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# 139. Seikichi Suzuki, Fumihiko Sano, and Hidetada Yuki*3 : Studies on Antiviral Agents. Ⅳ.*1 Biological Activity of Tenuazonic Acid Derivatives.

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Antiviral activity of tenuazonic acid derivatives against adenovirus type 5 was examined. Some compounds showed the significant chemotherapeutic indexes. This antiviral activity was not antagonized by the amino acid from which the test compounds were derived.

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In 1964, inhibitory activity of tenuazonic acid for growth of human adenocarcinoma-1 in the embryonated egg was first communicated by Kaczka, *et al.*,[1] and then the detail was reported by the same workers[2] comparing with the activity of other antitumor agents. Miller, *et al.*[3] synthesized the tenuazonic acid from ethyl L-isoleucinate, and found that sodium tenuazonate reduced 50% plaque production of Measles (Enders), Vaccinia, Herpes simplex (HF), ECHO-9, and 'B' viruses at the concentration of 500, 500, 160, 500, and 500 μg./ml. respectively, but it was essentially inactive against bacteria, yeasts, and *Trichomonas vaginalis in vitro.* Shigeura, *et al.*[4] reported that tenuazonic acid inhibited the incorporation of various amino acids into proteins both *in vivo* and *in vitro.* In the part Ⅱ of this series,[5] synthesis of tenuazonic acid derivatives was reported. In the current work their antiviral activity was investigated with following results.

## Screening Tests of Antiviral Activity

**Materials and Methods**——Adenovirus type 5, strain Ch and HeLa cell were used. To prepare the monolayer of this cell line, YLG medium (1.0 g. of yeast extract and 5.0 g. of lactalbumin hydrolysate were dissolved in 1000 ml. of Gey's balanced salt solution; to this solution were added 100 μg./ml. of streptomycin 100 units/ml. of penicillin, and calf serum in 20 per cent) was used throughout the experiments. For the cultivation of virus, YLG medium supplemented with calf serum in 3 per cent was used.

For the determination of cell toxic concentration of the compounds, HeLa cell cultures were preincubated at 37° for about 3 days to obtain a monolayer cell sheet. After establishment of the cell sheet, the growth medium was removed and then to the tube were added 0.9 ml. of maintenance medium and 0.1 ml. of the serial two-fold dilutions of the test compounds. The tubes were incubated at 37° for 5 days to observe microscopically the cytotoxic concentration of the compounds.

For the determination of inhibitory activity of the compounds on the growth of virus, to the HeLa cell cultures were added 0.8 ml. of maintenance medium, 0.1 ml. of serial two-fold dilution of the maximum tolerable concentration for HeLa cells of the test compounds, and 0.1 ml. of $10^5 TCID_{50}$/ml. virus suspension. After the cell cultures were incubated at 37° for 9 days, inhibition of viral cytopathic effect was determined by microscopic observation. Thereafter, they were stocked at −20° for the determination of viral yield.

For the determination of viral yield, one or two log dilution of the viral materials, which had been subjected to freezing and thawing 6 times, was inoculated to tubes in which the cell sheet had been established, and those cell cultures were incubated at 37°. The viral cytopathic effects were determined by daily microscopic observation. The previous experiments[6] showed that infective dose ($TCID_{50}$) had a linear

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1) E. A. Kaczka, *et al.*: Biochem. Biophys. Res. Comm., **14**, No. 1, 54 (1964).
2) C. O. Gitterman, *et al.*: Cancer Research, **24**, 440 (1964).
3) F. A. Miller, *et al.*: Nature, **200**, 1338 (1964).
4) H. T. Shigeura, *et al.*: Biochemistry, **2**, 1132 (1963).
5) This Bulletin, **15**, 1101 (1967).
6) H. Yuki, *et al.*: *Ibid.*, **14**, 139 (1966).

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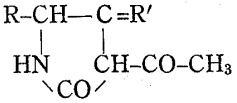
relation with the incubation periods for appearance of viral cytopathic effect in the initial three weeks. Accordingly, the viral infectivity was calculated from the prolonged days of appearance of viral cytopathic effect comparing with the control without the test compounds.

**Test of the Effect of Amino Acids on the Antiviral Activity of Tenuazonic Acid Derivatives**—— Seven tenths ml. of maintenance medium, 0.1 ml. of a half of cytotoxic concentration of test compounds, 0.1 ml. of amino acid solution, and 0.1 ml. of $10^5 TCID_{50}$/ml. virus suspension were added to HeLa cell cultures. The cell cultures were incubated at 37°, and the viral activity was determined comparing with that of control which did not contain the amino acid.

Table I. Activity of Tenuazonic Acid Derivatives against Adenovirus Type 5



| | R | R′ | HeLa cell maximum tolerable concn. (μg./ml.) | Minimum inhibitory concn. of CP effect[a] (μg./ml.) | Minimum inhibitory concn. of viral multiplication[b] (μg./ml.) | Chemotherapeutic index[c] |
|---|---|---|---|---|---|---|
| I | H | O | 250 | — | — | |
| II | H | N-NH-CS-$NH_2$ | 250 | — | — | |
| III | $(CH_3)_2$>CH- | O | 1000 | 500 | 1000 | 2 |
| IV | $(CH_3)_2$>CH- | NOH | 125 | 125 | nt[e] | 1 |
| V | $(CH_3)_2$>CH- | N-NH-$C_6H_5$ | 25 | — | — | |
| VI | $(CH_3)_2$>CH- | N-NH-CO-$NH_2$ | 250 | — | — | |
| VII | $(CH_3)_2$>CH- | N-NH-CS-$NH_2$ | 500 | 62.5 | 500 | 8 |
| VIII | $(CH_3)_2$>CH-$CH_2$ | O | 250 | 125 | 250 | 2 |
| IX | $(CH_3)_2$>CH-$CH_2$ | NOH | 25 | — | — | |
| X | $(CH_3)_2$>CH-$CH_2$ | N-NH-CO-$NH_2$ | 125 | — | — | |
| XI | $(CH_3)_2$>CH-$CH_2$ | N-NH-CS-$NH_2$ | 12.5 | — | — | |
| XII | $C_2H_5$-CH($CH_3$)[d] | O | 25 | — | — | |
| XIII | $CH_3$-S-$CH_2$-$CH_2$- | O | 1000 | 500 | 500 | 2 |
| XIV | $CH_3$-S-$CH_2$-$CH_2$- | N-$NH_2$ | 125 | — | — | |
| XV | $CH_3$-S-$CH_2$-$CH_2$- | N-NH-$C_6H_5$ | 25 | — | — | |
| XVI | $C_2H_5$-S-$(CH_2)_2$- | O | 3.1 | — | — | |
| XVII | $CH_3O$-CO-$CH_2$- | O | 2000 | 2000 | 1000 | 1 |
| XVIII | $CH_3O$-CO-$CH_2$- | N-NH-$C_6H_5$ | 25 | — | — | |
| XIX | $CH_3O$-CO-$CH_2$- | N-NH-CO-$NH_2$ | 1000 | — | — | |
| XX | $CH_3O$-CO-$CH_2$- | N-NH-CS-$NH_2$ | 25 | — | — | |
| XXI | HOOC-$CH_2$- | O | 250 | — | — | |
| XXII | $C_6H_5$-$CH_2$- | O | 250 | — | — | |
| XXIII | $C_6H_5$-$CH_2$- | NOH | 25 | — | — | |
| XXIV | $C_6H_5$-$CH_2$- | N-NH-$C_6H_5$ | 25 | — | — | |
| XXV | $C_6H_5$-$CH_2$- | N-NH-CS-$NH_2$ | 25 | — | — | |
| XXVI | HO-$C_6H_4$-$CH_2$- | O | 1000 | 1000 | 1000 | 1 |
| XXVII | HO-$C_6H_4$-$CH_2$- | N-$NH_2$ | 25 | — | — | |
| XXVIII | HO-$C_6H_4$-$CH_2$- | N-NH-CO-$NH_2$ | 62.5 | — | — | |

*a*) 50% inhibitory concentration of cytopathic effect compared with control at the 9th day.
*b*) Half log (70%) inhibitory concentration of viral infectivity (viral yield) compared with control.
*c*) Expressed as a ratio of the maximum tolerable concentration for cells and the minimum effective concentration for virus.
*d*) N,N′-dibenzylethylenediamine salt.
*e*) Not tested.

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## Results and Discussion

The antiviral activity of the tenuazonic acid derivatives was shown in Table I. The compounds, which exhibited the antiviral activity, had the maximum tolerable concentration for HeLa cell of 800 μg./ml. in average of eight compounds, and the compounds, which did not exhibit the antiviral activity, had the cell toxic concentration of 140 μg./ml. in average of twenty-three compounds. From this result, one can agree that less toxic compounds of this series have antiviral activity in non-toxic concentrations, but comparatively highly toxic compounds can not exhibit antiviral activity in non-toxic concentrations. However, it remains uncertain whether this argument can be applied to other compounds of this type. The Most notable compound is thiosemicarbazone of valine derivative (Ⅶ), which has chemotherapeutic index of 8, and is eight times as active as its carbonyl-free derivative in inhibition of viral cytopathic effect, while other thiosemicarbazones failed to exhibit the similar effect. Tenuazonic acid N,N′-dibenzylethylenediamine salt (Ⅻ) did not reveal the activity under these experimental conditions described above. The inhibitory activity of viral multiplication and the cytopathic effect were approximately correlated. In order to study the mechanism of action of these compounds against the adenovirus type 5, some active compounds which were not condensed with carbonyl reagents were tested in the presence of amino acids from which they were synthesized. Results are summarized in Table Ⅱ. Any compound tested was not antagonized even by five times equimolar amount of the original amino acid. Therefore their virus inactivating activity should not be due to the antagonistic effect to the amino acids. Anti-tumor activity of these compounds is now under examination at Cancer Chemotherapy National Service Center, N.I.H.

Table Ⅱ. Effect of Amino Acids on the Antiviral Activity of Tenuazonic Acid Derivatives

| Compounds | Concn. ($M$) | Amino acid | Concn. ($M$) | Inhibition of virus |
|---|---|---|---|---|
| XIII | $4.6\times10^{-3}$ | L-methionine | 0 | + |
| | $4.6\times10^{-3}$ | L-methionine | $4.6\times10^{-3}$ | *a*) |
| | $4.6\times10^{-3}$ | L-methionine | $2.3\times10^{-2}$ | *a*) |
| XVI | $4.0\times10^{-3}$ | L-tyrosine | 0 | + |
| | $4.0\times10^{-3}$ | L-tyrosine | $4.0\times10^{-3}$ | *b*) |
| | $4.0\times10^{-3}$ | L-tyrosine | $2.0\times10^{-2}$ | *b*) |
| XVII | $8.8\times10^{-3}$ | L-aspartic acid | 0 | + |
| | $8.8\times10^{-3}$ | L-aspartic acid | $8.8\times10^{-3}$ | + |
| | $8.8\times10^{-3}$ | L-aspartic acid | $4.4\times10^{-2}$ | + |
| Ⅷ | $1.2\times10^{-3}$ | L-leucine | 0 | + |
| | $1.2\times10^{-3}$ | L-leucine | $1.2\times10^{-3}$ | + |
| | $1.2\times10^{-3}$ | L-leucine | $6.0\times10^{-3}$ | + |
| Ⅲ | $2.0\times10^{-3}$ | L-valine | 0 | + |
| | $2.0\times10^{-3}$ | L-valine | $2.0\times10^{-3}$ | + |
| | $2.0\times10^{-3}$ | L-valine | $1.0\times10^{-2}$ | + |

*a*) Methionine was toxic to HeLa cells at the concentration tested.
*b*) Tyrosine was insoluble at the concentration tested.

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