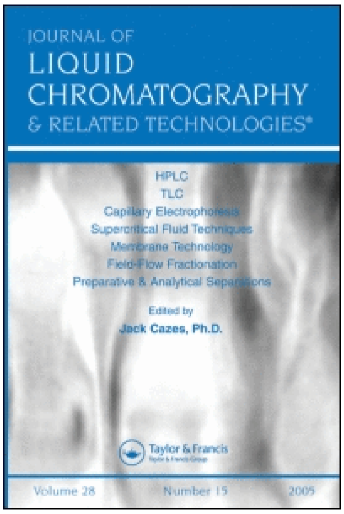


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

EARLY EXPERIMENTATION IN THE LIQUID CHROMATOGRAPHY OF NATURAL AND SYNTHETIC PRODUCTS

A. A. Durrani[a]; A. Garrett[a]; R. A. Johnson[a]; S. K. Sood[a]; J. H. P. Tyman[a]
[a] Department of Chemistry, Brunel University, Uxbridge, Middlesex, UK

To cite this Article Durrani, A. A. , Garrett, A. , Johnson, R. A. , Sood, S. K. and Tyman, J. H. P.(2002) 'EARLY EXPERIMENTATION IN THE LIQUID CHROMATOGRAPHY OF NATURAL AND SYNTHETIC PRODUCTS', Journal of Liquid Chromatography & Related Technologies, 25: 10, 1543 — 1559
To link to this Article: DOI: 10.1081/JLC-120005703
URL: http://dx.doi.org/10.1081/JLC-120005703

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

J. LIQ. CHROM. & REL. TECHNOL., 25(10&11), 1543–1559 (2002)

EARLY EXPERIMENTATION IN THE LIQUID CHROMATOGRAPHY OF NATURAL AND SYNTHETIC PRODUCTS

A. A. Durrani, A. Garrett, R. A. Johnson, S. K. Sood,
and J. H. P. Tyman*

Department of Chemistry, Brunel University, Uxbridge,
Middlesex, UK

ABSTRACT

Some experiences in practical aspects of TLC are reviewed, including the role of particle size of silica gel G, the use of different oxide adsorbent layers, the employment of TLC-type silica gel H alone and with silver nitrate in argentation column chromatography; also the use of silica gel G containing silver ion in sesquiterpene separations and of boric acid and sodium arsenite impregnated layers for derivatives of vicinal diols from hexoses.

Key Words: Oxide adsorbents; Particle size (SiO_2); Silver ion TLC; Boric acid TLC; CC with TLC; Sesquiterpene separations

INTRODUCTION

The role of the particle size of the adsorbent in TLC may well be overlooked at present when commercial plates, of excellent quality and performance for

---

*Corresponding author. E-mail: jhptyman@hotmail.com analytical and preparative TLC and HPTLC, are readily available. Even in the days prior to this era, when plates were largely self-prepared and spreaders often self-constructed,[1] particle size, for example of commercial silica gel G, was usually tacitly accepted and unquestioned as perfectly adequate. Nontheless, commercial TLC plates are an invaluable acquisition in quantitative work[2,3] compared with the self-prepared variety, although, for very extensive qualitative analytical and monitoring use these have still remained the inexpensive choice for many investigators. In a major text[4] remarkably little information is given on particle size, surface area, pore size, and pore volume of silica gel for TLC, although, subsequent accounts[5] have given general specifications, as for example, that for TLC the average particle diameter is 10–20 μm and for HPTLC, 5–10 μm. Nevertheless, in 1976, silica gel G produced by Merck was described as possessing a particle size range of 3–50 μm, a surface area of 400 m^2/g, a pore size of 40 Å, and pore volume of 0.7–0.8 cm^3/g.

Our experimental work, before and at that time, was directed to the use of a range of silica gels of different surface areas, pore volumes, and average particle sizes, which were used to prepare standard TLC plates and then examined for the separation of a wide variety of organic substrates, including oil soluble dyes, the component phenols of cashew, synthetic food colors, pesticides, and isomeric nitroanilines and phenols.[6] Argentation experiments were conducted with the monene, diene, and triene constituents of cardanol, a major component phenol of technical cashew nutshell liquid (CNSL).

The colorless oxides of the metals in Period 3 of group IV(a and b), namely stannic, titanium, and zirconium oxides and in group IIb, zinc oxide, had not, in the early seventies, been examined for TLC use apart from isolated experimentation[7] and our own work then[6] with new adsorbents benefited from the initial study of the influence of particle size with silica gel used as a control with the same range of solutes.

Our findings on the advantages of using silica gel of high surface area and low, narrow range particle size, led to a potential application in column chromatography[8] for obtaining greater band resolution and the usage of much higher solute/adsorbent ratios, since generally, the prevalent practice at that time involved relatively large particle size adsorbents and, consequently, very low ratios. The use of a pressurized column was obligatory to accelerate the separation and avoid diffusion of bands encountered in slower operations, both of which factors are essentially those operative in ‘flash chromatography’ which was described at that time.[9] The same methodology[8] was also applied in argentation column chromatography, in which dry-packed silver nitrate/silica gel mixtures were used. In both practices, much greater amounts of solute were separated and, also, column reuse proved feasible.

Argentation TLC, when applied to complex mixtures, can result in many overlapping bands and, advisedly, is assisted by a preliminary separation of the mixture on an ordinary plate.[10] In the case of the sesquiterpenes found in *Gosweilerodendron balsiferum*, combined argentation TLC/GC separation or vice versa proved necessary[11] to locate and identify some of the many sesquiterpenes present.

The TLC behaviour of cyclic-1,2-diols and of derivatives from α- and β-glucose, galactose and α-mannose, α-altrose and α-allose on boric acid and on sodium arsenite impregnated plates is described.

All these aspects of TLC methodology were investigated in the sixties and early seventies but remained unpublished at the time.

EXPERIMENTAL

Experiments with Different Particle Size Silica Gel G

A range of silica gel samples, which possessed the properties listed in Table 1 were obtained from Unilever Ltd, Port Sunlight Cheshire. They were of type H (without binder), and for the preparation of coated microscope slides the following steps were used: (1) 10% Calcium sulfate was incorporated. (2) A mixture was prepared with water to afford a cream, which was ground in a mortar for 5 min, allowed to stand for 2 min, and then spread (Ref. 1), in the usual way, on cleaned microscope slides. (3) The resultant slides were dried at 50°C for 30 min and then at 110°C for 30 min. (4) The dried slides were cooled and stored in a desiccator.

TLC separations were conducted with slides marked with a groove 1 cm from the edge and developed until the particular solvent reached that line. All the solutes with the exception of CNSL were standard laboratory chemicals supplied by Koch Light, Colnbrook, Bucks., UK. CNSL was obtained from British Coco Mills, Hull, UK. Visualization of colorless solutes was effected with iodine vapour.

Table 1. Properties of Silica Gel of Different Types

Sample Size	Surface Area (m^2/g)	Pore Volume (mL/g)	Average Particle (μm)
Gasil 200	800	0.3	4–5
Sorbsil U-30	400	0.7	5
Gasil 35	300	1.2	3–4
Neosyl ES-992	55	–	14

The solutes (solvent) were as follows: Oil Soluble Dyes, Rhodamine B, Sudan Black, Sudan 4 (chloroform); Cardanol, technical CNSL, and cardol (chloroform); synthetic food colours, Orange II, Brilliant black BN, Amaranth (2.5% aq. sodium citrate–25% aq. ammonia, 80 : 20); pesticides, dieldrin, DDT, aldrin (cyclohexane–chloroform, 80 : 20); nitroaniline isomers, ortho, meta, para (chloroform); nitrophenol isomers, ortho, meta, para (chloroform–ethylacetate, 90 : 10).

The silica gels were impregnated with 15% silver nitrate and saturated cardanol, and a mixture of the monoene, diene, and triene constituents examined in chloroform–ethyl acetate, 85 : 15 and visualized with dichlorofluorescein.

Experiments with New Adsorbents, Titanium Dioxide, Zinc Oxide, Zirconium Dioxide, and Stannic Dioxide

Titanium dioxide was obtained from British Titan and zirconium oxide from Mr A. K. Rahman, Surface Chemistry Laboratory, Brunel University.

Titanium dioxide in the size range 53–90 μm and found, subsequently, to be of low surface area, proved ineffective. Since it was desirable to employ an oxide layer with particle size comparable to Merck silica gel (3–50 μm), the titanium dioxide sample was milled for 5 min in a centrifugal ball mill and then sieved through an Endecotts (53 μm) sieve, resulting in the majority of the oxide laying well below 53 μm. After several trials, titanium dioxide, zinc oxide, zirconium dioxide, and stannic oxide TLC slides were all prepared by the same procedure as that given for silica gel layers. Titanium dioxide produced a tenacious layer, while those from zinc oxide and zirconium dioxide proved mechanically weak, and stannic dioxide layers tended to chip. Iodine was used for visualization, since fluorescent indicators were ineffective with ZnO or TiO_2.

The range of solutes used was as follows: the same three oil soluble dyes, the CNSL series, pesticides, the isomeric nitroanilines and nitrophenols.

In the $AgNO_3$ impregnated plate series, ZnO and ZrO_2 proved reactive, giving dark layers, although, incorporation was satifactory with SnO_2 and TiO_2.

Experiments on the Use of TLC Grade Silica Gel H in Column Chromatography

The improved TLC separations found with low particle size silica gel G led to column chromatographic use.[8] Silica gel H (type 60 article 7736 and article 9385 E. Merck, Darmstadt, West Germany) was used, and for comparison, silica gel MFC (from BDH Ltd., Poole, Dorset, UK). Technical CNSL was obtained from Minnesota 3 M Research, Harlow, Essex, UK. Column chromatography was carried out with wide bore glass columns (i.d. 6.5 cm × 50 cm) equipped with a sintered disc (porosity 2), PTFE stopcock, and tightly fitting bung or stopcock having a glass entry tube for admission of solvent and for air pressurization, either manually or by pump (Hyflo model B, Metcalf Bros., London, UK). Column packing and consolidation with a known weight of silica gel and volume of light petroleum was concluded by capping of the adsorbent with three circles of filter paper to ensure even descent of the solute. Gradient elution with light petroleum (60–80°C)/diethyl ether was used, and all fractions were monitored by analytical TLC as described. Comparative separations with silica gel MFC were carried out with unpressurized columns (i.d. 2.5 cm × 65 cm) following similar separations[12] on Davison grade silica gel (60–120 mesh).

Dry incorporated argentation columns were prepared from silica gel H and finely ground silver nitrate (in sieve distribution, 94.6% passed through 500 μm, 87.1% through 355 μm, 68.1% through 210 μm, and 9.4% through 53 μm). Gradient elution was effected with light petroleum/diethyl ether and all fractions were monitored. The cardanol used contained the saturated (3.01%), monene (25.1%), diene (18.2%), and triene (43.4%) constituents.

Separation of Sesquiterpenes by Argentation TLC/GC and by GC/TLC

Agba gum resin from trees of the Balsaminaceae species, *G. balsiferum*, was obtained originally from the United Africa Co. Ltd. The first GC was carried out on a Griffin and George instrument equipped with glass columns containing celite coated with carbowax 20 M and detection by katharometer. Preparative GC was effected on a glass column (6′ × 1/8″) containing Chromosorb W coated with 15% carbowax 20 M at 135°C, with helium as carrier gas, with the help of the Development Laboratory, Perkin Elmer Ltd., Beaconsfield, Bucks, UK. The collection of fractions was confined to four main peaks, which were then separated by preparative argentation TLC on silica gel G with 15% silver nitrate. Subsequently, GC/MS was carried out on a Perkin Elmer 880 instrument (coupled to an MS9 mass spectrometer) equipped with a similar column.

Caryophyllene, was purified by preparative TLC and preparative argentation TLC on silica gel G, with 15% $AgNO_3$. Isocaryophyllene was prepared from caryophyllene by nitrosation and by isomerization, and TLC purification effected in the same way. α-Copaene and α-humulene were separated by argentation and preparative TLC from hop oil obtained from the Department of Hop Research, Wye College, Ashford, Kent, UK. ^1H NMR spectra were carried out on a Varian T60 instrument and on a 220 mHz instrument by the Physico Chemical

Measurements Unit (PCMU). Infrared spectra were obtained on a SP200 spectrometer with solutes on KBr discs.

TLC of Cyclic Vicinal Diols on Boric Acid and Sodium Arsenite Impregnated Layers

Cis and *trans*-cyclohexane-1,2-diols were obtained from cyclohexene by a variety of syntheses and purified by preparative TLC. 4,6-O-Benzylidene-2,3-dihydroxy-1-methoxy derivatives of α- and β-glucose, α- and β-galactose, α-mannose, α-altrose and α-allose were used, and the migration behaviour of this series was examined on silica gel G impregnated first with 15% of sodium arsenite and then with 15% boric acid.

RESULTS AND DISCUSSION

Experiments with Different Particle Size Silica Gel

The results from the use of TLC plates made with different particle grade silica gel are summarized in Table 2 for the solutes listed in the Experimental section.

The results show, that in all cases, relatively better separations were achieved with the use of higher surface area silica gel G, and graphical plots of R_f/surface area indicated, that in many cases, the relationship was linear. Departures from linearity might be anticipated with the adsorbents of higher pore volume leading to trapping of the solute and a lower R_f value, but the effect was only partially demonstrable, e.g., in the case of the large dye Brilliant Black BN(2) compared with smaller dyes Orange II(1) and Amaranth(3) on the Sorbsil layer compared with Gasil 200. Gasil 200 containing silver ion afforded the best separation of saturated cardanol from the 8′-monoene.[10] Smaller particle size adsorbents led to increased times for ascent of the solvent, a factor slightly detrimental to the speed of obtaining results, a key factor in TLC monitoring. It was noted in graphical plots, particularly with the pesticides, synthetic food colors, and the components of CNSL, that for small changes in surface area between 300 and 500 m^2/g, marked variation of R_f values occurred.

At the time this work was carried out, the commercial Merck description of laboratory grade indicated that silica gel, 250 g of particle size 3–50 μm, surface area 400 m^2/g, pore size 40 Å, and pore volume 0.7–0.8 cm^3/g was mixed with 50 g silica gel having particle size 3–15 μm and surface area 380 m^2/g. Thus, the product was within the critical range observed notably for the above mentioned three solutes. Our observations were passed to Mr Osman, Merck Ltd.,

Table 2. The Effect of Particle Size on TLC Migration

		Oil Soluble Dyes		
Adsorbent	Rhodamine B	Sudan Black		Sudan 4
Gasil 200	0	70	26	76
Sorbsil U-30	0	73	39	75
Gasil 35	0	70	40	78
Neosyl ES-992	4	86	58	88
		CNSL (Technical)		
Adsorbent	Cardanol	2-Methylcardol		Cardol
Gasil 200	66	32		11
Sorbsil U-30	73	52		14
Gasil 35	60	–		12
Neosyl ES-992	73	–		41
		Synthetic Food Colours		
Adsorbent	Orange II	Brilliant Black		Amaranth
Gasil 200	87	78		22
Sorbsil U-30	98	36		18
Gasil 35	98	85		55
Neosyl ES-992	98	91		56
		Pesticides		
Adsorbent	Aldrin	DDT		Dieldrin
Gasil 200	43	21		3
Sorbsil U-30	45	28		5
Gasil 35	38	24		5
Neosyl ES-992	78	53		33
		Nitroanilines		
Adsorbent	0	m		p
Gasil 200	64	33		31
Sorbsil U-30	36	22		22
Neosyl ES-992	29	22		22
		Nitrophenols		
Adsorbent	0	m		p
Gasil 200	98	67		55
Sorbsil U-30	85	56		55
Neosyl ES-992	72	57		54
		The Unsaturated Constituents of Cardanol on 15% Argentated Adsorbents		
Adsorbent	Saturated	Monoene	Diene	Triene
Gasil 200	92	90	79	60
Sorbsil U-30	83	83	65	37
Neosyl ES-992	89	89	68	60

Hampshire, UK at the time we were examining the role of particle size in both TLC and in column chromatography. Further work is desirable with a range of homologous and isomeric solutes and a selected range of silica gels to examine, in depth, the relationship between particle size, pore volume, and size with TLC migration behavior.

Gasil 200 silica gel, and to some extent Gasil 35 and Sorbsil U-30, do resemble the material favoured for HPTLC, which was introduced in the late seventies with particle size 5–15 μm by comparison with TLC material of particle size 10–60 μm having a 20 μm mean. The influence of particle size in chromatography has been discussed.[13]

Experiments with New Adsorbents, Titanium Dioxide, Zinc Oxide, Zirconium Dioxide, and Stannic Dioxide

The results from the use of new adsorbents are summarized in Table 3. The results with the same range of solutes as with the particle size studies, indicate that titanium dioxide and stannic dioxide were more effective for TLC separations than zinc oxide or zirconium dioxide. The separations of Sudan black from Sudan 4 with either TiO_2 or SnO_2 of the isomeric nitroanilines with TiO_2, and of the saturated from the unsaturated constituents of cardanol with ether oxide with 15% incorporation of silver nitrate, were specific improvements compared to silica gel G. Titanium and stannic dioxides were the easiest to use of the four adsorbents, since the layers were formed readily, streaking was not observed, and solute spots did not become large or blurred. The initial poor performance with laboratory grade TiO_2 of low surface area, $8\,m^2/g$, was greatly enhanced after milling to $167\,m^2/g$, although a corresponding treatment of ZnO failed, yet proved satisfactory with SnO_2. Titanium and stannic dioxides were brittle and more readily formed a favorable particle size distribution with more than 70% less than 25 μm in size, whereas with zinc oxide the same proportion of particles was greater than 50 μm. Our experience with TiO_2 differed from that described,[7] in which TiO_2 of surface area $38.3\,m^2/g$ and pore volume $0.35\,cm^3/g$ was used and led to a development time of 13 min, relative to 28 min and 39 min for SiO_2 and Al_2O_3, respectively, for layer thicknesses of 120–260 μm. Nevertheless, the ready availability, cheapness, density, and low toxicity (TLV) of TiO_2 are favourable compared with the other oxides. The surface areas of the oxides used, development times, costs, densities, and relative toxicities are summarised in Table 4.

In summary, TiO_2 gave separations equal to those obtained with silica gel G, and better with TiO_2 containing silver ion, but excluding boric acid impregnated plates. Visualization in the present work involved the use of either iodine or sulfuric acid charring.

Table 3. Migration Properties of Solutes on New Adsorbents

		Oil Soluble Dyes		
Adsorbent	Rhodamine B	Sudan Black		Sudan 4
Silica Gel G	0	60	31	60
Titanium dioxide	0	5	5	75
Zinc oxide	(0–56)	98	93	98
Zirconium oxide	(0–27)	98	96	98
Stannic oxide	6	68	6	93
		CNSL (Technical)		
Adsorbent	Cardanol	2-Methylcardol		Cardol
Silica Gel G	80	32		9
Titanium dioxide	64	–		5
Zinc oxide	88	–		10
Zirconium dioxide	95	–		25
Stannic oxide	82	–		5
		Pesticides		
Adsorbent	Aldrin	DDT		Dieldrin
Silica Gel G	54	32		4
Titanium dioxide	60	20		6
Zinc oxide	87	56		(0–23)
Zirconium dioxide	85	65		(0–33)
Stannic oxide	60	35		6
		Nitroanilines		
Adsorbent	0	m		p
Silica Gel G	44	50		70
Titanium dioxide	22	4		60
Zinc oxide	–	–		–
Zirconium dioxide	72	81		91
Stannic oxide	53	60		82
		Nitrophenols		
Adsorbent	0	m		p
Silica Gel G	85	54		48
Titanium dioxide	75	57		55
Zinc oxide	–	–		0
Zirconium dioxide	48	30		30
Stannic oxide	96	36		36
		The Unsaturated Constituents of Cardanol		
Adsorbent	Saturated	Monoene	Diene	Triene
Silica Gel G	88	84	60	37
Titanium dioxide	87	67	25	9
Zinc oxide	–	–	–	–
Zirconium dioxide	–	–	–	–
Stannic oxide	94	88	54	21
Alumina	88	76	50	22

Table 4. Characteristics of Different Adsorbents

Adsorbent	Surface Area (m^2/g)	Devt. (min)	Layer	Price (£/0.5 k)	Density	TLV (mg/m^3)
TiO_2	167	45	Strong	0.68	4.0	15
ZnO	6	30	Weak	0.57	5.6	5
ZrO_2	117	10	Weak	0.93	5.6	5
SnO_2	155	10	Strong	2.40	6.6	2
SiO_2 gel	350	5	Strong	3.43	2.6	20

Experiments on the Use of TLC Grade Silica Gel H in Column Chromatography

The practical separation of the component phenols in technical CNSL by liquid chromatography,[8,14] on silica gel H (TLC type) with solute/adsorbent ratios of 1/4, is depicted in Table 5. Cardanol (I), 2-methylcardol (II), and cardol (III) (Fig. 1) have unsaturated constituents, namely the 8(Z)-monoene, 8(Z), 11(Z)-diene, and the 8(Z), 11(Z), 14-triene.

For the first three separations mentioned in Table 5 with TLC grade silica gel H, 30.2 g, 34.97 g, and 35.6 g of semi-purified CNSL were used, and in the fourth, 25.6 g technical CNSL was chromatographed. Analytical monitoring

Table 5. Column Chromatography of Technical CNSL (25.6 g) on Silica Gel H TLC Type (Article 7736, 125 g, 4th reuse)

Combined Fractions	Weight (g, %)	Solvent	Volume (mL)	Component Phenol
1	17.68, 69.1	P	650	Cardanol
		P/E(12 : 1)	650	Cardanol
		P/E(10 : 1)	650	Cardanol
2	0.90, 3.5	P/E(7 : 1)	650	Cardanol, 2-Mecardol
3	0.98, 3.9	P/E(3 : 1)	650	Cardanol, 2-Mecardol
4	0.80, 3.1	P/E(1 : 1)	650	2-Mecardol, Cardol
5	2.84, 11.1	P/E(1 : 1)	650	Cardol
6	2.40, 9.3	M	650	Polymer

P, Light Petroleum, 60–80°C; E, Diethyl ether; M, Methanol.
Adapted from Table 2, Ref. 8 with permission from the publishers.

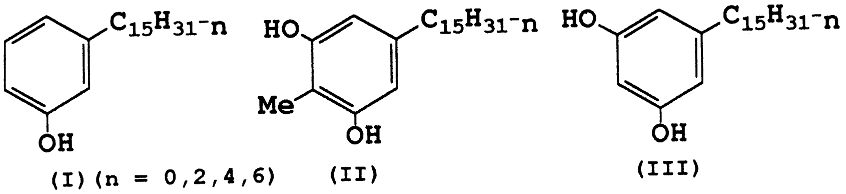


Figure 1. Formulae of cardanol, 2-methylcardol, and cardol.

(with iodine visualisation) of alternate samples of the 141 fractions collected, indicated a clear demarcation between the main component phenols. For reuse of the column, the traces of residual polymeric material were removed with acetic acid, which itself was washed out prior to reuse with the next batch of technical CNSL. Initial separations with larger particle size Davison grade silica gel or Silica gel MFC at solute/adsorbent ratios of 1/50, 1/45, 1/30, respectively resulted in resolution of the component phenols, but with a ratio of 1/16 separation was inadequate. Thus, Silica gel MFH was only equally effective if employed in a tenfold greater proportion. Silica gel H (type 60 article 7736) particle size (0.01–0.04 mm) has a surface area at least twenty times greater than silica gel MFC (0.075 mm), while silica gel H (article 9385) by contrast, with particle size (0.040–0.063 mm), is intermediate and probably more uniform.

For argentation column chromatography, dry incorporation of 15% silver nitrate with silica gel H gave excellent separations of the unsaturated constituents of cardanol, as shown in Table 6.

Table 6. Argentation TLC Separation of the Constituents of Cardanol (10 g) on Silica Gel G (100 g) Containing 15% Dry Incorporated Silver Nitrate

Combined Fractions	(%)*	Constituent	Solvent	Fraction Range	Volume (mL)
1 (33–64)	42.30	Monoene	P	(1–12)	100
			P/E(20 : 1)	(1–12)	500
2 (65–82)	14.82	Monoene/diene	P/E(15 : 1)	(13–24)	300
3 (83–104)	13.32	Diene,monoene	P/E(12 : 1)	(25–46)	300
4 (105–122)	6.46	Triene,diene	P/E(10 : 1)	(47–70)	400
5 (123–140)	6.50	Triene	P/E(8 : 1)	(71–90)	300
			P/E(5 : 1)	(91–146)	1000
6 (141–166)	16.60	Triene	P/E(2 : 1)	(147–166)	600

P, Light petroleum 60–80°C; E, Diethyl ether; *, % after evapn. to constant weight. Adapted from Table 3, Ref. 8 with permission from the publishers.

All 166 fractions were monitored by analytical TLC. The total recoveries of monoene, diene, and triene constituents with solute/adsorbent ratios of 1 : 20 and 1 : 10 were 76.7% and 77.2%, respectively, the remaining material consisting of mixed fractions. Subsequent analysis by HPLC, somewhat later, indicated the monoene, diene, and triene fractions to be 90%, 83%, and 97% pure, respectively. By comparison, separations at a solute/adsorbent ratio of 1 : 900 carried out with 100% silver ion silica gel (60–100 mesh), which is larger in particle size than the MFC grade, led to the complete separation of the three constituents.[15]

Separation of Sesquiterpenes by Argentation TLC/GC and by TLC/GC

The sesquitepenes were recovered either by steam distillation, by solvent extraction with light petroleum from an alkaline solution, or by column chromatography on alumina and elution with light petroleum (40–60°C). From these procedures, Agba gum resin was found to comprise a mixture of steam volatile sesquiterpenes (29.3%), non-acidic non-steam volatile terpenoid material (12.5%), and acidic rosin acids related to dihydroabietic acid (56.5%). The terpenes had bp 257–270°C and the acidic fraction, mp 170–175°C. TLC on ordinary plates, only partially revealed the complexity of the components present (except for a major component which formed a nitrosite and appeared to be caryophyllene). For greater resolution, preparative plates with silica gel G incorporating 15% silver nitrate were employed. Development with light petroleum (40–60°C)/diethyl ether (90 : 10) indicated eight bands with the relative R_f values (size) indicated from the top of the plate: fraction (8), 0.67 (large), (7) 0.61 (small), (6) 0.48 (medium), (5) 0.41 (medium), (4) 0.24 (small), (3) 0.21 (small), (2) 0.12 (large, caryophyllene), (1) 0.05 (small), all of which were eluted with methanolic ether, freed from silver nitrate by aqueous washing, dried, and recovered for spectral and GC examination.

For larger quantities, prep GC on carbowax 20 M was used and material from four of the main peaks 2, 3, 5, 8 collected at the half height points. These four collected materials were then separated further by argentation TLC, and the resultant analytical TLC indicated the presence of eight components in agreement with the initial TLC examination by argentation TLC alone. Band 1, (α-humulene) (VI), band 2 (caryophyllene, formerly termed *β*-caryophyllene) (IV), band 7 (isocaryophyllene, formerly, termed *γ*-caryophyllene) (V), and band 8 (α-copaene) (VII). The formulae of the sesquiterpenes are depicted in (Fig. 2). GC/MS conducted with a smaller sample showed 15 components to be present; several very minor and further identifications are required.

Argentation TLC comparison with authentic samples, spectroscopic identification by ^{1}H NMR, and infrared spectral examination confirmed the

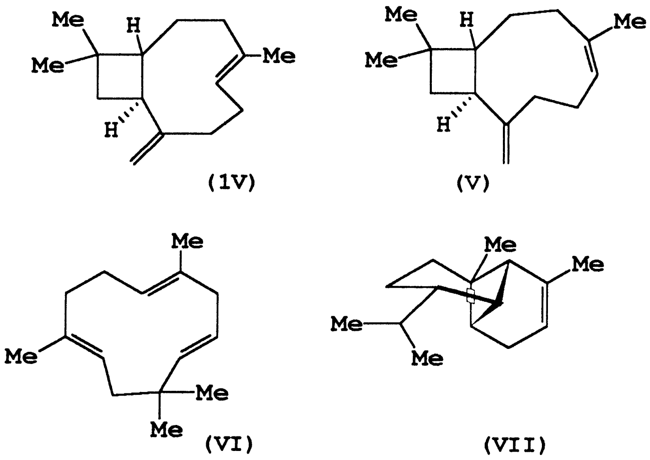


Figure 2. Formulae of sesquiterpenes.

presence of caryophyllene (IV), isocaryophyllene (V), α-humulene (VI) and α-copaene (VII) in the gum rosin. Whereas, in the argentation TLC of acyclic systems, *cis* compounds always have lower R_f value than their trans isomers in the sesquiterpene series, similar separations of isomers, e.g., caryophyllene and isocaryophyllene respond differently and are generally apparently influenced mainly by the number of double bonds present and not on their configuration. On ordinary silica gel G isocaryophyllene (cis) has a considerably higher R_f value than caryophyllene (trans) in a wide range of non-polar solvents[16] and it seems that the same holds for a system containing silver ion, although, this has not been described elsewhere.

The application of different systems of TLC/GC in the analysis of terpenoid mixtures has been described.[17]

TLC of Vicinal Cyclic Diols on Boric Acid and Sodium Arsenite Impregnated Layers

The separation of the racemic *erythro* and *threo* methyl 9,10-dihydroxyoctadecanoates and 13,14-dihydroxyoleates has been described[18] on silica gel G impregnated with boric acid and with sodium arsenite. However, cyclic diols have received no similar examination. *cis*- and *trans*-cyclohexane-1,2-diols and a range of 1-methoxy-4,6-benzylidene derivatives of five isomeric hexoses having 2,3-

dihydroxy groups, have been examined on silica gel G containing 15% of either boric acid or 15% sodium arsenite.

Cis- and *trans*-cyclohexane-1,2-diols, when developed with methanol–chloroform (50 : 50) on silica gel G containing 15% of either boric acid or sodium arsenite, were clearly separated; the cis isomer having R_f 0.79 and the *trans* R_f 0.73. In the cis-isomer, the hydroxyl groups have an e, a (equatorial axial) and an e,e (diequatorial) configuration in the trans isomer.

With the 2,3-diols from 4,6-benzylidene-1-methoxy derivatives of α-glucose (VIII), β-glucose (IX), α-galactose (X), β-galactose (XI), α-mannose (XII), α-allose (XIII), and α-altrose (XIV) (Fig. 3) on silica gel G containing either 15% of boric acid or sodium aresenite, the glucose and galactose derivatives, in which the hydroxyl groups have an 2e, 3e configuration had the lowest set of R_f values, α-mannose with 2a, 3e groups and α-allose with

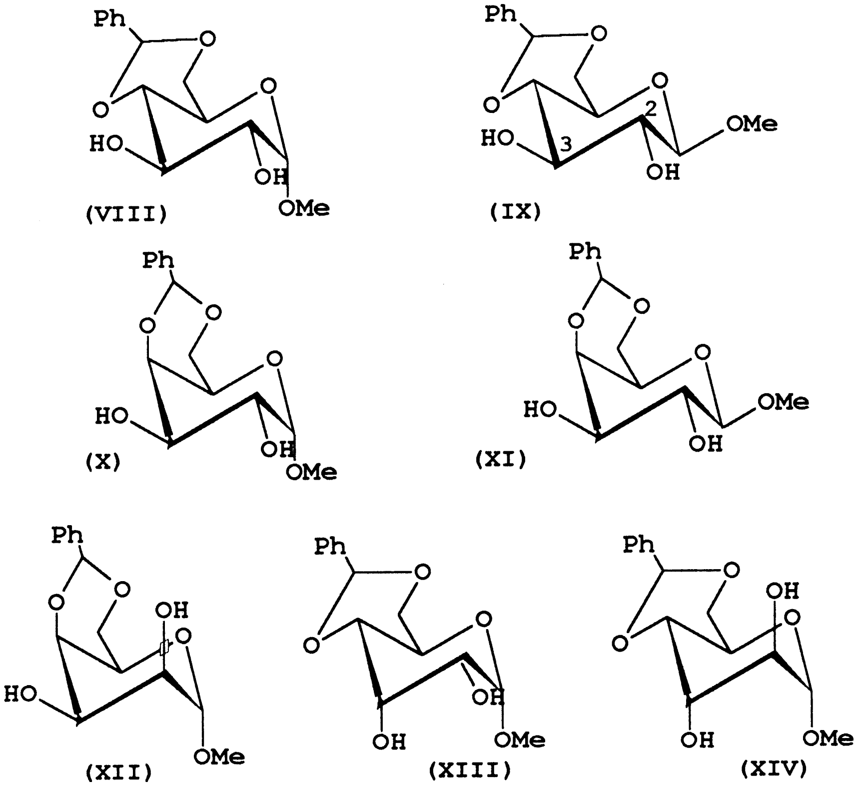
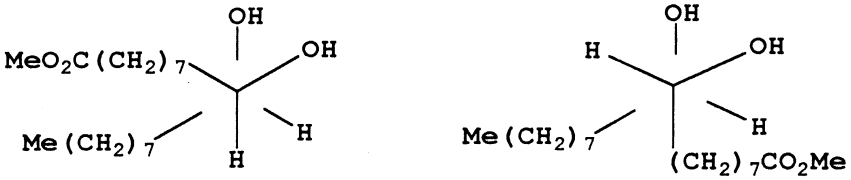


Figure 3. Formulae of 4,6-benzylidene-2,3-dihydroxy-1-methoxyhexoses.



(XV) (Erythro) (Threo) (XVI)

Figure 4. Newman configurations of an erythro and a threo form of methyl 9,10-dihydroxystearates.

2e, 3a groups both had higher R_f values, and α-altrose with 3a,4a hydroxyl groups the highest R_f value. Thus, to summarize, R_f values followed the order e, e > a, e or e, a > a, a, the same as found with the parent diols.

In the cyclic diols complexed with boric acid or sodium arsenite, the hybridization state is sp^3 and boric acid or sodium arsenite may be considered to lead to a cyclic derivative or, more likely, to a hydrogen-bonded chelation association. By contrast in argentation TLC, the complexation state is essentially planar, commencing with an sp^2 alkene.

In the case of the 9,10-dihydroxystearates, Newman representations of one of the *erythro* pair (XV) and one of the *threo* pair (XVI) are shown (Fig. 4) in the *gauche* conformations, the most favorable for borate complexation. The *threo* form appears to be less sterically hindered, which may account for its interaction with the borate ion and higher migration than the *erythro* form in TLC, if indeed as has been suggested,[18] *threo* compounds complex ‘much more readily and the complexes are less polar than the original diols’.

In the case of the cyclic 2,3-diols from the hexoses, where conformational restrictions impose some limitations on the existence of the *anti* and *gauche* conformations compared to the acyclic series, the extent of boric acid complexation clearly varies. *Trans* forms, namely α-glucose and α-altrose show wide difference in TLC migration and steric factors apparently predominate, leading to uncertainty concerning the nature of the structural situation in borate complexation.

ACKNOWLEDGMENTS

Mr M. Harding, Perkin Elmer Ltd., Beaconsfield, Bucks, UK is thanked for prep GC. Dr C. Baker, of the PCMU, Harwell, Oxon, UK and Dr C. Simpson, formerly of Department of Chemistry, University of Sussex, UK are thanked for MS/GC; and Dr R. E. Day, Tioxide Ltd. for surface area measurements. Samples of hop oil were obtained with the help of Dr C. Green of Wye College, University of London. Prof. R. D. Guthrie (University of Sussex) kindly provided samples of hexose derivatives. Prof. C. Heathcock is thanked for the ^{1}H NMR spectrum of α-copaene. Certain HPLC analyses were carried out after completion of this work by Mrs V. Tychopoulos.

REFERENCES

1. Tyman, J.H.P; Higdon, A. An Inexpensive Spreader for Thin Layer Chromatography. Lab. Pract. **1972**, *21*, 559–560.
2. Tyman, J.H.P. Quantitative Analysis of the Phenolic Composition of Natural Cashew Nutshell Liquid (*anacardium occidentale*) by Thin Layer Chromatography, Densitometry, and Ultraviolet Spectrophotometry J. Chromatogr. **1978**, *166*, 169–172.
3. Tyman, J.H.P. Analysis of Long Chain Phenols. In *Quantitative Thin-Layer Chromatography and its Industrial Applications*; Treiber, L.R., Ed.; Marcel Dekker Inc.: New York, 1987; 125–161.
4. Kohlschutter, H.W.; Unger, K. The Historical Development of the Method. In *Thin-Layer Chromatography*; Stahl, E., Allen and Unwin: London, 1969; 1–21.
5. Sherma, J.; Basic Techniques, Materials and Apparatus. In *Handbook of Thin-Layer of Chromatography*, 2nd Ed.; Fried, B., Sherma, J., Eds.; Marcel Dekker Inc.: New York, 1995; 3–41.
6. Garrett, A.; Tyman, J.H.P. (unpublished work, 1975).
7. Grace, W.R. and Co. Plates for Thin Layer Chromatography, Brit. Pat. 1,181,089, Feb 11, 1970.
8. Durrani, A.A.; Johnson, R.A.; Sood, S.K.; Tyman, J.H.P. Practical Liquid Chromatographic Separation of the Phenols in Technical Cashew Nutshell Liquid from *Anacardium occidentale*, Lipids **1986**, *21*, 241–246.
9. Still, W.C.; Kahn, X.; Mitra, A. J. Org. Chem. **1978**, *43*, 2923–2925.
10. Tyman, J.H.P.; Morris, L.J. The Composition of Cashew Nutshell Liquid and the Detection of a Novel Phenolic Ingredient. J. Chromatogr. **1967**, *27*, 287–288.
11. Tyman, J.H.P. (unpublished work, 1969–1970); Tyman, J.H.P. Chromatographic Separation of the Sesquiterpenes from the Gum Rosin of *Gosweilerodendron balsiferum.* Symposium on the Chemistry of Natural Products, International Union of Pure and Applied Chemistry, Delhi, February, 1972; Abstract C39, 197–198.

12. Tyman, J.H.P. Identification of the Components of a Novel Phenolic Fraction in *Anacardium occidentale* (Cashew Nut-Shell Liquid) and Synthesis of the Saturated Member. J. Chem. Soc. Perkin Trans. **1973**, *1*, 1639–1647.
13. Halpaap, H. Achievement of Reproducible Separations by the Application of Standardized Sorbents in Specified Systems. J. Chromatogr. **1973**, *78*, 77–87.
14. Tyman, J.H.P. Phenols, Aromatic Carboxylic Acids and Indoles. In *Handbook of Thin Layer Chromatography*, 2nd. Ed.; Fried, B., Sherma, J., Eds; Marcel Dekker Inc.: New York, 1995; 877–920.
15. Murthy, B.J.K.; Sivasamban, S.; Aggarwal, A. J. Chromatogr. **1968**, *32*, 519–528.
16. Miller, J.G.; Kirchner, J.G. Chromatostrips for Identifying Constituents of Essential Oils. Anal. Chem. **1953**, *25*, 1107–1109.
17. Humphrey, A.M. The Examination of Volatile Oils by Combined Gas–Liquid Chromatography and Thin-Layer Chromatography, J. Chromatogr. **1970**, *53*, 375–378.
18. Morris, L.J. Separation of Higher Fatty Acid Isomers and Vinylogues by TLC. Chem. and Ind. (London) **1962**, 1238–1240.

Received December 5, 2001
Accepted January 14, 2002
Manuscript 5794L