Antibacterial Agents from the Cashew *Anacardium occidentale* (Anacardiaceae) Nut Shell Oil

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Sixteen phenolic compounds have been isolated from the cashew *Anacardium occidentale* (Anacardiaceae) nut shell oil. Their antimicrobial activity has been tested against four typical microorganisms, *Bacillus subtilis*, a Gram-positive bacterium; *Escherichia coli*, a Gram-negative bacterium; *Saccharomyces cerevisiae*, a yeast; and *Penicillium chrysogenum*, a mold. Most of them exhibited potent antibacterial activity against only Gram-positive bacteria, among which *Streptococcus mutans*, one of several bacteria responsible for tooth decay, and *Propionibacterium acnes*, one of the bacteria responsible for acne, were the most sensitive bacteria. Anacardic acids also showed weak activity against molds. Their structure–activity relationships are also described.

INTRODUCTION

We now can control many human and animal pathogenic microorganisms with antibiotics that are derived from either microorganism metabolites or synthesized chemicals. Nevertheless, the need for new antibiotics still exists. Similarly, we can control many troublesome microorganisms that contaminate foods and cosmetics with antimicrobial agents. Many of these agents are plant secondary metabolites and their derivatives such as hinokitiol, benzoic acid and its sodium salt, salicylic acid, and *p*-hydroxybenzoic acid and their various esters, etc. The need for new antimicrobial agents for foods and cosmetics also still remains. The use of preservatives to control microorganisms that putrefy nutritious food and cosmetic products is one of the major problems to be resolved. In the case of cosmetics, the control of specific microorganisms that cause skin, hair, and tooth problems is becoming even more important.

In contrast to medicine, preservatives for foods are consumed continuously and often for long duration by healthy people, so that safety is the first consideration. Similarly, since the preservatives in cosmetics are repeatedly applied to healthy skin, hair, and teeth, safety has top priority. It seems that edible plants may be a good source of new antimicrobial agents, especially for use in food and cosmetics. Keeping this in mind, we have screened various tropical vegetables and fruits that have been continuously eaten by many people since ancient times for antimicrobial activity.

For our first routine screening, the four microorganisms *Bacillus subtilis* ATCC 9372, a Gram-positive bacterium, *Escherichia coli* ATCC 9637, a Gram-negative bacterium, *Saccharomyces cerevisiae* ATCC 7754, a yeast, and *Penicillium chrysogenum* ATCC 10106, a mold, were selected.

In our preliminary screening the cashew *Anacardium occidentale* (Anacardiaceae) nut shell oil exhibited an activity *B. subtilis*, although it lacked activity against the other three. This result seems reasonable since the cashew nut shell oil was once used in Brazil for treatment of leprosy caused by *Mycobacterium leprae*, a Gram-positive bacterium (Vasconcelos, 1987). We also tested several additional microorganisms including *Streptococcus mutans*, which is known to be one of the main bacteria that causes tooth decay, *Staphylococcus aureus*, which causes suppuration, food poisoning, and toxic shock syndrome, and *Propionibacterium acnes*, one of the bacteria responsible for acne.

Since the cashew nut shell oil was a mixture of structurally similar phenolic compounds (Tyman, 1979), the final purification of these compounds was achieved by recycle high-performance liquid chromatography (R-HPLC) (Kubo et al., 1987a).

This paper describes the antimicrobial activity of these phenolic compounds, and their structure–activity relationships are briefly discussed.

MATERIALS AND METHODS

Microorganisms and Media. All microorganisms for the antimicrobial assay were purchased from American Type Culture Collection, Rockville, MD. They are *B. subtilis* ATCC 9372, *Brevibacterium ammoniagenes* ATCC 6872, *P. acnes* ATCC 11827, *S. aureus* ATCC 12598, *S. mutans* ATCC 25175, *Enterobacter aerogenes* ATCC 13048, *E. coli* ATCC 9637, *Pseudomonas aeruginosa* ATCC 10145, *S. cerevisiae* ATCC 7754, *Candida utilis* ATCC 9226, *P. chrysogenum* ATCC 10106, *Rhizopus stolonifer* ATCC 6227B, *Mucor mucedo* ATCC 20094, and *Aspergillus niger* ATCC 16404. Nutrient broth (BBL) (0.8%) yeast extract (Difco) (0.5%), and glucose (0.1%) were used for the culture of bacteria except for *S. mutans*. Brain heart infusion broth (Difco) (3.7%) was used for the culture of *S. mutans*. Malt extract broth (BBL) (2.5%) was used for the culture of yeasts and molds.

Chemicals. Anacardic acids (**5–7**), cardols (**13–15**), 2-methylcardols (**9–11**), and cardanols (**1–3**) were previously isolated from the cashew *A. occidentale* (Anacardiaceae) nut shell oil which was collected both near Mombasa, Kenya, and near Salvadol, Brazil (Kubo et al., 1986). Four additional structurally similar phenolics (**4**, **8**, **12**, and **16**) were also isolated from the same sources by R-HPLC (Kubo et al., 1987a). Two commercial cashew nut shell oils were purchased in Sao Paulo. Salicylic acid (**17**), resorcinol (**18**), and *p*-hydroxybenzoic acid (**19**) were purchased from Sigma Chemical Co., St. Louis, MO. The structures of these compounds are shown in Figure 1. For antimicrobial assay experiments, all chemicals were first dissolved in *N*,*N*-dimethylformamide.

Cultivation of Microorganisms. *B. subtilis*, *S. cerevisiae*, *C. utilis*, *P. chrysogenum*, *R. stolonifer*, *M. mucedo*, and *A. niger* were cultured with shaking at 30 °C, *B. ammoniagenes* and *E. aerogenes* were cultured stationarily at 30 °C, and the other bacteria were cultured stationarily at 37 °C.

Antimicrobial Assay. The minimal inhibitory concentration (MIC) against microorganisms was measured by twofold serial broth dilution (Taniguchi and Satomura, 1972). Microorganisms

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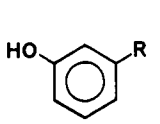
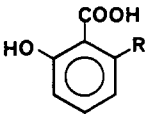
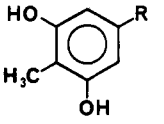


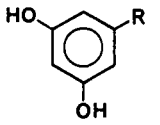
1: $C_{15:3}$, 3-[8(Z),11(Z),14-pentadecatrienyl]phenol
2: $C_{15:2}$, 3-[8(Z),11(Z)-pentadecadienyl]phenol
3: $C_{15:1}$, 3-[8(Z)-pentadecenyl]phenol
4: $C_{15:0}$, 3-pentadecylphenol



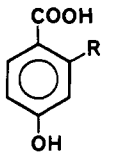
5: $C_{15:3}$, 6-[8(Z),11(Z),14-pentadecatrienyl]salicylic acid
6: $C_{15:2}$, 6-[8(Z),11(Z)-pentadecadienyl]salicylic acid
7: $C_{15:1}$, 6-[8(Z)-pentadecenyl]salicylic acid
8: $C_{15:0}$, 6-pentadecylsalicylic acid
17: H , salicylic acid



9: $C_{15:3}$, 2-methyl-5-[8(Z),11(Z),14-pentadecatrienyl]resorcinol
10: $C_{15:2}$, 2-methyl-5-[8(Z),11(Z)-pentadecadienyl]resorcinol
11: $C_{15:1}$, 2-methyl-5-[8(Z)-pentadecenyl]resorcinol
12: $C_{15:0}$, 2-methyl-5-pentadecylresorcinol



13: $C_{15:3}$, 5-[8(Z),11(Z),14-pentadecatrienyl]resorcinol
14: $C_{15:2}$, 5-[8(Z),11(Z)-pentadecadienyl]resorcinol
15: $C_{15:1}$, 5-[8(Z)-pentadecenyl]resorcinol
16: $C_{15:0}$, 5-pentadecylresorcinol
18: H , resorcinol



19: H , p-hydroxybenzoic acid

Figure 1. Structures of 16 phenolic compounds from the cashew nut shell oil and salicylic acid, resorcinol, and *p*-hydroxybenzoic acid.

Table I. Antimicrobial Activity of the Raw and Heated Cashew Nut Shell Oil

microorganisms tested	MIC, μg/mL raw	MIC, μg/mL heated
B. subtilis ATCC 9372	6.25	12.5
B. ammoniagenes ATCC 6872	3.13	100
S. aureus ATCC 12598	12.5	400
S. mutans ATCC 25175	3.13	3.13
E. aerogenes ATCC 13048	>1600	>1600
E. coli ATCC 9637	>1600	>1600
P. aeruginosa ATCC 10145	>1600	>1600
S. cerevisiae ATCC 7754	>1600	>1600
C. utilis ATCC 9226	>1600	>1600
P. chrysogenum ATCC 10106	>1600	>1600

were cultured in a series of tubes containing a broth medium with different concentrations of the test compound. For the antimicrobial assay, all microorganisms were cultured stationarily expect molds, which were cultured with shaking. After 48 h (5 days for molds), the growth of bacteria and yeasts was examined as turbidity (OD at 660 nm) and that of molds with the naked eye. The lowest concentration of the test compound in which no growth occurred was considered the MIC.

RESULTS AND DISCUSSION

In our first routine screening against four typical microorganisms, the cashew nut shell oil showed activity against *B. subtilis*, a typical Gram-positive bacterium, but not against the other three, *E. coli*, *S. cerevisiae*, and *P. chrysogenum*, at the concentration of 1600 μg/mL (Table I). Therefore, we have further examined the activity of this oil against three additional Gram-positive bacteria, *B. ammoniagenes*, *S. aureus*, and *S. mutans*. As shown in Table I, the cashew nut shell oil also exhibited activity against all the additional Gram-positive bacteria. Among them, the most sensitive bacteria were *S. mutans* and *B. ammoniagenes* (MIC, 3.13 μg/mL), and the least sensitive bacterium was *S. aureus* (MIC, 12.5 μg/mL). Almost all cashew nut shell oil that is commercially available has previously been treated by a heating process; therefore, the antimicrobial activity of the heated cashew nut shell oil was also tested. Once it is heated, the carboxyl group in anacardic acids is usually lost, converting them to the corresponding cardanols (**5** → **1**, **6** → **2**, **7** → **3**, and **8** → **4**). As a result, the strongest antibacterial anacardic acids in the cashew nut shell oil are converted to the least active cardanols (Table II). As shown in Table I, the antibacterial activity of the cashew nut shell oil itself has been weakened if it is processed by heating. The activity against *S. aureus* and *B. ammoniagenes* was especially decreased, 32-fold.

The bioassay guided fractionation has led to the isolation of antibacterial phenolic compounds (**1–3**, **5–10**, and **13–15**) from the cashew nut shell oil. Four additional structurally similar phenolics (**4**, **11**, **12**, and **16**) were also isolated to check the activity for comparison purpose. Most of them have been previously isolated from the same source as potent molluscicides against an aquatic snail *Biomphalaria glabratus*, one of the vectors of schistosomiasis (Kubo et al., 1986). Incidentally, we have also previously reported that two anacardic acids, including anacardic acid itself (**5**), isolated from another Anacardiaceae plant, *Ozoroa mucronata*, have potent prostaglandin synthetase inhibitory activity (Kubo et al., 1987b).

The MICs against 11 microorganisms of these phenolic compounds are shown in Table II. Since the shell oil showed rather narrow spectra against only Gram-positive bacteria, one additional Gram-positive bacterium, *P. acnes*, was also tested. *P. acnes*, one of the bacteria responsible for acne, produces a lipase that hydrolyzes sebum triglycerides to free fatty acids which cause inflammation and comedones. Similar to the shell oil itself, all the isolated phenolic compounds did not exhibit any activity against three Gram-negative bacteria, two yeasts

Table II. Antimicrobial Activity of the Principal Components of the Cashew Nut Shell Oil

components	MIC against microorganisms[a] tested, μg/mL										
	Bs	Ba	Pac	Sa	Sm	Ea	Ec	Pae	Sc	Cu	Pc
1	50	50	1.56	>100	1.56	>100	>100	>100	>100	>100	>100
2	>100	50	0.78	>100	3.13	>100	>100	>100	>100	>100	>100
3	>100	100	1.56	>100	>100	>100	>100	>100	>100	>100	>100
4	>100	>100	50	>100	>100	>100	>100	>100	>100	>100	>100
5	3.13	3.13	0.78	6.25	1.56	>100	>100	>100	>100	>100	>100
6	12.5	6.25	1.56	25	3.13	>100	>100	>100	>100	>100	>100
7	6.25	12.5	0.39	100	3.13	>100	>100	>100	>100	>100	>100
8	12.5	25	0.39	100	3.13	>100	>100	>100	>100	>100	>100
9	3.13	3.13	0.78	3.13	0.78	>100	>100	>100	>100	>100	>100
10	6.25	12.5	0.78	>100	1.56	>100	>100	>100	>100	>100	>100
11	>100	>100	0.39	>100	>100	>100	>100	>100	>100	>100	>100
12	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
13	1.56	12.5	1.56	6.25	0.78	>100	>100	>100	>100	>100	>100
14	3.13	6.25	0.78	>100	1.56	>100	>100	>100	>100	>100	>100
15	6.25	25	1.56	>100	1.56	>100	>100	>100	>100	>100	>100
16	>100	>100	50	>100	>100	>100	>100	>100	>100	>100	>100

[a] Bs, *B. subtilis* ATCC 9372; Ba, *B. ammoniagenes* ATCC 6872; Pac, *P. acnes* ATCC 11827; Sa, *S. aureus* ATCC 12598; Sm, *S. mutans* ATCC 25175; Ea, *E. aerogenes* ATCC 13048; Ec, *E. coli* ATCC 9637; Pae, *P. aeruginosa* ATCC 10145; Sc, *S. cerevisiae* ATCC 7754; Cu, *C. utilis* ATCC 9226; Pc, *P. chrysogenum* ATCC 10106.

and a mold tested at the concentration of 100 μg/mL. Again similar to the shell oil, among Gram-positive bacteria tested, *P. acnes* and *S. mutans* were the most sensitive bacteria (MICs, 0.39–50 and 0.78–3.13 μg/mL, respectively) and *S. aureus* was the least sensitive bacterium (MIC, 3.13–100 μg/mL) to these phenolic compounds.

The antibacterial activity of the cashew nut shell oil against Gram-positive bacteria was due not to one component but to a broad number of principal components. All phenolics identified in the shell oil have a C_{15}-alkyl side chain with 0–3 double bonds. By comparison of the simplest cardanols and use of them as standards, the following points can be summarized: (1) An additional hydroxy group on the cardanols changes them to the corresponding cardols (**1** → **13**, **2** → **14**, **3** → **15**, and **4** → **16**), and an increase in the antibacterial activity occurs. (2) An additional alkyl (methyl) group on the cardols converts them to the corresponding methylcardols (**13** → **9**, **14** → **10**, **15** → **11**, and **16** → **12**), and a small decrease in the activity results. (3) An addition of a carboxylic group to the cardanols changes them to anacardic acids (**1** → **5**, **2** → **6**, **3** → **7**, and **4** → **8**), and a dramatic increase in the activity occurs. (4) In general, an increase in the number of double bonds in the side chain also increases the activity.

Oral diseases caused by bacteria are a serious problem (Hamada and Slade, 1980), and *S. mutans* is one of several bacteria responsible for tooth decay. Since the cashew nut shell oil exhibited strong activity against *S. mutans*, it may be used for protection from the infection of this bacterium without further purification.

Salicylic acid (**17**) and resorcinol (**18**) have been used as cosmetic preservatives. As shown in Table III, these two simple phenolic compounds, which have no alkyl side chain, exhibited weak but broad antimicrobial activity against almost all of the microorganisms tested. In other words, their antimicrobial activity is not potent enough to be used exclusively for the control of specific microorganisms (such as those causing tooth decay) but is broad enough to be utilized as cosmetic preservatives. Similarly, *p*-hydroxybenzoic acid (**19**), one of the most common commercial cosmetic and food preservatives, has weak but broad antimicrobial activity. On the other hand, anacardic acid (**5**) and cardol (**13**), both possessing a C_{15}-alkyltriene side chain in addition to salicylic acid and resorcinol, respectively, exhibited a narrow spectrum of activity, against only Gram-positive bacteria; however, their activity against Gram-positive bacteria was dramatically increased compared to that of salicylic acid and resorcinol. For example, the activity of **5** against *S. mutans* and *S. aureus* was increased 2048 and 64 times, respectively. Similarly, that of **13** against *S. mutans* and *S. aureus* was increased 4096 and 1024 times, respectively. Since they have a narrow antimicrobial spectrum, they cannot be used as preservatives. However, they may be used for tooth and skin problems caused by specific Gram-positive bacteria such as *S. mutans*, *S. aureus*, and *P. acnes*.

The pure phenolics identified in the cashew nut shell oil were all assayed at the concentration of 100 μg/mL against all microorganism including yeasts and molds. Salicylic acid (**17**) and resorcinol (**18**) were assayed at a higher concentration because of their weak activity. Therefore, two major antibacterial phenolics in the shell oil, anacardic acid (**5**) and cardol (**13**), were retested at higher concentrations against four typical microorganisms to be able to compare their activities with those of salicylic acid and resorcinol, respectively. Although cardol (**13**) did not

Table III. Antimicrobial Activity of Salicylic Acid (17), Anacardic Acid Triene (5), Resorcinol (18), Cardol Triene (13), and *p*-Hydroxybenzoic Acid (19)

microorganisms tested	MIC, μg/mL				
	17	5	18	13	19
B. subtilis ATCC 9372	400	3.13	6400	1.56	800
B. ammoniagenes ATCC 6872	400	3.13	3200	12.5	400
S. aureus ATCC 12598	400	6.25	6400	6.25	800
S. mutans ATCC 25175	3200	1.56	3200	0.78	3200
E. aerogenes ATCC 13048	800	>1600	3200	>1600	3200
E. coli ATCC 9637	800	>1600	6400	>1600	800
P. aeruginosa ATCC 10145	800	>1600	3200	>1600	1600
S. cerevisiae ATCC 7754	400	>1600	>6400	>1600	>3200
C. utilis ATCC 9226	400	>1600	>6400	>1600	1600
P. chrysogenum ATCC 10106	200	200	6400	>1600	1600

Table IV. Antifungal Activity of Anacardic Acid Triene (5), Diene (6), and Monoene (7), Saturated Anacardic Acid (8) and Salicyclic Acid (17)

microorganisms tested	MIC, μg/mL				
	5	6	7	8	17
P. chrysogenum ATCC 10106	200	200	>1600	>1600	200
M. mucedo ATCC 20094	400	400	>1600	>1600	200
R. stolonifer ATCC 6227B	200	>1600	>1600	>1600	200
A. niger ATCC 10106	>1600	>1600	>1600	>1600	800

exhibit any activity even at higher concentration, anacardic acid (**5**) showed activity against not only *B. subtilis* but also *P. chrysogenum*. Therefore, four anacardic acids (**5–8**) were assayed against four common molds at higher concentration. Although **7** and **8** did not exhibit any activity even at the concentration of 1600 μg/mL, **5** and **6** showed activity even against the molds as shown in Table IV. It appears that the C_{15} side chain increases antibacterial activity, but it decreases the broad antimicrobial activity, and also that an increase of the number of double bonds in the side chain increases the antifungus activity.

Although the cashew nut shell oil itself is not edible, these 16 phenolic compounds isolated from this oil were also found in the nut and in the fruit juice, which have long been consumed by many people as both food and drink. Therefore, it would appear that their potential for human oral toxicity either is not serious or has been overlooked.

LITERATURE CITED

Hamada, S.; Slade, H. D. Biology, immunology, and cariogenicity of *Streptococcus mutans*. *Microbiol. Rev.* **1980**, *44*, 331–384.

Kubo, I.; Komatsu, S.; Ochi, M. Molluscicides from the cashew *Anacardium occidentale* and their large-scale isolation. *J. Agric. Food Chem.* **1986**, *34*, 970–973.

Kubo, I.; Kim, M.; DeBore, G. Efficient isolation of insect growth inhibitory macrolide alkaloids using recycle high-performance gel permeation chromatography. *J. Chromatogr.* **1987a**, *354*, 7778–7781.

Kubo, I.; Kim, M.; Naya, K.; Yamagiwa, Y.; Ohashi, K.; Sakamoto, Y.; Hirakawa, S.; Kamikawa, T. Prostaglandin synthetase inhibitors from an African medicinal plant *Ozoroa mucronata*. *Chem. Lett.* **1987b**, 1101–1104.

Taniguchi, M.; Satomura, Y. Structure and physiological activity of carbostyril compounds. *Agric. Biol. Chem.* **1972**, *36*, 2169–2175.

Tyman, J. H. P. Non-isoprenoid long chain phenols. *Chem. Soc. Rev.* **1979**, *8*, 499–537, and references cited therein.

Vasconcelos, B. Caju. *Globo Rural* **1987**, 70–77.

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