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LONG-CHAIN PHENOLS

XXI*. QUANTITATIVE ANALYSIS OF THE PHENOLIC LIPIDS IN TECHNICAL CASHEW NUT-SHELL LIQUID, FROM *ANACARDIUM OCCIDENTALE*, BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

J. H. P. TYMAN*, V. TYCHOPOULOS and B. A. COLENUTT
School of Chemistry, Brunel University, Uxbridge, Middlesex UB8 3PH (Great Britain)
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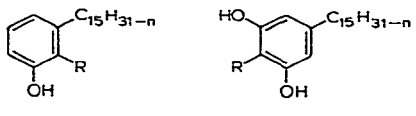
SUMMARY

The novel separation of the constituent phenols in technical cashew nut-shell liquid from the industrial processing of *Anacardium occidentale* has been effected by high-performance liquid chromatography.

The adsorption mode on columns of 5-μm and 10-μm Partisil and the reversed-phase partition mode with 5-μm and 10-μm Spherisorb bonded with octadecylsilane have been investigated. Determination of relative molar response values for the main constituent phenols and the use of an internal standard have led to a quantitative procedure by isocratic elution under reversed-phase partition, preferably with the solvent acetonitrile–water. Gradient elution with tetrahydrofuran and acetonitrile has also enabled the polymeric material to be estimated in the various types of technical cashew nut-shell liquid examined.

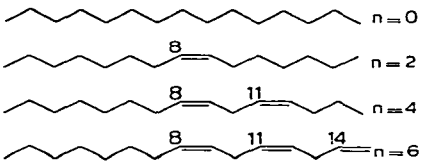
INTRODUCTION

The principal component phenols of technical cashew nut-shell liquid (CNSL) from the industrial processing of *Anacardium occidentale* are cardanol (I; R = H, n = 0, 2, 4 or 6) and cardol (II; R = H, n = 0, 2, 4 or 6), and earlier contributions[1–5] have been concerned with various techniques for quantitative chromatographic determination. Although the trimethylsilylated material can be analysed by gas–liquid chro-



I

II



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matography (GLC) on polyethyleneglycol adipate[6], the derivative process and the possibility of polymerisation of the highly susceptible diene and triene constituents led us to examine quantitative analysis by high-performance liquid chromatography (HPLC). A further requirement[7] to monitor rapidly reaction mixtures involving I and II made it desirable to devise a "cold" method. No previous analyses have been described in the field of phenolic lipids, although the basis of the method was established qualitatively some time ago[8].

The separation by HPLC of certain homologous 5-*n*-alkylresorcinols has been described and of C_8–C_{10} alkylphenols[10]. Acetylated urushiol obtained by the use of acetic anhydride under acidic conditions has been examined[11] by HPLC. In any area of natural and other products, quantitative HPLC[12] has received little attention.

With the reversed-phase partition mode under isocratic conditions and the use of an internal standard the relative molar response values of the constituents of the principal phenols in technical CNSL have been determined, and thence the total quantitative composition without the need for any derivative. By gradient elution following this procedure the polymeric material has been separated. The results agree with those of a previous GLC method[13].

The HPLC method revealed a number of hitherto undetected* minor components probably consisting of geometrical and structural isomers of the principal C_{15} unsaturated constituents together with the unsaturated constituents of the C_{17} homologue of cardanol since upon hydrogenation of the technical CNSL the principal phenolic materials observed were only (15:0)-cardanol, (17:0)-cardanol and (15:0)-cardol.

EXPERIMENTAL

Equipment

A self-constructed liquid chromatograph was used, consisting of a Perkin-Elmer ultraviolet variable-wavelength spectrophotometer (Model LC55) equipped with a flow-through cell, an Altex (Anachem) metering pump (Model 110A), a Rheodyne injection system (Model 7120) with a 20-μl loop, a Servoscribe recorder (Model 1S) and an Infrotonics programmable digital integrator (Model CRS 20-1) with a Monroe (1310) printer. Subsequently a Hewlett-Packard printer–plotter (Model 3909) was used as computing integrator giving results in agreement and without the erratic and sometimes unpredictable behaviour of the former system. The units were interconnected in the usual way with standard stainless-steel tubing and unions.

Gradient elution was carried out with a second Altex metering pump of similar type and an Altex programmer (Model 420). Stainless-steel columns (Altex) were 250 × 4.6 mm I.D. For adsorption conditions they had been packed with silica gel (Partisil) and for reversed-phase partition, Spherisorb bonded with octadecylsilane (ODS). In both types columns with particle sizes of 5 μm and 10 μm were used.

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* The presence of unsaturated constituents with double bonds at other positions than the 8, 11 and 14 has been shown chemically[14].

Conditions

Generally for detection of phenolic constituents the wavelength used was 275 nm since the absorption maxima were in the range 275–280 nm[15]. The pressure in the system depended on the viscosity of the solvent but was usually in the range 1000–1500 p.s.i. and the flow-rate was generally in the range 1–3 ml/min. Solute was made up in chloroform, and generally 5 μl of a 10% solution was chromatographed, with recorder sensitivity in the middle (5 mV) of the range, and a chart speed of 4 mm/min. In quantitative determinations all HPLC analyses were conducted six times to obtain representative peak areas for integration. Reproducibility was excellent and the standard deviations for each constituent were low. Isocratic elution with the reversed-phase partition mode and solvent acetonitrile–water (66:34) was used for simplicity in the final analysis of the phenolic constituents and subsequently gradient elution for the polymeric material (as discussed later).

Materials

All solvents for HPLC were of liquid chromatography grade. Technical CNSL of three types was used. The first (sometimes termed "raw" CNSL) was kindly made available by 3M Co. (St. Paul, MN, U.S.A.) and was believed to be of Brazilian origin. Distilled CNSL, representing vacuum-distilled "raw" CNSL, was from the same source. Blend 3, initially more polymerised, was material obtained five years ago but stored at 0°C, sealed up and in the dark in the interim.

Cardanol and cardol required for the preparation of a standard calibration solution were obtained by column chromatography as described[16] followed by further purification by preparative thin-layer chromatography (TLC). Analytical and preparative TLC were carried out with silica gel G (type 60) as previously described. The constituents of cardanol were separated by preparative argentation TLC on silica gel G containing 15% silver nitrate with the solvent ethyl acetate–chloroform (20:80) and those of cardol with ethyl acetate–chloroform (50:50). Bands were located by spraying a narrow strip resulting from a spot separate from the rest of the plate with 50% aqueous sulphuric acid and warming in a stream of hot air. The phenolic constituents were eluted with ether–methanol (90:10), isolated by filtration, concentration under reduced pressure, ethereal extraction of the residue, water-washed to remove some silver nitrate, dried and recovered. These materials were then all repurified by ordinary preparative TLC with the solvent ethyl acetate–chloroform (5:95). The purity of all six materials was determined by HPLC examination. All gave single peaks*. Saturated cardanol and saturated cardol were obtained as described[1,16] and purified by recrystallisation from light petroleum (b.p. 40–60°C). *p*-Cresol (BDH, Poole, Great Britain) gave a single peak by HPLC.

For the preparation of the standard containing the unsaturated phenols, cardanol monoene (11.36 mg), cardanol diene (1.55 mg), cardanol triene (11.51 mg), cardol diene (0.75 mg), cardol triene (10.30 mg) and the internal standard, *p*-cresol (2.1 mg), were weighed on a five-place balance. (Unfortunately in the final standard prepared, following a number of trial runs, insufficient cardol monoene was available.) The mixture was prepared in chloroform (2 ml) and stored under nitrogen

* Cardanol monoene contained a trace of peak B1. Preparative HPLC should ideally be used for obtaining pure constituents.

TABLE I

RETENTION TIMES AND RETENTION VOLUMES OF CASHEW-NUT PHENOLS UNDER ADSORPTION CONDITIONS ON PARTISIL (5 μm)

t_R = Retention time (min); V_R = retention volume (ml).

Solvent	*Retention parameter*	*Flow-rate (ml/min)*	*Cardanol*				*Cardol*				*2-Methylcardol*			*Mixed*		
			15:0	*15:1*	*15:2*	*15:3*	*15:0*	*15:1*	*15:2*	*15:3*	*15:1*	*15:2*	*15:3*	*Cardanol*	*Cardol*	*2-Methyl-cardol*
n-Hexane–methanol (100:4)	t_R	1	3.75	4	–	–		22	26.75	32.5	20.3	24.5	30.5	–	–	–
	V_R		3.75	4	–	–		22	26.75	32.5	20.3	24.5	30.5			
n-Hexane–methanol (100:2)	t_R	2	2.5	2.75	3	3.37	–	21.75	25.5	30.25	24	26	31.25	–	–	–
	V_R		5.0	5.5	6	6.74		43.50	51.0	60.5	48	52	62.5			
n-Hexane–ethyl acetate (100:5)	t_R	2	6	6.25	6.75	7.5	–	–	–	–	–	–	–	–	–	–
	V_R															
n-Hexane–iso-propanol (100:4)	t_R	1.5	–	–	–	–		–	–	–	–	–	–	3.5	10.0	5.87
	V_R													5.25	15.0	8.80
Isooctane–methanol (100:4)	t_R	1	3.75	4.25	4.87	5.12	17	22.0	28.1	36.25	26.75	30.1	38.0	–	–	–
	V_R		3.75	4.25	4.87	5.12	17	22.0	28.1	36.25	26.75	30.1	38.0			
Isooctane–methanol–diethyl ether (100:3.6:1.2)	t_R	1	4.75	5.25	5.75	6.5	–	26.25	31.0	37.25	25.75	30	36.25	–	–	–
	V_R		4.75	5.25	5.75	6.5		26.25	31.0	37.25	25.75	30	36.25	–	–	–

at −20°C when not required. A separate standard of saturated cardanol (11.70 mg), saturated cardol (9.84 mg) and *p*-cresol (1.92 mg) was prepared and made up similarly in chloroform solution.

A linearity check with the six constituents and *p*-cresol was made to check the validity of Beer's law for the concentration ranges encountered, and substantially straight-line plots were found for volume (*μl*) *versus* peak area in all cases.

RESULTS AND DISCUSSIONS

Retention volumes of the constituents of the component phenols

Adsorption mode. A large number of solvents were examined for the separation of the component phenolic constituents of technical CNSL. In these experiments *n*-hexane or isooctane was the major component and a minor proportion of methanol, ethyl acetate, or isopropanol was used. The results in terms of retention time and volume are summarised in Table I. Reduction of polarity in the solvent led generally to the expected increase in retention volume, the minor, more polar component of the binary combination exerting the greater effect. Thus a change from *n*-hexane to isooctane did not greatly affect the complete resolution of the four constituents of cardanol, cardol and 2-methylcardol (II; R = CH_3, n = 0, 2, 4 or 6), but replacement of methanol by a similar proportion of isopropanol resulted in no resolution of the constituents of each phenol, and three collective peaks were observed for cardanol, cardol and 2-methylcardol. Departures from the combinations shown in the table led to exceedingly long retention times or loss of resolution of the constituents of each component phenol. *n*-Hexane, dichloromethane–ethyl acetate (90:10), chlorohexane–ethyl acetate (96:4), *n*-hexane–acetonitrile (100:2) were all ineffective. A typical chromatogram obtained with *n*-hexane–methanol (100:4) is shown in Fig. 1.

Partition mode. The results for a number of reversed-phase partition experiments under isocratic conditions are given in Table II. The use of methanol–water compared with acetonitrile–water resulted generally in lower retention volumes for all the constituents, but frequently lack of resolution of minor components. The resolution of minor constituents was used as a criterion for the effectiveness of the particular binary combination under examination. The solvent acetonitrile–water (66:34) was an improvement generally in giving resolution of the minor components B1 to B7 as in the typical chromatogram illustrated in Fig. 2. Fig. 3 shows the separation in methanol–water (80:20). The lower viscosity of the former solvent enabled the pressure to be reduced considerably by comparison with aqueous methanol combinations. On account of the better resolution effected, the reversed-phase partition mode was the preferred method for use in quantitative analysis. Furthermore, a profusion of large peaks in the adsorption mode at the commencement of the chromatographic run complicated the choice of internal standard. The average height equivalent to a theoretical plate (HETP) for the separations in Fig. 2 was 0.005 cm.

Determination of relative molar response (RMR) values

The reversed-phase partition mode simplified the choice of an internal standard and enabled a lower alkylphenol to be used. *p*-Cresol was available in pure form and was generally more suitable than *m*-cresol, *p*-ethylphenol, *p*-isopropylphenol, or *p-tert.*-butylphenol. The internal standard and the pure monoene, diene and triene

TABLE II

RETENTION TIMES AND RETENTION VOLUMES OF CASHEW NUT-SHELL PHENOLS UNDER PARTITION (REVERSED-PHASE) CONDITIONS ON SPHERISORB BONDED WITH OCTADECYLSILANE (5 μm)

A = Acetonitrile; E = ethanol; M = methanol; W = water; t_R = retention time (min); V_R = retention volume (ml); NR = not resolved.

Solvent	*Retention parameter**	*Flow-rate (ml/min)*	*Cardol*				*2-Methylcardol 15:3*	*Cardanol*				*Other constituents (minor)*						
			15:3	*15:2*	*15:1*	*15:0*		*15:3*	*15:2*	*15:1*	*15:0*	*B1*	*B2*	*B3*	*B4*	*B5*	*B6*	*B7*
A–W (75:25)	t_R	1.5	5.2	9.25	13.75	–	7.62	16.25	22.5	35	68	15.37	17.75	20.75	24.5	27.37	30.25	
	V_R		7.8	13.87	20.62		11.43	24.37	33.75	52.5	102	23.05	26.62	31.12	36.75	41.05	45.37	–
A–W (70:30)	t_R	1.0	11.25	15	22.4	–	12.55	26.5	37.5	58.5	–	25.4	29.75	34.75	41.25	46	51.25	–
	V_R		11.25	15	22.4		12.55	26.5	37.5	58.5	–	25.4	29.75	34.75	41.25	46	51.25	
A–W (68:32)	t_R	1.5	8.0	10.75	16	–	8.87	19.12	27	42.5	–	18.25	21	24.75	29.25	32.75	36.5	–
	V_R		12.0	16.12	24	–	13.30	28.68	40.2	63.75	–	27.37	31.5	37.12	43.87	49.12	54.75	–
A–W (65:35)	t_R	1.5	10.87	14.75	22.75	–	12.37	28.0	40.25	64.5	–	26.5	33.5	–	43.87	50.0	–	61.75
	V_R		16.30	22.12	34.12	–	18.55	42.0	60.37	96.75	–	39.75	50.25	–	65.80	75.0	–	92.6
A–W (66:34)	t_R	1.7	8.75	12.12	18.5	–	10.12	22.5	32.25	51.62	–	21.25	25.12	30.0	35.5	40.0	44.75	49.75
	V_R		14.87	20.60	31.45	–	17.20	38.25	54.82	87.75	–	36.12	42.70	51.0	60.35	68.0	76.07	84.57
A–W (66:34), average (11 runs)	V_R	1.7	14.69	20.11	30.84	55.45	16.86	37.52	53.56	85.85	165.35	35.31	41.77	49.81	58.73	66.59	74.37	81.94
M–W (90:10)	t_R	1.0	5.5	6.37	7.75	–	NR	9.0	10.75	13.87	20.75	NR	–	–	–	–	–	–
	V_R		5.5	6.37	7.75			9.0	10.75	13.87	20.75							
M–W (85:15)	t_R	1.0	8.5	10.5	13.25		NR	16.5	21.12	29.25	49.5	15.5	18.75	NR	NR	25.8	27.87	NR
	V_R		8.5	10.5	13.25			16.5	21.12	29.25	49.5	15.5	18.75			25.8	27.87	
M–W (82.5:17.5)	t_R	1.5	7	7.87	12.5	–	NR	15.0	19.75	28.5	51.25	14.25	16.0	17.25	NR	25.0	26.5	27.25
	V_R		10.5	11.8	18.75	–		22.5	29.62	42.75	76.87	21.37	24.0	25.87		37.5	39.75	40.87
M–W (83:17)	t_R	1.8	6.0	7.5	10.5	–	NR	12.25	16.0	23.12	40.5	11.75*	NR	14.25	NR	20.25	21.5	–
	V_R		10.8	13.5	18.9			22.05	28.8	41.61	72.9	21.15		25.65		36.45	38.7	–
M–W (80:20), average (8 runs)	t_R	1.8	8.1	10.64	15.14	–	NR	18.34	24.72	36.77	75.05	17.32	19.76	20.62	26.64	32.36	33.92	–
	V_R		14.58	19.15	27.25			33.01	44.49	66.19	135.09	31.18	35.57	37.12	47.95	58.25	61.05	–
E–W (70:30)	t_R	0.9	10.75	13.0	17.25	–	NR	23.37	29.5	40.0	68.0	NR	25.25	NR	30.25	36.35	38.0	–
	V_R			11.7	15.52			21.03	26.55	36.0	61.2		22.72		37.22	32.62	34.2	–

* Marginal separation.

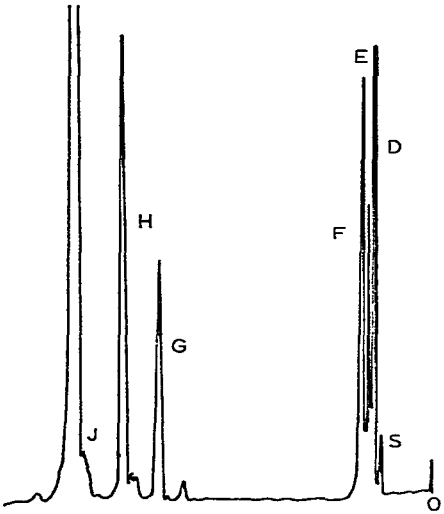

Fig. 1. HPLC separation of mixed cardanol and cardol under adsorption conditions on Partisil (5 μm) with *n*-hexane–methanol (100:4). Flow-rate, 1.0 ml/min. Peaks: D = cardanol monoene; E = cardanol diene; F = cardanol triene; G = cardol monoene; H = cardol diene; I = cardol triene.

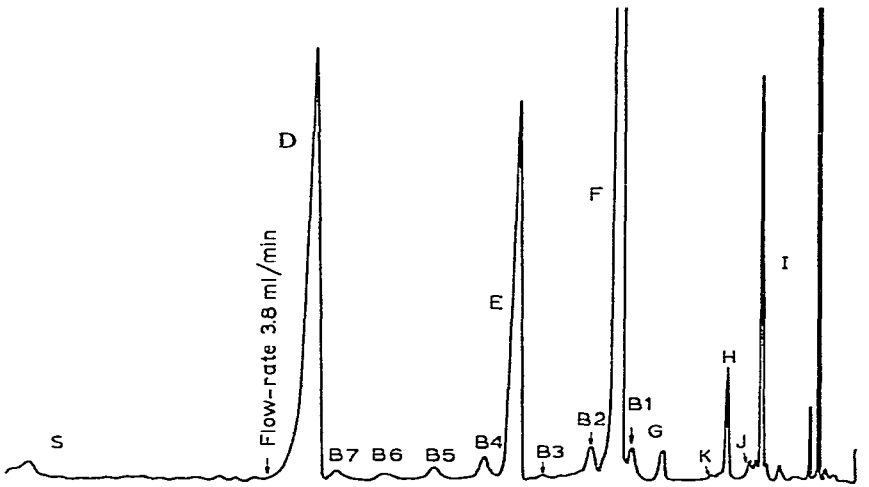

Fig. 2. HPLC separation of distilled CNSL under reversed-phase partition conditions on Spherisorb ODS (5 μm) with acetonitrile–water (66:34). Flow-rate, 1.7 ml/min. Peaks: D–I as in Fig. 1; J = 2-methylcardol triene; K = 2-methylcardol diene; S = saturated cardanol.

constituents of cardanol and cardol were incorporated in one solution which was chromatographically examined twelve times in order to select six representative results. To obtain a measure of agreement between the relative molar response (RMR) values for the monoene, diene and triene it proved necessary for chromatographic reasons or owing to integrator problems to examine four different standards before the required accuracy could be achieved, so that a considerable number of chromatograms were carried out to achieve the final result. A second standard of saturated

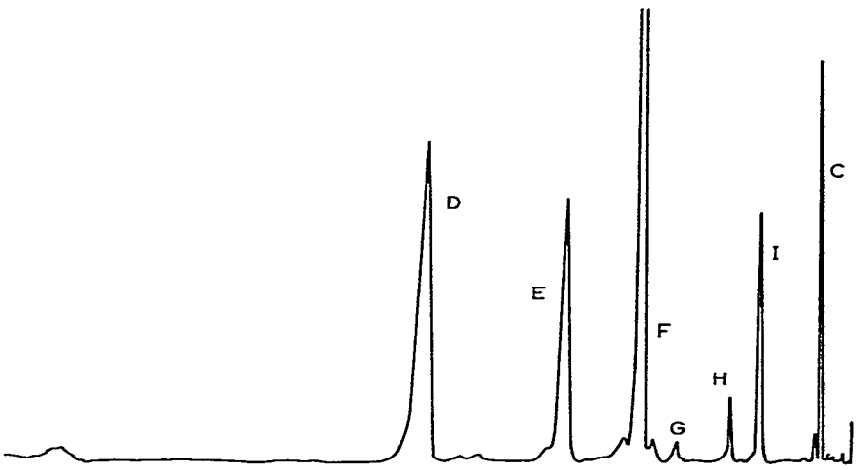

Fig. 3. HPLC separation of distilled CNSL on Spherisorb ODS (5 μm) with methanol–water (80:20). Flow-rate, 1.8 ml/min. Peaks as in Fig. 2. C = *p*-Cresol.

cardanol and saturated cardol with *p*-cresol was prepared, and the results for both are given in Table III. The lower RMR values for the cardol constituents compared with those of cardanol are expected from their respective ε values[14]. RMR values for the constituent phenol $(RMR)_p$ in relation to that for *p*-cresol ($RMR_c = 1$) were calculated from

$$RMR_p/RMR_c = \frac{(\text{peak area})_p}{(\text{g mole})_p} \Big/ \frac{(\text{peak area})_c}{(\text{g mole})_c}$$

TABLE III

RELATIVE MOLAR RESPONSE VALUES OF CASHEW NUT-SHELL CONSTITUENT PHENOLS (WITH REFERENCE TO *p*-CRESOL AS INTERNAL STANDARD)

Phenol	*Weight (mg)*	*Peak areas (normalised %)*	*Relative molar response value*
p-Cresol	2.10	10.35 ± 0.14	1.000
(15:3)-Cardol (cardol triene)	10.30	17.32 ± 0.13	0.992
(15:2)-Cardol (cardol diene)	0.74	1.25 ± 0.014	0.997
(15:1)-Cardol (cardol monoene)	—	—	0.997*
(15:3)-Cardanol (cardanol triene)	11.36	26.29 ± 0.39	1.296
(15:2)-Cardanol (cardanol diene)	7.55	17.13 ± 0.44	1.279
(15:1)-Cardanol (cardanol monoene)	11.51	26.47 ± 0.22	1.305
p-Cresol	1.92	18.07 ± 1.04	1.000
(15:0)-Cardol (cardol saturated)	11.70	31.29 ± 0.08	1.001
(15:0)-Cardanol (cardanol saturated)	9.84	50.61 ± 1.05	1.294

* Calculated value.

TABLE IV

QUANTITATIVE ANALYSIS OF DIFFERENT TYPES OF TECHNICAL CASHEW NUT-SHELL LIQUID

Parameter	*Internal standard (1)*	*(15:3) Cardanol (2)*	*(15:2) Cardol (3)*	*(15:1) Cardol (4)*	*(15:3) Cardanol (5)*	*(15:2) Cardanol (6)*	*(15:1) Cardanol (7)*	*(15:0) Cardanol (8)*	*Total (mg)*
Technical CNSL (new material) (128.86 mg)									
Relative molar response value	1.000	0.992	1.003	0.997	1.296	1.279	1.305	1.294	–
Peak area (normalised %)	7.47 ± 0.186	11.31 ± 0.100	2.92 ± 0.061	0.89 ± 0.077	38.29 ± 0.142	13.81 ± 0.190	22.03 ± 0.240	3.23 ± 0.369	–
Wt. calc. (mg)	(wt. used 3.94)	17.48	4.46	1.38	43.01	15.82	24.90	3.76	110.84
Percentage present (cols. 2–8)	–	13.56	3.48	1.07	33.38	12.28	19.32	2.91	86.02
Distilled CNSL (123.48 mg)									
Peak area (normalised %)	6.45 ± 0.106	5.83 ± 0.118	1.84 ± 0.017	0.75 ± 0.037	33.85 ± 0.287	16.33 ± 0.142	31.05 ± 0.303	3.88 ± 0.532	–
Wt. calc. (mg)	(wt. used 3.60)	9.54	2.96	1.23	40.23	19.80	37.14	4.58	115.61
Percentage present (cols. 2–8)	–	7.72	2.40	0.99	32.58	16.03	30.07	3.81	93.63
Technical CNSL (old material) (128.32 mg) (blend 3)									
Peak area (normalised %)	8.81 ± 0.205	6.36 ± 0.121	2.49 ± 0.085	1.03 ± 0.198	27.75 ± 0.356	16.36 ± 0.334	33.10 ± 0.210	4.05 ± 0.548	–
Wt. calc. (mg)	(wt. used 4.00)	8.46	3.30	1.37	28.83	16.09	32.20	3.99	94.24
Percentage present (cols. 2–8)	–	6.59	2.57	1.06	22.47	12.54	25.09	3.11	73.44

Quantitative analysis of different types of technical CNSL

The reversed-phase partition mode was used to obtain the quantitative composition of a typical good-quality technical CNSL, a vacuum-distilled material and a somewhat polymerised specimen, in terms of the constituents of cardanol and of cardol*. *p*-Cresol was incorporated with each of the types of CNSL and chromatograms obtained under isocratic conditions with the solvent acetonitrile–water (66:34). The objective in each case was to obtain six determinations with reproducible peak areas and low standard deviations. In early experiments sets of asymmetrical peaks were encountered, but subsequently when it was found possible to elute adsorbed polymeric material with 100% tetrahydrofuran, symmetrical peaks were obtained. From the peak areas obtained $(g\ mole)_p$ for each constituent was calculated from its known RMR_p value, $(peak\ area)_p$, $(peak\ area)_c$, $(g\ mole)_c$ and thence the percentage contribution. The total material accounted for is shown in the final column of Table IV. That unaccounted for includes the minor constituents B1 to B7, 2-methylcardol constituents, the C_{17} bis homologue of cardanol (in the form of the monoene, diene, and triene), polymeric material, and traces of anacardic acid (I; R = COOH, n = 0, 2, 4 or 6)**.

TABLE V

COMPOSITION OF TECHNICAL CNSL SAMPLES IN TERMS OF CARDANOL, CARDOL, 2-METHYLCARDOL, MINOR MONOMERIC CONSTITUENTS AND POLYMERIC MATERIAL

CNSL type	*(Found) cardanol (%)*	*(Found) cardol (%)*	*(Calc.**) 2-methyl-cardol (%)*	*(Calc.*) minor constituents (%)*	*(Calc.) polymeric material (%)*
New	67.82	18.20	3.32	3.28	7.38
Distilled	82.38	11.25	2.05	3.98	0.34
Old (blend 3)	63.13	10.31	1.88	3.05	21.63

* Based on triangulation and proportion of cardanol present. The same RMR value as for cardanol was assumed.

** The same RMR as for cardol was used.

Minor monomeric and polymeric material

Minor monomeric constituents. It was not found possible to use the particular integrator available to determine the peaks B1 to B7, or 2-methylcardol triene and diene. From previous work[2] on the analysis of hydrogenated and methylated technical CNSL the proportion of 2-methylcardol associated with cardol is fairly constant. On the basis of cardol having an associated 18% of 2-methylcardol, the calculated proportion of 2-methylcardol in the three samples of CNSL is given in Table V. By triangulation, the contribution of the minor peaks B1 to B7, due to monomeric substances, in the case of distilled CNSL was found to be 3.97% (in relation to the

* Generally the results show the instability of triene constituents, particularly that of cardol, towards distillation and the increase of the percentage of polymer with age of the material accompanied by diminution of both cardanol and cardol triene constituents.

** Anacardic acid was eluted prior to cardol, the constituents giving tailing peaks. The proportion was negligible in comparison with that reported earlier[17] as 1–1.5%.

peak area of cardanol diene, 16.03%). The final column in the table gives an estimate by difference of the proportion of polymeric material present in the three samples examined.

For a total quantitative analysis ideally it would be preferable to integrate the peak areas of B1 to B7 and to establish their chemical identity. Our work in this direction is as yet incomplete. From their retention data they are mostly related to C_{15} cardanol constituents and to the C_{17} bis homologue, the presence of which in the monoene, diene and triene form has been shown by mass spectroscopy[4].

Upon hydrogenation of the CNSL sample, peaks B1 to B7 substantially disappeared. A new large peak appeared after that for (15:0)-cardanol at the retention expected for saturated C_{17} (17:0)-cardanol, and a small peak preceded that for (15:0)-cardanol. From the retention data found for 3-butylphenol, 3-undecylphenol (log retention time, 1.36) available from synthesis[18] and (15:0)-cardanol (3-pentadecylphenol) (log retention time, 2.05) together with the linear relationship between methylenic carbon chain length and log (retention), the expected relative retention (1.51) compared to that of (15:0)-cardanol, the retention time for (17:0)-cardanol was found. On the reasonable basis that a similar relative retention holds for (17:1)-, (17:2)- and (17:3)-cardanol it seems most probable that peaks B7 and B4, respectively, correspond to the two latter constituents. It is considered that the remaining minor constituents are probably structural and geometrical isomers of (15:1)-, (15:2)- and (15:3)-cardanol. In a study of the effect of using different wavelengths for detection it was observed that at 240 nm certain of the B1 to B7 peaks exhibited maximum absorption consistent with the presence of conjugated side-chains. The small peak preceding (15:0)-cardanol is probably ascribable to C_{13} chain-length material.

Polymeric material. The presence of polymeric material has previously[5] been inferred from quantitative GLC analysis, but by gradient elution it proved possible

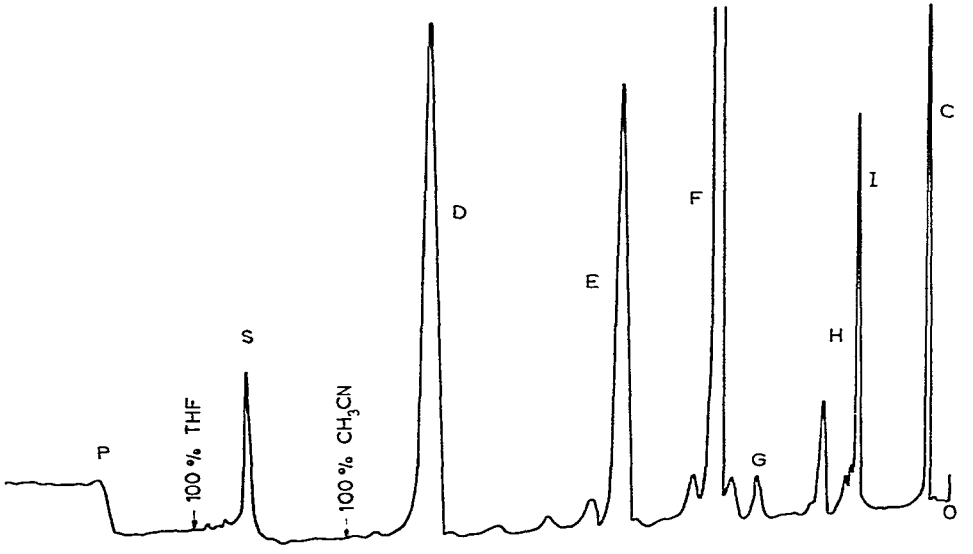

Fig. 4. HPLC separation of distilled CNSL on Spherisorb ODS (5 μm) by gradient elution, starting with acetonitrile–water (66:34). Flow-rate, 1.7 ml/min. Peaks D–I and S as in Fig. 2; P = polymer; C = *p*-cresol.

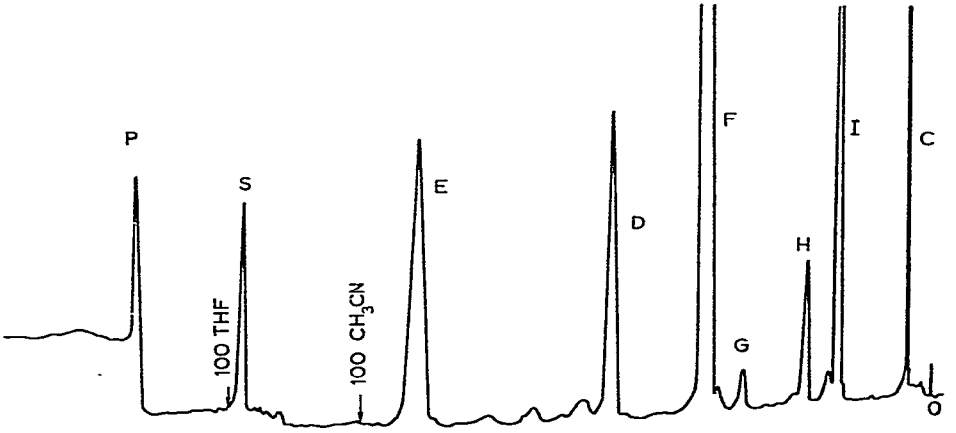

Fig. 5. HPLC separation of new CNSL on Spherisorb ODS (5 μm) by gradient elution, starting with acetonitrile–water (66:34). Flow-rate, 1.7 ml/min. Peaks as in.Fig. 4.

by HPLC to demonstrate its presence. Gradient elution was also desirable because of the long retention of (15:0)-cardanol. In the absence of gradient elution, stepwise elution of (15:0)-cardanol was effected with acetonitrile (100%)* and of polymeric material with tetrahydrofuran (100%). With gradient elution a progressive programmed change from acetonitrile–water (66:34) to tetrahydrofuran (THF) (100%) was effected. Figs. 4, 5, and 6 show the complete chromatograms for distilled, new and old (blend 3) technical CNSL, respectively**. The heterogeneous nature of the polymeric material is clear since a number of peaks are produced upon elution with acetonitrile–tetrahydrofuran. The retention information suggests that the material is probably

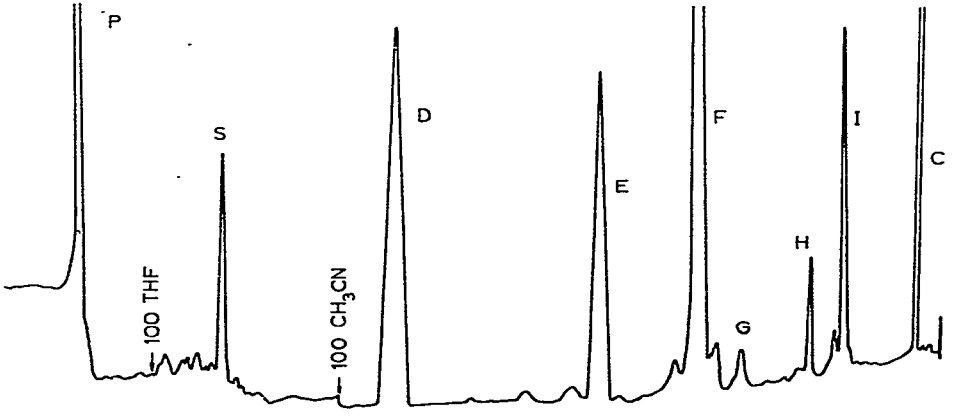

Fig. 6. HPLC separation of old CNSL on Spherisorb ODS (5 μm) by gradient elution, starting with acetonitrile–water (66:34). Flow-rate, 1.7 ml/min. Peaks as in Fig. 4.

* Rapid analysis of hydrogenated technical CNSL was effected with acetonitrile (100%).

** Adsorption conditions were not suitable for demonstrating the presence of polymer.

dimeric and trimeric and relatively saturated. Recovery of the eluted polymeric material and TLC examination (Fig. 7) indicated an increase of complexity and polarity with level of polymerisation.

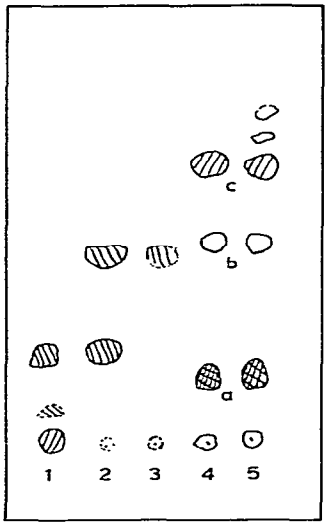

Fig. 7. TLC separation on silica gel G of THF eluted polymer. 1 = From old CNSL; 2 = from new CNSL; 3 = from distilled CNSL; 4 = new CNSL; 5 = old CNSL. Solvent, chloroform–ethyl acetate (90:10). a = Cardol; b = 2-methylcardol; c = cardanol.

Comparison of HPLC with other methods of analysis for phenolic lipids

Previous analyses based essentially on GLC have consisted of two stages, the component phenols being determined after hydrogenation and methylation[2] and the unsaturated constituents of each analysed by preliminary TLC separation of each component phenol followed by GLC on the methyl ethers[1], or mass spectrometry[4] on the component phenols. More recently[6], trimethylsilylated technical CNSL has been examined by GLC on polyethyleneglycol adipate and all the unsaturated constituents were separated. This procedure is somewhat similar to the HPLC adsorption mode in the order of emergence of constituents. The need for derivatisation and the relatively high temperature involved, which may cause some polymerisation, particularly in the preheater of the GLC apparatus, are disadvantages of the "hot" method. The HPLC method, particularly, in the reversed-phase partition mode, avoids the preceding difficulties and gives a higher degree of resolution of all the constituents, although good integration equipment is necessary for all the minor constituents to be determined. A rapid method is now available for the industrial evaluation of technical CNSL in terms of the principal phenols and polymeric material and for this purpose the internal standard is not absolutely necessary.

We have examined natural CNSL and urushiol by HPLC and derivative formation in the latter case appears unnecessary. Bearing in mind the increasing numbers of phenolic lipid types being found[19], most of which contain some highly unsaturated constituents, there is little doubt that for their analysis HPLC is the method of choice.

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