

CHEMICAL CONSTITUENTS AND CYTOTOXICITY OF SOME TANZANIAN WILD MUSHROOMS

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ABSTRACT

The sterol ergosterol and ergosta-4,22-diene-3 β ,7 α -diol, together with 4-hydroxybenzaldehyde were isolated from the mushroom species Polyporus molluscensis, Cantharellus isabelinus, C. symoensii and a Podaxis species. Their structures were established on the basis of spectroscopic data. The ethanol extracts of mushroom samples of Agaricus sp. and Termitomyces letestui also showed cytotoxicity against the brine shrimp larvae.

INTRODUCTION

Extensive basic and clinical research on mushrooms over the past 20 years has revealed that these fungi have an unfolding number of properties that seemingly provides remarkable health benefits (Nkunya 2002, Konno 2003). In some way, all mushrooms appear to have immunomodulatory, antiviral, antimicrobial and antitumor activities (Konno 2003). These biological activities are attributable primarily to the constituent polysaccharides (or glucans), which have different types of glycosidic linkages (Konno 2003). Some of the polysaccharides are bound to protein residues, forming polysaccharide-protein complexes. The so formed biopolymers are considered to be responsible for the immunomodulatory and anticancer properties of a wide array of mushrooms. Terpenes and steroids are also found in certain mushrooms. These compounds also have anti-inflammatory and anticancer properties (Nkunya 2002). We report the isolation and characterization of two sterols and a benzenoid from some Tanzanian mushrooms, including the cytotoxic properties of some of the crude samples in this investigation.

MATERIAL AND METHODS

General remarks

Mps: uncorrected. Analytical TLC was performed on precoated plates (silica gel 60 F₂₅₄ Merk) using petrol-ethyl acetate as the

eluent; detection by UV and anisaldehyde reagent. For column chromatography (CC) silica gel 60 (Merck) or Sephadex LH-20 (Pharmacia) were used. Vacuum liquid chromatography (VLC) was performed with silica gel (Merck) using petrol with increasing amounts of EtOAc as eluant. FTIR: KBr discs. UV spectra were obtained in MeOH. IR spectra were recorded in CHCl₃ or KBr. NMR spectra were run in CDCl₃ and CD₃COCD₃ solutions at 25^oC, 300 MHz for ¹H NMR and 90 MHz for ¹³C NMR. EIMS were recorded at 70 eV.

Mushroom material

The fruiting bodies used in this study were collected from Iringa, Tanga and parts of the coastal region of Tanzania. The identification was confirmed at the Herbarium of Department of Botany, University of Dar es Salaam where voucher specimens were deposited.

Extraction and Isolation

The fruiting bodies of mushroom species were oven-dried in the field, pulverized and then soaked in hexane, dichloromethane and ethanol for extraction. The filtrates were then dried on a rotary evaporator and subjected to chromatographic fractionation procedures. The crude extracts were separated by vacuum liquid chromatography (VLC) on silica gel, eluting with n-hexane containing increasing amounts of ethyl acetate. Each of the four mushroom species

upon concentration yielded white solids in most fractions, which were purified further by recrystallization to form white needles either containing pure ergosterol (**1**) or mixtures of sterols. Recrystallization using ethyl acetate/pet ether of the combined 13th and 14th VLC fractions of *Cantharellus isabelinus* yielded white crystals of Ergosta-4,22-diene-3 β ,7 α -diol (**2**). The crude extract of *Polyporus moluscensis* was fractionated by VLC on silica gel, eluting with n-hexane containing increasing amounts of ethyl acetate, then ethyl acetate containing increasing amounts of methanol. The combined 5th and 6th VLC fractions of the ethanol extract, on repeated column chromatography on silica gel and further purification on Sephadex[®] LH-20, yielded 4-hydroxybenzaldehyde (**3**).

Ergosterol (1). White needles, m.p. 155-158°C, yield, 27 mg; anisaldehyde: Brick red; MS, m/z (rel. abundance) 396 (M⁺, 100), 363 (85) and 337 (45).

Ergosta-4,22-diene-3 β ,7 α -diol (2). White crystals, m.p. 208-220°C, yield 7 mg; anisaldehyde: Light green; MS, m/z (rel. int.); 414 (M⁺, 3), 384 (68), 379 (41), 337 (6), 269 (20) and 139 (18).

4-Hydroxybenzaldehyde (3). Brown gum, yield, 3.5 mg; anisaldehyde: Red; MS, m/z (rel. int) 122 (M⁺, 80), 121 (100), 93 (45) and 65 (23); ¹H NMR, d 7.85 (d, J = 9 Hz, 2H), 6.95 (d, J = 9 Hz, 2H) and 9.88 (s, 1H); ¹³C NMR, d 130.2 (C-1), 132.3 (C - 2, C - 6), 115.9 (C - 3, C - 5), 161 (C - 4) and 190.7 (CHO).

Brine Shrimp Lethality Bioassays

The cytotoxicity of samples were tested using a test based on brine shrimp (*Artemia salina* Leach) larvae (Mayer 1982).

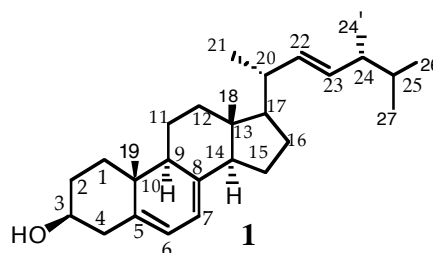
RESULTS AND DISCUSSIONS

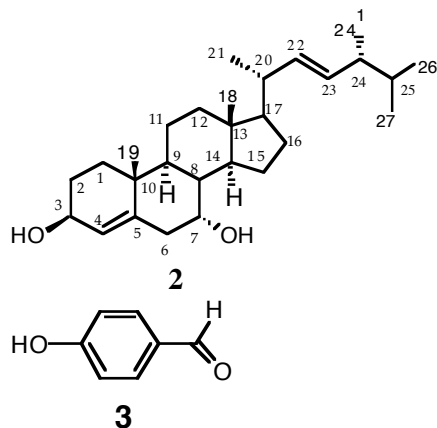
The structures of ergosterol (**1**) and Ergosta-4,22-diene-3 β ,7 α -diol (**2**) and 4-Hydroxybenzaldehyde (**3**) were established on the basis of a combination of

spectroscopic analyses (¹H and ¹³C NMR, and MS) and upon comparison of the observed spectral data with those reported in the literature for this or similar compounds (Chobot 1997). The MS of ergosterol (**1**) exhibited the M⁺ peak at m/z 396 and this, as well as the ¹³C NMR spectrum led to the deduction of the molecular formula C₂₈H₄₄O. For compound **2**, the M⁺ peak at m/z 414 was in agreement with the literature, hence molecular formula C₂₈H₄₆O₂.

Both the ¹H and ¹³C NMR spectra exhibited signals that were consistent with the structure of ergosterol (**1**). The two proton doublet of doublets at d 5.20 could be assigned to olefinic protons of the side chain at C-22 and C-23 as the signal portrayed the characteristic splitting pattern typical of olefinic proton resonances in the side chain moiety in sterols (Chobot 1997).

The ¹³C NMR spectrum of compound **1** consisted of six signals due to sp² carbon atoms at δ 141.36, 139.79, 135.57, 132.99, 119.59 and 116.30, indicating the presence of three double bonds, as in ergosterol. Assignment of the rest of the ¹³C NMR signals was made by comparison of these resonances with those reported in the literature for ergosterol (Chobot 1997).





Both the ^1H and ^{13}C NMR spectra of compound **2** exhibited signals that suggested the presence of two olefinic double bonds instead of three as shown in ergosterol. The ^{13}C NMR spectrum, unlike that of ergosterol, consisted of four signals due to sp^2 carbon atoms at δ 117.92, 132.57, 135.77 and 144.44, indicating the presence of two double bonds instead of three such bonds found in compound **1**.

4-Hydroxybenzaldehyde was another compound obtained from *Polyporus molluscensis*. Quite often wild edible mushrooms possess interesting natural flavours that may be associated with low molecular weight carbonyl compounds. Therefore, 4-hydroxybenzaldehyde may be one of the compounds responsible for the natural flavour of *Polyporus molluscensis*. The crude extracts exhibited significant activity with brine shrimp bioassays. The value that corresponded with the lethal dose that was required to kill 50% of the the shrimp larvae was 19.54 mg/ml, and 59.93 mg/ml for ethanol extracts of *Agaricus sp.* and *Termitomyces letestui* respectively. The high cytotoxicity of *Agaricus* species suggests the reason why this mushroom is not edible in most parts of Tanzania. Further investigations need to be done to follow up

the agents that are responsible for this cytotoxicity.

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