Biosynthetic Route to the *Ephedra* Alkaloids[1]

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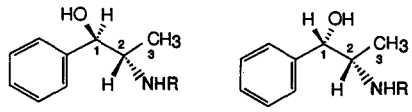
Abstract: It is shown by ^{13}C NMR spectroscopy that [*carboxyl*-^{13}C]benzoic acid rather than [*carbonyl*-^{13}C,^{2}H]-benzaldehyde supplies the benzylic fragment of the *Ephedra* alkaloids, norephedrine, ephedrine, norpseudoephedrine, and pseudoephedrine, in growing plants of *Ephedra gerardiana sikkimensis*. [1,2,3-$^{13}C_3$]-1-Phenylpropane-1,2-dione and (*S*)-[1,2,3-$^{13}C_3$]-2-amino-1-phenylpropan-1-one ([1,2,3-$^{13}C_3$]cathinone) serve as precursors of the *Ephedra* alkaloids. (*R*)- and (*S*)-[1-^{13}C,1-^{2}H]-1-hydroxy-1-phenylpropan-2-one are excluded as intermediates. Cathinone is shown to be a constituent of *E. gerardiana*.

Introduction

The C_6-C_3 skeleton of the *Ephedra* alkaloids (**1**–**4**) is generated by union of two discrete subunits, a benzylic C_6-C_1 moiety and a C_2 unit. The identity of these basic building blocks of the two subunits is known.

It was shown more than 35 years ago by Edward Leete[2] and confirmed 10 years later by Shibata and his students[3,4] that the benzylic C_6-C_1 unit of the alkaloids is derived from the benzylic C_6-C_1 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 C_6-C_1 unit of cinnamic acid is also incorporated[3,4] and since benzoic acid and benzaldehyde serve as precursors of the benzylic C_6-C_1 unit of ephedrine (**1**).[3,4]



1 R = CH_3 **3** R = CH_3

2 R = H **4** R = H

The origin of the remaining C_2 unit of the alkaloid skeleton remained obscure until recently, when we identified the CH_3CO moiety of pyruvic acid as its source in the four alkaloids, (1*R*,2*S*)-(−)-ephedrine (**1**), (1*R*,2*S*)-(−)-norephedrine (**2**), (1*S*,2*S*)-(+)-pseudoephedrine (**3**), and (1*S*,2*S*)-(+)-norpseudoephedrine (**4**), in *E. gerardiana sikkimensis*.[6,7]

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, C_6-C_1 and C_2, 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 C_6-C_1 precursor whose reaction with the pyruvate-derived C_2 unit yields the first C_6-C_3 intermediate on route to the noralkaloids.

Plausible hypotheses may be advanced to implicate either benzaldehyde or benzoic acid as the ultimate C_6-C_1 precursor.

There is ample biochemical precedent for the reaction of benzaldehyde with pyruvate (Scheme 1, path A). If benzaldehyde were the ultimate C_6-C_1 precursor, its condensation with the pyruvate-derived C_2 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 transamination[20] of each of the two α-ketols would yield the corresponding noralkaloid. However, attempts to demonstrate incorporation into the *Ephedra* alkaloids of ^{14}C-labeled samples of (*RS*)-1-hydroxy-1-phenylpropan-2-one have been unsuccessful.[21]

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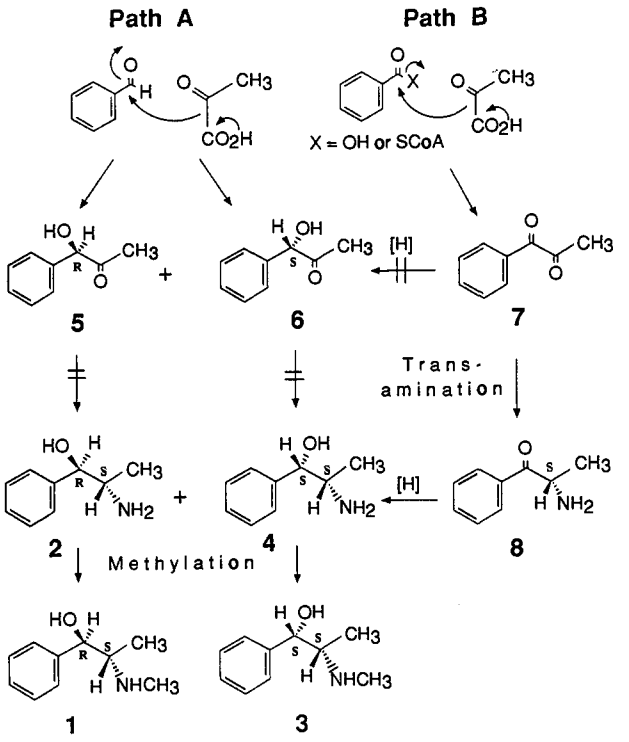
Scheme 1. Proposed Biogenetic Pathways to the *Ephedra* Alkaloids



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 C_6-C_1 unit (Scheme 1, path B). Such a reaction, although unprecedented, would be expected to yield 1-phenylpropane-1,2-dione (**7**) as the first C_6-C_3 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 C_6-C_1 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 ^{13}C 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 C_6-C_1 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 ^{13}C and ^{2}H from (*RS*)-[1-^{13}C,1-^{2}H]-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*-^{13}C,^{2}H]benzaldehyde (experiment 1) and with (*RS*)-[1-^{13}C,1-^{2}H]-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*-^{13}C,^{2}H]benzaldehyde was administered to *Ephedra* shoots. However, $^{2}H/^{13}C$ coupling was not detectable in the carbinol signals of the ^{13}C NMR spectrum of the alkaloids derived from [*carbonyl*-^{13}C,^{2}H]benzaldehyde. Intact incorporation of the ^{13}C,^{2}H-labeled benzaldehyde, as demanded by the hypothesis that benzaldehyde is the ultimate C_6-C_1 intermediate, would have maintained deuterium at the carbinol carbon of the alkaloids, giving rise to multiplicity of the enriched carbinol signals in the ^{13}C NMR spectrum. The incorporation of ^{13}C 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 C_6-C_1 intermediate but is oxidized to benzoic acid prior to incorporation.

This inference was corroborated by the result of the experiment with (*RS*)-[1-^{13}C,1-^{2}H]-1-hydroxy-1-phenylpropan-2-one (**5** + **6**)(experiment 2). This compound would be the first C_6-C_3

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	(*RS*)-[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	(*S*)-[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

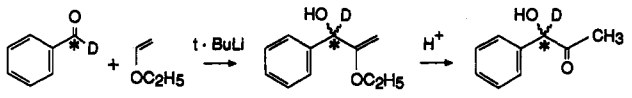
[a] Tracer was dissolved in water (5 mL) with Triton X-165[31] and applied with a fine paint brush in five equal portions over five days. [b] In experiments 2–5 the plants were sprayed with water each day for four days after the administration of the last portion of precursor. [c] During the last three 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	18:11:27:9:35[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 nonreacted precursor is included in the molar ratio.

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



[1-^{13}C,1-^{2}H]-**5** + [1-^{13}C,1-^{2}H]-**6**

* = ^{13}C

intermediate generated by condensation of benzaldehyde with pyruvate. A ^{13}C,^{2}H doubly labeled sample of the compound was synthesized by modification of a published method[25] (Scheme 2). The lithium salt of ethyl vinyl ether was condensed with [*carbonyl*-^{13}C,^{2}H]benzaldehyde and the resulting enol ether was hydrolyzed with dilute hydrochloric acid to give the desired mixture of (*R*)- and (*S*)-[1-^{13}C,1-^{2}H]-1-hydroxy-1-phenylpropan-2-one in 20% yield. In confirmation of the results of K. Yamasaki[21] who failed to detect incorporation into ephedrine of radioactivity from ^{14}C-labeled 1-hydroxy-1-phenylpropan-2-one, no enrichment of either ^{13}C or ^{2}H was observed in any of the four alkaloids when (*RS*)-[1-^{13}C,1-^{2}H]-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 C_6-C_1 substrate of the sequence leading to the alkaloids (Scheme 1, path B). The C_6-C_3 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 ^{13}C-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-$^{13}C_3$]-1-phenylpropane-1,2-dione was the condensation of [*carboxyl*-^{13}C]benzoic acid with the lithium salt of [1,2-$^{13}C_2$]vinyl ethyl ether, followed by acid hydrolysis of the resulting enol ether[25] (Scheme 3). The labeled vinyl ethyl ether was formed by reaction of [$^{13}C_2$]acetaldehyde with hydrogen chloride and ethanol to yield [1,2-$^{13}C_2$]-1-chloroethyl ethyl ether,[27] followed by base-promoted elimination of hydrogen chloride.[28]

(*S*)-[1,2,3-$^{13}C_3$]-2-Amino-1-phenylpropan-1-one was prepared by Friedel–Crafts reaction of benzene with *N*-(ethoxycarbonyl)-L-[1,2,3-$^{13}C_3$]alanine,[26] followed by acid hydrolysis (Scheme 4).

Label from sodium [*carboxyl*-^{13}C]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 ^{13}C into the four alkaloids was higher than that observed in the experiment with [2,3-$^{13}C_2$]pyruvate,[6,7] the precursor of the C_2 unit, C-2,3. This shows that benzoic acid is an efficient precursor of the C_6-C_1 unit of the *Ephedra* alkaloids.

According to Scheme 1, path B, the first C_6-C_3 intermediate on the route to the noralkaloids is 1-phenylpropane-1,2-dione (**7**). Indeed, as expected, label from [1,2,3-$^{13}C_3$]-1-phenylpropane-1,2-dione was incorporated into the alkaloids (experiment 5)(see Table 2). The ^{13}C NMR spectrum (Figure 1d) of the alkaloid mixture from this experiment showed the characteristic coupling pattern for the presence of a $^{13}C_3$ 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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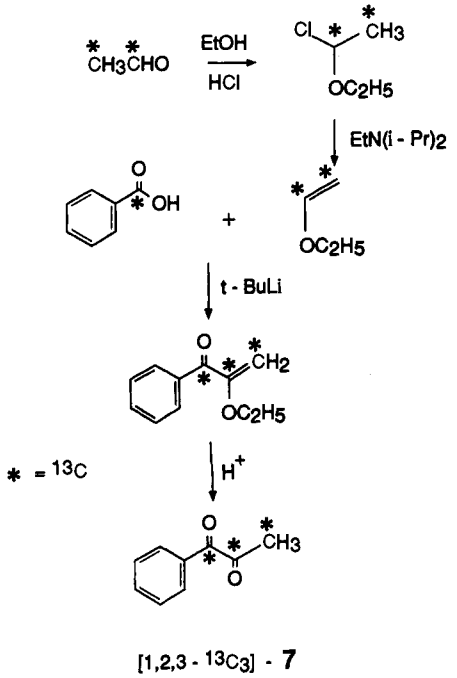
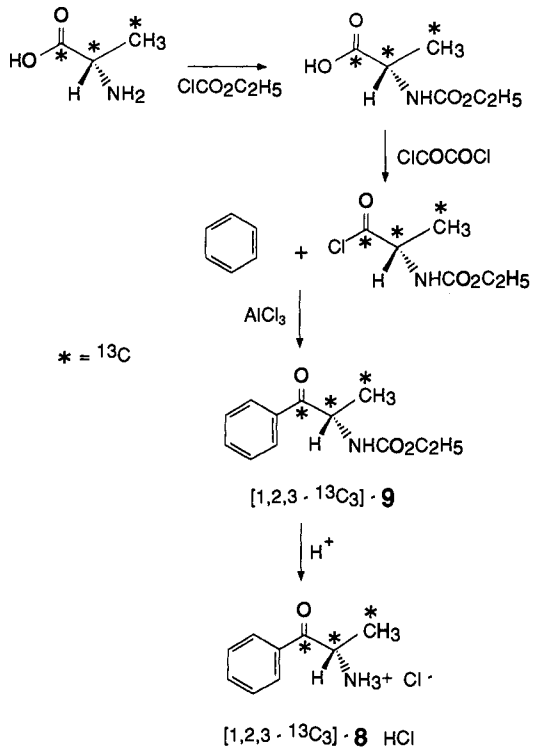
Scheme 3. Synthesis of [1,2,3-$^{13}C_3$]-1-Phenylpropane-1,2-dione



[1,2,3 - $^{13}C_3$] - **7**

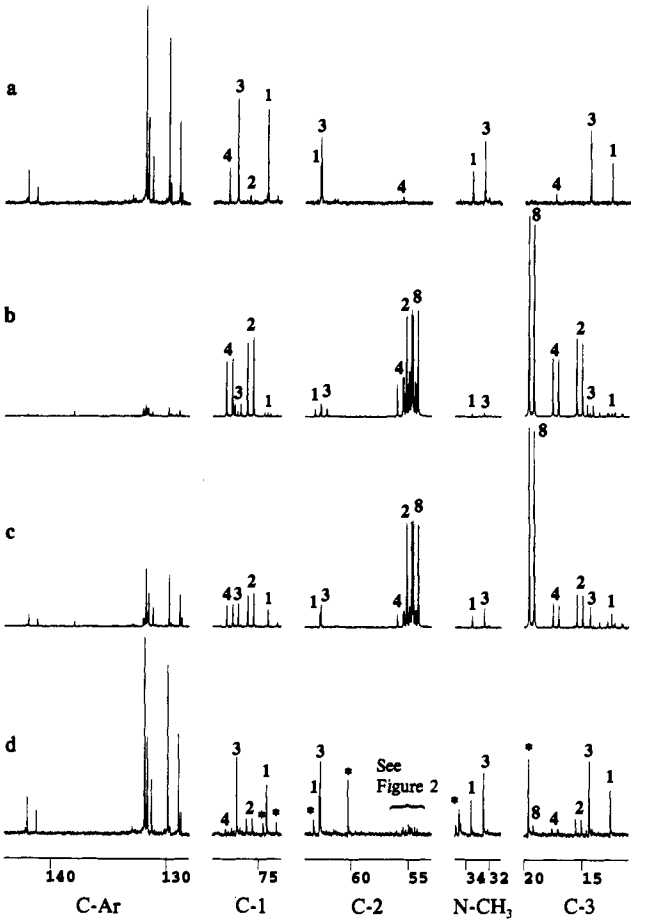
Scheme 4. Synthesis of (*S*)-[1,2,3-$^{13}C_3$]-2-Amino-1-phenylpropan-1-one ([1,2,3-$^{13}C_3$]cathinone) Hydrochloride



[1,2,3 - $^{13}C_3$] - **9**

[1,2,3 - $^{13}C_3$] - **8** HCl

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-$^{13}C_3$]cathinone is unambiguously established by the exact match with signals in the ^{13}C NMR spectrum of authentic [1,2,3-$^{13}C_3$]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-$^{13}C_3$]cathinone hydrochloride, experiment 4a; (c) from nonpainted aerial parts, from [1,2,3-$^{13}C_3$]cathinone hydrochloride, experiment 4b; (d) from [1,2,3-$^{13}C_3$]-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.

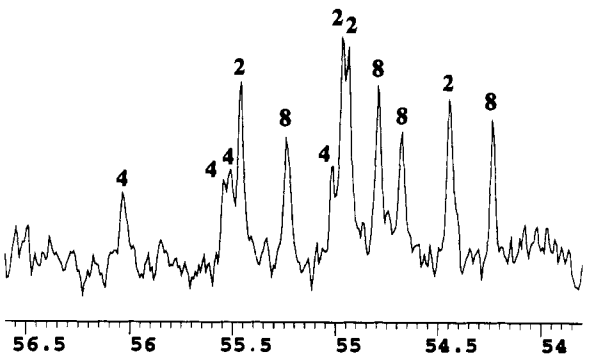
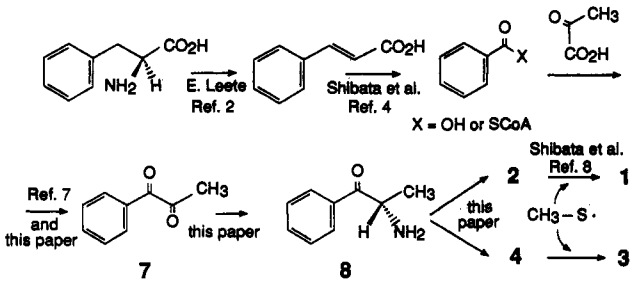


Figure 2. Expanded 54.0–56.5-ppm region of the 75.47-MHz ^{13}C NMR spectrum of the alkaloids from the tracer experiment with [1,2,3-$^{13}C_3$]-1-phenylpropane-1,2-dione (experiment 5, Figure 1d), showing three doublets of doublets, the signals due to C-2 of norephedrine (**2**), norpseudoephedrine (**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.

Scheme 5. Established Major Steps on the Route from Phenylalanine into the Four *Ephedra* Alkaloids



Intermediacy of cathinone on the biosynthetic route into the noralkaloids (Scheme 1, path B) was confirmed by a further experiment. [1,2,3-$^{13}C_3$]Cathinone was administered, as described above, by applying the tracer solution to freshly grown aerial parts (experiment 4). The yield of mixed alkaloid hydrochlorides isolated separately from the painted shoots (experiment 4a), on the one hand, and from the untreated older aerial parts of the plants (experiment 4b), on the other, was comparable (see Table 1). The ^{13}C NMR spectra (Figure 1b,c) show incorporation of the $^{13}C_3$ unit into all four alkaloids in each of the two samples. However, the specific incorporation of label was quite distinct in the two samples: As expected, the highest specific incorporation was observed in the alkaloids from the fresh parts of the plants and hardly any dilution of label was observed within the two noralkaloids **2** and **4** (experiment 4a) (see Table 2). In the case of the noralkaloids isolated from the nonpainted parts (experiment 4b) specific incorporation was also unusually high (87 and 43%, respectively for **2** and **4**). It is evident from these results that the reduction of cathinone to yield the two noralkaloids in an almost 1:1 ratio is remarkably efficient. This facile transformation of cathinone into the noralkaloids explains why cathinone had not hitherto been detected in or isolated from any *Ephedra* species.

The specific incorporation of label into ephedrine and pseudoephedrine in the two experiments 4a and 4b differed by a factor of 40 (see Table 2). This might be due to one or more of several possible causes: (i) Lack of transmethylase activity in the older shoots, (ii) slow migration of ephedrine and pseudoephedrine from young to old shoots, and (iii) storage of ephedrine and pseudoephedrine (but not of the noralkaloids) in the old but not the young shoots, so that newly biosynthesized material in the old shoots is more heavily diluted with preformed, unlabeled material. An experiment in which only the old aerial parts of the plants are painted with tracer solution, and the painted and nonpainted parts of the plants are then harvested and worked up separately, may, if successful, locate the site of biosynthetic activity.

The major steps on the route from phenylalanine into the four alkaloids (Scheme 5) are now defined. The enzymology of the process is as yet unexplored.

Experimental Section

General. ^{13}C NMR spectra (75.47 MHz) were obtained on a Bruker AC 300 spectrometer. Chemical shift (δ) values are given relative to TMS at 0.00 ppm for $CDCl_3$ solutions and relative to sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionate at 0.00 ppm for D_2O solutions. Coupling constants (J) are given in hertz (numerical values).

THF was dried by distillation from sodium/benzophenone. DMF was dried by vacuum distillation from sodium hydride.

Labeled Compounds. All labeled compounds were obtained from MSD Isotopes, Montreal, Canada. Regrettably, this company is no longer in business. [*carbonyl*-^{13}C,^{2}H]Benzaldehyde (99 atom% ^{13}C; 99.6 atom% ^{2}H)(experiment 1) was used directly. Sodium [*carboxyl*-^{13}C]benzoate (99 atom % ^{13}C)(experiment 3) was prepared from [*carboxyl*-^{13}C]benzoic acid by neutralization with aqueous sodium hydroxide. The other labeled substrates were prepared as follows.

(*RS*)-[1-^{13}C,1-^{2}H]-1-Hydroxy-1-phenylpropan-2-one ([1-^{13}C,1-^{2}H]-**5** + [1-^{13}C,1-^{2}H]-**6**) (*cf.*, ref 25) (experiment 2). A mixture of dry THF (2.5 mL) and ethyl vinyl ether (0.27 mL, 203 mg, 2.8 mmol) under argon was cooled to −70 °C. *tert*-Butyl lithium in pentane (1.06 mL, 1.7 M, 1.8 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature until the yellow color had disappeared, when it was immediately cooled, again to −70 °C.

A solution of [*carbonyl*-^{13}C,^{2}H]benzaldehyde (99 atom % ^{13}C, 99.6 atom % ^{2}H, 196 mg, 1.81 mmol) in dry THF (0.5 mL) was added and the mixture was stirred for 10 min at 0 °C. An aqueous solution (6 mL) of ammonium chloride (1.5 g) was added and the mixture was extracted with ether (2 × 10 mL). The combined extracts were washed successively with saturated aqueous sodium hydrogen carbonate (10 mL) and saturated aqueous sodium chloride (10 mL). The organic solution was dried over magnesium sulfate and evaporated to dryness *in vacuo*. The oily residue was stirred with a mixture of hydrochloric acid (0.1 M, 1.50 mL), water (1.35 mL), and methanol (6.0 mL) for 50 min at room temperature and the mixture was then concentrated *in vacuo*. Ether (20 mL) was added and the mixture was washed successively with saturated aqueous sodium hydrogen carbonate (10 mL) and saturated aqueous sodium chloride (10 mL). The organic phase was dried over magnesium sulfate and evaporated to dryness *in vacuo*. The oily residue was chromatographed on silica gel (20 g, 200–400 μm) with ethyl acetate/dichloromethane 5:95 (v/v) to give the title compound as a colorless oil: yield 50 mg (0.37 mmol, 20%); ^{1}H-NMR (δ ppm) 2.06 (d, J = 1.8, 3H, H-3), 4.33 (d, J = 3.0, 1H, OH), 7.26–7.41 (m, 5H, H-Ar); ^{13}C-NMR (δ ppm) 24.9 (d, J(C-3,C-1) = 14.0, C-3), 79.4 (t, J(C-1,D) = 22.3, C-1), 127.0 (s, C-Ar), 128.3 (s, C-Ar), 128.7 (s, C-Ar), 137.6 (d, J(C-1′, C-1) = 47.1, C-1′), 206.9 (d, J(C-2, C-1) = 36.7, C-2).

The ^{13}C NMR spectrum did not show signals due to α-^{13}C enriched benzaldehyde, benzoic acid, or benzyl alcohol.

[1,2,3-$^{13}C_3$]-1-Phenylpropane-1,2-dione ([1,2,3-$^{13}C_3$]-**7**) (*cf.*, ref 25) (experiment 5). Hydrogen chloride gas was bubbled through a mixture of ethanol (1.33 mL, 22.7 mmol) and [$^{13}C_2$]acetaldehyde (99 atom % per position, 1.00 g, 21.7 mmol) at 0 °C for 15 min (until no more gas was absorbed). The lower aqueous phase of the reaction mixture was removed and the remaining organic phase was dried by stirring with anhydrous calcium chloride for 10 min. The mixture was filtered and excess HCl gas removed *in vacuo* (for 5 min at 20 torr) (*cf.*, ref 27). The yield of crude [1,2-$^{13}C_2$]-1-chloroethyl ethyl ether was 1.96 g. This crude compound was added dropwise at 0 °C with stirring to ethyldiisopropylamine (8.0 mL). The mixture was refluxed (bath temperature 110 °C) for 30 min and was then cooled to −70 °C, when the condenser was replaced with bulbs for Kugelrohr distillation, which was carried out at 110 °C (for 40 min) with dry ice cooling of the distillate. Redistillation in the Kugelrohr apparatus at 75 °C (25 min) gave [1,2-$^{13}C_2$]vinyl ethyl ether (*cf.*, ref 28) (328 mg, 4.43 mmol). This (very volatile!) compound was dissolved in dry THF (2.5 mL) under argon at −70 °C. *tert*-Butyllithium (1.7 M, 1.5 mL, 2.55 mmol) in pentane was added dropwise. The mixture was kept at 20 °C until the yellow color had disappeared (*ca.* 15 min). After cooling to −70 °C, a solution of [*carboxyl*-^{13}C]-benzoic acid (99 atom % ^{13}C) (122 mg, 0.99 mmol) in dry THF (1.0 mL) was added. After stirring for 30 min at 20 °C the mixture was washed with 20% aqueous ammonium chloride (2 × 2.5 mL) and saturated sodium hydrogen carbonate (1.0 mL). Drying (magnesium sulfate) and evaporation of the solvent *in vacuo* gave crude [1,2,3-$^{13}C_3$]-2-ethoxy-1-phenylprop-2-en-1-one as an oil (72 mg). This compound was mixed with methanol (1.25 mL) and 1.0 M hydrochloric acid (0.16 mL) and stirred at 64 °C in a sealed flask for 3 h. Water (10 mL) was added at room temperature and the mixture was extracted with ether (10 mL). The ether extract was dried (magnesium sulfate) and evaporated. The oily residue was chromatographed on silica gel (5 g, 63–200 μm) with dichloromethane/ethyl acetate 98:2 (v/v) as eluent, yielding [1,2,3-$^{13}C_3$]-1-phenylpropane-1,2-dione (22 mg, 0.15 mmol, 15% from benzoic acid) as a yellow oil: ^{1}H-NMR (δ ppm) 2.52 (ddd, J(H-3,C-3) = 129.1, J(H-3,C-2) = 6.3, J(H-3,C-1) = 1.2, 3H, H-3), 7.47–7.52 (m, 2H, H-Ar), 7.61–7.67 (m, 1H, H-Ar), 7.99–8.04 (m, 2H, H-Ar); ^{13}C-NMR (δ ppm) 26.4 (dd, J(C-3,C-2) = 41.8, J(C-3,C-1) = 13.3, C-3), 128.9 (d, J(C-3′,C-1) = 3.9, C-3′), 130.3 (d, J(C-2′,C-1) = 2.8, C-2′), 131.8 (d, J(C-1′,C-1) = 56.0, C-1′), 134.6 (d, J(C-4′,C-1) = 1.1, C-4′), 191.4 (dd, J(C-1, C-2) = 49.0, J(C-1, C-3) = 13.3, C-1), 200.5 (dd, J(C-2, C-1) = 49.0, J(C-2, C-3) = 41.8, C-2). The ^{13}C NMR spectrum did not show a signal due to [*carboxyl*-^{13}C]benzoic acid.

(*S*)-[1,2,3-$^{13}C_3$]-2-Amino-1-phenylpropan-1-one Hydrochloride ([1,2,3-$^{13}C_3$]-**8**, HCl) (cf., ref 26, 29) (experiment 4). Three portions of ethyl chloroformate (3 × 187 μL, 3 × 212 mg, 3 × 1.96 mmol) were added,

in 5-min intervals, to a stirred solution of L-[$^{13}C_3$]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 × 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 × 7 mL), drying with calcium chloride, and evaporation of the solvent *in vacuo* gave crude *N*-(ethoxycarbonyl)-L-[$^{13}C_3$]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 × 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-$^{13}C_3$]-2-amino-1-phenylpropan-1-one ([1,2,3-$^{13}C_3$]-**9**)[26] (653 mg, 2.91 mmol, 54%): mp 57–58 °C; ^{1}H-NMR (D_2O)(δ ppm) 1.26 (t, J = 7.1, 3H, CH_2CH_3), 1.43 (dm, J(H-3,C-3) = 129.7, 3H, H-3), 4.14 (q, J = 7.1, 2H, CH_2CH_3), 5.33 (dm, J(H-2,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}C-NMR (D_2O)(δ ppm) 14.6 (s, CH_2CH_3), 20.0 (d, J(C-3,C-2) = 34.7, C-3), 51.2 (dd, J(C-2,C-1) = 42.0, J(C-2,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(C-1,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 × 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 × 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-$^{13}C_3$]-2-amino-1-phenylpropan-1-one ([1,2,3-$^{13}C_3$]-**8**) hydrochloride (66 mg, 0.35 mmol, 12% from (*S*)-*N*-(ethoxycarbonyl)-[1,2,3-$^{13}C_3$]-2-amino-1-phenylpropan-1-one): mp 185–186 °C (lit.[29] mp 188–190 °C); ^{1}H-NMR (D_2O)(δ ppm) 1.47 (dm, J(H-3,C-3) = 131.1, 3H, H-3), 4.66 (s, HOD), 5.08 (dm, J(H-2,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}C-NMR[30] (D_2O)(δ ppm) 19.5 (d, J(C-3,C-2) = 33.3, C-3), 54.8 (dd, J(C-2,C-1) = 42.0, J(C-2,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(C-1,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-165^R.[31] 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 × 50 mL) and was then basified with solid potassium carbonate and extracted with ether (4 × 50 mL). The ether extracts were extracted with hydrochloric acid (1 M, 2 × 5 mL), and the aqueous phase was evaporated to dryness *in vacuo* to yield a mixture of alkaloid hydrochlorides. This was analyzed by ^{13}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-hydroxypoly(oxy-1,2-ethanediyl) in 70% aqueous solution; trademark of the Rohm and Haas Co.