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N^{ε}-(CARBOXYMETHYL)-L-LYSINE IN THE ACID HYDROLYSATE OF *SAGITTARIA PYGMAEA*

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Key Word Index—*Sagittaria pygmaea*; Alismataceae; isolation; structural determination; synthesis; amino acid; N^{ε}-(carboxymethyl)-L-lysine: NMR.

Sagittaria pygmaea Miq. (Japanese epithet: Urikawa) is a wild plant of the Alismataceae growing in paddy fields of Japan. The plants were collected in Fukui Prefecture between late June and early July. In the acid hydrolysate of the whole plant, diaminopimelic acid and an unknown amino acid were found, besides the usual amino acids. The unknown amino acid showed an almost indistinguishable retention time to that of methionine or diaminopimelic acid under the usual conditions of amino acid analysis. Its ^{1}H NMR spectrum was similar to that of lysine except for the additive presence of a singlet for 2H at δ 4.06 which can be assigned to N-$\underline{CH_2}$-CO function. Moreover, the signals of δ 4.21 in ^{1}H NMR and δ 54.5 in ^{13}C NMR indicated the α-amino acid structure, N-$\underline{CH}$R-COOH. Furthermore, from its ^{13}C NMR, it was revealed that 5 × CH_2, 1 × CH, and 2 × CO carbons are present in the molecule. The neutral behaviour on paper electrophoresis and the elemental analysis indicated the presence of two NH_2 (or NH) groups and two COOH groups. Thus a tentative structure was deduced; either N^{ε}-carboxymethyl(CM)-Lysine (1) or N^{α}-CM-lysine (2).

In order to confirm the structure, both amino acids were then synthesized starting from L-lysine according to the procedures shown in the Experimental. By comparison with the two synthetic compounds thus obtained, the natural amino acid was found to be identical with N^{ε}-CM-L-lysine 1) and different from N^{α}-CM-derivative (2) in respect of TLC, amino acid analysis, ^{1}H NMR and $[\alpha]_D$. This is a first finding of N^{ε}-CM-L-lysine in plants, although its occurrence as a free amino acid in human urine has been recently reported [1]. The amino acid was never found as a free form in the plant, but only in the hydrolysate. This may mean that the enzymical carboxymethylation may occur at the ε-NH_2 group of the lysine residue in a conjugated form. Since nothing is known about the biosynthetic formation of this amino acid, the possibility of its artificial formation by means of either a herbicide or a pesticide supplied to the paddy field cannot be excluded.

$$R_2NH(CH_2)_4CH\begin{matrix}NHR_1\\CO_2H\end{matrix}$$

1 $R_1 = CH_2CO_2H$; $R_2 = H$
2 $R_1 = H$; $R_2 = CH_2CO_2H$

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EXPERIMENTAL

For NMR, chemical shifts are recorded using tetramethylsilane as standard. Off resonance features of each signal are given in parentheses. Abbreviations used: Boc, *t*-butoxycarbonyl; CM, carboxymethyl; Z, benzyloxycarbonyl.

Isolation of the unknown amino acid from hydrolysate of Sagittaria pygmaea. The whole plant (9.2 kg) was washed and air-dried at room temp. The dried material (740 g) was hydrolysed with 6 N HCl (20 l.) at 100° for 30 hr and then refluxed for an additional 30 hr. After decolorization with active charcoal, the mixture was filtered and condensed *in vacuo*. A soln of the residue in Py–HCOOH buffer (pH 3.1, 660 ml) was subjected to an Amberlite CG 120 column and eluted with the same buffer soln followed by Py–CH_3COOH buffer (pH 4.4). The later fraction containing the unknown amino acid was repeatedly applied to the same column, which was eluted with the gradient concn of HCl. The eluate with 4 N HCl contained only diaminopimelic acid and the unknown amino acid, which were finally separated by means of PLC of Si gel G on PhOH–H_2O (4:1). Crude wt 52 mg, mp 265–270° (decomp.), R_f on Si gel TLC (MeOH–H_2O–Py, 77:20:10): 0.44, $[\alpha]_D^{26}$ + 18.8° (*c* 0.5, 6N HCl). (Found: C, 45.77; H, 7.77; N, 13.24. $C_8H_{16}O_4N_2 \cdot 1/4H_2O$ requires: C, 46.03; H, 7.97; N, 13.42%). ^{1}H NMR (100 MHz, 3 N DCl–D_2O): δ 1.4–2.2 (6H, *m*), 3.23 (2H, *t*, J = 8 Hz), 4.06 (2H, *s*), 4.21 (1H, *t*, J = 6 Hz). ^{13}C NMR (25.05 MHz, D_2O): δ 21.64 (*t*), 25.24 (*t*), 29.97 (*t*), 47.07 (*t*), 49.22 (*t*), 54.58 (*d*) 171.34 (*s*), 174.50 (*s*).

Synthesis of N^{ε}-*CM*-L-*lysine* (1) [cf. 1]. N^{α}-Boc-L-lysine was prepared starting from N^{ε}-Z-L-lysine according to the usual procedure [2]. However, a new reagent, 2-(*t*-butoxycarbonyloximino)-2-phenylacetonitrile [3] was employed for *t*-butoxycarbonylation. To a soln of N^{α}-Boc-L-lysine (300 mg, 1.2 mM) in H_2O (10 ml), a neutralized soln of monobromoacetic acid (260 mg, 1.9 mM) with aq. 0.2 N NaOH was added. The reaction mixture was subjected to Amberlite IRCG 120 Type 1 (70 ml) and NaOH. After acidification with 6 N HCl to pH 2, the soln was stirred at 60–70° for 1 hr to remove the Boc group. The reaction mixture was subjected to Amberlite IRCG 120 Type 1 (70 ml) and then Dowex 1 × 8 (20 ml) columns to remove inorganic salts. The desired product, N^{ε}-CM-L-lysine was separated from lysine and N^{ε},N^{ε}-diCM-lysine by paper electrophoresis using Toyo No. 50 paper and a buffer soln of pH 6.9. Aq. extract from paper was condensed *in vacuo* and crystallized from H_2O–EtOH. Recrystallization was carried out from the same solvent. Yield, 30.9 mg (17.7%), mp *ca* 280° (decomp.). $[\alpha]_D^{22}$ + 19.1° (*c* 1, 6 N HCl). (Found: C, 46.05; H. 7.75; N, 13.32. Calc. for $C_8H_{16}O_4N_2 \cdot 1/4H_2O$: C, 46.03; H, 7.97; N, 13.42%).

Synthesis of N^{α}-*CM-*L-*lysine* (2). N^{α}-CM-L-lysine was prepared by carboxymethylation of N^{ε}-Z-L-lysine (2.00 g, 7.1 mM) according to a similar procedure to that for N^{ε}-CM-lysine described above [1]. Mp 237–240° (decomp.). (Found: C, 46.17; H, 7.83; N, 13.30. Calcd for $C_8H_{16}O_4N_2 \cdot 1/4H_2O$: C, 46.03; H, 7.97; N, 13.42%.

REFERENCES

1. Wadman, S. K., De Bree, P. K., Van Sprang, F. J., Kamerling, J. P., Haverkamp, J. and Vliegenthart, J. F. G. (1975) *Clin. Chim. Acta* **59**, 313.
2. Schwyzer, R., Costopanagiotis, A. and Sieber, P. (1963) *Helv. Chim. Acta* **46**, 870.
3. Itoh, M., Hagiwara, D. and Kamiya, T. (1977) *Bull. Chem. Soc. Jpn* **50**, 718.

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MUCILAGE IN CALLUS CULTURES OF HIGHER PLANTS

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Key Word Index—Callus cultures; mucilage; monosaccharides; galactose; mannose.

INTRODUCTION

Mucilage commonly occurs in higher plants [1, 2] but, so far, this class of natural products has not received any attention by workers studying plant cell cultures. Since mucilage finds wide industrial application [3], it was considered worthwhile to investigate callus cultures of some medicinally and economically important plants for their mucilage contents and for the composition of constituent monosaccharides in the mucilage. The results of this study are summarized in the present communication.

RESULTS AND DISCUSSION

The data presented in Table 1 indicate that callus cultures of higher plants are relatively rich in mucilage, which commonly makes up 8–10% of the dry wt. In callus cultures of *Trigonella foenum-graecum*, 21.2% mucilage is found. The seeds and 3-week-old seedlings of *Trigonella foenum-graecum* contain 26.3 and 10.8% mucilage, respectively.

Galactose and mannose appear to be the common constituent monosaccharides in mucilage of callus cultures. In mucilage from cultures of *Catharanthus roseus*, glucose instead of galactose, is detected and in mucilage from *Nicotiana glauca*, only galactose is present. In the case of *T. foenum-graecum* and *Glycine max*, a striking similarity between the qualitive and quantitative composition of mucilage in callus cultures (Table 1) and mucilage in organs of the intact plant [4, 5] is observed.

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Table 1. Mucilage contents* of callus cultures and monosaccharides of hydrolysates

Callus culture	Mucilage content	Monosaccharides (ratio)
Trigonella foenum-graecum	21.2	Galactose:mannose (1:1·4)
Catharanthus roseus	8.4	Glucose:mannose (1·2:1)
Cicer arietinum	8.9	Galactose:mannose (1·5:1)
Glycine max	8.1	Galactose:mannose (1:1·2)
Nicotiana glauca	9.4	Galactose

* Values are expressed in % dry wt.

EXPERIMENTAL

Callus cultures of *T. foenum-graecum* and *C. roseus* were established from sterile seedlings on MS medium [6]. Cultures of *Cicer arietinum*, *G. max* and *N. glauca* were kindly provided by Prof. W. Barz, University of Münster, Münster, Germany. Eight-week-old cultures were dried *in vacuo* at 60° for 12 hr. Mucilage was extracted with 5% aq. HOAc, precipitated from the conc soln by excess EtOH, purified, dried to constant wt and determined gravimetrically [7]. Mucilage was hydrolysed by heating in a sealed tube with 2 N H_2SO_4 at 100° for 1 hr. The soln was cooled, neutralized with $BaCO_3$, passed through Amberlite IR-120 and concd *in vacuo* [8]. The monosaccharides formed by hydrolysis were identified by TLC together with authentic samples on Kieselguhr G plates impregnated with Pi buffer (pH 5) using n-BuOH–Me_2CO–Pi buffer, pH 5 (4:5:1) as the developing solvent [9]. The monosaccharides were also analysed qualitatively and quantitatively by GLC of their TMSi derivatives [10] using a glass column (2 m × 6 mm) packed with Chromosorb W, 80–100 mesh, coated with 15% Carbowax 20 M. Column temp. was 220°, N_2 was carrier gas. The proportions of the constituent monosaccharides were determined from their rel. peak areas.

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