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# AB-400, a New Tetraene Macrolide Isolated from *Streptomyces costae*

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In our screening program for new antifungal compounds produced by microorganisms, a new natural tetraene macrolide AB-400 has been isolated from culture broth of *Streptomyces costae*. In addition, this strain produces the known tetraene antibiotic pimaricin. Both antibiotics were found to have very similar structure and were compared by physico-chemical, spectral and microbiological methods.

The compound supressed the growth of fungi and the activity is compared with another polyene macrolide, amphotericin B, nystatin, candicidin and pimaricin. The structure of AB-400 and its *N*-acetyl derivative (Fig. 1) were determined by comparative physico-chemical and spectral analysis with pimaricin and *N*-acetylpimaricin.

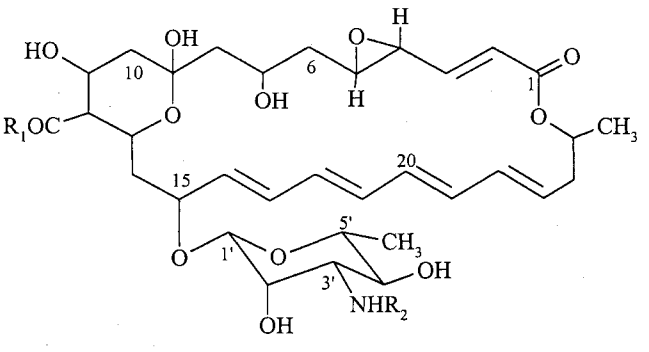
## Experimental

The microorganism was isolated from a soil sample collected in the surrounding of Madrid, Spain. Taxonomic studies indicated that it belonged to the genus *Streptomyces*.

A suspension of spores of the microorganism was inoculated into 1 liter Erlenmeyer flask containing 200 ml of a seed medium composed of glucose 5%, cotton seed meal 2%, corn steep liquor 1%, dextrin 1% and $CaCO_3$ 0.1%. The pH was adjusted to 6.2 before sterilization and incubated at 28°C for 5 days on an orbital shaking table. The thus prepared inoculum was used to seed 8-liter jar fermenter containing 4 liters of the production medium composed of soybean meal 4%, glucose 6%, soybean oil 0.5% and $CaCO_3$ 1%. The pH was adjuted to 6.2 before sterilization. The fermentation was carried out at 28°C for 90 hours, after this time the amount of the antibiotic has reached its maximum and the culture broth was harvested. The production of the antibiotic during the fermentation and the following extraction and purification processes were performed by HPLC. Spherisorb ODS-2 C18 (Waters, 10 $\mu$m) column was used for HPLC analysis. The solvent system, acetonitrile - 0.06 M citrate buffer (3 : 7, pH 4.8), was used as the mobile phase. The flow rate was 1.5 ml/minute and the monitored wavelength was 305 nm. HPLC analysis gave the following retention times (minute): 4.5 (**2**), 6.6 (**1**), 4.1 (**4**) and 6.1 (**3**).

The fermentation broth (2 liters) was adjusted to pH 8 with 5 N NaOH and it was filtrated to separate mycelium and supernatant. The micelial cake was extracted twice with 400 ml of 2-propanol, the extract was concentrated *in vacuo* to 200 ml and was diluted with 200 ml of $H_2O$. The

Fig. 1. The structures of AB-400 (**1**), pimaricin (**2**) and their *N*-acetyl derivatives (**3**) and (**4**).




| | | |
|---|---|---|
| AB-400 (**1**) | $R_1 = NH_2$ | $R_2 = H$ |
| Pimaricin (**2**) | $R_1 = OH$ | $R_2 = H$ |
| *N*-acetyl AB-400 (**3**) | $R_1 = NH_2$ | $R_2 = COCH_3$ |
| *N*-acetylpimaricin (**4**) | $R_1 = OH$ | $R_2 = COCH_3$ |

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Table 1. Comparison of $^{13}C$ NMR chemical shifts ($\delta$, ppm) for **3** and **4** (125 MHz, pyridine-$d_5$).

| Carbon No. | 3 | 4 |
|---|---|---|
| 1 | 165.2 | 165.2 |
| 2 | 125.3 | 125.2 |
| 3 | 145.2 | 145.1 |
| 4 | 54.6 | 54.5 |
| 5 | 59.2 | 59.1 |
| 6 | 41.9 | 41 8 |
| 7 | 67.6 | 67.6 |
| 8 | 47.8 | 47.7 |
| 9 | 98.3 | 98.4 |
| 10 | 39.7 | 39.6 |
| 11 | 66.6 | 66.8 |
| 12 | 59.5 | 59.4 |
| 13 | 66.3 | 66.5 |
| 14 | 37.9 | 38.3 |
| 15 | 75.4 | 75.5 |
| 16 | 134.2 | 134.0 |
| 17 | 129.1 | 129.3 |
| 18 | 131.8 | 132.0 |
| 19 | 132.1 | 132.2 |
| 20 | 132.6 | 132.5 |
| 21 | 136.4 | 136.3 |
| 22 | 137.6 | 137.8 |
| 23 | 128.8 | 128.8 |
| 24 | 45.9 | 45.7 |
| 25 | 70.1 | 70.0 |
| 26 | 20.4 | 20.3 |
| 27 | 176.7 | 176.8 |
| 1′ | 98.0 | 98.1 |
| 2′ | 71.0 | 71.0 |
| 3′ | 56.4 | 56.4 |
| 4′ | 72.6 | 72.7 |
| 5′ | 74.8 | 74.6 |
| 6′ | 18.9 | 18.5 |
| 3′-NHCOCH$_3$ | | |
| $CH_3CO$ | 171.4 | 171.2 |
| $CH_3CO$ | 23.2 | 23.1 |

Assignments to the numbering system shown in Fig. 1.

resultant aqueous alcohol extract was adjusted to pH 10 with NaOH and it was stood one overnight at −5°C. The precipitate was filtered and washed with water to yield 1.2 g of AB-400 crude powder. Pimaricin was concentrated in the supernatant of that precipitation. The crude of AB-400 was chromatographed on a silicagel column packed with $CHCl_3$. The column after washing with $CHCl_3$-MeOH 1 : 1 was eluted with MeOH. The eluate was monitored by HPLC and the fractions containing AB-400 as a peak by HPLC were collected and concentrated to dryness in the dark to yield 125 mg of fine yellow powder. To enhance the solubility of the antibiotic, AB-400 was derivated like *N*-acetyl derivative and purified by FLC on silica gel using $CHCl_3$-MeOH 75 : 25 as eluting solvent.

## Results and Discussion

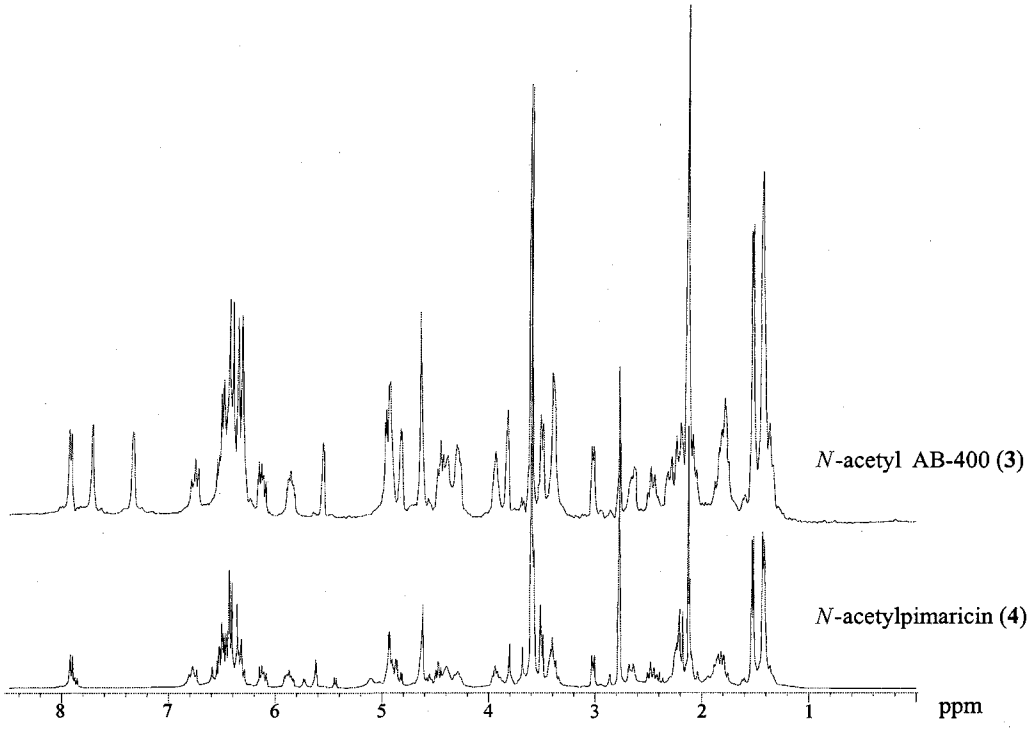
AB-400 and pimaricin were transformed into their *N*-acetyl derivatives (**3** and **4**, Fig. 1) by the reported method[1], which facilited the purification process. The used pimaricin was a comercially available authentic sample from Sigma Chem. Com. Pimaricin (**2**) and its *N*-acetyl derivative (**4**) were identificated by comparison of their NMR and Mass spectra with data given as reference[2~5]. AB-400 (**1**) and *N*-acetylAB-400 (**3**), exhibited the physico-chemical properties similar to pimaricin (**2**) and *N*-acetylpimaricin (**4**), these components are distinguished from each other by their HPLC mobilities. The UV spectrum of **1** showed characteristic polyene-type absortion very similar to that of **2** with maxima at 290, 304, and 318 nm corresponding to an all-*trans*-tetraene moiety[6]. The IR spectrum of **1**, $\nu_{max}$ (KBr) $cm^{-1}$: 3448, 1068 indicated the presence of hydroxyl group, a lactone unit at 1721, double bonds at 1630, there is no band at 1579 of an ionized carboxyl unit and it is shown a new band at 1655 of an amide group. The molecular formula of **1** $C_{33}H_{48}N_2O_{12}$ was determined by HRFAB-MS experiment, which gave a $(M+H)^+$ ion at *m/z* 665.3280 (calcd. *m/z* 665.3286 for $C_{33}H_{49}N_2O_{12}$)

The molecular formula of **3** was determined to be $C_{35}H_{50}N_2O_{13}$ from the FAB-MS peaks at *m/z* 706.9 $(M+H)^+$ and *m/z* 705.3 $(M-H)^-$ and *m/z* 687.5 $(M-H-H_2O)^-$ and from HRFAB-MS [found *m/z* 705.3225 $(M-H)^-$, calcd. *m/z* 705.3235 for $C_{35}H_{49}N_2O_{13}$], which was supported by the number of carbons in the $^{13}C$ NMR spectrum (Table 1). The molecular formula of **4** was determined to be $C_{35}H_{49}NO_{14}$ from the FAB-MS peaks at *m/z* 707.9 $(M+H)^+$ and *m/z* 706.2 $(M-H)^-$ and *m/z* 688.4 $(M-H-H_2O)^-$ and from HRFAB-MS [found *m/z* 706.3094 $(M-H)^-$, calcd. *m/z* 706.3075 for $C_{35}H_{48}NO_{14}$]. The molecular weight of both compounds differs in one mass unit. The molecular formula of **3** shows one nitrogen and one hydrogen plus than **4** and one oxygen less than **4**.

The $^{13}C$ NMR spectra of **3** and **4** recorded in pyridine-$d_5$ solution are virtually superimposable (Table 1). HMQC experiments allowed the specific $^{13}C$ assignments and multiplicities were determined by DEPT experiments. Assignments in $^{13}C$ NMR chemical shifts of **3** and **4** are compared in Table 1.

The $^1H$ NMR spectra of **3** and **4** were almost identical when the spectra were recorded in pyridine-$d_5$. However,

Fig. 2. $^1$H NMR spectra of **3** and **4** in DMSO-$d_6$ (500 MHz).




when the spectra were recorded in DMSO-$d_6$, a striking difference can be noticed in the behavior at 7~8 ppm (Fig. 2). The $^1$H NMR of **3** shows signals of amide group, two broad singlets at 7.28 and 7.68 ppm which disappear by adding NaOD. In the $^1$H NMR spectrum of **4** no signals of the amide protons could be detected (Fig. 2). The proton correlations from $^1$H-$^1$H COSY support the structure of **3** and were very similar to those observed on the $^1$H-$^1$H COSY of **4**, the only difference was that the COSY of **3** showed that the protons at 7.28 and 7.68 ppm were coupled. The structure assignment of **3** is consistent with a compound with amide group in place of the corresponding carboxylic group in **4** as shown in Fig. 1. This assignment was confirmed by methylation experiments of **3** and **4**, the methyl esters were prepared by the reported method[7]. The compound **4** was treated with $CH_2N_2$ in THF and the methyl ester was obtained which gave a clear molecular ion $(M-H)^-$ at *m/z* 720.3. In the case of **3**, the methyl ester was not yielded in the same conditions. For all of these findings, the total planar structure of **1** was established as shown in Fig. 1.

## Biological Activity

*In vitro* antifungal activity of AB-400 and *N*-acetyl AB-400 was tested against *Candida albicans* and *Candida tropicalis*, in comparison with other polyene antibiotics such as nystatin, amphotericin B, candicidin and pimaricin. The results obtained with the antibiotics in the serial dilution test[8] are compared in Table 2. AB-400 was active against the organisms tested with potency comparable to those of other tetraene antibiotics such as nystatin and pimaricin. Acylation of AB-400 drastically reduces the activity.

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Table 2. Antifungal activity of AB-400, *N*-acetyl AB-400 and other polyene macrolides (MIC, $\mu$M).

| STRAIN | AB-400 | *N*-acetyl AB-400 | AMPH-B | CAND | NYS | PIM |
|---|---|---|---|---|---|---|
| *Candida albicans* | 18.0 | >75 | 0.86 | 0.21 | 3.3 | 18.0 |
| *Candida tropicalis* | 4.6 | >75 | 1.7 | 0.43 | 3.3 | 9.3 |

AMPH-B: amphotericin B, CAND: candicidin, PIM: pimaricin, NYS: nystatin

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