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Synthesis of racemic [methyl-d_3]-labeled *cis*- and *trans*-3'-hydroxycotinine

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Summary

A method is described for the synthesis of the racemic [methyl-d_3] forms of the nicotine metabolites *cis*-3'-hydroxycotinine and *trans*-3'-hydroxycotinine. The key intermediate was [methyl-d_3]-N-methylhydroxylamine, obtained from a selective hydrogenation of d_3-nitromethane. This intermediate was converted to [methyl-d_3]-α-3-pyridyl-N-methylnitrone, which was condensed with methyl acrylate to give a mixture of isomeric isoxazolidines. The hydrogenolysis of this mixture afforded a 70:30 mixture of [methyl-d_3] *cis*- and *trans*-3'-hydroxycotinine, from which the pure *cis*- isomer could be isolated by recrystallization from acetone. [Methyl-d_3]-*trans*-3'-hydroxycotinine could be prepared in high yield from the *cis*-isomer via chiral inversion utilizing a Mitsunobu reaction, or by chromatographic separation from a mixture of the *cis*- and *trans*-3'-benzoyloxycotinine, followed by O-debenzoylation in methanolic NaOH.

Key words: Deuterated nicotine metabolites, [methyl-d_3]-3'-hydroxycotinine.

INTRODUCTION

S(-)-Nicotine (**1**), the major alkaloid present in tobacco, is extensively metabolized in man to more than twenty known biotransformation products[1]. The principal metabolite of S(-)-nicotine found in the urine of smokers is 3'-hydroxycotinine (**2a/2b**)[2,3], which is presumably formed from the further metabolism of the primary oxidation product, S(-)-cotinine (**2c**)[4]. Both the *cis* and *trans*-isomers of 3'-hydroxycotinine (**2a** and **2b**, respectively) have been detected as metabolites of S(-)nicotine, however, *cis*-3'-hydroxycotinine is only a minor metabolic product[5,6]. 3'-Hydroxycotinine has been identified as a potential marker for determining exposure to tobacco smoke based upon its favorable pharmacokinetic profile[6,7]. A glucuronide conjugate of

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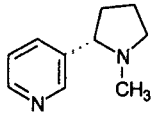
3'-hydroxycotinine, which appears to be a relatively long-lived metabolite of S(-)-nicotine in man, has a kinetic disposition that is unaltered by cigarette smoking, suggesting that it could also be used as a sensitive and reliable index for determining exposure to tobacco smoke[1]. Thus the availability of the deuterium-labeled forms of *cis*- and *trans*-3'-hydroxycotinine should be useful in the development of direct LC-MS analytical methods for quantifying these polar nicotine metabolites in smokers' urine.

While the syntheses of deuterium labeled forms of nicotine, nornicotine and cotinine have been described previously[8-11] , no reports on the preparation of the [methyl-d_3]-labeled form of 3'-hydroxycotinine are available. In this communication, we report the total synthesis of both the *cis*- and *trans*-isomers of [methyl-d_3]-3'-hydroxycotinine based on a modification[12] of Dagne and Castagnoli's method[13] .



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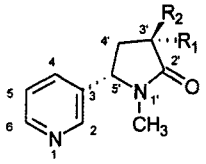


2a R^1=OH , R^2=H

2b R^1=H , R^2=OH

2c R^1=R^2=H

EXPERIMENTAL

[Methyl-d_3]-nitromethane 99.0% gram atom D was obtained from Merck, Sharp and Dohme Isotopes (St. Louis, MO), all other chemicals and solvents were purchased from the Aldrich Chemical Company (Milwaukee, WI).

Melting points were recorded on a Fisher Johns melting point apparatus and are uncorrected. Silica gel plates (2.5 x 7.5 cm), fluorescent at 254 nm, were purchased from Diamond Whatman International Ltd. (Hillsboro, OR). Column chromatographic separations were carried out using silica gel, 200-400 mesh, 60Å from the Aldrich Chemical Company. ^{1}H-NMR, ^{13}C-NMR, DEPT and two-dimensional NMR spectra were obtained on a Varian

VXR-300 MHz spectrometer (Palo Alto, CA); spectra were run at 21°C in either $CDCl_3$, D_2O or DMSO-d_6 using tetramethyl silane (TMS) or the sodium salt of 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid (TSP) as internal standard. Mass spectra were recorded on a Kratos Concept 1H spectrometer by either fast atom bombardment or electron impact. Isotopic purity of the deuterated metabolites was determinated by high resolution mass spectrometry.

[Methyl-d_3]-N-methylhydroxylamine sulfate (3)

A solution of [methyl-d_3]-nitromethane (4g, 62 mmole) and concentrated sulfuric acid (3.1g, 31 mmole) in 35 mL of water was hydrogenated at 50 psi H_2 in the presence of 5% Pd-on-carbon (130 mg) at room temperature in a Parr apparatus for 15 hours. The catalyst was filtered off over a bed of celite, and washed with water (2x10 mL). The resulting aqueous filtrate was washed with CH_2Cl_2 (2x5 mL), and evaporated to dryness to give (**3**) as a white solid, which was washed with cold MeOH (2x2 mL) and dried under high vacuum. Yield 5.1g (83%); mp=127°-131°C; CMR (D_2O + TSP): δ 38.2 ppm (heptuplet).

[Methyl-d_3]-α-3-pyridyl-N-methylnitrone (4)

A solution of 3-pyridine carboxaldehyde (2.8g, 26.1 mmole) and [methyl-d_3]-N-methyl-hydroxylamine sulfate (1.94g, 9.78 mmole) in absolute EtOH (20 mL) was stirred for 30 minutes under reflux, then for 6 hours at room temperature. The resulting white solid (1.7g) was collected, and the mother liquor was stirred for 20 additional hours, during which time a second crop of crystals (195 mg) was collected. An additional batch of crystals (834 mg) was obtained by stirring the filtrate for 48 hours at room temperature, concentrating to dryness and washing the residue with Et_2O (2x5 mL). The combined sample of [methyl-d_3]-α-3-pyridyl- N-methyl- nitrone sulfate (2.7g, 9.6 mmole); mp=135°-137°C, was dissolved in water (75 mL) and saturated with solid K_2CO_3. The basic solution was concentrated to 20 mL and extracted with $CHCl_3$ (4x25 mL). The extracts were dried over $MgSO_4$, filtered and concentrated to give 1.5g (56% yield) of (**4**) as an oil which slowly crystallized on cooling (-20°C). PMR ($CDCl_3$ + TMS) δ 9.05-9.00 (1H, m, H-6), 8.95 (1H, s,H-2), 8.63-8.6 (1H, m, H-4), 7.42-7.38 (2H, m,CH=N

and H-5) ppm. CMR ($CDCl_3$ + TMS) δ 150.2 (C-6), 149.8 (C-2), 134.6 (C-3),132.5 (CH=N), 127.1 (C-4), 123.7 (C-5),53.9 (m, CD_3) ppm.

(±)-[methyl-d_3]-*cis*-3'-hydroxycotinine (7)

A solution of nitrone (**4**) (1.8g, 12.9 mmole) in methyl acrylate (7 mL) was stirred at room temperature for 5 days. The residue obtained after removing the solvent, was mixture of the deuterated isoxazolidine isomers (**5**). This mixture of isoxazolidines (2.85g, 12.6 mmole) was hydrogenated (Parr apparatus, 50 psi) in absolute EtOH (130 mL) over freshly prepared Raney nickel catalyst for 3 days. The reaction mixture was filtered, the catalyst washed with absolute EtOH (2x10 mL), and the solvent was removed by rotary evaporation to give a crude product, which was purified by silica column chromatography (100g) by elution with 2% methanol in chloroform to yield 1.6g (67%) of a mixture of [methyl-d_3]-*cis*-and *trans*-3'-hydroxycotinine (**6**) (70/30, respectively). The pure *cis* isomer (**7**) was obtained after three recrystallizations from acetone (590 mg, 0.3 mmole); mp=149°-150°C. PMR (DMSO-d_6 + TMS) δ 8.57-8.53 (2H,m, H-2 and H-6), 7.74-7.70 (1H, m, H-4), 7.47-7.43 (1H, m, H-5), 5.74 (1H, dd, J=6.1,1.5Hz, OH), 4.48 (1H, t, J=7.1Hz, H-5'), 4.26-4.18 (1H, m, H-3'), 2.8-2.7 (1H, m, H-4'a), 1.63-1.54 (1H, m, H-4'b) ppm. CMR (DMSO-d_6+ TMS) δ 174.4 (C=O), 149.2 (C-2), 148.8 (C-6), 136.1 (C-3), 134.6 (C-4), 123.9 (C-5), 68.4 (C-3'), 57.2 (C-5'), 38.0 (C-4'), 27.0 (m, CD_3) ppm. EI-MS: 195 (37, M+), 106 (100) m/z.

Lit.[13] PMR ($CDCl_3$) δ 5.5 (1H, b exchangeable, OH), 4.5 (2H, m, H-3' and H-5'), 2.9 (1H, m, H-4' *trans* to pyridyl), 2.64(3H, s, NCH_3), 2.0 (1H, m, H-4' *cis* to pyridyl) ppm.

(±)-[methyl-d_3]-*trans*-3'-benzoyloxycotinine (8)

Method 1. The pure *cis*-isomer (**7**) (300 mg, 1.6 mmole), benzoic acid (245 mg, 2.04 mmole) and triphenylphosphine (540 mg, 2.04 mmole) were dissolved in dry THF (40 mL) at 25°C, dry toluene (40 mL) was added, and the solution cooled to -50°C under N_2. Diethyl azodicarboxylate (DEAD) (0.323 mL, 2.04 mmole) was added *via* a syringe over a 1 minute period and the reaction was stirred for 1.5 hours at -50°C. After removal of the solvents *in vacuo*, the residue was chromatographed on a silica column (12g) with chloroform-acetone

(4:1 v/v) as elution solvent. The required product was recrystallized from acetone:ether to afford 410 mg (77 %) of [methyl-d_3]-*trans*-3'-benzoyloxycotinine (**8**); m.p.=146°-148°C; PMR ($CDCl_3$ + TMS) δ 8.65-8.63 (1H, m, H-6), 8.53 (1H, d, J=2.2Hz, H-2), 8.11-8.07 (1H, m, H-5), 7.62-7.26 (6H, m, H-4 and ArH), 5.7 (1H, dd, J=8.4,6.7Hz, H-3'), 4.75 (1H, dd, J=8.4,4.0Hz, H-5'), 2.65-2.50 (2H, m, H-4') ppm. EI-MS 299 (52, M+) m/z.

Method 2. The mother liquors from the recrystallization of the *cis* isomer (**7**) were evaporated to dryness (920 mg, 4.7 mmole), mixed with benzoic anhydride (1.4g, 6.2 mmole) and triethylamine (630 mg, 6.2 mmole) under nitrogen and the mixture stirred at room temperature for 20 hours. The reaction mixture was diluted with water (5 mL) and washed with CH_2Cl_2 (2x10 mL). The aqueous solution was neutralized with a saturated solution of $NaHCO_3$ and extracted with CH_2Cl_2 (3x15 mL). The organic solution was dried over $MgSO_4$, filtered and concentrated to give a mixture of [methyl-d_3]-*cis* and [methyl-d_3]-*trans*-3'- benzoyloxycotinine (**9**). The pure *trans* isomer (**8**) (333 mg, 1.1 mmole) was isolated by silica column chromatography (80g) by elution with chloroform:acetone (7:3 v/v). The oily product crystallized slowly on cooling (0° C) and was identical to the product obtained by method 1 above.

(±)-[methyl-d_3]-*trans*-3'-hydroxycotinine (**10**)

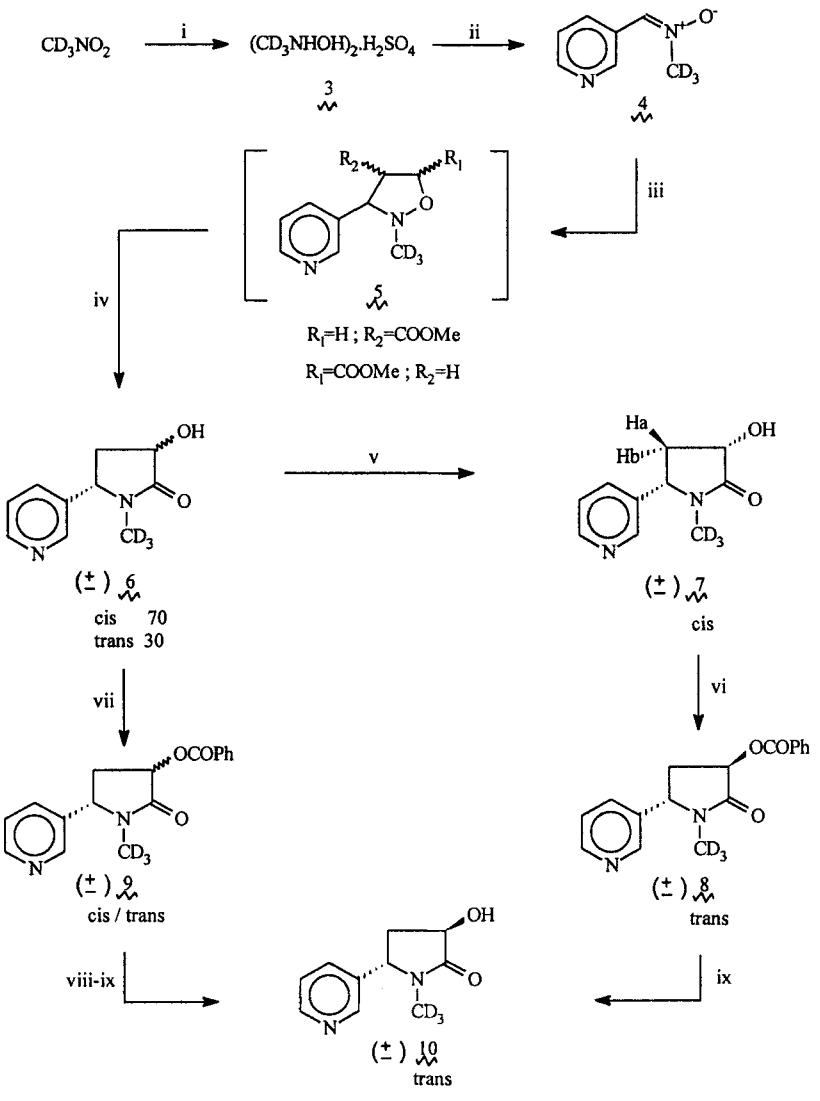
A solution of [methyl-d_3]-*trans*-3'-benzoyloxycotinine (**8**) (200 mg, 0.67 mmole) in methanolic NaOH (10 mL, 1% w/v) was stirred at room temperature for 15 minutes. The solution was evaporated to dryness, and chromatographed on a silica column (7 g) with chloroform as eluent, followed by elution with 2% methanol in chloroform. The fractions containing the product were evaporated to give an oil, which was dissolved in a mixture of acetone-ether (2:1 v/v) and stored at -20°C for 20 hours. An initial crop of white crystals (54 mg) was collected, and after concentration of the mother liquor to half its volume and cooling for 20 hours at -20°C, a second crop was collected to yield a total of 99.6 mg (78%) of [methyl-d_3]- *trans*-3'-hydroxycotinine (**10**). PMR (DMSO-d_6 + TMS) δ 8.58-8.52 (1H, m, H-6), 8.50-8.46 (1H, m, H-2), 7.66-7.59 (1H, m, H-4), 7.44-7.39 (1H, m, H-5), 5.7 (1H, d, J=6.1Hz,

OH), 4.73-4.68 (1H, m, H-5'), 4.38-4-31 (1H, m, H-3'), 2.25-2.12 (2H, m, H-4') ppm. EI-MS: 195 (50, M+), 106 (100) m/z.

Lit.[14]: PMR ($CDCl_3$) δ 8.6-8.52 (1H, m, H-6), 8.48 (1H, d, H-2), 7.5-7.4 (1H, m, H-5), 7.38-7.3 (1H, m, H-4), 4.6-4.4 (2H, m, H-3' and H-5'), 2.8 (3H, s, NCH_3), 2.6-2.4 (1H, m, H-4' *trans* to pyridyl), 2.3-2.2 (1H,m, H-4' *cis* to pyridyl) ppm.

RESULTS AND DISCUSSION

The preparation of racemic [methyl-d_3]-3'-hydroxycotinine was carried out *via* the synthetic routes illustrated in the Scheme. Initially, commercially available [methyl-d_3]-nitromethane was hydrogenated in acidic media over 5% palladium-on-carbon, to afford [methyl-d_3]-N-methyl-hydroxylamine sulfate (**3**), which could then be condensed with 3-pyridine-carboxaldehyde to form the [methyl-d_3]-α-3-pyridyl-N-methylnitrone (**4**). Reaction of the nitrone with methyl acrylate afforded a mixture of the intermediate deuterated isoxazolidine isomers (**5**). PMR analysis of the crude reaction product suggested the presence of three isomeric forms, since three singlets assignable to OCH_3 ester resonances in the ration 9:1:1 were observed. This mixture was immediately hydrogenated over freshly prepared Raney nickel catalyst, to afford after chromatography, 67% of a 70:30 mixture of the *cis* and *trans* isomers, respectively, of racemic [methyl-d_3]-3'-hydroxycotinine (**6**). The *cis* isomer was only sparingly soluble in acetone and could be obtained in a pure crystalline form after three recrystallizations of the *cis/trans* mixture from acetone. The PMR spectrum reported in the literature [12,14] for the unlabeled *cis*-3'-hydroxycotinine, using $CDCl_3$ as solvent, shows one overlapping multiplet centered near 4.5 ppm and assigned to the signals for H-3' and H-5'. The PMR spectrum of (**7**) under the same condition gave the same result , however when the spectrum was run in DMSO-d_6, the signals for H-3' and H-5' occur as separate multiplets at 4.48 (t, H-3') and 4.26-4.18 (m, H-5').The proton assignments were made with the assistance of the two-dimensional heteronuclear correlation (hetcor) spectrum of (**7**). The pure *cis* isomer (**7**) was treated with benzoic acid, triphenylphosphine and diethyl azodicarboxylate at -50°C to afford the benzoate ester of racemic [methyl-d_3]-*trans*-3'-hydroxycotinine (**8**), which was converted to



i) H_2 , H_2O-H_2SO_4 , Pd/C , 50 psi ii) 3-Pyridine carboxaldehyde , abs. EtOH
iii) methyl acrylate iv) H_2 , abs. EtOH , Raney Ni , 50 psi v) recryst. acetone
vi) PhCOOH , $(Ph)_3$ P , DEAD, THF-toluene vii) $(PhCO)_2O$, Et_3N , room temp.
viii) chromatography ix) 1% NaOH in MeOH , room temp.

Synthesis of(±)-[methyl-d 3] *cis* and *trans*-3'-hydroxycotinine

racemic[methyl-d_3]- *trans*- 3'-hydroxycotinine (**10**) by treatment with 1% methanolic NaOH. The *trans* isomer could also be obtained by benzoylation of the *cis/trans* mixture (**9**) with benzoic anhydride, followed by separation of the *trans* benzoate ester by silica gel chromatography, and O-debenzoylation in methanolic NaOH. The structure of (**10**) was established from a hetcor experiment. Isotopic purity was determinated on methanolic solutions of (**7**) and (**10**) by GC-EI mass spectral analysis. Isotopic purity of the [methyl-d_3]-labeled *cis* and *trans*-3'-hydroxy-cotinine isomers was determined by comparing the relative integrated peak areas of unlabeled and d_3 labeled compounds for the M^+, $(M-1)^+$, $(M-2)^+$ and $(M-3)^+$ ions, and correcting the values for losses of H from the molecular ion, based on the unlabeled compounds behaviour. The *cis*-isomer (**7**) afforded a value of 97.2 atom % D. Values of 97.4 and 96.8 atom % D for *trans* isomer (**10**) were obtained when prepared from (**8**) and (**9**) respectively.

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REFERENCES

1. Kyerematen, G.A., Vesell, E.S. - Drug Metab. Rev. 23, 3 (1991).
2. Crooks, P.A. - Biochemistry and Metabolism of Nicotine and Related Alkaloids (Gorrod, J.W. and Wahren, J., Eds.), Chapman Hall, London, p.81 (1993).
3. Benowitz, N.L. - The Pharmacology of Nicotine (Rand, M.J. and Thurau, K. Eds.), IRL press, Oxford, p.3 (1988).
4. Cundy, K.C. and Crooks, P.A. - Xenobiotica 17, 785 (1987).
5. Adlkofer, F., Scherer, G., Jarczyk, L., Heller, W.D. and Neurath, G.B. - The pharmacology of Nicotine (Rand, M.J. and Thurau, K. Eds.), IRL press, Oxford, p.25 (1988).
6. Jacob, P. III, Benowitz, N.L. and Shulgin, A.T. - Pharmacol. Biochem. Behav. 30, 249 (1988).
7. Voncken, P., Rustemeier, K. and Schepers, G. - Xenobiotica 20, 1353 (1990).
8. Nguyen, T.-L. and Castagnoli, N. Jr - J. Labelled Compounds Radiopharm. 14, 919 (1978).
9. Seeman, J.L., Secor, H.N. and Forrest, G. - J. Labelled Compounds Radiopharm. 14, 387 (1978).
10. Pitner, T.P., Edwards, W.B. III, Bassfield, R.L. and Whidby, J.F. - J. Amer. Chem.Soc.100, 246 (1978).

11. Jacob, P. III, Benowitz, N.L. and Shulgin, A.T. - J. Labelled Compounds Radiopharm. 25, 1117, (1988).

12. Crooks, P.A., Bhatti, B.S., Ravard, A., Riggs R.M. and Caldwell W.S. - Med. Sci. Res. 20, 881 (1992).

13. Dagne, E. and Castagnoli, N. Jr - J. Med. Chem. 15, 356 (1972).

14. Desai, D. and Amin, S. - Chem. Res. Toxicol. 3, 47 (1990).