Chem. Pharm. Bull. 19(8)1664—1668(1971)

UDC 547.745.07 : 581.192

Studies of Tenuazonic Acid Analogs. I. Synthesis of 5-Substituted 3-(1'-Anilinoethylidene)pyrrolidine-2,4-dione

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(Received February 15, 1971)

Derivatives of tenuazonic acid, 3-acetyl-5-*sec*-butyltetramic acid, were synthesized. 5-Substituted 3-acetyl tetramic acids were condensed with primary aromatic amines refluxing in alcohol. The structure of the condensation products were determined as 5-substituted 3-(1'-anilinoethylidene)pyrrolidine-2,4-dione. The stability of the one of the condensation products was investigated, and it was found to be stable in acidic and neutral, but unstable in alkaline medium.

Tenuazonic acid was first isolated from the culture filtrate of alternaria tenuis[2] and its structure was determined by Stickings as 3-acetyl-5-*sec*-butyltetramic acid (I).[3] In 1963, Kaczka, *et al.*[4] reported the growth inhibitory action against human tumors growing in the embryonated eggs. Miller, *et al.*[5] reported the activity against several viruses. Harris, *et al.*[6] synthesized a various tenuazonic acid derivatives, but none of them were found to be more effective against the tumor cells.[7] The authors are interested in further structural modification of this antibiotic to clarify the structure-activity relationship. This paper deals with the synthesis of 5-substituted 3-(1'-anilinoethylidene)pyrrolidene 2,4-dione.[8]

5-Substituted 3-acetyltetramic acids(II) were synthesized from L-amino acids according to the Lacey's method.[9] Amino acid esters were reacted with diketene to give N-acetoace-

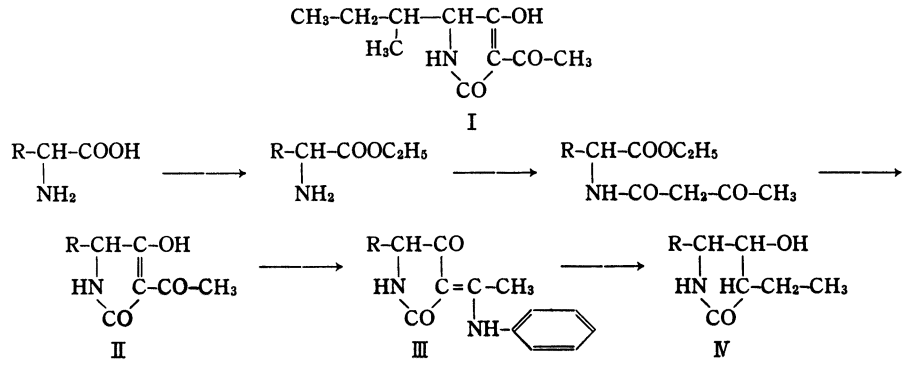


Chart 1

1) Location: 5, *Toneyama 6 chome, Toyonaka, Osaka.*
2) T. Rosett, R.S. Sankhala, C.E. Stickings, E.U. Taylor and R. Thomas, *Biochem. J.*, **67**, 390 (1957).
3) C.E. Stickings, *Biochem. J.*, **72**, 332 (1959).
4) E.A. Kaczka, C.O. Gitterman, E.L. Delaney, M.C. Smith, D. Hendlin, H.B. Woodruff and K. Folkers, *Biochem. Biophys. Res. Comm.*, **14**, 54 (1964).
5) F.A. Miller, W.A. Rightsel, B.J. Sloan, J. Ehrlich, J.C. French and Q.R. Barte, *Nature*, **200**, 1338 (1964).
6) S.A. Harris, L.A. Fisher and K. Folkers, *J. Med. Chem.*, **8**, 478 (1965).
7) C.O. Gitterman, *J. Med. Chem.*, **8**, 483 (1965).
8) A part of this paper has been communicated previously. H. Yuki, K. Kariya and Y. Hashimoto *Chem. Pharm. Bull.* (Tokyo), **15**, 727 (1967).
9) *a*) R.N. Lacey, *J. Chem. Soc.*, **1954**, 850; *b*) H. Yuki, Y. Tohira, B. Aoki, T. Kano, S. Takama and T. Yamazaki, *Chem. Pharm. Bull.* (Tokyo), **15**, 1107 (1967).

tylated derivatives, which were cyclized by refluxing with sodium methoxide in benzene to II. When isoleucine was used as the starting material, tenuazonic acid is synthesized by this method. To modify the structure of II, these tetramic acids were reacted with aniline and its derivatives refluxing in alcohol giving the condensation products (III) (Chart 1).

The elemental analysis of the products indicated that II condensed with one molecule of aniline. In order to determine the position where the condensation took place, one of the product (III: R=benzyl) was catalytically hydrogenated under a pressure over Raney nickel. The elemental analysis of the reduced compound (IV) indicated the disappearance of the aniline moiety and hydrogenation of one =CO to =CH-OH. The nuclear magnetic resonance spectrum of IV in $CDCl_3$ also indicated the disappearance of one benzene ring, and a signal of the methyl group appeared as a triplet (J=7 cps) at τ value of 8.9. If 4-hydroxyl group of the pyrrolidine ring condensed with aniline, the signal of the methyl group would appear as a doublet due to -CH(OH)-CH_3. Moreover, the mass spectrum of III (R=benzyl) did not give the deacetylated peak (M-42) while II (R=benzyl) gave a strong peak of M-42. From these data, it is evident that aniline condensed with the carbonyl group of 3'-acetyl group of tetramic acid, and III took the enamine form of the three possible tautomeric forms (Chart 2). This was further supported by the measurement of the ultraviolet (UV) absorption spectra. The UV spectrum of III (R=benzyl) gave the absorption maximum at 318 mμ (ε=28800) which suggests the enamine form. The UV spectrum of 5-benzyl-3-(1'-N-me-

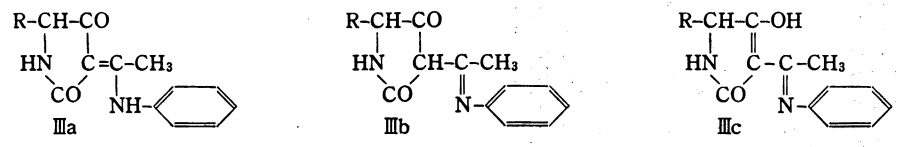


Chart 2

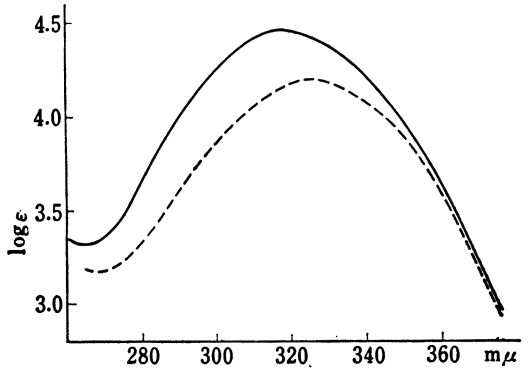
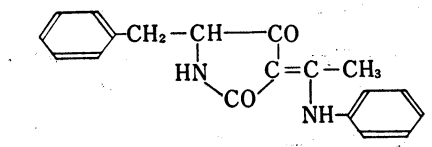
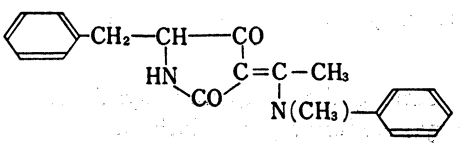


Fig. 1. UV Spectra of III (R=benzyl), and V in EtOH

———: III (R=benzyl) ----------: V



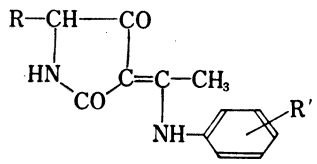
III

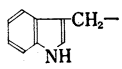


V

thylanilinoethylidene)pyrrolidine-2,4-dione (V), which was prepared by condensing II (R=benzyl) with N-methylaniline and has unambiguously the structure of the enamine form (III-a), showed similar absorption spectrum (λ_{max} 327 mμ, ε=16300) (Chart 2). III (R=benzyl) was insoluble in sodium hydroxide solution. From these data, the structure of III (R=benzyl) was determined as to have an enamine form (III-a). Although the structure III-b has been presented for this compound in the previous communication, this is now corrected. The elemental analysis and melting points of III are listed in Table I.

TABLE I. 5-Substituted 3-(1′-Anilinoethylidene)pyrrolidin-2,4-dione



R	R′	Formula	mp (°C)	Analysis (%) Calcd. C	H	N	Found C	H	N
Benzyl-	H	$C_{19}H_{18}O_2N_2$	173—176	74.47	5.95	9.14	74.48	6.00	9.12
Benzyl-	*o*-F	$C_{19}H_{17}O_2N_2F$	104	70.35	5.28	8.63	70.66	5.64	8.23
Benzyl-	*m*-F	$C_{19}H_{17}O_2N_2F$	182—183	70.35	5.28	8.63	70.38	5.34	8.54
Benzyl-	*p*-F	$C_{19}H_{17}O_2N_2F$	184—185	70.35	5.28	8.63	70.08	5.29	8.80
Benzyl-	*m*-Cl	$C_{19}H_{17}O_2N_2Cl$	165—166	66.93	5.03	8.23	66.96	5.03	8.39
Benzyl-	*p*-Cl	$C_{19}H_{17}O_2N_2Cl$	195—197	66.93	5.03	8.23	66.80	4.91	8.39
Benzyl-	*p*-Br	$C_{19}H_{17}O_2N_2Br$	189	59.22	4.95	7.27	59.17	4.29	7.32
Benzyl-	*p*-I	$C_{19}H_{17}O_2N_2I$	189—191	52.78	3.97	6.48	52.76	3.85	6.56
Benzyl-	*o*-CH_3	$C_{20}H_{20}O_2N_2$	112—113	74.97	6.29	8.74	74.90	6.14	8.60
Benzyl-	*m*-CH_3	$C_{20}H_{20}O_2N_2$	158—160	74.97	6.29	8.74	74.57	6.19	8.74
Benzyl-	*p*-CH_3	$C_{20}H_{20}O_2N_2$	191—193	74.97	6.29	8.74	74.77	6.19	8.94
Benzyl-	*o*-OH	$C_{19}H_{19}O_2N_2$	267—269	67.44	5.36	8.28	67.27	5.27	8.22
Benzyl-	*p*-CH_3O-	$C_{20}H_{20}O_3N_2$	154—156	71.61	5.72	8.35	71.31	5.87	8.26
Benzyl-	3-=N-	$C_{18}H_{17}O_2N_3$	168—169	70.33	5.58	13.67	70.41	5.57	13.80
CH_3-S-CH_2-CH_2-	*m*-NO_2	$C_{15}H_{17}O_4N_3S$	151—153	53.71	5.11	12.53	53.71	4.94	12.57
C_2H_5-S-CH_2-CH_2-	H	$C_{16}H_{20}O_2N_2S$	133	63.14	6.62	9.21	63.21	6.72	9.26
CH_3-CH_2-CH(-CH_3)-	H	$C_{16}H_{20}O_2N_2$	40	70.58	7.35	10.29	69.92	7.38	10.18
$(CH_3)_2$=CH-	H	$C_{15}H_{18}O_2N_2$	176—177	69.74	7.02	10.85	70.37	6.97	11.24
HO-C$_6$H$_4$-CH_2-	H	$C_{19}H_{18}O_3N_2$	219—221	70.79	5.59	8.69	70.47	5.51	8.77
	H	$C_{21}H_{19}O_2N_3 \cdot \frac{1}{2}H_2O$	159—160	71.18	5.65	11.86	71.41	5.70	11.99
HOOC-C$_6$H$_4$-CH_2-	H	$C_{20}H_{18}O_4N_2 \cdot \frac{1}{2}H_2O$	293—297	66.84	5.33	7.79	67.02	5.09	7.61
$(CH_3)_2$=N-C$_6$H$_4$-CH_2-	H	$C_{21}H_{23}O_2N_3$	184—186	72.18	6.63	12.03	72.22	6.58	11.96
		$C_{21}H_{23}O_2N_3 \cdot HCl$	163—167	65.36	6.25	10.89	65.26	6.25	10.52
CH_3O-C$_6$H$_4$-CH_2-	H	$C_{20}H_{20}O_3N_2$	173—174	71.41	5.99	8.33	71.26	5.90	8.22

Among the compounds synthesized, two compounds (III: R=benzyl, and *p*-hydroxybenzyl) exhibited particularly strong antitumor activity *in vitro* and *in vivo* as was reported in the previous communication.[8] The both compounds, however, were insoluble in water, so that they had to be administrated in suspended form. Synthesis of water soluble derivatives is desired, and they might be expected to give the stronger antitumor activity. As one of the water soluble derivatives, the hydroxyl group of III (R=*p*-hydroxybenzyl) was sulfated by treatment with pyridine–sulfur trioxide. The sulfate ester was identified as crystalline benzyl thiuronium salt. The synthesis of other derivatives, which have hydrophilic group at *para*-position of the benzyl group such as COOH and -N=$(CH_3)_2$, was also undertaken. The starting materials, *p*-substituted phenylalanine, were synthesized by usual way. *p*-Dimethylaminobenzaldehyde and terephthalic acid aldehyde were condensed with hyppuric acid followed by hydrogenation and hydrolysis in the presense of red phosphorus

and hydroiodic acid (Chart 3). The amino acids thus prepared were derived to tetramic acids, then condensed with aniline as mentioned above. In order to test the relationship between solubility and antitumor activity, a hydrophobic derivative was also prepared starting from *p*-methoxyphenylalanine, which was prepared according to the literature.[10]

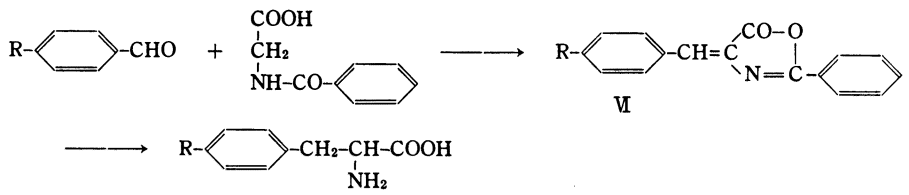


Chart 3

The stability of the compounds is an important factor to reveal the biological activity when they are applied to a living tissue. The buffer solutions of compound III (R=benzyl) at various pH were allowed to stand for 24 hr at room temperature. The UV spectra of the solutions before and after standing were shown in Fig. 2. The absorption spectra did not change in acidic and neutral solutions. In alkaline solutions, however, the absorption maximum (317 mμ) shifted to shorter wave length (λ_{max}: 280 mμ), which corresponds to that of compound II (R=benzyl). The crystalls isolated from a concentrated solution of III (R=benzyl) after standing 24 hr at pH 13 was identified as II (R=benzyl) by measuring the infrared spectrum. Thus, III (R=benzyl) was shown to be unstable in the strong alkaline solution, but it is supposed to be able to remain unchanged in the pH range of body fluids.

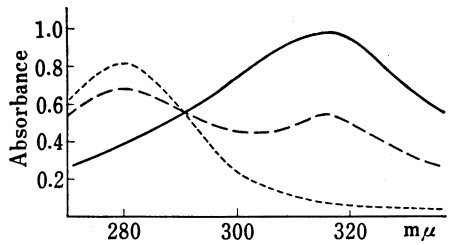


Fig. 2. Spectral Change of III (R=benzyl) in Various pH Solutions

Sample solutions (10 μg/ml) of each pH were allowed to stand at room temperature for 24 hr UV spectra were measured before and after the standing.
———: before standing at each pH, and after standing at pH 1 (0.1M HCl solution) and pH 7 (0.1M phosphate buffer solution)
– – – –: after standing at pH 11 (0.1M borate buffer solufon)
----------: after standing at pH 13 (0.1M NaOH solution)

The detail of the antitumor activity of the compounds synthesized will be reported in a separate paper.[11]

Experimental[12]

Synthesis of III——Compound II was dissolved in 10 parts of EtOH, and equimolar quantity of aromatic primary amine was added. The mixture was refluxed for 30 to 60 min, and then cooled. Crystals separated were collected and recrystallized from EtOH–H_2O. If crystals did not separate on cooling, water was added to the mixture. The oily substance separated solidified after washing with dil. HCl and NaOH solution. Yield, 40—80%.

Reduction of III (R=benzyl)——III dissolved in EtOH was catallytically reduced over Raney nickel at 50° in 65 atms for 1 hr. The hydrogenated mixture was filtered, and the filtrate was evaporated to dryness under a reduced pressure. The residue was recrystallized from benzene, mp 121—122°. *Anal.* Calcd. for $C_{13}H_{17}O_2N$: C, 70.93; H, 7.91; N, 6.12. Found: C, 71.20; H, 7.82; N, 6.39.

Sulfation of III (R=*p*-hydroxybenzyl)——5.27 g of III (R=*p*-hydroxybenzyl) and 7.81 g of pyridine-SO_3 were dissolved in 53 ml of pyridine, and the mixture was heated at 70° for 5 hr with stirring. The sus-

10) B.R. Baker, J.P. Joseph and J.H. Williams, *J. Am. Chem. Soc.*, **77**, 4 (1955).
11) H. Yuki, E. Kitanaka, A. Yamao, K. Kariya and Y. Hashimoto, *Gann* **62**, 199 (1971).
12) All melting points were not corrected.

pension turned to a pale yellow solution. Pyridine was removed off in a reduced pressure, and 200 ml of water was added to the residue. The undissolved materials was removed by filtration. The filtrate was brought to pH 9.0 by addition of NaOH solution, and was evaporated to dryness *in vacuo*. The residue was extracted with 100 ml of hot EtOH. 100 ml of ether was added to the extract. After cooling, 6.54 g of yellow crystals were obtained, which were purified by dissolving in water and treated with active carbon. Carbon was removed by filtration, and the filtrate was evaporated to dryness. The residue was recrystallized by treatment with EtOH followed by ether to give Na salt of the sulfate ester, which was very hygroscopic substance. Elemental analysis was performed as benzylthiuronium salt.

S-benzylthiuronium Salt of Sulfate Ester of III (R=benzyl)——The purified sodium salt of sulfate ester and equimolar quantity of S-benzylthiuronium chloride were dissolved in EtOH, and kept standing for several hours. NaCl precipitated was filtered off, and the filtrate was evaporated to dryness. The residue was recrystallized from water, mp 216—217° (decomp.). *Anal.* Calcd. for $C_{27}H_{28}O_6N_4S_2$: C, 57.03; H, 4.96; N, 9.85. Found: C, 56.92; N, 5.00; N, 9.81.

5-Benzyl-3-(1′-N-methylanilinoethylidene)pyrolidine-2,4-dione (V)——A mixture of 5 g of II (R=benzyl), 2.5 g of N-methylaniline, 0.25 g of *p*-toluenesulfonic acid, 20 g of molecular sieves 4A 1/16, and 120 ml of anhyd. toluene was refluxed for 100 hr in a soxlet extracting apparatus containing $CaCl_2$ in the extraction thimble to remove water produced. Molecular sieves was removed by filtration. After cooling, crystals separated were recrystallized from toluene. Yield, 3 g (43.3%), mp 187°. *Anal.* Calcd. for $C_{20}H_{20}O_2N_2$: C, 74.97; H, 6.29; N, 8.74. Found: C, 75.18; H, 6.23; N, 8.73.

***p*-Dimethylaminophenylalanine**——A mixture of *p*-dimethylaminobenzoldehyde (15 g), hyppuric acid (20 g), Ac_2O (30 g), and anhyd. AcONa (4 g) was heated in a water bath for 30 min being protected from moisture. During the heating the mixture melted once and then solidified again. The reaction mixture was washed with 150 ml of boiling water twice and filtered. A mixture of the residue, 9 g of red phosphorus, 140 ml of AcOH, and 100 ml of constant boiling HI solution ($d=1.70$, $bp_{760}=127°$) was refluxed for 90 min. The reaction mixture was filtered hot, and the residue was washed with hot AcOH. The filtrate and washings were combined and evaporated to dryness *in vacuo*. Water was added to the residue, and evaporated again. This procedure was repeated for several times. The residue was dissolved in a small volume of boiling water and cooled. Benzoic acid separated and other unreacted mateials were extracted with ether. The water layer was concentrated to about 30 ml, and neutralized with NH_4OH. After standing over night, crystals separated were collected and washed with ether, mp 210—215°. Yield, 35%. *Anal.* Calcd. for $C_{11}H_{16}O_2N_2$: C, 63.44; H, 7.74. Found: C, 63.78; H, 7.99.

***p*-Carboxyphenylalanine**——Synthesis of this compound was essentially following that of *p*-dimethylaminophenyl alanine, but terephthalic acid aldehyde was used in place of *p*-dimethylaminobenzaldehyde, mp 300°. Yield, 53%. *Anal.* Calcd. for $C_8H_{11}O_4N$: C, 57.41; H, 5.30; N, 6.69. Found: C, 56.88; H, 5.27; N, 6.41.

Isolation of II (R=benzyl) from NaOH Solution of III (R=benzyl)——To 200 ml of NaOH solution of pH 13.0, was added 200 mg of III (R=benzyl), and the mixture was stirred at room temperature for 24 hr, and neutralized by addition of dil. HCl. The solution was extracted with $CHCl_3$. The solvent was evaporated to dryness, and the residue was recrystallized from EtOH. Crystals separated were identified as II (R=benzyl) by measurement of melting point and infrared spectrum.

Acknowledgement The authors are grateful to Dr. M. Fukuda for the elemental analysis, and Dr. M. Suzuki for measurement of NMR spectra.