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Chemical Constituents from the Fruit Body of
Chlorophyllum molybdites

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A new glycoxyuracil and 15 known compounds were isolated from the fruit body of *Chlorophyllum molybdites*. The structures were determined from their NMR and MS data and by comparison with literature data. Compounds **2–5**, **10–11**, **13**, and **15–16** were isolated from the genus *Chlorophyllum* for the first time. Compounds (**1–9**) did not show cytotoxicity against two human cancer cell lines, A549 and Panc-28 (GI₅₀ > 50 μ M).

Keywords: *Chlorophyllum molybdites* (Meyer: Fr.) Massee, Agaricaceae, Glycoxy uracil, Chemical constituents, Steroids, Cytotoxicity.

Chlorophyllum molybdites (Meyer. ex Fr.) Mass. (Agaricaceae) is known as false parasol or green-spored parasol. It is a widespread poisonous mushroom of the subtropics and usually grows in lawns and parks [1]. This fungus is a common agent of mushroom poisoning in many countries and is the most commonly consumed poisonous mushroom in North America [2]. It has been reported as a gastrointestinal irritant for over a century [3]. There are numerous and increasing reports of mushroom poisoning worldwide each year [4,5]. Chemical investigations have reported nine steroid derivatives [6a–b], two pyrrolidine alkaloids lepiothins A and B [6c], and α -methylglyceric acid [6d]. Molybdophyllysin, a toxic protein, was also isolated from this mushroom [7]. Some steroids isolated from *C. molybdites* showed cytotoxicity against human gastric carcinoma Kato III cells with IC₅₀ values of 2.69 to 14.27 μ g/mL [6a]. In the course of screening for novel bioactive agents from plants, the new (*S*)-glycoxyuracil (**1**), along with 15 known compounds including three nitrogen heterocycles (**2–4**), five steroids (**5–9**), and seven fatty acids and their derivatives (**10–16**) (Figure 1), were isolated from the fruit body of *C. molybdites*. This is the first time that compounds **2–5**, **10–11**, **13**, and **15–16** were isolated from the genus *Chlorophyllum*. The known compounds were identified as uracil (**2**) [8a], thymine (**3**) [8b], nicotinamide (**4**) [8c], ergosterol (**5**) [8d], ergosterol-3-*O*- β -D-glucopyranoside (**6**) [6a], (22*E*,24*R*)-ergosta-7,22-diene-3 β ,5 α ,6 β -triol (**7**) [6a], (22*E*,24*R*)-5 α ,8 α -epidioxy-ergosta-6,9,22-triene-3 β -ol (**8**) [6a], (22*E*,24*R*)-5 α ,8 α -epidioxyergosta-6,9,22-triene-3 β -ol-3-*O*- β -D-glucopyranoside (**9**) [6a], oleic acid (**10**) [8c], palmitoleic acid (**11**) [8e], palmitic acid (**12**) [8f], palmityl-1-*O*- β -D-glucopyranoside (**13**) [8g], linoleic acid (**14**) [8f], 1-linoleylglycerol (**15**) [8h] and (9*Z*,12*Z*)-1-(9,12-octadecadienoate)- β -D-glucopyranose (**16**) [8g]. None of the tested compounds (**1–9**) showed toxicity against two human cancer cell lines (A549 and Panc-28).

Compound **1** was obtained as a light yellow powder with $[\alpha]_D^{20}$ -34 (c 0.10, MeOH). Its molecular formula was established as C₆H₈N₂O₄ by HR ESIMS. The ¹H NMR spectrum exhibited signals of a pair of olefinic protons at δ_H 7.35 (d, *J* = 8.0) and 5.40 (d, *J* = 8.0), one oxymethine proton at δ_H 3.37 (dd, *J* = 5.4, 5.0), and two oxymethylene protons at δ_H 3.23 (dd, *J* = 11.0, 5.4) and 3.32 (dd, *J* = 11.0, 5.0) that were assigned by HSQC correlations. The ¹³C NMR spectrum with DEPT experiments indicated six C-atom resonances comprising of one OCH₂ group at δ_C 63.6, one OCH

Figure 1: Structures of the isolated compounds (**1–16**).

group at δ_C 73.0, a pair of olefinic carbons at δ_C 100.7 and 142.8, and two carbonyl groups at δ_C 152.1 and 164.9. Detailed analysis of its ¹H- and ¹³C-NMR data, revealed that **1** was a uracil derivative with a glycoxy group. The glycoxy group was attached to N-1 by the ROESY correlation of H-1'/H-6 and the whole structure of **1** supported by the HMBC spectrum (Figure 2). The (*S*)-configuration at position 1' of **1** was determined by comparison of optical rotation values ($[\alpha]_D^{25}$ - 34) with the known compound (*S*)-1-(2,3-dihydroxypropyl) uracil ($[\alpha]_D^{25}$ - 22.3) [9]. Therefore, compound **1** was identified as (*S*)-glycoxyuracil.

Figure 2: Selected HMBC correlations (→) and selected ROESY correlation (↔) of (**1**).

Experimental

General: NMR experiments were performed using a JEOL ECS 400 spectrometer, with spectroscopic data referenced to the solvent used. HR-mass spectra were acquired using a MDS Sciex API

QStar Pulsar mass spectrometer. UV spectra were recorded on a UV 210A spectrophotometer. Optical rotation values were measured on a JASCO P-1010 polarimeter. Octadecyl-functionalized silica gel, silica gel, and TLC plates were purchased from Aldrich Chemical Co. HPLC analysis was performed on an Agilent 1260 HPLC system using Agilent ODS columns (Zorbax SB-C18, 4.6 × 250 mm, 3.5 μm). Doxorubicin (98%) was purchased from Sigma-Aldrich Chemical Co.

Plant material: The fresh fruit bodies of *C. molybdites* were collected in Nacogdoches, Texas, USA, identified by Dr. Shiyu Li and the voucher specimen (TX-Nac-20111115) deposited at the National Center for Pharmaceutical Crops at Stephen F. Austin State University, USA.

Extraction and isolation: Air-dried powdered fruit bodies (965 g) of *C. molybdites* were extracted three times for 3 days each with 10L CHCl₃/MeOH (1:1, v/v) at room temperature in a glass column. After removal of the solvent, the crude extract (60 g) was obtained as brown semi-solid. The residue (52 g) was separated on silica gel with an n-hexane/acetone gradient system to give six fractions (Fr.1–Fr.6). Fr.2 (5 g) was separated on silica gel with a CH₂Cl₂/MeOH gradient system to give three subfractions (Fr.2.1–Fr.2.3). Fr.2.1 (352 mg) was separated on analytical HPLC (CH₃CN/0.1% HOAc in H₂O: 85/15, v/v, 0.7 mL/min) to afford compounds **14** (34 mg, *t_R* 9.7 min) and **10** (22 mg, *t_R* 16.3 min). Compounds **12** (26 mg, *t_R* 19.6 min) and **11** (62 mg, *t_R* 22.1 min) were obtained from subfraction Fr.2.3 (1.1 g) by analytical HPLC (CH₃CN/0.1% HOAc in H₂O: 83/17, v/v, 0.7 mL/min). Repeated crystallization of Fr. 3 (4.3 g) with n-hexane/acetone provided white needles (m.p.=158–160°C) of **5** (785 mg). Fr. 4 (2.2 g) was chromatographed over a silica gel column using CH₂Cl₂/MeOH (30:1 v/v) as eluent to yield compounds **8** (14 mg) and **15** (8 mg). Fr. 5 (8.1 g) was separated by Si gel CC by CHCl₃/MeOH gradient

system to afford seven subfractions Fr.7.5.1–Fr.7.5.7. Compounds **4** (21 mg) and **6** (13 mg) were separated from Fr.5.4 and Fr.5.5, respectively. Compounds **2** (21 mg) and **3** (9 mg) were obtained from Fr.5.6 by silica gel column using acetone as eluent. Fr.5.7 (2.9 g) was separated on RP-C18 gel CC with MeOH/H₂O (50:50 to 100:0, v/v) to afford three subfractions Fr.5.7.1–Fr.5.7.3. Fr.5.7.1 was separated on a Sephadex LH-20 column using MeOH as eluent to yield compounds **7** (6 mg) and **9** (12 mg). Fr.5.7.2 was purified by Si gel CC with n-hexane/acetone to afford compounds **13** (7 mg) and **16** (24 mg). Compound **1** (15 mg) was purified from Fr.5.7.3 on a Sephadex LH-20 column eluting with MeOH.

(S)-glycoxyuracil (**1**)

Light yellow powder.

[α]_D²⁵: -34 (c 0.10, MeOH).

UV/Vis λ_{max} (MeOH) nm (log ε): 208 (3.48), 262 (0.98).

¹H NMR (400 MHz, DMSO-*d*₆): δ 5.40 (*d*, *J* = 8.0 Hz, H-5), 7.35 (*d*, *J* = 8.0 Hz, H-6), 3.37 (*dd*, *J* = 5.4, 5.0, H-1'), 3.23 (*dd*, *J* = 11.0, 5.4, H-2a'), 3.32 (*dd*, *J* = 11.0, 5.0, H-2b').

¹³C NMR (100 MHz, DMSO-*d*₆): δ 152.1 (*s*, C-1), 164.9 (*s*, C-4), 100.7 (*d*, C-5), 142.8 (*d*, C-6), 73.0 (*d*, C-1), 63.6 (*t*, C-2).

HR ESIMS: *m/z* 173.0557 ([*M* + *H*]⁺, C₆H₉N₂O₄, calc. 173.0562).

Cytotoxicity assay: Compounds (**1**–**9**) were assayed for their cytotoxicity against human non-small cell lung cancer cells A549 and human pancreatic cancer cells Panc-28, by WST-8 method, with doxorubicin as a positive control [10].

Supplementary Material: Spectral data and physical properties of Compounds **1**–**16** are available in the online edition of this article.

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