Hydrolysis of GS-E′ (I) with 2 N H_2SO_4 in EtOH——A solution of I (20 mg) in EtOH (10 ml) was treated with 2 N H_2SO_4 (10 ml) and the mixture was worked up in the same way as described for II. Glucose, arabinose, xylose and rhamnose were identified by TLC and PPC, and echinocystic acid was identified by comparison with an authentic sample.

Permethylation of I——Compound (I) (100 mg) was methylated in the same way as II to afford the permethylate (VI) (80 mg), as a white powder, $[\alpha]_D^{10}$ −35.4° (c=0.67, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730 (COOR). ^{1}H-NMR δ: 4.17 (1H, d, J=3 Hz, anomeric H), 4.29 (1H, d, J=7 Hz, anomeric H), 4.61 (1H, d, J=7 Hz, anomeric H), 4.70 (1H, d, J=7 Hz, anomeric H), 4.82 (1H, broad s, anomeric H), 5.17 (1H, broad s, anomeric H), 5.54 (1H, d, J=7 Hz, anomeric H). *Anal.* Calcd for $C_{88}H_{150}O_{34}\cdot 2H_2O$: C, 59.11; H, 8.64. Found: C, 58.85; H, 8.49.

Methanolysis of VI——A solution of VI in methanolic 1 N HCl was worked up in the same way as described for III. GLC t_R values; 2′30″ (methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside), 1′49″, 2′09″ (methyl 2,3,4-tri-O-methyl-D-xylopyranoside), 3′25″ (methyl 2,3,4-tri-O-methyl-L-arabinopyranoside), 4′36″, 6′20″ (methyl 2,3-di-O-methyl-L-rhamnopyranoside), 4′38″, 5′54″ (methyl 2,4-di-O-methyl-D-xylopyranoside), 7′50″, 10′40″ (methyl 2,3,4-tri-O-methyl-D-glucopyranoside), 13′25″, 15′20″ (methyl 3,4-di-O-methyl-D-glucopyranoside). The identities of these products were confirmed by comparison with authentic samples.

Reduction of VI with $LiAlH_4$——The permethylate (VI) (70 mg) was dissolved in anhydrous THF (10 ml), and worked up in the same way as described for III. Compound VII (20 mg) was obtained as a colorless syrup from the ether extract of the reaction mixture, $[\alpha]_D^{10}$ −15.2° (c=0.76, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH). ^{1}H-NMR δ: 0.84, 0.88, 0.90, 0.93, 0.99, 1.03, 1.25 (3H, each s, 7×CH_3), 3.27, 3.44, 3.48, 3.50, 3.56, 3.61, 3.62 (3H, each s, 7×OCH_3), 4.17 (1H, d, J=3 Hz, anomeric H), 4.29 (1H, d, J=7 Hz, anomeric H), 5.24 (1H, triplet-like). *Anal.* Calcd for $C_{48}H_{82}O_{12}\cdot 2H_2O$: C, 64.97; H, 9.77. Found: C, 65.41; H, 9.36. The compound obtained from the AcOEt extract was identical with V as judged by TLC, IR and ^{1}H-NMR comparisons.

Methanolysis of VII——A solution of VII in methanolic 1 N HCl was refluxed for 3 h and the reaction mixture was worked up in the same way as described for III. Methyl 2,3,4-tri-O-methyl-L-arabinopyranoside, and methyl 2,3,4-tri-O-methyl-D-glucopyranoside were identified by TLC and GLC comparisons with authentic samples.

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A Convenient Preparation of N-Acylpyroglutamic Acid

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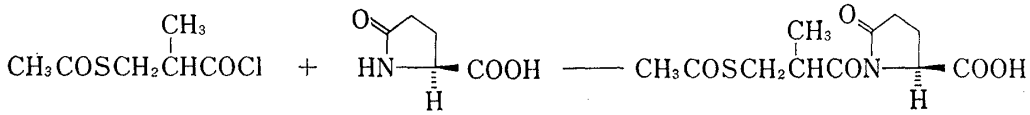
Pyroglutamic acid reacted with acyl chloride in the presence of triethylamine in acetonitrile, yielding N-acylpyroglutamic acid without epimerization by way of mixed anhydride formation followed by intramolecular N-acylation.

Keywords——angiotensin-converting enzyme; pyroglutamic acid; N-acylpyroglutamic acid; (2*S*)-1-[(2*S*)-3-acetylthio-2-methylpropanoyl]pyroglutamic acid; N-acetyl-L-pyroglutamic acid

During the course of research on angiotensin-converting enzyme inhibitors,[1] we required a procedure for N-acylation of pyroglutamic acid. Several methods for the preparation of N-acylpyroglutamic acid have been reported.[2] In these methods, N-acylpyroglutamic acids were prepared from glutamic acid *via* N-acylglutamic acids and some epimerization occurred.

We found that pyroglutamic acid reacted with acyl chloride in the presence of triethylamine in aprotic solvents under mild conditions to afford N-acylpyroglutamic acid without epimerization.

We carried out the N-acylation of L-pyroglutamic acid II with (2*S*)-3-acetylthio-2-methylpropanoyl chloride I (Chart 1). The results are summarized in Table I.



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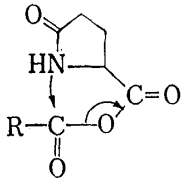

IV

Chart 1

TABLE I. Preparation of (2*S*)-1-[(2*S*)-3-Acetylthio-2-methylpropanoyl]pyroglutamic Acid

I (mmol)	II (mmol)	Base (mmol)	Solvent	Temp., time	Yield
1.0	1.0			120°C	No reaction
1.0	1.0	Pyridine 1.0	$CHCl_3$	Reflux 20 h	20%
1.0	1.0	NEt_3 2.0	CH_2Cl_2	40°C, 2 h	55%
1.0	1.0	NEt_3 2.0	CH_3CN	r.t., 2 h	65%

As shown in Table I, no reaction was observed when II was treated with I in the absence of base. However II reacted with I in the presence of triethylamine (2 eq) in dry methylene chloride (at reflux temperature) or acetonitrile (at room temperature) without epimerization, affording (2*S*)-1-[(2*S*)-3-acetylthio-2-methylpropanoyl]pyroglutamic acid III in a moderate yield.

On the other hand, it was observed that *tert*-butyl L-pyroglutamate did not react with I in the presence of triethylamine under the same conditions (acetonitrile, room temperature, 20 h).

Thus, N-acylation of pyroglutamic acid under these conditions probably proceeds by way of the formation of a mixed anhydride such as IV, followed by intramolecular N-acylation. Other results of N-acylation of II with various acyl chlorides are summarized in Table II.

Table II. N-Acylation of L-Pyroglutamic Acid

Compound	Yield	$[\alpha]_D$ (MeOH)	mp
N-Acetyl-L-pyroglutamic acid	95%	−25.9° (*c*, 1.00)	Oil
N-Pivaloyl-	54%	−32.3° (*c*, 1.00)	149—150°C
N-Acryloyl-	78%	Easily polymerized	
N-Methacryloyl	43%	−22.6° (*c*, 1.00)	137—138°C
N-Benzoyl-	21%	+22.2° (*c*, 1.00)	166—167°C
N-Benzyloxycarbonyl-[2]	20%	−29.5° (*c*, 1.00)	135—136°C

These reactions were carried out in dry acetonitrile at room temperature for 1 h.

Experimental

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian XL-100 machine and signals are given in δ units downfield from TMS as an internal standard. Infrared (IR) spectra were measured with a Hitachi 269-30 spectrometer. Mass spectra and specific rotations (25°C) were taken on JMS-01SG and on JASCO DIP-4 machines, respectively.

(2*S*)-1-[(2*S*)-3-Acetylthio-2-methylpropanoyl]pyroglutamic Acid III; General Procedure——A solution of (2*S*)-3-acetylthio-2-methylpropanoyl chloride (1.80 g, 10.0 mmol) in dry acetonitrile (2 ml) was added to a solution of L-pyroglutamic acid (1.29 g, 10.0 mmol) and triethylamine (2.02 g, 20.0 mmol) in dry acetonitrile (30 ml) at 0°C. After being stirred for 1 h at room temperature, the reaction mixture was filtered to remove precipitates, concentrated *in vacuo* and diluted with water (20 ml). The aqueous layer was washed with ethyl acetate, acidified with 1 N HCl and extracted with ethyl acetate.

The extract was dried over Na_2SO_4 and concentrated *in vacuo* to afford an oil. The oil was chromatographed on silica gel ($CHCl_3$: THF: AcOH/100: 10: 1) to afford 1.77 g of the title compound. $[\alpha]_D$ −100° (*c*=1.65, MeOH). NMR ($CDCl_3$) ppm: 1.24 (3H, d, *J*=7.0 Hz), 2.30 (3H, s), 2.10—2.90 (4H, m), 3.05 (1H, dd, *J*=7.0, 14.0 Hz), 3.21 (1H, dd, *J*=5.5, 14.5 Hz), 3.70—4.05 (1H, m), 4.76 (1H, dd, *J*=4.0, 8.0 Hz). IR (film) cm^{-1}: 1750, 1695. Calcd for $C_{11}H_{15}NO_5S$: C, 48.34; H, 5.53; N, 5.13; S, 11.73. Found: C, 48.41; H, 5.51; N, 5.01; S, 11.77.

N-Acetyl-L-pyroglutamic Acid; General Procedure——A solution of acetyl chloride (236 mg, 3.0 mmol) in dry acetonitrile (2 ml) was added to a solution of L-pyroglutamic acid (387 mg, 3.0 mmol) and triethylamine (606 mg, 6.0 mmol) in dry acetonitrile (20 ml) at 0°C. After being stirred for 1 h at room temperature, the reaction mixture was filtered, concentrated *in vacuo* and dissolved in water (10 ml). The aqueous layer was washed with ethyl acetate, acidified with 1 N HCl and extracted with ethyl acetate. The extract was dried over Na_2SO_4 and concentrated *in vacuo* to afford an oil.

The oil was chromatographed on silica gel ($CHCl_3$: THF: AcOH/20: 2: 1) to afford 488 mg (95% yield) of the title compound. $[\alpha]_D$ −25.9° (*c*=1.00, MeOH). NMR ($CDCl_3$) ppm: 1.53 (3H, s), 1.0—1.8 (4H, m), 3.77 (1H, dd, *J*=3.5, 8.5 Hz). IR (film) cm^{-1}: 1740, 1700, 1660. Calcd for $C_7H_9NO_4$: C, 49.12; H, 5.30; N, 8.18. Found: C, 49.03; H, 5.39; N, 8.13.

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