Thin Layer, Column, and Gas Liquid Chromatography of Resin Acid Esters and Related Terpenes

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The ability of olefins to form co-ordination compounds with silver ions has been used for the chromatographic separation of various unsaturated compounds (*e.g.* Refs. [1-3]). A simple method for the separation of various lipids using silica impregnated with silver nitrate has recently been described [4-6]. This highly selective silver nitrate-silica adsorbent has now been found to be very useful for the separation and identification of various terpenes, particularly those of "rosin" products for which simple chromatographic methods are needed (*cf.* Ref. [2]).

On thin layer chromatography using the silver nitrate-silica adsorbent all the common resin acid methyl esters separate almost completely when the chromatogram is developed with benzene (Table 1). The compounds appear as round spots. The R_F-values vary somewhat with the quality and the dryness of the adsorbent. It is therefore advisable to run reference substances in order to identify the components of a mixture.

Thin layer chromatographic data for some other diterpenoid compounds are given in Table 2. Generally the chromatograms of hydrocarbons, esters, ketones, and aldehydes can be developed with light petroleum-benzene mixtures and those of the alcohols with isopropyl ether or ethyl ether.

Separations achieved with the above thin layer chromatographic method can usually be done on a preparative scale according to the general method of de Vries [3]. The examples (see Experimental) chosen to illustrate the applicability of this preparative method have been taken from an investigation on the wood constituents of *Pinus silvestris* in progress [7] in this Laboratory.

The above method for thin layer and column chromatography has a wide applicability for the separation and purification of many other terpenes and we have used it successfully in a number of cases.

Gas liquid chromatography (G.L.C.) is a valuable qualitative complement to the above methods of analysing "rosin" products. The facile thermo-isomerisations of methyl levopimarate and methyl palustrate have previously [8] limited the use of this technique for the analyses of resin acid ester mixtures. This disadvantage has now been overcome by using a carefully deactivated silicon rubber gum column of low liquid loaded type [9] operating at a low column temperature. Under these conditions the resin acid methyl esters with pimarane skeleton are completely separated (Table 1). Two of the esters with abietane skeleton, methyl levopimarate and methyl palustrate, have identical retention times. A mixture, in which these two compounds occur together (*e.g.* as indicated by thin layer chromatographic data), can be analysed as follows. A sample of the free acids is heated at 155° for 4 h under an atmosphere of dry nitrogen in a sealed tube *. Under these conditions palustric acid does not isomerise to any measurable extent while levopimaric acid is isomerised to a mixture of palustric (35%), abietic (50 %), and neoabietic (15 %) acid [11]. Methylated samples of the heated and non-heated acid mixtures are then analysed by G.L.C. An approximate estimation of the amounts (in per cent) of levopimaric (C_L) and palustric (C_P) acids in the non-heated mixture is obtained from the expressions:

$$C_L = (C_1 - C_2)/0.65; C_P = (C_2 - 0.35\,C_1)/0.65$$

C_1 and C_2 are the levopimarate-palustrate peak area in percentage of the total peak areas before and after the heat treatment, respectively.

Experimental. Thin layer chromatography. The plates were prepared according to the method of Barret *et al.* [5]. They were dried at 120° for 1 h and could be stored in a black painted desiccator without darkening for a few weeks. Slight darkening has very little influence on the efficiency of the plates The chromatograms were developed according to the usual procedure with ascending solvent. The compounds were detected by spraying the plates

* In order to obtain reproducible results in this thermo-isomerisation process, the resin acid mixture should be freed from other acids by precipitation with cyclohexylamine in light petroleum and the salts decomposed with boric acid [11].

Table 1. Gas liquid chromatography (G.L.C.) and thin layer chromatography (T.L.C.) data for resin acid methyl esters.

Type of skeleton	Methyl ester	G.L.C. Relative retention time	T.L.C. R_F-value*	T.L.C. Colour of the spot
Pimarane	Pimarate	1.00**	0.40	Blue
	Sandaracopimarate	1.09	0.27	Violet
	Isopimarate	1.21	0.32	Brown
Abietane	Levopimarate	1.33	0.50	Grey
	Palustrate	1.33	0.60	Yellow
	Dehydroabietate	1.53	0.83	Yellow-brown
	Abietate	1.82	0.75	Blue-grey
	Neoabietate	2.13	0.73	Grey

* Developed with benzene.

** Methyl pimarate (reference) has the retention time 26.2 min at a column temperature of 150° and a gas flow of 54 ml/min.

Table 2. Gas liquid chromatography (G.L.C.) and thin layer chromatography (T.L.C.) data for some diterpene aldehydes, alcohols, and hydrocarbons.

Compound	G.L.C. Retention time min *	T.L.C. R_F-value	T.L.C. Solvent
Pimarinal	17.7	0.36	Benzene
Isopimarinal	20.9	0.24	
Dehydroabietinal	26.9	0.56	
Abietinal	30.1	0.52	
Pimarinol	24.4	0.47	Isopropyl ether
Isopimarinol	30.2	0.36	
Abietinol	39.9	0.47	
Pimaradiene	10.8	0.73	Benzene
Isopimaradiene	13.1	0.52	

* Column temperature, 150°; gas flow, 54 ml/min.

with 10 % solution of antimony pentachloride in chloroform. The colours of the spots darkened when the plates were heated at 100° for 10 min. Eluents and R_F-values are given in Tables 1 and 2.

Column chromatography. Silver nitrate (125 g) in water (300 ml) was added to silica (450 g, particle size 0.15–0.30 mm). The slurry was shaken for 5 min. The water was then evaporated on a steam bath under reduced pressure in a rotatory evaporator. The residue was dried in an oven at 130° for 15 h. The resulting powder was almost white and could be stored in a dark bottle for a few months without significant darkening. Columns were packed in the same way as ordinary silica columns and wrapped in dark paper. Most mixtures were separated using 50 g of adsorbent for each gram of mixture.

Resin acid methyl esters. A crude ester mixture (9.0 g) prepared from a resin acid fraction of *Pinus silvestris* [7] was adsorbed on silver nitrate-silica (150 g). Elution with the solvents indicated gave the following fractions:

(*1*) Light Petroleum (L P., b.p. 40–60°; 1200 ml), 0.03 g; (*2*) 2 % Ether (E.) in L.P. (300 ml), 1.04 g; (*3*) 2 % E. in L.P. (400 ml), 3.03 g; (*4*) 2 % E. in L.P. (300 ml), 1.04 g; (*5*) 5 % E. in L.P. (300 ml), 1.01 g; (*6*) 5 % E. in L.P. (900 ml), 2.04 g; (*7*) 10 % E. in L.P. (2100 ml), 1.26 g.

On rechromatography on 150 g of adsorbent and elution with ether (2 %) in light petroleum fraction (*3*) gave methyl dehydroabietate in the first fractions and methyl abietate in the latter fractions. Fraction (*4*) was a mixture of methyl abietate, neoabietate, palustrate, pimarate, and levopimarate. These constituents could, however, be separated by rechromatography on 100 g of adsorbent using ether (2 %) in light petroleum as eluent. Fraction (*5*) consisted of almost pure methyl levopimarate. M.p. and mixed m.p., 64–65°, infrared and ultraviolet spectra were identical with those of an authentic sample.

Diterpene aldehydes. An aldehyde mixture [7] (1.0 g) was adsorbed on silver nitrate-silica (30 g). Light petroleum (1000 ml) with a linear concentration gradient (0 → 4 %) of ether was used to elute the following main fractions (25 ml fractions were collected): (*15–16*) 0.03 g dehydroabietinal; (*17–18*) 0.04 g abietinal. (*20–25*) 0.29 g pimarinal; (*32–40*) 0.18 g isopimarinal.

Diterpene alcohols. The alcohol mixture [7] (1.0 g) was adsorbed on silver nitrate-silica (30 g). Light petroleum (1800 ml) with a linear concentration gradient (0 → 20 %) of ether was used to elute the following main fractions (50 ml fractions were collected): (*24–25*) 0.16 g pimarinol; (*27*) 0.03 g abietinol; (*31–35*) 0.07 g isopimarinol. Of interest is that abietinol and pimarinol separate on column chromatography whereas on thin layer chromatography these two alcohols have identical R_F-values.

Diterpene hydrocarbons. The hydrocarbon mixture [7] (1.0 g) was adsorbed on silver nitrate-silica (30 g). Light petroleum (850 ml) with a linear concentration gradient (0→1 %) of ether was used to elute the following main fractions (50 ml fractions were collected): (*8–9*) 0.23 g pimaradiene; (*13–17*) 0.12 g isopimaradiene.

Gas liquid chromatography. The analyses were made on a Pye Argon Chromatograph, Cat. No 12000 (column length, 1.2 m; inner diameter, 4 mm) equipped with an inlet heater which in all the experiments was adjusted to about 200°. The stationary phase was made according to a procedure described by Horning *et al.* [9] The acid washed and pre-siliconized support material (100–120 mesh Gas-Chrom P; Applied Science Laboratories, INC, State College Pennsylvania, USA) was coated with silicon rubber gum (E 301; Imperial Chemical Industries, Nobel Division, Glasgow, U.K.; 1 % of the weight of the support material). The packed column was conditioned with a slow nitrogen flow at 230° for 15 h and was then ready for use. Relative and absolute retention times, column temperature, and gas flow for the various compounds are given in Tables 1 and 2.

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Added in proof. After the completion of our work we have learned that Zinkler *et al.* (D. F. Zinkler, *Private communication*) have carried out a similar investigation on the separation of resin acid methyl esters by thin layer chromatography (Zinkler, D. F. and Rowe, J. W. *J. Chrom. In the press*) and by gas liquid chromatography (Zinkler, D. F. and Nestler, F. H. M. *Anal. Chem. In the press*).

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