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Astraodoric Acids A−D: New Lanostane Triterpenes from Edible Mushroom *Astraeus odoratus* and Their Anti-*Mycobacterium tuberculosis* $H_{37}Ra$ and Cytotoxic Activity

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Supporting Information

ABSTRACT: Tuberculosis (TB) is one of the chronic infectious diseases caused by *Mycobacterium tuberculosis* that causes about 2−3 million deaths per year. Isoniazid and rifampicin are examples of first line drugs used for TB treatment; however, they are potentially hepatotoxic. More effective and safer drugs are urgently needed, especially from natural products. Basidiomycete mushrooms are known as important sources of pharmaceutically active metabolites including an anti-TB agent. In this work, the chemical constituents of the edible mushroom *Astraeus odoratus* were isolated and investigated for antibacterial activity against *M. tuberculosis* $H_{37}Ra$. The cytotoxic activity against cancerous cell lines was also evaluated. Four new lanostane triterpenes, astraodoric acids A−D, and new 5-hydroxyhypaphorine have been isolated together with four known compounds. The structures were elucidated by NMR spectroscopic methods, HR-ESI-MS results, and X-ray crystallographic analysis. Astraodoric acids A and B exhibited moderate antibacterial (MICs of 50 and 25 μg/mL) and cytotoxic activities (IC_{50} values of 34.69 and 18.57 μg/mL against KB and 19.99 and 48.35 μg/mL against NCI-H187), respectively. The results of this study show that *A. odoratus* could be a significant natural source for safer antitubercular and anticancer agents.

KEYWORDS: *Astraeus odoratus, lanostane triterpene, astraodoric acids A−D, hypaphorine, antituberculosis, cytotoxic activity*

INTRODUCTION

Tuberculosis (TB) is a condition caused by an infection of *Mycobacterium tuberculosis.* One third of the world's population is latently infected, and it is responsible for the death of at least 2−3 million per year.[1,2] The treatment of TB requires the long-term usage of multidrugs, that is, at least two of first-line antibiotics, for example, isoniazid, rifampicin, pyrazinamide, and ethambutol, to prevent the risk of drug resistance. Recent studies[3,4] have shown that this could lead to drug-induced hepatotoxicity, which would be more severe in patients with hepatitis or HIV. The emergence of multidrug-resistant strains has also raised concerns for the difficulty of treating TB in the future.[5] Therefore, the need for new antitubercular agents is urgent, especially those from natural products, to overcome the obstacle of side effects. Medicinal mushrooms[6] have been long exploited throughout Asia. Mushrooms such as *Gonoderma*, *Lentinus*, *Tramete*, and *Argaritus* spp. have played an important role in medicinal remedies, producing a variety of biological activities such as antimicrobial, anticancer, antidiabetes, and hepatoprotective activities.[6−8] There are approximately 7000 unidentified species[7] of mushrooms that have never been investigated for their pharmaceutically active metabolites, and very little is known so far for those that have been identified. Therefore, basidiomycete-edible mushrooms are crucial sources of biological metabolites that have yet to be discovered. Our work is a part of the search for bioactive compounds including antitubercular agents from the edible mushrooms of *Astraeus* spp. in Thailand.

Astraeus[9] belonging to Astraeaceae family (Sclerodermatineae suborder, Boletales order, Gasteromycetes class, and Basidiomycota phylum) can be found in many areas of the world. The most well-known distinctive feature of *Astraeus* spp. is the star-shaped split of the exoperidium that occurs when it reaches maturity. This is why it is also known as the false earthstar mushroom. There are currently five known species including *Astraeus hygrometicus*, which is found throughout the world; *Astraeus pteridis*, which is found only in North America and the Canary Islands; and *Astraeus asiaticus* (similar to *Astraeus thailandicus*), *Astraeus odoratus*, and *Astraeus koreanus* (also known as *Astraeus hygrometricus* var. *koreanus*), which are mainly found in Asia.[10] The chemical constituents and biological activities of *Astraeus* spp. were mostly studied in *A. hygrometicus* and *A. pteridis*. *A. hygrometicus*[11,12] has been used as a hemostatic in Chinese folk medicine, and its constituents exhibited versatile biological activities such as free radical

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scavenging and hepatoprotective activities. Recent studies[13] of *A. pteridis* showed that their chemical constituents exhibited anti-TB activity.

A. asiaticus and *A. odoratus*,[9] known as "Hed Phor Fai and Hed Phor Nang" in the Northeast of Thailand, are widely known for their popularity in Thai food dishes and are one of the most expensive edible mushrooms available. The chemical constituents from *A. asiaticus* have never been reported. The only report[14] of the constituents in *A. odoratus* was C8 volatile compounds, which have never been studied for their biological activities. This paper reports the isolation and structure elucidation of four new lanostane triterpenes, astraodoric acids A–D, one new 5-hydroxyhypaphorine, and four known compounds, namely, ergosterol, astraodorol (artabotryols A), nicotinic acid, and hypaphorine from the edible mushroom *A. odoratus*. The isolated compounds were evaluated for anti-*M. tuberculosis* $H_{37}Ra$. The screening for cytotoxicity against human epidermoid carcinoma (KB), human small cell lung cancer (NCI-H187), and human breast cancer cell (MCF-7) was also investigated.

MATERIALS AND METHODS

General Procedures. Melting points were determined on a Gallenkamp SANYO MPU250BM 3.5 melting point apparatus. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. The chemical structures were analyzed by a combination of spectroscopic methods (UV, IR, and NMR). Functional groups were analyzed using UV and IR techniques. UV spectra were recorded on an Agilent 8453 UV–visible spectrophotometer. IR spectra were recorded on a Perkin-Elmer Spectrum One FT-IR spectrometer. The nuclear magnetic resonance (NMR) spectroscopic data were used to characterize resonance signals of hydrogen 1 (^{1}H) and carbon 13 (^{13}C) within molecules. The resonance signals were recorded as a chemical shift (δ) in ppm. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury Plus 400 spectrometer. The molecular formula was determined in terms of mass to charge ratio (m/z), using electrospray ionization (ESI) mass spectra, which were measured on a micrOTOF Bruker mass spectrometry. X-ray diffraction data were collected at 293(2) K on a Bruker Smart CCD area detector diffractometer using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). Flash column chromatography (FCC) was performed on silica gel 60 (0.063–0.200 and 0.040–0.063 mm, Merck, Germany). Thin-layer chromatography (TLC) was performed using silica gel 60 F_{254} precoated sheets (Merck, Germany). All solvents used in the chromatographic separation were commercial grade and were distilled before being used. Analytical grade solvents were used for crystallization.

Fungus Material. The fresh fruit bodies of *A. odoratus* mushroom were purchased from a local market in Yasothon Province, Northeast of Thailand, in July 2009. It is recognized by its strong odor when it is fresh and has a smooth outer peridium.[9,15] The specimens were deposited at the Microbiology program, Faculty of Science and Technology, Phibulsongkram Rajabhat University, Pitsanulok, Thailand.

Extraction and Isolation. The mushrooms were dried at room temperature and then ground to a powder using a blender. The air-dried powder (1.4 kg) was soaked and extracted with hexane (2 L × 3), and the solutions were filtered and evaporated under reduced pressure to yield crude hexane extract (20.8 g, 1.49%). The extraction of the mushroom residue was continued with EtOAc and then MeOH to yield the EtOAc (13.5 g, 0.96%) and MeOH (95.9 g, 7.41%) crude extracts. All crude extracts were repeatedly fractionated on silica gel using FCC (silica gel, 0.063–0.200 mm) with various stepwise gradient elution and recrystallization to yield the pure compounds. The crude hexane extract was subjected to FCC using silica gel (0.063–0.200 mm) and eluted with a gradient system of hexane/CH_2Cl_2 (0–100%), followed by CH_2Cl_2/EtOAc (0–100%) and then EtOAc/MeOH (0–100%). The collected solutions were combined by TLC analysis into eight fractions (H1–H8). The crude EtOAc and MeOH extracts were individually subjected to silica gel (0.063–0.200 mm) FCC, eluted with stepwise gradient elution of hexane/EtOAc (0–100%), and then eluted with stepwise gradient elution of with EtOAc/MeOH (0–100%). The collected solutions were combined by TLC analysis into fractions E1–E8 and M1–M6, respectively.

Fraction H2, obtained by eluting the crude hexane with CH_2Cl_2/EtOAc (4:1), appeared as a mixture of a white solid and a trace amount of yellow oil and was purified by adding hexane. The yellowish solution was filtered out, and the remaining white solid was recrystallized in MeOH to yield compound **1**. Compound **1** (406 mg, 0.029%) was also obtained from fractions E1, M1, and M2 when fractionated on silica gel (0.040–0.063 mm) FCC, eluted with hexane/EtOAc (1:2). Compound **2** (1.2 g, 0.086%) was obtained from fractions H4, H5, E1, E2, E5, and M1 when eluted with 15% of EtOAc in CH_2Cl_2 on FCC. Fraction H6 was repeatedly purified on FCC, eluted with hexane/EtOAc (3:2) to yield compound **3** (158.1 mg, 0.011%). Compound **4** (352.2 mg, 0.025%) was recrystallized in MeOH directly from fraction E4, which was obtained by eluting crude EtOAc with hexane/EtOAc (3:7). Compound **5** (127.3 mg, 0.009%) was obtained by eluting fraction E6 with EtOAc/MeOH (9:1) on FCC and recrystallizing in MeOH. Compound **6** (58.7 mg, 0.004%) was obtained from fractions M2 and M3 when eluted with hexane/EtOAc (2:1). Compounds **7** (45 mg, 0.003%) and **8** (1.55 g, 0.111%) were obtained when fraction M5 was fractionated on silica gel FCC and then eluted with EtOAc/MeOH (3:7) and EtOAc/MeOH (1:9), respectively. Purification of fraction M6 on silica gel FCC and elution with EtOAc/MeOH (2:8) yielded compound **9** (53.1 g, 0.004%). The physical properties and spectroscopic and HR-ESI-MS data of the isolated compounds are as follows.

Ergosterol (1). White amorphous solid; mp 156–158 °C. IR (KBr): ν_{max} 3428, 1655, 1605, 1459, 1381, 1055 cm^{-1}. ^{1}H and ^{13}C NMR data, see ref 16.

Artabotryols A (2). (3α,22*S*,25*R*)-3-Hydroxy-22,26-epoxylanost-8-en-26-one): white solid; mp 241–243 °C; $[\alpha]_D^{27}$ −24.6 (c = 0.5, $CHCl_3$). UV (CH_2Cl_2): λ_{max} 229 nm. IR (KBr): ν_{max} 3562, 1725, 1457, 1381, 1242, 1184 cm^{-1}. ^{1}H and ^{13}C NMR data, see ref 17. HR-ESI-MS m/z 457.3666 $[M + H]^+$ (calcd for $C_{30}H_{48}O_3$, 457.3666).

Astraodoric Acid A (3). (24*E*,22α)-3-Oxo-22-acetoxylanosta-8,24-dien-26-oic acid: white solid; mp 135–137 °C; $[\alpha]_D^{27}$ −6.6 (c = 0.5, $CHCl_3$). UV (CH_2Cl_2): λ_{max} 231 nm. IR (KBr): ν_{max} 3444, 1735, 1707, 1686, 1644, 1456, 1424, 1373, 1240 cm^{-1}. ^{1}H and ^{13}C NMR data, see Tables 1 and 2 and ref 13. HR-ESI-MS m/z 535.3398 $[M + Na]^+$ (calcd for $C_{32}H_{48}O_5Na$, 535.3398).

Astraodoric Acid B (4). (24*E*,22α)-3-Oxo-22-hydroxylanosta-8,24-dien-26-oic acid: colorless needles; mp 105–107 °C; $[\alpha]_D^{27}$ −9.5 (c = 0.5, $CHCl_3$). UV (CH_2Cl_2): λ_{max} 231 nm. IR (KBr): ν_{max} 3447, 1698, 1650, 1457, 1433, 1376, 1288 cm^{-1}. ^{1}H and ^{13}C NMR data, see Tables 1 and 2. HR-ESI-MS m/z 493.3283 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_4Na$, 493.3283).

Astraodoric Acid C (5). (24*E*,3β,22α)-3,22-Dihydroxylanosta-8,24-dien-26-oic acid: white solid; mp 187–190 °C; $[\alpha]_D^{27}$ −23.6 (c = 0.5, MeOH). UV (MeOH): λ_{max} 203 nm. IR (KBr): ν_{max} 3445, 1692, 1644, 1455, 1427, 1371, 1290 cm^{-1}. ^{1}H and ^{13}C NMR data, see Tables 1 and 2. HR-ESI-MS m/z 495.3450 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_4Na$, 495.3450).

Astraodoric Acid D (6). (24*E*,3α,22α)-3-Hydroxy-22-acetoxylanosta-8,24-dien-26-oic acid: white solid; mp 176–179 °C; $[\alpha]_D^{27}$ −23.5 (c = 0.5, $CHCl_3$). UV (CH_2Cl_2): λ_{max} 230 nm. IR (KBr): ν_{max} 3449, 1723, 1690, 1648, 1454, 1373, 1240 cm^{-1}. ^{1}H and ^{13}C NMR data, see Tables 1 and 2. HR-ESI-MS m/z 537.3545 $[M + Na]^+$ (calcd for $C_{32}H_{50}O_5Na$, 537.3545).

Nicotinic Acid (7). Pyridine-3-carboxylic acid: red amorphous solid; mp 223–224 °C. IR (KBr): ν_{max} 3435, 1712, 1596, 1494 cm^{-1}. ^{1}H and ^{13}C NMR data, see ref 18.

Hypaphorine (8). Pale yellow solid; mp 245–247 °C; $[\alpha]_D^{27}$ +238.9 (c 0.125, H_2O). IR (KBr): ν_{max} 3736, 1630, 1454, 1351 cm^{-1}. ^{1}H and ^{13}C NMR data, see Table 3 and ref 19.

Table 1. ^{13}C NMR Data of Astraodoric Acids A–D (3–6) (400 MHz, $CDCl_3$)

	δ_C of ^{13}C NMR			
no.	3	4	5	6
1	36.0	36.0	35.6	30.1
2	34.6	34.6	27.3	27.9
3	217.9	218.0	78.6	76.0
4	47.4	47.4	38.7	37.6
5	51.2	51.2	50.4	44.2
6	19.4	19.4	20.9	18.1
7	26.3	26.3	27.5	26.0
8	135.2	135.2	134.2	133.9
9	133.2	133.2	134.5	134.7
10	36.9	36.9	36.9	36.9
11	21.0	21.0	18.2	20.9
12	30.9	30.8	26.4	25.6
13	44.4	44.4	44.3	44.4
14	49.9	49.9	49.8	49.9
15	30.8	31.0	31.0	31.0
16	27.9	27.6	30.7	30.7
17	46.9	46.8	46.6	46.9
18	15.7	15.8	15.5	15.5
19	18.7	18.7	18.9	19.0
20	39.6	41.2	41.1	39.6
21	12.9	11.7	11.5	12.9
22	74.7	72.8	72.3	74.8
23	31.8	35.1	34.8	31.8
23′	31.8	35.1	34.8	31.8
24	139.6	141.4	140.1	139.5
25	129.1	128.8	128.8	129.1
26	172.6	172.6	170.5	172.5
27	12.2	12.3	12.2	12.2
28	24.3	24.4	24.0	24.2
29	26.2	26.2	27.7	28.0
30	21.3	21.3	15.2	22.1
31	170.7			170.7
32	21.0			21.0

Table 2. ^1H NMR Data of Astraodoric Acids A–D 3–6 (400 MHz, $CDCl_3$)

	δ_H[a] of ^1H NMR (J, Hz)			
no.	3	4	5	6
1	1.97 (m)	1.96 (m)	1.66 (m), 1.17 (m)	1.42 m
2	2.55 (m), 2.40 (m)	2.60 (m), 2.40 (m)	1.58 (m)	1.92 m, 1.30 m
3			3.16 (dd, 9.6, 6.0)	3.42 brt
4				
5	1.59 (m)	1.60 (m)	1.03 (m)	1.50 m
6	1.60 (m)	1.63 (m)	1.99 (m)	1.59 m, 1.45 m
7	2.06 (m)	2.07 (m)	1.93 (m), 1.29 (m)	2.01 m
8				
9				
10				
11	2.04 (m)	1.99 (m)	1.62 (m), 1.48 (m)	2.02 m
12	1.66 (m)	1.70 (m), 1.24 (m)	2.00 (m)	1.68 m
13				
14				
15	1.21 (m)	1.77 (m)	1.70 (m)	1.73 m, 1.62 m
16	1.99 (m), 1.36 (m)	1.98 (m), 1.35 (m)	1.16 (m)	1.18 m
17	1.63 (m)	1.88 (m)	1.86 (m)	1.55 m
18	0.70 (s)	0.71 (s)	0.66 (s)	0.67 s
19	1.10 (s)	1.10 (s)	0.94 (s)	0.98 s
20	1.56 (m)	1.45 (m)	1.37 (m)	1.52 m
21	0.98 (d, 6.8)	0.94 (d, overlap)	0.89 (d, overlap)	0.97 d (overlap)
22	5.08 (brt, 7.1)	3.85 (brt, 6.2)	3.77 (brt, 6.2)	5.08 brt (7.6)
23	2.52 (m)	2.48 (m)	2.40 (quint, 7.6),	2.54 quint (7.6)
23′	2.33 (m)	2.25 (m)	2.25 (quint, 7.6)	2.36 quint (7.6)
24	6.81 (brt, 7.4)	6.93 (brt, 7.0)	6.81 (brt, 7.0)	6.80 brt (7.2)
25				
26				
27	1.86 (s)	1.85 (s)	1.80 (s)	1.85 s
28	0.86 (s)	0.91 (s)	0.87 (s)	0.86 s
29	1.08 (s)	1.08 (s)	0.94 (s)	0.96 s
30	1.05 (s)	1.06 (s)	0.76 (s)	0.85 s
31				
32	2.04 (s)			2.03 s

[a]Chemical shift values are in ppm.

*5-Hydroxyhypaphorine (**9**).* Colorless needles; mp 253–255 °C; $[\alpha]_D^{27}$ +253.4 (*c* 0.125, H_2O). UV (H_2O): λ_{max} 217, 277 nm. IR (KBr): ν_{max} 3465, 3270, 1634, 1588, 1486, 1389 cm^{-1}. ^1H and ^{13}C NMR data, see Table 3. HRESIMS *m/z* 263.1384 $[M + H]^+$ (calcd for $C_{14}H_{19}N_2O_5$, 263.1384).

Antimycobacterial Assay. Anti-*M. tuberculosis* $H_{37}Ra$ was evaluated at the National Centre for Genetic Engineering and Biotechnology (BIOTEC), Thailand, using Green Fluorescent Protein (GFP)-based fluorescent detection established by Changsen et al.[20] Briefly, $H_{37}Ra$ *gfp* was cultivated on 7H10 agar containing 30 μg/mL kanamycin at 37 °C for 4 weeks or until growth was observed. The starter cultures were further diluted to 1:10 by 7H9 broth and incubated at 37 °C in a 200 rpm shaker until the optical density (OD) at 550 nm was approximately 0.5–1. Titer stocks were determined by colony-forming unit (cfu) assay, and the inoculum density for anti-TB assay was optimized by serial dilution. An assay was carried out in 384-well format in duplicate; the inoculum was approximately 2×10^4 to 1×10^5 cfu/mL/well. Five microliters of test sample (the maximum concentration was 50 μg/mL) was serially diluted in 5% dimethyl sulfoxide (DMSO), followed by addition of 45 μL of cell suspension and then incubated at 37 °C for 7 days. The fluorescence signals were measured on day 7 at wavelengths of 485 and 535 nm. The lowest concentration that inhibited cell growth by 90% was reported as the minimum inhibitory concentration (MIC). Isoniazid (MIC = 0.023–0.046 μg/mL), rifampicin (MIC = 0.003–0.012 μg/mL), streptomycin (MIC = 0.156–0.313 μg/mL), and ofloxacin (MIC = 0.391–0.781 μg/mL) were used as positive controls, and 0.5% DMSO was used as a negative control.

Cytotoxicity Assay. Cancer cell growth inhibition was performed at BIOTEC, Thailand, using a resazurin microplate assay (REMA) against three cancerous cell lines of human epidermoid carcinoma (KB), human small cell lung cancer (NCI-H187), and human breast cancer cell (MCF-7), as described by Brien et al.[21] In brief, cells at the logarithmic growth phase were harvested and diluted in fresh medium to 7×10^4 cells/mL for KB and 9×10^4 cells/mL for NCI-H187 and MCF-7. Next, 5 μL of test samples (the maximum concentration was 50 μg/mL) was added to 384-well plates and serially diluted in 5% DMSO, followed by adding 45 μL of cell suspension and then incubated at 37 °C in a 5% CO_2 incubator. After the incubation period (3 days for KB and MCF-7 and 5 days for NCI-H187), 12.5 μg/mL of 62.5 μg/mL resazurin solution was added to each well, and the plates were further incubated at 37 °C for 4 h. Fluorescence signals were measured on day 7 at wavelengths of 530 and 590 nm. The sample

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Table 3. ^{1}H and ^{13}C NMR Data of Hypaphorine and 5-Hydroxyhypaphorine (400 MHz, CD_3OD)

	hypaphorine		5-hydroxyhypaphorine	
no.	δ_H[a]	δ_C	δ_H[a]	δ_C
1				
2	125.2	8.56 (s)	126.3	8.52 (s)
3	108.8		108.0	
3′	128.2		128.8	
4	119.0	8.98 (d, 8)	103.4	8.36 (d, 2.4)
5	120.1	8.42 (dt, 6.8, 1.2)	150.8	
6	122.7	8.48 (dt, 6.8, 1.2)	112.8	8.10 (dd, 8.8,2.4)
7	112.5	8.72 (d, 8)	113.5	8.61 (d, 8.8)
7′	138.0		132.9	
8	24.6	4.77 (d, 7.6)	24.6	4.69 (d, 8.4)
9	80.5	5.25 (dd, 9.2, 4.8)	80.3	5.20 (dd,8.4, 6)
10	171.7		172.0	
N^+Me_3	52.8	4.62 (s)	53.1	4.62 (s)

[a]Chemical shift values are in ppm, and J values (in Hz) are presented in parentheses.

concentrations that inhibited cell growth by 50% were reported as the inhibitory concentration (IC_{50}). These values were derived from a dose−response curve using six concentrations of 2-fold serially diluted samples by the SOFTMax Pro software. Ellipticine and doxorubicine were used as a positive control for KB (IC_{50} of 0.32 and 0.282 μg/mL) and NCI-H187 (IC_{50} of 1.12 and 0.067 μg/mL). Tamoxifen and doxorubicine were used as a positive control for MCF-7 (IC_{50} of 0.058 and 0.091 μg/mL).

X-ray Diffraction. Astraodoric acid B was recrystallized in MeOH to colorless needles, and the X-ray crystallographic was analyzed. The details are as follows: Light brown prisms 0.21 × 0.21 × 0.24 mm^3 were mounted on a quartz fiber with protection oil. Cell dimensions and intensities were measured at 200 K on a Stoe IPDS diffractometer with graphite monochromated Mo[KR] radiation (λ= 0.71073 Å); θ_{max} = 25.8°; 19655 measured reflections, 4213 independent reflections (R_{int} = 0.072) of which 1819 had $|F_o| > 4\ \sigma\ (F_o)$. Data were corrected for Lorentz and polarization effects and for absorption (T min, max = 0.9703, 0.9839). The structure was solved by direct methods using SIR97; all other calculations were performed with the XTAL system and ORTEP programs. A full-matrix least-squares refinement based on F using the weight of $1/[\sigma^2(F_o) + 0.00015(F_o^2]$ gave final values of R = 0.039, ωR = 0.038, and GOF(F) = 1.46(3) for 352 variables and 2037 contributing reflections. The maximum shift/error = 0.0003 and the max/min residual electron density = 0.61/−0.48 eÅ^{-3}. Hydrogen atoms were observed and refined with a fixed value of their isotropic displacement parameter.

RESULTS AND DISCUSSION

Air-dried powder of *A. odoratus* was successively extracted with organic solvents to yield crude hexane, ethyl acetate, and methanol extracts. All crude extracts were purified repeatedly on FCC, and nine compounds were obtained (Figure 1). Their structures were elucidated on the basis of spectroscopic data including 1D and 2D NMR, HR-ESI-MS spectral data, and X-ray crystallographic analysis.

Compound **1** was obtained as a white amorphous solid. The IR absorption bands at 3428 and 1655 cm^{-1} corresponded to hydroxy (OH) and olefinic bond (C═C) functional groups. The ^{1}H and ^{13}C NMR spectra showed the characteristic signals of a steroid unit, containing 28 carbons with six olefinic carbon signals were observed at δ_C 116.3 (C-7), 119.7 (C-6), 131.9 (C-23), 135.6 (C-22), 139.8 (C-8), and 141.3 (C-5). The ^{1}H NMR spectral data also confirmed olefinic protons at δ_H 5.58 (1H, dd, J = 5.6, 2.4 Hz, H-6), 5.38 (1H, dd, J = 5.6, 2.4 Hz, H-7), 5.22 (1H, dd, J = 15.6, 7.0 Hz, H-22), and 5.18 (1H, dd, J = 15.6, 7.0 Hz, H-23), respectively. The observed resonance signal of oxygenated carbon at δ_C 70.4 (C-3) associated with an oxymethine proton at δ_H 3.66 (1H, m, H-3). A comparison of the ^{1}H and ^{13}C NMR spectral data of compound **1** and known steroids reported in literature[16] confirmed that compound **1** was an ergosterol.

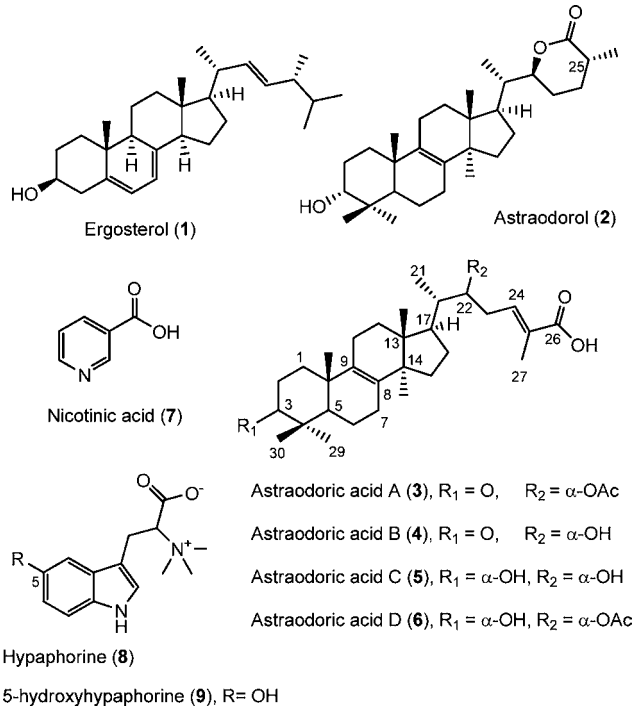

Figure 1. Chemical structures of nine isolated from fruit body of *A. odoratus*.

Compound **2** was obtained as a white amorphous solid. The molecular formula $C_{30}H_{48}O_3$ was deduced from the $[M + H]^+$ peak at m/z 457.3666 in the HR-ESI-MS. The IR spectrum showed the main absorption bands for a hydroxy group (3562 cm^{-1}), a carbonyl of ester (1725 cm^{-1}), an olefinic bond (1650 cm^{-1}), and two bands of C−O of ester (1242 and 1184 cm^{-1}). The ^{13}C NMR spectrum indicated 30 carbons. The signals at δ_C 133.9 (C-8) and 134.7 (C-9) were two olefinic carbons, and 174.9 (C-26) was an ester carbonyl. Two signals were characterized as oxygenated carbons at δ_C 75.9 (C-3) and 84.1 (C-22), which corresponded to the resonance signal of olefinic protons at δ_H 3.41 (1H, brs, H-3) and 4.39 (1H, dd, J = 11.6, 2.4 Hz, H-22). The ^{1}H NMR showed the signals of two secondary methyls at δ_H 1.28 (3H, d, J = 6.8 Hz, H-27) and 0.94 (3H, d, J = 6.4 Hz, H-21) together with five tertiary methyls at δ_H 0.98 (3H, s, H-19), 0.96 (3H, s, H-29), 0.90 (3H, s, H-28), 0.86 (3H, s, H-30), and 0.67 (3H, s, H-18). The ^{1}H and ^{13}C NMR spectra of compound **2** were compared to related literature,[11] and the results suggested a structure similar to that of 3-*epi*-astrahygrol. However, there were differences in the ^{1}H and ^{13}C NMR signals at C-22 to C-25. Therefore, the ^{1}H and ^{13}C NMR data were carefully analyzed and compared to the work done by Fujimoto and co-workers.[22] In their report, the stereochemistries of steroidal lactone derivatives isolated from turtle bile were determined by comparing their ^{1}H and ^{13}C NMR data with the reference compounds of four possible stereoisomers, particularly at C-22 and C-25 on lactone moiety. The result showed that the ^{1}H NMR of compound **2** agreed

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with 22*S*, 25*R* isomers, δ_H 4.39 (1H, dd, J = 11.6, 2.4 Hz, H-22) and 2.38 (1H, ddq, J = 12.3, 6.0, 6.8 Hz, H-25) from the literature.[21] As a result, compound **2** was identified to be a C-25 epimer of 3-*epi*-astrahygrol named astraodorol, found for the first time in *Astraeus* spp. However, a similar structure had been reported as an Artabotryols A,[17] isolated from the seeds of *Artabotrys odoratissimus*. Therefore, compound **2** is a known compound.

Compound **3** was obtained as a white amorphous solid with a molecular formula of $C_{32}H_{48}O_5$, according to the $[M + Na]^+$ peak at m/z 535.3398 in the HR-ESI-MS. The IR spectrum indicated the presence of hydroxy (3444 cm^{-1}), three carbonyl groups of ester (1735 cm^{-1}), ketone (1707 cm^{-1}), conjugated carboxylic acid (1686 cm^{-1}), and an olefinic bond (1644 cm^{-1}). The ^{13}C NMR data (Table 1) suggested that compound **3** possessed a triterpene structure containing a total of 32 carbons. The signals at δ_C 170.7 (C-31), 172.6 (C-26), and 217.9 (C-3) correspond to carboxylic acid, ester, and ketone carbonyl groups. The olefinic carbons signals were observed at δ_C 129.1 (C-25), 133.2 (C-9), 135.2 (C-8), and 139.6 (C-24). The ^1H NMR spectrum (Table 2) showed a high number of methyl groups at δ_H 2.04 (3H, s, H-32), 1.86 (3H, s, H-27), 1.10 (3H, s, H-19), 1.08 3H, s, H-29), 1.05 (1H, s, H-30), 0.98 (3H, d, J = 6.8 Hz, H-21), 0.86 (3H, s, H-28), and 0.70 (3H, s, H-18). The resonance signal of an olefinic proton appeared at δ_H 6.81 (1H, brt, J = 7.4 Hz, H-24), and an oxymethine proton displayed at δ_H 5.08 (1H, brt, J = 7.1 Hz, H-22), which corresponds to oxygenated carbon at δ_C 72.8 (C-22). The comparison of ^1H and ^{13}C NMR spectral data of compound **3** with related literature[11,12] established a lanostane type triterpenes skeleton. The 2D NMR of **3** was analyzed to confirm its structure. The CIGAR spectrum displayed a correlation of H-1 to C-3, and C-10; H-2 to C-1, C-3, and C-10; H-29 to C-5 and C-30; and H-30 to C-3, C-4, and C-29. This confirms that compound **3** consists of a C-3 carbonyl ketone on lanostane ring A. The correlation of H-22 to C-17, C-20, C-21, C-23, C-24, and C-31 and H-32 to C-31 defined the acetyl group attached to C-22 on a side chain. The α-orientation of an acetate group was established by the NOESY correlations of H-18 with H-20 and H-20 with H-22. The correlation of H-23 to C-22, C-24, and C-25; H-24 to C-26, and C-27; H-27 to C-24, C-25, and C-26 represented a conjugated carboxylic acid at the end of a side chain. The absence of a NOESY correlation between olefinic H-24 and H_3-27 suggested the relative configuration of 24*E*. According to the above data, compound **3** (Figure 1) was assumed to be a new compound, which was named astraodoric acid A. There was a report[23] by Yang and co-workers of a similar lanostane triterpenoid structure named kadnanosic acid B. However, there were a few differences in the ^{13}C NMR data that have to be addressed. In their ^{13}C NMR assignment, they assigned the signals at δc 26.3, 21.0, 44.4, and 49.9 to C-11, C-7, C-14, and C-13. In our report, they were assigned to C-7, C-11, C-13, and C-14, respectively. The 1D and 2D NMR data for compound **3** corresponded to the literature[22−24] that reported the related structures that could be explained, for example, at C-14, which bonded to olefinic carbon C-8 (Figure 2). Therefore, C-14 was deshielded according to sp^2 hybridization and some diamagnetic anisotropy from the C-8 double bond, which shifted the C-14 signal more downfield than C-13.[24]

Compound **4** was obtained as colorless needles. Its molecular formula was established as $C_{30}H_{46}O_4$ from the HRESIMS (m/z 493.3283 $[M + Na]^+$). The IR spectrum suggested the presence of hydroxy (3447 cm^{-1}), carbonyl of ketone and carboxylic acid (1698 cm^{-1}), and olefinic (1650 cm^{-1}) groups. The ^1H and ^{13}C NMR (Tables 1 and 2) spectra suggested a lanostane triterpene structure, very similar to astraodoric acid A, except for the absence of an acetyl group resonance signal, which had been replaced by a hydroxyl group at C-22. This was confirmed by an oxymethine proton observed at δ_H 3.85 (1H, br, t, J = 6.2 Hz, H-22) in **4** that appeared at a lower field than that of astraodoric acid A [δ_H 5.08 (1H, brt, J = 7.1 Hz, H-22)]. The relative configuration of the hydroxy group at C-22 was assigned as α on the basis of the NOESY correlations of H-22 with H-18 and H-20. Compound **4** was recrystallized to colorless needles in absolute methanol, and the single crystal X-ray crystallographic was studied. X-ray crystallographic data confirmed the relative configuration at C-22 to be 22*S* and the geometric double bond of 24*E*. The structure of compound **4** was unambiguously confirmed and named astraodoric acid B (Figures 1 and 2b).

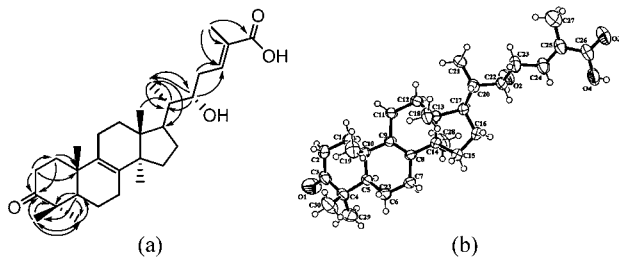

Figure 2. Key long-range correlations (a) and ORTEP drawings (b) of astraodoric acid B.

Compound **5** was obtained as a white amorphous solid. The HR-ESI-MS spectrum exhibited a molecular ion peak at m/z 495.3450 $[M + Na]^+$, which corresponds to the molecular formula $C_{30}H_{48}O_4$. The IR absorption bands indicated the presence of hydroxy (3445 cm^{-1}), carbonyl (1692 cm^{-1}), and olefinic (1644 cm^{-1}) groups. The ^{13}C NMR (Table 1) indicated 30 carbons, which is comparable to astraodoric acid B, except for the oxygenated carbon signal at C-3 that appeared to be shielded and shifted to the higher field region. This suggested that the carbonyl at C-3 in astraodoric acid B was reduced to a hydroxy group in **5**. This in turn was confirmed by two oxymethine protons observed at δ_H 3.85 (1H, br, t, J = 6.2 Hz, H-22) and 3.16 (1H, dd, 9.6, 6.0 Hz, H-3), which corresponded to two signals of oxygenated carbons at δ_C 72.3 (C-22) and 78.6 (C-3). The correlations of H-3 to C-29 and C-30; H-29 to C-3, C-4, C-5, and C-30; H-30 to C-3, C-4, C-5, and C-29 in CIGAR also verified the existence of a hydroxy group at C-3. The orientations of the C-3 and C-22 hydroxy substitutes were assigned as β and α. These assignments came from the comparison of ^1H and ^{13}C NMR to astraodoric acid B and the NOESY correlations of H-3 with H-29 and H-22 with H-20. The β-orientation at C-3 was confirmed by the fact that the C-3 substitute configuration had an impact on the ^{13}C chemical shift of the adjacent carbon.[25] This had a more steric effect on the C-30 signal than on the C-29 signal. Therefore, the β-hydroxy at C-3 shielded C-30 and shifted its signal from about 21 to upfield at 15 ppm. According to the analysis of the above data, compound **5** was then identified as a new lanostane type triterpene, called astraodoric acid C.

Compound **6** was obtained as a white amorphous solid. The HR-ESI-MS indicated the molecular formula to be $C_{32}H_{50}O_5$ from the $[M + Na]^+$ ion peak at m/z 537.3545. The IR bands

indicated the existence of hydroxy (3449 cm^{-1}), carbonyl of ester (1723 cm^{-1}), carbonyl of carboxylic acid (1690 cm^{-1}), and olefinic (1648 cm^{-1}) groups. The ^1H and ^{13}C NMR (Tables 1 and 2) spectral data showed a similar resonance pattern to that of astraodoric acid C, except for an additional two carbons of acetyl moiety at δ_C 21 (C-31) and 170.1 (C-30). The HMBC correlation of H-22 to C-31 and a comparison of ^1H and ^{13}C NMR data to astraodoric acid C confirmed the existence of a α-orientated acetyl group at C-22. An α-orientated hydroxy at C-3 was established through the HMBC correlations of H-3 to C-1 and C-5 and NOESY correlation of H-3 with H_3-30. A comparison of the ^{13}C NMR signal of compound **6** to related literature[25] was confirmed by the fact that the substitute bearing axial was more shielded than the equatorial, which shifted the δ_C of C-3 and C-5 from 78.6 and 50.4 ppm in called astraodoric acid C to 76 and 44.2 ppm in compound **6**. However, the effect on C-30 was less because the resonance signals appeared at δ_C 21.1 ppm. Consequently, compound **6** was a new compound and was named astraodoric acid D.

Compound **7** was obtained as a red amorphous solid. The IR spectrum showed absorption bands that suggested the presence of hydroxy (3435 cm^{-1}), carbonyl of conjugated carboxylic acid (1712 cm^{-1}), and aromatic double bond (1596 cm^{-1}) groups. The ^{13}C NMR spectrum indicated six carbons at δ_C 123.8 (C-5), 127.2 (C-3), 138.1 (C-4), 150.3 (C-2), 152.3 (C-6), and 166.8 (C-7). The ^1H NMR spectrum showed four aromatic protons at δ_H 9.12 (1H, s, br, H-2), 8.67 (1H, d, J = 4.8 Hz, H-6), 8.34 (1H, d, J = 8 Hz, H-4), and 7.45 (1H, dd, J = 7.6, 4.8 Hz, H-5). A comparison of the ^1H and ^{13}C NMR spectral data with related literature[18] led to the conclusion that compound **7** was a nicotinic acid or vitamin B_3.

Compound **8** was obtained as a pale yellow solid. The IR spectrum showed absorption bands of N−H secondary amine (3411 cm^{-1}), carbonyl of carboxylic acid (1620 cm^{-1}), and aromatic double bond (1544 cm^{-1}) groups. The ^1H and ^{13}C NMR spectroscopic data (Table 3) indicated 14 carbon signals. The signal at δ_C 171.7 (C-10) indicated a carboxylic acid carbon. The signals at δ_C 108.8 (C-3), 112.5 (C-7), 119.0 (C-4), 120.1 (C-5), 122.7 (C-6), 125.2 (C-2), 128.2 (C-3a), and 138.0 (C-7a) were characterized to aromatic carbons, which corresponded to resonance signals of aromatic protons at δ_H 8.98 (1H, d, J = 8 Hz, H-4), 8.72 (1H, d, J = 8 Hz, H-7), 8.56 (1H, s, H-2), 8.48 (1H, t, J = 8.0 Hz, H-6), and 8.42 (1H, t, J = 7.6 Hz, H-5). A methine proton appeared at δ_H 5.25 (1H, dd, J = 9.2 Hz, 4.8 Hz, H-9), which corresponded to a nitrogenated carbon at δ_C 80.5 (C-9). Three methyl carbons attached to the nitrogen atom were observed as a singlet signal at δ_C 52.8, and their methyl protons showed resonance signals at δ_H 4.62 (N^+Me_3). By comparing the ^1H and ^{13}C NMR spectra data with those reported from literature,[19] compound **8** was identified as a known hypaphorine.

Compound **9** was obtained as colorless needles. It gave a molecular ion peak at m/z 263.1384 $[M + H]^+$ in the HRESIMS spectrum, which is consistent with the molecular formula $C_{14}H_{18}N_2O_3$. The IR spectrum displayed an N−H secondary amine (3465 cm^{-1}), hydroxy (3270 cm^{-1}), carbonyl of carboxylic acid (1634 cm^{-1}), and aromatic double bond (1588 cm^{-1}) groups. The ^{13}C NMR spectral data (Table 3) suggested 14 carbons with a comparable resonance signal to hypaphorine, except at C-5, which was substituted by a hydroxy group and shifted the carbon signal from δ_C 120.1 to 150.8. This hydroxylation also affected the signals of C-4 and C-5, shifting to upfield according to the resonance effect from a lone pair of electrons on the oxygen of the hydroxy group. According to the analysis of the above data and a comparison of the ^1H and ^{13}C NMR spectra data to hypaphorine, as well as the assignment made by 2D NMR experiments, compound **9** was then identified to be a new compound, a product of hydroxylation of hypaphorine, which was then named as 5-hydroxyhypaphorine.

Nine compounds were achieved from the isolation and purification of the edible mushroom *A. odoratus*. Four of them were known: ergosterol, astraodorol (artabotryols A), nicotinic acid, and hypaphorine. Nicotinic acid has been used to help in the fight against various diseases such as elevated fasting glucose, diabetes, metabolic syndrome, and the treatment of dyslipidemia.[26] In fungi, hypaphorine is an antagonist against an IAA (indole-3-acetic acid) that inhibits the root hair tip growth and hair elongation.[19] Recent studies[27] have shown that hypaphorine extracted from *Impatiens niamniamensis* seeds was an antihyperglycemic agent and showed some activities in streptozotocin-induced diabetic rats. This paper[27] also reviewed previous work where hypaphorine promoted sleep in mice and stimulated the fermentation process in normal male Long−Evans rats that lead to changes in phosphoenolpyruvate carboxykinase activity. Astraodorol and hypaphorine have never been isolated from any *Astraeus* spp. The remaining five new isolated compounds were four lanostane triterpenes astraodoric acids A−D and 5-hydroxyhypaphorine. Lanostane type triterpenes have been found in *A. hygrometicus*[11] and *A. pteridis*,[13] and now, for the first time, in this work, lanostane triterpenes have also been found in *A. odoratus*. The diversity of astraodoric acids A−D resides in the functional group at C-3 (OH or C═O), C-22 (OH or CH_3−C═O), and conjugated carboxylic acid side chain. 5-Hydroxyhypaphorine is a hypaphorine derivative that has a substituted hydroxy group at C-5 on the aromatic ring. It would appear that lanostane triterpenes are common chemical constituents within *Astraeus* spp. However, to confirm this, the chemical constituents of the rest of *Astraeus* spp.[10] need to be studied.

Some of selected compounds were tested for anti *M. tuberculosis* $H_{37}Ra$ and cytotoxic activity against cancerous cell lines (KB, NCI-H187, and MCF-7). The results (Table 4)

Table 4. Anti-*M. tuberculosis* $H_{37}Ra$ and Cytotoxicity of Selected Compounds

		cytotoxicity (IC_{50}, μg/mL)		
compds	anti-TB (MIC, μg/mL)	KB[a]	NCI-H187[b]	MCF7[c]
2	inact[d]	inact[d]	inact[d]	inact[d]
3	50.00	34.69	18.57	inact[d]
4	25.00	19.99	48.35	inact[d]
5	inact[d]	inact[d]	inact[d]	inact[d]
6	inact[d]	31.55	34.15	40.15
8	inact[d]	inact[d]	inact[d]	inact[d]
9	inact[d]	inact[d]	inact[d]	inact[d]
isoniazid[e]	0.023−0.046			
ellipticine		0.320	1.210	
doxorubicin		0.280	0.067	0.058
tamoxifen		0.320	1.210	0.096

[a]Human epidermoid carcinoma in the mouth. [b]Human lung cancer cell. [c]Human breast cancer cell. [d]Inactive at >50.0 μg/mL. [e]Rifampicin (MIC = 0.003−0.012 μg/mL), streptomycin (MIC = 0.156−0.313 μg/mL), and ofloxxacin (MIC = 0.391−0.781 μg/mL).

showed that astraodoric acids A and B exhibited moderate anti-*M. tuberculosis* $H_{37}Ra$ with MICs of 50 and 25 μg/mL, respectively. They also exhibited cytotoxicity against KB and NCI-H187 with IC_{50} values of 34.69 and 18.57 μg/mL for astraodoric acid A and 19.99 and 48.35 μg/mL for astraodoric acid B. Astraodoric acid D has no anti-*M. tuberculosis* $H_{37}Ra$ activity; however, it exhibited slight cytotoxicity against KB, NCI-H187, and MCF-7 with IC_{50} values of 31.55, 34.15, and 40.15 μg/mL, respectively. It should be noted that their cytotoxic activity against normal cell lines was not tested in this work due to the low yield of some of the isolated compounds.

There are well-documented reviews[1,2,28,29] on the diverse classes of natural products that have exhibited anti-TB activity such as polyketides, flavonoids, terpenoids, alkaloids, and peptides that have been discovered from plant, microorganism, and marine sources. These reports mainly covered compounds with anti-TB activity at MICs from 5 to 50 μg/mL. Various triterpene carbon skeletons,[1,28] for example, hopane, cycloartane, ursane, seco-taraxane, oleanan, and lanostane-types, were among the group of potential anti-TB candidates. 3-*epi*-Astrahygrol and 3-*epi*- astrapteridiol, lanostane triterpenes found in *A. ptertidis*[13] with anti-*M. tuberculosis* $H_{37}Rv$ (ATCC27294) at MICs of 34 and 58 mg/mL, were also included. In this report, similar lanostane type skeletons of astraodoric acids A and B were discovered with better anti-*M. tuberculosis* $H_{37}Ra$ activity (Table 4). Interestingly, astraodorol, an epimer of 3-*epi*-astrahygrol, has shown no anti-TB activity. This might be due to the differences in their stereochemistry. Stereochemistry[30] in a chiral pharmacologically active molecule is one of the crucial factors for their specific mode of action. The structures of astraodorol and 3-*epi*-astrahygrol are only different in the C-25 relative configuration. Astraodorol has a 25*R*-configuration and 3-*epi*-astrahygrol has a 25*S*-configuration. To confirm the significance of C-25 stereochemistry to anti-TB activity, astraodorol must be tested against the same *M. tuberculosis* $H_{37}Rv$ (ATCC27294). Modes of action of first line, second line,[31] and clinical trial[32,33] anti-TB drugs have been exclusively studied. However, there is still little or no knowledge of the mechanisms of many antitubercular agents from natural products. Most anti-TB drugs act on inhibiting the enzymes involved in bacterial metabolism, for example, in fatty acid biosynthesis, arabinogalactam, and peptidoglycan biosynthesis, DNA, RNA, and amino acid and protein synthesis.[5,31−33] There have been no studies on the mechanisms of how triterpene antitubercular agents target mycobacteria. With regard to the similarities in the chemical structures of astraodoric acids A−D, the functional groups at C-3 and C-22 appeared to have an influence on their biological activities. A carbonyl group at C-3, in both astraodoric acids A and B, is crucial for their anti-*M. tuberculosis* activity, which may explain the inactivity in astraodoric acids C and D. This implies that the hydroxy or acetyl groups at C-22, in conjunction with a carbonyl group at C-3 in astraodoric acids A and B, correspond to an anti-*M. tuberculosis* ability. In this case, the hydroxy group made astraodoric acid B more active than the acetyl group in astraodoric acid A. However, the hydroxy and acetyl groups at C-22 had no effect or coeffect to anti-TB activity when in conjunction with an OH group at C-3, as in astraodoric acids C and D.

This is the first report on the chemical constituents isolated from *A. odoratus*; four of them were known, that is, ergosterol, astraodorol (artabotryols A), nicotinic acid, and hypaphorine. There were five new compounds, astraodoric acids A−D and 5-hydroxyhypaphorine, none of which have ever been isolated from any other natural source, including *Astraeus* spp. Of the nine compounds isolated, astraodoric acids A and B exhibited antimycobacterial against *M. tuberculosis* $H_{37}Ra$ and cytotoxicity against KB and NCI-H187. Astraodoric acid C also showed slight cytotoxicity against KB, NCI-H187, and MCF-7. On the basis of this preliminary work, it can be concluded that the edible mushroom *A. odoratus* is a potential source of anti-TB and anticancer agents. Further studies are necessary to validate the possibility of pharmaceutical use such as the evaluation of cytotoxicity against normal cell lines, in vivo experiments, and mode of mechanism study.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

IR, 1D and 2D MMR, and MS spectra of astraodoric acids A−D and 5-hydroxyhypaphorine. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) García, A.; Bocanegra-García, V.; Palma-Nicolás, J. P.; Rivera, G. Recent advances in antitubercular natural products. *Eur. J. Med. Chem.* **2012**, *49*, 1−23.

(2) Rogoza, L. N.; Salakhutdinov, N. F.; Tolstikov, G. A. Antitubercular activity of natural products: Recent developments. *Opportunity, Challenge and Scope of Natural Products in Medicinal Chemistry*; Research Signpost: India, 2011; pp 103−120.

(3) Sharma, S. K. Antituberculosis Drugs and Hepatotoxicity. *Infect., Genet. Evol.* **2004**, *12*, 167−170.

(4) Park, W. B.; Kim, W.; Lee, K. L.; Yim, J.-J.; Kim, M.; Jung, Y. J.; Kim, N. J.; Kim, D. H.; Kim, Y. J.; Yoon, J. H.; Oh, M.-D.; Lee, H. S. Antituberculosis drug-induced liver injury in chronic hepatitis and cirrhosis. *J. Infect.* **2010**, *61*, 323−329.

(5) Zhang, Y.; Post-Martens, K.; Denkin, S. New drug candidates and therapeutic targets for tuberculosis therapy. *Drugs Discovery Today* **2006**, *11*, 21−27.

(6) Elisashvili, V. Submerged cultivation of medicinal mushrooms: bioprocesses and products (review). *Int. J. Med. Mushrooms* **2012**, *14*, 211−239.

(7) Lindequist, U.; Niedermeyer, T. H. J.; Julich, W.-D. The Pharmacological Potential of Mushrooms. *Evid. Based Complement. Altern. Med.* **2005**, *2*, 285−299.
(8) Wasser, S. P. Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. *Appl. Microbiol. Biotechnol.* **2011**, *89*, 1323−1333.
(9) Phosri, C.; Martín, M. P.; Sihanonth, P.; Watling, R.; Whalley, A. J. S. Molecular study of the genus *Astraeus*. *Mycol. Res.* **2007**, *111*, 275−286.
(10) Fangfuk, W.; Petchang, R.; To-aanun, C.; Fukuda, M.; Yamada, A. Identification of Japanese *Astraeus*, based on morphological and phylogenetic analyses. *Mycoscience* **2010**, *51*, 291−299.
(11) Lai, T. K.; Biswas, G.; Chatterjee, S.; Dutta, A.; Pal, C.; Banerji, J.; Bhuvanesh, N.; Reibenspies, J. H.; Acharya, K. Leishmanicidal and Anticandidal Activity of Constituents of Indian Edible Mushroom *Astraeus hygrometricus*. *Chem. Biodiversity* **2012**, *9*, 1517−1524.
(12) Biswas, G.; Sarkar, S.; Acharya, K. Hepatoprotective activity of the ethanolic extract of *Astraeus hygrometricus* (Pers.) Morg. *Dig. J. Nanomater. Bios.* **2011**, *6*, 637−41.
(13) Stanikunaite, R.; Radwan, M. M.; Trappe, J. M.; Fronczek, F.; Ross, S. A. Lanostane- type triterpenes from the mushroom *Astraeus pteridis* with antituberculosis activity. *J. Nat. Prod.* **2009**, *71*, 2077−2079.
(14) Kakumyan, P.; Matsui, K. Characterization of volatile compounds in *Astraeus* spp. *Biosci., Biotechnol., Biochem.* **2009**, *73*, 2742−2745.
(15) Phosri, C.; Martín, M. P.; Whalley, A. J. S. The genus *Astraeus* in Thailand. *Mycotaxon.* **2004**, *89*, 453−463.
(16) Soubias, O.; Jolibois, F.; Massou, S.; Milon, A.; Reat, V. Determination of the Orientation and Dynamics of Ergosterol in Model Membranes Using Uniform ^{13}C Labeling and Dynamically Averaged ^{13}C Chemical Shift Anisotropies as Experimental Restraints. *Biophys. J.* **2005**, *89*, 1120−1131.
(17) Gupta, C.; Prasad, S.; Sahai, M.; Asai, T.; Hara, N.; Fujimoto, Y. Artabotryols A-E, new lanostane triterpenes from the seeds of *Artabotrys odoratissimus*. *Helv. Chim. Acta* **2010**, *93*, 1925−1932.
(18) Patel, N. B.; Shalkh, F. M. New 4-Thiazolidinones of Nicotinic Acid with 2-Amino-6-methylbenzothiazole and their Biological Activity. *Sci. Pharm.* **2010**, *78*, 753−765.
(19) Bel-Kassaoui, H.; Lamnaouer, D.; Abdennebi, E. H.; Charrouf, Z.; Bodo, B.; Jossang, A. Toxicity of hypaphorine from *Astragalus lusitanicus*. *Biol. Santé.* **2007**, *7*, 12−19.
(20) Changsen, C.; Franzblau, S. G.; Palittapongarnpim, P. Improved green fluorescent protein reporter gene-based microplate screening for antituberculosis compounds by utilizing an acetamediase promoter. *Antimicrob. Agents Chemother.* **2003**, *47*, 3682−3687.
(21) Brien, J. O.; Wilson, I.; Orton, T.; OPognan, F. Investigation of the alamar blue (resazurin) fluorescent for the assessment of mammalian cell cytotoxicity. *Eur. J. Biochem.* **2000**, *267*, 5421−5426.
(22) Kuramoto, T.; Kameyama, Y.; Kaneda, M.; Shiro, M.; Hoshita, T.; Une, M. Structure and Stereochemistry of the Higher Bile Acid Isolated from Turtle Bile: (22*S*,25*R*)-3α,12α, 15α, 22-Tetrahydroxy-5 -cholestan-26-oic Acid. *Chem. Pharm. Bull.* **2000**, *48*, 53−55.
(23) Yang, J. H.; Wen, J.; Du, X.; Li, X. N.; Wang, Y. Y.; Li, Y.; Xiao, W. L.; Pu, J. X.; Sun, H. D. Triterpenoids from the stems of *Kadsura ananosma*. *Tetrahedron* **2010**, *66*, 8880−8887.
(24) Kim, Y. J.; Park, J.; Min, B. S.; Shim, S. H. Chemical constituents from the sclerotia of *Inonotus obliquus*. *J. Korean Soc. Appl. Biol. Chem.* **2011**, *52*, 287−294.
(25) Ko, H.-H.; Hung, C.-F.; Wang, J.-P.; Lin, C.-N. Antiinflammatory triterpenoids and steroids from *Ganoderma lucidum* and *G. tsugae*. *Phytochemistry* **2008**, *69*, 234−239.
(26) Mcgovern, M. E. Use of nicotinic acid in patients with elevated fasting glucose, diabetes, or metabolic syndrome. *Br. J. Diabetes Vasc. Dis.* **2004**, *4*, 78−85.
(27) Chand, K.; Akanksha; Rahuja, N.; Mishra, D. P.; Srivastava, A. K.; Maurya, R. Major alkaloidal constituent from *Impatiens niamniamensis* seeds as antihyperglycemic agent. *Med. Chem. Res.* **2011**, *20*, 1505−1508.
(28) Okunade, A. L.; Elvin-Lewis, M. P. F.; Lewis, W. H. Natural antimycobacterial metabolites: Current status. *Phytochemistry* **2004**, *65*, 1017−1032.
(29) Kishore, N.; Mishra, B. B.; Tripathi, V.; Tiwari, V. K. Alkaloids as potential anti-tubercular agents. *Fitoterapia* **2009**, *80*, 149−163.
(30) Peepliwal, A. K.; Bagade, S. B.; Bonde, C. G. A Review: Stereochemical consideration and eudismic ratio in chiral drug development. *J. Biomed. Sci. Res.* **2010**, *2*, 29−45.
(31) Janin, Y. L. Antituberculosis drugs: Ten years of research. *Bioorg. Med. Chem.* **2007**, *15*, 2479−2513.
(32) Rivers, E. C.; Mancera, R. L. New anti-tuberculosis drugs in clinical trials with novel mechanisms of action. *Drug Discovery Today* **2008**, *13*, 1090−1098.
(33) Shi, R.; Sugawara, I. Developement of new anti-tuberculosis drug candidates. *Tohoku J. Exp. Med.* **2010**, *221*, 97−106.