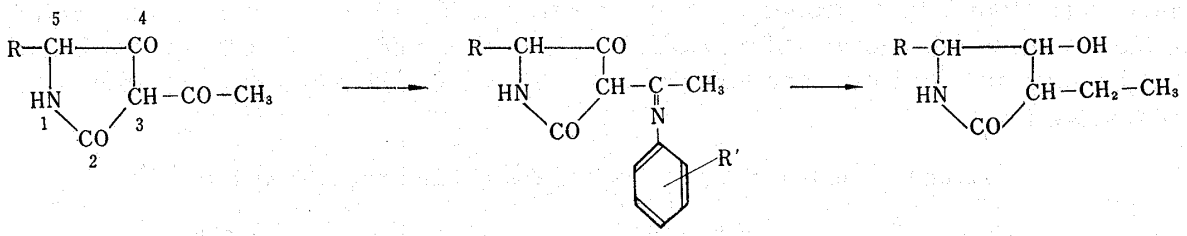
[Chem. Pharm. Bull. 15(5) 727~729 (1967)]
UDC 615.771.7-07 : 547.745.09

Synthesis and Anti-tumor Activity of Tenuazonic Acid Analogues

In 1964, the anti-tumor activity of tenuazonic acid (3-acetyl-5-*sec* butyltetramic acid) was first reported by Kaczka, *et al.*[1] Until now, however, attempts[2,3] to find active analogous compounds have been unsuccessful. We wish to report briefly the synthesis and anti-tumor activity *in vitro* and *in vivo* of tenuazonic acid analogues.

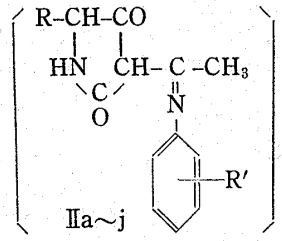
5-Substituted-3-acetylpyrrolidine-2,4-dione (I) was synthesized from α-amino acid according to Lacey's method[4]. I was then condenced with primary aromatic amines by



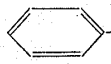
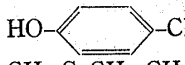
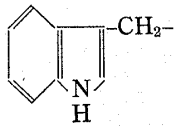
I II(a–j) III

Chart 1.

Table I. 5-Substituted-3-(N-phenylacetimidoyl)pyrrolidine-2,4-dione



IIa~j

Compound	R	R′	m.p. (°C)	Analysis (%) Calcd. C	H	N	Found C	H	N
IIa		H	173~176	74.47	5.93	9.14	74.48	6.00	9.12
IIb	〃	*p*-Cl	195~197	66.93	5.03	8.23	66.80	4.91	8.39
IIc	〃	*m*-Cl	165~166	66.93	5.03	8.23	66.96	5.03	8.39
IId	〃	*p*-CH_3	191~193	74.97	6.29	8.74	74.77	6.19	8.94
IIe	〃	*p*-F	184~185	70.35	5.28	8.63	70.08	5.29	8.80
IIf	〃	*m*-F	182~183	70.35	5.28	8.63	70.38	5.34	8.54
IIg		H	219~221	70.79	5.59	8.69	70.47	5.51	8.77
IIh	CH_3-S-CH_2-CH_2-	*m*-NO_2	151~153	53.71	5.11	12.53	53.71	4.94	12.75
IIi	C_2H_5-S-CH_2-CH_2-	H	133	63.14	6.62	9.21	63.21	6.72	9.26
IIj		〃	159~160	71.18	5.65	11.86[a]	71.41	5.70	11.99

[a] Calculated as ½ mole water of cystallization.

1) E. A. Kaczka, *et al.*: Biochem. Biophys. Res. Commun., **14**, No. 1, 54 (1964).
2) S. A. Harris, *et al.*: J. Med. Chem., **8**, 478 (1965).
3) C. O. Gitterman: *Ibid.*, **8**, 483 (1965).
4) R. N. Lacey: J. Chem. Soc., **1954**, 850.

refluxing in alcohol. To decide the position of the condensation, one of the products (II-a, $R=C_6H_5-CH_2-$, $R'=H$) was catalytically reduced in high pressure over platinum oxide. The integral of NMR (nuclear magnetic resonance) spectra of the reduced compound (III) indicated disappearance of one benzene ring, and the signal of the methyl group appeared as a triplet at τ value of 8.9. These facts suggest that the amino group condensed with the acetyl group at position 3 of the pyrrolidin ring to give the compounds of structure II.

The antitumor activity of these compounds was first tested against Yoshida sarcoma cells *in vitro*. As is shown in Table II, growth of Yoshida sarcoma cells was inhibited by the compounds. Fifty percent inhibition concentration (IC_{50}) of a compound was calculated as reported by Moriwaki.[5] Minimum concentration of a compound in which more than 95% of tumor cells was destroyed after 48 hours was designated as complete inhibition concentration (CIC). The compound IIa is nearly as active as nitrogen mustard, and other compounds are also more active than 6-mercaptopurine in this test system.

Table II. Antitumor Activity against Yoshida Sarcoma Cells *in Vitro*

Compound[a]	CIC (48 hr.) (mM)	IC_{50} (mM)	IC_{50} range (mM)
IIa	5.8×10^{-5}	2.5×10^{-5}	$(2.6\sim2.2)\times10^{-5}$
IIb	5.8×10^{-3}	1.7×10^{-3}	$(1.8\sim1.7)\times10^{-3}$
IIc	1.0×10^{-3}	2.5×10^{-4}	$(2.8\sim2.2)\times10^{-4}$
IId	3.2×10^{-3}	9.0×10^{-4}	$(9.0\sim8.8)\times10^{-4}$
IIe	1.0×10^{-4}	5.2×10^{-5}	$(5.5\sim5.0)\times10^{-5}$
IIg	1.8×10^{-4}	8.5×10^{-5}	$(9.0\sim7.4)\times10^{-5}$
IIi	3.2×10^{-2}	4.3×10^{-3}	$(5.7\sim1.8)\times10^{-3}$
IIj	1.0×10^{-2}	——	——
Nitrogen mustard[b]		1.2×10^{-5}	$(2.0\sim0.7)\times10^{-5}$
6MP[b]		8.3×10^{-3}	$(14.6\sim4.7)\times10^{-3}$

The numbers of non-treated Yoshida sarcoma cells at 0, 24, and 48 hrs. of culture were 50,000, 138,000±41,000, and 406,000±57,000, respectively.
a) Shown in Table I. *b*) Cited from Moriwaki's data.[5]

Compounds IIa and IIg were tested against Ehrlich ascites and solid tumors in mice. The results were shown in Table III. Administraion of 50 or 100 mg./kg. of IIa prolonged the survival time of mice bearing ascites tumor for about three times than non-treated group. To obtain the information about the toxicity of this compound toward the host

Table III. Antitumor Activity against Ehrlich Carcinoma in Mice

Compound[a]	Dose (mg./kg.)	Tumor Form	Mean Survival Time (days)	Mortality	Host Weight Change (g.)	Tumor Weight (mg./mouse)	Ratio to Control (%)
Control	—	ascites	17.7				100
IIa	50	〃	50.5				285
IIa	100	〃	52.6				297
Control	—	solid		0/8	+2.1	2789	100
IIa	50	〃		0/8	+2.6	2569	92
IIa	100	〃		0/8	+4.2	2334	83
IIg	50	〃		2/8	+2.6	947	34
Mit. C[b]	2	〃		1/8	+2.1	861	31

LD_{50} i.p. of IIa and IIg were 681 and 584 mg./kg., respectively (Calculated by Horn's method[6]).
a) Shown in Table I. *b*) Mitomycin C.

5) A. Moriwaki: This Bulletin, **10**, 462 (1962).
6) H. J. Horn: Biometrics, **12**, 311 (1956).

animals, the body weight change of the treated mice bearing ascites tumor was observed through the experiment, but a significant decrease of the body weight was not recorded even by a large level of the continuous intraperitoneal administration. This compound (IIa), however, showed little or no activity against the solid form of the same tumor. On the other hand, 50 mg./kg. dose of IIg was as active against the solid tumor as Mitomycin C at the dose level of 2 mg./kg.

Synthesis and biological tests of other analogous compounds are now under investigation.

Experimental

Synthesis of II——Compound I was dissolved in 10 parts of EtOH, and equimolar quantity of aromatic amine was added. The mixture was refluxed for 30 mins., then cooled. The crystals separated were recrystallized from EtOH or EtOH-H_2O. Yield 40～80%.

Reduction of IIa——IIa dissolved in EtOH was catalytically reduced over Adams' PtO_2 at 50° in 65 atms for 1 hr. The solvent was removed off under a reduced pressure, and the residue was recrystallized from benzene. m.p. 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.

***In Vitro* Test**——Basal medium used was Eagle minimum essential medium containing 0.01% sodium pyruvate,[7] penicillin G (100 U/ml.), and dihydrostreptomycin (125 μg./ml.). Yoshida sarcoma cells were suspended in the basal medium containing 40% heat inactivated horse serum to make a suspension of 1×10^5 cells/ml.

The test compound was dissolved in ethanol and diluted with physiological saline to give a necessary concentration. Ethanol concentration in the final culture medium was less than 0.3%.

To 0.5 ml. of the cell suspension in a test tube were added 0.4 ml. of the basal medium and 0.1 ml. of the serially diluted test solution or as the control, 0.1 ml. of physiological saline containing the same percentage of ethanol as the test solution. Three sets of each test suspension were prepared and they were agitated with a vibrater and a part of the suspension was withdrown for cell counting. The numbers of living Yoshida sarcoma cells were counted under a phase contrast microscope with a Buerker hemocytometer.

***In Vivo* Test**——Animals used in these experiments were male ddO strain mice weighing 20±1 g. and antitumor activity was assayed on the mice bearing Ehrlich carcimoma.

In the group of ascites tumor, each animal was given intraperitoneal injection of 0.2 ml. of ascitic fluid containing 2×10^6 tumor cells. In the group of solid tumor, 4×10^6 cells were inoculated subcutaneously into the inguinal region on both sides of mouse.

The compounds tested were dissolved or suspended in saline containing 0.5% carboxymethylcellulose and administered intraperitoneally. Treatment of the mice bearing ascites or solid tumor was started 24 or 72 hr., respectively, after the tumor inoculation, and was continued once daily for 6 days.

The effect of the compounds on the ascites tumor was expressed as the ratio of the mean survival time of the treated group to that of the control. In the case of the solid tumor, it was expressed as the ratio of the tumor weight on the 12th day after inoculation.

The authors wish to express their gratitude to Professors K. Takiura and H. Iwata for their kind guidances and encouragements throughout this investigation. Thanks are also due to Miss Y. Shiratsuchi for assistance in the synthetic work, and to Dr. Y. Fukuda for the elemental analysis.

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Received February 3, 1967

7) T. Kuroki, *et al.*: Gann, **57**, 35 (1965).