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Susceptibility of Oral Bacterial to Sophoraflavanone G isolated from the Root of Sophora flavescens

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Objective : The aim of this work is to investigate the antibacterial activity of the Sohporaflavanone G isolated from Sophora flavescens (S. flavescens), as the development of microbial resistance to antibiotics make it essential to constantly look for new and active compounds effective against pathogenic bacteria.

Method : Sophoraflavanone G was isolated from the dried roots of Sophora flavescens Aiton (Leguminosae) by bioassay?guided fractionation. We investigated the effect of sophoraflavanone G on oral bacterial at various concentrations after incubation of 24 h in strains in the dose?dependent manner.

Results : The structure of active compound, Sophoraflavanone G having a lavandulyl group at C?8, was elucidated on the basis of spectral data especially 1H?NMR and 13C?NMR. The antimicrobial activity showed that Sophoraflavanone G exhibited antimicrobial activities against all the bacteria tested (MICs, 0.39 ~ 6.25 μg/ml). Sophoraflavanone G showed the strong antimicrobial activity against all the facultative bacteria and microaerophilic bacteria (MICs, 0.78 ~ 1.56 μg/ml) and also Sophoraflavanone G showed the strong antimicrobial activity against obligate anaerobic bacteria (MICs, 0.39 ~ 6.25 μg/ml).

Key words : S. flavescens, Antibacterial activity

Introduction

Sophorae Radix, the dried roots of S. flavescens Aiton (Leguminosae) is oriental traditional medicine well known to have antibacterial, anti?inflammatory, antipyretic, antiarrhythmic, antiasthmatic, antiulcerative, and antineoplastic effects and is used as an insecticide and for the treatment of skin and mucosal ulcers, sores, diarrhea, gastrointestinal hemorrhage, arrhythmia and eczema[1-4].

In earliest investigations, the phytochemical constituents of this plant investigated so for were sophoraflavanoside I, soyasapogenol[5] sophoraflavoside II-IV[6], matrine as quinolizidine alkaloids, flavonoids, and triterpenoides. In addition to flavonoids with the above regular prenyl side chains, S. flavescens produced diverse flavanones with lavandulyl chains,

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irregular monoterpenoid groups, such as kurarinone and sophoraflavanone G[7,9]. It is structurally characteristic of these flavanones to possess a hydroxy group at C-2' together with a lavandulyl group at C-8 or C-6 [10].

In the course of screening for antibacterial activity from medicinal plants, the methanolic extract of this plant was found to have a significant activity. In this paper, we report on the isolation, the identification and the antibacterial activity of constituent from the roots of S. flavescens.

Materials and Methods

1. Plant material

The roots of Sophora flavescens Ait. were collected at Jin-an, Chonbuk province, Korea, in February 2002. The voucher specimen (JS-02-A23) was deposited at the Herbarium of the College of Oriental Medicine, Woosuk University.

2. Isolation of antibacterial active constituent

The dried roots of S. flavescens (1.2 kg) were crushed and extracted with MeOH (8 l) for 7days at room temperature. The MeOH extract was concentrated, suspended in H2O, and sequentially partitioned with CH2Cl2, EtOAc and BuOH. The bioactive EtOAc-soluble fraction (6.3g) was subjected to silica gel (Merck Kieselgel 60; 0.063-0.04 mm particle size; 3 x 50 cm) column chromatography. The column was eluted with a gradient elution using mixtures of MeOH in CH2Cl2 (10% with 700 ml, 20% with 500 ml, 30% with 300 ml, 40% with 200 ml), followed by 300 ml of MeOH. Fractions were combined based on their TLC pattern to yield fractions designated as S1-S6. Fraction S3(1.4g) retained the activity, thus fractionated further by octadecyl-functionalized silica gel (Aldrich) column chromatography [3 x 50 cm; stepwise gradient of 30%, 50%, and 100% (v/v) CH3CN in H2O, followed by 300ml of MeOH; collecting 50ml fraction]. A portion (275 ㎎) of the active fraction (JS 2) was subjected to recycling preparative HPLC (eluent; MeOH) to yield sophoraflavanone G [purity; 97 %, tr = 58min. 129 ㎎, 0.03% w/w, m.p. 173-17 5℃;[α]D25 -49° (c 1.0 in MeOH). The chemical structure of sophoraflavanone G (Fig 1) was determined by analysis of [α], 1H-NMR, 13C-NMR and DEPT data and in comparison with literature values[1],[2].

Sophoraflavanone G; 1H-NMR (600 MHz, CD3OD); δ 1.50 (3H, s, H-6"), 1.57 (3H, s, H-7"), 1.65 (3H, s, H-10"), 2.06 (2H, m, H-3"), 2.53 (2H, m, H-2"), 2.62 (2H, m, H-1"), 2.75 (1H, dd, J=17.2, 2.9 Hz, Ha-3), 3.06 (1H, dd, J=17.2, 13.2 Hz, Hb-3), 4.54 (1H, s, Ha-9"), 4.58 (1H, s, Hb-9"), 5.00 (1H, m, H-4"), 5.66 (1H, d, J=12.9Hz, H-2), 6.02 (1H, s, H-6), 6.47 (1H, m, H-5'), 6.49 (1H, m, H-3'), 7.40 (1H, m, H-6'), 12.18(1H, s, C5-OH). 13C-NMR (175 MHz, CD3OD); δ 17.9 (C-2), 43.4 (C-3), 199.2 (C-4), 163.3 (C-5), 96.4 (C-6), 166.6 (C-7), 108.7 (C-8), 162.8 (C-9), 103.4 (C-10), 118.5(C-1'), 156.8 (C-2'), 103.5 (C-3'), 159.7 (C-4'), 107.9 (C-5'), 128.9 (C-6'), 28.1 (C-1"), 49.2 (C-2"), 32.5 (C-3"), 124.9 (C-4"), 132.2 (C-5 "), 26.0 (C-6"), 17.9 (C-7"), 149.9 (C-8"), 111.3 (C-9"), 19.3 (C-10").

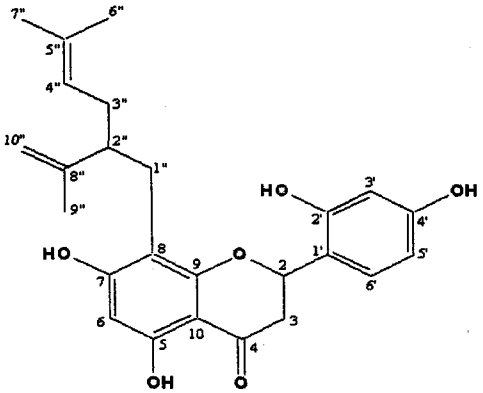

Fig. 1. Structure of sophoraflavescen G

3. Bacterial strains

Antimicrobial activites of Sophoraflavanone G against some oral bacteria were determined by the broth dilution method. The oral bacterial strains used in this study were: Streptococcus mutans ATCC 25175, Streptococcus sanguinis ATCC 10556, Streptococcus sobrinus ATCC 27607, Streptococcus ratti KCTC (Korean collection for type cultures) 3294, Streptococcus criceti KCTC 3292, Streptococcus anginosus ATCC 31412, Streptococcus gordonii ATCC 10558, Actinobacillus actinomycetemcomitans ATCC 43717, Fusobacterium nucleatum ATCC 51190, Prevotella intermedia ATCC 49046, and Porphylomonas gingivalis ATCC 33277. Brain-Heart Infusion broth supplemented with 1% yeast extract (Difco Laboratories, Detroit, MI) was used for all bacterial strains except P. intermedia and P. gingivalis. For P. intermedia and P. gingivalis, Brain-Heart Infusion broth containing hemin and menadione was used.

4. Minimum inhibitory concentrations assay

The minimum inhibitory concentrations (MICs) were determined for Sophoraflavanone G by the broth dilution method, and were carried out in triplicate. The antibacterial activities were examined after incubation at 37℃ for 18 h (facultative bacteria), for 24 h (microaerophilic bacteria), and for 1-2 days (obligate anaerobic bacteria) under anaerobic conditions. MICs were determined as the lowest concentration of test samples that resulted in a complete inhibition of visible growth in the broth. Following anaerobic incubation of MICs plates were determined on the basis of the lowest concentration of the essential oil that kills 99.9% of the test bacteria by plating out onto each appropriate agar plate. Ampicillin and Gentamicin were used as standard antibiotics in order to compare the sensitivity with Sophoraflavanone G against test bacteria.

Results and Discussion

1. Identification of the antibacterial compound.

Sophoraflavanone G was obtained as pale yellow needles, m.p 173-175℃. The proton nuclear magnetic resonance (1H-NMR) spectrum of sophoraflavanone G showed the signals of one lavandulyl group [δ 1.50, 1.57 and 1.65 (each 3H, each s, CH3 -), δ 2.06-3.33 (5H, m, =CH2×2, CH), δ 4.58 (2H, brs, =CH2), δ 5.00 (1H, brt, J=7.0Hz, =CH-CH2-)], three hydroxyls [δ 8.6 (OH×2) and δ 9.5 (OH×1)], one chelated hydroxyl (δ 12.20) and an ABX type grouping due to the C-2 (δ 5.68) and C-3 protons (δ 2.77 and δ 3.08). In the aromatic region of the spectrum, the signals of the remaining four protons appeared as a singlet (δ 6.02, 1H), due to the A-ring proton, and an ABC type grouping [δ 6.47 (1H, dd, J=8.1, 2.2 Hz), δ 6.49 (1H, d, J=2.2Hz) and δ 7.40 (1H, d, J=8.1 Hz)] due to the C-5', C-3'and C-6' protons in the B-ring. Thus, the

structure of sophoraflavanone G was concluded to be 5,7,2',4'-tetrahydroxy-8-lavandulyl flavanone(Fig. 1).

2. Antimicrobial activity

The results of the antimicrobial activity (Table 1) showed that Sophoraflavanone G exhibited antimicrobial activities against all the bacteria tested (MICs, 0.39 ~ 6.25 μg/ml). Sophoraflavanone G showed the strong antimicrobial activity against all the facultative bacteria and microaerophilic bacteria (MICs, 0.78 ~ 1.56 μg/ml) and also Sophoraflavanone G showed the strong antimicrobial activity against obligate anaerobic bacteria (MICs, 0.39 ~ 6.25 μg/ml). The results suggested that Sophoraflavanone G showed more antimicrobial activity than standard drugs, penicillin and gentamicin.

Several studies have reported on the antibacterial effects of chewing sticks on cariogenic bacteria and periodontal pathogens, particularly bacteroides speciesand inhibitory action on dental plaque formation11-12). Several reports have shown that Sophoraflavanone G can possess antimalarial activity, tyrosinase inhibitory, anti-inflammatory, antiulcer[11], and antibacterial activity.

These findings suggest that Sophoraflavanone G fulfills the conditions required of a novel cariogenic bacteria and periodontal pathogens, particularly bacteroides species drug and may be useful in the future in the treatment of oral bacteria infection. However, for medicinal purposes, the safety and toxicity of this compound need to be addressed. The difference in susceptibility may allow formulation of products that will selectively kill or inhibit certain organisms while having a minimum effect on the commensal microorganisms.

Table 1. Minimum inhibitory concentrations (μg/ml) of Sophoraflavanone G for some oral bacteria.

Strains	Sophoraflavanone G	Ampicillin	Gentamicin
Streptococcus mutans ATCC 25175	1.56	4	8
Streptococcus sanguinis ATCC 10556	1.56	32	8
Streptococcus sobrinus ATCC 27607	0.78	2	4
Streptococcus ratti KCTC 3294	1.56	4	4
Streptococcus criceti KCTC 3292	1.56	4	8
Streptococcus anginosus ATCC 31412	1.56	4	16
Streptococcus gordonii ATCC 10558	0.78	1	2
Actinobacillus actinomycetemcomitans ATCC 43717	0.78	64	2
Fusobacterium nucleatum ATCC 10953	1.56	0.25	16
Prevotella intermedia ATCC 25611	6.25	32	0.5
Porphylomonas gingivalis ATCC 33277	0.39	0.5	256

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