Communication to the editor

THE ISOLATION AND STRUCTURES OF HYGROLIDIN AMIDE AND DEFUMARYLHYGROLIDIN

Sir:

In a previous paper[1] we described the isolation and structure of hygrolidin (**1**), an antibiotic produced by *Streptomyces hygroscopicus* D-1166 which is specifically active against *Valsa ceratosperma*, the pathogen of apple canker disease. **1** belongs to a new group of 16-membered macrolide antibiotics. Recently, additional members of this group such as bafilomycins[2], a new macrolide substance[3] and L-681,110[4] have been reported. Further screening for minor components of **1** resulted in the isolation of two metabolites named hygrolidin amide (**2**) and defumarylhygrolidin (**3**). In this paper, we wish to describe the isolation, structural elucidation and biological activities of these two minor components.

Isolation

S. hygroscopicus D-1166[1] was maintained on agar slants consisting of glucose 0.4, malt extract 1.0, yeast extract 0.4 and agar 1.5%, pH 7.3. Fermentation was initiated by transferring a loopful of surface growth to large test tubes (2×20 cm) each containing 15 ml of PC-I medium (starch 1.0, Polypepton 1.0, molasses 1.0 and beef extract 1.0%, pH 7.2) and incubated at 30°C for 2 days on a reciprocal shaker.

A 2.0% (v/v) transfer of this culture growth was used to inoculate 500-ml Erlenmeyer flasks each containing 100 ml of N medium with the following composition; starch 2.5, soybean meal 1.5, dry yeast 0.2, $(NH_4)_2SO_4$ 0.2, NaCl 0.5 and $CaCl_2$ 0.4%, pH 7.0. The fermentation was continued for 72 hours at 28°C on a rotary shaker.

Since the production yields of **2** and **3** were very low, and since the organism produced several antibacterial substances such as azalomycin B, azalomycin F_{4a} and geldanamycin, antibiotic isolation was monitored by TLC analysis detecting compounds which showed UV absorption similar to that of **1**. **2** and **3** were isolated from mycelium of *S. hygroscopicus* as summarized in Fig. 1. The production yields of **2** and **3** were 0.07 mg/liter and 0.1 mg/liter, respectively.

Structures of **2** and **3**

2, $C_{38}H_{59}O_{10}N$, SIMS, $(M+Na)^+$ m/z 712, $[\alpha]_D^{25}$ +43.4° (c 0.1, MeOH), UV λ_{max}^{MeOH} 245 nm (ε 14,000), 276 (5,800), IR ν_{max}^{KBr} 3400, 1720, 1685, 1675, 1650, 1615, 1250 cm^{-1}, was obtained as rod

Fig. 1. Isolation procedures for hygrolidin amide (**2**) and defumarylhygrolidin (**3**).

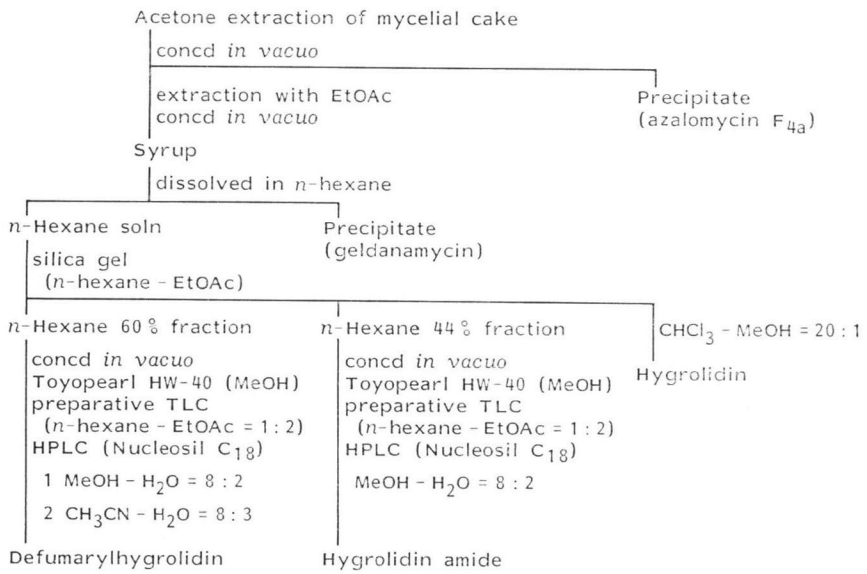


Table 1. ^{13}C NMR chemical shifts of hygrolidin amide (**2**) and defumarylhygrolidin (**3**).

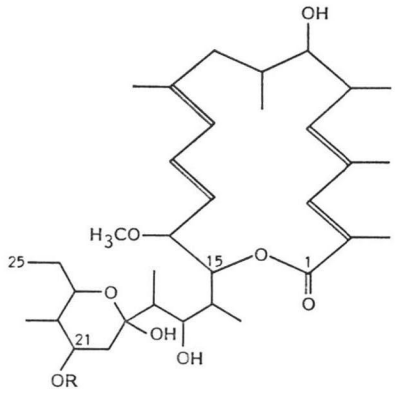
Carbon	2	3	Carbon	2	3
1	171.9	171.9	20	34.4	37.5
2	122.2	122.2	21	73.1	68.0
3	146.4	146.4	22	35.2	38.1
4	134.3	134.3	23	71.3	71.6
5	144.4	144.5	24	25.2	25.3
6	36.7	36.6	25	10.7	10.7
7	81.2	81.1	26	13.7	13.6
8	39.7	39.7	27	15.1	15.1
9	41.2	41.1	28	17.4	17.4
10	142.4	142.5	29	21.5	21.5
11	125.1	125.2	30	20.0	20.0
12	132.5	132.3	31	9.6	9.6
13	127.3	127.3	32	6.9	6.8
14	82.3	82.5	33	4.8	3.9
15	76.0	76.0	34	55.5	55.5
16	37.8	37.8	35	163.9	
17	70.3	70.3	36	132.5	
18	41.6	41.5	37	135.3	
19	99.3	99.2	38	165.0	

Spectra were taken in $CDCl_3$ solution and the chemical shifts were expressed in ppm from internal TMS.

crystals which melted at 138~139°C. The molecular ion peak 712 $(M+Na)^+$ was shifted to 728 $(M+K)^+$ when the mass spectrum was taken in the presence of KI.

The 100 MHz ^{13}C NMR spectrum of **2** is almost completely identical with that of **1**[1] except for the upfield shift of the carbonyl carbon (C-38) of the fumaric acid residue from 168.5 ppm to 165.0 ppm (Table 1). This phenomenon together with the molecular formula of **2** suggested that **2** is an amide derivative of **1**. In agreement with this structure, broad singlet amide protons (area 2H) were observed at 5.91 ppm in addition to three hydroxyl protons at 1.62 (d, J=4.0 Hz), 4.85 (d, J=4.5 Hz) and 5.80 ppm (br. s) in the 400 MHz ^1H NMR spectrum of **2** in $CDCl_3$. When the ^1H NMR spectrum was taken at −10°C, the amide protons changed to two unequivalent broad singlets at 6.15 and 6.28 ppm and three hydroxyl protons were observed at 1.85 (d, J= 4.0 Hz), 4.92 (d, J=4.5 Hz) and 5.99 ppm (s). On the other hand, hydroxyl protons could not be observed in the ^1H NMR spectrum of **1** probably due to rapid exchange of these two protons

Fig. 2. The structures of hygrolidin (**1**), hygrolidin amide (**2**) and defumarylhygrolidin (**3**).



Hygrolidin (**1**) R=COCH=CHCOOH
Hygrolidin amide (**2**) R=COCH=CHCONH$_2$
Defumarylhygrolidin (**3**) R=H

catalyzed by the free carboxylic acid in **1**. Thus, **2** is concluded to be an amide derivative of hygrolidin as shown in Fig. 2.

3, $C_{34}H_{56}O_8$, SIMS, $(M+Na)^+$ m/z 615, was obtained as a white amorphous powder, mp 95~96.5°C, $[\alpha]_D^{25}$ +49.4° (c 0.1, MeOH), UV λ_{max}^{MeOH} 246 nm (ε 18,400) and 278 (7,500), IR ν_{max}^{KBr} 3400, 1700 (sh), 1670, 1250 cm^{-1}.

The structure of **3** was determined by detailed analysis of its ^1H and ^{13}C NMR spectral data. As shown in Fig. 3, the 400 MHz ^1H NMR spectrum of **3** is very close to that of **2**. The only difference observed was upfield shift of H-21 from 5.4 ppm in **2** to 3.85 ppm in **3** together with the absence of the characteristic AB-type quartet (6.82 and 6.90 ppm, J=16.0 Hz) ascribed to the fumaric acid moiety. The ^{13}C NMR spectral data of **3** (Table 1) were in accord with this structural modification; the resonances due to the fumaric acid moiety disappeared and the C-21 signal showed upfield shift by 5.1 ppm in the ^{13}C NMR spectrum of **3**. Thus, **3** is the defumaryl derivative of hygrolidin as shown in Fig. 2 and named as defumarylhygrolidin.

Biological Activities

2 and **3** exhibited selective antifungal activity against *V. ceratosperma* but their activities are much weaker than **1**. The relationship between the concentration and the diameter of inhibition zone is summarized in Table 2. **2** and **3** were inactive against *Bacillus subtilis* and *Escherichia coli* at the concentration of 100 μg/ml. Cyto-

Fig. 3. 400 MHz ^1H NMR spectra of hygrolidin amide (**2**, A) and defumarylhygrolidin (**3**, B).

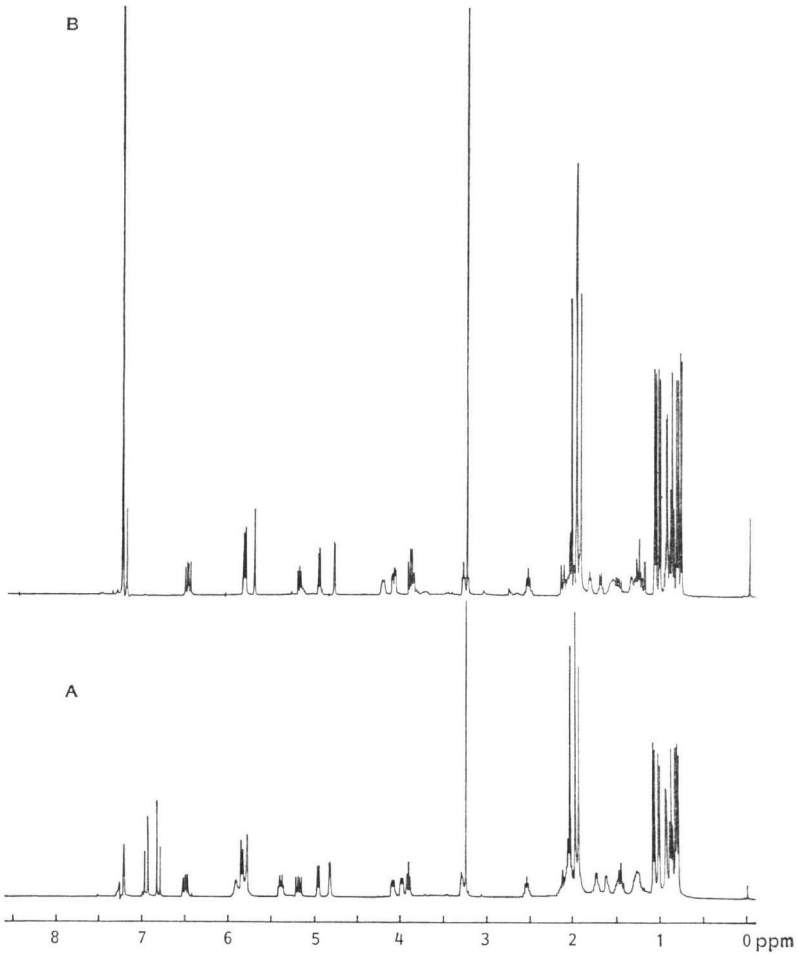


Table 2. Antifungal activity of hygrolidin amide (**2**) and defumarylhygrolidin (**3**) against *V. ceratosperma*.

Concentration (μg/ml)	Inhibition zone (diameter, mm)	
	2	**3**
25.0	20	45
12.5	19	40
6.25	18	37
3.13	17	31.5
1.56	16	27.5
0.78	14	22

Inhibition zone of hygrolidin (**1**) at 5 μg/ml was 50 mm.

toxicity of **3** against Friend cell was not observed at 10 μg/ml, while **2** was toxic at a level of 2 μg/ml (IC_{50}). Thus, the fumaric acid residue in hygrolidin derivatives seems to play an important role for the biological activities.

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