Antibacterial Activity of Flavonoids against 
Staphylococcus epidermidis, a Skin Bacterium

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An investigation was carried out on Okinawan plants to find antibacterial compounds against a human skin bacterium, Staphylococcus epidermidis, which causes acne vulgaris. A medicinal plant, Elaeagnus glabra, showed significant activity, and (−)-epigallocatechin (27) was isolated from the plant as an antibacterial constituent against the bacterium. Twenty-six flavonoids related to 27 were tested for the activity, galangin (7) being the most active species. Although a structure–activity study was attempted, no clear structural factor was deduced.

The human skin bacterium, Staphylococcus epidermidis (Gram-positive), is assumed to be a cause of the skin disease, acne vulgaris (pimples).1 Although antibiotics2,3 have been reported to be effective in clinical use against acne vulgaris, antibacterial agents against S. epidermidis have not yet been described. This led us to isolate such antibacterial constituents from methanol extracts of Okinawan plants. Among twelve Okinawan plants tested, Elaeagnus glabra Thunb. demonstrated significant activity against the skin bacterium.

Infusion of the bark of E. glabra, which is an Okinawan medicinal plant belonging to the Elaeagnaceae family, is utilized by Okinawan people to treat such diseases as diarrhoea, tetanus and asthma. However, no chemical work has been carried out to confirm the constituents of this plant.

(−)-Epigallocatechin (27) was isolated in our work as an antibacterial constituent against S. epidermidis, and 26 flavonoids related to 27 (structures shown in Fig. 1) were tested for their activity against the skin bacterium. This paper reports the antibacterial results for the flavonoids, and also describes the antibacterial activity of 27 against Proteus vulgaris (Gram-negative) and Staphylococcus aureus, as well as the cytotoxicity against HeLa cells in our extended biological tests.

MATERIALS AND METHODS

Isolation of the antibacterial constituent [(−)-epigallocatechin (27)] from Elaeagnus glabra. The isolation of the antibacterial constituent was monitored by its activity against S. epidermidis. The bark (1.4 kg) of E. glabra, collected in Yagaji in Okinawa Prefecture, was extracted with methanol (20 liters) for two weeks at room temperature. Removal of the solvent (at 30°C) and complete drying of the methanol extract gave a brown powder (74.9 g), which was chromatographed over silica gel (400 g, Silica gel 60, Merck) by successive elutions with 1 : 1 (4 liter) and 7 : 3 (4 liter) mixture of n-hexane and ethyl acetate (EtOAc). The activity was observed in the latter fraction (5.3 g). Silica gel (400 g) chromatography of the active fraction was eluted with a 3 : 2 : 0.01 solvent mixture of n-hexane, EtOAc and acetic acid (AcOH). The active fraction was eluted with a 3 : 2 : 0.01 solvent mixture of n-hexane, EtOAc and acetic acid (AcOH), and was collected in fractions of 15 ml each to give the active fractions (Nos. 141 ~ 283 of 335 fractions). The concentrate of the active fractions afforded a crude crystalline material, which was recrystallized from the solvent mixture (n-hexane:EtOAc:AcOH = 3 : 2 : 0.01) to give pure crystals of compound I (1.02 g), mp 211°C; [α]D20 = −64.0° (c 1.0, ethanol); FD-MS: m/z 306 (C15H14O7). From the inactive fractions (Nos. 95 ~ 120) compound II (0.95 g) was afforded by similar recrystallization, mp 210°C; [α]D20 =
compounds having the absolute structures shown in Fig. 1.

**Antibacterial test.** A sensitive meat extract broth [E-MC68, Eiken Chemical Co. (Tokyo)] was used for culturing *S. epidermidis* ATCC 12228. An 18 hr cultured fresh broth (at 30°C) was diluted by the same broth to indicate a 0.4 value of optical density. The diluted broth was further diluted to a 1/5000 concentration which was served as a MIC (minimal inhibitory concentration, µg/ml) measuring both. From a dimethylsulfoxide (100 µl) solution of a compound (2 mg) or chromatographic fraction (2 mg of the concentrate), two-fold serial dilutions (3.1 ~ 200 µg/ml) were made by adding the meat extract broth. After incubating the measuring broth containing a test sample for 18 hr at 30°C, MIC values were determined by turbidity. In the case of tests using *Proteus vulgaris* OX-19 and *Staphylococcus aureus* FDA 209 PJC-1, a similar method was performed at 37°C.

**Cytotoxicity test.** The method was essentially that of Kurobane et al.9) The uterine carcinoma HeLa-S3 cells were maintained in a suspension culture of RPMI 1640 [Nissui Seiyaku Co. (Tokyo)] that was supplemented with 10% calf serum [Gibco 200–6170, Gibco Co. (USA)] containing L-glutamine and a penicillin-streptomycin mixture (Gibco 600–5070, 5000 U/ml). Into a well of a 96-well microtiter plate [Costa Co. (USA)], a 100 µl aliquot of 1 x 10^5 cells/ml suspension was distributed, before a 100 µl aliquot of two-fold serially diluted (-)-epigallocatechin (27) was added. The plate was incubated for 96 hr at

-64.0° (c 1.0, ethanol); FD-MS m/z 290 (C_{15}H_{14}O_6).

Compounds I and II were respectively identified to be (+)-epigallocatechin (27) and (-)-epicatechin (26) by comparing their melting points, specific rotations, IR and ^1H-NMR spectra with those in the literatures.4~6)

**13C-NMR data of 26 and 27.** Since no ^13C-NMR data have been reported for 26 and 27, the data are described here.

**(-)-Epicatechin (26).** δ(C) (125 MHz): 28.7 (t, C-4), 67.0 (d, C-3), 79.4 (d, C-2), 95.7 (d, C-8), 96.3 (d, C-6), 99.9 (s, C-4a), 114.8 (d, C-2'), 115.1 (d, C-5'), 119.1 (d, C-6'), 131.5 (s, C-1'), 132.9 (s, C-3'), 146.1 (s, C-3 and C-5'), 157.1 (s, one of C-5, C-7 and C-8a), 157.5 (s, two of C-5, C-7 and C-8a).

**(-)-Epigallocatechin (27).** δ(C) (125 MHz): 28.8 (t, C-4), 67.0 (d, C-3), 79.4 (d, C-2), 95.7 (d, C-8), 96.3 (d, C-6), 99.9 (s, C-4a), 107.0 (d, C-2' and C-6'), 131.5 (s, C-1'), 132.9 (s, C-3'), 146.1 (s, C-3 and C-5'), 157.1 (s, one of C-5, C-7 and C-8a), 157.5 (s, two of C-5, C-7 and C-8a).

**Other flavonoids.** The flavonoids used were commercially obtained. Compounds 1, 10 and 16~18 were from Aldrich Chemical Co. (USA), 2~9, 11, 12, 14, 15 and 19~24 were from Laboratories Sarret (France), and 13 and 25 were from Wako Pure Chemical Co. and Tokyo Kasei Co. (Tokyo), respectively. From optical rotations (Table I) and the Cotton effects of CD curves7) (1 x 10^-4 mol in methanol) of the flavonoids, it was revealed that the flavanones 17~21 were racemates, and that flavanons 22~24 and 25 were optically active

Fig. 1. Structures of Flavonoids Tested for Antibacterial Activity against *Staphylococcus epidermidis*. 

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37°C in a moist chamber under a CO₂ atmosphere, and the cells were then stained with gentian violet. The stained cells were solubilized with ethanol (100 µl/well), and the absorbance of the colored ethanol was measured at 620 nm. Cytotoxicity is described by the IC₅₀ value (the concentration of a compound required to reduce the absorbance to 50% of) on the calibration curve.

RESULTS

(−)-Epigallocatechin (27) showed antibacterial activity against both *P. vulgaris* (Gram-negative) and *S. aureus* (Gram-positive) at 100 µg/ml (MIC). Cytotoxicity was also exhibited by 27 against HeLa cells at 18.6 µg/ml (IC₅₀).

The MIC values of 27 and the other flavonoids against *S. epidermidis* are listed in Table I. Among the flavonoids tested, galangin (7) demonstrated the strongest activity (6.3 µg/ml), and significant activity (50~100 µg/ml) was observed in several flavones (1~3) and flavonols (8~10 and 16); however, all the flavanones (17~21) and flavanons (22~24) were inactive. In the catechins (25~27), only (−)-epigallocatechin (27) showed the activity (50 µg/ml).

DISCUSSION

*Staphylococcus epidermidis,* an apathogenic bacterium of resident flora of the human skin, acts in some cases as a pathogenic bacterium inducing acne vulgaris, together with other skin microorganisms. Tetracyclins, clindamycins, benzyl peroxide and other antibiotics (cephalosporins, erythromycin, etc.) are known to be applicable for both or either oral and ointment administration against the skin disease. However, there is no example of plant-derived antibiotics for the skin disease and against the skin microorganisms.

Flavonoids are ubiquitously distributed in plants and have not been reported for serious

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<tr>
<th>Flavonoid</th>
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<tr>
<td>Flavone</td>
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<td>Flavone (1)</td>
<td>50</td>
<td>(±)-Flavanone (17)</td>
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<tr>
<td>7,8-Dihydroxyflavone (2)</td>
<td>100</td>
<td>(±)-4',5,7-Trihydroxy-flavanone (18)</td>
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<td>50</td>
<td>(±)-Eriodictyol (19)</td>
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<tr>
<td>Chrysin (4)</td>
<td>—</td>
<td>(±)-Homoeodictyol (20)</td>
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<td>Luteolin (5)</td>
<td>—</td>
<td>(±)-Hesperitin (21)</td>
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<td>Apigenin-7,4’-dimethylether (6)</td>
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<td>Flavanonol b</td>
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<td>Flavonol</td>
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<tr>
<td>Galangin (7)</td>
<td>6.3</td>
<td>(−)-Fustin (22)</td>
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<tr>
<td>Datiscetin (8)</td>
<td>50</td>
<td>(±)-Taxifolin (23)</td>
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<td>(±)-Dihydrorobinetin (24)</td>
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<td>Fisetin (10)</td>
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<td>Catechin c</td>
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<td>Quercetin (11)</td>
<td>—</td>
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<tr>
<td>Rhamnetin (12)</td>
<td>—</td>
<td>(−)-Epicatechin (26)</td>
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<tr>
<td>Morin (13)</td>
<td>—</td>
<td>(−)-Epigallocatechin (27)</td>
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<td>Robinetin (15)</td>
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<tr>
<td>Myricetin (16)</td>
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a (−) means inactive at 200 µg/ml in MIC.

b [α]D values (c 1.0, 50% acetone–water, 25°C) are: 22, −14°; 23, +49°; and 24, +17°.

c [α]D values (c 1.0, ethanol, 25°C) are: 25, +59°; 26, −64°; and 27, −64°.
toxicity. The low toxic nature and rather lipophilic properties of flavonoids were anticipated to be advantageous for repetitive administration as ointment to the human skin.

In the present study, although the following fragmentary information can be provided for structure-activity relationships, precise relationships were impossible to deduce.

Most of the active flavonoids were found in flavones and flavonols, whose A/C rings take planar structures. Hence, the antibacterial activity against S. epidermidis seems to be related to the molecular planarity of the A/C ring component of the flavonoid structure. On the other hand, in flavonoids which possess a non-planar structure for the A/C rings, only 27 was active. In the case of flavanols and catechins, cis substitution at the 2 and 3 positions (2α and 3α) may be important for the activity [all the trans-substituted compounds (22 ~25) were inactive].

The role of hydroxy groups (OHs) on the flavane skeleton was ambiguous for the antibacterial activity. When we compared activity employing defined pairs of compounds, some trends were expected for the OH roles [for example, comparisons between 7 and 4, and 26 (11) and 27 (16), respectively, suggested the importance of 3-OH and 5′-OH]. However, such inspections could not be extended to the whole structure-activity relationship of all the flavonoids tested. Nevertheless, it was evident that the active flavonoids contained OHs in their structures. A greater number of flavonoids would be required to elucidate the significance of the OHs.

The lipase of S. epidermidis has been known to hydrolyze triglycerides in the sebaceous organ to free fatty acids,1) which sometimes cause kelatinization1) and inflammation1) of the organ. This inflammation and over-kelatinization are considered to cause acne vulgaris.3) Hence, the inhibitory action of flavonoids on the bacterial lipase may be of interest to investigate.

For the flavones and isoflavones, antibacterial activity has often been reported14~19) against various microorganisms other than S. epidermidis, as well as their pharmacological activities.20)

In this study, since (−)-epigallocatechin (27) exhibited antibacterial activity against both Gram-positive and -negative bacteria, as well as cytotoxicity against a carcinoma cell, catechins should be further surveyed for their biological activity.

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