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Article

Crystal Structure Characterization of Natural Allantoin from Edible Lichen *Umbilicaria esculenta*

Baojun Xu [1,*], Changkeun Sung [2] and Byunghee Han [3]

[1] Food Science and Technology Program, Beijing Normal University–Hong Kong Baptist University United International College, Zhuhai, Guangdong 519085, China

[2] Department of Food Science and Technology, Chungnam National University, Taejon 305-764, South Korea

[3] Department of Chemistry, Chungnam National University, Taejon 305-764, South Korea

* Author to whom correspondence should be addressed; E-Mail: baojunxu@uic.edu.hk; Tel.: +86-756-3620636; Fax: +86-756-3620882.

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Abstract: In China, Korea, and Japan, *Umbilicaria esculenta* is considered as both food and medicinal resources from lichen. In the current study, a prismatic crystal was first isolated from edible lichen *U. esculenta* via solvent fractionation. The structure of the crystalline compound was elucidated as allantoin using single-crystal X-ray crystallographic and spectroscopic techniques. In light of the wide use of synthesized allantoin in cosmetic industry as a skin protectant, the biological origin of the allantoin isolated from natural food stuff edible lichen has great potential to be developed into functional cosmetics. Current findings also provided useful information for ecologists to further explore the role of lichen and allantoin in nitrogen metabolism.

Keywords: *Umbilicaria esculenta*; edible lichen; crystal; allantoin; X-ray crystallography

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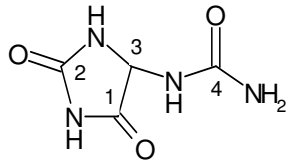
1. Introduction

Traditionally, lichens are used as food, medicine, dye, perfume, and decoration. Medicinally, lichens have been used by many early civilizations. In Europe, records from around the 15th century

suggested that several lichens were in regular medicinal usage. *Umbilicaria esculenta* (Miyoshi) Minks (Umbilicariaceae) is one kind of edible and medicinal lichen. In China, Korea, and Japan, *U. esculenta* is considered a delicacy where it is eaten as a soup or in salads. Previous researches indicated that secondary metabolites of *U. esculenta* possessed broad bioactivities. For instance, anti-tumor cell growth activity of polysaccharide (GE-3) [1], cholesterol synthesis inhibitory activity of gyrophoric acid derivates [2], insect-growth inhibitory activity of atranorin and vulpinic acid [3], anti-HIV activity of polysaccharide (GE-3S) [4], phospholipase A2 inhibitory activity and antitumor activity of lecanoric acid [5,6]. Our investigations on *U. esculenta* led to the first isolation of natural allantoin (**1**) (Figure 1) from this lichen.

Figure 1. Planar structural formula of allantoin.



(**1**)

Allantoin is a pharmacologically active compound [7]. It is a component of the pathway of purine catabolism. Based on the wide use and clinical acceptance of allantoin, as well as published reports in the literature [8-10], allantoin has long been known to enhance the efficacy and desirability of cosmetic creams and lotions through its actions as a skin protectant. The Merck Index lists the therapeutic applications of allantoin as a topical vulnerary and treatment for skin ulcers [11]. Application of allantoin to intact skin on the face and the body leads to a soft, smooth and healthy appearance. The details of the mechanisms of action of allantoin are not fully understood. It is believed that allantoin causes a transient local increase in leucocytes and possibly also improves lymph flow. The US FDA's Tentative Final Monograph on skin protectant drug products for Over-The-Counter (OTC) human use has classified allantoin as a Category I (safe and effective) active ingredient skin protectant. The FDA has approved allantoin skin creams (0.5–2.0%) as non-prescription drug products for: (1) the temporary protection of minor cuts, scrapes, burns, and sunburn; (2) preventing and protecting skin and lips against chapping, chafing, cracking, and wind-burn; and (3) relieving dryness and softening cold sores and fever blisters. It works to promote the healing of tissues within the body. The allantoin is taken internally to promote cell proliferation. It protects tissues in the stomach, accelerates the healing processes throughout the stomach and bowels, and promotes increased tissue repair throughout the entire gastrointestinal tract. The current investigation yielded large amounts of natural crystalline allantoin. Because of the biological origin, it may differ from chemically synthesized allantoin in chemical structure as well as their biological functions. To address our hypothesis, the crystal structure of allantoin from edible lichen was first elucidated here.

2. Results and Discussion

Compound (**1**), was obtained as colorless prismatic crystals (Figure 2B). Its molecular weight 158.13 was determined from its positive FAB-MS spectrum at *m/z* 180.9 $[M + Na]^+$ in DMSO with

NBR (Na), and its X-ray crystal data (Tables 1 and 2): $C_4H_6N_4O_3$, MW = 158.13, crystal size 0.397 × 0.324 × 0.184 mm, orthorhombic a = 8.0004 (6), b = 5.1487 (4), c = 14.7501(10) Å, T = 232 K. The ^{1}H-NMR spectrum gave 6 proton signals at δ 8.06 (1H, s, N_4-$\underline{H}_4$), 6.90 (1H, d, N_3-$\underline{H}_3$), 5.79 (2H, s, N_1-$\underline{H}$1a, 1b), 5.25 (1H, t, N_2-$\underline{H}_2$), and 3.35 (1H, s, C_3-$\underline{H}_3$). The ^{13}C-NMR spectrum gave 4 carbon signals at δ 173.60 (C-4), 157.35 (C-2), 156.76 (C-1), and 62.41 (C-3).

X-ray crystal structure was determined using single crystals and was refined by the least-squares method to give a final R indices R_1 = 3.2%. The molecular structure is presented clearly in Figure 3 as determined by X-ray diffraction analysis. The molecule of (**1**) consists of one five-membered ring, and the selected bond lengths and angles are given in Table 1. The spectral data of compound (**1**) were identical with those of previously reported allantoin (5-ureidohydantoin) [12,13]. The crystal data of natural allantoin isolated from edible lichen were almost identical with those of crystalline structure of *R*,*S*-allantoin (produced by uric acid oxidation) obtained by X-ray diffraction analysis [14], in which the crystal was found to be belong to the $P2_1/C$ space group (a = 8.024, b = 5.153, c = 14.797 Å, β = 93.01), with four molecules in the unit cell, exhibiting an intricate three-dimensional H-bond network in which the three carbonyl oxygens, three imido, and two amino hydrogen atoms take part. The monomeric unit in the crystal was found to have the C_4-N_{10}-C_{11}-O_{14} axis in the *cis* conformation and a conformation around the C_4-N_{10} bond that directs the O_{14} atom to above the heterocyclic ring, so that the molecule assumes a 'scorpion like geometry [14]. The crystal data of natural allantoin isolated from lichen in the current study were also very close to those of crystalline structure of another natural allantoin from plant *Oryza sativa* (rice) [15], in which the crystal was found to be belong to space group $P2_1/c$, with unit cell dimension parameters a = 8.013 (2) Å, b = 5.1458(15) Å, c = 14.770(4) Å, Volume = 608.1(3) Å^3, Z = 4, Calculated density = 1.727 mg/m^3; F (000) = 328, Absorbance coefficient = 0.148 mm^{-1}, final R indices R_1 = 0.0324, wR_2 = 0.0794. The crystal data of natural allantoin from lichen were a little different from those of the allantoin which were chemically synthesized by the oxidation and condensation reactions with ethandial and urea as starting materials [12], the crystalline structure of this synthesized allantoin was also determined by X-ray diffraction. The compound crystallized in the monoclinic space group $P2_1/c$ with the unit-cell parameters: a = 8.025(3) Å, b = 5.160(9) Å, c = 14.795(7) Å, β = 93°, Volume = 611.96 Å^3 and Z = 4. The block least- square refinement for all structural parameters gave final discrepancy factor R = 0.0361.

Figure 2. Photo of experimental materials and isolated crystals: (**A**) fruit bodies of lichen *U. esculenta* (Miyoshi) Minks; (**B**) microscopic photo of isolated crystals.

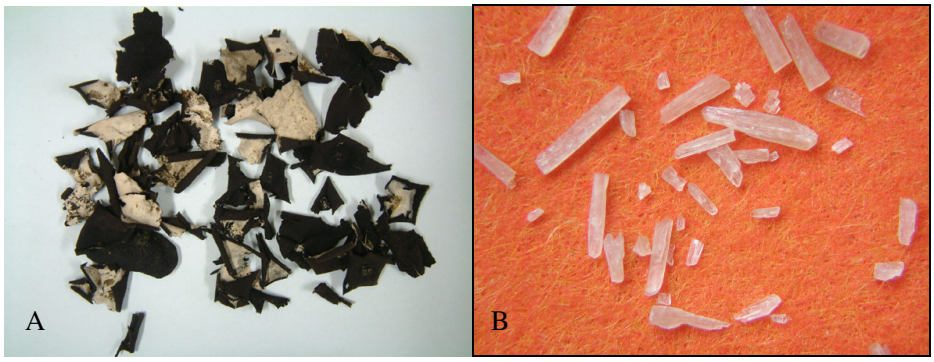


Table 1. Bond lengths [Å] and angles [°] for allantoin from *U. esculenta*.

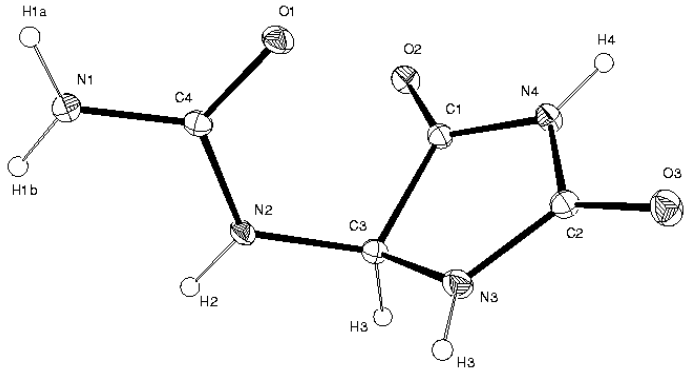
O(1)-C(4)	1.2480(13)	C(3)-N(2)-H(2)	119.8
O(2)-C(1)	1.2167(14)	C(2)-N(3)-C(3)	112.42(9)
O(3)-C(2)	1.2254(14)	C(2)-N(3)-H(3)	123.8
N(1)-C(4)	1.3350(14)	C(3)-N(3)-H(3)	123.8
N(1)-H(1A)	0.8600	C(1)-N(4)-C(2)	111.71(9)
N(1)-H(1B)	0.8600	C(1)-N(4)-H(4)	124.1
N(2)-C(4)	1.3627(14)	C(2)-N(4)-H(4)	124.1
N(2)-C(3)	1.4276(13)	O(2)-C(1)-N(4)	126.89(10)
N(2)-H(2)	0.8600	O(2)-C(1)-C(3)	126.30(10)
N(3)-C(2)	1.3424(15)	N(4)-C(1)-C(3)	106.77(9)
N(3)-C(3)	1.4597(14)	O(3)-C(2)-N(3)	127.87(11)
N(3)-H(3)	0.8600	O(3)-C(2)-N(4)	124.25(10)
N(4)-C(1)	1.3585(14)	N(3)-C(2)-N(4)	107.87(9)
N(4)-C(2)	1.3975(14)	N(2)-C(3)-N(3)	116.17(9)
N(4)-H(4)	0.8600	N(2)-C(3)-C(1)	113.63(9)
C(1)-C(3)	1.5393(15)	N(3)-C(3)-C(1)	100.88(8)
C(3)-H(3)	0.9800	N(2)-C(3)-H(3)	108.6
C(4)-N(1)-H(1A)	120.0	N(3)-C(3)-H(3)	108.6
C(4)-N(1)-H(1B)	120.0	C(1)-C(3)-H(3)	108.6
H(1A)-N(1)-H(1B)	120.0	O(1)-C(4)-N(1)	122.91(10)
C(4)-N(2)-C(3)	120.42(9)	O(1)-C(4)-N(2)	119.99(10)
C(4)-N(2)-H(2)	119.8	N(1)-C(4)-N(2)	117.09(9)

Symmetry transformations used to generate equivalent atoms.

Table 2. Crystal data and structure refinement for allantoin from *U. esculenta*.

Empirical formula	$C_4 H_6 N_4 O_3$
Formula weight	158.13
Temperature	232(2) K
Wavelength	0.71073 Å
Crystal system, space group	monoclinic, $P2_1/c$
Unit cell dimensions	a = 8.0004(6) Å; α = 90°
	b = 5.1487(4) Å; β = 92.9080(10)°
	c = 14.7501(10) Å; γ = 90°
Volume	606.80(8) A^3
Z, Calculated density	4, 1.731 mg/m^3
Absorption coefficient	0.149 mm^{-1}
F(000)	328
Crystal size	0.397 × 0.324 × 0.184 mm
Theta range for data collection	2.55° to 28.33°
Limiting indices	$-10 \le h \le 10$, $-6 \le k \le 6$, $-19 \le l \le 19$
Reflections collected / unique	5873/1505 [R(int) = 0.0142]
Completeness to theta = 28.33	99.7%
Absorption correction	multiple scan
Max. and min. transmission	0.973 and 0.7956
Refinement method	full-matrix least-squares on F^2
Data / restraints / parameters	1505/0/100
Goodness-of-fit on F^2	1.073
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0320$, $wR_2 = 0.0873$
R indices (all data)	$R_1 = 0.0361$, $wR_2 = 0.0918$

Figure 3. Molecular structure of natural allantoin from *U. esculenta*.



Allantoin occurs widely in nature, where it arises from the oxidation of uric acid, while uric acid is created when the organism breaks down purine nucleotides. In humans, uric acid is the final oxidation product of purine metabolism and is excreted in urine. In most other mammals, plants and microorganisms, the enzyme urate oxidase (uricase) oxidizes uric acid to 5-hydroxyisourate, the latter is further oxidized to allantoin by enzyme 5-hydroxyisourate hydrolase (HIUase) [16]. The conversion of uric acid to allantoin is elucidated as the following scheme:

$$\text{Uric acid} + O_2 + H_2O \xrightarrow{\text{Uricase}} \text{5-hydroxyisourate} + H_2O_2 \xrightarrow{\text{HIUase}} \text{allantoin} + CO_2$$

Although it is widely believed that allantoin is the product of the urate oxidase reaction, it has been demonstrated that it is not the immediate product of the enzyme reaction, and the details of the biogenesis of allantoin remain unknown [17]. Non-enzymatic decomposition of optically active 5-hydroxyisourate generate racemic allantoin [18], and it has been reported that enzyme allantoinase, which catalyzes the conversion of allantoin to the allantoate, is specific for the *S*-enantiomer of allantoin [19]. Allantoin was first found in edible lichen in our current investigation. We deduce that lichen *U. esculenta* may be rich in active enzymes uricase and HIUase which convert uric acid to allantoin, while absent in allantoinase which is responsible for the degradation of *S*-allantoin; meanwhile certain non-enzymatic decomposition reactions of 5-hydroxyisourate may occur in this lichen, therefore this kind of edible lichen riches in racemic allantoin. However, such deductions need to be further validated from experimental data.

Allantoin plays an essential role in the assimilation, metabolism, transport, and storage of nitrogen in plants; its ecological implications are largely unknown. A few studies have shown that allantoin may act as an agent participating in chemical interactions between plants and other species [15]. Allantoin has been found in numerous plant species [20]. However, to the best of our knowledge, allantoin has not been reported in lichens according to the literature review conducted in the current investigation. Lichens are commensal organisms consisting of a symbiotic association of a fungus with a photosynthetic partner, usually either a green alga or cyanobacterium. Chemical interactions including nitrogen metabolism between fungus and its symbiont are quite complex. The potential mechanism to explain the reason edible lichen is enriched in allantoin will be the subject of further studies. Chemically synthesized allantoin is widely applied in the cosmetic and pharmaceutical sectors; however, the biological origin natural allantoin from edible lichen possesses much higher qualities for use in the cosmetic and pharmaceutical industry.

3. Experimental

3.1. Materials

Edible lichen *Umbilicaria esculenta* (Miyoshi) Minks (Figure 2A) were provided by Illssan Co., South Korea, and identified by Dr. Changtian Li at College of Chinese Medicinal Materials, Jilin Agricultural University, Changchun, China.

3.2. Extraction and isolation of natural allantoin from U. esculenta

The dried powdered *U. esculenta* (500 g) were extracted with 75% ethanol at room temperature for 48 h, the ethanol extract was concentrated *in vacuum* to gum, the concentrated gum was dissolved in 1 liter of water, 5 fold volume of anhydrous ethanol was added into water solution, and then was deposited overnight to make precipitation. After removing precipitation by centrifugation, the supernatant was concentrated *in vacuum* to 500 mL, the concentrated solution was deposited at room temperature for a couple of days. A few days later, colorless prismatic crystals were found at the bottom of the flask, the crystals were collected (4.98 g) and re-crystallized from aqueous DMSO. The twice-crystallized colorless prismatic crystals (**1**) were used for X-ray crystallography and spectral analyses.

3.3. X-ray crystallographic analysis

X-ray diffraction analysis was performed on a Multi Purpose X-ray Diffractometer (D/MAX-2200 Ultima, Rigaku International Corporation, Japan). Data collection and structure determinations of X-ray diffraction analysis are listed in Table 2 and the supplemental tables. The crystallographic data for compound (**1**): $C_4H_6N_4O_3$, *FW* = 158.1, monoclinic, space group $P2_1/c$, a = 8.0004(6) Å, b = 5.1487(4) Å, c = 14.7501(10) Å, α = 90°, β = 92.9080 (10)°, γ = 90°, V=606.80(8) Å^3, Z = 4, D_c = 1.731 mg/cm^{-3}, μ (Mo K_α) = 0.149 mm^{-1}, 5873 reflections collected, 1505 independent reflections, $R_1[I > 2\sigma(I)]$ = 0.032, $wR_2[I > 2\sigma(I)]$ = 0.0873, crystal dimensions 0.397 × 0.324 × 0.184 mm^3. Some bond lengths and angels are listed in Table 1.

3.4. Spectral analyses

^{1}H-NMR and ^{13}C-NMR spectra were recorded (in supplemental spectral data) using a Varian Unity 600 NMR Spectrometer (600 MHz for ^{1}H, 150 MHz for ^{13}C) at room temperature and chemical shifts in DMSO-d_6 were given in ppm relative to tetramethylsilane as internal reference. Mass spectra were determined with a Jeol Mass Spectrometer (JMS-HX110/110A, Jeol Ltd., Japan) equipped with FAB ion source. FT-IR spectrum was recorded on a Jasco FT/IR-5300 spectrometer using potassium bromide (KBr) pellets. Compound (**1**): $C_4H_6N_4O_3$, colorless prismatic crystal (in water), m.p. 219–220 °C. Positive FAB-MS: *m/z* 180.9 $[M + Na]^+$ in DMSO with NBR (Na). IR νmax (KBr) cm^{-1}: 3425, 3340, 3125, 3060, 1810, 1740, 1680, and 1560. ^{1}H-NMR (600 MHz, DMSO-d_6) chemical shift assignments were δ 8.06 (1H, s, N_4-$\underline{H}_4$), 6.90 (1H, d, N_3-$\underline{H}_3$), 5.79 (2H, s, N1-$\underline{H}$1a, 1b), 5.25 (1H, t, N_2-$\underline{H}_2$), and 3.35 (1H, s, C_3-$\underline{H}_3$). ^{13}C-NMR (125 MHz, DMSO-d_6) chemical shift assignments were δ 173.60 (C-4), 157.35 (C-2), 156.76 (C-1), and 62.41 (C-3).

4. Conclusions

To summarize, a natural crystalline compound (1) was first isolated from lichen *U. esculenta*. The molecular structure of the crystalline compound was elucidated as allantoin using X-ray crystallographic analysis along with spectral analyses. The biological origin allantoin from edible lichen possesses much higher qualities for use in the cosmetic and pharmaceutical sectors as compared to chemically synthesized allantoin. The current finding of allantoin in lichen may attract ecologists' attention to further investigate the roles of lichen in nitrogen cycle and the roles of allantoin in nitrogen metabolism. The potential mechanism to explain the reason lichen is enriched in allantoin will be the subject of further studies.

Supplementary Material

Supplementary data associated with this article can be found in the online version at doi:10.3390/cryst1030128.

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