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THE STRUCTURE OF PINNATANINE, A NEW AMINO ACID AMIDE FROM STAPHYLEA PINNATA L.

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During extensive analyses of seeds from angiospermous plants, high concentrations of an unidentified amino acid were noted in the acid hydrolysate of seed meals from Staphylea pinnata L. (European bladdernut, Staphyleaceae) and Hemerocallis fulva L. (common orange day lily, Liliaceae) (1). This finding prompted an investigation of unhydrolyzed S. pinnata seed extract for novel ninhydrin-reactive compounds. We now report the isolation and structure of "pinnatanine," N^5-(2-hydroxymethylbutadienyl)-L-allo-γ-hydroxyglutamine (I). Pinnatanine, which we have also found in H. fulva, appears closely related to a compound occurring in Phlox decussata and Hemerocallis sp. (2).

Defatted seed meal (10 g) from S. pinnata was extracted at room temperature with EtOH-H_2O (7:3). Fractionation of the concentrated extract by column chromatography on Adsorbosil (3) CAB-60/100 (Applied Science Laboratories) gave a major fraction, which eluted with 35-50% H_2O in i-PrOH. Decolorization with charcoal and Polyclar AT (General Aniline and Film Corp.) followed by partial evaporation of the solvent afforded 300 mg of crystalline pinnatanine (I): m.p. dec. starts 165°; $[\alpha]_D^{27}$ +3.2° (C = 0.5 H_2O); $C_{10}H_{16}N_2O_5$ by elemental analysis; Silica Gel TLC R_f 0.45, i-PrOH-EtOAc-H_2O-HOAc (40:38:20:2); paper chromatography R_f 0.28, n-BuOH-EtOH-H_2O (4:1:4); IR (Nujol) 3390, 3340 (amide NH); 1660 (amide I C=O); 1605 ($-CO_2^-$, C=C); 1500 cm^{-1} (amide II C=O); UV (H_2O) λ_{max} (ε) 262 nm (24,200).

Ten of the 16 hydrogens of pinnatanine were observed in the NMR spectrum (4) (D_2O): δ2.28 2H m; 3.94 1H q (J=5 and 6 Hz); 4.29 2H s; 4.45 1H q (J=5 and 8 Hz); 5.31 1H d (J=11 Hz); 5.45 1H d (J=17 Hz); 6.65 1H q (J=11 and 17 Hz); 6.74 1H s. Irradiation of the signals at δ3.94 or 4.45 sharpened the multiplet at δ2.28. Likewise, irradiation at δ2.28 collapsed the δ3.94 and 4.45 quartets. These double resonance experiments suggested a $-\overset{|}{C}H-CH_2-\overset{|}{C}H-$ group. Such a group is present in γ-hydroxyglutamic acid, an acid that is known to occur in

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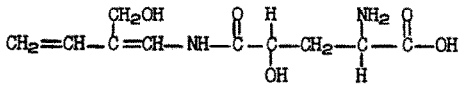
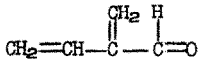
Hemerocallis sp. (5) and Staphylea colchica (6). A CH_2=CH- group was indicated by the pair of doublets at δ5.31 and 5.45 coupled to the δ6.65 signal. The singlet at δ6.74, attributed to a fourth vinyl proton, plus the UV spectrum suggested the presence of a butadienyl system CH_2=CH-C=CH-.



I



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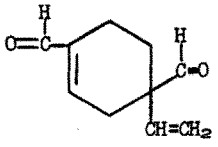
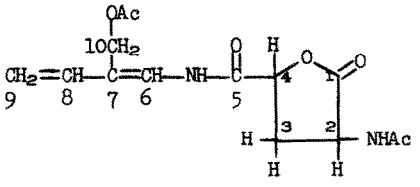


III



IV

Acid hydrolysis of pinnatanine (I) (2N H_2SO_4, reflux 3 hr) afforded ammonia, a CH_2Cl_2 soluble fraction and an amino acid ($C_5H_9NO_5$ by elemental analysis). The amino acid was identified as L-allo-γ-hydroxyglutamic acid (7): m.p. 182-185° dec.; $[\alpha]_D^{27}$ -13.2° (C = 0.5 H_2O); NMR (D_2O) δ2.31 2H; 3.92 1H; 4.32 1H. An IR spectrum of the acid was superimposable on that of an authentic sample (8), and the compounds migrated the same upon TLC (EtOH-H_2O 7:3). The CH_2Cl_2 soluble portion of the acid hydrolysate was a yellow oil: TLC single spot R_f 0.4, acetone-benzene (5:95); IR ($CHCl_3$) 2815, 2715 (aldehyde CH); 1725 (saturated aldehyde); 1680 (unsaturated aldehyde); 1645 (C=C); 994, 917 cm^{-1} (CH deformation of CH_2=CH-); UV (EtOH) λ_{max} (ϵ) 230 nm (14,550). Verification of a CH_2=CH- group was shown by NMR ($CDCl_3$) signals at δ5.14 1H q (J=1 and 17 Hz), 5.32 1H q (J=1 and 11 Hz), and 5.74 1H q (J=11 and 17 Hz). A fourth vinyl proton appeared as a multiplet at δ6.80. Two aldehyde protons were present as singlets at δ9.36 and 9.40. With the observation of six additional protons as a complex at δ1.62-2.96, 12 hydrogens are accounted for.

The presence of the parent ion at m/e 164 in the mass spectrum plus NMR data suggests a molecular formula of $C_{10}H_{12}O_2$ for the hydrolysis product. This compound could arise via Diels-Alder dimerization of the transient 2-methylene-3-butenal (II) from I to give 4-vinyl-1-cyclohexen-1,4-dicarboxaldehyde (III). Dialdehyde III was synthesized by a sodium

acetate-catalyzed condensation of crotonaldehyde with formaldehyde (9). Synthetic III was identical to the hydrolysis product in all respects (TLC, IR, UV and NMR, and mixed m.p. of the dioximes). Acid hydrolysis of pinnatanine under milder conditions with Bio-Rad 50W cation exchange resin (room temperature, 3 hr) also gave dialdehyde III.

Acetylation of pinnatanine (I) (acetic anhydride-pyridine) afforded a noncrystalline diacetyl derivative (IV): TLC R_f 0.39, acetone-$CHCl_3$ (1:1); IR ($CHCl_3$) 3460, 3430 (amide NH); 1795 (δ-lactone); 1735 (acetate C=O); 1690 (amide C=O); 1660 cm^{-1} (unsaturated amide C=O); UV (EtOH) λ_{max} 266 nm. Two acetyl methyl singlets (total 6H) at δ2.01 and 2.04 were observed in the NMR spectrum ($CDCl_3$). Two C_3 protons of the γ-hydroxyglutamyl moiety appeared as multiplets at δ2.30 and 3.00 coupled to the C_2 and C_4 protons at δ4.51 and 4.93, respectively. Irradiation at δ4.51 collapsed an NH proton doublet at δ7.20 (J=7 Hz) to a singlet. Likewise, irradiation at δ7.20 sharpened the δ4.51 multiplet to a triplet. The presence of a primary allylic alcohol in pinnatanine was indicated by a downfield shift of the two-proton singlet at δ4.29 to 4.73 in the acetylated product. Two C_8 protons appeared as a pair of doublets at δ5.22 (J_{cis}=11 Hz) and 5.33 (J_{trans}=17 Hz) coupled to the C_8 proton quartet centered at δ6.66. A doublet at δ6.92 (J=11 Hz) was assigned to the C_6 vinyl proton and is coupled to the low-field NH proton at δ8.87. Formation of IV indicates that the butadienyl moiety is linked to the amide nitrogen rather than to C_2 nitrogen or C_4 oxygen of γ-hydroxyglutamine.

Evidence that pinnatanine is an α-amino acid and not a γ-amino acid, in which the C_2 amino and C_4 hydroxyl groups are reversed, was obtained in two ways. First, the apparent dissociation constant of the amino group (pK_2') is 9.1. The pK_2' of glutamine is 9.13 and of isoglutamine, 7.88 (10). Secondly, at pH 1 the signal for the proton on carbon bearing the amino group was shifted downfield from δ3.94 to 4.20, while the proton on carbon bearing the hydroxyl group remained at δ4.45. This shift demonstrated that the δ3.94 proton is nearer the ionized carboxyl group (11).

Structural studies are in progress on a second amino acid also present in both S. pinnata and H. fulva.

Acknowledgments

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3. Mention of firm names or trade products is for identification only and does not imply endorsement by the U.S. Department of Agriculture.

4. NMR spectra were determined on a Varian HA-100 instrument in D_2O or $CDCl_3$ with 2,2,3,3-tetradeutero-3-(trimethylsilyl)-propionic acid sodium salt (TSP) or TMS, respectively, as internal standards.

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