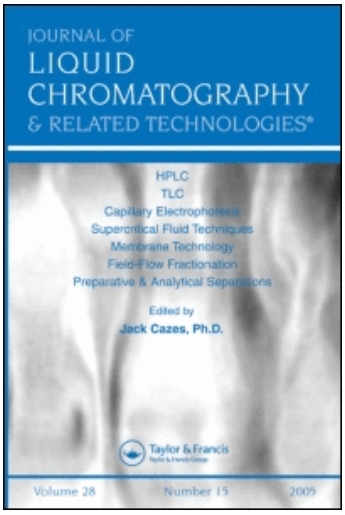

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF BRYOSTATINS 1-12

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ABSTRACT

A reversed-phase high performance liquid chromatographic (RP-8; acetonitrile-water gradient) separation procedure was developed for detecting bryostatins 1-12, using a photodiode array detector system. While bryostatins 6 and 9 were found to co-elute they were easily separated using a silica gel column with 9:1 n-hexane-n-propanol as eluent.

INTRODUCTION

The very promising antineoplastic,[1] antitumor promoting,[2-4] immunomodulating[5,6] and normal bone marrow growth stimulating [7]

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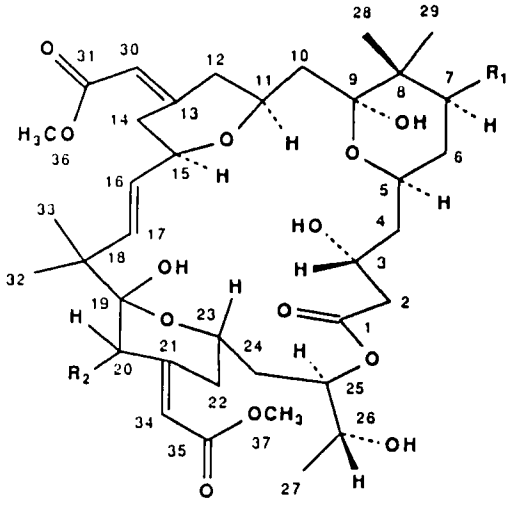

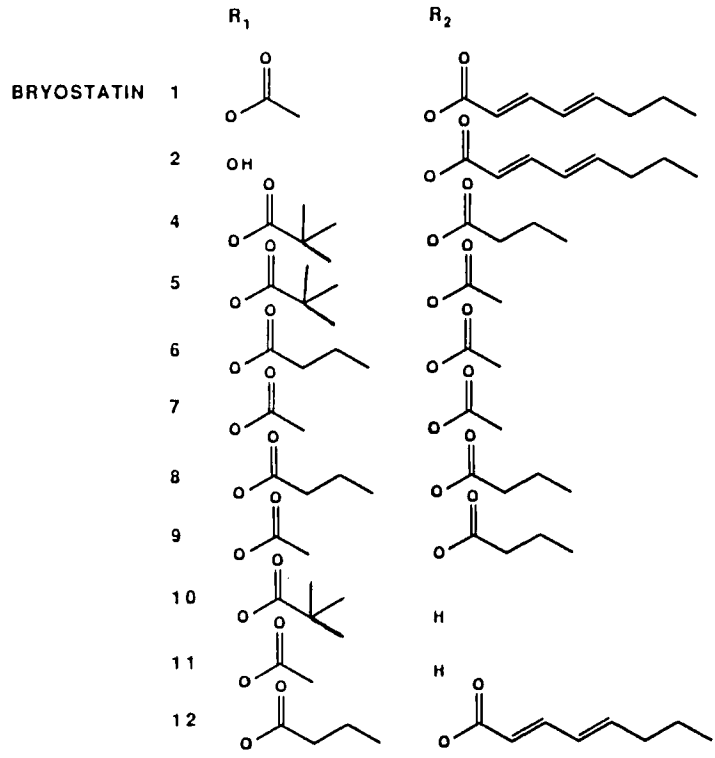

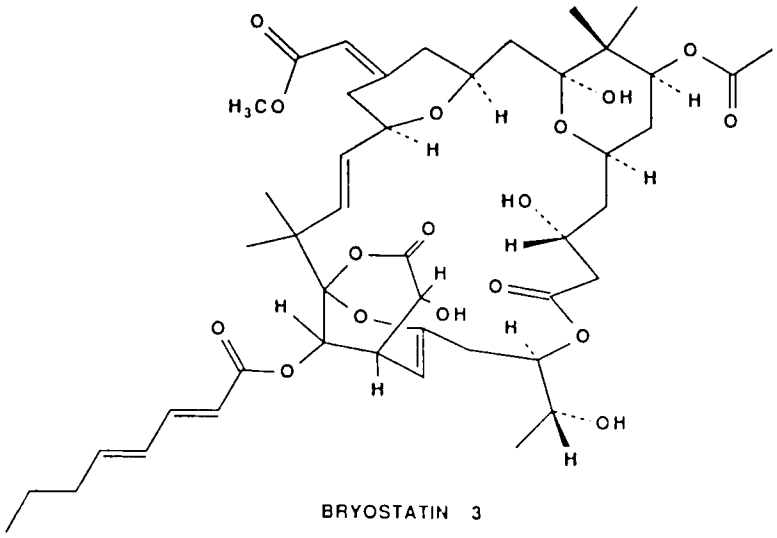

BRYOSTATIN 3

properties of the bryostatins (1) from the marine bryozoa Bugula neritina[8] has encouraged extensive preclinical studies directed at eventual clinical trials. In turn these efforts and further research aimed at discovery of new members of the series has required the development of analytical methods for their rapid separation and recognition. In the following report we describe a fast and selective HPLC separation of pure bryostatins 1-12, using a photodiode array detector (HPLC-UV/vis).[9,10]

RESULTS AND DISCUSSION

The reversed phase and normal phase silica gel separations originally developed for separation of bryostatins 4-8[11] was further simplified. The Eastern Pacific Bugula neritina contains primarily bryostatins 1-3 and 12 and Fig. 1a shows the RP-HPLC separation

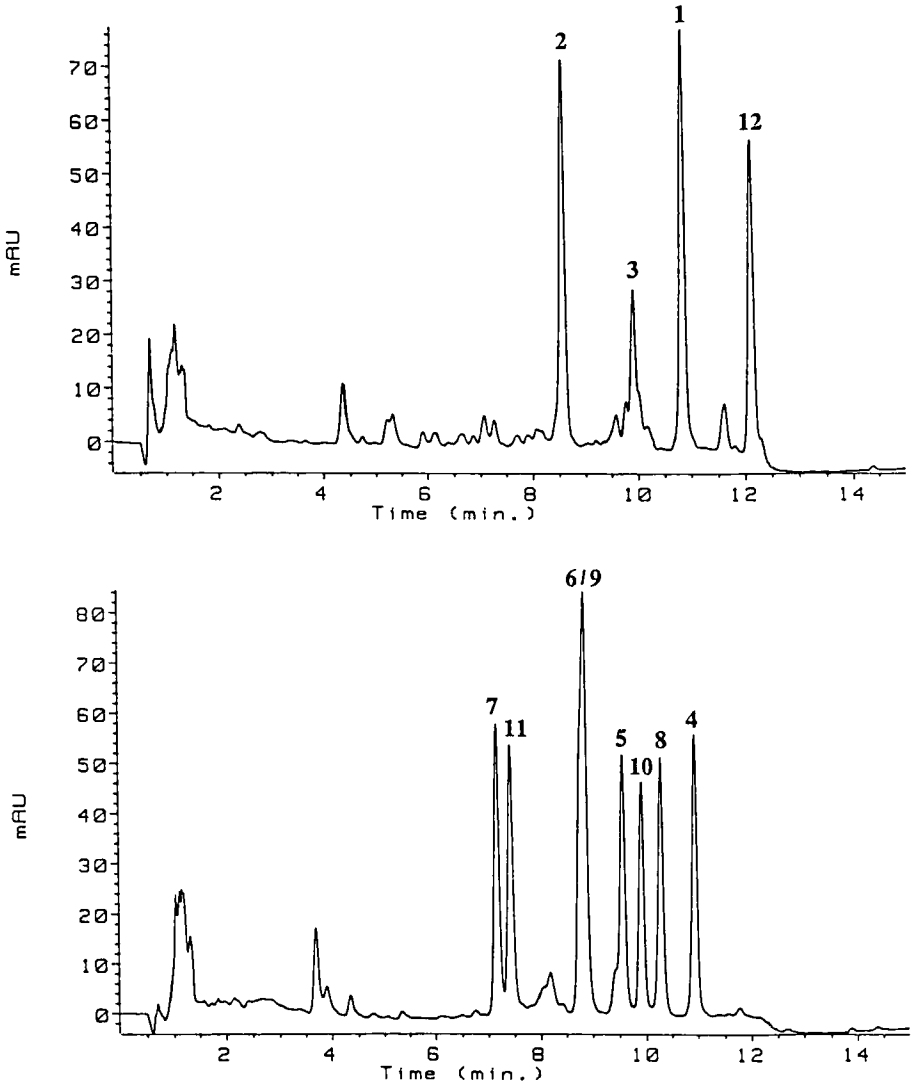

Figure 1 HPLC-UV/vis of bryostatins 1-3,12 (a) and and 4-11 (b); conditions: RP-8 (3 μm, 100 x 4.6 mm I.D.); 1:1 water-acetonitrile to pure acetonitrile in 15 min; 1 ml/min; 230 nm; spectra see Fig. 2.

realized here. A complete separation of bryostatins 1-3 and 12 was achieved on a short analytical column in less than 15 minutes, using linear gradient elution with acetonitrile-water. The increasing length of the C-7 side chain for bryostatins 2 (free hydroxyl), 1 (acetyl) and 12 (butyl) resulted in increasing retention times. The UV spectra of bryostatins 1-3 and 12 were recorded on-line with a photodiode array detector. The UV spectra were identical and displayed absorption maxima at 265 and 235 nm (Fig. 2).

Next, a complex mixture of bryostatins 4-11 (from B. neritina collected in the Gulfs of California and Mexico)[11] differing only in their C-7 and -20 substituents was separated under identical HPLC conditions. Except for bryostatins 6 and 9, they were conveniently separated by reversed phase HPLC using acetonitrile-water as illustrated by Fig. 1b. The UV spectra of bryostatins 4-11 were all characterized by a single absorption maximum at 229 nm (Fig. 2). The two bryostatin structural isomers 6 and 9 co-eluted under these experimental conditions. Attempts to separate the two isomers employing different systems such as RP-18 silica with a methanol-water gradient were not successful. But bryostatins 6 and 9 were found to easily separate on silica gel using n-hexane-n-propanol (9:1) as eluent (Fig. 3).

As noted above, HPLC separations were monitored with a photodiode array detector which allowed the on-line recording of UV spectra as each bryostatin separated. The conjugated octadienate side-chains of bryostatins 1-3 and 12 were responsible for the observed maxima at 265 and 235 nm. Bryostatins 4-11 lack the

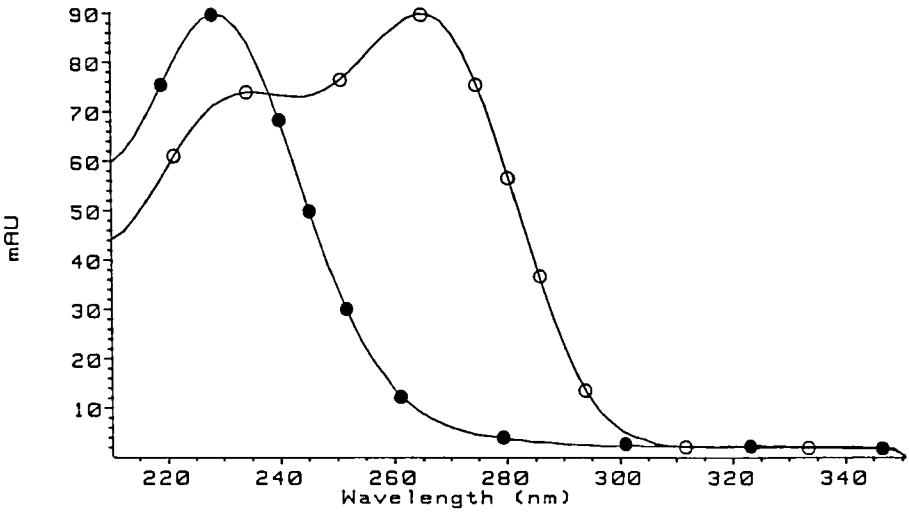

Figure 2 UV spectra of bryostatins 1-3,12 (—o—) and bryostatins 4-11 (—●—) recorded in acetonitrile-water (Photodiode array detector).

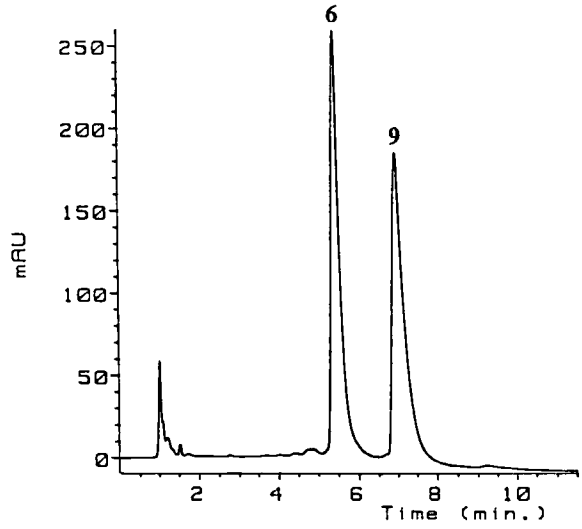

Figure 3 HPLC-UV/vis of isomeric bryostatins 6 and 9; conditions: silica gel (3 µm, 100 x 4.6 mm I.D.); n-hexane-n-propanol (9:1); 1 ml/min; 230 nm.

octadienate ester and displayed absorption maxima near 229 nm. As both classes of bryostatins showed overlapping retention times, photodiode array detection proved to be a method of choice for differentiating between bryostatins 1-3 and 12 and bryostatins 4-11.

EXPERIMENTAL

Solvents and Instruments.

HPLC grade solvents (Omnisolv) were purchased from EM Science. Freshly distilled water was purified over a Norganic cartridge (Millipore) and filtered (0.45 μm). Helium was used for solvent degassing. Solvents were delivered by two Gilson Model 302 pumps controlled by an Apple IIe programmer through a Rheodyne 7161 injector and a 0.5 μm in-line precolumn filter (Rainin). Chromatograms and UV spectra were recorded with a HP 1040A photodiode array detector equipped with a HP 79994A work station (Hewlett-Packard).

Natural Products and Chromatographic Conditions.

Bryostatins 1-12 were isolated from Bugula neritina as earlier described.[1,8,9] Samples (approx. 0.1 mg/1 ml) were dissolved in methanol (RP separations) or 1:1 n-hexane-n-propanol (normal phase separations) and the injection size was 10 μl. Reversed phase separations were performed with a RP-8 3 μm Ultremex column (100 x 4.6 mm I.D.; Phenomenex, Rancho Palos Verde Ca.) using a linear gradient of 1:1 aqueous acetonitrile to pure acetonitrile in 15 min at a flow rate of 1 ml/min. Normal phase separations were achieved on a silica gel 3 μm Ultremex columnn (100 x 4.6 mm I.D.; Phenomenex) employing

isocratic elution with 9:1 n-hexane-n-propanol (a flow rate of 1 ml/min). Chromatograms were recorded at 230 nm (± 2 nm) and spectra measured over a 210-350 nm range.

ACKNOWLEDGMENTS

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