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LONG-CHAIN PHENOLS

IV*. QUANTITATIVE DETERMINATION OF THE OLEFINIC COMPOSITION OF THE COMPONENT PHENOLS IN CASHEW NUT-SHELL LIQUID

J. H. P. TYMAN
School of Chemistry, Brunel University, Kingston Lane, Uxbridge, Middlesex (Great Britain)
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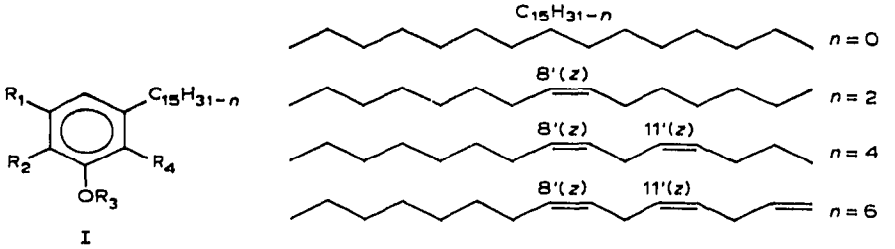
SUMMARY

By means of combined thin-layer chromatography–gas–liquid chromatography, the quantitative analysis has been achieved of the unsaturated constituents of the long-chain component phenols in cashew nut-shell liquid from *Anacardium occidentale*. The method is applicable to both the natural and the industrially heated and decarboxylated material.

INTRODUCTION

The component phenols of cashew nut-shell liquid (CNSL) from *Anacardium occidentale*, namely anacardic acid (I: $R_1 = R_2 = H$; $R_3 = H$; $R_4 = COOH$; $n = 0, 2, 4, 6$), cardol (I: $R_2 = R_4 = H$; $R_1 = OH$; $R_3 = H$; $n = 0, 2, 4, 6$), cardanol (I: $R_1 = R_2 = R_3 = R_4 = H$; $n = 0, 2, 4, 6$) and 2-methylcardol[1,2] (I: $R_1 = OH$; $R_2 = CH_3$; $R_3 = R_4 = H$; $n = 0, 2, 4, 6$), are predominantly polyunsaturated, and technical CNSL, obtained by the thermal decarboxylation of the anacardic acid in the natural product, owes its industrial usefulness[3,4] to its highly unsaturated character. It was of interest, in connection with structure–property correlations on cardanol[5], cardol[6] and anacardic acid[7], to determine the olefinic distribution of the phenols in natural CNSL and in the industrially decarboxylated product (technical CNSL**).



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* Part III, *J. Chem. Soc., Perkin Trans. I*, (1973) 1693.

** Some confusion exists in the literature between the natural and the industrially decarboxylated product. In this work, they are referred to as natural and technical CNSL, respectively.

By the use of a combined argentation thin-layer chromatographic (TLC) and an ultraviolet spectroscopic procedure, anacardic acid[8] was analyzed.

It was found that the argentation TLC procedure could be replaced by the direct use of ^1H nuclear magnetic resonance (NMR) spectroscopy and the proportions of the unsaturated constituents could be determined. However, when applied to the other component phenols (cardanol, cardol and 2-methylcardol), the method[9] necessitated the use of a 100-MHz, and preferably a 220-MHz, spectrometer. This procedure was devised because the three phenols, but not the phenolic acid, interacted with the silver nitrate in argentation TLC. Since the original work, alternative chromatographic procedures with derivatives of the phenols have been examined and work is still continuing.

Nevertheless, a simpler and less time-consuming procedure has been sought in order to avoid individual separation and spectrophotometric determination of the olefinic constituents of the component phenols. A combined TLC–gas–liquid chromatographic (GLC) method has now been evolved for the quantitative analysis of all of the components.

In this procedure, work on which was commenced four years ago, a preliminary TLC separation of natural or technical CNSL has been carried out. Under adsorption conditions, the component phenols contained collectively in each band the saturated, monoene, diene and triene constituents. The component phenols were converted into their methyl ethers and then submitted to the partition conditions of GLC analysis. On the stationary phase polyethylene glycol adipate, complete resolution was obtained. The phenols themselves were not sufficiently volatile with the latter stationary phase for direct analysis. Partition TLC analysis was of only qualitative use for indicating the presence of saturated, monoene, diene and triene constitutents, and for quantitative work GLC had to be employed.

EXPERIMENTAL

Cashew nuts were obtained from the principal source, Mozambique, and technical CNSL through British Cocoa Mills Ltd., Hull, Great Britain. Previously[2], it had been found convenient to separate the cashew shells from the kernel by initial treatment with solid carbon dioxide followed by cracking. A simpler method was to place the whole nuts in liquid nitrogen contained in a Dewar flask followed by the cracking procedure. Comminution of the shells in a Waring blender was essential for complete extraction of the CNSL with diethyl ether containing 0.1% of 2,6-di-*tert.*-butyl-4-methylphenol. All extracts were concentrated at ambient temperature and stored at 0° under nitrogen in the dark in tightly stoppered flasks with a small free space above the solvent. Technical CNSL was kept in a cool place in the dark in a sealed container. All solvents were re-distilled.

Thin-layer chromatography

The previous procedure[8] has been modified and justifies description. TLC was carried out on analytical plates (10 × 8 × 0.25 cm) and preparative plates (20 × 20 cm × 1 mm) coated with silica gel G (Merck, Darmstadt, G.F.R.). All sample applications and evaporations of solvent from the plates after development were carried out under nitrogen. Bands were rendered visible with rhodamine 6G and

ultraviolet irradiation, and were eluted under nitrogen with diethyl ether containing 10% of methanol for 16 h in the dark at ambient temperature. After recovery by filtration and rotary evaporation, samples were restored to atmospheric pressure under nitrogen. Trace amounts of indicator were removed by washing solutions in light petroleum (b.p. 40–60°) with 0.5% aqueous sulphuric acid. Natural CNSL (*ca.* 0.2 g) was separated on a preparative plate by development first in chloroform (95 ml) and ethyl acetate (5 ml) with the addition of concentrated ammonia solution (2 ml). In addition to the usual paper liner, a liner was placed in the base of the tank so as to absorb any water.

After development to the top of the plate and location of the bands by spraying (they were sometimes visible without spraying, particularly 2-methylcardol), the plate was developed in chloroform (80 ml) and ethyl acetate (20 ml) containing 2% of concentrated ammonia solution so that the solvent front did not reach the 2-methylcardol band, in order to separate cardol from anacardic acid.

For technical CNSL (*ca.* 0.283 g), which contained a negligible amount of anacardic acid, no multiple development was necessary and a single separation in chloroform (95 ml) and ethyl acetate (5 ml) was carried out. Owing to tailing of the cardanol band, it was important not to overload the plate and to cause a less precise separation from 2-methylcardol. In all preparative TLC experiments, the material recovered from bands was weighed and the adsorbent between the bands was also eluted so as to ensure completeness of separation. In instances when preparative plates had been overloaded, re-purification of the eluted materials became necessary. By the use of diethyl ether–light petroleum–formic acid, in which anacardic acid behaves as a non-polar intramolecularly bonded substance[1], complete separation from cardol was obtained. Cardanol and 2-methylcardol were purified without the need for addition of formic acid. All preparatively separated materials were checked by analytical TLC.

Conversion of the phenols into methyl ethers

Trial procedures established that quantitative O-methylation of cardanol, cardol, 2-methylcardol and anacardic acid (to the methyl ester, methyl ether) took place by refluxing a 10% solution of the phenol in benzene with dimethyl sulphate (5 molar proportions) and anhydrous potassium carbonate (10 molar proportions) for 3 h under nitrogen on a steam-bath. The recovered, alkali-washed and dried products were kept in the dark under nitrogen in benzene solution.

In the methylation procedure, O-methylation was predominant and C-methylation[10] did not occur as no 2-methylcardol or 4-methylcardol was produced from cardol.

For anacardic acid, complete methylation proved unnecessary and methylation with excess of ethereal diazomethane at 0° gave the phenolic methyl ester having the correct ^{1}H NMR absorption spectrum with a hydrogen-bonded OH group at low field.

Gas–liquid chromatography

Chromatographic determinations were carried out with a Pye 104 apparatus equipped with a flame ionization detector (FID) and a Fisons Vitatron integrator–recorder. Acid-washed and silanized Celite (80–100 mesh) was coated with 2% polyethylene glycol adipate. Silanized columns (5 ft. × 3/16 in.) were maintained at 200°

and a nitrogen flow-rate of 45 ml/min (7 p.s.i.) was used. At 180° (the recommended upper temperature for this stationary phase), retention times of the methyl ethers were excessive; at 220°, the signal to noise ratio was poorer and the life of the column decreased. It was found convenient to inject benzene solutions of the methyl ethers (usually 0.5 or 1.0 μl). Three or more chromatograms were obtained for each sample and at least two integrations were carried out. Results by integration and triangulation were compared, the latter subsequently being used as integration led to the use of excessive amounts of chart paper because of the desirability of operating at high chart speeds. The phenols themselves (even cardanol) were not used because of their exceedingly long retention times.

Elution and recovery of bands from argentation TLC experiments on the phenols followed by methylation and GLC examination corroborated the assigned retention times of the monoene, diene and triene constituents. In addition, mass spectrometry–GLC gave confirmatory information from the molecular ions exhibited.

The FID was calibrated in order to allow for its different response towards the saturated, monoene, diene and triene constituents. Methyl anacardate was separated by argentation TLC (15% silver nitrate) with ethyl acetate–chloroform (1:9) on preparative plates and the purity of the recovered constituents (eluted with methanol–diethyl ether, 1:9) examined by GLC and NMR spectroscopy. Each fraction was found to be completely pure. A chloroform solution of the four components in proportions that resembled their occurrence in the natural product was prepared (weighings were carried out on a five-place balance). Four repetitions enabled the average peak area per gram to be obtained for the saturated, monoene, diene and triene constituents.

RESULTS AND DISCUSSION

Relative retentions of saturated, monoene, diene and triene constituents of the CNSL phenols

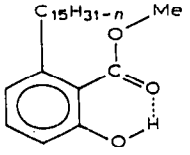
The retention distances (with standard deviations) and the relative retentions of the various substances on 2% polyethylene glycol adipate at 200° are summarized in Table I. The monoene, diene and triene peaks were completely resolved although the saturated and monoene peaks overlapped slightly. For methyl anacardate, evidence was obtained of a small peak (1.07% of the total unsaturation at a relative retention of 6.70 (triene 5.85, Table I) corresponding to a tetraene, but it has not been taken into consideration in the calculations. Homologous (C_{13} and C_{17}) members of the saturated, monoene, diene and triene constituents of each component phenol would be isolated with the main C_{15} constituent in adsorption TLC, and would be separated only under the partition conditions of GLC analysis. Nevertheless, this method revealed only a minor proportion of the C_{17} homologue and C_{13} in negligible amounts. The numbers of theoretical plates calculated in the usual way for cardanol monoene, diene and triene were 2115, 2190 and 2270, respectively, and indicated that the column was reasonably efficient. The methyl ethers represented the most useful derivative for GLC examination. The acetates of the phenols (from acetic anhydride and pyridine) were slightly less volatile and less thermally stable and as the methyl ester was required in the case of anacardic acid, the use of the methyl group throughout the series was convenient. Other protective groups such as tetrahydropyranyl, al-

TABLE I

RETENTION DISTANCES (R.D.) AND RELATIVE RETENTIONS (R.R.) OF METHYL ETHERS OF CNSL PHENOLS ON 2% POLYETHYLENE GLYCOL ADIPATE AT 200°
Retention distances are given in millimetres.

Compound	*Olefinic constituent*							
	Saturated		*Monoene*		*Diene*		*Triene*	
	R.D.	*R.R.*	*R.D.*	*R.R.*	*R.D.*	*R.R.*	*R.D.*	*R.R.*
Cardanol methyl ether	140±2.7	0.90	155±4.8	1.0*	189±6.4	1.22	230±8.4	1.48
Cardol dimethyl ether	—	2.71	442±9.1	2.88	535±16.1	3.52	663±14.9	4.31
2-Methylcardol dimethyl ether	—	2.33	410±15.6	2.58	502±17.7	3.17	613±22.2	3.88
Methyl anacardate	525±65.2	3.54	573±47.1	3.87	720±69.7	4.75	909±67.6	5.85
Cardanol	—	5.58	—	5.94	—	7.24	—	8.85
Methyl oleate	—	—	—	0.28	—	—	—	—

* Retention time 15.5 min.



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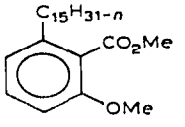

III

though invaluable in synthetic work[5,6] for alcohols rather than phenols, do not have the required thermal stability. The trimethylsilyl group has been widely used for phenols[11,12] and our own work in this field will be reported elsewhere. In the present work, it was found sufficient for volatilization to form the methyl ester of anacardic acid (II) rather than the O-methyl methyl ester (III), as the former was more volatile owing to intramolecular hydrogen bonding. Although the GLC analysis of the methyl esters of the fatty acids and O-methyl methyl anacardate (no preliminary separation of the shell liquid from the kernel was made) has been described[13], a stationary phase that is not readily available, β-cyclodextrin acetate, was used and the resolution achieved was less satisfactory than in the present method. Owing to the similar retention times of the methylated cardol and 2-methylcardol constituents, the complete analysis of natural and technical CNSL is not possible without preliminary TLC separation of these two closely related phenols. Work is proceeding, however, on the analysis, by GLC alone, of CNSL after hydrogenation and methylation, and this work will be reported elsewhere.

Comparison of different quantitation procedures

Both triangulation and integration gave similar results. Of thirty individual results, four agreed exactly, four within 0.1%, eight within 0.3%, four within 0.6%, two within 0.9%, four within 1.2% and four within 2%. Owing to the slight overlap of the saturated and the monoene components, triangulation was useful. The close similarity of most of the results by triangulation and integration vindicates the continued popularity[14] of the former method.

TABLE II

TOTAL OLEFINIC COMPOSITION OF METHYL ETHERS OF THE COMPONENT PHENOLS OF NATURAL AND TECHNICAL CNSL

Parent phenol	*Uncorrected*			*Corrected*			
	Monoene	*Diene*	*Triene*	*Saturated*	*Monoene*	*Diene*	*Triene*
Natural cardanol	—	—	—	2.68	29.5	16.6	51.2
Technical cardanol	40.99±1.03	21.06±0.64	37.94±1.39	3.11	36.1	20.1	40.6
Natural cardol	9.08±1.41	22.96±3.01	67.95±3.74	0.31	8.1	21.9	69.7
Technical cardol	11.00*	26.70*	62.30*	—	9.6	25.2	65.2
Natural 2-methylcardol	18.38±1.13	19.87±1.57	61.74±1.97	1.66	13.9	18.5	65.9
Technical 2-methylcardol	18.54±1.16	22.64±0.66	59.15±0.71	2.43	15.3	20.4	61.9
Methyl anacardate**	43.35±2.33	18.71±0.40	37.98±1.60	4.05	38.3	17.3	40.4

* Only two results available, which agreed exactly.

** As methylation is quantitative, this represents the composition of anacardic acid in natural CNSL. None is present in technical CNSL.

The normalized composition results obtained by the triangulation method for the monoene, diene and triene (from six determinations in each instance) together with the standard deviations are summarized on the left-hand side of Table II. Most of the standard deviations lie within the range 0.5–1.5%.

Proportions of the olefinic constituents in the component phenols

The monoene, diene and triene olefinic compositions shown on the left-hand side of Table II were derived on the basis of an equal response of the FID towards the four constituents. Calibration experiments were carried out with the individual constituents of methyl anacardate separated by argentation TLC. The peak areas, weights of constituents used in the standard solution and the relative response factors for four experiments are shown in Table III.

The results indicate the necessity for correction of the peak areas in each instance[15] in order to obtain a quantitative analysis. Different levels of unsaturation in hydrocarbons have been found to result in different detector response[16]. Because

TABLE III

RELATIVE DETECTOR RESPONSE FOR THE CONSTITUENTS OF METHYL ANACARDATE

Parameter	*Olefinic constituent*			
	Saturated	*Monoene*	*Diene*	*Triene*
Proportion of constituent (%)	1.848±0.44	39.36±0.33	14.86±0.18	44.81±0.25
Peak area × 10^{-3}	0.0567	1.181	0.4451	1.343
Weight taken (g)	0.0391	0.6649	0.2720	0.9078
Relative response factor	1.449	1.776	1.636	1.479

the ring is identical and only the side-chains differ in the other phenolic components, as with methyl anacardate, it was assumed that similar correction factors can be applied with respect to the constituents of cardanol, cardol and 2-methylcardol.

The complete results (the average of at least four determinations in each instance) for the saturated, monoene, diene and triene constituents of the phenols in natural and technical CNSL are shown on the right-hand side of Table II as the corrected results after applying the appropriate relative response factor (Table III) and normalizing the results in each instance.

The results for natural CNSL in Table II quantitatively support the previous qualitative (argentation TLC) observations[1] on the similarity in composition between cardanol and anacardic acid and between cardol and 2-methylcardol. Nevertheless, there are significant differences between the two sets that suggest that the cardanol did not arise by natural decarboxylation or the 2-methylcarbol by methylation of cardol without subsidiary changes in each instance.

Biological methylation is believed to occur at the polyketide stage of biosynthesis[17] and the significance of the present results, which suggest that it could occur after cyclization of the polyketide, will be discussed elsewhere. It can be simply noted that if this is the sequence, a side-chain saturation–desaturation process is simultaneously taking place.

Together with the described quantitative TLC–UV spectrophotometric procedure[1], which indicated a composition of 73.3% anacardic acid, 19.1% cardol, 2.8% 2-methylcardol and 4.8% cardanol for natural CNSL of Tanzanian origin, a total analysis is now available.

The results for technical CNSL in Table II indicate a close similarity in unsaturated composition between the precursor anacardic acid and technical cardanol. The composition of natural CNSL used to prepare the technical CNSL was not available but it seems probable that it is likely to be similar to the natural CNSL used in this present work. It is apparent that the decarboxylation process has been effected with little alteration of the triene unsaturation. Experience of the distillation of both natural and technical CNSL and prolonged decarboxylation of anacardic acid has shown that the triene constituent is most vulnerable to polymerization and decomposition. The diene and the monoene are progressively less influenced.

The preparative TLC separation of technical CNSL indicated gravimetrically the presence of 88.1% of volatile material (comprising 64.8% cardanol, 20.5% cardol and 2.8% 2-methylcardol) and 11.9% of non-volatile polymeric metarial. As the saturated and monoene components are more stable, it is of interest to express the results from natural and technical cardol and 2-methylcardol in terms of the same percentage of monoene. In each instance, the percentage of triene in the technical material is lower than in the natural product.

The results for methyl anacardate obtained by the TLC–GLC method, namely 38.3% monoene, 17.3% diene and 40.4% triene, agree substantially with those found by the argentation TLC–UV spectrophotometric method[8], which were, to quote one set, 37.8% monoene, 18.2% diene and 44.1% triene. The natural CNSL was of slightly different origin in each instance. Possible regional variation in the phenolic composition of *Anacardium occidentale* is currently being examined. The TLC–GLC method has the advantage that it is applicable to all of the phenols (in the form of their ethers) and a total analysis can be achieved within a comparatively short time.

The method was applied to cardanol without the methylation stage but the retention time of the phenol relative to the methyl ether was unfavourable at the moderate temperature required for operation with polyethylene glycol adipate. With developments in polar stationary phases for use well in excess of 200°, it is possible that the constituents of the phenols could be examined directly and the methylation omitted. Instead of the present indirectly linked TLC–GLC procedure, it is probable that the component phenols could be investigated by the direct linked procedure[18] and a rapid method of total analysis obtained for natural and technical CNSL.

CONCLUSION

By combination of a preliminary quantitative TLC separation of the component phenols anacardic acid, cardol, 2-methylcardol (from natural CNSL) and cardanol, cardol and 2-methylcardol (from technical CNSL) with the GLC of the methyl ethers of the constituents of the phenols, a total analysis of the two products is now possible. The present work is the first described attempt to achieve a total analysis.

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