in a small vol. of H_2O and subsequently applied to a Dowex 1 × 4 resin column (OAc^- form, 2 × 8 cm). A ninhydrin positive product **2** detected in the 0.3 M HOAc eluate of the column was further purified by fractionation using a Dowex 50w × 2 resin column (pyridine form, 2 × 80 cm). **2** was pptd by adding EtOH to the concd relevant fractions, yielding 29 mg; $[\alpha]_D^{25}$ + 2.7 (H_2O, *c* 0.5), +8.8 (3 M HCl, *c* 0.25); SIMS *m/z*: 174 $[M+1]^+$; ^1H NMR (90 MHz, D_2O, TSP): δ 1.2–1.8 (4H, *m*, H-4, 5), 1.7–2.1 (2H, *m*, H-3), 2.23 (3H, *s*, H-8), 2.63 (2H, *t*, *J* = 7 Hz, H-6), 3.87 (1H, *t*, *J* = 6 Hz, H-2); IR ν_{max}^{KBr} cm^{-1}: 3420, 2940, 1710, 1580, 1512, 1440, 1410, 1320, 1160, 1095, 850, 805, 655, 549.

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β-HYDROXY-L-VALINE FROM *PLEUROCYBELLA PORRIGENS*

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Key Word Index—*Pleurocybella porrigens*; basidiomycetes; mushroom; non-protein amino acid; β-hydroxy-L-valine.

Abstract—β-Hydroxy-L-valine was isolated from fruiting bodies of *Pleurocybella porrigens* (Fr.) Sing.

In a previous paper [1], we reported the free amino acid contents of fruiting bodies of 113 mushroom species, as determined by automatic amino acid analysis of their 70% ethanol extracts. In the course of the experiment, some of the mushrooms were found to contain several unusual amino acids. This paper describes the isolation and identification of an unknown ninhydrin positive compound, hereafter termed as **1**, from the fruiting bodies of the edible mushroom, *Pleurocybella porrigens* (Fr.) Sing.

Compound **1** was detected on the chromatogram of the amino acid analyser (Li-citrate buffer system) and was completely overlapped with threonine. The two were distinguishable by TLC (silica gel and Avicel cellulose). The isolation of **1** was accomplished by chromatography using several ion-exchange resins, Avicel cellulose and silica gel, followed by crystallization. From the elemental analysis and SIMS, the molecular formula of **1** was estimated to be $C_5H_{11}NO_3$. The ninhydrin reaction of **1** on paper was completely inhibited by Cu^{2+}, showing that **1** is a α-monoamino acid. On the ^1H NMR of **1** (in D_2O), one *gem*-dimethyl group (δ 1.26 and 1.47) and one α-methyne singlet (δ 3.65), which indicated the β carbon atom to be fully substituted, were observed. The absorption band at 1167 cm^{-1} in the IR showed the presence of a tertiary hydroxyl group in the molecule [2]. These facts strongly suggested that **1** is β-hydroxyvaline. This was further supported by the formation of valine from the reduction with HI-red P [3], and the detection of glycine in the degradation product by barium hydroxide [4].

Optical rotation measurements on **1** performed in H_2O and 6 M HCl solution showed the shift to more positive rotations in acid associated with an L configuration. The IR of **1** correlated well with that of a synthetic specimen of β-hydroxy-L-valine [5].

Although β-hydroxy-L-valine was first found as a constituent of the antibiotic YA-56 [6, 7], it is thought that the evidence presented here is the first to demonstrate its natural occurrence in a free form.

EXPERIMENTAL

Mushroom. Fruiting bodies of *P. porrigens* were collected from a forest in Nagano prefecture during autumn 1985. The fresh fruiting bodies were washed with H_2O and then freeze-dried. They were kept at 4° until use.

General. Chromatography solvents were *n*-BuOH–HOAc–H_2O (8:1:1, by vol.; solvent 1), *n*-BuOH–HOAc–H_2O (4:1:2, by vol.; solvent 2), PrOH–H_2O (7:3, by vol.; solvent 3).

Isolation. The freeze-dried fruiting bodies (670 g) were extracted with 70% EtOH (× 3) and filtered. The filtrate (30 l) was passed through a column (5 × 90 cm) of Amberlite IR-120 (H^+). After the resin was washed with 70% EtOH and H_2O, the amino acids were eluted with 2 M NH_4OH. The eluate was evapd to dryness and dissolved in 200 ml of H_2O. The concentrate was applied to a column (5 cm × 75 cm) of Dowex 1 × 4 (OAc^-). Compound **1** was detected in the neutral and basic amino acid fractions eluted with 0.1 M HOAc from the column. The amino acid fraction was concentrated and subsequently chromato-

graphed on a Dowex 50w × 2 resin column (pyridine form, 3 cm × 100 cm). Elution was performed with a linear gradient system made by 1.5 l of pyridine–HOAc buffer (pH 3.1, 0.2 M pyridine) and 1.5 l of the same buffer (pH 5.0, 2.0 M pyridine). The fractions containing **1** were combined and concd to a small vol. The concentrate was then chromatographed on an Avicel cellulose column (4 cm × 43 cm) with solvent 1. As compound **1** fraction thus obtained was contaminated with various substances, including proline, further purification was conducted by chromatography using a silica gel column (Wako C-200, 4 × 40 cm) with solvent 2 and by recrystallization from H_2O–Me_2CO (× 3). The yield of pure **1** was *ca* 1.2 g, mp 196–197°; $[\alpha]_D^{23}$ +4.0 (H_2O; *c* 1.6), +13.0 (6 M HCl; *c* 0.8); (Found: C, 44.92; H, 8.44; N, 10.42. Calc. for $C_5H_{11}NO_3$: C, 45.10; H, 8.33; N, 10.52%); ^{1}H NMR (90 MHz, D_2O, TSP): δ 1.26 and 1.47 (3H, *s* and 3 H, *s*, respectively, *gem*-dimethyl group), 3.65 (1H, *s*, H-2); IR ν_{max}^{KBr} cm^{-1}: 3410, 3075, 1635, 1576, 1492, 1400, 1327, 1167, 1107, 972, 902, 740; SIMS *m/z*: 134 $[M+1]^+$.

HI–*red* P *reduction.* A small amount of **1** (10 mg) was heated with 1 ml of HI (57%) and 10 mg of red P in a sealed tube at 153° for 5 hr. After purification according to ref. [3], the reaction mixture was analysed by TLC (silica gel and Avicel cellulose; solvent 2 and 3) and an amino acid analyser. Valine was detected as the reaction product.

Degradation by $Ba(OH)_2$. A small amount of **1** was heated with satd $Ba(OH)_2$ soln at 120° for 20 hr. The reaction mixture was adjusted to pH 3 by 1M H_2SO_4 and then filtered. The filtrate was evapd to dryness, dissolved in H_2O and then analysed by TLC and an amino acid analyser. Glycine was detected together with unreacted **1**.

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ACETYLENIC COMPOUNDS AND OTHER CONSTITUENTS FROM *CINERARIA* SPECIES

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Key Word Index—*Cineraria britteniae, C. geifolia*; Compositae; acetylenic compounds; sesquiterpenes; myrcene derivatives.

Abstract—From two *Cineraria* species three new diacetylenes and two myrcene derivatives were isolated in addition to known compounds.

INTRODUCTION

From the South African genus *Cineraria* some species have been studied chemically [1–4]. Most characteristic are the unusual C_{11}-acetylenes and special eremophilanes. We have studied a further species and reinvestigated another one and the results are reported in this paper.

RESULTS AND DISCUSSION

The aerial parts of *Cineraria britteniae* Hutch. et R. A. Dyer afforded large amounts of the triyne **2** [1]. Furthermore, the eremophilanes **5** [1] and **6** [1], spathulenol, the *E*/*Z*-isomers **7a**/**b** and the diyne **1** were present. The structure of the latter followed from the molecular formula ($C_{11}H_{14}$) and from the NMR spectral data. In the ^{1}H NMR spectrum characteristic signals of a vinyl end group were visible (δ5.03 *dd*, 5.08 *dd* and 5.83 *ddt*). Spin decoupling showed that two methylene groups were attached to the vinyl group (δ2.26 *dt* and 2.22 *tt*). The last signal showed a long range coupling with a broadened triplet at δ2.34 which is typical for diynes. The remaining signals required a *n*-propyl group. In agreement with the fragmentation pattern in the mass spectrometer therefore