SYNTHESIS OF SPECIFICALLY LABELED (S)-NICOTINE-5-^{3}H AND (S)-COTININE-5-^{3}H BY CARRIER FREE TRITIOLYSIS OF THE CORRESPONDING 5-BROMO DERIVATIVES

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SUMMARY

The synthesis of high specific activity tritium labeled (S)-nicotine and (S)-cotinine has been achieved. (S)-5-Bromonornicotine of high enantiomeric purity was converted to the corresponding (S)-5-bromonicotine by reductive amination with formaldehyde and sodium borohydride. Tritiolysis of this intermediate with carrier free tritium in the presence of triethylamine using 10% Pd/C catalyst provided (S)-nicotine-5- H with a specific activity of 32 Ci/mmol in ethanol solvent and 28 Ci/mmol in tetrahydrofuran solvent. Similarly, tritiation of (S)-5-bromocotinine, obtained by oxidation of (S)-5-bromonicotine with bromine followed by reduction of the intermediate (S)-3',3',5-tribromonicotine with zinc dust, yielded the corresponding (S)-cotinine-5- H (22.8 Ci/mmol). All reactions proceeded in good to excellent yields.

Key Words: carrier free tritiolysis, (S)-nicotine-5-^{3}H, (S)-cotinine-5-^{3}H

INTRODUCTION

In addition to the toxic effects mediated by its cholinergic stimulating properties (1), the tobacco alkaloid (S)-nicotine (1a) has been reported to be a co-carcinogen when tested with benzo(a)pyrene in a mouse skin assay (2). Furthermore, the reports that (S)-nicotine induces injury to endothelial cells

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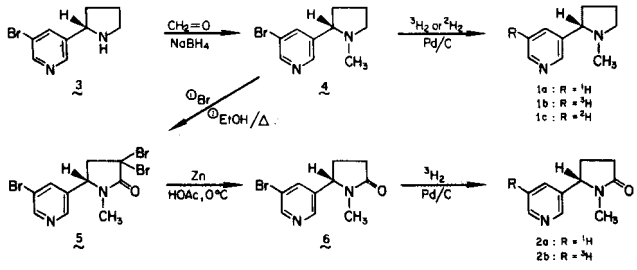
of the aortic arch (3) and hypersensitization reactions (4) indicate the possibility that this alkaloid may cause irreversible biochemical lesions in certain cells. These considerations plus concern about the health effects associated with long term exposure to tobacco products have prompted workers to speculate that (S)-nicotine may be metabolically activated to chemically reactive species capable of alkylating biomacromolecules (5). In order to investigate the formation of such covalent adducts, we required a high specific activity tritium labeled analog of (S)-nicotine. Currently available tritium labeled (S)-nicotine analogs [N-methyl (6) and 4'-labeled (7)] are not suitable for our studies since the label may be lost as a result of known metabolic transformations (8,9). Since the pyridyl moiety of (S)-nicotine does not appear to undergo any bond cleaving metabolic reactions in mammals (10), we have developed a route which has led to the synthesis of high specific activity (S)-nicotine-5-^{3}H (1b). The approach also has been applied to the synthesis of the corresponding analog of (S)-cotinine (2a), namely (S)-cotinine-5-^{3}H (2b). Both products were prepared in enantiomerically pure form.

RESULTS AND DISCUSSION

Our approach to this problem focused on the carrier free tritiolysis of (S)-5-bromonicotine (4) and (S)-5-bromocotinine (6). The key intermediate in these syntheses was (S)-5-bromonornicotine (3) which was available in a state of high enantiomeric purity (>98%) by total synthesis and resolution of the corresponding racemate with (+)-α-methoxy-α-trifluoromethylphenylacetic acid (11). The conversion of 3 to (S)-5-bromonicotine (4) was accomplished by reductive amination using formaldehyde and sodium borohydride (Scheme 1). Reaction of 4 with excess bromine in acetic acid for 1 hour produced (S)-3',3',5-tribromocotinine hydrogen tribromide (5 HBr_3) which subsequently was obtained as the corresponding free base 5. Conversion of 5 to (S)-5-bromocotinine (6) was achieved by careful reduction with zinc dust in acetic acid. It was important to carry out the reaction at 0° C and to monitor the progress of the reaction by TLC in order to prevent reductive cleavage of the bromo group from the pyridine ring.



Scheme I

Prior to performing the carrier free tritiolysis reactions, a series of preliminary deuterolysis studies was conducted to optimize reaction conditions. The course of these reactions was monitored by an HPLC assay which provided quantitative estimations of both starting material and product (Table 1). Deuterolysis of 4 in tetrahydrofuran using a Pd/C catalyst was found to proceed at a rate of 0.67 mmol/min at room temperature. In the presence of triethylamine the same reaction proceeded at a rate of 1.8 mmol/min. This observation as well as that of others (12) point to the importance of neutralizing the reaction generated acid (^{2}HBr) which has been shown to poison the Pd/C catalyst.

A comparison of reaction rates in tetrahydrofuran and ethanol in the presence of triethylamine showed that the deuterolysis proceeded approximately 5 times faster in ethanol (9.2 vs 1.8 mmol/min) and that the yield of (S)-nicotine-5-^{2}H (1c) in the ethanol reaction mixture peaked at about 15 minutes compared to 30 minutes in the tetrahydrofuran reaction mixture. We also

Table 1. Deuterolysis of (S)-5-Bromonicotine to (S)-Nicotine-5-^{2}H Under Various Conditions.

Reaction	(S)-5-Bromonicotine* (mM)	kcat (mM min^{-1})	% Conversion 5 min	% Conversion 60 min
THF - TEA	52	0.67	8.2	55.8
THF + TEA	33	1.8	17.0	93.8
EtOH + TEA	50	9.2	92.0	>99.0
EtOH + TEA	166	26.0	73.0	99.0

observed that the ratio of deuterium consumed to product formed in these reactions increased with time. These observations are consistent with the possibility that the initially formed deuterolysis product may undergo further reduction. The absence of detectable levels of side products by HPLC, capillary column GC and UV analysis of the reaction mixture suggests that, if formed, such overreduction products may undergo spontaneous oxidation during workup (13). Support for this suggestion comes from the observation that the peak height ratios of 1c to starting material analyzed in a reaction mixture that had been stopped short of completion increases upon storage overnight in the presence of air. Furthermore, CI-MS analysis (Table 2) showed the presence of a dideuteronicotine species to the extent of 4% and 7% in the tetrahydrofuran and ethanol reaction mixtures, respectively. This observation is not trivial since overreduction of the pyridine ring by tritium could lead to undesired radiochemical contaminants.

The tritiolysis experiment was carried out both in ethanol and tetrahydrofuran at slightly less than one atmosphere of tritium gas using a custom built manifold. The isolation of (S)-nicotine-5-^{3}H was accomplished by partial lyophilization of the solvents followed by the addition of ethanol to the ethanol containing vessel and further lyophilization to remove exchangeable tritium atoms present in this reaction mixture. Purification of both reaction mixtures was accomplished by semipreparative normal phase HPLC. The purity of the tritium labeled (S)-nicotine-5-^{3}H was confirmed by HPLC analysis employing a UV diode array detector which showed superimposable UV spectra, identical to those of a reference standard, at different positions along the eluting peak (upward inflection, apex, and downward inflection). Concurrent analysis with a radioactivity flow detector established that all of the radioactivity co-eluted with the (S)-nicotine peak. The specific location of the tritium label at the 5-position of the pyridyl moiety was confirmed by ^{3}H NMR analysis which displayed a single signal at δ 7.33 ppm which corresponds to the chemical shift value for the C-5 proton resonance of (S)-nicotine (14). The radiochemical purity of the product isolated from these reactions was estimated by HPLC to be >99%. The specific activities of the 1b isolated from the ethanol and

tetrahydrofuran reaction mixtures were 32 Ci/mmol and 28 Ci/mmole, respectively. The higher than theoretical (29 Ci/mmol) incorporation of tritium in the ethanol reaction may be due to overreduction followed by oxidation of the initially formed product. A similar reaction in ethanol provided pure (S)-cotinine-5-^{3}H (2b) with a specific activity of 22.8 Ci/mmol.

In the past, commercially available tritium labeled nicotine was prepared by N-methylation of naturally occuring nornicotine, a tobacco plant product which is a 60:40 mixture of the (S)- and (R)-isomers. Since the synthesis of enantiomerically pure (R)- and (S)-nornicotine has been accomplished (11), radiolabeled (S)-nicotine of high enantiomeric purity may be prepared by this route. Recently, an alternative synthetic approach has provided both

Table 2. Deuterium Incorporation by Catalytic Deuteration of (S)-5-Bromonicotine

Mass of Parent Ion	Relative Peak Intensity		Distribution of Label	
	THF	ETOH	THF	ETOH
163 (d_0)	63.2	63.0	37.2	24.7
164 (d_1)	100.0	100.0	58.8	68.7
165 (d_2)	6.9	9.6	4.0	6.6

enantiomers of nicotine-4'-^{3}H (7). Unfortunately, the label of both of these analogs of (S)-nicotine is metabolically labile and therefore not suitable to our needs. The synthetic route described in this paper provides a third approach to tritium labeled (S)-nicotine which also is applicable to the synthesis of tritium labeled (S)-cotinine and which leads to analogs in which the tritium label is metabolically stable.

EXPERIMENTAL

General

Melting points (Mel-Temp apparatus) are uncorrected. The tritiations were performed at the National Tritiation Facility, University of California, Berkeley using a custom made tritiation apparatus. Analysis of the radiolabeled products was achieved by HPLC/UV diode array and radiochemical

detection. Instrumentation included the following: Waters model 680 automated gradient controller, M-45 HPLC pump, Z-module, and a Whatman 10 μ Porasil silica cartridge; Hewlett Packard model 85 computer linked to a 1040A UV diode array detector; Berthold ratemeter model BF2304, LSC-module model BF2240, flowmeter model D7547, and a Tracor Northern model TN7200 oscilloscope. Purification of radiolabeled nicotine was accomplished by semi-preparative HPLC utilizing a Rainin microsorb silica column (10 mm x 25 cm) coupled to the controller and pump described above. A Spectroflow model 757 UV detector was utilized and all absorbances (AU setting = 2.0) were monitored at 259 nm. The ^{3}H NMR experiments were performed on a custom built 288 MHz NMR instrument equipped with Nicolet data aquisition software. CI mass spectra were taken on an AEI MS 902S double-focus mass spectrometer equipped with a direct-inlet system and modified for chemical ionization. The reagent gas was isobutane at a pressure of 0.5 to 1.0 torr. The optical purity of (S)-5-bromonornicotine was determined by capillary column (5% methylphenylsilicone) gas chromatography (HP 5889A, temperature program 90 - 275° C at 25°/min) of the camphanic acid amide derivative (11).

Methods

(S)-5-Bromonicotine (4). Sodium borohydride (1 g, 26.5 mmol) was added portionwise with vigorous stirring over a period of 10 minutes to a solution of the (S)-5-bromonicotine as its (+) α-methoxy-α-trifluoromethylphenylacetic acid salt (1 g, 2.2 mmol >98% enantiomeric purity) and 5 mL of 30% aqueous formaldehyde in 40 mL 10:1 isopropanol:acetic acid. Following the addition, most of the solvent was removed on a rotary evaporator and the crude product was dissolved in 100 mL of 5% aqueous sulfuric acid. After washing twice with 50 mL portions of methylene chloride, the aqueous phase was made basic with sodium hydroxide and extracted with methylene chloride (2 x 50 mL). The extracts were combined, dried, evaporated and the residue distilled (Kugelrohr oven, 90-100° C at 10 mm) to give 0.35 g (67%) of a colorless liquid displaying identical physical and spectral properties to those reported for the racemic material (15).

(S)-3',3',5-Tribromocotinine (5). Bromine (1.35 mL, 26.3 mmol) was added

to a solution of 4 (0.7 g, 2.9 mmol) in 10 mL of 80% aqueous acetic acid. The solution was heated at 80° C for 15 minutes, diluted with 25 mL ethanol to reduce excess bromine, and allowed to stand overnight. Most of the solvent was removed on a rotary evaporator and the residue in 100 mL water, pH adjusted to 7-8 with potassium bicarbonate, was extracted with methylene chloride (2 x 50 mL). Evaporation of the solvent yielded an orange gum which was purified by column chromatography on silica (1 x 20 cm column) eluting with ethyl acetate to give 0.8 g of solid. This material had an Rf value on silica gel TLC [ethyl acetate:methanol:58% ammonia (85:10:1)] identical with that of racemic 3',3'-5-tribromocotinine prepared in the same manner. The product was recrystallized from ethanol (fine white needles): mp 145-150° C (dec); ^{1}H NMR ($CDCl_3$) δ 2.75 (s, CH_3), 3.25 (ABX, J_{AB} = 16 Hz, C_4,H_2), 4.62 (m, C_5,H), 7.70 (t, C_4H), 8.45 (d, C_2H), and 8.70 ppm (d, C_6H).

Anal. Calcd for $C_{10}H_9N_2Br_3O$: C, 29.07; H, 2.20; N, 6.79. Found: C, 29.22; H, 2.25; N, 6.88

(S)-5-Bromocotinine (6). Zinc dust (2 g, 31 mmol) was added to a vigorously stirred solution of 5 (0.4 g, 1 mmol) in 20 mL of 95% aqueous acetic acid with external ice bath cooling. After 7 minutes the mixture was filtered through Celite and the filter cake was washed with 10 mL glacial acetic acid. The filtrate was diluted with 50 mL water and the resulting solution was made basic with aqueous ammonia and extracted with methylene chloride (2 x 50 mL). The combined extracts were dried, evaporated on a rotary evaporator, and the residue distilled (Kugelrohr oven, 140-150° C at 0.1 mm Hg) to give 0.24 g of a light yellow liquid that crystallized on standing: mp 89-92° C. Recrystallization from 3:1 methylcyclohexane:toluene provided 0.15 g (0.59 mmol, 59%) of a white crystalline solid which melted at 94.5-95.4° C: ^{1}H NMR ($CDCl_3$) δ 2.25-2.50 (m, C_3,H_2 + C_4,H_2), 2.65 (s, CH_3), 4.40 (m, C_5,H), 7.60 (t, C_4H), 8.30 (d, C_2H), and 8.65 ppm (d, C_6H).

Anal. Calcd for $C_{10}H_{11}N_2BrO$: C, 47.06; H, 4.35; N, 10.99. Found: C, 47.18; H, 4.36; N, 11.17.

Interestingly, racemic 5-bromocotinine prepared in the same manner melted at 81.5-82.5° C after two recrystallizations. Both the racemate and the (S)-

isomer had identical Rf values on silica gel TLC [ethyl acetate:methanol: 58% ammonia (85:10:2)] and contained traces of (S)-cotinine.

Deuterolysis of (S)-5-Bromonicotine. A septum stoppered two-necked reaction vessel (10 mL) containing 1.0 mL anhydrous ethanol, 5.3 mg anhydrous triethylamine (52 µmol), and 6.3 mg of 10% Pd/C was flushed 4 times with 100% deuterium gas. Compound 4 (12.5 mg, 52 µmol) was introduced neat by syringe and the resulting mixture was stirred vigorously in the presence of approximately 5 mmol of deuterium gas. During the course of the reaction 5 µL aliquots were withdrawn at 5 minute intervals through the septum and resuspended in acetonitrile (72 µL) to give a total final concentration of reactant plus product of approximately 0.8 µg/mL. The samples were analyzed by HPLC on a 5 µ Alltech silica column using a mobile phase consisting of 1% propylamine in acetonitrile. The eluent was monitored at 254 nm with a Beckman model 330 fixed wavelength UV detector. Peak heights of the starting compound 4 and the product 1c were compared to peak heights of standard calibration curves to estimate relative amounts of reactant and product. These results as well of those of additional deuteration studies are summarized in Table 1.

(S)-Nicotine-5-^{3}H (1b). The tritiation apparatus was arranged with a glass dual manifold which allowed us to carry out two tritiolysis reactions with the same tritium source. To one reaction vessel (25 mL) was added 4 (43.4 mg, 0.18 mmol) in 1 mL anhydrous ethanol containing 18.2 mg (0.18 mmol) of anhydrous triethylamine and 20 mg of 10% Pd/C. To the second reaction vessel (10 mL) was added 4 (150 mg, 0.62 mmol) in 2.5 mL anhydrous tetrahydrofuran containing 83 mg (0.82 mmol) anhydrous triethylamine and 40 mg of 10% Pd/C. The flasks, under nitrogen, were cooled with liquid nitrogen and placed under vacuum (30 mtorr). The flasks were flushed an additional two times in this way. The vapor pressure of water measured on a Varian Model 810 tc vacuum gauge was less than 10 mtorr following this procedure. Carrier free tritium gas (58 Ci/mmol) was introduced into the reaction vessels to a pressure of 735 and 670 torr for the ethanol and tetrahydrofuran solutions, respectively. The liquid nitrogen baths were removed and the progress of the

tritiation monitored by noting the decrease in the partial pressure of tritium gas. After 25 minutes the partial pressure of tritium had plateaued at about 677 and 413 torr, respectively. The vessels were cooled once again in liquid nitrogen following which remaining tritium gas was removed by flushing with nitrogen gas. The cooled reaction mixtures were concentrated to about 1/2 initial volume under vacuum (50 mtorr for the ethanol and 90 mtorr for the tetrahydrofuran mixture). In order to insure complete degassing, 5 mL of anhydrous ethanol was added to the ethanol reaction mixture which again was concentrated to 1/2 initial volume. Additional anhydrous ethanol or tetrahydrofuran (2 mL) was added to the respective reaction mixture which then was filtered through 0.2 μ nylon to remove the Pd/C catalyst. The reaction flasks were rinsed with ethanol (2 mL) which was filtered and combined with the appropriate initial filtrate.

Purification of (S)-Nicotine-5-^3H (1b). The reaction mixtures initially were analyzed by UV diode array and radiochemical detectors following separation on a 10 μ Porasil silica HPLC cartridge (Whatman). By both UV and radiochemical criteria the product obtained from the ethanol reaction appeared to be pure while the tetrahydrofuran reaction mixture contained approximately 10% starting material. To insure UV and radiochemical purity, the following procedure was adopted. From each stock reaction mixture injections equivalent to approximately 650 μg of the product were made on a semipreparative silica HPLC column (Rainin) with acetonitrile--1% propylamine as mobile phase. Fractions corresponding to the elution time of (S)-nicotine were collected, reanalyzed by HPLC, and pooled. The pooled fractions (50 mL) were concentrated to 5 mL under a slight vacuum at 48° C. To this concentrate was added 10 mL of 0.05 N HCl. Most of the remaining acetonitrile was removed by rotary evaporation. The remaining solution was transfered to a 25 mL volumetric flask. Ethanol (2.5 mL) was added to the aqueous solution of radiolabeled product as a radical trapping agent. Final UV and radiochemical purity were confirmed by HPLC on silica employing a combined UV diode-array/radiochemical flow detector. The UV purity was estimated as 99.4 and 100% for the ethanol and tetrahydrofuran reaction products, respectively, by comparison of

absorption ratios at 260 nm (λ_{max}) to 240 and 280 nm. Radiochemical purity in both instances was >99.0%. The final specific activity for the preparations were 32 Ci/mmol for the ethanol reaction product and 28 Ci/mmol for the tetrahydrofuran reaction product. For ^{3}H NMR analysis, an aliquot of approximately 100 mCi of 1b purified from the tetrahydrofuran reaction was evaporated to dryness under vacuum and then dissolved in $CDCl_3$. The 288 MHz spectrum displayed a singlet at δ 7.33 ppm as the only detectable signal.

(S)-Cotinine-5-^{3}H (2b). A solution of 6 (100 mg, 0.4 mmol) in 1 mL anhydrous ethanol containing 0.1 mL triethylamine and 50 mg of 10% Pd/C was exposed to carrier free tritium under conditions analogous to those described above for the synthesis of 1b. After 1 hour the solvent was removed by lyophilization. The residue in 2 mL ethanol containing 100 mL triethylamine was centrifuged to remove the catalyst. After degassing, the catalyst was washed with 1 mL ethanol. The combined supernatant fractions were lyophilized to give a white solid which was triturated with 8 mL of benzene. TLC analysis [ethyl acetate:methanol:58% ammonia] (80:20:2) indicated that the product was essentially pure (S)-cotinine. The radiochemical purity was estimated to be >98% by TLC with the following two solvent systems: ethyl acetate:methanol:58% ammonia (80:20:2) and chloroform:methanol:acetic acid (6:9:1). The specific activity was 22.8 Cimmol.

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