

# IDENTIFICATION OF SOME NATURALLY OCCURRING ALKYL-SUBSTITUTED PHENOLS IN CASHEW-NUT SHELL LIQUID BY CHROMATOGRAPHIC TECHNIQUES\*

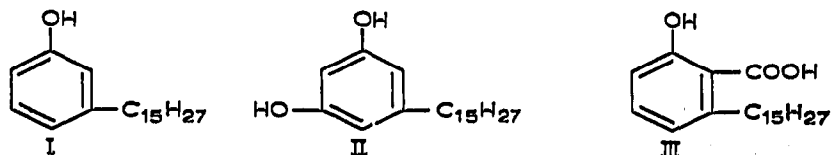
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## INTRODUCTION

The presence of a considerable amount of alkyl-substituted phenols in cashew-nut shell liquid (CNSL) makes it an important raw material in the manufacture of resins. Commercial CNSL contains a monophenol, cardanol (I) and a dihydric phenol, cardol (II), as major constituents and small amounts of anacardic acid (III). Analysis of these three materials has shown them to contain both saturated and unsaturated compounds. By careful chromatographic and degradation studies of the methyl ether of cardanol, SYMES<sup>1</sup> has shown in it, the presence of 3-pentadecylanisole, 3-(pentadecenyl-8')-anisole, 3-(pentadecadienyl-8',11')-anisole and 3-(pentadecatrienyl-8',11',14')-anisole.



It is likely that some structural changes might take place during the formation of the methyl ether of cardanol. Therefore, it was felt that separations based on cardanol itself would yield useful results. This paper describes the separation and identification of cardanol and cardol fractions using paper, thin-layer and column chromatographic methods. Separation of cardanol according to the degree of unsaturation was achieved by chromatography on a silica gel-silver nitrate column<sup>2,3</sup>. The separated fractions were identified by determining the analytical constants, such as iodine and hydrogen absorption values. Infrared spectra of the compounds helped in assigning structures for individual components. The column separations were monitored by paper and thin-layer chromatographic procedures.

## MATERIALS

(i) Technical cardanol containing a small quantity (less than 5%) of cardol was obtained by the rapid vacuum distillation of CNSL using the procedure of MURTHY *et al.*<sup>4</sup>.

\* Taken in part from the Ph.D. Thesis of B. G. K. MURTHY, submitted to the Osmania University, Hyderabad, India, in 1965.

(ii) Cardol and anacardic acid were prepared by the lead salt method, using the procedure of BACKER AND HAACK<sup>5</sup>, from solvent (commercial hexane) extracted CNSL obtained from the raw shells in the laboratory.

(iii) 3-Pentadecyl-phenol was obtained by the hydrogenation of cardanol in a medium pressure Parr hydrogenator using Pd/C (10%) catalyst according to the method of WASSERMAN AND DAWSON<sup>6</sup>.

## METHODS

### *Paper chromatography*

Preliminary trials for separating technical cardanol and commercial CNSL into their components were made by a paper chromatographic technique using 2,040 chromatographic paper strips (Selecta, Schleicher and Schüll). About 10  $\mu\text{g}$  of the material in chloroform solution were spotted on the paper strips leaving a margin of about 3 mm from the sides. The chromatogram was developed in about 8 h using the solvent system petroleum ether (40°–60°)—ethyl ether (1:1, v/v), and the solvent front was allowed to move about 20–25 cm from the base line. The papers were dried and the spots were developed by spraying a solution of 5% phospho-tungstomolybdic acid in dilute hydrochloric acid solution and exposing the paper to ammonia vapour.

In a separate experiment using reversed-phase chromatography, the resolution of the technical cardanol and commercial CNSL into their components was effected on papers impregnated with a 10% solution of paraffin oil in benzene and developed with 80% aqueous acetic acid using the descending technique. 3-Pentadecyl-phenol (m.p. 51.5°) was used as a reference compound.

### *Thin-layer chromatography*

The separation of pure cardanol and cardol from technical cardanol was carried out using the following TLC technique:

Glass plates (20 × 20 cm) were coated with a well stirred slurry of Silica Gel G (E. Merck, Darmstadt, 30 g in 60 ml of water) using a Desaga thin-layer applicator to give a 250  $\mu$  thick layer as described by MANGOLD<sup>7</sup>. The plates were activated by heating at 110° for 30 min and preserved in a desiccator. A chloroform solution of the sample (about 10  $\mu\text{g}$ , 1%) was spotted on the TLC plate using a micropipette. A solvent mixture of benzene–ethyl acetate (95:5, v/v) was used for the separation of the components and yellow spots were obtained by spraying with diazotised sulphonic acid in 2% sodium bicarbonate solution.

### *Silica gel–silver nitrate thin-layer chromatography*

The separation of pure cardanol into its components was carried out by silica gel–silver nitrate TLC. The silica gel plate prepared as above was impregnated by spraying with 10 ml of a 6.25% solution of silver nitrate in aqueous ethanol (1:1, v/v). It was kept at room temperature for 5 min and activated at 110° and preserved in a desiccator covered with black paper. The material was spotted as described above in chloroform solution. The chromatographic plate was developed in about 40 min using a solvent system of benzene–ethyl acetate (85:15, v/v). The spots were obtained by spraying the plate with concentrated sulphuric acid and charring.

### *Column chromatography*

Having developed a method for following the separation of components of technical cardanol by TLC, attempts were made to isolate the pure cardanol (monohydric component) using a silica gel column as described below.

The adsorbent column was prepared by pouring a slurry of silica gel (50 g, 100–200 mesh) in petroleum ether (40–60°) into a glass column (2 cm diam., 200 cm long).

A sample of technical cardanol (1.0 g) dissolved in 5 ml petroleum ether (40–60°) was introduced into the column. The column was developed successively with the following solvent systems: (1) petroleum ether, (2) benzene; (3) benzene containing 3% (v/v) ethyl acetate; (4) benzene containing 20% (v/v) ethyl acetate.

20 ml fractions of eluant were collected and the solvents were removed by heating over a water bath under reduced pressure (20–30 mm Hg). The column separations were monitored by TLC as described earlier. A change in the solvent system was made only when no material was detected in the last fraction when monitored by TLC.

### *Silica gel–silver nitrate column chromatography*

After isolation of pure cardanol from technical cardanol, attempts were made to separate its constituents by silica gel–silver nitrate column chromatography and following the separation by TLC. The column was prepared following the directions of DE VRIES<sup>8</sup>. Silica gel (100 g, 100–200 mesh) was suspended in a 50% aqueous solution of silver nitrate (200 ml). The mixture was stirred well and heated at 100° for 3 min. After cooling it was filtered through a Buchner funnel. The impregnated material was dried at 120°. The silver nitrate impregnated silica gel (30 g) was made into a slurry with benzene (50 ml) and transferred to a glass column (1.5 cm × 150 cm) with the addition of 100 ml of benzene. The material was packed to a height of about 20 cm. The column was protected from light by wrapping it in black paper.

A pure sample of cardanol (113 mg) dissolved in a small amount of benzene was introduced on to the column and the rate of flow of the eluant was adjusted to between 0.5 ml and 0.75 ml/min. Successive elutions were carried with 5, 10 and 20% solutions of ethyl acetate in benzene (v/v).

10 ml fractions of the eluant were collected in 24 ml weighed flasks. The solvent was evaporated over a water bath in an inert atmosphere of nitrogen. The weight of the material obtained in each of the fractions was determined. When no material was present in the eluant fraction as indicated by TLC, the solvent was drained off and further elution was continued with the next solvent system.

### *Determination of olefinic unsaturation*

Iodine values were determined by the modified ROSENKUND AND KUHNHENN method<sup>8</sup>. Hydrogen values were obtained<sup>9</sup> by hydrogenation of the samples in a small scale hydrogenator, using Pd-C (10%) as catalyst and glacial acetic acid as solvent.

*Infrared spectra.* For assignment of structures to the different components of cardanol their infrared spectra were taken. Spectral grade carbon tetrachloride was used as solvent and the infrared spectra were recorded with a Perkin Elmer Model 221 Spectrophotometer using a sodium chloride prism

## RESULTS AND DISCUSSION

*Preliminary trials by paper chromatography*

Preliminary trials for separating technical cardanol and commercial CNSL by paper chromatography showed that both materials gave two spots. Based on considerations of polarity, the lower one was considered to be cardol and the one which moved close to the solvent front, cardanol.

When these two materials were spotted on the paraffin oil impregnated paper they gave five definite spots (A, B, C, D and E, Fig. 1). The  $R_F \times 100$  values are given in Table I. Spot A, which had the lowest  $R_F$  value obtained both with technical cardanol and commercial CNSL, corresponded to that of 3-pentadecyl phenol. The other spots (B, C and D) with increasing  $R_F$  values may be considered to be due to mono-, di- and tri-olefinic components of cardanol and CNSL, respectively. Spot E, which had the highest  $R_F$  value, may be attributed to the dihydric component, cardol. A small portion, probably a polymerised fraction of CNSL, did not move from the base line.

TABLE I

SEPARATION OF TECHNICAL CARDANOL AND COMMERCIAL CNSL ON PARAFFIN OIL IMPREGNATED PAPERS

No.	Material	$R_F \times 100$				
		Spot A	Spot B	Spot C	Spot D	Spot E
1	Technical cardanol	28	43	55	66	90
2	Commercial CNSL	29	44	58	69	92
3	3-Pentadecyl-phenol	28	—	—	—	—

*Isolation of pure samples of cardanol and cardol from technical cardanol by column chromatography*

The TLC technique for the separation of pure cardanol and cardol from technical cardanol was employed to monitor their separation by column chromatography. Technical cardanol, commercial CNSL and solvent-extracted CNSL samples were spotted on the TLC plates. Samples of 3-pentadecyl-phenol, anacardic acid, and cardol were employed as reference compounds. The  $R_F$  values of the spots (Fig. 2) are given in Table II. The spots with lower  $R_F$  values which correspond to that of cardol (sample 6, Fig. 2) appeared brighter with higher colour intensity than the spots with high  $R_F$  values, which may be attributed to its dihydric nature. Technical cardanol (sample 1, Fig. 2) gave three spots which have the same  $R_F$  values as those obtained from commercial CNSL (sample 2, Fig. 2). 3-Pentadecyl-phenol (sample 4, Fig. 2) had the maximum  $R_F$  value and the spots with  $R_F$  values nearly the same were considered to be those of monohydric components. Anacardic acid (sample 5, Fig. 2) remained at the base line in the form of a streak because of its highly polar nature due to the carboxylic group. This kind of streaking due to anacardic acid is also noticed in solvent-extracted CNSL which gave pronounced streaking at the base line (sample 3, Fig. 2). With commercial CNSL the streaking was very faint, and can

TABLE II

SEPARATION OF TECHNICAL CARDANOL, COMMERCIAL AND SOLVENT EXTRACTED SAMPLES OF CNSL INTO THEIR COMPONENTS BY TLC.

No.	Material	$R_F \times 100$			Remarks
		Lower spot	Middle spot	Higher spot	
1	Technical cardanol	10	47	70	—
2	Commercial CNSL	9	47	70	—
3	Solvent-extracted CNSL	Oval-shaped streak	46	69	The lowest spot is oval shaped and did not move from the base line
4	3-Pentadecyl-phenol	—	Faint	70	—
5	Anacardic acid	Oval-shaped streak	—	—	The product did not move from the base line
6	Cardol	10	Faint	70	—

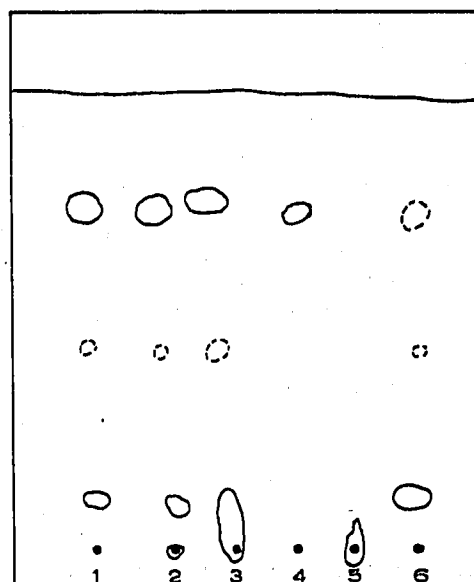
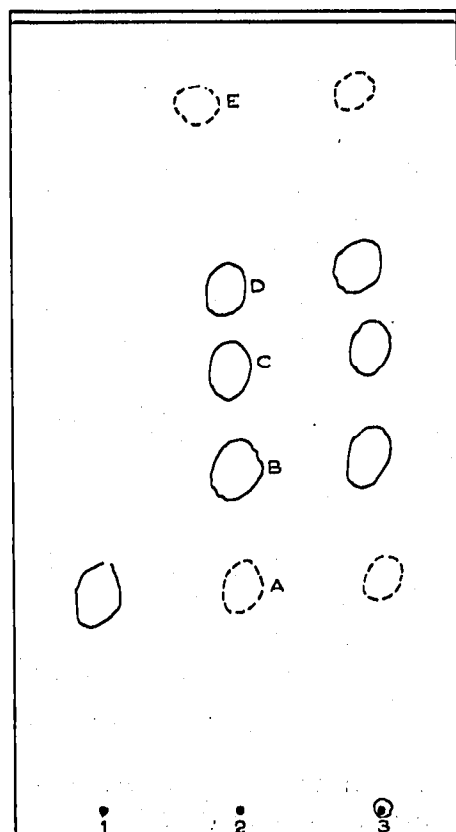


Fig. 1. Reversed-phase paper chromatography of technical cardanol and CNSL. 1 = 3-Pentadecyl phenol; 2 = cardanol (technical); 3 = commercial CNSL.

Fig. 2. Silica gel TLC of CNSL and its components. 1 = Cardanol (technical); 2 = commercial CNSL; 3 = solvent extracted CNSL; 4 = 3-pentadecyl-phenol; 5 = anacardic acid; 6 = cardol.

be attributed to the presence of some polymerised matter. With all the test samples 1-3, a faint yellow middle spot appeared at about  $R_F$  46, which may be due to the presence of small amounts of some phenolic component other than cardanol and cardol.

Having standardised a TLC technique for monitoring the separation of components, attempts were made to isolate pure cardanol by column chromatography using silica gel as described earlier. The results given in Table III show that the monohydric component comprising 94.6 % has been completely eluted with 250 ml benzene. Likewise, the unknown phenolic fraction (1.2 %, giving the middle spot on TLC) and the cardol fraction (dihydric component, 3.8 %) were obtained in 100 ml and 200 ml of 3 % and 20 % ethyl acetate in benzene solutions, respectively.

The monohydroxy component of technical cardanol thus isolated with benzene alone, had a hydroxyl value of 186.7 (calc. 187.0), refractive index  $n_D^{20}$  of 1.050 and an iodine value ( $RK$ ) of 390.8 and corresponded to pure cardanol.

TABLE III

ISOLATION OF PURE CARDANOL AND CARDOL FROM TECHNICAL CARDANOL BY COLUMN CHROMATOGRAPHY

No.	Eluant	Eluant collected (ml)	Material as identified by TLC	Yield of the material (%)
1	Petroleum ether (40-60°)	50	Nil	Nil
2	Benzene	250	Cardanol(mono-hydric component)	94.6
3	Ethyl acetate in benzene (3 %)	100	Unknown compound (giving middle spot)	1.2
4	Ethyl acetate in benzene (20%)	200	Cardol (dihydric component)	3.8

#### *Isolation of the different components of the cardanol fraction by silica gel-silver nitrate column chromatography*

Before isolating the components of pure cardanol using a silica gel-silver nitrate column, it was first resolved on silver nitrate impregnated silica gel TLC plates. A pure sample of 3-pentadecyl-phenol was also used for reference purposes. Pure cardanol gave four spots (Fig. 3) of which spot A with the highest  $R_F$  value of 90 corresponded to that given by 3-pentadecyl-phenol. The use of silver nitrate as impregnant for silica gel results in the formation of loose  $\pi$ -complexes<sup>10,11</sup> with unsaturated compounds. Materials with higher unsaturation are known to be retained to a greater extent on silver nitrate-silica gel adsorbent. It may therefore be reasonably assumed that spots B, C and D, which have  $R_F$  values in the descending order (81, 64 and 28), correspond to the mono-, di-, and tri-olefinic components of cardanol, respectively.

For the sake of comparison, technical cardanol and commercial CNSL were spotted on a silver nitrate-silica gel plate using the same solvent system. Both these compounds resolved into four components each. The  $R_F$  values of the four spots obtained with cardanol corresponded to those of the four spots of commercial CNSL, respectively (Fig. 4). A faint spot on the base line was obtained with cardanol, which most probably is due to the dihydric component, cardol. The spot is more

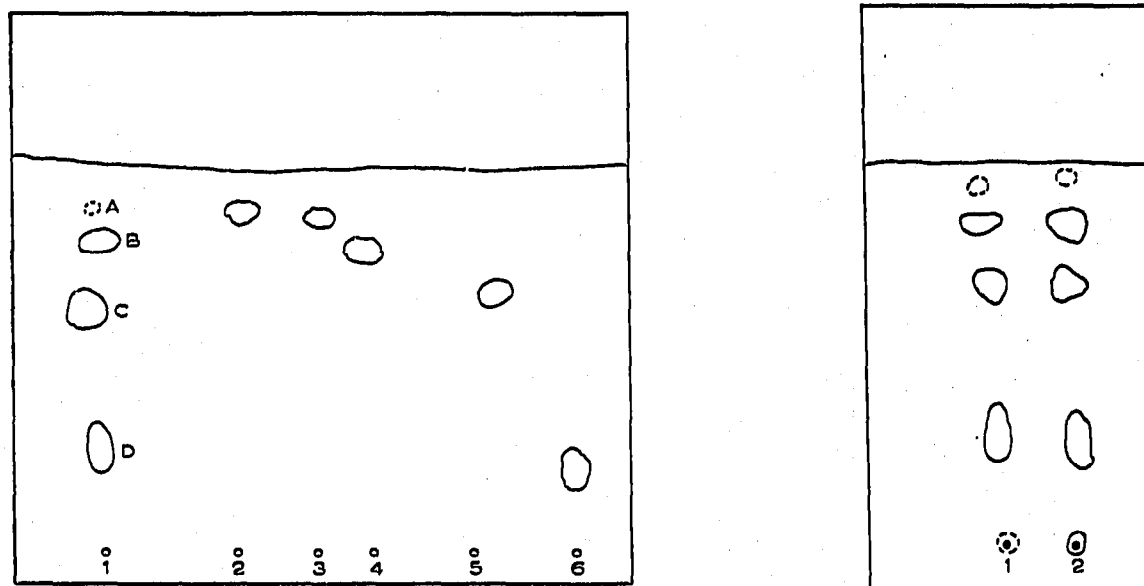


Fig. 3. Silica gel-silver nitrate TLC of pure cardanol and its components (components 3 to 6 were separated by silica gel-silver nitrate column chromatography). 1 = Cardanol (pure); 2 = 3-pentadecyl-phenol; 3 = saturated component of cardanol; 4 = mono-olefinic component of cardanol; 5 = di-olefinic component of cardanol; 6 = tri-olefinic component of cardanol.

Fig. 4. Silica gel-silver nitrate TLC of technical cardanol and commercial CNSL. 1 = Cardanol (technical); 2 = CNSL commercial.

predominant in the case of commercial CNSL as it contains polymerised material besides cardol.

The components of pure cardanol were isolated using the silica gel-silver nitrate column and monitoring the separations by silica gel-silver nitrate TLC. The separations are shown diagrammatically in Fig. 5. The results indicate that pure cardanol (iodine value (*RK*) 390.8, olefinic unsaturation 1.73), consists of the four components as given in Table IV. It is seen from the above results that the total weight of the four components (113 mg) is in good agreement with the weight of the four components taken (113 mg), leading to the conclusion that no other material is present except the above four components.

TABLE IV

ISOLATION OF THE COMPONENTS OF CARDANOL BY CHROMATOGRAPHY ON A SILICA GEL-SILVER NITRATE COLUMN

No.	Eluant	Wt. of the material (g)	Weight (%)	Probable compound	Double bond value
1	Benzene	0.0057	5.4	Saturated	—
2	Ethyl acetate in benzene (5%)	0.0534	48.5	Mono-olefin	0.485
3	Ethyl acetate in benzene (10%)	0.0178	16.8	Di-olefin	0.336
4	Ethyl acetate in benzene (20%)	0.0361	29.3	Tri-olefin	0.879
		0.1130	100.0		1.700

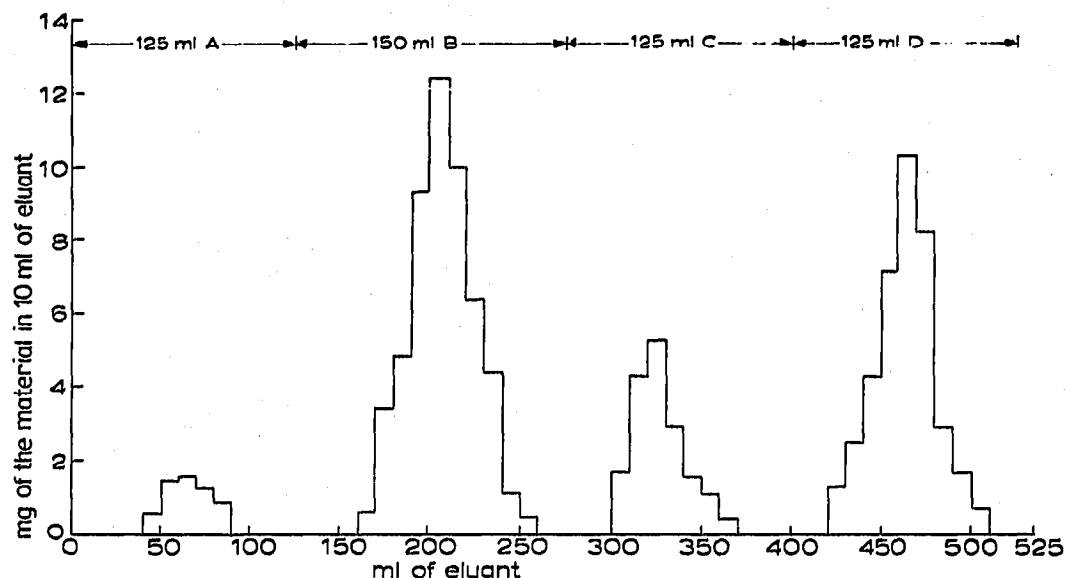


Fig. 5. Quantitative separation of the components of pure cardanol on a silica gel-silver nitrate column.

#### Characterisation of the components of cardanol

The results of hydrogenation of cardanol and its components are given in Table V.

From the results it may be seen that, as expected, the saturated component has not absorbed any hydrogen. The identity of the other three components as mono-, di-, and tri-olefins is seen to be definitely established by consideration of their double bond values as obtained by the hydrogenation method and reported in the last column of Table V.

#### Infrared studies

Samples of the four components of cardanol *viz.*, saturated, mono-, di-, and tri-olefins were taken for study. For the sake of comparison, studies were also made on other related phenolic compounds; simple phenol, 3-pentadecyl-phenol and cyclo-

TABLE V

#### HYDROGENATION DATA ON CARDANOL AND ITS COMPONENTS

No.	Component of cardanol	Wt. of the material taken (mg)	Hydrogen absorbed at N.T.P. (ml)	Theoretical mol.wt. of component	Moles of H <sub>2</sub> absorbed/mole of the compound	No. of double bonds per mole of the compound
1	Saturated	14.80	Nil	304	Nil	Nil
2	Mono-olefinic	15.40	1.193	302	1.03	One
3	Di-olefinic	17.75	2.705	300	2.02	Two
4	Tri-olefinic	12.30	2.664	298	2.90	Three
5	Cardanol	21.64	1.400	300 (Average)	1.73	1.70 (Average)



hexanol, and completely hydrogenated cardanol (*i.e.* the hydrogenated product of 3-pentadecyl-phenol using Raney Nickel catalyst at 200° and 500 p.s.i.g.). Infrared spectra of the mono- and di-hydric components and the unknown middle fractions have also been taken.

All the spectra indicate a common general pattern with the exception of a few characteristic bands. The absorption bands have all been assigned<sup>12</sup> to different characteristic groups which revealed the nature of the compound and confirmed the earlier findings.

Some interesting features were noticed while comparing the spectra of the above materials:

(1) Among the mono-, di- and tri-olefinic components of cardanol, only the last-mentioned compound is characterised by the presence of a vinyl type of double bond as indicated by a strong absorption band at 900  $\text{cm}^{-1}$ .

(2) Unlike the mono-, di-, and tri-olefinic components, 3-pentadecyl-phenol showed absolutely no absorption at 1700  $\text{cm}^{-1}$  suggesting the absence of olefinic unsaturation. However, in common with the other components, it exhibits the band at 1580  $\text{cm}^{-1}$  characteristic of the phenyl group.

(3) The spectra of the hydrogenated 3-pentadecyl-phenol indicated the absence of the phenyl nucleus (absence of absorption at 1580  $\text{cm}^{-1}$  and 3015  $\text{cm}^{-1}$ ). The lower intensity of the 3610  $\text{cm}^{-1}$  band indicates the loss of hydroxyl to some extent during hydrogenation. Similar observations have been made with cyclohexanol.

(4) In a comparison of the spectra of cardanol and cardol it is seen that the 1140  $\text{cm}^{-1}$  band is very broad in cardol, signifying that the C-OH stretching frequency of the phenol is stronger, due to the presence of the two —OH groups.

(5) Comparison of the spectra of the middle unknown fraction of technical cardanol with cardol showed considerable similarity.

After this manuscript was prepared for publication a short note entitled "The composition of cashew-nut shell liquid CNSL and the detection of a novel phenolic ingredient" by J. H. P. TYMAN AND L. J. MORRIS, appeared in *J. Chromatog.*, 27 (1967) 287. Earlier, a brief report of our work was published in the *Annual Report of the Regional Research Laboratory, Hyderabad, India, for the year 1964-1965*.

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#### SUMMARY

Pure samples of the mono- and di-hydric components, *viz.* cardanol and cardol, have been separated from technical cardanol using silica gel thin-layer chromatography and silica gel column chromatography. The presence, in small amounts, of a third phenolic component in addition to cardanol and cardol has been observed. Cardanol, the main constituent of technical cardanol, has been successfully separated into four components, *viz.* saturated, mono-, di-, and tri-olefins using silver nitrate

impregnated silica gel, thin-layer and column chromatographic techniques. The four components have been characterised as saturated, mono-, di-, and tri-olefins by consideration of their properties and their hydrogenation values. Further elucidation of their structures has been made by interpretation of their infrared spectra. The presence of a vinyl double bond in the tri-olefins has been confirmed.

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