The di-N-oxide compounds, however, were ineffective against the solid form of Ehrlich carcinoma.

Other phenazine derivatives exhibited no effect on the growth of tumor cells.

The degrees of inhibition of glycolysis (aerobic or anaerobic) and respiration of tumor cells by these compounds were in the same order as their antitumor activities.

So, the following general structure-activity relationships were observed with the series of compounds tested: appreciable antitumor activity was found only among compounds with the $\geqslant N \rightarrow O$ moiety.

Possible parallels between the antitumor activities of these compounds and the degrees of their inhibition of energy-generating systems of tumor cells were shown.

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16. Masahiro Nakadate, Yoshihiro Takano, Tadamasa Hirayama, Setsuko Sakaizawa, Tadashi Hirano, Kenji Okamoto, Kennichi Hirao, Tadao Kawamura, and Michiya Kimura: Janovsky Reaction of Nitropyridines. II.*1 Preparation of 5-Nitronicotinic Acid and its Related Compounds.

(*Faculty of Pharmaceutical Sciences, School of Medicine, Hokkaido University**2)

During the course of an investigation of the color reaction of polynitrobenzene derivatives with active methylene compounds, it was found that the same kind of reaction could similarly occur on the mononitro-benzene derivatives in which the electron-attracting substituents such as CN, $COOCH_3$, COOH occuppied at the *meta*-position of nitro groups.[1] This finding prompted us to explore the Janovsky color reaction of nitropyridine derivatives. The present paper deals with the preparation of several β-nitro-β'-substituted pyridine derivatives.

At the earlier stages of our investigation some attempts to obtain 5-nitronicotinic acid were carried out unsuccessfully as shown in Chart 1. Both nitration and amination of nicotinic acid with the mixture of fuming nitric acid and 30% fuming sulfuric acid and with sodium amide in N,N-dimethylaniline respectively, did not proceed so that the starting material was recovered. Alternatively 8-quinolinol was submitted to the degradation into 2,3-pyridinedicarboxylic acid with fuming nitric acid, which was then converted to the imide through anhydride. Hofmann rearrangement of 2,3-pyridinedicarboximide thus formed gave 3-aminopicolinic acid alone in place of the desired 2-amino isomer.*3 According to Wallis and Lane[2] the Hofmann rearrangement of substituted phthalic imide can afford two kinds of isomer, that is, the one possessing a

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*1 Previous paper, "Synthesis of 3-Nitropyridine and its Related Compounds," Yakugaku Zasshi, **79**, 549 (1959), represents Part I of this series.

*2 Nishi-5-Chome, Kita-12-Jo, Sapporo (中舘正弘，高野良宏，平山忠允，堺沢節子，平野 正，岡元賢二，平尾健一，河村忠男，木村道也).

*3 Sucharda obtained these two isomers from the same reaction using hypochlorite in the yields of 67.2 and 23.2% respectively.[3]

1) M. Kimura, M. Tohma: Yakugaku Zasshi, **78**, 1401 (1958).
2) E. Wallis, J. Lane: "Org. Reactions" Vol. III, p. 277 (1946), John Wiley & Sons inc., New York.
3) E. Sucharda: Ber., **58**, 1727 (1925).

carboxyl group at the position of *para* or *ortho* to the substituent which has an electron-attracting effect, and the other one possessing an amino group at the same position when the substituent has an electron-donating effect. It seemed reasonable that in the case of quinolinic imide the electron-attracting tertiary nitrogen of pyridine affected on the rearrangement into 3-amino derivative.

The second project to obtain 5-nitronicotinic acid through pyridine-nucleus-formation as shown in Chart 2 was fairly successful with the result of poor yields. On the course of obtaining 2-hydrazino derivative (I), reddish crystals, m.p. 261°, were separated as a by-product. As Schmidt, *et al.*[4] reported the formation of 3-amino-6-dimethylamino-1*H*-pyrazolo[3,4-*d*]pyrimidine (Ⅳ) in the case of hydrazination of 2-dimethylamino-4-chloro-5-pyrimidinecarbonitrile as shown in Chart 3, this unknown product showing no infrared absorption due to cyano group seemed to have a chemical constitution shown in the formula Ⅴ that was also considered to be reasonable from the result of elemental analysis. When methyl alcohol was used as a solvent for the hydrazination, however, 2-hydrazino derivative (I) was obtained almost quantitatively. On an attempt for removing hydrazino group from I several salt of heavy metals such as silver acetate, copper sulphate, mercuric chloride, and ferric chloride were employed and it was found that silver acetate was the most suitable oxidizing agent to prepare 5-nitronicotinonitrile (Ⅱ) which was converted to 5-nitronicotinic acid (Ⅲ) by the following hydrolysis with diluted sulfuric acid. In this oxidation procedure I should be used immediately after preparation and in such a smaller scale as less 1 g. in order to obtain Ⅱ in better yields.

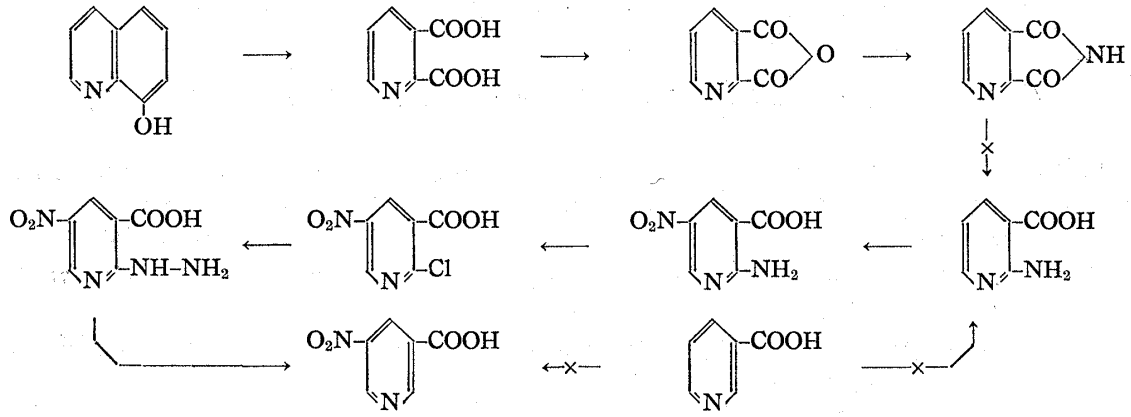

Chart 1.

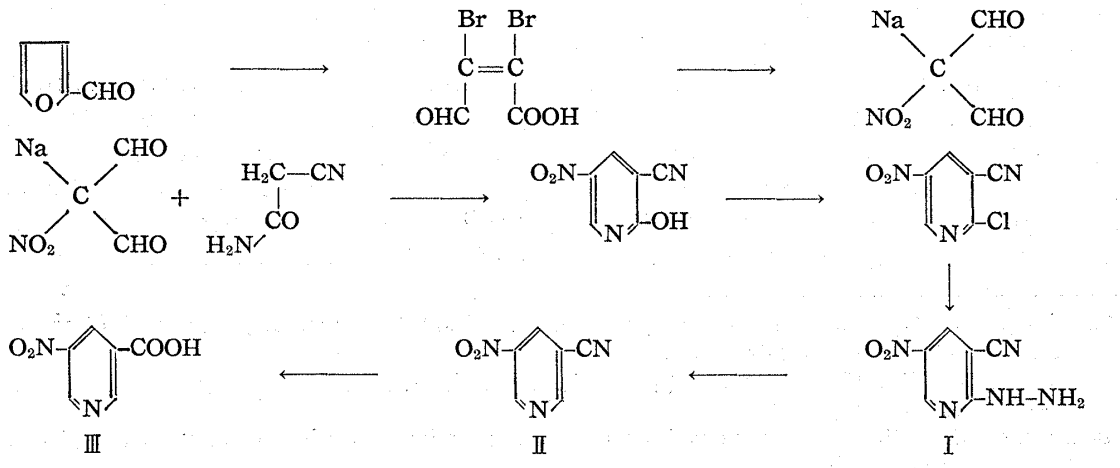

Chart 2.

4) P. Schmidt, K. Eichenberger, M. Wilhelm, J. Druey : Helv. chim. Acta., 42, 763 (1959).

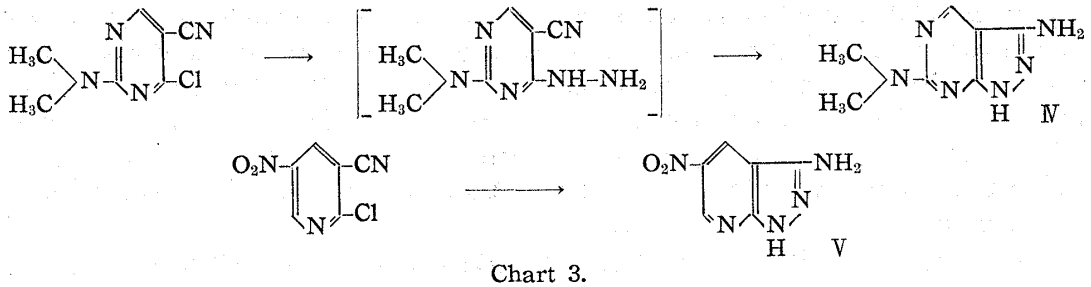

Chart 3.

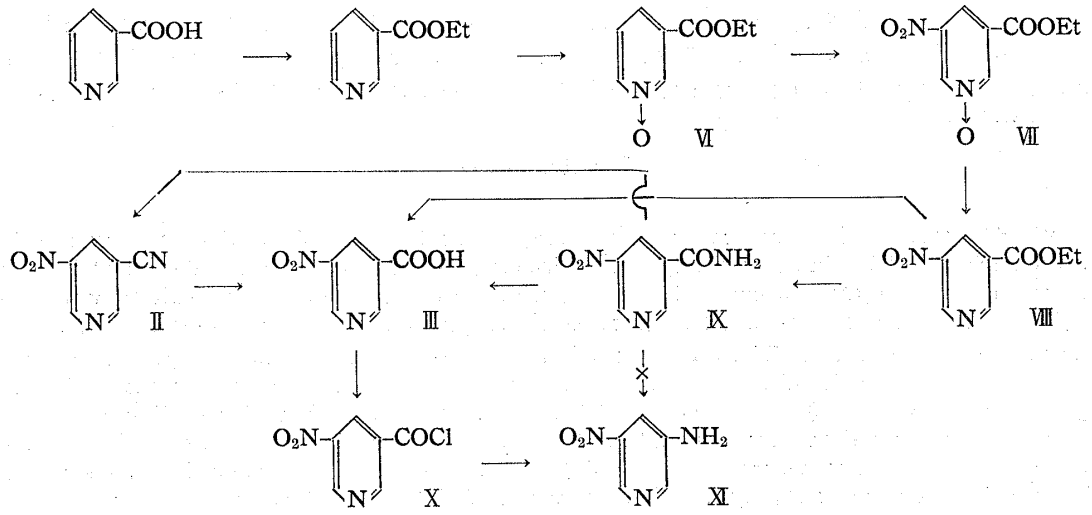

Chart 4.

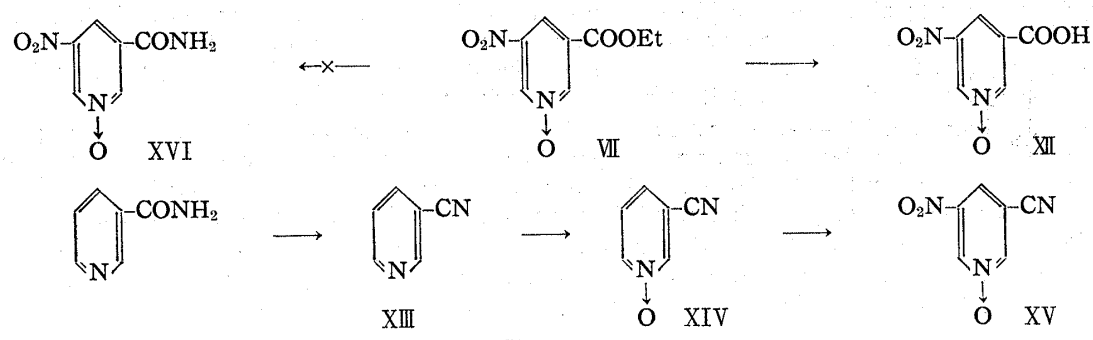

Chart 5.

Ochiai, *et al.*[5] reported a novel method for introducting a nitro group to the β-position of pyridine through N-oxide. This finding encouraged us for the preparation of a series of nitropyridine derivatives, on some of which had been studied almost unsuccessfully with several synthetic trials as described above. The starting material, nicotinic acid, was converted into ethyl nicotinate 1-oxide (VI) by the usual methods, from which ethyl 5-nitronicotinate 1-oxide (VII) was then obtained quite easily by the method of Ochiai, *et al.* in 45~50% yields. The nitrated N-oxide (VII) thus obtained was deoxygenated into ethyl 5-nitronicotinate (VIII) by the method of Hamana[5] with phosphorustrichloride. From the ester (VIII) thus formed were derived 5-nitronicotinamide (IX) by the action of conc. ammonia, 5-nitronicotinonitrile (II) by the dehydration[6] with phosphoruspentoxide, and 5-nitronicotinic acid (III) by the hydrolysis with 10% aq. sodium

5) E. Ochiai, C. Kaneko: This Bulletin, 8, 28 (1960).
6) M. Hamana: Yakugaku Zasshi, **71**, 263 (1951).
7) F. B. LaForge: J. Am. Chem. Soc., **50**, 2477 (1928).

hydroxide, which was also obtained by the action of nitrous acid on Ⅸ, respectively. Of these nitropyridine derivatives, Ⅱ and Ⅲ were proved to be identical respectively with those obtained by the ring closure reaction shown in Chart 2, in respects to melting points, infrared spectra*4 and elemental analysis. While the Hofmann rearrangement of 5-nitronicotinamide (Ⅸ) was unsuccessful, 3-amino-5-nitropyridine (Ⅺ) was obtained by the Curtius reaction of 5-nitronicotinic acid (Ⅲ) through the chloride without separating its azide.

On the preparation of the remained N-oxides derived from these nitropyridines some attempts were made successfully except 5-nitronicotinamide 1-oxide (XVI) as shown in Chart 5. The β-nitropyridine 1-oxides thus obtained in this study showed generally intensive colorations by alkali. The mechanism of this phenomena and the Janovsky reactions of nitropyridine derivatives will be described in the following paper.

Experimental*4

The following compounds were prepared according to previously established procedures: 2,3-Pyridinedicarboxylic acid,[4] 2,3-Pyridinedicarboxylic anhydride,[4] 2,3-pyridinedicarboximide,[4] mucobromic acid,[8] sodium nitromalonic aldehyde,[9] 3-cyano-5-nitro-2-pyridone,[10] 2-chloro-3-cyano-5-nitropyridone,[10] ethyl nicotinate,[11] ethyl nicotinate 1-oxide (Ⅵ),[12] nicotinonitrile (XIII),[6] nicotinonitrile 1-oxide (XIV).[13]

Hofmann Rearrangement of 2,3-Pyridinedicarboximide——To a solution of 2,3-pyridinedicarboximide (5 g.), dissolved in 10% NaOH solution (80 ml.), was added dropwise sodium hypobromite solution which was prepared freshly with 12% NaOH solution (28 ml.) and bromine (6 g.), under cooling with ice-water. The reaction mixture was then heated at 80° for 30 min. After cooling, saturated $CuSO_4$ solution was added to the reaction mixture neutralized with 50% H_2SO_4. Cu salt thus produced was suspended in hot water, followed by introducing H_2S gas. After removal of CuS, the filtrate was evaporated to dryness under reduced pressure and residue was recrystallized to give 3-aminopicolinic acid, m.p. 210°. Yield: 18%.

2-Hydrazino-5-nitronicotinonitrile (I)——To a solution of 2-chloro-5-nitronicotinonitrile (1 g.) dissolved in CH_3OH (50 ml.), was added hydrazine hydrate (1 g.) dropwise with stirring. After the reaction mixture was allowed to stand for 1 hr. at room temperature, the dark brown solid separated was collected by filtration, followed by wash with CH_3OH to give I as dark brown crystalline powder. Yield: 100%.

5-Nitronicotinonitrile (II)——Preparation 1. To a solution of silver acetate (300 mg.) suspended in water (10 ml.) was added I (1 g.) portionwise with stirring and moderately boiling for 30 min. After cooling, the reaction was neutralized with NH_4OH and was added sodium acetate, followed by extracting with benzene. Evaporation of benzene solution dried over anhyd. Na_2SO_4 gave a crystalline residue. Recrystallization from ethanol afforded Ⅱ (180 mg.) as pale yellow plates, m.p. 112~113°. Yield: 22%.

Preparation 2. Ⅸ (1 g.) was distilled with phosphoruspentoxide (0.9 g.) under reduced pressure to give pale yellow solid, b.p_{30} 130°, which gave Ⅱ as pale yellow plates from ethanol, m.p. 111~112°. Yield: 73%.

IR spectra and mixed melting point of these two products thus obtained proved their mutual identity. *Anal.* Calcd. for $C_6H_3O_2N_3$: C, 48.30; H, 2.01; N, 28.18. Found: C, 47.90; H, 1.89; N, 28.29. IR ν_{max}^{Nujol} cm^{-1}: 2240 (CN), 1528 (nitro), 1365 (nitro), 1607 (aromatic C=C), 1570 (aromatic C=C), 1416 (aromatic C=C), 1221 (CH in plane or skeletal), 1151 (CH in plane or skeletal), 1024 (CH in plane or skeletal), 798 (CH out of plane), 750 (CH out of plane)

5-Nitronicotinic Acid (III)——Preparation 1. A solution of Ⅱ (200 mg.) dissolved in 50% H_2SO_4 (2 ml.), was refluxed for 1.5 hr. After cooling, water (28 ml.) was added to the reaction mixture and extracted

*4 H. Shindo (This Bulletin, **6**, 117 (1958)) assigned the IR spectra due to α-, β-and γ-substitutions in monosubstituted pyridine derivatives. Almost same absorptions due to the CH out-of and in plane vibrations and skeletal vibrations were observed on these β,β'-disubstituted pyridine derivatives obtained in this study.

*5 All melting points are uncorrected.

8) M. G. Chavanne: Compt., **153**, 185 (1911).
9) H. B. Hill: Am. Chem. J., **22**, 89 (1899).
10) P. E. Fanta, R. A. Stein: J. Am. Chem. Soc., **77**, 1045 (1955).
11) H. Gilman, H. S. Broadbent: *Ibid.*, **70**, 2757 (1948).
12) A. R. Katritzky: J. Chem. Soc., **1956**, 2404.
13) E. Ochiai, Z. Sai: Yakugaku Zasshi, **65B**, 18 (1945).

with benzene. Evaporation of benzene solution dried over anhyd. Na_2SO_4 gave white crystals. Recrystallization from aq. ethanol afforded III as white crystalline powder, m.p. 171~172°, Yield: 52%.

Preparation 2. A solution of VIII (100 mg.) in 10% aq. NaOH (2 ml.) was heated for 30 min. on a boiling water bath. After cooling, the mixture was acidified with conc. HCl and extracted with ether. Evaporation of ether solution dried over Na_2SO_4 gave a crystalline residue that was recrystallized from aq. ethanol to give III of m.p. 170~171°. Yield: 65%.

Preparation 3. To a solution of IX (500 mg.), dissolved in conc. H_2SO_4 was added dropwise aq. solution (5 ml.) of $NaNO_2$ (600 mg.) under cooling with ice-water. The reaction mixture was bubbled vigorously and turned blue, to which conc. NH_4OH was then added to cause complete precipitation at 40°. Recrystallization of the precipitate from aq. ethanol gave III as colorless crystalline powder, m.p. 170~171°. Yield: 70%.

No depression in melting point was observed in admixture of any pair of three products thus obtained. Their IR spectra were also identical with each other. *Anal.* Calcd. for $C_6H_4O_4N_2$: C, 42.86; H, 2.40; N, 16.67. Found: C, 42.87; H, 2.23; N, 16.60. IR ν_{max}^{Nujol} cm^{-1}: 2320 (pyridine carboxylic acid), 1880 (broad; pyridine carboxylic acid), 1700 (C=O), 1526 (nitro), 1362 (nitro), 1611 (aromatic C=C), 1592 (aromatic C=C), 1460 (aromatic C=C), 1179 (CH in plane or skeletal), 1117 (CH in plane or skeletal), 1028 (CH in plane or skeletal), 819 (CH out of plane), 741 (CH out of plane), 720 (CH out of plane).

Ethyl 5-Nitronicotinate 1-Oxide (VII)——To a solution of ethyl nicotinate 1-oxide (6.7 g.), dissolved in $CHCl_3$ (100 ml.) was added portionwise $CHCl_3$ solution (65 ml.) of *p*-nitrobenzoyl chloride (7.5 g.) with stirring at −3~−7° for 10 min., followed by adding at a time finely powdered $AgNO_3$ (8.5 g.) dried over NaOH. The reaction mixture was further stirred for 1 hr. at −3~−7°, 2 hr. at room temperature, 2.5 hr. at 40~50° and 2 hr. at 55° respectively. After the mixture was allowed to stand overnight, AgCl precipitated was collected and washed with $CHCl_3$. The combined solution of the filtrate and washed $CHCl_3$, dried over anhyd. Na_2SO_4, were evaporated to give yellow crystalline residue. A suspension of this yellow crystals in abs. C_2H_5OH (200 ml.) was made clear by refluxing under the introduction of dried HCl gas for 1 hr. After the reaction mixture was allowed to stand overnight, pale yellow crystals were obtained. Recrystallization from ethanol gave VII as pale yellow needles, m.p. 154°. Yield: 50%. *Anal.* Calcd. for $C_8H_8O_5N_2$: C, 45.28; H, 3.77; N, 13.21. Found: C, 45.44; H, 3.82; N, 13.58. IR ν_{max}^{Nujol} cm^{-1}: 1723 (C=O), 1535 (nitro), 1360 (nitro), 1248 (N-oxide), 1621 (aromatic C=C), 1568 (aromatic C=C), 1455 (aromatic C=C), 1136 (CH in plane or skeletal), 1086 (CH in plane or skeletal), 1006 (CH in plane or skeletal), 934 (CH in plane or skeletal), 819 (CH out of plane), 808 (CH out of plane), 770 (CH out of plane).

Ethyl 5-Nitronicotinate (VIII)——To a solution of VII (500 mg.), dissolved in $CHCl_3$ (15 ml.) was added dropwise $CHCl_3$ solution (15 ml.) of PCl_3 (1.5 g.) at room temperature for 10 min. The reaction mixture was then refluxed for 1 hr. After cooling, the mixture was poured into water, followed by alkalifing with conc. NH_4OH. The reaction mixture was then extracted with $CHCl_3$. Evaporation of $CHCl_3$ solution dried over anhyd. Na_2SO_4 gave yellow needles. Recrystallization from MeOH afforded VIII as colorless plates, m.p. 88~89°. Yield: 70%. *Anal.* Calcd. for $C_8H_8O_4N_2$: C, 48.98; H, 4.11; N, 14.28. Found: C, 48.80; H, 4.22; N, 14.97. IR ν_{max}^{Nujol} cm^{-1}: 1718 (C=O), 1533 (nitro), 1363 (nitro), 1605 (aromatic C=C), 1576 (aromatic C=C), 1459 (aromatic C=C), 1178 (CH in plane or skeletal), 1127 (CH in plane or skeletal), 1020 (CH in plane or skeletal), 840 (CH out of plane), 782 (CH out of plane), 738 (CH out of plane).

5-Nitronicotin Amide (IX)——A suspension of VIII (5.3 g.) in conc. NH_4OH (50 ml.) was allowed to stand for 3 days in an ice box. The suspended plates were changed into colorless needles which were recrystallized from acetone or ethanol to give IX as colorless needles. m.p. 179~180°. Yield: 100%. *Anal.* Calcd. for $C_6H_5O_3N_2$: C, 43.12; H, 3.02; N, 25.15. Found: C, 43.23; H, 3.36; N, 25.31. IR ν_{max}^{Nujol} cm^{-1}: 3220 (NH), 3365 (NH), 1668 (C=O), 1533 (nitro), 1359 (nitro), 1606 (aromatic C=C), 1572 (aromatic C=C), 1460 (aromatic C=C), 1190 (CH in plane or skeletal), 1130 (CH in plane or skeletal), 1020 (CH in plane or skeletal), 795 (CH out of plane), 755 (CH out of plane), 730 (CH out of plane).

3-Amino-5-nitropyridine (XI)——A solution of III (300 mg.) in $SOCl_2$ (30 ml.) was refluxed for 3.5 hr. After evaporation of $SOCl_2$, 5-nitronicotinoyl chloride (X) was obtained as colorless needles, m.p. 162~162.5°. To a suspended benzene solution of the crude chloride (200 mg.) thus obtained was added finely powdered NaN_3 (200 mg.) and refluxed the mixture for 12 hr. at 80~90° on a water bath. After cooling, conc. H_2SO_4 (10 ml.) was added to the reaction mixture, which was then heated for 3 hr. on a water bath showing vigorous outbreak of gas. As no residue was obtained from benzene layer, H_2SO_4 layer was poured into ice-water and the mixture was made alkaline with NH_4OH followed by extraction with ether. Evaporation of ether solution dried over Na_2SO_4 gave brown solid which was recrystallized from ethanol to give XI as yellow needles, m.p. 185~185.5°. *Anal.* Calcd. for $C_5H_5O_2N_2$: C, 43.17; H, 3.62. Found: C, 42.97; H, 3.34.

5-Nitronicotinic Acid 1-Oxide (XII)——A solution of VII (100 mg.), dissolved in 50% H_2SO_4 (10 ml.) was heated for 5 hr. on a water bath. After cooling, the reaction mixture was extracted with $CHCl_3$ to remove the remained starting materials and then extracted with ethyl acetate. Yellow crystals were obtained from ethyl acetate extracts which gave XII from methanol as yellow prisms, m.p. 230~232° (decomp.), Yield: 50%. *Anal.* Calcd. for $C_6H_4O_5N_2$: C, 39.14; H, 2.19; N, 15.22. Found: C, 39.09; H, 2.22; N,

15.55. IR ν_{max}^{Nujol} cm^{-1} : 1855 (broad; pyridine carboxylic acid), 1701 (C=O), 1525 (nitro), 1359 (nitro), 1285 (N-oxide), 1610 (aromatic C=C), 1590 (aromatic C=C), 1459 (aromatic C=C), 1176 (CH in plane or skeletal), 1089 (CH in plane or skeletal), 1027 (CH in plane or skeletal), 937 (CH in plane or skeletal), 813 (CH out of plane), 743 (CH out of plane), 710 (CH out of plane),

5-Nitronicotinonitrile 1-Oxide (XV)——To a solution of XIV (3 g.), dissolved in $CHCl_3$ (150 ml.) was added dropwise $CHCl_3$ solution (60 ml.) of *p*-nitrobenzoyl chloride (4.65 g.) with stirring at −5～−7° for 5 min. followed by adding finely powdered $AgNO_3$ (6 g.) at a time. The reaction mixture was stirred for 1 hr. at −5～−7°, 2 hr. at room temperature, 2.5 hr. at 40～50° and 2 hr. at 55° respectively. After the mixture was allowed to stand overnight, AgCl precipitated was collected by filtration and washed with $CHCl_3$. Combined solution of the filtrate and washed $CHCl_3$ was concentrated to 4/5 volume and allowed to stand overnight. Yellow crystals separated was recrystallized from acetone to give XV as yellow needles, m.p. 205° (decomp.). Yield : 36.5%. *Anal.* Calcd. for $C_6H_3O_3N_3$: C, 43.64; H, 1.83; N, 25.45. Found : C, 43.73; H, 1.78; N, 24.76. IR ν_{max}^{Nujol} cm^{-1} : 2240 (CN), 1543 (nitro), 1365 (nitro), 1270 (N-oxide), 1631 (aromatic C=C), 1571 (aromatic C=C), 1455 (aromatic C=C), 1150 (CH in plane or skeletal), 1032 (CH in plane or skeletal), 1015 (CH in plane or skeletal), 945 (CH in plane or skeletal), 1015 (aromatic C=C), 935 (aromatic C=C), 815 (CH out of plane), 724 (CH out of plane).

The authors express their deep gratitudes to Prof. Emeritus E. Ochiai, University of Tokyo, and Prof. Y. Mizuno, Hokkaido University, for their valuable suggestions. They are also indebted to Mr. K. Narita, Mrs. T. Tohma and Miss A. Maeda of the analytical laboratory of this Faculty for elemental analysis.

Summary

As a part of studies on the Janovsky reaction of nitropyridines, 3-substituted-5-nitropyridines such as 2-hydrazino-5-nitronicotinonitrile (I), 5-nitronicotinonitrile (II), 5-nitronicotinic acid (III), ethyl 5-nitronicotinate (VIII), 5-nitronicotinamide (IX), and 3-amino-5-nitropyridine (XI) were prepared. Some of their N-oxides such as ethyl 5-nitronicotinate 1-oxide (VII), 5-nitronicotinic acid 1-oxide (XII) and 5-nitronicotinonitrile 1-oxide (XV) were also prepared.

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UDC 547.538.2.07

17. Issei Iwai, Kazuo Tomita, and Junya Ide : Studies on Acetylenic Compounds. XL. The Addition Reaction of Nitrosyl Chloride and Nitryl Chloride to Acetylenic Compounds.*1

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The reaction of nitrosyl chloride or nitryl chloride with olefinic compounds has been investigated extensively, and the reaction of nitrosyl chloride with olefins has especially been a useful method for the structural determination of terpenes.[1] However, there have been only a few reports[2～5] on the addition of these reagents to acetylenic compounds, and such investigations have not been systematic.

The present investigation was carried out to obtain information concerning with the direction and mode of addition of nitrosyl chloride or nitryle chloride to the carbon-carbon triple bond.

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*1 Part XXXIX : This Bulletin, **12**, 1446 (1964).

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2) R. Perrot, R. Berger; Compt rend., **235**, 185 (1952).
3) W. Steinkopf, M. Kuhnel : Ber., **75B**, 1323 (1942).
4) J. P. Freeman, W. D. Emmons : J. Am. Chem. Soc., **79**, 1712 (1957).
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