CONSTITUENTS OF A NEW GUINEA BOLETUS

ISOLATION AND IDENTIFICATION OF A NEW UNSATURATED α-AMINO ACID

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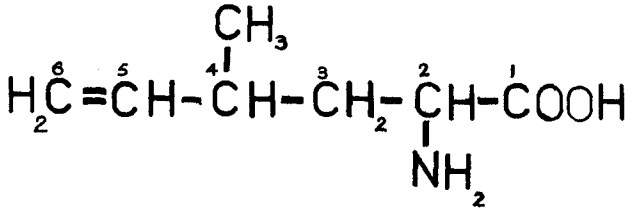
SUMMARY

A new unsaturated α-amino acid identified as L-2-amino-4-methyl-5-hexenoic Acid has been isolated from a New Guinea fungus, tentatively identified as Boletus, section Ixocomus, group Nudi.

A study of the free amino acids of a New Guinea fungus, tentatively identified as Boletus, section Ixocomus, group Nudi (1), has led to the isolation of a new unsaturated α-amino acid (I) (0.04% dry weight). Through the application of ion-exchange (Zeocarb 225 (H^+) and preparative paper chromatography (R_f 0.80, butanol-acetic acid-water (2:1:1)), (I) was obtained as colourless plates (m.p. 240-2° (dec.), aqueous ethanol), possessing the empirical formula $C_7H_{13}NO_2$ (Found: C, 58.5;



[I]

H, 9.2, N, 9.6%. Calc. for: C, 58.7; H, 9.2; N, 9.8%.). The migration characteristics of (I) in paper electrophoresis at several pH values were typical of a neutral amino acid and its ability to chelate with Cu^{2+} (2) showed it to be an α-amino acid. On the basis of a prominent peak in its mass spectrum, (I) has a molecular weight of 143, in excellent agreement with the above formula. The specific rotation of (I) in water was -9.6° (c = 1.777) and this became more positive in acid solution ($+5.7^{\circ}$, 1N HCl, c = 0.7), indicating that the amino acid belongs to the L-series at the α asymmetric center (3). This assignment was confirmed independently by a gas liquid chromatographic method, which relies on the consistency in the order of retention behaviour of diastereoisomeric trifluoracetyl-L-prolyl-DL-amino acid esters (4). The infrared spectrum showed absorption bands at 1580 and 1405 cm^{-1}, characteristic for zwitterionic amino acids (5) and in addition it contained peaks at 990 (medium intensity) and 920 cm^{-1} (strong intensity), which could be assigned to -CH- out of plane deformation of a terminal vinyl group ($R - CH = CH_2$) (6). The presence of a double bond received strong support from the result of catalytic hydrogenation of (I), when one equivalent of hydrogen was taken up (Found: 15.35 ml; Calculated: 15.65 ml). Further evidence favouring the presence of a terminal vinyl group was provided by the detection of formaldehyde as a product of the periodate-permanganate oxidation of (I) (7). The nuclear magnetic resonance spectrum showed a three proton doublet at δ 1.52 (J = 7 c/s) due to the protons of the methyl group coupling with the C_4 proton; a two-proton multiplet at δ 2.30 (J = 3 c/s) due to the magnetically non equivalent protons at C_3 coupling with the C_2 proton; a one proton multiplet centered at δ 2.70 due to the C_4 proton and a one proton pair of

doublets (AB Quartet) at δ 4.14 (7 c/s) which is assigned to the C_2 proton signal split by the adjacent C_3 protons. The two proton multiplet at δ 5.56 is due to the terminal methylene group and the one proton multiplet at δ 6.22 is assigned to the vinylic proton, which is deshielded by the other C_5 substituent. The analysis, spectral data and physical properties of (I) indicated that the compound is L-2-amino-4-methyl-5-hexenoic acid (the absolute configuration at C^4 is as yet not known). Further confirmation for this structural assignment was obtained from low resolution mass spectrometric analysis of the amino acid. This showed prominent peaks at m/e 143 $(M)^+$; 98 $(M\text{-}COOH)^+$; 74 $(M\text{-}(CH_2{=}CH\text{-}\overset{CH_3}{\overset{|}{C}}\text{-}CH_2))^+$; 69 $(CH_2{=}CH\text{-}\overset{CH_3}{\overset{|}{C}}H\text{-}CH_2)^+$ and 55 $(CH_2{=}CH\text{-}\overset{CH_3}{\overset{|}{C}}H)^+$. The isolation of (I) from a mushroom source is not entirely unexpected, since three other unsaturated α-amino acids have been discovered previously in the fruiting bodies of mushrooms (8) (9).

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