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Preparation of Some Acetylated, Reduced and Oxidized Derivatives of 2,4-Diaminotoluene and 2,6-Dinitrotoluene

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4-Acetylamino-2-hydroxylaminotoluene (4AA2HAT) and 2-hydroxylamino-6-nitrotoluene (2HA6NT) were prepared from 4-acetylamino-2-nitrotoluene (4AA2NT) and 2,6-dinitrotoluene (2,6-DNT), respectively, by reduction with zinc dust and NH_4Cl. 2,6-Dinitrobenzylalcohol (2,6-DNB) and 2,6-dinitrobenzoic acid (2,6-DNBA) were prepared from 2,6-dinitrobenzaldehyde (2,6-DNBA1) by reduction with $NaBH_4$ and by oxidation with $KMnO_4$, respectively. 2-Acetylamino-4-aminobenzoic acid (2AA4ABA) was prepared from 4-nitroanthranilic acid (4NAA) by acetylation followed by reduction with $NaBH_4$ and Pd–C. 2,4-Diacetylaminobenzoic acid (2,4-DAABA) was prepared from 4NAA by reduction with $NaBH_4$ and Pd–C followed by acetylation. 4-Acetylamino-2-aminobenzoic acid (4AA2ABA) was prepared from 4NAA by reduction and acetylation followed by chelation with $Cu(AcO)_2$.

Keywords——4-acetylamino-2-hydroxylaminotoluene; 2-hydroxylamino-6-nitrotoluene; 2,6-dinitrobenzylalcohol; 2,6-dinitrobenzoic acid; 2-acetylamino-4-aminobenzoic acid; 4-acetylamino-2-aminobenzoic acid; 2,4-diacetylaminobenzoic acid; carcinogenicity; mutagenicity ; preparation

2,4-Diaminotoluene (2,4-DAT)[1] and 2,6-dinitrotoluene (2,6-DNT),[2] intermediates in polyurethane foam and elastomer production, have carcinogenic and/or mutagenic effects, requiring metabolic activations. Acetylation, oxidation, reduction or mixed reactions are possible metabolic routes of 2,4-DAT and 2,6-DNT, and the purpose of this study was to prepare various acetylated, oxidized and reduced derivatives of these compounds for determining spectral and other physicochemical characteristics (see Experimental). These data are required to identify metabolites of 2,4-DAT and 2,6-DNT as well as to help define the relationship of chemical structure to the carcinogenicity and mutagenicity.

Two hydroxylaminonitrotoluenes, 2-hydroxylamino-6-nitrotoluene (2HA6NT) and 4-acetylamino-2-hydroxylaminotoluene (4AA2HAT), were prepared from 2,6-DNT and 4-acetylamino-2-nitrotoluene (4AA2NT), respectively, by reaction with zinc dust and NH_4Cl as described for the preparations of 2(4)-hydroxylamino-4-(2)-nitrotoluenes.[3] These hydroxylamino compounds were stable on storage at room temperature, but the solutions exposed to air were unstable due to a tendency to form the azoxy compounds. Attempts to prepare 2-acetylamino-4-hydroxylaminotoluene were not successful. Two oxidized compounds of 2,6-DNT, 2,6-dinitrobenzylalcohol (2,6-DNB) and 2,6-dinitrobenzoic acid (2,6-DNBA), were prepared from 2,6-dinitrobenzaldehyde (2,6-DNBAl) by reaction with $NaBH_4$ at room temperature for 1 h and by reaction with $KMnO_4$ at 60 °C for 1 h without particular difficulty. Three acetylaminobenzoic acids, 2-acetylamino-4-aminobenzoic acid (2AA4ABA), 4-acetylamino-2-aminobenzoic acid (4AA2ABA) and 2,4-diacetylaminobenzoic acid (2,4-DAABA) were prepared stepwise from 4-nitroanthranilic acid (4NAA) as follows (Chart 1): 2AA4ABA was prepared from 4NAA by acetylation followed by reduction with $NaBH_4$ and Pd–C; 2,4-DAABA was prepared from 2,4-diaminobenzoic acid (2,4-DABA)[1a] by reaction

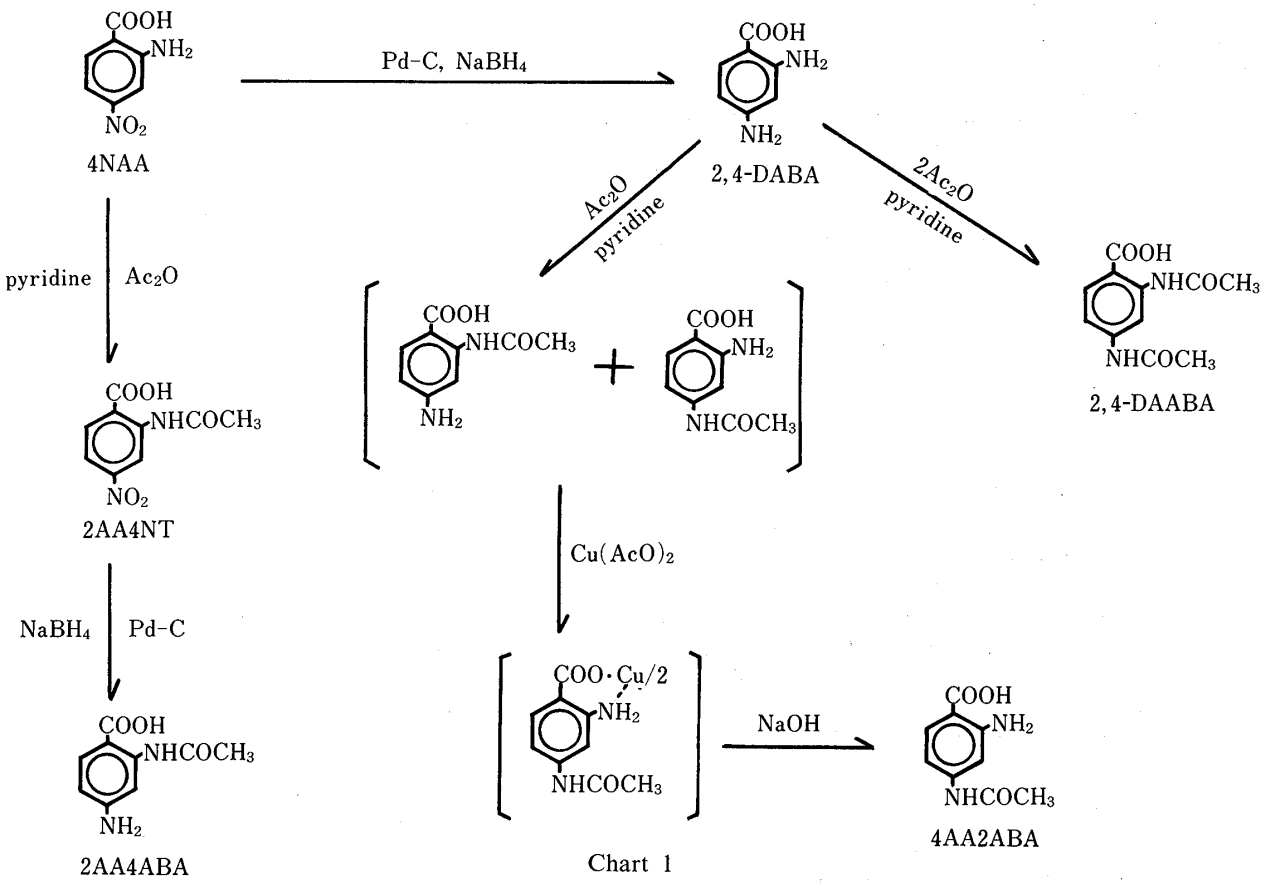

Chart 1

with 2 mol of Ac_2O in pyridine; and 4AA2ABA was prepared by the hydrolysis of the copper salt of 4AA2ABA isolated from the reaction mixture containing two acetylaminobenzoic acids, formed by the reaction of 2,4-DABA with Ac_2O in pyridine.

Experimental

All melting points were obtained on a Yanaco micro-melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded with JASCO IRA-2. Mass spectra (MS) were recorded on a JMS D-200 at 75 eV ionizing potential. ^{1}H-Nuclear magnetic resonance (^{1}H-NMR) were recorded on a Varian XL-200 and were reported as parts per million downfield from Me_4Si ($\delta=0$). Abbreviations used: s=singlet, d=doublet, t=triplet, br=broad, m=multiplet. Column chromatography was performed with silica gel (Wakogel C-200). 4-Acetylamino-2-nitrotoluene (4AA2NT)[4] and 2,4-diaminobenzoic acid (2,4-DABA)[1a] were prepared as previously described.

2-Hydroxylamino-6-nitrotoluene (2HA6NT)——A solution of NH_4Cl (0.1 mol, 5.3 g) in H_2O (20 ml) was added to 2,6-DNT (0.08 mol, 14.1 g) dissolved in ethanol (400 ml). The mixture was stirred, and zinc dust (0.12 mol, 8.0 g) was added in portions. Stirring was continued at 40 °C for 30 min after all the zinc dust had been added. The solution was filtered and the filtrate was evaporated *in vacuo*. The residue was chromatographed on silica gel (150 g) and eluted with ether–methanol (99:1). The residue from the eluate, after recrystallization from chloroform, gave 2.2 g of 2HA6NT as pale yellow plates, mp 121 °C. The yield was 16.9%. *Anal.* Calcd for $C_7H_8N_2O_3$: C, 50.00; H, 4.80; N, 16.66. Found: C, 49.95; H, 4.89; N, 16.98, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560 (NH), 3310 (OH), 1525 (NO_2). MS *m/z*: 168 (M^+), 121, 77 (base peak). ^{1}H-NMR (in $CDCl_3$)δ: 2.25 (3H, s, CH_3), 5.25 (1H, br, OH), 7.28—7.56 (3H, m, aromatic).

4-Acetylamino-2-hydroxylaminotoluene (4AA2HAT)——4AA2HAT (2.5 g, mp (dec.) 243 °C) was prepared from 4AA2NT (0.11 mol, 20 g) by reaction with zinc dust (0.13 mol, 8.5 g) and NH_4Cl (0.11 mol, 5.8 g) in ethanol (500 ml) under conditions similar to those described above, with ether–methanol (97:3) as the solvent for column chromatography. The yield was 12.6%. *Anal.* Calcd for $C_9H_{12}N_2O_3$: C, 59.99; H, 6.71; N, 15.54. Found: C, 60.18; H, 6.73; N, 15.24 IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1655, 1543 (NHCO). MS *m/z*: 180 (M^+), 164, 122, 121 (base peak). ^{1}H-NMR (in$(CD_3)_2SO$) δ: 2.02 (6H, s, CH_3 and $COCH_3$), 6.86 (1H, d, J=8.0 Hz, aromatic 6-H), 7.06 (1H, dd, J=8.0, 2.0 Hz, aromatic 5-H), 7.28 (1H, d, J=2.0 Hz, aromatic 3-H), 7.92 (1H, s, OH), 8.26 (1H, s, NHOH), 9.76 (1H, s, NHCO).

2,6-Dinitrobenzylalcohol (2,6-DNB)——A solution of $NaBH_4$ (0.08 mol, 2.9 g) in methanol (20 ml) was added

dropwise to a cooled solution of 2,6-DNBAl (0.05 mol, 10 g) in methanol. The mixture was stirred at room temperature for 1 h, then filtered. The filtrate was evaporated *in vacuo*. The residue was chromatographed on silica gel and eluted with chloroform–methanol (90 : 10). The residue from the eluate, after recrystallization from ethanol, gave 3.0 g of 2,6-DNB as pale yellow plates, mp 96 °C. The yield was 30.0%. *Anal.* Calcd for $C_7H_6N_2O_5$: C, 42.43; H, 3.05; N, 14.14. Found: C, 42.63; H, 3.09; N, 14.04. IR ν_{max}^{KBr} cm^{-1}: 3200 (OH), 1520 (NO_2). MS *m/z*: 197 ($M-1^+$), 120 (base peak). ^{1}H-NMR (in $(CD_3)_2SO$) δ: 4.81 (2H, d, J=4.0 Hz, CH_2), 5.74 (1H, br, OH), 7.80 (1H, d, J=9.0 Hz, aromatic 4-H), 8.22 (2H, d, J=9.0 Hz, aromatic 3-H and 5-H).

2,6-Dinitrobenzoic Acid (2,6-DNBA)——A solution of $KMnO_4$ (0.025 mol, 4 g) in H_2O (40 ml) was added dropwise to a stirred suspension of 2,6-DNBAl (0.01 mol, 1.9 g) in H_2O (40 ml). The mixture was stirred at 60 °C for 1 h, then acidified, and extracted with ether. The ether extract was washed with water, dried (Na_2SO_4) and concentrated *in vacuo*. Recrystallization of the residue from ether gave 1.3 g of 2,6-DNBA as colorless needles, mp (dec.) 208 °C. The yield was 63.3%. *Anal.* Calcd for $C_7H_4N_2O_6$: C, 39.64; H, 1.90; N, 13.21. Found: C, 39.48; H, 2.00; N, 13.44. IR ν_{max}^{KBr} cm^{-1}: 1730 (CO), 1545 (NO_2). MS *m/z*: 212 (M^+), 168, 122, 94, 75 (base peak). ^{1}H-NMR (in $(CD_3)_2SO$) δ: 8.02 (1H, t, J=9.0 Hz, aromatic 4-H), 8.58 (2H, d, J=9.0 Hz, aromatic 3-H and 5-H).

2-Acetylamino-4-nitrobenzoic Acid (2AA4NBA)——A mixture of 4NAA (0.01 mol, 1.8 g), pyridine (0.19 mol, 15 g) and Ac_2O (0.05 mol, 5 g) was left to stand at room temperature overnight. The reaction mixture was poured into ice and water. The precipitate obtained by acidification of the reaction mixture with 5% HCl was collected. Recrystallization of the precipitate from ethanol gave 2.1 g of 2AA4NBA, as colorless needles, mp 200 °C. The yield was 92.1%. *Anal.* Calcd for $C_9H_8N_2O_5$: C, 48.22; H, 3.60; N, 12.50. Found: C, 48.38; H, 3.58; N, 12.67. IR ν_{max}^{KBr} cm^{-1}: 3420 (NH_2), 1655, 1525 (NHCO).

2-Acetylamino-4-aminobenzoic Acid (2AA4ABA)——2AA4NBA (0.01 mol, 2 g) was added in portions to a suspension of 5% Pd–C (0.2 g) and $NaBH_4$ (0.04 mol, 1.5 g) in H_2O (50 ml). The mixture was left to stand at room temperature for 1 h, then filtered, and the filtrate was neutralized with 5% HCl. The precipitate thus obtained was recrystallized from ethanol, giving 1.3 g of 2AA4ABA as colorless needles, mp 217 °C. The yield was 69.7%. *Anal.* Calcd for $C_9H_{10}N_2O_3$: C, 55.67; H, 5.19; N, 14.43. Found: C, 55.89; H, 5.11; N, 14.35. IR ν_{max}^{KBr} cm^{-1}: 3500 (NH_2), 1650, 1530 (NHCO). MS *m/z*: 194 (M^+), 176, 152, 134 (base peak). ^{1}H-NMR (in $(CD_3)_2SO$) δ: 2.04 (3H, s, CH_3), 6.60 (1H, dd, J=9.5, 2.0 Hz, aromatic 5-H), 7.22 (1H, d, J=2.0 Hz, aromatic 3-H), 7.62 (1H, d, J=9.5 Hz, aromatic 6-H), 8.50 (1H, br, OH).

2,4-Diacetylaminobenzoic Acid (2,4-DAABA)——A mixture of 2,4-DAB (6.6 mol, 1.0 g), pyridine (0.19 mol, 15 g) and Ac_2O (0.05 mol, 5 g) was left to stand at room temperature overnight. The reaction mixture was poured into ice and water. The precipitate obtained by acidification of the mixture with 5% HCl was collected. Recrystallization of the precipitate from ethanol gave 1.2 g of 2,4-DAABA as colorless needles, mp 248 °C. The yield was 77.4%. *Anal.* Calcd for $C_{11}H_{12}N_2O_4$: C, 55.93; H, 5.12; N, 11.86. Found: C, 56.04; H, 5.07; N, 11.71. IR ν_{max}^{KBr} cm^{-1}: 1670, 1550 (NHCO). MS *m/z*: 236 (M^+), 194, 176, 152, 134, 107, 43 (base peak). ^{1}H-NMR (in $(CD_3)_2SO$) δ: 2.08 (3H, s, CH_3), 2.15 (3H, s, CH_3), 7.64 (1H, dd, J=10.0, 2.0 Hz, aromatic 3-H), 10.32 (1H, s, NH), 11.29 (1H, s, NH).

4-Acetylamino-2-aminobenzoic Acid (4AA2ABA)——A mixed solution of Ac_2O (0.02 mol, 1.9 g) and AcOH (0.02 mol, 1.2 g) was added to 2,4-DABA (0.02 mol, 2.8 g) dissolved in Ac_2O (0.1 mol, 10 g). The mixture was left to stand at room temperature overnight, then neutralized with 10% NH_4OH. A copper salt (2.5 g) obtained by addition of $Cu(AcO)_2$ (3.5 mmol, 0.7 g) dissolved in H_2O (5 ml) to the neutralized solution was collected, and suspended in water (10 ml). The suspension was alkalized with 10% NaOH, and filtered to remove copper hydroxide liberated. The precipitate obtained by acidification of the filtrate with 5% HCl was recrystallized from ethanol, giving 1.5 g of 4AA2ABA as colorless needles, mp (dec.) 207 °C. The yield was 42.0%. *Anal.* Calcd for $C_9H_{10}N_2O_3$: C, 55.67; H, 5.19; N, 14.43. Found: C, 55.38; H, 5.24; N, 14.35. IR ν_{max}^{KBr} cm^{-1}: 3470 (NH_2), 1665, 1500 (NHCO). MS *m/z*: 194 (M^+), 176, 152, 134 (base peak), 107. ^{1}H-NMR (in $(CD_3)_2SO$) δ: 2.04 (3H, s, CH_3), 6.60 (1H, dd, J=9.5, 2.0 Hz, aromatic 5-H), 7.22 (1H, d, J=2.0 Hz, aromatic 3-H), 7.62 (1H, d, J=9.5 Hz, aromatic 6-H), 8.50 (1H, br, OH).

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