Synthesis of the 3-Methyl and 4-Methyl Derivatives of 3-Amino-3,4-dihydro-1-hydroxycarbostyril and Related Compounds

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The 3-methyl and 4-methyl derivatives of 3-amino-3,4-dihydro-1-hydroxycarbostyril were synthesized by the reductive cyclization of α-methyl-β-(*o*-nitrophenyl)alanine and α-amino-β-(*o*-nitrophenyl)butyric acid hydrohalides, respectively, under conditions of catalytic hydrogenation in acidic solution. The free bases of the latter two *o*-nitroaromatic amino acids were also catalytically hydrogenated under neutral conditions to yield the respective α-methyl-β-(*o*-aminophenyl)alanine and α-amino-β-(*o*-aminophenyl)butyric acid which were converted to the corresponding lactams, 3-methyl- and 4-methyl-3-amino-3,4-dihydrocarbostyrils. α-Methyl-β-(*o*-nitrophenyl)alanine was obtained by acid hydrolysis of 5-methyl-5-(*o*-nitrobenzyl)hydantoin which was prepared by treatment of *o*-nitrophenylacetone with potassium cyanide and ammonium carbonate. α-Amino-β-(*o*-nitrophenyl)butyric acid was synthesized by condensation of α-bromo-*o*-nitroethylbenzene with diethyl acetamidomalonate, followed by acid hydrolysis of the condensation product. The 4-methylated compounds were obtained as synthetic mixtures of two diasteromeric racemates in nearly the same amounts as shown by nmr spectral analysis. Unlike the demethylated parent compound, 3-amino-3,4-dihydro-1-hydroxycarbostyril, neither the 3-methyl nor 4-methyl analog was found to possess any antibacterial activity.

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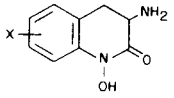
In this laboratory we have been concerned for some time with the modification of the 3-amino-3,4-dihydro-1-hydroxycarbostyril molecule (I) and the effects that these modifications have on biological activity (1-6) and on chemical rearrangement (7-9). Among the analogs of I



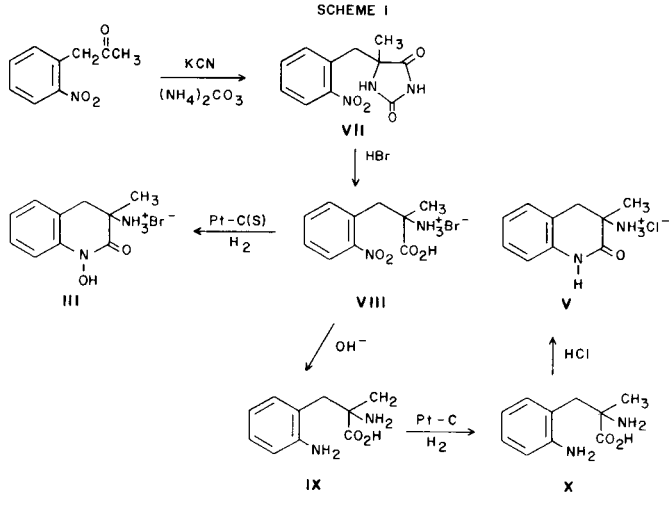
I: X = H
II: X = Cl

reported previously, the 5-, 6-, 7-, and 8-chlorosubstituted derivatives (II) have been studied most extensively for their antibacterial activities (5,6) and for their rearrangement in hydrohalic acids (9). Such studies have shown that the position of the chloro group on the benzene ring has a marked effect on the level of inhibitory activity against microbial growth and on the orientation of the halide nucleophile in the heteroaromatic rearrangement.

In order to obtain further information on the relationship between structural modification and biological activity, some addition analogs of I which contain a methyl group at positions 3 and 4 in the heterocyclic portion of the carbostyril ring have been synthesized for microbiological assay. Therefore, the purpose of this paper is to describe the synthesis of the 3-methyl (III) and 4-methyl (IV) analogs of I and to disclose the finding that both methylated analogs are devoid of antibacterial activity. For purposes of comparison, the present study also includes the synthesis of the related lactams, the 3-methyl (V) and 4-methyl (VI) substituted 3-amino-3,4-dihydrocarbostyrils.

The syntheses of the 3-methyl cyclic hydroxamate III

SCHEME I



and 3-methyl lactam V are depicted in Scheme I. *o*-Nitrophenylacetone was treated with potassium cyanide and ammonium carbonate in aqueous ethanol to give 5-methyl-5-(*o*-nitrobenzyl)hydantoin (VII), which was hydrolyzed in concentrated hydrobromic acid to afford α-methyl-β-(*o*-nitrophenyl)alanine hydrobromide (VIII). Reductive cyclization of the latter compound VIII to yield the desired 3-amino-3,4-dihydro-1-hydroxy-3-methylcarbostyril hydrobromide (III) was effected under acidic conditions of catalytic hydrogenation in the presence of platinum on carbon, sulfided catalyst. The hydrobromide salt VIII was converted to the corresponding free base (IX) by neutralization with aqueous sodium hydroxide solution. Catalytic hydrogenation of the free base (IX) in the presence of platinum on carbon catalyst gave *o*-amino-α-methylphenylalanine (X). The *o*-aminoaromatic amino acid (X)

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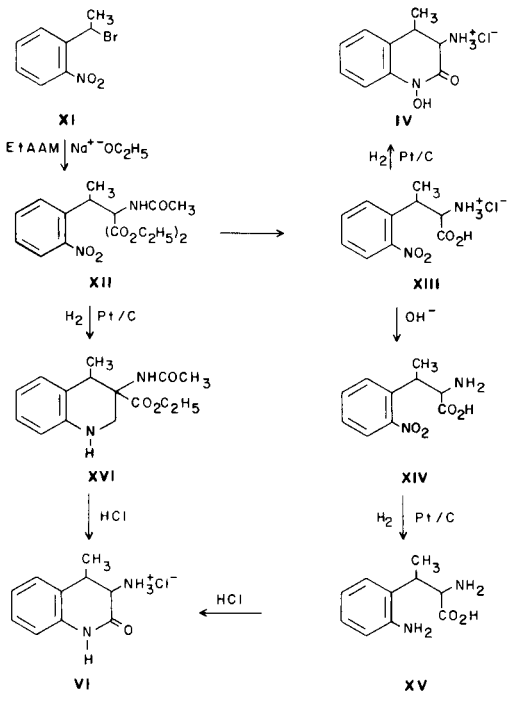
SCHEME II



rapidly cyclized in acidic solution to give the lactam, 3-amino-3,4-dihydro-3-methylcarbostyril (V).

The 4-methyl analog (IV) of the carbostyril hydroxamic acid was obtained as a mixture of two diastereomeric racemates. The synthetic method leading to the diastereomeric mixture is shown in Scheme II. *o*-Nitroethylbenzene was brominated with *N*-bromosuccinimide in the presence of benzoyl peroxide to yield α-bromo-*o*-nitroethylbenzene (XI). This compound was obtained as a low-melting solid which gave an acceptable elemental analysis, whereas no analytical data was reported in a previous paper which described its isolation as an oil (10). Condensation of XI with ethyl acetamidomalonate (Et AAM) in ethanolic sodium ethoxide gave ethyl 2-acetamido-2-[α-(*o*-nitrophenethyl)]malonate (XII). The latter compound XII was hydrolyzed in a refluxing mixture of concentrated hydrochloric acid and glacial acetic acid to afford α-amino-β-(*o*-nitrophenyl)butyric acid hydrochloride (XIII). Compound XIII was reductively cyclized by catalytic hydrogenation under acidic conditions in the presence of platinum on carbon catalyst to afford 3-amino-3,4-dihydro-1-hydroxy-4-methylcarbostyril hydrochloride (IV).

The 4-methyl analog (VI) of the lactam was also prepared as a mixture of diastereomers by two different methods as presented in Scheme II. The hydrochloride salt of α-amino-β-(*o*-nitrophenyl)butyric acid was converted to the free base XIV prior to catalytic hydrogenation under neutral conditions using platinum on carbon as catalyst whereby α-amino-β-(*o*-aminophenyl)butyric acid (XV) was produced. The latter compound XV underwent lactamization in acidic solution to form 3-amino-3,4-dihydro-4-methylcarbostyril hydrochloride (VI). The other method leading to VI resulted from acid hydrolysis of

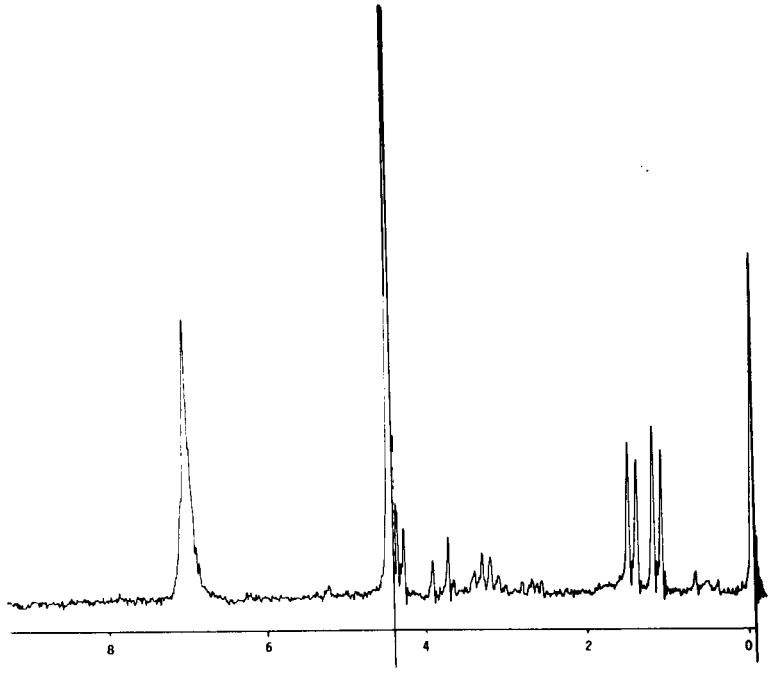


Figure 1. The nmr spectrum of the diasteromeric 3-amino-3,4-dihydro-1-hydroxy-4-methylcarbostyril (IV).

3-acetamido-3-carboethoxy-3,4-dihydro-4-methylcarbostyril (XVI), which was produced by reductive cyclization of XII under conditions of catalytic hydrogenation.

The nmr spectra were used to determine the presence and the relative amounts of the two diastereomers in the synthetic mixtures of the carbostyril compounds IV and VI. As shown in Figure 1, the nmr spectrum of IV shows two distinct doublets in the δ 1 to 2 region resulting from the magnetic nonequivalence of the methyl groups as based on the chemical shifts of the protons. The absorption of each methyl group appears as a doublet as a result of coupling with the neighboring proton at position 4. Since the relative intensities of the nmr absorptions resulting from each diastereomer were observed to be nearly the same, it was concluded that the synthetic mixture contained almost equal amounts of the two diastereomers.

Neither the 3-methyl (III) nor the 4-methyl (IV) analogs of 3-amino-3,4-dihydro-1-hydroxycarbostyril were active against the growth of *Escherichia coli* 9723 and *Lactobacillus plantarium* 8014 even at concentrations of 200 μg./ml. Therefore, substitution of a methyl group at position 3 or 4 of 3-amino-3,4-dihydro-1-hydroxycarbostyril nullifies growth inhibitory activity in the microorganisms studied.

In previous work, the various chloro-substituted carbostyril hydroxamates were uniformly more effective antimicrobial agents than the corresponding lactams (5,6). This difference in antimicrobial activity was ascribed to the presence of the hydroxamate function as part of the heterocyclic ring. However, such is not the case with the methylated carbostyrils which are inactive as growth inhibitors in spite of the hydroxamate group of III and IV. It appears that methylation of the 3 position of I sterically interferes with the 3-amino group and that methylation of the 4 position substantially alters some key conformational feature in the molecule to markedly decrease its affinity for an enzyme or receptor site which is necessary for its inhibitory effect on microbial growth. A study describing the separation and preparation of the two diasteromeric 4-methylated 3-amino-3,4-dihydro-1-hydroxycarbostyrils and the 3-amino-3,4-dihydrocarbostyrils by preparative liquid chromatography is forthcoming.

EXPERIMENTAL

General.

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were recorded on a Beckman Model IR-10 spectrophotometer (potassium bromide) and were calibrated with polystyrene film. Nmr spectra were recorded on a Perkin-Elmer Model R12-B spectrometer at 60 MHz. The nmr spectrum of the diastereomeric mixture of 3-amino-3,4-dihydro-1-hydroxy-4-methylcarbostyril (30 mg.) was recorded in about 0.5 ml. of 20% deuterium chloride in deuterium oxide solution with 1% sodium 2,2-dimethyl-2-silapentane-5-sulfinate (DSS) as the reference standard. Microanalyses were performed by M-H-W Laboratories, Phoenix, Arizona.

5-Methyl-5-(*o*-nitrobenzyl)hydantoin (VII).

A suspension of 11.2 g. (0.0621 mole) of (2-nitrophenyl)acetone, 29.13 g. (0.303 mole) ammonium carbonate, and 8.45 g. (0.130 mole) potassium cyanide in 200 ml. of 50% aqueous ethanol was heated at 55-60° in a water bath for 4 hours. The solution was acidified with concentrated hydrochloric acid, reduced to dryness *in vacuo*, and the resulting residue was washed with water and benzene to give 12.57 g. (81.2%) of product. An analytical sample, m.p. 188-190°, was obtained by recrystallization from ethanol.

Anal. Calcd. for $C_{11}H_{11}N_3O_4$: C, 53.01; H, 4.45; N, 16.86. Found: C, 53.13; H, 4.55; N, 17.00.

o-Nitro-α-methylphenylalanine Hydrobromide (VIII).

A 6.0 g. (0.024 mole) sample of 5-methyl-5-(*o*-nitrobenzyl)hydantoin (VII) was refluxed in 150 ml. of redistilled (48%) hydrogen bromide for 48 hours. The hot solution was treated with darco, filtered through celite, and cooled to give 5.1 g. (69.7%) product. Recrystallization from 50% aqueous alcohol gave an analytical sample, m.p. 243-246°.

Anal. Calcd. for $C_{10}H_{12}N_2O_4 \cdot HBr$: C, 39.36; H, 4.29; N, 9.18. Found: C, 39.13; H, 4.44; N, 9.03.

o-Amino-α-methylphenylalanine (X).

A 400 mg. (0.0018 mole) sample of *o*-nitro-α-methylphenylalanine (IX), m.p. 229° dec., prepared from VIII by treatment of an aqueous solution with sodium hydroxide, was dissolved in 40 ml. of 75% aqueous methanol and hydrogenated at 3.67 kg./cm^2 of hydrogen pressure in the presence of 70 mg. of 5% platinum on carbon catalyst for 4 hours. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo* to about 3 ml. and cooled at $-17°$ overnight to give 240 mg. (66.3%) of product, m.p. 153-154°.

Anal. Calcd. for $C_{10}H_{14}N_2O_2 \cdot \frac{1}{2}H_2O$: C, 59.10; H, 7.44; N, 13.78. Found: C, 58.94; H, 7.33; N, 13.87.

3-Amino-3,4-dihydro-1-hydroxy-3-methylcarbostyril Hydrobromide (III).

A 500 mg. (0.0016 mole) sample of *o*-nitro-α-methylphenylalanine hydrobromide (VIII) was dissolved in 3 ml. of 50% aqueous methanol, 0.5 ml. of 48% hydrogen bromide was added, and the solution was hydrogenated at 3.67 kg/cm^2 of hydrogen pressure in the presence of 50 mg. of 5% platinum on carbon sulfided catalyst for 3 hours. The resulting precipitate was dissolved in water and the catalyst was removed by filtration. The filtrate was reduced in volume *in vacuo* until cloudiness appeared and 20 ml. of hydrogen bromide was added to help facilitate precipitation. After chilling, the solution was filtered, and the resulting precipitate was washed with acetone to give 250 mg. (58.0%) of product, m.p. 315-316° dec. The ir spectrum showed major absorption bands at 2950 (broad), 1660, 1595, 1515, 1500, 1470, 1425, 1345, 755, and 695 cm^{-1}.

Anal. Calcd. for $C_{10}H_{12}N_2O_2 \cdot HBr$: C, 43.97; H, 4.80; N, 10.26. Found: C, 43.94; H, 4.94; N, 10.14.

3-Amino-3,4-dihydro-3-methylcarbostyril Hydrochloride (V).

To a 200 mg. (0.00098 mole) sample of *o*-amino-α-methylphenylalanine (X), dissolved in water was added 1 ml. of concentrated hydrochloric acid. The volume of the solution was reduced *in vacuo* until precipitation started to occur, and the solution was chilled to give 205 mg. (98.4%) product. Recrystallization from methanol-ether gave an analytical sample, m.p. 311-313° dec. The ir spectrum showed major absorption bands at 3420, 3050 (broad), 1715, 1605, 1510, 1405, 1370, 1335, 1300, 1275, 780 and 765 cm^{-1}.

Anal. Calcd. for $C_{10}H_{12}N_2O \cdot HCl$: C, 56.47; H, 6.16; N, 13.17. Found: C, 56.60; H, 6.23; N, 13.04.

α-Bromo-*o*-nitroethylbenzene (XI).

To a refluxing solution of 45 g. (0.30 mole) of freshly distilled *o*-nitroethylbenzene and 150 ml. of anhydrous carbon tetrachloride were added 54 g. (0.30 mole) of *N*-bromosuccinimide and 1 g. of benzoyl peroxide.

The reaction mixture was treated with 15 g. of F-20 alumina, filtered, and the filtrate was passed through a 1 × 10 cm column charged with 40 g. of F-20 alumina. The column was eluted with 100 ml. of anhydrous carbon tetrachloride and the combined elements were distilled *in vacuo* to remove the unreacted starting material and residual carbon tetrachloride and then chilled to yield 62.4 g. (90.4%) of product, m.p. 10-12°.

Anal. Calcd. for $C_8H_8BrNO_3$: C, 41.76; H, 3.50; N, 6.09. Found: C, 41.57; H, 3.37; N, 6.06.

Ethyl 2-Acetamido-2-[α-(*o*-nitrophenethyl)]malonate (XII).

To a 57 g. (0.26 mole) sample of ethyl acetamidomalonate dissolved in 500 ml. of anhydrous ethanol containing 6.0 g. (0.26 mole) of sodium was added 60 g. (0.26 mole) of α-bromo-*o*-nitroethylbenzene (XI), and the reaction mixture was stirred at 50° for 5 hours. Water was added to reaction mixture, and the resulting precipitate was filtered, air-dried, and washed with ether. Additional product was recovered from work-up of the filtrates and washings to give a total of 81.1 g. (85.1%) of product, m.p. 190-191°.

Anal. Calcd. for $C_{17}H_{22}N_2O_7$: C, 55.74; H, 6.05; N, 7.65. Found: C, 56.00; H, 5.99; N, 7.50.

α-Amino-β-(*o*-nitrophenyl)butyric acid Hydrochloride (XIII).

A 20 g. (0.055 mole) sample of ethyl acetamido-2-[α-(*o*-nitrophenethyl)]-malonate (XII) was suspended in 180 ml. of concentrated hydrochloric acid and 120 ml. of glacial acetic acid, and the solution was refluxed for 16 hours. The reaction mixture was taken to dryness *in vacuo* and upon repeated addition and evaporation of anhydrous ethanol there was obtained 12.14 g. (84.7%) of product, m.p. 203-209°. A small portion was recrystallized from ethanol-acetone (1:1) to give an analytical sample.

Anal. Calcd. for $C_{10}H_{12}N_2O_4 \cdot HCl$: C, 46.07; H, 5.03; N, 10.75. Found: C, 45.82; H, 4.89; N, 10.63.

α-Amino-β-(*o*-aminophenyl)butyric Acid (XV).

A 1.0 g. (0.0045 mole) sample of the free base XIV of α-amino-β-(*o*-nitrophenyl)butyric acid, m.p. 201-202°, prepared by treatment of XIII with NaOH, was dissolved in 30 ml. of 95% aqueous methanol and hydrogenated at 3.67 kg./cm² of hydrogen pressure in the presence of 100 mg. of 5% platinum on carbon catalyst for 3 hours. The catalyst was removed by filtration, and the filtrate was reduced in volume *in vacuo* to give 0.71 g. (81.2%) of product, m.p. 180-181°.

Anal. Calcd. for $C_{10}H_{14}N_2O_2$: C, 61.82; H, 7.26; N, 14.42. Found: C, 61.69; H, 7.28; N, 14.28.

3-Amino-3,4-dihydro-1-hydroxy-4-methylcarbostyril Hydrochloride (IV).

A 1.0 g. (0.0038 mole) sample of α-amino-β-(*o*-nitrophenyl)butyric acid hydrochloride (XIII) was suspended in 6 ml. of 50% aqueous methanol and 1 ml. of concentrated hydrochloric acid and hydrogenated at 3.67 kg./cm² of hydrogen pressure in the presence of 100 mg. of 5% platinum on carbon catalyst for 3 hours. The catalyst was removed by filtration, and the volume of the filtrate was reduced *in vacuo* almost to dryness. Addition of 25 ml. of acetone and chilling caused precipitation of 0.69 g. (79.4%) of product, m.p. 249-255°. Recrystallization from methanol gave an analytical sample. The ir spectrum showed major absorption bands at 2960 (broad), 1670, 1605, 1580, 1490, 1460, 1400, 970, and 750 cm^{-1}.

Anal. Calcd. for $C_{10}H_{12}N_2O_2 \cdot HCl$: C, 52.52; H, 5.73; N, 12.25. Found: C, 52.59; H, 5.74; N, 12.05.

3-Acetamido-3-carboethoxy-3,4-dihydro-4-methylcarbostyril (XVI).

A 1.7 g. (0.0046 mole) sample of ethyl 2-acetamido-2-[α-(*o*-nitrophenethyl)]malonate (XII) was suspended in 10 ml. of 95% aqueous ethanol and hydrogenated at 3.67 kg./cm² of hydrogen pressure in the presence of 170 mg. of 5% platinum on carbon catalyst for 3 hours. The catalyst was removed by filtration, and the solution was reduced in volume *in vacuo* to give 1.25 g. (92.6%) of product, m.p. 118-120°.

Anal. Calcd. for $C_{15}H_{18}N_2O_4$: C, 62.06; H, 6.25; N, 9.65. Found: C, 62.10; H, 6.10; N, 9.62.

3-Amino-3,4-dihydro-4-methylcarbostyril Hydrochloride (VI). Method A.

A suspension of 100 mg. (0.00051 mole) of α-amino-β-(*o*-aminophenyl)-butyric acid (XV) dissolved in 5 ml. of 95% aqueous ethanol was treated with 5 drops of concentrated hydrochloric acid to effect solution. The volume of the solution was reduced *in vacuo* almost to dryness, and the addition of 5 ml. of acetone resulted in precipitation of 90 mg. (83%) of product, m.p. 256-258°. The ir spectrum showed major absorption bands at 3410, 2950 (broad), 1695, 1590, 1485, 1410, 1310, and 750 cm^{-1}.

Anal. Calcd. for $C_{10}H_{12}N_2O \cdot HCl$: C, 56.47; H, 6.16; N, 13.17. Found: C, 56.23; H, 6.17; N, 12.96.

Method B.

A 1.0 g. (0.0034 mole) sample of 3-acetamido-3-carboethoxy-3,4-dihydro-4-methylcarbostyril (XVI) was heated at reflux in 10 ml. of concentrated hydrochloric acid for 3 hours. The volume of the reaction mixture was reduced *in vacuo* almost to dryness and the solution was chilled to give 630 mg. (87.1%) of product. The products from Methods A & B were shown to be identical by a comparison of their infrared spectra.

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