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137. Hidetaka Yuki,*2 Yasuo Tohira, Bunya Aoki, Tokio Kano, Shin-ichi Takama, and Tsuyoshi Yamazaki : Studies on Antiviral Agents. III.*3 Synthesis of Tenuazonic Acid Derivatives.

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Tenuazonic acid derivatives were synthesized from amino acid esters by N-acetoacetylation with diketene followed by cyclization with sodium alkoxides. Some L-amino acid produced a small amount of DL-compounds in this process. N-Acetoacetyl group of diethyl aspartate cyclized to the α-ester group to form five-membered ring compound selectively. These compounds were condensed with carbonyl reagents.

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Tenuazonic acid (I) is an antibiotic, first isolated by Rosett, *et al.*[1] from *Alternaria tenuis*, and its structure has been established by Stickings[2] as 3-acetyl-5-sec. butyl-4-hydroxy-3-pyrrolin-2-one. Shigeura, *et al.*[3] investigated its biological activity *in vivo* with rats and *in vitro* with Ehrlich ascites tumor and rat liver cells, and found that tenuazonic acid inhibited the incorporation of amino acid into proteins *in vivo* and *in vitro*. Kaczka, *et al.*[4,5] discovered its growth inhibitory effect on human adenocarcinoma-1 in the embryonated eggs. The inhibitory activity against Measles, Vaccinia, Herpes simplex, ECHO-9, and 'B' viruses was also reported by Miller, *et al.*,[6] who synthesized tenuazonic acid from ethyl L-isoleucinate and diketene according to Lacey's method.[7] Harris, *et al.*[8] reported the synthesis of tenuazonic acid and congeneric tetramic acids, and Gitterman[9] examined the antitumor, cytotoxic, and antibacterial activities of them. From these facts, it is of interest to examine the biological activities, particularly antivirus and antitumor activities of other amino acid derivatives, whose structure resemble tenuazonic acid.

In the present study, Lacey's method[7] was followed with some modification for the synthesis of tenuazonic acid derivatives. α-Amino acids (II) were first esterified to III by hydrochloric acid in ethanol, and then the amino groups were reacted with diketene in ether to give N-acetoacetylamino acid ethyl ester (IV), which was then cyclized by sodium methoxide or ethoxide in benzene to V. Thus, L-valine (V-1), L-phenylalanine (V-2), L-methionine (V-3), glycine (V-4), L-tyrosine (V-5), L-ethylaspartate (V-6), L-methylaspartate (V-7), L-leucine (V-8), L-ethionine (V-9), L-tryptophane (V-10),

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and L-isoleucine (I) derivatives (Table I) were prepared successfully. The compound V-1 could not be obtained in a pure crystalline form, so that it was identified as thiosemicarbazone. In the case of V-5 and V-8, a small amount of DL-compound has been isolated, which was identified by elemental analysis and by measurement of specific optical rotation. Aebi[10] reported that melting points of V-5 and V-8 were 186~187°, and 133.5~134.5°, respectively, without quoting the rotation of the compounds, but it is evident from the melting points in the present experiment (L-V-5, 114~114.5°; DL-V-5, 193°; L-V-8, 114°; DL-V-8, 134°) that the compounds described by him are DL-compounds. Melting points of V-1,2,3,4 are almost coincidental indicating that these compounds reported by Aebi are L-compounds. γ-Amino-*n*-butyric acid, DL-norvaline, DL-ε-amino-*n*-caproic acid, L-lysine, L-arginine, and L-cysteine did not give the desired products.

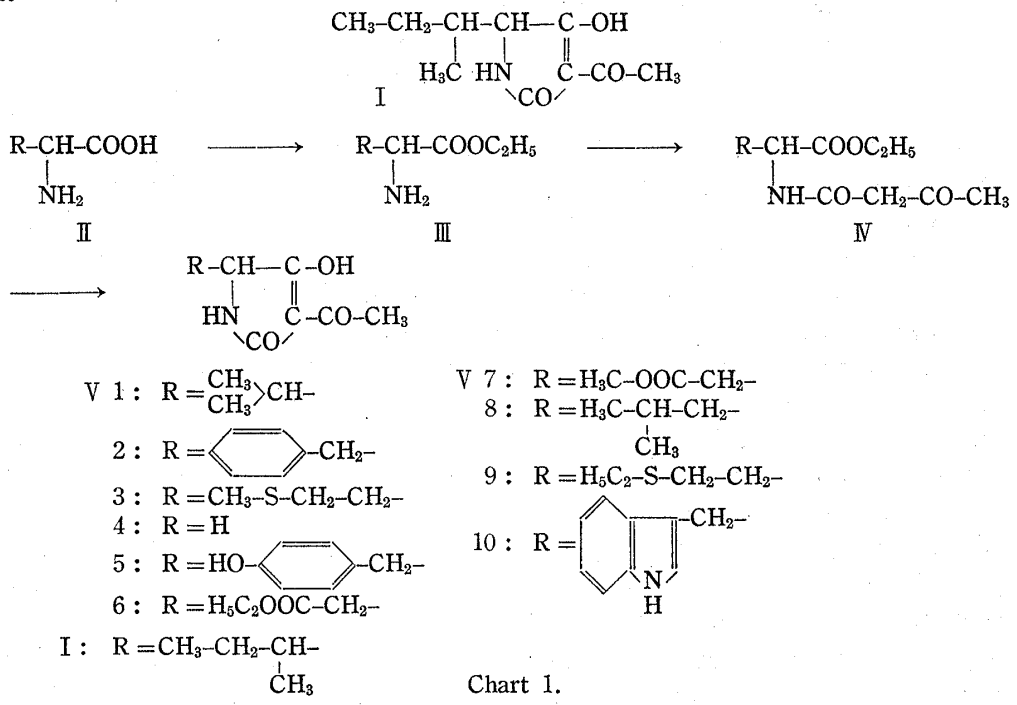

Chart 1.

TABLE I. Tenuazonic Acid Derivatives

Compounds	Method	m.p. (°C)	Recrystn. solvents	Analysis (%) Calcd. C	H	N	Found C	H	N
V 1[a]	A	74~75.5	ether-petr. ether						
2	A	151~151.5	MeOH	67.52	5.67	6.06	67.68	5.68	6.05
3	A, B	97~99	benzene-petr. ether	50.22	6.09	6.51	50.60	6.19	6.41
4	A	155	AcOEt	51.06	5.00	9.93	50.87	5.04	9.77
5[b]	A	114~114.5	EtOH-H_2O	58.86	5.70	5.28	58.75	5.90	5.31
6	B	95	EtOH-hexane	52.86	5.77	6.17	52.80	5.67	6.20
7	A, B	111~112	EtOH-hexane	50.70	5.20	6.75	50.86	5.22	6.68
8	B	114	benzene-hexane	60.88	7.67	7.11	60.57	7.92	7.37
9	A	102~104	benzene-petr. ether	52.30	6.59	6.10	52.16	6.85	5.99
10	A	171~173	benzene-EtOH	66.65	5.22	10.30	66.66	5.41	10.34
I[c]	B	158~158.5	acetone-benzene	67.26	8.15	8.72	67.00	8.26	8.68

a) Analyzed as thiosemicarbazone (Table II).
b) Calculated as one molar water of crystallization.
c) N,N'-dibenzylethylenediamine salt.

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N-Acetoacetyl group of diethyl aspartate (Ⅵ) can cyclize to two directions, namely to α-ester or β-ester group giving five-membered ring compound (V-6) or six-membered ring compound (Ⅶ) respectively. To decide which cyclization has preferentially occurred, β-monoethyl aspartate (Ⅸ)[11] was synthesized, and reacted with diketene to X followed by cyclization to obtain unambiguous structure of six membered ring compound (Ⅺ) bearing a free carboxyl group. The cyclized compound (V-6 or Ⅶ) prepared from diethylaspartate was hydrolized by dil. sodium hydroxide solution to Ⅷ or Ⅺ. However, this compound could not be identified with the authentic compound (Ⅺ). Consequently, it was found that the cyclization of diethyl N-acetoacetylaspartate has exclusively been directed to α-ester group producing the five-membered ring compound (V-6) as well as other α-amino acid esters. Cyclization of diethyl aspartate by sodium ethoxide afforded an ethyl ester compound (V-6), but cyclization by sodium methoxide afforded a methyl ester compound (V-7) by exchange of the ester group during the reaction. Ethyl ε-amino-n-caproate yielded the N-acetoacetylated free acid (Ⅻ) at the final step by hydrolysis of the ester group. Ring closure of ethyl N-acetoacetyl L-threonate was unsuccessful, but it formed crystalline thiosemicarbazone (XIII).

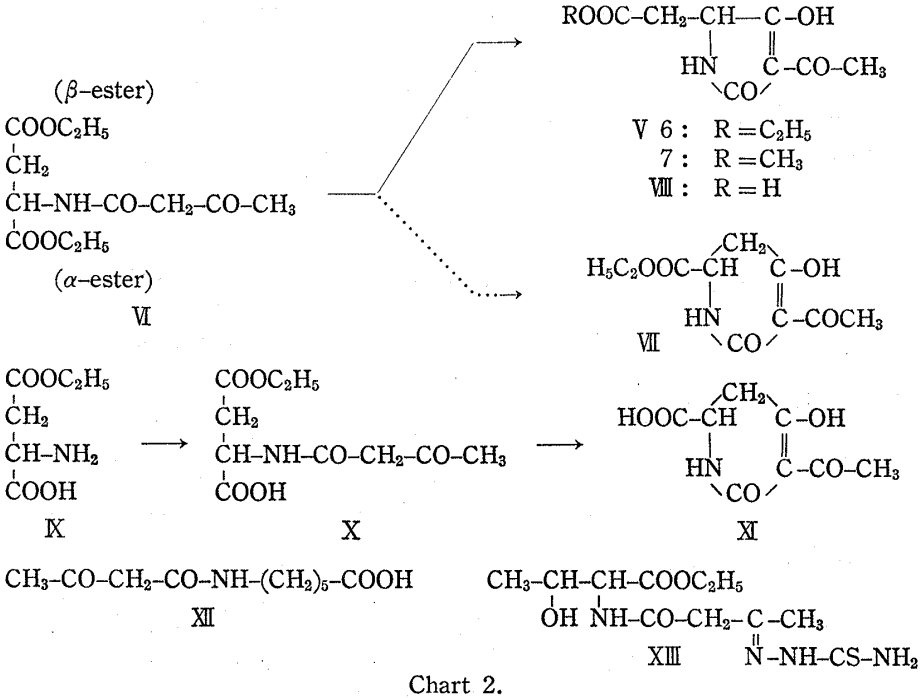

Chart 2.

Thiosemicarbazone of some compounds, particularly that of isatine, has strong inhibitory effect against certain viruses.[12,13] Hence, the compounds synthesized above were reacted with thiosemicarbazide and other carbonyl reagents, being hoped to exhibit a greater antiviral activity. Condensation of these compounds with the carbonyl reagents can take place at 1′-carbonyl group of position 3 to yield XIV and ketonized carbonyl group at position 4 to yield XV. Aebi[10] demonstrated that the condensation of tenuazonic acid derivatives with the carbonyl reagents took place at the carbonyl group of position 4 basing upon analysis of infrared spectra. So, the structure of the products is expressed as XV.

Antiviral activities of the compounds described above will be reported in a later paper.

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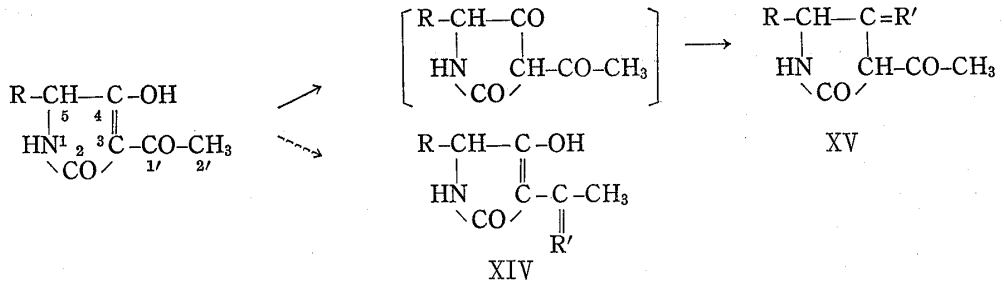

XV 1 : R=H R′=N-NH-CS-NH$_2$
2 : R=$(CH_3)_2$CH- R′=N-NH-CS-NH$_2$
3 : R=$(CH_3)_2$CH- R′=N-NH-CO-NH$_2$
4 : R=$(CH_3)_2$CH- R′=N-OH
5 : R=$(CH_3)_2$CH- R′=N-NH-C$_6$H$_5$
6 : R=$(CH_3)_2$CH-CH$_2$- R′=N-NH-CS-NH$_2$
7 : R=$(CH_3)_2$CH-CH$_2$- R′=N-OH
8 : R=$(CH_3)_2$CH-CH$_2$- R′=N-NH-CO-NH$_2$
9 : R=CH$_3$-CH$_2$-CH(CH$_3$)- R′=N-NH-CS-NH$_2$
10 : R=CH$_3$OOC-CH$_2$- R′=N-NH-C$_6$H$_5$
XV 11 : R=CH$_3$OOC-CH$_2$- R′=N-NH-CO-NH$_2$
12 : R=CH$_3$-S-CH$_2$-CH$_2$- R′=N-NH-C$_6$H$_5$
13 : R=CH$_3$-S-CH$_2$-CH$_2$- R′=N-NH$_2$
14 : R=C$_6$H$_5$-CH$_2$- R′=N-NH-CS-NH$_2$
15 : R=C$_6$H$_5$-CH$_2$- R′=N-NH-C$_6$H$_5$
16 : R=C$_6$H$_5$-CH$_2$- R′=NOH
17 : R=C$_6$H$_5$-CH$_2$- R′=N-NH-CO-NH$_2$
18 : R=HO-C$_6$H$_4$-CH$_2$- R′=N-NH-CO-NH$_2$
19 : R=HO-C$_6$H$_4$-CH$_2$- R′=N-NH$_2$

Chart 3.

TABLE II. Tenuazonic Acid Derivatives condensed with Carbonyl Reagents

Compounds	m.p. (°C)	Recrystn. solvents	Analysis (%) Calcd. C	H	N	Found C	H	N
XV 1	187~188	90% MeOH	39.24	4.70	26.15	39.36	5.18	26.04
2	161	MeOH	46.86	6.29	21.86	46.61	6.65	21.58
3	207~208	EtOH-H_2O	49.99	6.71	23.32	49.82	6.58	23.65
4	159~160	EtOH	54.53	7.12	14.13	54.67	7.04	14.27
5	158~159	EtOH	65.91	7.01	15.37	66.28	6.86	15.47
6	202	MeOH	48.86	6.71	20.72	49.15	6.28	20.73
7	152~153	H_2O	56.86	7.15	13.26	56.59	7.44	13.37
8	204~206	EtOH-H_2O	51.95	7.14	22.03	51.89	6.99	21.97
9	143~147	EtOH-H_2O	48.87	6.71	20.72	48.98	6.81	20.41
10	164~166	EtOH-H_2O	59.39	5.65	13.86	59.57	5.34	14.00
11	206	70% EtOH	44.44	5.22	20.73	44.49	5.58	20.99
12	147.5~148	EtOH	58.98	6.27	13.76	58.63	6.39	13.56
13	147~148	EtOH	47.12	6.59	18.33	46.78	6.36	18.26
14	222	EtOH	55.25	5.30	18.40	55.82	5.46	18.40
15	199~201	EtOH	71.01	5.96	13.08	71.34	5.92	13.37
16	119~121	EtOH	63.40	5.73	11.38	63.64	5.71	11.64
17	191~192	EtOH	58.32	5.59	19.44	58.31	5.55	19.32
18	213~214	EtOH-H_2O	55.25	5.30	18.41	55.28	5.34	18.51
19	202	EtOH	59.76	5.79	16.08	60.06	5.75	16.30

Experimental

General Method (A)——Amino acid was esterified by hydrochloric acid in EtOH as usual. The reaction mixture was evaporated to dryness, and the residue was dissolved in EtOH. Equimolar quantity of alc.

EtONa soln. was added, then precipitated NaCl was removed by filtration. Diketene (1.2 mol) was added dropwise to the filtrate keeping the temperature below 5°, then stirring was continued for further 30 mins. at room temperature. Evaporation of the solvent under a reduced pressure gave oily residue of N-acetoacetyl amino acid ester. 1.1 mol of NaOMe in approx. 15 times volume of MeOH was added to the residue, and the mixture was refluxed in benzene for 3 hrs. After standing over night at room temperature, the cyclized product was extracted with small volume of water for three times. The water extract was brought to pH 2~3, and the separated product was extracted with ether or AcOEt. After drying the solvent over Na_2SO_4, solvent was removed off under a reduced pressure. The residue was recrystallized from the solvent listed in the Table I.

General Method (B)——The hydrochloride of the amino acid ester, obtained by evaporation of the reaction mixture as described in the method (A), was treated with K_2CO_3 soln., and the liberated amine was extracted with benzene or $CHCl_3$. After drying the extract over Na_2SO_4, the solvent was removed under a reduced pressure. The residue, amino acid ester, was dissolved in ether. Diketene (1.1 mol) was added to the solution dropwise with stirring at room temperature for 30 mins. Cooling with water was sometimes required to maintain the temperature properly. Removal of the solvent by distillation under a reduced pressure gave N-acetoacetyl amino acid ester, which was then cyclized as described in the method (A).

DL-Compound of V-5——The crude product of V-5 was recrystallized from EtOH-H_2O, and filtered. Concentration of the filtrate afforded colorless crystals, which were recrystallized from EtOH-H_2O. $[\alpha]_D^{20}$ 0° (c=1.0, C_2H_5OH), while $[\alpha]_D^{20}$ of V-5 was −213° (c=1.0, C_2H_5OH). *Anal.* Calcd. for $C_{13}H_{13}O_4N$: C, 63.15, H, 5.30; N, 5.67. Found: C, 62.88; H, 5.42; N, 5.85.

DL-Compound of V-8——The crude product of V-8 was recrystallized from benzene-hexane, and filtered. Concentration of the filtrate afforded colorless crystals, which were recrystallized from benzene-hexane. $[\alpha]_D^{20}$ 0° (c=3.9, C_2H_5OH), while $[\alpha]_D^{20}$ of V-8 was −117.3° (c=1.2, C_2H_5OH). *Anal.* Calcd. for $C_{10}H_{15}O_3N$: C, 60.88; H, 7.67; N, 7.11. Found: C, 61.27; H, 7.78; N, 7.21.

3-Acetyl-4-hydroxy-5-methoxycarbonylmethyl-3-pyrrolin-2-one (V-7)——Aspartic acid was treated by method A or B to yield the above compound.

3-Acetyl-5-ethoxycarbonylmethyl-4-hydroxy-3-pyrrolin-2-one (V-6)——Cyclization by EtONa instead of MeONa in the above treatment resulted in production of this compound.

3-Acetyl-5-carboxymethyl-4-hydroxy-3-pyrrolin-2-one (VIII)——V-6 was dissolved in 20 ml. of 5% NaOH soln. and kept at room temperature for 3 days. After acidification with sulfuric acid the mixture was extracted with AcOEt, and the extract was washed with water and dried. Removal of the solvent under a reduced pressure gave crystalline residue, which was recrystallized from AcOEt, m.p. 168°. *Anal.* Calcd. for $C_7H_9O_5N$: C, 48.25; H, 4.55; N, 7.03. Found: C, 48.49; H, 4.67; N, 7.15.

3-Acetyl-6-carboxy-4-hydroxy-1,2,5,6-tetrahydropyrid-2-one (XI)——β-Ester of aspartic acid was synthesized according to the literature.[11] It was then treated by method (A) yielding XI. m.p. 203~204°. *Anal.* Calcd. for $C_8H_9O_5N$: C, 48.25; H, 4.55; N, 7.03. Found: C, 48.36: H, 4.58; N, 7.05.

N-Acetoacetyl-ε-amino-*n*-caproic Acid (XII)——ε-Amino-*n*-caproic acid was esterified and treated with the procedure of the cyclization. The elemental analysis indicated that the product was not the desired one but N-acetoacetyl-ε-amino-*n*-caproic acid. m.p. 169~170°. *Anal.* Calcd. for $C_{10}H_{17}O_4N \cdot \frac{1}{2}H_2O$: C, 53.80; H, 7.68; N, 6.27. Found: C, 53.75; H, 7.27; N, 6.35.

Ethyl N-Acetoacetyl-L-threonate Thiosemicarbazone (XIII)——L-Threonine was esterified and reacted with diketene according to the method (A). Removal of the solvent gave yellow crystalline residue. This was reacted with thiosemi carbazide as usual. m.p. 146.5°. *Anal.* Calcd. for $C_{11}H_{20}O_4N_4S$: C, 43.41; H, 6.62; N, 18.41. Found: C, 43.05; H, 6.74; N, 18.35.

Condensation with Carbonyl Reagents——A mixture of V and carbonyl reagent in EtOH-H_2O was warmed in a water bath for several minutes, and cooled. Crystals separated were collected and recrystallized.

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