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Evaluation of the mass spectrometric fragmentation of codeine and morphine after ^{13}C-isotope biosynthetic labeling

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Abstract

All major fragment ions of codeine and morphine were elucidated using LC–electrospray MS/MS and high resolution FT-ICR-MS combined with an IRMPD system. Nanogram quantities of labeled codeine were isolated and purified from *Papaver somniferum* seedlings, which were grown for up to 9 days in the presence of [ring-$^{13}C_6$]-L-tyrosine, [ring-$^{13}C_6$]-tyramine and [1,2-$^{13}C_2$], [6-*O*-methyl ^{13}C]-(*R*,*S*)-coclaurine. The labeling degree of codeine up to 57% into morphinans was observed.

Keywords: *Papaver somniferum*; Morphine; Codeine; Mass spectral fragmentation; Electrospray ionization; LC–MS/MS; FT-ICR-MS; IRMPD

1. Introduction

Stable isotopes play a crucial role in biochemistry for the elucidation of biosynthetic pathways and reaction mechanisms. The enrichment of the content of isotopic atoms in various positions of a labeled molecule can be determined by NMR, mass spectrometry or chemical/enzymatic degradation. Among these methods, mass spectrometry represents the most advanced technique because of its high sensitivity and specificity and the possibility to determine the desired substance in not highly purified samples by using LC–MS/MS. Specific labeling with stable isotopes (^2H, ^{13}C, ^{15}N and ^{18}O) is also of importance in mass spectrometry for the investigation of fragmentation mechanisms (Hesse et al., 1997). However, in many cases, the synthesis of isotopically labeled compounds necessitates strong efforts, especially in case of a complex natural compound skeleton. In this respect the biochemical incorporation of a labeled precursor leading to a specifically labeled derivative represents an elegant alternative to conventional chemical synthesis.

Codeine and morphine are two highly esteemed, pharmacologically active natural products, representing complex low molecular weight molecules. Their biosynthesis in the opium poppy plant (*Papaver somniferum*) is highly complicated and has not yet been elucidated entirely, though it has been investigated at the biochemical and molecular level for more than 50 years (Kutchan, 1998). The possible endogenous occurrence of morphine in minute amounts in mammals is still an open question (Laurent et al., 2000; Spector, 1990; Stefano et al., 2000) and needs to be unequivocally solved by biosynthetic approaches.

Hence, we attempted to labeled codeine and morphine with ^{13}C-labeled precursors under conditions allowing high incorporation into poppy seedlings. Using these labeled alkaloids, an extensive mass spectrometric investigation revealed the identification of all major fragment ions of codeine as well as morphine. Thus, the combination of high resolution MS/MS and isotopically labeling, in particular by means of biochemical incorporation,

Abbreviations: ESI, Electrospray ionization; CID, Collision-induced dissociation; LC–MS/MS, Liquid chromatography–mass spectrometry/mass spectrometry; FT-ICR-MS, Fourier transform ion cyclotron resonance mass spectrometry; IRMPD, Infrared multiphoton dissociation.

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represents a powerful tool, that can be used for the determination of fragmentation pathways and ion structures. The groundwork for sophisticated experiments to study the biosynthesis of morphinan alkaloids using mass spectrometry has been laid.

2. Results and discussion

2.1. Application experiment

Biosynthetic experiments using in vivo application of labeled precursors usually suffer from low incorporation rates into the target molecules. In most experiments, differentiated, mature plants or plant organs are used. The main reason for the observed low incorporation rates are the sluggish metabolism, compartmentation and transport problem as well as an already high content of the metabolite in question, which may lead to dilution of the concentration of labeled metabolite and may even result in down regulation of the specific secondary biosynthetic pathway.

In order to increase the rate of specific incorporation into the target molecule, we decided to allow the poppy seeds to germinate and subsequently grow in a solution containing the isotopically labeled precursor. It has been shown that the dormant seed of *P. somniferum* does not contain morphinan alkaloids, however that upon germination these alkaloids are rapidly synthesized (Wieczorek et al., 1986). During the first two days of germination, no alkaloids were formed. Then a rapid synthesis of salutaridine, thebaine and codeine took place until day 8 while the formation of morphine occurred at a later stage, which was beyond the time frame of the first week. Differential labeling of the codeine was achieved by using [ring-$^{13}C_6$]-L-tyrosine and [ring-$^{13}C_6$]-tyramine. As reviewed by Spenser (1968), the carbon skeleton of morphinans is derived from two molecules of L-tyrosine, while tyramine is exclusively incorporated into only the dopamine derived isoquinoline ring. In addition, the well established precursor [1,2-$^{13}C_2$], [6-*O*-methyl ^{13}C]-(*R*,*S*)-coclaurine was used, and the codeine, formed from this intermediate, was also subjected to extensive mass spectrometric analysis, to follow the fate of the individual heavy isotope atoms (Fig. 1). Supplying separately all three ^{13}C-labeled precursors to poppy seedlings, the labeling degree of codeine was calculated based on LC–MS full scan data (see Section 3) as follow: [ring-$^{13}C_6$]-L-tyrosine into codeine: 30%; [ring-$^{13}C_6$]-tyramine into codeine: 38%; [1,2-$^{13}C_2$], [6-*O*-methyl ^{13}C]-(*R*,*S*)-coclaurine into codeine: 57%. The specific incorporation rates, reported here, range among the highest one achieved so far in higher plants and were the basis for the planned mass spectrometric fragmentation experiment.

2.2. Elucidation of fragment ions

The electron impact (EI) mass spectra of morphine and related morphinans are well described (Audier et al., 1965; Hesse and Bernhard, 1975; Wheeler et al., 1967). During the past few years soft ionization methods were intensively used for investigation of morphine and its metabolites (Baumann et al., 2000; Schanzle et al., 1999; Slawson et al., 1999; Weinmann and Svoboda, 1998). Recently, based on investigations with an ion trap mass spectrometer and a triple quadrupole system the fragmentation pathways of the $[M + H]^+$ ions of morphinans could be elucidated in our group (Raith et al., 2003). The electrospray CID mass spectra of morphine (**1**) and codeine (**2**) display a series of key ions reflecting the substructures of the morphinan skeleton. However, because of the complexity of the CID spectra an assignment of the different carbon atoms in the morphinan skeleton to these key ions is difficult. Combination of ^{13}C-labeling experiments with high resolution mass data can provide more information with respect to the ion structures, making the assignment of the ring carbons to the major fragment ions originated by collision-induced dissociation should be possible. Scheme 1 summarizes the results based on the ^{13}C-labeled compounds, **2a**–**2c** and the high resolution IRMPD mass measurements (Table 1). The IRMPD spectra of morphine (**1**) and codeine (**2**) (Fig. 2) shows a similar fragmentation behavior as the corresponding CID spectra obtained from a triple quadrupole instrument (Raith et al., 2003). Therefore, the elemental composition of the key ions of morphine (**1**) and codeine (**2**) could be determined unambiguously. The mass spectral decomposition of the two morphinans starts with the loss of the nitrogen-containing ring leading to ions of type **a** and [**a**-2H] and a further loss of water affording ions of type **b** and [**b**-2H] which represent highly conjugated systems (Scheme 1, Tables 1 and 2). The loss of CO from ion **a** formed ion **c** (**1**: m/z 201, **2**: m/z 215) is realized by loss of one carbon atom from ring C as shown by a comparison of the ^{13}C-labeled compounds **2a** and **2b** (Fig. 3). The nature of the removed oxygen could not be clarified. An interesting feature of the fragmentation is shown by the formation of ions **d** and **h**. The inspection of the CID spectra of the ^{13}C-labeled compounds **2a**–**2c** clearly shows that the ring C is lost during this fragmentation, as indicated by the corresponding mass shifts (Fig. 3, Scheme 1). It should be pointed out, that the key ion at m/z 173 in morphine appears as a doublet in the IRMPD spectrum, reflecting the fragments **h** and [**c**-CO], in contrast to codeine, where only an ion of type **h** is observed at m/z 187 (Table 1). Further degradation of ions **b** and **c** leads to the key ion **e** at m/z 183 comprising the indicated carbon atoms (Scheme 1). Ion **e** is decomposed by loss of H_2O to the base peak ion at m/z 165 [**e**-H_2O] in the ESI CID spectrum of morphine and m/z 155

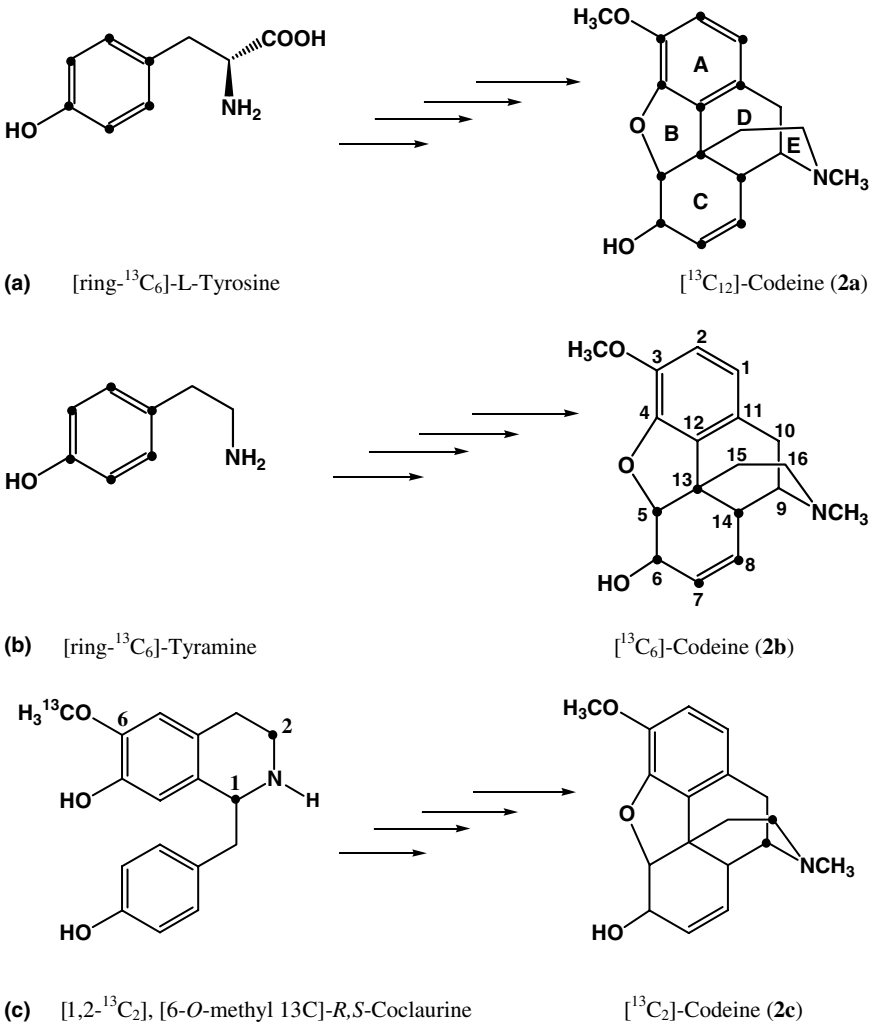
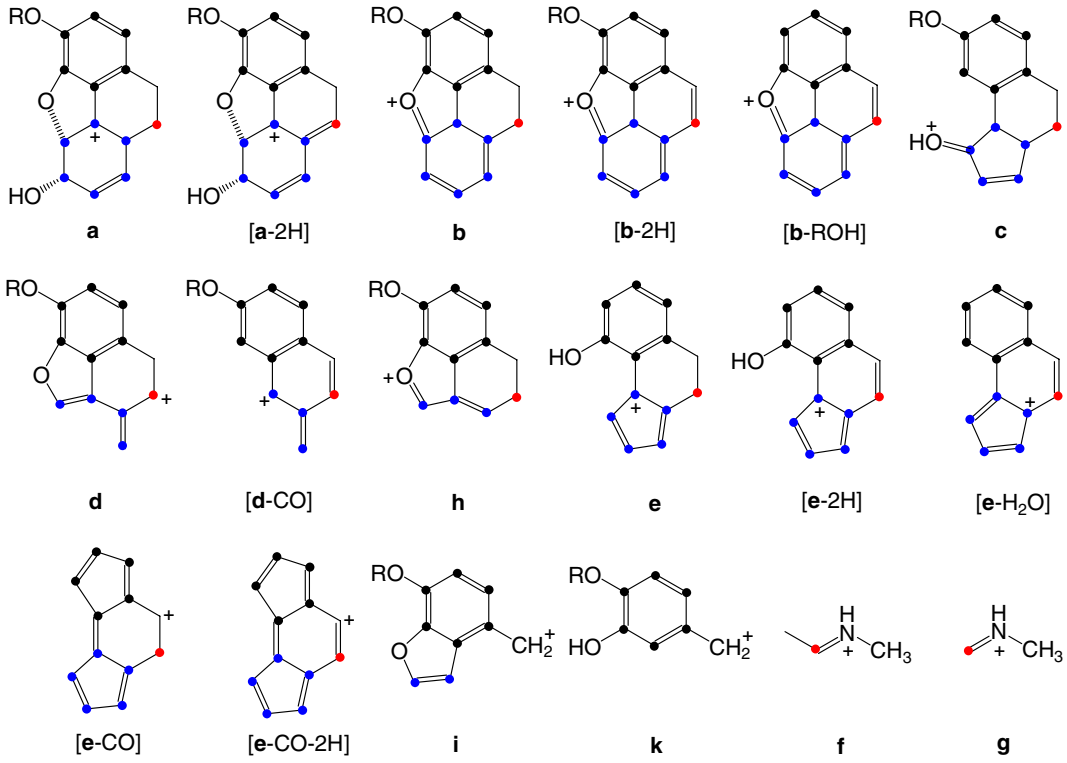


Fig. 1. Labeled codeine biosynthetically derived from [ring-$^{13}C_6$]-tyrosine (a), [ring-$^{13}C_6$]-tyramine (b) and [1,2-$^{13}C_2$], [6-*O*-methyl ^{13}C]-coclaurine (c).

([**e**-CO]), respectively. The ion of type [**e**-H_2O] ($C_{13}H_9$) is a highly conjugated resonance-stabilized ion. $C_{13}H_9^+$ ions are also known from the EI mass spectra of a variety of unsaturated hydrocarbons, e.g., by loss of methyl from ionized stilbene and 9,10-dihydrophenanthrene (Kuck, 1990). In case of the $C_{13}H_9^+$ ion, formed by electrospray CID, an isomerization and/or hydrogen and carbon scrambling cannot be excluded. However, the nature of the carbon atoms could be clearly determined by the ^{13}C-labeled derivatives (Scheme 1, Table 2). Two important key ions comprising the ring A are **i** and **k**. Both ions possess the functional group at C-3 and the epoxy oxygen. Ions **f** and **g** represent key ions resulting from the isoquinoline ring, displaying the corresponding mass shift of 1 a.m.u. in the ESI CID mass spectrum of [$^{13}C_2$]-codeine (**2c**).

The collision-induced dissociation (CID) mass spectra of the $[M+H]^+$ ions of morphine and codeine display a similar fragmentation behavior, which are quite different from those of the electron impact mass spectra. The observed complex pattern shows a series of key ions reflecting the several substructures of the morphinan skeleton. The elemental composition of the important fragments could be established by accurate mass determination based on IRMPD mass data from a FT-ICR mass spectrometer. The previously described fragmentation pathways (Raith et al., 2003) are now supported by ^{13}C-labeled derivatives of codeine, obtained by feeding experiments with seedlings of *P. somniferum*. The CID spectra of these specifically labeled codeine derivatives allow an unambiguous assignment of the carbon atoms in the morphinan skeleton to the corresponding key ions of these two important morphinan alkaloids. It could be shown that during the mass spectral degradation of the morphinan skeleton the ions of type **d** (**1**: *m/z* 185, **2**: *m/z* 199) and **h** (**1**: *m/z* 173, **2**: *m/z* 187) comprises the complete AB ring system and not the rings AC which one could also expect. In the abundant, highly conjugated key fragment at *m/z* 165 ([**e**-H_2O], $C_{13}H_9^+$) exclusively a carbon atom of the



Scheme 1. Proposed ion structures of morphine (**1**, R = H) and codeine (**2**, R = CH_3) based on ^{13}C-labeled codeine derivatives and high resolution MS/MS experiments (origin of the ^{13}C-atoms: black and blue = [$^{13}C_{12}$]-codeine, **2a**; blue = [$^{13}C_6$]-codeine, **2b**; red = [$^{13}C_2$]-codeine, **2c**), see also Table 2.

Table 1
High resolution mass data of morphine (**1**) and codeine (**2**) obtained by an ESI-FT-ICR mass spectrometer combined with an IRMPD system

Ion	Morphine (**1**)			Codeine (**2**)		
	Composition	*m/z*	Error (ppm)	Composition	*m/z*	Error (ppm)
$[M+H]^+$	$C_{17}H_{20}NO_3$	286.14366	0.4	$C_{18}H_{22}NO_3$	300.16015	2.4
a	$C_{14}H_{13}O_3$	229.08613	0.9	$C_{15}H_{15}O_3$	243.10204	1.9
b	$C_{14}H_{11}O_2$	211.07542	0.3	$C_{15}H_{13}O_2$	225.09190	4.0
c	$C_{13}H_{13}O_2$	201.09111	0.5	$C_{14}H_{15}O_2$	215.10739	3.4
d	$C_{12}H_9O_2$	185.05987	0.9	$C_{13}H_{11}O_2$	199.07616	4.0
(**b**-ROH)	$C_{14}H_9O$	193.06470	0.5	$C_{14}H_9O$	193.06555	4.0
(**c**-CO)	$C_{12}H_{13}O$	173.09604[a]	0.3	–	–	–
h	$C_{11}H_9O_2$	173.05978[a]	0.4	$C_{12}H_{11}O_2$	187.07568	1.7
e	$C_{13}H_{11}O$	183.08052	0.5	$C_{13}H_{11}O$	183.08100	3.0
(**e**-2H)	$C_{13}H_9O$	181.06504	1.4	$C_{13}H_9O$	181.06527	2.7
(**e**-H_2O)	$C_{13}H_9$	165.06992	0.3	$C_{13}H_9$	165.07049	3.7
(**d**-CO)	$C_{11}H_9O$	157.06479	0.7	–	–	–
(**e-CO**)	$C_{12}H_{11}$	155.08556	0.2	$C_{12}H_{11}$	155.08598	2.9
(**e**-CO–2H)	$C_{12}H_9$	153.06991	0.2	$C_{12}H_9$	153.07028	2.6
i	$C_9H_7O_2$	147.04413	0.5	$C_{10}H_9O_2$	161.06000	1.8
k	$C_7H_7O_2$	123.04417	1.0	$C_8H_9O_2$	137.06030	4.3

[a] Doublet detected by a MS/MS experiment using an FT-ICR mass spectrometer coupled with an IRMPD system.

six-membered ring C is lost. Therefore, the biosynthetically formed ^{13}C-labeled codeine derivatives can be used for detailed mass spectrometric investigations to get a better insight and understanding of the key ion structures of morphine and codeine formed by ESI-CID mass spectrometry. The dissection of the codeine/morphine molecule in single atoms or groups of atom of known origin by mass spectrometry will allow future insight into the biosynthesis of these morphinan alkaloids in plants and possibly animals as well.

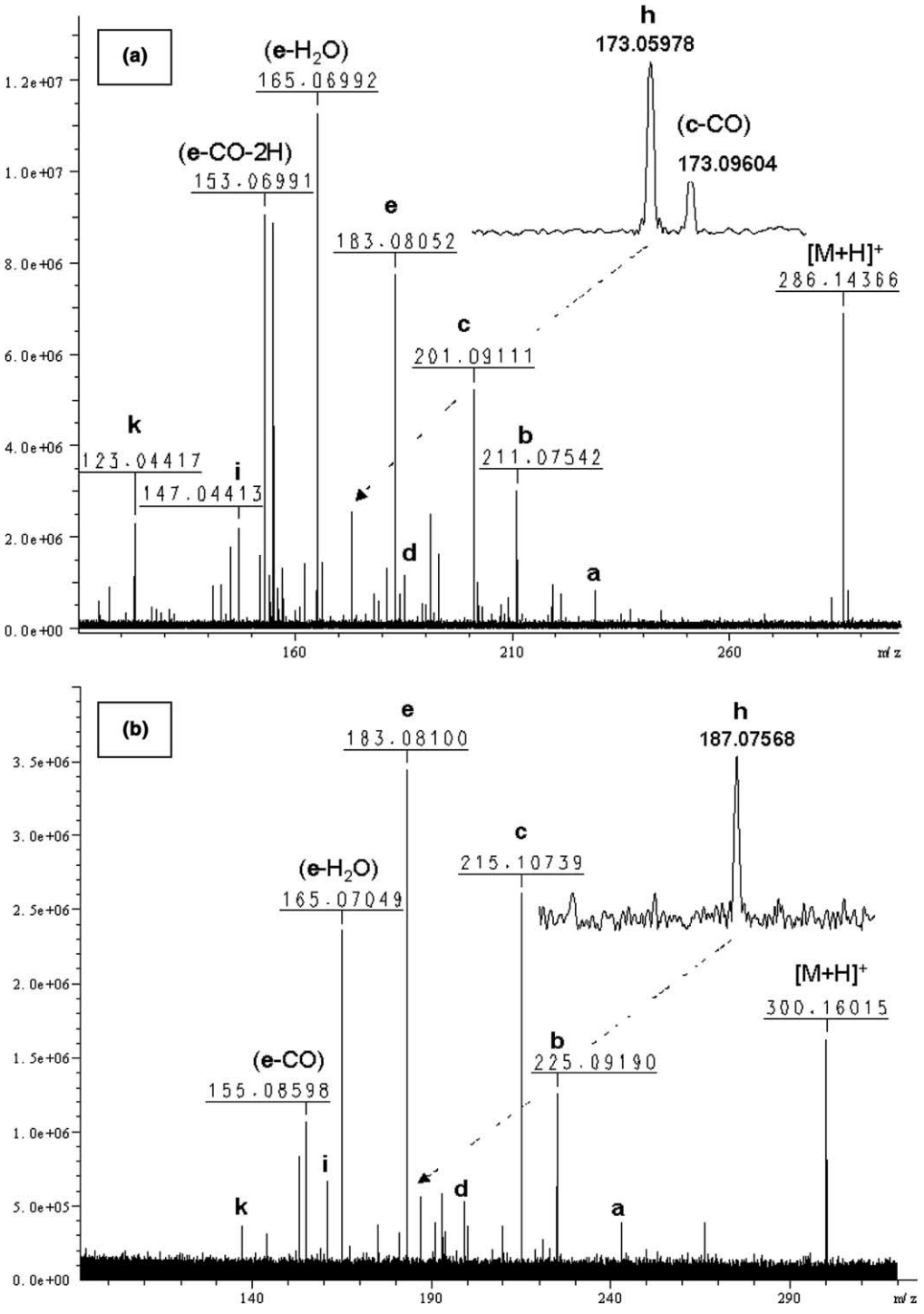


Fig. 2. IRMPD mass spectra of (a) morphine (**1**) and (b) codeine (**2**) obtained from an ESI-FT-ICR mass spectrometer.

3. Experimental

3.1. Plant material and growth conditions

Seeds of an inbred *P. somniferum* variety "Munich" were used throughout these experiments. The seeds were surface sterilized and sown into round plastic boxes (69×40 mm) supported by one layer of filter paper. The filter sterilized isotopic labeled compounds were added at a concentration of 1 mM. The plastic boxes were closed and exposed to constant illumination at 2000 lx (warm white fluorescent lamps). The seedling were harvested after 9

Table 2
ESI-CID mass spectra (38 eV) of morphine (**1**), codeine (**2**) and its ^{13}C-labeled derivatives **2a–2c** (main mass shift with respect to non-labeled codeine in brackets)

Ion	Morphine (m/z)		Codeine (m/z)		
	1	**2**	**2a**	**2b**	**2c**
$[M + H]^+$	286	300	312 (+12)	306 (+6)	302 (+2)
$[M + H–H_2O–CH_4]^+$	252	266	278 (+12)	272 (+6)	268 (+2)
a	229	243	255 (+12)	249 (+6)	244 (+1)
(**a**-2H)	227	241	253 (+12)	247 (+6)	242 (+1)
b	211	225	237 (+12)	231 (+6)	226 (+1)
(**b**-2H)	209	223	235 (+12)	229 (+6)	224 (+1)
c	201	215	226 (+11)	220 (+5)	216 (+1)
d	185	199	209 (+10)	203 (+4)	200 (+1)
(**b**-ROH)	193	193	205 (+12)	199 (+6)	194 (+1)
(**c**-CO)	173[a]	–	–	–	–
h	173[a]	187	196 (+9)	190 (+3)	188 (+1)
e	183	183	194 (+11)	188 (+5)	184 (+1)
(**e**-2H)	181	181	192 (+11)	186 (+5)	182 (+1)
(**e**-H_2O)	165	165	176 (+11)	170 (+5)	166 (+1)
(**d**-CO)	157	171	180 (+9)	174 (+3)	172 (+1)
(**e**-CO)	155	155	165 (+10)	160 (+5)	156 (+1)
(**e**-CO–2H)	153	153	163 (+10)	158 (+5)	154 (+1)
i	147	161	169 (+8)	163 (+2)	161 (0)
k	123	137	143 (+6)	137 (0)	137 (0)
f	58	58	58 (0)	58 (0)	59 (+1)
g	44	44	44 (0)	44 (0)	45 (+1)

[a] Doublet detected by a MS/MS experiment (IRMPD) using an FT-ICR mass spectrometer coupled with an IRMPD.

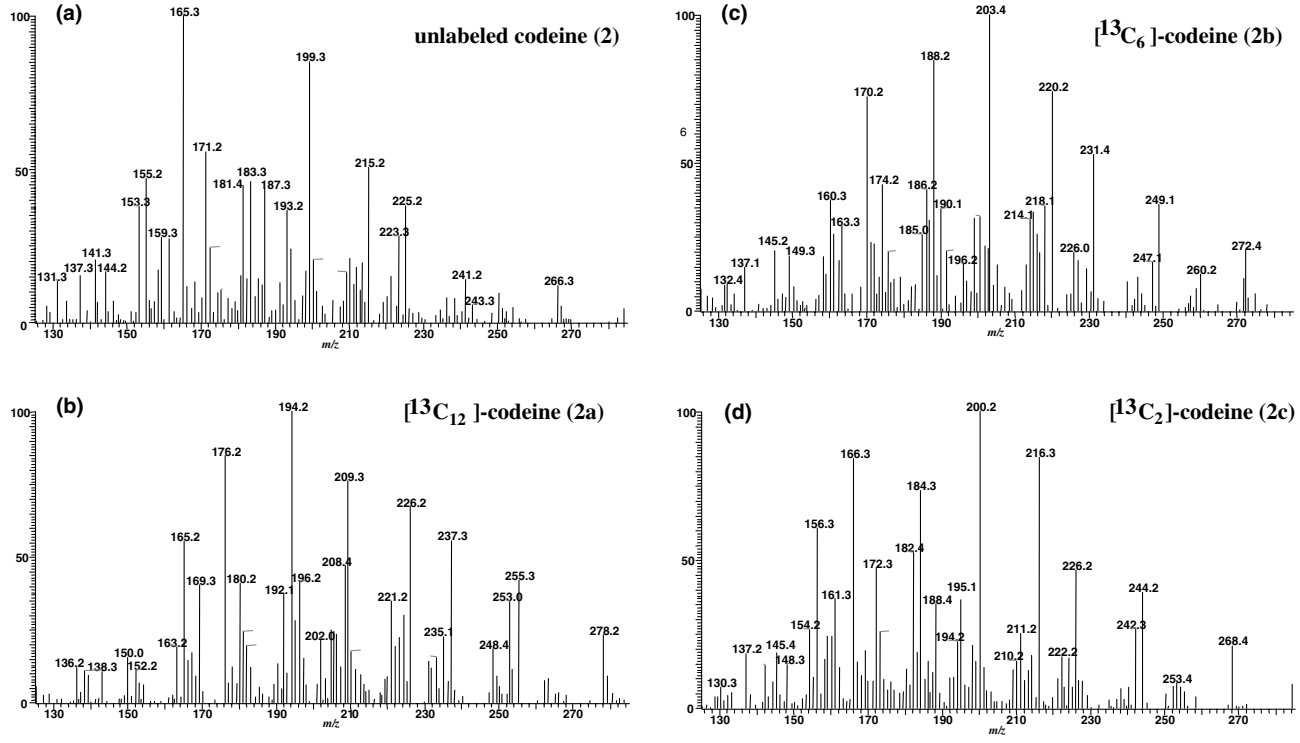


Fig. 3. CID mass spectra of codeine (**2**) (a), [$^{13}C_{12}$]-codeine (**2a**) (b), [$^{13}C_6$]-codeine (**2b**) (c) and [$^{13}C_2$]-codeine (**2c**) isolated from poppy seedling (d), normalized to the most abundant peak in the mass range m/z 125–285.

days, frozen in liquid nitrogen, ground to a fine powder and extracted with boiling 80% ethanol. The extract was filtered, evaporated to dryness, taken up in 80% (v/v) ethanol and purified by TLC (toluene/acetone/ethanol/NH_4OH, 45:45:7:3 (v/v/v/v)) yielding codeine (R_f value = 0.3), which was for mass spectrometric experiments.

3.2. Chemicals

Preparation of [ring-$^{13}C_6$]-l-tyrosine. [ring-$^{13}C_6$]-L-tyrosine was synthesized according to the principle described by Yamada et al. (1972). *Citrobacter freundii* ATCC 6750 was grown in 0.5% peptone, 0.5% yeast extract, 0.2% NaCl, pH 7.0; for the induction of β-tyrosinase, 0.2% L-tyrosine were added. A 240 ml culture was grown over night in the induction medium to OD_{600} 2.5. The bacteria were harvested by centrifugation and added to a mixture of 1 g [U-$^{13}C_6$]-phenol (Cambridge Isotope Labs, Woburn, USA), 3.76 g Na-pyruvate, 4 g ammonium acetate, pH 8.0, in a total volume of 80 ml. The incubation was performed at 30 °C with shaking (240 rpm) for 4 h. The precipitated tyrosine was separated by filtration and further purified by precipitation and recrystallization. The yield was 1.84 g [ring-$^{13}C_6$]-L-tyrosine (95.5%); $[\alpha_D^{20}] = -9.2$; CI-MS: m/z (rel. int.), 188 (100) $[M + H]^+$; ^{13}C NMR (D_2O): δ (ppm) = 156.6 (C-7), 132.5 (C-5), 128.4 (C-4), 117.5 (C-6).

Preparation of [ring-$^{13}C_6$]-tyramine. [$^{13}C_6$]-Tyramine was synthesized from [$^{13}C_6$]-L-tyrosine using tyrosine decarboxylase (Fluka Chemie GmbH, Germany) with a yield of 99%.

Preparation of [1,2-$^{13}C_2$], [6-O-methyl^{13}C-(R,S)-coclaurine. [1,2-$^{13}C_2$], [6-*O*-Methyl ^{13}C]-(*R*,*S*)-coclaurine was synthesized according to standard techniques (Stadler and Zenk, 1990).

3.3. Mass spectrometry

The high resolution ESI-IRMPD mass spectra of morphine (**1**) and codeine (**2**) were obtained from a Bruker BioApex 70e Fourier transform ion cyclotron resonance mass spectrometer (Bruker Daltonics, Billerica, MA, USA) equipped with an Infinity^{TM} cell, a 7.0 T superconducting magnet (Bruker, Karlsruhe, Germany), an RF-only hexapole ion guide and an external electrospray ion source (Agilent electrospray source, off-axis spray, voltages: endplate, −3.700 V; capillary, −4.200 V; capillary exit, 50 V; skimmer 1, 10 V; skimmer 2, 10 V) and an infrared multiphoton dissociation (IRMPD) system (cooling gas argon, laser pulse delay time 0.15 s). Nitrogen was used as drying gas at 150 °C. The sample solutions were introduced continuously via a syringe pump with a flow rate of 120 μl h^{-1}. All data were acquired with 512 k data points and zero filled to 2048 k by averaging 32 scans.

The positive ion electrospray (ES) mass spectra were obtained from a Finnigan MAT TSQ 7000 instrument (electrospray voltage 4.5 kV; heated capillary temperature 220 °C; sheath gas: nitrogen) coupled with a Surveyor MicroLC system equipped with a RP18-column (5 μm, 1 × 100 mm, Ultrasep). For the HPLC a gradient system was used starting from H_2O/CH_3CN, 85:15 (each containing 0.2% HOAc) to 10:90 within 15 min, followed by a 15 min isocratic period; flow rate 25 μl min^{-1}. The collision-induced dissociation (CID) mass spectra of morphine (**1**) and codeine (**2**) were recorded with a collision energy of 38 eV. The CID spectra of the ^{13}C-labeled codeine derivatives (**2a**–**2c**) were performed during the HPLC run of the corresponding experiment by recording alternating a spectrum of the $[M + H]^+$ ion at m/z 300 and the corresponding $[M + H]^+$ ions at m/z 312 (**2a**), 306 (**2b**) and 302 (**2c**), respectively; collision gas: argon, collision pressure: 1.8×10^{-3} Torr (for the 38 eV CID mass spectra of **1** and **2,** see Raith et al., 2003). A typical CID mass spectrum of unlabeled codeine (**2**) obtained from the same experiment as for **2c** is shown below, to allow a comparison.

Thirty-eight eV CID mass spectrum of codeine (**2**), m/z (rel. int., %): 300 ($[M + H]^+$, 62), 266 ($[M + H–H_2O–CH_4]^+$, 11), 243 (**a**, 5), 241 ([**a**-2H], 14), 225 (**b**, 39), 223 ([**b**-2H], 29), 221 (13), 215 (**c**, 51), 213 (20), 212 (18), 211 (13), 210 (20), 209 (17), 201 (11), 200 (20), 199 (**d**, 82), 198 (18), 194 (24), 193 ([**b**-MeOH], 38), 191 (12), 187 (**h,** 43), 186 (13), 185 (15), 183 (**e**, 47), 182 (12), 181 ([**e**-2H], 46), 171 ([**d**-CO], 54), 168 (12), 166 (11), 165 ([**e**-H_2O], 100), 161 (**k,** 27), 159 (28), 158 (17), 155 ([**e**-CO], 48), 153 ([**e**-CO–2H], 35), 144 (17), 141 (17), 137 (**i** 13), 58 (**f**, 75), 44 (**g**, 55).

Thirty-eight eV CID mass spectrum of [1,2,3,4,5,6,7,8,11,12,13,14-$^{13}C_{12}$]-codeine (**2a**), m/z (rel. int., %): 312 ($[M + H]^+$, 100), 278 ($[M + H–H_2O–CH_4]^+$, 9), 255 (**a**, 16), 253 ([**a**-2H], 13), 237 (**b**, 21), 235 ([**b**-2H], 10), 226 (**c**, 25), 209 (**d**, 28), 208 (18), 206 (9), 205 ([**b**-MeOH], 9), 202 (8), 196 (**h**, 17), 195 (10), 194 (**e**, 37), 192 ([**e**-2H], 14), 180 ([**d**-CO], 15), 176 ([**e**-H_2O], 32), 169 (**k**, 15), 165 ([**e**-CO], 21), 163 ([**e**-CO–2H], 7), 150 (6), 143 (**i**, 4), 87 (5), 58 (**f**, 50), 44 (**g**, 18).

Thirty-eight eV CID mass spectrum of [5,6,7,8,13,14-$^{13}C_6$]-codeine (**2b**), m/z (rel. int., %): 306 ($[M + H]^+$, 100), 272 ($[M + H–H_2O–CH_4]^+$, 12), 249 (**a**, 21), 247 ([**a**-2H], 10), 231 (**b**, 31), 229 ([**b**-2H], 10), 227 (10), 226 (12), 220 (**c**, 47), 218 (21), 217 (12), 216 (15), 215 (20), 214 (21),205 (10), 203 (**d**, 60), 202 (14), 201 (19), 199 ([**b**-MeOH], 19), 191 (12), 190 (**h**, 25), 189 (7), 188 (**e**, 51), 187 (19), 186 ([**e**-2H], 24), 185 (16), 177 (10), 176 (12),174 ([**d**-CO], 26), 172 (13), 171 (14), 170 ([**e**-H_2O], 47), 163 (**k**, 17), 162 (11), 161 (15), 160 ([**e**-CO], 24), 159 (7), 158 ([**e**-CO–2H], 12), 145 (14), 137 (**i**, 8), 58 (**f**, 59), 44 (**g**, 58).

Thirty-eight eV CID mass spectrum of [9,16-$^{13}C_2$]-codeine (**2c**), (m/z (rel. int., %): 302 ($[M + H]^+$, 86), 268 ($[M + H–H_2O–CH_4]^+$, 20), 244 (**a**, 34), 242 ([**a**-2H], 27), 226 (**b**, 46), 224 ([**b**-2H], 16), 222 (18), 216 (**c**, 78), 214 (18), 213 (12), 212 (9), 211 (24), 210 (15), 209 (11), 201 (13), 200 (**d,** 95), 199 (15), 198 (19), 195 (34), 194 ([**b**-MeOH], 22), 193 (10), 188 (**h**, 36), 187 (11), 186 (16), 184 (**e**, 69), 183 (17), 182 ([**e**-2H], 51), 180 (14), 173 (23), 172 ([**d**-CO], 43), 169 (17), 168 (10), 167 (16), 166 ([**e**-H_2O], 80), 162 (12), 161 (**k**, 34), 160 (21), 159 (25), 158 (17), 156

([**e**-CO], 56), 155 (10), 154 ([**e**-CO–2H], 27), 148 (15), 145 (18), 142 (14), 137 (**i**, 17), 59 (**f**, 100), 45 (**g**, 69).

The ^{13}C-content of **2a**–**2c** was calculated from the LC–MS full scan data.

2a ([$^{13}C_{12}$]-codeine): 54.1% $^{13}C_0$, 0.8% $^{13}C_1$, 1.1% $^{13}C_5$, 28.2% $^{13}C_6$, 1.4% $^{13}C_{11}$, 13.9% $^{13}C_{12}$, others 0.5%.

2b ([$^{13}C_6$]-codeine): 59.0% $^{13}C_0$, 3.2% $^{13}C_1$, 2.2% $^{13}C_5$, 35.0% $^{13}C_6$, others 0.6%.

2c ([$^{13}C_2$]-codeine): 42.2% $^{13}C_0$, 5.4% $^{13}C_1$, 49.6% $^{13}C_2$, others 2.8%.

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