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Studies on Chemical Carcinogens. XVIII.[1] Acetylation of Carcinogenic 4-(N-Hydroxy-N-methylamino)quinoline 1-Oxide

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Treatment of carcinogenic and mutagenic 4-(N-hydroxy-N-methylamino)quinoline 1-oxide with acetic anhydride gave 4-(N-acetoxy-N-acetoxymethylamino)quinoline and 4-(N-acetoxy-N-methylamino)quinoline as the main products, with evolution of methane and carbon dioxide. This reaction is discussed in relation to the carcinogenicity and mutagenicity.

Keywords——N-demethylation; N-deoxygenation; homolysis of N–O bond; acetoxy radical; polonovski reaction; mutagen; carcinogen

Experimental evidence has accumulated that arylhydroxylamines are carcinogenic and mutagenic to a greater or lesser extent probably due to the formation of nitrene or nitrenium intermediates derived by the N–O bond heterolysis of the hydroxyamino substituent in these molecules.[3] It is considered that the N–O bond cleavage may occur in neutral media via the O-acyl derivatives conjugated enzymatically with sulfuric acid, carboxylic acids, *etc.* Such acid-conjugates of some arylhydroxylamines were synthesized as model compounds for the activated forms of the carcinogens.[3,4,5]

The present work was undertaken to prepare the acetyl derivatives of 4-(N-hydroxy-N-methylamino)quinoline 1-oxide (I), which was recently shown to be potently carcinogenic[6] and mutagenic.[7] Contrary to our expectation, the O-acetyl derivative of I was not found in the mixture of products obtained by treatment of I with acetic anhydride. It is of interest that the crude reaction mixture thereby produced was still potently mutagenic on *Salmonella typhimurium* TA 100, even through the starting material had been entirely consumed. This finding prompted us to identify the products obtained by the acetylation of I. The reaction mechanism is also described.

Results

When a suspension of I in acetic anhydride was stirred at room temperature, crystals of I readily went into solution. The PMR spectrum of the chloroform extract of the neutralized reaction mixture indicated that it consisted mainly of two components, II and III, in a molar ratio of 1.0 to 1.7. Analysis of the PMR spectrum suggested that II included an acetyl group

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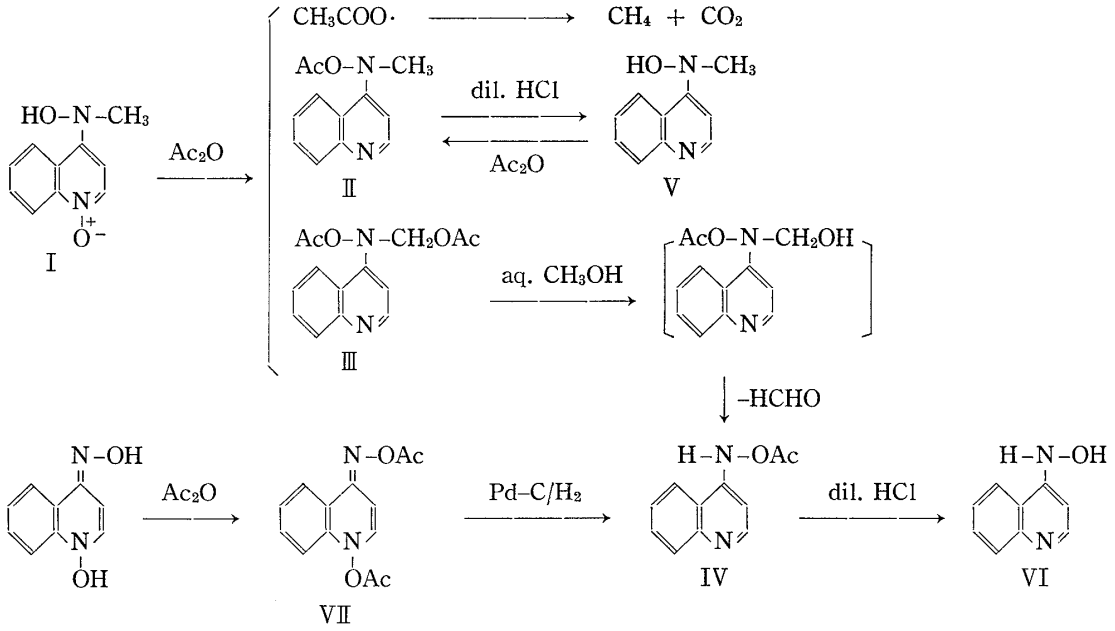

Chart 1

and an N-methyl group, while III contained two acetyl groups and a methylene group magnetically more deshielded (δ 5.6) than would be expected for methylene adjacent to an amino group. Attempts at the chromatographic isolation of II and III were without success.[8] Next, the chloroform extract of the neutralized reaction mixture was dissolved in aqueous methanol and warmed in a water bath for 1 hr. The PMR spectrum of the hydrolysate indicated that, while II remained unchanged, III was converted to a partially hydrolyzed monoacetyl compound (IV). The PMR spectrum of IV showed only one acetyl methyl signal in the aliphatic proton region. Compounds II and IV were readily isolated by silica gel chromatography, eluted with 2% methanol in chloroform. Acid-hydrolyses of II and IV gave 4-(N-hydroxy-N-methylamino)quinoline (V)[9] and 4-hydroxyaminoquinoline (VI),[10] respectively, in quantitative yields. These results strongly suggest that II, III, and IV are 4-(N-acetoxy-N-methylamino)quinoline, 4-(N-acetoxy-N-acetoxymethylamino)quinoline, and 4-acetoxyaminoquinoline, respectively, as formulated in Chart 1. The structures of these products were further supported by the following results. Compound II was identical (PMR and IR) with an authentic specimen prepared by acetylation of 4-(N-hydroxy-N-methylamino)quinoline (V)[9] with acetic anhydride. The partially hydrolyzed product IV was identical (thin-layer chromatography and PMR spectroscopy) with an authentic specimen synthesized by hydrogenation of 1-acetoxy-4-acetoxyimino-1,4-dihydroquinoline (VII)[5] in the presence of palladium on charcoal.[11] In addition to the PMR analysis, the structure of III was supported by the observation that formaldehyde was liberated during the acid-hydrolysis of the acetylation reaction mixture. The yield of formaldehyde was almost equimolar with respect to the amount of III formed.

The product ratio, III/II, was strongly dependent on the reaction temperature employed; the results are shown in Table I.[12] Formaldehyde was quantitatively analyzed by colori-

8) Compound III was readily converted to the partially hydrolyzed product (IV) even on a silica gel TLC plate. This is one reason why chromatographic separation failed.
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12) Yields of II and III were estimated by quantitative analyses of the PMR signal intensities referred to the internal standard signal due to the reference compound (CH_2Cl_2 and/or benzene) added directly to the reaction mixture at a known molar concentration after completion of the reaction.

Table I. Yields of Products in the Acetylation of 4-(N-Hydroxy-N-methylamino)quinoline 1-Oxide (I) with Acetic Anhydride

Reaction temperature	II(%)	III(%)	HCHO(%)	Product ratio (III/II)
0°	12	60	65	5.0
	10	59	64	5.9
	10	62	65	6.2
	11	60	63	5.5
	11	62	64	5.6
15°	18	50	58	2.8
	15	49	52	3.2
	17	50	54	2.9
30°	25	40	45	1.6
	27	40	44	1.5
	25	41	46	1.6
75°	34	30	40	0.88
	27	25	34	0.93
	30	26	37	0.87

II: 4-(N-acetoxy-N-methylamino)quinoline.
III: 4-(N-acetoxy-N-acetoxymethylamino)quinoline.
HCHO: Analysis was done after acid-hydrolysis of the reaction mixture.

metric analysis using Formaldehyde-Test Wako,[13] after hydrolyzing the reaction mixture with dilute hydrochloric acid. The results indicate that formation of the deoxygenated product II takes place more easily at higher temperatures, in preference to the formation of III.

The product ratio is not very dependent on the acid-base character of the solvent used for the reaction, as shown in Table II.

Table II. Dependence of Product Ratio, III/II, on the Reaction Solvent in the Acetylation of I at Room Temperature

Reaction solvent	Product ration (III/II)
aq. 5% H_2SO_4	1.3[a]
aq. 5% AcONa	1.3[a]
MeOH	1.6
Acetic anhydride	1.7
Pyridine	2.6

a) The ratio was estimated from the yields of the hydrolyzed products, V and VI, respectively.

Liberation of Carbon Dioxide and Methane during Acetylation

During the acetylation process, bubbles were evolved even at 0° and appeared more vigorously at higher reaction temperatures. Only methane was detected in the gas by gas-chromatography. Carbon dioxide was also identified as barium carbonate (about 0.8 molar equivalent relative to II). These results suggest that the reaction is accompanied by formation of acetoxy free radical which is degraded to carbon dioxide and methyl radical, the latter being stabilized as methane.[14]

Further Evidence for Free Radical Formation

When aqueous potassium iodide was added to the reaction mixture, the mixture turned a red-brown color, and, on addition to aqueous starch, it changed to intense blue. The iodide

13) Purchased from Wako Pure Chemical Industries, Ltd., Osaka. See the technical manual for Formaldehyde-Test *Wako*. Refer also to R.G. Dickinson and N.W. Jacobsen, *Chem. Commun.*, **1970** 1719.
14) Y. Kawazoe and M. Araki, *Chem. Pharm. Bull.*, **19**, 1278 (1971).

oxidation thus observed started several seconds after I was dissolved in acetic anhydride at room temperature or even at 0°. This may be an indication that free radical formation is involved in the reaction process.

Thus, in order to detect the methyl radical directly, the PMR spectrum of the reaction mixture was taken during the reaction; however no signals due to chemically induced dynamic nuclear polarization (CIDNP) were observed. The measurement conditions for the CIDNP spectrum were the same as those used for the pyrolysis of 1-acetoxy-4-acetoxyimino-1,4-dihydroquinoline.[14] Thus, no direct evidence is available for formation of the methyl radical.

Acetylation of Some Related Compounds

The N-demethylation and deoxygenation observed in the acetylation of I do not occur with the related compounds examined in the present study. Thus, 4-(N,N-dimethylamino)-quinoline 1-oxide did not react with acetic anhydride even at 90°, while V reacted, without accompanying N-demethylation, to give II in quantitative yield. The acetylation of IV gave 1-acetoxy-4-acetoxyimino-1,4-dihydroquinoline, as already reported.[11] The acetylation of these three compounds did not show either the color reaction upon addition of iodide or the evolution of carbon dioxide. Thus, it seems that ready N-demethylation and homolytic deoxygenation are characteristic of the acetylation of I.

Discussion

The reaction mechanism of this acetylation can be postulated on the basis of the product analysis described in this study. Thus, I reacts with acetic anhydride to afford the intermediate A, which is then deprotonated to give a kind of nitron B. Hence, the second acetylation takes place to give the intermediate C, which is converted to the products through two different

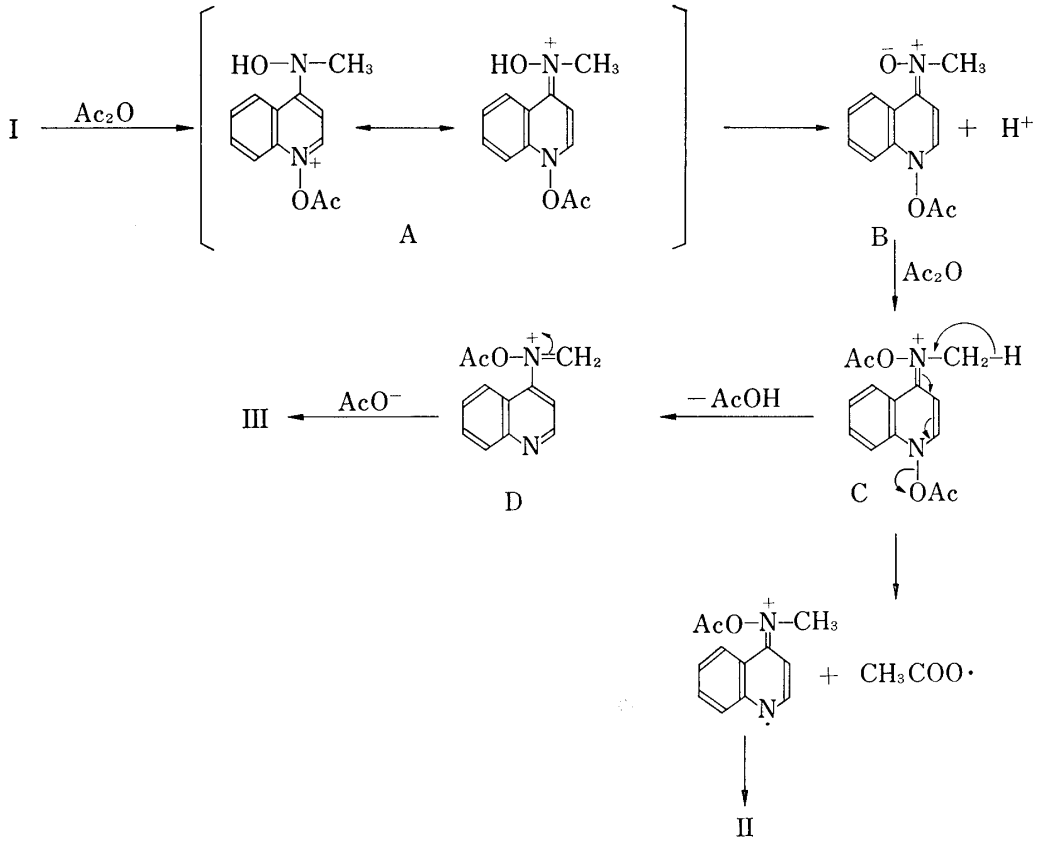

Chart 2

reaction pathways. Thus, C is converted to D by a process similar to that of the Polonovski reaction:[15] leaving of an acetate ion from the ring nitrogen and deprotonation from the substituent methyl group due to the attack of an acetate ion. Next, D must be attacked by an acetate ion to afford III.

It is interesting that the intermediate C has a structure similar to that of 1-acetoxy-4-acetoxyimino-1,4-dihydroquinoline (VII),[5] which is distinguished by its high thermal homolytic reactivity, resulting in the formation of an acetoxy radical.[14,16] The similarity in structure suggests that C may undergo ready homolytic fission between the ring nitrogen and OAc group. The yield of II became higher as the reaction temperature was increased. This appears to support the view that II is a product of thermal homolysis.

We will next comment briefly on the mutagenic properties of the acetylation product mixture. The isolated products, II, IV, V, and VI, were assayed for mutagenicity using *Salmonella typhimurium* TA 100, and it was found that only IV was potently mutagenic. Compound IV is also known to be carcinogenic.[11] It is, therefore, possible that the mutagenic and carcinogenic activities of I stem from its enzymic acylation, followed by chemical degradation as desribed in this paper. However, the possibility that I is activated through an alternative enzymic process to a metabolite such as a 4-(N-acyloxy-N-methylamino)quinoline 1-oxide cannot be ruled out. The results of a biological study will be reported elsewhere.

Experimental

Spectral Measurements——PMR spectra were measured in Me_2SO-d_6 with a JNM-MH-100 spectrometer (100 MHz). IR spectra were recorded with a Hitachi EPI-G3 spectrometer.

Acetylation of I with Ac_2O without Solvents——Ac_2O (0.4 ml) was added to 40 mg of I and the reaction mixture was kept at an appropriate temperature (0°, 15°, 30°, or 75°) with stirring. It was poured into 10 ml of ice-water when the suspended crystals had all gone into solution. After stirring for 1 hr to decompose excess Ac_2O, it was extracted 5 times with 20 ml of $CHCl_3$ each. The extracts were combined and washed thoroughly with 20 ml of saturated aqueous $NaHCO_3$. After drying over anhydrous Na_2SO_4, it was evaporated to dryness under reduced pressure. The residue was treated as follows (procedures *a* and *b*).

a) The crude products thus obtained were developed on a silica gel thin-layer plate with $CHCl_3$. The *Rf* values of the main spots were 0.24 and 0.03. The fraction of *Rf* 0.24 was extracted with $CHCl_3$. This was identified by PMR spectroscopy as a mixture of II and III. This mixture was dissolved in 50% aqueous CH_3OH and warmed at 60° for 2 hr. The solution was concentrated under reduced pressure. The residue was identified as a mixture of II and IV by PMR spectroscopy and thin-layer chromatography on silica gel with various eluting solvent systems. The lower *Rf* fraction (0.03) of the crude acetylation mixture was extracted with $CHCl_3$ and the extract was recrystallized from CH_2Cl_2–Et_2O to give crystals, mp 178°; this material was shown to be identical with IV by PMR and IR spectroscopies.[11]

b) The crude acetylation products were dissolved in 5 ml of 3% HCl and warmed at 60° for 30 min. After evaporation to dryness, the residue was chromatographed on a preparative silica gel TLC plate with 20% MeOH in $CHCl_3$. The main products thus isolated were identified as V (V HCl, mp 200—201°) and VI (VI HCl, mp 262—263°).

Acetylation of I with Ac_2O in Solvents——Ac_2O (0.4 ml) was added to an ice-chilled solution of 40 ml of I in 0.4 ml of MeOH with stirring. The reaction mixture was stirred at room temperature for 10 min, by which time the starting material was no longer detectable by TLC. The solution was concentrated under reduced pressure. The residue was neutralized with saturated aqueous $NaHCO_3$ and extracted with $CHCl_3$. The extract was analyzed by PMR spectroscopy for quantitative product analysis. The same procedure was used in experiments in other reaction solvents, *i.e.*, in pyridine, 5% aqueous H_2SO_4, and 5% aqueous AcONa.

Quantitative Analysis of Acetylation Products, II and III, by PMR Spectroscopy——A solution of 100 mg of I in 0.9 ml of Ac_2O was kept at an appropriate reaction temperature for 30 min under stirring, then 0.10 ml of Ac_2O containing 16.7 μl of CH_2Cl_2 and/or 7.79 μl of benzene was added. The yields of the acetylation products, II and III, were evaluated by PMR spectroscopy with reference to the signal intensities of the CH_2Cl_2 and/or benzene added.

Preparation of II——A solution of 0.02 g of V in 2 ml of Ac_2O was kept at 75° under stirring. After

15) D.H. Kim, *J. Heterocycl. Chem.*, **13**, 179 (1976).
16) M. Araki, Y. Kawazoe, and C. Nagata, *Chem. Pharm. Bull.*, **17**, 1344 (1969).

stirring for 1 hr, the reaction mixture was poured into 20 ml of ice-water, stirred for 1 hr, and made neutral with $NaHCO_3$. The neutralized solution was extracted 5 times with 20 ml of $CHCl_3$ each. The extract was washed with a small volume of sat. NaCl solution and dried over $MgSO_4$. Concentration of the solution *in vacuo* gave a yellow viscous oil. IR: ν_{max}^{film} (cm^{-1}); 1773, 1203, and 768. PMR: δ (Me_2SO-d_6); 8.83 (1H, d, J=5.5 Hz for C_2–H), 8.05 (2H, d, J=9 Hz for C_5–H and C_8–H), 7.77 (1H, t, J=9 Hz for C_7–H), 7.60 (1H, t, J=9 Hz for C_6–H), 7.36 (1H, d, J=5.5 Hz for C_3–H), 3.30 (3H, s, for N–Me), and 2.17 (3H, s, for OAc). MS: *m/e*, 216 (M^+). When this oil was warmed at 60° in 1 N HCl for 1 hr, the residue obtained on evaporation of the solution was the almost pure HCl salt of V.

Preparation of IV——The method was very similar to that reported by Sato *et al.*[11] Compound VII (0.10 g) was dissolved in a small volume of MeOH and hydrogenated in an H_2 atmosphere in the presence of 50 mg of 5% Pd–C. When one equivalent of H_2 (0.86 ml) had been absorbed, the reaction was interrupted. After removing the catalyst, the reaction mixture was concentrated and the residue was chromatographed on a silica gel column with $CHCl_3$. The main fraction eluted with $CHCl_3$ was concentrated and recrystallized from CH_2Cl_2–Et_2O to give 30 mg of yellow needles. mp 175—178 (dec.). *Rf* value (silica gel TLC) with 15% MeOH–$CHCl_3$: 0.45. IR: ν_{max}^{KBr} (cm^{-1}); 3225, 1730, 1648, and 1256. PMR: δ (Me_2SO-d_6); 10.95 (1H for NH), 8.06 (1H, d, J=8 Hz for C_2–H), 7.51 (1H, t, J=7.5 Hz for C_7–H), 7.30 (2H, d, J=7.5 Hz for C_5–H and C_8–H), 7.18 (1H, t, J=7.5 Hz for C_6–H), 6.12 (1H, d, J=8 Hz for C_3–H), and 2.17 (3H, s, for OAc). This was hydrolyzed with 1 N HCl to give the HCl salt of VI[10] in quantitative yield.

Quantitative Analysis of CO_2——The quantitative analysis of CO_2 evolved by the treatment of 100 mg of I with 1.0 ml of Ac_2O was carried out by the EDTA–chelatometric titration reported by Schwarzenbach.[17]

Gas-Chromatographic Detection of the Methane evolved——The gas evolved was analyzed with a JGC-100 (JEOL) apparatus: column, Porapak Q; carrier gas, N_2; detector, FID; column temperature, 38°; t_R, 1.2 min. Ethane and higher homologs were not detected.

Quantitative Analysis of the HCHO produced by Hydrolysis of the Acetylation Mixture——Acetylation of 100 mg of I with 0.9 ml of Ac_2O was carried out at an appropriate temperature for 30 min. A part of this reaction mixture (0.10 ml) was sealed in a tube together with 3.7 ml of H_2O and 0.20 ml of conc. HCl and the tube was warmed at 80° for 2 hr. After ice-cooling of the reaction mixture, the content was diluted 50 times with H_2O. Colorimetric analysis of HCHO was done with 2.0 ml of this diluted solution using Formaldehyde-Test Wako,[13] which involved the condensation of HCHO with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole in 5 N NaOH, followed by its oxidation with KIO_4 and then measurement of the optical density at 550 nm. The amount of HCHO was obtained from the OD values thus obtained by reference to the calibration plots obtained for standard HCHO solutions. It is worth noting that a small amount of HCHO (less than 4% in all the cases examined) was detected without acid-hydrolysis of the acetylation products. It is possible that III is hydrolyzed in part even on shaking of the reaction mixture diluted with CH_2Cl_2, or alternatively that the acetylation process studied here is accompanied by HCHO formation due to some minor process.[15,18]

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