

The biosynthesis of ephedrine

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It is shown by ¹³C nuclear magnetic resonance spectroscopy that the labelled C₂ fragment of [2,3-¹³C₂]pyruvic acid is transferred intact into the C-methyl group and the adjacent carbon atom of the Ephedra alkaloids, norephedrine, ephedrine, norpseudoephedrine, and pseudoephedrine, in growing plants of *Ephedra gerardiana*. This finding serves to identify pyruvate as the elusive precursor of the aliphatic C₂ terminus of the skeleton of the alkaloids. In earlier experiments with ¹⁴C-labelled substrates, label from [3-¹⁴C]pyruvic acid was incorporated mainly, but not exclusively, into the C-methyl group of ephedrine, and label from [2-¹⁴C]pyruvate was incorporated similarly into the carbon atom adjacent to the C-methyl group. A C₆-C₁ unit related to benzaldehyde or benzoic acid has long been known to generate the benzylic fragment of the carbon skeleton of the Ephedra alkaloids. It is likely that the carbon skeleton of ephedrine is generated from pyruvate and either benzaldehyde or benzoic acid, by a reaction analogous to the formation of acetoin or diacetyl from pyruvate and acetaldehyde or acetic acid, respectively.

Key words: biosynthesis of ephedrine, Ephedra alkaloids, ¹³C NMR spectra, ephedrine, biosynthesis of pyruvic acid, incorporation into ephedrine ¹³C NMR spectra.

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Utilisant la résonance magnétique nucléaire du ¹³C, on a démontré que, dans les plants d'*Ephedra gerardiana* en croissance, le fragment en C₂ de l'acide pyruvique-[¹³C₂-2,3] est transféré tel quel au groupement C-méthyle et à l'atome de carbone adjacent de la noréphédrine, de l'éphédrine de la norpseudoéphédrine et de la pseudoéphédrine, des alcaloïdes de l'Ephedra. Sur la base de ces résultats, on peut identifier le pyruvate comme le précurseur de la portion aliphatique en C₂ du squelette des alcaloïdes. Dans des expériences antérieures réalisées à l'aide de substrats marqués au ¹⁴C, le marqueur provenant de l'acide pyruvique-[¹⁴C-3] avait été incorporé principalement, mais pas exclusivement, dans le groupement C-méthyle de l'éphédrine alors que le marqueur du pyruvate-[¹⁴C-2] s'était lui aussi incorporé dans l'atome de carbone adjacent au groupement C-méthyle. On sait depuis longtemps que l'unité C₆-C₁ reliée au benzaldéhyde ou à l'acide benzoïque génère le fragment benzylique du squelette de carbone des alcaloïdes de l'Ephedra. Il est probable que le squelette carboné de l'éphédrine est généré, à partir du pyruvate et du benzaldéhyde ou de l'acide benzoïque, par une réaction analogue à celle conduisant à la formation de l'acétoïne ou du diacétyle à partir du pyruvate et respectivement de l'acétaldéhyde ou de l'acide acétique.

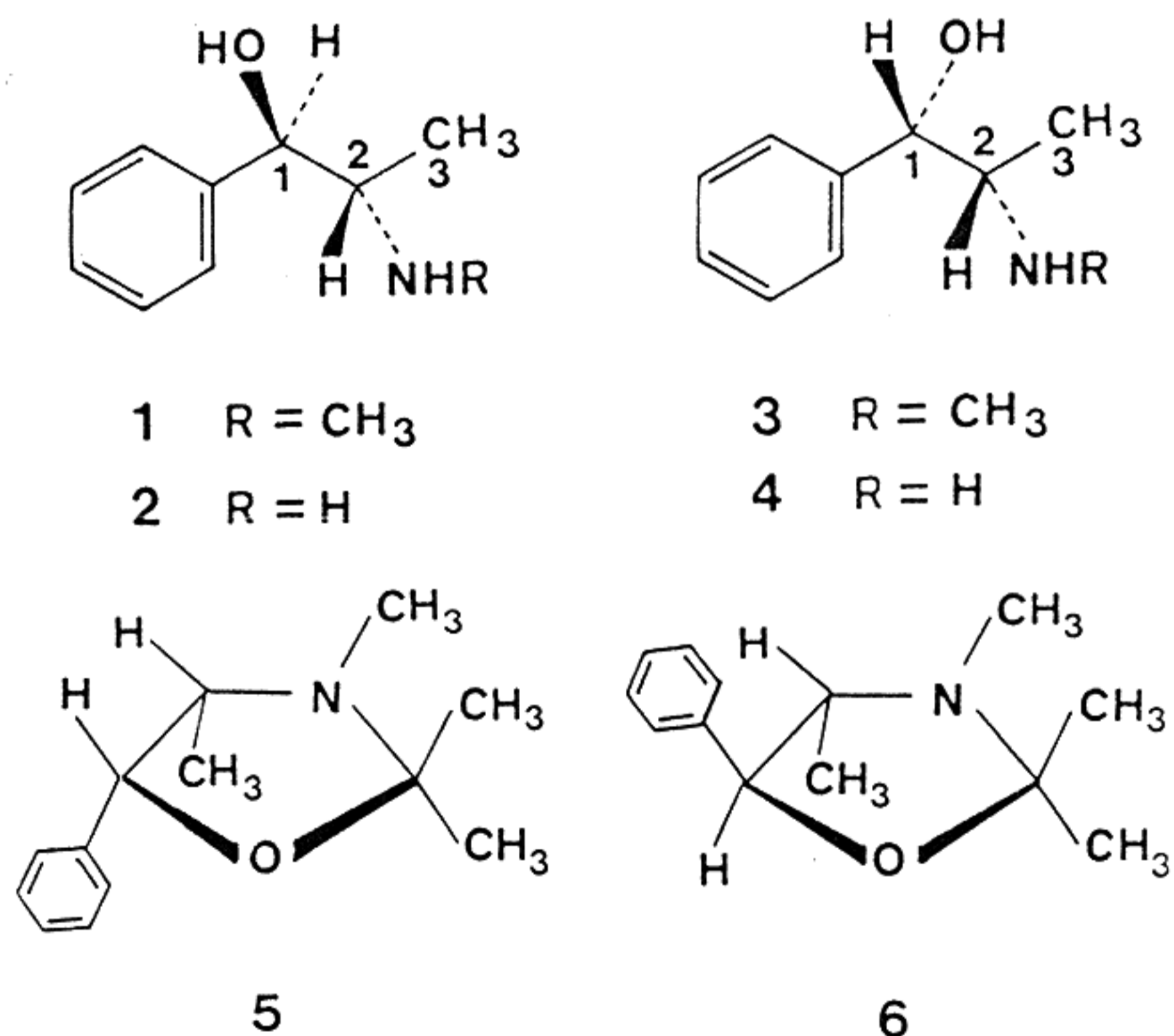
Mots clés : biosynthèse de l'éphédrine, alcaloïdes de l'Ephedra, RMN du ¹³C, éphédrine, biosynthèse de l'acide pyruvique, incorporation dans les spectra RMN du ¹³C de l'éphédrine.

[Traduit par la revue]

Introduction

(-)-Ephedrine (**1**) is the active principle of the herb "Ma Huang," which has been used in Chinese medicine for centuries (1). The compound has received much attention by pharmacologists because of its action on the central nervous system (2), but despite its simple chemical structure the biosynthetic origin of its carbon skeleton is still not established. In plants of the genus *Ephedra* (-)-ephedrine is usually found together with (-)-norephedrine (**2**), (+)-pseudoephedrine (**3**), and (+)-norpseudoephedrine (**4**) (1, 3), and it is likely that the phenylpropanoid skeleton of these four alkaloids is derived from common precursors. *Catha edulis*, a plant of an unrelated genus, which is used as a stimulant in many parts of the Middle East (4), contains (+)-norpseudoephedrine (**4**). It was shown some 30 years ago (5) that, in this plant, radioactivity from the benzylic carbon atom of phenylalanine was transferred into the benzylic carbon atom of (+)-norpseudoephedrine (**4**), and that, in *E. distachya*, the N-methyl group of **1** is derived from the S-methyl group of methionine (6).

It was originally thought that the aminophenylpropanoid system of ephedrine was derived directly from phenylalanine (7) or by reaction of a phenylalanine-derived phenethylamine with a formate-derived one-carbon unit (8-10). These views had to be abandoned when it was found (11, 12) that label from [2-



¹⁴C]phenylalanine did not enter ephedrine, and that label from [2,3-¹⁴C]phenylalanine was found solely at the benzylic carbon atom, C-1, of ephedrine (from [3-¹⁴C]phenylalanine) but not at C-2, the site predicted for entry of label from [2-¹⁴C]phenylalanine. It thus became evident that phenylalanine supplies neither the intact phenylpropanoid system of the alkaloids, nor a C₆-C₂ moiety, but merely a C₆-C₁ unit. Benzoic

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acid and benzaldehyde, whose side-chain carbon atom enters the benzylic carbon atom of ephedrine (11, 12), are presumably intermediates on the route from phenylalanine into the C₆-C₁ unit of the alkaloids.

The origin of the C₂ unit, C-2,-3, of ephedrine remained unknown. None of a wide range of ¹⁴C-labelled substrates ([2-¹⁴C]glycine (12), [U-¹⁴C]alanine (12), [U-¹⁴C]serine (12), [U-¹⁴C]aspartic acid (12), [2-¹⁴C]propanoic acid (12), [¹⁴C]formic acid (8, 12), [6-¹⁴C]glucose (12)) delivered radioactivity preferentially into this unit.

We have investigated the origin of this C₂ unit. Experiments with ¹⁴C-labelled substrates did not lead to unequivocal results. It was only with the advent of high field ¹³C nmr spectroscopy that definitive proof emerged that the nonbenzylic C₂ unit of the Ephedra alkaloids is derived intact from pyruvic acid.³

Methods and results

Specifically ¹⁴C-labelled samples of serine (in admixture with [ring-³H]phenylalanine), of pyruvic acid, and of acetic acid were administered to *Ephedra gerardiana* and *E. distachya* plants by infusion into their stems through cotton wicks or by external application (Table 1, Expts. 1-8). Extraction of the plants yielded a mixture of the diastereomeric alkaloids, (-)-ephedrine ((1*R*,2*S*)-2-methylamino-1-phenylpropan-1-ol) (1) and (+)-pseudoephedrine ((1*S*,2*S*)-2-methylamino-1-phenylpropan-1-ol) (3). Separation was achieved by conversion into the corresponding acetonides, (4*S*,5*R*)-2,2,3,4-tetramethyl-5-phenyloxazolidine (5) and (4*S*,5*S*)-2,2,3,4-tetramethyl-5-phenyloxazolidine (6), which were separated by liquid chromatography and reconverted into the alkaloids by acid hydrolysis.

The *Ephedra* species used were *E. distachya* (Expts. 1-6) and *E. gerardiana sikkimensis* (Expts. 7-9). The early experiments (Expts. 1-6), with mature plants, were performed in the greenhouse at McMaster University. Radioactive tracers, dissolved in glass distilled water, were administered by the wick method and were kept in contact with the plants for 4-5 days before harvesting (see Table 1). For the more recent experiments (Expts. 7-9) small plants were used, which were grown from one year old cuttings, obtained from The Geographic Garden, Kolding, Denmark. In these experiments a solution of the tracers was painted onto the growing aerial parts of the plants over a period of 3-5 days. The plants were in contact with the tracer for a total of 7-8 days before being harvested (see Table 1). Only the aerial parts of the plants were extracted.

The procedures for the isolation of the alkaloids varied to some extent from experiment to experiment. In Expts. 1-3 the plant material was dried (50°C, 1 week) before extraction with methanol, whereas in Expts. 4-9 the fresh plant material was extracted immediately with methanol. In Expts. 1-8 a Soxhlet apparatus was used for the extraction and in Experiment 9 methanol was percolated through a column containing the plant material until extraction was complete.

In Expts. 1-6 the methanolic extracts were evaporated *in vacuo*, whereas in Expts. 7-9 the methanolic extracts were acidified with hydrochloric acid before evaporation of solvent.

After the usual work-up, with acid/base partitioning between water and ether, the crude alkaloids were purified in several ways.

In Expts. 1-3 the Ephedra bases were treated with acetone/silica gel (14) to convert ephedrine (1) and

pseudoephedrine (3) into their acetonides 5 and 6, respectively. Facile separation of 5 and 6 by liquid chromatography followed by acidic demasking gave ephedrine and pseudoephedrine as their hydrochlorides (see Table 2). The radioactive (-)-ephedrine was further purified by recrystallization after addition of unlabelled (-)-ephedrine hydrochloride. In Expts. 4-8 addition of radioinactive (-)-ephedrine hydrochloride to the crude mixture of alkaloid hydrochlorides, followed by repeated recrystallization, gave radiochemically pure (-)-ephedrine hydrochloride. In Expts. 2 and 3 (-)-ephedrine hydrochloride was converted to (-)-ephedrine sulfate via the free base.

It is apparent from Table 2 that both the amount of isolated (-)-ephedrine as well as the relative amounts of (-)-ephedrine and (+)-pseudoephedrine isolated from individual experiments varied from experiment to experiment. Whether this reflects a natural variation or is a consequence of different work-up procedures is not known, but some loss of (-)-ephedrine during standard isolation procedures has been observed (15). In most cases (-)-ephedrine was the more abundant alkaloid. The amount of either alkaloid isolated was too small for the chemical manipulation required to determine the distribution of radioactivity. Carrier dilution was thus essential to obtain enough material for a complete degradation. Since, of the two alkaloids, (-)-ephedrine invariably had the higher specific activity, this compound was chosen for carrier dilution and degradation.

Even so, the carrier dilution that was required to obtain sufficient material for determination of the sites of labelling within an alkaloid sample was such that the degradation experiments had to be performed with samples whose specific radioactivity (cpm per mg above background) was close to the limits of detectability. As a result, the confidence limits of the values for the molar specific activity (dpm per mmol) of the individual degradation products were unusually wide.

The distribution of label within (-)-ephedrine derived from radioactive precursors (Expts. 1-8) was determined by a degradation sequence (Schemes 1 and 2) that permitted individual assay of activity at C-1, C-2, C-3, N-methyl, and at the phenyl group. Dehydration with 50% (v/v) sulfuric acid⁴ at 110°C gave, within 15 min, a mixture of ephedrine and pseudoephedrine (ca. 1:1 by ¹H nmr) (Scheme 1) that on standing 24 h was converted almost quantitatively into methylamine, isolated by conversion into the *N*-phthaloyl derivative (9), and 1-phenyl-2-propanone (7), which was recovered as the semicarbazone (8) in unexpectedly poor yield.

Chromic acid oxidation of 1-phenyl-2-propanone (7), or of ephedrine (1) itself (12), gave a mixture of benzoic acid and acetic acid. A portion of this mixture was converted into a mixture of the corresponding 1-acylaminonaphthalenes (10, 11), which were then separated by liquid chromatography. Another portion of the mixture of benzoic and acetic acids was reacted with azide in concentrated sulfuric acid (8) at 25°C for 3 h to give aniline (isolated as its hydrochloride) (aromatic C-atoms), or at 100°C for 2 h to give methylamine (C-3) (isolated as *N*-methylphthalimide (9)). The degradation sequence is shown in Scheme 2.

The sample of (-)-ephedrine obtained from plants that had been fed with a mixture of L-[3-¹⁴C]serine and L-[ring-2,6-³H]phenylalanine (Expt. 1) showed a much higher incorpora-

⁴More concentrated sulfuric acid (e.g., 85% (v/v)) (cf. ref. 8) led to sulfonation in the *para* position of the aromatic nucleus, as shown by the appearance of an AB system in the aromatic region of the ¹H nmr spectrum of the reaction mixture.

³An account of the conclusive experiment has appeared (13).

TABLE 1. Administration of tracers to *Ephedra distachya* L. and *E. gerardiana sikkimensis*

Expt. no.	Date	Precursor	Nominal total activity (mCi)	Nominal specific activity (mCi/mmol)	Ephedra	Tracer application method	Tracer administered over followed by water for	Weight of aerial parts of plant material (g)	
									Fresh wt.	Dry wt.
1	June 1978	L-[ring-2,6- ³ H]Phenylalanine ^a L-[3- ¹⁴ C]Serine ^a	1.0 0.1	54 × 10 ³ 56	<i>distachya</i> L.	Wick	1 day	3 days	56	20
2	Sept 1978	Sodium [2- ¹⁴ C]pyruvate ^{b,e}	0.1	6.5	<i>distachya</i> L.	Wick	1 day	4 days	191	73
3	Sept 1978	Sodium [3- ¹⁴ C]pyruvate ^{b,e}	0.1	18.4	<i>distachya</i> L.	Wick	1 day	4 days	169	63
4	Sept 1979	Sodium [1- ¹⁴ C]acetate ^{a,f}	0.25	57.2	<i>distachya</i> L.	Wick	1 day	4 days	80	—
5	Sept 1979	Sodium [2- ¹⁴ C]pyruvate ^{b,f}	0.25	7.7	<i>distachya</i> L.	Wick	1 day	4 days	84	—
6	Sept 1979	Sodium [3- ¹⁴ C]pyruvate ^{b,f}	0.25	21.0	<i>distachya</i> L.	Wick	1 day	4 days	102	—
7	July 1982	Sodium [2- ¹⁴ C]pyruvate ^b	0.15	15.2	<i>gerardiana sikkimensis</i>	Painting	3 days	5 days	60	—
8	July 1982	Sodium [3- ¹⁴ C]pyruvate ^b	0.15	19.0	<i>gerardiana sikkimensis</i>	Painting	3 days	5 days	68	—
9	Sept 1987	Sodium [2,3- ¹³ C ₂]pyruvate ^c	—	—	<i>gerardiana sikkimensis</i>	Painting	5 days	2 days	66	—

^aRadiochemical Centre, Amersham, England.^bNew England Nuclear, Boston, Mass., U.S.A.^cMerck, Sharp and Dohme, Point-Claire, P.Q., Canada.^dMeasured ³H/¹⁴C ratio of the mixture, 13:1.^eIn 10 ml glass distilled water.^fIn 20 mL glass distilled water. Three days before administration of the tracer solution, a solution containing 0.6 mg thiamin chloride hydrochloride and 1 mg sodium benzoate in 20 mL glass distilled water was administered to the plants. The pH was adjusted to pH 7 with dilute sodium hydroxide solution.

TABLE 2. Incorporation of labelled precursors into *Ephedra* alkaloids

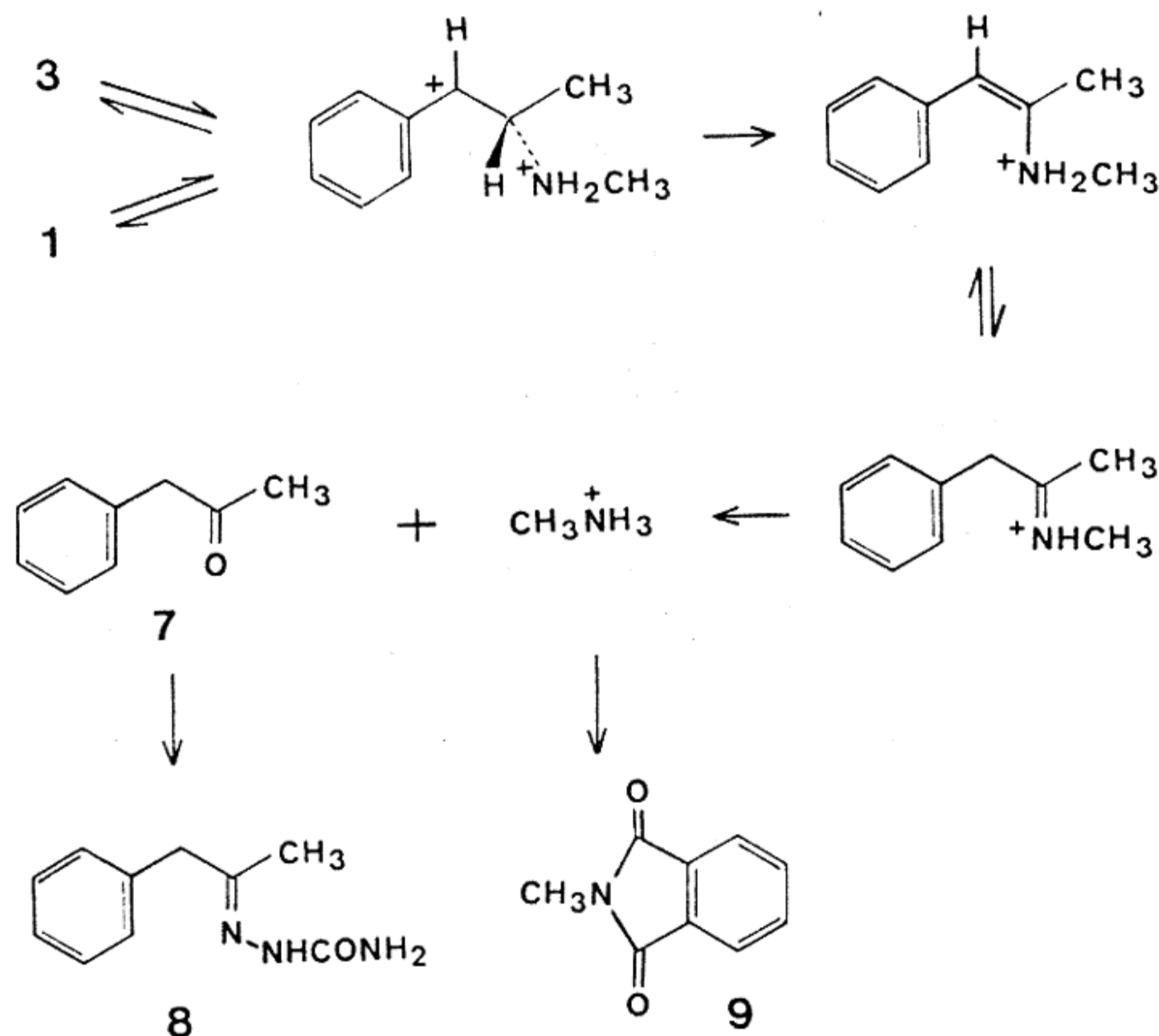
Expt. no.	Precursor	Yield ^a (mg)				Specific activity (dpm/mmol) ($\times 10^{-4}$)	³ H/ ¹⁴ C	Total incorporation ^b (%)	Specific incorporation ^c (%)
		1	3	2	4				
1	L-[ring-2,6- ³ H]Phenylalanine ^a	18				³ H: 2.1	3.0	8.5×10^{-5}	1.8×10^{-8}
	L-[3- ¹⁴ C]Serine ^a					¹⁴ C: 0.7		2.8×10^{-4}	5.6×10^{-6}
2	Sodium [2- ¹⁴ C]pyruvate	15	3			0.8	2.7×10^{-4}	5.5×10^{-5}	
			18			—			
3	Sodium [3- ¹⁴ C]pyruvate	47				2.0	2.1×10^{-3}	4.9×10^{-5}	
			19			—			
4	Sodium [1- ¹⁴ C]acetate	9				0.1	8.1×10^{-6}	7.9×10^{-7}	
5	Sodium [2- ¹⁴ C]pyruvate	6				1.5	8.1×10^{-5}	8.8×10^{-5}	
6	Sodium [3- ¹⁴ C]pyruvate	41				0.3	1.1×10^{-4}	6.4×10^{-6}	
7	Sodium [2- ¹⁴ C]pyruvate	87				10.8	1.4×10^{-2}	3.2×10^{-4}	
8	Sodium [3- ¹⁴ C]pyruvate	127				13.5	2.6×10^{-2}	3.2×10^{-4}	
9	Sodium [2,3- ¹³ C ₂]pyruvate	20 ^d					7.1×10^{-3}	3.2×10^{-1}	
			31 ^d					7.6×10^{-3}	2.2×10^{-1}
				2 ^d				3.0×10^{-3}	1.4
				6 ^d			7.0×10^{-3}	1.0	

^aAlkaloid hydrochlorides.

^b $100 \times$ (total activity within product/total activity within substrate) (Expts. 1–8); $100 \times$ (g-atom ¹³C within product/g-atom ¹³C within substrate) (Expt. 9).

^c $100 \times$ (specific activity of product/specific activity of substrate) (Expts. 1–8); $100 \times$ (at.% ¹³C above natural abundance within product/at.% ¹³C above natural abundance within substrate) (Expt. 9) (see Table 6).

^dCalculated from the ¹³C nmr spectrum of the alkaloid mixture.



SCHEME 1

tion of ¹⁴C than of ³H (Table 2). Both the benzoic acid and the acetic acid obtained on degradation contained ¹⁴C (Table 3), but more than half of the ¹⁴C was located within the N-methyl group (ephedrine minus (benzoic plus acetic acid)). Tritium was confined to the benzoic acid (Table 4).

The sample of (-)-ephedrine from the experiment with [1-¹⁴C]acetic acid (Expt. 4) was essentially inactive (Table 2).

The results of the degradation of the samples of ephedrine obtained from the experiments with [2-¹⁴C]- and [3-¹⁴C]pyruvic

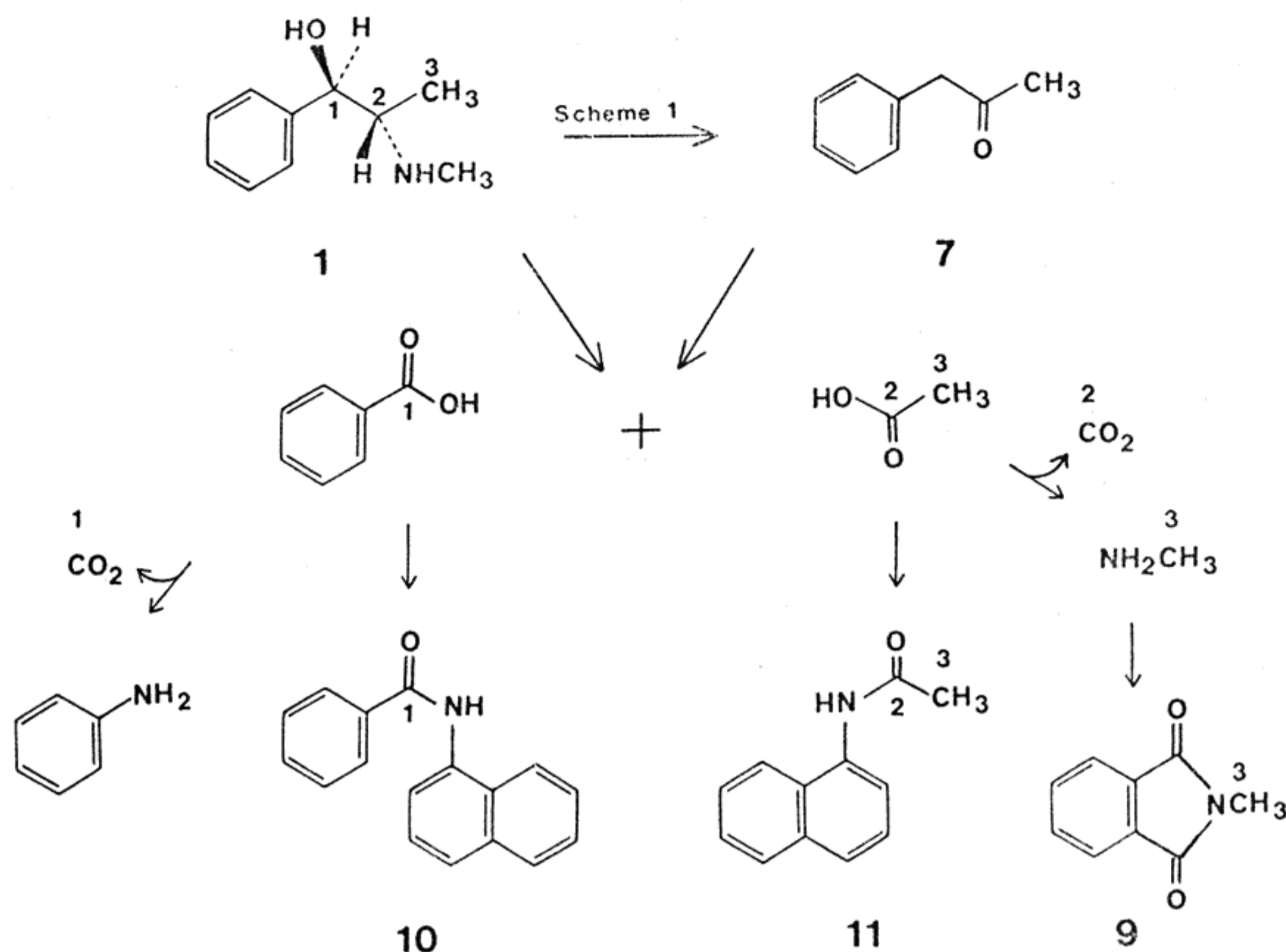
acid (Expts. 2, 3, 5–8) are summarized in Table 3. In each case, the acetic acid obtained by degradation, representing the C-methyl group of (-)-ephedrine and the adjacent carbon atom, contained between one and two thirds of the activity of the intact ephedrine, the N-methyl group contained ca. one tenth, and the C₆-C₁ unit accounted for a significant portion ranging from one quarter to more than one half of the total activity.

The distribution of the activity within the acetate differed in the experiments with [3-¹⁴C]- and those with [2-¹⁴C]pyruvic

Table 3. Incorporation of ^{14}C into ephedrine

Expt. no.	Precursor	Specific activity ^{a,b} (relative specific activity) ^{c,d}							
		Ephedrine HCl ^a	Ephedrine sulfate ^b	N-Methyl-phthalimide ^a	1-Phenyl-2-propanone semicarbazone ^a	1-Benzamido-naphthalene ^a	1-Acetamido-naphthalene ^a	N-Methyl-phthalimide ^a	Aniline HCl ^a
1	L-[3- ^{14}C]Serine	3.4 (100±12)			0.7 (22±13)		0.3 (8±9)		
2	Sodium [2- ^{14}C]pyruvate	2.5 (100±4)	2.4 (97±9)	0.3 (11±8)		1.1 (44±29)	1.2 (49±8)	0.2 (6±9)	
5	Sodium [2- ^{14}C]pyruvate	0.9 (100±5)				0.2 (21±9)	0.6 (63±6)	0.1 (10±8)	
7	Sodium [2- ^{14}C]pyruvate	12.8 (100±2)				7.7 (60±2)	3.2 (25±2)	1.0 (8±2)	6.7 (52±2)
3	Sodium [3- ^{14}C]pyruvate		8.6 (100±2)		5.7 (67±4)	2.0 (23±3)	4.6 (55±2)	3.5 (41±2)	
6	Sodium [3- ^{14}C]pyruvate	1.3 (100±6)				0.8 (57±8)	0.8 (58±12)	0.6 (44±19)	
8	Sodium [3- ^{14}C]pyruvate	17.3 (100±2)		2.0 (12±2)		9.4 (54±2)	6.5 (38±2)	4.0 (23±2)	7.0 (40±2)
	C-atoms of ephedrine:	All	All	N-CH ₃	All except N-CH ₃	Aromatic, C-1	C-2, C-3	C-3	Aromatic

^aSpecific activity (dpm/mmol) × 10⁻³.^bSpecific activity (dpm/mmol) × 0.5 × 10⁻³.^cRelative specific activity (per cent) (ephedrine HCl or ephedrine sulfate = 100%).^dConfidence limits shown are standard deviation of the mean.



SCHEME 2

acid. In the former case (Expts. 3, 6, and 8) the greater part of the activity (approximately three quarters) was recovered in *N*-methylphthalimide representing the C-methyl group of (–)-ephedrine. In the latter case (Expts. 2, 5, and 7) the greater part of the activity (approximately three quarters) was located in the carboxyl group of acetate, i.e., within the carbon of (–)-ephedrine adjacent to the C-methyl group.

The distribution of label within the four Ephedra bases 1–4, derived from plants to which [2,3-¹³C₂]pyruvic acid had been administered (Expt. 9), was determined by ¹³C nmr.

To facilitate the analysis of the ¹³C nmr spectrum of the mixture of ¹³C-enriched alkaloid hydrochlorides, the individual natural abundance ¹³C nmr spectra of the four compounds were determined under similar conditions (Table 5). Spectra of three of the compounds had previously been reported and assigned (16–18).⁵

The ¹³C nmr spectrum of the mixture of alkaloid hydrochlorides from plants to which sodium [2,3-¹³C₂]pyruvate had been administered (Expt. 9) (see Fig. 1 and Table 2) shows that the sample consists of pseudoephedrine (3), ephedrine (1), norpseudoephedrine (4), and norephedrine (2), in the molar ratio

52:35:10:3 (based on the peak integrals of the central lines of the C-methyl signals). The C-methyl signal (C-3) of each of the four alkaloids consists of a central line (δ 12.49 (1), 14.39 (3), 15.24 (2), and 17.36 (4) ppm) straddled by a doublet ($J = 37$ Hz). A similar pattern is discernible in each of the signals due to C-2 (δ 54.95 (2), 55.51 (4), 62.61 (3), and 62.69 (1) ppm). The benzylic carbon atoms (C-1) (δ 74.16 (1), 75.69 (2), 76.81 (3), and 77.55 (4) ppm) and the phenyl carbon atoms (δ 128–142 ppm) give signals that appear as singlets. The relative intensity of all singlet signals was identical with that in the spectra of authentic non-labelled alkaloids. This indicates that all detectable ¹³C enrichment in the nmr spectrum of the four alkaloids from Expt. 9 originates from intact ¹³C₂ units. The average specific incorporation of C-2,3 of sodium [2,3-¹³C₂]pyruvate into norephedrine (2), norpseudoephedrine (4), ephedrine (1), and pseudoephedrine (3) was 1.4, 1.0, 0.32, and 0.22%, respectively (see Table 6).

Discussion

It was shown by Leete (5) and by Shibata and his students (11, 12) that the benzylic fragment of the carbon skeleton of the Ephedra alkaloids originates from a C₆–C₁ unit derived from phenylalanine. In plant cuttings, radioactivity from DL-[3-¹⁴C]phenylalanine entered C-1 and no other sites of (+)-norpseudoephedrine (5) and of (–)-ephedrine (11, 12), and label from DL-[ring-³H]phenylalanine entered the benzylic fragment of (–)-ephedrine (11, 12). Label from DL-[2-¹⁴C]phenylalanine was not incorporated (11, 12).

Similarly, label from [3-¹⁴C]cinnamic acid, from [carboxyl-¹⁴C]benzoic acid, and from [carbonyl-¹⁴C]benzaldehyde also entered C-1 of (–)-ephedrine (11, 12). Thus, it is likely that a C₆–C₁ unit related to benzaldehyde or benzoic acid and derived from L-phenylalanine by the phenylalanine ammonia lyase route (22) is the direct precursor of the benzylic fragment of the Ephedra alkaloids (see Scheme 3).

The search for the source of the remaining C₂-unit of the alkaloid skeleton had so far proved fruitless. Less than one third of the total activity within the samples of (–)-ephedrine derived

⁵The assignment of two signals (C-2',6' and C-3',5') in the spectra has not been settled (cf., refs. 16–18). Assignment is now made on the basis of the following considerations:

Comparison of the chemical shifts of the signals in the spectrum of benzyl alcohol (19) with those of the substituted benzyl alcohols, 1–4, permits the prediction that the signals due to C-2',6' be 1–2 ppm upfield from those due to C-3',5'. The observed chemical shift differences between C-2',6' and C-3',5' are (1) 2.73, (2) 2.51, (3) 2.00, and (4) 2.12 ppm (see Table 5). Secondly, the magnitude of two-, three-, and four-bond carbon–proton coupling constants in monosubstituted benzenes permits a distinction to be made between C-2',6' and C-3',5' (20, 21). It is predicted that, for C-2',6', the numerical values of two of the carbon–proton coupling constants ($J_{2',3'}$ and $J_{2',5'}$) would be <2 Hz and two ($J_{2',4'}$ and $J_{2',6'}$) would be 4.5–8 Hz. For C-3',5', the numerical values of three of the carbon–proton coupling constants ($J_{3',2'}$, $J_{3',4'}$, and $J_{3',6'}$) are predicted to be <2 Hz and one ($J_{3',5'}$) to be 7–9 Hz.

The assignments shown in Table 5 are the only ones consistent with these predictions.

TABLE 4. Incorporation of ^3H into ephedrine

Expt. no.	Precursor	Specific activity ^a (relative specific activity) ^b		
		Ephedrine HCl	1-Benzamidonaphthalene	1-Acetamidonaphthalene
1	L-[ring-2,6- ^3H]Phenylalanine	10.2 (100±7)	9.0 (88±9)	0.0 (0±5)
	H-atoms of ephedrine:	All	Aromatic	At C-3

^aSpecific activity (dpm/mmol) × 10⁻³.^bRelative specific activity (per cent) (ephedrine HCl = 100%).TABLE 5. The ^{13}C nmr chemical shifts^a of *Ephedra* alkaloids^b

	C-1'	C-2',6'	C-3',5'	C-4'	C-1	C-2	C-3	N-CH ₃
Ephedrine (1) HCl	141.21	128.85	131.58	131.17	74.15	62.68	12.49	33.53
Norephedrine (2) HCl	141.09	129.10	131.61	131.36	75.68	54.90	15.24	—
Pseudoephedrine (3) HCl	141.97	129.83	131.83	131.83	76.81	62.60	14.39	32.49
Norpseudoephedrine (4) HCl	142.07	129.65	131.77	131.68	77.55	55.50	17.36	—

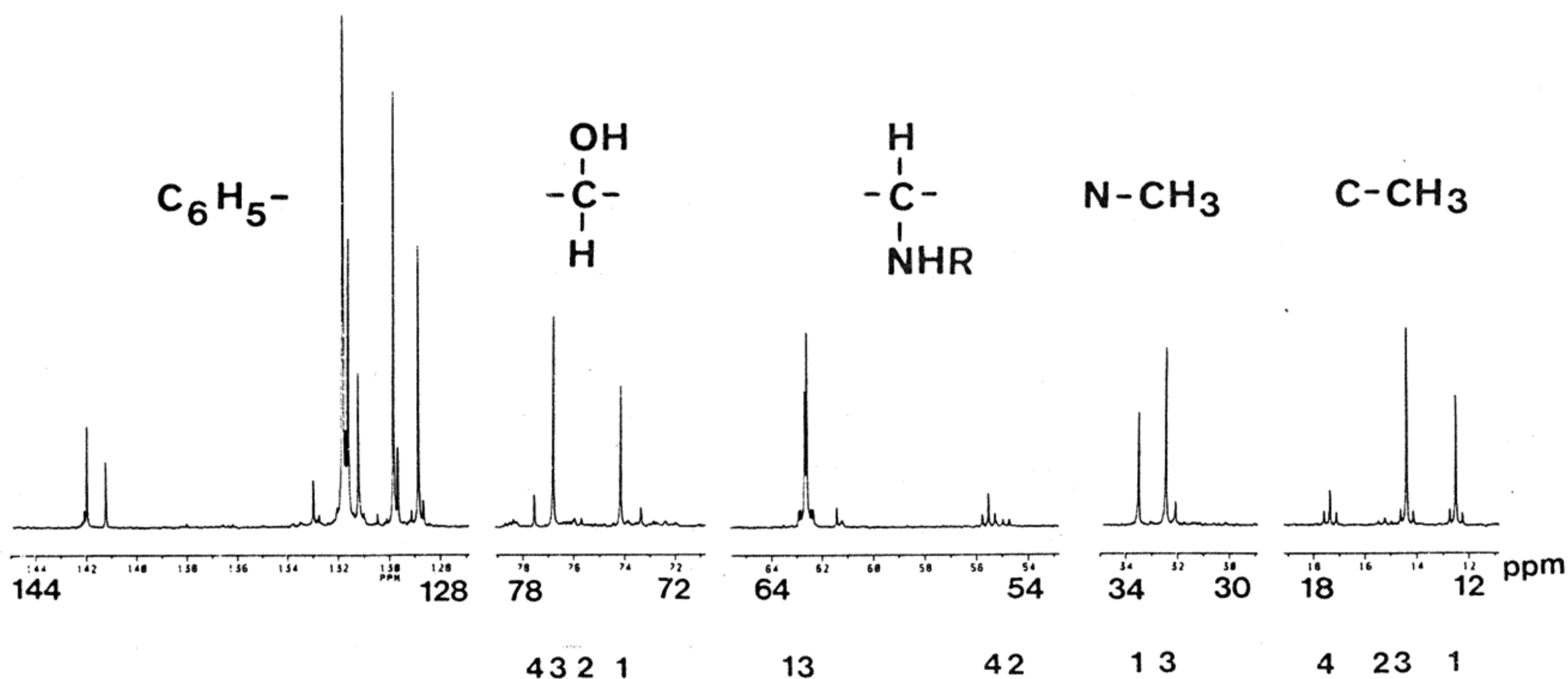
^aRelative to sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionate (δ 0.00 ppm).^bFifty milligrams alkaloid HCl in D₂O (0.6 mL).

FIG. 1. Proton noise decoupled ^{13}C nmr spectrum (75.47 MHz, 5000 transients) of alkaloid hydrochlorides (57 mg in 0.6 mL D₂O) obtained from plants of *Ephedra gerardiana* after administration of sodium [2,3- $^{13}\text{C}_2$]pyruvate. The spectrum was recorded on a Bruker AC 300 spectrometer under standard conditions, with sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionate (δ 0.00 ppm) as internal reference. The numbers below the δ (ppm) scale show assignments of the signals to the four compounds 1-4.

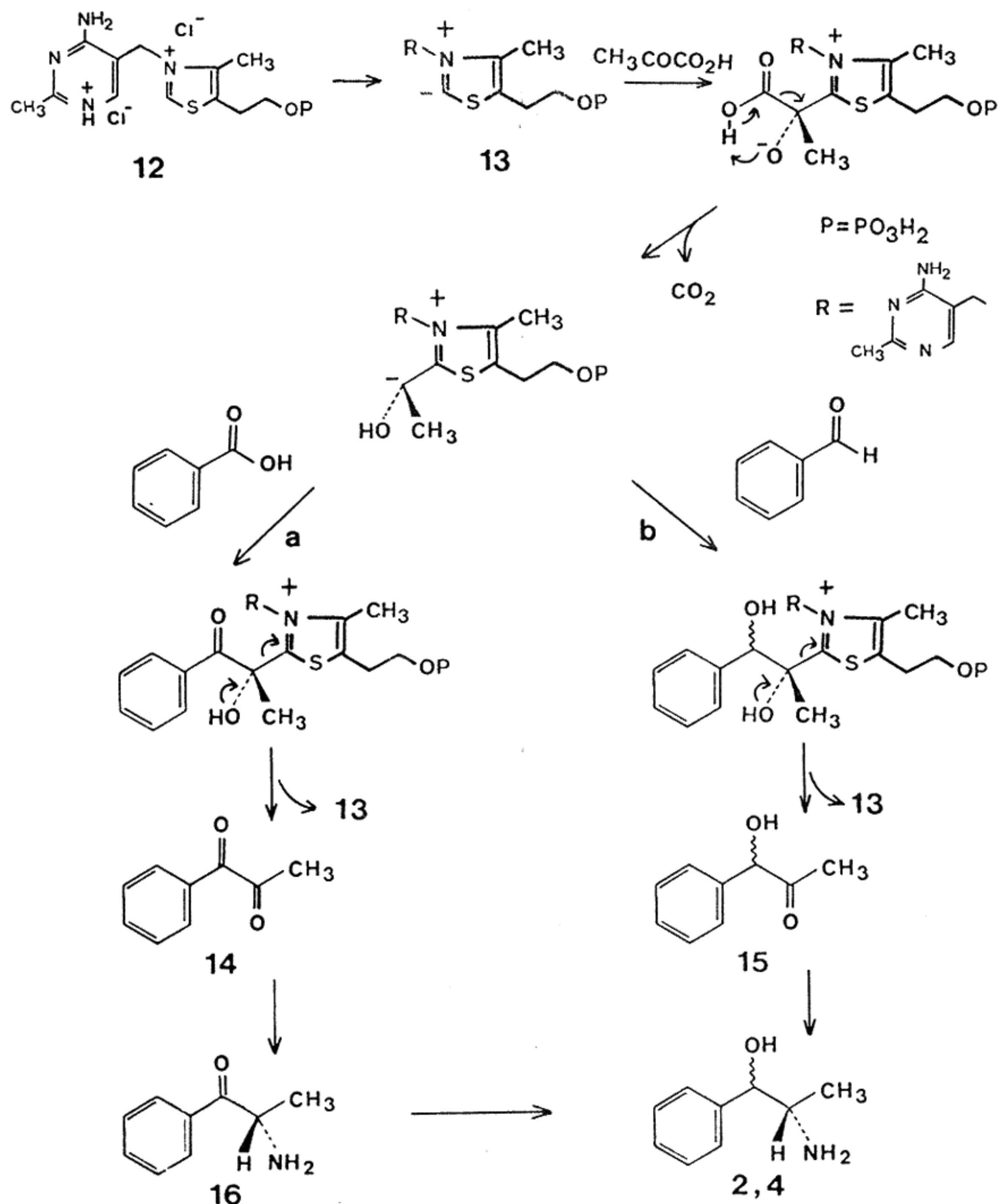
from a variety of radioactive substrates was present within this unit; 70% or more of the label was distributed over the rest of the molecule.

The multicarbon substrates that delivered the highest fraction (ca. 25%) of the total activity of ephedrine into the C₂ unit were D-[6- ^{14}C]glucose, and L-[U- ^{14}C]aspartic acid and L-[U- ^{14}C]alanine, two amino acids closely related to the tricarboxylic acid cycle. But in each case at least an equal fraction of label entered the C₆-C₁ unit and twice as much was incorporated into the N-methyl group (12).

[2- ^{14}C]Glycine and L-[U- ^{14}C]serine delivered ca. 75% of

activity into the N-methyl group, 20% into the benzylic fragment, and only ca. 5% into the C₂ unit (12). This distribution of label was maintained (see Tables 2 and 3) when L-[3- ^{14}C]serine was administered in the presence of L-[ring- ^3H]phenylalanine ($^3\text{H}/^{14}\text{C}$ ratio 13:1) (Expt. 1). Even though the ephedrine that was isolated showed a $^3\text{H}/^{14}\text{C}$ ratio 3:1, indicating that ^{14}C (i.e., serine) had been incorporated almost four times as efficiently as ^3H (i.e., phenylalanine), the presence of phenylalanine had not caused a redirection of ^{14}C from the C₆-C₁ into the C₂ unit.

Evidently, in all the above experiments metabolic fragmenta-



SCHEME 3

TABLE 6. Incorporation of sodium [2,3-¹³C₂]pyruvate into *Ephedra* alkaloids; ¹³C nmr analysis (Expt. 9)

	1	2	3	4
R(C-2) ^a	0.31	1.5	0.22	0.9
R(C-3) ^a	0.26	1.0	0.17	0.9
Average specific incorporation: ^b	0.32%	1.4%	0.22%	1.0%

^aR(C-*n*) = ¹³C nmr doublet area of C-*n*/¹³C nmr singlet area of C-*n*.

^bAverage specific incorporation of ¹³C from sodium [2,3-¹³C₂]pyruvate into C-2 and C-3 of the *Ephedra* alkaloids = (E_E/E_P) × 100%, where E_E = average enrichment above natural abundance (at. % ¹³C) at C-2 and C-3 = [(R(C-2) + R(C-3))/2] × 1.1% and E_P = average at. % ¹³C at C-2 and C-3 of sodium [2,3-¹³C₂]pyruvate = 99%.

tion of the precursors had occurred prior to incorporation of label into the alkaloid. Glycine and serine are known to participate in one-carbon metabolism (23). Another one-carbon donor, [¹⁴C]formic acid (9, 12), supplied 50% of the total activity into the N-methyl group and ca. 20% into the phenyl nucleus. In this instance the C₂ unit contained 30% of the total activity. Most of this activity was present in the C-methyl group (9).

The scrambling of label and, in particular, the predominant

presence of label in the N-methyl group that was observed in every one of the above experiments points to rapid metabolic turnover in the course of the feeding experiments. The distribution of label observed in these experiments tended to obscure rather than to illuminate the origin of the C₂ fragment.

Since the carbon skeleton of the *Ephedra* bases does not appear to be derived directly from an amino acid, it is likely that the nitrogen atom is introduced at a late stage of biosynthesis. Indeed, the stereospecific transfer of the amino group of L-glutamic acid into (±)-1-hydroxy-1-phenyl-2-propanone (15), to yield a mixture of (-)-norephedrine (2) as the major and (+)-norpseudoephedrine (4) as the minor product, is catalyzed by transaminases that have been obtained from several plant species (24). The same enzyme system catalyzes the reverse reaction, converting (-)-norephedrine into (-)-1-hydroxy-1-phenyl-2-propanone.

The latter compound has been reported to be the major product when yeast (*Saccharomyces cerevisiae*) is incubated with benzaldehyde (25–35) in the presence of fermentable sugars (25, 27, 30, 33–35), pyruvic acid (25, 29, 31, 32), or acetyl phosphate (31). The relative amounts of (*S*)- and (*R*)-1-hydroxy-1-phenyl-2-propanone that are formed are determined by the stereoselectivity of this reaction.

The reaction is catalyzed by "α-carboxylase" (E.C. 4.1.1.1,

2-oxo acid carboxylase, pyruvate decarboxylase), and requires thiamin as a cofactor (31, 32). The course of the reaction (Scheme 3) is evidently analogous to that of the carboxylase-catalyzed formation of acetoin from acetaldehyde and pyruvate (36–38).

If the carbon skeleton of the Ephedra alkaloids were generated in Ephedra plants by a reaction analogous to the formation of 1-hydroxy-1-phenyl-2-propanone in yeast, the stereoselectivity of the process would ultimately determine the relative amounts of (1*R*) alkaloids ((–)-norephedrine (2) and (–)-ephedrine (1)) and (1*S*) alkaloids ((+)-norpseudoephedrine (4) and (+)-pseudoephedrine (3)) present in the plants.

The primary objective of this investigation was to test whether, in analogy with the condensation reaction that takes place in yeast, the C₂ unit of the Ephedra alkaloids is derived from pyruvate.

Samples of sodium [2-¹⁴C]pyruvate (Expts. 2, 5, 7) and sodium [3-¹⁴C]pyruvate (Expts. 3, 6, 8) were administered to plants of two different Ephedra species by two different methods, under various conditions (Table 1). In some cases (Expts. 5, 6) thiamin and sodium benzoate were administered 3 days before the solution containing the pyruvate was applied, in an attempt to enhance the condensation reaction (thiamin) and to suppress incorporation of label into the benzylic fragment of the alkaloid (benzoic acid). The yield of ephedrine, the per cent incorporation, and the specific incorporation of radioactivity into the alkaloid (Table 2) as well as the distribution of label over the three fragments, N-methyl, C₆–C₁, and C₂, within the alkaloid (Table 3) varied from experiment to experiment and no consistent pattern was evident. As in the earlier experiments, label was heavily scrambled over the three fragments, indicating rapid metabolic turnover of pyruvate (Table 3).

Even though label from [2-¹⁴C]- and [3-¹⁴C]pyruvate did not necessarily enter the C₂ unit predominantly, the distribution of activity within this C₂ unit showed a strong trend (Table 3): In each of the three experiments with [3-¹⁴C]pyruvate most of the label (ca. 75%) within the C₂ unit was located at the C-methyl group (C-3) of ephedrine (recovered as methylamine by Schmidt degradation of the Kuhn–Roth acetate), whereas in each of the three experiments with [2-¹⁴C]pyruvate most of the label (ca. 75%) within the C₂ unit was located at the adjacent carbon atom, C-2 (Kuhn–Roth acetate minus Schmidt methylamine). Even though this distribution of label tends to be in accord with that expected if pyruvate were the source of the C₂ unit (Scheme 3), these results could not be regarded as conclusive evidence in support of the proposition that the C₂ unit was generated from an acetyl unit derived from pyruvate by decarboxylation.

Incorporation of radioactivity into ephedrine from acetate itself (Expt. 4) was too low to attempt the chemical degradation required to determine the distribution of label. This was not altogether surprising. The kinases required to convert acetate into its biologically active species, acetyl CoA or acetyl phosphate, are absent from many biological systems. The conversion of pyruvate into acetyl CoA, on the other hand, takes place in most living species. Thus, for example, pyruvate served as the direct precursor of the non-tyrosine derived C₂ unit of anhalonidine and pelletine, alkaloids of the peyote cactus (39), whereas acetate was incorporated only indirectly, with scrambling of label (40). In yeast, acetate did not serve as a precursor of the nonbenzylic C₂ unit of 1-hydroxy-1-phenyl-2-propanone, whereas acetyl phosphate or pyruvate did (31). Even so, in the present instance the experiments with ¹⁴C-labelled samples of pyruvate (Expts. 2, 3, 5–8), while indicative, were inconclu-

sive, since label from these substrates was not confined to the predicted sites.

Conclusive evidence was obtained, almost 10 years after our first experiments with ¹⁴C-labelled precursors, by means of an experiment with [2,3-¹³C₂]pyruvic acid. The distribution of label within the four alkaloids, 1–4, derived from this precursor in *E. gerardiana sikkimensis*, is evident from the high field ¹³C nmr spectrum of the mixture of alkaloid hydrochlorides (Fig. 1). The signals due to the C-methyl group and the adjacent carbon atom of each of the four alkaloids show satellites, due to ¹³C–¹³C coupling, a clear demonstration of the incorporation of the intact ¹³CH₃–¹³CO unit of the precursor into each of the four alkaloids. A full interpretation of the spectrum has appeared (13).

The question arises why the experiments with [¹⁴C]pyruvate gave results that were inconclusive whereas the experiment with [¹³C₂]pyruvate gave an unequivocal result, even though the sensitivity of detecting ¹⁴C within the product⁶ is approximately 3 orders of magnitude greater than that of detecting ¹³C (cf. ref. 41).⁷

It is probable that it is this sensitivity which permitted the detection of radioactivity in all parts of the ephedrine molecule when [¹⁴C]pyruvate served as the substrate, indicating rapid metabolic turnover of pyruvate. There is no reason to think that in the experiment with [¹³C₂]pyruvate a similar rapid turnover did not also occur. But since such turnover would have led to enrichment at all carbon atoms of ephedrine, leading to a small increase in the height of every peak in the ¹³C nmr spectrum of the product, these increases were not detectable.

What was detectable were the satellites due to intact transfer

⁶Radioactivity of carrier free ¹⁴C-labelled precursor: Specific activity = ca. 62 mCi per mmol, i.e., ca. 1.4×10^{11} disintegrations per minute per mmol, i.e., ca. 1.2×10^{11} counts per minute per mmol, when radioactivity is assayed in a modern liquid scintillation counter at ca. 90% efficiency. Radioactivity of a product of MW 200 (say), determined on a weighed sample of 0.1 mg (say) to be 50 counts per minute above background (say). Specific activity of product is 1.0×10^5 counts per minute per mmol, i.e., 1.1×10^5 disintegrations per minute per mmol. Thus a dilution of radioactivity of six orders of magnitude is detectable.

⁷To detect, in unequivocal fashion, incorporation of ¹³C from a singly ¹³C-labelled substrate, the peak height of the ¹³C nmr signal due to the enriched carbon atom of the product must show an enhancement of at least 20–30% over that of the corresponding signal of a sample containing only natural abundance ¹³C. This enhancement, which corresponds to an incorporation of 0.2–0.3% ¹³C above natural abundance (1.1%), must be determined relative to the peak height of signals due to unenriched carbon atoms in the sample containing the enriched carbon.

Thus the maximum permissible dilution of ¹³C of a precursor containing 100% ¹³C at a single position, en route to a biosynthetic product, is less than three orders of magnitude.

If the ¹³C-labelled substrate is enriched in ¹³C at a pair of contiguous carbon atoms, i.e., the substrate is "bond-labelled", and if this enriched C₂ unit is transferred intact into a product, detection of incorporation no longer depends on observing the increase in peak height of a single signal in the ¹³C nmr spectrum, but on the appearance of signal satellites due to ¹³C–¹³C coupling. The use of such bond-labelled substrates thus permits much greater dilution of the administered precursor.

The limit of detection of these satellites depends less on the per cent incorporation of ¹³C than on the field strength of the spectrometer and the quality of the probe used in recording the spectrum. The field strength controls the signal dispersion while the quality of the probe determines the signal-to-noise ratio of the spectrum, for a given amount of sample.

of pairs of contiguous carbon atoms from substrate into each of the products.

Thus the less sensitive tracer technique provided an unequivocal result whereas the more sensitive technique gave information that was not readily interpretable.

A further difference between the experiments with ^{14}C and ^{13}C -labelled pyruvate is the enhanced specific incorporation of label into the alkaloids in the latter case (Expt. 9) (10^3 -fold) compared to that in the ^{14}C -pyruvate experiments (Expts. 7, 8), in the same *Ephedra* species (Table 1). This may be related to the fact that, whereas in the ^{14}C experiments (Expts. 7, 8) only tracer amounts of sodium pyruvate (ca. 1 mg) were administered, in the ^{13}C experiment (Expt. 9) a massive dose of substrate (500 mg) was applied.

The final question that must be raised concerns the status of 1-hydroxy-1-phenyl-2-propanone (**15**) in the biosynthesis of the *Ephedra* alkaloids. Attempts to trap this compound in the plant extracts from our tracer experiments, or to demonstrate specific incorporation into ephedrine, of label from ^{14}C -labelled samples of 1-hydroxy-1-phenyl-2-propanone by means of feeding experiments with *E. distachya*,⁸ did not meet with success.

There is thus no experimental evidence supporting the intermediacy of this compound in ephedrine biosynthesis. The supposition that it is implicated is based entirely on chemical analogy (28, 33, 35).

A closely related compound, (*S*)-(-)-2-amino-1-phenyl-1-propanone ((-)-cathinone) (**16**) is a major constituent of *Catha edulis* Forsk., where it occurs together with (+)-norpseudoephedrine (**4**) and (-)-norephedrine (**2**) (42, 43). It is, of course, possible that (-)-cathinone (**16**) is derived from (+)-norpseudoephedrine or (-)-norephedrine by enzymic dehydrogenation. However, it is also conceivable that (-)-cathinone might, in fact, serve as the substrate for stereospecific enzymic reduction and represent the precursor of (+)-norpseudoephedrine and of (-)-norephedrine (42). If so, the compound from which the alkaloids are derived would not be 1-hydroxy-1-phenyl-2-propanol (**15**) but 1-phenyl-1,2-propandione (**14**).

This compound, which has been detected in *Catha edulis* (42), in turn would be generated in the presence of thiamin by the carboxylase-catalyzed reaction of pyruvate with benzoic acid (rather than with benzaldehyde) (Scheme 3), analogous to the carboxylase-catalyzed formation of butan-2,3-dione (diacetyl) (44, 45).

Such a biosynthetic sequence would be consistent with the apparent lack of participation of 1-hydroxy-1-phenyl-2-propanone in ephedrine biosynthesis. It would also explain the observation that benzoic acid as well as benzaldehyde serves as the precursor of the $\text{C}_6\text{-C}_1$ unit of the *Ephedra* alkaloids (12). Whereas the biological oxidation of benzaldehyde to benzoic acid is likely to be a facile process (46), the reverse reaction, reduction of benzoic acid to benzaldehyde, does not appear to have been observed in higher plants (46).

Experiments are in hand to examine the possible intermediacy of 1-hydroxy-1-phenyl-2-propanone (**15**), 1-phenyl-1,2-propandione (**14**), and cathinone (**16**) in the biosynthesis of the *Ephedra* alkaloids.

Experimental

Labelled compounds

The labelled compounds employed in the nine feeding experiments were obtained from commercial suppliers (see Table 1).

Tracer experiments

Administration of labelled compounds to *Ephedra distachya* L. and *E. gerardiana sikkimensis*

Experiments 1-6

Stocks of *Ephedra distachya* L. were obtained from Hillier Nurseries, Winchester, England. The plants were propagated in the greenhouse at approximately 24°C . Large stocks were split in the spring. Labelled compounds were administered to actively growing plants by the wick method. The plants were kept in contact with tracer for 4-5 days. Alkaloids were isolated from the plants as described below.

Experiments 7-9

Plants of *E. gerardiana sikkimensis* were propagated in a greenhouse at $25\text{-}30^\circ\text{C}$ for 1 year, from cuttings obtained from The Geographic Garden, Kolding, Denmark. Labelled compounds were administered in an aqueous solution of Tween 80 (1% w/v). This solution (1.0 mL per day) was applied to the light green shoots with a fine paint brush. After administration of tracer the plants were painted with 1.0 mL of water per day (see Table 1). In Experiment 9 a total of 500 mg sodium [$2,3\text{-}^{13}\text{C}_2$]pyruvate (99 at. % ^{13}C per position) was administered over 5 days.

Details of the feeding experiments are summarized in Table 1.

Isolation of ephedrine and pseudoephedrine

Extraction of the alkaloid mixture

Expts. 1-3: The aerial parts of the plants were cut into pieces (approximately 1 cm long) and dried at 50°C for 1 week. The weight loss was 60-65%. The dry plant material was finely ground in a blender and extracted with methanol in a Soxhlet extractor until the extract was no longer coloured (ca. 8 h). Expts. 4-8: The fresh aerial parts of the plants were ground in a blender and extracted with methanol in a Soxhlet apparatus. Experiment 9: The fresh aerial parts of the plants were ground in a blender and extracted with methanol at 20°C . In each case the extraction of the alkaloids was considered complete when chlorophyll had been completely extracted from the plant material.

In Expts. 1-6, the extract was then concentrated *in vacuo*, sulfuric acid (0.1 M, 70 mL) was added to the oily residue, the mixture was extracted with ether (4×35 mL), and the ether extract discarded. The aqueous phase was basified to pH 10 with solid potassium carbonate and then extracted with ether (5×30 mL). In Expts. 7-9, 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), basified with potassium carbonate, and extracted with ether (4×50 mL).

In Expts. 1-3, the ether extract was dried (anhydrous sodium sulfate) and concentrated *in vacuo* to yield an oily residue that contained the crude alkaloids, which were then separated via the acetonides (*vide infra*). In Expts. 4-9, 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 crude alkaloid hydrochlorides. In Expts. 4-8, radioinactive (-)-ephedrine hydrochloride (60-220 mg) was then added as carrier and the product was recrystallized from ethanol/ether, mp 217°C (lit. (12) mp 220°C). In Expt. 9 the 75.5-MHz ^{13}C nmr spectrum (see Fig. 1) of the mixture of alkaloid hydrochlorides (57 mg) showed that the sample consisted of the hydrochlorides of pseudoephedrine (**3**), ephedrine (**1**), norpseudoephedrine (**4**), and norephedrine (**2**) in the molar ratio 52:35:10:3 (see Table 2). An analysis of the ^{13}C nmr spectrum is presented in Tables 5 and 6.

Separation of ephedrine and pseudoephedrine via their acetonides 5 and 6 (Expts. 1-3)

The residue containing the crude alkaloids was dissolved in acetone (50 mL). Silica gel 60 (60-200 mesh, 700 mg) and a few boiling chips were added, the mixture was refluxed 20 h, filtered, and concentrated *in vacuo* (14). The oily residue was dissolved in a mixture of ethyl acetate (2.5 mL) and petroleum ether (bp $30\text{-}60^\circ$) (2.5 mL), and residual solid was removed by centrifugation. The supernatant liquid was applied to a column (Merck Silica Gel 60, prepacked, size B) and eluted with a mixture of ethyl acetate and petroleum ether (bp $30\text{-}60^\circ\text{C}$) (1:1 v/v). The ultraviolet absorption of the effluent was monitored continuously at 254 nm. The fractions containing the two products, (4*S*,5*R*)-2,2,3,4-

⁸K. Yamasaki. Personal communication.

tetramethyl-5-phenyloxazolidine (i.e., (-)-ephedrine acetonide (5)) and (4*S*,5*S*)-2,2,3,4-tetramethyl-5-phenyloxazolidine (i.e., (+)-pseudoephedrine acetonide (6)) were collected separately (tlc (ethyl acetate:petroleum ether (bp 30–60°C) 1:1, v/v): R_f 0.6 and 0.5, respectively) and concentrated *in vacuo*.

Each acetonide was hydrolyzed without further purification. Each residue was dissolved in ethanol (1 mL) and hydrochloric acid (0.25 M, 1 mL), and the solution kept 1.5 h at 70°C. Each solution was evaporated *in vacuo*, the residue was dried over P₂O₅, an inactive sample of (-)-ephedrine hydrochloride or (+)-pseudoephedrine hydrochloride, respectively, was added as carrier, and the products were recrystallized from ethanol/ether: (-)-ephedrine hydrochloride, mp 217°C (lit. (12) mp 220°C; (47*a*) mp 218°C; (+)-pseudoephedrine hydrochloride, mp 180–181°C (lit. (48) mp 179–181°C; (47*a*) mp 182–182.5°C).

(-)-Ephedrine sulfate

A sample of (-)-ephedrine hydrochloride (50 mg) was dissolved in water (3 mL), solid potassium carbonate was added (to pH 10), and the solution was continuously extracted with ether (20 h). Ether was evaporated and sulfuric acid (0.1 M) was added dropwise to the residue (to pH 4). The solution was concentrated and the solid residue recrystallized (water/ethanol/ether) to give (-)-ephedrine sulfate (44 mg, 0.10 mmol, 83%); mp 259°C (lit. (48) mp 257°C).

Determination of radioactivity

Radioactivity was assayed by liquid scintillation counting (Mark 1 liquid scintillation computer, Model 6860, Nuclear Chicago (Expts. 1–6) or Beckman LS 9000 liquid scintillation system (Expts. 7, 8). Activity due to ³H and ¹⁴C was determined simultaneously, by external standardization counting with ¹³³Ba.

Samples (0.5–1.0 mg) were dissolved in ethanol or water, and the solution dispersed in Aquasol (New England Nuclear). The incorporation of the radioactive precursors into the alkaloids (Expts. 1–8) is summarized in Table 2.

Degradation of labelled ephedrine

The radioactively labelled samples of ephedrine (Expts. 1–8) were degraded by the reactions outlined below. The purity of all labelled samples was checked by thin-layer chromatography and melting point determination. All samples were recrystallized to constant specific activity. The molar specific activities of the samples of ephedrine and its degradation products, obtained from individual tracer experiments, are summarized in Tables 3 and 4. Confidence limits shown in Tables 3 and 4 are standard deviation from the mean.

Carbon atoms 1–3 and the aromatic carbon atoms as 1-phenyl-2-propanone semicarbazone

(-)-Ephedrine sulfate (27 mg, 0.06 mmol) or an equivalent quantity of the hydrochloride was dissolved in sulfuric acid (50% (v/v), 0.5 mL), and kept 24 h in a sealed flask at 110°C. Water (1 mL) was added and the mixture was extracted with ether (3 × 1 mL). The ether extract was washed with water (2 × 1 mL) and concentrated in a stream of nitrogen. The residue was dissolved in a solution of potassium acetate (20 mg, 0.20 mmol) in ethanol (0.5 mL). A solution of semicarbazide hydrochloride (20 mg, 0.18 mmol) in water (0.5 mL) was added, the mixture was heated 1 min on a steam bath, solvent was removed *in vacuo*, and the residue was sublimed at 140°C and 0.027 Torr. The sublimate was applied to a column (Merck Silica Gel 60 prepacked, size A), which was eluted with ethanol. The fraction containing 1-phenyl-2-propanone semicarbazone (detected by continuous monitoring of the effluent at 254 nm) (tlc (ethanol) R_f 0.7) was concentrated *in vacuo*. Yield 3 mg (0.02 mmol, 25%); mp 181°C (from ethanol) (lit. (49) mp 177°C).

This degradation of ephedrine to 1-phenyl-2-propanone was a modification of an established method (8).

The *N*-methyl group of ephedrine as *N*-methylphthalimide

(-)-Ephedrine sulfate (71 mg, 0.16 mmol) or an equivalent quantity of the hydrochloride was dissolved in sulfuric acid (50% (v/v), 1 mL) and kept 24 h in a sealed flask at 110°C. The flask was cooled and a solution of potassium carbonate (2.0 g) in water (6 mL) was added. The mixture was heated until 4 mL distillate had been trapped in hydro-

chloric acid (1 M, 0.5 mL). The acidified distillate was concentrated to dryness *in vacuo*. The crystalline residue of methylamine hydrochloride was redissolved in water (0.5 mL), the solution was basified with solid sodium carbonate (to pH 9), and *N*-carboethoxyphthalimide (62 mg, 0.28 mmol) was added. The reaction mixture was stirred until precipitation of *N*-methylphthalimide was complete (15 min). The product was sublimed at 70°C and 0.025 Torr and recrystallized (methanol/water). Yield 11 mg (0.07 mmol, 44%), mp 133°C (lit. (47*b*) mp 133–134°C; (50) mp 134°C).

Carbon atoms 2 and 3 as acetic acid and carbon atom 1 and the aromatic carbons as benzoic acid (isolated as 1-acylamino-naphthalenes)⁹ (12)

(-)-Ephedrine hydrochloride (50 mg, 0.25 mmol) was dissolved in a solution of chromium trioxide (750 mg, 7.5 mmol) in concentrated sulfuric acid (0.7 mL) and water (10 mL). The mixture was distilled, with simultaneous addition of water to maintain constant volume, until 50 mL distillate had been collected. The distillate was neutralized with dilute sodium hydroxide and the solution was divided in two portions in a ratio of 1:3. Both portions were concentrated to dryness *in vacuo*. The larger portion was kept for a Schmidt degradation of benzoic acid and acetic acid (*vide infra*).

The smaller portion was dissolved in water (2 mL) and 1-naphthylamine hydrochloride (53 mg, 0.34 mmol) and *N*-ethyl-*N'*-[3-(dimethylamino)propyl]carbodiimide hydrochloride (170 mg, 0.89 mmol) were added and the solution stirred for 1 h. Hydrochloric acid (1 M, 1 mL) was then added and the mixture was extracted with ether (3 × 3 mL). The ether extracts were dried (sodium sulfate) and evaporated to dryness *in vacuo* and the residue was chromatographed on silica gel 40 (70–230 mesh, 15 g) with ether as eluent. The fractions containing 1-benzamidonaphthalene and 1-acetamidonaphthalene were collected (tlc (ether) R_f 0.8 and 0.4, respectively) and evaporated, and the products were recrystallized. 1-Benzamidonaphthalene, yield 5 mg (0.02 mmol, 32%), mp 162–163°C, after sublimation at 135°C and 0.025 Torr, and recrystallization from benzene/hexane (lit. (47*c*) mp 162°C; (52) mp 157–159°C). 1-Acetamidonaphthalene, yield 7 mg (0.04 mmol, 61%), mp 158–159°C, after sublimation at 95°C and 0.025 Torr, and recrystallization from benzene/hexane (lit. (47*c*) mp 160°C; (53) mp 159–160°C).

The *C*-methyl group of ephedrine (*C*-3) as methylamine (isolated as *N*-methylphthalimide) and the aromatic carbon atoms as aniline hydrochloride

The solid residue containing the sodium salts of benzoic acid and acetic acid obtained from the major portion of the distillate from the chromic acid oxidation, which had been kept for a Schmidt degradation (*vide supra*), was dissolved in concentrated sulfuric acid (1.8 mL). Sodium azide (120 mg, 1.85 mmol) was added. When a homogeneous solution was obtained the mixture was divided into two equal portions.

One portion was kept 3 h at 25°C. The mixture was added to water (8 mL) and solid sodium hydrogen carbonate was added, to pH 8. The mixture was extracted with ether (3 × 3 mL) and the extracts were dried (sodium sulfate). Hydrochloric acid (1 M, 0.5 mL) was added and the mixture was concentrated to dryness *in vacuo*. Recrystallization from ethanol/ether gave aniline hydrochloride: 3 mg (0.02 mmol, 34%), mp 194°C (lit. (54) mp 198°C).

⁹These simple compounds do not appear to have a unique name assigned to them: IUPAC nomenclature rules (51) permit any one of four alternative names: (i) IUPAC rule C-824-1: *N*-1-naphthalenylacetamide and -benzamide. This is the nomenclature used in Chem. Abstr. (registry numbers 575-36-0 and 634-42-4, respectively). (ii) IUPAC rule C-824-2: 1-acetyl- and 1-benzoylaminonaphthalene or, since the name 1-naphthylamine is permissible for 1-aminonaphthalene (see rule C-812), acetyl- and benzoyl-1-naphthylamine. These are the names found in the Dictionary of Organic Compounds (47*c*). (iii) IUPAC rule C-824-3: 1-acetamido- and 1-benzamidonaphthalene. Furthermore, IUPAC rule C-823-1 refers to either (ii) or (iii), but not to (i). So much for uniformity of chemical nomenclature.

The second portion was heated on a steam bath for 2 h. After cooling, the mixture was added to water (10 mL). Solid sodium hydroxide was then added to pH 13. The mixture was heated until 5 mL distillate had been collected in hydrochloric acid (1 M, 2 mL). The acidified distillate was concentrated to dryness *in vacuo* and the solid residue of methylamine hydrochloride was converted to *N*-methylphthalimide, as described above. Yield: 5 mg (0.03 mmol, 47%).

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