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NOTE

A New HPLC Condition for the Analysis of Aclacinomycins A and Y in the Mixtures of Aclacinomycins

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As the previous HPLC method (Ogasawara *et al.*, 1983) was described for the analysis of aclacinomycin A and some of its analogue compounds but not for aclacinomycin Y, we developed novel HPLC condition by optimizing solvent system. The newly developed solvent system allowed a complete separation of aclacinomycins Y and A, as opposed to the incomplete resolution of these two compounds in conventional method. The amounts of aclacinomycins Y and A could be accurately determined when the fermentation broth of *Streptomyces lavendofoliae* DKRS was analyzed by the newly developed method.

Aclacinomycins, anthracycline antibiotics (4), were produced as a mixture of aclacinomycin A and its analogue compounds in the fermentation broths of *Streptomyces* species (7, 9, 10). Aclacinomycin A has been analyzed by the HPLC method of Ogasawara *et al.* (6). Although this method could resolve aclacinomycin A from many other aclacinomycins, it was difficult to measure accurately the amount of either aclacinomycins A or Y when aclacinomycin Y was coproduced with aclacinomycin A due to their similar retention time in HPLC analysis (1, 2, 3). We studied the production of aclacinomycin A by fermentation with *Streptomyces lavendofoliae* (*S. lavendofoliae*) DKRS, a mutant strain derived from *S. lavendofoliae* ATCC15872 (See accompanying paper). This strain produced considerable amount of aclacinomycin Y as well as aclacinomycin A during fermentation. It was necessary to have an accurate analytical method for exact quantification of both compounds in order to monitor fermentation and purification processes. Therefore, we modified the solvent system of HPLC to optimize the separation method of aclacinomycins A and Y.

S. lavendofoliae DKRS was cultured in a medium consisting of potato starch 4.0%, soybean meal 1.5%, NaCl 0.1%, $CaCO_3$ 0.3%, $MgSO_4 \cdot 7H_2O$ 0.05%, $FeSO_4 \cdot 7H_2O$ 0.001%, $CuSO_4$ 0.001% and K_2HPO_4 0.1%. The fermentation was carried out using 3 liters of medium in a 5 liter jar fermentor (B. Braun) at 28°C for 144 hours with aeration rate of 0.5 liter per min. Two 10 ml of samples of the fermentation broth were sampled for HPLC analysis by conventional method and by newly developed method. The culture broth was centrifuged at 5,000 rpm for 10 min. The mycelium cake obtained was extracted with 10 ml of acetone with intensive shaking for 1 hour and centrifuged again. The supernatant was mixed with 4 ml of chloroform and the organic layer was analyzed by HPLC(Beckman Gold). For reference standards, aclacinomycin A was purchased from Sigma and aclacinomycin Y which was not commercially available was prepared in-house and characterized to confirm its identity. 20 μl of sample was injected on a silica column (Toyosoda Silica-60, 5 μm, 4.6×25) and the column was eluted at a flow rate of 1.0 ml/min. Elution was monitored at 436 nm.

Eluting solvent system of two different compositions was tested. First, the solvent system of chloroform-methanol-acetic acid-water-triethylamine (68 : 20 : 10 : 2 : 0.01) of the conventional method was used. As shown in the HPLC chromatogram of the sample prepared from the culture broth (Fig. 1), the peak eluted at 11.90 min contained aclacinomycin A as judged by the same retention time of the authentic aclacinomycin A. However, it was noted that an analogue, aclacinomycin Y was also eluted exactly at the same retention time when analyzed under the same running condition. This seems to be due to the fact that a-

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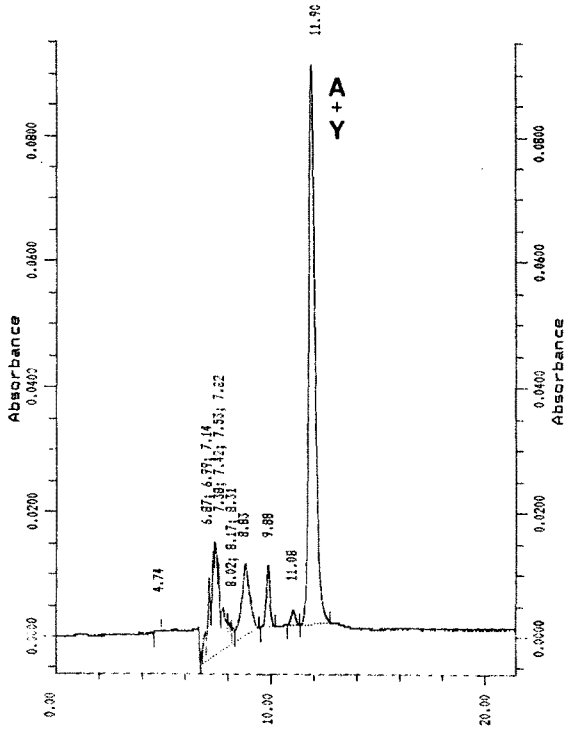

Fig. 1. HPLC analysis of fermentation broth by the previously published method (6).
Solvent system of chloroform-methanol-acetic acid-water-trietylamine (68 : 20 : 10 : 20 : 0.01) of the conventional method was used.

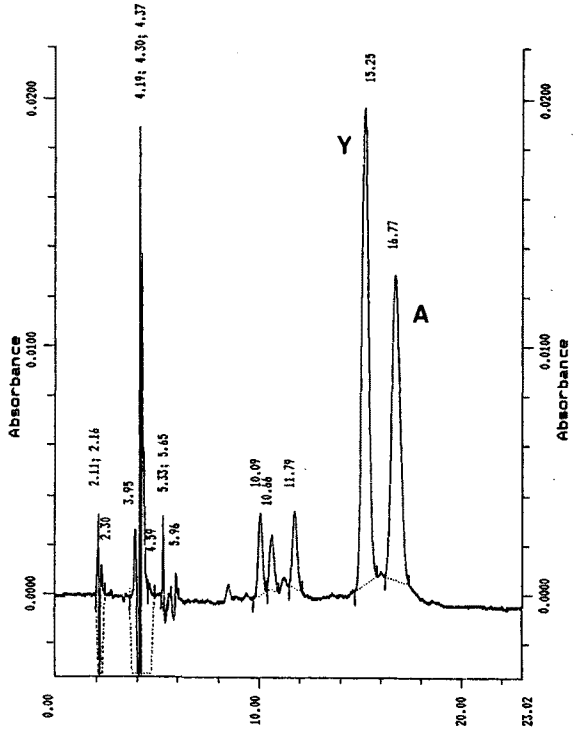

Fig. 2. HPLC analysis of fermentation broth by the newly developed method.
Solvent system of toluene-ethyl acetate-benzene-methanol-formic acid-water-triethylamine (20 : 20 : 20 : 5 : 3 : 0.5 : 0.3) was used.

clacinomycin Y shows structural similarity to aclacinomycin A more than other analogues do. It is known that aclacinomycins A and Y show very similar Rf values on a TLC plate owing to their structural similarities (5). When a new solvent system of toluene-ethyl acetate-benzene-methanol-formic acid-water-triethylamine (20 : 20 : 20 : 5 : 3 : 0.5 : 0.3) was used for the analysis of the same sample, the aclacinomycins A peak which was eluted at 11.90 min in Fig. 1 was separated into two peaks which were eluted at 15.25 min and 16.77 min (Fig. 2). By running authentic samples, these two peaks were confirmed as aclacinomycin Y (15.25 min) and aclacinomycin A (16.77 min).

In order to confirm the accuracy of the analysis of aclacinomycins A and Y in their mixture, a series of dilutions of the two samples were made and analyzed for both aclacinomycins by the newly developed method. As shown in Fig. 3, the plots of concentration versus peak area showed linear relationships over the concentration range of 1-30 mg/l of aclacinomycins. Thus, the new solvent system is well suited for the HPLC analysis of a mixture of different forms of aclacinomycins, especially when aclacinomycins A and Y coexist in the sample.

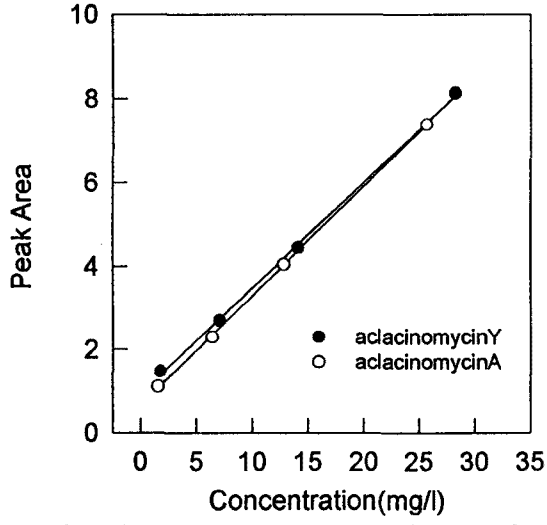

Fig. 3. Plot of concentration versus peak area of aclacinomycins A and Y in fermentation broth sample.
A series of different concentrations of sample was analyzed by HPLC developed in this study.

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