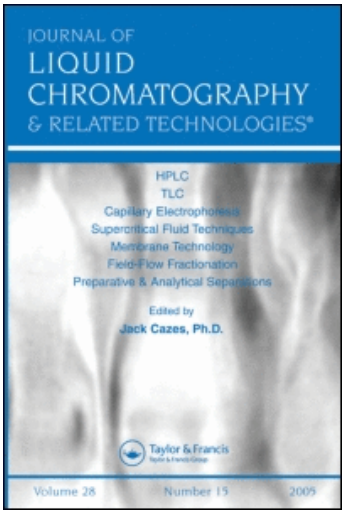

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Separation and Identification of Labile Polyacetylenes by RP-HPLC

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SEPARATION AND IDENTIFICATION OF LABILE POLYACETYLENES BY RP-HPLC

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ABSTRACT

Traditional methods of isolation and identification of labile polyacetylenes often lead to substantial loss of material. Reverse-phase HPLC coupled with a diode array detector offers a fast and convenient way to isolate and identify polyacetylenes from plant sources. Minimizing the number of manipulations reduces the potential for losses, and makes a more accurate quantitative analysis of the polyacetylenes possible.

INTRODUCTION

Although a multitude of polyacetylenes have been isolated from a variety of plant sources(1), the vast majority are found in the Asteracea family(1). Historically, these compounds have been isolated by a combination of column and thin layer chromatography on silica or alumina using a variety of solvent

systems. Often repetitive procedures are used to sufficiently purify the compounds for spectroscopic examination. Due to the labile nature of polyacetylenes(2), many of which are sensitive to heat, light, oxygen and concentration factors, especially in the crystalline form, any repetitive procedure or handling technique increases the risk of losing a portion of the material if not all of the more labile substances. For example, compound 11 polymerizes to a black amorphous mass when crystallized and stored at sub-zero temperatures.

Our work on the allelopathic nature of certain noxious weeds from the genus Centaurea(3), and more recently on the chemotaxonomy of species from the thistle family, led us to investigate the polyacetylene content of a number of plant species. Different plant parts(roots, flowers, etc.) from several different species were surveyed for polyacetylenes. Traditional methodology, which often results in substantial degradation of many polyacetylenes, necessitated the need to find a more rapid, direct and accurate method of isolation and identification of these compounds. Previous work by Towers et al(4) and McLachlan et al(5) demonstrated the potential of reverse phase liquid chromatography for the separation of polyacetylenes. However, identification of the individual components still required the isolation of the polyacetylene from the eluting solvent followed by a spectroscopic investigation. These manipulations often result in substantial degradation of the desired polyacetylenes.

EXPERIMENTAL

Materials

The chemicals used were as purchased. Methylene chloride and methanol (HPLC grade) were obtained from Mallinckrodt. Water was deionized and used without further treatment. All solutions were degassed and filtered through 0.22μm Millipore filters prior to use.

Instrumentation

The HPLC analyses were run isocratically using a Waters HPLC system with a Waters reverse phase C-18 u-BondaPac semi-prep column(7.8 mm x 30 cm). The column was operated at ambient temperature. Detection and identification of the eluting components was accomplished with a Hewlett-Packard Diode-Array detector (model 1040A) coupled to a Hewlett-Packard data acquisition and data processing workstation (model 79994A). Emerging components were monitored by recording at 260μm with a 100μm bandwidth.

EXTRACTION AND HPLC METHOD

Fresh plant material was usually processed within a few hours of collection. Plants were divided into component parts, *viz*. flowers, roots etc. and ground in a commercial Waring blender with methylene chloride. Mixtures were allowed to stand at room temperature for 18 hours then filtered. The methylene chloride extracts were dried over anhydrous magnesium sulfate then reduced in volume on a rotary evaporator at less than 30 C. All procedures were conducted in subdued light. An aliquot of each

concentrated extract was filtered through a C-18 Sep-Pac cartridge then injected on the HPLC column. The HPLC apparatus was operated at 2.5 ml/min. at approximately 1000 psi. The solvent was methanol/water (77.5 : 22.5).

RESULTS AND DISCUSSION

Most extracts contained a large number of compounds with a wide differential in polarity, e.g., hydrocarbons, esters, alcohols etc. Thus it was necessary to choose a solvent system that would elute the components over a large enough time span to obtain sufficient resolution. After much experimentation, it was found that 22.5% water in methanol afforded the best compromise for resolution of the individual compounds and retention times.

The extraction procedure undoubtedly removed compounds from the plant other than polyacetylenes. However, the extinction coefficient of these multi-conjugated systems are typically well over 100,000. In most cases, however, the other compounds are not "seen" because of their weak extinction coefficients.

Fig. 1 is a typical example of a methylene chloride extract of Russian knapweed root extract(Centaurea repens). Under these conditions, the hydrocarbons and thiophene hydrocarbons are eluted after 28 min., the more polar ethers, acetates and chloroacetates between 15 and 28 min.; and the alcohols between 12 and 15 min. By using the diode-array detector, a complete UV spectrum of each compound was obtained. Usually this was sufficient to characterize the chromophore of the emerging

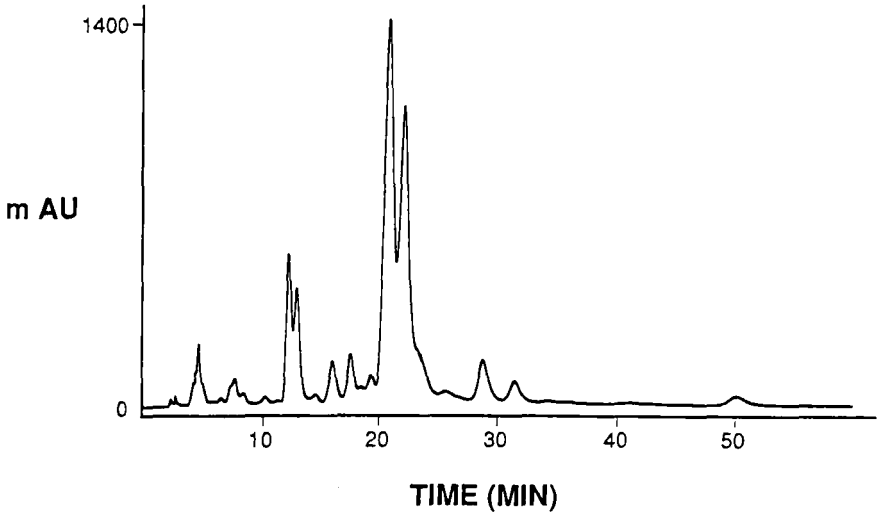


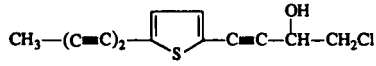
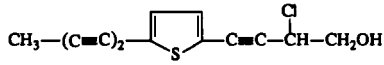
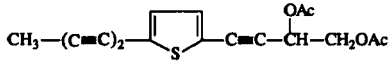
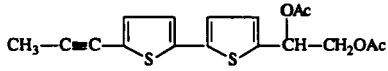
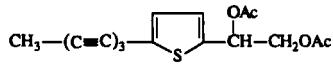
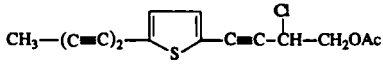
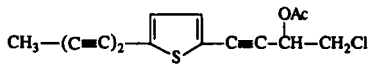
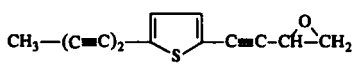
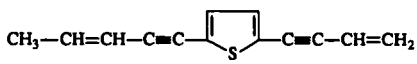
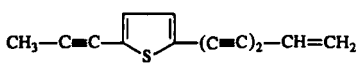
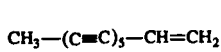
Fig. 1. Liquid chromatogram of Russian knapweed extract. Monitored at 260 μm with a 100 μm bandwidth.

compound. This information coupled with the retention times of known standards allowed the identifications shown in Table 1.

The identification of the individual compounds can often be accomplished by the UV spectrum alone, especially compounds such as 9, 10 and 11. Constituents such as 1-8 require further spectroscopic information which can be obtained from isolates from the semi-prep column or other preparative methods. For example, sufficient material can be obtained from the semi-prep column to obtain mass spectral information which often suffices for a complete identification coupled with the UV data. Once the compound has been identified, the UV spectrum along with its retention time is usually sufficient to identify the component in

TABLE I

The Retention Times and Structures of the Polyacetylenes from Russian Knapweed Root Extract

Retention Time	Structure	
12.5		(1)
13.2		(2)
16.4		(3)
17.9		(4)
19.7		(5)
21.2		(6)
22.5		(7)
26.1		(8)
29.3		(9)
32.0		(10)
50.9		(11)

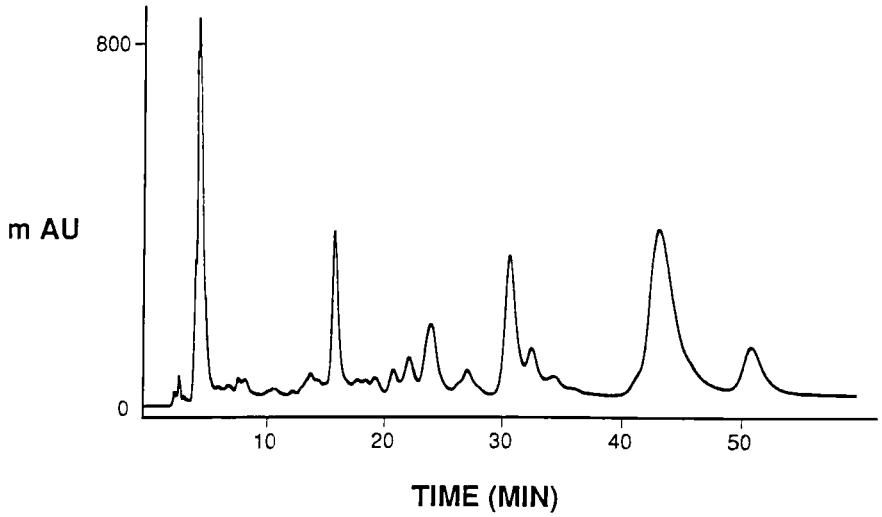


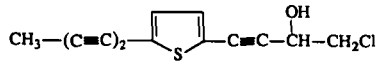
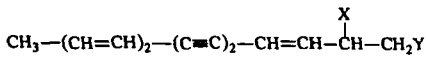
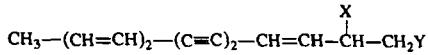
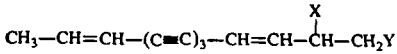
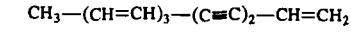
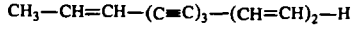
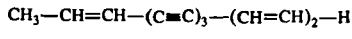
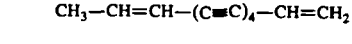
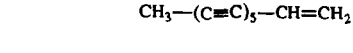
Fig. 2. Liquid chromatogram of yellow starthistle extract. Monitored at 260 μm with a 100 μm bandwidth.

various plant parts and plant sample. One such comparison is shown in Figure 2 which is a methylene chloride extract of yellow starthistle roots(Centaurea solstitialis). The hydrocarbons are eluted between 25 and 50 min.(identifications are shown in Table 2). The more polar substituents are eluted between 11 and 25 min.(Table 2).

The most obvious differences between the two Centaurea species are the presence of a large number of thiophene compounds in Russian knapweed and their scarcity in yellow starthistle. Thiophenes are usually considered to be polyacetylenes because they can be formally thought to arise from the addition of hydrogen sulfide to two acetylenic units(1). Using this method we have been able to readily screen and compare a number of plant

TABLE II

The Retention Times and Structures of the Polyacetylenes from Yellow Starthistle Extract

Retention Time	Structure
10.9	
16.0	
22.4	
24.2	
26.4	
27.3	
30.9	
32.8	
43.5	
51.1	

species for the production of an accumulation of polyacetylenes in the various plant parts. By minimizing the number of manipulations in the workup and analyses, the unstable compounds are less likely to be degraded resulting in a more accurate estimation of relative amounts of polyacetylenes.

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