MECHANISTIC STUDY ON EXCHANGE BETWEEN LABELED CYANIDE AND NITRILES

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SUMMARY

The potential of a clean, rapid exchange between the nitrile function of mandelonitrile and cyanide was examined for the preparation of labeled mandelonitrile which could be subsequently rapidly reduced with borane to labeled phenylethanolamine (PEOH). The mandelonitrile exchange (CN-CN) was studied using [^{13}C]-NaCN with crown ethers in THF, monitoring the results with ^{13}C-NMR. A large increase in the intensity of the signal due to [^{13}C]-nitrile was observed. The exchange was also carried out using [^{14}C]-NaCN, and the exchanged nitrile was reduced to [^{14}C]-PEOH. The chemical yield for the reduction of [^{14}C]-mandelonitrile to [^{14}C]-PEOH was 60% and the overall radiochemical yield of the cyanide-exchange and borane reduction (based on [^{14}C]-NaCN used) was 20%. Mechanisms are proposed which were found to be consistent with results of cyanide exchange of appropriately selected nitriles.

Key Words: Cyanide-nitrile exchange, labeled cyanohydrins, phenylethanolamine.

INTRODUCTION

There is not much information in the literature on the exchange between cyanide ion and the nitrile function of organic nitriles. Kourim and Zikmund (1) reported the synthesis of [^{14}C]-serine and [^{14}C]-cycloserine by the exchange of the nitrile function of the appropriate cyanohydrin compound with [^{14}C]-NaCN and hydrolysis of the resulting [^{14}C]-cyanohydrin to the corresponding [^{14}C]-acid. Jay, et.al. (2) reported the exchange between the nitrile function of acetonitrile and the [^{14}C]-cyanide of [^{14}C]-NaCN as the result of an attempt to synthesize [^{14}C]-phenylacetonitrile from benzyl chloride and [^{14}C]-NaCN using acetonitrile as the solvent. The loss of the [^{14}C]-cyanide to the solvent was reported to be 48%. Our aim was to use exchange reactions in the synthesis of radiolabeled amines and to study the mechanism(s) by which these reactions proceed.

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EXPERIMENTAL

Reagents

2-Amino-1-phenylethanol (phenylethanolamine), benzoyl chloride, sodium cyanide, 18-crown-6, 15-crown-5, tetrahydrofuran (gold label), borane-tetrahydrofuran complex, mandelonitrile, thionyl chloride, 1-adamantanecarbonitrile, 1-adamantanemethylamine, anhydrous sodium sulfate, and deuterated chloroform were purchased from Aldrich Chemical Company. Ninhydrin and DL-α-methoxyphenylacetic acid were purchased from Sigma Chemical Company. Toluene, chloroform, cyclohexane, hydrochloric acid, sodium hydroxide, sodium chloride, ammonium hydroxide, ethyl ether, heptane, ammonium acetate (HPLC grade) and sodium acetate (HPLC grade) were purchased from Fisher Scientific Company. Cation-exchange resin (AG50W-X2) was purchased from Bio-Rad Laboratories. [^{14}C]-Sodium cyanide (7.9 mCi/mmol) and Aquasol-2 were purchased from New England Nuclear Corp. LK5F silica gel thin layer chromatography plates were purchased from Whatman, Inc. Sulfamide (99% pure) and sulfolane were purchased from Fluka Chemical Corporation. Cab-O-Sil (grade 5) was purchased from Cabot Corporation. [^{13}C]-Sodium cyanide (93% enriched) was purchased from B.O.C., Ltd. Methanol (Omni Solv reagent, HPLC grade) was purchased from MCB Manufacturing Chemists, Inc.

Instruments.

Infrared spectra were recorded using a Perkin-Elmer Model 567 infrared spectrophotometer, Perkin-Elmer Co. Proton NMR spectra were recorded using a Varian model EM-360 spectrometer, Varian Associates.

HPLC analyses were carried out using a solvent delivery pump (Model 110A, Beckman Instruments, Inc.); a loop injector (model 7125), Rheodyne; UV detection (Varichrom, Varian or a Beckman detector model 153, Beckman Instruments, Inc.); and a strip chart recorder (Omniscribe). Two types of HPLC columns were employed: Partisil PXS 10/25 SCX strong cation exchange column with a particle size of 10 μm, and an Ultrasphere ODS 5 μm Altex (4.6 mm x 25 cm) reverse phase column.

Radioactive samples containing carbon-14 were counted in a Packard

model 3375 Tri-carb Liquid Scintillation Spectrometer, Packard Instruments Co.

Carbon-13 NMR Spectra for the cyanide exchange reactions were determined using a VN XL-200 spectrometer, Varian Associates.

Exchange between [^{13}C]-sodium cyanide and mandelonitrile as determined by ^{13}C-NMR.

A mixture of [^{13}C]-NaCN (0.028 g, 0.575 mmol), 18-crown-6 (2.5 g, 9.5 mmol), 15-crown-5 (0.5 g, 2.2 mmol) and THF (5 ml) in a closed vial was sonicated for 10 min, then mandelonitrile (1.5 g, 11.2 mmol) was added to the mixture and the volume was adjusted to 10 ml with THF and sonicated for an extra 3 min. Using the same concentration, control solutions of 1) Na^{13}CN and crown ethers in THF and 2) mandelonitrile and crown ethers in THF were prepared. The ^{13}C-NMR spectra of these solutions were recorded using tetramethylsilane (TMS) as a standard reference.

Exchange between [^{14}C]-NaCN and the nitrile function of mandelonitrile, and reduction to [1-^{14}C]-phenylethanolamine.

A mixture of 18-crown-6 (1.25 g, 4.7 mmol), 15-crown-5 (0.25 g, 1.1 mmol), NaCN (2 mg, 4 x 10^{-2} mmol), [^{14}C]-NaCN (3.6 μCi, 7.9 mCi/mmol) and THF (3 ml) was sonicated for 10 min which was followed by the addition of mandelonitrile (118 mg, 0.88 mmol). The mixture was further sonicated for 10 min and transferred to a 3-neck flask (preheated to 80° using a heating mantle). The flask was fitted with a condenser and the mixture was refluxed for 10 min after which the 3-neck flask was fitted with an addition funnel containing 3 ml of 1 M BH_3/THF. The borane solution was added dropwise with stirring to the reaction mixture over a period of 2 min. Heating under reflux was continued for 10 min after which the system was connected in series to a blank trap and a NaOH trap (100 ml, 0.2N) and the excess borane was destroyed by the addition via the addition funnel of 1N HCl (18 ml) containing NaCl (0.33 g, 5.8 mmol). (The 1N HCl was added very slowly at first.) Nitrogen gas was bubbled through the quenched reaction mixture for 15 min. The reaction mixture was then passed through a cation-exchange column prewashed with 1N HCl (100 ml). The column was then washed with H_2O (30 ml) and eluted with 0.2N NaOH until the resin was neutral as indicated by a darkening of

of the resin color. A volume of 40 ml of the subsequent basic eluate was then collected.

Part of the basic eluate (30 ml) from the cation-exchange column from the mandelonitrile reduction experiment (30 ml) was acidified, lyophilized and reconstituted in H_2O (6 ml). The water-reconstituted product was qualitatively analyzed by thin layer chromatography on a Whatman LK5F silica gel plate 25 x 5 cm. The reconstituted product was spotted along the TLC plate and eluted with ethanol: ammonium hydroxide (4:1) solution. After the plate was developed and dried, one side of the plate was covered with a sheet of paper and the exposed portion was sprayed with a ninhydrin solution (1,2,3-indantrione monohydrate) in ethanol (1 mg/ml). The ninhydrin spot with an R_f value of 0.72 corresponded to the R_f value exhibited by authentic phenylethanolamine when chromatographed under identical conditions. A radiochromatogram was run by scraping 1.5 cm strips from the side which was not sprayed with ninhydrin, suspending the silica gel in Cab-O-sil and Aquasol-2 cocktail and counting in the liquid scintillation counter. The radiochromatogram showed that all the radioactivity corresponded to the R_f of phenylethanolamine.

The HPLC system consisted of a solvent delivery pump[1], a loop injector[2] with a 20 µl sample loop, a variable wavelength detector[3] and a strip chart recorder[4]. Separation was effected at ambient temperature on a 25 cm x 4.6 mm stainless steel column prepacked with octadecylsilane-coated 5 µ particles[5]. The mobile phase consisted of 33% (v/v) methanol in 0.1 M aqueous ammonium acetate and was delivered at 1 ml/min. The detector was set at 254 nm and a sensitivity of 0.1 absorbance units at full scale. At a flow rate of 1 ml/min, phenylethanolamine exhibited a retention time of 5.5 min.

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[1]Pump; Altex, model 110A, Beckman Instruments, Inc.

[2]Injector; Rheodyne, model 7125.

[3]Detector; Varichrom, Varian.

[4]Recorder; Omniscribe recorder, Houston Instruments.

[5]Column; Ultrasphere-ODS-5 µm; Altex Instruments, Inc.

The lyophilized water-reconstituted product was injected into the HPLC system. One ml (1 min) fractions were collected in scintillation vials and counted in Aquasol-2 liquid scintillation cocktail in a liquid scintillation counter. A plot of radioactivity (dpm) versus retention time was constructed. The concentration of the [^{14}C]-phenylethanolamine was calculated by fitting the peak height into the standard curve, and by using the radioactivity corresponding to the phenylethanolamine peak, the specific activity (dpm/mmol) was calculated.

[^{14}C]-Phenylethanolamine benzamide.

The benzamide derivative was prepared (3) from a portion of the basic eluent from the cation exchange column. The melting point of the benzamide derivative was 145.5-146.5°, lit 147.5 (4). The specific activity of the benzamide after crystallizing from 15% ethanol to constant activity was found to be equal to the specific activity of [^{14}C]-phenylethanolamine calculated from the HPLC chromatogram.

α-Methoxyphenylacetonitrile

α-Methoxyphenylacetonitrile was synthesized by the general method of Hulkenberg et al (5) for the synthesis of nitriles. The product distilled at b.p. 124° (20 mm). The yield was found to be 65%. NMR and IR spectra were recorded and corresponded to the desired product; NMR ($CDCl_3$) δ 3.55 (s, 3H, $-OCH_3$), 5.25 (s, 1H, -CH-), 7.53 (s, 5H, aromatic H); IR (film) showed an absorption at 2230 cm^{-1} (CN). Analytical calculation for C_9H_9NO: C, 73.45; H, 6.16; N, 9.5. Found: C, 73.67; H, 5.94; N, 9.27.

Exchange between [^{14}C]-sodium cyanide and α-methoxyphenylacetonitrile and reduction of [^{14}C]-α-methoxyacetonitrile to [α-^{14}C]-β-methoxy-β-phenylethylamine.

The cyanide-nitrile exchange, reduction and purification of α-methoxyphenylacetonitrile were run as described under the mandelonitrile-[^{14}C]-cyanide exchange.

The thin layer radiochromatogram was run as described under the analysis of [^{14}C]-phenylethanolamine synthesized by the cyanide-nitrile exchange between

[^{14}C]-cyanide and mandelonitrile with subsequent reduction to [^{14}C]-phenylethanolamine. The ninhydrin spot with an R_f value of 0.78 corresponded to the R_f value exhibited by a characterized sample prepared by borane reduction of α-methoxyphenylacetonitrile when chromatographed under identical conditions. The prepared authentic sample was analyzed by IR ($CHCl_3$ film): 3370, 3300, 3025, 2820-2940, 1600, 1585, 1100 cm^{-1} and NMR ($CDCl_3$) δ 1.63 (broad s, 2H, $-NH_2$), 2.7-2.96 (m, 2H, $-CH_2-$), 3.2 (s, 3H, $-OCH_3$), 4.06 (t, H, -CH-), 7.16 (s, 5H, aromatic H). The hydrochloride salt (m.p. 156-157°) exhibited the following elemental analytical calculation for $C_9H_{14}NOCl$:C, 57.6; H, 7.52; N, 7.46. Found: C, 57.48; H, 7.56; N, 7.43. The radiochromatogram showed that 20% of the total radioactivity on the TLC plate had an R_f value of 0.78.

The high pressure liquid chromatographic system consisted of the same pump, injector and recorder used in the characterization of [^{14}C]-phenylethanolamine prepared from mandelonitrile, and a fixed wavelength UV detector[6]. Separation was effected at ambient temperature on a 25 cm x 4.6 mm stainless steel column prepacked with 10 μ strong cation exchange particles[7]. The mobile phase consisted of 50% (v/v) methanol in 0.01 M, pH 4.5 aqueous sodium acetate and was delivered at 2 ml/min and the detection was at 254 nm. Under these conditions β-methoxy-β-phenyloethylamine exhibited a retention time of 9.0 min. A standard curve of authentic β-methoxy-β-phenylethylamine over different concentrations was constructed by plotting concentration (mg/ml) versus peak height (cm).

The water-reconstituted product from lyophilization was injected into the HPLC system and 2 ml (1 min) fractions were collected in scintillation vials and counted in Aquasol-2 liquid scintillation cocktail. The specific activity (dpm/mmol) was calculated. About 20% of the total radioactivity in the chromatogram coincided with the β-methoxy-β-phenylethylamine peak.

β-Methoxy-β-phenylethylamine benzamide

The derivatization of the ^{14}C-β-methoxy-β-phenylethylamine with benzoyl

[6]Detector: Altex; model 153, Beckman Instruments, Inc.

[7]Column; Partisil 10-SCX, Whatman, Inc.

chloride was run as discussed under ^{14}C-phenylethanolamine. Authentic non-radioactive β-methoxy-β-phenylethylamine benzamide was prepared under identical conditions (m.p. 92°) and verified by IR (KBr) 3340, 1630, 1580, 1530, 1110 cm^{-1} and proton NMR ($CDCl_3$) δ 3.27 (s, 3H, OCH_3); 3.3-4.15 (m, 2H, $-CH_2$); 4.39 (dd, 1H, J = 8.5, 4 Hz, -CH-); 6.5-6.95 (m, 1H, -NH); 7.3 (s, 5H, aromatic H); 7.3-7.5 (m, 3H, aromatic H) and 7.6-7.9 (m, 2H aromatic H). Elemental analytical calculation for $C_{16}H_{17}NO_2$: C, 75.27; N, 6.71; N, 5.49. Found: C, 75.01; H, 6.73; N, 5.43.

Exchange between [^{14}C]-sodium cyanide and 1-adamantanecarbonitrile.

The exchange, reduction and purification were run as discussed under the mandelonitrile-[^{14}C]-cyanide exchange study. The TLC radiochromatogram was similarly constructed as described under [^{14}C]-phenylethanolamine. Ninhydrin detection showed the presence of 2 amines at R_f values of 0.71 and 0.76. The amine with the R_f value of 0.76 corresponded to the R_f value exhibited by authentic 1-adamantanemethylamine when chromatographed under identical conditions and represented 36% of the total radioactivity on the TLC plate. The other unidentified amines represented 31% of the total radioactivity on the TLC plate.

RESULTS AND DISCUSSION

It was reported (2) that acetonitrile undergoes nitrile-cyanide exchange using [^{14}C]-KCN in the presence of crown ethers. The possibility of the exchange between the nitrile function of mandelonitrile and [^{14}C]-cyanide was examined for the preparation of [^{14}C]-mandelonitrile for subsequent reduction to [^{14}C]-PEOH.

The exchange was studied first with carbon-13 NMR using [^{13}C]-NaCN (93% enriched) and the results were monitored by ^{13}C-NMR. An increase in the intensity of the signal due to the [^{13}C]-nitrile carbon was observed. The product of the reaction in which [^{13}C]-sodium cyanide was sonicated with mandelonitrile showed an enhancement of this signal relative to the unreacted mandelonitrile and also relative to mandelonitrile of the reaction in which [^{12}C]-sodium cyanide

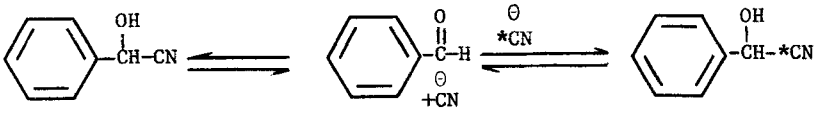
was sonicated with mandelonitrile. From the carbon-13 NMR data, it was found that the ratio of the NMR signal intensity of the nitrile carbon of the ^{13}C-cyanide-exchanged mandelonitrile to the intensity of the quaternary carbon was 3.24. The ratio obtained using natural sodium cyanide (1.1% ^{13}C) was 0.815. So, the intensity of the nitrile ^{13}C-resonance of the product compared to the unreacted mandelonitrile increased by a factor of 3.98.

The exchange reaction was then tried with [^{14}C]-NaCN in the presence of crown ethers using THF as the solvent. After running the exchange reaction and reducing with borane, the product, [^{14}C]-PEOH was analyzed by TLC. A TLC radiochromatogram was constructed which showed that nearly all the radioactivity appeared at the R_f value corresponding to PEOH. The product was also analyzed and quantified by HPLC. The isolated chemical yield for the reduction of mandelonitrile to PEOH was 60% and the overall radiochemical yield of the cyanide exchange and borane reduction (based on [^{14}C]-NaCN used) was 20%.

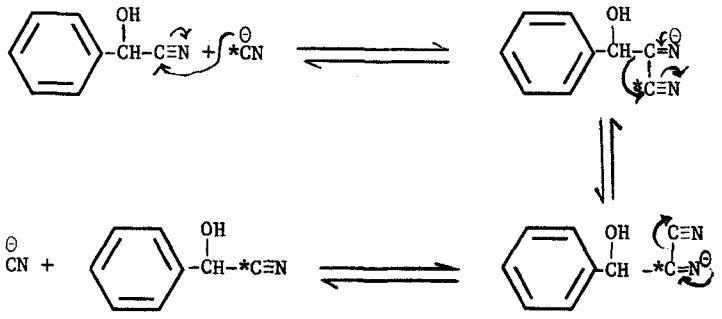
Furthermore, the amine group of the product was derivatized with benzoyl chloride. The benzamide derivative was recrystallized to constant radioactivity and specific activity of the derivative was determined to be equal to the specific activity calculated from the HPLC radiochromatogram for the corresponding [^{14}C]-PEOH.

After this exchange was found to be successful, mechanistic studies were initiated on how nitrile functions exchange with cyanide. For the nitrile-cyanide exchange of mandelonitrile one or more of the following mechanisms could apply:

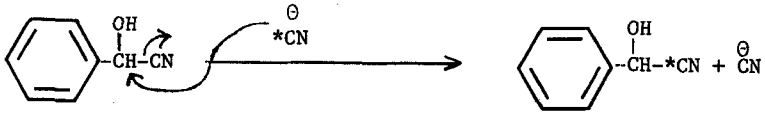
(1) An equilibrium could exist between mandelonitrile and benzaldehyde.



(2) A benzilic acid-type rearrangement mechanism.



(3) A simple SN2 type (cyanide-cyanide displacement) mechanism.



If mechanism 3 is exclusively operating, then a compound like 1-adamantanecarbonitrile in which the nitrile function is on a bridgehead carbon should not exchange (7). When the exchange between 1-adamantanecarbonitrile and [^{14}C]-NaCN was attempted and the reaction product was reduced, TLC analysis and ninhydrin treatment showed the presence of [^{14}C]-1-adamantanemethylamine and an unidentified [^{14}C]-labeled amine in approximately equal amounts of radioactivity.

To exclude mechanism 1 as the sole route of cyanide exchange a similar compound, α-methoxyphenylacetonitrile, which is not amenable to the suggested benzaldehyde-cyanohydrin equilibrium, was studied. The exchange reaction between α-methoxyphenylacetonitrile and [^{14}C]-NaCN was run and the exchanged product was reduced to the corresponding amine, β-methoxy-β-phenylethylamine. The TLC radiochromatogram in conjunction with ninhydrin detection showed the presence of the [^{14}C]-β-methoxy-β-phenylethylamine. Additionally, there were

other radioactive spots on the TLC plate which did not correspond to the [^{14}C]-β-methoxy-β-phenylethylamine. The desired product was characterized by HPLC with an ion-exchange system. The isolated chemical yield for the reduction of α-methoxyphenylacetonitrile to the corresponding amine was 50% and the overall radiochemical yield of the cyanide exchange and borane reduction (based on ^{14}C-cyanide used) was 7%. A standard curve was constructed using authentic β-methoxy-β-phenylethylamine hydrochloride prepared by the borane reduction of the corresponding nitrile, and the product was found by HPLC and melting point to be pure and was further characterized by IR, NMR and elemental analysis. The [^{14}C]-β-methoxyophenylethylamine was derivatized with benzoyl chloride, the [^{14}C]-benzamide derivative was recrystallized to constant radioactivity, and the specific activity (dpm/mmole) was found to be the same as the specific activity determined from the HPLC radiochromatogram of the parent amine.

Comparison of the results of the separate reaction between [^{14}C]-sodium cyanide and excess equimolar amounts of mandelonitrile and α-methoxyphenylacetonitrile followed by borane reduction revealed that the exchange and subsequent reduction of the mandelonitrile resulted in approximately three times more of the carbon-14 labeled amine product than when α-methoxyphenylacetonitrile was used.

The fact that the reaction of α-methoxyphenylacetonitrile with ^{14}C-cyanide followed by reduction yielded the corresponding labeled amine obviously does not exclude mechanism 1 but lends support to mechanisms 2 and/or 3 for the mandelonitrile-cyanide exchange. The reaction of 1-adamantanecarbonitrile with ^{14}C-cyanide followed by borane reduction to labeled 1-adamantanemethylamine lends support to mechanism 2.

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