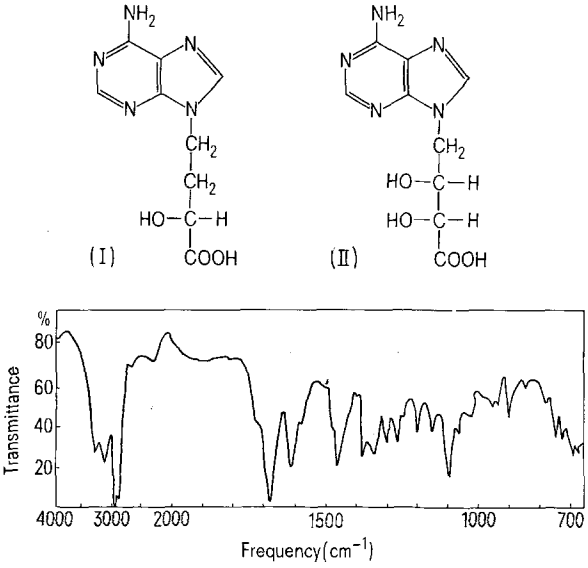
Isolation of Intermediate in Biosynthesis of Eritadenine from Adenine

A new hypocholesterolemic substance, eritadenine[1], was isolated from an edible mushroom 'Shiitake' (*Lentinus edodes*), and its chemical structure was confirmed in our laboratory[2] to be 4-(6-amino-9H-purine-9-yl)-2,3-dihydroxybutyric acid (II). Eritadenine is the first example of the adenine 9-substituted with hydroxy acid in natural products and its biosynthesis is of special interest in our investigation. In view of the structural relationship of eritadenine to adenine, the latter substance is presumed to be a possible precursor of eritadenine. To prove this hypothesis, adenine-8-C^{14} was infused into a Shiitake. From the distribution of C^{14} it was suggested that eritadenine was synthesized from adenine via the intermediate, substance A. This intermediate was obtained as crystalline powder and identified as 4-(6-amino-9H-purine-9-yl)-3-hydroxybutyric acid (I). SAITO et al.[3] and TOKITA et al.[4] isolated the same substance, but its biological role in Shiitake has not been discussed.



IR-spectrum of substance A (in Nujol).

In the cap of growing Shiitake on trunks of 'Kunugi' (*Quercus acutissima*), a small hole was made with corkborer. 20 μCi of adenine-8-C^{14} dissolved in a small volume of 0.9% sodium chloride solution was gradually infused into the hole and then the hole was blocked up. The Shiitake was harvested at 6 and 24 h after infusion and extracted with 80% ethanol. The extract was fractionated on an amino acid analyzer, Hitachi KLA-3, specially equipped UV-detector. The effluent peaks having absorption at 254 nm were collected. Radioactivity of the respective peaks was determined on a Beckman liquid scintillation spectrometer.

In the Table are shown the radioactivities of major peaks. The most radioactive substance A in 6 h sample was eluted in the fraction between 208 and 212 ml. At 24 h after infusion, the radioactivity of substance A decreased and that of eritadenine increased. Therefore, substance A could be expected to be one of the intermediates in the sequence of the biosynthesis of eritadenine from adenine.

Isolation of the substance from dried Shiitake was made by preparative amino acid analyzer in a manner similar to that previously employed for the isolation of eritadenine[2]. Approximately 70 mg were obtained from 2 kg of dried Shiitake, Anal. C, 45.20; H, 4.62; N, 29.25. Calcd. for $C_9H_{11}O_3N_5$: C, 45.57; H, 4.67; N, 29.53, mp 271-5° (dec.); $[\alpha]_D^{20}$ +17.5° (C, 0.5 in 0.1 *N* NaOH). The substance exhibits a characteristic UV-absorption of 9-substituted adenine $\lambda_{max}^{0.1\,N\,HCl}$ 261 nm (ε, 12824), $\lambda_{max}^{0.1\,N\,NaOH}$ 262 nm (ε, 14038). The IR-spectrum is shown in the Figure. The NMR-spectrum of the sodium salt had the following signals: (60 MHz in D_2O) multiplet at δ (ppm) 2.5 (2 protons), broad triplet at 4.3 (1 proton), triplet at 4.5 (2 protons), and singlet at 8.3 (2 protons). On the basis of these data, the structure of substance A was proposed as 4-(6-amino-9H-purine-9-yl)-3-hydroxybutyric acid.

Radioactivities of fractions isolated by an amino acid analyzer[a]

Peaks	Effluent (ml)	Radioactivity (cpm × 1000) Time of culture after infusion 6 h	24 h
Eritadenine	178–185	39.8	64.6
Substance A	208–212	52.7	30.5
B	245–252	29.9	20.8
Adenine	361–375	33.7	18.8

Adenine-8-C^{14} (22 mCi/m mol) was purchased from Daiichi Pure Chemical Company (Japan). [a] The column contained 0.9 × 50 cm of resin (Amberlite CG-120). It was operated at 50° using buffer flow rate of 30 ml/h. The buffer change (PH 3.25 to 4.25) was made after 2.5 h, and the change to pH 5.28 after 5 h.

Zusammenfassung. Zur Biosynthese von Eritadenin in einem Speisepilz «Shiitake» wurde 4-(6-amino-9H-purine-9-yl)-3-hydroxybuttersäure als ein Zwischenprodukt isoliert und identifiziert.

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[1] The trivial name 'lentinacin' was used in the previous paper[2].
[2] I. CHIBATA, K. OKUMURA, S. TAKEYAMA and K. KOTERA, Experientia *25*, 1237 (1969).
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Effects of Fruit on Ribulosediphosphate Carboxylase Activity in *Citrus madurensis* Leaves

Previous measurements of photosynthetic rates of *Citrus madurensis* Loureiro (Tanaka, 1954) cuttings showed intensified CO_2-uptake in fruiting as compared with non-fruiting plants[1], and other workers have reported stimulatory effects of various 'metabolic sinks' on CO_2-uptake[2,3]. WAREING et al.[2], also suggested that hormones derived from these sinks may stimulate formation of carboxylating enzymes in neighbouring leaves. In