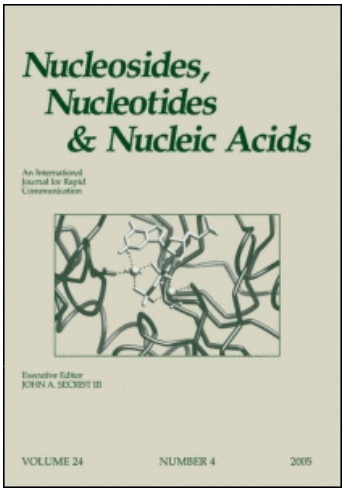

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SYNTHESIS AND CHARACTERIZATION OF ADENOSINE ADDUCTS OF ARYLAMINES

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SYNTHESIS AND CHARACTERIZATION OF ADENOSINE ADDUCTS OF ARYLAMINES

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ABSTRACT

The synthesis of adducts of arylamines with adenosine are reported.

Key Words: Adenosine adducts; Synthesis; Characterization

Aromatic amines and nitroarenes are very important industrial intermediates. Several scientists[1,2] have extensively studied the covalent binding of a number of aromatic amines to DNA and identified various products by studying *in vivo* and *in vitro* reactions of esters of aryl hydroxylamine.[3] Aromatic amines can be metabolised to highly reactive N-hydroxy aromatic amines. These are highly reactive intermediates which are responsible for the genotoxic effects of this class of compounds. DNA adducts of arylamines have been found in several organs of exposed experimental animals. With the advancement in analytical technique, scientists have quantified the DNA adducts of arylamines in human tissue. Therefore, the reference standards of DNA adducts have been synthesized. The deoxyguanosine adducts of arylamines by synthesis are known[4–7] but not the adenosine adducts. Hence, this study of the reaction of arylamines with adenosine is undertaken.

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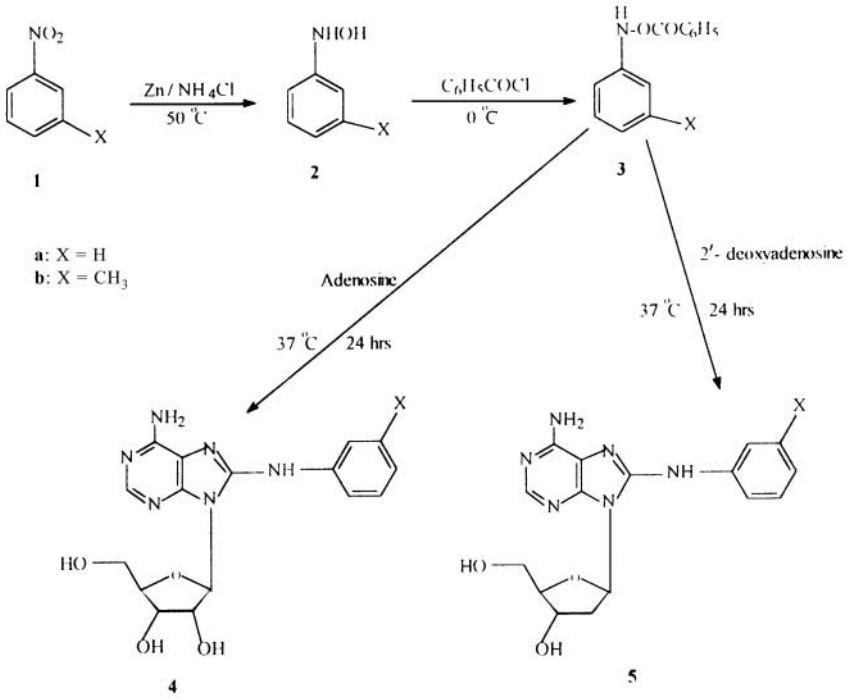

Scheme 1.

The synthetic route was based on the observations by Lobo et al.[8,9] on the reaction of N-benzoyloxy-N-arylamines with nucleophiles under basic conditions. The DNA adducts of arylamines are synthesized from the corresponding N-benzoyloxy arylamines and nucleoside. These N-benzoyloxy compounds are obtained by treating the corresponding N-hydroxy arylamines with benzoyl chloride below 0°C. The freshly prepared solution of the N-benzoyloxy-N-arylamine was added to the aqueous solution of adenosine. The resulting mixture was purified by solvent extraction (Sch. 1) and are characterized by NMR and IR spectral data. NMR characterization was performed with d_6-DMSO as solvent. The peaks were assigned according to the spectra of adenosine. In the ^{1}H NMR spectra of the adenosine adducts, the peak of 8H disappears and the NH proton of the arylamine shifted downfield by 4 ppm. The C-8 peak for unmodified adenosine is 150 ppm where as the C-8 peak shifted to 160–164 ppm for the adducts.

^{13}C NMR spectra reveals that the C-2′ peak of the sugar in the adducts is shifted slightly upfield by 1 ppm and the resonance of C-5 is shifted by 3 ppm. It is important to note that, the C-1 peak of arylamine is shifted upfield by about 8 ppm. The UV spectra (methanol/water) of adenosine adducts were shifted to longer wavelength by 10–45 nm compared to adenosine alone.

The adducts formation of aniline and 3-methyl aniline with adenosine were carried out to know whether the adduct can be synthesized or not, so that, the reaction pathway of forming the adduct can be deducted. The possibility of synthesizing the 2′-deoxyadenosine adducts would be similar and these can be used to develop ^{32}P post-labeling and mass spectrometric methods for low level detection in biological materials. The synthetic route for the synthesis of 2′-deoxyadenosine adducts with aniline and 3-methyl aniline (**5**) is similar to that of **4**. However, we are interested in the synthesis of **4** as reference standard adducts. The characterization of **5** is also under progress. It is important that the mechanism of formation of these adducts will be known so that, the required reference adducts can be synthesized for quantification.

EXPERIMENTAL

Synthesis and Characterization of N-(Adenosin-8-yl)-aniline (4a)

To a stirred solution of nitro benzene (**1a**, 8 mmol, 0.985 g), ammonium chloride (0.5 g) and ammonium hydroxide (2 mL) were added in water (40 mL). The solution was heated to 50°C. Zinc (17.6 mmol, 1.1509 g) was added in such a way that the temperature does not rise above 60°C. Reaction was monitored by thin layer chromatography (TLC). After the reduction of nitro benzene, reaction mixture was cooled to room temperature, and filtered. The filtrate was saturated with sodium chloride and cooled in ice bath. This was extracted with ether:benzene (1:1 v/v, 4 × 35 mL). The organic layer was dried over anhydrous magnesium sulphate and the solvent was evaporated. The product, N-phenyl hydroxylamine (**2a**), so obtained was further purified by washing with n-hexane followed by cyclohexane. The pure hydroxylamine (**2a**) was characterized by m.p., NMR and IR, which is in confirmation with the reported data.[10]

To a solution of aryl hydroxylamine (**2a**, 2 mmol, 0.2183 g) in dry ether (cooled in ice bath), benzoyl chloride (2.4 mmol, 0.3374 g) in dry ether (ice cold) was added. The rate of addition of benzoyl chloride was such that the temperature does not rise above 0°C. The solution was stirred vigorously for 3 h, and the reaction mixture was stirred for another 30 min so that it attains room temperature. Finally, this was neutralized with saturated solution of sodium bicarbonate. The organic layer was dried over anhydrous sodium acetate and the solvent was evaporated. The product was purified by recrystallization with water. Pure N-benzoyloxy-N-phenyl hydroxylamine (**3a**) was characterised by mp, NMR and IR. Data obtained are in good agreement with the reported data.[10]

To the ester (**3a**, 0.206 mmol, 43.98 mg) in chloroform:alcohol:water (7:3:4 v/v, 35 mL), was added triethylamine (0.2 mL). The temperature was

maintained at 37°C. Adenosine (0.206 mmol, 55.05 mg) was added to a stirred reaction mixture and the reaction was monitored by TLC. After 24 h, the reaction mixture was evaporated to dryness and redissolved in water. This was extracted with diethyl ether (4×50 mL) and n-butanol (3×25 mL). The n-butanol extract was concentrated *in vacuo*. Yield = 4.4 mg (5.9%). Analysis of the product (**4a**) by HPLC [Chloroform:Methanol (50:50 v/v), $\lambda = 264$ nm] showed 98.7% purity. This was characterised by ^{1}H and ^{13}C NMR.

Elemental analysis of **4a**: (Calcd.) C 53.63%, H 5.06%, N 23.45% (found) C 53.66%, H 5.07%, N 23.42%.

IR of **4a** (in cm^{-1}) 672.4, 1087.7, 1151.6, 1221.7, 1309.0, 1436.8, 1643.3, 1697.5, 3184.2, 3341.4, 3508.9.

^{1}H NMR of **4a** (400 MHz, d_6-DMSO):

δ (ppm): 3.72 (m, 2H, 5′H); 3.91 (m, 1H, 4′H); 3.96 (m, 2H, NH_2); 4.24 (m, 1H, 3′H); 4.51 (m, 1H, 2′H); 4.62 (s, 1H, 2′OH); 4.69 (s, 1H, Ar-NH); 4.81 (s, 1H, 5′OH); 4.94 (s, 1H, 3′OH); 6.18 (q, 1H, 1′H); 6.62 (d, 2H, Ar-2H, Ar-6H); 6.79 (t, 1H, Ar-4H); 7.02 (t, 2H, Ar-3H, Ar-5H); 8.42 (d, 1H, 2H).

^{13}C NMR of **4a** (75.5 MHz, d_6-DMSO):

δ (ppm): 63.89 (5′C); 72.93 (3′C); 77.86 (2′C); 82.27 (1′C); 92.06 (4′C); 119.34 (Ar-2C, Ar-6C); 120.78 (Ar-4C); 122.32 (C5); 128.17 (Ar-3C, Ar-5C); 141.24 (Ar-1C); 147.76 (4C); 151.39 (2C); 153.19 (6C); 161.23 (8C).

Synthesis and Characterisation of N-(Adenosin-8-yl)-3-methylaniline (4b)

The procedure for the synthesis of **4b** is similar to that of **4a**. In this case, 8 mmol of **1b**, 2 mmol of **2b** and 0.206 mmol of **3b** were used to get 6.7 mg (8.7%) of the product **4b**. Analysis of the product (**4b**) by HPLC [Chloroform:Methanol (50:50 v/v), $\lambda = 264$ nm] showed 99.4% purity. This was characterised by ^{1}H and ^{13}C NMR.

Elemental analysis of **4b**: (Calcd.) C 54.83%, H 5.41%, N 22.57% (found) C 54.85%, H 5.44%, N 22.55%.

IR of **4b**(in cm^{-1}) 669.1, 1091.9, 1152.2, 1224.8, 1304.7, 1439.3, 1648.4, 1691.5, 3187.6, 3345.3, 3511.7.

^{1}H NMR of **4b** (400 MHz, d_6-DMSO):

δ (ppm): 2.28 (s, 3H, Ar-CH_3); 3.77 (m, 1H, 5′H); 3.86 (m, 1H, 4′H); 4.01 (m, 2H, NH_2); 4.29 (m, 1H, 3′H); 4.47 (m, 1H, 2′H); 4.66 (s, 1H, 2′OH); 4.76 (s, 1H, Ar-NH); 4.80 (s, 1H, 5′OH); 4.93 (s, 1H, 3′OH); 6.17 (q, 1H, 1′H); 6.71 (d, 1H, Ar-2H); 6.74 (d, 1H, Ar-6H); 6.79 (d, 1H, Ar-4H); 7.11 (d, 1H, Ar-5H); 8.44 (d, 1H, NH).

^{13}C NMR of **4b** (75.5 MHz, d_6-DMSO):

δ (ppm): 19.24 (Ar-CH_3); 63.92 (5′C); 72.92 (3′C); 77.96 (2′C); 82.57 (1′C); 92.16 (4′C); 118.72 (Ar-6C); 121.38 (Ar-4C); 122.37 (5C); 123.99 (Ar-3C);125.94 (Ar-5C); 128.41 (Ar-2C); 142.27 (Ar-1C); 147.77 (4C); 151.32 (2C); 156.71 (6C); 163.89 (8C).

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