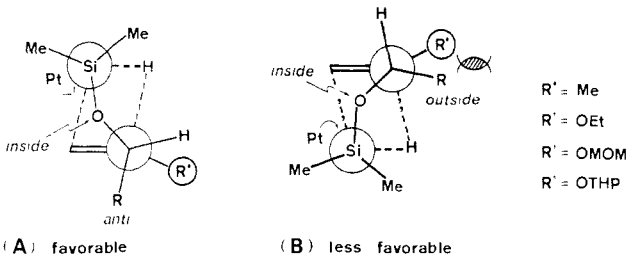

glyceraldehyde 7[13] was converted into a mixture of stereoisomers **8** and **9** (1/1.4).[14] The isomeric mixture was subjected to the intramolecular hydrosilation–oxidation sequence to form **10** and **11** in 64% combined yield. The acetonides were, fortunately, readily separated into two optically pure stereoisomers, **12** (R_f 0.5) and **13** (R_f 0.29), by simple column chromatography (silica gel, hexane/EtOAc, 2.5/1).[11] No 2,3-erythro isomers were obtained, if any only trace, indicative of the perfect syn stereoselective hydrosilation.[15] These products, **12** and **13**, were deprotected to free pentitols which were converted into, respectively, optically pure D-arabinitol pentaacetate (**14**) and xylitol pentaacetate (**15**).[16,17] It may be mentioned that the present pentitol synthesis is among the shortest pathways together with the highest stereoselection ever reported,[4a,d,g] starting with optically active glyceraldehyde derivatives.[2a] Refinement and further applications as well as development of the procedure for the opposite stereoselection, 2,3-erythro (anti), are now under investigation.

Acknowledgment. We thank the Ministry of Education, Japan, for a Grant-in-Aid for Scientific Research (no. 60550605) and H. Fujita for measurements of 400 MHz ^{1}H NMR spectra.

Supplementary Material Available: Data and/or copies of 400-MHz ^{1}H NMR spectra of acetonides **4a** and **4b** and **8**, **9**, **12**, and **1** (7 pages). Ordering information is given on any current masthead page.

(13) Sugiyama, T.; Sugawara, H.; Watanabe, M.; Yamashita, K. *Agric. Biol. Chem.* **1984**, *48*, 1841.
(14) We have examined several reagents, such as copper, magnesium, or zinc reagents, derived from the lithiated species **6** to improve both the selectivity and the yield, but have not gotten any satisfactory results yet. The two stereoisomers, **8** and **9**, were separated only with difficulty by medium pressure liquid chromatography.
(15) This point has also been confirmed by similar transformations starting with each isomer, **8** and **9**, separated as above.[14]
(16) *Rodd's Chemistry of Carbon Compounds*; Coffey, S., Ed.; Elsevier: New York, 1976; Vol. 1F, pp 18 19. For ^{1}H and ^{13}C NMR data, see the following literatures: Moore, R. E.; Barchi, J. J., Jr.; Bartolini, G. *J. Org. Chem.* **1985**, *50*, 374. Angyal, S. J.; Le Fur, R. *Carbohydr. Res.* **1980**, *84*, 201.
(17) Optical rotations, $[\alpha]_D^{20}$, of compounds prepared in this study are as follows: **8**, +1.82° (*c* 1.10, benzene); **9**, −2.97° (*c* 1.01, benzene); **12**, −10.0° (*c* 1.56, EtOH); **13**, +21.1° (*c* 1.28, EtOH); **14**, +37.25° (*c* 1.02, $CHCl_3$); **15**, ±0.00° (*c* 1.07, $CHCl_3$).

Biosynthesis of Ephedrine

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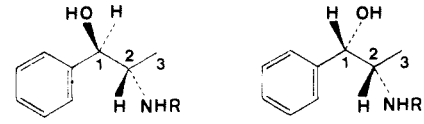
It is remarkable that the biosynthetic origin of the simple phenylpropanoid skeleton of the Ephedra alkaloids (e.g., (−)-ephedrine (**1**) and (+)-pseudoephedrine (**3**)) is still not established.

Label from [3-^{14}C]phenylalanine was shown,[1] 30 years ago, to be specifically incorporated into C-1 of (+)-norpseudoephedrine (**4**) in *Catha edulis* and, 10 years later, into C-1 of (−)-ephedrine



1 R = CH_3 **3** R = CH_3
2 R = H **4** R = H

(**1**) in *Ephedra distachya*.[2,3] Tritium from [ring-^{3}H]phenylalanine also entered the alkaloids.[2,3] It was originally thought[4-6] that the aminophenylpropanoid system of the alkaloids was derived either directly from the aminophenylpropanoid system of phenylalanine[4] or by reaction of a phenylalanine-derived phenylethylamine moiety with a one-carbon unit.[5-7] These views had to be abandoned when it was found[2,3] that label from [2-^{14}C]phenylalanine did not enter (−)-ephedrine (**1**) and that label from [2,3-^{14}C]phenylalanine was found solely at C-1 of ephedrine (from 3-^{14}C) but not at C-2, the site predicted for entry of label from [2-^{14}C]phenylalanine. It thus became evident that phenylalanine supplies neither the intact C_6–C_3 skeleton of the alkaloids nor a C_6–C_2 moiety but merely a C_6–C_1 unit. Benzoic acid and benzaldehyde, whose sidechain carbon atom enters C-1 of (−)-ephedrine,[2,3] are presumably intermediates on the route from phenylalanine into the C_6–C_1 unit of the alkaloids.

The origin of the C_2 unit, C-2,-3, of the alkaloids remained unknown. None of a wide range of ^{14}C-labeled substrates ([2-^{14}C]glycine,[3] [U-^{14}C]alanine,[3] [U-^{14}C]serine,[3] [U-^{14}C]aspartic acid,[3] [2-^{14}C]propionic acid,[3] [^{14}C]formic acid,[3,7] [6-^{14}C]glucose[3]) delivered radioactivity preferentially into this unit.

We now report that this C_2 unit is derived from the intact CH_3CO– group of pyruvic acid.

A freshly prepared solution of sodium [2,3-$^{13}C_2$]pyruvate (99.0 atom % ^{13}C, 100 mg, MSD Isotopes, Montreal, Canada) in demineralized water (1 mL) containing Tween 80 (0.01 mL) was applied with a fine paint brush to the growing stems of mature plants of *Ephedra gerardiana*, on each of 5 successive days (September 1987). Thus, a total of 500 mg of labeled pyruvate was administered. The plants were allowed to grow for 2 more days and were then harvested. The aerial parts (66 g fresh weight) were macerated in methanol (100 mL), hydrochloric acid (4 M, 3 mL) was added, methanol was removed, the residue was suspended in dilute hydrochloric acid (0.1 M, 50 mL), and the aqueous suspension was washed with ether (4 × 50 mL) and was then basified with K_2CO_3. The alkaloids were extracted into ether (4 × 50 mL) and reextracted into hydrochloric acid (1 M, 2 × 5 mL). Evaporation of the acid extracts gave a residue containing base hydrochlorides. The 75.47 MHz proton noise decoupled ^{13}C NMR spectrum of this sample (57 mg in 0.6 mL of D_2O) is presented in Figure 1.

The spectrum shows that the sample consists of the hydrochlorides of pseudoephedrine[8] (**3**), ephedrine[8,9] (**1**), norpseudoephedrine (**4**), and norephedrine[8-10] (**2**) in the ratio 52:35:10:3

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(1) Leete, E. *Chem. Ind. (London)* **1958**, 1088–1089.
(2) Yamasaki, K.; Sankawa, U.; Shibata, S. *Tetrahedron Lett.* **1969**, 4099–4102.
(3) Yamasaki, K.; Tamaki, T.; Uzawa, S.; Sankawa, U.; Shibata, S. *Phytochemistry* **1973**, *12*, 2877–2882.
(4) Shibata, S.; Imaseki, I. *Chem. Pharm. Bull.* **1956**, *4*, 277–280.
(5) Imaseki, I.; Shibata, S.; Yamazaki, M. *Chem. Ind. (London)* **1958**, 1625.
(6) Shibata, S.; Imaseki, I.; Yamazaki, M. *Chem. Pharm. Bull.* **1959**, *7*, 449–452.
(7) Shibata, S.; Imaseki, I.; Yamazaki, M. *Chem. Pharm. Bull.* **1957**, *5*, 594–597.
(8) Yamasaki, K.; Fujita, K. *Chem. Pharm. Bull.* **1979**, *27*, 43–47.
(9) Patra, A.; Mitra, A. K.; Mukhopadhyay, A. K. *J. Indian Chem. Soc.* **1979**, *56*, 1245–1247.
(10) Johnson, L. F.; Jankowski, W. C. *Carbon-13 NMR Spectra*; Wiley Interscience: New York, 1972; spectrum 355.

0002-7863/88/1510-3714$01.50/0

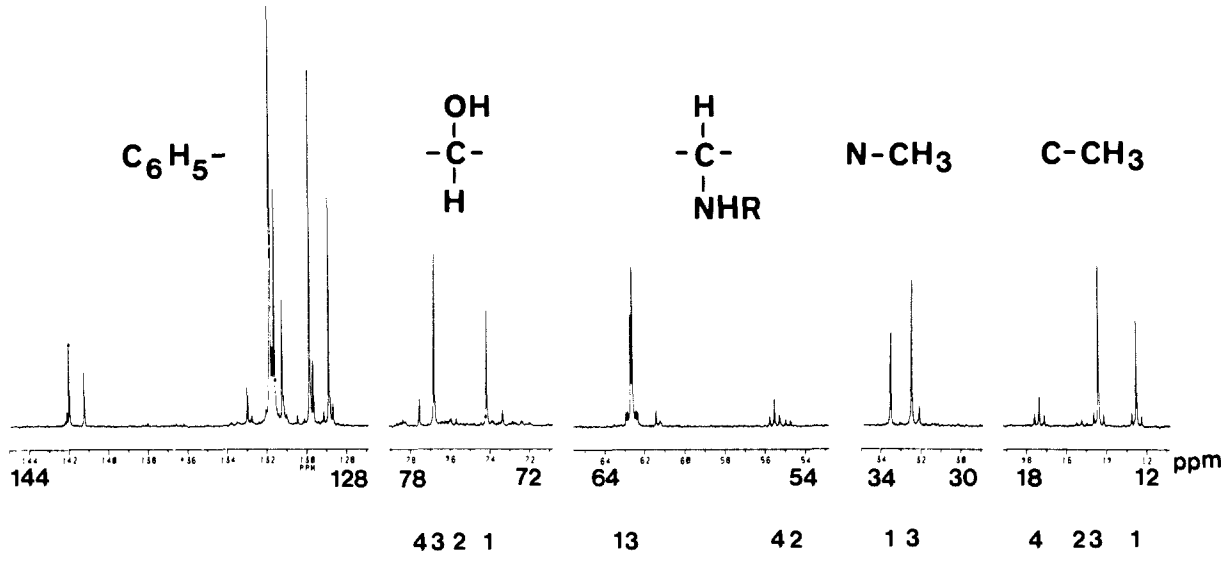
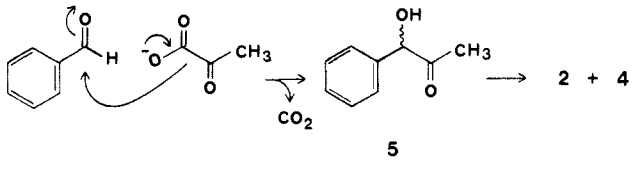


Figure 1. Proton noise decoupled ^{13}C NMR spectrum (75.47 MHz, 5000 transients) of alkaloid hydrochlorides (57 mg in 0.6 mL of D_2O) obtained from plants of *Ephedra gerardiana* after administration of sodium [2,3-$^{13}C_2$]pyruvate. The spectrum was recorded on a Bruker AC 300 spectrometer under 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**.

Scheme I



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(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**), 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**), 62.69 (**1**) ppm), i.e., the carbon atom to which the amino group is attached. The benzylic carbon atoms (δ 74.16 (**1**), 75.69 (**2**), 76.81 (**3**), 77.55 (**4**) ppm) and the phenyl carbon atoms (δ 128–142 ppm) give signals which appear as singlets.

The relative intensities of the central lines of the multiplets, and of the singlet signals, in each of the four components of the spectrum of the enriched sample, are identical within experimental error with the relative intensities of the signals in the natural abundance spectra of authentic alkaloid hydrochlorides. Thus, only the doublets in the spectrum of the enriched sample are due to ^{13}C-enrichment. The specific incorporation of pyruvate into each of the alkaloids can thus be calculated from the relative intensities of the satellite and central peaks in the signals due to the coupled carbon atoms, C-2 and C-3 (percent incorporation = 1.1 × (area of doublet/area of central peak)): pseudoephedrine (**3**) 0.22%, ephedrine (**1**) 0.32%, norpseudoephedrine (**4**) 1.0%, norephedrine (**2**) 1.4%.

It can be concluded on the basis of the spectrum that pyruvic acid supplies an intact CH_3CO– group from which the C_2 unit, comprising the C-methyl group and the neighboring carbon atom, of the four Ephedra alkaloids, **1**–**4**, is derived. Thus, the carbon skeleton of the Ephedra alkaloids is generated from two fragments, a C_6–C_1 unit related to benzaldehyde and a CH_3CO– moiety derived from pyruvate.

The enzyme-catalyzed condensation of precisely these fragments is known to occur in yeast and has been the subject of many investigations:[11-21] Carboxylase (EC 4.1.1.1) catalyzed condensation[18] of benzaldehyde and pyruvic acid in the presence of thiamin pyrophosphate leads, with loss of carbon dioxide, to the two enantiomers of 1-hydroxy-1-phenylpropan-2-one (**5**). Stereospecific amination of the two enantiomers at the carbonyl group, catalyzed by a transaminase, leads to (–)-norephedrine (**2**) and (+)-norpseudoephedrine (**4**), respectively.[22] It is tempting to speculate that a similar sequence of steps is responsible for the biosynthesis of these two compounds in *Ephedra* species.

Methylation of the two norbases, **2** and **4**, by transfer of an *S*-methyl group from methionine,[23] yields (–)-ephedrine (**1**) and (+)-pseudoephedrine (**3**), respectively, in the final step of the biosynthetic sequence.

Attempts to trap the putative intermediate, 1-hydroxy-1-phenylpropan-2-one (**5**) or to demonstrate[24] specific incorporation into the alkaloids of label from ^{14}C-labeled samples of this compound have so far been unsuccessful, however.

The present finding, that the nonbenzylic C_2 unit of the *Ephedra* alkaloids is derived from pyruvate, solves a biosynthetic problem of long standing. The solution, so long delayed, turns out to be anticlimactic.

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Registry No. **1**, 299-42-3; **2**, 492-41-1; **3**, 90-82-4; **4**, 492-39-7; HO_2CCOCH_3, 127-17-3.

(11) Neuberg, C.; Hirsch, J. *Biochem. Z.* **1921**, *115*, 282–310; *Chem. Abstr.* **1921**, *15*, 1205.
(12) Neuberg, C.; Ohle, H. *Biochem. Z.* **1922**, *127*, 327–339; *Chem. Abstr.* **1923**, *17*, 1484.
(13) Neuberg, C.; Ohle, H. *Biochem. Z.* **1922**, *128*, 610–618. *Chem. Abstr.* **1923**, *17*, 1484.
(14) Kamlet, J. *Wallerstein Lab. Commun.* **1941**, *4*, 213–214; *Chem. Abstr.* **1942**, *36*, 3820[2].
(15) Shimazu, Y. *Nippon Kagaku Zasshi* (*J. Chem. Soc. Jpn., Pure Chem. Sect.*) **1950**, *71*, 503–505; *Chem. Abstr.* **1951**, *45*, 9004d.
(16) Shimazu, Y. *J. Inst. Polytech. Osaka City Univ., Ser. C.* **1952**, *2*, 125–130; *Chem. Abstr.* **1953**, *47*, 12517e.
(17) Smith, P. F.; Hendlin, D. *J. Bacteriol.* **1953**, *65*, 440–445.
(18) Hanč, O.; Kakáč, B. *Naturwissenschaften* **1956**, *43*, 498.
(19) Gröger, D.; Schmauder, H. P.; Mothes, K. *Z. Allg. Mikrobiol.* **1966**, *6*, 275–287.
(20) Voets, J. P.; Vandamme, E. J.; Vlerick, C. *Z. Allg. Mikrobiol.* **1973**, *13*, 355–366.
(21) Subramanian, P. M.; Chatterjee, S. K.; Bhatia, M. C. *J. Chem. Technol. Biotechnol.* **1987**, *39*, 215–218.
(22) Hasse, K.; Schmid, G. *Biochem. Z.* **1963**, *337*, 69–79.
(23) Shibata, S.; Imaseki, I.; Yamazaki, M. *Chem. Pharm. Bull.* **1957**, *5*, 71–73.
(24) Yamasaki, K., personal communication.