Journal of Chromatography, 303 (1984) 137–150
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,967

LONG-CHAIN PHENOLS

XXV*. QUANTITATIVE ANALYSIS OF NATURAL CASHEW NUT-SHELL LIQUID (*ANACARDIUM OCCIDENTALE*) BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY**

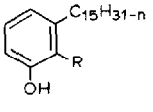
J. H. P. TYMAN*, V. TYCHOPOULOS and P. CHAN
Department of Chemistry, Brunel University, Uxbridge, Middlesex UB8 3PH (U.K.)
(Received June 14th, 1984)

SUMMARY

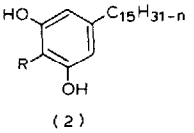
The phenolic constituents of natural cashew nut-shell liquid (CNSL) from *Anacardium occidentale* have been separated by high-performance liquid chromatography with 5-μm ODS Spherisorb by the reversed-phase method. The relative molar response values for the saturated component phenols have been determined and the use of an internal standard has led to a quantitative procedure. Isocratic elution with acetonitrile-water-acetic acid and gradient elution with acetonitrile followed by tetrahydrofuran has enabled a total analysis to be effected. Various regional sources of natural CNSL have been examined.

INTRODUCTION

The principal component phenols of natural cashew nut-shell liquid (CNSL)[2] (*Anacardium occidentale*) anacardic acid (1; R = CO_2H, n = 0, 2, 4, 6), cardol (2; R = H, n = 0, 2, 4, 6), 2-methylcardol (2; R = CH_3, n = 0, 2, 4, 6) and cardanol (1; R = H, n = 0, 2, 4, 6) have previously been analysed quantitatively by gas–liquid chromatography (GLC)[3], thin-layer chromatography (TLC)[4] and TLC–mass spectrometry[5]. A quantitative method of total analysis has been sought avoiding derivatisation, free from thermal polymerisation side-reactions and the problems of combined techniques or the use of two different GLC columns. Increasing interest in the utilisation of the natural product[6,7] has required the availability of a rapid procedure



(1)



(2)

* For Part XXIV, see ref. 1.

** Presented at the *14th IUPAC Symposium on the Chemistry of Natural Products, Poznan, Poland, July 1984.*

0021-9673/84/$03.00

by which both the quality of the raw material and the monitoring of reaction mixtures containing it could be effected.

The method[8] for the total analysis of the related technical CNSL, the industrial product obtained by decarboxylation, was not found to be suitable for the natural product and it was essential to develop a different high-performance liquid chromatographic (HPLC) procedure for the analysis of the mixed phenolic acids and phenols. The presence of these two functional types precluded the adaptation of the method for mixed anacardic acid described by Lloyd and co-workers[9] to the more complex natural CNSL.

By the reversed-phase technique and initially isocratic elution with acetonitrile–water–acetic acid followed by acetonitrile and finally tetrahydrofuran, a total quantitative analysis of natural CNSL has been effected revealing the constituents of the component phenols, the minor constituents and polymeric material.

Although complex mixtures of hydroxybenzoic and polyphenolic acids from plant extracts[10] and their metabolic and decomposition products[11], of hydroxynaphthalenes and hydroxybenzophenones[12] have been analysed by HPLC and the HPLC analysis of phenolic materials has been reviewed[13], the phenolic lipids have received no attention apart from studies on acetylated urushiol[14], the catechol analogue of the cashew phenols (1; R = OH).

EXPERIMENTAL

Equipment

A similar assembly was used essentially as in the previously described work[8] with a Perkin-Elmer LC 55 variable-wavelength ultraviolet spectrophotometer, but with two Altex metering pumps (Model 110A), a Rikadenki recorder and a Columbia Scientific Instruments Supergrator 3A computing integrator. Reversed-phase partition experiments were conducted with 5-μm Magnusphere and Spherisorb bonded with ODS in 250 × 4.6 mm stainless-steel columns.

Conditions

For the detection of phenols and phenolic acids the wavelength for detection was 275 nm. Experiments at 255, 270 and 310 nm were conducted as discussed in the section on Results. The solutes were made up in ethereal rather than chloroform solution and generally 5–10 μl of a 5% solution was used. It was found essential to use a gradient elution system according to the following typical programme in the acetonitrile series with solvent A, acetonitrile-water-acetic acid (66:33:2) and solvent B, tetrahydrofuran (100%)*:

Time	Flow (ml/min)	Composition
0	2.7	A (100%) B (0%) (duration 3 min)
25	2.7	A (70%) B (30%) (duration 10 min)
38	2.7	A (0%) B (100%)
52	2.7	A (100%)

* For the calibration, 100% A was used for 54 min to ensure comparable UV detection of (15:0)-cardanol.

At the end of a series of runs the column was purged with water to remove the acetic acid, followed by pumping through with methanol or acetonitrile. After a succession of many runs it was found essential to clean the column by passing through solvent in the order, tetrahydrofuran, water, methanol, acetonitrile, dichloromethane and iso-octane followed by a reverse of this sequence. Occasionally it was found desirable to remove the top 4 mm of the column and to "redome" it with fresh adsorbent.

Materials and procedure

All solvents (except acetic acid) for HPLC were of liquid chromatography grade. Cashew nuts of known Mozambique, Brazilian and Kenyan origin were obtained originally from Mr. J. O. Duce, Wigglesworth & Co, London, U.K.

For the extraction of the natural CNSL from each source, the particular source of nuts (50 g) was stored overnight in a freezer to make the shell brittle. The nuts were bisected by light hammering along the axis of the junction of the two halves of the shell. The intact kernel in the testa lining was separated and the two halves of the shells were placed in diethyl ether (100 cm^3) containing 0.1% of the antioxidant 4-methyl-2,6-di-*tert.*-butylphenol. After 24 h the ethereal solution was decanted and the ground-up residual shell material re-extracted with more diethyl ether (100 cm^3) during a further 24 h. The combined extracts were filtered and evaporated to constant weight.

Anacardic acid, cardol and cardanol required for the preparation of a standard calibration solution were separated as described[15,16], or by "flash" chromatography[17,18] of the phenolic filtrate after removal of anacardic acid as lead anacardate. Since the monoene, diene and triene constituents had, from previous experiments, almost identical extinction coefficients in the ultraviolet to the saturated component it was found more convenient to make a stable calibration standard of (15:0)-anacardic acid, (15:0)-cardol and (15:0); cardnol. The saturated compounds were prepared by catalytic hydrogenation of the mixed materials in ethanolic solution with palladium-carbon catalyst and hydrogen as previously described[15,19]. *p-tert.*-Butylphenol (BDH) was used as an internal standard in preference to *p*-cresol.

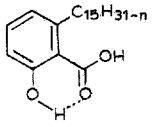
For the preparation of the standard, (15:0)-cardol (134.4 mg), (15:0)-cardanol (127.7 mg), (15:0)-anacardic acid (146.1 mg) and the internal standard (16.8 mg) were weighed on a five-place balance. The mixture was prepared in diethyl ether solution (10 cm^3) and stored at 0°C under nitrogen away from the light. For the HPLC analyses of natural CNSL samples in ethereal solution (10 cm^3), the internal standard was incorporated with each material. At least five analytical determinations were made on each of the CNSL samples, and ten on the calibration standard for determination of the relative molar response valves. Standard deviations were obtained in the usual way. The % composition determined from peak height recorded as a digital read-out on the spectrophotometer agreed with that found by the computing integrator*.

* The gradient elution programme affected the integration and peak width, height, and sloping base line changes were continuously taken into account. Changes of base line due to solvent alteration only were checked by runs without solute. Attempts to use three solvents with manual change at selected times tended to lead to abrupt and less predictable base line variation than with automatic gradient elution and two solvents. Ideally the integration programme should not be dependent on the chromatogram.

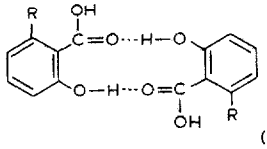
RESULTS AND DISCUSSION

Retention volumes of the constituents of the phenolic components

The normal adsorption mode was not found suitable for the HPLC separation of natural CNSL and experiments were therefore conducted by reversed-phase partition, initially with methanol-water in which it was expected that the polarity of anacardic acid would lead to its early elution in the chromatogram. Increase in polarity of the solvent mixture led to increased retention volumes for both cardol and anacardic acid (Table I), the former always emerging first although the constituents of each were not resolved. The elution of the more acidic anacardic acid after cardol suggests that it is present in the intramolecularly hydrogen-bonded form (4) or the dimeric intermolecular type (5).



(4)



(R = $C_{15}H_{31-n}$)

(5)

TABLE I

RETENTION TIMES (t_R) AND RETENTION VOLUMES (V_R)* OF PHENOLIC CONSTITUENTS (OF NATURAL CNSL) UNDER PARTITION (REVERSED-PHASE) CONDITIONS ON MAGNUSPHERE (5 μm) BONDED WITH OCTADECYLSILANE

NR = not resolved. A_2 = (15:3)-2-methylcardol; A_3 = (15:2)-2-methylcardol; A_6 = (17:1)-anacardic acid; A = acetic acid; C = cyanomethane; W = water.

Solvent	*Retention*	*Flow-rate (ml/min)*	*Cardol*			*Anacardic acid*	
			15:3	*15:2*	*15:1*	*15:3*	*15:2*
C–W–A	t_R	1.7	6.63	8.56	12.74	11.07	15.07
66:33:2	V_R		11.27	14.55	21.66	18.82	25.62
C–W–A	t_R	2.7	4.17 ± 0.086	5.40 ± 0.117	7.96 ± 0.193	6.91 ± 0.152	9.44 ± 0.218
66:33:2	V_R		11.26	14.58	21.49	18.66	25.49
M–W	t_R	2.0 (NR)	2.40	NR	NR	4.10 (NR)	NR
70:10	V_R		4.80			8.20	
M–W–A	t_R	1.5	5.35	–	–	9.35 (NR)	NR
85:15:0.04	V_R		8.02			14.03	
M–W–A	t_R	1.75	11.70	NR	NR	17.70 (NR)	NR
80:20:0.04	V_R		20.48			30.96	

* These are corrected values since the column is permanently solvent saturated.

The solvent system, methanol–water–acetic acid (85:15:0.04) and detection wavelength, 313 nm, reported[9] for the separation of mixed anacardic acid were not found suitable for natural CNSL. Cardanol and cardol exhibited almost zero absorption at that wavelength[4]. Change to 275 nm offered no improvement since with that solvent resolution was inadequate. Accordingly the solvent acetonitrile–water[8] with the addition of acetic acid, was examined. After much experimentation the mixture acetonitrile–water–acetic acid (66:33:2) was adopted and gave resolution of the constituents of the major component phenols, cardol, anacardic acid and cardanol and of the minor materials (peaks A1–A6) (Table I), the identity of which is discussed later. High relative retentions in the acetonitrile solvent compared to the methanol system are in conformity with the relative k' of the former in reversed-phase chromatography[20]. The reproducibility of retention volumes was approximately ±2% either in a given series of runs or with different flow-rates within the range 1.7–2.8 ml/min. All peaks in chromatograms were identified by HPLC examination of the pure constituent, the structures of which have been established[15].

Acetic acid appears to exert two opposing effects on anacardic acid although this did not affect the other component phenols. As a polar organic solvent an increasing proportion would lead to a decrease in retention volume for anacardic acid whereas its acidity would suppress that of anacardic acid through formation of 4 and 5 and thence cause an increase in retention volume.

Cardanol				*Other constituents (minor)*					
15:1	*15:3*	*15:2*	*15:1*	A_1	A_2	A_3	A_4	A_5	A_6
23.24	14.28	19.91	32.35	–	–	–	–	–	–
39.51	24.28	33.85	55.00	–	–	–	–	–	–
14.46 ± 0.369	8.99 ± 0.225	12.52 ± 0.328	20.01 ± 0.548	1.19	4.68	6.15	10.08	11.2	13.2
39.04	24.27	33.80	54.03	3.21	12.64	16.61	27.22	30.24	35.6
6.00	NR	NR	NR	NR	NR	NR	NR	NR	NR
12.00									
15.20	NR	NR	NR	NR	NR	NR	NR	NR	NR
(NR)									
22.80									
32.0	NR	NR	–	NR	NR	NR	NR	NR	NR
(NR)									
56.0									

The mixtures, acetonitrile–water–acetic acid, 70:30:0, 70:29:1 and 65:33:2 gave as expected a progressive increase in retention volume for cardol while anacardic acid exhibited non-linear behaviour. This effect is still being studied. In the absence of acetic acid, anacardic acid gave tailing peaks and progressive increase in the proportion of acetic acid resulted in sharper less broad peaks apparently through ionisation suppression. The addition of acetic acid not only enhanced the symmetry of peaks for the anacardic acid constituents but increased the resolution of the minor peaks A1–A6. A further advantage of the acetonitrile–water solvent system was its lower viscosity and resultant pressure in the system.

Ultraviolet wavelength for detection

Anacardic acid exhibits a UV absorption maximum at 308 nm while for cardanol, cardol and 2-methylcardol this is generally at 275 nm[4]. UV detection at 255, 270, 275 and 310 nm was examined. At 255 nm and 310 nm the intense absorption of the phenolic acid dominated the chromatogram obscuring the minor peaks of the constituents of cardol and of cardanol. At 270 nm and 275 nm similar patterns of peaks were observed but the ratio of peak height and peak area was different. Generally the latter wavelength afforded greater sensitivity.

Choice of solvent for solute injection

In the analysis of the solute (natural CNSL) the solvent for solute dissolution was found, somewhat unusually, to have a significant effect on the resolution observed in the resultant HPLC chromatogram. Although in previous work[8] with technical CNSL chloroform appeared to be completely satisfactory, it proved to be unexpectedly deleterious in the present work. The effect is shown in Fig. 1, from which it can be discerned that although the main peaks have the same general order of retention, they are no longer symmetrical compared with those in Fig. 3 and furthermore they are considerably diminished in peak height. The effect was highly reproducible. Chloroform may well influence the solvent composition at the site of partition–separation although no obvious explanation can be advanced. With diethyl

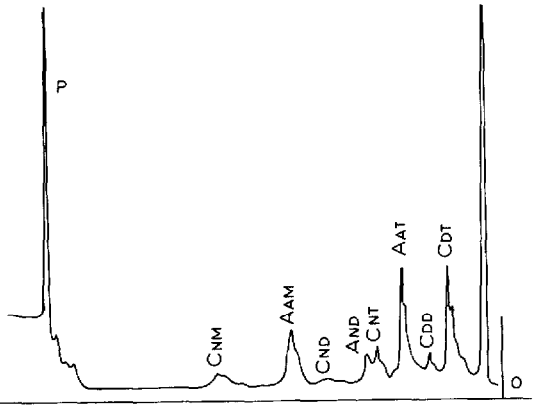


Fig. 1. Effect of chloroform as solute solvent in the HPLC separations of natural CNSL. Cd = cardol; Cn = cardanol; Aa = anacardic acid; T = triene; D = diene; M = monoene; S = saturated.

ether no comparable problems were found and it was adopted throughout these analyses.

Determination of relative molar response (RMR) values

The reversed-phase mode facilitated the choice of an internal standard and enabled a lower alkylphenol to be used. *p*-Cresol, *m*-cresol, 2,6-di-*tert.*-butylphenol, acetophenone and *p*-bromophenol all gave too short a retention time of less than 1.5 min (at a flow-rate of 2.7 ml/min) whereas *p-tert.*-butylphenol afforded a single sharp peak (t_R 2 min) which just preceded the first of the minor components in the chromatogram.

It was found convenient to employ the saturated component phenols obtained by hydrogenation of the mixed component phenols since the unsaturated side chain contributed nothing to the UV absorption of the phenol in the region 275 nm[4]. In the early stages of the work it was found necessary to make up five calibration standards to ensure reproducible results. The molar ratio of the component phenol to that of internal standard was kept in the region of 10:1 to ensure linearity of response. At the molar ratio (component phenol:internal standard) 1:1.5 departures from linearity were observed which were attributed to the very sharp peak for *p-tert.*-butylphenol and overloading of the micro flow-through cell. Fig. 2 depicts the linearity achieved by adopting a considerably lower molar proportion of internal standard in the calibration solution. Quantitative analysis appeared to be possible only at the

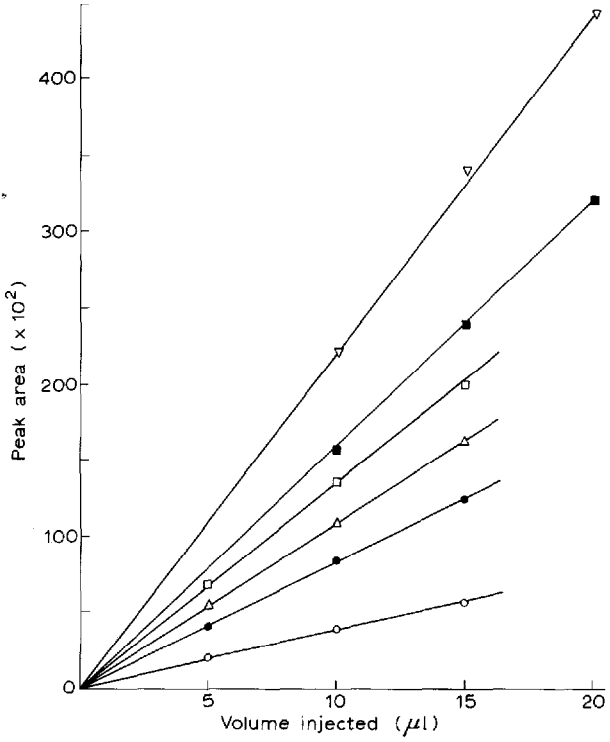


Fig. 2. Linearity of UV detection. ○, (15:3)-Cardanol (Cnt); ●, internal standard; △, (15:3)-cardol (Cdt); □, (15:3)-anacardic acid (Aat); ■, (15:0)-anacardic acid (Aas); □, (15:0)-cardol (Cds).

molar ratios and concentrations indicated. The proximity of peaks precluded the use of a higher alkylphenol since it was also desirable for the internal standard to be eluted with the main constituents in the isocratic portion of the elution programme.

RMR values for the saturated phenols (RMR_p) in relation to *p-tert.*-butylphenol ($RMR_b = 1$) were calculated from eqn. 1.

$$RMR_p/RMR_b = \frac{(\text{peak area})_p}{(g \cdot \text{mol})_p} \Big/ \frac{(\text{peak area})_b}{(g \cdot \text{mol})_b} \quad (1)$$

The results are shown in Table II and follow the expected numerical trend from their known extinction coefficients at 275 nm[4]. The standard deviation for a given run was ±2% and for a series of calibration standards was similar.

Quantitative analysis of different types of natural CNSL

To achieve quantitative analysis of all the components present, the gradient elution programme listed in the Experimental was convenient since at a flow-rate of 2.7 ml/min the complete chromatogram occupied 55 min. The internal standard, *p-tert.*-butylphenol, was incorporated with regional sources of natural CNSL of Brazilian, Kenyan and Mozambique origin. A typical chromatogram or two is shown in Fig. 3. Four chromatograms following the initial run were run in each case to ensure reproducibility and to provide information for the computing integrator which was programmed to include all the minor peaks referred to as A1–A6. Of the 32 peaks recorded nine contributed more than 85% of the total in each case, two other appreciable components being (15:0)-anacardic acid and polymeric material. The % compositional results with respect to the three constituents of cardol, anacardic acid, and cardanol are shown in Table III, and were calculated from the known values of RMR_p, $(\text{peak area})_p$, $(\text{peak area})_b$ and $(g \cdot \text{mol})_b$ by the use of eqn. 1. The RMR_p values for the unsaturated constituents were calculated by assuming that an equal weight of the respective material had been used in place of the saturated (Table II).

The unaccounted material comprises the minor materials A1–A6, of which A2 and A3 represent (15:3)-2-methylcardol and (15:2)-2-methylcardol, respectively,

TABLE II

RELATIVE MOLAR RESPONSE VALUES OF (15:0) PHENOLS* FROM NATURAL CASHEW NUT-SHELL LIQUID (AT 275 nm)

With reference to *p-tert.*-butylphenol as internal standard.

(15:0) Phenol	*Retention time (min)*	*Retention volume** (cm^3)*	*Weight (mg)*	*Peak areas (normalised %)*	*Relative molar response value*
p-tert.-Butylphenol	1.83	4.94	16.8	9.36 ± 0.24	1.0000
Cardol	15.39	41.55	134.4	26.29 ± 0.47	0.749
Anacardic acid	28.83	77.84	146.1	18.77 ± 0.435	0.535
Cardanol	43.47	117.37	127.7	43.68 ± 0.39	1.246

* Only minor constituents in the natural product and obtained by hydrogenation of the mixed component phenols.

** Flow-rate 2.7 cm^3/min. Chart speed 30 cm/h.

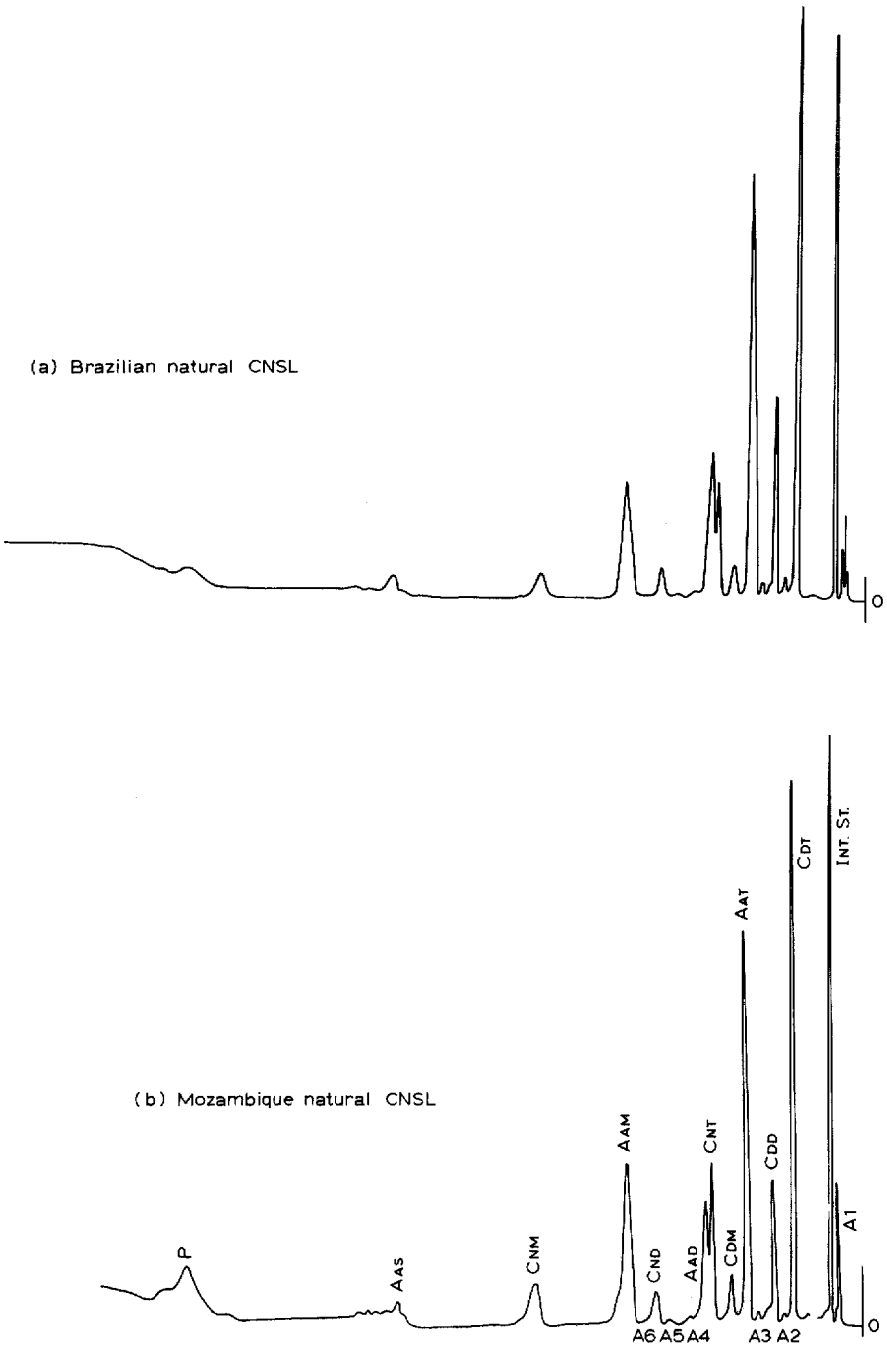


Fig. 3. HPLC separations of natural CNSL from different sources. (a) Brazilian, (b) Mozambique.

TABLE III

QUANTITATIVE ANALYSIS OF NATURAL CASHEW NUT-SHELL LIQUID FROM DIFFERENT REGIONAL SOURCES

Parameter	*Internal standard*	*Cardol*			*Anacardic acid*			*Cardanol*			*Total (mg)*
		15:3	*15:2*	*15:1*	*15:3*	*15:2*	*15:1*	*15:3*	*15:2*	*15:1*	
Relative molar response value*	1.000	0.735	0.7395	0.744	0.526	0.529	0.532	1.221	1.229	1.237	
Brazilian CNSL (443.1 mg) (internal standard, 16.9 mg)											
Peak area normalised (%)	11.53 ± 0.105	18.32 ± 0.178	0.018 ± 6.03	2.02 ± 0.088	20.94 ± 0.173	8.87 ± 0.017	11.84 ± 0.145	4.33 ± 0.139	2.32 ± 0.076	2.76 ± 0.01	
Wt. calc. (mg)	–	76.47	25.17	8.43	133.04	56.36	75.24	10.32	5.53	4.98	375.54
% Present (cols. 3–11)		17.26	5.68	1.90	30.02	12.72	16.98	2.33	1.25	1.13	89.27
Kenyan CNSL (432.3 mg) (internal standard, 16.9 mg)											
Peak area normalised (%)	11.23 ± 0.052	13.88 ± 0.106	4.55 ± 0.045	2.39 ± 0.031	18.01 ± 0.103	7.30 ± 0.137	15.34 ± 0.135	4.38 ± 0.073	2.07 ± 0.043	3.83 ± 0.243	
Wt. calc. (mg)	–	59.49	19.50	10.24	117.48	47.62	100.09	10.72	5.06	8.22	379.02
% Present (cols. 3–11)	–	13.76	4.51	2.37	27.17	11.01	23.15	2.48	1.17	2.04	87.675
Mozambique CNSL (431.4 mg) (internal standard, 16.9 mg)											
Peak area normalised (%)	10.75 ± 0.175	13.76 ± 0.307	4.09 ± 0.027	2.21 ± 0.063	17.26 ± 0.112	7.08 ± 0.306	14.46 ± 0.075	4.96 ± 0.173	2.15 ± 0.109	4.32 ± 0.093	
Wt. calc. (mg)	–	61.61	18.31	9.90	117.61	48.25	98.56	12.69	5.50	8.42	380.85
% Present (cols. 3 11)	–	14.28	4.24	2.29	27.26	11.18	22.85	2.94	1.275	1.95	88.28

* The RMR values should, in fact, be identical and equal to that of the (15:0) constituent on the basis that equal peak area results from equimolarity of the constituent (eqn. 1). The % differences resulting from using the RMR value of the (15:0) constituent in each case are however within the standard deviations generally found.

TABLE IV

COMPOSITION (%) OF NATURAL CNSL FROM DIFFERENT REGIONAL SOURCES

The values in parenthesis denote the amounts in mg.

Natural CNSL Type (mg)	*Anacardic acid*		*Cardol*	*Cardanol*	*2-Methylcardol***	*Total*		*Polymer*§*
	(15:3) (15:2) (15:1)	*(15:0)**	*(15:3) (15:2) (15:1)*	*(15:3) (15:2) (15:1)*	*(15:3) (15:2)*	*Accounted*	*Unaccounted****	
Brazilian (443.1)	59.71 (264.6)	0.96 (6.09)	24.81 (110.07)	4.70 (20.83)	1.61 (7.16)	92.25 (408.75)	7.75	1.61
Mozambique (431.4)	61.34 (264.1)	1.27 (8.07)	20.82 (89.82)	6.17 (26.61)	0.97 (4.20)	91.05 (392.8)	8.95	8.49
Kenyan (432.3)	61.32 (265.09)	1.21 (7.69)	20.64 (89.23)	5.69 (24.6)	0.88 (3.82)	90.31 (390.43)	9.69	7.03

* RMR value for (15:0)-anacardic acid = 0.535.

** RMR value for (15:3)- and (15:2)-2-methylcardol calculated as 0.533.

*** This includes material and minor constituents. The % is based on gravimetry.

§ This is obtained from the normalised % composition by integration and is uncorrected.

(15:0)-anacardic acid and polymeric material as well as other constituents. The total % composition for each component phenol is shown in Table IV in which it can be seen that 7.75, 9.69 and 8.95% unaccounted material is present in Brazilian, Kenyan and Mozambique CNSL, respectively. This contains polymeric material for which an RMR value could not be easily obtained and its % listed in Table IV is that obtained directly from the uncorrected % composition.

Amongst the minor components, A6 is considered to be (17:3)-anacardic acid (t_R 13.2 min). The calculated value (t_R 13.3 min) is based on the calculated retention for the (17:2) constituent (t_R 18.2 min), and the experimental value for the (17:1) found from an HPLC examination of the shell oil from *Pistacia vera*[1,21], in relation to the known relative retentions for the (15:3)-, (15:2)- and (15:1)-anacardic acids.

Peaks A1, A4, A5 and numerous others have not as yet been identified although they appear to be phenolic from their UV absorption and are of decreasing polarity. They appear to be mainly attributable to stereo and structural isomers of the principal component phenols since upon hydrogenation of any of the natural CNSL samples, substantially only three peaks for saturated cardanol, cardol and anacardic acid (C15 and C17) remained (Fig. 4). A number of minor peaks were present at retention times similar to the polymeric material suggestive of the presence of related molecular weight substances of different adduct structure[3]. Another group of minor substances was associated with the peak for (15:0)-anacardic acid and they presumably represent related saturated substances.

Generally it can be concluded from these analytical results that Brazilian CNSL is richer in the more unsaturated constituents than the other two sources. This compositional difference is reflected in the resultant technical CNSL obtained by industrial processing[22]. The % polymer is higher in the Kenyan and Mozambique sources and the lower % cardol in these two latter sources is indicative also of the susceptibility of this reactive component to polymerisation and deterioration.

Comparison of HPLC analysis with other chromatographic techniques

The present HPLC results have been compared with those determined by GLC[22,23] and by TLC–UV[4]. Table V shows a comparison of the % composition of Mozambique and Kenyan type natural CNSL sources. Because of the partial decarboxylation of the major component, anacardic acid, since the time of the original TLC and GLC determinations, the results have been compared on an equal % cardanol basis assuming that the only deterioration has been that of decarboxylation

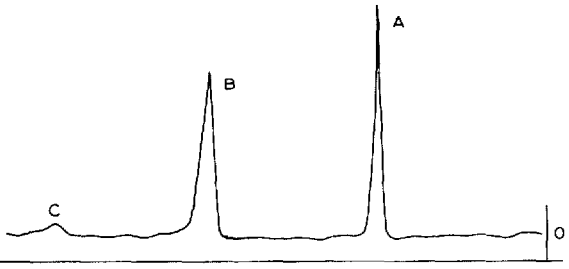


Fig. 4. HPLC separation of hydrogenated natural CNSL. A, (15:0)-cardol; B, (15:0)-anacardic acid; C, (15:0)-cardanol.

TABLE V

COMPARISON OF COMPOSITION (%) OF COMPONENT PHENOLS (IN VARIOUS SOURCES) DETERMINED BY DIFFERENT METHODS

Source	*Method*	*Anacardic acid*	*Cardol*	*Cardanol*	*2-Methylcardol*
Mozambique	HPLC	(a) 69.29*	22.87	6.77	1.07
		(b) 71.19	22.87	5.12	1.07
		(c) 71.65 ± 0.493	22.17 ± 0.398	5.13 ± 0.374	1.08 ± 0.049
	TLC–UV	71.37 ± 1.25	20.28 ± 0.71	5.12 ± 0.74	3.25 ± 0.74
	GLC	71.51 ± 0.23	22.34 ± 0.11	3.27 ± 0.11	2.75 ± 0.72
Kenya	HPLC	(a) 69.55	22.85	6.30	0.98
		(b) 73.80	22.85	2.59	0.98
		(c) 73.64 ± 0.375	22.80 ± 0.181	2.58 ± 0.359	0.98 ± 0.012
	GLC	75.72 ± 0.486	19.34 ± 0.178	2.59 ± 0.09	2.37 ± 0.987

* (a) Represents the normalised % composition for the four components calculated from Table IV; (b) represents an adjustment of the % anacardic for that decarboxylated to cardanol. For the Mozambique sample the % cardanol (5.12), and for the Kenyan sample the % cardanol (2.59) have been used as the basis for calculation. (c) Normalisation of (b).

with no change of unsaturation. The normalised HPLC results (c), following the calculation step (b), show reasonably good agreement with those from GLC and TLC–UV, with the exception of the % cardol in the Kenyan material*.

The derivatisation step in GLC, the thermal conditions involved and the determination of the % polymer, essentially by difference, all combine to complicate this method and there must also be reservations concerning the accuracy of determining the minor constituents. Probably trimethylsilylation[24] represents the most suitable method of derivatisation but unfortunately it was not possible to make a comparison of this approach with HPLC in the present experiments.

The TLC–UV method while giving quite good agreement with HPLC is essentially an adsorption method in which minor components cannot reliably be determined as can be seen from the higher standard deviations found. The improvement in integration procedures and the partition nature of the reversed-phase HPLC method clearly reveals a detailed composition which is only likely to be matched by the capillary GLC technique. The revelation of polymeric material in the HPLC method is a further advantage however. The combination of HPLC with MS could enable all the minor constituents to be structurally determined and quantified.

The present results show that part of the polymeric material in technical CNSL is certainly originally present in the natural product and the industrial decarboxylation is probably effected quite efficiently. The % cardol in natural CNSL is higher than generally appreciated and indicated in review literature while the % anacardic acid is substantially lower than generally quoted. HPLC analysis both of natural and technical CNSL is a valuable quality control operation indicating the efficiency of the decarboxylation process and affords a useful guide to the potential chemical reactivity to be expected in for example polymerisation reactions.

* The lower % cardol shown by GLC analysis is probably due to the derivatisation step and thermal instability in the analysis.

REFERENCES

1 M. Yalpani and J. H. P. Tyman, *Phytochemistry*, 22 (1983) 2263.
2 J. H. P. Tyman, *Chem. Soc. Rev.*, 8 (1979) 499.
3 J. H. P. Tyman, *J. Chromatogr.*, 156 (1978) 255.
4 J. H. P. Tyman, *J. Chromatogr.*, 166 (1978) 159.
5 J. H. P. Tyman, *J. Chromatogr.*, 136 (1979) 289.
6 A. J. Hawkes, A. A. Durrani and J. H. P. Tyman, *Eur. Pat. Appl.*, 80 300702.0 (1980).
7 J. H. P. Tyman, *U.K. Patent Appl.*, 82 20097 (1982).
8 J. H. P. Tyman, V. Tychopoulos and B. A. Colenutt, *J. Chromatogr.*, 213 (1981) 287.
9 H. A. Lloyd, C. Denny and G. Krishna, *J. Liq. Chromatogr.*, 3 (1980) 1497.
10 F. Villeneuve, G. Abbravanel, M. Montouret and G. Alibert, *J. Chromatogr.*, 234 (1982) 131.
11 R. D. Hartley and H. Buchan, *J. Chromatogr.*, 180 (1979) 139.
12 G. Chiavari, V. Concialini and P. Vitali, *J. Chromatogr.*, 249 (1982) 385.
13 J. B. Harborne, in E. Heftmann (Editor), *Chromatography, Part B*, Elsevier, Amsterdam, Oxford, New York, 1983, p. 407.
14 Y. Yamauchi, R. Oshima and J. Kumanotani, *J. Chromatogr.*, 198 (1980) 49.
15 J. H. P. Tyman, *J. Chem. Soc.*, Perkin I (1973) 1639.
16 Lam Soot Kiong and J. H. P. Tyman, *J. Chem. Soc., Perkin I* (1982) 1942.
17 V. Tychopoulos and J. H. P. Tyman, unpublished results.
18 V. Tychopoulos, *Ph.D. Thesis*, Brunel University, 1983.
19 J. H. P. Tyman, *J. Chromatogr.*, 111 (1975) 285.
20 K. Karel, *J. Chromatogr.*, 98 (1976) 156.
21 V. Tychopoulos and J. H. P. Tyman, unpublished results.
22 J. H. P. Tyman and S. K. Lam, *Lipids*, 13 (1978) 525.
23 S. K. Lam, *M. Phil. Thesis*, Brunel University, 1976.
24 J. H. P. Tyman, K. H. Tam and A. P. France, unpublished results.