Bioactive Components of the Traditionally used Mushroom Podaxis pistillaris

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In the course of an ethnobotanical study on fungi used in Yemeni ethnomedicine the fungus Podaxis pistillaris (Podaxales, Podaxaceae, Basidiomycetes) was found to exhibit antibacterial activity against Staphylococcus aureus, Micrococcus flavus, Bacillus subtilis, Proteus mirabilis, Serratia marcescens and Escherichia coli. In the culture medium of P. pistillaris three epidithiodiketopiperazines were identified by activity-guided isolation. Based on spectral data (NMR, ESI-MS and DCI-MS) their identity was established as epicorazine A (1), epicorazine B (2) and epicorazine C (3, antibiotic F 3822), which have not been reported as constituents of P. pistillaris previously. It is assumed that the identified compounds contribute to the antibacterial activity of the extract.

Keywords: Epicorazines – antibacterial – cytotoxic – Yemen

Introduction

In our continuing study of plants and fungi used in traditional medicine in Yemen (1–5), we selected the fungus Podaxis pistillaris (L. Pers.) Morse agg. for an in depth investigation. This fungus is spread in semideserts of Africa, Asia, Australia and America. In Yemen it can be found in arid zones. No species of this genus has been found in Europe and Japan (6).

The fruiting bodies of P. pistillaris are used in some parts of Yemen for the treatment of skin diseases, in South Africa as folk medicine against sunburn and in China to treat inflammation (5,7,8). In other countries, e.g. India, Afghanistan and Saudi Arabia, they are used as food (9,10). In Australia, the fungus was used by many desert Aborigines to darken the white hair in the whiskers of old men, for body painting and as a fly repellent (11). The fruiting bodies are known to be rich in proteins containing all essential amino acids, carbohydrates, lipids and minerals (12). Antimicrobial activities against Pseudomonas aeruginosa and Proteus mirabilis have been found (13). On the other hand, there is information from Nigeria and South Africa about a possible toxicity (14). A high value for total lanthanides was measured in this mushroom (75 mg kg⁻¹ dry weight) (15). Cultivation assays of the fruiting bodies have been successful (16,17). But no phytochemical studies of P. pistillaris have been reported till now.

In order to get enough and reproducible material for phytochemical and biological studies we established mycelial cultures of P. pistillaris, expecting that the cultures will produce the same metabolites as the fruiting bodies. Here, we report on the antibacterial and cytotoxic activities of extracts and isolated metabolites, the isolation of three antibacterial epicorazines from the culture medium and the possible implications on safety.

Materials and Methods

General

Thin layer chromatography (TLC) was carried out on silica gel 60 F₂₅₄ plates (Merck) with the solvent EtOAc/toluene/HOAc (7:3:0.1). Chromatograms were inspected under UV light at wavelengths 254 and 366 nm, and by spraying with anisaldehyde–H₂SO₄ reagent. CC was carried out on Sephadex...
LH-20 (Pharmacia Biotech). IR spectra were recorded in KBr on a Perkin-Elmer FTIR 1650 spectrometer and UV spectra in MeOH with a Uvikon 930 spectrometer. 1H- and 13C-NMR spectra were obtained on a Bruker Spectrometer AM 400 with the solvent signal as internal reference. MS spectra were recorded on a Finnigan MAT 95 spectrometer (Finnigan MAT GmbH; Bremen), CD spectra on a circular dichrograph J-710 (Jasco Corp., Tokyo). Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter at room temperature. M.p.s were recorded on a Kofler apparatus.

Fruiting Bodies

Fruiting bodies of *P. pistillaris* were collected from Aden region, Yemen, during the rainy season in February 1999. They were identified by Prof. H. Kreisel (Institute of Microbiology, Ernst-Moritz-Arndt-University, Greifswald). A voucher specimen is deposited at the Department of Pharmaceutical Biology (Ernst-Moritz-Arndt-Universität, Greifswald, Germany).

Pieces of the fruiting bodies were isolated and placed on solid HAGEM medium (18) in Petri dishes. After cultivation for 3 weeks at room temperature, the mycelium was removed from the agar plates and transferred into a liquid medium containing 5% glucose and 5% malt extract in distilled water adjusted to pH 5.4. The cultures were incubated for 3 weeks at 25°C on a rotary shaker (125 r.p.m.).

Extraction and Isolation

The culture medium was separated from the mycelium by filtration and extracted with ethyl acetate. The extract was dried over Na2SO4 and evaporated under reduced pressure. The resulting concentrate (0.5 g) was passed through Sephadex LH-20 using *n*-hexane/CH2Cl2 (1:2) as eluent to give five fractions (A–E). Fraction B (50 mg) was separated on a Sephadex LH-20 column with MeOH as the eluent to yield 2.5 mg of epicorazine A (1) (Fig. 1). Further purification of fraction C (30 mg) on Sephadex LH-20 with CH2Cl2/MeOH (2:1) yielded (1.5 mg) epicorazine B (2) (Fig. 2). Fraction E was separated on a Sephadex LH-20 column with CH2Cl2/MeOH (2:1) and yielded epicorazine C (3) (Fig. 3).

Epicorazin A (1), white crystals, melting point (mp) 197–198°C, TLC: EtOAc/toluene/HOAc (7:3:0.1) *Rf* = 0.72 (brown spot after spraying with anisaldehyde–H2SO4 reagent and heating); UV (MeOH) *λ*max (lg ε): 215 (4.42) and 260 (3.65) nm. IR (KBr) νmax: 3360, 1690, 1410, 1380, 1365, 1250, 1195, 1185, 1140, 1095, 1030 and 810 cm–1; NMR (Table 2); MS: EI-MS (200°C): m/z (%): 420 (0.5) M+ 356 (100) (M – S2)–, (+)-DCI (NH3): m/z (%) = 420 (100) (M+) 356 (22) (M – S2)–, (+)-DCI (NH3): m/z (%) = 438 (21) (M + NH3)+, 421 (5) (M + H)+, 357 (43) (M + H – S2)+, 296 (100).

Epicorazin B (2), white crystals, mp 192–193°C, TLC: EtOAc/toluene/HOAc (7:3:0.1) *Rf* = 0.65 (brown spot after spraying with anisaldehyde–H2SO4 reagent and heating); UV (MeOH) *λ*max (lg ε): 220 (2.83) nm. IR (KBr) νmax: 3450,
Table 1. Antibacterial activity of the EtOAc extract from the culture medium of Podaxis pistillaris and of epicorazines in agar diffusion assay

| Strain                           | EtOAc extract 1 mg per disk | Epicorazine A 100 µg per disk | Epicorazine B 100 µg per disk | Epicorazine C 100 µg per disk |
|----------------------------------|-----------------------------|--------------------------------|--------------------------------|--------------------------------|
| Staphylococcus aureus ATCC 29213| 30 ± 3                      | 35 ± 4                          | 30 ± 3                         | 25 ± 3                         |
| Micrococcus flavus SBUG 16       | 20 ± 2                      | 32 ± 3                          | 30 ± 2                         | 22 ± 2                         |
| Bacillus subtilis SBUG 14        | 18 ± 2                      | 35 ± 3                          | 32 ± 2                         | 20 ± 2                         |
| Proteus mirabilis SBUG 47       | 19 ± 3                      | 25 ± 2                          | 24 ± 2                         | 20 ± 2                         |
| Serratia marcescens SBUG 9      | 18 ± 1                      | 20 ± 1                          | 20 ± 2                         | 17 ± 2                         |
| Escherichia coli ATCC 25922     | 18 ± 1                      | 25 ± 2                          | 24 ± 3                         | 18 ± 3                         |

n.d. non detected; n = 9.

Table 2. NMR-data of epicorazine A (1) in CDCl₃ (¹H: 300 MHz, ¹³C: 75 MHz)

| H     | δ_H (ppm) | J (Hz)   | C     | δ_C (ppm) | HMBC-correlated H | δ_C" (ppm) |
|-------|-----------|----------|-------|-----------|------------------|-------------|
| —     | —         | —        | —     | —         | —                | —           |
| 2     | 6.14      | dd       | 2.4, 10.2 | 129.30 | d             | 4-OH (163 Hz)b | 129.5       |
| 3     | 6.94      | dd       | 1.9, 10.2 | 150.75 | s             | 4-OH, 4 (165 Hz)b | 151.3       |
| 4     | 4.79      | ddt      | 8.2, 1.6, 1.9 | 71.13 | d             | 4-OH, 5, 6   | 71.3        |
| 4-OH  | 5.84      | d        | 1.3    | —         | —                | —           |
| 5     | 3.81      | dd       | 12.8, 8.2 | 69.43  | d             | 6, 7b (3)    | 69.9        |
| 6     | 3.26      | dd       | 5.6, 12.8 | 48.97  | d             | 7a+b (2, 5)  | 49.2        |
| 7a    | 3.03      | dd       | 12.8, 14.8 | 31.08  | t             | —           | 31.0        |
| 7b    | 2.59      | dd       | 5.6, 14.8 | —      | —             | —           |
| —     | —         | —        | —     | 8         | 75.81           | d           | 7a+b   |
| —     | —         | —        | —     | 9         | 164.27          | s           | 7a      |

a In dioxane see (20), the ¹H-NMR data given by Deffieux et al. were obtained in acetone-d₆, where several signals overlap especially at 90 MHz. According to our spectra their assignment of the partially overlapping 2- and 2'-protons has to be exchanged; b direkt ¹J_B,C coupling.

3360, 1690, 1670 and 1370 cm⁻¹; NMR (Table 3); MS: EI-MS (200°C); m/z (%): 356 (100) (M – S₂)⁺, (–)DCI (NH₃): m/z (%) = 420 (100) (M)⁺, 355 (16) (M – H – S₂)⁺, (–)DCI (NH₃): m/z (%) = 438 (7) (M + NH₄)⁺, 110 (100).

Epicorazin C (3), glassy material, TLC: EtOAc/toluene/HOAc (7:3:0.1) Rᵢ = 0.45 (brown spot after spraying with anisaldehyde–H₂SO₄ reagent and heating), NMR (Table 4); MS: EI (200°C): m/z (%) = 374 (91), 356 (100). (–)DCI (NH₃): m/z (%) = 438 (100), 420 (17), 373 (15). (–)DCI (NH₃): m/z (%) = 456 (100) (M + NH₄)⁺, 150 (100).

Determination of Antibacterial Activity

The following bacterial strains were used: Staphylococcus aureus (ATCC 29213), Micrococcus flavus (SBUG 16), Bacillus subtilis (SBUG 14), P. mirabilis (SBUG 47), Serratia marcescens (SBUG 9) and Escherichia coli (ATCC 25922).

A modified agar diffusion method (19) was used to determine the antibacterial activity. Sterile nutrient agar (Innogenetic, Zellik, Belgium) was poured into Petri dishes to give a solid plate. An aliquot of 40 µl of methanol was added to 1 mg of the dried extract or 100 µg pure compounds) was applied on sterile paper discs (6 mm diameter; Schleicher and Schuell, Ref. No 321860). Methanol was allowed to evaporate and the discs were deposited on the surface of inoculated agar plates. The plates were kept for 3 h in a refrigerator at 6°C to enable prediffusion of the substances into the agar and were then incubated for 24 h at 37°C. Amoxicillin and gentamycin were used as positive and the solvent methanol as negative control. At the end of the incubation time inhibition zone diameters around each of the disc (diameter of inhibition zone minus diameter of the disc) were measured and recorded. An average zone of inhibition was calculated for the three replicates. An inhibition zone of 8 mm or greater was considered as good antibacterial activity.

The minimum inhibitory concentration (MIC) values were determined by standard serial broth microdilution assay in nutrition medium II (SIFIN, Berlin), starting from a 250 µg ml⁻¹ solution. The end point in this assay was indicated by the absence of detectable growth after 18 h of incubation at 37°C.

Determination of Cytotoxic Activity

Cytotoxicity was measured by the neutral red uptake assay using FL-cells, a human amniotic epithelial cell line (1,20). Only living cells are able to manage the active uptake of neutral red. FL-cells were cultivated in a 96-well microtiter plate...
Table 3. NMR data of epicorazine B (3)

| H  | δH (ppm) | m | J (Hz) | C | δC (ppm) | m | HMBC-correlated H | δH (ppm) | m | J (Hz) |
|----|----------|---|--------|---|----------|---|------------------|----------|---|--------|
|    | —        | — | —      | 1 | 193.70   | s | 6, 7a/b          | —        | — | —      |
| 2  | 6.14     | dd | 10.3, 2.0 | 2 | 129.28   | d | —               | 6.07     | dd | 10.3, 2.3 |
| 3  | 6.94     | dd | 10.3, 1.8 | 3 | 150.77   | d | 4-OH, 4         | 6.91     | dd | 10.3, 1.9 |
| 4  | 4.79     | ddt | 8.1, 1.8, 1.8c | 4 | 71.16   | d | 4-OH, 5, 6      | 4.77     | ddt | 8.5, 2.0, 2.0c |
| 4-OH | 5.88     | s | —      | — | —        | — | —               | 5.22     | s | —      |
| 5  | 3.79     | dd | 13.0, 8.1 | 5 | 69.51   | d | 7a/b, 6, 3      | 3.98     | dd | 13.1, 8.5 |
| 6  | 3.26     | dt | 5.7, 12.7 | 6 | 49.02   | d | 7a/b, 2         | 3.41     | dt | 5.8, 12.7 |
| 7a | 3.01     | dd | 15.0, 12.5 | 7 | 30.81   | t | 6               | 2.98     | dd | 14.5, 12.7 |
| 7b | 2.60     | dd | 15.0, 5.7 | 8 | 76.19   | s | 7a/b           | 2.55     | dd | 14.5, 5.8 |
|    | —        | — | —      | 9 | 164.67  | s | 7a              | —        | — | —      |
| 1' | —        | — | —      | 1' | 193.36  | s | 6', 7a/b        | —        | — | —      |
| 2' | 6.17     | dd | 10.4, 2.0 | 2' | 128.41  | d | —               | 6.11     | dd | 10.3, 1.1 |
| 3' | 6.88     | dd | 10.4, 2.3 | 3' | 148.77  | d | 4'             | 6.97     | dd | 10.3, 4.1 |
| 4' | 4.90     | dd | 6.5, 2.4 | 4' | 67.73   | d | 5', 2', 6'      | 5.23     | m | —      |
| 4'-OH | —        | — | —      | — | —        | — | —              | 4.78     | d | 5.7      |
| 5' | 4.68     | dd | 9.7, 6.5 | 5' | 65.82   | d | 7'b, 3', 6'     | 4.67     | dd | 7.2, 4.9 |
| 6' | 3.38     | dddd | 9.7, 9.7, 8.0 | 6' | 44.47   | d | 7'a/b, 2'       | 3.44     | dddd | 8.5, 7.2, 5.6 |
| 7'a | 2.60     | dd | 8.0, 14.6 | 7' | 33.68   | t | 5', 6'         | 2.55     | dd | 5.6, 14.4 |
| 7'b | 3.55     | dd | 9.7, 14.6 | 7' | 33.68   | t | 5', 6'         | 3.36     | dd | 8.5, 14.4 |
|    | —        | — | —      | 8' | 75.88   | s | 5', 7a/b       | —        | — | —      |
|    | —        | — | —      | 9' | 163.76  | s | 7a/b          | —        | — | —      |

Notes:
- In CDCl3 (1H: 400 MHz, 13C: 100 MHz); in acetone-d6 (600 MHz); broad.
- a corresponding OH signal was not observed.

(10^5 cells per ml EAGLE-MEM, Sifin, Berlin; 150 µl per well) at 37°C in a humidified 5% carbon dioxide atmosphere. The EAGLE-MEM was completed by L-glutamine (0.1 g l⁻¹), HEPES (2.38 g l⁻¹), penicillin G (10^6 IE l⁻¹), streptomycin sulfate (0.10 g l⁻¹) and FCS (Gibco; 80 ml l⁻¹). After 24 h, 50 µl of the solution of test substance or medium with equal amounts of ethanol (control) were added. After a further incubation for 72 h the cells were washed three times with phosphate buffered saline (PBS) solution. An aliquot of 100 µl of neutral red solution (SERVA, 0.3% in EAGLE-MEM) was added per well. The cells were then incubated for 3 h at 37°C, followed by another three times washing with PBS. Hundred microliters of a solution of acetic acid (1%, v/v) and ethanol (50%, v/v) in distilled water were added. After shaking for 15 min the optical density was measured at 540 nm with an ELISA-Reader-HT II (Anthos Labtec Instruments, Salzburg). The mean of four measurements for each concentration was determined (n = 3). IC₅₀ values (concentration that caused a 50% inhibition of growth compared with controls) were calculated with the help of micro cal. Origin program.

**Results**

**Biological Activities**

Unlike the extracts from the mycelium (DMC, EtOH, aqueous) the EtOAc extract from the culture medium of *P. pistillaris* showed a strong antibacterial activity against several Gram-positive and Gram-negative bacteria (*S. aureus* ATCC 29213, *M. flavus* SBUG 16, *B. subtilis* SBUG 14, *P. mirabilis* SBUG 47, *S. marcescens* SBUG 9 and *E. coli* ATCC 25922; Table 1). Bioactivity-guided fractionation of this extract led to the isolation and structural elucidation of three pure substances (1–3) related to the antibacterial activity. The compounds were identified as epicorazines A, B and C (see below). In the agar diffusion assay the epicorazines are active against all bacteria tested (Table 1). The MIC against *S. aureus* is 25 µg ml⁻¹ for epicorazine A (1), 50 µg ml⁻¹ for epicorazine B (2) and 75 µg ml⁻¹ for epicorazine C (3) (MIC ampicillin 0.05 µg ml⁻¹). For a further evaluation of the biological activities of extract and isolated compounds their cytotoxicity was measured in the neutral red assay using cultivated human amnion epithelial cells (FL cells). All tested samples showed remarkable cytotoxicity. Ten microgram per milliliter of the extract caused the death of all FL cells. The IC₅₀ against FL cells is 10 µg ml⁻¹ for epicorazin A (1) and B (2).

**Isolation and Structure Elucidation of Epicorazines**

Compound 1 was obtained as white crystals melting at 197–198°C. All well-resolved NMR signals of 1 in CDCl₃ could be assigned and correlated by 1H, 1H-COSY, 1H, 13C-HMQC and 13C-HMBC NMR spectroscopy (Table 2), and thus provided the carbon skeleton from C-1 to C-9. DCI-MS
Table 4. $^1$H-NMR data of epicorazine C (3) (antibiotic F 3822)

| H | DMSO$^a$ | DMSO/(D$_2$O)$^b$ | DMSO$^b,c$ |
|---|---|---|---|
| | $\delta$ | m | J (Hz) | $\delta$ | m | J (Hz) | $\delta$ | J (Hz) |
| 2 | 6.07 | dd | 10, 2 | 6.06 | dd | 10, 1.2 | 6.05 | 6.2 |
| 3 | 6.90 | dd | 10, 2 | 6.89 | dd | 9.9, 1.8 | 6.88 | 10.2 |
| 4-OH | 5.87 | s | — | — | — | — | — | — |
| 4 | 4.68 | d | 9 br. | 4.67 | ddd | 8.5, 2.2, 2.2 | 4.67 | 8.2 |
| 5 | 3.98 | dd | 13, 8 | 3.94 | dd | 13.7, 9.2 | 3.96 | 13.8 |
| 6 | 3.34 | ddd | 13, 12, 6 | 3.34 | ddd | 12.9, 12.6, 6.0 | 3.33 | 13, 12, 6 |
| 7b | 2.53 | m | —$^d$ | 2.54 | m | —$^d$ | 2.55 | 17.5 |
| 7a | 2.84 | dd | 14, 12 | 2.84 | dd | 14.2, 12.7 | 2.86 | 14, 12 |
| 2a | 2.70 | dd | 16, 10 | 2.70 | dd | 16.3, 10.2 | 2.69 | 17, 10 |
| 2′b | 2.53 | m | —$^d$ | 2.54 | m | —$^d$ | 2.51 | 14, 6 |
| 3′ | 3.71 | ddd | 10, 5, 5, 2 | 3.71 | ddd | 10.4, 4.3, 1.8 | 3.76 | 10, 5, 2 |
| 3′-OH | 5.14 | d | 5 | — | — | — | — | — |
| 4′ | 4.78 | ddd | 5, 5, 2, 1 br | 4.77 | d | 4.6 br | 4.73 | 5, 2 |
| 4′-OH | 5.46 | d | 5 | — | — | — | — | — |
| 5′ | 4.40 | dd | 7, 5 | 4.41 | dd | 7.1, 4.6 | 4.43 | 8, 5 |
| 6′ | 3.17 | dddd | 8, 7, 2, 2 | 3.18 | ddd | 7.8, 7.5, 2.4 | 3.18 | 8, 3 |
| 7a | 2.99 | dd | 15, 8 | 2.99 | dd | 14.2, 8.1 | 3.02 | 15, 8, 2 |
| 7b | 2.79 | dd | 15, 2 | 2.79 | dd | 14.5, 2.3 | 2.79 | 15, 3 |

$^a$600 MHz; $^b$400 MHz; $^c$Japan. Pat. 91-227 991; $^d$overlap of 2′b- and 7b-H.

spectrometry indicated a molecular mass of 420. This mass was rarely present in the EI mass spectrum, which was dominated by a fragment ion at $m/z$ 356 corresponding to the loss of $S_2$. With these data, 1 was recognized as the symmetrical epidithiodiketopiperazine antibiotic epicorazine A (1), previously isolated from the fungus *Epicoccum nigrum* (21). Comparison of our data with the $^{13}$C-NMR data of 1 in dioxane-$d_8$ (10) and $^1$H-NMR data in acetone-$d_6$ (22) confirmed this identification, including the same relative configuration, which was indicated by the vicinal coupling constants. Additionally, 1 furnished the same CD curve as given for epicorazine B (2) in (22) and thus showed that these compounds from different sources have the identical absolute configuration.

A second compound 2, isolated as white crystals melting at 192–193°C showed the same molecular mass and fragmentation behavior during mass spectrometry. It was identified as epicorazine B (2) (23) from its NMR data given in Table 3. Although the molecule halves are identical except their chirality, all $^1$H-NMR signals of 2 were well resolved at 400 MHz in CDCl$_3$, and thus provided the complete proton coupling data indicating the relative configuration. The absolute configuration was confirmed by CD spectroscopy.

Further traces of another variant 3 were isolated as glassy material. The molecular ion at $m/z$ 438 suggested the formal addition of water. This was supported by the $^1$H-NMR spectra of 3 in DMSO-$d_6$ and in DMSO-$d_6$ with D$_2$O for H/D exchange (Table 4), where one part of the molecule showed the signals known from 1 while the remaining $^1$H-NMR signals and their correlations from a $^1$H, $^1$H-COSY spectrum identified the other part of 3 as a saturated β-hydroxy-variant. Thus, 3 was recognized as epicorazine C, which has been isolated from *Stereum hirsutum* (24) and as antibiotic F 3822 from a strain of *Epicoccum purpurascens* (25). In order to designate the relative configuration, the coupling constants were studied. The sharp dd signal of 5′-H shows a coupling constant of 5 Hz with 4′-H and of 7 Hz with 6′-H. This indicates a cis-configuration of protons 5′-H and 6′-H. As in 1 the trans-configuration of protons 5-H and 6-H in the other part of 3 is evident by a coupling constant of 13 Hz. Biological activities of isolated compounds.

**Discussion**

Herbal remedies used in the traditional folk medicine provide an interesting and still largely unexplored source for the creation and development of potentially new drugs (26). But it is necessary to reveal the active principles by isolation and characterization of their constituents and to validate their possible toxicity. Up to date, very little research was done to investigate the traditional medicinal plants and fungi in Yemen (1–4).

During our study of mycelial cultures of the widely used mushroom *P. pistillaris*, which has not been phytochemically investigated previously, its antimicrobial constituents were identified as epicorazines A (1), B (2) and C (3). These substances belong to the group of epipolythiopiperazine-2,5-diones (ETPs), an important class of biologically active metabolites produced only by fungi [reviewed in (26)]. They occur as monomers or dimers and are characterized by the presence
of an internal disulphide bridge. Epicorazines could be found only in some strains of *E. nigrum* (21–23), *E. purpurascens* (25,27) and in *S. hirsutum* (24).

ETPs, their mostly investigated representative is gliotoxin, show a broad range of biological activities including antifungal, antibacterial and antiviral activities. In mammalian systems they display potent *in vitro* and *in vivo* immunosuppressive activity, induce apoptosis and possess anticancer activity. They may be produced *in vivo* during the course of fungal infections and contribute to the etiology of the disease. Gliotoxin appears to be a virulence factor associated with invasive aspergillosis of immunocompromised patients. The mode of actions of ETPs seems to be the inhibition of thiol invasive aspergillosis of immunocompromised patients. The broad-spectrum of activities and the toxicity of most members of the ETP class ruled them out for clinical use as antimicrobial, immunosuppressive or anticancer agents till now.

With respect to the traditional medicine of Yemen our investigations confirm the biological activity and justify the ethnomedicinal use of *P. pistillaris*. We assume that the identified epocorazines contribute to the observed antibacterial effects of this mushroom. Because of cytotoxic and other undesired effects of epocorazines the results raise doubts about the usefulness of *P. pistillaris* as food (additive) and medicine. *In vivo* investigations of possible toxicity are strongly recommended. Such cautions are equally applicable to compounds derived from mushrooms (29) and white cedar (30).

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