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.

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L- 4-HYDROXY-3-MÉTHOXYPHENYLALANINE A PARTIR DE *CORTINARIUS BRUNNEUS*

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Key Word Index—*Cortinarius brunneus*; Basidiomycete; non-protein amino acid; ^1H and ^{13}C NMR L-4-hydroxy-3-methoxyphenylalanine.

Ce travail s'inscrit dans une série d'études sur la répartition des acides aminés et peptides libres des champignons‡. Le nombre des acides aminés libres dérivés de la phénylalanine ne cesse d'augmenter. Ainsi, Bell *et al.* et Mwauluka *et al.* [1,2] ont isolé érécemment de graines de *Combretum zeyheri*, la L-*N*-méthyltryosine et la 3-aminoéthylphénylalanine. Dans le champignon *Cortinarius brunneus*, nous avons isolé, pour la première fois dans le règne végétal, la L-3-hydroxy-4-méthoxyphénylalanine. Cet acide aminé a été identifié comme un métabolite formé, en faible quantité, dans le sang des patients recevant la dopa comme traitement de la maladie de Parkinson [3]. Il est également considéré comme un précurseur biologique possible de l'acide homovanilique, un des principaux métabolites urinaires chez l'homme et l'animal [4].

RESULTATS ET DISCUSSION

La 2D-PC de la fraction soluble dans l'éthanol aqueux de *C. brunneus* a révélé la présence d'un spot non identifiable, violet–brun avec la ninhydrine, migrant comme la tyrosine au butanol et légèrement plus loin que l'acide γ aminobutyrique au phénol. Par HVE à pH 3,6, la substance est neutre. Après purification de l'extrait sur une colonne de Lewâtit S 1080, les acides aminés ont été adsorbés sur une colonne de Dowex 1 × 2, forme Cl^- et élués par H_2O. La substance passe en même temps que la tyrosine. Elle a été séparée de ce composé aromatique par chromatographie préparative sur papier et ensuite purifiée sur une très petite colonne de Lewatit S 1080. La substance a été recristallisée dans l'eau où elle est particulièrement insoluble. L'analyse élémentaire conduit à la formule moléculaire $C_{10}H_{13}NO_4$ en accord avec la SM. L'acide aminé est aromatique (test de Le Rosen) et possède une fonction αNH_2 (Van Slyke). Le spectre IR du composé présente les bandes caractéristiques d'un acide α aminé aromatique monocarboxylique et aussi des bandes hydroxyles à 3510 et 3245 cm^{-1}. Dans le SM de l'ester méthylique, le pic moléculaire se trouve à m/e = 225,0990 ($C_{11}H_{15}NO_4$ = 225,1001). Les fragmentations observées sont caractéristiques des acides aminés aromatique substitués en para par un groupe électrodonneur [5] (voir Partie Experimentale). Elles sont identiques à celles relevées pour la 3-hydroxy-4-méthoxyphénylalanine (**1**) et pour son isomère, la 4-hydroxy-3-methoxyphénylalanine (**2**). Leur distinction est impossible par SM. L'attribution d'une de ces deux structures à l'acide aminé de *C. brunneus* est corroborée par ^1H RMN (voir Partie Experimentale) et par déméthylation par l'acide iodhydrique [7,8]. Celle-ci fournit en effet un composé qui migre comme la dihydroxy-3,4-phénylalanine (dopa) à la chromatographie sur papier et sur couche mince avec de nombreux solvants très différents.

La valeur du pK_a du groupe phénolique (9,8), déterminée par spectroscopie UV [9], est en faveur de l'isomère **2**. (pK_a des produits synthétiques DL: **1** = 10,4;

‡Dardenne *et al.* Public. 11 Acides aminés et peptides des champignons. Public. 10, voir Welter *et al.* (1976) *Phytochemistry* **15**, 1984.