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LONG-CHAIN PHENOLS

IX*. COMPOSITIONAL STUDIES ON THE UNSATURATED PHENOLS OF *ANACARDIUM OCCIDENTALE* (CASHEW NUT-SHELL LIQUID)

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SUMMARY

An extensive gas chromatographic investigation of alternative stationary phases to polyethyleneglycol adipate has revealed two which are suitable for the quantitative analysis of hydrogenated and methylated natural and technical cashew nut-shell liquid. Relative response factors have been obtained for the saturated (15:0), monoene (15:1), diene (15:2) and triene (15:3) constituents of all the component phenols and have been used to correct the gas chromatographic results described in Part IV. A comparison has been drawn between the ionisation processes in the flame ionisation detector and in mass spectrometry.

INTRODUCTION

In Part IV[1] of this series a thin-layer–gas chromatographic procedure was described for the quantitative determination of the saturated (15:0), monoene (15:1), diene (15:2) and triene (15:3) constituents of the component phenols as their methyl ethers in natural and technical cashew nut-shell liquid (*Anacardium occidentale*). A preliminary account of the analysis of the phenols in hydrogenated natural and technical cashew nut-shell liquid (CNSL) was given in Part V[2], which was subsequently improved (Part VI)[3] by the use of the methylated hydrogenated phenols with polyethyleneglycol adipate (PEGA) as the stationary phase. The present contribution describes modifications and refinements of the preceding methods.

The stationary phases SE-52 and OV-17 have been found to be useful alternatives to PEGA, which at 200° was being used at the maximum temperature recommended for this material. Subsequently a stabilised type of PEGA[4] has become available usable up to 250° and experiments are currently being conducted with this material.

In the original work[1], it had been assumed that, since the constituents of the

* Part VIII, ref. 8; Part VII, ref. 9; Part VI, ref. 3; Part V, ref. 2.

component phenols have structurally identical side chains, the relative response factors for all the (15:0), (15:1), (15:2) and (15:3) substances would be identical. Detailed examination of standard mixtures has, however, revealed differences between the constituents of the component phenols and indicated the need to correct the earlier results.

Although the linearity of response of the flame ionisation detector (FID) has been established[5–7], the wide differences in the proportions of the component phenols present in natural and technical CNSL made it desirable to confirm this.

Further examination of alternative volatile derivatives of the hydrogenated phenols has been made. While the acetates of the phenols can be readily obtained and a rapid method for the analysis of acetylated technical CNSL has been devised, which will be described elsewhere, the methyl ethers are intrinsically more stable. The trimethyl silyl ethers were apparently less stable and also quantitative conversion of the phenols was not quite obtained.

The significance of the relative response factors of the constituents of the component phenols determined by gas chromatography with a FID in relation to those of the same constituents determined by the mass spectroscopic (MS) method[8] is briefly discussed. The results support the chemi-ionisation mechanism for the FID.

EXPERIMENTAL

Thin-layer chromatography

Analytical thin-layer chromatography (TLC) was carried out with silica gel G (Merck) on plates, (10 × 8 cm), with a 0.25-mm layer and preparative TLC on (20 × 20 cm) plates with a 1-mm layer as described[1–3,8]. For preparative argentation TLC 15% silver nitrate was used, and bands were visualised with 0.1% ethanolic dichlorofluorescein. Bands were eluted with 10% methanolic diethyl ether (overnight) at ambient temperature in stoppered flasks in the dark and under nitrogen. They were isolated as described[8]. The purity of the recovered material was checked on analytical plates and by gas–liquid chromatography (GLC). If impure, further purification was carried out and the finally recovered materials were stored under nitrogen in stoppered flasks at −20°.

Cardol, 2-methylcardol and cardanol were separated on ordinary plates from natural CNSL, with ethyl acetate–chloroform (5:95, v/v) by preparative TLC, on the concentrate from the filtrate obtained in the modified lead salt separation[3] of anacardic acid (to be published). From the three phenols their constituents were separated by argentation TLC.

Cardanol (0.03 g/plate, 8 plates) was separated with ethyl acetate–chloroform (1:4) as developing solvent. The sample was applied to the plate in a nitrogen box and the solvent remaining after development and visualisation removed in a stream of nitrogen. At low concentrations on the plates the bands were visible as green fluorescent zones, but at higher concentrations the fluorescence was minimal and the bands were located through the partial formation of silver at the surface of the plate. Filtration of the elution mixture, washing the silica gel with eluting solvent, evaporation of the combined filtrates at ambient temperature (rotary evaporator), addition of water, light petroleum extraction followed by washing with dilute aqueous alkali to remove the dichlorofluorescein, afforded after drying ($MgSO_4 \cdot H_2O$) the olefinic

constituent. In this way from cardanol, the following constituents [wt. (g); R_F] were obtained: triene (0.01018; 0.1), diene (0.025; 0.3), and monoene (0.099; 0.6).

The constituents of 2-methylcardol were separated in ethyl acetate–chloroform (1:3). Approximately 0.2 g was separated (six plates) and the following constituents [wt. (g); R_F] isolated: triene (0.0638; 0.075), diene (0.0163; 0.25) and monoene (0.0136; 0.6).

The constituents of cardol were separated in ethyl acetate–chloroform (1:1). In Part V[2], a distinction was made between the component phenol containing the (15:0), (15:1), (15:2) and (15:3) constituents and the hydrogenated material which was termed the H phenol. Since in all cases only a very small proportion of the (15:0) (saturated) constituent is present in the natural product it is more convenient to obtain it by hydrogenation of the natural mixture of four constituents. Careful examination has indicated no difference between the (15:0) material and the so-called H phenol and the latter terminology has been abandoned.

Gas–liquid chromatography

A Pye Unicam Model 104 gas chromatograph equipped with a FID and a Fisons/Vitatron recorder was used[1–3]. Columns, carrier gas flow-rate and chart speed were as previously used. The particular instrument used was not equipped with mass/flow controllers and it was found necessary to check occasionally the hydrogen and air flow-rates to ensure constancy of the detector response. In a later series of experiments a Pye Unicam GC-D apparatus having mass/flow controllers was used. Flow-rates and other settings were the same as with the Model 104, but a new 2% PEGA column was used with stabilised stationary phase.

Samples were used in benzene or chloroform solution (10%) and injections were in the range 0.1 to 5 μl made. Peak sizes were controlled by appropriate attenuation adjustment, the objective being to obtain large peaks for integration/triangulation purposes. Peak areas were generally obtained by accurate triangulation from at least six determinations made on each sample and standard deviations calculated in the usual way.

Methyl ether formation

A molar proportion of the particular phenolic constituent was refluxed in dry benzene solution containing anhydrous potassium carbonate (20 mole proportion) with dimethyl sulphate (10 mole proportion) for 3 h. The cooled reaction mixture was diluted with water and the benzene layer, after thorough washing with water, was dried and made up to 10% concentration. The methylation was monitored by GLC and TLC. By a similar procedure it was shown that methylation of mixtures of phenols proceeded uniformly.

Detector linearity experiment

A standard mixture of (15:0) cardanol methyl ether (0.05511 g), (15:0) cardol dimethyl ether (0.05812 g) and dimethyl anacardate (0.07519 g) in benzene solution was used. Four injections of 0.5, 1.0, 2.0 and 10 μl, and an appropriate dilution so as to obtain 0.05 μl of the original, were made and the peak areas of the three methyl derivatives found, appropriate attenuation changes being made to ensure comparable peak size, since the attenuation control was known and confirmed to be linear. For

TABLE I

RETENTION TIMES (RT) AND RELATIVE RETENTIONS (RR) OF THE METHYL ETHERS OF THE COMPONENT PHENOLS OF CNSL

RR_1 = Relative retention with respect to (15:0) cardanol methyl ether at that particular temperature. RR_2 = Relative retention with respect to (15:0) cardanol methyl ether at 200°.

Column	*Temperature (°C)*	*(15:0) Cardanol methyl ether*			*(15:0) Cardol dimethyl ether*			*(15:0) 2-Methylcardol dimethyl ether*			*(15:0) Dimethyl anacardate*		
		R_1	*RT*	RR_2	RR_1	*RT*	RR_2	RR_1	*RT*	RR_2	RR_1	*RT*	RR_2
2% PEGA	180	1	50.4	2.18	3.23	163.2	7.06	2.91	146.9	6.36	5.53	279.2	12.09
2% PEGA	190	1	32.2	1.39	3.11	100.2	4.34	2.81	90.4	3.91	5.16	166.0	7.19
2% PEGA	200	1	23.1	1	2.83	65.3	2.83	2.56	59.1	2.56	4.59	106.1	4.59
2% PEGA	220	1	12.0	0.52	2.64	31.9	1.38	2.28	27.4	1.19	3.88	46.8	2.03
3% Dexsil	220	1	49.8	2.16	2.15	107.2	4.64	2.47	123.2	5.34	2.73	136.0	5.89
3% Dexsil	230	1	34.7	1.51	2.07	72.0	3.12	2.36	82.1	3.56	2.59	90.2	3.91
3% Dexsil	250	1	18.1	0.78	1.90	34.4	1.49	2.12	38.4	1.67	2.33	42.2	1.83
3% Dexsil	290	1	6.8	0.28	1.63	11.1	0.48		—*		19.1	13	0.57
3% SE-30	220	1	12.8	0.56	1.89	24.2	1.05	2.12	27.2	1.18	2.32	29.85	1.29
5% SE-52	200	1	41.1	1.77	2.13	87.4	3.78	2.52	103.5	4.48	2.79	114.6	4.96
	220	1	20.7	0.89	1.94	40.1	1.73	2.25	46.6	2.02	2.48	51.4	2.23
3% OV-17	220	1	40.5	1.75	2.28	92.4	4.00	2.56	103.5	4.48	3.34	135.2	5.85

* Not resolved from the cardol dimethyl ether peak.

calculation of results, peak areas were brought to the same attenuation value before normalisation. The average areas were obtained in each case after accurate triangulation.

Preparation and examination of calibration standards for obtaining relative response factors (RRFs)

For the cardanol standard, the (15:0), (15:1), (15:2) and (15:3) phenolic constituents (prepared as described) were each accurately weighed on a five-place balance, made up to 5 ml, and the mixture methylated in the way described. A 10% solution was used for GLC experiments on the methyl ethers.

With cardol, a similar procedure and methylation was used with the pure (15:0) (0.00287 g), (15:1) (0.00469 g), (15:2) (0.00158 g) and (15:3) (0.00638 g) phenolic constituents. Some doubt arose concerning the validity of the peak area of the diene methyl ether constituent. Accordingly, a second standard was prepared and methylated comprising the (15:0), (15:2) and (15:3) cardol constituents together with (15:0) cardanol to enable the results to be interrelated with the first standard and with the cardanol series.

Although a 2-methylcardol standard was prepared and methylated, the GLC results obtained presented some ambiguities that have not yet been resolved. When opportunity permits, this will be investigated and reported.

A standard containing (15:0), (15:1), (15:2) and (15:3) methyl anacardate was prepared as previously described[1].

Owing to the difficulty of obtaining, at the microlitre level, reproducible volumes of the sample, to different concentrations from standard to standard and, sometimes, variations in attenuation, the peak area (percent constituent per gram) found for a given material was not an absolute or constant value and the results therefore had to be interrelated.

To enable the methyl anacardate to be interrelated with the cardol and cardanol series, a third standard of (15:0) cardanol methyl ether, (15:0) cardol dimethyl ether, (15:0) methyl anacardate and (15:2) methyl anacardate was prepared and examined, in the usual way, gas chromatographically. To check further on the accuracy of this, a fourth final standard of (15:0) cardanol methyl ether, (15:0) cardol dimethyl ether, (15:0) methyl anacardate, (15:2) methyl anacardate and *n*-octacosane (C_{28}) was made up and examined to enable Kováts retention indices to be calculated.

In all cases, six chromatograms of each standard were obtained.

RESULTS AND DISCUSSION

Relative retentions of the (15:0) component phenolic methyl ethers

The retention times and relative retentions of the (15:0) component phenolic methyl ethers on 5% SE-52 and 3% OV-17 in comparison with 2% PEGA and 3% Dexsil 300 are given in Table I. 3% OV-17 resembled 3% Dexsil although the separation of (15:0) 2-methylcardol dimethyl ether from (15:0) dimethyl anacardate was better with the former. 5% SE-52 gave a similar type of separation to 3% Dexsil with slightly less resolution of the two peaks referred to. 2% PEGA was the only stationary phase on which 2-methylcardol dimethyl ether emerged before cardol dimethyl ether.

Figs. 1–3 show tracings of the (15:0) component phenolic methyl ethers of natural CNSL on 2% PEGA, 5% SE-52 and 3% OV-17. A small advantage of PEGA was its ability to reveal minor proportions of the methyl ethers of the (15:1), (15:2) and (15:3) constituents, but the greatest disadvantage was the need to use it at the maximum recommended temperature of 200° in order to obtain useful retention times. SE-52 and OV-17 have considerable superiority in this respect and both can be used up to 250°.

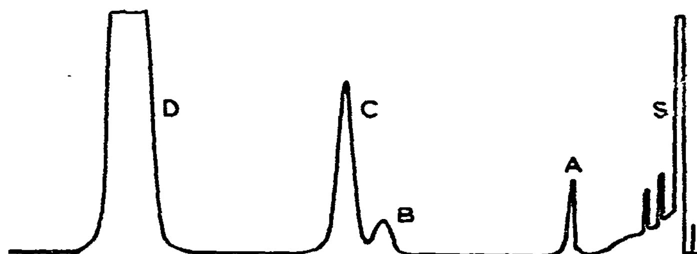

Fig. 1. Gas–liquid chromatogram of natural CNSL on 2% PEGA at 190°. A = (15:0) Cardanol methyl ether; B = 2-methylcardol dimethyl ether; C = (15:0) cardol dimethyl ether; D = (15:0) dimethyl anacardate; S = solvent.

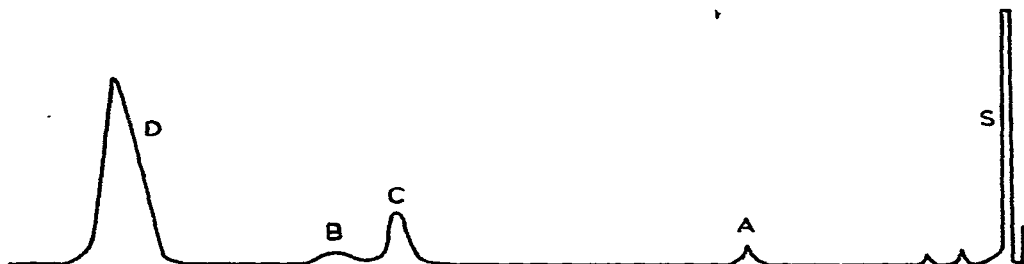

Fig. 2. Gas–liquid chromatogram of natural CNSL on 5% SE-52 at 200°. Peaks as in Fig. 1.

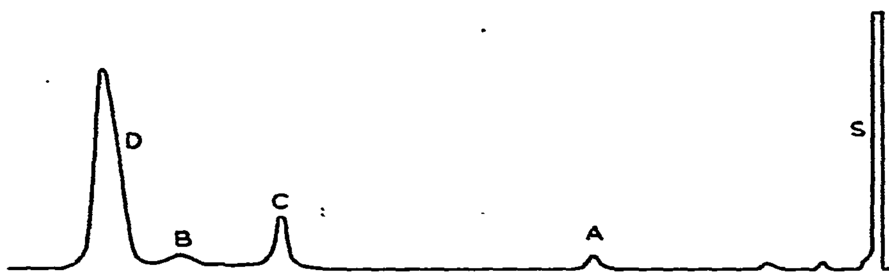

Fig. 3. Gas–liquid chromatogram of natural CNSL on 3% OV-17 at 220°. Peaks as in Fig. 1.

The average retention distances (and standard deviations) with 2% PEGA at 200° for hexacosane (46.9 ± 0.99 mm), octacosane (85 ± 1.95 mm), (15:0) cardanol methyl ether (114.5 ± 0.65 mm) and (15:0) methyl anacardate (44.71 ± 3.10 mm) determined with the 104 chromatograph show a greater measure of reproducibility than those reported earlier, largely, it is believed, because they were examined over a short period of time. Other stationary phases gave a similar level of reproducibility.

The retention times reported in Table I are uncorrected. With the GCD equipment (2% PEGA, new column on Diatomite M 60–80, nitrogen flow-rate 45 ml/min) more accurate retention distances were obtained, *e.g.* with (15:0) cardanol methyl ether (37.13 ± 0.345 mm), (15:0) cardol dimethyl ether (107.7 ± 0.49 mm) and (15:0) dimethyl anacardate (160.94 ± 0.51 mm).

To enable Kovàts retention indices to be obtained the n-C_{26}, C_{28}, C_{30}, C_{32} and C_{36} alkanes in n-hexane were run under identical conditions to the previous three materials on 2% PEGA at 200° (nitrogen 45 ml/min). Since not all the n-alkanes were available, the average log (retention distance) was plotted against the number of carbon atoms and the values for the unavailable ones were obtained by extrapolation. The indices were calculated from the expression,

$$I = \frac{100\,[\log(R_{ts}) - \log(R_{tA})]}{\log(R_{t(A+1)}) - \log(R_{tA})} + 100\,A$$

where R_{ts} is the corrected retention distance of the substance, $R_{t(A+1)}$ that of the nearest higher alkane and R_{tA} that of the nearest lower alkane with A carbon atoms, where,

$$R_{tA} \leqslant R_{ts} \leqslant R_{t(A+1)}$$

In this way, the indices found were 2842 for (15:0) cardanol methyl ether, 3197 for (15:0) cardol dimethyl ether and 3334 for (15:0) dimethylanacardate. The following log (corrected retention) were found: C_{26}, 1.257; C_{28}, 1.513; C_{30}, 1.777; C_{32}, 2.037; C_{36}, 2.565; (15:0) cardanol methyl ether, 1.5705; (15:0) cardol dimethyl ether, 2.0323; and (15:0) dimethyl anacardate, 2.2052.

Composition of the C_{15} component phenols of natural CNSL

The composition of hydrogenated and methylated natural CNSL was examined on 3% OV-17 and on 5% SE-52.

The uncorrected results have been summarised in Table II alongside those found previously for 3% Dexsil and for 2% PEGA. The results for all four columns are closely similar and the standard deviations of the average values shown are, with one exception, considerably smaller than those found for 2% PEGA.

It is quite clear that for the analysis of natural and technical CNSL by the

TABLE II

COMPOSITION OF HYDROGENATED AND METHYLATED NATURAL CNSL DETERMINED BY GLC ON DIFFERENT STATIONARY PHASES

The results are for the methyl ethers and are uncorrected (by use of RRF values).

Stationary phase	*(15:0) Cardanol methyl ether*	*(15:0) Cardol dimethyl ether*	*(15:0)2-Methylcardol dimethyl ether*	*(15:0) Dimethyl anacardate*
3% OV-17	1.94	14.37	1.98	81.70
5% SE-52	1.86	14.23	2.19	82.55
3% Dexsil 300	1.74	14.12	2.24	82.1
2% PEGA	1.76 ± 0.17	14.03 ± 0.79	2.53 ± 0.16	81.68 ± 1.05
Average	1.83 ± 0.093	14.19 ± 0.147	2.24 ± 0.227	82.01 ± 0.410

hydrogenation–methylation procedure, the use of OV-17 and of SE-52 is a practical alternative. The minor advantage of PEGA that it permits both the hydrogenated (15:0) and the saturated (15:0) monoene (15:1) diene (15:2) and (15:3) triene methyl ethers to be separately determined is outweighed by the fact that these constituents can also be readily analysed by TLC–mass spectrometry[8].

Determination of the linearity of the FID

In view of the wide disparity of the percent composition of the components it was of interest to check the linearity of the FID. For this purpose a standard mixture of the three principal (15:0) phenolic methyl ethers was examined over the concentration range present in solutions of (15:0) natural CNSL. Application of the least squares method to the results shown in Table III, with the basic assumption that the (log) plot of detector response *versus* sample size[7] passed through the origin, gave the values of $m(\Sigma xy/\Sigma x^2)$ shown. The FID is linear over a greater range than most detectors and the present results indicate clearly that such errors and deficiencies as are apparent in the hydrogenation–methylation procedure are not ascribable to this cause.

TABLE III

DETECTOR LINEARITY EXPERIMENTS WITH COMPONENT PHENOLIC METHYL ETHERS

Component phenol	*Detector response (peak area) (x)* *Sample size (μl) (y)*					*m*
	0.5	*0.5*	*1.0*	*2.0*	*10.0*	
Cardanol methyl ether	142.5	1343.7	2781	5157.6	30669.6	1.00049
Cardol dimethyl ether	118.2	1111.9	2470.8	4558.7	25840	0.9964
Dimethyl anacardate	187.2	1455.5	3223.7	5921.1	32596	1.0018

Determination of the RRFs for the constituents of the component phenolic methyl ethers

In the original quantitative procedure for the constituents it was assumed that the structural similarity of the (15:1), (15:2) and (15:3) side chains justified the applicability of the RRFs found for methyl anacardate to the constituents of the other phenolic methyl ethers. Examination of standard solutions of the four constituents (as methyl ethers) of cardanol and of cardol however has revealed differences. Effectively, the RRF values for the methyl ethers represent the peak area/g (percent constituent/weight (g)$\cdot 10^3$) and tend to fall in value from the (15:0) to the (15:3) constituent as shown in Table IV in which the values for methyl anacardate (%/wt.$\cdot$10) have been given for comparison. It was found desirable to separate the phenolic constituents rather than the methyl ethers by argentation TLC and then methylate the standard mixture of phenols. For calculation purposes the RRF values for each constituent methyl ether proved useful in correcting the compositional results but the set of values for one component phenolic methyl ether had not been related to those of other components. For interrelation, use was made of values[3] found for (15:0) cardanol methyl ether and (15:0) cardol dimethyl ether, namely 6.5643 and 5.7911, respectively, together with information derived from three further standards. For this, cardanol (0.007165 g, derived methyl ether 0.007494, 19.9684% present by GLC) was also used in the cardol standard referred to in Table IV, and this enabled the RRF values

TABLE IV

RRF VALUES FOR THE CONSTITUENTS OF CARDANOL METHYL ETHER, CARDOL DIMETHYL ETHER AND METHYL ANACARDATE

Constituent	*Cardanol methyl ether standard*					*Cardol dimethyl ether standard*					*Methyl anacardate standard*			
	Normalised (%)	*Phenol wt. (g)*	*Methyl ether wt. (g)*	*RRF*	*Inter-related RRF*	*Normalised (%)*	*Phenol wt. (g)*	*Methyl ether wt. (g)*	*RRF*	*Inter-related RRF*	*Normalised (%)*	*Methyl ester wt. (g)*	*RRF*	*Inter-related RRF*
15:0 Saturated	15.68 ± 0.34	0.00278	0.00291	5.3920	6.5643 (1.0000)	33.23 ± 0.44	0.01354	0.01472	22.578 (12.395)	5.7911 (0.8822)	1.85 ± 0.44	0.0391	4.7314	3.2379 (0.4932)
15:1 Monoene	46.02 ± 0.29	0.01040	0.01088	4.2298	5.1493 (0.7844)	23.52 ± 0.22*	0.00469	0.00510	(4.6089)	2.1533 (0.3280)	39.36 ± 0.33	0.6649	5.9196	3.9686 (0.6045)
15:2 Diene	14.21 ± 0.21	0.00340	0.00356	3.9951	4.8636 (0.7409)	19.73 ± 0.27	0.02138	0.02318	8.5114 (4.672)	2.1830 (0.3325)	14.86 ± 0.18	0.2730	5.4432	3.6558 (0.5569)
15:3 Triene	24.08 ± 0.61	0.00756	0.00792	3.0428	3.7044 (0.5643)	27.07 ± 0.17 30.62 ± 0.85*	0.03093 0.00638	0.03368 0.00694	8.0364 (4.412)	2.0613 (0.3140)	44.81 ± 0.25	0.9078	4.936	3.3049 (0.5034)

* These results taken from a first standard and the two values related to a second standard giving the total set shown in btackets.

of 26.645 (15:0) cardanol methyl ether to be obtained in relation to 22.578 (15:0) cardol dimethyl ether, 8.5114 (15:2) cardol dimethyl ether and 8.0364 (15:3) cardol dimethyl ether. Relative to the selected RRF value of 5.7911 for (15:0) cardol dimethyl ether, that of (15:0) cardanol methyl ether was 6.8342.

From another standard containing (15:0) cardanol methyl ether, (15:0) cardol dimethyl ether, (15:0) methyl anacardate and (15:2) methyl anacardate, the methyl anacardate was interrelated with the cardanol and cardol methyl ether series. A final standard containing for reference purposes *n*-octacosane, (15:0) cardanol methyl ether, (15:0) cardol dimethyl ether, (15:0) and (15:2) methyl anacardate was made up since there had been some doubt about the validity of the (15:0) methyl anacardate result in the preceeding standard. The RRF values determined have been considered in relation to the quantitative evaluation of response characteristics recently summarised[10] in order to interrelate those found in the present work. The influence of unsaturation upon detector response has been considered in earlier work[11]. From the value of R (coulombs/mole), 1.880 for *n*-octane, and an incremental value of 0.24 for each additional carbon atom an approximate value of 6.580 C/mole for *n*-octacosane can be assigned. The present arbitrary value of 6.688 has been used together with the average value 6.6869 for (15:0) cardanol methyl ether to calculate R for (15:0) cardanol methyl ether (6.5789), (15:0) cardol dimethyl ether (5.8038), (15:0) dimethyl anacardate (5.7591) and (15:0) 2-methylcardol dimethyl ether (5.4874), taking into account the relative values given in Table IV[3].

The effective carbon number[10], N_c, for (15:0) cardanol methyl ether ($C_{22}H_{38}O$) can be calculated as 21.97 on the basis of *n*-octacosane ($C_{28}H_{58}$) being assigned the value 28. The expression shown and the relevant values (Table V) were used

$$N_c = \frac{28 \cdot \text{Peak area of phenolic methyl ether/peak area of } C_{28}}{\text{Molar ratio of (phenolic methyl ether/}C_{28}\text{)}}$$

In a similar way with the additional use of information from Table II[3], the values for (15:0) cardol dimethyl ether ($C_{23}H_{40}O_2$) and (15:0) dimethyl anacardate ($C_{24}H_{40}O_3$) were found to be 21.78 and 23.49, respectively. These findings indicate that the N_c contribution[10] for an ether (—1) has little effect in the case of a large molecule unless

TABLE V

INTERRELATION OF RRF VALUES BY THE USE OF STANDARDS

(i) and (ii) represent two different standards.

Constituent	*wt. (g)*	*Normalised (%)*	*RRF*	*Interrelated RRF*
(i) (15:0) cardanol methyl ether	0.01344	37.221 ± 0.370	2.7694	6.8453
(ii) (15:0) cardanol methyl ether	0.03938	46.287 ± 1.806	1.1754	6.5038
(i) (15:0) cardol dimethyl ether	0.01554	36.408 ± 0.573	2.3429	5.7911
(ii) (15:0) cardol dimethyl ether	0.00648	6.782 ± 0.1376	1.0466	5.7911
(i) (15:0) methyl anacardate	0.00290	—	—	—
(ii) (15:0) methyl anacardate	0.01418	7.95	0.5608	3.103
(i) (15:2) methyl anacardate	0.01654	24.463 ± 0.392	1.4790	3.6558
(ii) (15:2) methyl anacardate	0.01139	6.99 ± 0.299	0.6138	3.3963
(i) *n*-octacosane	0.02656	32.104 ± 1.124	1.2087	6.6880

two methoxy groups are present or a methoxy group with a methyl ester group. The calculated values for the methyl anacardates are lower and this aspect is being examined further.

The weights used, normalised percent composition, RRF values found and the interrelated values are shown in Table V, the value of 5.7911 for cardol dimethyl ether being used as before for this purpose. The value 3.6558 for (15:2) methyl anacardate was then used to obtain the interrelated values for the constituents of methyl anacardate: (15:0), 3.2379; (15:1), 3.9668; (15:2), 3.6558; and (15:3) 3.3049; as shown in Table IV. Thence the value for (15:0) methyl anacardate could be related with the previous values found for the phenols[2] (15:0) cardanol, 1.384; (15:0) cardol, 0.8162; (15:0) 2-methylcardol, 0.8128; giving a final correlation of (15:0) methyl anacardate, 3.2379; (15:0) cardanol, 5.073; (15:0) cardol, 2.992; and (15:0) 2-methylcardol, 2.979. The peak areas per gram for the phenols are lower in value than those for the methyl ethers.

As pointed out earlier, the general trend for RRF values is to decrease with progressive increase in the unsaturation, except in the case of (15:2) cardol dimethyl ether, which has a higher value than its neighbours the (15:1) and (15:3) constituent methyl ethers. It was the finding of this higher value which led to the use of a second standard in order to confirm or disprove this observation.

The RRF values show a striking similarity to those found in MS determinations (Part VIII)[8] where the relative response factors were obtained as peak height per gram with a decrease in value from the (15:0) to the (15:3) phenolic constituent except for cardol where a maximum value for the (15:2) constituent was observed.

It is apparent that the ionisation processes in the FID and MS are related. The original view was that in the former, thermal ionisation[12] was mainly involved through the low ionisation potential (4.3 eV) of carbon aggregates and that certain substances were not detected because of their high ionisation potentials[13]. Later evidence has indicated that thermal ionisation plays a minor part in the overall process which is based mainly on "chemi"-ionisation[14,15]. The present results lend some support for this mechanism. In the FID the chemi-ionised species subsequently suffers an oxidative sequence and in the mass spectrometer becomes fragmented. Lower ionisation conditions in mass spectrometry (*e.g.* 7 eV) can result in preservation of the molecular ion.

No theoretical reason can be given for the maximum value of (15:2) cardol dimethyl ether and (15:2) cardol by the two methods and it may conceivably simply be coincidental. At the present time no interrelated figures for RRF values by the MS method are available.

From the RRF values for (15:0) cardanol methyl ether, namely 6.5643, 6.8342, and the two values given in Table V (6.8453 and 6.5038) an average of 6.6869 and thence a standard deviation of ± 0.178 can be calculated. The level of accuracy is thus approximately $\pm 2\%$. This is probably attributable to the inherent difficulties in the purification and manipulation of these easily autoxidised substances and to the method of quantitative peak area determination employed. The effect of a small variation in the RRF value upon the compositional results is considered in the next section.

Corrected compositional results for the constituents of the component phenolic methyl ether in natural and in technical CNSL

In Table VI, the original uncorrected results are shown in the left hand of the table and the corrected values obtained by using the RRF values (Table IV) (percent composition/RRF value, followed by normalisation) at the right. With 2-methylcardol, the RRF values could not be determined satisfactorily and the figures shown have been obtained by using the cardol values. Comparatively small differences can be seen for cardol and somewhat larger variations for cardanol in relation to the original ones described[1]. Values for (15:3) constituents in the component phenols of technical CNSL are lower, consistent with thermal decomposition during industrial decarboxylation. The GLC results relate to the volatile portion of technical CNSL and both TLC and molecular distillation have indicated the presence of as much as 10% non-volatile polymer material to be present in the original technical CNSL. Compositional results referred to the original material should therefore be multiplied by a factor of approximately 0.90.

TABLE VI

TOTAL COMPOSITIONAL RESULTS FOR THE CONSTITUENTS OF THE COMPONENT PHENOLIC METHYL ETHERS IN NATURAL AND TECHNICAL CNSL

Component phenol and type	*Uncorrected results*				*Corrected results using RRF values given in Table IV*			
	15:0 Saturated	*15:1 Monoene*	*15:2 Diene*	*15:3 Triene*	*15:0 Saturated*	*15:1 Monoene*	*15:2 Diene*	*15:3 Triene*
Cardanol (natural)	2.14	37.47	16.17	44.22	1.42	31.83	14.54	52.21
Cardanol (technical)	2.79	39.6	20.3	37.1	1.91	34.49	18.72	44.89
Cardol (natural)	0.29	9.33	23.3	67.1	0.001	9.10	22.44	68.36
Cardol (technical)	nil	11.0	26.7	62.3	nil	10.74	25.71	63.55
2-Methylcardol (natural)	1.54	15.7	19.3	62.1	0.57*	15.67	19.00	64.76
2-Methylcardol (technical)	2.29	17.6	21.6	59.3	0.84*	17.30	20.95	60.91
Methyl anacardate	3.62	41.9	17.5	36.8	4.05	38.3	17.3	40.4

* Calculated using the RRF values of the cardol constituents. Gravimetry (prep. TLC) indicated 15:0 (nil), 15:1 (14.5%), 15:2 (17.4%), and 15:3 (68.1%) for the natural component.

The determination of RRF values has indicated an inherent error of approximately $\pm 2\%$. On the assumption of an error of 2% in the RRF for (15:3) cardanol methyl ether and with a value of 3.6304 in place of 3.7044, the others being the same, the composition found was (15:0) 1.41%, (15:1) 31.49%, (15:2) 14.39%, and (15:3) 52.71%. The values differ by up to 0.5%, which is still within the standard deviation value found for the method. It is thus apparent that some small inaccuracy in the RRF does not greatly affect the final compositional results.

Comparison of corrected GLC and MS compositional results for the constituents of natural CNSL

Table VII gives a comparison of the compositional results for the GLC and MS methods. In all cases they show a fairly close conformity and the differences lie within the standard deviations for the GLC method[1] and are comparable to those of the MS procedure[8]. The latter suggest that the MS method is more accurate,

since for one reason a single resolved peak has to be determined compared with a peak area, sometimes overlapping, in the GLC method. It is hoped, when opportunity permits, to investigate both the GLC and MS analytical procedure with the phenolic methyl ethers from the commencement of the method and to incorporate various other refinements in peak area and peak heights determination.

TABLE VII

COMPARISON OF THE RESULTS OF GLC AND OF TLC–MS METHODS OF ANALYSIS OF NATURAL CNSL

Component phenol	*GLC corrected results using RRF values (Table IV)*				*TLC–MS corrected results*[8]			
	15:0	*15:1*	*15:2*	*15:3*	*15:0*	*15:1*	*15:2*	*15:3*
Cardanol	1.42	31.83	14.54	52.21	1.98	31.31	15.23	51.47
Methyl anacardate	4.05	38.3	17.30	40.4	3.65	38.19	16.50	41.65
Cardol	0.001	9.10	22.44	68.36	0.24	10.74	20.64	68.39
2-Methyl cardol*	1.63	18.08	19.55	60.74	3.9	18.43	20.15	57.50

* These results (GC and MS) have not been corrected with respect to RRF in either set although the MS figures have been corrected for the P+2 peak contribution.

The MS technique is fundamentally a rapid one with quite reasonable accuracy and it requires the use of rather less material than the GLC method. This has to be considered also from the aspect that mass spectrometers are generally inoperative for an appreciably greater time than gas chromatographs.

CONCLUSION

The saturated (15:0) phenolic methyl ethers obtained by the hydrogenation–methylation procedure[3] may conveniently be determined by GLC on the stationary phases OV-17 and on SE-52. For the GLC analysis of the (15:0), (15:1), (15:2) and (15:3) constituents of the component phenols, relative response factors have been determined and applied to correct earlier results.

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