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15. Takanobu Itai and Shozo Kamiya : Potential Anti-cancer Agents. II.*2 4-Azidoquinoline and 4-Azidopyridine Derivatives. (1).

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Inorganic azides have been known as powerful inhibiting agents on enzymes such as oxidase, peroxidase, catalase, and aspartase. Also it is said that organic azides have more potent bacteriostatic action than inorganic azides, though the former does not separate azide ion in biological conditions.[1] Actually, diaryl sulfone[2] or sulfonamide compounds[3] with azide group have been described as antimicrobial agents in literature and 6-azidopurine was recently reported to be carcinostatic.[4] As the chemistry of azido radical on heterocyclic compounds are not studied so widely,[5] it seems very interesting to study them and to examine their anti-cancer action. Therefore, attempt was made to synthesize 4-azidoquinoline, 4-azidopyridine, and their derivatives. Some of their photolysis and pyrolysis reactions were also examined.

Quinoline Derivatives

When 4-chloroquinoline 1-oxide (II) was allowed to stand at room temperature with hydrazine hydrate, 4-hydrazinoquinoline 1-oxide (III) was produced as yellow-green needles, explosive on heating, and reducing ammoniacal silver nitrate solution. It formed a hydrochloride, picrate, and benzaldehyde hydrazone. Although analytical data of the free base did not agree with the calculated values, its salts and benzaldehyde hydrazone were identified by analysis.

Diazotization of (III) gave 4-azidoquinoline 1-oxide (IV) in a good yield. (IV) was also obtained by reaction of (II) with sodium azide at about 120°, but the latter reaction was not so convenient owing to its side reaction. Excessive heating often yielded a considerable amount of resinous substance, from which 4-aminoquinoline 1-oxide separated out. If (II) was heated with hydrazine, 4-hydrazinoquinoline (VI) was obtained, attributable to the reducing action of hydrazine hydrate. (VI) was also prepared by a reaction of 4-chloroquinoline and hydrazine hydrate in a good yield. (VII) was prepared by diazotization of (VI), and (VII) yielded (IV) in 33% yield by N-oxidation with hydrogen peroxide in acetic acid. It was found that the most convenient preparation method for (IV) was an approach through (II)→(III)→(IV). (IV) occurs as yellowish needles when recrystallized in a dim place, but usually as pale brown needles. They turn reddish brown on exposure to light. It was

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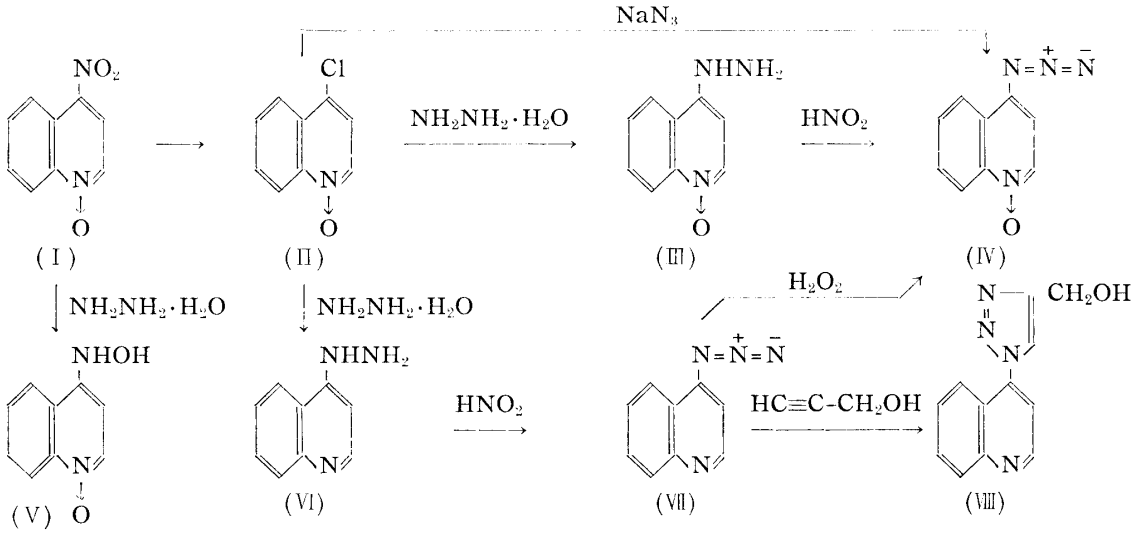
*2 Part I. T. Itai, S. Suzuki : This Bulletin, 8, 999 (1960).
1) E. Werle : Biochem. Z., **322**, 507 (1952).
2) C. K. Banks, O. M. Gruhzit : J. Am. Chem. Soc., **70**, 1268 (1948).
3) R. O. Jr. Roblin : U. S. Pat. 2,254,191 (C. A., **35**, 8214 (1941)).
4) Ciba Foundation Symposium, Chem. and Biol. of Purines, 8 (1957).
5) J. H. Boyer, F. C. Canter : Chem. Revs., **54**, 1 (1954).

identified by its analytical data and strong absorption at 2110 cm^{-1}, characteristic to azide group.

In order to prove azido group, (IV) was submitted to addition reaction to propargyl alcohol on heating, but it failed, maybe on account of fast decomposition by itself. Next, the same reaction was carried out with (VII), by which only one isomer of 1-(4-quinolyl)-1*H*-[1,2,3]triazole-4-methanol (VIII) was formed. Its hydroxymethyl in triazole ring might be presumed to be located at 4-position, analogous to the reaction of phenylazide and propargyl alcohol, but it has not yet been determined.

On adding hydrazine hydrate to 4-nitroquinoline 1-oxide (I), heat was vigorously evolved and 4-hydroxyaminoquinoline 1-oxide (V) was obtained from the reaction mixture, which was found identical by mixed melting point determination with a specimen synthesized by Ochiai and his colleagues' procedure.[6]



Pyridine Derivatives

Similar to quinoline derivatives, the corresponding azidopyridines and their derivatives were obtained. Namely, 4-hydrazinopyridine 1-oxide[7] (X) was led to 4-azidopyridine 1-oxide (XI) by diazotization, but repeated attempts to obtain (XI) from 4-chloropyridine 1-oxide (IX) and sodium azide in various conditions met with failure, only giving (XI) in a low yield (10%).

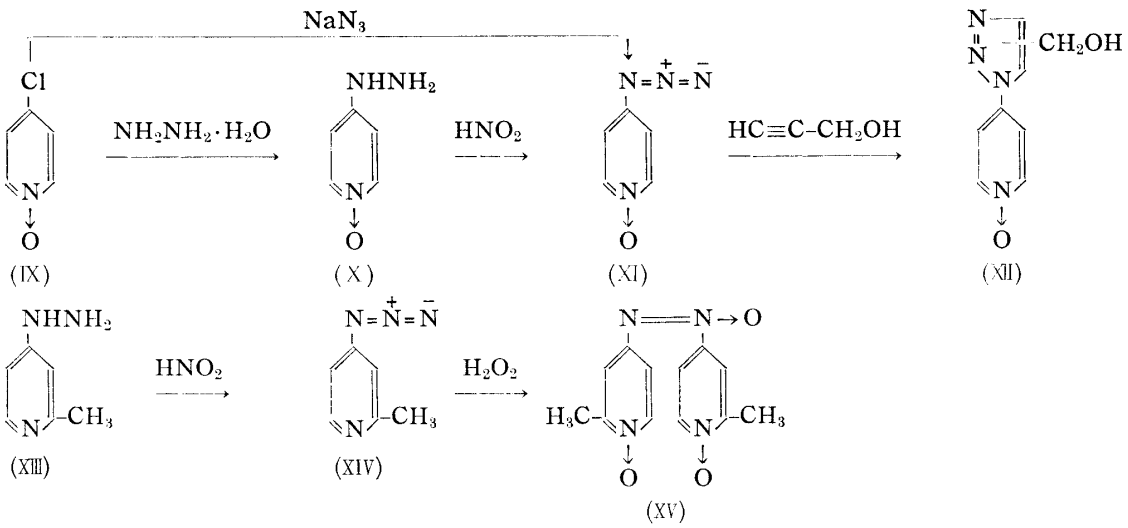
4-Azido-2-picoline was similarly prepared from 4-hydrazino derivative (XIII). In order to lead it to its 1-oxide, (XIV) was warmed with hydrogen peroxide-acetic acid but it gave, contrary to expectations, 4,4'-azoxy-di-2-picoline 1,1'-dioxide[8] (XV). No experiment was made with milder conditions.

On addition reaction of propargyl alcohol with (XI), two compounds, giving similar analytical data, were obtained in a 10:1 ratio. They were considered as isomers of 4-(hydroxymethyl-1*H*-[1,2,3]triazol-1-yl)pyridine 1-oxide, due to the difference in the positions of hydroxymethyl, which have not yet been determined.

Photolysis

No reaction occurred on leaving (XI) in acetone in a room, but on exposure to sunlight for 10 days, many little bubbles began to rise and reddish orange needles precipitated, which on examining by infrared spectroscopy, seemed to be 4,4'-azoxydipyridine 1,1'-

6) E. Ochiai, A. Ohta, H. Nomura : This Bulletin, **5**, 312 (1957).
7) A. R. Katritzky : J. Chem. Soc., **1956**, 2404.
8) E. Ochiai, I. Suzuki : Yakugaku Zasshi, **67**, 30 (1946).



dioxide. 4,4′-Azoxydipyridine 1,1′-dioxide was synthesized according to the method of Ochiai and Katada,[9] and both infrared spectra and melting points agreed completely. This reaction may be produced by way of evolution of nitrogen by light from the azido group, two of the free radicals formed would combine, and the product is oxidized to azoxy compound by oxygen in air. In nitrogen atmosphere, almost pure 4,4′-azodipyridine 1,1′-dioxide was isolated from the solution in 28% yield after three hours' decomposition.

When (IV) was similarly treated, it was led more rapidly to 4,4′-azodiquinoline 1,1′-dioxide. It may be concluded qualitatively that the stability of azido compounds is in the descending order of (XI), (VII), and (IV).

It has been reported that azido group shows a strong absorption band at 2130 cm^{-1} due to its asymmetric stretching vibration and a second weaker one at 1343~1177 cm^{-1}.[10] Absorption bands of the compounds synthesized in the present series appeared at 2110 cm^{-1} in (IV), 2120 cm^{-1} in (VII), 2110 cm^{-1} in (XIV), and 2100 cm^{-1} in (XI), and the second weaker band was not distinctly recognized. These absorptions were very strong and could be used for their identification.

On bacteriostatic screening tests *in vitro*, (IV), (XI), and (XV) showed a strong action against *Staphylococcus aureus* (FDA-209P), *Escherichia coli* O-1, and *Candida albicans*.*3

Tests on cancerostatic and cancerogenetic actions are now in progress and the results will be reported elsewhere.

Experimental

4-Hydrazinoquinoline 1-Oxide (III)—To a solution of 1 g. of 4-chloroquinoline 1-oxide suspended in 7 cc. of EtOH, 5 cc. of 80% $NH_2NH_2 \cdot H_2O$ was added and the mixture was allowed to stand at room temperature with frequent stirring. The solution solidfied after 20~40 hr. and 0.63 g. (67%) of 4-hydrazinoquinoline 1-oxide was obtained as yellowish green needles (from EtOH), m.p. 168° (decomp.). Explosive! Hydrochloride : Yellowish brown needles (from EtOH), m.p. 235.5° (decomp.). *Anal.* Calcd. for $C_9H_9ON_3 \cdot HCl$: N, 19.85. Found : N, 20.38.
Benzaldehyde Hydrazone : Yellowish brown needles (from EtOH), m.p. 220.5° (decomp.). *Anal.* Calcd. for $C_{16}H_{13}ON_3$: C, 72.98; H, 4.98; N, 15.96. Found : C, 73.24; H, 4.98; N, 15.82.
Picrate : Yellowish brown plates (from MeOH), m.p. 177° (decomp.). *Anal.* Calcd. for $C_9H_9ON_3 \cdot C_6H_3O_7N_3$: N, 20.79. Found : N, 20.85.

4-Azidoquinoline 1-Oxide (IV)—a) Reaction of 4-Chloroquinoline 1-Oxide and NaN_3 : A mixture of 4.5 g. of 4-chloroquinoline 1-oxide, 4.5 g. of NaN_3, 10 cc. of H_2O, and 10 cc. of EtOH was heated

*3 The tests were performed by Dr. M. Nakamura and Mr. F. Miyazawa, Division of Microbiology in this Institute, and the results will be published in detail elsewhere.

9) E. Ochiai, M. Katada : Yakugaku Zasshi, **63**, 186 (1943).

10) L. J. Bellamy : "The Infra-red Spectra of Complex Molecules," 2nd ed., 273 (1958). Methuen & Co., London.

in a sealed tube at 115~125° for 5 hr. After cool, the brown solution was extracted with $CHCl_3$ and the solvent was evaporated after drying over Na_2SO_4. Yield, 2.3 g. (45%). Pale brown needles (from Me_2CO), m.p. 141~142° (decomp.). Explosive! *Anal.* Calcd. for $C_9H_6ON_4$: C, 58.06; H, 3.25. Found: C, 58.28, H, 3.32.

Picrolonate: Pale yellow powder (from MeOH), m.p. 168° (decomp.). *Anal.* Calcd. for $C_9H_6ON_4 \cdot C_{10}H_8O_5N_4$: N, 24.88. Found: N, 24.23.

Hydrochloride: Pale brown needles (form EtOH), m.p. 179° (decomp.).

b) Diazotization of 4-Hydrazinoquinoline 1-Oxide: To a solution of 0.20 g. of 4-hydrazinoquinoline 1-oxide dissolved in 10 cc. of 10% HCl, a solution of 0.10 g. of $NaNO_2$ dissolved in 3 cc. of H_2O was added dropwise with cooling. After standing for 30 min., the solution was basified with Na_2CO_3 and extracted with $CHCl_3$. The $CHCl_3$ residue was recrystallized from Me_2CO. Yield, 0.16 g. (72%). Reddish brown needles (from Me_2CO), m.p. 139° (decomp.). Infrared spectrum ($CHCl_3$ solution) of this product was identical with that of 4-azidoquinoline 1-oxide obtained by the foregoing method.

c) Oxidation of 4-Azidoquinoline: To a solution of 0.20 g. of 4-azidoquinoline (Ⅶ) in 14 g. of AcOH, 0.8 g. of 30% H_2O_2 solution was added and the mixture was warmed at 60~70° for 8 hr. The solution was evaporated under a reduced pressure, the residue was dissolved in a small amount of H_2O, basified with 10% Na_2CO_3 solution, and extracted with benzene. After drying over Na_2SO_4, the residue from benzene extract was recrystallized twice from Me_2CO. Yield, 77 mg. (33%). Reddish brown needles, m.p. 139° (decomp.). Infrared spectrum ($CHCl_3$ solution) of this product was identical with that of 4-azidoquinoline 1-oxide, obtained by the method (a).

4-Hydrazinoquinoline (VI)—a) Reaction of 4-Chloroquinoline and Hydrazine Hydrate: A mixture of 1.3 g. of 4-chloroquinoline, 6.5 cc. of 80% $NH_2NH_2 \cdot H_2O$, and 6.5 cc. of EtOH was heated on a water bath for 8 hr. and the solution was evaporated under a reduced pressure. After the residue was dissolved in 10 cc. of H_2O, the solution was again evaporated. The residue was dissolved in a small amount H_2O, basified with Na_2CO_3, and extracted with $CHCl_3$. The solvent was evaporated after drying over Na_2SO_4 and 0.8 g. (62%) of crude crystals were obtained, which strongly reduced $AgNO_3$-NH_4OH and Fehling solution. Pale yellow needles (from benzene), m.p. 158° (decomp.). *Anal.* Calcd. for $C_9H_9N_3$: C, 67.90; H, 5.77. Found: C, 67.86; H, 5.59.

Benzaldehyde Hydrazone: Yellow prisms (from EtOH), m.p. 226~228°. *Anal.* Calcd, for $C_{16}H_{13}N_3$: C, 77.71; H, 5.30; N, 16.99. Found: C, 77.59; H, 5.10; N, 17.11.

Hydrochloride: Yellowish needles (from MeOH + EtOH), m.p. 301° (decomp.).

Picrate: Reddish brown needles (from EtOH), m.p. 204° (decomp.).

b) Reaction of 4-Chloroquinoline 1-Oxide and Hydrazine Hydrate: A mixture of 1 g. of 4-chloroquinoline 1-oxide, 10 cc. of 80% $NH_2NH_2 \cdot H_2O$, and 10 cc. of EtOH was refluxed on a water bath for 5 hr. The excess $NH_2NH_2 \cdot H_2O$ was distilled off under a reduced pressure and the mixture was treated as in (a). To the dried $CHCl_3$ extract equivalent amount of benzene was added and the mixture was chromatographed on alumina column. After washing with benzene, 4-hydrazinoquinoline was eluted with $CHCl_3$ and 0.2 g. of pale yellowish needles, m.p. 154° (decomp.), were obtained. No depression of m.p. was observed on admixture of its benzaldehyde hydrazone with the one obtained from 4-hydrazinoquinoline described in (a).

4-Azidoquinoline (VII)—To a solution of 0.21 g. of 4-hydrazinoquinoline in 5 cc. of 5% HCl, a solution of 0.10 g. of $NaNO_2$ dissolved in 3 cc. of H_2O was added dropwise with cooling and the mixture was allowed to stand for 30 min. at room temperature. The reaction mixture was basified with Na_2CO_3, and extracted with $CHCl_3$. After drying over Na_2SO_4, the solvent was evaporated. Yield, 0.16 g. (71%). Slightly yellow prisms (from petr. ether), m.p. 76~78°. *Anal.* Calcd. for $C_9H_6N_4$: C, 63.52; H, 3.55; N, 32.93. Found: C, 63.29; H, 3.22; N, 32.75.

Picrate: Yellowish sands (from MeOH), m.p. 172° (decomp.). *Anal.* Calcd. for $C_9H_6N_4 \cdot C_6H_3O_7N_3$: N, 24.56. Found: N, 24.41.

1-(4-Quinolyl)-1*H*-[1,2,3]triazole-4-methanol (VIII)—A mixture of 0.50 g. of 4-azidoquinoline, 0.25 g. of propargyl alcohol, and 20 cc. of toluene was refluxed in an oil bath for ca. 40 hr. The hot solution was filtered and fine orange needles separated on standing. A solution of 0.43 g. of these crude crystals dissolved in benzene was poured into an alumina column, the column was developed with a mixture of 50 cc. of benzene and 1 cc. of MeOH, and eluted by a mixture of 50 cc. of benzene and 5 cc. of MeOH. The solvent was evaporated and the residue was recrystallized from benzene. Orange yellow needles, m.p. 148~150°. *Anal.* Calcd. for $C_{12}H_{10}ON_4$: C, 63.70; H, 4.46; N, 24.77. Found: C, 64.30; H, 4.40; N, 24.40.

Picrate: Yellowish needles (from EtOH), m.p. 161~162° (decomp.). *Anal.* Calcd. for $C_{12}H_{10}ON_4 \cdot C_6H_3O_7N_3$: C, 47.47; H, 2.88. Found: C, 47.90; H, 2.97.

Hydrochloride: Pale yellow pillars (from EtOH), m.p. 170° (decomp.). *Anal.* Calcd. for $C_{12}H_{10}ON_4 \cdot HCl$: N, 21.33. Found: N, 21.94.

4-Hydroxyaminoquinoline 1-Oxide (V)—To a solution of 2 g. of 4-nitroquinoline 1-oxide (I) suspended in MeOH, 10 cc. of 80% $NH_2NH_2 \cdot H_2O$ was added dropwise and the reaction became exothermic after 5 min., but was kept at 20~30° by intermittent swirling under the tap. A pale brown

precipitate started to form a few min. later. The suspension was allowed to stand over night at room temperature after evolution of bubbles had ceased. Pale brown precipitate was collected with suction and washed with EtOH. Pale brown needles or powder (form MeOH), m.p. ca. 190° (decomp.). Hydrochloride : Pale brown needles (from EtOH), m.p. 191~192° (decomp.). Yield, 0.9 g. (49%).

4-Azidopyridine 1-Oxide (XI)—a) Reaction of NaN_3 and 4-Chloropyridine 1-Oxide : A mixture of 0.5 g. of 4-chloropyridine 1-oxide, 0.4 g. of NaN_3, and 5 cc. of H_2O was heated in a sealed tube at 175~185° for 8 hr. After cool, the reaction mixture was extracted with $CHCl_3$ and the solvent was evaporated after drying. The $CHCl_3$ residue was dissolved in benzene, poured into alumina column, and the column was eluted with benzene-MeOH (50 cc. + 2 cc.). After evaporation of the solvent, orange yellow residue remained which recrystallized from benzene. Yield, 55 mg. (10%). Yellowish prisms, m.p. 142~143° (decomp.). *Anal.* Calcd. for $C_5H_4ON_4$: C, 44.12; H, 2.96; N, 41.17. Found: C, 43.64; H, 3.04; N, 41.45.
Picrolonate : Orange yellow needles (from EtOH), m.p. 177° (decomp.). *Anal.* Calcd. for $C_5H_4ON_4 \cdot C_{10}H_8O_5N_4 \cdot H_2O$: N, 26.79. Found : N, 26.59.

b) Diazotization of 4-Hydrazinopyridine 1-Oxide : To a solution of 0.50 g. of 4-hydrazinopyridine 1-oxide dissolved in 10 cc. of 5% HCl, a solution of 0.28 g. of $NaNO_2$ dissolved in 3 cc. of H_2O was added dropwise with cooling. After 30 min., the reaction mixture was treated as in (b) for 4-azidoquinoline 1-oxide. Yield, 0.30 g. (55%). Orange needles, m.p. 139~140° (decomp.). Infrared spectrum of this product was identical with that of 4-azidopyridine 1-oxide obtained by the method (a).

4-(Hydroxymethyl-1*H*-[1,2,3]triazol-1-yl)pyridine 1-Oxide (XII)—A mixture of 1.0 g. of 4-azidopyridine 1-oxide, 1.0 g. of propargyl alcohol, and 50 cc. of benzene was refluxed for 18 hr. Brown precipitate was collected, washed with benzene, and recrystallized twice from EtOH. Yield, 0.55 g. Pale yellow pillars or pale brown powder, m.p. 248° (decomp.). *Anal.* Calcd. for $C_8H_9O_2N_4$: N, 29.16. Found : N, 28.76.

From the above-mentioned alcoholic filtrate, 56 mg. of pale yellow pillars was obtained which were recrystallized from EtOH, m.p. 206~208° (decomp.). *Anal.* Calcd. for $C_8H_8O_2N_4$: N, 29.16. Found : N, 29.25.

4-Hydrazino-2-picoline (XIII)—A mixture of 5 g. of 4-chloro-2-picoline, 7.5 g. of 80% $NH_2NH_2 \cdot H_2O$, and 15 cc. of EtOH was heated in a sealed tube at 120~140° for 15 hr. After removal of excess $NH_2NH_2 \cdot H_2O$ under a reduced pressure as mentioned in (a) for 4-hydrazinoquinoline, a white crystalline residue of hydrochloride was obtained. Yield, 4.0 g. (69%). White needles (from EtOH), m.p. 267~268° (decomp.). *Anal.* Calcd. for $C_6H_9N_3 \cdot HCl$: N, 26.38. Found : N, 27.04.
Picrate : Yellowish needles (from EtOH), m.p. 188° (decomp.). *Anal.* Calcd. for $C_6H_9N_3 \cdot C_6H_3O_7N_3$: N, 23.86. Found : N, 23.66.

4-Azido-2-picoline (XV)—To a solution of 0.6 g. of 4-hydrazino-2-picoline hydrochloride dissolved in 10 cc. of 5% HCl, 0.4 g. of $NaNO_2$ dissolved in 5 cc. of H_2O was added dropwise with cooling and the mixture was allowed to stand for 30 min. The reaction mixture was basified with Na_2CO_3, extracted with $CHCl_3$, and $CHCl_3$ residue was distilled *in vacuo*. Slightly yellow liquid, $b.p_6$ 74~78° (bath temp.). Yield, 0.2 g. (40%).
Picrate : Yellowish needles (from EtOH), m.p. 137° (decomp.). *Anal.* Calcd. for $C_6H_6N_4 \cdot C_6H_3O_7N_3$: N, 27.00. Found : N, 27.17.

4,4′-Azoxy-di-2-picoline 1,1′-Dioxide from 4-Azido-2-picoline—To a solution of 0.1 g. of 4-azido-2-picoline dissolved in 5 cc. of AcOH, 0.6 g. of H_2O_2 was added, and the mixture was warmed on a water bath at 60~70°. The reaction mixture was evaporated under a reduced pressure, basified with with 10% Na_2CO_3 solution, and extracted with $CHCl_3$. $CHCl_3$ residue was allowed to stand for ca. 10 days. Orange needles separated, which were dissolved in benzene, chromatographed on alumina column, and eluted with benzene-MeOH (50 cc. + 1 cc.). Yield, 26 mg. Orange yellow needles (from benzene), m.p. 218° (decomp.). Its infrared spectrum was identical with that of 4,4′-azoxy-di-2-picoline 1,1′-dioxide, synthesized by I. Suzuki.

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Summary

4-Azidoquinoline (VII) and its N-oxide (IV) were prepared from the corresponding hydrazino compounds (VI and III) with sodium nitrite and also from chloro compounds (II) with sodium azide. Similarly, 4-azidopyridine 1-oxide and 4-azido-2-picoline were obtained.

The addition reaction of their azido group to propargyl alcohol, photolysis, and thermal decomposition were examined. (Received May 6, 1960)