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THE COMPOSITION OF THE UNSATURATED PHENOLIC COMPONENTS OF ANACARDIC ACID

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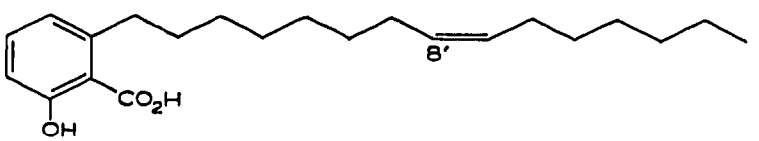
SUMMARY

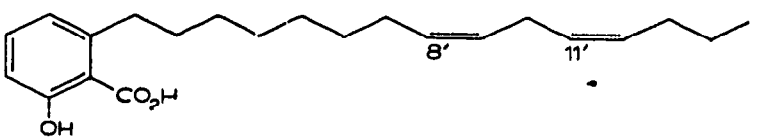
The monoene, diene, and triene components of anacardic acid have been quantitatively separated by argentation–thin-layer chromatography, and determined by ultraviolet spectrophotometry and gravimetry. Some progress has been made in the direct determination of the composition without separation of the component acids by use of nuclear magnetic resonance spectroscopy.

INTRODUCTION

The unsaturated components of anacardic acid*, the principal constituent of cashew nut-shell liquid (*Anarcardium occidentale*)[1–4], have been examined indirectly through chromatography of cardanol methyl ether[5], obtained through decarboxylation and methylation, and by low temperature acetone crystallisation[6] of anacardic acid itself. These qualitative studies have revealed the existence of 8′-monoene (I),



(I)



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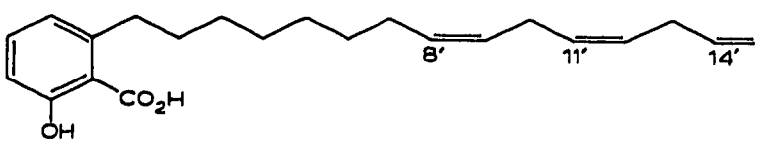

(III)

* The name anacardic acid although originally retained for the main component of cashew nut-shell liquid has more recently been used for 2-hydroxy-6-alkylbenzoic acids where the alkyl group (C_{11} and higher) has the relevant saturation and/or unsaturation.

8',11'-diene (II), and 8',11',14'-triene unsaturation (III) in a pentadecyl side chain. Recently, small proportions of a component having a saturated tridecyl side chain and four components arising from a heptadecyl side chain have been described[7] in cashew nut-shell liquid from an unstated source.

In the course of a quantitative investigation of the composition of cashew nut-shell liquid by a spectrophotometric method[8], qualitative observations on the unsaturated ingredients of the individual phenols were made by the argentation–TLC method[9–11]. The present paper describes work, completed in 1966, on the quantitative separation of the monoene, diene, and triene components of anacardic acid and more recent spectroscopic work; the method was not so successful when applied to the cardanol, cardol, and 4-methylcardol constituents of cashew nut-shell liquid and these results will be described subsequently.

A method for the determination of the composition of purified anacardic acid was sought to obviate the chromatographic separation of the component acids. Although infrared (IR) absorption and mass spectrometry were extremely useful procedures when applied qualitatively for confirming the presence of certain structural features and determination of molecular weights respectively there were difficulties in their quantitative application. NMR spectroscopy was, however, more useful in this latter respect provided the class of the component was qualitatively known. Further work is required to improve the procedure.

Quantitative information on the distribution of unsaturation in natural cashew nut-shell liquid phenols and in the decarboxylated material is of interest both with regard to the biosynthesis of these materials and to possible side reactions occurring in the industrial decarboxylation process.

EXPERIMENTAL

Analytical (8 × 10 cm) and preparative TLC plates (20 × 20 cm) were prepared in the usual way with the applicator set to 0.250 mm and 1 mm, respectively. Silver nitrate-impregnated plates (% $AgNO_3$, w/w on Silica Gel G), prepared by incorporation of the former in the slurry, were stored in glass containers in the dark. All plates were activated in an oven at 110° for 1 h and then cooled to ambient temperature. All operations were carried out in the absence of direct sunlight. TLC applications and solvent evaporations were carried out in an atmosphere of nitrogen. For recording TLC results, analytical plates were visualised with 50% w/w aqueous H_2SO_4 by charring at 150°.

UV absorption spectra were recorded automatically on an Optica (Great Britain) and a Perkin Elmer Model 137 spectrophotometer and manually on a Unicam SP 500 instrument. Methanol (A.R. grade) was used throughout. All optical density measurements were the average of two readings.

Cashew nuts (*Anacardium occidentale*) were of Tanganyikan origin.

Separation of cashew nut-shell liquid (CNSL) components

Disintegrated cashew nut-shells separated from the kernel by fracturing at −70° (solid CO_2) were extracted with diethyl ether for 24 h with occasional shaking at ambient temperature. After filtration the solid residue was comminuted in a blender and re-extracted with diethyl ether. The solvent in the combined filtrates was

evaporated at ambient temperature under reduced pressure in the presence of nitrogen to avoid decarboxylation, until the residue was constant in weight.

CNSL (0.1957 g) in chloroform (2 ml) was distributed along the baseline of two preparative plates and developed in 200 ml of diethyl ether–petroleum ether (40–60°) (50:150) containing 10 ml aqueous ammonia (0.880 S.G.) until the solvent front was 1 cm from the top of the plate. After removal of the organic solvents in a stream of nitrogen, the plates were developed in diethyl ether (150 ml) containing aqueous ammonia (0.880; 10 ml) until the solvent front had travelled 8 cm from the baseline, dried and sprayed with 0.01% ethanolic Rhodamine 6G. Under UV light seven bands of increasing R_F value were discernible as follows: (1) baseline material, ammonium anacardate, (2) anacardic acid, (3) cardol, (4) unknown minor ingredient, (5) 4-methylcardol, (6) cardanol, (7) unknown minor ingredients. Bands (1) and (2) were combined, eluted with methanol (25 ml) for 24 h, filtered, the solid washed with methanol (2 × 25 ml) and diethyl ether (2 × 25 ml) and after evaporation at room temperature of the combined filtrates, the residual material in chloroform (3 ml) was purified further on a preparative plate by development in 200 ml of diethyl ether–petroleum ether (40–60°) (50:150) containing 95% formic acid (1.5 ml). Anacardic acid formed an intense band near the solvent point, while traces of cardol were left nearer the baseline, and was recovered as before. The Rhodamine 6G indicator was removed by washing an ethereal solution of the evaporated product with 0.01 *N* aqueous sulphuric acid (6 × 15 ml) followed by water (6 × 15 ml). The dried solution ($MgSO_4 \cdot H_2O$) was evaporated and residual material which was homogeneous was stored in chloroform (concentration, approx. 15 mg/ml).

Separation of the unsaturated components of anacardic acid on silver nitrate-impregnated Silica Gel G

Owing to the relatively low ε value of anacardic acid at λ_{max} 308 nm it was necessary to use larger amounts than could easily be obtained on the analytical scale.

TABLE I

R_F VALUES OF THE COMPONENTS OF ANACARDIC ACID

Solvent system No.	*Solvent proportions*			*R_F values*		
	Petroleum ether	*Diethyl ether*	*Formic acid*	*Monoene*	*Diene*	*Triene*
1	50	—	1		all at baseline	
2	50	—	3		all at baseline	
3	50	10	1		poor separation	
4	50	10	3		poor separation	
5	50	20	3	0.69	0.51	0.33
6	50	20	5	0.69	0.52	0.33
7	50	30	3	0.90	0.75	0.59

The use of this wavelength precludes interference from non-phenolic material. Plates prepared with 10% silver nitrate were less effective on the preparative than on the analytical scale whereas 20% silver nitrate gave almost as good results at both levels and was accordingly used. Reproducible R_F values were not always achieved. Table I

summarises the values (on plates having 20% silver nitrate) found with different proportions of petroleum ether (40–60°), diethyl ether and formic acid.

Ethyl acetate–chloroform mixtures were not so useful and acetic acid or mono-, di, or trichloroacetic acids (to avoid possible reducing action) were not as effective as formic acid. Solvent system (5) was generally used throughout. For quantitative work, Rhodamine 6G was unsatisfactory for detection of separated components, as it faded rapidly due to the formic acid present. Dichlorofluorescein and 3,5-dihydroxypyrene-8,10-disulphonic acid were both suitable for visualisation but the former could only be removed by water washing from petroleum solutions, whereas diethyl ether was generally the preferred solvent for fractions. Water was an effective indicator since the bands showed up as hydrophobic areas.

Quantitative experiments

Anacardic acid in chloroform solution (1 ml) was applied to a 20 × 20 cm preparative plate and developed with solvent system (5). After evaporation of nearly all the solvent in a nitrogen atmosphere, the bands were visualised by means of pyrenesulphonic acid. The solvent front, the three main bands, and the baseline were each eluted with methanol (25 ml) during 2 h with intermittent shaking. After filtration and washing the silica gel with methanol (5 × 10 ml), the combined filtrates were evaporated nearly to dryness at ambient temperature *in vacuo* and the concentrate was extracted with ether. The small amount of insoluble material (Ag anacardate) was warmed with a small volume of dilute nitric acid and re-extracted with ether and the combined ethereal solutions were washed with water until neutral. The residual material left after evaporation of the ether was dissolved and made up to 25 ml in methanol. The optical densities of the pure solutions were measured at 308–309 nm. Material from the solvent front and from the baseline showed no absorption

TABLE II

UV ABSORPTION OF THE COMPONENTS OF ANACARDIC ACID FROM CASHEW NUT-SHELL LIQUID

Optical density observed			*Optical density corrected*		*Total optical density*	*Total optical density (%)*		
Triene (T)	*Diene (D)*	*Monoene (M)*	*Diene*	*Monoene*	*(T + D + M)*	*Triene*	*Diene*	*Monoene*
0.581	0.180	0.498	0.181	0.504	1.266	45.9	14.3	39.8
0.995	0.408	0.842	0.410	0.852	2.257	44.1	18.2	37.8
0.987	0.356	0.830	0.357	0.840	2.184	45.2	16.4	38.5

maximum, while the monoene, diene, and triene showed clear maxima. Examined on $AgNO_3$ analytical plates each fraction gave a single spot of different R_F value, while on unimpregnated plates all the components had the same R_F value corresponding to that of the fully hydrogenated material.

Minor variations in the work-up procedure in or after elution were made, such as the direct use of ether and dilute nitric acid or elution with methanol containing

hydrochloric acid to remove silver nitrate, but none of these altered the optical density found. The results of three experiments are summarised in Table II. (Since only the molar extinction coefficients are uniform for the mono, di and triene the optical densities were corrected using molecular weights 346, 344 for the monoene, diene, respectively and c, concentration = optical density × mol. wt./molar extinction coefficient × 10. The correction is in fact trivial.)

The average result was 45.1% triene, 16.3% diene and 38.7% monoene.

A negligible proportion of the saturated component was present. Elution of a progressive series of strips from above the monoene band to the top of the plate failed to yield any material having a maximum absorption at 308 nm. It was considered that the saturated component could be present with cardol in band (3) of the initial separation but careful purification failed to yield any such material.

Separation and estimation of component acids by gravimetry

A total of eleven plates impregnated with 20% silver nitrate was developed and the combined bands eluted giving the triene 0.0889 g (44.1%), the diene 0.0352 g (17.4%) and the monoene 0.0774 g (38.4%) affording a good measure of agreement with the spectroscopic method. The physical properties of the separated components agreed with those described[6]. The monoene was a solid, m.p. approx. 50°. The diene solidified on cooling to 0° and had a melting point just above ambient temperature, while the triene was an oil which only solidified on cooling with acetone–carbon dioxide.

The monoene was the only sample which could be analysed. Found: C, 76.20%; H, 9.83%; calculated for $C_{22}H_{34}O_3$; C, 76.25%; H, 9.89%.

Spectroscopic characterisation of the components of anacardic acid

IR absorption. All three unsaturated materials showed characteristic weak absorption in the region 700–710 cm^{-1}, characteristic of *cis* C–H (out-of-plane bend) although this was partly obscured by the $(CH_2)_n$ rocking frequency. In all cases the much stronger *trans* C–H absorption at 965–975 cm^{-1} was absent (for reference purposes oleic and elaidic acids were used). Although the triene showed strong absorption at 900 cm^{-1} due to the terminal $C=CH_2$ group its determination in anacardic acid was rendered difficult by overlapping adjacent bands from the monoene and the diene.

UV light irradiation. Irradiation of the monoene, diene and triene in petroleum ether (40–60°) solutions in silica cells during 24 h caused rapid isomerisation in the presence of 1% iodine to the all *trans* configurations in each case with strong absorption at 965 cm^{-1}. In the absence of iodine, isomerisation was considerably slower. The whole process with or without iodine could be monitored by TLC. In the absence of a catalyst the diene and triene showed two additional spots of higher R_F value than the starting material corresponding, it is thought, to *cis/trans* and *trans/cis* configuration of the 8′ and 11′ double bonds.

Mass spectra. The parent ions for the saturated monoene, diene and triene components were at m/e 348, 346, 344 and 342 corresponding to the molecular formulae $C_{22}H_{36-n}O_3$, where n = 0, 2, 4, 6, respectively. Higher masses corresponding to possible C_{17} components were present in traces.

Proton magnetic resonance spectra. Spectra were initially run on a Varian T60 and subsequently on an HA100 instrument in order to obtain better resolution between benzylic protons and those adjacent to two double bonds. Carbon tetra-

TABLE III

NMR ABSORPTION OF ANACARBIC ACID AND ITS HYDROGENATION PRODUCT
s = singlet; d = doublet; t = triplet; q = quarter; m = multiplet.

Type of group		*τ value*	*Type of splitting*
Anacardic acid			
OH, CO_2H	(internally bonded)	−0.92	broad s
HAr	(two types)	2.63–2.8, 3.16–3.35	m
—HC = CH—	(olefinic)	4.6–4.79	m
CH_2 = C	(olefinic)	4.93–5.13	m
—CH_2—Ar	(benzylic)	6.94–7.11	t
—CH=CH—CH_2—CH=CH—	(methylenic 2 double bonds)	7.17–7.27	m
—CH_2—CH=CH—	(methylenic 1 double bond)	7.82–8.14	m
—$(CH_2)_2$	(methylenic chain)	8.2—8.98	double s
—CH_3	(methyl)	9.0–9.3	t
6-Pentadecylsalicylic acid			
3HAr	(3 aromatic H)	2.54–2.72, 3.04–3.34	m
—CH_2Ar	(2-benzylic H)	6.92–7.18	t
—$(CH_2)_2$—	(2 methylenic H)	8.2–8.9	s
—CH_3	(3 methyl H)	9.04–9.22	t

chloride was the preferred solvent as deuterochloroform contained sufficient chloroform to interfere with the integration in the region of aromatic protons.

The spectra of the monoene, diene, triene, and the saturated component obtained by hydrogenation were first obtained. Bands were identified by the use of appropriate reference compounds such as 6-methylsalicyclic acid, salicylic acid, eugenol, oleic acid, elaidic acid (and their esters) and by application of the Shoolery rules. It was found desirable to use freshly prepared specimens free of autoxidised products. The spectrum of a freshly prepared sample of anacardic acid was next determined and bands due to the individual components identified. The values for anacardic acid and its hydrogenation product are listed in Table III. In the monoene the coupling constants (4.6 c.p.s.) and sharpness of the peaks due to –CH=CH– protons indicated that *cis* and none of the *trans* isomer was present. The complex olefinic peaks in the diene and triene precluded similar observations but the IR absorption of these two components indicates the *cis* configuration of the double bonds. From scale expansion of the integration the average relative areas (from three runs) were 78 for the –CH=CH– protons and 20 for $=CH_2$ protons. By comparison (see DISCUSSION) of the theoretical values from different mixtures of the monoene, diene and triene, the composition of anacardic acid itself was found by trial and error to approximate to 45% triene, 15% diene and 40% monoene.

DISCUSSION

Anacardic acid from cashew nut-shell liquid was separated on silver nitrate-impregnated silica gel plates and no material remained at the baseline through silver salt formation or autoxidation. Reduction was observed with cardanol and cardol and

its absence with the acid may be possibly due to the internal bonding of the –OH and $-CO_2H$ groups. The method separates the components of the acid according to the degree of unsaturation but not chain length. (This, however, in the case of the present CNSL appeared to be substantially C_{15}.)

The lack of IR absorption at 965 cm^{-1} indicated that the unsaturated components were present in the *cis* form[12]. The configuration of the constituent acids has previously been inferred from experiments carried out with cardanol methyl ether. Upon irradiation of anacardic acid with UV light under mild conditions the monoene was partly transformed into the *trans* form while the diene and triene gave, it was believed, the partly isomerised *cis*-8′, *trans*-11′ and *trans*-8′, *cis*-11′ products of higher R_F values than starting material but lower than the all *trans* product. The existence of partly *trans* found in the natural product would in fact have rendered the argentation–TLC separation almost impossible due to overlapping bands.

The NMR spectra of the component acids and of the saturated acid afforded confirmation of certain groupings. The coupling constant (4.6 c.p.s.) in the triplet attributable to the –CH=CH– protons of the monoene is similar to the value for oleic acid and larger than that for elaidic (3.6 c.p.s.) and is consistent with a *cis* configuration. The spectrum for the triene clearly indicated the presence of $=CH_2$ protons. Although at higher τ values it was not easy to ascribe exactly to given groups the areas found from integration, in the region of the olefinic protons the areas due to –CH=CH– and $=CH_2$ groups could be distinguished. A trial and error fitting procedure has indicated a composition in agreement with the spectrophotometric method. By the additional use of another part of the spectrum a more exact mathematical solution should be possible. The region where the methylenic protons of the $-CH=CH-CH_2-CH=CH-$ group and the benzyl group (a common structural feature for all the component acids) overlap would in theory be suitable. In practice some difficulty was experienced in obtaining reproducible areas from integration and further work is required. (Nevertheless an NMR procedure appeared to be the only one for determining the composition of anacardic acid without preliminary separation of the component acids.)

No monoethenoid fatty acids were found in CNSL although GELLERMAN AND SCHLENK[7] have reported their occurrence. In a typical chromatogram on analytical plates with solvent 5, oleic acid (R_F 0.54) and elaidic acid (R_F 0.61) were well separated from the monoene (R_F 0.66), and the diene (R_F 0.47). The presence of monoethenoid acids, although non-absorbing at the wavelength used for determining the component anacardic acids, would have led to a discrepancy between the gravimetric and spectrophotometric methods. The agreement between the two procedures was however, satisfactory.

There are wide differences in the compositional figures found by PAUL AND YEDDANAPALLI[6], by GELLERMAN AND SCHLENK[7] and the present authors. The acetone crystallisation technique would not be expected to give sharp separation of the component acids and the figures found for anacardic acid of Indian origin namely, 4% saturated, 37% monoene, 44% diene, and 15% triene, are not likely to be accurate. The acids isolated were characterised by C/H analysis. The theoretical values for the monoene and the triene differ by only 0.89% for C and 1.16% for H, while the C_{16} and C_{18} monoethenoid fatty acids have an almost identical %C with %H slightly higher.

The relative proportion of component acids found by GELLERMAN AND SCHLENK[7], 0.9% saturated, 14.3% monoene, 20% diene, and 38.4% triene (the remainder comprising unsaturated fatty acids and traces of C_{13} and C_{17} anacardic acids) are lower in the case of the monoene and diene than those found in the present work. The method of extraction of the cashew nut-shell liquid used was not described and the nuts were from an unstated source.

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