STRUCTURE AND SYNTHESIS OF MEDICANINE, A NEW AMINO ACID FROM MEDICAGO SATIVA

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Abstract —— A new amino acid derivative was isolated from seedlings of Medicago sativa. The structure of this compound, medicanine has been determined as (S)-N-(3-hydroxypropyl)azetidine-2-carboxylic acid (1) on the basis of nmr analysis and chemical synthesis.

Recently several amino acid derivatives containing an azetidine-2-carboxylic acid moiety, such as mugineic acid (2)[1] and related substances were isolated from root washings of barley, wheat and oat cultured under iron free conditions. These compounds were shown to play a role in iron uptake and transport in Gramineae plants. Nicotianamine (3) closely related to 2 was obtained both from Beta vulgaris and Medicago sativa as a phytosiderophore which plays a role in cellular iron transport and/or metabolism.[2] In the course of investigations on these types of amino acid derivatives, we have isolated from the extract of Medicago sativa a new azetidine containing amino acid named medicanine, the structure and synthesis of which are described in this communication. From the ethanol extracts of Medicago sativa[3] a neutral amino acid (Rf, 0.11 on pc[4] ; Rt, 6.8 min on hplc[5]) showing a brown to violet coloration on testing with ninhydrin was obtained as a colorless non-crystalline material through purification with a Dowex 50 W column, preparative hplc and a Sephadex G-10 column.

The amino acid, medicanine (1), $[\alpha]_D$ -92.2° (c, 0.13, H_2O) had the molecular formula $C_7H_{13}NO_3$; Fd-ms, m/e 160 $(M+1)^+$ and showed ir absorption (KBr disk) at 3350 (NH and OH), 1623 and 1404 cm^{-1} (COO^-). The pmr spectrum of 1 (in D_2O) revealed the presence of an azetidine-2-carboxylic acid moiety by the signals at 3.76-4.24 (2H, m), 2.32-2.94 (2H, m) and 4.69 (1H, t, J=9.5) which was confirmed by a decoupling experiment. The compound 1 exhibited also methylene signals at 1.84 (2H, m), 3.32 (2H, m) and 3.68 (2H, t, J=6.0) attributed to the partial atructure $HOCH_2$-CH_2-CH_2-N-. The cmr spectrum of 1 showed a signal due to a carboxyl carbon at 173.0 and six signals at 20.8 (t), 26.4 (t), 50.2 (t), 52.1 (t), 58.5 (t) and 66.6 (d). These findings revealed that medicanine has a structure of 1, which was confirmed by the following synthesis.[6]

A protected amino acid 4 derived from L-azetidine-2-carboxylic acid (i, CBZ-S, Et_3N/dioxane-H_2O ; ii, isobutylene, H_2SO_4/dioxane-CH_2Cl_2 ; iii, H_2, Pd-C/MeOH-HCl) was reductively coupled[7] with 3-hydroxypropanal derivative 5[8] by the action of $NaBH_3CN$ (MeOH/pH 6.8 phosphate buffer : 3/1) giving rise to a product 6, $[\alpha]_D$ -103.6° (c, 0.40, $CHCl_3$) in 71 % yield. Deprotection of 6 with 1N HCl followed by chromatographic purification furnished the compound 1, mp 124-126°C, $[\alpha]_D$ -100.5° (c, 0.45,

H_2O) in 72 % yield as colorless hygroscopic crystals. The synthetic 1 was shown to be identical with the natural substance in all respects including the sign of optical rotation. Medicanine (1) might be the first substance in which the nitrogen of azetidine-2-carboxylic acid is linked with an alkyl unit other than amino acids. The biosynthetic relationship of 1 to the aforementioned iron chelating substances is under investigation.

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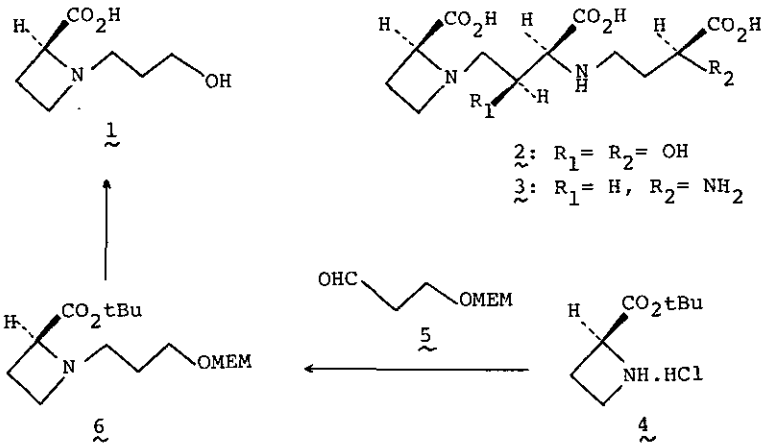

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2. M. Budesinsky, H. Budzikiewicz, Z. Prochazka, H. Ripperger, A. Romer, G. Scholz and K. Schreiber, Phytochemistry, 1980, 19, 2295 ; Tetrahedron, 1981, 37, 191.
3. Medicago sativa was cultured in the dark and extracted with ethanol for a pilot production of phyto-proteins. The precipitate produced by adding acetone to the extract was filtered off, and the filtrate was used for this experiment.
4. PC was performed using n-BuOH-AcOH-H_2O (4:1:5 upper layer) by the ascending technique on Toyo No.50 paper.
5. A stainless steel column (2.6 mm ϕ x 50 cm) packed with Hitachi gel 2618 (cation exchange resin) was used and eluted with an NH_3-HCO_2H buffer (pH 2.90) at a rate of 0.5 ml/min.
6. Satisfactory spectral data (pmr, ms and ir) were obtained for all intermediates.
7. This reductive coupling method was used for the synthesis of avenic acids and nicotianamine ; S. Fushiya, S. Nakatsuyama, Y. Sato and S. Nozoe, Heterocycles, 1981, 15, 819 ; Chemistry Letters, 1981, 909.
8. The aldehyde 5 was prepared from 3-butene-1-ol, (i) MEM chloride, N,N-diisopropylethylamine, CH_2Cl_2, rt, (ii) O_3, MeOH, -78°C, (iii) H_2, 5 % Pd-C.

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