Notes

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Takanobu Itai and Shigeru Sako : Potential Anti-cancer Agents. III.[1] 3,6-Disubstituted 4-Nitropyridazine 1-Oxides. (1).

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As far as is known, N-oxidation of pyridazine derivatives has only been reported by Itai and Igeta,[2] Koelsch and Gumprecht,[3] and by Igeta,[4] but, other monocyclic diazines, i.e. pyrimidine and pyrazine, were more widely studied for their N-oxidation.[5,6] In several of these reports, steric effect of α-substituted groups to N-oxidation was discussed. As 3,6-dialkoxy- and dimethylpyridazine had already been synthesized in this Institute, their N-oxidation and nitration of compounds were examined.

The synthetic methods were about the same as in the previous report.[2] Mono-N-oxides of 3,6-dialkoxypyridazines (ethoxy, propoxy, butoxy) and 3,6-dimethylpyridazines were prepared as shown in Table I, but di-N-oxides were not obtained.

Table I. 3,6-Disubstituted Pyridazine 1-Oxides

Compd. No.	Substituents at 3,6-	Crystal form	Solvent of recrystn.	m.p. (°C)	Yield (%)	IR absorption (N-O) (in KBr, cm^{-1})
(I)	C_2H_5O-	plates	benzine	71~72	40	1350
(II)	C_3H_7O-	flakes	〃	54~55	30	1345
(III)	C_4H_9O-	needles	〃	52~53	47	1345
(IV)	CH_3-	〃	ligroine	113~114	52	1330

When 3,6-dibenzyloxypyridazine was heated with hydrogen peroxide-acetic acid, colorless needles, m.p. 171~172°, were isolated in 65% yield. Its analytical data corresponded to $C_{11}H_{10}O_2N_2$ and its infrared spectrum showed absorption bands at 3200 (-NH-), 1670 (strong, -CONH-), 1290, and 1000, cm^{-1} (-OR). From these results, it seemed likely that the product is 6-benzyloxy-3(2*H*)-pyridazinone (V). Then, dibenzyloxypyridazine was heated only with acetic acid and (V) was gained in almost quantitative yield. It became clear, therefore, that 6-benzyloxy-3(2*H*)-pyridazinone had been produced by hydrolysis at first and N-oxidation did not take place.

Di-*tert*-butoxypyridazine was decomposed further with hydrogen peroxide in acetic acid and white prisms, m.p. 298°, were obtained in 100% yield. It was identified as 6-hydroxy-3(2*H*)-pyridazinone (VI) by its analytical data, its color reaction with ferric ion, and infrared spectrum, which showed absorptions at ca. 3200 (broad), 1670, 1290, and 1000 cm^{-1}. When di-*tert*-butoxypyridazine was heated in acetic acid alone, 6-*tert*-butoxy-3(2*H*)-pyridazinone (VII) was gained in 91% yield. Its infrared absorption bands were at ca. 3200 (broad) and 1670 cm^{-1}, attributable to -NH- and -CONH-.

In order to prevent the above-mentioned hydrolysis, N-oxidation with phthalic monoperacid in ether was examined, but N-oxide compound was not obtained.

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These 3,6-dialkoxypyridazine N-oxides were nitrated with nitric acid-sulfuric acid with chilling the flask in ice-water and the reaction mixture was allowed to stand for 1 or 2 days in the same condition. In the case of 3,6-dimethylpyridazine, it was necessary to warm on a water-bath and to use fuming nitric acid and sulfuric acid. The products obtained are listed in Table II.

Table II. 3,6-Disubstituted 4-Nitropyridazine 1-Oxides

Compd. No.	Substituents at 3,6-	Crystal form	Solvent of recrystn.	m.p. (°C)	Yield (%)	IR absorption (N-O) (in KBr, cm^{-1})
(VIII)	C_2H_5O-	prisms	ligroine	75~76	44	1320
(IX)	C_3H_7O-	plates	benzine	67~68	35	1340
(X)	C_4H_9O-	flakes	〃	54~56	54	1340
(XI)	CH_3-	needles	ligroine-benzene	117~118	54	1325

Position of the introduced nitro group was not determined but it is probably in 4-position, analogous to nitration of pyridine and quinoline 1-oxides. On this problem, experiments will be made in future.

3,6-Dimethoxy-4-nitropyridazine 1-oxide had been found to have cancerostatic action by Dr. D. Mizuno, National Institute of Health, using INK method. The above-mentioned compounds were also tested by INK method and by unstained cell count method in this Institute.*2 They were also carcinostatic except for 3,6-dibutoxy-4-nitropyridazine 1-oxide. All of them showed bacteriostatic action and especially were active against Staphylococcus.

Table III. Results of Screening Tests

	(VIII)	(IX)	(X)	(XI)
Anticancer action	+	+	−	+
Antibacterial action	+	+	+	+

Experimental

General Procedure of N-Oxidation—To a solution of 1 part of 3,6-dialkoxypyridazine in 7 volumes of glacial AcOH, 2 volumes of 30% H_2O_2 was added and the mixture was heated at 70° for 3 hr. One volume of 30% H_2O_2 was added to the mixture and heating was continued for 2 or 3 hr. more. After distilling off the solvent in vacuum, the residue was neutralized with $NaHCO_3$, adding simultaneously some water. The solution was extracted with several portions of $CHCl_3$, the combined extract was dried over Na_2SO_4 and the solvent was distilled off. Almost colorless crystals were recrystallized from benzine or ligroine.

Table IV. Analytical Data of N-Oxides

Compd. No.	Formula	Calcd. (%)			Found (%)		
		C	H	N	C	H	N
(I)	$C_8H_{12}O_3N_2$	52.16	6.57	15.21	52.62	6.70	14.86
(II)	$C_{10}H_{16}O_3N_2$	56.59	7.60	13.20	57.10	7.56	13.45
(III)	$C_{12}H_{20}O_3N_2$	59.98	8.39	11.66	59.77	8.09	11.87
(IV)	$C_6H_8O_3N_2$	58.05	6.50	22.57	58.67	6.33	22.74

N-Oxidation of 3,6-Dibenzyloxypyridazine : Formation of (V)—A solution of 2 g. of 3,6-dibenzyloxypyridazine dissolved in 40 cc. of glacial AcOH was warmed at 70° for 8 hr. and the solution was treated as above.

Hydrolysis of 3,6-Dibenzyloxypyridazine : Formation of (V)—A mixture of 2 g. of 3,6-dibenzyloxypyridazine in 40 cc. of glacial AcOH was treated as for N-oxidation.

N-Oxidation of 3,6-Di-*tert*-butoxypyridazine : Formation of (VI)—500 mg. of 3,6-di-*tert*-butoxypyridazine was treated as mentioned above. White crystals were obtained, which were almost insoluble in H_2O, Et_2O, $CHCl_3$, Me_2CO, and benzene. Its solution turned dark red on adding $FeCl_3$

*2 This screening test was performed by Dr. M. Nakamura and Mr. F. Miyazawa, Division of Microbiology in this Institute. The results will be published elsewhere in detail.

solution. Crystals were collected and recrystallized from H_2O. White prisms, m.p. 295° (decomp.). Yield, 250 mg. (100%, calcd. as hydroxypyridazinone). No depression was seen on mixed m.p. determination with an authentic substance.

Hydrolysis of 3,6-Di-*tert*-butoxypyridazine with Acetic Acid : Formation of (VII)—57.1 mg. of 3,6-di-*tert*-butoxypyridazine was warmed at 70° with 0.5 cc. of AcOH for 9 hr., the solvent was removed by vacuum distillation, and the residue was dried in evacuated desiccator. Yield, 39 mg. (91%), m.p. 154~160°. Recrystallization from ligroine-benzene gave colorless plates, m.p. 161~162°.

Table V. Analytical Data of the Decomposition Products

Compd. No.	Formula	Calcd. (%)			Found (%)		
		C	H	N	C	H	N
(V)	$C_{11}H_{10}O_2N_2$	65.33	4.98	13.86	65.73	5.18	13.55
(VI)	$C_4H_4O_2N_2$	42.86	3.60	24.99	42.91	3.67	24.55
(VII)	$C_8H_{12}O_2N_2$			16.66			16.92

General Procedure for Nitration of 3,6-Dialkoxypyridazine 1-Oxide—To a solution of 2 g. of 3,6-dialkoxypyridazine 1-oxide dissolved in 6 cc. of 80% H_2SO_4, a mixture of 1.5 cc. of HNO_3 (d=1.38) and 1.5 cc. of conc. H_2SO_4 was added dropwise at below 10° and the solution was allowed to stand in a same condition for 1 or 2 days. The mixture was poured on ice-water, extracted with $CHCl_3$, and the extract was dried over Na_2SO_4. $CHCl_3$ was distilled off, the residue was dissolved in benzene, and the solution was poured on alumina column for chromatography. The absorbed substances were eluted with benzene and a yellow solution was collected. After removal of the solvent, the residue was recrystallized from benzine or ligroine.

Nitration of 3,6-Dimethylpyridazine 1-Oxide—A solution of 500 mg. of 3,6-dimethylpyridazine 1-oxide dissolved in 1.5 cc. of conc. H_2SO_4 was cooled, 0.3 cc. of fuming HNO_3 was added in drops, and the mixture was heated on a boiling water-bath for 5 hr. This was treated as above. Pale yellow crystals, m.p. 116~117°. Yield, 0.39 g. They were recrystallized from ligroine-benzene to pale yellow needles, m.p. 117~118°. Yield, 365 mg. (54%).

Table VI. Analytical Data of Nitrated N-Oxides

Compd. No.	Formula	Calcd. (%)			Found (%)		
		C	H	N	C	H	N
(VIII)	$C_8H_{11}O_5N_3$	41.92	4.84	18.34	42.28	4.74	18.68
(IX)	$C_{10}H_{15}O_5N_3$	56.69	5.88	16.34	46.41	5.55	16.49
(X)	$C_{12}H_{19}O_5N_6$	50.52	6.71	14.73	51.09	6.76	15.02
(XI)	$C_6H_7O_3N_3$	42.60	4.17	24.85	43.23	3.99	24.58

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