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The Antibacterial Component from Cinnamomi Cortex against a Cariogenic Bacterium *Streptococcus mutans* OMZ 176

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Abstract □ The methanol extract of Cinnamomi Cortex showed antibacterial action against cariogenic bacterium, *Streptococcus mutans* OMZ 176. The active principle of the extract was identified to be *trans*-cinnamaldehyde, which was bactericidal in the minimal inhibitory concentration (MIC) of 100 μg/m*l* against the strain. From the results of antibacterial activity of cinnamaldehyde and its derivatives, the acrolein group in the cinnamaldehyde was elucidated to be an essential element for the activity.

Keywords □ Cinnamomi Cortex, cinnamaldehyde, cinnamic acid, cinnamic alcohol, *Streptococcus mutans* OMZ 176

The increase of the relative amount of *Streptococcus mutans* in dental plaque in connection with the intake of sucrose appears to be correlated with the production of adhesive polysaccharides[1]. Although many researchers studied inhibitors of plaque formation, there are few reports on inhibition by components in natural products. Namba *et al.*[2] found that magnolol and honokiol, isolated from Magnoliac Cortex, inhibit plaque formation due to their antibacterial action. Southard *et al.*[3] noted that sanguinarin, a component of a Sanguinaria extract, prevented dental caries by its antibacterial action and retention in oral cavity. For the purpose of developing of antiplaque agents, we previously investigated the antibacterial activities of methanol and benzene extracts of 55 higher plant leaves against a cariogenic bacterium, *Streptococcus mutans* OMZ 176[4]. Among them, the methanol and benzene extracts of 12 species showed the activity. Furthemore, β-liriodenolide was elucidated as an antibacterial component from *Liriodendron tulipifera* L.,[5] and emodin from Polygonati Radix[6].

S. mutans strains are physiologically and morphologically homogenous, but they are classified serologically into seven types (a-g). The types c and d are most frequently detected in Japnaese, European and Australian dental caries, they differed genetically from each other[2]. In the present study, we isolate and identify an antibacterial component from Cinnamomi Cortex against *S. mutans* OMZ 176, also examine the chemical structure and activity relationships.

EXPERIMENTAL METHODS

Experimental material

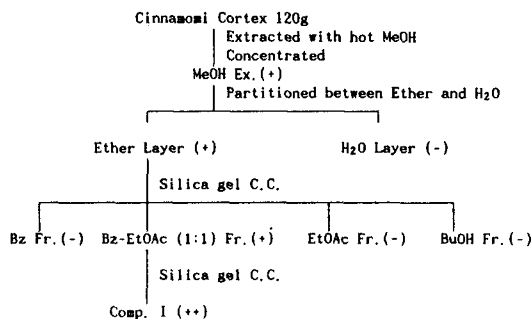
Commercially available Cinnamomi Cortex was purchased from Jungdo crude drug shop, Taejon (July, 1989).

Chemicals

Cinnamic acid and cinnamic alcohol were purchased from Aldrich, Bacto-brain heart infusion (BHI) from Difco and paper disk (8 mm) from Toyo Co.

Analytical instruments

The infrared spectra were carried out in nujol on a Perkin-Elmer model 783 spectrophotometer. The ^{1}H-NMR spectra were recorded on a Varian 360 A (90 MHz) with TMS as internal standard.



Scheme 1. Schematic fractionation process and antibacterial activity against *Streptococcus mutans* OMZ 176

(++) : Inhibitory zone of 11-15 mm was formed in the concentration 100 μg/disk
(+) : Inhibitory zone of 8.5-11 mm was formed
(−) : No inhibitory zone was formed.

Extraction and isolation of antibacterial component

To isolate and identify the active component, the methanol extract of Cinnamomi Cortex was fractionated into gross chemical classes as described in Scheme 1. The distribution and purification of the active component were monitored by the paper disk method[2]. Each fraction was adjusted 100 μg per a disk for antibacterial assay.

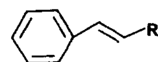
Powdered Cinnamomi Cortex 120g was extracted with 1.6 *l* of methanol using a water bath. After filtration, the filtrate was evaporated to dryness and the extract was partitioned between ether and water. The ether layer was applied to a column of silica gel. The column was eluted with benzene, benzene-ethyl acetate (1 : 1), ethyl acetate, and butanol. The benzene-ethyl acetate fraction was applied to a column of silica gel using benzene-ethyl acetate (20 : 1) to give oily substance. Further purification was conducted simiarly with silica gel column using benzene-ethyl acetate (10 : 1) to yield 980 mg of a pure compound.

Test organisms

Streptococcus mutans OMZ 176 (serotype d) was given from Prof. Namba, Toyama Medical and Pharmaceutical University, Japan.

Antibacterial assay

BHI agar 40 m*l* was overlaid with the seed BHI broth (turbidity of the cell suspension, 0.07 OD units/m*l*) previously inoculated with precultured *S. mutans* cells to yield a lawn of growth. Thereafter, filter paper disks (8 mm in diameter, 1.5 mm in thickness, Toyo filter) containing various amounts of extract or component were carefully placed on the seeded Petri dishes. The cultivation was then carried out at 37° for 16 hours.



1, R = CHO
2, R = COOH
3, R = CH_2OH

Fig. 1. The structures of cinnamaldehyde (1), cinnamic acid (2) and cinnamic alcohol (3).

Determination of minimal inhibitory concentration (MIC)

Test compound 1mg was dissolved in a minimum amount of methanol for a series of diluted solutions. The solution 0.1 m*l* was then added to 4.9 m*l* of sterile liquid BHI broth containing about 10^6 cells/m*l* of *S. mutans* in a test tube. The tube was mixed throughly and incubated at 37° for 48 hours. The MIC was determined by judging visually the bacterial growth in the series of test tubes.

RESULTS AND DISCUSSION

Isolation and identification of antibacterial component

As shown in Scheme 1, the isolation process of active component was monitored by the paper disk assay. Benzene-ethyl acetate fraction showed activity, therefore, the fraction was applied on silica gel column using benzene-ethyl acetate (20 : 1) to give only substance 980 mg, 0.82% overall yield (Comp. **1**).

Comp. **1**, colorless oily liquid, gave a orange coloration with 2,4-dinitrophenylhydrazine. Its IR spectrum showed the presence of α, β-unsaturated C=O (1690 cm^{-1}) and a double bond (1630 cm^{-1}). Its ^{1}H-NMR spectrum showed two olefinic protons at δ=6.68 ppm (1H, dd, J=7 and 18 Hz) and 7.43 ppm (1H, d, J=18 Hz), 7.0-8.0 ppm (5H, m, aromatic-H), and 9.65 ppm (1H, d, J=7 Hz, CHO).

The comparison of these data with those of an authentic specimen confirmed the active compound to be *trans*-cinnamaldehyde (Fig. 1, **1**)[7].

Comparing with the antibacterial activities of

Table I. The antibacterial activities and minimal inhibitory concentration of cinnamaldehyde and its derivatives against *S. mutans* OMZ 176

Compounds	Diameter of inhibitory zone (mm)[1]					MIC[4]
	5[2]	10	20	40	80	(ug/m*l*)
Cinnamaldehyde	–[3]	8.5	10.5	11.5	13.0	100
Cinnamic acid	–	–	9.0	10.5	11.5	200
Cinnamic alcohol	–	–	–	–	–	>200

1 Mean values from three observations
[2]Added amounts (ug) per a disk
[3]No inhibitory zone was formed
[4]Minimal inhibitory concentration

magnolol and honokiol (MIC, 6.3 μg/ml) isolated from the stem bark of *Magnolia obavata*[2], the antibacterial activity of cinnamaldehyde (Fig. 1, **1**) was weaker than that of magnolol and honokiol (Table I and Literature 2). But cinnamaldehyde showed as same activity as berberine[2], β-liriodenolide[5] and emodin[6], in the test of MIC (100 μg/m*l*).

Cinnamaldehyde **1** and cinnamic acid **2**, a derivative of cinnamaldehyde, showed the antibacterial activity but no activity was observed in cinnamic alcohol **3**, another derivative of it. As shown in Fig. 1, the presence of the acrolein group (α,β-unsaturated carbonyl one) in the structures of cinnamaldehyde and cinnamic acid is thought to be an essential element for the antibacterial activity.

CONCLUSION

1. The active antibacterial component of Cinnamomi Cortex against a cariogenic bacterium, *Streptococcus mutans* OMZ 176 was identified to be cinnamaldehyde, which was bactericidal in the minimal concentration (MIC) of 100 μg/m*l*.

2. The acrolein group (α, β-unsaturated carbonyl one) in the structure of cinnamaldehyde is thought bo be an essential element for the antibacterial activity.

ACKNOWLEDGEMENT

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