Journal of Chromatography, 166 (1978) 159–172

CHROM. 11,223

LONG-CHAIN PHENOLS

XIII*. QUANTITATIVE ANALYSIS OF THE PHENOLIC COMPOSITION OF NATURAL CASHEW NUT-SHELL LIQUID (*ANACARDIUM OCCIDENTALE*) BY THIN-LAYER CHROMATOGRAPHY, DENSITOMETRY AND ULTRAVIOLET SPECTROPHOTOMETRY

J. H. P. TYMAN

School of Chemistry, Brunel University, Uxbridge, Middlesex (Great Britain)

(First received January 20th, 1978; revised manuscript received May 29th, 1978)

SUMMARY

Natural cashew nut-shell liquid has been analysed by thin-layer chromatography (TLC) combined with spectrophotometry. In a direct method the bands were examined *in situ* by an absorbance method involving a "flying spot" scanning procedure and densitometry. The indirect method, on which a limited amount of work has been carried out, involved elution of bands and examination of their absorbance by ultraviolet spectrophotometry at the absorption maximum for the phenolic moiety. A comparison of the procedures with gas–liquid chromatographic analysis has been made and reasonable agreement shown. TLC–densitometry applied to phenolic substances is a useful method which requires extensive preliminary experimentation and carefully controlled conditions for its successful operation.

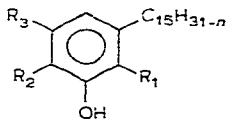
INTRODUCTION

A considerable amount of work has been carried out by thin-layer chromatography (TLC) in conjunction with ultraviolet (UV) spectrophotometry for quantitative TLC analysis. In early work[1] the emphasis was upon indirect procedures involving elution of bands and spectrophotometric determination. More recent work[2,3] has concentrated on direct determination without elution by absortiometry or fluorimetry. Generally there has been more preoccupation with theoretical, instrumental and computing aspects and rather less information has been available on current instrumental equipment and on the comparison of different chromatographic methods of quantitative analysis.

The present work has been concerned with the analysis of natural and technical cashew nut-shell liquid, CNSL (*Anacardium occidentale*) by TLC–UV spectrophotometry.

* Part XII, *J. Chromatogr.*, 156 (1978) 255; Part XI, *J. Amer. Oil Chem. Soc.*, in press; Part X, ref. 16.

Natural CNSL contains the component phenols cardanol (I; $R_1 = R_2 = R_3 = H$, $n = 0, 2, 4, 6$), cardol ($R_1 = R_2 = H$, $R_3 = OH$, $n = 0, 2, 4, 6$), 2-methylcardol ($R_1 = H$, $R_2 = CH_3$, $R_3 = OH$, $n = 0, 2, 4, 6$) and anacardic acid ($R_1 = CO_2H$, $R_2 = R_3 = H$, $n = 0, 2, 4, 6$) which is decarboxylated industrially to yield cardanol, the major component of technical CNSL. Other components are present apart from cardol and 2-methylcardol, such as minor amounts of C9, C11, C13 and rather more of the C17 homologue.



I

The partition conditions of gas–liquid chromatography (GLC) are suitable for the analysis of the component phenols after hydrogenation[4] and methylation[5] or of the unsaturated trimethylsilyl ethers[6]. In adsorption TLC the homologues and unsaturated constituents of each component phenol travel collectively as one band and their subsequent elution and spectrophotometric determination by the indirect method gives the basis for quantitative analysis[7]. It seemed however that the complex mixture of phenolic lipids in CNSL could be analysed more easily by a direct method. This was investigated in 1971 by the use of a "flying spot" densitometer but there has been little opportunity until now to describe the results obtained. They have been compared with GLC analysis.

EXPERIMENTAL

Materials

Cashew nuts were obtained from Tanzania and in later experiments a Mozambique source was used (from British Coco Mills, Hull, Great Britain). Natural CNSL was extracted and the component phenols separated as described[8,9].

All solvents were redistilled. For indirect spectrophotometric work methanol was used (optically transparent from 240 nm to 320 nm). Following exploratory experiments in the direct method, five further plates (series A) were examined and standards of cardol (0.0688 g per 10 ml chloroform), anacardic acid (0.2726 g per 10 ml chloroform) and a solution of natural CNSL (0.1962 g per 10 ml chloroform) were used. Then a further thirteen plates (series B) were examined with a standard of anacardic acid (0.2371 g), cardol (0.0636 g), 2-methylcardol (0.0204 g) and cardanol (0.0224 g) made up in chloroform (10 ml) in order to avoid syringe errors involved with four separate standards. The weights resembled those in natural CNSL. The solutions to be analysed contained natural CNSL (0.2136 g per 10 ml chloroform) and technical CNSL (0.1356 g per 10 ml chloroform).

Thin-layer chromatography

TLC was carried out initially on silica gel G (Merck, Darmstadt, G.F.R.; nach Stahl, type 60) with self-prepared preparative plates (20 × 20 cm × 1 mm). Later for quantitative work pre-coated commercial silica gel G (F254) preparative

plates (20 × 20 cm × 0.25 mm, Merck) believed to contain starch as well as the binder were used. Solutions carefully freed of all air bubbles were accurately dispensed with a 100-μl Hamilton syringe. Circular spots were applied to the plate in a nitrogen box which was used to prevent oxidation and assist evaporation of the solvent during sample application and after development. This was carried out at 20° and at low humidity in two stages to separate cardanol and 2-methylcardol with light petroleum (b.p. 40–60°)–diethyl ether (7:3) with 2% concentrated ammonia and with diethyl ether with 2% concentrated ammonia added to separate cardol from anacardic acid. It was necessary to absorb water from the ammoniated solvent at the base of the tank with a filter paper liner although some water probably remained in equilibrium. A third solvent system was used for both natural and for technical CNSL and comprised light petroleum–diethyl ether (7:3) with 2% (98–100%) formic acid. Alternative solvent systems were based on firstly ethyl acetate–chloroform (5:95) with 2% concentrated ammonia followed by a second development with ethyl acetate–chloroform (1:4) (and 2% ammonia). A very precise separation of cardol from anacardic acid was obtained with silica gel G or H containing 10% of sodium carbonate incorporated at the layer preparation stage with development in ethyl acetate–chloroform (10:90), provided a second development was carried out with a more polar solvent, ethyl acetate–chloroform (40:60). Such systems were superior to the use of organic bases or ammonia in the solvent and it is likely they may have an application to other mixtures of acidic and neutral, or less acidic solutes.

After development and prior to visualisation, the plates, partially freed of solvent in the nitrogen box, were placed firstly in an oven at 100° to remove the remaining solvent and most of the ammonia and immediately afterwards in a warm tank containing iodine crystals and iodine vapour. The plates were kept in this tank for at least half an hour, removed and the free iodine in the background allowed to evaporate. A second blank plate was then placed over the silica gel and the edges sealed with self-adhesive tape. TLC plates were also visualised for absorptiometry by spraying with 50% aqueous sulphuric acid and charring at 180°.

For fluorimetry the plates were sprayed with 0.1% ethanolic rhodamine 6G or dichlorofluorescein. In the indirect procedure all bands were visualised with 0.1% ethanolic rhodamine 6G. Materials were recovered subsequently as previously described[8].

UV spectrophotometry

Direct procedure using a densitometer. The Fisons-Vitatron "flying spot" scanning apparatus (TLD 100) was used equipped with a Fisons-Vitatron recorder/integrator. For transmission no filter was used. The eccentricity was adjusted so that the whole spot or band and the adjacent area were scanned. To obtain peak areas with speed, the digital read-out was used rather than "notch" counting at the baseline. The two agreed. The former was facilitated by a constant baseline since all areas above the starting baseline were automatically counted. The direct read-out was started from a counting rate of one notch/sec at the commencement and conclusion of the peak. Integration was carried out at a high chart speed to obtain large peaks. For the present analyses, six rolls of chart paper were used for the work. Where symmetrical peaks were obtained, triangulation could also be used.

Absorption, reflectance and fluorescence measurements were made and the transmission mode with iodine-impregnated plates was found to be the more satisfactory method (see Results and discussion).

Indirect procedure. In this method a Unicam SP500 was employed with manual scanning throughout the region, 230 to 330 nm. The 1-cm cells were interchanged and the results averaged. The UV absorption of the eluted bands was attributable to the main components since silica gel G eluted between the bands indicated no absorption of any significance.

RESULTS AND DISCUSSION

Exploratory experiments with direct spectrophotometry

In some preliminary experiments with self-prepared and commercial silica gel

TABLE I
PRELIMINARY EXPERIMENTAL PROCEDURES

Expt. No.	*Type of TLC plate*	*Visualisation procedure*	*Mode**	*Observations*
1	Pre-coated commercial Merck silica gel PF254 (analytical type) (20 × 20 cm × 0.25 mm)	PF254	Fluorescence	Fluorescence quenching of lower band. Upper bands fluorescent
2	Pre-coated commercial Merck silica gel PF254 (analytical type) (20 × 20 cm × 0.25 mm)	H_2SO_4	Transmission	Whole plate background absorbing due to charring of binder (starch)
3	Silica gel 60G (nach Stahl) Self-prepared (analytical type) (20 × 20 cm × 0.25 mm)	Rhodamine 6G	Fluorescence	All band fluorescent but with different colours
4	Silica gel 60G (nach Stahl) Self-prepared (analytical type) (20 × 20 cm × 0.25 mm)	Dichloro-fluorescein	Fluorescence	All bands quenching fluorescence, lower band giving different colour from the remainder
5	Silica gel 60G (nach Stahl) Self-prepared (prep. type) (20 × 20 cm × 1 mm)	H_2SO_4	Transmission	Lower band (anacardic acid) small and showing low light absorption
6, 7	Silica gel 60G (nach Stahl) Self-prepared (prep. type)	H_2SO_4	Transmission	Development to increase size of band. Increased light absorption
8	Silanised silica gel 60H Self-prepared (prep. type) (20 × 20 cm × 1 mm)	H_2SO_4	Transmission	Silanised layer used to stop tailing of bands

* Fluorescence (fluorimetric), transmission (absorptiometric).

G plates, absorptiometric and fluorimetric procedures were examined. These have been briefly summarised in Table I. All the fluorescent visualising reagents presented difficulties in the measurement of fluorescence intensity due either to the simultaneous occurrence of fluorescence and quenching of the different bands or to the appearance of differently coloured bands. Measurement of absorption of plates charred with sulphuric acid was considerably easier. Subsequently iodine impregnation was found to be the best visualising procedure. The peak areas obtained in absorption measurements with charred plates were found to be dependent on the R_F value since more polar solvents gave greater mobility and size to the bands. The dependence of the measured peak area upon the extent of sulphuric acid charring, the difficulty of uniform spraying of the plate and the presence of an increasingly dark background towards the solvent front were disadvantages of this visualising procedure. The major difficulty with self-prepared plates was variation in thickness of the layer, and non-linearity of area of spots *versus* concentration. The constancy of the baseline was examined by transmission prior to chromatographic determinations to check the uniformity of the plates. Reflectance gave no advantage. Although commercial plates were largely free from this defect and gave linearity, the starch binder believed to be present caused excessive darkening at the charring stage and led to the need for an improved visualising technique. With the cashew phenols multiple development was necessary to adequately resolve the bands and to position them widely between the baseline and the solvent front. The least solvent polar was used last in sequence since otherwise a faint solvent-front band tended to interfere with the integration measurement. A consistent observation was the dependence of integration results on the development procedure. Others have commented upon this[10,11] and found quantitation to be most accurate within a specified range of R_F values. We believe that the greatest inaccuracy occurs only with bands of high R_F value. It was vital to use calibration standards applied from the same solvent and of approximately the same sample magnitude. Slight streaking and tailing caused minor inconstancy of the baseline and a simple remedy was to scan the bands in a direction parallel to the baseline (horizontal rather than vertical scan); all bands of the same components were successively scanned commencing with the unknown sample and proceeding through a series of standard amounts. At the commencement of each horizontal series of bands of the given component, the instrument was rezeroed and linearity of peak area *versus* amount of component phenol was in most cases observed. Departures from linearity were generally attributed to patchy or inhomogeneous charring, partly due to the residual effect of ammonia from the developing solvent and the inherent defect of manual spraying. Accordingly vapour impregnation by the use of iodine or bromine seemed likely to be more efficient.

Use of iodine for visualisation and quantitation in direct spectrophotometry

A further series of five plates (series A)* was examined with a solution of natural CNSL (20 μl, 30 μl) alongside separate standard solutions of anacardic acid and cardol (10, 20, 30, 40 and 50 μl). Although both charring with 50% sulphuric acid and iodine visualisation resulted in linear plots of area *versus* weight which passed through the origin, iodine was easier to apply and gave consistent results. Visualisa-

* There results have not been tabulated.

TABLE II

SUMMARY OF TLC PLATES (SERIES B) DEVELOPED, EXPERIMENTAL CONDITIONS AND OBSERVATIONS

Plate No.	*Material*	*Solvents*, in order of use*	R_F				*Amount of CNSL used (μl)*			*Amounts of standard used*								*Observations*
			*A***	*B*	*C*	*D*				*D (μl)*				*C (μl)*				
1	Nat. CNSL (Mozambique)	I, II	0.79	0.74	0.41	0	30			20	30	40	50	20	30	40	–	Standard commercial plate used for the series of experiments with plates 1–13 (Series B)
2		II, I	0.83	0.76	0.45	0	28			20	30	40	50	20	30	–		Standard commercial plate used for the series of experiments with plates 1–13 (Series B)
3		II only	0.95	0.95	0.66	0	30			18	30	40	–	20	30	–		A and B not separated
4		II, I	0.83	0.66	0.37	0	30			20	30	40	50	20	30	40	50	Quite good sharp spots. Suitable for integration
5		II, I	0.59	0.50	0.35	0	30			*(Standard of A, B, C, D used)*								New 4-component standard. Separation of A and B just sufficient for horizontal and vertical scan
										10	20	30	40	50			60	
6		III only	0.79	0.55	0.26	0.79	20	20	30	10	–	20	–	30			40	A and D not separated. Plate suitable for horizontal and vertical scan
7		II, I	0.63	0.57	0.43	0	10	20	30	10	16	20	24	30			–	Separation of A and B not sufficient. Tailing; horizontal but not vertical scan
8		I, II	0.71	0.61	0.36	0	10	20	30	10	16	20	24	30			–	Separation of A and B. Streaking of D, spots all good size
9		II, I	0.72	0.63	0.58	0	–	–	30	10	16	20	–	30			–	Reasonably good separation of A and B. Good plate for horizontal and vertical scan
10	Technical CNSL	II, I	0.82	0.52	0.24	–	–	–	30	10	16	20	–	30			–	Excellent separation of A and B. Bow-shaped solvent front
11		III	0.89	0.66	0.34	–	–	–	30	10	16	20	24	30			–	Broad A spot. Too wide for scan. Bow-shaped solvent front
12		III	0.67	0.59	0.40	–	10	20	30	–	–	20	–	40			60	Insufficient separation of A and B for horizontal and vertical scan
13	Nat. CNSL (Tanzanian)	II, I	0.73	0.63	0.48	0	–	20	30	10	16	20	20	20			–	Separation of A and B reasonably good for horizontal and vertical scan

* I = Light petroleum (b.p. 40–60°)–diethyl ether (7:3) with 2% conc. ammonia; II = diethyl ether with 2% conc. ammonia; III = light petroleum–diethyl ether (7:3) with 2% (98–100%) formic acid.

** A, Cardanol; B, 2-methylcardol; C, cardol; D, anarcardic acid.

tion with bromine was not so effective and the bands were fugitive. Although it has been commented[12] that iodine can be used for compounds possessing no absorption in the UV or visible region, we have found that it is highly satisfactory for materials with UV absorption but no colour. The interaction of iodine with components has been investigated in some detail by Šaršúnová *et al.*[13]. A broad wavelength of absorption occurs with the phenolic lipids and the resultant quantitation amounts to densitometric determination as with sulphuric acid charring. It seems likely that iodine interacts partially with the phenolic ring. Upon standing, some slight loss of iodine took place and it was found convenient to place a blank glass plate over the one under examination and seal the edges with self-adhesive tape. Absorption measurements on freshly impregnated plates and those from which the iodine had partially evaporated still showed linearity (Fig. 1).

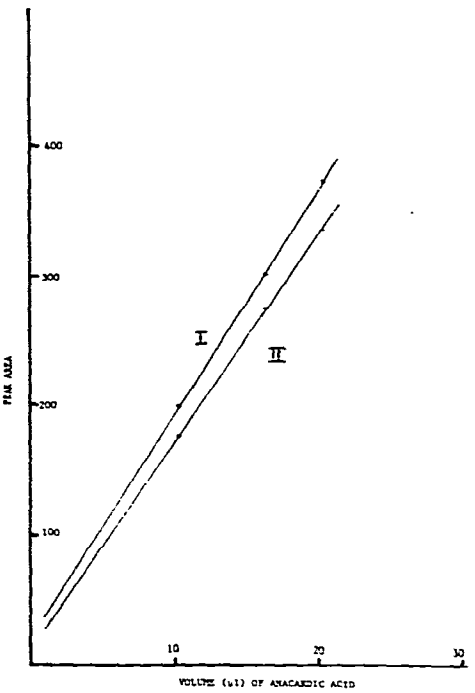


Fig. 1. Plot of effect of time on UV absorption of iodine-impregnated anacardic acid. I, Direct measurement; II, measurement after 6 h.

To locate at the baseline highly polar material distinct from anacardic acid the alternative solvent system light petroleum (b.p. 40°–60°)–diethyl ether (7:3) containing 2% formic acid[7] was used in which anacardic acid behaves as a hydrogen-bonded substance with high R_F values. Resolution from cardanol was insufficient for good integration to be obtained and further investigation is required. The general difficulty with substances of high R_F value, particularly with major components, was the greater size of bands towards the solvent front and the eccentricity could not be adjusted adequately to cover both background and band.

With the iodine visualisation technique followed by densitometry, an initial

group of four plates was developed to confirm the findings with the two principal components, anacardic acid and cardol. For the remaining plates in series B the chromatographic separation of the four components in the ammoniated solvent system was perfected and results (Table II) obtained for natural Mozambique CNSL on plates 1–9, technical CNSL of Mozambique origin on 10, 11 and 12 and natural Tanzanian CNSL on plate 13 are given.

For plates 5–13 a combined calibration standard of anacardic acid, cardol, 2-methylcardol and cardanol was used and eight samples were applied at the baseline, three of the unknown CNSL solution and five of the standard mixture. The plates were multiply developed so as to resolve the four components which were present as comparatively faint spots until visualisation. Although doubtful separations could

TABLE III

ANALYSIS OF NATURAL CNSL BY TLC AND UV SPECTROPHOTOMETRY

Plate No.	*Component phenol*	*Volume (μl) of unknown CNSL and peak areas*			*Volume (μl) of standards and peak areas*					*Slope (m)*
		10	*(i) 20*	*(ii)* 30 μl*	*10*	*16*	*20*	*24*	*30 μl*	
8	anacardic acid	125	205	296	180	270	330	401	513	1.3546
	cardol	187	291	388	253	371	430	497	—	1.7414
	2-methylcardol	—	128	185	201	323	422	535	—	1.7092
	cardanol	256	272	345	309	505	610	713	—	2.4314
		(i)a 10	*(ii)b 20*	*(iii)c 30 μl*	*10*	*16*	*20*	*24*	*30 μl*	
8	anacardic acid	97	176	255	153	227	294	343	440	1.1639
	cardol	135	249	334	219	315	367	434	512	1.4461
	2-methylcardol	61	98	147	146	247	331	414	522	1.3487
	cardanol	122	172	244	205	370	429	514	615	1.6966
		—	—	*(i)a 30 μl*	*10*	*16*	*20*	—	*30 μl*	
9	anacardic acid	—	—	270	178	277	337	—	451	1.2793
	cardol	—	—	273	160	255	324	—	446	1.2338
	2-methylcardol	—	—	141	64	172	375	—	419	1.1334
	cardanol	—	—	243	158	284	384	—	472	1.3509
		—	—	*(i)b 30 μl*	*10*	*16*	*20*	—	*30 μl*	
9	anacardic acid	—	—	336	199	303	375	—	510	1.4318
	cardol	—	—	315	186	300	381	—	519	1.4420
	2-methylcardol	—	—	171	136	273	372	—	648	1.1573
	cardanol	—	—	211	114	297	410	—	609	1.6208
		—	*(i) 20*	*(ii) 30 μl*	*10*	*16*	*20*	*20*	*20 μl*	
13	anacardic acid	—	227	322	192	297	350	354	410	1.4912
	cardol	—	270	383	290	424	479	443	506	2.0322
	2-methylcardol	—	218	351	282	466	588	662	672	2.4432
	cardanol	—	665	889	580	889	1022	—	—	4.2819

* The headings (i), (ii) etc. refer to the third column in Table IV.

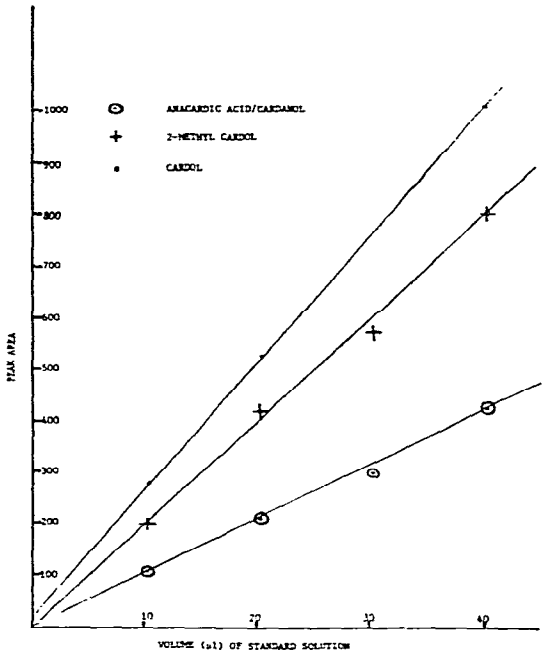


Fig. 2. Plots of area for iodine-impregnated component phenols (plate 6 in Table II) in the standard solution. ○, Anacardic acid/cardanol; +, 2-methylcardol; ●, cardol (without least squares treatment).

be remedied by multiple development this procedure prolonged the analysis and led to band spreading. Of the nine plates, five were suitable for integration and the results for three have been listed in Table III. Fig. 2 shows a typical plot (without least squares treatment). Plates 8 and 9 were reexamined for the reproducibility of the analysis. Since linearity of calibration plots, most of which passed through the origin, had been established, the application of the least squares procedure was considered justified and the slope m ($= \Sigma(xy)/\Sigma(x^2)$) calculated. From plots of the slope for the four component phenols, anacardic acid, cardol, 2-methylcardol and cardanol, the axes representing integrated area and volume (μl) of the four standards, the volume of unknown CNSL corresponding to the area measured for the particular component was found. From these equivalent volumes the composition of the natural CNSL in terms of the four principal components was readily calculated. If the volume with respect to anacardic acid (0.2371 g present in the standard) was found to be $x\,\mu$l and the volume with respect to cardol (0.0636 g in the same standard) was $y\,\mu$l then the relative amount of cardol present was $0.0636\,\text{g}\cdot y/x$ and similarly for the other components, 2-methylcardol ($z\,\mu$l, present $0.0204\,\text{g}\cdot z/x$), and cardanol ($w\,\mu$l, present $0.0224\,\text{g}\cdot w/x$). The weights were used to obtain normalised results and Table IV shows the per cent composition of component phenols in the natural CNSL sample. The results for plates 10, 11 and 12 used for technical CNSL have not been included. They will be discussed in a forthcoming publication[6] together with other methods of analysis of technical CNSL.

In one detailed study[14] on preliminary theoretical aspects, rather than a particular analysis, with a similar densitometer it was concluded that corrections

TABLE IV

COMPOSITION OF COMPONENT PHENOLS IN NATURAL CNSL FROM (NON-ELUTION) TLC AND UV SPECTROPHOTOMETRY

Vol. of CNSL (μl)	*Plate No.*	*Expt. No.*	*Equiv. μl*				*Wt. component phenol found*				*Total wt.*	*Composition (%)*				*% (CNSL found / weight taken)*
			An-acardic acid	*Cardol*	*2-Methyl-cardol*	*Cardanol*	*An-acardic acid*	*Cardol*	*2-Methyl-cardol*	*Cardanol*		*An-acardic acid*	*Cardol*	*2-Methyl-cardol*	*Cardanol*	
20	8	(i)	12.16	13.20	5.88	8.76	0.1442	0.0420	0.0060	0.0098	0.2020	71.38	20.79	2.97	4.86	102.6
30		(ii)	17.44	17.92	8.60	11.36	0.1378	0.0380	0.0058	0.0085	0.1901	72.48	19.98	3.07	4.46	96.6
10		(i)a	6.6	7.36	3.76	6.56	0.1565	0.0468	0.0077	0.0147	0.2257	69.35	20.74	3.39	6.51	–
20	8	(ii)b	12.08	13.6	6.24	9.04	0.1432	0.0432	0.0064	0.0101	0.2029	70.57	21.31	3.13	4.99	103.1
30		(iii)c	17.44	18.24	9.36	13.00	0.1378	0.0387	0.0064	0.0097	0.1926	71.57	20.08	3.30	5.04	97.8
30	9	(i)a	16.96	17.76	10.08	14.24	0.1340	0.0376	0.0068	0.0106	0.1892	70.85	19.90	3.62	5.62	96.1
30	9	(i)b	18.64	18.24	9.8	11.8	0.1473	0.0387	0.0067	0.0088	0.2015	73.13	19.19	3.30	4.37	102.3
												Av. 71.33 ±1.25	20.28 ±0.71	3.25 ±0.22	5.12 ±0.74	Av. 99.75
20	13	(i)	12.24	10.63	7.2	12.48	0.1451	0.0038	0.0073	0.0140	0.2002	72.46	16.89	3.67	6.98	93.7
30		(ii)	17.28	15.14	11.52	16.64	0.1366	0.0321	0.0078	0.0124	0.1889	72.30	16.99	4.15	6.58	–
												Av. 72.38	16.94	3.91	6.78	Av. 98.9

for background fluctuation and light scattering should be incorporated*. The present results suggest that, with uniform commercial plates, these two factors may be of less importance, but become more significant for minor compounds and those of high R_F value.

Indirect spectrophotometry

The elution procedure followed by UV spectrophotometry was originally carried out some years ago and stemmed from attempts to use gravimetry for quantitation with the desirability of having some characterisation of the eluted bands since the total recovered material was greater than 100%. In this quantitative procedure, the visualising agent rhodamine 6G was carefully removed, redistilled solvents were employed and a good grade of silica gel G was used. Two forms of correction were used. Firstly the carefully purified component phenols were examined and their extinction coefficients at $\lambda_{max.}$ determined. The UV absorption maximum measured is attributable to the substituted phenolic ring and the unsaturated sidechains do not absorb and thus interfere. The purity of materials recovered from the natural CNSL was expressed as a percentage of the standard material. Secondly to correct for background absorption[15], a corrected optical density was used in calculations for each of the component phenols. The absorption spectra of the four component phenols are shown in Fig. 3. The nature of the correction is shown in Fig. 4.

The optical density, O.D. at $\lambda_{max.}$ was adjusted to $O.D._{\lambda max.} - (O.D._{\lambda A} + O.D._{\lambda B})/2$ where $O.D._{\lambda A}$ and $O.D._{\lambda B}$ are the optical densities at λ_A and λ_B. While insufficient results have been carried out to determine the accuracy of the procedure they afford some comparison with those obtained by direct spectrophotometry. The average results of two determinations are given in Table V. They differ from the results obtained by the direct method but the latter is based on peak area while the indirect method is based on peak height (optical density or absorbance) at the absorption maximum, the traditional procedure in UV spectrophotometry. Area determination is less straightforward but could clearly be of more relevance where the absorption curves are not symmetrical. The derivation of response factors in terms of area/g from standard solutions and their application to the peak areas of the mixture being analysed would probably be a more legitimate way of comparing the direct and the indirect spectrophotometric procedures.

Comparison of different analytical procedures

GLC analysis[16,17] of natural CNSL of Mozambique origin was compared with UV densitometric analysis and moderately good agreement shown. In a similar way, GLC, UV densitometric, UV indirect and gravimetric analyses were compared for Tanzanian natural CNSL, but less work was carried out on the UV procedures with this material. Agreement again is reasonably good for the two major components by the first two methods. Some deficiencies are apparent as far as the minor components are concerned and it is here that errors due possibly to background absorption and light scattering may be more significant in the UV densitometric procedure.

* The present procedure of assuming "$y = mx$" plots approximates to a correction for irrelevant absorption.

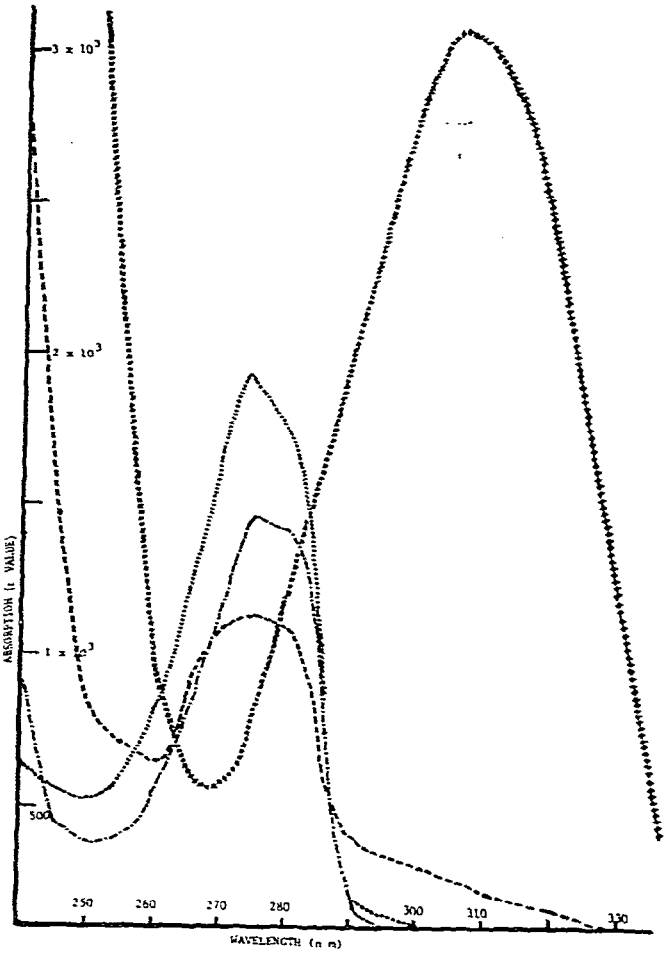


Fig. 3. UV absorption spectra of the four component phenols (methanol solution) from natural CNSL of Tanzanian origin. ◆◆◆, Anacardic acid; . . ., cardanol; · – ·, cardol; – – –, 2-methylcardol.

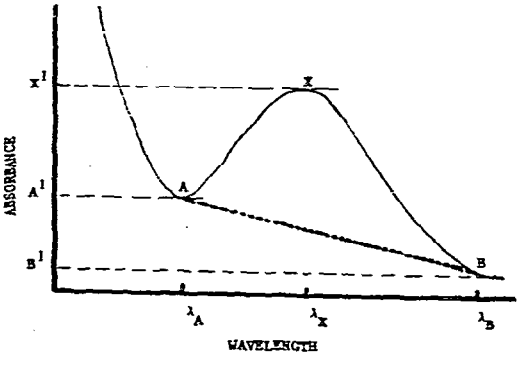


Fig. 4. Typical correction procedure, used in UV indirect spectrophotometric method for determining irrelevant background absorption. For details, see text.

TABLE V

DETERMINATION OF % COMPONENT PHENOLS IN NATURAL CNSL* BY (ELUTION) AND UV SPECTROPHOTOMETRY

Component phenol	$\lambda_{max.}$ *(nm)*	ε** *found*	ε *pure (Av.)*	*Purity (%)*	*Fraction wt.*** *(g)*	*Fraction wt. × purity (1)*	*Optical density (O.D.) correction factor*§ *(2)*	*(1) × (2)*	*Composi (%) (normali*
Anacardic acid	306	2914	2998	97.19	0.0924	0.0898	0.919	0.1626	74.64
		2907		96.96	0.0898	0.0871			
Cardol	277	1400	1545	90.65	0.0512	0.0464	0.904	0.0419	19.25
2-Methylcardol	275	882.3	1100	80.18	0.0083	0.0066	0.546	0.0036	1.65
Cardanol	275	1653	1893	87.29	0.0133	0.0116	0.837	0.0097	4.46

* Material of Tanzanian origin was used.

** $E_{1\%1cm} \times MW \times 10^{-1}$. MW anacardic acid = 344, cardol = 314, 2-methylcardol = 328 and carc = 300.

*** Only the four components have been included and minor materials ignored.

§ $\left[O.D._{\lambda max.} - \left(\frac{O.D._{\lambda_A} + O.D._{\lambda_B}}{2}\right)\right] \Big/ O.D._{\lambda max.}$. O.D. = Absorbance.

GLC analysis is probably overall more accurate for all components. However the reproducibility of the UV densitometric analysis is excellent as judged by the low standard deviations. The collected results are summarised in Table VI.

It can be concluded that UV densitometry is a useful method which does appear to deserve more attention than it has received. Considerable patience and TLC expertise are required and the method may sometimes probably have been prematurely abandoned as a chromatographic quantitation procedure. More work is undoubtedly required with regard to the accurate determination of minor components possibly by some form of double-beam instrument.

TABLE VI

COMPARISON OF DIFFERENT ANALYTICAL METHODS

Source	*Method**	*Composition (%)*			
		Anarcadic acid	*Cardol*	*2-Methyl-cardol*	*Cardanol*
Mozambique	UV densitometry	71.37	20.28	3.25	5.12
		±1.25	±0.71	±0.22	±0.74
	GLC	71.51	22.34	2.78	3.37
		±0.23	±0.11	±0.72	±0.11
Tanzania	UV densitometry	72.38	16.94	3.90	6.70
	GLC	74.01	15.02	1.74	9.23**
		±0.68	±0.51	±0.24	±0.09
	UV indirect	74.64	19.25	1.65	4.46
	Gravimetric	71.99	19.94	2.98	5.09

* UV densitometric, UV indirect and gravimetric methods determine in addition to C15 components, the C17 and traces of C9, C11, C13, while GLC simply determines C15 components.

** This sample of material had been kept over a period of six years between the methods of GLC and the other analytical methods. Some decarboxylation of anacardic acid, resulting in a higher percentage of cardanol, has occurred.

ACKNOWLEDGEMENTS

The author thanks Mr P. Dalton of Fisons Scientific Instruments Ltd. for the use of loaned equipment and The Tropical Products Institute for making available a similar apparatus.

REFERENCES

1 E. Stahl (Editor), *Thin Layer Chromatography, A Laboratory Handbook*, Allen and Unwin, London, 1969, p. 133.
2 J. C. Touchstone, S. S. Levin and T. Murawee, in J. C. Touchstone (Editor), *Quantitative Thin Layer Chromatography*, Wiley-Interscience, New York, 1974, pp. 1, 95.
3 *J. Chromatogr.*, 63 (1971) (special issue, First Camag International Symposium on Advanced Thin-Layer Chromatography).
4 J. H. P. Tyman, *J. Chromatogr.*, 111 (1975) 285.
5 J. H. P. Tyman, *Anal. Chem.*, 48 (1976) 30.
6 J. H. P. Tyman and M. A. Kashani, to be publihsed.
7 J. H. P. Tyman and L. J. Morris, *J. Chromatogr.*, 27 (1967) 277.
8 J. H. P. Tyman, *J. Chromatogr.*, 136 (1977) 289.
9 J. H. P. Tyman, *J. Chem. Soc., Perkin Trans. I*, (1973) 1639.
10 J. C. Touchstone, S. S. Levin and T. Murawee, in J. C. Touchstone (Editor), *Quantitative Thin Layer Chromatography*, Wiley-Interscience, New York, 1974, p. 6.
11 O. S. Privett, K. A. Dougherty and W. L. Erdhahl, in J. C. Touchstone (Editor), *Quantitative Thin Layer Chromatography*, Wiley-Interscience, New York, 1974, p. 72.
12 R. R. Goodall, *J. Chromatogr.*, 123 (1976) 5.
13 M. Šaršúnová, B. Kakáč and L. Krasnec, *J. Chromatogr.*, 48 (1970) 353.
14 J. B. Shute, H. J. de Jong and H. A. Dingjan, *Pharm. Weekbl.*, 105 (1970) 1025.
15 E. S. Stern and C. J. Timmons, *Introduction to Electronic Absorption Spectroscopy in Organic Chemistry*, Arnold, London, 3rd ed., 1970, p. 202.
16 S. K. Lam, *M. Phil. Thesis*, Brunel University, Uxbridge, 1976.
17 J. H. P. Tyman and S. K. Lam, *Lipids*, 13 (1978) in press.