IDENTIFICATION OF COMPONENTS IN THE BLEOMYCIN COMPLEX BY DIRECT TLC/FABMS

Sir:

A fast method is described for obtaining mass spectral identifications of components of the bleomycin complex by a new thin-layer chromatography/fast atom bombardment mass spectrometry (TLC/FABMS) procedure. This method provides rapid analyses of antibiotics in mixtures and the bleomycins represent a difficult example since they are very polar, high molecular weight and thermally unstable compounds.

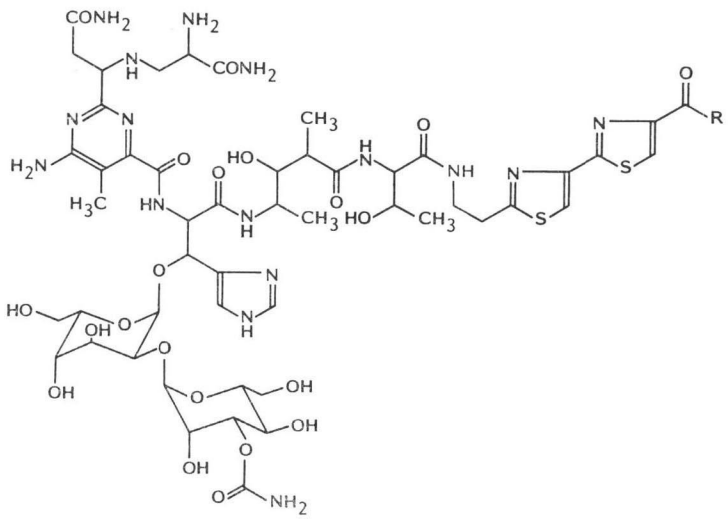
The bleomycins are a family of glycopeptide antibiotics produced by *Streptomyces verticillus*[1,2]. Bleomycins are effective against various human neoplasms, particularly against squamous cell carcinoma, sarcoma, and malignant lymphoma[3~5]. Bleomycin complex used was a product of Bristol Laboratories, sold under the trade name of Blenoxane. The major components are bleomycins A_2 (**1**) and B_2 (**2**).

Field desorption mass spectrometry (FDMS) and FABMS have been demonstrated to be useful in the identification and structure determination of bleomycin-type antibiotics[6~8]. Recently, CHANG *et al.* described in detail the procedure for the direct examination of TLC spots by FABMS[6], a new technique which showed fast analysis for mixtures. Application of this new technique to bleomycins is discussed in this paper.

The TLC system for this study of the bleomycin complex was methanol and 10% ammonium acetate in 1:1 ratio with plastic-backed TLC sheets, IB2-F Bakerflex, from the J.T. Baker Chemical Co., Phillipsburg, N.J. Prior to spotting the sample, all TLC plates were cleaned by solvent development and drying. A 10 μg/μl solution was prepared dissolving 1 mg of Blenoxane in 100 μl of 1:1 H_2O - MeOH solution. Most analysis were based on 10 μg or 20 μg spotting.

The FAB probe tip was covered with double-faced-masking tape and pressed against the TLC spot to transfer the adsorbent. After addition of 1~2 μl of solvent and 2~5 μl of FAB matrix liquid, thioglycerine, to the adsorbent, the probe was introduced into the mass spectrometer in the usual manner.

A Kratos MS-50 high resolution mass spectrometer was used in these studies. The instrument was equipped with a FAB source and gun supplied by M-Scan, Ltd., Ascot, Berkshire, England. The FAB probe tip was made of copper with a 2 mm × 7 mm cross section and xenon was used as the gas for the FAB gun.



1 R = $NHCH_2CH_2CH_2\overset{+}{S}(CH_3)_2$

2 R = $NH(CH_2)_4NHCNH_2$ (NH)

3 R = $NHCH_2CH_2CH_2SCH_3$

Fig. 1. Fast atom bombardment mass spectrometry of Blenoxane.

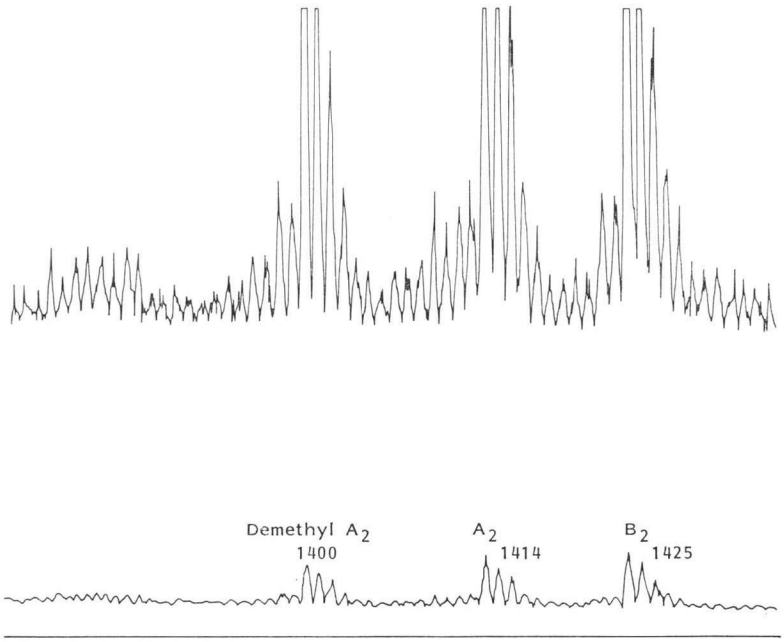
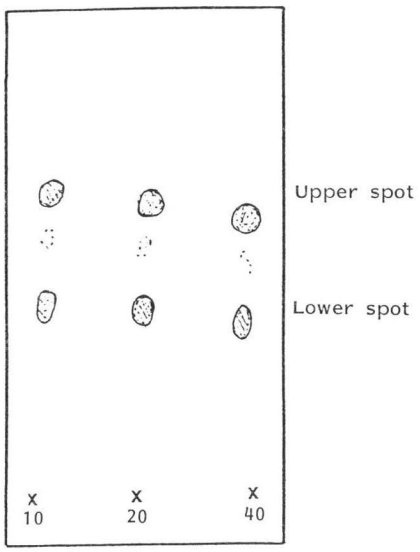


Fig. 2. TLC separation of Blenoxane.

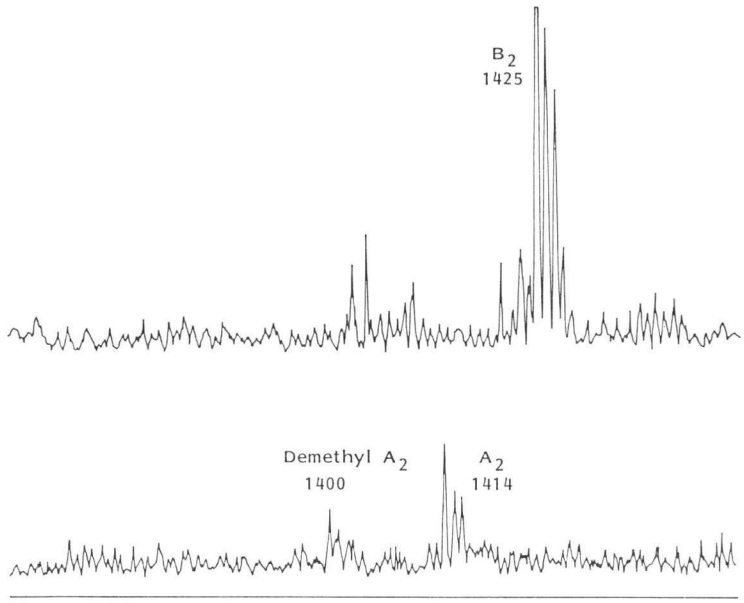


Mass spectrometric investigation of bleomycins requires considerable attention and experience. Bleomycins produced weak and short lived signals, usually lasting less than a minute, during FD and FAB examination. Therefore, it is preferable to make several repetitive narrow mass range scans on the molecular weight region than attempt to obtain a full mass range scan.

Blenoxane produced a positive FABMS consisting of three major ions, *m/z* 1,400, 1,414 and 1,425 assigned to MH^+ of demethylbleomycin A_2 (**3**), M^+ of bleomycin A_2 (**1**) and MH^+ of bleomycin B_2 (**2**), respectively (Fig. 1). The rather intense signal of demethylbleomycin A_2 has been attributed to an artifact due to demethylation of bleomycin A_2 under FAB conditions[8]. Negative FAB examination of bleomycins was unsuccessful. Only the positive FAB, therefore, was employed for the following studies.

The bleomycin components are easily separated with the described TLC system and detected by UV quenching. As shown in Fig. 2, Blenoxane is composed of two major components. The TLC/FABMS results of these two components are shown in Fig. 3. The FABMS of the upper TLC spot shows the MH^+ ion of bleomycin B_2 at *m/z* 1,425. The FABMS of the lower TLC spot produces two major ions, *m/z* 1,400 and 1,414, corresponding to the MH^+ ion of demethylbleomycin A_2 and M^+ of bleomycin A_2, respectively. The fact that both *m/z* 1,400 and 1,414 appeared on the same TLC spot, again confirms the demethylation of A_2 under the FAB conditions. This spot was identified as bleo-

Fig. 3. Fast atom bombardment mass spectrometry of TLC spots from the resolution of Blenoxane.



mycin A_2.

These findings are comparable to the analysis of bleomycins collected after HPLC separation[10,11]. Nevertheless, the direct TLC/FABMS procedure is a much simpler, more economical, and less laborious technique. It has, therefore, proven to be an efficient and useful confirmation technique for bleomycins.

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