Short Paper

Enzymatic Preparation of 5-(3-Amidinoureido)-2-oxovaleric Acid from L-Gigartinine*¹

In the previous paper¹⁾ we have reported that the red alga *Gymnogongrus flabelliformis* had an enzyme which specifically catalyzed oxidative deamination of basic L-amino acids. We describe here the preparation method of a new keto acid, 5-(3-amidinoureido)-2-oxovaleric acid(II) from L-gigartinine(I) by the enzyme of the seaweed and some properties of the keto acid.

Forty g of the freeze-dried powder of *G. flabelliformis* were extracted with 480 ml of 0.1 M Tris-HCl buffer pH 8.0. The supernatant obtained by centrifugation at 10,000 rpm for 30 min was dialyzed against distilled water for 24 h. This enzyme solution, 230 ml of 0.05 M borate buffer pH 8.0 and 230 ml of the substrate solution containing 644 mg of L-gigartinine nitrate isolated from *G. flabelliformis* were combined. After 8 h of incubation at 30°C under shaking, the reaction mixture was deproteinized with the addition of 2 vol. of ethanol. The filtrate was passed through a column of Dowex 50X8 (H⁺, 3×35 cm). From the column washed with water, the adsorbed material was eluted with N aqueous ammonia until the SAKAGUCHI's test indicated complete elution. After removal of ammonia, the eluate was brought to pH 3 with N HCl and left stand for a few days in an ice-box. The precipitates of crude 5-(3-amidinoureido)-2-oxovaleric acid were filtered off and decolorized with charcoal in 100 ml of N aqueous ammonia. When the colorless filtrate was condensed to 10 ml and kept overnight in an ice-box, the keto acid crystallized out in prisms. The repeated crystallization from hot water yields 104 mg (21%). m.p., 199°C (decompn.). Drying *in vacuo* at 60°C over P₂O₅, the crystals were degraded to amorphous powder. *Anal.* Found: C, 38.80; H, 5.66; N, 25.83. Calcd. for C₇H₁₂N₄O₄: C, 38.89; H, 5.59; N, 25.91%. This compound gave an orange color with the SAKAGUCHI's reagent, unlike the orange-red given by arginine and the typical yellow-green under UV light with the o-phenylenediamine reagent.²⁾ With ninhydrin it gave reddish violet color the intensity of which was lower than those of usual amino acids. The R_f values in TLC and the IR spectrum are shown in Table 1 and Fig. 1, respectively.

This compound gave gongrine (III) on oxidation with hydrogen peroxide as follows: To a solution of 100 mg of the keto acid in 100 ml of 0.1 N aqueous ammonia was added 0.1 ml of 30% hydrogen peroxide solution. After maintaining at 30°C for 10 min, the

Fig. 1. IR spectrum of 5-(3-amidinoureido)-2-oxovaleric acid (Nujol).

Table 1. R_f values of 5-(3-amidinoureido)-2-oxovaleric acid and related compounds in TLC*¹

Compound	Solvent* ²				
	1	2	3	4	5
5-(3-amidinoureido)-2-oxovaleric acid	0.54	0.37	0.57	0.40	0.69
gigartinine	0.34	0.24	0.43	0.06	0.47
gongrine	0.72	0.64	0.81	0.43	0.82

*¹ Merck cellulose plate, the SAKAGUCHI's reagent.

*² 1) 1-BuOH: AcOH: pyridine: H₂O(4: 1: 1: 2), 2) 1-BuOH: AcOH: H₂O(4: 1: 2), 3) pyridine: AcOH: H₂O(10: 7: 3), 4) MeOH: H₂O: pyridine(20: 5: 1), 5) phenol: H₂O(4: 1).

reaction mixture was evaporated to 10 ml and kept in an ice-box. Colorless prisms of gongrine appeared. Yield, 42 mg (48%). The R_f values in TLC and the IR spectrum of the isolate were in good agreement with those of authentic gongrine. These reactions are summarized as follows;

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References

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*¹ L-gigartinine から 5-(3-amidinoureido)-2-oxovaleric acid の酵素による調製.

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