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LONG-CHAIN PHENOLS

VIII*. QUANTITATIVE ANALYSIS OF THE UNSATURATED CONSTITUENTS OF PHENOLIC LIPIDS BY THIN-LAYER CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

A thin-layer chromatographic–mass spectrometric procedure has been devised for the quantitative analysis of phenolic lipids. Correction for different response factors of the constituents of each component phenol and for isotopic abundances is necessary and leads to results showing excellent agreement with those from gas chromatographic analysis. No special instrumental requirements are needed and the method is successful due to the reproducible scanning obtainable.

INTRODUCTION

The composition of the unsaturated constituents of the complex phenolic shell liquid from *Anacardium occidentale* has engaged considerable attention since the finding of the inhomogeneity of so-called cardanol diene and the separation by adsorption chromatography of its methyl ether into saturated, monoene, diene and triene constituents**,[1] followed by structural elucidation. The constituents of cardol were similarly treated[2], and of those anacardic acid by low-temperature crystallisation[3]. Later, argentation thin-layer chromatography (TLC)–ultraviolet spectrophotometry was used quantitatively[4]. More recently, quantitative separations of the constituents[5] of the component phenols of *Anacardium occidentale*[6] and of *Rhus toxicodendron*[7] have been effected by TLC–gas–liquid chromatography (GLC). The composition of the unsaturated constituents in the latter case differs from a reported analysis[8] for *Rhus vernicifera* and the comparative results are interesting in view of the dermatological properties[9] of the active substance, urushiol (I; $n = 0, 2, 4, 6$), and studies made in the Rhus and Anacardium species. In both also, quantitative knowledge of un-

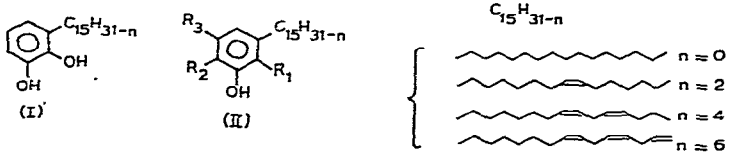
* Part VI, see ref. 6; Part VII, *J. Org. Chem.*, 41 (1976) 894.

** The term constituent is used for the individual saturated, monoene, diene and triene substances and component phenol for the natural mixture of the four constituents.

saturation is necessary for an understanding of certain industrial polymerization processes involving the component phenols.

During the course of work in 1966–67 on argentation TLC and on the identification of 2-methylcardol[10] in cashew nut-shell liquid (*Anacardium occidentale*) it became clear that the saturated, monoene, diene and triene constituents gave well defined mass spectra and, moreover, those of mixtures were remarkably reproducible. Opportunity was not then available for examining the potentiality of a mass spectral method of analysis.

Subsequently in a study of regional variations in composition of *Anacardium occidentale*, the examination of a large number of samples by the TLC–GLC method[5] was considered to be too lengthy on the limited stationary phases available. Attention was turned to TLC–mass spectrometry and it has been found that quantitative analysis can be both rapid and accurate. The results for the constituents of the component phenols in the natural product, namely anacardic acid (II; $R_1 = CO_2H$, $R_2 = R_3 = H$, $n = 0, 2, 4, 6$), cardol (II; $R_3 = OH$, $R_1 = R_2 = H$, $n = 0, 2, 4, 6$), cardanol (II; $R_1 = R_2 = R_3 = H$, $n = 0, 2, 4, 6$) and 2-methylcardol (II; $R_3 = OH$, $R_2 = CH_3$, $R_1 = H$, $n = 0, 2, 4, 6$) have been found to agree closely with those obtained by the TLC–GLC procedure[5].



The applicability of the method depends firstly on the almost identical vapour pressures of the saturated, monoene, diene and triene constituents and secondly on the difference of two mass units between each successive constituent with the effect that the P+2 peak contribution of the preceding constituent makes a minimal contribution to the peak height of the next. No correlation with the results by GLC could be obtained, however, unless sensitivity factors (response factors) were determined for each constituent.

Generally, there has been more interest in the fragmentation than the separatory aspect of mass spectrometry. Quantitative analysis of mixtures of known structure by mass spectrometry[11] has to some extent been overlooked. It has been somewhat displaced by GLC in recent years although there are numerous examples to which the latter procedure can be applied only with difficulty. Thus, a quantitative mass spectral procedure[12] for the analysis of component glycerides in natural oils and fats has been described in which various corrective rather than sensitivity response factors were employed. TLC–mass spectrometry is in our experience a reliable quantitative analytical procedure for the unsaturated constituents of long-chain phenolic lipids.

EXPERIMENTAL

Materials

On ordinary silica gel G plates the saturated, monoene, diene and triene

constituents of each component phenol migrate as a single band and separate only on argentated silica gel G into four bands.

Whole cashew nuts were obtained from Mozambique and were extracted as described earlier[10]. The component phenols were separated by preparative TLC with ammoniated ethyl acetate–chloroform (5:95, v/v) or, as described[4], ammoniated diethyl ether–light petroleum (b.p. 40–60°) (30:70, v/v). The bands made visible with rhodamine 6G were eluted (approximately 25 cm^3 of eluting solvent per gram of adsorbent) with diethyl ether–methanol (90:10, v/v) containing 0.1% of 2,6-di-*tert.*-butyl-4-methylphenol, and examined for purity on analytical plates. Solvent of the above composition was found to remove all of the adsorbed constituents without co-extraction of the detection reagent (in early experiments[4], pure methanol was employed and after a short period both the phenolic material and dye passed into solution). After standing for 1–2 h with occasional swirling, the mixture was filtered to remove the silica gel G that was washed with the eluting solvent. The combined filtrates were evaporated (rotary evaporator) *in vacuo* to remove the solvents. As only 1-mg amounts were required for mass spectral examination, the TLC separation could be effected on analytical plates (10 cm × 8 cm × 0.25 mm) but generally preparative plates and a sample size in the range 0.1–0.25 g were employed. Within 3 h the component phenols were available for mass spectral examination. All phenolic materials isolated were stored in closed flasks at −20° under nitrogen. Anacardic acid in ethereal solution at 0° was converted into the methyl ester by treatment at 0° with ethereal diazomethane[5].

An alternative TLC separation of cashew nut-shell liquid involved treatment with ethereal diazomethane at 0°, to esterify anacardic acid, followed by initial development with light petroleum to obtain methyl anacardate near the solvent front and subsequently use of a more polar solvent, chloroform-ethyl acetate (95:5, v/v), to separate cardanol, 2-methylcardol and cardol. Recoveries were effected as previously described[4].

For the preparation of standards the component phenols were each separated into their saturated, monoene, diene and triene constituents by argentation TLC[4] with chloroform–ethyl acetate in the proportions 90:10 (v/v) for methyl anacardate, 80:20 (v/v) for cardanol, 75:25 (v/v) for 2-methylcardol and 50:50 (v/v) for cardol. Eluted and recovered materials freed from trace amounts of silver nitrate were examined on ordinary and argentation analytical plates and were re-purified when necessary on ordinary plates. All materials purified by this sequence were stored at −20° in the dark under nitrogen. As the natural product contained insufficient of the saturated (15:0) component phenol for the standard, in each instance it was supplemented by hydrogenation of the side-chain[10] of the appropriate component phenol. All standards comprising the four constituents of each component phenol were made up in benzene solution (2 ml) from the relevant material weighed on a five-place balance.

*Mass spectra**

Mass spectra were determined on an AEI MS9 and on a Hitachi–Perkin-

* Mass spectra on the MS9 instrument were carried out under the ULIRS scheme at the School of Pharmacy, University of London; the RMS4 spectra were obtained at Brunel University.

Elmer RMS4 instrument by direct insertion as for solids under standard conditions (70 eV, temperature in the range 160–180°). Samples were used at a concentration of 20% in diethyl ether or chloroform. A standard capillary mounted in the probe was used for the RMS4 and the quartz tip of the probe for the MS9. Scans were made at ½, 1, 2, 3, 4 and 5 min after injection (and sometimes longer) until the sample was exhausted. As much of the width of the chart paper as possible was used to obtain the maximum peak height on scale in the first scan. Scale expansion could be used also but generally was not required. In every instance after the final scan no background material was found in the instrument. In order to find the reproducibility, several runs were carried out on a further sample.

The mass spectra of the individual constituents of each component phenol, the natural component phenol and the relevant standard mixture were all examined.

The chart records were sprayed with a stabilising lacquer. The peak heights were accurately measured by inspection under powerful magnification. The percentage composition was then obtained for both the natural component phenol and the standard mixture. From the weights taken of the consituents of each component phenol, sensitivity factors (relative response factors) were obtained in each instance. These were applied to correct the results obtained for the natural component phenol. The peak heights for the diene, monoene and saturated consituents were corrected for the theoretical P+2 peak contribution from the triene, diene and monoene, respectively. The theoretical P+2 peak contribution was calculated from the usual binomial expression[13,14] according to the molecular formula of the relevant phenol, taking into consideration the carbon, hydrogen and oxygen atoms. The observed P+2 peak contribution was also used as a basis for calculation. In all instances standard deviations were calculated in the usual way.

The RMS4 instrument was used for the analysis of cardanol in order to find the performance of an instrument with more limited resolution than the MS9.

RESULTS AND DISCUSSION

General procedure

The analytical method depends for its applicability on a preliminary TLC separation on ordinary silica gel G plates of the component phenols each containing saturated, monoene, diene and triene constituents. In this way materials of comparable volatility were then available for mass spectral examination. Attempts to run unseparated natural cashew nut-shell liquid in which the anacardic acid had been converted into methyl anacardate were not as successful.

The volatility of the phenols at the temperature attained by the probe and at the operative vacuum in the mass spectrometers used was adequate and it was unnecessary to use volatile derivatives. The dimethyl ether of urushiol[8] has been used for the analysis of its constituents but in our work this did not prove to be a requirement. Anacardic acid could be used without conversion into methyl anacardate but its known decarboxylation at temperatures slightly above that of the probe, possibly also catalyzed by the metal surface, led to the use of the derivative[5].

Reproducibility of results

In the early experiments, the variation in results was greater than in the final

procedure. The standard deviations for the percentage composition of the triene, diene and monoene constituents of cardol (65.14 ± 1.26%, 24.91 ± 1.07% and 9.91 ± 0.88%) and of cardanol (29.94 ± 1.61, 21.99 ± 0.77 and 40.87 ± 2.70) were higher than those given in Table II. The reproducibility of results on a given sample examined at widely different times was excellent. Thus, for cardol two sets of results for the triene, diene and monoene were 65.38%, 24.31% and 10.30%, and 65.65%, 24.60% and 10.09%.

Determination of relative response factors

The mass spectral analytical procedure depended on the absence of P—6, P—4 and P—2 ions in the mass spectrum of the 15:0 constituent, P—4, P—2 ions in that of the monoene and a P—2 ion in that of the diene. The four separated pure constituents of each component phenol were found not to contain interfering peaks at the above masses, nor did P+2 ions constitute more than a few per cent, which would not have been the case had the purified constituents contained vinylogous impurities. Formation of P—1, P—2, P—3 and P—4 ions has been observed with some compounds[15] and makes the examination of pure constituents necessary. The molecular ion peaks were strong but not base peaks[10]. The basis for quantitative mass spectral analysis has been generally discussed[11] and the molecular ion intensities for the 15:0 (s), 15:2 (m), 15:2 (d) and 15:3 (t) constituents are proportional to $S_s \cdot p_s$, $S_m \cdot p_m$, $S_d \cdot p_d$, and $S_t \cdot p_t$, respectively, where S represents the sensitivity (relative response factor) of the detector and p the vapour pressure of the relevant constituent. It was not expected that relative response factors would be similar but the vapour pressures are known to be comparable and the small standard deviations clearly demonstrate this. No further correction for minor differences in volatility was made and the relative response factors obtained from the calibration experiments were used to correct the results from the natural product.

The peak heights for the four constituents were accurately measured, normalised to give the percentage composition and the standard deviations calculated. The results were corrected for the theoretical contribution of the P+2 peak and the relative response factor calculated as in grams per unit peak height. The results for the standards, methyl anacardate, cardol and cardanol are shown in Table I.

Results for the unsaturated constituents of natural cashew nut shell liquid

The mass spectral interpretative aspects of the spectra obtained are the subject of a forthcoming report on the synthesis of the unsaturated constituents of cardanol[16] and are therefore not discussed here. Fig. 1 shows the mass spectrum of methyl anacardate. The molecular ion peaks for the triene (*m/e* 356), diene (*m/e* 358), monoene (*m/e* 360) and saturated (*m/e* 362) constituents appear at the right-hand side of the spectrum. In Fig. 2 the molecular ion peaks of the four constituents resulting from scans at ½, 1-, 2-, 3- and 4-min intervals are given. The total results for the constituents of the natural component phenols are summarised in Table II. The uncorrected results (i), their correction for the P+2 peak contribution (ii) and for the differing relative response factors (iii), the final normalised percentage (iv) and a comparison with the GLC values are given. Close similarity is apparent between the two methods for methyl anacardate, cardol and cardanol. For 2-methylcardol, the purity of the constituents of the material was doubtful owing to the long period that

TABLE I

RELATIVE RESPONSE FACTORS FOR THE FOUR CONSTITUENTS OF THE COMPONENT PHENOLS

Phenol	*Parameter*	*15:0 (saturated)*	*15:1 (8'z) monoene*	*15:2 (8'z, 11'z) diene*	*15:3 (8'z, 11'z, 14') triene*
Methyl anacardate	(i) Standard (%)	7.29 ± 0.52	50.66 ± 3.19	13.47 ± 1.02	28.576 ± 1.91
	(ii) P + 2 peak (% of P)	3.17	3.17	3.17	3.17
	(iii) Corrected standard (%)	5.86	51.76	12.95	29.43
	(iv) Wt. (g)	0.00391	0.06647	0.02720	0.09078
	(v) Relative response factor (RRF):(iv) × 10^3/(iii)	0.66736	1.2842	2.1004	3.0843
Cardol	1st standard (%)	47.19 ± 0.89	16.84 ± 0.38	11.50 ± 0.37	24.46 ± 0.64
	P + 2 peak (% of P)	2.63	2.63	2.63	2.63
	Corrected standard (%)	47.41	16.77	11.01	24.81
	Wt. (g)	—	0.00469	—	0.00636
	Relative response factor (RRF)	—	2.7964	—	2.5636
	2nd standard (%)*	54.708	—	20.582	24.703
	Corrected standard (%)	55.070	—	19.436	24.867
	Wt. (g)	0.01245	—	0.01958	0.02840
	Relative response factor (RRF)	0.2261	—	1.0074	1.1421
	Interrelation of the two sets	0.5074	2.7964	2.2612	2.5636
Cardanol	Standard (%)	29.04 ± 0.65	45.98 ± 0.84	13.45 ± 0.33	11.53 ± 0.23
	P + 2 peak (% of P)	2.63	2.63	2.63	2.63
	Corrected standard (%)	28.359	46.492	13.397	11.752
	Wt. (g)	0.00278	0.0104	0.00340	0.00756
	Relative response factor (RRF)	1.95730	2.2621	2.5279	6.5557

* For a number of reasons it became necessary to use separate standards and to interrelate the two sets of results.

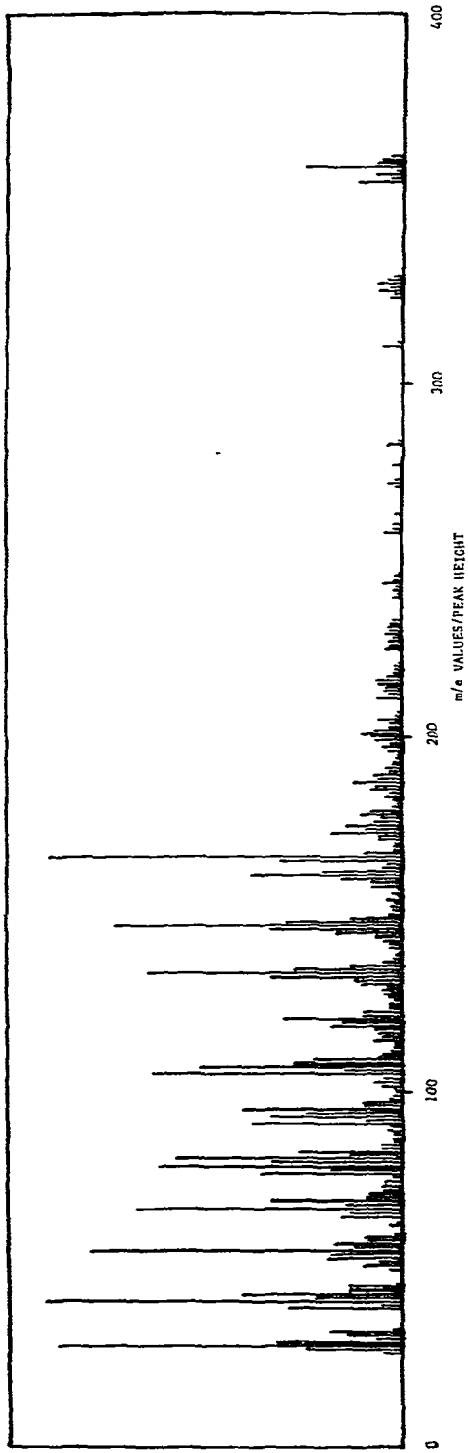


Fig. 1. Mass spectrum of methyl anacardate (triene, diene, monoene and saturated constituents).

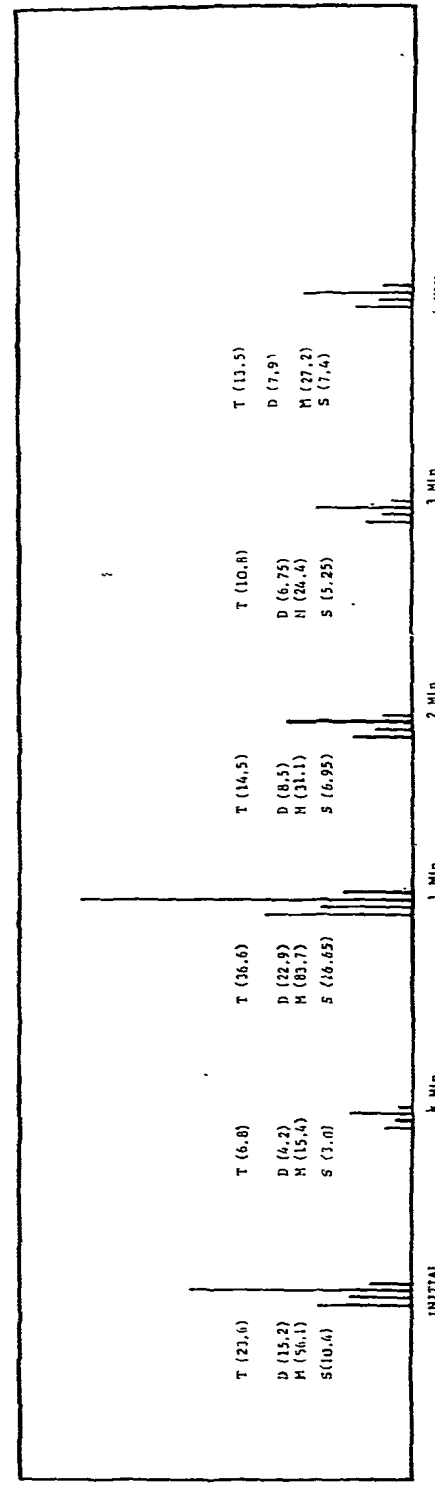


Fig. 2. Peak heights of the molecular ions from mass spectral scans of methyl anacardate at intervals up to 4 min.

TABLE II

COMPOSITION OF OLEFINIC CONSTITUENTS OF COMPONENT PHENOLS IN NATURAL CNSL BY MASS SPECTROMETRY

Phenol	*Parameter*	*Saturated*	*Monoene*	*Diene*	*Triene*
Methyl anacardate	(i) Composition of natural product (uncorrected) (%)	11.04 ± 1.15	51.54 ± 1.65	14.23 ± 0.18	23.20 ± 0.62
	(ii) Corrected (for P + 2 peak)	9.40	51.99	13.49	23.20
	(iii) Corrected for relative response factor [(ii) × RRF]	6.2733	65.605	28.341	71.544
	(iv) Normalized (%)	3.65	38.19	16.50	41.65
	(v) GLC analysis (%)	4.05	38.3 ± 2.33	17.3 ± 0.4	40.4 ± 1.6
Cardol	(i) Composition of natural product (uncorrected) (%)	1.41 ± 0.11	9.95 ± 0.56	23.93 ± 0.11	64.74 ± 0.51
	(ii) Corrected (for P + 2 peak)	1.15	9.32	22.22	64.74
	(iii) Corrected for relative response factor [(ii) × RRF]	0.598	26.742	51.403	170.353
	(iv) Normalized (%)	0.24	10.74	20.64	68.39
	(v) GLC analysis (%)	0.31	8.1. ± 1.41	21.9 ± 3.01	69.7 ± 3.74
Cardanol	(i) Composition of natural product (uncorrected) (%)	7.79 ± 0.6	46.03 ± 1.25	20.19 ± .41	25.99 ± 0.41
	(ii) Corrected (for P + 2 peak)	6.57	45.49	19.50	25.99
	(iii) Corrected for relative response factor [(ii) × RRF]	6.604	104.264	50.726	171.412
	(iv) Normalized (%)	1.98	31.31	15.23	51.47
	(v) GLC analysis (%)	2.68	29.5 ± 1.03	16.6 ± 0.64	51.2 ± 1.39
2-Methylcardol*	(i) Composition of natural product (uncorrected) (%)	4.36 ± 0.57	18.54 ± 0.88	21.21 ± 0.51	55.91 ± 1.17
	(ii) Corrected (for P + 2 peak)	3.81	17.93	19.59	55.91
	(iii) Normalized (%) (uncorrected)	3.92	18.43	20.15	57.50
	(iv) GLC analysis (uncorrected)	1.63	18.08	19.55	60.74

* Relative response factors for the constituents of this component phenol to be reported in later work.

had elapsed between isolation and examination and corrected results have not been obtained in this instance. Nevertheless, the uncorrected mass spectral and GLC results show reasonable similarity.

Further experiments with technical CNSL, the product of industrial decarboxylation of the natural product, have indicated the applicability of the mass spectral procedure to that product.

Results for different mass spectrometers

In Table III, the results for experiments carried out with a Hitachi–Perkin-Elmer RMS4 and with an AEI MS9 are given for cardanol.

The agreement was acceptable although the reproducibility was not so good with the former instrument. Mass spectral tracings for the standard followed by the natural product could usually be obtained in a few minutes, and the measurement of peak heights and calculation of corrections occupied most of the procedure. No attempt was made to enhance the molecular ion by reduction of the ionization voltage or by the use of the field ionization technique, and both instruments were used essentially as set up for standard mass spectral examination at 70 eV. The consistency of the results obtained was to some extent unexpected in view of the proverbial variation sometimes associated with mass spectra. It would have been advantageous to have a digital display of maximum peak height and numerous other improvements suggest themselves.

Comparison of results obtained with different extents of correction

It was clear that, apart from gross correction for the differing relative response factors of the constituents, different extents of correction for the P+2 ion peak could be applied. Where no correction was used the percentages of monoene, diene and triene constituents agreed tolerably well with those obtained by GLC but the percentage of saturated constituents was too high as shown in Table IV for methyl anacardate. Consequently, it was generally desirable to correct both the standard and the natural product for the P+2 peak contribution.

At first it seemed more logical to correct the results in either instance by the use of the observed P+2 peak contribution, obtained from the mass spectral examination of the individual constituents, rather than the theoretical value. The values for the observed P+2 peak were always slightly higher than the theoretical value but no simple explanation is evident as each constituent was checked analytically for purity by argentation TLC and by GLC, to ensure homogeneity. For quick results and certain applications where the percentage of unsaturated constituents only is of primary interest the uncorrected standard and natural product results may be acceptable.

Influence of homologous phenols on the analytical procedure

GLC examination has shown that homologous phenols are present particularly in the case of anacardic acid in natural CNSL and cardanol in technical CNSL. The C-17 contribution is the greatest, although constituents with chain lengths of C-9, C-11 and C-13 are also present. For cardol, 2-methylcardol and cardanol in natural CNSL, C-17 homologues are present in smaller proportions. The detection of the C-17 homologue is laborious by GLC but straightforward by mass spectroscopy. In the adsorption TLC separation used in the initial stage, homologues migrate together.

TABLE III

COMPARISON OF RESULTS FOR THE CONSTITUENTS OF CARDANOL DETERMINED ON HITACHI-PERKIN-ELMER RMS4 AND AEI MS9 INSTRUMENTS

Instrument	*Parameter*	*Saturated*	*Monoene*	*Diene*	*Triene*
RMS4	Standard (%)	26.60 ± 0.51	45.24 ± 0.59	14.76 ± 0.48	13.46 ± 0.58
	Corrected standard (for P + 2)	25.411	44.851	14.403	13.458
	Normalized (%)	25.897	45.709	14.679	13.715
	Wt. (g)	0.00278	0.0104	0.00340	0.00756
	RRF ($g \times 10^4$) (%)	1.0735	2.2752	2.3162	5.5122
	Natural product (%)	8.995 ± 1.230	42.996 ± 1.057	20.446 ± 2.043	27.347 ± 1.267
	Corrected (for P + 2)	7.862	42.457	19.725	27.347
	Normalized (%)	8.073	43.594	20.254	28.079
	RRF × normalized (%)	8.666	98.959	46.911	154.773
	Normalized (%)	2.81	32.04	15.19	50.12
MS9	Totally corrected (%)	1.98 ± 0.66	31.31 ± 1.25	15.23 ± 0.41	51.47 ± 0.41

TABLE IV

COMPARISON OF RESULTS FOR CONSTITUENTS OF COMPONENT PHENOLS WITH DIFFERING EXTENTS OF CORRECTION

Phenol	*Parameter*	*Saturated*	*Monoene*	*Diene*	*Triene*
Methyl anacardate	Standard (corrected with observed P + 2)	4.07	49.99	11.79	28.57
	Natural product (%) (corrected with observed P + 2)	4.19	37.88	16.62	41.31
	Standard (uncorrected)	7.29	50.66	13.47	28.57
	Natural product (%) (uncorrected)	3.36	38.42	16.32	41.89
	Standard (uncorrected)	7.29	50.66	13.47	28.57
	Natural product (%) (corrected with observed P + 2)	1.02	39.90	14.56	44.52
	Standard (corrected with theoretical P + 2)	5.86	51.76	12.95	29.43
	Natural product (%) (corrected with theoretical P + 2)	3.65	38.19	16.50	41.65
	% by GLC	4.05	38.3	17.3	40.4
Cardol	1st standard (uncorrected)	47.19	16.84	11.50	24.46
	2nd standard (uncorrected)	54.71	–	20.58	24.70
	Natural product (%) (uncorrected)	0.30	11.16	20.74	67.80
	% by GLC	0.31	8.1	21.9	69.7
Cardanol	Standard (uncorrected)	29.04	45.98	13.45	11.53
	Natural product (%) (uncorrected)	2.24	31.26	15.32	51.18
	% by GLC	2.68	29.5	16.6	51.2

The importance of precise TLC separation of the component phenols is shown by reference to cardol (molecular weight of 15:0 constituent = 320) where the presence of anacardic acid (molecular weight of 15:0 constituent = 348) would contribute a mass coincident with the C-17 homologue of cardol. In the method used in this work, the methylation of anacardic acid clearly avoids this occurrence. In Table V the molecular weights of the C-13, C-15 and C-17 homologues of the saturated constituents of the component phenols are shown. This indicates that homologues do not interfere in the analytical procedure and furthermore, the examination of the pure constituents of each component phenol ensures the validity of the method.

TABLE V

MOLECULAR WEIGHTS OF SATURATED HOMOLOGOUS COMPONENT PHENOLS

Chain length	*Cardanol*	*Cardol*	*2-Methylcardol*	*Methyl anacardate*
C-13	276	292	306	334
C-15	304	320	334	362
C-17	332	348	362	390

Table V indicates the difficulties inherent in a direct mass spectral examination with no initial TLC separation. An attempt to analyze technical CNSL is being examined but much further work remains to be done. While the different proportions of the component phenols present and their different volatilities might be overcome by the use of appropriate standard mixtures, the reproducibility of repeated scans remains a difficulty. Another possibility with a combined TLC–MS method would be to use direct recovery[17] from the chromatographic plate rather than the present indirect technique involving elution of bands and recovery of the component phenol containing its four constituents. Experimental work is also in progress to develop a GLC–MS procedure by means of which the component phenols could be analyzed quantitatively[5] simultaneously with the unsaturated constituents.

General observations

The close similarity of analytical results obtained by the GLC and TLC–MS procedures, despite wide differences in relative response factors between individual unsaturated constituents, is a strong recommendation for the latter approach. Without the application of relative response factors the results show no such conformity. The success of the mass spectral method is inherently associated with the comparable vapour pressure of the four constituents of each component phenol. Hites[12], in his work on glycerides, employed corrective rather than relative response factors and this entailed using information obtained from molecular distillation characteristics to allow for differing volatilities.

In biological, biosynthetic and technological areas[18], where the rapid analysis of methyl esters of polyethenoid fatty acids may be required, and indeed GLC is readily applicable, the quantitative usefulness of mass spectrometry has to some extent been overlooked. It is our experience that, like the phenolic lipids, the polyethenoid C-18 methyl esters with similar vapour pressures may be rapidly and quantitatively determined provided that relative response factors are employed. The ex-

treme rapidity of multiple scanning in mass spectrometry from a single sample has much in its favour compared with the repeated injection and time-consuming equilibration–elution process in GLC. The stability and reproducibility of the detector response in the mass spectral procedure are further strong recommendations for this technique.

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