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N-Methylputrescine Oxidation during Cocaine Biosynthesis: Study of Prochiral Methylene Hydrogen Discrimination Using the Remote Isotope Method†

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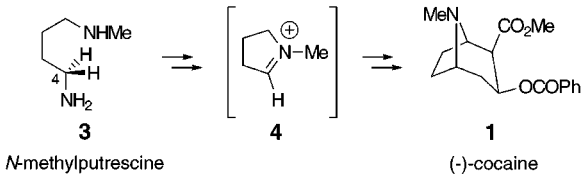
ABSTRACT



3 *N*-methylputrescine **4** **1** (-)-cocaine

The stereoselectivity of *N*-methylputrescine (3) oxidation to pyrrolinium ion 4 in *Erythroxylum coca* during cocaine (1) biosynthesis was studied. The remote isotope method was used to advantage. Each enantiomer of 4-monodeuterated *N*-methylputrescine served as a precursor for plant feeding. To facilitate mass-spectrometric analysis of products, a $^{2}H_{3}{}^{13}C$-methyl group was also incorporated into the 4-deuterio-*N*-methylputrescines. Oxidative deamination of *N*-methylputrescine was found to be stereoselective; the *pro-S* hydrogen atom is removed with 6–10:1 selectivity.

The biosynthesis of cocaine (and related tropane alkaloids) was investigated for over a decade by Leete,[1] and the latest hypothesis[1c] for the assembly of cocaine in *Erythroxylum coca* is summarized in Scheme 1. It starts with l-ornithine (**2**) and passes through *N*-methylputrescine (**3**), which is then oxidized to form *N*-methyl-Δ^{1}-pyrrolinium ion (**4**). Addition of acetyl coenzyme A (**5**) gives **6**. Acylation of another acetyl coenzyme A unit leads to the 4-acetoacetyl coenzyme A derivative **7**. Pyrrole oxidation and cyclization lead to the tropane derivative **9** via **8**. Ketone reduction provides ecgonine methyl ester (**10**), which is benzoylated to give cocaine (**1**). Benzoic acid required for esterification of **10** en route to cocaine is synthesized from phenylalanine via cinnamic acid.[1h] A similar biosynthetic pathway has been proposed for tropane alkaloid biosynthesis in *Datura innoxia*.[2] We have now studied the question of whether the initial oxidation of the primary amine carbon in *N*-methylputrescine (**3**) is stereoselective with respect to removal of the *pro-R* or *pro-S* methylene hydrogen atom.[3]

Isotopic labeling strategies of various types play important

† Dedicated to the memory of our former colleague and mentor, Professor Edward Leete, whose long career in plant alkaloid biosynthesis and overall zeal for life continue as inspiration.

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(1) (a) Huang, M. N.; Abraham, T. W.; Kim, S. H.; Leete, E. *Phytochemistry* **1996**, *41*, 767−773. (b) Bjorklund, J. A.; Leete, E. *Phytochemistry* **1992**, *31*, 3883−3887. (c) Leete, E.; Bjorklund, J. A.; Couladis, M. M.; Kim, S. H. *J. Am. Chem. Soc.* **1991**, *113*, 9286−9292. (d) Couladis, M. M.; Friesen J. B.; Landgrebe, M. E.; Leete, E. *Phytochemistry* **1991**, *30*, 801−805. (e) Leete, E. *Planta Med.* **1990**, *56*, 339−352. (f) Leete, E. *Heterocycles* **1989**, *28*, 481−487. (g) Leete, E.; Kim, S. H. *J. Am. Chem. Soc.* **1988**, *110*, 2976−2978. (h) Leete, E.; Bjorklund, J. A.; Kim, S. H. *Phytochemistry* **1988**, *27*, 2553−2556. (i) Leete, E. *J. Nat. Prod.* **1987**, *50*, 30−35. (j) Leete, E. *Phytochemistry* **1983**, *22*, 699−704. (k) Leete, E. *J. Am. Chem. Soc.* **1983**, *105*, 6727−6728. (l) Leete, E. *Rev. Latinoam. Quim.* **1983**, *14*, 1−6. (m) Leete, E. *J. Am. Chem. Soc.* **1982**, *104*, 1403−1408. (n) Leete, E. *J. Chem. Soc. Chem. Comm.* **1980**, *23*, 1170.

(2) Abraham, T. W.; Leete, E. *J. Am. Chem. Soc.* **1995**, *117*, 8100−8105.

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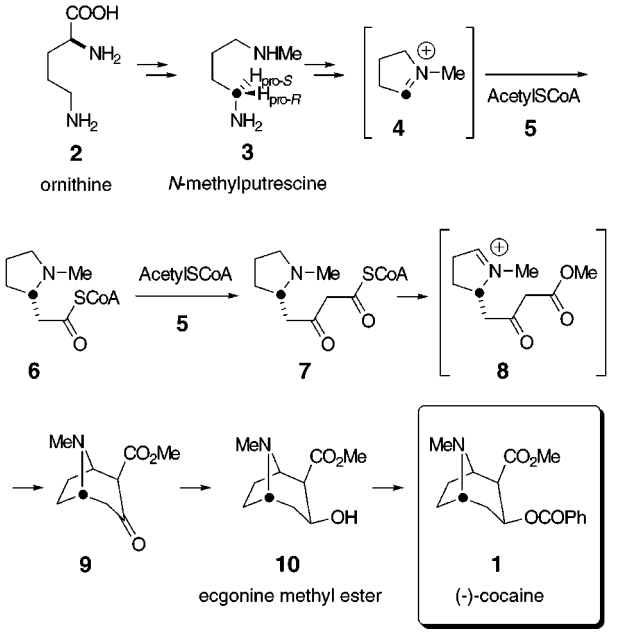
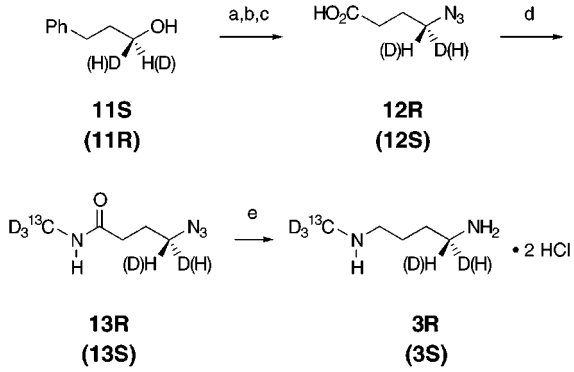
Scheme 1



roles in the study of biosynthetic pathways. Feeding experiments using precursors bearing either radioactive atoms or two adjacent, NMR-active nuclei are two examples of these powerful methods. The specific incorporation of the labeled precursor into the final natural product is a factor that dictates the choice of strategy. While direct mass spectrometric determination of a label in the natural product is often a potentially very convenient method, its use has been limited. There are many complications inherent in accurately determining the extent of incorporation of labeled precursors bearing a single heavy atom (e.g., ^{2}H or ^{13}C) vis-à-vis the natural abundance P + 1 peaks. One solution to this problem has been to apply the remote isotope method, wherein a second, remote (spectator) label is introduced.[4] The mass of those molecules that have incorporated labeled precursor is moved to a region of the spectrum where there is no interference from the natural abundance peaks of the unlabeled product.[5]

To use the remote isotope method to probe the oxidation of C(4) in *N*-methylputrescine (**3**), we identified the enantiomeric monodeuterated species **3R** and **3S**, each of which also contains a $^{2}H_{3}{}^{13}C$ labeled *N*-methyl group as attractive candidates for feeding to *E. coca*. These precursors were

Scheme 2[a]



[a] (a) MsCl, Et_3N, CH_2Cl_2, 81−86%; (b) NaN_3, DMF, 63−77%; (c) $RuCl_3{\cdot}H_2O$, $NaIO_4$, 4 Å ms, 60−97%; (d) isobutyl chloroformate, *N*-methylmorpholine, CH_2Cl_2; $^{13}CD_3NH_2{\cdot}HCl$, NaOH, K_2CO_3, CH_2Cl_2; (e) $LiAlH_4$, THF; HCl, i-PrOH, 73−77% (for two steps).

prepared in parallel as shown in Scheme 2 starting from 1-^{2}H-3-phenylpropanols **11S** and **11R**.[6] Mesylation and azide displacement (assumed to proceed with inversion of configuration) and oxidation of the phenyl ring gave the monodeuterated 4-azidobutanoic acids **12**. Amide formation using $^{2}H_{3}{}^{13}CNH_2$ introduced the remote label and provided amides **13**, each of which was finally reduced to the enantiomers of *N*-methylputrescine **3R** and **3S**.[7]

The results are summarized in Table 1. An initial feeding in November led to a barely detectable level of specific incorporation (columns 2 and 4). This was evidenced by the low intensity of the P + 4 (0.03% vs P) and P + 5 (0.02%

Table 1. Relative Intensity of Cocaine Molecular Ion Peaks[a,b]

		3S feeding		**3R** feeding	
mass	natural abundance	Nov	May	Nov	May
303 (P)	100.000	100.00	100.00	100.00	100.00
304 (P + 1)	18.397	18.51	17.28	19.00	17.69
305 (P + 2)	2.558	2.17	2.31	2.23	2.58
306 (P + 3)	0.239	0.14	0.27	0.19	0.26
307 (P + 4)	0.019	**0.03**	**1.07**	0.00	0.18
308 (P + 5)	0.006	0.00	0.30	**0.02**	**1.42**
309 (P + 6)	0.004	0.00	0.08	0.00	0.36

[a] Data from low-resolution EI (70 eV) mass spectra. [b] Although the relative intensities for the peaks associated with the array of molecular ions are only reported here, analogous patterns can be observed for various of the fragment ions, including the array associated with the base peak at *m*/*z* 182 (see Supporting Information).

(3) This oxidation has been studied and found to be selective for removal of the *pro-S* hydrogen at C(4) of *N*-methylputrescine in the biosynthesis of nicotine in *Nicotiana tabaccum* and *N. glutinosa*: Wigle, I. D.; Mestichelli, L. J. J.; Spencer, I. D. *J. Chem. Soc., Chem. Commun.* **1982**, 662−664.

(4) A "remote label technique" has been used extensively to facilitate the study of various *kinetic* isotope effects: (a) O'Leary, M. H.; Marlier, J. F. *J. Am. Chem. Soc.* **1979**, *101*, 3300−3306. (b) Kiick, D. M. In *Enzyme Mechanisms from Isotope Effects*; Cook. P. F., Ed.; CRC Press: Boca Raton, FL, 1991; Chapter 12.

(5) For recent examples, see: (a) Li, Y.; Alanine, A. I. D.; Vishwakarma, R. A.; Balachandran, S.; Leeper, F. J.; Battersby, A. R. *J. Chem. Soc., Chem. Commun.* **1994**, 2507−2508. (b) Barrot, M.; Fabrias, G.; Camps, F. *Tetrahedron* **1994**, *50*, 9789−9796. (c) Kumar, P.; Chilton, S. *Tetrahedron Lett.* **1994**, *35*, 3247−3250. (d) Walker, K. D.; Floss, H. G. *J. Am. Chem. Soc.* **1998**, *120*, 5333−5334.

(6) Enantiomers **11S** and **11R** were prepared, respectively, by *S*- and *R*-Alpine-Borane reduction (Midland, M. M.; Tramontano, A.; Zderic, S. A. *J. Am. Chem. Soc.* **1977**, *99*, 5211−5213) of ^{2}H-3-phenylpropanal (e.g., Trost, B. M.; Kulawiec, R. J. *J. Am. Chem. Soc.* **1993**, *115*, 2027−2036. For an alternative preparation, see: Keck, G. E.; Krishnamurthy, D. *J. Org. Chem.* **1996**, *61*, 7638−7639). The enantiomeric purity was verified by formation of the Mosher ester of each of **11S** and **11R**. Barely perceptible ^{1}H NMR resonances for the minor diastereomer of each MTPA ester were too small to be integrated.

vs P) molecular ions in the mass spectrum of cocaine isolated from the feeding of precursors **3R** and **3S**, respectively. Although it is dangerous to draw quantitative conclusions about the degree of stereoselectivity on the basis of these data, it is noteworthy that the use of the isotopic spectator group permitted detection of specific incorporation of approximately 0.01% (1 part in 10 000) using direct mass spectrometric analysis of the natural product. Such information would have been impossible to obtain without the use of the remote (spectator) label.[8]

A subsequent pair of feeding experiments was carried out in May, when the *E. coca* plants were clearly growing more vigorously. A substantially higher level of incorporation (>1%) was observed (Table 1, columns 3 and 5). The oxidation of the primary amine carbon in *N*-methylputrescine (**3**) is stereoselective for removal of the *pro-S* enantiotopic hydrogen. That is, the predominant cocaine molecule resulting from enantiomer **3S** is *four* mass units (P + $^2H_3{}^{13}C$) higher than the most abundant parent ion. The major species emanating from the **3R** enantiomer is *five* mass units (P + $^2H_4{}^{13}C$) higher. We estimate that oxidative loss of the *pro-S* vs *pro-R* hydrogen atom occurs with a ratio of 6−10:1.[9] Thus, the sense of enantioselectivity of *N*-methylputrescine oxidation is the same in both nicotine biosynthesis in tobacco plants[3] and cocaine biosynthesis in *E. coca*.

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Supporting Information Available: The contents include experimental procedures and characterization data for compounds **12R/12S**, **13R/13S**, **3R/3S**, and intermediates leading to each. Details of the feeding experiments with **3** and of the mass spectrometric determinations of the isolated cocaine are also provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) The bis-MTPA amides derived from chiral deuterated amines analogous to **3R** and **3S** (containing unlabeled rather than $D_3{}^{13}C$-labeled methyl groups) verified no measurable (^1H NMR) loss of enantiomeric excess during the conversion of **11** to **3**.

(8) Notice that there is significant variation in the P/(P + 1) peak ratios, presumably arising from experimental issues such as self-chemical ionization. This variability (cf., row 2 of Table 1) is significantly larger than the inherent noise in the total ion count present across the entire spectrum.

(9) Results from the May feeding of **3S** show a relative intensity of 307/308 ions of 1.07/0.30. Corrected for contribution from natural abundance ^{13}C, the ratio of *pro-S* vs *pro-R* hydrogen atom removal becomes 1.03/0.16 {i.e., [1.07 − (0.27 × 1.1% × 13)]/[0.30 − (1.07 × 1.1% × 12)]} = 6.5. Similarly for the May feeding of **3R**, the 308/307 ratio of 1.42/0.18 is corrected to 1.40/0.14 {i.e., [1.42 − (0.18 × 1.1% × 12)]/[0.18 − (0.26 × 1.1% × 13)]} = 9.8.