

THIN-LAYER CHROMATOGRAPHY OF RESIN ACID METHYL ESTERS

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INTRODUCTION

The problem of separating and identifying resin acids has been the subject of considerable research effort. A partition chromatographic system has been employed but is rather tedious and complete separations are not obtained¹. Partition paper chromatography has been applied with limited success^{2,3}. Gas chromatography has been used with partial success⁴; in the Forest Products Laboratory, resin acid methyl esters have been gas chromatographed without isomerization of the sensitive abietadienic acid esters⁵. Mass spectroscopy has been used successfully⁶. However, a much simpler method is desirable.

DANIELS AND ENZELL⁷ have successfully applied a method, developed by WICKBERG⁸ for the separation of unsaturated hydrocarbons by partition of their silver π -complexes between hexadecane and aqueous methanol, to the separation of resin acid methyl esters. It seemed quite possible from this work that a more convenient system could be developed having the silver salt in the stationary phase. Indeed, thin-layer chromatography using silver nitrate impregnated silica gel has been successfully applied to some difficult separations of unsaturated fatty acid esters⁹ and triglycerides¹⁰. The acetic acid containing solvent system of BARRETT, DALLAS AND PADLEY¹⁰ was ruled out because of the probability of acid isomerization of the resin acid esters. The ethyl ether-hexane system of MORRIS⁹, however, offered promise.

EXPERIMENTAL

Glass plates (200 mm \times 200 mm) were coated with a slurry of alumina (aluminum oxide G with binder, Research Specialties Co.) and silver nitrate solution using a spreader set for a thickness of 250 μ . A solution of 12 g silver nitrate in 20 ml water was diluted with 40 ml methanol and the resulting solution added to 30 g alumina and mixed by shaking. The plates prepared with this coating were allowed to air dry overnight. Before use, the plates were dried at 110° for 30 min.

The resin acid methyl esters were prepared using a freshly distilled ethyl ether solution of diazomethane in a similar manner to that of SCHLENK AND GELLERMAN¹¹. The diazomethane solution was added dropwise to 1% solutions of pure or mixed resin acids in 9:1 (v/v) ethyl ether-methanol at 0° until a slight excess of diazome-

* Maintained at Madison, Wis. (U.S.A.), in cooperation with the University of Wisconsin.

thane solution was discernible against a white background. The solution was then concentrated under a stream of purified nitrogen. A mixture of methyl stearate, oleate and linoleate (Applied Science Laboratories, State College, Pa.) was run on all plates for comparison. The methyl esters of commercial pine gum rosin and resin acids obtained unaltered from longleaf pine oleoresin by amino-cellulose ion exchange¹² were dissolved in hexane and filtered through alumina (Woelm, neutral, activity I) to remove oxidized materials.

Best results were obtained when 25 % by volume peroxide-free anhydrous ethyl ether in petroleum ether (b.p. 30–60°) was used as the developing solvent. It was important that the developing solvent be added to the tank just before inserting the plate. The thin-layer plates were allowed to develop (ascendingly) the length of the plate. The thin-layer plates were allowed to develop (ascendingly) the length of the plates (180 mm) in a constant temperature room held at 30°. Development time ranged from 80 to 90 min.

The developed plates were sprayed with 1:4 (v/v) conc. sulfuric acid–ethyl ether solution. Sulfuric acid diluted with ether has the additional advantage of being easier to spray than conc. sulfuric acid alone. After heating in an oven at 110° for 15 min, all spots except the fatty acid and saturated resin acid esters could be readily seen under ultraviolet light. All spots could be observed by charring at 200° for 1 h.

RESULTS

Fig. 1 shows the results that were obtained with the described chromatographic system. Plates 200 mm × 500 mm with descending development were also used for

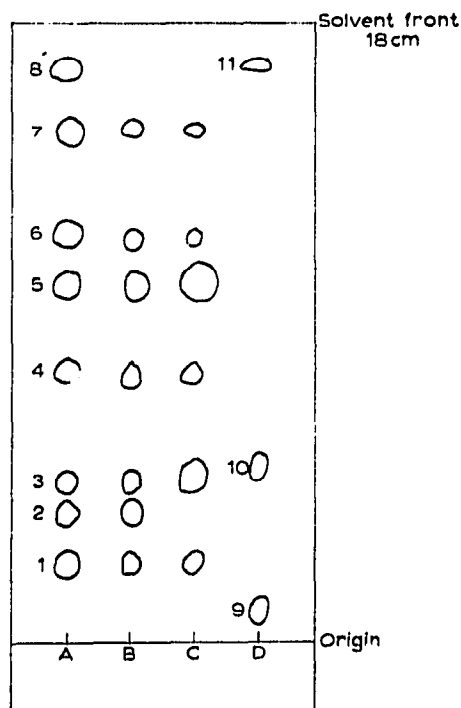


Fig. 1. Thin-layer chromatogram of fatty and resin acid methyl esters. Methyl (1) isopimarate, (2) levopimarate, (3) palustrate, (4) pimarate, (5) abietate, (6) neoabietate, (7) dehydroabietate (8) dihydro and tetrahydro resin acids, (9) linoleate, (10) oleate, and (11) stearate. (A) Resin acid methyl ester standard mixture prepared from pure resin acids, (B) resin acid methyl esters from oleoresin, (C) resin acid methyl esters from gum rosin, and (D) fatty acid methyl esters.

the separation of methyl dihydroisopimarate or methyl dihydrolevopimarate from methyl tetrahydroabietate (prepared by hydrogenation of levopimaric acid with Adams catalyst in acetic acid). The use of a less polar solvent, *e.g.*, petroleum ether alone, on standard plates also gave good separation of the dihydro from the tetrahydro resin acid esters, although some of the other resin acid esters were no longer resolved under these conditions.

The developing solvent should be placed in the tank just before use. Successful separation apparently depends on a combination of gradient development and an extended development resulting from the evaporation of solvent from the plate during the development. Attempts to operate under a saturated solvent atmosphere by the use of blotters did not approach the separation attained under the described conditions even though various solvent components and proportions were tried.

The use of hazardous and expensive silver fluoroborate as per DANIELS AND ENZELL⁷ gave no improvement over the use of silver nitrate. Doubling the silver nitrate content did not improve separations. Incorporation of ethylene glycol in the stationary phase to effect a liquid-liquid partition chromatographic system gave inferior results.

Silica with calcium sulfate binder was tried as a support for the silver nitrate, but the binder was not effective in the presence of silver nitrate and, consequently, it was difficult to keep the layer intact while spraying and handling these plates. The alumina-silver nitrate plates, on the other hand, can be sprayed without special precautions and are easily handled.

The maximum loading of each resin acid ester is about 10 μg per spot. Minimum detection limits of the sulfuric acid-ether spray reagent for unsaturated acid esters when heated and observed under ultraviolet light are from 0.5 to 1 μg , whereas these minimum limits are from 1 to 2 μg when charring at 200° is used as a means of detection. Both of these methods are far more sensitive than using antimony pentachloride or any of the standard lipid reagents such as 2',7'-dichlorofluorescein or rhodamine B. Permanent records of the plates were made with a Xerox 914 copying machine.

Methyl abietate-neoabietate and methyl palustrate-levopimarate are the most difficult pairs to separate, especially when too large a sample size is used. The methyl palustrate-levopimarate separation can be specifically improved by increasing the amount of ethyl ether in the solvent system to 40%. Separation of methyl abietate-neoabietate when one of these esters is present to a much larger extent than the other is more difficult. A longer plate may be required under some circumstances.

Since the R_F or R_{solute} values vary somewhat from plate to plate, it is advisable to run a set of standards when identifying the components of a mixture. However, the order of elution remains as shown in Fig. 1. This order is somewhat different from that of the partition system of DANIELS AND ENZELL⁷.

Oxidized resin acid esters can interfere with the identification. This is particularly true of the methyl abietate or neoabietate oxidation products which make it difficult to establish the presence of methyl isopimarate, levopimarate, or palustrate.

Application of this method to methylated resin acids from gum rosin and oleoresin reveals the presence of the primary resin acids in oleoresin and the expected absence of methyl levopimarate among the resin acids of gum rosin.

Confirmation of the identity of resin acid esters has been achieved by recovery

and spectral examination of the esters. A solution of the unknown was streaked onto the plate by an adaptation of the streaking apparatus of MCKIBBINS *et al.*¹³ and developed. The plate was then masked and several 1 mm-wide strips parallel to the direction of development sprayed with the sulfuric acid-ether reagent. The zones on the unsprayed portions of the plates corresponding to the spots on the sprayed areas were removed from the plate and the esters eluted in a microapparatus developed at the Forest Products Laboratory¹⁴. The esters were then additionally characterized by infrared and ultraviolet spectroscopy.

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SUMMARY

A procedure has been developed in which all of the common pine resin acids may be cleanly separated by thin-layer chromatography of their methyl esters on alumina impregnated with silver nitrate.

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NOTE ADDED IN PROOF

We are grateful to T. NORIN and L. WESTFELT for sending us a copy of their paper, "Thin-layer, column, and gas-liquid chromatography of resin acid esters and related terpenes," *Acta Chem. Scand.* (in press) in which they describe the use of silver nitrate on silica gel plates and a benzene-ether solvent system for the separation of resin acid esters.