

CHROMATOGRAPHY OF ORGANIC COMPOUNDS

I. THIN-LAYER CHROMATOGRAPHY OF OLEFINS*

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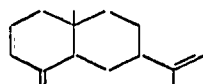
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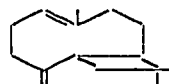
In recent years, thin layer chromatography¹⁻³ has acquired an important place in organic laboratory practice, especially due to its versatility and the speed with which separations can be effected. Though various attempts⁴⁻⁷ at thin-layer chromatography of olefins have been made, results have not been encouraging with olefinic hydrocarbons, due to their rapid movement even with a non-polar solvent like *m*-hexane. In this communication we describe the use of silica gel containing silver nitrate for the thin-layer chromatography of olefinic hydrocarbons, a procedure which we have used with considerable success for the past year or so. Though the results reported in this paper only cover the sesquiterpene hydrocarbons, the method has been used with equal success with other unsaturated hydrocarbons and olefins with functional groups.

The capacity of olefins to complex selectively with the silver cation⁸⁻¹¹ has been exploited for the separation of olefins in column¹², paper¹³ and gas-liquid chromatography^{14,15} and the present work constitutes its extension to thin-layer chromatography**.

During the exploratory phase of this problem, it was found that a mixture of β -selinene (I) and caryophyllene (II) could be separated satisfactorily on silica gel



(I)



(II)

containing 25 % silver nitrate with a solvent system consisting of benzene and 5 % acetone. This system was employed to investigate and standardise the various variables.

Even after taking the usual precautions¹⁷ for ensuring reproducibility in R_F values, the results were not satisfactory and it was soon found out that the mode of preparation of the silver nitrate-silica gel mixture was also an important factor determining the reproducibility of R_F values. A method, as described under procedure, was evolved which always yielded a silver nitrate-silica gel mixture of uniform

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** Recently BARRETT, DALLAS AND PADLEY¹⁸ have described the use of silica impregnated with silver nitrate for the thin-layer chromatography of glycerides.

quality. Furthermore, it was found that it was better to record the data in terms of movement of a standard substance^{18,19} rather than that of the solvent front; two dyes, Sudan III (*p*-phenylazoaniline \rightarrow 2-naphthol) and azo-benzene were found useful for this purpose and the data are given in terms of R_S or R_A values (R_{Dye} values) depending on whether the reference substance is Sudan III (S) or azo-benzene (A) respectively. R_{Dye} is defined by the expression:

$$R_{Dye} (R_S \text{ or } R_A) = \frac{\text{Movement of substance from start in mm}}{\text{Movement of dye from start in mm}}$$

In all experiments the solvent was allowed to rise to a height of 10 cm.

EXPERIMENTAL

Effect of mode of activation on R_S values

The absolute value of R_{Dye} (for a given compound) and the degree of deviation in a set of experiments was found to be dependent on the mode of activation of the plates. Table I summarises the data. On the basis of these results, the method of activation as used in the last set of experiments was adopted for subsequent work. Plates activated in this way could be stored in a desiccator in the dark for two to three weeks without affecting their performance.

TABLE I
EFFECT OF MODE OF ACTIVATION ON R_S VALUES

Mode of activation* (after initial drying in air for 3 h)	Caryophyllene			β -Selinene		
	Number of experiments	Mean R_S value	Maximum deviation	Number of experiments	Mean R_S value	Maximum deviation
$1/2$ h at 105° ; cool 1 h	4	0.289	+ 0.021	4	0.698	+ 0.022
			— 0.007			— 0.007
2 h at 105° ; cool 12 h	24	0.282	+ 0.029	24	0.601	\pm 0.027
			— 0.066			
$2^{1/2}$ h at 105° ; cool 1 h	4	0.257	+ 0.027	4	0.587	+ 0.040
			— 0.016			— 0.020
$2^{1/2}$ h at 105° ; cool 12 h	15	0.259	+ 0.011	15	0.586	+ 0.019
			— 0.012			— 0.015

* Cooling was carried out in a desiccator (silica gel).

Silver nitrate content and R_S values

In order to determine the dependence of the R_S values on the silver nitrate content of the silica gel, a series of mixtures of silica gel containing different amounts of silver nitrate was prepared and the R_S values for caryophyllene and β -selinene (in a mixture) determined. Fig. 1 depicts the data graphically. As anticipated the R_S value is dependent on the silver nitrate content, but the ΔR_S for the two compounds does remain more or less constant. For subsequent work silica gel containing 15 % silver nitrate was selected as it offered a better distribution of the spots for a solvent rise of 10 cm.

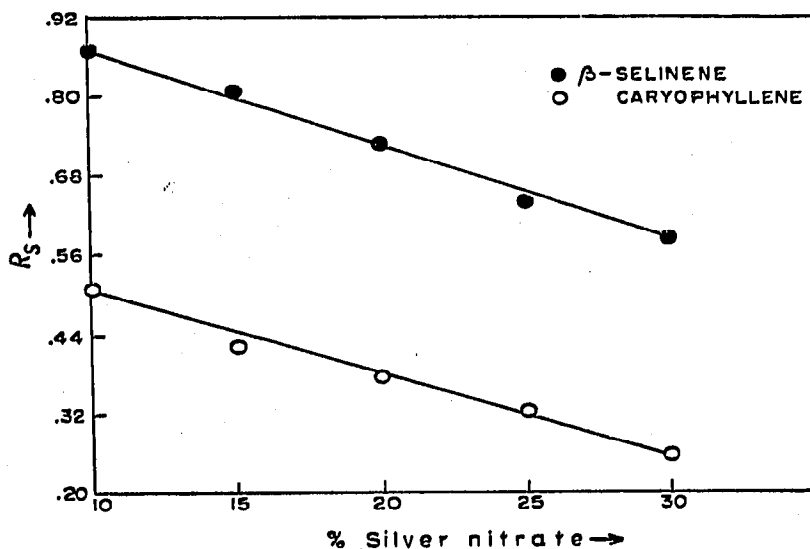


Fig. 1. Variation of R_S with the silver nitrate content.

Solvent system

Having standardised the mode of activation of the plates and the method of preparation of silica gel-silver nitrate mixture, attention was given to the determination of R_S values in different solvents so as to arrive at the most suitable solvent system. Based on the results in Table II benzene containing 5% acetone was selected as the

TABLE II
APPROXIMATE R_S VALUES OF CARYOPHYLLENE AND β -SELINENE IN
DIFFERENT SOLVENTS
(at $28^\circ \pm 2^\circ$)

Solvent	Caryophyllene	β -Selinene
Benzene	0.285	0.807
Toluene	0.299	0.966
Acetone	0.712	0.896
Ethyl acetate	0.609	0.848
Dioxane	0.249	0.623
Di-isopropyl ether	0.764	1.065
Hexane	No movement of dye and compounds	
CH_2Cl_2	No movement of compounds	
CHCl_3	Very slight movement of compounds	
CCl_4	Very slight movement of compounds	
Benzene + 5% acetone	0.422	0.805
Toluene + 5% acetone	0.470	0.844

most suitable solvent mixture for determining the R_S values of some other sesquiterpenes.

R_{Dye} values for some sesquiterpenes

The thin-layer chromatography of olefins, under the conditions described above, was next applied to a larger number of sesquiterpenes, when it was found that certain sesquiterpenes moved along with the solvent front (benzene + 5% acetone). How-

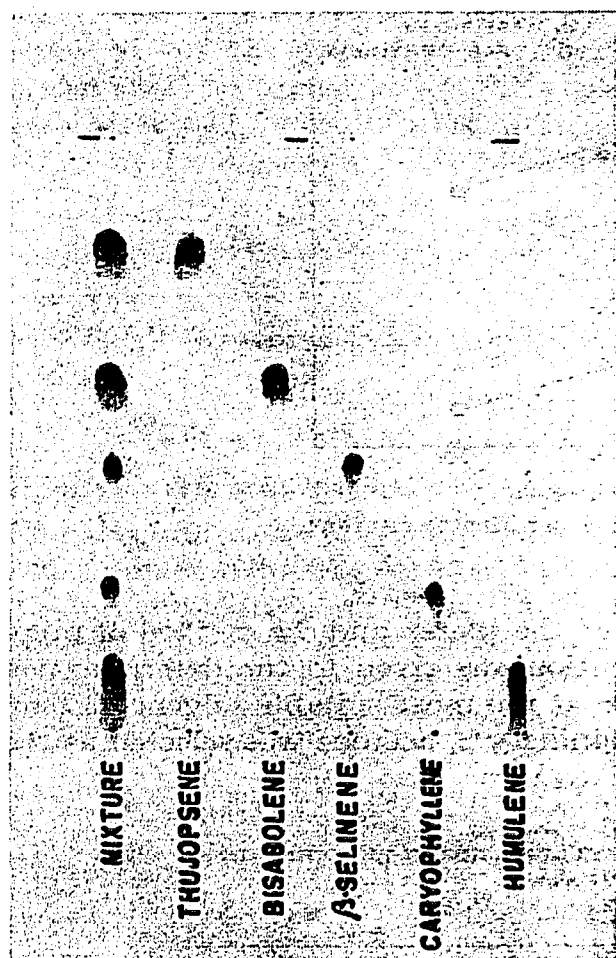


Fig. 2. Thin-layer chromatogram of some sesquiterpenes.

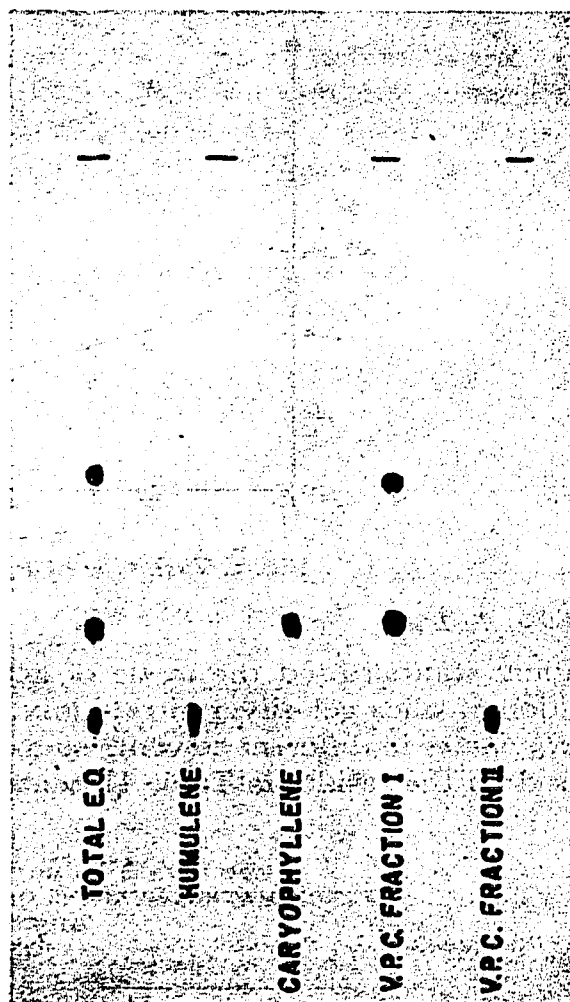


Fig. 3. Thin-layer chromatogram of the essential oil of *Dipterocarpus pilosus* and its fractions.

ever, these compounds could be separated satisfactorily by using *n*-hexane as the developing solvent; since Sudan III does not move at all with the solvent, azobenzene was used as the reference dye and as stated earlier these data are recorded as R_A values. The R_S and R_A values of some sesquiterpenes are given in Table III and Fig. 2 shows a typical thin-layer chromatogram of a mixture of sesquiterpenes.

Thin layer chromatography of sesquiterpenes on silica gel-silver nitrate, in conjunction with gas-liquid chromatography (GLC), has proved of value in the study of essential oils, both in identifying the various components and also in indicating whether a single peak in a gas-liquid chromatogram represents one component or, possibly, is still a mixture. For example, Fig. 3 shows a thin-layer chromatogram of the essential oil from *Dipterocarpus pilosus*; its GLC over diethylene glycol showed only two peaks, which were perfectly symmetrical, however, thin-layer chromatography showed that the first fraction contained another component, besides caryophyllene. The identification of humulene and caryophyllene in the essential oil was aided by determining the retention times and the R_S values of its components and comparing them with standard values.

TABLE III
R_{Dye} VALUES OF SOME SESQUITERPENES
 (at 28° ± 2°)

Compound	No. of olefinic linkages in the molecule	<i>R_S</i> *	<i>R_A</i> †
Humulene	3	0.189	—
β-Elemene	3	0.331	—
Caryophyllene	2	0.422	—
β-Selinene	2	0.805	—
β-Bisabolene	3	0.936	—
Thujopsene	1	1.149	—
Copaene	1	1.161	—
<i>α</i> -Himachalene	one aromatic ring	1.164	—
α-Gurjunene	1	1.168	—
β-Himachalene	2	—	3.155
α-Himachalene	2	—	5.190
Longifolene	1	—	5.357
Cuparene	one aromatic ring	—	5.391
Iso-longifolene	1	—	7.447
Longicyclene	nil	—	8.399

* Solvent system: Benzene + 5% acetone.

† Solvent system: *n*-Hexane.

PROCEDURE

Materials

Silica gel used for this work was "Silica gel less than 0.08 mm for chromatography"*. Sieve analysis showed that 97% passed through 200 mesh.

The terpene hydrocarbons were pure samples, either available in this laboratory or supplied by the courtesy of other workers.

Azobenzene (1 g) was purified by passing its benzene solution through alumina (activity I, 30 g) and further eluting with benzene. Sudan III was first crystallised from ethanol and then purified as above. Purified materials gave single spots in thin-layer chromatography (silica gel/benzene).

Only purified anhydrous solvents were employed.

Silica gel-silver nitrate mixture

An aqueous solution of silver nitrate (National Refinery, India) 7.5 g in 7.5 ml water, was diluted with 125 ml alcohol and 50 g silica gel was added gradually to this, with stirring. After stirring for a further 15 min, the aqueous alcohol was removed by suction (water-bath) with constant shaking and the residue finally dried in vacuum to constant weight. Plaster of paris (—200 mesh) was added to this dry powder, 7.5 g for every 57.5 g of silica gel-silver nitrate, and this was well mixed by thoroughly shaking in a mechanical shaker. The tan-coloured preparation, if stored in the dark, will keep well for a few months.

* E. Merck A.G., Darmstadt, Germany.

General conditions

Smooth glass plates (thickness 0.3 cm) in two sizes (3.5×15 cm and 10.0×15 cm) were used. The silica gel-silver nitrate mixture (1 part) was slurried with water (2.5 parts) by gently rubbing in a pestle and mortar and the plates coated with the slurry with the aid of an applicator (Fig. 4). Two different sizes of applicators were used for the two sizes of glass plates. The applicators, which can be cheaply and easily constructed, are made in stainless steel. The design is based on "Sheen's film applicator for paints and varnishes"*. The applicators give a uniform film of 0.5 mm and 0.3 mm thickness with the smaller and the large applicator respectively, and the hopper dimensions are such that with a slurry made from 4.0 g or 6.0 g of the material, four smaller or four larger plates can be made with the appropriate applicator.

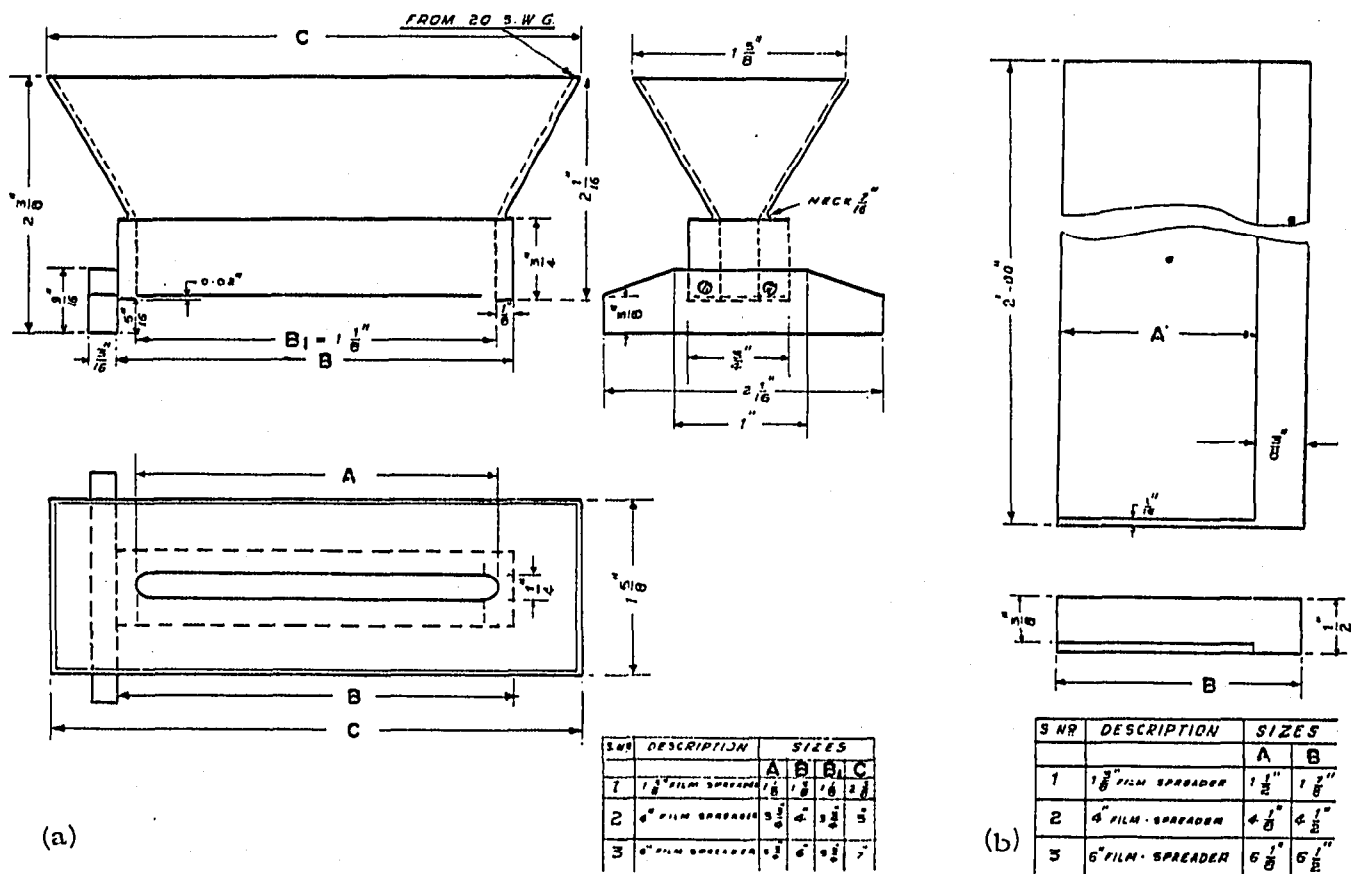


Fig. 4. Stainless steel applicator.

The plates were dried in the air for 3 h and then activated at 105° for $2\frac{1}{2}$ h and finally stored in a desiccator over silica gel until needed.

The starting points were marked on the plates at 2 cm from the lower edge with a distance of 1 cm in between.

1-10 μ l of a 1% solution of the substance in hexane (dyes were applied as 0.2% solution in acetone) was applied at the starting points. After a few minutes, the plates were placed in glass cylinders (18.5 cm \times diam. 5 cm) having a 1 cm high segment of a

* Sheen Instruments Ltd., London.

glass tube (diam. 3 cm) to act as a stand for the plate. The cylinders contained the appropriate solvent to a height just below that of the plate stand. These were tightly closed with a cork with a small plugged hole, through which more solvent could be introduced later. The jars, also, contained a semi-cylindrical strip of filter-paper of height almost equal to that of the cylinder in order to help in the saturation of the cylinder atmosphere with the solvent vapours¹⁸. The larger plates were placed in a glass jar (9.8 cm × 15.0 cm × 19.8 cm) fitted with a suitable glass stand to hold two such plates, and carrying a filter-paper strip along the sides (height 15 cm) immersed in the solvent, and closed by a ground-glass cover. After the plates had been allowed to stand for 15 min to equilibrate, more solvent was added so as to wet the glass plates to a height of about 0.5 cm. Development was stopped when the solvent reached the pre-marked height of 10 cm; development took about 20–25 min.

After development the plates were dried in air (20 min) and the dried plates were sprayed with a solution of chlorosulphonic acid in acetic acid (1:2)²⁰ and heated for 10 min at 130°. Usually different coloured spots were observed, e.g. humulene, brown; caryophyllene, blue; longifolene, pink.

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SUMMARY

A method for the thin-layer chromatography of olefins over a silica gel–silver nitrate mixture is described.

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