Efficient Medium-Scale Chromatographic Group Separation of Anacardic Acids from Solvent-Extracted Cashew Nut (*Anacardium occidentale*) Shell Liquid

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Biologically active anacardic acids as a group have been separated from the toxic phenolic constituents (cardols and cardanols) of the solvent-extracted cashew nut shell liquid (CNSL) by a novel two-stage chromatographic method. Advantage was taken of the difference in acidity of the two groups of compounds, namely, the carboxylic acids and the phenolics, and the ability of silica gel to selectively retain amines and amine salts. CNSL (1 part) was loaded onto a silica gel bed (5 parts), prepared in a solvent system comprising ethyl acetate–hexane (25:75) and an organic base (triethylamine, 0.5%). Continued irrigation of the bed with the same eluent resulted in the elution of all the non-acid components. The anacardic acids were subsequently eluted by changing the basic eluent to acidic by replacing the triethylamine with acetic acid (1%). This procedure was shown to be of general applicability for group separation of carboxylic acids.

Keywords: *Anacardic acids; cardols; cardanols; chromatographic separation; cashew nut shell liquid; Anacardium occidentale; carboxylic acids; group separation*

INTRODUCTION

The cashew nut kernel (*Anacardium occidentale* L.) is a well-known and highly prized food material. The nut comprises the kernel of nutritive value, enclosed in a hard shell. The shell contains about 30% by weight of long-chain (pentadecyl) phenols and phenolic acids, based on phenol, resorcinol, and salicylic acid; the structures of 16 of the major constituents are given in Figure 1 (Kubo et al., 1986). The cashew nut shell liquid (CNSL), which is a byproduct of the cashew nut processing industry, is obtained as a dark brown, viscous, vesicant liquid and is produced to the extent of 75 000 tonnes in India annually. Other major producers of the cashew nut are Tanzania, Brazil, Mozambique, and Kenya. Two distinct commercial types of CNSL are available: (A) the hot-processed CNSL, which oozes out of the shells during roasting of the nuts for separation of the kernels, and (B) the cold-processed CNSL, obtained by solvent extraction of the cashew nut shells. The major components of the hot-processed CNSL (A) are cardanols (Figure 1, **1**–**4**; 60–70%) and cardols (Figure 1, **5**–**8**; 20–25%) with minor quantities of 2-methylcardols (Figure 1, **9**–**12**). The cardols and cardanols are separable by distillation, and cardanols find several applications in the form of phenol–formaldehyde resins in varnishes, paints, and brake linings (Tyman, 1980). The major component of the cold-processed CNSL (B) are anacardic acids (Figure 1, **13**–**16**; 60–70%) and cardols (20–25%). While the cardols are known to be toxic and cause contact dermatitis, anacardic acids have a variety of uses; for example, they are known to be potent molluscicides and may be used for control of the snail population and thus, indirectly, for control of schistosomiasis (Kubo et al., 1986). They are also inhibitors of medicinally important enzymes such as prostaglandin synthase (Grazzini et al., 1991), lipoxygenase (Shobha et al., 1994), and tyrosinase (Kubo et al., 1994a); they also exhibit antitumor (Kubo et al., 1993a), antimicrobial (Kubo et al., 1993b), and antiacne (Kubo et al., 1994b) properties. A clean-cut separation method, capable of large-scale application, is therefore required, particularly in view of the known toxicity and allergenic properties of the cardols and the usefulness of the anacardic acids.

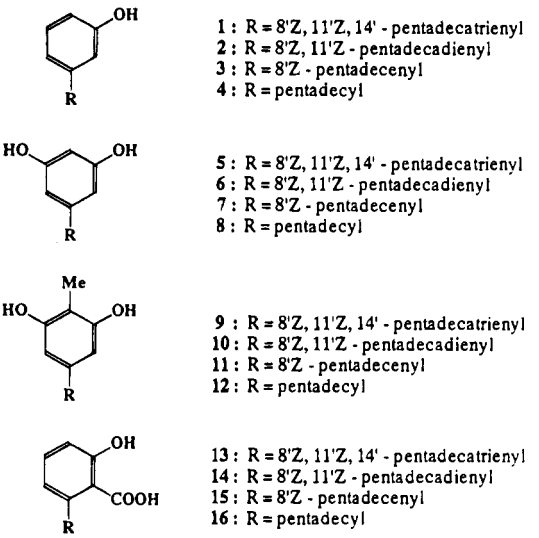

Figure 1. Structures of the major constituents of CNSL.

Anacardic acids are thermolabile and decompose on heating to cardanols and carbon dioxide and, therefore, distillation methods cannot be used (Tyman et al., 1989). Traditional chromatographic methods (Kubo et al., 1986), useful in the laboratory for separation of anacardic acids and cardols, are too time-consuming and expensive for use on a large scale. The CNSL components are highly hydrophobic and, thus, aqueous solvent systems, reversed-phase chromatography, and ion-exchange chromatography also are not useful. The

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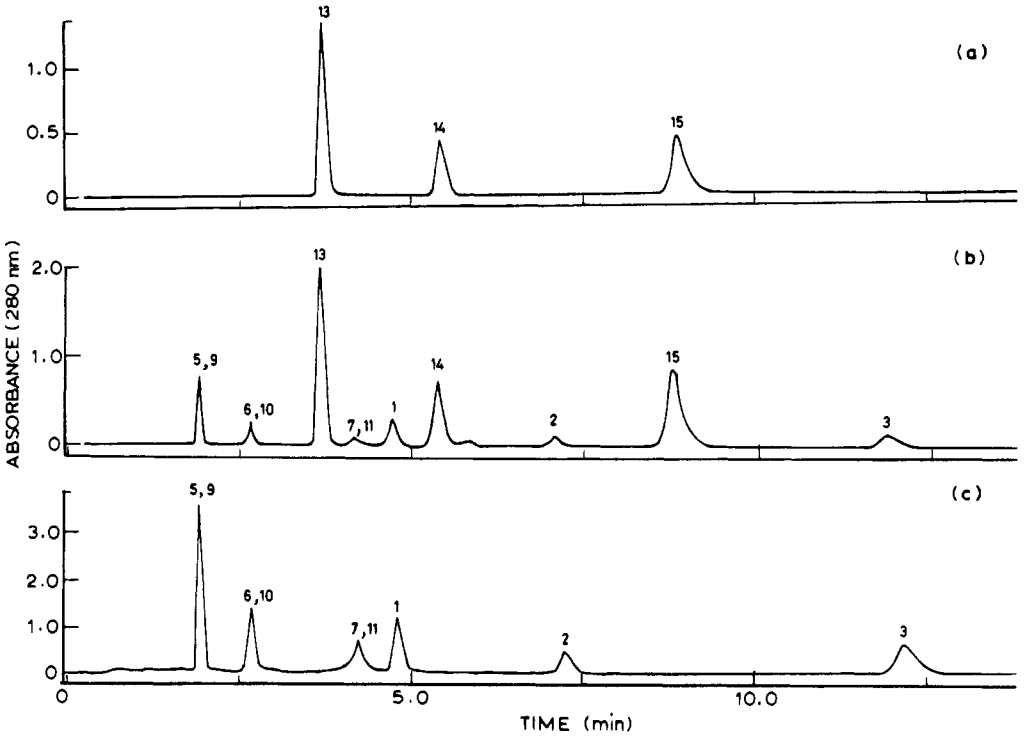

Figure 2. HPLC of (a) anacardic acid fraction, (b) solvent-extracted CNSL, and (c) non-acid fraction. For identity of peak numbers, see Figure 1.

practical separation method is thus required to be based on the use of nonpolar organic solvents and, thus, normal-phase chromatography. We now describe an efficient and convenient chromatographic method to achieve the above objective on a preparative scale (100 g of CNSL). The method is also amenable to large-scale application.

MATERIALS AND METHODS

Solvent-Extracted CNSL. This was obtained by extraction of raw cashew shells, as described earlier (Kubo et al., 1986).

Chemicals and Solvents. Silica gel for column chromatography (100–200 mesh) was obtained from Acme Chemical Co., Bombay (India). Solvents and chemicals were purchased from BDH, Bombay (India).

High-Performance Liquid Chromatography. A modular HPLC instrument comprising two 510 reciprocating pumps, a 481 variable-wavelength detector, and a U6K injector, all from Waters Associates, Milford, MA, was used for analytical chromatography [column, Novapak C_{18} (4 mm × 150 mm); mobile phase, 80% aqueous acetonitrile (v/v) containing 1% acetic acid at a flow of 1.8 mL/min; detection, UV at 280 and 310 nm for phenols and phenolic acids, respectively].

Separation of Anacardic Acids from CNSL. A column (10 cm diameter) of silica gel was prepared by slurrying the gel (500 g) in 1.5 L of hexane, containing 30 mL of triethylamine. A mixture of 100 g of CNSL, 30 mL of triethylamine, and 100 mL of hexane was loaded onto the column, using a separatory funnel as the delivery system, and the column was irrigated with 4 L of ethyl acetate–hexane mixture (25:75 v/v), containing 0.5% triethylamine. The column effluent was evaporated on a rotary vacuum evaporator, yielding 27 g of phenols, consisting essentially of cardols (Figure 1, **5**–**8**) and 2-methylcardols (**9**–**12**). The column was then irrigated with 1.5 L of ethyl aetate–hexane mixture (25:75 v/v), containing 1% acetic acid. The effluent on evaporation as above yielded 70 g of the product consisting of anacardic acids (**13**–**15**) in the approximate ratio of 40:20:40, along with traces of saturated anacardic acid (**16**). The identity of the compounds was confirmed by HPLC and comparison with standard samples (Shobha and Ravindranath, 1991).

Group Separation of Carboxylic Acids from Synthetic Mixtures. A column (2 cm diameter) of silica gel (30 g) was prepared as above in hexane containing about 3.2% triethylamine. A mixture of benzoic acid, cinnamic acid, phenylacetic acid, acetophenone, benzyl alcohol, and phenethyl acetate (1 g each) in hexane–triethylamine (10:3, 13 mL) was loaded onto the column and the column irrigated with 125 mL of ethyl acetate–hexane (25:75 v/v) containing 0.5% triethylamine. The effluent on evaporation gave a 1:1:1 mixture of the neutral compounds (2.89 g; 96%). The column was then irrigated with 150 mL of ethyl acetate–hexane mixture (25:75 v/v) containing 1% acetic acid, yielding, on evaporation, the mixture of the three carboxylic acids (2.91 g; 97%).

RESULTS AND DISCUSSION

The present method is based on the facts that (a) silica gel is acidic and can strongly retain basic substances such as amines as well as amine salts; (b) the anacardic acids, being relatively more acidic, selectively form salts with weakly basic amines; and (c) cardols and cardanols, being phenolic and weakly acidic, are unretained on weakly basic surfaces. After a series of experiments different solvent, acid, and base combinations and solute to sorbent ratios, the following method has been developed.

A bed of silica gel was first modified by irrigation with a nonpolar solvent containing an organic-solvent-soluble base, preferably an amine (0.1–5%). Among the various bases studied, namely, ammonia, a primary (*n*-butylamine) or tertiary amine (triethylamine), and aromatic amines (e.g., pyridine), triethylamine was the most effective. CNSL, treated with an equivalent quantity of the base, was then loaded onto the bed and irrigated with a medium polar organic solvent mixture containing the base (0.1–5%). CNSL to silica gel ratios of 1:1 to 1:20 were examined, but a ratio of 1:5 was found to be adequate. The organic solvent was chosen to have sufficient polarity or strength to elute cardols and other phenols from the column. A combination of ethyl

acetate and hexane in the ratio of 25:75 (v/v) was found to be optimum; a mixture of hexane and acetone (85:15) could also be used but less effectively. Halogenated solvents were unsuitable.

After elution of the cardols and other phenols, the column was irrigated with suitable organic solvent or solvent mixture (ethyl acetate and hexane, 25:75), containing an organic-solvent-miscible acid such as acetic acid to obtain the anacardic acids in good yield (70–75%). Formic acid could also be used with comparable efficiency. The HPLC of the product showed that it was totally devoid of the phenols. Figure 2 shows the HPLC traces of (a) total cold-processed (solvent-extracted) CNSL and (b) the anacardic acid fraction obtained according to the present method.

In principle, the above described method may be used not only for the separation of anacardic acids from CNSL but also for carboxylic acids in general from plant extracts as well as synthetic mixtures containing carboxylic acids. This was demonstrated when a mixture of carboxylic acids, alcohols, esters, and aldehydes was separated using the same principle, yielding 96–97% recovery of both groups of compounds, namely, the carboxylic acids and the neutral compounds. Low silica gel to solute ratio, rapid operation, low solvent consumption, and nearly complete recovery of both groups of compounds are the advantages of the method.

A notable feature of the above developed method, particularly in relation to its large-scale application, is the use of the same organic solvent composition (namely, ethyl acetate and hexane in 25:75 (v/v) ratio) throughout the separation process to facilitate easy recovery and recycling of the solvent(s).

NOMENCLATURE

Anacardic acid, cardol, and cardanol are the common names of 2-hydroxy-6-pentadecylbenzoic acid, 5-pentadecylresorcinol, and 3-pentadecylphenol, respectively. The side-chain nomenclature of the unsaturated analogues that occur in the cashew nut shell are given in Figure 1. They may also be designated, for example, cardanol-8′(*Z*),11′(*Z*),14-triene (**1**); see also Shobha and Ravindranath (1991).

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