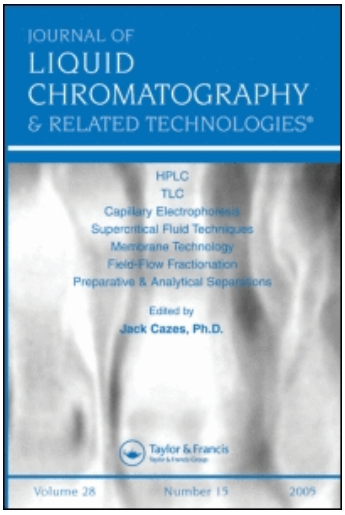

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A Novel Solvent Strategy for Sep-Pak C_{18} Elution of Hydrophobic Compounds: Applications to Lacinilenes and Cadalenes from Cotton Bract

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A NOVEL SOLVENT STRATEGY FOR SEP-PAK C_{18} ELUTION OF HYDROPHOBIC COMPOUNDS: APPLICATIONS TO LACINILENES AND CADALENES FROM COTTON BRACT

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ABSTRACT

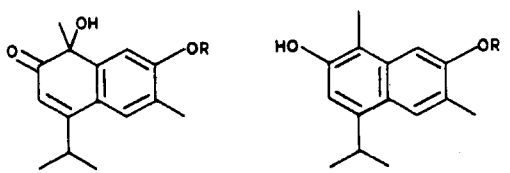
Lacinilene C 7-methyl ether (an implicated causative agent of byssinosis), lacinilene C, and their immediate cadalene precursors were extracted from dried green cotton bracts into water. These compounds were then quantitatively adsorbed from water and concentrated on SEP-PAK C_{18} Cartridges. The lacinilenes, and their cadalene precursors were then separated on a μBONDAPAK C_{18} column. A new sample preparation strategy for SEP-PAK C_{18} Cartridges was developed, in which the loaded Cartridge was dried with N_2 gas and eluted with the most non-polar solvent that would totally release the compound(s) in question. Consequently, due to insolubility, many adsorbed undesirable compounds were not eluted. This strategy is highly selective and allows for a quick, convenient, yet quantitative recovery, and should have wide applicability to the isolation of hydrophobic compounds.

INTRODUCTION

Byssinosis, an occupational respiratory disease that affects some cotton mill workers, is apparently caused by the inhalation of dust generated during fiber processing. Lacinilene C 7-methyl ether, (C), has been implicated as one component in

dust capable of causing the disease (2,3). This compound, lacinilene C, (A), and their immediate biosynthetic precursors, 2-hydroxy-7-methoxycadalene, (D), and 2,7-dihydroxycadalene, (B), (Figure 1), have been isolated from cotton bracts and characterized (4,5). The cadalenes can also be considered as possible byssinotic causatives because of their potentially high chemical reactivity. The biosyntheses of both A and B have been induced in the cotton plant by infection, and the latter compound is a potent bacteriocide (6).

A reliable detection method and preparative scheme was required before initiating an intensive study of these compounds. Previously, Gilbert et al. (7) indicated that HPLC using a μBONDAPAK C_{18} (8) column could be used to separate C from other components isolated from preparative thin-layer plates. Wall et al. (9) attempted reverse phase HPLC for the separation of C but maintained that "its residence time on a C_{18} column (6 hr) was too long and allowed decomposition." They then used silica, normal phase, and injected crude ether extracts directly on to the column. Although their system was very simple, it lacked selectivity since "a conventional column displayed varying solvent synergism effects" (9,10). They had to spike each sample with the known to determine which peak corresponded to C. Therefore, we decided to investigate other methods to resolve the compounds.



(A) R = H (B) R = H

(C) R = Me (D) R = Me

FIGURE 1. Lacinilene C (A), 2,7-Dihydroxycadalene (B), Lacinilene C 7-methyl ether (C), and 2-Hydroxy-7-methoxycadalene (D).

Wall et al. (9) described C as being insoluble in water; however, their data showed that C has some water solubility. Further, A and C were previously isolated from cotton bracts, for preparative work, by extraction with water followed by partitioning into ether (4). Because the lacinilenes and cadalenes are only slightly soluble in water, the extraction procedure becomes an enormous task. Therefore, a simple method for extracting these compounds from aqueous solutions was needed.

Various hydrophobic compounds have been extracted from aqueous solutions with the use of SEP-PAK C_{18} Cartridges (11). Presently, the sample preparation strategy consists of eluting the loaded Cartridge with successively less polar solvents until the compound(s) of interest is removed.

In this paper, we demonstrated that A and C can be almost quantitatively extracted with water from dried green cotton bracts. SEP-PAK C_{18} Cartridges can then be used to quantitatively extract and concentrate these compounds and their immediate precursors from aqueous solutions. Further, we have utilized an alternative sample preparation strategy that consists of first eluting the loaded Cartridge with the most non-polar solvent applicable until the compound(s) of interest is removed. This method markedly enhances selectivity during elution of SEP-PAK C_{18} Cartridges. We have also shown that a μBONDAPAK C_{18} column can be used, with excellent reproducibility, for separating the lacinilenes and their immediate precursors.

MATERIALS AND INSTRUMENTATION

SEP-PAK C_{18} Cartridges were obtained from Waters Associates, Inc. Aqueous solutions were pumped through the Cartridges with a 4-channel Buchler Polystaltic Pump. Solvents used for HPLC were distilled Certified A.C.S. grade filtered through 0.2 μm Millipore filters. A 950 Chromatographic Pump (Tracor Instruments) was used to deliver the solvent through a 3.9 mm ID x 30 cm μBONDAPAK C_{18} column (Waters Associates, Inc.). Samples were introduced via a 20 μl loop injector, Model 7120

(Rheodyne Inc.). Column effluent was monitored by a Tracor 970A detector at 250 nm, and recorded with a Hewlett-Packard 3380A Integrator or an OmniScribe recorder (Houston Instrument). Silica-gel (Silicar TLC-7G) was obtained from Mallinckrodt, Inc.

METHODS

All procedures were conducted in minimum light conditions. Dried green cotton bracts, cultivar Stoneville 256, were used throughout this study. Extracts were prepared by stirring overnight (16 hr), 40 mesh cotton bract powder (0.5 g) and oxalic acid (30 mg) in distilled water (1 liter). Solids were removed by suction filtration through Whatman No. 3 filter paper. The filtrate was brought to 10% saturation with NaCl and pumped (10 ml/min) through a SEP-PAK C_{18} Cartridge previously washed stepwise with methanol (2 ml) and water (5 ml). Two distilled water washes (20 ml) were also pumped through the Cartridge. The Cartridge was then dried with a stream of N_2 (10 psi for 10 min), and eluted with n-hexane/acetone (85:15 v/v, 8 ml). Methanol/water (85:15 v/v, 0.5 ml) was added to the n-hexane/acetone eluate, and the n-hexane/acetone was removed by rotary evaporation (30°C). The resulting solution was then passed through another prewashed C_{18} Cartridge followed by rinsing with 85% methanol in water (3 x 0.25 ml). All fractions passed through the second Cartridge were collected in a 5 ml volumetric flask. A final Cartridge wash with sufficient 85% methanol to fill the volumetric flask removed the compounds. This solution was directly injected onto a μBONDAPAK C_{18} column being pumped with methanol/water (67:33 v/v) at a flow rate of 0.8 ml/min. The flow rate was increased to 1.8 ml/min after C had been completely eluted. Samples were also two-dimensionally chromatographed on silica-gel (0.5 mm layers) with benzene/methanol (95:5 v/v); A, hR_f = 19; B, hR_f = 30; C, hR_f = 58; D, hR_f = 68, and diethyl ether/n-hexane (50:50 v/v); A, hR_f = 39; B, hR_f = 57; C, hR_f = 61; D, hR_f = 71. A and C were visualized by long

wave UV. Their immediate precursors were visualized in the same manner after heating (100°C for 10 min) the TLC plates (12).

RESULTS AND DISCUSSION

In the process of extracting freeze dried or air dried cotton bracts with water, we found a distinct difference between extracts in regard to the relative amounts of A and its immediate precursor, B. It appeared that B was being converted to A. We attempted to prevent this conversion by adding various compounds during extraction (sodium azide, sodium bisulfite and oxalic acid). Sodium azide or sodium bisulfite had no effect; however, oxalic acid appeared to stop the conversion. The ratios of (B) to (A) and (D) to (C) demonstrate the conversion of B in the absence of oxalic acid (Table 1).

Based upon the complete extraction of these four compounds with methanol/acetone (80:20 v/v)(13), extraction efficiencies have been determined (Table 1). Since approximately 80% of compounds A, B, and C were removed with water, this appears to be a simple, expedient method to isolate sufficient quantities of

TABLE 1

Extraction Efficiency for Lacinilene C (A), 2,7-Dihydroxycadalene (B), Lacinilene C 7-Methyl Ether (C), and 2-Hydroxy-7-methoxycadalene (D) from 0.5 g Dried Green Bract Powder (40 mesh) in Water (1 liter) for 16 hrs (n = 3 or 4 Extractions).

	EXTRACTION EFFICIENCY (%)				EFFICIENCY RATIOS	
oxalic acid (30 mg/L)	A	B	C	D	B/A	D/C
+	79±8	76±6	78±10	31±4	0.96	0.39
-	148±7[a]	27±4	92±4	39±6	0.18	0.42

[a]This large percentage reflects chemical conversion from B to A.

these compounds for biological testing. Compound D, however, was not efficiently removed by water extraction.

SEP-PAK C_{18} Cartridges were used to extract and concentrate compounds A, B, C, and D from water extracts. By using tandem Cartridges, a second Cartridge connected to the first with a glass tube (4 mm OD), no leakage was observed from the first Cartridge when a 1 liter extract from 2 g of bract powder was processed. Further, the lacinilenes and cadalenes from an extract of 1/4 g bract powder in 4 liters of water exhibited no leakage in the tandem system.

The normal sample preparation strategy for SEP-PAK C_{18} Cartridges utilizes polar solvents to remove high polarity compounds. Systems of decreasing polarity are then used to remove other adsorbed compounds. This can, and often does, result in the elution of a substantial number of impurities along with the desired compound. Our preparation strategy was devised to improve elution selectivity, resulting in greater fractionation (Table 2).

TABLE 2

Outline of the New Sample Preparation Strategy for SEP-PAK C_{18} Cartridges.

NEW SAMPLE PREPARATION STRATEGY
1 The sample is adsorbed onto the C_{18} Cartridge from an aqueous solution.
2 The Cartridge is dried in a stream of N_2 gas (10 psi for 10 min).
3 The compound(s) of interest is removed by washing the Cartridge with the most non-polar solvent applicable. Most water soluble compounds are retained.
4 The solvent polarity can then be increased to elute other compounds.

TABLE 3

Solvents Used for Elution of SEP-PAK C_{18} Cartridges by the New Sample Preparation Strategy; from n-Hexane to Methanol by Increasing Polarity.

ELUTION SOLVENTS
n-hexane < n-hexane/acetone[a] < acetone < acetone/methanol[a] < methanol

[a]The solvent ratio is dependent upon the individual application.

This strategy not only takes advantage of the C_{18} Cartridges ability to quantitatively adsorb and concentrate hydrophobic compounds, but also takes advantage of solubilities. Many of the compounds concentrated on the SEP-PAK are insoluble in non-polar systems, i.e., n-hexane/acetone (85:15 v/v), and therefore are retained on the SEP-PAK.

Our general approach to the type of solvents used for C_{18} Cartridge elution is presented in Table 3. By selecting the appropriate solvents, it is possible to make cleaner fractional cuts. We used n-hexane/acetone (85:15 v/v) to elute A, B, C, and D from C_{18} Cartridges.

Figure 2 shows a HPLC trace of a typical water extract processed by our method. Unlike Wall et al. (8,9), we observed no synergism (variability in retention times). The reproducibility of retention times in this system was excellent, as shown in Table 4. As a result, sample spiking with authentic C and repetitive injections are not necessary. More importantly, the disadvantage of extensive column washing following crude extract injection (to maintain theoretical plate efficiency) is eliminated.

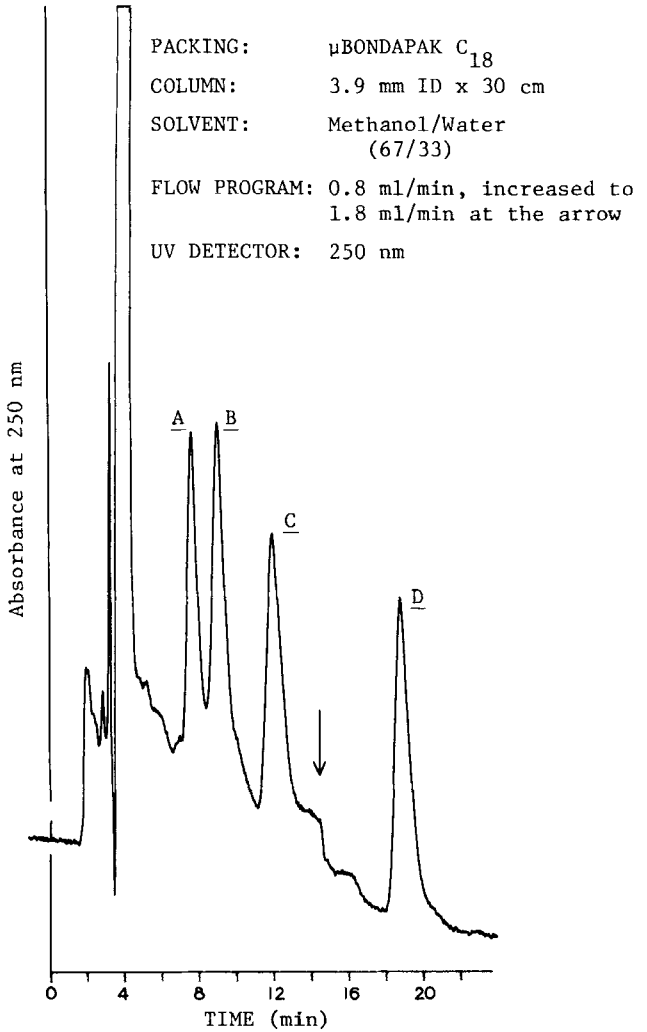


FIGURE 2. HPLC Chromatograph of Lacinilene C (A), 2,7-Dihydroxycadalene (B), Lacinilene C 7-methyl ether (C), and 2-Hydroxy-7-methoxycadalene (D) Obtained by Water Extraction of Dried Green Cotton Bracts, Cultivar Stoneville 256.

TABLE 4

Reproducibility Data ($\bar{x} \pm \delta$) Over a Two Month Period, for Retention Times of Lacinilene C (A), 2,7-Dihydroxycadalene (B), Lacinilene C 7-Methyl Ether (C), and 2-Hydroxy-7-methoxycadalene (D) Obtained on a 3.9 mm ID X 30 cm μBONDAPAK C_{18} Column Using Methanol/Water (67:33 v/v).

Reproducibility Data ($\bar{x} \pm \delta$)				
RETENTION TIME (min)				
A	B	C	D	Number Of Injections
7.9±0.07	9.3±0.09	12.4±0.12	19.1±0.28	27

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