Table III. Molecular Weight Distribution of Peanut Skin Polymeric Procyanidins

	GPC			
	M_n	M_w	VPO M_n	dispersivity[a]
flaked skins				
1-stage purifcn	1803	3407	2206	1.70
2-stage purifcn	2123	3769	2013	1.82
pelletized skins				
1-stage purifcn	1701	3834	2176	1.98
2-stage purifcn	2120	3861	2284	1.75
average	1937	3718	2170	1.81

[a] Determined by average M_n by GPC and VPO and M_w by GPC. On the basis of a peracetate of a flavan unit (i.e. M_w = 500), this corresponds to a P_n of 3.9 and a P_w of 7.4. Because of the presence of some (4β→8;2β→O→7) linkages in the polymer, this underestimates the number of flavan units per number average of weight average chain.

were also determined by gel permeation chromatography and by vapor pressure osmometry of the peracetate derivatives. These analyses (Table III) showed that the molecular weights of peanut skin tannins are comparatively low and the molecular weight distributions is remarkably narrow in comparison with tannins from a broad spectrum of plants (Williams et al., 1982). Neither the elemental composition nor the molecular weight distribution was altered by a second purification of Sephadex LH-20. Furthermore, there was no significant difference in elemental composition or molecular weight distribution between water-soluble polymeric procyanidins isolated from the freshly prepared flaked or pelletized material.

Registry No. 1, 154-23-4; 2, 490-46-0; 3, 20315-25-7; 4, 103883-03-0; 5, 41743-41-3.

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Molluscicides from the Cashew *Anacardium occidentale* and Their Large-Scale Isolation

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A large-scale isolation of the constituents of the cashew shell oil (*Anacardium occidentale*) was performed by medium-pressure liquid chromatography. A series of 12 components was isolated, including derivatives of anacardic acid, cardol, 2-methylcardol, and cardanol that differ in their side chain. These were tested against *Biomphalaria glabratus*, one of the snail vectors of schistosomiasis, and their structure–activity relationship is discussed. The principal components in the shell oil, nuts, and fruit juice were also quantitatively determined by high-performance liquid chromatography.

INTRODUCTION

Schistosomiasis, a parasitic disease carried by snail vectors, is one of the largest of human health problems in the tropics. During the past decade, numerous schistosomiasis control projects in the Third World have shown that control of snail vectors by molluscicides can be a rapid and efficient means for reducing or eliminating the transmission of this disease.

In a previous report molluscicidal activity against the South American freshwater snail *Biomphalaria glabratus* was found in the hexane extract of cashew [*Anacardium occidentale* (Anacardiacea)] nut shells (Pereira and DeSouza, 1974). The principal components of the oil of cashew nut shells are derivatives of 6-pentadecylsalicyclic acid (anacardic acid), 5-pentadecylresorcinol (cardol), 2-methyl-5-pentadecylresorcinol (2-methylcardol), and 3-pentadecylphenol (cardonol), which differ in their type of C_{15} side chains: saturated, monoene, diene, and triene (Tyman, 1979). Among these components, anacardic acid was separated by high-performance liquid chromatography (HPLC) in relatively large amounts (200 mg) (Lloyd et al., 1980). The molluscicidal activity of these anacardic acid derivatives was quite strong (LC_{50}: triene, 0.35 ppm; diene, 0.9 ppm; monoene 1.4 ppm) (Sullivan et al., 1982). The other main constituents, cardol, 2-methylcardol, and cardanol, were separated quantitatively by HPLC (Tyman et al., 1981). However, the molluscicidal activity and large-scale separation of these phenolic compounds have not been examined.

This paper describes the efficient isolation of a series of 12 molluscicidal compounds in the oil of cashew nut shells by large-scale medium-pressure liquid chromatography and an analysis of the main components from the

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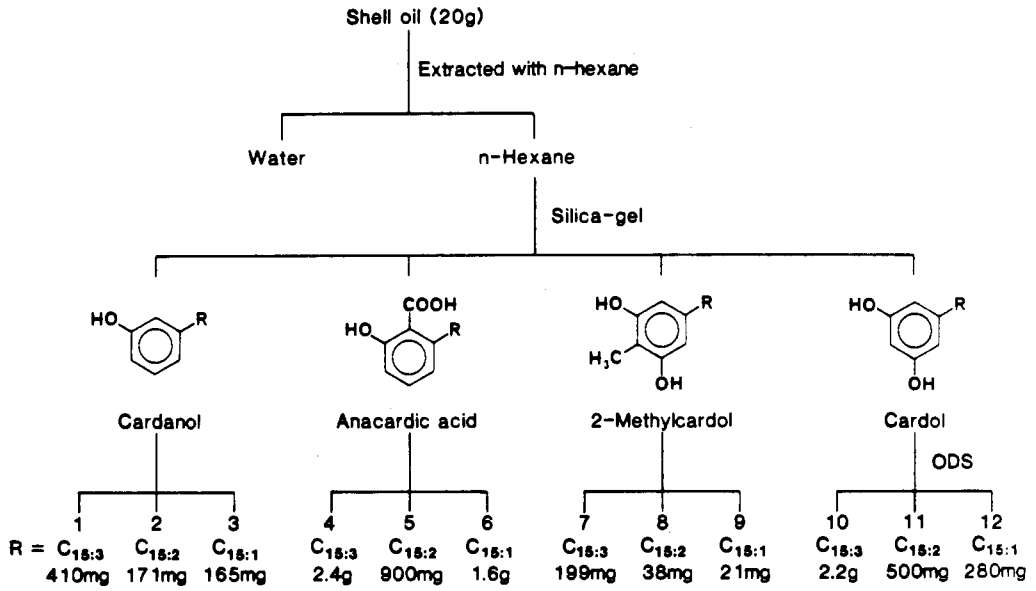

1: 3-[8(*Z*),11(*Z*),14-pentadecatrienyl]phenol
2: 3-[8(*Z*),11(*Z*)-pentadecadienyl]phenol
3: 3-[8(*Z*)-pentadecenyl]phenol
4: 6-[8(*Z*),11(*Z*),14-pentadecatrienyl]salicylic acid
5: 6-[8(*Z*),11(*Z*)-pentadecadienyl]salicylic acid
6: 6-[8(*Z*)-pentadecenyl]salicylic acid
7: 2-methyl-5-[8(*Z*),11(*Z*),14-pentadecatrienyl]resorcinol
8: 2-methyl-5-[8(*Z*),11(*Z*)-pentadecadienyl]resorcinol
9: 2-methyl-5-[8(*Z*)-pentadecenyl]resorcinol
10: 5-[8(*Z*),11(*Z*),14-pentadecatrienyl]resorcinol
11: 5-[8(*Z*),11(*Z*)-pentadecadienyl]resorcinol
12: 5-[8(*Z*)-pentadecenyl]resorcinol

Figure 1. Schematic diagram for the separation of the components of the cashew nut shell oil.

cashew shell oil, nuts, and fruit juice by HPLC.

The 12 compounds isolated were then tested for any molluscicidal activity against *B. glabratus*. Their structure–activity relationship is discussed.

EXPERIMENTAL SECTION

Plant Material. The cashew shell oil was collected in Bahia, Brazil. The cashew nuts were commercially available. The freeze-dried fruit juice was obtained from Bahia, Brazil.

HPLC. Analyses were carried out with a Rainin Rabbit HP solvent delivery system (Rainin Instrument, Woburn, MA) equipped with a Du Pont variable-wavelength ultraviolet (UV) spectrophotometer at 280 nm. A YMC pack ODS column (15 cm × 6 mm i.d.) (Yamamura Chemical, Kyoto, Japan) equipped with an Uptight precolumn (Upchurch Scientific, 2 cm × 2 mm i.d.) packed with pellicular Watman Co. Pell ODS was used. Samples were injected into the column with a Rheodyne rotary valve 7120 syringe-loading injector. The mobile phase used was methanol–10% aqueous acetic acid (90:10, v/v) at a flow rate of 1.0 mL/min. All solvents were of HPLC grade. The shell oil (3 mg) and freeze-dried fruit juice (500 mg) were extracted with *n*-hexane, and the hexane portion was evaporated to an oil. The residue was dissolved in the mobile phase (200 μL), and an aliquot (10 μL) was injected into HPLC. The nuts (10 g) were ground with *n*-hexane in a mortar and then filtered. The filtrate was evaporated and applied onto silica gel (open-column chromatography, 100 g) and eluted with *n*-hexane–ethyl acetate (9:1, v/v, 100 mL; 5:5, v/v, 50 mL). The *n*-hexane–ethyl acetate (5:5, v/v, 50 mL) fraction was evaporated to dryness. The residue was dissolved in the mobile phase (50 μL), and an aliquot (10 μL) was injected onto the HPLC system described above.

Isolation. The cashew nut shell oil (20 g) was applied to column chromatography on silica gel (Merck; 230–400 mesh, 300 g) and eluted with *n*-hexane–ethyl acetate–acetic acid (90:10:1, v/v/v, 1 L80:20:1, v/v/v, 1 L; 50:50:1; v/v/v, 1 L). Fractions (20 mL) were collected and monitored by thin-layer chromatography. Four major fractions containing derivatives of anacardic acid, cardanol, cardol, and 2-methylcardol were collected. Each fraction was then applied to medium-pressure column chromatography on a Pharmacia K column (100 cm × 26 cm i.d.) packed with ODS (40–63-μm particle size). The mobile phase used was methanol–10% aqueous acetic acid (88:12, v/v) and delivered with a Pharmacia Peristaltic Pump P-1 at 2 mL/min. The effluent was monitored by a Pharmacia UV-1 monitor at 280 nm. The isolation method is schematically illustrated in Figure 1. The identification of compounds was carried out by ^{1}H and ^{13}C NMR, mass, UV, and IR spectroscopy.

Molluscicidal Activity. Molluscicidal activity was monitored as previously described (Nakanishi and Kubo, 1977). Snails of uniform size (average diameter of the shell 12 mm) were placed, with two snails used at each concentration, into deionized water solutions containing known concentrations of the compounds.

RESULTS AND DISCUSSION

The gram-scale separation of the cashew nut shell oil was attempted with medium-pressure ODS column chromatography. The maximum sample capacity of a typical ODS preparative HPLC column (50 cm × 10 mm i.d.) is a few hundred milligrams. Gram separations are available on larger bore preparative HPLC columns. However, this method requires an extremely expensive column and an efficient high-volume solvent delivery system. In this case medium-pressure liquid chromatography, using an inex-

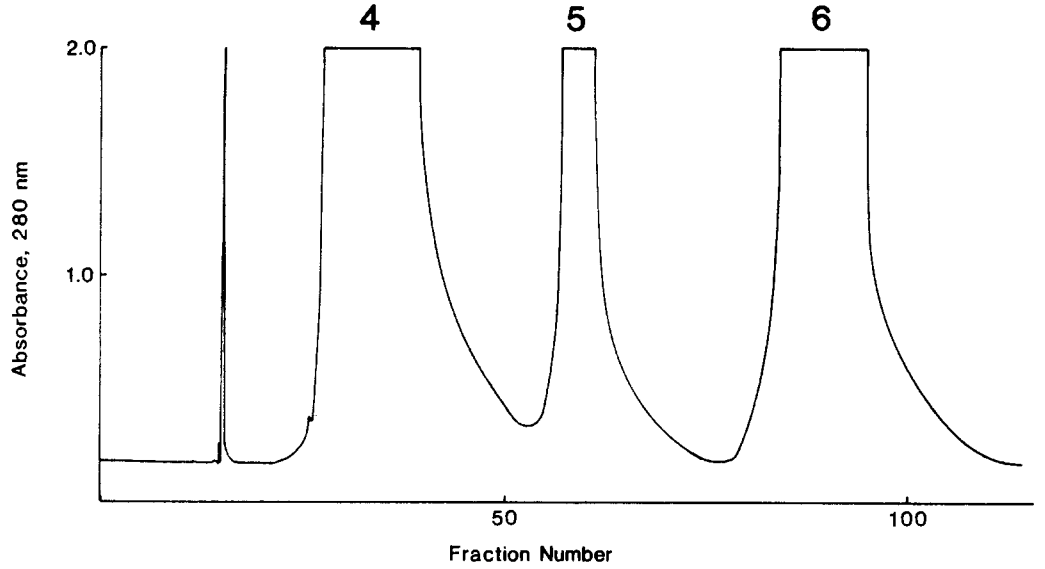

Figure 2. Medium-pressure liquid chromatogram of anacardic acids: column, Pharmacia K column (100 cm × 26 mm) packed with handmade 40-μm ODS; mobile phase, methanol–10% aqueous acetic acid (88:12), 2 mL/min; detection, UV 280 nm. Peak numbers are as in Figure 1.

Table I. Concentration of the Components in *A. occidentale*

constituents	R	shell oil, %	nuts, % × 10^{-5}	freeze-dried fruit juice, %
anacardic acid	$C_{15:3}$	17.50		0.02
	$C_{15:2}$	8.75		0.01
	$C_{15:1}$	13.13		0.02
cardol	$C_{15:3}$	15.75	39.0	
	$C_{15:2}$	4.38	9.0	
	$C_{15:1}$	1.31	2.5	
2-methylcardol	$C_{15:3}$	1.09	5.0	
	$C_{15:2}$	0.22	1.0	
	$C_{15:1}$	0.22	1.0	
cardanol	$C_{15:3}$	4.38		
	$C_{15:2}$	1.75		
	$C_{15:1}$	1.75		

pensive and commercially available glass column and 40–63-μm ODS packing material, offered an efficient large-scale separation at a much lower cost.

The oil of cashew nut shells (20 g) was partitioned between *n*-hexane and water. The *n*-hexane layer was evaporated, giving 17.5 g of an oily residue. The residue was chromatographed on silica gel into four principal groups: an anacardic acid type fraction (6.5 g); a cardanol type fraction (510 mg); a 2-methylcardol type fraction (980 mg); a cardol type fraction (3.3 g). Each group was applied to large-scale medium-pressure column chromatography using an ODS support. A typical chromtogram is shown in Figure 2. The final yields of these compounds from 20 g of shell oil are given in Figure 1.

Chromatograms from a quantitative study on the oil of the shell, nuts, and fruit juice are illustrated in Figure 3. The ratio of cardol and 2-methylcardol type compounds in the oil of the shell and in the nuts was almost the same. No anacardic acid and cardanol type compounds were found in the nuts. Only anacardic acid type compounds were found in the fruit juice. The results, given in percentages, are listed in Table I.

The LD_{50} values, the dose for 50% fatality due to these 12 compounds against the snail *B. glabratus*, were examined and are shown in Table II. The molluscicidal activity of anacardic acid had already been examined, and strong activity was reported (Sullivan et al., 1982). It had also been pointed out that the activity is increased in direct proportion to the degree of unsaturation in the alkyl side chain (Sullivan et al., 1982). Our study gave the same

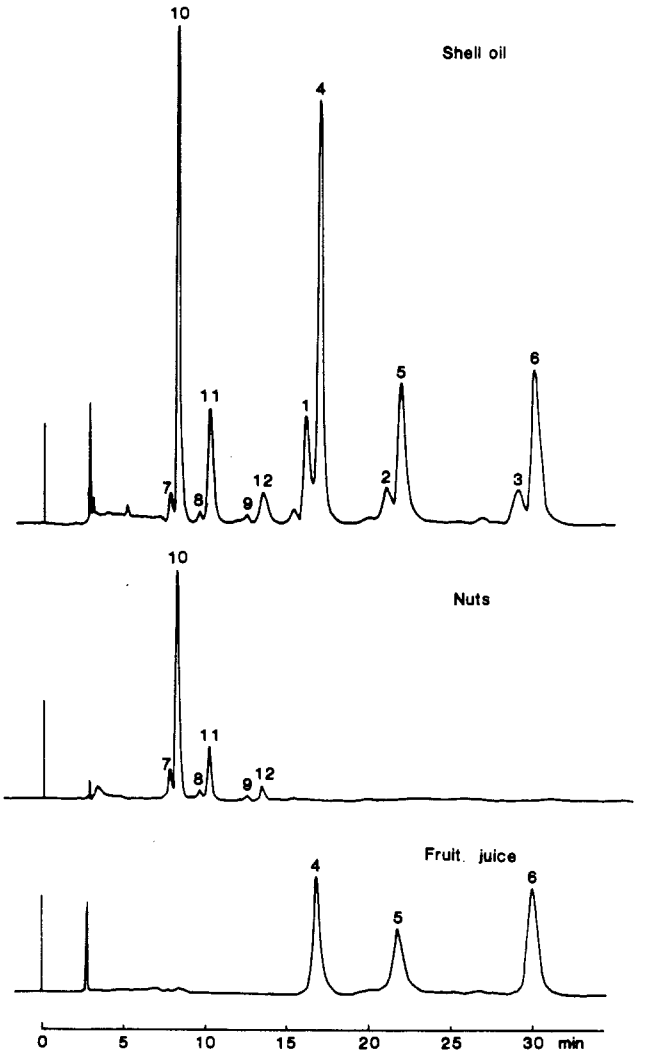

Figure 3. HPLC analyses of the components from the cashew shell oil, nuts, and fruit juice: column, YMC pack ODS (15 cm × 6 mm); mobile phase, methanol–10% aqueous acetic acid (90:10), 1 mL/min; detection, UV 280 nm. Peak numbers are as in Figure 1.

results concerning anacardic acid. However, in the cases of cardol and 2-methylcardol type compounds, the activity

Table II. LD_{50}[a] of the Components in Cashew Shell Oil against the Snail *B. glabratus*

constituents	R	LD_{50}, ppm	constituents	R	LD_{50}, ppm
anacardic acid	$C_{15:3}$	0.3	2-methylcardol	$C_{15:3}$	20
	$C_{15:2}$	0.6		$C_{15:2}$	15
	$C_{15:1}$	1.0		$C_{15:1}$	10
cardol	$C_{15:3}$	15	cardanol	$C_{15:3}$	80
	$C_{15:2}$	7		$C_{15:2}$	80
	$C_{15:1}$	7		$C_{15:1}$	>100

[a] LD_{50} values are the lethal doses for 50% mortality.

decreased in inverse proportion to the degree of unsaturation in the side chain. The activity–side-chain relationship concerning the cardanol type compounds was less clear. The activity decreased in the order of cardol > 2 methylcardol ≫ cardanol. It seems that the degree of saturation in the alkyl side chain gives a relatively small change in activity, while the activity is dramatically changed by a change of hydrophilic groups on the ring. Moreover, an additional carboxy group on the ring results in a large increase in activity.

Molluscicides of plant origin are currently receiving considerable attention due to their relatively harmless biodegradative properties (Kools and McCullogh, 1981). Recently, many saponins have been reported as naturally occurring molluscicides (Domon and Hostettmann, 1984; Hostettmann et al., 1982; Sati et al., 1984). Unfortunately, many molluscicidal saponins are also toxic against fish. The toxicity of molluscicides against fish should be considered, since fish are a very important protein source in the countries where schistosomiasis is a problem. However, an application of these nonpolar compounds around the shoreline, the location favored by these snails, would more likely remain in this location than more polar compounds such as saponins. Hence, the physical property of solubility suggests an application of such nonpolar compounds will be less available to fish than an application of saponins.

These compounds were found in the nuts and in the fruit juice of the cashew, which are used for both food and drink. These compounds have also been reported in pistachio nuts *Pistachia vera* (Yalpini and Tyman, 1983) and in several varieties of cereals (Salek and Brudzyński, 1981). Therefore, it would appear that their potential for human oral toxicity either is not serious or has been overlooked.

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Registry No. 1, 79353-39-2; 2, 51546-63-5; 3, 501-26-8; 4, 103904-73-0; 5, 103904-74-1; 6, 22910-60-7; 7, 50423-15-9; 8, 50423-14-8; 9, 50423-13-7; 10, 79473-24-8; 11, 79473-25-9; 12, 22910-86-7.

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Scilliroside and Other Scilla Compounds in Red Squill

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Scilliroside and other bufadienolide glycosides in red squill, *Urginea maritima* (L.) Baker, were identified by isolation, high-performance liquid chromatography and thin-layer chromatography. Scilliroside, the major toxic glycoside, occurs in all plant parts including leaves, flower stalk, scales, and especially the roots and core of this bulbous plant. Other scilla compounds detected include desacetylscilliroside, scillaren A, and the aglycon scillirosidin. A new glucosylscilliroside and a phenolic nonscilla glycoside were also isolated and partially characterized. Scilliroside content of bulbs is highest in late summer after a dormancy period and does not appear to change with age. The scilliroside content of seed-derived varieties differs substantially, indicating a genetic factor affecting toxicant levels in the individual seedling plants. Toxicity of the bulbs is due principally to their content of scilliroside. The 6-acetoxy group of scilliroside contributes substantially to this toxicity.

INTRODUCTION

Red squill is a large onionlike plant whose bulb extracts and dried powders have been used in rodent control since the 13th century (Chitty, 1954; Marsh and Howard, 1975). The bulb and other plant parts contain scilliroside, a high-toxicity bufadienolide glycoside. Scilliroside affects the cardiovascular and central nervous systems, causing convulsions and death. Red squill preparations are emetic to humans (Belt, 1944), dogs and cats (Gold et al., 1950), and pigeons (Crabtree, 1947; Marsh and Verbiscar, 1986). However, rats and mice are unable to vomit, and they die within a few hours after ingesting lethal doses of scilliroside

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