Fellow (1984-1985). K.P.C.V. is a Miller Research Professor in Residence (1985-1986).

Supplementary Material Available: Complete yield, melting point, and spectral and/or analytical data of all new compounds reported (11 pages). Ordering information is given on any current masthead page.

Identification and Structure Assignment of Components of Leucinostatin and CC-1014 by Directly Coupled Liquid Chromatography/Fast Atom Bombardment Mass Spectrometry[1]

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We recently described the coupling of liquid chromatography (LC) with fast atom bombardment mass spectrometry (FABMS) via a moving belt interface.[3] While successful in dealing with peptides to molecular weight 1900, the interface showed considerable breakup in the total ion current (TIC) trace, so that separations were difficult to follow and the limit of the spectrometer's sensitivity was nearly reached. In that version of the LC/FAB interface, the sample was deposited from a column onto the moving belt via a jet spray.[4] We have now modified our interface to include a frit in place of the jet at the end of the liquid chromatograph, and we now use microbore (1-mm i.d.) columns at flow rates up to 100 μL/min. With these modifications, component peak shapes resemble much more closely those from the LC/UV detector and sensitivity is no longer a problem. Additional improvement in the peak shape can be effected by measuring the TIC only above a preset mass, which eliminates most of the background ions arising from the belt. One advantage of coupled LC/FAB over isolation of components from the liquid chromatograph and subsequent analysis by FABMS is that no matrix need be included with the effluent (since the moving belt presents the atom beam with a continuously changing surface) and no matrix peaks are observed. We describe here the use of LC/FAB in the identification of a number of new components of the peptide antibiotics leucinostatin and CC-1014.

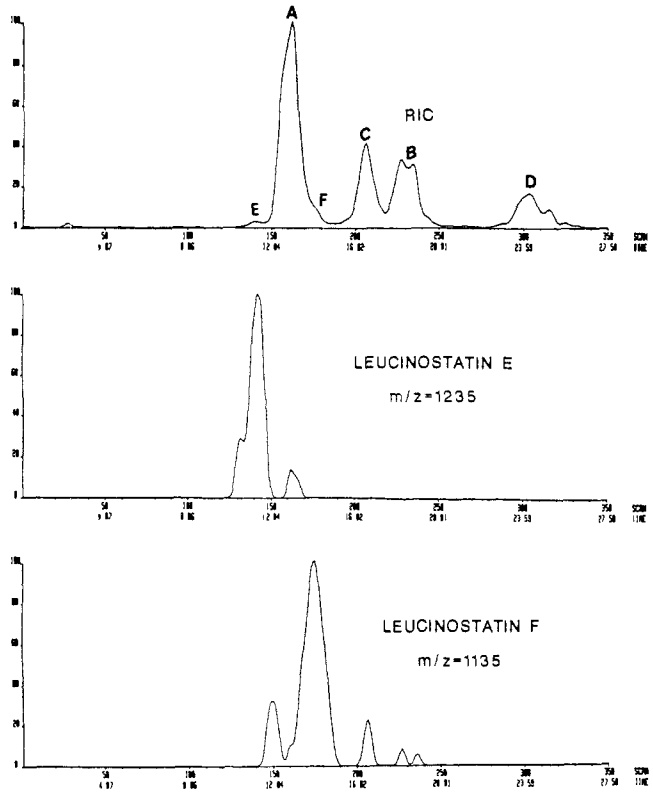
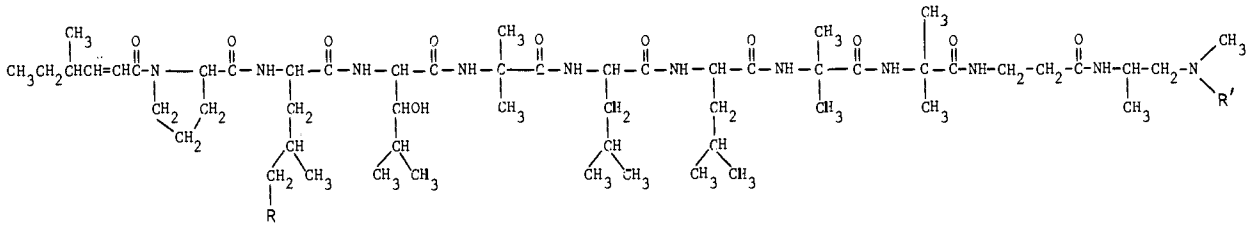


Figure 1. Top trace: limited reconstructed ion chromatogram of leucinostatin mixture. Solvent conditions: methanol:2-propanol:water: acetonitrile:carbon dioxide/diethylamine, 40:30:20:10:0.1. Flow rate: 100 μL/min, 1-mm i.d. C_{18} column, 5 μg injected. Middle trace: single-ion trace of m/z 1235 (leucinostatin E). Bottom trace: single-ion trace of m/z 1135 (leucinostatin F).

The structures of leucinostatins A and B were recently assigned by Fukushima et al.,[5] as shown in Scheme I. Our studies carried out on very limited samples of CC-1014[6,7] suggested that CC-1014 might be very closely related to or perhaps identical with leucinostatin A, and the latter appears to be identical with P168[8] and 1907.[9] Consequently, the two antibiotics were reinvestigated by LC/FABMS and the TIC trace of leucinostatin is shown in Figure 1. Components A and B were identified as leucinostatins A and B, respectively, from their FAB spectra, that of leucinostatin A from the LC/FAB experiment being shown in Figure 2. The remaining components of the leucinostatin mixture, however, had not been previously reported. They were identified as the new leucinostatins C and D and assigned the structures shown in Scheme I on the basis of their FAB spectra. Two more components, leucinostatins E and F, were observed in such low abundance that only molecular weight information was obtained and the

Scheme I



Leucinostatin A:[5] R = $CHOHCH_2COCH_2CH_3$; R' = CH_3
B:[5] R = $CHOHCH_2COCH_2CH_3$; R' = H
C: R = H; R' = CH_3
D: R = H; R' = H

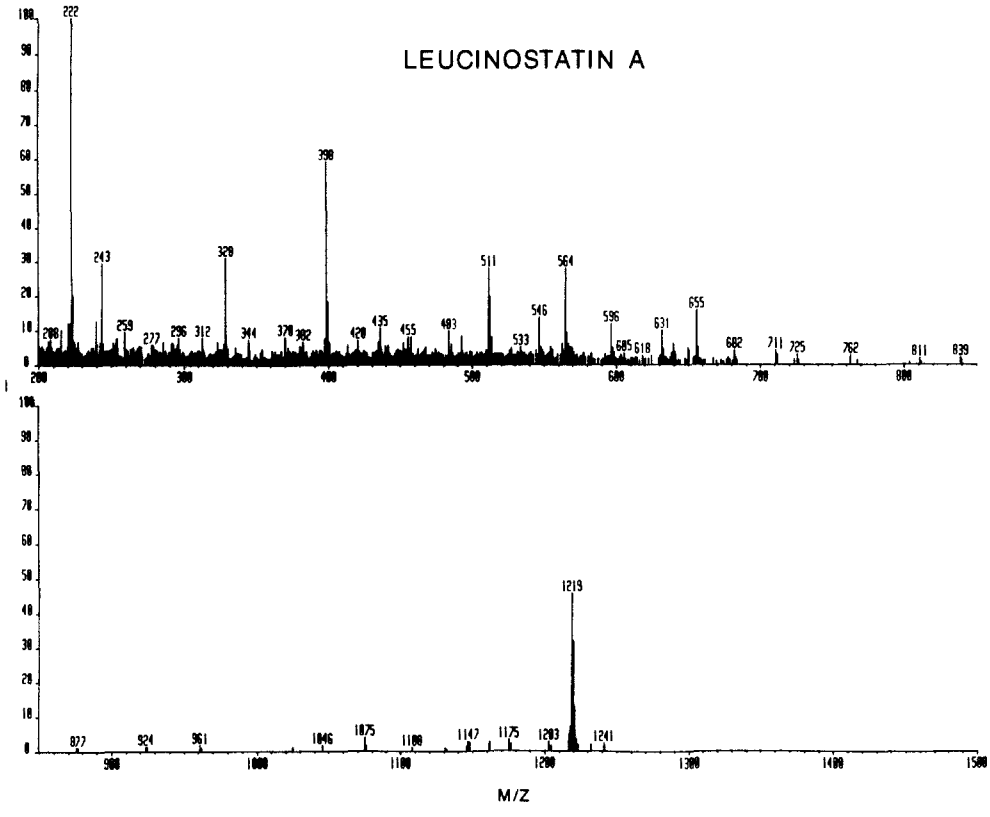


Figure 2. LC/FAB mass spectrum of leucinostatin A. The computer-generated ion at 1219 (M + H, accurate mass) corresponds to a nominal mass of 1218 (M + H).

components were located by single-ion computer-reconstructed plots (Figure 1). Leucinostatin E corresponds to leucinostatin A with an extra oxygen and leucinostatin F to leucinostatin C with another oxygen.

The antibiotic CC-1014 has been obtained in pure form and its normal FAB spectrum is identical with that of leucinostatin A. As further confirmation of the identity of the antibiotics, CC-1014 was coinjected with leucinostatin, whereupon leucinostatin A and CC-1014 coeluted. In addition, CC-1014B[6] eluted with leucinostatin E, though the latter very small peak was broad.

The coupling of a liquid chromatograph to a fast atom bombardment mass spectrometer holds great promise for dealing with mixtures of peptides as exemplified by the present report. We are currently testing other classes of compounds with the technique.

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