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189. Tatsuya Horie: Studies on Pyridazine Derivatives. V.[1] Syntheses of 5-Substituted Derivatives of 3-Amino-6-alkoxypyridazine 2-Oxide.*1

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As described in the preceding reports,[1,2] the author clarified that the N-2-oxides should be mainly formed by the N-oxidation of 3-aminopyridazine and its 6-substitutes with hydrogen peroxide in acetic acid. It is of interest to investigate the ring substitution of the pyridazine N-2-oxide derivatives, since α- and γ-position of these derivatives are considered apt to electrophilic reagents on account of the mesomeric effect of N-oxide group, inferred from the analogy to the reactivity of other heterocyclic N-oxides. Accordingly, the author studied on the substitution of 3,6-disubstituted pyridazine derivatives, employing 3-acetamido-6-alkoxypyridazine 2-oxide as the starting material and were able to obtain several new compounds of 3,5,6-trisubstituted pyridazine.

This report describes the syntheses of 5-substituted 3-amino-6-alkoxypyridazine derivatives.

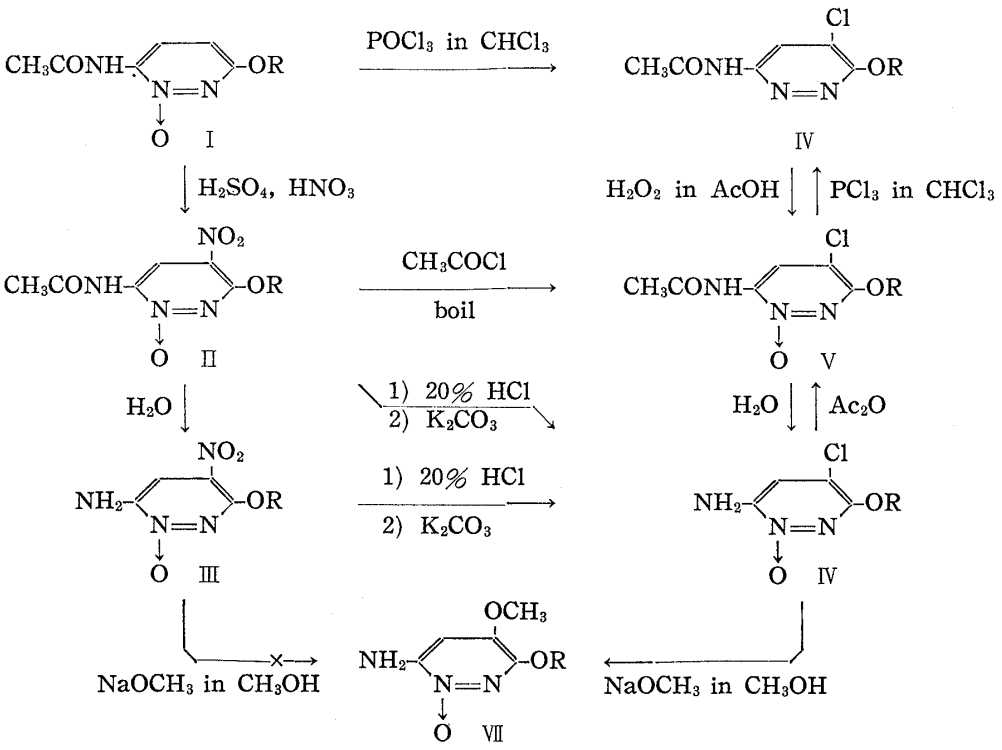

Chart 1.

Synthesis of 3-Amino-5-nitro-6-alkoxypyridazine 2-Oxide

3-Acetamido-6-methoxypyridazine (I) was found to be converted to mononitro substitutes by the reaction with an excessive mixture of sulfuric and nitric acids. By the method shown in Chart 3, the hydrogenation of this nitro substitute afforded the

*1 This paper was read before the 82nd Annual Meeting of Pharmaceutical Society of Japan (1962).

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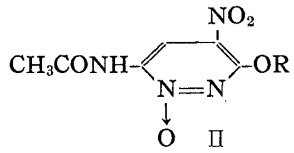
1) Part IV. T. Horie, T. Ueda: This Bulletin, **11**, 114 (1963).

2) T. Horie: Yakugaku Zasshi, **82**, 627 (1962).

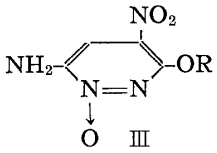
TABLE I-a. General formula CH_3CONH-  -OR

II

No.	R	m.p. (°C)	Appearance	Recryst. sorvent	UV λ_{max}^{EtOH} (mμ)	
II-a	CH_3	211	yellow needles	MeOH	262	383
II-b	C_2H_5	209	〃	〃	263	386
II-c	C_3H_7	181	〃	〃	263	286.5
II-d	C_4H_9	152	〃	〃	262.5	386
II-e	iso-C_5H_{11}	142	〃	〃	262.5	
II-f	C_5H_{11}	133	〃	〃	262.5	386
II-g	C_6H_{13}	136	〃	〃		
II-h	C_8H_{17}	131	slight yellow n.	〃	262.5	386
II-i	$C_{10}H_{21}$	123	〃	〃		

TABLE I-b. Analytical Date

No.	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
II-a	$C_7H_8O_5N_4$	36.85	3.53	24.77	36.91	3.40	24.59
II-b	$C_8H_{10}O_5N_4$	39.62	4.16	23.14	39.92	4.16	22.94
II-c	$C_9H_{12}O_5N_4$	42.19	4.72	21.88	41.95	4.94	21.89
II-d	$C_{10}H_{14}O_5N_4$	44.44	5.22	20.74	44.18	5.19	20.69
II-e	$C_{11}H_{16}O_5N_4$	46.47	5.67	19.72	46.58	5.48	19.58
II-f	$C_{11}H_{16}O_5N_4$	46.47	5.67	19.72	46.29	5.46	19.81
II-g	$C_{12}H_{18}O_5N_4$	—	—	18.12	—	—	17.98
II-h	$C_{14}H_{22}O_5N_4$	—	—	17.17	—	—	17.02
II-i	$C_{16}H_{26}O_5N_4$	—	—	15.82	—	—	15.69

TABLE II-a General formula NH_2-  -OR

III

No.	R	m.p. (°C)	Appearance	Recryst. solvent
III-a	CH_3	181	orange prisms	EtOH
III-b	C_2H_5	156	〃	〃
III-c	C_3H_7	150	yellow needles	〃
III-d	C_4H_9	132	〃	dil. EtOH
III-e	iso-C_5H_{11}	126	〃	〃
III-f	C_5H_{11}	112	〃	〃
III-g	C_6H_{13}	120	〃	〃
III-h	C_8H_{17}	92	〃	〃
III-i	$C_{10}H_{21}$	110	〃	〃

TABLE II-b. Analytical Data

No.	Formula	Calcd. N (%)	Found N (%)
III-a	$C_5H_6O_4N_4$	30.10	29.88
III-b	$C_6H_8O_4N_4$	27.99	28.09
III-c	$C_7H_{10}O_4N_4$	26.16	26.33
III-d	$C_8H_{12}O_4N_4$	24.55	24.28
III-e	$C_9H_{14}O_4N_4$	23.13	22.90
III-f	$C_9H_{14}O_4N_4$	23.13	22.93
III-g	$C_{10}H_{16}O_4N_4$	21.87	21.66
III-h	$C_{12}H_{20}O_4N_4$	19.71	19.49
III-i	$C_{14}H_{24}O_4N_4$	17.94	17.80

diamino compound (XIV), which was found not to form triazoro ring-closure with the addition of nitrous acid. Accordingly, this nitro substitute (II) should have the structure of 3-acetamido-5-nitro-6-methoxypyridazine 2-oxide, inferred from the mesomeric effect of N→O in the nitration of pyridine N-oxide[3] and quinoline N-oxide.[4] Analogously, the other compounds of 3-acetamido-6-alkoxypyridazine 2-oxide were converted to the corresponding 5-nitro substitutes in good yields with the nitration reagent, just as the nitration of 3-acetamido-6-methoxypyridazine 2-oxide. The nitro substitutes thus obtained are listed in Table I.

The above compounds of 3-acetamido-5-nitro-6-alkoxypyridazine 2-oxide (II) were converted in good yields into the corresponding compounds of 3-amino-5-nitro-6-alkoxypyridazine 2-oxide by the hydrolysis with hydrochloric acid in ethanol. The compounds obtained are listed in Table II.

Syntheses of 5-Chloro Derivatives of 3-Amino-6-alkoxypyridazine

It was reported by the Itai, *et al.* that all of 4-nitropyridine N-oxide,[5] 4-nitroquinoline N-oxide[5] and 4-nitro-3,6-dialkoxypyridazine 1-oxide[6] gave the corresponding 4-chloro substitutes by the reaction with acetyl chloride. Analogously, the author also found that the reaction of the compound (II) with acetyl chloride easily afforded 3-acetamido-5-chloro-6-methoxypyridazine 2-oxide in good yield.

At next, it is already made clear by Ochiai[7] that the reaction of an aromatic N-oxide with phosphoryl chloride gives a chloro substitute which possesses a chlorine atom at the position of *ortho* or *para* to N-oxide group in the original N-oxide, and it was reported by Igeta[8,9] that this reaction, also, hold true in the cases of pyridazine N-oxide derivatives. It is of interest to examine how 3-acetamido-6-methoxypyridazine 2-oxide responses to phosphoryl chloride.

The author found that a compound (IV) decomposing at 247° was obtained in poor yield by refluxing the compound (I) in chloroform with phosphoryl chloride, but in a better yield of ca. 50% by the reaction at 50°.

The compound (IV) was found to form 3-acetamido-5-chloro-6-methoxypyridazine 2-oxide (V) by the reaction with hydrogen peroxide in glacial acetic acid, since the compound (V) was prepared through the reaction between the known compound, 3-acetamido-5-nitro-6-methoxypyridazine 2-oxide (II) and acetyl chloride. Therefore, the structure of the compound (IV) should conform to 3-acetamido-5-chloro-6-methoxypyridazine. 3-Acetamido-5-chloro-6-methoxypyridazine 2-oxide (V) was found to be reversely converted to the compound (IV) by the deoxylation with phosphorus trichloride.

In addition of the above findings, yellow needles melting at 204° were obtained as the main-product instead of orange prisms of III, when the compound (II) was heated with 15~20% hydrochloric acid on a steam bath and then neutralized. This product was found to conform to 3-amino-5-chloro-6-methoxypyridazine 2-oxide (VI), which was prepared through the deacetylation of 3-acetamido-5-chloro-6-methoxypyridazine 2-oxide (V). Just as the chlorination of the compound (II) with hydrochloric acid, the compounds of 3-amino-5-chloro-6-alkoxypyridazine 2-oxide (VI) were prepared from the chlorination of 3-acetamido- and 3-amino-5-nitro-6-alkoxypyridazine 2-oxide (II and III) with hydrochloric acid. The compounds prepared are listed in Table III. Through the

3) E. Ochiai, M. Ishikawa, K. Arima : Yakugaku Zasshi, **63**, 79 (1943).
4) E. Ochiai, M. Ishikawa, Z. Sai : *Ibid.*, **63**, 186 (1943).
5) T. Itai : *Ibid.*, **65**, 70 (1945).
6) T. Itai, H. Igeta : *Ibid.*, **75**, 966 (1955).
7) E. Ochiai : J. Org. Chem., **18**, 534 (1953).
8) H. Igeta : This Bulletin, **7**, 938 (1959).
9) *Idem* : *Ibid.*, 8, 368 (1960).

analogous replacement of nitro group with halogen atom was also observed in 4-nitropyridine[10] and 4-nitropicoline[11] N-oxides, the author found that the compounds (II) and (III) could be converted to the corresponding chloro derivatives more easily than the above cited pyridine and picoline N-oxide derivatives by the action of hydrochloric acid.

Synthesis of 3-Amino-5,6-dimethoxypyridazine 2-Oxide

It is already shown that 4-nitropyridine N-oxide could be converted to the corresponding 4-alkoxy- or 4-phenoxy derivatives by the reaction with metal alkoxide or phenoxide.[12,13] The author examined the substitution of this type using 3-amino-5-nitro-6-alkoxypyridazine 2-oxide (III) as the starting material. It was however, found that the reaction between the compound (III) and metal alkoxide did not give any product except a black resinous product under any reaction condition. On the other hand, when the compound (IV) was refluxed with sodium methoxide in methanol, the objective 3-amino-5,6-dimethoxypyridazine 2-oxide (VII) was produced in good yield. It is of interest that the present results are contradictory with the findings on the alkoxylation of 3,6-dialkoxypyridazine 1-oxide derivatives reported by Itai[6] and Igeta,[9] who could

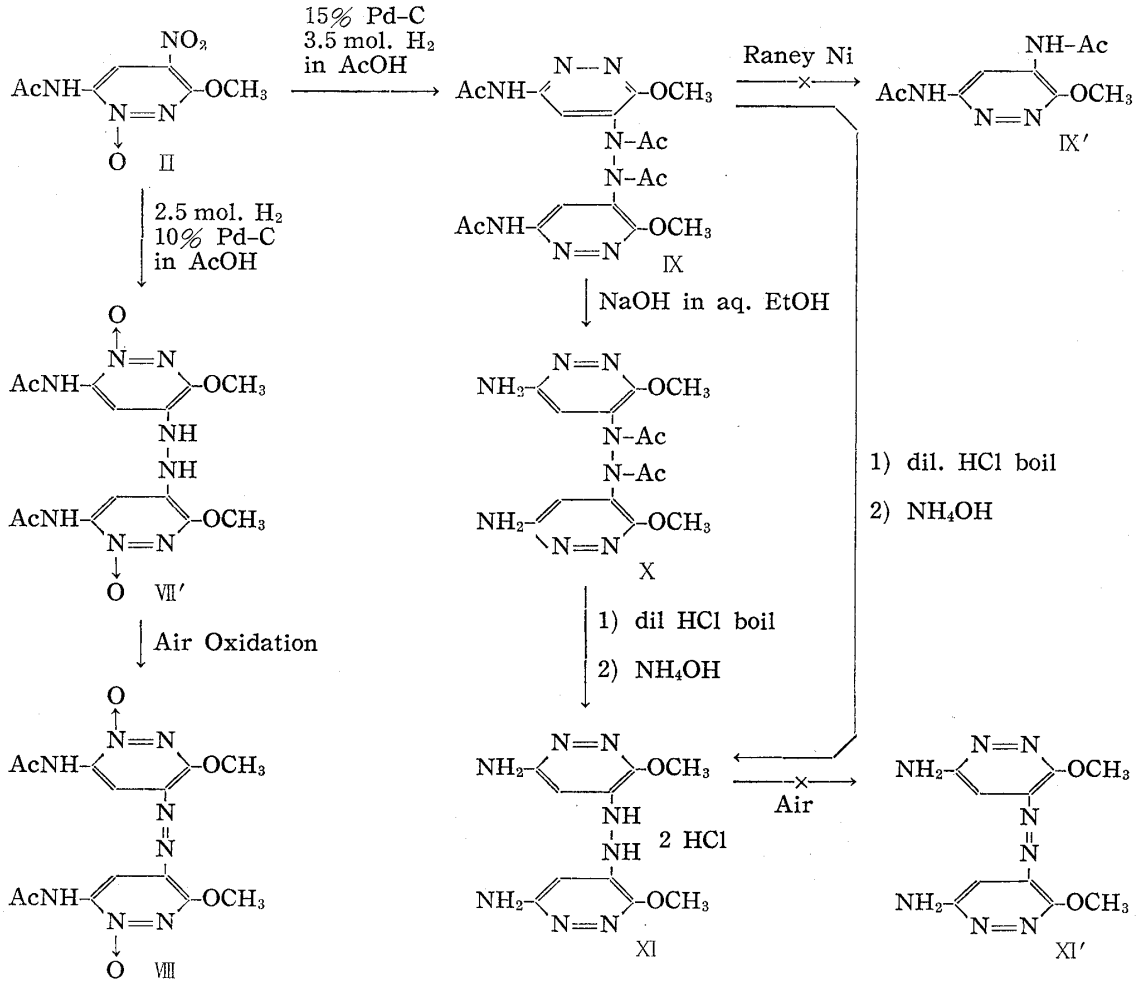


Chart 2.

10) E. Ochiai, T. Ito, S. Okuda : Yakugaku Zasshi, **71**, 591 (1951).
11) I. Suzuki : *Ibid.*, **68**, 126 (1948).
12) E. Ochiai, M. Katada : Yakugaku Zasshi, **63**, 265 (1943).
13) M. Katada : *Ibid.*, **67**, 25 (1947).

not obtained 3,4,6-trimethoxypyridazine 1-oxide from 4-chloro-3,6-dimethoxypyridazine 1-oxide but obtained from 4-nitro-3,6-dimethoxypyridazine 1-oxide by the reactions with sodium methoxide.

Hydrogenation of 3-Amino-5-nitro-6-methoxypyridazine 2-Oxide and Its Derivatives

Since 3-amino-5-nitro-6-methoxypyridazine 2-oxide and its acetyl derivatives have some aromaticity and reducible nitro and N-oxide groups, it is interesting to examine as to their behaviors in the hydrogenation under various reaction conditions.

At first, it was found that the compound (II) absorbed an amount of hydrogen corresponding to 2.5 moles by the hydrogenation with 20% palladium-carbon catalyst in acetic acid. In this reaction, a compound of intensively red needles (VIII) was obtained, after air had been sufficiently passed into the reaction mixture. Considered from the analogy to the findings on 4-nitropyridine N-oxide and from the data of the elementary analysis, the compound (VIII) was found to conform to 3,3′-bis(acetamido)-6,6′-dimethoxy-5,5′-azodipyridazine 2,2′-dioxide.

At next, the compound (II) in the acetic anhydride was found to absorb an amount of hydrogen corresponding to 3.5 moles by the hydrogenation with 20% palladium-carbon. In this reaction, a compound melting at 266° under the decomposition, was obtained from the reaction mixture. This compound seemed to be identical with 1,2-diacetyl-1,2-bis(3-acetamido-6-methoxy-5-pyridazinyl)hydrazine (IX) from the data of elementary analysis. This compound was surveyed as to its chemical property as follows. It was found to liberate two moles of acetyl group by the treatment of alcoholic solution of sodium hydroxide to afford the compound of X, and four moles of acetyl group by the hydrolysis with alcoholic hydrochloric acid to give the compound (XI). The hydrochloride of the compound (X) gave its free base by the addition of an excess of ammonia, while the hydrochloride of the compound (XI) did not. These facts also support the structures of the compounds (IX), (X), and (XI). In addition of the above findings, the compound (XI) was found not to afford the corresponding azo compound (XI′) by the air oxidation and the compound (IX) not to give diacetamido derivative (IX′) by the reaction with Raney nickel.

Moreover, the compound (III) was examined as to its behavior to the hydrogenation. The hydrogenation of the compound (III) in 5% hydrochloric acid with 20% palladium-

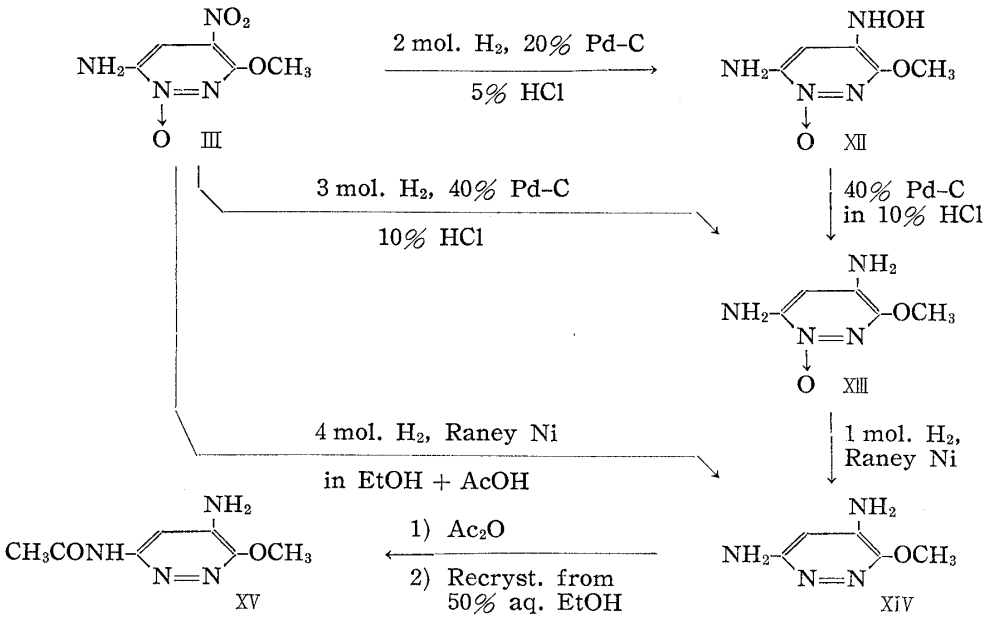


Chart 3.

carbon afforded 3-amino-5-hydroxyamino-6-methoxypyridazine 2-oxide (XII) under the absorption of hydrogen corresponding to two moles, while the treatment of the compound (III) in 10% hydrochloric acid with 40% palladium-carbon, 6-methoxy-3,5-diaminopyridazine 2-oxide (XIII) under the absorption of hydrogen corresponding to three moles. The compound (XIII), however, found not to be reduced with palladium-carbon under any reaction condition. These findings showed that the hydrogenation of the compound (III) with palladium-carbon was apt to induce the reduction of the nitro group, but not to the redution of N-oxide group.

On the contrary, the hydrogenation of the compound (III) with Raney nickel gave 6-methoxy-3,5-diaminopyridazine (XIV) under the absorption of hydrogen corresponding to four moles. Analogously, the compound (XIII) was reduced to the compound (XIV) by mean of Raney nickel with hydrogen.

The hydrogenation processes from the compounds (II) and (III) are shown in Charts 2 and 3.

As described above, it may be inferred that the hydrogenation of compounds of nitropyridazine N-oxide should give rise to the reduction of nitro group with palladium-carbon and the reduction of nitro and N-oxide groups with Raney nickel. Moreover, it may be added that the hydrogenation of nitropyridazine N-oxide should afford monomolecular reduction-products in strong acidic medium, while bimolecular reduction-products in weakly acidic medium. These tendencies coincide well with those already observed in hydrogenation of compounds of heteroaromatic N-oxides having nitro group.[6,14~20]

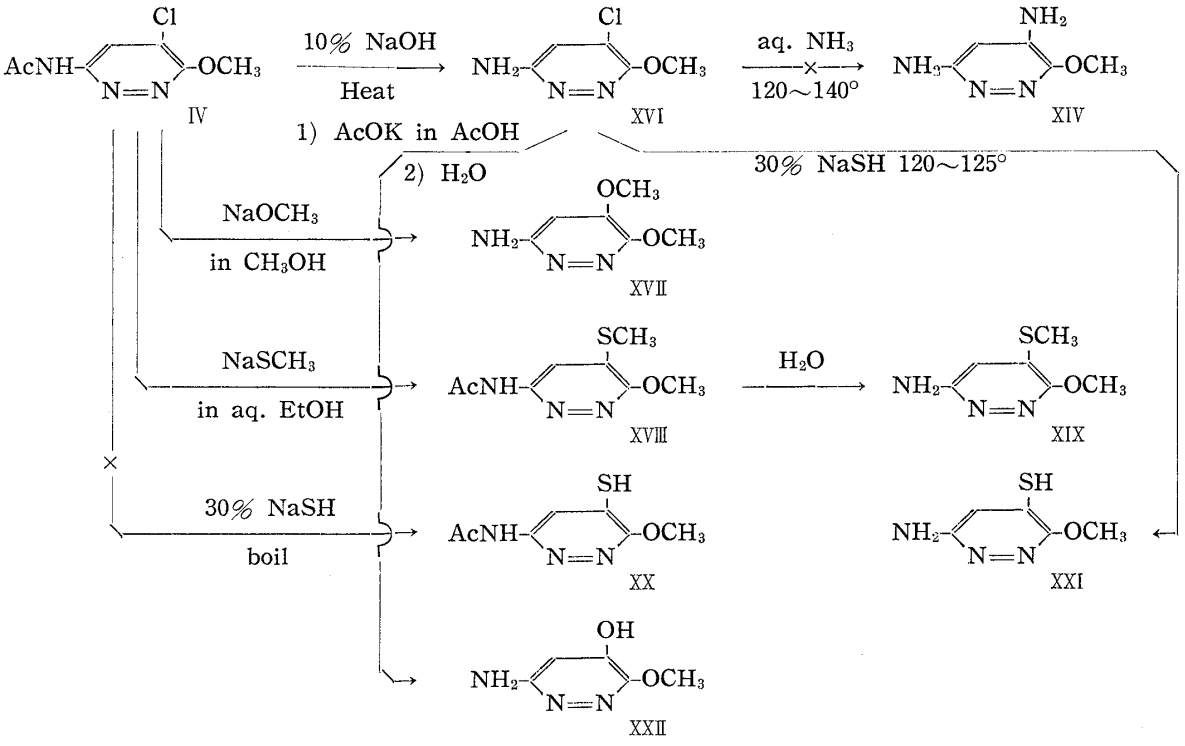
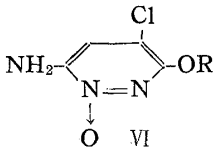


Chart 4.

14) E. Ochiai, M. Katada : Yakugaku Zasshi, **63**, 186 (1943).
15) E. Ochiai, T. Naito : *Ibid.*, **64**, 206 (1944).
16) E. Ochiai, E. Hayashi : *Ibid.*, **67**, 151 (1947).
17) E. Ochiai, H. Nomura : This Bulletin, **5**, 310 (1957).
18) H. Igeta : *Ibid.*, 8, 550 (1960).
19) T. Nakagome : Yakugaku Zasshi, **81**, 554 (1961).
20) E. Hayashi, H. Yamanaka : This Bulletin, **7**, 141, 146, 149 (1959).

Nucleophilic Substitution of 3-Acetamido-5-chloro-6-methoxypyridazine (IV)

It is of interest to conduct the nucleophilic substitution of 3-acetamido-5-chloro-6-methoxypyridazine (IV), since it is known that the chlorine atom at 5-position is more active than at 3- or 6-position in pyridazine ring.[21,22] As shown in Chart 4, the author attempted reactions of 3-acetamido-5-chloro-6-methoxypyridazine (IV) with various kinds of nucleophilic reagents. The chlorine atoms of the compounds (IV) and (XVI) were resistant to the warming with 10% aqueous solution of sodium hydroxide and 30% sodium bisulfide solution, but susceptible to the solvolysis with anhydrous potassium acetate in acetic acid, to give 3-amino-5-hydroxy-6-methoxypyridazine (XXII). The compound (IV) was converted to 3-amino-5,6-dimethoxypyridazine (XVII) by the simultaneous deacetylation and methoxylation with sodium methoxide in anhydrous methanol. On the other hand, 3-acetamido-5-methylthio-6-methoxypyridazine (XVIII) was obtained as the intermediate product by the reaction of the compound (IV) with sodium methylmercaptide in 50% aqueous ethanol, and the former (XVIII) was deacetylated with aqueous solution of sodium hydroxide to 3-amino-5-methylthio-6-methoxypyridazine (XIX). To obtain 5-pyridazinethiol derivative, the compound (XVI) was heated in an autoclave with 30% aqueous solution of sodium bisulfide at 120~125° for 6 hours, and the objective 3-amino-6-methoxy-5-pyridazinethiol (XXI) was obtained. However, 3,5-diamino-6-methoxypyzidazine (XIV) could not be produced by the reaction of 3-amino-5-chloro-6-methoxypyridazine (XVI) with ammonia even at 130~140° in an autoclave, but the starting material (XVI) was almost quantitatively recovered.

Table III-a. General formula 

No.	R	Form	m.p. (°C)	Appearance	Recryst. solvent
VI-a	CH_3	Free base	204 (decomp.)	yellow needles	75% EtOH
		HCl salt	196 (〃)	white powder	EtOH
VI-b	C_2H_5	〃	187 (〃)	white powder	〃
VI-c	C_3H_7	〃	169 (〃)	faint yellow powder	〃
VI-d	C_4H_9	〃	161 (〃)	white powder	〃

Table III-b. Analytical Data

No.	Form	Formula	Calcd. (%)			Found (%)		
			C	H	N	C	H	N
VI-a	Free base	$C_5H_6O_2N_3Cl$	34.18	3.42	23.93	34.28	3.71	23.78
	HCl salt	$C_5H_7O_2N_3Cl_2$	28.33	3.33	19.81	28.29	3.18	19.93
VI-b	〃	$C_6H_9O_2N_3Cl_2$	—	—	18.58	—	—	18.84
VI-c	〃	$C_7H_{11}O_2N_3Cl_2$	—	—	17.50	—	—	17.32
VI-d	〃	$C_8H_{13}O_2N_3Cl_2$	—	—	16.53	—	—	16.50

As described above, it was found that new derivatives of pyridazine were synthesized through the substitution of the chlorine atom at 5-position of the compounds (IV) and (XVI) with nucleophilic reagents. These 3-amino-5,6-disubstituted pyridazine here obtained might be interesting as the components of sulfanilamides and the work on this problem will be published in the future.

Screening Test with the Compounds Synthesized

The compounds above synthesized were tested as to their antimicrobial activity against various bacteria and fungi. The microbes employed were as follows : *E. Coli*

21) T. Kuraishi : This Bulletin, **4**, 137 (1956).
22) K. Eichenberger, R. Rometsch, J. Druery : Helv. Chem. Acta., **39**, 1755 (1956).

K12, *E. Coli* C14, *E. Coli* K12 CTS, *E. Coli* UEDA, *S. enteritides* No. 11, *Staph. aureus* TERASHIMA, *Candida albicans* 40, *Candida tropicalis, Candida stellatidis, Tricophyton asteridws, Tricophyton interigitale* 841, *Tricophyton rubrum* 825 and *Xyantomonas oryzae.* The experimental procedures were the same to those described in the previous reports.[23,24]

Among these compounds, it was found that 3-acetamido- and 3-amino-5-nitro-6-alkoxypyridazine 2-oxide exerted an excellent effect *in vitro* against the bacteria and fungi. Especially 3-amino-5-nitro-6-methoxypyridazine 2-oxide showed the strongest antibacterial effect *in vitro,* inhibiting in concentration of 10^{-5} mole/ml. the growth of intestinal pathogenic bacteria and *Staph. aureus.* However, any of the reduction of these 5-nitropyridazine derivatives and the derivatives of 5-halopyridazine did not show any antimicrobial activity.

As described above, the author synthesized many pyridazine derivatives of new types to find new antimicrobial agents. From the screening tests with these compounds, the author found several new antimicrobial agents. These finding are useful not only for the chemistry of pyridazine and its related, but also for the search of antimicrobial agents.

Experimental

General Method for the Synthesis of 3-Acetamido-5-nitro-6-alkoxypyridazine 2-Oxide (II)——To an ice cold solution consisting of 3.0 ml. of HNO_3 (d=1.50) and 1.0 ml. of conc. H_2SO_4, 10 g. of 3-acetamido-6-alkoxypyridazine 2-oxide was added portionwise under agitation, while the reaction temperature should be kept below 10°. After the reaction mixture was allowed to stand for 30 min. at room temterature, the resulting reddish mixture was poured into 30 g. of crushed ice. The deposited yellow precipitate was collected and recrystallized from EtOH to obtain long yellow needles. Yield: 72～83%.

The compounds synthesized are listed in Table I.

General Method for the Synthesis of 3-Amino-5-nitro-6-alkoxypyridazine 2-Oxide (III)——A solution of 1.0 g. 3-acetamido-5-nitro-6-alkoxypyridazine 2-oxide in 10 ml. of 50% EtOH was refluxed with 3.0 ml. of conc. HCl for 2 hr. After EtOH was removed *in vacuo,* the mixture was neutralized with 10% K_2CO_3 to pH 5～6, and extracted with $CHCl_3$ repeatedly. The $CHCl_3$ extract was dried with anhyd. Na_2SO_4 and concentrated to deposit yellow or orange crystals. Recrystallization from EtOH or dil. EtOH gave orange or yellow needles. Yield: 55～65%.

The compounds synthesized are listed in Table II.

3-Acetamido-5-chloro-6-methoxypyridazine (IV)——i) From 3-acetamido-6-methoxypyridazine 2-oxide (I): To a mixture of 15 ml. of chloroform and 10 ml. of $POCl_3$, 5.0 g. of I was added and warmed on a water bath at 50° for 6 hr. under occasional shaking. The resulting reddish black solution was concentrated *in vacuo* and the residue was poured into crushed ice. After the mixture was made alkaline with NH_4OH, the deposited muddy precipitate was collected by filtration. Recrystallization from AcOH, followed by an active charcoal treatment, gave white prisms of m.p. 245～247°(decomp.). *Anal.* Calcd. for $C_7H_8O_2N_3Cl$: C, 41.68; H, 3.97; N, 20.84. Found: C, 41.51; H, 4.03; N, 20.95. Yield: 2.3 g.

When the basic filtrate obtained above was extracted with chloroform, there was recovered ca. 2.0 g. of rather contaminated starting material, which was hydrolyzed with dil. NaOH to give 3-amino-6-methoxypyridazine, m.p. 132～134° alone and on admixture with an authentic sample.

ii) From 3-acetamido-5-chloro-6-methoxypyridazine 2-oxide (V): A mixture of 4.0 ml. of PCl_3, 4.0 ml. of chloroform and 0.3 g. of V was refluxed on a steam bath for 30 min. Then the crystals were separated from the reddish black solution. After evaporation of the volatiles, the residue was added to ca. 10 ml. of H_2O. The precipitate was collected and recrystallized from AcOH to give white fine prisms of m.p. 242～245°(decomp.). No depression was observed on admixture with the authentic sample obtained above. Yield: 0.12 g.

3-Acetamido-5-chloro-6-methoxypyridazine 2-Oxide (V)——i) From 3-acetamido-5-nitro-6-methoxypyridazine 2-oxide (II-a): A suspension of 3.0 g. of II-a in 20 ml. of AcOCl was heated on a steam bath for 1.5 hr. under reflux. The volatiles were removed *in vacuo* and the residue was poured into ice H_2O. The resulting white precipitate was collected and recrystallized from EtOH to afford white

23) T. Makino: This Bulletin, **10**, 576 (1962).
24) Part I. T. Horie, K. Kinjo, T. Ueda: *Ibid.*, **10**, 580 (1962).

long needles of m.p. 233~234° (decomp.). *Anal.* Calcd. for $C_7H_8O_3N_3Cl$: C, 38.62; H, 3.68; N, 19.29. Found : C, 38.45; H, 3.88; N, 19.18. Yield : 2.1 g.

ii) From 3-acetamido-5-chloro-6-methoxypyridazine (IV) : A suspension of 0.5 g. of IV in a mixture of 15 ml. of AcOH and 0.5 ml. of 30% H_2O_2 was heated on a steam bath for 3 hr. After concentration *in vacuo*, the solid residue was recrystallized from EtOH to afford long white needles of m.p. 233~234° (decomp.). No depression was observed when mixed with the sample synthesized from II-a as described in the procedure i). Yield : ca. 0.4 g.

General Method of the Synthesis of 3-Amino-5-chloro-6-alkoxypyridazine 2-Oxide (VI)——A mixture of 1.0 g. of 3-acetamido-5-nitro-6-methoxypyridazine 2-oxide (II) and 1.0 g. of 15% HCl was heated on a steam bath for 8 hr. and concentration *in vacuo* to dryness. The residue was recrystallized from EtOH to afford white or faint yellow needles. Yield : ca. 60~71%.

The free base : The hydrochlorides obtained above were dissolved into dil. NH_4OH and concentrated *in vacuo* to leave a solid residue, which was extracted with abs. EtOH. After evaporation of EtOH, the deposited crude crystals were recrystallized from aq. EtOH.

The compounds obtained above are shown in Table III.

The same compound (IV) was also produced by treating the compound (III) with HCl using the same method as described above.

3-Amino-5,6-dimethoxypyridazine 2-Oxide (VII)——To a solution consisting of 0.1 g. of sodium metal and 10 ml. of anhyd. MeOH, 0.45 g. of 3-amino-5-chloro-6-methoxypyridazine 2-oxide (VI-a) was added and refluxed on a steam bath for 3 hr. After the deposited NaCl was filtered off, the filtrate was acidified with AcOH and then basified with NH_4OH again. The mixture was concentrated to dryness and the residue was extracted with $CHCl_3$. After the solvent was evaporated to leave a solid mass, which was recrystallized from a mixture of EtOH and Me_2CO. Yield : 0.32 g. White needles of m.p. 194~195° (decomp.). *Anal.* Calcd. for $C_6H_9O_3N_3$: C, 58.14; H, 5.23; N, 24.41. Found : C, 58.40; H, 5.46; N, 24.39.

Hydrogenation of 3-Acetamido-5-nitro-6-methoxypyridazine 2-Oxide (II-a)

3,3'-Bis(acetamido)-6,6'-dimethoxy-5,5'-azodipyridazine 2,2'-Dioxide (VIII)——A suspension of 5.0 g. of II-a in 70 ml. of anhyd. AcOH was hydrogenated with 1.0 g. of 10% Pd-C in atmospheric pressure. About 2.5 mol. (1200 ml.) of H_2 was consumed. After the catalyst was removed by filtration, air was passed into the filtrate on a steam bath, then intensively red needles were separated from the reaction mixture. They were collected by filtration and washed with H_2O. Yield : 3.5 g., m.p. over 280°. *Anal.* Calcd. for $C_{14}H_{16}O_6N_8$, C, 42.85; H, 4.08; N, 28.57. Found : C, 42.59; H, 3.81; N, 28.75.

1,2-Diacetyl-1,2-bis(3-acetamido-6-methoxy-5-pyridazinyl)hydrazine (IX)——A mixture of 10.0 g. of II-a, 2.0 g. of 15% Pd-C and 100 ml. of Ac_2O was placed in a shaking flask and hydrogenated at atmospheric pressure on warming. Reduction was almost completed after 3.5 mol. of H_2 had been absorbed (8 hr.). After the catalyst was filtered off, the solvent was evaporated *in vacuo*. The residue was added with MeOH to obtain white precipitate, which was recrystallized from AcOH-EtOH (1:2) to give white fine powder of m.p. 266° (decomp.). *Anal.* Calcd. for $C_{18}H_{22}O_6N_8$ (IX) : C, 48.43; H, 4.93; N, 25.33. Found : C, 48.32; H, 4.93; N, 25.14.

1,2-Diacetyl-1,2-bis(3-amino-6-methoxy-5-pyridazinyl)hydrazine (X)——To a mixture of 2.0 ml. of 10% NaOH and 15 ml. of EtOH, 1.0 g. of 1,2-diacetyl-1,2-bis(3-acetamido-6-methoxy-5-pyridazinyl)-hydrazine (IX) was added. When the suspension was heated on a steam bath, the crystals were dissolved in a few minutes and soon white fine needles were separated again, which were collected and recrystallized from aq. EtOH. m.p. 261~263° (decomp.). Yield : ca. 0.6 g. *Anal.* Calcd. for $C_{14}H_{18}O_4N_8$: N, 30.94. Found : N, 31.27.

When X was treated with acetic anhydride, the compound (IX), m.p. 266° (decomp.), was regenerated in a good yield.

1,2-Bis(3-amino-6-methoxy-5-pyridazinyl)hydrazine Dihydrochloride (XI)——A mixture of 5 ml. of conc. HCl, 5 ml. of H_2O, 5 ml. of EtOH and 5.0 g. of IX was refluxed for 2 hr. on a steam bath. After the solution was made alkaline with 28% NH_4OH, the volatiles were evaporated to dryness *in vacuo*. The residue was extracted with abs. EtOH to remove insoluble salts and then the extract was concentrated to obtain crude crystals. Recrystallization from aq. EtOH gave white powder of m.p. 190~191°. Positive to Beilstain test. Yield : 3.1 g. *Anal.* Calcd. for $C_{10}H_{14}O_2N_8 \cdot 2HCl$ (XI) : C, 33.99; H, 4.50; N, 31.72. Found : C, 33.49; H, 4.24; N, 31.82.

The compound (XI) was also obtained by the hydrolysis of the compound (X) using the same method as described above.

Hydrogenation of 3-Amino-5-nitro-6-methoxypyridazine 2-Oxide (III)

3-Amino-5-hydroxyamino-6-methoxypyridazine 2-Oxide (XII)——A mixture of 0.55 g. of III and 20 ml. of 5% HCl was hydrogenated with 0.25 g. of 20% Pd-C in atmospheric pressure. After ca. 2.0 mol. of H_2 had been absorbed, the reduction was completed. The catalyst was removed and the filtrate was concentrated to obtain white prisms. Recrystallization from EtOH gave colorless prisms

of m.p. 222～223°(decomp.). Positive to $FeCl_3$ test. Yield; 0.2 g. *Anal.* Calcd. for $C_5O_8O_3N_4 \cdot HCl$ (XII): N, 26.85. Found : N, 26.47.

3,5-Diamino-6-methoxypyridazine 2-Oxide (XIII)——A mixture of 1.86 g. of III and 40 ml. of 10% HCl was hydrogenated with 1.0 g. 20% Pd-C under atomospheric pressure. The reduction was completed after 3 mol. of H_2 had been absorbed (ca. 3 hr.). The catalyst was filtered off and the filtrate was concentrated to afford white crystals, which were collected. Recrystallization from EtOH gave colorless fine prisms of m.p. 233～234°(decomp.). *Anal.* Calcd. for $C_5H_8O_2N_4 \cdot HCl$: C, 31.16; H, 4.67; N, 29.09; Found: C, 31.36; H, 4.43; N, 29.14. Yield : 1.1 g. Positive to $FeCl_3$ test.

3,5-Diamino-6-methoxypyridazine (XIV)——A mixture of 1.25 g. of III, 50 ml. of 90%. EtOH and 2.5 ml. of AcOH was hydrogenated with Raney Ni prepared from 2.5 g. of 50% Ni-Al alloy. The reduction was completed after 4 mol. of H_2 had been absorbed (3.5 hr.). The reaction mixture was filtered and the filtrate was concentrated to obtain white precipitate, which was recrystallized from 75% EtOH. The colorless prisms of m.p. 198～199° were obtained. Yield : 0.8 g. Negative to $FeCl_3$ test. *Anal.* Calcd. for $C_5H_8ON_4 \cdot CH_3CO_2H$: C, 30.00; H, 4.00; N, 28.00. Found : C, 30.23; H, 3.88; N, 27.87.

The mono-acetate (XV)*[3] was obtained by treating the compound (XIV) with Ac_2O, followed by the repeated recrystallization from 50% EtOH. White needles of m.p. 238～240°. *Anal.* Calcd. for $C_7H_{10}O_2N_4$: N, 30.76. Found : N, 30.48.

3-Amino-5-chloro-6-methoxypyridazine (XVI)——A mixture of 2.0 g. of IV and 1.2 g. (corresponding to 3 mol.) of NaOH and 12 ml. of H_2O was heated on a steam bath for 3 hr. In the course of the reaction, the material (IV) was dissolved into solution and in the meanwhile white needles were separated again. After cooling, the deposited white silky crystals were collected to obtain white amorphous crystals of m.p. 151～152°. Yield : 1.1 g. *Anal.* Calcd. for $C_5H_6ON_3Cl$: C, 37.61; H, 3.76; N, 26.33. Found : C, 37.87; H, 3.61; N, 26.11.

3-Amino-5,6-dimethoxypyridazine (XVII)——To a solution consisting of 2.3 g. of Na and 100 ml. of anhyd. MeOH, 10 g. of IV was added and the mixture was heated on a steam bath for 5 hr. under reflux. After cooling, the deposited NaCl was removed by filtration and the filtrate was concentrated to its half volume. The solution was refluxed with 10 ml. of 20% NaOH for 1 hr. and then concentrated *in vacuo* to obtain crystalline residue. Recrystallization from 75% EtOH gave colorless prisms of m.p. 211～212°. Yield : 6.0 g. *Anal.* Calcd. for $C_6H_9O_2N_3$: C, 46.44; H, 5.85; N, 27.08. Found : C, 46.05; H, 6.10; N, 27.14.

Acetate (XVII') : White fine needles of m.p. 240～242°(decomp.). Recrystallized from EtOH. *Anal.* Calcd. for $C_8H_{11}O_3N_3$: C, 48.72; H, 5.62; N, 21.31. Found : C, 48.29; H, 5.74; N, 21.51.

3-Acetamido-5-methylthio-6-methoxypyridazine (XVIII)——A mixture of 2.0 g. of IV, 3.0 ml. of 30% $NaSCH_3$ and 20 ml. of MeOH was heated on a steam bath for 3 hr. The crystal from of the material was changed from prisms to needles in the course of the reaction. After cooling, the deposited crystals were collected and recrystallized from EtOH to obtain white needles of m.p. 269～271° (decomp.). Yield: crude 1.9 g. *Anal.* Calcd. for $C_8H_{11}O_2N_3S$: C, 45.07; H, 5.20; N, 19.71. Found : C, 45.38; H, 5.27; N, 19.59.

3-Amino-5-methylthio-6-methoxypyridazine (XIX)——To a mixture of 2 ml. of 20% NaOH and 15 ml. of EtOH, 1.5 g. of XVIII was added the mixture was boiled for 1 hr. under reflux. The reaction mixture was acidified with conc. HCl and basified again with 28% NH_4OH. Then the mixture was concentrated to leave a solid residue, which was extracted with Me_2O. The Me_2O extract was dried over anhyd. K_2CO_3 and concentrated to obtain white needles of m.p. 125～135°. Recrystallization from Me_2O gave white needles of m.p. 155～156°. Yield : 0.8 g. *Anal.* Calcd. for $C_6H_9ON_3S$: C, 42.10; H, 5.30; N, 26.41. Found : C, 42.54; H, 5.01; N, 26.70.

3-Amino-5-hydroxy-6-methoxypyridazine (XXII)——A mixture of 0.16 g. of IV, 0.11 g. of freshly fused AcOK and 10 ml. of anhyd. AcOH was heated on a oil bath for 6 hr. under reflux. After evaporation of the solvent, the residue was heated with 2 ml. of 10% HCl on a steam bath for 1 hr. The reaction mixture was treated with active charcoal and concentrated to leave a solid residue, which was added to a small quantity of dil. NH_4OH and allowed to stand overnight. The crystals deposited were collected and recrystallized from 75% EtOH. Yield : crude 0.1 g. White needles of m.p. 129～130°. *Anal.* Calcd. for $C_5H_7O_2N_3$: C, 42.55; H, 5.00; N, 29.78. Found : C, 42.19; H, 4.78; N, 29.88.

3-Amino-6-methoxy-5-pyridazinethiol (XXI)——A suspension of 2.0 g. of XVI in 10 ml. of 30% NaSH solution was heated in an autoclave at 120～125° for 6 hr. After the reaction mixture was allowed to stand for few days, the deposited brown prisms were collected and recrystallized from H_2O. Yield: 0.8 g. Slight yellow prisms of m.p. 207～209° (decomp.). *Anal.* Calcd. for $C_5H_7ON_3S$: N, 26.75. Found : N, 26.89.

*[3] This compound is supposed to be 3-acetamido-5-amino-6-methoxypyridazine, the analogious example revealed in 6.

The author wishes to express his deepest thanks to Prof. Takeo Ueda for the kind leading in these studies.

Summary

In order to find antimicrobial agents, the substitution of 3-acetamido-6-alkoxypyridazine 2-oxide was investigated. As the results obtained, it was made clear that a number of 3-acetamido-6-alkoxypyridazine, 3-amino-6-alkoxypyridazine and their 2-oxides having such a substituent group as nitro, amino, hydroxyamino, azo, hydrazo, chloro, methoxy, methylthio, hydroxy, and mercapto at 5-position of the pyridazine rings, were synthesized. This finding is of use to develope the chemistry of pyridazine.

Among these new compounds, 3-acetamido- and 3-amino-5-nitro-6-alkoxypyridazine 2-oxides were found to exert excellent *in vitro* effects on pathogenic bacteria.

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190. Kikuo Yasuda : Oxidation of 3-Enol Derivatives of 4-En-3-oxo-steroids by *tert*-Butyl Chromate.

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Several works[1] on *t*-butyl chromate oxidation of steroids, especially 3β-acetoxy- and 3-ethylenedioxy-Δ^5-steroids to the corresponding 7-ones have been carried out, since Oppenauer and Oberrauch[2] reported on the oxidation of cholesteryl acetate. There has been, however, no report on *t*-butyl chromate oxidation of 3-enol derivatives of 4-en-3-oxo-steroids, although Fieser obtained 6β-hydroxycholest-4-en-3-one by sodium dichromate oxidation of cholest-4-en-3-one enol acetate.[3] Some observations on *t*-butyl chromate oxidation of such derivatives are described in the present paper.*2

When testosterone enol diacetate (Ia) was oxidized with *t*-butyl chromate in the presence of acetic anhydride, there was obtained the corresponding 7-one (IIa). The structure of IIa was confirmed because the same substance was given by acetylation of 3,17β-dihydroxyandrosta-3,5-dien-7-one 17-acetate (Va), which was prepared from 3-ethylenedioxy-17β-acetoxyandrost-5-en-7-one (VIa) by the method of Marshall and his coworkers.[4] Their assignment of structure (Va) was based upon analogy to 3-hydroxycholesta-3,5-dien-7-one[5] for which, however, the possibility of another enolic form, 7-hydroxycholesta-4,6-dien-3-one, was suggested. Now this possibility can be evidently excluded from the above-mentioned observation. IIa (UV : λ_{max} 282 mμ) was treated

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*2 Presented in part at the 14th Annual Meeting of the Pharmaceutical Society of Japan (1961).

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