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# Antitumor Activity of 4-Nitropyridazine 1-Oxides and Related Compounds for AH-13[1]

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Antitumor activity of 4-nitropyridazine 1-oxides and related compounds was tested with AH-13 system. Among these nitro compounds, 3,6-dimethoxy-4-nitropyridazine 1-oxide (III) and 4-nitrocinnoline 1-oxide (XIII) were the most effective. 4-Nitropyridazine 1-oxide (I), 3,6-dimethyl-4-nitropyridazine 1-oxide (II) and 3-alkoxy-4-nitro-6-chloropyridazine 1-oxides (VI, VII) were effective to some extent.

**Keywords**——4-nitropyridazine 1-oxides; 3,6-dimethoxy-4-nitropyridazine 1-oxide; 4-nitrocinnoline 1-oxide; antitumor activity for AH-13; alkylating type antitumor activity; reactive 4-nitro groups of 1,2-diazine N-oxides

In these ten years a number of pyridazine and cinnoline derivatives were synthesized in this laboratory. Among them, 4-nitropyridazine 1-oxides and 4-nitrocinnoline 1-oxides are highly reactive toward nucleophilic and electrophilic reagents, for their nitro groups are activated by the N-oxide groups. Their biological actions are also of interest in connection with their chemical reactivities.

This paper describes the antitumor activity of these nitro compounds for a rat ascites hepatoma, AH-13 cells.

## Experimental

**Materials**——4-Nitropyridazine 1-oxides (I—IX), 3,6-dimethyl-4-hydroxy-5-nitropyridazine 1-oxide (X) and 4-nitrocinnoline 1-oxide (XIII) were prepared by nitration of the corresponding 1-oxides with a mixture of fuming nitric acid and concentrated sulfuric acid, according to the literatures.[3-7] 3,6-Dimethyl-5-nitropyridazine 1-oxide (XII) and 3-nitrocinnoline 1-oxide (XIV) were prepared by nitration of the corresponding 1-oxides with benzoyl nitrate, which was produced by the reaction of benzoyl chloride and silver nitrate, according to the literatures.[8,9]

Their physical properties and the literatures used are as follows.

4-Nitropyridazine 1-Oxide (I): Pale yellow plates (from benzene), mp 149—150° (lit.,[3] mp 151°).

3,6-Dimethyl-4-nitropyridazine 1-Oxide (II): Yellow plates (from benzene), mp 117—118° (lit.,[4] mp 117—118°).

3,6-Dimethoxy-4-nitropyridazine 1-Oxide (III): Yellow plates (from acetone), mp 112—113° (lit.,[5] mp 114°).

3,6-Diethoxy-4-nitropyridazine 1-Oxide (IV): Yellow needles (from a mixture of benzene and diethyl ether), mp 74—75° (lit.,[4] mp 75—76°).

3,6-Dipropoxy-4-nitropyridazine 1-Oxide (V): Yellow flakes (from diethyl ether), mp 65—67° (lit.,[4] mp 67—68°).

1) Presented at the 95th Annual Meeting of the Pharmaceutical Society of Japan, April 1974, Kobe.
2) Location: *Kamiyoga 1-18-1, Setagaya-ku, Tokyo.*
3) T. Itai and S. Natsume, *Chem. Pharm. Bull.* (Tokyo), **10**, 643 (1962).
4) T. Itai and S. Sako, *Chem. Pharm. Bull.* (Tokyo), **9**, 149 (1961).
5) T. Itai and H. Igeta, *Yakugaku Zasshi*, **75**, 966 (1955).
6) T. Itai and S. Sako, *Chem. Pharm. Bull.* (Tokyo), **10**, 934 (1962).
7) H. Igeta, *Chem. Pharm. Bull.* (Tokyo), **8**, 550 (1960).
8) T. Itai and S. Natsume, *Chem. Pharm. Bull.* (Tokyo), **12**, 288 (1964).
9) I. Suzuki, T. Nakashima, N. Nagasawa and T. Itai, *Chem. Pharm. Bull.* (Tokyo), **12**, 1090 (1964)

3-Methoxy-4-nitro-6-chloropyridazine 1-Oxide (VI): Yellow needles (from ethanol), mp 145—146° (lit.,[6] mp 144—145°).

3-Ethoxy-4-nitro-6-chloropyridazine 1-Oxide (VII): Yellow needles (from ethanol), mp 124—125°. Yield, 54%. NMR (ppm, $CDCl_3$): 8.50 (s, $H^5$), 4.60 (q, $\underline{CH_2}CH_3$), 1.51 (t, $CH_2\underline{CH_3}$). *Anal.* Calcd. for $C_6H_6O_4N_3Cl$: C, 32.82; H, 2.75; N, 19.14. Found: C, 32.87; H, 2.64; N, 19.23.

3-Methoxy-4-nitropyridazine 1-Oxide (VIII): Yellow needles (from methanol), mp 102° (lit.,[7] mp 103°).

3-Methoxy-4,6-dinitropyridazine 1-Oxide (IX): Yellow leaflets (from methanol), mp 130° (lit.,[7] mp 130°).

3,6-Dimethyl-4-hydroxy-5-nitropyridazine 1-Oxide (X): Yellow needles (from acetone), mp 184—185° (decomp.) (lit.,[10] mp 184—185°).

1-Methoxy-3,6-dimethyl-5-nitro-4(1H)-pyridazinone (XI): Yellow needles (from diisopropyl ether), mp 78—79° (lit.,[10] mp 78—79°).

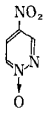
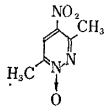
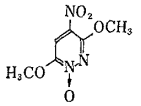
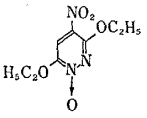
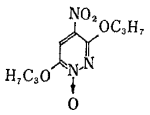
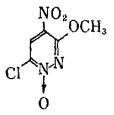
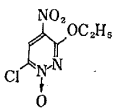
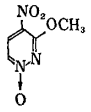
3,6-Dimethyl-5-nitropyridazine 1-Oxide (XII): Yellow needles (from diisopropyl ether), mp 85—86° (lit.,[8] mp 85—86°).

4-Nitrocinnoline 1-Oxide (XIII): Yellow needles (from acetone), mp 161—162° (lit.,[9] mp 161—162°).

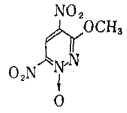
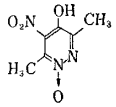
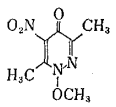
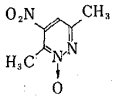
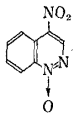
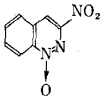
3-Nitrocinnoline 1-Oxide (XIV): Yellow needles (from chloroform), mp 214—215° (lit.,[9] mp214—215°).

**Screening Method**——Ascites hepatoma, AH-13 cells ($1\times10^6$) were intraperitoneally inoculated into Donryu rats. On the 3rd day the growth of the ascitic fluide was checked, and then the compound of different doses was injected intraperitoneally. The administration was usually continued once daily for 5 days, the surviving animals were checked daily, and the evaluation of each compound was based on the number of survivors on the 30th day and on the 60th day. One dose group consisted of 6 female Donryu rats of body weight 100—150 g.

Table I. Effect of 4-Nitropyridazine 1-Oxides and Related Compounds on Survival Time Prolongation of The Hosts Transplanted with AH-13 Cells

| Compd. No. | Compound | MTD[a] (mg/kg) | MED[b] (mg/kg) | Dose (mg/kg) | T/C[c] | No. of survivors 30 days | No. of survivors 60 days |
|---|---|---|---|---|---|---|---|
| I |  | >500 | — | 50 | 154 | | |
| | | | | 100 | | 1/6 | 1/6 |
| II |  | >500 | — | 25 | 193 | 1/6 | 0/6 |
| | | | | 50 | 195 | 0/6 | 0/6 |
| | | | | 100 | 217 | 1/6 | 0/6 |
| III |  | 250 | 5 | 25 | | 4/6 | 3/6 |
| | | | | 50 | | 4/6 | 3/6 |
| | | | | 100 | 104 | | |
| IV |  | 250 | — | 20 | 83 | 0/6 | |
| | | | | 40 | 100 | 0/6 | |
| | | | | 80 | 100 | 0/6 | |
| V |  | 250 | — | 20 | 84 | 0/6 | |
| | | | | 40 | 87 | 0/6 | |
| | | | | 80 | 91 | 0/6 | |
| VI |  | 100 | — | 15 | 124 | | |
| | | | | 30 | | 1/6 | 1/6 |
| | | | | 60 | 317 | 2/6 | 0/6 |
| VII |  | 100 | — | 15 | 123 | | |
| | | | | 30 | 151 | 0/5 | |
| | | | | 60 | | 1/6 | 1/6 |
| VIII |  | >500 | — | 100 | 235 | 0/6 | |
| | | | | 200 | 246 | 1/5 | |

10) S. Kamiya and M. Tanno, *Chem. Pharm. Bull.* (Tokyo), **23**, 1879 (1975).

| Compd. No. | Compound | MTD[a] (mg/kg) | MED[b] (mg/kg) | Dose (mg/kg) | T/C[c] | No. of survivors 30 days | No. of survivors 60 days |
|---|---|---|---|---|---|---|---|
| IX |  | 5 | — | 1<br>2 | 108<br>121 | 0/6<br>0/6 | |
| X |  | >500 | — | 25<br>50<br>100 | 97<br>106<br>94 | 0/5<br>0/5<br>0/5 | |
| XI |  | >500 | — | 25<br>50<br>100 | 100<br>103<br>97 | 0/6<br>0/6<br>0/6 | |
| XII |  | 100 | — | 15<br>30<br>60 | 117<br>157<br>123 | 0/6<br>0/6<br>0/6 | |
| XIII |  | 50 | 10 | 25<br>50<br>100 | <br><br>128 | 2/6<br>1/6<br>0/6 | 2/6<br>1/6 |
| XIV |  | >500 | — | 50<br>100 | 101<br>104 | 0/6<br>0/6 | |

*a*) maximum tolerated dose: single dose on Donryu rats bearing AH-13 cells
*b*) minimum effective dose: single dose on Donryu rats bearing AH-13 cells
*c*) mean survival time of controled group/mean survival time of treated group

The minimum effective doses (MED) of III and XIII on AH-13 cells were estimated as follows. Different doses diluted at the ratio 1/2, were separately injected once intraperitoneally into a rat on the 3rd day after intraperitoneal inoculation of AH-13 cells. Ascitic smear preparations were made on the 1st, 2nd, and 3rd day after the treatment. They were fixed, stained with the Giemsa's solution, and cytological effects of the compound were checked under a microscope. When abnormal mitosis such as scattering or disarrangement of chromosomes in metaphase and/or bridge formation in anaphase were observed in more than 50% of mitotic figures, the cytological effect was estimated as positive. Giant-cell formation and karyorrhexis in resting cells after the treatment were also taken into consideration of the final judgement.

## Results

The results obtained were summarized in Table I. Among these nitropyridazines and nitrocinnolines, 3,6-dimethoxy-4-nitropyridazine 1-oxide (III) and 4-nitrocinnoline 1-oxide (XIII) showed the most marked effects on prolongation of survival time. However, the ethoxy and propoxy derivatives (IV, V) of compound III were not effective. 4-Nitropyridazine 1-oxide (I), 3,6-dimethyl-4-nitropyridazine 1-oxide (II) and 3-methoxy(or ethoxy)-6-chloro-4-nitropyridazine 1-oxide (VI, VII) showed moderate effects.

The MED of III and XIII on AH-13 cells was estimated as 5 and 10 mg/kg, respectively. From morphological point of view, the damage seemed to be more alkylating type in XIII rather than in III, when compared with that previously observed in a typical alkylating agent, nitrogen mustard.

Subsequently, compound III and XIII were tested for mouse leukemia, L-1210, but they were not effective.

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