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# Antitumor Activity of 3-(Substituted cinnamoyl)pyridine and 1-Oxide

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Some azachalcones (3-(substituted cinnamoyl)pyridine and its 1-oxide) were synthesized. Their antitumor activity on Yoshida sarcoma and rat ascites hepatoma AH-13, AH-66 and AH-7974 was determined. 2-Chloroazachalcone, 3-chloroazachalcone, 2-chloroazachalcone 1-oxide and 3-chloroazachalcone 1-oxide were found to be curative to AH-13, AH-66 and AH-7974 but negative to Yoshida sarcoma. A few experiments to elucidate the mechanism of antitumor effect of azachalcones were performed. Effects of 3-chloroazachalcone on the growth and on the content of DNA, RNA and protein of ascites hepatoma AH-66 was observed.

It is well known that chalcones are one of a constituent of plant glycosides, and some analogs exhibit interesting pharmacological activities. Several compounds of chalcone type have been prepared and reported to have antibacterial,[2,3] antifungal,[3] insecticidal,[4] spasmolytic,[5] choleretic,[6] coronary dilating,[7] antihormonal[8] and antitumor[9] activities. Many reports relate to the antibacterial activity but literature dealing with the study of antitumor activity is few. Acrylophenone type derivatives of chalcones are listed on the antitumor screening data of the Cancer Chemotherapy National Service Center (CCNSC).[10] However, these compounds show no effect against Sarcoma 180, Carcinoma 755, and Leukemia L1210 of mouse tumor. Sometimes antimicrobial activity is parallel to antitumor activity, therefore, it is interesting at this point to examine the antitumor activity of chalcone type derivatives.

This paper deals with the results of an investigation on antitumor activity of some azachalcones (cinnamoyl pyridine types) on Yoshida sarcoma and other rat ascites hepatomas.

## Results and Discussion

Primary screening data of azachalcones on Yoshida sarcoma and ascites hepatoma AH-66 which is known to be resistant to most of antitumor agents[11] are listed in Table I. Some

1) Location; *Ukima 1-3, Kita-ku, Tokyo.*
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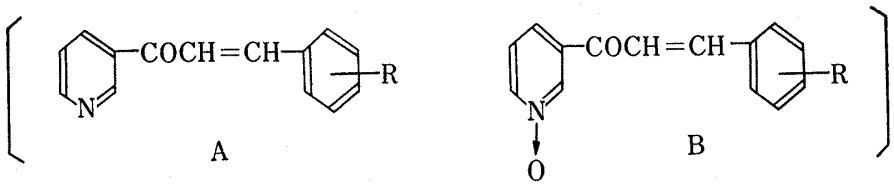
Table I. Antitumor Activity of 3-(Substituted cinnamoyl)pyridine (A) and 3-(Substituted cinnamoyl)pyridine 1-Oxide (B)




| Compound No. | R | Yoshida sarcoma MTD (mg/kg) | Yoshida sarcoma MED (mg/kg) | Yoshida sarcoma MTD/MED | AH-66 MTD (mg/kg) | AH-66 MED (mg/kg) | AH-66 MTD/MED | Reference for preparation |
|---|---|---|---|---|---|---|---|---|
| A | | | | | | | | |
| I | H | 500 | 250 | 2 | 500 | 250 | 2 | 6*b*) |
| II | 2-OH | >500 | 50 | >10 | >500 | 250 | >2 | 6*b*) |
| III | 2-OMe | >1000 | 250 | >4 | >250 | 250 | >1 | 6*b*) |
| IV | 2-Cl | 500 | 50 | 10 | >1000 | 25 | >40 | 6*c*) |
| V | 2-Br | >500 | 100 | >5 | >500 | 250 | >2 | *a*) |
| VI | 3-OH | >500 | 50 | >10 | >500 | — | | 6*b*) |
| VII | 3-OMe | 500 | 250 | 2 | not tested | | | 6*b*) |
| VIII | 3-$NO_2$ | >1000 | 500 | >2 | >1000 | 1000 | >1 | 6*c*) |
| IX | 3-Cl | >1000 | 50 | >20 | >1000 | 50 | >20 | 6*c*) |
| X | 3-Br | not tested | | | >1000 | 50 | >20 | *a*) |
| XI | 4-OH | >1000 | 500 | >2 | 500 | 500 | 1 | 6*b*) |
| XII | 4-OMe | 250 | 100 | 2.5 | 100 | 100 | 1 | 6*b*) |
| XIII | 4-$NO_2$ | >1000 | 50 | >20 | >1000 | 250 | >4 | 6*d*) |
| XIV | 4-Cl | >500 | 50 | >10 | >1000 | 250 | >4 | 6*b*) |
| XV | 4-Br | >500 | 50 | >10 | >500 | 50 | >10 | 6*c*) |
| XVI | 2-OH, 3-OMe | 500 | 500 | 1 | not tested | | | 6*b*) |
| XVII | 2,3-di-OMe | 500 | 100 | 5 | 500 | 50 | 10 | 6*b*) |
| XVIII | 3,4-di-OMe | >1000 | — | | >1000 | 250 | >4 | 6*b*) |
| XIX | 2,4-di-Cl | >1000 | 500 | >2 | >1000 | 250 | >4 | *a*) |
| XX | 3,5-di-Br,2-OH | >500 | 500 | >1 | >500 | 500 | >1 | *a*) |
| B | | | | | | | | |
| XXI | 2-Cl | >1000 | 100 | >10 | >1000 | 25 | >40 | *a*) |
| XXII | 3-Cl | >1000 | 100 | >10 | >1000 | 100 | >10 | *a*) |
| XXIII | 3,4-di-Cl | >1000 | 250 | >4 | >1000 | 250 | >4 | *a*) |
| XXIV | 4-OH, 3-OMe | >1000 | — | | >500 | — | | *a*) |
| XXV | 3,5-di-Br,2-OH | >1000 | 100 | >10 | >1000 | 100 | >10 | *a*) |

—: negative MTD-: maximum tolerant dose MED: minimum effective dose
*a*) new compounds, see Experimental

azachalcones were active against AH-66 than Yoshida sarcoma. For example, MTD/MED value of 2-chloroazachalcone (IV) against Yoshida sarcoma was only 10 but the value against AH-66 was over 40. There was no clear relationship between chemical structures and antitumor activities. 2-Chloroazachalcone (IV), 3-chloroazachalcone (IX), 2-chloroazachalcone 1-oxide (XXI) and 3-chloroazachalcone 1-oxide (XXII) show relativey high activity against AH-66. So these four compounds were examined on life-span prolongation effects. Fig. 1 and 2 illustrate the life-span prolongation effects of compounds (IX) and (XXI) on various experimental tumors, respectively. Compounds (IX) and (XXI) had an almost complete curative activity against AH-13, but they were negative against Yoshida sarcoma. It is interesting that they were shown to be curative to rats bearing AH-66 or AH-7974 which are known to be more refractory than Yoshida sarcoma.

In these experiments AH-7974 was found to be more sensitive to these derivatives than other tumors except AH-13 which is known to be very sensitive to most of the antitumor agents. Then all four compounds were applied to AH-7974 and compared their life-span

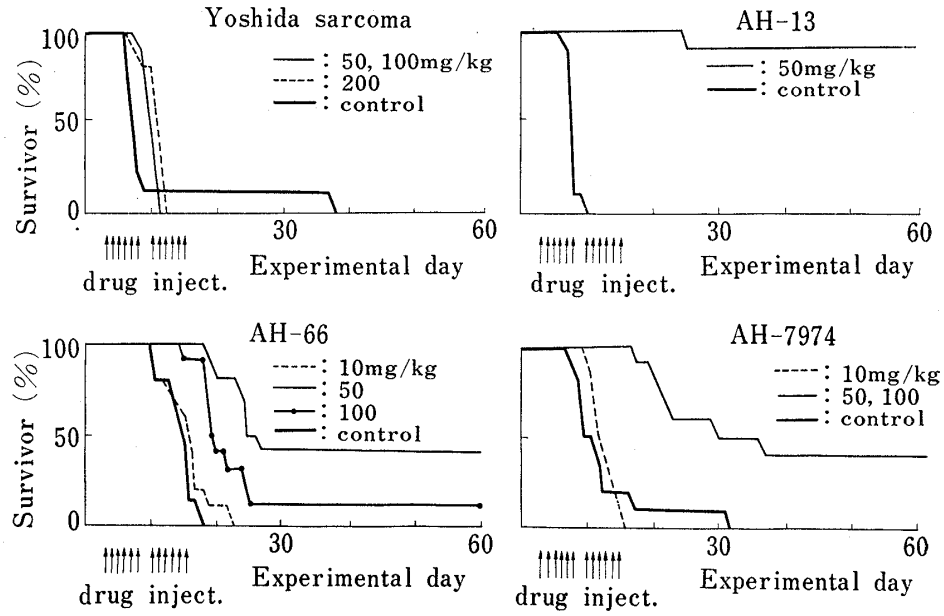



Fig. 1. Life Prolongation Experiments on Various Experimental Tumors by Intraperitoneal Injection of Compound (IX)

ten animals in one group

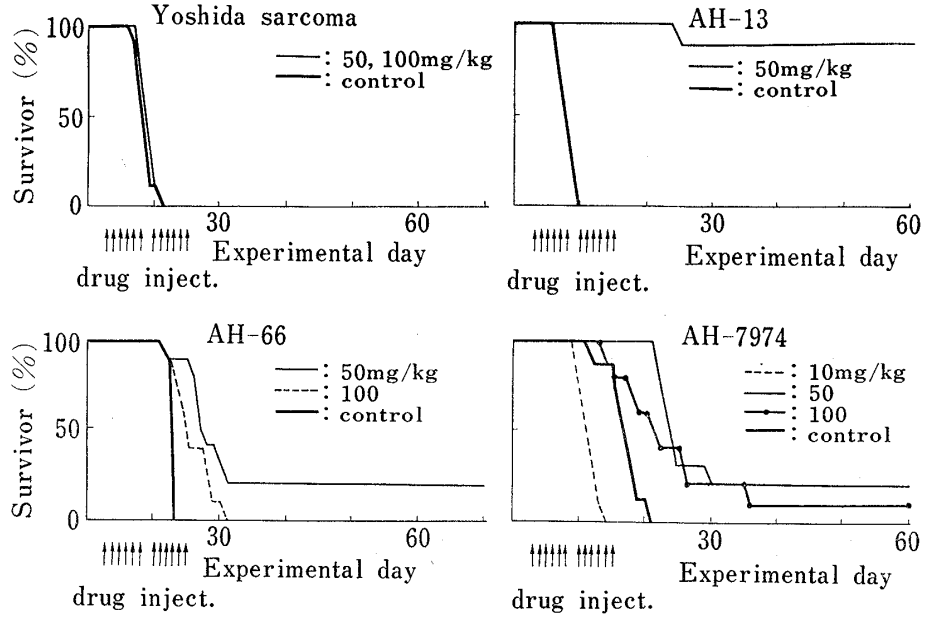



Fig. 2. Life Prolongation Experiments on Various Experimental Tumors by Intraperitoneal Injection of Compound (XXI)

ten animals in one group

prolongating activities as shown in Fig. 3. There was, however, no clear differences between them.

A few experiments were performed to elucidate the mechanism of these compounds against tumor cells. Effects of compound (IX) on the growth of ascites hepatoma AH-66 *in vivo* was observed. In the abdominal cavity, tumor cells grew exponentially and their growth rate fell when their total cell number reached about $10^9$. The maximum tolerant number of AH-66 in Donryu rat seemed to be about $10^9$ because animals bearing over $10^9$ cells died soon. Three days after inoculation of $10^9$ cells of AH-66, about $8\times10^7$ cells were found in

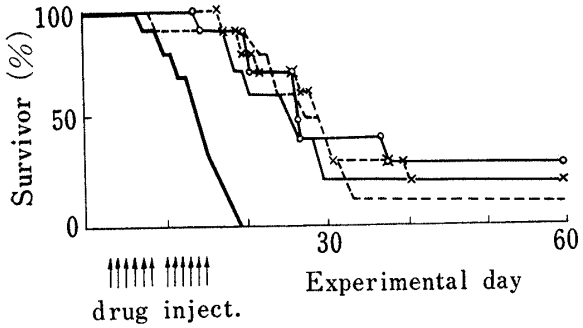



Fig. 3. Life Prolongation Experiments on Ascites Hepatoma AH-7974 by Intraperitoneal Injection of Chemicals

ten animals in one group

| | compound No. | dose (mg/kg) |
|---|---|---|
| ——: | IX | 50 |
| ---------: | XXII | 50 |
| —○—: | IV | 50 |
| —×—: | XXI | 50 |
| ——: | control | — |

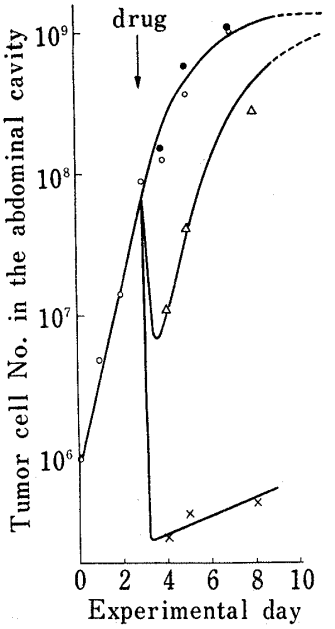



Fig. 4. Effect of Compound (IX) on the Growth of Ascites Hepatoma AH-66 Cells *in Vivo*

Each point represents the mean of 3 determinants.
—○—: no treated, —●—: 5 mg/kg (*i.p.*)
—△—: 50 mg/kg (*i.p.*) —×—: 500 mg/kg (*i.p.*)

Table II. Effect of Compound (IX) on the Growth of Ascites Hepatoma AH-66

| Time after inoculation (hr) | Time after administration | Total treated cell No. | |
|---|---|---|---|
| | | No treated | 100 mg/kg |
| 0 | — | 7.5[a] | — |
| 44 | 0 | 83.0[b] | — |
| 48 | 4 | 88.0 | 73.2[b] |
| 52 | 8 | 102.4 | 50.0 |

*a*) tumor cell number incoculated *b*) mean value of three experiments

abdominal cavity. Then administered compound (IX), the growth of tumor cells were disturbed as shown in Fig. 4.

There was no considerable effects when administered 5 mg/kg of test compound, but rapid diminution was observed when administered 50 mg/kg. Two days after injection of 50 mg/kg, growth rate recovered as fast as before treatment. In the case of 500 mg/kg, the rate of diminution of cell number was more rapid and the rate of recovery was more slow. The diminution of cell growth was tested in detail. AH-66 tumor cells of $7.5\times10^6$ were transplanted intraperitoneally and about $83.0\times10^6$ cells were found in 44 hours. Then administered 50 mg/kg of compound (IX), rapid diminution of total tumor cell number was observed in about 4 hours as illustrated in Table II.

The fashion of growth inhibition of the azachalcones seemed to be different from that of mitomycin C reported by Terawaki, *et al.*[12] Mitomycin C was found to diminish total cell number of Ehrlich carcinoma not so rapid as visible within a few hours. To elucidate the difference, effects of compound (IX) on DNA, RNA and protein content of AH-66 cell

12) A. Terawaki, H. Kontani, J. Dohi and T. Taguchi, *Osaka Med. J.*, 12, 837 (1960).

Table III. Effect of Compound (IX) on DNA, RNA and Protein Content of Ascites Hepatoma AH-66

| Treatment | Exptl. time (hr) | Cell No.[a] ($\times 10^6$) | DNA[b] ($\mu$g/cell) | RNA[b] ($\mu$g/cell) | Protein[b] ($\mu$g of N/cell) |
|---|---|---|---|---|---|
| — | 0 | 215.7 | $11.4\times10^{-6}$ | $21.9\times10^{-6}$ | $5.04\times10^{-6}$ |
| No treated | 6 | 229.1 | 11.5 | 22.1 | 5.47 |
| MT-C[c] | 6 | 216.0 | 8.3 | 18.6 | 4.02 |
| Compd. (IX)[d] | 6 | 92.1 | 16.1 | 29.5 | 7.14 |

*a*) mean value of three determination *b*) mean value of duplicate determination
*c*) dose: 0.5 mg/kg (*i.p.*) MT-C=Mitomycin C *d*) dose: 50 mg/kg (*i.p.*)

was examined. As shown in Table III, compound (IX) increased DNA, RNA and protein content in a parallel fashion, different from that of mitomycin C which diminished especially DNA content.[12]

From the results of these experiments, it is not clear but the azachalcones seem to affect on tumor cells in a particular manner. At present stage, there are many uncertain points, but it may be said that azachalcone derivatives have a very interesting antitumor activity as described and they might have some effects on certain human neoplasms.

## Experimental

### General Preparation of Azachalcones

**3-(Substituted cinnamoyl)pyridine**[7]——To the solution of 2.4 g (0.02 mole) of 3-acetylpyridine and 0.02 mole of aromatic aldehyde in 50—60 ml of anhydrous ethanol was added 30 drops of piperidine. The solution was refluxed on a water bath for 30—50 hours. The reaction mixture was then concentrated to a small volume under reduced pressure. The resulting mateial was recrystallized from the suitable solvent.

**3-(Substituted cinnamoyl)pyridine 1-Oxide**——2.5 g (0.02 mole) of 3-acetylpyridine 1-oxide,[13] 0.02 mole of aromatic aldehyde and 20 drops of piperidine were dissolved in 100 ml of anhydrous ethanol. The solution was refluxed on a water bath for 10 hours. Ethanol was evaporated off under reduced pressure and the resulting solid was recrystallized from the suitable solvent.

The results of new compounds are shown in Table IV.

Table IV. Analytical Data of 3-(Substituted cinnamoyl)pyridine and 3-(Substituted cinnamoyl)pyridine 1-Oxide

| Comp. No. | mp (°C) | Yield (%) | Recrystl. solvent | Formula | Analysis (%) Calcd. C | H | N | Found C | H | N |
|---|---|---|---|---|---|---|---|---|---|---|
| V | 106—108 | 32 | EtOH | $C_{14}H_{10}ONBr$ | 58.36 | 3.50 | 4.86 | 58.34 | 3.56 | 4.58 |
| X | 109—110 | 27 | EtOH | $C_{14}H_{10}ONBr$ | 58.36 | 3.50 | 4.86 | 57.90 | 3.43 | 4.95 |
| XIX | 140—141 | 52 | MeOH–$H_2O$ | $C_{14}H_{10}ONCl_2$ | 60.46 | 3.26 | 5.04 | 60.39 | 3.42 | 5.06 |
| XX | 191—193 | 17 | DMF–$H_2O$ | $C_{14}H_9O_2NBr_2$ | 43.90 | 2.37 | 3.66 | 44.30 | 2.44 | 2.76 |
| XXI | 200—202 | 73 | MeCN–MeOH | $C_{14}H_{10}O_2NCl$ | 64.75 | 3.88 | 5.39 | 64.80 | 3.96 | 5.18 |
| XXII | 232—233 | 45 | MeCN–MeOH | $C_{14}H_{10}O_2NCl$ | 64.75 | 3.88 | 5.39 | 64.97 | 3.93 | 5.19 |
| XXIII | 266—267 | 62 | DMF | $C_{14}H_9O_2NCl_2$ | 57.17 | 3.08 | 4.76 | 57.32 | 3.05 | 4.44 |
| XXIV | 243 | 66 | MeOH–$H_2O$ | $C_{15}H_{13}O_4N$ | 66.42 | 4.83 | 5.16 | 66.19 | 4.84 | 5.09 |
| XXV | 230 (decomp.) | 45 | MeCN–$H_2O$ | $C_{14}H_9O_3NBr_2$ | 42.14 | 2.27 | 3.51 | 42.13 | 2.28 | 3.49 |

13) S. Kanno, *Yakugaku Zasshi*, 73, 120 (1953).

**Biological Procedure**

**Animals and Tumors**——All animal studies were made on female of Donryu rat, weighing 90—120 g, obtained from Nippon Rat Co. The rats were fed with standard diet CE-2 of CLEA Japan Co., and water **ad libitum**. In these experiments Yoshida sarcoma, ascites hepatoma AH-13, AH-66 and AH-7974 were used. All tumors were supplied from Cancer Institute and have been kept in our laboratory by successive transplantation in ascites form. Tumor cells of 4-7-day-old were used throughout.

**Screening Test System**——Primary screening were performed with Yoshida sarcoma and ascites hepatoma AH-66 by the method of Yoshida, *et al.*[14] Donryu rats were inoculated with $10^6$ tumor cells intraperitoneally and three days after inoculation, they were injected with test compounds at serial dilution intraperitoneally. Antitumor activity was showed by the value of MTD/MED: Minimum effective dose (MED) was determined by the cytomorphological changes of the ascites tumors at 24, 48 and 72 hours after the injection of test compound. Maximum tolerant dose (MTD) was determined after one week observation.

Life-span prolongation studies were performed with the selected compounds. Animals were inoculated with $10^6$ cells of Yoshida sarcoma or ascites hepatomas. Three days after the inoculation, test compounds were given once a day for two weeks. Mortality was observed during 2 months.

**Cytological Experiments**——Determination of total cell number in abdominal cavity was made by the following methods. An animal was killed by cervical dislocation and a small hole was cut in the exposed abdominal musculature. Physiological saline containing 2.5% of EDTA was added into the peritoneal fluid suitable volume. Then the fluid contents of peritoneum was transferred with a pipette to a beaker. Tumor cells were washed three times with physiological saline to make them free from red blood cells and other impurities. Cell number was determined by the method of McIntire and Smith.[15] Tumor cells fractionated into DNA, RNA and protein and measured by the method of Schneider.[16]

14) T. Yoshida, H. Satoh, B. Nagasawa, A. Atsumi, H. Sato, K. Kobayashi, K. Hosokawa, K. Nakamura, M. Ishidate and Y. Sakurai, *Gann.*, **41**, 93 (1950).
15) F.C. McIntire and M.F. Smith, *Proc. Exptl. Biol. Med.*, **98**, 76 (1958).
16) W.C. Schneider, "Methods in Enzymology," Vol. III, ed by S.P. Colowick and N.O. Kaplan, Academic Press, Inc., New York, 1957, p. 680.