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The use of silver nitrate impregnated silica gel layers in the separation of monoterpene hydrocarbons

Silver nitrate impregnation of silica gel and other adsorbent layers has been used by a number of workers to separate unsaturated compounds. This technique has been especially useful in the field of lipids¹⁻³ where it has been used to separate *cis*- and *trans*-isomers and other compounds according to their number of double bonds. Recently^{4,5}, more interest has been shown in the separation of positionally isomeric unsaturated compounds.

In the field of essential oil and resin constituents, a number of workers have used the technique to aid them in generally difficult separations. A list of these is given in Table I. In this study the technique is used to determine the best impregnation for the separation of monoterpene hydrocarbons.

TABLE I

SILVER NITRATE THIN-LAYER CHROMATOGRAPHY OF ESSENTIAL OIL AND RESIN CONSTITUENTS

<i>Type of compounds analyzed</i>	<i>Percentage of silver nitrate impregnation</i>	<i>Reference</i>
Sesquiterpene hydrocarbons	15	7
Resin acid esters and related terpenes	12.5	8
Resin acid methyl esters	20	9
Sesquiterpene alcohols	10	10
Sesquiterpene hydrocarbons	12.5	11
Oxygenated terpenes	15	12
Sesquiterpene hydrocarbons	15	13
General essential oil constituents	8	14
Terpene and sesquiterpene alcohols	3	15
Terpene hydrocarbons	12.5	16
Allyl and propenyl phenol ethers	12.5	17
General essential oil constituents	6	18

Method

The slurries were prepared using 40 g of Silica Gel G (E. Merck) and 80 cc of a series of aqueous solutions of reagent grade silver nitrate (B.D.H.). The strength of the silver nitrate solutions used were 0, 6.25 %, 12.5 %, 18.75 % and 25.0 % respectively. Each set of layers was prepared using the Desaga equipment as described by LAWRENCE⁶. The layers were activated in a drying oven at 100-110° for 1 h and then stored in a desiccator over phosphorus pentoxide until used. It was found that differences in R_F value were within experimental error for the three day lifetime of the layers. (It is general procedure used by the author that all activated plates are given a three-day lifetime.)

The monoterpenes were spotted in 1 microlitre quantities with the aid of a 10 μ l syringe (Hamilton) from 10 % (v/v) hexane solutions. All separations were carried out in a standard chromatographic tank containing a filter-paper wick liner using benzene as the eluent. For detection purposes a 10 % chloroform solution of antimony pentachloride proved to be the most useful reagent tried. The plates were

all developed for a standard 15 cm and R_F measurements were made from the most intense part of the detected spot. As a number of runs were made on each compound the $R_F \times 100$ values were recorded as a range rather than a single value.

Results and conclusions

The results obtained from this study (see Table II) show that the R_F value of a monoterpene hydrocarbon varies with percentage impregnation of silver nitrate, and from these results some interesting findings follow:

(1) The R_F value of a monoterpene hydrocarbon can be predicted within the range 0–25% impregnation from a graphical representation of the results (see Table III).

(2) Certain separations which are difficult to achieve with gas chromatography can be readily carried out with AgNO_3 -impregnated TLC, e.g. the separation of sabinene from β -pinene.

Now, the usefulness of the above separation stems from the ability of un-

TABLE II

THE EFFECT OF SILVER NITRATE IMPREGNATION ON THIN-LAYER CHROMATOGRAPHIC SEPARATIONS OF MONOTERPENES

Benzene was the eluent used in all cases.

Compounds	Percentage impregnation of silver nitrate				
	Zero	6.25	12.5	18.75	25.0
<i>p</i> -Cymene	72.5–73.5*	72.5–73.5	72.5–73.5	66.5–67.5	60.5–61.5
δ -3-Carene	71.5–72.5	71.5–72.5	71.5–72.5	65.5–66.5	59.5–60.5
α -Pinene	70.5–71.5	70.5–71.5	70.5–71.5	64.5–65.5	58.5–59.5
Terpinolene	70.5–71.5	70.5–71.5	67.5–68.5	60.5–61.5	52.5–53.5
γ -Terpinene	70.5–71.5	70.5–71.5	65.5–66.5	57.5–58.5	49.5–50.5
<i>allo</i> -Ocimene	69.5–70.5	68.5–69.5	66.5–67.5	62.5–63.5	56.5–57.5
Camphene	67.5–68.5	58.5–59.5	54.5–55.5	52.5–53.5	50.5–51.5
α -Terpinene	69.5–70.5	57.5–58.5	50.5–51.5	46.5–47.5	42.5–43.5
β -Pinene	69.5–70.5	57.5–58.5	49.5–50.5	45.5–46.5	41.5–42.5
α -Phellandrene	70.5–71.5	57.5–58.5	48.5–49.5	43.5–44.5	39.5–40.5
Limonene	71.5–72.5	51.5–52.5	40.5–41.5	34.5–35.5	30.5–31.5
Ocimene	70.5–71.5	41.5–42.5	34.5–35.5	31.5–32.5	29.5–30.5
Myrcene	70.5–71.5	39.5–40.5	32.5–33.5	29.5–30.5	27.5–28.5
Sabinene	70.5–71.5	37.5–38.5	29.5–30.5	26.5–27.5	24.5–25.5

* Refers to $R_F \times 100$.

TABLE III

THE PREDICTED VERSUS THE EXPERIMENTAL $R_F \times 100$ VALUES OF SOME MONOTERPENES ON A 3.125% SILVER NITRATE IMPREGNATED LAYER USING BENZENE AS THE ELUENT

Compounds	Predicted $R_F \times 100$ value	Experimental value
Limonene	61.5–62.5	62.5
α -Phellandrene	62.5–63.5	63.0
α -Terpinene	69.5–70.5	70.5
Ocimene	51.5–52.5	52.0
δ -3-Carene	71.5–72.5	72.0
Myrcene	49.5–50.5	50.5

saturated compounds to form π -complexes with the silver ion. Considering this fact, some possible conclusions can be drawn from these results.

(1) Cyclic terpenes with single internal double bonds do not readily form π -complexes.

(2) Cyclic or acyclic terpenes with two non-terminal double bonds do not readily form π -complexes unless the double bonds are *cis* conjugated.

(3) Cyclic or acyclic terpenes with exocyclic or terminal double bonds do form π -complexes.

Recently, NANO AND MARTELLI¹⁷ obtained a concurrent conclusion in their study of the separation of allylic and propenylic derivatives of benzene and cyclohexene. They found that only allylic isomers readily formed π -complexes and were thus able to carry out clean separations of the isomeric pairs studied. Hence, it can be seen that this impregnation technique is very useful in the separation of unsaturated compounds, however, for maximum separation the percentage impregnation is dependent on the types of compounds to be separated.

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