position still allows a relatively high cytokinin activity. In this connection, it is of interest to note that in our preliminary experiment 3-methyl-N^6-isopentenyladenine that was chemically synthesized was as active as kinetin. Secondly, discadenine may be converted to N^6-isopentyladenine in the callus tissue by some unknown enzymatic systems. However, we presume that this latter possibility is very unlikely. Earlier [5], we showed that discadenine inhibits spore germination of *D. discoideum* at concentrations of *ca* 10^{-8} M. In the case of chemically synthesized zeatin and N^6-isopentenyladenine inhibition occurred at *ca* 10^{-5} M. These results suggest that substitution of the purine skeleton at the 3 position, possibly with an amino acid, is necessary for high inhibitory activity against spore germination. It was suggested that this inhibitor may act on the plasma membrane of the spores [5]. In this context, it should be noted that LéJohn and his associates reported that N^6-isopentenyladenine regulates uptake of amino acids, Ca^{2+}, nucleosides and glucose in the water mold *Achlya* [7, 8]. It was also shown that N^6-isopentenyladenine binds to a glycopeptide which is localized on the cell membrane of the sporangiospores of this organism [9].

EXPERIMENTAL

Discadenine was isolated from *Dictyostelium discoideum* (NC-4) by the procedure of refs [4, 10]. Discadenine crystallized twice was used in this expt. Callus, derived from the pith of tobacco plant (*Nicotiana tabacum* var. Wisconsin No. 38), was cultured aseptically on the revised agar medium (RM-1962) of ref. [11] with 2 mg/l. of IAA and the discadenine soln. The inhibitor soln was added through a sterilized millipore filter syringe to avoid breakdown by heating. Three pieces of callus, each *ca* 50 mg, were planted per flask with 20 ml of agar medium and 5 flasks were used for each expt. These flasks were incubated at 30° in the dark for 30 days and fr. wt yields of callus were determined.

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α-METHYLENE-γ-AMINOBUTYRIC ACID FROM *MYCENA PURA**

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Key Word Index—*Mycena pura*; Tricholomataceae; Basidiomycetes; α-methylene-γ-aminobutyric acid.

Abstract—α-Methylene-γ-aminobutyric acid was isolated and characterized from fruit bodies of *Mycena pura*. It was the decarboxylation product of L-γ-methyleneglutamic acid by L-glutamic acid decarboxylase.

Previously we reported the isolation of 3 unsaturated amino acids, L-γ-methylene-, L-γ-ethylidene- and L-γ-propylideneglutamic acid from fruit bodies of *Mycena pura* (Fr.) Kummer [1]. During this work we observed the presence of another unsaturated amino acid (ninhydrin-yellow) in the neutral amino acid-fraction.

The result of elementary analysis, as well as the PMR spectrum of the isolate suggested the structure, α-methylene-γ-aminobutyric acid, which has been reported in groundnut (*Arachis hypogaea*) [2]. Our isolate was, as expected from the above structure, optically inactive and oxidation with $KMnO_4$ gave β-alanine.

For direct comparison, we prepared α-methylene-γ-aminobutyric acid from L-γ-methyleneglutamic acid by L-glutamic acid decarboxylase. Mp, IR, colour reaction with ninhydrin, as well as chromatographic behaviour of the decarboxylation product were in good agreement with those of the natural amino acid.

EXPERIMENTAL

Fungal material. The fruit bodies of *Mycena pura* (Fr.) Kummer were collected in Sept. 1974 in Nagano Prefecture. Voucher specimens are deposited in the Department of Biology, College of General Education, The University of Tokyo.

Isolation of amino acid. Fruit bodies (9.4 kg) were crushed and extracted × 4 with 80% EtOH and the filtered extract (80 l.) was treated with a column of Amberlite IR-120 (H^+, 1 l.). After the resin was washed with aq. EtOH and H_2O, successively, the amino acids were eluted with 2M NH_4OH (14 l.).

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The eluate was concd *in vacuo* to a small vol. (150 ml), applied to a column of Dowex 1 × 4 (200–400 mesh, OAc^-, 5 × 117 cm), and fractionated with 0.2 M HOAc. The fractions containing the unsaturated amino acid (525 ml) were combined and each half of the concentrate was further fractionated on a cellulose column (5 × 116 cm) with the solvent *n*-BuOH–HOAc–H_2O (63:10:27). The relevant fractions were extracted × 4 with H_2O and the H_2O layers concd. Since a large amount of valine was still present in these fractions, the aliquots were applied to a column of Dowex 50W × 8 (200–400 mesh, H^+, 1.5 × 88 cm) and fractionated with 1.5 M HCl, to give pure fractions. On repeated concn with the addition of a small amount of H_2O, crystals were separated (1.56 g) which were recrystallized × 3 from H_2O–EtOH. Mp 170–9° (decomp.), optically inactive in H_2O and 3 M HCl. Found: C, 51.58; H, 7.96; N, 12.03. Calc. for $C_5H_9NO_2$: C, 52.16; H, 7.88; N, 12.17%. PMR (100 MHz, D_2O, TSP): δ 2.57 (2H, *t*, *J* = 7Hz, βC), 3.05 (2H, *t*, *J* = 7 Hz, γ C), 5.4 (H, *s*, H *trans* to CO_2D), 5.82 (H, *s*, H *cis* to CO_2D).

Preparation of authentic α-methylene-γ-aminobutyric acid. L-γ-Methyleneglutamic acid (1 g), isolated earlier from the same fungus [1], was dissolved in 0.2 M Py–HCl buffer, pH 4.5 (100 ml). Commercial L-glutamic acid decarboxylase (Kyowa Hakko Kogyo Co. Ltd., preparation from *E. coli*, 47 mg) in the same buffer (2 ml) was added and the mixture incubated at 37.5°. After 8 hr more enzyme (50 mg) was added and incubation was continued for a further 16 hr. The reaction was stopped by heating at 100° for 15 min. The pptd proteins were removed by filtration through a Celite-layer. The filtrate was concd, applied to a column of Dowex 1 × 4 (200–400 mesh, OAc^-, 1.5 × 54 cm), and fractionated with 0.2 M HOAc. Fractions containing the decarboxylation product were combined and purified further on a cellulose column (2.4 × 90 cm) and *n*-BuOH–HOAc–H_2O (63:10:27). The relevant fractions were combined and extracted × 4 witB H_2O. The H_2O extract was concd to a syrup and treated with EtOH–Me_2CO, to give crystals (65 mg). They were recrystallized once from H_2O–Me_2CO. Mp 170–9° (decomp.) Found: C, 51.37; H. 7.79; N, 12.01. Calc. for $C_5H_9NO_2$: C, 52.16; H, 7.88; N, 12.17%.

Chromatographic comparison. The isolated amino acid and authentic α-methylene-γ-aminobutyric acid had the same R_f with *n*-BuOH–HOAc–H_2O (63:10:27), PhOH–H_2O (25:9), *t*-AmOH–MeCOEt–NH_4OH–H_2O (72:45:20:10) and *t*-AmOH satd with H_2O.

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OCCURRENCE OF ALBIZZIINE IN *DIALIUM* SPECIES (CAESALPINIACEAE)

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Key Word Index—*Dialium* species; Caesalpiniaceae; amino acid; albizziine.

The presence of albizziine in plant species of the Leguminosae has been recorded [1–6], but only in members of Mimosaceae. The occurrence of albizziine in a plant species not belonging to the Mimosaceae is recorded for the first time in this report. 2-D PC of 70% ethanolic extracts of the seeds of *Dialium ovoideum*. The (endemic to Sri Lanka [7]), *D. engleranum*, *D. guineense* Willd, *D. dinklagei* Harms showed the occurrence of very high concentrations of albizziine. Albizziine is the dominant component of the free amino acid pool of all 4 species of *Dialium* investigated. A partial isolation of albizziine in a crude form, from *D. ovoideum* showed it to be present in the order of 2.5% by wt in the dry seeds. A comparison of the 2-D PC of the other 3 species studied with that of *D. ovoideum*, showed the spot corresponding to albizziine to be present in *ca* the same concentration.

The vegetative and floral morphology of the 4 *Dialium* species studied is 'Caesalpinaceous' in character. However the free amino acid distribution pattern as seen on 2-D PC is very similar to that of many *Acacia* species e.g. *A. decurrens* Willd [3]. The presence of albizziine in *Dialium* may be of taxonomic value within the family Caesalpiniaceae. Shewry and Fowden [10], in reporting the non-protein amino acids of the genera *Julbernardia*, Pellegr, *Isoberlinia* Craib & Stapf, *Brachystegia* Benth, and *Cryptosepalum* Benth which belong to the sub family Brachystegioideae of the family Caesalpiniaceae, do not record the presence of albizziine, in any of these genera. The presence of albizziine in *Dialium* may also provide some evidence for the view that the Mimosaceae or part of them are derived from the Caesalpinaceae. It is hoped to extend this study to other members of the group 1 of the sub-family Caesalpinioideae [9] of which the genus *Dialium* is a member.

EXPERIMENTAL

Solvents for PC were as follows: (1) PhOH–H_2O, 4:1, (w/w) in an atmosphere of NH_4OH (2) *n*-BuOH–HOAc–H_2O, 90:10:29. (3) EtOAc–Py–H_2O, 2:1:2, (upper phase). (4) *n*-BuOH–NH_4OH–H_2O, 5:1:4, (upper phase). (5) *tert*-amyl alcohol–HOAc–H_2O, 20:1:20, (upper phase).

Partial isolation of albizziine. Seeds of *D. ovoideum* (10 g) were ground and extracted with 70% EtOH (25 ml/g). The extract was applied to a column of Zeokarb 325 and the amino acid fraction eluted with ethanolic 2 N ammonia. Fractions containing albizziine were concd and crystallised. Further purification was by PC developing in solvent 3, eluting and crystallising. From 10 g of seed material 262 mg of albizziine were obtained. Albizziine was identified by eluting from the PC and co-chromatographing with authentic albizziine in the 5 different solvent systems given above. Albizziine gave a positive Ehrlich's test [8], showing the presence of the ureido group.

Amino acid distribution. The ground seed was extracted with 70% EtOH and the amino acid fraction separated using a small Zeokarb 325 column. The individual components were resolved on 2-D PCs in solvents 1 and 2.