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D$_s$-*ERYTHRO*-2-AMINO-3,4-DIHYDROXYBUTANOIC ACID, A CONSTITUENT IN THE EDIBLE MUSHROOM, *LYOPHYLLUM ULMARIUM*

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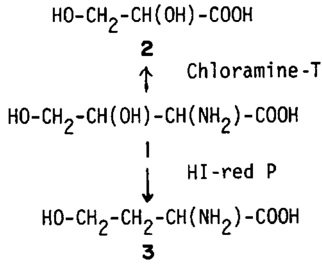
Key Word Index—*Lyophyllum ulmarium*; Tricholomataceae; mushroom; amino acid; D$_s$-*erythro*-2-amino-3,4-dihydroxybutanoic acid.

Abstract—An unusual amino acid occurring in the fruiting bodies of *Lyophyllum ulmarium* was identified as D$_s$-*erythro*-2-amino-3,4-dihydroxybutanoic acid, which is a compound found for the first time in biological materials.

An unidentified ninhydrin-positive compound (**1**) occurring in the fruiting bodies of the edible mushroom, *Lyophyllum ulmarium*, was isolated by ion-exchange chromatography and prep. PC. From elemental analysis and the field desorption mass spectrum (FDMS) (m/z 136 $[M+1]^+$), the molecular formula of **1** was estimated as $C_4H_9NO_4$. The findings that the relative mobility to serine was 1.0 on paper electrophoresis (PE) and **1** became ninhydrin-negative in the presence of Cu^{2+} on PC [1] indicated **1** was a neutral α-amino acid. From this fact and the ^1H and ^{13}C NMR spectra, **1** was deduced to be a 2-aminobutanoic acid derivative (C-3, methine; C-4, methylene).

On GC/MS, TMSi-**1** gave the molecular ion at m/z 423, which indicated the formation of tetra-TMSi-**1** by trimethylsilylation. The spectrum also showed the fragment peak at 218 $[TMSiNH{=}CH{-}COOTMSi]^+$, a diagnostic ion for common α-amino acids [2]. This ion was the half part of the above 2-aminobutanoic acid derivative (C-1 and C-2 unit). The other part, that is, the C-3–C-4 unit, appeared as the fragment ion at m/z 205 $[TMSiOCH_2CHOTMSi]^+$, which showed the presence of OTMSi groups, namely, hydroxyl groups, on the methine and methylene carbon atoms. The presence of the hydroxyl group was supported by the IR spectrum (3300 and 1035 cm^{-1}). Thus, **1** was identified as 2-amino-3,4-dihydroxybutanoic acid. The structure was further confirmed by the chemical degradation shown in Scheme 1, and glyceric acid (**2**) and homoserine (**3**) were formed by the degradation reactions, as expected.

As C-2 and C-3 of **1** are the asymmetric carbon atoms, there are four stereoisomers, D$_s$†-*erythro*- (**1a**), L$_s$-*erythro*- (**1b**), D$_s$-*threo*- (**1c**) and L$_s$-*threo*-**1** (**1d**). These stereoisomers could be readily distinguished from one another by characterization of the optical properties of the products **2** and **3** formed by the above-mentioned chemical degradation reactions. The combinations of the optical isomers of **2** and **3** to be formed from the four stereoisomers of **1** by the degradation are as follows: D$_g^*$-**2**–D$_s$-**3** from **1a**, L$_g$-**2**–L$_s$-**3** from **1b**, L$_g$-**2**–D$_s$-**3** from **1c** and D$_g$-**2**–L$_s$-**3** from **1d**. In the case of the isolated compound, **2** and **3** were proved to have the D$_g$- and D$_s$-configurations, respectively (Table 1). This combination, D$_g$-**2**–D$_s$-**3**, showed the isolated compound to be **1a**. The identity was confirmed by comparison with synthetic **1a**. On the chemical degradation, synthetic **1a** showed the same behaviour as that of the isolated compound. The optical rotations of synthetic **1a** were consistent with those of the isolated one. Further evidence was obtained by the



Scheme 1. Chemical degradation. For detailed procedures, see the Experimental.

Table 1. Optical properties of the degradation products of the isolated compound

Product	Optical property	Ratio to the theoretical value (%)*
2	D$_g$	95.4†
3	D$_s$	94.5

For analytical methods, see the Experimental.

*Ratio to the amounts of the product used for the measurement.

†Values are means of duplicate determinations.

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†Fischer's projection formula.

chromatographic method of Manning and Moore [3], which is a useful tool for the resolution of optical isomers. In this analysis, the L$_s$-leucyl peptides of synthetic **1a** and the isolated compound co-eluted on the amino acid analyser. The other properties of synthetic **1a** coincided with those of the isolated compound in all respects (IR, ^{1}H and ^{13}C NMR, GC/MS and various types of co-chromatography). On the basis of the above evidence, the structure of the isolated compound was established as **1a**, namely, D$_s$-*erythro*-2-amino-3,4-dihydroxybutanoic acid.

Compound **1a** existed in the mushroom in relatively high concentrations (*ca* 1 mg/g fr. wt of the fruiting bodies at the edible stage). The presence of **1d**, that is, the L$_s$-*threo* form of **1**, in a strain of *Streptomyces* [4] and Actinomycin Z_1 [5] has been reported, but this is the first report on the occurrence of the D$_s$-*erythro* form of **1** (**1a**) in nature. This finding may provide some useful knowledge in the fields of investigations of non-protein amino acids, especially of D$_s$-amino acids in biological materials. Moreover, it seems interesting that 2-amino-2-deoxy-D$_g$-erythrose, the corresponding aldehyde of **1a**, was found in the glycoprotein fraction of the cell wall of the fruiting bodies of the cultivated mushroom, *Agaricus bisporus* [6], though **1a** could not be detected in *A. bisporus* in our previous work [7].

EXPERIMENTAL

General methods. GC/MS of TMSi derivatives was carried out under the following conditions: OV-17 (1%, 3 mm × 1 m); He, 30 ml/min; temp. programmed 90–200° at 10°/min; injector and separator at 230° and 260°, respectively; MS, 20 eV. Trimethylsilylation was performed with MeCN–BSTFA (1:1) at 80° for 15 min. Amino acids were determined by the amino acid analyser equipped with the resin-buffer systems I or II described previously [7]. The solvent systems used for PC were as follows: *n*-BuOH–HOAc–H_2O (4:1:1, by vol.; solvent 1), PhOH–H_2O (4:1, w/w; solvent 2). For TLC (cellulose), solvent 3 (*n*-BuOH–Me_2CO–conc NH_4OH–H_2O, 50:6.25:6.25:37.5, by vol. [8]) was used. PE was carried out with a voltage gradient of 15 V/cm in a pyridine–HOAc buffer, pH 3.1.

Mushroom. The freshly harvested fruiting bodies of *Lyophyllum ulmarium* (edible stage: cap diameter, 1.5–2.0 cm; one fruiting body, *ca* 2.5 g fr. wt) were obtained from a commercial grower.

Isolation of **1**. The fruiting bodies of the mushroom (300 g fr. wt) were homogenized and were extracted with EtOH (900 ml). The extract was applied to a column of Amberlite IR-120B (H^+, 500 ml) and the amino acids adsorbed were eluted with 2 M NH_4OH. The amino acid fraction thus prepared was applied to a column of Dowex 1 × 4 (AcO^-, 100 ml) to remove acidic amino acids. The neutral and basic amino acids which passed through the column were chromatographed on a column of Dowex 50 W × 8 (H^+, 50 ml). After washing the column with H_2O (300 ml) and 0.1 M HCl (300 ml), **1** was eluted from the column with 0.12 M HCl (300 ml). The eluate was evaporated to dryness and then the residue was dissolved in a small quantity of H_2O, and was subjected to prep. PC with solvent 1. Compound **1** was further purified by prep. PC with solvent 2 to eliminate serine, a minor contaminant, and was crystallized from H_2O–EtOH (105 mg), mp 195–196° (uncorr.); $[\alpha]_D^{25}$ +11.9° (H_2O; *c* 0.9), $[\alpha]_D^{25}$ −21.8° (2.4 M HCl; *c* 0.9); (Found: C, 36.14; H, 6.95; N, 10.09. $C_4H_9NO_4$ requires: C, 35.55; H, 6.71; N, 10.37%); ^{1}H NMR (100 Mz, D_2O, TMS): δ3.67 and 3.87 (1H, *dd*, *J* = 5, 12 Hz and 1H, *dd*, *J* = 4, 12 Hz, respectively, H-4), 3.91 (1H, *d*, *J* = 4 Hz, H-2), 4.15 (1H, *ddd*, *J* = 4, 4, 5 Hz, H-3); ^{13}C NMR (25.05 MHz, D_2O, TMS): δ57.6 (*d*, C-2), 62.6 (*t*, C-4), 69.4 (*d*, C-3), 171.5 (*s*, C-1); IR ν_{max}^{KBr} cm^{-1}: 1035 (*s*, OH), 1040 (*s*, OH), 1310 (*s*), 1340 (*s*), 1430 (*m*), 1450 (*m*), 1500 (*s*), 1590 (*s*), 1620 (*s*), 1670 (*s*), 3300 (*s*, OH); FDMS *m/z* 136 $[M+1]^+$ (100%); EIMS (GC) of TMSi-1: 20 eV, *m/z* (rel. int.); 423 $[M]^+$ (0.2), 408 $[M-15]^+$ (6), 306 $[M-COOTMSi]^+$ (35), 219 $[TMSiNH{=}CH{-}COOTMSi+1]^+$ (100), 218 $[TMSiNH{=}CH{-}COOTMSi]^+$ (65), 205 $[TMSiOCH_2CHOTMSi]^+$ (44); PC: R_fs 0.18 (solvent 1), 0.20 (solvent 2); TLC: R_f 0.10 (solvent 3); RR_ts of the isolated compound and its L$_s$-leucyl peptide (see the text) on the amino acid analyser: 0.71 (system I, relative to L$_s$-serine) and 0.65 (system II, relative to L$_s$-leucine), respectively.

Chemical degradation. The oxidative degradation of **1** to **2** with chloramine-T and Br_2–HCl and the conversion to **3** with HI–red P were carried out according to ref. [9]. The products **2** and **3** were isolated from the reaction mixtures by prep. PC and ion-exchange chromatography, and were identified by GC/MS as the TMSi derivatives. The quantitative analysis of **2** was performed by MS-fragmentography (*m/z* 408 $[M-15]^+$, the ion derived from its TMSi derivative). The amount of **3** were determined by the amino acid analyser.

Determination of the optical properties of **2** *and* **3**. D$_g$-**2** was assayed with D$_g$-glycerate dehydrogenase (EC 1.1.1.29) according to ref. [10]. The optical isomers of **3** were determined by the method of ref. [3].

Synthesis of **1a**. Synthetic **1a** was prepared from 2-amino-3-hydroxy-4-benzyloxybutanoic acid (**4**) according to ref. [11] with the following modifications. The racemic mixture of D$_s$- and L$_s$-*erythro*-**4** was separated from the mixture of D$_s$- and L$_s$-*threo*-**4** by ion-exchange chromatography (Dowex 50 W × 8, 0.4 M Na-citrate buffer, pH 4.25). By this chromatographic method, both of the racemic mixtures could be completely resolved from each other. Aminoacylase (EC 3.5.1.14) prepared from *Aspergillus oryzae* No. 9 [12] was used for the optical resolution of the *N*-acetyl derivatives of D$_s$- and L$_s$-*erythro*-**4**, instead of the enzyme from Takadiastase. Hydrogenation of D$_s$-*erythro*-**4** prepared by the above method gave **1a**, mp 195–196° (uncorr.) (lit. [11] mp 193–194°); $[\alpha]_D^{25}$ +11.2° (H_2O; *c* 1.0) (lit. [11] +11.3°), $[\alpha]_D^{25}$ −21.3° (2.1 M HCl; *c* 1.0); (Found C, 35.05; H, 6.89; N, 10.15. Calc. for $C_4H_9NO_4$ C, 35.55; H, 6.71; N, 10.37%.) Synthetic **1a** was shown to be identical with the isolated compound by IR, ^{1}H and ^{13}C NMR, MS and chromatographic comparison.

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THE ISOLATION OF LANOSTA-7,9(11),24-TRIEN-3β,21-DIOL FROM THE FUNGUS *GANODERMA AUSTRALE*

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Key Word Index—*Ganoderma australe*; Polyporaceae; wood-rotting fungus; ergosterol palmitate; ergosta-7,22-dien-3-one; ergosterol; lanosta-7,9(11),24-trien-3β,21-diol.

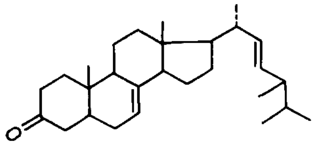
Abstract—The lipid-soluble fraction of the fungus *Ganoderma australe* belonging to the family Polyporaceae has yielded ergosterol palmitate, ergosta-7,22-dien-3-one, ergosterol and lanosta-7,9(11),24-trien-3β,21-diol. This fungus is the second reported natural source of the latter compound whose structure is now established on the basis of spectral data.

INTRODUCTION

The fungus infecting some local trees of a medicinally important host tree *Emblica officinalis*, and identified as *Ganoderma australe* (Specimen No. 8443, incorporated in Forest Research Institute Herbarium, Dehradun, India) belongs to the Polyporaceae. We now report the first chemical study of this fungus, although two other species of the genus *Ganoderma* have been studied recently in some detail. *G. lucidum* yielded L-mannitol, ergosterol and eight polyoxygenated triterpenic acids belonging to the lanostane series [1, 2] which all contain a terminal carboxylic group and some of them display cytotoxic activity *in vitro* on hepatoma cells [2]. The second fungus, *G. applanatum* afforded ergosta-4,6,8(14),22-tetraen-3-one [3], ergosta-7,22-dien-3β-ol, ergosta-7,22-dien-3-one [4], ergosterol and palmitic acid besides the two pentacyclic triterpenes, friedoolean-5-en-3-one and friedelin [5].

RESULTS AND DISCUSSION

The petrol and benzene extracts of *G. australe* were found to have similar components on TLC and were therefore mixed. The total residue was 0.4 % of the weight of the fungus. On column chromatography, four compounds were isolated. The first three compounds eluted were identified as ergosterol palmitate, ergosta-7,22-dien-3-one (**1**) and ergosterol, respectively. The identity of ergosterol palmitate was established by its mass spectrum and hydrolysis to ergosterol. The UV spectrum of compound **1** showed the absence of a conjugated chromophore and the IR band at 1710 cm^{-1} indicated the presence of a carbonyl group. The colour reaction and the general features of its ^{1}H NMR spectrum pointed out its steroidal nature. The mass ion at *m/z* 396 in the mass spectrum gave the molecular formula as $C_{28}H_{44}O$. Three important features were noteworthy in the mass spectrum; the intense peak at *m/z* 43 corresponding to an isopropyl group, the fragment of *m/z* 298 showing the presence of double bond in the C-22 position [6] and a peak at *m/z* 244 indicating the presence of a double bond in the C-7 position [7]. The ^{1}H NMR spectrum also supported the presence of three olefinic protons by a multiplet at δ5.12 and further, it showed the absence of typical signals of a 3α-proton at δ3.25. Finally, the mp of **1** agreed with the lit. mp [4].



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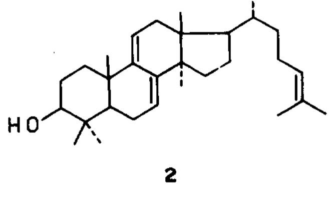

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