Synthesis and Characterization of the Food-Derived Carcinogens 2-(Hydroxylamino)-α-carboline and 2-(Hydroxylamino)-3-methyl-α-carboline

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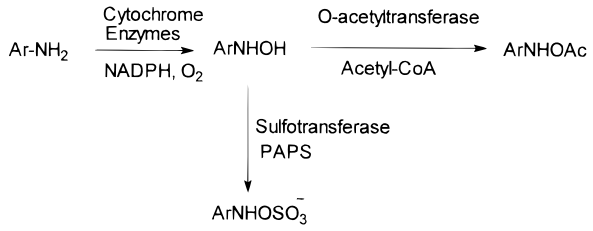
Received September 25, 1997

Introduction

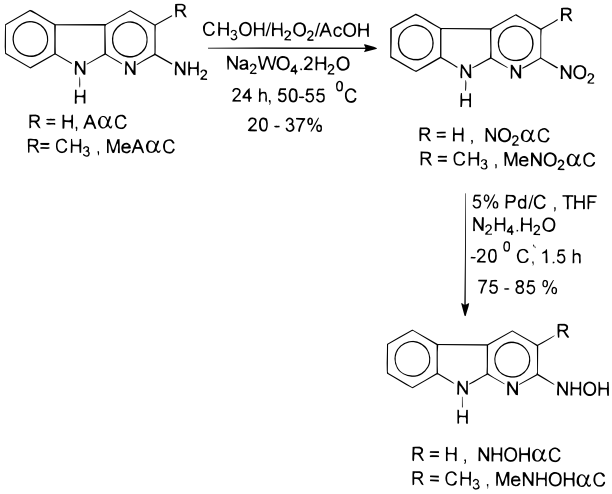
2-Amino-α-carboline (AαC) and 2-amino-3-methyl-α-carboline (MeAαC) are representative of a large group of heterocyclic amines formed by pyrolysis of proteins and amino acid mixtures.[1] These materials have also been isolated in parts per billion concentrations from broiled and fried meats, fish, and protein-rich plant material and have been shown to be mutagenic to *Salmonella* in the presence of rat liver homogenates.[1] The compounds are also carcinogenic in mice and rats, with cancers of the liver and intestine being most prominent.[2] These heterocyclic amines are now considered to be probable human carcinogens.[2,3] Average daily intake of heterocyclic amines is diet-dependent but appears to range from 0.1 to 15 μg/person in most developed countries.[4] At these levels it is believed that heterocyclic amines constitute a significant cancer risk to human populations.[5]

The heterocyclic amines are promutagens/procarcinogens that require oxidative metabolism for activation. The likely activation processes are shown in Scheme 1.[6] Although the heterocyclic hydroxylamines are central to this process, in many cases these compounds have not been isolated or are incompletely characterized in the literature. In the case of the α-carbolines, the synthesis of 2-(hydroxylamino)-α-carboline (NHOHαC) has recently been reported from 2-nitro-α-carboline (NO_2αC) with few experimental details and incomplete product characterization.[7] The nitro derivative of MeAαC, $MeNO_2$αC, has also been reported in the literature, but attempts to synthesize the hydroxylamine, MeNHOHαC, from the nitro compound were unsuccessful.[8] Since we have embarked on a study of the chemical basis of heterocyclic amine carcinogenesis, we needed to develop reliable procedures for the synthesis and purification of the hydroxylamine derivatives of the heterocyclic amines. Herein we report the synthesis and purification of NHOHαC and MeNHOHαC and complete spectral characterization of both compounds.

Scheme 1



Scheme 2



Results and Discussion

The parent amines (AαC and MeAαC) were synthesized, with minor modifications, from published procedures.[9] The amines are also commercially available, but expensive.[10] Our synthetic procedures for the formation of the hydroxylamines are outlined in Scheme 2.

Many different routes were attempted for the synthesis of NO_2αC and $MeNO_2$αC, but most failed, presumably

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S0022-3263(97)01786-6 CCC: $15.00
Published on Web 01/14/1998

due to the relatively strongly acidic conditions employed. This oxidation step has proven to be a significant synthetic problem in the chemistry of these carcinogens.[7,8,11,12] We tried to take advantage of a procedure using mildly acidic conditions published by Grivas.[11] In this procedure the amine is dissolved in a 50:50 mixture of DMF/AcOH, which is then added to a stirred concentrated solution of $NaNO_2$ at room temperature. This method provides NO_2αC and $MeNO_2$αC in very low yields (less than 10%). Later, our attention was focused on a procedure, reported by Saito et al., in which Trp-P-2 (a heterocyclic amine carcinogen structurally similar to AαC) was oxidized to its nitro derivative.[12] With a few modifications, this method provided $MeNO_2$αC and NO_2αC in 20−37% yield. The yields are low but are superior to the other methods in our hands.

Both $MeNO_2$αC and NO_2αC were synthesized in the following manner: The amine (AαC or MeAαC) was dissolved in a mixture of CH_3OH and acetic acid (500:1, v/v). To this solution were added 30% H_2O_2 and $Na_2WO_4{\cdot}2H_2O$. The mixture was stirred for 24 h at 50−55 °C. Reaction progress was monitored by HPLC. After workup and column chromatography, a yellow solid product was isolated. Complete spectral characterization of these compounds is provided in the Experimental Section and Supporting Information.

^{1}H NMR spectra of $MeNO_2$αC and NO_2αC show the disappearance of the singlet for NH_2 protons observed near 6.0 ppm for AαC and MeAαC. All aromatic proton peaks are shifted downfield due to the deshielding effect of the nitro group. In the case of $MeNO_2$αC, there is also a distinct shift of the methyl group from 2.15 to 2.58 ppm.

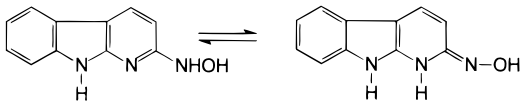
Reduction of $MeNO_2$αC and NO_2αC by $N_2H_4{\cdot}H_2O$ using a modification of a procedure reported by Westra[13] generated NHOHαC and MeNHOHαC in good yield. These hydroxylamines are not very stable neat or in solution at room temperature but are stable enough in DMSO that ^{1}H and ^{13}C NMR spectra can be obtained. No decomposition of these products was observed when the hydroxylamines were kept under nitrogen at −80 °C for a period of 60 days. Both compounds are subject to air oxidation in solution and also undergo apparent acid-catalyzed decomposition in H_2O.

Generally, ca. 100 mg of the nitro compound was dissolved in 100 mL of dry, peroxide-free (freshly distilled) THF in the presence of 135 mg of 5% Pd/C at −20 °C. Then 200 μL of $N_2H_4{\cdot}H_2O$ was added to the mixture in four portions of 50 μL each. About 15 min after each addition of $N_2H_4{\cdot}H_2O$, the temperature was allowed to increase from −20 to 0 °C and remain at 0 °C for ca. 1 min. A sample was removed for HPLC, the temperature was reduced again to −20 °C, and the next portion of $N_2H_4{\cdot}H_2O$ was added to the mixture. After 1.5 h the Pd/C was filtered off, and the solvent was removed by rotary evaporation. The product was rapidly recrystallized from EtOAc and hexanes at −20 °C to provide a 75−85% yield of the hydroxylamine.

Further reduction of the hydroxylamine to the amine was observed when the reaction was performed at uniform higher temperatures (−5 to 0 °C). Mixtures of both NHOHαC and AαC were obtained, even with shorter reaction times and reduced amounts of $N_2H_4{\cdot}H_2O$.

NHOHαC and MeNHOHαC were fully characterized by ^{1}H,^{13}C, DEPT, NOESY, and COSY NMR, MS, and IR. Copies of ^{1}H and ^{13}C NMR spectra and tables of NOESY and COSY NMR correlations are provided in the Supporting Information.

The ^{1}H NMR spectra of NHOHαC and MeNHOHαC show the appearance of two new exchangeable protons at 8.3−8.8 ppm that correspond to the NH and OH protons of the hydroxylamines. All aromatic protons are shifted upfield as expected. In the case of MeNHOHαC, there is also an upfield shift of the methyl group from 2.58 to 2.17 ppm. ^{1}H and ^{13}C NMR spectra of NHOHαC and MeNHOHαC are nearly identical with those of the corresponding amines, AαC and MeAαC, with the exception of the NHOH protons of the hydroxylamines and the NH_2 protons of the amines. This indicates that the hydroxylamine tautomer, not oxime tautomer (eq 1), is the correct structural representation for these compounds.



(1)

We are attempting to improve the yields of the nitro compounds and are testing the applicability of these procedures to the synthesis of hydroxylamine derivatives of the other carcinogenic heterocyclic amines. The results of these studies and characterization of the aqueous solution chemistry of the hydroxylamines and their carboxylic or sulfuric acid esters will be presented elsewhere.

Experimental Section

Caution: All compounds reported in this section are known[8] or likely mutagens and should be treated as probable human carcinogens.

2-Nitro-α-carboline (2-Nitro-9*H*-pyrido[2,3-*b*]indole) and 3-Methyl-2-nitro-α-carboline (3-Methyl-2-nitro-9*H*-pyrido-[2,3-*b*]indole). General Procedure. In a 250-mL round-bottom flask, 0.5 mmol of AαC (92 mg) or MeAαC (99 mg) was dissolved in a mixture of 50 mL of MeOH and 0.1 mL of concentrated acetic acid. To this solution was added 50 mL of 30% H_2O_2. While stirring, 1.0 g (3 mmol) of $Na_2WO_4{\cdot}2H_2O$ was added to the solution, and the temperature was elevated and kept at 50−55 °C for 24 h. To maintain solution homogeneity, 25 mL of MeOH was added to the reaction mixture ca. 5 h after addition of the $Na_2WO_4{\cdot}2H_2O$. The reaction progress was monitored by HPLC using a C_{18} reverse-phase column at λ = 255 nm; 60:40 MeOH/H_2O, 0.05 M 1:1 KOAc/AcOH buffer was used as the mobile phase. After 24 h, the MeOH was removed by rotary evaporation at room temperature and the solution was neutralized with 10% $NaHCO_3$. The product was extracted three times with 50-mL portions of $CHCl_3$. The organic layer was washed with 5% $NaHCO_3$ and distilled water and dried over $MgSO_4$. After filtration, the $CHCl_3$ was removed by rotary evaporation and the crude product was purified by column chromatography (silica gel) using 85:15 CH_2Cl_2/THF to yield 24−42 mg (20−37%) of products.

2-Nitro-9*H*-pyrido[2,3-*b*]indole (NO_2αC): mp >270 °C; IR (KBr) 3299, 1540, 1343, 1309 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6), δ 12.49 (1H, s, exchangeable), 8.87 (1H, d, J = 8.2 Hz), 8.32 (1H, d, J = 7.9 Hz), 8.19 (1H, d, J = 8.2 Hz), 7.60−7.58 (2H, m), 7.35−7.30 (1H, m); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 153.3 (C), 149.5 (C), 141.4 (C), 131.3 (CH), 129.2 (CH), 122.8 (CH), 120.9 (CH), 120.9 (C), 119.5 (C), 112.2 (CH), 109.2 (CH); high-resolution MS *m/e* 213.0536, $C_{11}H_7N_3O_2$ requires 213.0539.

3-Methyl-2-nitro-9*H*-pyrido[2,3-*b*]indole ($MeNO_2$αC): mp >270 °C; IR (KBr) 3244, 1528, 1319 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.24 (1H, s, exchangeable), 8.73 (1H, s), 8.23 (1H,

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d, J = 7.9 Hz), 7.56−7.55 (2H, m), 7.33−7.27 (1H, m), 2.58 (3H, s); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 154.1 (C), 147.9 (C), 141.3 (C), 134.0 (CH), 128.8 (CH), 122.4 (CH), 120.7 (CH), 119.8 (C), 119.3 (C), 117.0 (C), 112.1 (CH), 18.3 (CH_3); high-resolution MS *m/e* 227.0695, $C_{12}H_9N_3O_2$ requires 227.0696.

2-(Hydroxylamino)-α-carboline (2-(Hydroxylamino)-9*H*-pyrido[2,3-*b*]indole) and 2-(Hydroxylamino)-3-methyl-α-carboline (2-(Hydroxylamino)-3-methyl-9*H*-pyrido[2,3-*b*]-indole). General Procedure. In a two-neck 125-mL round-bottom flask, 100 mg of the nitro compound was dissolved in 100 mL of dry, peroxide-free THF (freshly distilled). While stirring under nitrogen, 140 mg of 5% Pd/C was added to the solution that was then cooled to −20 °C in a dry ice−ethylene glycol bath. After 10 min, 200 μL of $N_2H_4 \cdot H_2O$ in four portions of 50 μL was added to the reaction mixture by syringe over a period of 1 h. About 15 min after each addition the temperature was allowed to increase from −20 to 0 °C and remain at 0 °C for 1 min. While at 0 °C, an aliquot was taken for HPLC analysis, the temperature was lowered to −20 °C, and the next portion of $N_2H_4 \cdot H_2O$ was added to the reaction mixture. During this time, the aliquot was analyzed by HPLC at λ = 255 nm using 60:40 MeOH/H_2O, 0.05 M 1:1 KOAc/AcOH buffer and C_{18} reverse-phase column. About 1.5 h after the first addition of $N_2H_4 \cdot H_2O$, the reaction was stopped by filtering the Pd/C. The filtered solution was kept in an EtOH−dry ice bath, and then the solvent was removed by rotary evaporation at 20 °C. The product was recrystallized from EtOAc/hexanes at −20 °C to provide 70−80 mg of white solid (75−85% yield).

2-(Hydroxylamino)-9*H*-pyrido[2,3-*b*]indole (NHOHαC): mp 140−148 °C dec; IR (KBr) 3278, 3226, 1629, 1608, 1578, 1419 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.30 (1H, s, exchangeable), 8.78 (1H, s, exchangeable), 8.63 (1H, d, J = 1.5 Hz, exchangeable), 8.24 (1H, d, J = 8.4 Hz), 7.90 (1H, d, J = 7.7 Hz), 7.35 (1H, d, J = 8.0 Hz), 7.25 (1H, m), 7.10 (1H, m), 6.72 (1H, d, J = 8.4 Hz); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 162.2 (C), 151.3 (C), 137.9 (C), 130.3 (CH), 124.2 (CH), 121.7 (C), 119.3 (CH), 119.2 (CH), 110.9 (CH), 108.2 (C), 99.7 (CH); high-resolution MS *m/e* 199.0749, $C_{11}H_9N_3O$ requires 199.0746.

2-(Hydroxylamino)-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeNHOHαC): mp 195−197 °C dec; IR (KBr) 3312, 1629, 1610, 1572, 1412, 1300 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.37 (1H, s, exchangeable), 8.70 (1H, s, exchangeable), 8.34 (1H, s, exchangeable), 7.97 (1H, s), 7.86 (1H, d, J = 7.6 Hz), 7.36 (1H, d, J = 7.9 Hz), 7.22 (1H, t, J = 7.5 Hz), 7.08 (1H, t, J = 7.4 Hz), 2.18 (3H, s); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 158.3 (C), 150.1 (C), 137.5 (C), 130.0 (CH), 123.7 (CH), 121.6 (C), 119.2 (CH), 119.0 (CH), 110.9 (CH), 109.3 (C), 107.1 (C), 16.8 (CH_3); high-resolution MS *m/e* 213.0886, $C_{12}H_{11}N_30$ requires 213.0903.

Acknowledgment. This work was supported by a grant from the American Cancer Society (CN-172).

Supporting Information Available: Tables of spectroscopic data and copies of ^{1}H and ^{13}C NMR spectra for all nitro and hydroxylamine compounds (12 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO971786V