Notes

ISOLATION AND STRUCTURES OF TWO NOVEL ANTI-MITE SUBSTANCES, AB3217-B AND C

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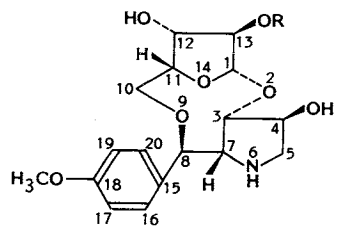
(Received for publication August 30, 1991)

We previously reported[1] the isolation and structure determination of a novel anti-mite substance, AB3217-A (**1**), one of the components of an anti-mite substance complex produced by *Streptomyces platensis* AB3217. Following improvements in the isolation method of AB3217-A from cultured broth, we found that the strain produced two new minor components, AB3217-B (**2**) and C (**3**), which were structurally related to AB3217-A. In this paper, we wish to report the isolation and structural studies of the two components.

A slant culture of strain AB3217 was inoculated into a 500-ml Sakaguchi flask containing 125 ml of a medium consisting of dextrin 20 g, galactose 20 g, corn-steep liquor 5 g, Bacto-soytone (Difco) 10 g, $(NH_4)_2SO_4$ 2 g and $CaCO_3$ 2 g in 1 liter of deionized water (pH 7.4 before autoclaving) and incubated at 27°C for 72 hours on a reciprocal shaker (135 rpm). For the production of AB3217-A, B and C, 1 ml of this culture was used as inoculum for a similar flask of medium and this was cultivated at 27°C for 96 hours.

Fig. 1. Structures of AB3217-A, B, C.



AB3217-A (**1**) R = H

AB3217-B (**2**) R = $-\overset{O}{\overset{\|}{C}}(CH_2)_4\overset{OH}{\overset{|}{C}}(CH_3)_2$

AB3217-C (**3**) R = $-\overset{O}{\overset{\|}{C}}(CH_2)_2CH(CH_3)_2$

The cultured broth filtrate (10 liters) adjusted to pH 9 was charged on a column of Diaion HP-20 (500 ml). After the column was washed with water (2 liters), the active principles were eluted with 0.05 N HCl - 50% aq acetone (2 liters). The active eluate was neutralized with 5 N NaOH and concentrated for removal of acetone. The concentrated solution was diluted to 500 ml with water, adjusted to pH 9 with 1 N NaOH, mixed with an equal volume of ethyl acetate and vigorously stirred, successively. The ethyl acetate layer contained **2** and **3** while **1** remained in water layer. Compounds **2** and **3** in the ethyl acetate layer was extracted with water at pH 2. The aqueous layer was adjusted to pH 5 with 1 N NaOH and concentrated to give 651 mg of crude powder. The crude powder containing **2** and **3** was dissolved in 10 ml of water and adjusted to pH 9 with 1 N NaOH. The solution was chromatographed on a column of Diaion CHP-20P (15 ml) by elution with a linearly increasing concentration of aq acetone (0% to 50%) containing 0.05 N HCl. Two active peak fractions were observed. After the first fractions containing **2** were collected and neutralized with Amberlite IRA-45 (OH^-), the solution was concentrated and lyophilized to give 121 mg of crude powder. The latter fractions containing **3** were also subjected to the same procedures and gave 72 mg of crude powder. Each substance was further purified by silica gel PTLC with CH_2Cl_2 - MeOH (4 : 1) as a development solvent (**2**, Rf 0.46 and **3**, Rf 0.60). Each active band was collected and extracted with CH_2Cl_2 - MeOH (3 : 2). Each eluate was concentrated to dryness. Each residue was dissolved in water (5 ml) and adjusted to pH 5 with 0.1 N HCl and lyophilized to provide the HCl salts of **2** (57.6 mg) and **3** (23.4 mg).

The physico-chemical properties of the HCl salts of **2** and **3** are as follows: **2**, mp 116°C; $[\alpha]_D^{25}$ −68.3° (*c* 1.0, H_2O); UV $\lambda_{max}^{H_2O}$ nm ($E_{1\,cm}^{1\%}$) 226 (233), 272 (20), 278 (17); FAB-MS *m/z* 496 $(M+H)^+$; **3**, mp 117°C; $[\alpha]_D^{25}$ −76.8° (*c* 1.0, H_2O); UV $\lambda_{max}^{H_2O}$ nm ($E_{1\,cm}^{1\%}$) 226 (288), 272 (28), 278 (25); FAB-MS *m/z*

452 $(M+H)^+$. The molecular formulas of **2** and **3** were established as $C_{25}H_{37}NO_9$ and $C_{23}H_{33}NO_8$, respectively, by HRFAB-MS and ^{13}C NMR spectra (Table 2). The IR spectra of **2** and **3** suggested the presence of the ester bond (**2**, 1730 cm^{-1} and **3**, 1740 cm^{-1}) in their structures (Fig. 2). The UV

Fig. 2. IR spectra of the HCl salts of AB3217-B and C (KBr).

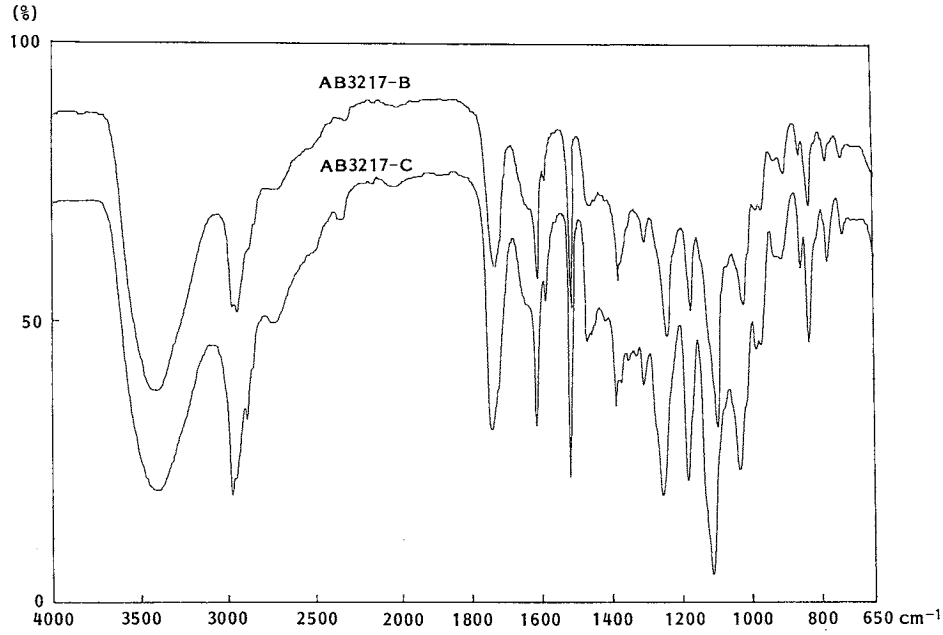


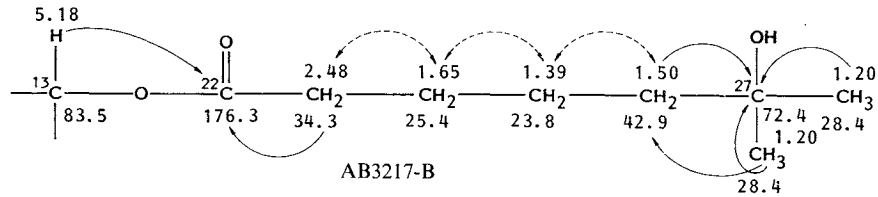
Table 1. ^1H NMR data for AB3217-A (**1**), B (**2**), C (**3**) HCl salts (400 MHz, D_2O).

Proton	Chemical shifts (δ value in ppm) and coupling constant (Hz)		
	1	**2**	**3**
1-H	5.31 s*	5.44 s	5.43 s
3-H	4.65 m	4.67 m	4.67 m
4-H	4.55 m	4.57 m	4.56 m
5-H_a	3.25 d, J=12.8	3.26 d, J=13.0	3.25 d, J=13.0
5-H_b	3.62 dd, J=4.2, 12.8	3.64 dd, J=4.0, 13.0	3.64 dd, J=4.0, 13.0
7-H	4.24 dd, J=4.2, 11.2	4.26 dd, J=4.0, 10.6	4.26 dd, J=4.0, 10.8
8-H	5.41 d, J=11.2	5.40 d, J=10.6	5.39 d, J=10.8
10-H_a	3.85 m	3.86 m	3.86 m
10-H_b	3.93 dd, J=2.4, 14.0	3.96 dd, J=2.6, 13.0	3.96 dd, J=2.6, 13.0
11-H	4.65 m	4.67 m	4.67 m
12-H	4.32 m	4.48 d, J=7.0	4.47 d, J=7.0
13-H	4.33 s	5.18 s	5.17 s
16-H	7.46 d, J=9.0	7.49 d, J=8.7	7.49 d, J=9.0
17-H	7.10 d, J=9.0	7.11 d, J=8.7	7.11 d, J=9.0
19-H	7.10 d, J=9.0	7.11 d, J=8.7	7.11 d, J=9.0
20-H	7.46 d, J=9.0	7.49 d, J=8.7	7.49 d, J=9.0
OCH_3	3.86 s	3.88 s	3.88 s
23-H		2.48 d, J=7.0	2.46 d, J=7.0
24-H		1.65 m	1.54 m
25-H		1.39 m	1.54 m
26-H		1.50 m	0.90 s
27-H			0.89 s
28-H		1.20 s	
29-H		1.20 s	

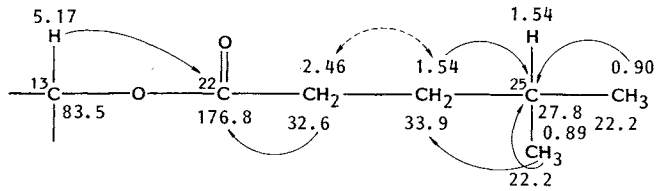
* Multiplicity.

Fig. 3. HMBC and ^1H-^1H COSY analyses for 6-methyl-6-hydroxyheptanyl moiety of AB3217-B and for 4-methylpentanyl moiety of AB3217-C.

HMBC cross peaks as solid-line arrows and COSY cross peaks as dotted-line arrows.



AB3217-B



AB3217-C

Table 2. ^{13}C NMR data for AB3217-B (**2**) and C (**3**) (100 MHz, D_2O).

Carbon	δ value in ppm	
	2	**3**
C-1	104.0 d*	104.0 d
C-3	78.4 d	78.4 d
C-4	73.3 d	73.4 d
C-5	52.5 t	52.5 t
C-7	64.8 d	64.8 d
C-8	75.2 d	75.3 d
C-10	64.2 t	64.2 t
C-11	81.1 d	81.1 d
C-12	74.7 d	74.7 d
C-13	83.5 d	83.5 d
C-15	129.9 s	129.9 s
C-16	130.3 d	130.3 d
C-17	115.5 d	115.6 d
C-18	160.7 s	160.7 s
C-19	115.5 d	115.6 d
C-20	130.3 d	130.3 d
C-22	176.3 s	176.8 s
C-23	34.3 t	32.6 t
C-24	25.4 t	33.9 t
C-25	23.8 t	27.8 t
C-26	42.9 t	22.2 t
C-27	72.4 s	22.2 s
C-28	28.4 q	—
C-29	28.4 q	—
OCH_3	56.2 q	56.2 q

* Multiplicity.

Table 3. Anti-mite activity of AB3217-A (**1**), B (**2**), C (**3**)[a].

Concentration (μg/ml)	Protection coefficient (%)[b]		
	1	**2**	**3**
100	91	100	90
10	93	93	20
1	63	67	31
0.1	32	51	27

[a] The anti-mite activity of **1**, **2**, and **3** against the two spotted spider mite (*Tetranychus urticae*) was examined by a pot test in green house. Twenty adult mites were released on leaves of kidney bean in the first stage. One day after the release, they were thoroughly sprayed with the diluted sample preparation. The number of adult mites surviving on the leaves were determined 14 days after treatment.

[b] The protection coefficient (%) was calculated from the following formula: 100 × (1-(number of the mites on treated leaves)/(number of the mites on non-treated leaves)).

spectra of **2** and **3** mentioned as above were closest to that of **1** and suggested these compounds have the same chromophore as **1**, that is, the anisole moiety.

As shown in Table 1, the ^1H NMR spectra (400 MHz, D_2O) of **2** and **3** were very similar to that of **1** measured in D_2O except for the proton signals in the high field region (about δ 1~2) and for that assigned to 13-H. The ^1H-^1H COSY, ^{13}C-^1H COSY and HMBC spectra, by which all carbons were assigned, suggested that the molecules of **2** and **3** had all of the structural features of **1**. In addition, the presence of a 6-methyl-6-hydroxyheptanyl moiety in **2** and the presence of 4-methylpentanyl moiety in **3** were indicated by these NMR spectral

analyses (Fig. 3). Comparing the ^{1}H NMR spectra of **2** and **3** with that of **1**, the 13-H signal in **2** and **3** is shifted downfield from the corresponding position in the spectrum of **1** (from δ 4.33 to δ 5.18 in the spectrum of **2** and to δ 5.17 in the spectrum of **3**, Table 1). Hence the 6-methyl-6-hydroxyheptanoic acid in **2** and the 4-methylpentanoic acid in **3** are linked to a hydroxyl group at the C-13 of **1** through an ester bond.

Thus, the structures of **2** and **3** were determined as shown in Fig. 1.

Anti-mite assay of **2** and **3** was performed by the procedure described in the previous paper[1]. As shown in Table 3, **2** and **3** showed more than 90% of the protection coefficient against the two spotted spider mite (*Tetranychus urticae*) on the kidney bean leaves at 10 ppm and 100 ppm. The protection coefficient against the mites of **2** was similar to that of **1**. Compound **3** was less active than the other two compounds.

Reference

1) KANBE, K.; Y. MIMURA, T. TAMAMURA, S. YATAGAI, Y. SATO, A. TAKAHASHI, K. SATO, H. NAGANAWA, H. NAKAMURA, T. TAKEUCHI & Y. IITAKA: AB3217-A, a novel anti-mite substance produced by a strain of *Streptomyces platensis*. J. Antibiotics 45: 458~464, 1992