

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

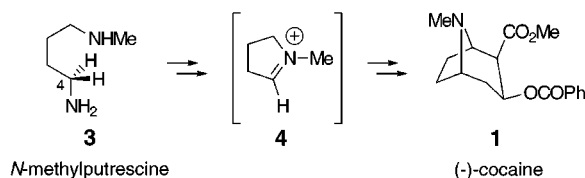
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ABSTRACT



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 ²H₃¹³C-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-Δ¹-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.^{1b} 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

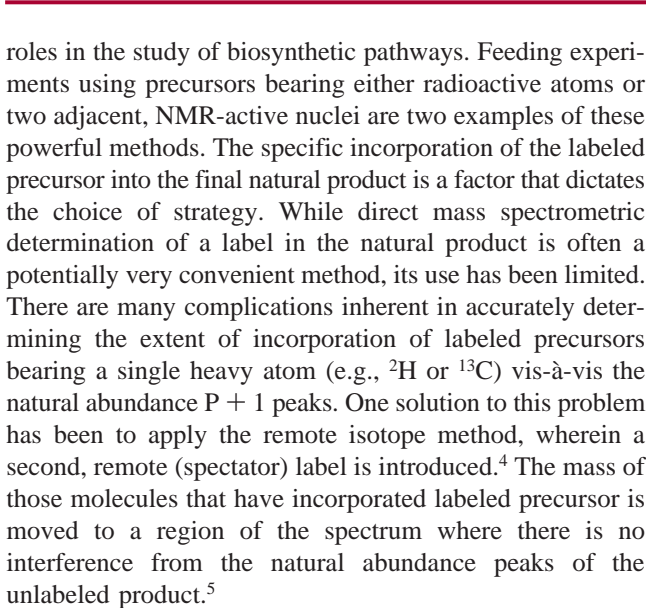
Isotopic labeling strategies of various types play important

[†] 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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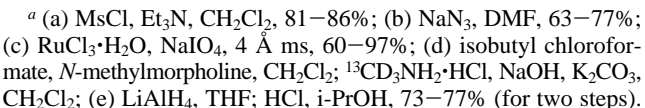
(1) (a) Huang, M. N.; Abraham, T. W.; Kim, S. H.; Leete, E. *Phytochemistry* **1996**, *41*, 767–773. (b) Bjorklund, J. A.; Leete, E. *Phytochemistry* **1992**, *31*, 3883–3887. (c) Leete, E.; Bjorklund, J. A.; Couladis, M. M.; Kim, S. H. *J. Am. Chem. Soc.* **1991**, *113*, 9286–9292. (d) Couladis, 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.



(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 tabacum* and *N. glutinosa*: Wigle, I. D.; Mestichelli, L. J. J.; Spencer, I. D. *J. Chem. Soc., Chem. Commun.* **1982**, 662–664.

(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.



The results are summarized in Table 1. An initial feeding in November led to a barely detectable level of specific

mass	natural abundance	3S feeding		3R feeding	
		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

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%

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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 ($P + {}^2\text{H}_3{}^{13}\text{C}$) higher than the most abundant parent ion. The major species emanating from the **3R** enantiomer is *five* mass units ($P + {}^2\text{H}_4{}^{13}\text{C}$) 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 plants³ 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 $\text{D}_3{}^{13}\text{C}$ -labeled methyl groups) verified no measurable (${}^1\text{H}$ 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 ${}^{13}\text{C}$, the ratio of *pro-S* vs *pro-R* hydrogen atom removal becomes 1.03/0.16 {i.e., $[1.07 - (0.27 \times 1.1\% \times 13)]/[0.30 - (1.07 \times 1.1\% \times 12)] = 6.5$ }. 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., $[1.42 - (0.18 \times 1.1\% \times 12)]/[0.18 - (0.26 \times 1.1\% \times 13)] = 9.8$ }.