*Phytochemistry*, Vol. 27, No. 3, pp. 931–932, 1988.
Printed in Great Britain.

0031-9422/88 $3.00+0.00


# ISOLATION OF (*S*)-(+)-α-METHYLSERINE AND γ-L-GLUTAMYL-L-ARGININE FROM *SPHAGNUM PALUSTRE*

HIDEJIRO MATSUTANI,* KUNIYUKI SETOGAWA,* TATEAKI WAKAMIYA, YUKIO KOBAYASHI, YOSHIAKI ODA, and TETSUO SHIBA†

*Fukui Technical College, Sabae, Fukui 916, Japan; Department of Chemistry, Faculty of Science, Osaka University, Toyonaka, Osaka 560, Japan

(*Received* 4 *August* 1987)

**Key Word Index**—*Sphagnum palustre*; Bryophyta; isolation; structural determination; synthesis; amino acid; peptide; (*S*)-(+)-α-methylserine; γ-L-glutamyl-L-arginine.

**Abstract**—(*S*)-(+)-α-Methylserine and γ-L-glutamyl-L-arginine were isolated from *Sphagnum palustre*. This is the first isolation of α-methylserine from the plants.

## INTRODUCTION

Two unusual compounds, (*S*)-(+)-α-methylserine (**1**) [1–5] and γ-L-glutamyl-L-arginine (**2**) [6], were isolated from an aqueous extract of *Sphagnum palustre* collected at Asahi-cho, Niu District in Fukui Prefecture. This is the first isolation of α-methylserine from the plants. γ-L-Glutamyl-L-arginine is also a very rare peptide found in nature. So far as we know, this compound has only been isolated from onion [6].

## RESULTS AND DISCUSSION

The structure of amino acid **1** was shown to be (*S*)-(+)-α-methylserine from the measurement of NMR spectrum, FD-mass spectrum of its methyl ester derivative, and $[\alpha]_D$. The same amino acid had already been found as a component of the antibiotic amicetin produced by *Streptomyces vinaceusdrappus* or *S. fasciculatis* [1] and the (*R*)-(−)-isomer was recently synthesized by Schöllkopf *et al.* [4, 5].

The amino acid of the peptide **2** was first determined by the DNP method. The γ-peptide bond linkage was suggested by the inability to form the PTH derivative of glutamic acid or glutamine after the Edman degradation. The peptide migrated in the same direction and distance as those of the neutral compound in paper electrophoresis. This fact indicated the presence of glutamic acid but not glutamine in the molecule. Furthermore the DNP-derivative of each constituent amino acid showed the same ORD curve as that of the authentic L-compound, respectively. Thus, the peptide **2** was assumed to be γ-L-glutamyl-L-arginine. In order to confirm this assumption, we synthesized γ-L-glutamyl-L-arginine, as well as α-L-glutamyl-L-arginine for comparison, as described in Experimental. The synthetic γ-peptide was identical with the natural peptide in respect of $R_f$ value on TLC, retention time in amino acid analysis, and NMR spectrum. The colour of the ninhydrin reaction of γ-L-glutamyl-L-arginine or the natural compound is purple, while α-L-glutamyl-L-arginine is yellowish brown initially and gradually changes to purple.

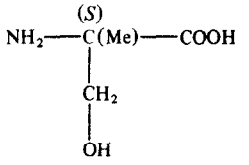
The occurrence of these two unusual compounds from *Sphagnum palustre* may have chemotaxonomic interest, although the biosynthesis of these compounds has not yet been elucidated.

## EXPERIMENTAL

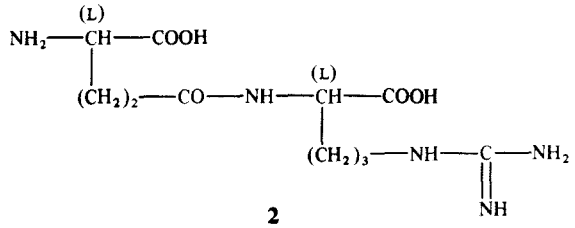
The mps are uncorr. Chemical shifts were given by use of Na 2,2-dimethyl-2-silapentane-5-sulphonate (DSS) as the external standard. TLC was carried out by the ascending method on silica-gel $60F_{254}$ plate (Merck, No. 5715) using *n*-BuOH–MeCOOH–$H_2O$ = 4:1:2 (solvent A) and PhOH–$H_2O$–15 M $NH_4OH$ = 30:10:1 (solvent B). Amino acid analysis was carried out with a Hitachi KLA-5 analyser under the following modified conditions; column: packed with a Hitachi No. 2618 resin (0.9 × 25 cm) at 55°, buffer; Na citrate buffer of 0.2 M (pH 3.25, 70 min), 0.2 M (pH 4.25, 34 min) and 0.8 M (pH 6.60, 110 min).

*Isolation of* (S)-α-*methylserine* (**1**) *and* γ-L-*glutamyl*-L-*arginine* (**2**). *Sphagnum palustre* (1.2 kg) was washed with water, air-dried, ground, and then boiled in $H_2O$ (*ca* 20 l). Insoluble material was filtered and washed with $H_2O$ several times. Each one third of the filtrate (*ca* 70 l) was separately applied to Amberlite IRCG-120 column ($H^+$ form, 6 × 40 cm) and eluted with 2 M $NH_4OH$ (1.5 l) after washing with $H_2O$ (10 l). The eluate (1.5 l × 3) was concd *in vacuo* and the residue was subjected to successive column chromatography as follows; (i) Amberlite IRCG-120 ($NH_4^+$ form, 6 × 40 cm, elution with $H_2O$); (ii) Amberlite IRCG-4B [$MeCOO^-$ form, 6 × 42 cm, elution with $H_2O$ (1.2 l) and 0.5 M MeCOOH (1.2 l)], (iii) Amberlite IRCG-120 [pyridine (Pyr) form, 6 × 60 cm, elution with 0.1 M Pyr–HCOOH (pH 3.1, 2 l) = $P_1$, 0.2 M Pyr–HCOOH (pH 3.1, 1 l) = $P_2$, 0.2 M Pyr–MeCOOH (pH 4.4, 3 l) = $P_3$, 1 M Pyr–MeCOOH (pH 5.1, 4 l) = $P_4$]. The first fraction containing **1** was concd *in vacuo* and rechromatographed on Amberlite IRCG-120 [Pyr form, 3 × 54 cm, elution with $P_1$ (1.8 l)]. Crude crystalline residue obtained was recrystallized from $H_2O$; yield 167 mg, mp

†Author to whom correspondence should be addressed.



1



2

242–247° (dec), $[\alpha]_D^{28}$ + 5.9° ($H_2O$, *c* 1.0) [cf. the amino acid from amicetin: mp 235–240° (dec), $[\alpha]_D^{25}$ + 6.3° ($H_2O$, *c* 1.0)]. [1] $^1H$ NMR (100 MHz, $D_2O$): δ1.40 (3H, *s*), 3.62 (1H, *d*, *J* = 12 Hz), 3.89 (1H, *d*, *J* = 12 Hz). $^{13}C$ NMR (22.5 MHz, $D_2O$): δ21.0 (C-2'), 65.6 (C-2), 67.9 (C-3), 179.9 (C-1), FDMS of methyl ester hydrochloride: *m/z* 134 $[M+H]^+$.

The second fraction containing **2** was concd *in vacuo* and rechromatographed on Amberlite IRCG-120 [Pyr form, 3 × 140 cm, elution with $P_1$ (1 l), $P_2$ (1.8 l), $P_3$ (1.8 l), and $P_4$ (1.3 l)]. Crude peptide obtained was finally purified by prep. TLC [prepared by coating with Merck silica gel 60 H (No. 7736); developed with solvent A], γ-L-Glutamyl-L-arginine was extracted with $H_2O$ from silica gel and isolated as a very hygroscopic powder by lyophilization, yield 114 mg. $R_f$ value on TLC: 0.08 (solvent A) and 0.21 (solvent B); amino acid analysis: 108 min; $[\alpha]_D^{23}$ + 9.9° (1 M HCl, *c* 1.0). Acid hydrolysis of **2** gave Glu (1.00) and Arg (1.22).

*Determination of configuration of glutamic acid and arginine.* The peptide **2** (8.1 mg, 27 mol) was hydrolysed with 6 M HCl (3 ml) in a sealed tube after evacuation. Hydrolysate was concd *in vacuo* and the concn was repeated × 3 after addition of $H_2O$ (each 3 ml). To the residue in $H_2O$ (1.5 ml) were added $NaHCO_3$ (30 mg, 0.34 mmol) and 2,4-dinitrofluorobenzene (25 mg, 0.13 mmol) in acetone (0.5 ml). The reaction mixture was stirred for 24 hr at 40° and then concd *in vacuo* after acidification. The crude product obtained was purified by prep. TLC (Merck silica gel plate, No. 5744) using the following developing solvent; $CHCl_3$–MeOH–MeCOOH = 19:1:2 for DNP-Glu and $CHCl_3$–MeOH–MeCOOH = 30:20:1 for DNP-Arg. DNP-Glu (5 mg) and DNP-Arg (6 mg) thus obtained were dissolved in 7 ml of 4% aq $NaHCO_3$, respectively. Optical rotation at 500 nm of each sample showed the negative value corresponding to the L-form. Optical purity was measured by comparison with the authentic sample; Glu 91% and Arg 98%.

*Synthesis of γ-L-glutamyl-L-arginine and α-L-glutamyl-L-arginine.* For the preparation of γ-L-glutamyl-L-arginine Z-L-Glu(OSu)-OBzl [7] (936 mg, 2 mmol) was coupled with H-L-Arg($NO_2$)-OBzl·2TosOH purchased from the Protein Research Foundation, Japan (1.31 g, 2 mmol) in THF in the presence of triethylamine (606 mg, 6 mmol). After general work-up, we obtained Z-L-Glu (L-Arg($NO_2$)-OBzl)-OBzl which was recrystallized from EtOH and hexane; yield 1.20 g (90%), mp 136.5°–138°, $[\alpha]_D^{23}$ − 105° ($CHCl_3$, *c* 1.00).

The protected peptide (500 mg, 0.755 mmol) in 90% MeOH (45 ml) and HOAc (8 ml) was hydrogenated in the presence of Pd catalyst for 3 hr. The catalyst was filtered off and the filtrate was concd *in vacuo*. To the residue in a small amount of $H_2O$ was added an excess of Pyr. The soln was concd in *vacuo* again and the residue was triturated with EtOH. The crude product obtained by centrifugation was repptd from $H_2O$ and EtOH to give a hygroscopic powder which did not show clear mp, yield 218 mg (95%). $R_f$ value: 0.08 (solvent A) and 0.21 (solvent B); amino acid analysis: 108 min; $[\alpha]_D^{23}$ + 9.2° (1 M HCl, *c* 1.00).

An authentic sample of α-L-glutamyl-L-arginine was prepared in a similar manner to that reported in ref. [8]. $R_f$ value: 0.11 (solvent A) and 0.18 (solvent B); amino acid analysis: 114 min.

## REFERENCES


1. Flynn, E. H., Hinman, J. W., Caron, E. L. and Woolf, D. O. Jr (1953) *J. Am. Chem. Soc.* **75**, 5867.
2. Hanessian, S. and Haskell, T. H. (1964) *Tetrahedron Letters* 2451.
3. Takamura, N., Terashima, S., Achiwa. K. and Yamada, S. (1967) *Chem. Pharm. Bull.* **15**, 1776.
4. Schöllkopf, U., Groth, U. and Hartwig, W. (1981) *Liebigs Ann. Chem.* 2407.
5. Groth, U., Chiang,Y. and Schöllkopf, U. (1982) *Liebigs Ann. Chem.* 1756.
6. Matikkala, E. J. and Virtanen, A. I. (1970) *Suomen Kemistilehti* **B43**, 435.
7. Hardy, P. M., Haylock, J. C. and Rydon, H. N. (1972) *J. Chem. Soc. Perkin I* 605.
8. Gibian, H. and Schröder, E. (1961) *Liebigs Ann. Chem.* **642**, 145.