

Biosynthetic Route to the *Ephedra* Alkaloids¹Gunnar Grue-Sørensen[†] and Ian D. Spenser^{*‡}

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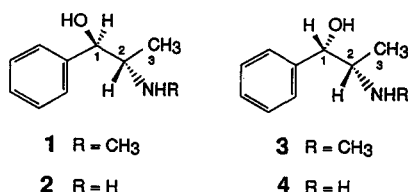
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Abstract: It is shown by ¹³C NMR spectroscopy that [carboxyl-¹³C]benzoic acid rather than [carbonyl-¹³C,²H]-benzaldehyde supplies the benzylic fragment of the *Ephedra* alkaloids, norephedrine, ephedrine, norpseudoephedrine, and pseudoephedrine, in growing plants of *Ephedra gerardiana sikkimensis*. [1,2,3-¹³C₃]-1-Phenylpropane-1,2-dione and (S)-[1,2,3-¹³C₃]-2-amino-1-phenylpropan-1-one ([1,2,3-¹³C₃]cathinone) serve as precursors of the *Ephedra* alkaloids. (R)- and (S)-[1-¹³C,1-²H]-1-hydroxy-1-phenylpropan-2-one are excluded as intermediates. Cathinone is shown to be a constituent of *E. gerardiana*.

Introduction

The C₆-C₃ skeleton of the *Ephedra* alkaloids (1–4) is generated by union of two discrete subunits, a benzylic C₆-C₁ moiety and a C₂ unit. The identity of these basic building blocks of the two subunits is known.

It was shown more than 35 years ago by Edward Leete² and confirmed 10 years later by Shibata and his students^{3,4} that the benzylic C₆-C₁ unit of the alkaloids is derived from the benzylic C₆-C₁ unit of phenylalanine: Radioactivity from the benzylic methylene carbon atom of DL-phenylalanine entered the benzylic carbon atom of (+)-norpseudoephedrine (4) in *Catha edulis*² and of ephedrine (1) in *Ephedra distachya*,^{3,4} and tritium from the tritium labeled phenyl nucleus of DL-phenylalanine was maintained in the phenyl nucleus of ephedrine in *E. distachya*.^{3,4} On the route to the alkaloids, phenylalanine presumably cleaves by the ammonia lyase route,⁵ since the C₆-C₁ unit of cinnamic acid is also incorporated^{3,4} and since benzoic acid and benzaldehyde serve as precursors of the benzylic C₆-C₁ unit of ephedrine (1).^{3,4}



The origin of the remaining C₂ unit of the alkaloid skeleton remained obscure until recently, when we identified the CH₃CO moiety of pyruvic acid as its source in the four alkaloids, (1R,2S)-(-)-ephedrine (1), (1R,2S)-(-)-norephedrine (2), (1S,2S)-(+)-pseudoephedrine (3), and (1S,2S)-(+)-norpseudoephedrine (4), in *E. gerardiana sikkimensis*.^{6,7}

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The final step in the biosynthesis of ephedrine (1) (and presumably also of pseudoephedrine (3)) is the methylation of the corresponding noralkaloid (2 and 4, respectively) by a methyl group supplied by methionine.⁸

The steps in the reaction sequence that lead from the two subunits, C₆-C₁ and C₂, into the noralkaloids (2 and 4) have not been defined, nor is it known whether it is benzaldehyde or benzoic acid that serves as the C₆-C₁ precursor whose reaction with the pyruvate-derived C₂ unit yields the first C₆-C₃ intermediate on route to the noralkaloids.

Plausible hypotheses may be advanced to implicate either benzaldehyde or benzoic acid as the ultimate C₆-C₁ precursor.

There is ample biochemical precedent for the reaction of benzaldehyde with pyruvate (Scheme 1, path A). If benzaldehyde were the ultimate C₆-C₁ precursor, its condensation with the pyruvate-derived C₂ unit would be analogous to the process that takes place when yeast is incubated with benzaldehyde in the presence of fermentable sugars^{9–14} or pyruvic acid.^{15–19} A reaction of benzaldehyde with pyruvate, accompanied by decarboxylation, would be expected to yield a mixture of two α-ketols, (R)-1-hydroxy-1-phenylpropan-2-one (5), the ketone corresponding to norephedrine (2), and (S)-1-hydroxy-1-phenylpropan-2-one (6), the ketone corresponding to norpseudoephedrine (4). If enzyme-catalyzed, this reaction would not necessarily lead to an equimolar mixture of the two isomers. In yeast, the major product of this thiamin-catalyzed reaction is (R)-(-)-1-hydroxy-1-phenylpropan-2-one (5).^{10,19} Stereospecific transamination²⁰ of each of the two α-ketols would yield the corresponding noralkaloid. However, attempts to demonstrate incorporation into the *Ephedra* alkaloids of ¹⁴C-labeled samples of (RS)-1-hydroxy-1-phenylpropan-2-one have been unsuccessful.²¹

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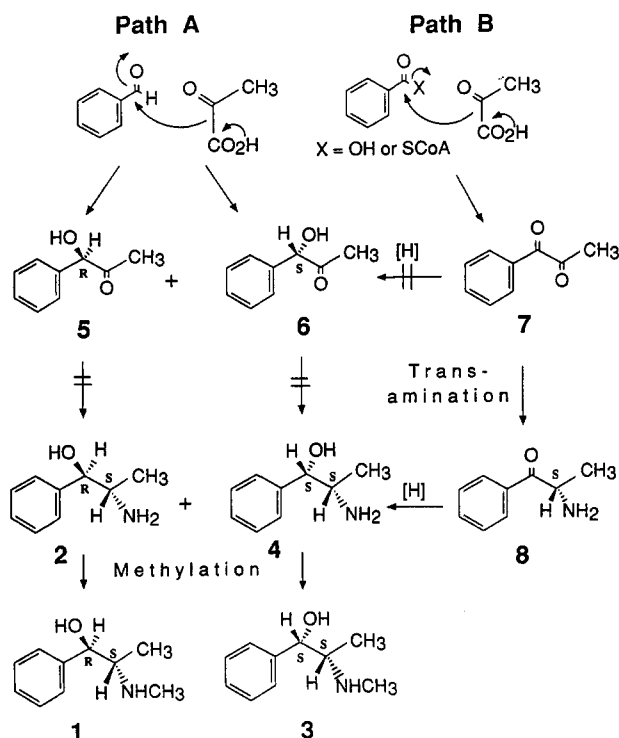
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Scheme 1. Proposed Biogenetic Pathways to the *Ephedra* Alkaloids

Even though there is biochemical precedent for this biogenetic sequence, addition and transamination, one aspect of this route is unattractive: Since not only benzaldehyde but also benzoic acid is incorporated into the alkaloids, this route demands that benzoic acid undergoes reduction to benzaldehyde. Whereas many instances have been recorded of the biological oxidation of the aldehyde to the acid, only a single report is available of the biological reduction of the acid to the aldehyde.²²

An alternative biogenetic sequence may be postulated: It involves the condensation of pyruvic acid with benzoic acid (or its CoA thioester) as the donor of the benzylic C₆-C₁ unit (Scheme 1, path B). Such a reaction, although unprecedented, would be expected to yield 1-phenylpropane-1,2-dione (7) as the first C₆-C₃ intermediate. This compound has not been detected in *Ephedra* species but occurs in *Catha edulis*, a plant that also contains (S)-(-)-2-amino-1-phenylpropan-1-one (cathinone) (8) as well as (+)-norpseudoephedrine (4) (also referred to as cathine) and (-)-norephedrine (2).^{23,24}

Conversion of 1-phenylpropane-1,2-dione (7) into the noralkaloids, norephedrine (2), and norpseudoephedrine (4), requires two steps, reduction of the C-1 oxo group and stereospecific transamination of the C-2 oxo group. Either of these two steps could precede or follow the other in the biosynthetic sequence. If reduction at C-1 preceded transamination at C-2, the intermediates of the sequence from 7 to the noralkaloids 2 and 4 would be predicted to be (R)- and (S)-1-hydroxy-1-phenylpropan-2-one (5 and 6, respectively). If transamination at C-2 preceded reduction at C-1, the intermediate would be cathinone (8). The cooccurrence, in *Catha edulis*, of cathinone (8) with 1-phenylpropane-1,2-dione (7) suggests that transamination at C-2 precedes reduction at C-1, at least in this plant species.

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We have examined the hypothetical sequences by means of five diagnostic experiments. On the basis of the results, to be reported in the sequel, we conclude that benzoic acid (or its CoA thioester), rather than benzaldehyde, represents the ultimate C₆-C₁ precursor, and that the biosynthetic route to the noralkaloids proceeds via 1-phenylpropane-1,2-dione (7) and cathinone (8), and excludes (R)- and (S)-1-hydroxy-1-phenylpropan-2-one (5 and 6).

Results and Discussion

The plant species used in this study was *Ephedra gerardiana sikkimensis*. Plants were grown from cuttings obtained from the Geographic Garden, Kolding, Denmark.

The labeled substrates were dissolved or suspended in glass-distilled water and a non-ionic detergent was added, partly to ensure homogeneity of the solutions and partly to facilitate the penetration of the substrates into the plant. The tracers were applied with a fine paint brush and application was confined to the most recent, fresh growth of the plants, where biosynthetic activity was assumed to be at a maximum. Details of the administration of the tracers are summarized in Table 1. All aerial parts were harvested and extracted and the yield of the four alkaloids was determined by ¹³C NMR. In experiment 4 harvesting was carried out in two batches: The portions of the plant where tracer had been applied (experiment 4a) were kept separate from the remaining older aerial parts of the plants which had not been painted with tracer solution (experiment 4b). The purpose of this exercise was to investigate the mobility of the substrate and/or of the newly biosynthesized alkaloids from the younger into the older parts of the plants. Details of the experiments are summarized in Table 1.

The general procedure for the isolation of the alkaloids has been reported.⁶ The yield in the five experiments (see Table 1) was in the range 0.7 to 2 mg alkaloid hydrochlorides per gram of fresh aerial plant material.

If benzaldehyde were the ultimate C₆-C₁ precursor on the route to the alkaloids (Scheme 1, path A), not only would labeled carbon from the carbonyl group of benzaldehyde be delivered into C-1 of the *Ephedra* alkaloids 1–4, as was indeed observed in the case of ephedrine,^{3,4} but, in addition, deuterium from the aldehyde group would be expected to be maintained at C-1 of the alkaloids. Furthermore, (R)- and (S)-1-hydroxy-1-phenylpropan-2-one (5 and 6) would be predicted intermediates on route to 2 (and 1) and to 4 (and 3), respectively, so that ¹³C and ²H from (RS)-[1-¹³C, 1-²H]-1-hydroxy-1-phenylpropan-2-one would be expected to enter the four alkaloids.

These predictions were tested by means of tracer experiments with [carbonyl-¹³C, ²H]benzaldehyde (experiment 1) and with (RS)-[1-¹³C, 1-²H]-1-hydroxy-1-phenylpropan-2-one (5 + 6) (experiment 2). In confirmation of the earlier result of Shibata et al.,^{3,4} the presence of labeled carbon was detected at C-1 of each of the four *Ephedra* alkaloids (Table 2) when [carbonyl-¹³C, ²H]benzaldehyde was administered to *Ephedra* shoots. However, ²H/¹³C coupling was not detectable in the carbinol signals of the ¹³C NMR spectrum of the alkaloids derived from [carbonyl-¹³C, ²H]benzaldehyde. Intact incorporation of the ¹³C, ²H-labeled benzaldehyde, as demanded by the hypothesis that benzaldehyde is the ultimate C₆-C₁ intermediate, would have maintained deuterium at the carbinol carbon of the alkaloids, giving rise to multiplicity of the enriched carbinol signals in the ¹³C NMR spectrum. The incorporation of ¹³C into the four alkaloids serves as an internal standard that confers significance on the lack of incorporation of deuterium. It follows that benzaldehyde does not serve as the ultimate C₆-C₁ intermediate but is oxidized to benzoic acid prior to incorporation.

This inference was corroborated by the result of the experiment with (RS)-[1-¹³C, 1-²H]-1-hydroxy-1-phenylpropan-2-one (5 + 6) (experiment 2). This compound would be the first C₆-C₃

Table 1. Administration of Tracers to *Ephedra gerardiana sikkimensis*

expt no.	date	precursor ^a	weight of precursor (mg)	Triton X-165 ³¹ (μL)	days between feeding and harvest ^b	weight of fresh plant material (g)	weight of alkaloid HCl mixture (mg)
1	May 90	[carbonyl- ¹³ C, ² H]benzaldehyde ^c	260	200	3	24	31
2	Aug 90	(<i>RS</i>)-[1- ¹³ C, 1- ² H]-1-hydroxy-1-phenylpropan-2-one	50	50	3	21	19
3	Sept 90	sodium [carboxyl- ¹³ C]benzoate	235	50	5	28	19
4a	June 92	(<i>S</i>)-[1,2,3- ¹³ C ₃]-2-amino-1-phenylpropan-1-one, HCl	64	50	4	15 ^d	26
4b						27 ^e	25
5	Aug 92	[1,2,3- ¹³ C ₃]-1-phenylpropane-1,2-dione	17	100	4	7	14

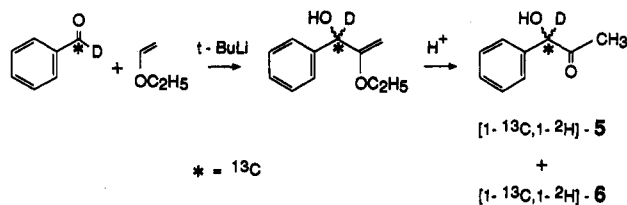
^a Tracer was dissolved in water (5 mL) with Triton X-165³¹ and applied with a fine paint brush in five equal portions over five days. ^b In experiments 2–5 the plants were sprayed with water each day for four days after the administration of the last portion of precursor. ^c During the last three days of administration of benzaldehyde a white layer/crust (presumably benzoic acid formed by air oxidation) was visible on the painted parts of the plants. ^d Weight of the fresh green shoots to which tracer had been applied. ^e Weight of older aerial parts to which tracer had not been applied.

Table 2. Incorporation of Labeled Precursors into *Ephedra* Alkaloids

expt no.	molar ratio ^a 1/2/3/4/8	specific incorporation of ¹³ C (%) ^b				
		1	2	3	4	8
1	27:1:64:8:0	0.2	0.9	0.2	1.5	
2	39:1:50:10:0	0.0	^c	0.0	0.0	
3	32:1:60:7:0	1.3	6.0	0.3	3.5	
4a	18:1:27:9:35 ^d	2	98	8	97	
4b	34:1:52:3:10 ^d	0.05	87	0.2	43	
5	36:2:61:1:<1	0.0	>25	0.15	10	>25

^a Molar ratio was determined by measuring peak height ratios of suitably selected natural abundance signals in the aromatic region of the spectra. ^b For experiments 1–3: Specific incorporation = % enrichment above natural abundance = 1.1% × (peak height of enriched C-1 signal minus calculated peak height of natural abundance C-1 signal)/(calculated peak height of natural abundance C-1 signal). For experiments 4 and 5: Specific incorporation = % enrichment above natural abundance = 1.1% × (2 × mean peak height of satellites at C-1)/(peak height of natural abundance singlet at C-1). ^c Not determined. ^d Reisolated non-reacted precursor is included in the molar ratio.

Scheme 2. Synthesis of (*RS*)-[1-¹³C, 1-²H]-1-Hydroxy-1-phenylpropan-2-one



intermediate generated by condensation of benzaldehyde with pyruvate. A ¹³C, ²H doubly labeled sample of the compound was synthesized by modification of a published method²⁵ (Scheme 2). The lithium salt of ethyl vinyl ether was condensed with [carbonyl-¹³C, ²H]benzaldehyde and the resulting enol ether was hydrolyzed with dilute hydrochloric acid to give the desired mixture of (*R*)- and (*S*)-[1-¹³C, 1-²H]-1-hydroxy-1-phenylpropan-2-one in 20% yield. In confirmation of the results of K. Yamasaki²¹ who failed to detect incorporation into ephedrine of radioactivity from ¹⁴C-labeled 1-hydroxy-1-phenylpropan-2-one, no enrichment of either ¹³C or ²H was observed in any of the four alkaloids when (*RS*)-[1-¹³C, 1-²H]-1-hydroxy-1-phenylpropan-2-one served as the labeled substrate (experiment 2).

If the keto alcohols 5 and 6 were precursors, a single step, transamination, would be required to produce the noralkaloids. Since incorporation of label from benzaldehyde, a compound at least two biosynthetic steps away from the noralkaloids, was high and readily detectable, it would be expected that if the keto alcohols were intermediates, label from these substrates would also be incorporated at readily detectable levels. Yet, incorporation was

not observed. The biogenetic sequence presented in Scheme 1, path A, is inconsistent with the lack of incorporation of the keto alcohols 5 and 6 and is therefore refuted by the results.

An alternative biogenetic pathway considers benzoic acid (or its CoA thioester), rather than benzaldehyde, as the ultimate C₆-C₁ substrate of the sequence leading to the alkaloids (Scheme 1, path B). The C₆-C₃ intermediates on this route must be 1-phenylpropane-1,2-dione (7) and its C-2 transamination product, cathinone (8). Based on the outcome of experiment 2, above, intermediacy of the reduction products of 7, (*R*)- and (*S*)-1-hydroxy-1-phenylpropan-2-one (5 + 6), need not be considered further.

To test Scheme 1, path B, three tracer experiments were performed, with ¹³C-labeled samples of sodium benzoate (experiment 3), 1-phenylpropane-1,2-dione (experiment 5) and cathinone (experiment 4) as the substrates. Bond-labeled samples of the latter two compounds were synthesized by minor modification of published procedures.^{25,26} The key step in the synthesis of [1,2,3-¹³C₃]-1-phenylpropane-1,2-dione was the condensation of [carboxyl-¹³C]benzoic acid with the lithium salt of [1,2-¹³C₂]vinyl ethyl ether, followed by acid hydrolysis of the resulting enol ether²⁵ (Scheme 3). The labeled vinyl ethyl ether was formed by reaction of [¹³C₂]acetaldehyde with hydrogen chloride and ethanol to yield [1,2-¹³C₂]-1-chloroethyl ethyl ether,²⁷ followed by base-promoted elimination of hydrogen chloride.²⁸

(*S*)-[1,2,3-¹³C₃]-2-Amino-1-phenylpropan-1-one was prepared by Friedel-Crafts reaction of benzene with *N*-(ethoxycarbonyl)-L-[1,2,3-¹³C₃]alanine,²⁶ followed by acid hydrolysis (Scheme 4).

Label from sodium [carboxyl-¹³C]benzoate (experiment 3) entered the carbinol carbon atom of each of the four alkaloids 1–4 (see Figure 1a and Table 2), in confirmation of the earlier results of Shibata et al.^{3,4} The specific incorporation of ¹³C into the four alkaloids was higher than that observed in the experiment with [2,3-¹³C₂]pyruvate,^{6,7} the precursor of the C₂ unit, C-2,3. This shows that benzoic acid is an efficient precursor of the C₆-C₁ unit of the *Ephedra* alkaloids.

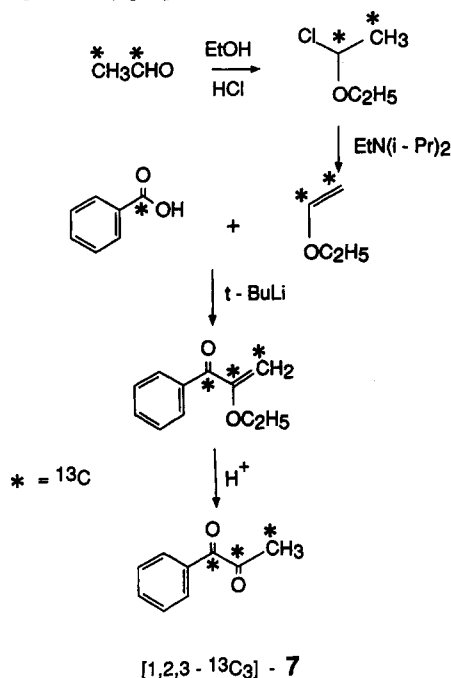
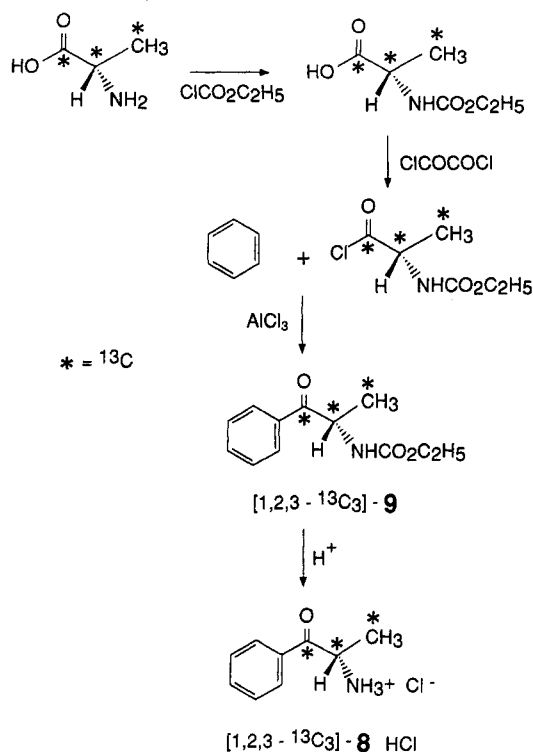
According to Scheme 1, path B, the first C₆-C₃ intermediate on the route to the noralkaloids is 1-phenylpropane-1,2-dione (7). Indeed, as expected, label from [1,2,3-¹³C₃]-1-phenylpropane-1,2-dione was incorporated into the alkaloids (experiment 5)(see Table 2). The ¹³C NMR spectrum (Figure 1d) of the alkaloid mixture from this experiment showed the characteristic coupling pattern for the presence of a ¹³C₃ unit in the signals from three of the alkaloids, norephedrine (2), norpseudoephedrine (4), and pseudoephedrine (3). Specific incorporation of label into pseudoephedrine was low (0.15%) and incorporation into ephedrine (1) was not detectable. However, incorporation into the noralkaloids was high (>10%) and the presence of a trace of

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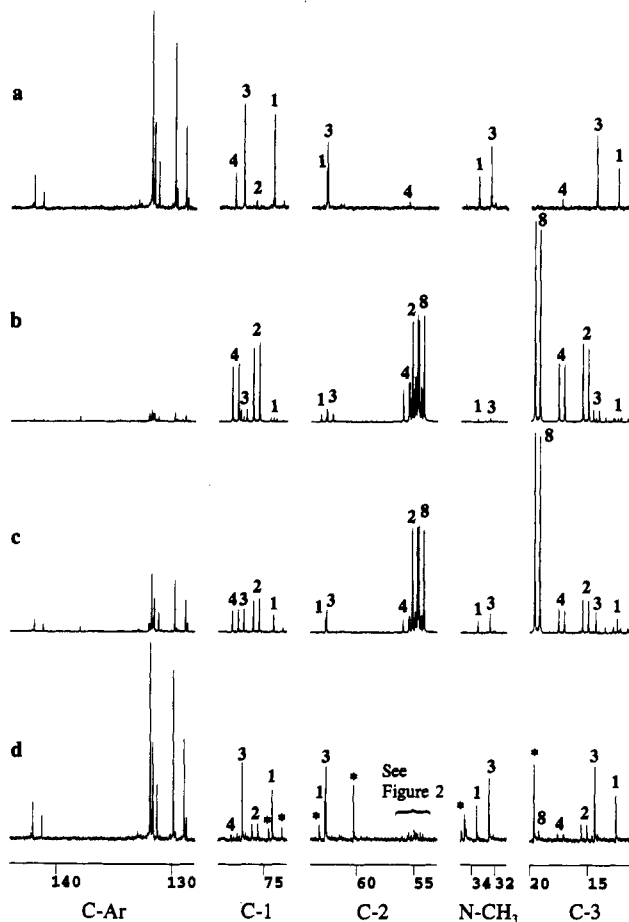
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Scheme 3. Synthesis of [1,2,3- $^{13}\text{C}_3$]-1-Phenylpropane-1,2-dione**Scheme 4.** Synthesis of (S)-[1,2,3- $^{13}\text{C}_3$]-2-Amino-1-phenylpropan-1-one ([1,2,3- $^{13}\text{C}_3$]cathinone) Hydrochloride

cathinone (**8**) (< 1% of the total alkaloid content), triply enriched in ^{13}C , was detected. Although a number of unidentified signals are present in the ^{13}C NMR spectrum from this experiment (see Figure 1d) the assignment of the dd-signal at 54.78 ppm (Figure 2) to C-2 of [1,2,3- $^{13}\text{C}_3$]cathinone is unambiguously established by the exact match with signals in the ^{13}C NMR spectrum of authentic [1,2,3- $^{13}\text{C}_3$]cathinone (see below). Also, even though half of the doublet at 19.45 ppm due to C-3 of the triply labeled cathinone is masked by a signal of an unidentified contaminant of the mixture, the other half is clearly visible (Figure 1d). This



• Unidentified signals

Figure 1. Proton noise-decoupled 75.47-MHz ^{13}C NMR spectra of the mixture of alkaloid hydrochlorides in D_2O . (a) from sodium [carboxyl- ^{13}C]benzoate, Experiment 3; (b) from painted aerial parts, from [1,2,3- $^{13}\text{C}_3$]cathinone hydrochloride, experiment 4a; (c) from nonpainted aerial parts, from [1,2,3- $^{13}\text{C}_3$]cathinone hydrochloride, experiment 4b; (d) from [1,2,3- $^{13}\text{C}_3$]-1-phenylpropane-1,2-dione, experiment 5. Spectral parameters: (a) 5871; (b) 3000; (c) 5000; (d) 50000 transients, spectral width 20833 Hz, 45° pulse flip angle, acquisition time 1.573 s, line broadening 2.0 Hz, memory size 64 K, digital resolution 0.636 Hz/data point. Chemical shift values for natural abundance *Ephedra* alkaloids are listed in ref 7.

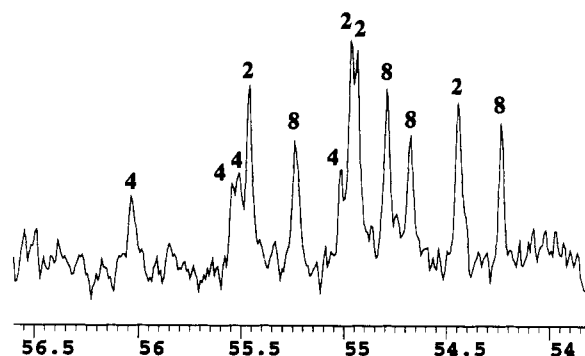


Figure 2. Expanded 54.0–56.5-ppm region of the 75.47-MHz ^{13}C NMR spectrum of the alkaloids from the tracer experiment with [1,2,3- $^{13}\text{C}_3$]-1-phenylpropane-1,2-dione (experiment 5, Figure 1d), showing three doublets of doublets, the signals due to C-2 of norephedrine (**2**), norpseudoephedrine (**4**), and cathinone (**8**), as their hydrochlorides.

is the first time that the presence of cathinone has been detected (see Figure 2) in an *Ephedra* species, providing additional evidence in support of path B, Scheme 1.

in 5-min intervals, to a stirred solution of L-[$^{13}\text{C}_3$]alanine (99 atom % per position, 500 mg, 5.43 mmol) in sodium hydroxide (1.0 M, 5.6 mL) at 12 °C, while the pH was kept between 9 and 10 by addition of 1 M sodium hydroxide, as required. When the pH had stabilized at pH 10 the mixture was cooled to 0 °C. The cold mixture was washed with ether (3 \times 7 mL), was then acidified to ca. pH 1 with 85% orthophosphoric acid and solid sodium chloride was added to saturation (still at 0 °C). Extraction with dichloromethane (3 \times 7 mL), drying with calcium chloride, and evaporation of the solvent *in vacuo* gave crude N-(ethoxycarbonyl)-L-[$^{13}\text{C}_3$]alanine (734 mg) as an oil. This compound (725 mg) was dissolved in a mixture of dichloromethane (13 mL) and dry DMF (22 μL) under argon at 0 °C, and oxalyl chloride (444 μL , 648 mg, 5.10 mmol) was added. The mixture was stirred at rt for 1.5 h. After cooling to -15 °C dichloromethane (6.5 mL), benzene (55 mL), and aluminium trichloride (1.29 g) was added and the mixture was stirred at 15 °C for 20 h. Hydrochloric acid (1.0 M, 13 mL) and water (9 mL) were added at 0 °C and stirring was continued for 10 min. The organic phase was separated, washed in succession with hydrochloric acid (1.0 M, 2 \times 13 mL), semisaturated sodium hydrogen carbonate (26 mL), and saturated sodium hydrogen carbonate (13 mL), dried over magnesium sulfate, and evaporated to dryness *in vacuo*, yielding a yellowish oil which crystallized upon cooling. Recrystallization from hexane gave (S)-N-(ethoxycarbonyl)-[1,2,3- $^{13}\text{C}_3$]-2-amino-1-phenylpropan-1-one ([1,2,3- $^{13}\text{C}_3$]-9)²⁶ (653 mg, 2.91 mmol, 54%): mp 57–58 °C; $^1\text{H-NMR}$ (D_2O) (δ ppm) 1.26 (t, J = 7.1, 3H, CH_2CH_3), 1.43 (dm, $J(\text{H}-3, \text{C}-3)$ = 129.7, 3H, H-3), 4.14 (q, J = 7.1, 2H, CH_2CH_3), 5.33 (dm, $J(\text{H}-2, \text{C}-2)$ = 140.5, 1H, H-2), 5.73 (bs, 1H, NH), 7.47–7.52 (m, 2H, H-Ar), 7.59–7.63 (m, 1H, H-Ar), 7.96–8.00 (m, 2H, H-Ar); $^{13}\text{C-NMR}$ (D_2O) (δ ppm) 14.6 (s, CH_2CH_3), 20.0 (d, $J(\text{C}-3, \text{C}-2)$ = 34.7, C-3), 51.2 (dd, $J(\text{C}-2, \text{C}-1)$ = 42.0, $J(\text{C}-2, \text{C}-3)$ = 34.7, C-2), 61.0 (s, CH_2CH_3), 128.7 (s, C-Ar), 128.9 (d, C-Ar), 133.8 (s, C-Ar), 199.1 (d, $J(\text{C}-1, \text{C}-2)$ = 42.4, C-1).

The signal for C-1' was not detectable in this spectrum (nonenriched position, low-intensity doublet).

The labeled [(ethoxycarbonyl)amino]-1-phenylpropan-1-one (645 mg, 2.88 mmol) was refluxed (bath temperature 115 °C) with a mixture of concd hydrochloric acid (10 mL) and acetic acid (4 mL) for 3 h. After cooling to 0 °C ice-cold water (10 mL) was added and the mixture was washed with ether (2 \times 10 mL). Solid potassium carbonate was added to the ice-cold aqueous phase to pH ca. 10 and the mixture was extracted with dichloromethane (2 \times 20 mL). After drying with magnesium sulfate, concd hydrochloric acid (0.5 mL) was added. Evaporation to dryness *in vacuo* gave a crude product which was recrystallized from 2-propanol/ether to give (S)-[1,2,3- $^{13}\text{C}_3$]-2-amino-1-phenylpropan-1-one ([1,2,3- $^{13}\text{C}_3$]-8) hydrochloride (66 mg, 0.35 mmol, 12% from (S)-N-(ethoxy-

carbonyl)-[1,2,3- $^{13}\text{C}_3$]-2-amino-1-phenylpropan-1-one): mp 185–186 °C (lit.²⁹ mp 188–190 °C); $^1\text{H-NMR}$ (D_2O) (δ ppm) 1.47 (dm, $J(\text{H}-3, \text{C}-3)$ = 131.1, 3H, H-3), 4.66 (s, HOD), 5.08 (dm, $J(\text{H}-2, \text{C}-2)$ = 146.6, 1H, H-2), 7.47–7.52 (m, 2H, H-Ar), 7.62–7.67 (m, 1H, H-Ar), 7.89–7.91 (m, 2H, H-Ar); $^{13}\text{C-NMR}$ (D_2O) (δ ppm) 19.5 (d, $J(\text{C}-3, \text{C}-2)$ = 33.3, C-3), 54.8 (dd, $J(\text{C}-2, \text{C}-1)$ = 42.0, $J(\text{C}-2, \text{C}-3)$ = 33.3, C-2), 131.7 (s, C-Ar), 132.0 (d, J = 3.9, C-Ar), 138.1 (s, C-Ar), 201.0 (d, $J(\text{C}-1, \text{C}-2)$ = 42.0, C-1).

The signal for C-1' was not detectable in this spectrum (nonenriched position, low intensity doublet).

Administration of Labeled Compounds to *Ephedra gerardiana sikkimensis*. Stocks of *E. gerardiana sikkimensis* were propagated in a greenhouse from cuttings obtained from The Geographic Garden, Kolding, Denmark. Labeled compounds were administered in aqueous solutions (5 mL) containing 1–4% (w/v) Triton X-165³¹. The solutions (1 mL/day) were applied to the green shoots with a fine paint brush. After administration of tracer the plants were sprayed with water twice a day for three to five days before harvesting. Details of the experiments are summarized in Table 1.

Isolation of the Alkaloid Mixture.⁶ The aerial parts of the plants were ground in a blender and extracted with methanol at 20 °C. The methanol extracts were acidified with hydrochloric acid (4 M, 3 mL) before concentration *in vacuo*. Hydrochloric acid (0.1 M, 50 mL) was added to the residue, and the aqueous mixture was washed with ether (4 \times 50 mL) and was then basified with solid potassium carbonate and extracted with ether (4 \times 50 mL). The ether extracts were extracted with hydrochloric acid (1 M, 2 \times 5 mL), and the aqueous phase was evaporated to dryness *in vacuo* to yield a mixture of alkaloid hydrochlorides. This was analyzed by $^{13}\text{C-NMR}$ (experiments 1–5). The data are summarized in Table 2.

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(31) Triton X-165 is α -[4-(1,1,3,3-tetramethylbutyl)phenyl]- ω -hydroxy-poly(oxy-1,2-ethanediyl) in 70% aqueous solution; trademark of the Rohm and Haas Co.