	80.9	4.82 (d, 9.6)
12	169.5	
13	71.5	5.83 (q, 6.9)
14	16.6	1.36 (d, 6.4)
15	39.5	1.49 (ddd, 13.9, 8.2, 4.1), 1.68[b]
16	24.5	1.72[b]
17	21.4[c]	0.92 (d, 6.4)
18	22.8[c]	0.87 (d, 6.9)
19	24.1[d]	1.25 (s)
20	19.8[d]	1.11 (s)
21	43.1	1.83 (ddd, 14.5, 10.5, 3.7), 1.74 (ddd, 14.5, 11.0, 3.7)
22	24.7	1.62[b]
23	23.6[e]	0.91 (d, 6.4)
24	21.0[e]	0.91 (d, 6.4)
25	36.6	2.07 (m)
26	14.4	0.98 (d, 6.9)
27	25.3	1.33 (m), 1.70[b]
28	10.4	0.96 (t, 7.3)
29	18.2	1.55 (d, 6.9)
1′	112.9	
2′	150.6	
3′	127.5	
4′	125.0	8.54 (dd, 8.3, 0.9)
5′	119.1	6.94 (t, 8.3)
6′	120.3	7.34 (dd, 8.3, 0.9)
7′	170.4	
8′	159.0	8.49 (d, 1.4)
2-NH		7.17 (d, 8.7)
9-NH		7.45 (d, 9.6)
2′-OH		12.51 (br)
3′-NH		7.96 (br)

[a] Coupling constants in J=Hz.
[b] Resonance obscured by overlapping signals.
[c~e] These assignments are interchangeable.

experiments showed the long range coupling of six methines (2-H, 3-H, 5-H, 9-H, 11-H, 13-H), two singlet methyls (19-H, 20-H) and two amino groups (2-NH, 9-NH) to seven quaternary carbons (C-1, C-4, C-6, C-7, C-8, C-10, C-12), and an 18-membered cyclodepsipeptide structure was determined. The long range couplings of 6′-H, 8′-H, 3′-NH and 2′-OH to C-1′, C-2′, C-3′, C-4′ and C-7′, respectively, were also observed by the HMBC experiments as shown in Fig. 2 and the connectivities of the chromophore moiety were confirmed. Further long range couplings of 2-H and 2-NH to C-7′ were also observed and the connectivity of the chromophore moiety and the 18-membered ring was confirmed. Thus, the covalent structure of **1** was established.

Fig. 2. Long range couplings observed by the HMBC experiments.

⟶ Long range coupling.

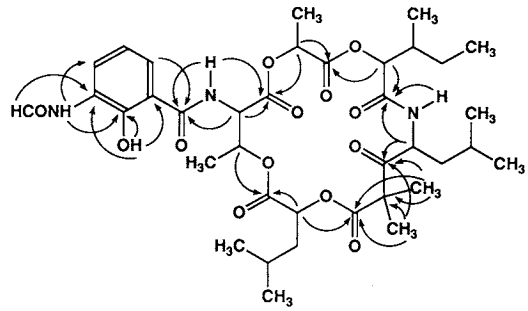


Table 3. Antimicrobial activities of respirantin.

Organism	Diameter of inhibition zone (mm)
Pyricularia oryzae	12
Alternaria mali	13
Rhizoctonia solani	12
Botrytis cinerea	12
Candida albicans	<10
Escherichia coli	0
Staphylococcus aureus	0

For the plate diffusion assay, 1 μg was applied to 8 mm paper disks. The disks were placed on plates seeded with the test microorganisms on the agar surface.

Respirantin (**1**) proved to contain a blastmycic acid[4] moiety as recognized in antimycin A[5] (**2**) and neoantimycin[6] (**3**). The presence of the moiety was also confirmed by the characteristic UV spectrum of **1**[5,6]. Though the chromophore of **1** was the same as those of **2** and **3**, the ring size and ring components of **1** were distinct from those of **2** and **3**, which are 9 and 15-membered cyclodepsipeptides with one peptide bond, respectively. A component of **1** containing a ketone, 4-amino-2,2,6-trimethyl-3-oxo-heptanoic acid, is unique among the known microbial products.

Respirantin (**1**) caused all the assay larvae to die

within 11 days at dose of 500 ppm and 18 days at dose of 100 ppm in the diet, respectively. The insecticidal activity of **1** was nearly equal to that of antimycin A (**2**). Furthermore **1** showed weak fungicidal activities as shown in Table 3.

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IKUMI URUSHIBATA[a,b]
AKIRA ISOGAI[a]
SHOGO MATSUMOTO[c]
AKINORI SUZUKI[a]

[a]Department of Agricultural Chemistry,
The University of Tokyo,
Bunkyo-ku, Tokyo 113, Japan
[b]K I Chemical Research Institute CO., LTD.,
Fukude, Shizuoka 408-1, Japan
[c]Insect Toxicology and Physiology Laboratory,
RIKEN,
The Institute of Physical and Chemical
Research,
Wako, Saitama 305-01, Japan

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