Argentation Resin Chromatography of Diterpene Resin Acids

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ABSTRACT

Argentation chromatography with silver ion macroreticular exchange resin was applied to diterpene resin acids and methyl esters. Best results for diterpene acids were obtained with silver-saturated ion exchange resin and solvent systems such as ethyl ether/acetone or acetone. The technique was applied in the isolation of a major component, identified as 20-nor-5,7,9-abietatrien-18-oic acid, from a commercial disproportionated rosin.

INTRODUCTION

Chromatographic methods using the complexing of unsaturated compounds with silver ion are well established. Argentation (Ag^+) thin layer separation of diterpene resin acid methyl esters has been described (1,2) and Ag^+ column chromatography has been used extensively in our laboratory for the isolation of new compounds and for purification of resin acid methyl esters for reference spectra (3).

Often, however, resin acids are preferred over the ester derivatives. Resin acids usually are more readily crystallized than esters for further purification; when the acid form is needed for further work, direct isolation and purification of a resin acid avoids the rigorous methods usually needed to hydrolyze the sterically hindered resin acid esters and possible attendant degradation. Interaction of unesterified carboxyl functionality with silica, alumina, or similar supports commonly used in Ag^+ thin layer or column chromatography is not compatible with effective separation of diterpenes as free acids. Further, we have often observed significant on-column losses of resin acid esters during prolonged chromatographic separations with $AgNO_3$-support columns. The use of silver ion as bound salts of macroreticular sulfonic acid ion-exchange resins would appear to avoid the problems associated with silica and alumina-type supports.

Early application of silver sulfonate resins was of limited success for chromatographic separation of unsaturated fatty acid esters (4). However, the greater surface area of new sulfonic acid ion-exchange resins results in significantly improved separations, particularly for geometric isomers (5). Further refinements have been made in the technique, including the application to unesterified fatty acids (6). This paper describes the extension of the silver sulfonate resin technique to the chromatographic separation of diterpene resin acids.

EXPERIMENTAL

Amberlyst XN-1010, a sulfonated polystyrene, cation-exchange resin (Rohm and Haas Co.) was used in this work. Product literature (7) indicates a cation exchange capacity of 3.1 meq/g dry resin, nominal particle size 16 to 50 mesh, a mean pore size of 50 Å and a surface area of 500 m^2/g. The resin as received was dry-sieved into several particle size fractions. A 100-mL portion of the largest-particle-size fraction (on 20-mesh screen) was added to 200 mL distilled water and ground for 5 min by a Waring blender at high speed. The ground resin from several such preparations was separated into 40/60, 60/80 and 80/100 mesh fractions by wet sieving in a manner similar to that described by Adlof et al. (8). The 60/80 mesh fraction was used in this work.

The 100% silver salt form of the resin was prepared as described by Adlof and Emken (6) with minor modifications. The resin, in distilled water, was packed into a column and 1 M $NaNO_3$ (aq.) added until the eluate was no longer acidic. After washing with distilled water, increments of aqueous silver nitrate alternating with water were applied to the column until the eluate formed a precipitate when added to a solution of KCl. The Ag^+ resin was then thoroughly washed with distilled water until all excess $AgNO_3$ was removed (KCl test). Because solvent changes affect swelling of the resin, the Ag^+ resin was repacked into a 0.9-cm id column after conditioning with the appropriate solvent.

Samples were introduced to the top of the resin bed in about 0.25 mL of 20% solutions and the column was eluted at a flow rate of 1 mL/min at gravity flow unless otherwise indicated. Elution of sample components was monitored by a Laboratory Data Control Model 1103 differential refractometer and by chromatography of collected fractions (acids were methylated with diazomethane) with an HP 5840 gas chromatograph using a 10-m glass capillary column coated with BDS. The void volume of the 96 × 0.9 cm Ag^+ resin bed for acetone as solvent is indicated by an elution volume of 27 mL for methyl palmitate (pentane and heptane eluted in ca. 28 mL) and 34.4 mL for D_6-acetone.

RESULTS AND DISCUSSION

Because of our prior experience with argentation chromatography of resin acid methyl esters with $AgNO_3$ on alumina or silica supports, resin acid esters were used in initial evaluations of the Ag^+ resin. The elution characteristics of methyl esters of resin acids common to pine oleoresins and rosins were evaluated for various solvent systems that are good solvents for resin acids. These solvents included representative aliphatic hydrocarbons (petroleum ether and solvents with 2-butene), aromatic hydrocarbons (toluene), ethers (diethyl ether) and ketones (acetone). Ethyl ether/petroleum ether and acetone offered the most promise as solvents for the chromatography of resin acid methyl esters. Partial argentation resin chromatography (PARC; columns prepared as per Adlof and Emken, [6]) was not as effective as 100% silver resin.

Elution with ethyl ether proved to be excessively slow, requiring 850 mL at a flow rate of 1 mL/min to elute methyl pimarate (ester has an exocyclic axial vinyl substituent) leaving sandaracopimarate and isopimarate (each has an exocyclic equatorial vinyl substituent) in the column. Not only was the elution slow, but separation of the other methyl esters was inadequate. Gradient elution with 25 to 35% ethyl ether in petroleum ether resulted in even greater elution volumes for the esters, as expected; but usable separation was achieved for the most rapidly eluting esters, methyl dehydroabietate, abietate and neoabietate. Acetone elution of methylated acids from a slash pine oleoresin is shown in Figure 1A. Elution of all components was completed in 8.5 hr at a flow rate of 0.3 mL/min. Although the more weakly Ag^+-π complexing esters (methyl dehydroabietate, abietate and neoabietate) were not separated, fractions were collected for levopimarate, pimarate, isopimarate and sandaracopimarate of >90% purity.

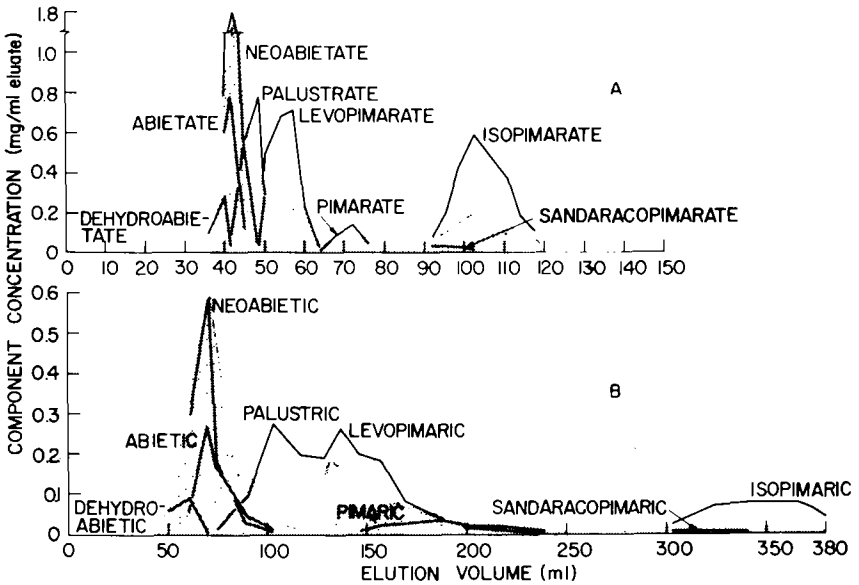

FIG. 1. Argentation separation of methylated resin acids (A), and resin acids as free acids (B), both with acetone as solvent. The shaded zones indicate the elution pattern as recorded by a differential refractometer. The solid lines denote the elution of the individual resin acids as determined by GLC of collected fractions.

Extension of the ethyl ether/petroleum ether system to free resin acids showed that development even with 100% ethyl ether resulted in excessively long elution time. Acetone elution, however, of the free resin acids was quite promising as can be seen in Figure 1B, a chromatogram of resin acids from slash pine oleoresin. As with the methyl esters, dehydroabietic, abietic and neoabietic acids were not resolved but useful separation was obtained of the stronger complexing acids. Chromatography of a gum rosin with acetone resulted in the isolation of palustric (rosins do not contain the readily isomerizable levopimaric acid), pimaric and isopimaric acids, each in >95% purity. Although the more weakly complexing resin acids cannot be separated with acetone as solvent, a stepwise gradient of 3.5 to 15% acetone in ethyl ether separated dehydroabietic acid (in 100% purity), neoabietic acid (97% purity) and abietic acid (95% purity) from a test mixture of the 3 compounds.

Preparative scale Ag^+ resin chromatography of a resin acid mixture was demonstrated on a commercial disproportionated (iodine-catalyzed) rosin, Resin 90 (Westvaco). Gas chromatography of methylated resin acids from the Resin 90 showed a compound, not identifiable by GC retention characteristics, that constituted about 10% of the product. A 1-g sample of the Resin 90 in 1.5 mL eluting solvent was applied to the analytical Ag^+ resin column and eluted with 10% acetone in ethyl ether. The unidentified component was enriched from 10 to 65% with 70% recovery in an 8-hr run. Rechromatography of the enriched material did not produce any significant further enrichment of the component. Contrary to general experience with resin acids of such quality, the component did not readily crystallize (methanol). Gel permeation chromatography with a column of 40 Å Styragel (9) and ethyl ether solvent was then used to enrich the concentration of the unidentified material to about 91%. Successive crystallizations from methanol produced the unknown in 99% purity. The acid had an mp of 147.5 to 150 C (evac. cap.) that was somewhat obscured by sublimation; $[\alpha]_D^{25}$ 59.3° (c 0.7, EtOH); UV (isooctane) $\lambda_{max}^{268.5}$ ϵ = 290, $\lambda_{max}^{203.3}$ ϵ = 46,800; IR (methyl ester, film) 1736 (C=O), 1255, 1112 and 815 cm^{-1}. GLC of methyl ester (10-m glass capillary columns, 190 C) $r_{pimarate}$= 1.358 (SE-30) and r_{pim} = 2.754 (BDS). The predominant features of the mass spectrum (methyl ester, Varian MAT 112 magnetic sector instrument) were (m/e, rel. intensity): 300.1, 8.7% (M^+); 241.3, 100% (M^+-$COOCH_3$); and 156.9, 22.5% ($C_{12}H_{13}$) with minor fragmentation ions at 185.1, 3.8%; 141.1, 4.3%; 129.1, 4.6%, and 114.9, 1.6%. The proton NMR (60 MHz) spectrum was characterized by resonances at δ 0.94 (6H, d, J=6), 1.53 (3H,s), 3.63 (3H,s), and 6.92 (2H,m). The spectral data agree with those reported for the methyl ester of a demethyldehydroabietic acid, methyl 20-nor-5,7,9-abietatrien-18-oate, as obtained from iodine disproportionation of abietic acid (10); Dr. Ishigami has informed us that the MS fragmentation he reported at m/e 147 (10) should be m/e 157. The 270 MHz spectrum had some surprising features in that the carbomethoxy methyl had chemical shifts at 3.654 and 3.649, the C-4 methyl at 1.545 and 1.535, and the isopropyl doublet at 0.958, 0.964, 0.933 and 0.940. Decoupling experiments show that the 270 MHz NMR phenomenon does not result from long-range coupling with the hydrogens at C-6 and C-7. An explanation that the isolated material consists of 2 closely related isomers (e.g., C-13 epimers) appears unlikely since there was no indication of 2 components on GC using a 30-m glass capillary, SE-30 column, as would be expected if 2 isomers were present (C-13 epimers of various resin acid methyl esters are readily separated on packed columns [3]).

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Effectiveness of Several Polyunsaturated Seed Oils as Boll Weevil Feeding Deterrents

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ABSTRACT

Prompted by the previous discovery that (9Z,11E,13E)-9,11,13-octadecatrienoic acid (α-eleostearic acid) was one of the components responsible for the boll weevil feeding deterrency of tung oil, the seeds of 11 other plant species were extracted with pentane and the oils were evaluated for their feeding deterrency in the laboratory. The oils of *Calendula suffruticosa*, *Centranthus macrosiphon*, *Jacaranda mimosifolia* and *Momordica cochinchinenis* were effective feeding deterrents for the boll weevil.

INTRODUCTION

(9Z,11E,13E)-9,11,13-octadecatrienoic acid (α-eleostearic acid), the principal fatty acid of tung oil from the seeds of *Aleurites fordii* Hemsl., has recently been identified (1) as one of the components responsible for boll weevil feeding deterrency ascribed to the oil in 1966 (2). The boll weevil, *Anthonomus grandis grandis* Boheman, is the most destructive insect pest of cotton in the U.S. and Mexico. Although α-eleostearic acid is too unstable for practical use as a feeding deterrent under field conditions, its methyl ester has been shown to be much more stable and equally effective as a deterrent (1,3). We were interested in determining whether a number of other seed oils known to contain conjugated polyunsaturated C_{18} fatty acids would also be effective deterrents. This paper reports the results of our investigation.

EXPERIMENTAL PROCEDURES

The dry whole seeds of each plant were ground in a blender at room temperature with several portions of pentane and the combined extracts were freed of solvent at 25 C and 15 mm pressure. The oils obtained were analyzed for conjugated unsaturation and geometric configuration by standard means of ultraviolet and infrared spectrophotometry, using absolute ethanol as UV solvent and KBr discs for IR determinations. Aliquots of each oil were tested in the laboratory against boll weevils by the method of Hardee and Davich (2) at 25-27 C and 30-42% relative humidity. Hexane was used to prepare a 1% (w/v) solution of the oil (except tung oil, which was tested in pentane), and an unpunctured, debracted square (bud) from a cotton plant was dipped momentarily in the solution. Ten 1- or 2-day-old adult weevils, unfed from time of emergence or starved for 24 hr, were placed in a petri dish with 1 treated bud and 1 control bud (dipped in solvent only) and held for 4 hr.

TABLE I

Yield and Boll Weevil Feeding Deterrency of Seed Oils

Plant name	Plant family	Oil yield/conjugated trienoic acid content (%)	Number of feeding punctures	
			Oil solution	Solvent
Aleurites fordii Hemsl.	Euphorbiaceae	30.0/50	2	21[a]
Calendula arvensis L.	Compositae	5.2/41	62	47
Calendula suffruticosa Vahl.	Compositae	6.1/62	18	50[a]
Catalpa bignonioides Walt.	Bignoniaceae	17.0/38	47	35
Centranthus macrosiphon Boiss.	Valerianaceae	22.6/56	16	87[a]
Impatiens balsamina L.	Balsaminaceae	4.9/22	46	77
Impatiens flemingii Hook. f.	Balsaminaceae	52.3/46	60	57
Jacaranda mimosifolia D. Don.	Bignoniaceae	23.1/37	21	52[a]
Momordica cochinchinensis Spreng.	Cucurbitaceae	22.5/58	27	74[a]
Punica granatum L.	Punicaceae	5.2/71	30	35
Santalum album L.	Santalaceae	33.7/69	38	45
Tricosanthes anguina L.	Cucurbitaceae	22.7/45	46	63

[a]These results differed significantly by the chi-square test ($p < 0.001\%$).