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TWO CYSTEINE DERIVATIVES IN ASPARAGUS SHOOTS

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Key Word Index—*Asparagus officinalis*; Liliaceae; cysteine derivatives; mixed disulfide of *S*-(2-carboxy-3-mercaptopropyl)-L-cysteine and 3-mercaptoisobutyric acid; disulfide of *S*-(2-carboxy-3-mercaptopropyl)cysteine.

Abstract—A mixed dusulfide of *S*-(2-carboxy-3-mercaptopropyl)-L-cysteine and 3-mercaptoisobutyric acid and the disulfide of *S*-(2-carboxy-3-mercaptopropyl)cysteine, not previously known in nature, have been isolated from asparagus (*Asparagus officinalis*) shoots. The former was converted to the latter by reduction with zinc powder in HCl followed by reoxidation with aeration.

INTRODUCTION

Asparagus (*Asparagus officinalis*) is one of the important vegetables cultivated in Hokkaido Island, Japan. The crops belonging to *Allium* of the same family are famous for containing a variety of cysteine derivatives, which have been discussed in relation to characteristic features such as flavor, antibacterial and lachrymatory activity [1–3]. Although some S-containing compounds such as asparagusic acid and its derivatives have been isolated from asparagus shoots [4], no cysteine derivative has been reported. We have isolated two acidic cysteine derivatives and six acidic dipeptides from asparagus shoots [5]. The present paper deals with the isolation and structure determination of two additional, new acidic cysteine derivatives.

RESULTS AND DISCUSSION

The acidic amino acid fraction of asparagus shoots contained several S-containing compounds which gave positive reactions with both iodoplatinate and ninhydrin reagents. Four of them were isolated using ion exchange chromatography, preparative PC and HVE. The structure and behaviors on PC, HVE and amino acid analyser of these four S-containing acidic amino acids are summarized in Table 1. Two of them were identified as *S*-(2-carboxy-*n*-propyl)-L-cysteine (**1**) and *S*-(1,2-dicarboxyethyl)-L-cysteine [5]. The structures of the other two compounds were established as the mixed disulfide of *S*-(2-carboxy-3-mercaptopropyl)-L-cysteine and 3-mercaptoisobutyric acid (**3**) and the disulfide of *S*-(2-carboxy-3-mercaptopropyl)cysteine (**4**), respectively, by spectral analysis and chemical reactions.

Mixed disulfide of S-(2-*carboxy*-3-*mercaptopropyl*)-L-*cysteine and* 3-*mercaptoisobutyric acid*(**3**).

Compound **3** obtained as a chromatographically pure evaporation residue had an unusual position on 2D-PC (Table 1) and gave a positive reaction in the –SS– test [6]. FD mass spectrometry and ^{1}H NMR spectra showed that **3** was a cysteine derivative. FD mass spectrum (*m/z*): 358 ($M^+ + 1$, 100%), 240 [$HOOCCH(NH_2)CH_2SCH_2CH(COOH)CH_2S^+ + 2$, 24%], 239 (21%), 238 (20%), 119 [$^+SCH_2CH(COOH)Me$, 15%]. FD-high resolution mass spectrum (*m/z*): 358.0518 (calcd. for $C_{11}H_{20}NO_6S_3$, 358.0453), 238.0167 (calcd. for $C_7H_{12}NO_4S_2$, 238.0207). ^{1}H NMR (200 MHz in D_2O): 1.18 (*d*, $J = 6.5$ Hz, 3 H), 2.76–2.91 [*m*, 8 H, $-SCH_2CH(COOD)CH_2SSCH_2CH(COOD)-$], 3.02 (*dd*, $J = 8, 14$ Hz, 1 H) and 3.18 *dd*, $J = 4$, 14 Hz, 1 H, $^-OOCCH(ND_3^+)CH_2S-$], 3.92 *dd*, $J = 4$, 8 Hz, 1 H, $^-OOCCH(ND_3^+)-$]. The presence of three carboxyl groups was confirmed by the FD mass spectrum after esterification with ethanolic HCl. Spectrum of the Et ester (*m/z*): 442 ($M^+ + 1$, corresponding to the tri Et ester of **3**, 100%), 296 [$EtOOCCH(NH_2)CH_2SCH_2CH(COOEt)CH_2S^+ + 2$, 8%], 295 (5%), 294 (26%), 147 [$^+SCH_2CH(COOEt)Me$, 17%]. Compound **3** was treated with Raney Ni. Only partial desulfurization occurred probably because the catalyst used was old and not very effective. The reaction mixture gave a spot for *S*-(2-carboxy-*n*-propyl)cysteine (**1**), a strong spot of unchanged starting **3** and a few weak spots of unidentified products on 2D-PC. Compound **3** was reduced with Zn powder in 1 N HCl and the reaction mixture was treated with Dowex 50 (H^+). The eluate from the column with 2 N NH_4OH was subjected to air oxidation. The product of reduction and reoxidation of **3** was identical to compound **4** by 2D-PC, FD and ^{1}H NMR spectra. The configuration of the cysteine moiety of **3** was tentatively determined as L by the Clough–Lutz–Jirgenson's rule [7]. $[\alpha]_D^{17}$ −39.4° (*c* 1, H_2O) and −35.3° (*c* 1, 2 N HCl). The configuration at the other two chiral centres is still unknown.

Disulfide of S-(2-*carboxy*-3-*mercaptopropyl*)*cysteine*(**4**)

Compound **4** was obtained as a chromatographically pure crystalline evaporation residue, but the yield was very low. A substantial proportion of **4** was lost during a purification step with preparative HVE. The reason for this loss is not known. Compound **4** also gave a positive reaction with the –SS– test [6]. It was suggested from ^{1}H NMR and FD spectra that compound **4** is a cysteine derivative with a symmetrical structure. The ^{1}H NMR spectrum of **4** was very similar within the range of 2.5–4.0 ppm to that of **3**. ^{1}H NMR (200 MHz in D_2O): 2.84–2.94 [*m*, 10 H, $2 \times -SCH_2CH(COOD)CH_2S-$], 3.01 (*dd*, $J = 8, 15$ Hz, 2 H) and 3.19 [*dd*, $J = 4, 15$ Hz, 2 H,

Table 1. Behaviors of four cysteine derivatives isolated from asparagus shoots by PC, HVE and amino acid analysers

	R_{Glu} on PC		Migration ratio to Glu on HVE		Elution time on amino acid analyser (hr)†
	Solvent 1*	Solvent 2*	Buffer 1*	Buffer 2*	
S-(2-Carboxy-*n*-propyl)-L-cysteine(**1**)‡	1.94	1.52	1.00	0.81	2.33
S-(1,2-Dicarboxyethyl)-L-cysteine (**2**)‡	1.05	0.22	4.10	1.43	1.41
Mixed disulfide of *S*-(2-carboxy-3-mercaptopropyl)-L-cysteine and 3-mercaptoisobutyric acid (**3**)	3.06	1.07	2.13	1.13	3.57
Disulfide of *S*-(2-carboxy-3-mercaptopropyl)cysteine (**4**)	0.98	0.18	1.69	0.97	3.47

* See text for solvent and buffer systems.

† Analytical conditions. Column: Amberlite CG-120 (400 mesh) (0.9 × 50 cm). Column temp: 50°. Eluant: 0.2 N Na citrate buffer (pH 3.25) followed by 0.2 N Na citrate buffer (pH 4.25) at 90 min. Flow rate: 30 ml/hr.

‡ See ref. [5] for compounds **1** and **2**.

$HOOCCH(NH_2)CH_2S–R$ **1** R = $–CH_2CH(COOH)Me$
2 R = $–CH(COOH)CH_2COOH$
3 R = $–CH_2CH(COOH)CH_2SSCH_2CH(COOH)Me$
4 R = $–CH_2CH(COOH)CH_2SSCH(COOH)CH_2SCH_2CH(NH_2)COOH$

$2 \times {}^{-}OOCCH(ND_3^+)C\underline{H}_2–$], 3.91 [*dd*, J = 4, 8 Hz, 2 H, $2 \times {}^{-}OOCC\underline{H}(ND_3^+)–$]. FD mass spectrum ($m/z$): 477 ($M^+ + 1$, 100%), 240 ($M^+/2 + 2$, 56%), 239 ($M^+/2 + 1$, 42%). Strong fragment ions m/z 240 and 239 were also observed in the FD mass spectrum of **3**. Compound **4** was treated with Raney Ni. Only partial desulfurization occurred as in the case of **3**. The reaction mixture gave a spot for *S*-(2-carboxy-*n*-propyl)cysteine (**1**), a strong spot of unchanged starting compound **4**, and a few spots of unidentified products on 2D-PC. The structure of compound **4** followed from the spectral analyses and chemical reactions and was confirmed by the fact that **4** was obtained upon reduction and re-oxidation of **3**. The configurations at the 4 chiral centres of **4** are unknown.

It has been proved that *S*-(2-carboxy-*n*-propyl)cysteine is formed from cysteine and methacrylic acid in *Allium sativum* [8]. 3-Mercaptomethacrylic acid and 3-mercaptoisobutyric acid have been detected in asparagus [9]. If cysteine reacts with 3-mercaptomethacrylic acid in asparagus in the same way as in *A. sativum*, *S*-(2-carboxy-3-mercaptopropyl)cysteine, which is a reasonable precursor of **3** and **4**, will be formed. It is also interesting that both **3** and **4** have the same skeleton as that of asparagusic acid [4], i.e., $–SCH_2CH(COOH)CH_2S–$.

EXPERIMENTAL

General methods. PC was carried out in *n*-BuOH–HOAc–H_2O(4:1:2) (solvent 1) and PhOH–H_2O–conc. NH_4OH (120:30:1, w/v/v) (solvent 2), 2D-PC with solvents 1 and 2. HVE was performed at pH 3.6 (pyridine–HOAc–H_2O, 1:20:200, 55 V/cm) (buffer 1) and pH 6.5 (pyridine–HOAc–H_2O, 25:1:500, 100 V/cm) (buffer 2).

Plant material and extraction. The lower part (bottom cut) (24 kg) of asparagus shoots supplied from the experimental farm of Hokkaido University was cut into small pieces and extracted with EtOH (20 l.). The residue was homogenized × 2 with 70% EtOH (25 l. each) and the extracts were combined.

Isolation of **3** *and* **4**. The extracts were concd and an equal amount of H_2O was added (total vol. *ca* 3.5 l.). After centrifugation, the supernatant was applied to a column of Amberlite IR-120 (H^+, 2.5 l.), which was thoroughly washed with H_2O. The amino acid fraction was eluted with 2 N NH_4OH, concd, adjusted to pH *ca* 4 with HOAc and centrifuged. The supernatant was applied to a column of Dowex 1 × 4 (AcO^-, 500 ml). After the neutral and basic fraction was washed out with H_2O, acidic amino acids were eluted with 0.2 N HOAc (11.2 l.), 2 N HOAc (13.6 l.) and 2 N HCl (2 l.). Fractions of 200 ml each were collected. With the aid of a combination of cation and anion exchange chromatography and prep. PC, **3** was isolated from fractions 79–124 as a chromatographically pure evaporation residue (21.8 mg). Compound **4**, isolated from fractions 57–78 by the same procedures was further purified by prep. HVE (buffer 1) to give a chromatographically pure crystalline evaporation residue (1.5 mg). A fairly large amount of **4** was lost during the purification by prep. HVE. For ^1H NMR, FDMS, FD-HR MS data and optical rotations, see Results and Discussion.

Esterification of **3**. EtOH (10 ml) was added to **3** (1.5 mg) cooled to 0°. After introduction of HCl gas for 15 min, the soln was heated under reflux for 1 hr and concd. See Results and Discussion for FDMS data of the tri Et ester obtained.

Raney Ni *treatment of* **3** *and* **4**. The mixture of **3** (1 mg) and Raney Ni (200 mg) in 80% EtOH (2 ml) was heated under reflux for 6 hr. The reaction mixture was examined by 2D-PC. Compound **4** (0.5 mg) was also treated by the same way with Raney Ni (100 mg, reflux for 4 hr).

Conversion of **3** *to* **4**. Compound **3** (1.9 mg) was dissolved in 1 N HCl (20 ml) and Zn powder (170 mg) added to the soln. After stirring at room temp. for 1 hr, the reaction mixture was filtered and the filtrate treated with Dowex 50 (H^+, 1 ml), which was thoroughly washed with H_2O and eluted with 2 N NH_4OH. The eluate was concd, dissolved in H_2O and aerated for 1 hr. The oxidation product which showed a positive reaction in the –SS– test [6] was concd to give a crystalline evaporation residue (0.6 mg). ^1H NMR (200 MHz in D_2O), FDMS and the R_f value on 2D-PC of the reaction product were consistent with those of **4**. FDMS (m/z): 477 (M^+, +1, 80%), 240 ($M^+/2$, +2, 21%), 239 ($M^+/2 + 1$, 100%).

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