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Difficulties in Differentiating Natural from Synthetic Alkaloids by Isotope Ratio Monitoring using ^{13}C Nuclear Magnetic Resonance Spectrometry

Authors

Richard J. Robins[1], Katarzyna M. Romek[1,2], Mathilde Grand[1], Pierrick Nun[1], Didier Diomande[1,3], Maxime Julien[1], Gérald S. Remaud[1]

Affiliations

1 Elucidation of Biosynthesis by Isotopic Spectrometry Group, CEISAM, CNRS – University of Nantes UMR6230, Nantes, France
2 Ecosystem Physiology, University of Freiburg, Freiburg, Germany
3 L'Université Félix Houphouët-Boigny, l'UFR Sciences des Structures de la Matière et de Technologie, Abidjan, Côte d'Ivoire

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Correspondence

Dr. Richard J. Robins
Elucidation of Biosynthesis by Isotopic Spectrometry Group, CEISAM, University of Nantes – CNRS UMR6230
BP 92208, 44322 Nantes, France
Phone: + 33 2 51 12 57 01, Fax: + 33 2 51 12 57 12
richard.robins@univ-nantes.fr

ABSTRACT

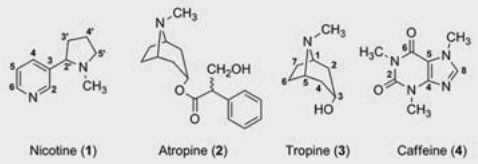
Within the food and pharmaceutical industries, there is an increasing legislative requirement for the accurate labeling of the product's origin. A key feature of this is to indicate whether the product is of natural or synthetic origin. With reference to this context, we have investigated three alkaloids commonly exploited for human use: nicotine, atropine, and caffeine. We have measured by ^{13}C nuclear magnetic resonance spectrometry the position-specific distribution of ^{13}C at natural abundance within several samples of each of these target molecules. This technique is well suited to distinguishing between origins, as the distribution of the ^{13}C isotope reflects the primary source of the carbon atoms and the process by which the molecule was (bio)synthesized. Our findings indicate that labeling can be misleading, especially in relation to a supplied compound being labeled as "synthetic" even though its ^{13}C profile indicates a natural origin.

Introduction

Alkaloids are known to have been exploited by people for several thousands of years in a variety of contexts: for poisons, stimulants, narcotics, or medicinal purposes [1]. Many of today's common drugs–both used and abused–are of alkaloid origin or based on natural alkaloids. Well-known examples of natural alkaloids used for recreational purposes include caffeine and nicotine, while examples of medicinally important alkaloids are morphine, atropine (hyoscyamine), and quinine. Drugs used for illegal purposes include cocaine and strychnine.

A significant proportion of exploited alkaloids is still obtained directly from natural sources or is synthesized from complex natural precursors (e.g., vinblastine). Others, however, such as ephedrine and pseudoephedrine, are mainly produced synthetically. Within the pharmaceutical industry, confirmation of the origin and method of production are essential aspects of drug control, both in relation to (bio)synthetic origin and geographical background. Traceability is also of importance in the control of drugs of abuse.

Among the range of methods for tracing chemical species, the use of intramolecular isotope distribution patterns has proved particularly powerful [2]. The advantage of this methodology is that the isotope ratios in the elements present are intrinsic to the target molecule and are established as a result of the precursors used, the fabrication process involved (be it synthetic or biosynthetic), and the environmental parameters extant at the time of (bio)synthesis. Furthermore, this holds true for all the elements in the compound, providing a number of parameters that can be determined to help provide a unique fingerprint for each batch of analyte.



▶ **Fig. 1** Structures of the alkaloids discussed in this paper: nicotine (**1**); atropine (**2**); tropine (**3**); caffeine (**4**).

This is because the isotope distribution pattern in a target compound is determined by the isotopic fractionation associated with steps in the (bio)synthesis of the compound [3]. This intramolecular variation can be due to a combination of factors, including flux variation and competition for precursors. These aspects of isotope fractionation are developed in [4]. The most effective method for accessing this intramolecular pattern is NMR spectrometry, using which both $^{2}H/^{1}H$ and $^{13}C/^{12}C$ ratios can be obtained [5]. Monitoring $^{2}H/^{1}H$ ratios with isotope ratio monitoring ^{2}H NMR (irm-^{2}H NMR) has been successfully exploited for some time, mainly in the food industry, although a smaller number of applications to pharmaceuticals has been made. The major drawbacks to ^{2}H monitoring are that the nucleus has poor NMR properties with a relatively limited dispersion in the frequency range and the isotope occurs at only low natural abundance (0.017%). In addition, some H-positions are exchangeable, leading to modifications that might depend on, for example, the extraction and work-up conditions. As an alternative, monitoring $^{13}C/^{12}C$ ratios using isotope ratio monitoring ^{13}C NMR (irm-^{13}C NMR) has been developed [2], with the advantages that the isotope has a greater dispersion in the frequency range and occurs at much higher abundance. In addition, provided certain conditions are respected, the positional $^{13}C/^{12}C$ ratios should not be modified during purification.

Irm-^{13}C NMR has now been developed successfully to analyze a number of alkaloids, including nicotine **1**, tropine **3**, caffeine **4** (▶ **Fig. 1**), and tramadol. For the solanaceous alkaloids, nicotine and tropine, it has proved possible to link the natural $^{13}C/^{12}C$ ratio patterns to the biosynthetic pathways [6]. Furthermore, for the recently discovered natural compound racemic (1*R*,2*R*)-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol, known by the trivial name tramadol, it has been possible to make a series of predictions as to its probable biosynthetic precursors based on the observed $^{13}C/^{12}C$ ratio in the natural product [7]. Within the pharmaceutical context, it has been clearly demonstrated that positional $^{13}C/^{12}C$ ratios can distinguish between different synthetic origins for ibuprofen [8], aspirin, and paracetamol [9]. However, two aspects remain underexplored. The first is the relationship during synthesis between the primary products, the synthetic process, and the final drug. The second is the degree to which natural compounds might be distinguished from those of synthetic origin on the basis of their position-specific $^{13}C/^{12}C$ ratios. Within this latter context, caffeine of synthetic origin has been shown to differ considerably from that of natural origin [10]. In this paper, we extend this approach to investigate the degree to which the measurement of $^{13}C/^{12}C$ ratios in several alkaloids differs with origin. We also highlight how labeling regulations may potentially lead to ambiguities in the designation of "natural" and "synthetic" for a given compound.

Results and Discussion

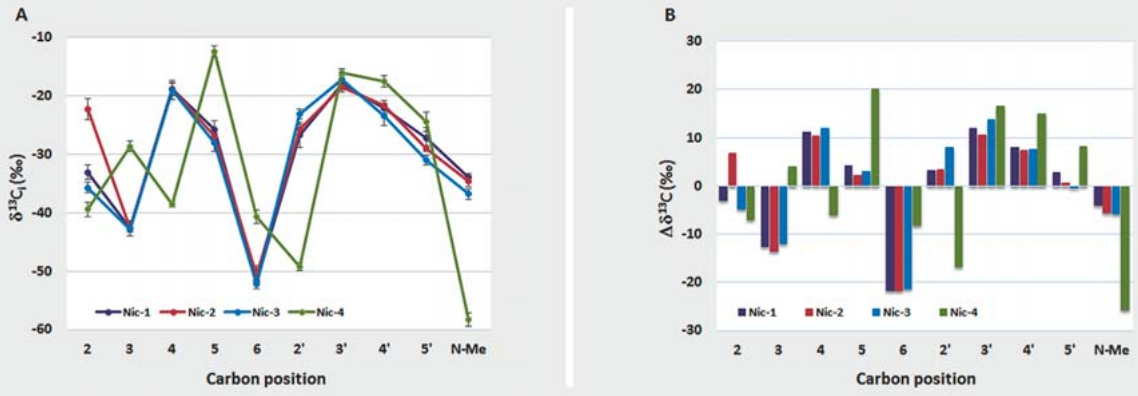
Nicotine **1** (▶ **Fig. 1**) is the dominant alkaloid in the leaves of a number of *Nicotiana* species (Solanaceae), including smoking tobacco, *Nicotiana tabacum* L. The positional $^{13}C/^{12}C$ ratios for four samples of nicotine from different sources (see ▶ **Table 1**) were determined using irm-^{13}C NMR spectrometry under quantitative conditions. The profiles obtained are presented in ▶ **Fig. 2**. Nic-3 is an authentic sample of (*S*)-nicotine guaranteed extracted from plants of *Nicotiana*. Nic-1 is designated by the supplier (Sigma-Aldrich) as "synthetic" and "manufactured from materials of plant origin" on the Certificate of Origin, while Nic-2 (Fluka) is designated "synthetic" and it is stated that "only synthetic materials were used in the manufacturing process." Nonetheless, Nic-1, Nic-2, and Nic-3 show very similar profiles, indistinguishable in a number of positions. Only the C-2 position of Nic-2 differs significantly: at all other positions, the maximum difference is 3.8‰. Notably, Nic-1 and Nic-2 are especially similar. Considering the similarity of the three profiles of these samples, it is difficult to conceive that their origins differ so much. All three samples show two key features that are also found in nicotine of natural origin, Nic-3 [6]. First, the C-2′ position is richer than the C-5′ position by a few ‰ (▶ **Fig. 2A**), a differentiation suggested as being introduced by the isotope effect associated with the nicotine synthase enzyme [6]. Second, the C-6 is very impoverished: again, a feature that can be explained by the biosynthetic route, as seen in the natural sample [6].

This similarity is emphasized when these samples of pure (*S*)-nicotine are compared with Nic-4, a pure synthetic sample of (*R,S*)-nicotine made in the laboratory using the method of Felpin et al. [11]. While the profile of (*R,S*)-nicotine will clearly vary depending on the exact source of the precursors used, the difference in its profile is very evident, with a variation of $\delta^{13}C_i$ values relative to the other samples of up to 25‰. A particularly notable difference is that in Nic-4 $\delta^{13}C_{C\text{-}5} > \delta^{13}C_{C\text{-}4}$, in complete contrast to the samples of natural origin.

A straightforward explanation of these observations is that the samples Nic-1 and Nic-2 are of natural origin but have undergone processing in such a way that they can no longer be labeled as "natural." The designation of Nic-1 as "manufactured from materials of plant origin" would fit with the suggestion that is an example of a case where the supplier is obliged to label a compound "synthetic" because the process used in its manufacture involved one or more chemical treatments so it cannot be labeled "natural". In this case, it can be reasonably assumed that the compound is, to all intents and purposes, extracted from a plant. The case of Nic-2 is more problematic, as the labeling specifically states that "only synthetic materials were used in the manufacturing process." Nonetheless, its profile is very similar to those of Nic-1 and Nic-3. This problem of the assignation of origin remains to be resolved. It should be noted, however, that it is commercially impractical to produce (*S*)-nicotine by laboratory synthesis (see [11]

▶ **Table 1** Details of the origin and characteristics of the compounds investigated.

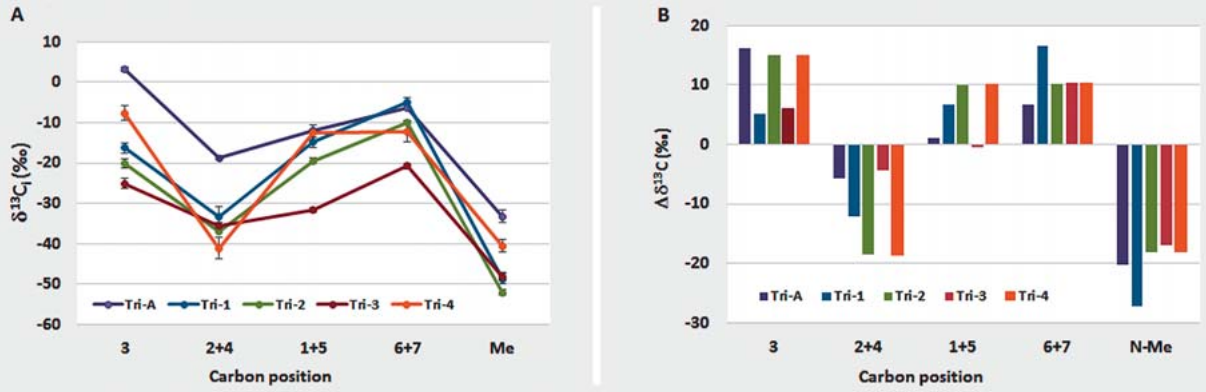
Compound	Sample identity	Supplier	Characteristic	Batch number	Purity	Designation (as per Certificate of Origin for purchased chemicals)
S-(−)-Nicotine	Nic-1	Sigma-Aldrich	Free base	1449194V	> 99 %	Synthetic, manufactured from materials of plant origin
	Nic-2	Fluka	Free base	SZBE245XV	Pestanal, > 99.1 %	Synthetic and only synthetic materials were used in the manufacturing process
	Nic-3	CHEMNOVATIC	Free base	Not available	PureNic99+ > 99.9 %	Guaranteed of natural origin extracted from *Nicotiana* sp.
	Nic-4	EBSI (by synthesis)	Free base	None	99 %	Guaranteed of synthetic origin
Tropine	Tri-1	Sigma-Aldrich	$2(H_2O)$	S21312-474	98 %	Not available
	Tri-2	Sigma-Aldrich	Free base	111F-0349	98 %	Not available
	Tri-3	Fluka	Free base	1212025	> 97 %	Not available
	Tri-4	Extrasynthese	Free base	1115/0	> 99 %	Guaranteed synthetic
Atropine	Tri-A	Acros Organics	Sulfate·H_2O	A0355240	> 97 %	Designated "synthetic"
Caffeine	Aldrich (EU)	Aldrich	Free base	001425	> 98 %	Synthetic
	Fluka (J)	Fluka	Free base	245781	> 99 %	Synthetic
	C. arabica	EBSI (by extraction)	Free base	None	> 99 %	Guaranteed natural
	C. robusta	EBSI (by extraction)	Free base	None	> 99 %	Guaranteed natural



▶ **Fig. 2** $\delta^{13}C_i$ (‰) values for 4 samples of nicotine **1**; **A** absolute values of $\delta^{13}C_i$ (‰); **B** relative values of $\Delta\delta^{13}C$ (‰) (i.e., the position-specific distribution relative to the mean value for the molecule), $\delta^{13}C_b$ (‰); Nic-1 = *S*-nicotine from Sigma-Aldrich (product N3876-5ML, batch 1449194V); Nic-2 = *S*-nicotine from Fluka (product 36733-1G, batch SZBE245XV); Nic-3 = *S*-nicotine from Chemnovatic (natural); Nic-4 = *R,S*-nicotine synthesized in the laboratory. Data shown are mean ± standard deviation (n = 5).

and references therein). The time and cost of such an operation is very much greater than obtaining (*S*)-nicotine through extraction from plant material, which is widely available.

A group of alkaloids much exploited pharmaceutically is the tropane family, present in a number of Solanaceae and extracted for commercial use from several *Datura* spp., *Hyoscyamus niger* L. and *Atropa belladonna* L. [1]. The whole molecules of hyoscyamine (atropine) or scopolamine are not well adapted to irm-^{13}C NMR spectrometry, so only tropine, the amine base of hyoscyamine (atropine), was studied (▶ **Fig. 1**). A potential drawback with tropine is the lack of resolution of six of the carbons: the (1 + 5), (2 + 4), and (6 + 7), giving only 3 resonance positions. Although these are just resolved in the 500 MHz ^{13}C NMR spectrum of hyoscyamine, the separation is insufficient for irm-^{13}C NMR spectrometry, making it impossible to measure the areas under the peaks with the required accuracy (see [12] for an explanation). Nonetheless, due to the symmetry of a number of the intermediates in the biosynthetic pathway, certain deductions can be made about the fractionation occurring during biosynthesis [6].



▶ **Fig. 3** $\delta^{13}C_i$ (‰) values for four samples of tropine **3**; **A** absolute values of $\delta^{13}C_i$ (‰); **B** relative values of $\Delta\delta^{13}C$ (‰) (see legend to ▶ **Fig. 2** for a definition); Tri-A = Tropine obtained by the hydrolysis of a commercial sample of atropine sulfate from Acros Organics (product N°22668, batch N°A0355240); Tri-1 = tropine from Sigma-Aldrich (batch N° S21312–474); Tri-2 = tropine from Sigma (batch N°111F-0349); Tri-3 = tropine from Fluka (product N°93550, batch N°1212025); Tri-4 = tropine from Extrasynthese (product No. 0672). Data shown are mean ± standard deviation (n = 5).

In the present set of analytes (▶ **Table 1**), Tri-A was made by the hydrolysis of commercially obtained atropine sulfate. While this is certified as "synthetic", it seems highly probable that the atropine used is of natural origin. As with nicotine, synthesizing atropine is not commercially competitive with obtaining it from a plant origin. Although the origin of the samples Tri-1, Tri-2, and Tri-3 is undefined, their profiles all show qualitative similarities to Tri-A, notably in their C3:(C2+C4) ratios and in having $\delta^{13}C_{(C1+C5)} < \delta^{13}C_{(C6+C7)}$ (▶ **Fig. 3A**). This latter feature is notably different in Tri-4, which was made by synthesis. Hence, the indications are that the samples Tri-A, Tri-1, Tri-2, and Tri-3 all have natural origins. However, it is not possible to determine the probable origin of unknown samples with so few authentic references. Nevertheless, the present data indicate that substantial variation can occur within the isotope ratios of this compound and that, again, labeling may not strictly represent the true origin of the compound.

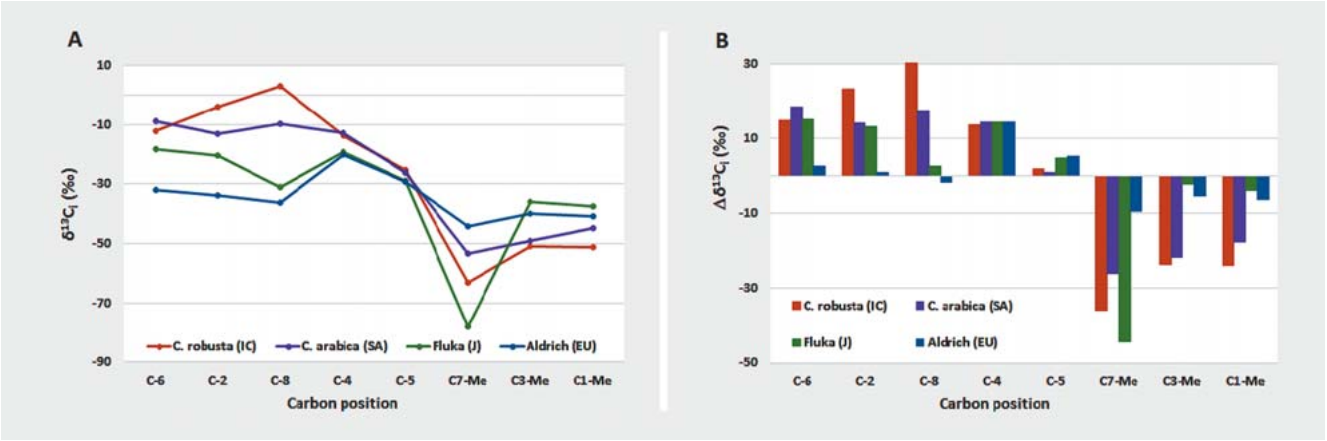
Caffeine **4** (▶ **Fig. 1**) is an alkaloid of considerable commercial interest, consumed in huge quantities in a number of natural and synthetic beverages as well as playing an important role as an adjuvant in many pharmaceutical preparations. Although a significant amount of caffeine is available from the decaffeination of coffee, this source does not satisfy demand and synthetic caffeine is also available. ▶ **Fig. 4** illustrates the profiles obtained from *Coffea robusta* L. Linden, *Coffea arabica* L., and two samples of commercial synthetic caffeine (see ▶ **Table 1**). As already discussed [10], the synthetic samples are readily distinguished from the natural ones by comparing their $^{13}C/^{12}C$ ratios in the C-2 and C-8 positions. In the natural product, the C-8 is systematically richer in ^{13}C than the C-2, while in the synthetic samples, the inverse is found. It is similar for the $^{13}C/^{12}C$ ratios in the C-4 and C-8 positions, with C-8 > C-4 in the natural product and C-8 < C-4 in the synthetic caffeine. The observed ratios in natural caffeine have partially been rationalized on the basis of the biosynthesis of xanthine alkaloids [13] and further insight based on additional data obtained by irm-^{13}C NMR will be the subject of another publication.

In the examples given here, the analysis of the $^{13}C/^{12}C$ ratios in three alkaloids that can be obtained from natural or synthetic sources indicates that differentiating "natural" from "synthetic" purely on the basis of the supplier's certification can be problematic. For (*S*)-nicotine, it is shown that samples that are labeled "synthetic" have the same ^{13}C isotopic fingerprint as a sample guaranteed to have been extracted from its natural source (*Nicotiana* spp. leaves), indicating that the certification may reflect not the real origin of the compound, but rather the treatment it has undergone. It is further shown that, for the base of the tropane alkaloids, differences can be seen between guaranteed synthetic and probably natural sources, again indicating a potential legislation-related cause for the certified origin. Clearly, measuring the position-specific $^{13}C/^{12}C$ ratios can help to define the history of a compound, even to the extent of giving clues that contradict those specified in its documentation. This study must be treated as a preliminary excursion into this field for the alkaloids presented. In order to assign compounds with confidence, a larger bank of samples must be analyzed, but this bank is difficult to construct on the basis of currently available commercial samples. Rather, as with caffeine [10], the target compounds need to be purified from defined sources in order to construct a validated inter-source comparison. Despite this, the data presented here strongly indicate that this technique has potential application in relation to alkaloids of natural origin.

Materials and Methods

Chemicals

S-(−)-Nicotine (3-[1-methylpyrrolidin-2-yl]pyridine) was purchased from Sigma-Aldrich, from Fluka, and from CHEMNOVATIC. Tropine ((3-endo)-8-methyl-8-azabicyclo[3.2.1]octan-3-ol) was purchased from Sigma-Aldrich, from Fluka, and from Extrasynthese. Atropine sulfate was purchased from Acros Organics. Caffeine



▶ **Fig. 4** $\delta^{13}C_i$ (‰) values for four samples of caffeine **4**; **A** absolute values of $\delta^{13}C_i$ (‰); **B** relative values of $\Delta\delta^{13}C$ (‰) (see legend to ▶ **Fig. 2** for a definition); *C robusta* (IC) = the mean value for eight acquisitions of *C. robusta* from the Ivory Coast; *C. arabica* (SA) = the mean value for five acquisitions of *C. arabica* from Central and South America; Fluka (J) = a commercial sample from Japan; Aldrich (EU) = a commercial sample from Europe. Data shown are mean values for the samples. Standard deviations are not included as, for the samples of natural origin, the plants represented are from various different locations. All data were obtained from five spectra per sample.

(1,3,7-triméthyl-1H-purine-2,6(3H,7H)-dione) was purchased from Sigma-Aldrich and from Fluka. Natural caffeine was extracted and purified from authentic samples of *C. arabica* and *C. robusta*. Details of these samples and of their preparation are given in [10]. Tris(2,4-pentadionato)chromium-(III) ($Cr(Acac)_3$) was purchased from Merck. Benzene-d_6, acetonitrile-d_3, and acetone-d_6 were purchased from Euriso-top, and silica gel was purchased from Merck.

Isotope ratio monitoring by ^{13}C NMR

Prior to preparation of samples for irm-^{13}C NMR, purity was confirmed higher than 98 % by recording a ^1H NMR spectrum. Tropine free base from Fluka (Tri-3) was recrystallized from diethyl ether before use.

For the analysis of nicotine, 250 µL of *S*-(−)-nicotine was homogenized in 500 µL of acetonitrile-d_3. To this was added 100 µL of a solution of relaxation agent $Cr(Acac)_3$ (0.1 M) prepared by dissolving 10.5 mg $Cr(Acac)_3$ in 300 µL of acetonitrile-d_3. Under these conditions, T_1^{max} = 3.74 s. Spectral acquisition was with AQ = 1.0 s for 152 scans, giving a signal-to-noise ratio of ~ 700.

For the analysis of tropine, 150 mg of tropine was dissolved in 600 µL of benzene-d_6. As T_1^{max} = 1.53 s is relatively short in these conditions, relaxation agent was not required. Spectral acquisition was with AQ = 0.95 s for 400 scans, giving a signal-to-noise ratio of ~ 550.

For the analysis of caffeine, 160 mg of caffeine and 1.75 mg of relaxant $Cr(Acac)_3$ were separately weighed into two small vials. The caffeine was dissolved in a mixture of 360 µL of pyrrole and 240 µL of toluene-d_8. The relaxant was dissolved in 100 µL of toluene-d_8. These two solutions were mixed and filtered to provide the analyte sample. Spectral acquisition was with AQ = 1.00 s for 35 scans, giving a signal-to-noise ratio of ~ 700 [10].

Quantitative ^{13}C NMR spectra were recorded as described previously for nicotine and tropine [6] and for caffeine [10]. Each measurement consisted of the average of five independently recorded NMR spectra.

Spectral data processing

Quantitative ^{13}C NMR spectra were recorded on a Bruker 500 Avance III spectrometer fitted with a 5-mm-i. d. $^{13}C/^1H$ dual cryoprobe carefully tuned to the recording frequency of 125.76 MHz. The temperature of the probe was set at 303 K. Spectra were processed as described previously [6, 10]. Spectra acquired on a Bruker 500 Avance III spectrometer were corrected by reference to the data obtained from an Avance III NMR spectrometer fitted with a broad band probe (see [14] for a detailed explanation).

The curve fitting was carried out with a Lorentzian mathematical model using Perch Software (Perch NMR Software). Data were processed as described previously, and the $\delta^{13}C_i$ [‰] values were calculated from the reduced molar fraction f_i/F_i and the $\delta^{13}C_b$ [‰] obtained by isotope ratio measurement by mass spectrometry, as described in [12].

Synthesis of (*R,S*)-nicotine

(*R,S*)-Nicotine was prepared from *N*-vinylpyrrolidinone and ethyl nicotinate as described in [11, 15].

Hydrolysis of atropine sulfate to obtain tropine

Atropine was hydrolyzed with warm $Ba(OH)_2$ solution. Atropine sulfate·H_2O (5 g) was dissolved in distilled water (50 g) in a 250-mL round-bottomed flask, and solid $Ba(OH)_2$ (4 g) was added (giving pH ≈ 13). This was left stirring at 75 °C for 16 h. Complete reaction was confirmed by TLC (Si-gel 60, CH_2Cl_2/EtOH/NEt_3 (46/46/1)) versus standards on a small aliquot extracted into CH_2Cl_2. Then the volume was reduced by rotary evaporation in vacuo (60 °C), and the remaining suspension was poured into a Soxhlet thimble and extracted exhaustively with MeOH (3 × 100 mL). The methanolic extract was dried over $MgSO_4$ (anhydr.) and MeOH re-

moved by rotary evaporation. The dry residue was taken into CH_2Cl_2 (leaving some white powder undissolved), filtered, and the solvent was again removed by rotary evaporation. The residue was taken into a minimum volume of hot acetone and left to crystallize (fridge). The crystals were collected and shown by ^1H and ^{13}C NMR to be pure tropine. The yield was essentially quantitative (1.95 g).

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Conflict of Interest

All authors declare that there is no financial or commercial conflict of interest associated with this article.

References

[1] Roberts M, Wink M, eds. Alkaloids: Biochemistry, Ecology and Medicinal Applications. New York: Plenum Press; 1998

[2] Remaud GS, Giraudeau P, Lesot P, Akoka S. Isotope Ratio monitoring by NMR. Part 1: recent Advances. In: Webb G, ed. Modern magnetic Resonance. 2nd ed. New York: Springer International; 2016: 1–25

[3] Robins RJ, Romek KM, Remaud GS, Paneth P. Non-statistical isotope fractionation as a novel “retro-biosynthetic” approach to understanding alkaloid metabolic pathways. Phytochem Lett 2017; 20: 499–506

[4] Schmidt HL, Robins RJ, Werner RA. Multi-factorial *in vivo* stable isotope fractionation: causes, correlations, consequences and applications. Isotop Env Health Stud 2015; 51: 155–199

[5] Robins RJ, Remaud GS, Billault I, Lesot P. Isotope Ratio monitoring by NMR. Part 2: New Applications in the Field of defining Biosynthesis. In: Webb GA, ed. Modern magnetic Resonance. 2nd ed. New York: Springer International; 2016: 1–26

[6] Romek KM, Remaud GS, Silvestre V, Paneth P, Robins RJ. Non-statistical ^{13}C fractionation distinguishes co-incident and divergent steps in the biosynthesis of the alkaloids nicotine and tropine. J Biol Chem 2016; 291: 16620–16629

[7] Romek KM, Nun P, Remaud GS, Silvestre V, Taïwe GS, Lecerf-Schmidt F, Boumendjel A, De Waard M, Robins RJ. A retro-biosynthetic approach to the prediction of biosynthetic pathways from position-specific isotope analysis as shown for tramadol. Proc Natl Acad Sci U S A 2015; 112: 8296–8301

[8] Remaud GS, Bussy U, Lees M, Thomas F, Desmurs JR, Jamin E, Silvestre V, Akoka S. NMR spectrometry isotopic fingerprinting: a tool for the manufacturer for tracking active pharmaceutical ingredients from starting materials to final medicines. Eur J Pharm Sci 2013; 48: 464–473

[9] Silvestre V, Mboula VM, Jouitteau C, Akoka S, Robins RJ, Remaud GS. Isotopic ^{13}C NMR spectrometry to assess counterfeiting of active pharmaceutical ingredients: site-specific ^{13}C content of aspirin and paracetamol. J Pharm Biomed Anal 2009; 50: 336–341

[10] Diomande DG, Martineau E, Gilbert A, Nun P, Murata A, Yamada K, Watanabe N, Tea I, Robins RJ, Yoshida N, Remaud GS. Position-specific isotope analysis of xanthines: a ^{13}C nuclear magnetic resonance method to determine the ^{13}C intramolecular composition at natural abundance. Anal Chem 2015; 87: 6600–6606

[11] Felpin FX, Girard S, Vo-Thanh G, Robins RJ, Villieras J, Lebreton J. Efficient enantiomeric synthesis of pyrrolidine and piperidine alkaloids from tobacco. J Org Chem 2001; 66: 6305–6312

[12] Robins RJ, Remaud G, Akoka S. Isotope Ratio monitoring ^{13}C nuclear magnetic Resonance Spectrometry for the Analysis of position-specific Isotope Ratios. In: Harris ME, Anderson VE, eds. Measurement and Analysis of kinetic Isotope Effects. Cambridge, MA: Elsevier; 2017: 369–401

[13] Weilacher T, Gleixner G, Schmidt HL. Carbon isotope pattern in purine alkaloids a key to isotope discriminations in C1 compounds. Phytochemistry 1996; 41: 1073–1077

[14] Bayle K, Gilbert A, Julien M, Yamada K, Silvestre V, Robins RJ, Akoka S, Yoshida N, Remaud GS. Conditions to obtain precise and true measurements of the intramolecular ^{13}C distribution in organic molecules by isotopic ^{13}C nuclear magnetic resonance spectrometry. Anal Chim Acta 2014; 846: 1–7

[15] Mesnard F, Girard S, Fliniaux O, Bhogal RK, Gillet F, Lebreton J, Fliniaux MA, Robins RJ. Chiral specificity of the degradation of nicotine by *Nicotiana plumbaginifolia* cell suspension cultures. Plant Sci 2001; 161: 1011–1018