Antibacterial and antifungal activity of scabronine G and H in vitro

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Two interesting cyathane-type diterpenoids, scabronines G and H, isolated from the fruiting bodies of the basidiomycete, Sarcodon scabrosus (L.: Fr.) Karst, were tested against the bacteria, Staphylococcus aureus, Bacillus thuringiensis, Escherichia coli, Bacillus megaterium and Bacillus subtilis. Both scabronine G and H showed promising activity in vitro against the five bacteria tested at 1 mg/ml and 100 μg/ml. The degree of antibacterial activity of both scabronine G and H was almost the same as streptomycin. Scabronines G and H were also tested against the pathogenic fungi, Gibberella zeae (Schwein.) Petch, Sclerotinia sclerotiorum (Lib.) de Bary, Fusarium moniliforme Sheldon, Fusarium oxysporum Schldl., Fusarium oxysporum f. sp. vasinfectum, and Fusarium oxysporum f. sp. capsicum. Both scabronines G and H showed promising activity in vitro against G. zeae, S. sclerotiorum, F. moniliforme, F. oxysporum at 1 mg/ml and showed weak activity in vitro against F. oxysporum f. sp. vasinfectum and F. oxysporum f. sp. capsicum at the same concentration.

Keywords: scabronine G; scabronine H; antibacterial activity; antifungal activity; diterpenoids; mushroom

Introduction
Basidiomycetes could be a good source of new antibacterials and antifungals (Lorenzen and Anke 1998; Luo et al. 2005). Already, a number of antibacterial compounds have been isolated from basidiomycete fungi, including illudin (McMorris and Anchel 1965), collybial (Simon et al. 1995), pleurotin (William et al. 1947), drosophilin A (Marjorie 1952) and frustulosin (Jordan 2004). Work has also shown that gram-positive bacteria are more sensitive to basidiomycete fungi than gram-negative species (Sevilla-Santos et al. 1964). Stroblurin A and oudemansin A are natural fungicidal products found in the basidiomycete fungus Strophilarus tenaceellus (Pers. Ex Fr.) Singer (Anke 1977) and Oudemansiella mucida Hoehn (Musilek et al. 1969), respectively. Mushrooms, as a biological resource, are still an unexplored source of new agricultural chemicals.

Sarcodon scabrosus (L.: Fr.) Karst, which has a strong bitter taste, is a mushroom belonging to the Thelephoraceae. Diterpenoids, sarcodonins A–H and sarcbronines B–F have been previously isolated from this mushroom as the bitter principles (Shibata et al. 1989; Kita et al. 1998). All these diterpenoids possess a cyathane skeleton consisting of angularly condensed five-, six- and seven-membered rings and show a high degree of stimulating activity on nerve growth factor (NGF) synthesis in vitro. Some cyathane-type diterpenoids were also reported to have antibacterial activity (Ayer and Lee 1979; Shibata et al. 1998). Recently, S. scabrosus was shown to be a source of cyathane-type diterpenoids. Scabronines G and H, and sarcodonin I were isolated from the same mushroom (Ma et al. 2004; Ma and Liu 2005). Scabronines G and H (compounds 1 and 2, Figure 1) have the same planar structure; scabronine H is the 11-epimer of scabronine G. As a part of our search for antibacterial compounds from mushrooms in He’nan Province, central China, we herein described the antibacterial and antifungal activities of the two cyathane-type diterpenoids.

Materials and methods
Materials
The dry fruiting bodies of S. scabrosus were immersed in 95% ethanol (EtOH) and left at room temperature for several days. Then, the EtOH extract was decanted and evaporated, and the residue extracted four times with CHCl3. The extract was fractionated by column chromatography (silica gel, petroleum ether/acetone 9:1, 8:2, 7:3 and 6:4, v/v). The fraction (eluted with petroleum ether/acetone 7:3, v/v) was submitted for further purification by reversed-phase column chromatography (RP-8, MeOH/H2O 85:15, v/v) and repeated Sephadex LH-20 (CHCl3/MeOH 1:1, v/v) to give the compounds scabronine G and H, and their structures were elucidated on the basis of spectroscopic studies.

Scabronines G, H and streptomycin were weighed and dissolved in H2O to 1 mg/ml, 100 μg/ml and 10 μg/ml final concentration, respectively, for antibacterial and antifungal tests in vitro.
Physico-chemical properties

Scabronine G, C_{27}H_{36}O_{5}, red oily solid, [α]_{D}^{20} = −18.53° (c = 0.2, CHCl_{3}). UV(CHCl_{3}): 207.8, 233.6 nm. IR(KBr): 3430, 2931, 2867, 1713, 1610, 1525, 1452, 1315, 1177, 1116, 1027, 714. HR-ESI–MS: 463.2460(C_{27}H_{36}O_{5}Na^{+}, calc. 463.2460). 13C-NMR(CD_{3}OD, 125MHz): δ 37.6(C–1), 28.6(C–2), 143.2(C–3), 134.5(C–4), 39.9(C–5), 42.1(C–6), 32.2(C–7), 36.3(C–8), 49.4(C–9), 33.4(C–10), 74.1(C–11), 142.1(C–12), 127.1(C–13), 75.5(C–14), 62.7(C–15), 16.4(C–16), 24.4(C–17), 34.9(C–18), 65.7(C–19), 15.6(C–20), 166.4(CO), 129.8(C–1′), 129.4(C–2′,6′), 128.2(C–3′,5′), 133.0(C–4′).

Scabronine H, C_{27}H_{36}O_{5}, yellow oily solid, [α]_{D}^{20} = −9.08° (c = 0.3, MeOH). UV(MeOH): 204.4, 228.0 nm. IR(KBr): 3421, 2934, 2867, 1719, 1610, 1525, 1452, 1373, 1315, 1176, 1114, 1016, 712. HR-ESI–MS: 463.2464(C_{27}H_{36}O_{5}Na^{+}, calc. 463.2460). 13C-NMR(CD_{3}OD, 125MHz): δ 39.2(C–1), 30.0(C–2), 143.7(C–3), 136.1(C–4), 41.4(C–5), 43.8(C–6), 33.0(C–7), 38.0(C–8), 50.6(C–9), 37.0(C–10), 71.6(C–11), 141.2(C–12), 131.6(C–13), 76.5(C–14), 67.2(C–15), 17.3(C–16), 25.0(C–17), 36.5(C–18), 67.4(C–19), 16.4(C–20), 167.8(CO), 128.6(C–1′), 130.6(C–2′,6′), 129.6(C–3′,5′), 134.2(C–4′).

Antibacterial test of scabronines G and H in vitro

The bacteria Staphylococcus aureus, Bacillus thuringiensis, Escherichia coli, Bacillus megaterium and Bacillus subtilis were inoculated into separate nutrient broths and incubated at 37 °C for 24 h. Then, broth of the test bacterium (0.1 ml) was evenly spread on a nutrient agar plate under sterile conditions.

The required stock solution (20 μl) was absorbed onto sterile filter paper discs (7 mm) and allowed to dry for a few minutes in a sterile Petri dish. Each disc was placed at the center of a nutrient agar plate, which was earlier inoculated with the appropriate bacterium. Filter paper discs having 20 μl of H_{2}O and streptomycin were used as controls. The Petri dishes were incubated at 37 °C. After 24 h, the diameter of any clear inhibition zone around the discs was measured. All experiments were triplicated.

Antifungal test of scabronines G and H in vitro

Scabronines G and H were tested for antifungal activity in vitro by poison food technique. Potato dextrose agar (PDA) was used as the medium for all test fungi. The test pathogenic fungi were Gibberella zeae (Schwein.) Petch, Sclerotinia sclerotiorum (Lib.) de Bary, Fusarium moniliforme Sheldon, Fusarium oxysporum Schltldl., Fusarium oxysporum f. sp. vasinfectum and Fusarium oxysporum f. sp. capsicum.

The medium, incorporating scabronines G and H at concentration of 1 mg/ml (DMSO concentration 1%), was inoculated at the centre with agar discs of test fungi (4 mm diameter), with five replicate plates for each fungus. After incubation for 3–6 days, until fungal growth in the control dishes was almost complete, the mycelial growth of fungi (mm) in both treated (T) and control (C) Petri dishes was measured diametrically in three different direction. The percentage of growth inhibition (I) was calculated using the formula (Sztejnberg et al. 1983):

\[ I(\%) = \left[ \frac{(C - T)}{C} \right] \times 100. \]

Results and discussion

Antibacterial activity of two cyathane-type diterpenoids, scabronines G and H, were tested by assaying against S. aureus, B. thuringiensis, E. coli, B. megaterium and B. subtilis. Both scabronines G and H showed significant activity in vitro against the five tested bacteria at concentrations of 1 mg/ml and 100 μg/ml. To determine the antibacterial sensitivity of scabronine G and H, they were compared with streptomycin under the same conditions. The degree of antibacterial activity of scabronine G and H was almost the same as streptomycin at 1 mg/ml and 100 μg/ml (Figure 2) and 10 μg/ml (Figure 3). Antibacterial sensitivity varied with species but streptomycin was still effective against all five bacteria at 10 μg/ml. However, only E. coli and B. megaterium were sensitive to scabronine G and H at 10 μg/ml. The inhibitory zone diameters of scabronine G against B. Megaterium and E. coli were 14.13 and 10.75 cm at 10 μg/ml. The
inhibitory zone diameters of scabronine H against *B. megaterium* and *E. coli* were 10.08 cm and 9.95 cm at the same concentration.

The antifungal activity of scabronines G and H was assayed against *G. zeae*, *S. sclerotiorum*, *F. moniliforme*, *F. oxysporum*, *F. oxysporum* f. sp. *vasinfectum* and *F. oxysporum* f. sp. *capsicum*. Both scabronine G and H showed strong inhibitory activity in vitro against *G. zeae*, *S. sclerotiorum*, *F. moniliforme*, *F. oxysporum* at 1 mg/ml but weak activity against *F. oxysporum* f. sp. *vasinfectum* and *F. oxysporum* f. sp. *capsicum* at the same concentration (Figure 4). However, scabronines G and H displayed weak antifungal activity to these four fungi only (inhibition of mycelial growth <20%) and were ineffective against the latter two fungal species at 100 μg/ml (inhibition of mycelial growth <5%).

Modern bactericides are generally selective, systemic and curative, achieving control with a limited number of applications and at low rates. However, the development of bactericide resistance is a major problem, leading to the search for new bactericides with different modes of action. In this respect, active cyathane-type diterpenoids isolated from mushrooms may be a potential candidate for development of a new bactericide. This is the first report of the antibacterial activity of scabronines G and H and their activity against plant phytenic fungi.

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