Biosynthesis of the Piperidine Nucleus. Incorporation of Chirally Labeled [1-^{3}H]Cadaverine[1]

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Abstract: The two enantiomeric chirally labeled [1-^{3}H]cadaverines were prepared and were employed to show that the incorporation of cadaverine into the piperidine nucleus of *N*-methylpelletierine takes place with stereospecific retention of one of the enantiotopic C-1 hydrogens and stereospecific loss of the other. Since it is inferred that diamine oxidase (EC 1.4.3.6) participates in the biosynthetic process, this result provides the first indication that this enzyme exhibits stereospecificity. Evidence is presented which disproves current hypotheses of the biosynthesis of the piperidine alkaloids. A new model is now advanced which is consistent with all available experimental data.

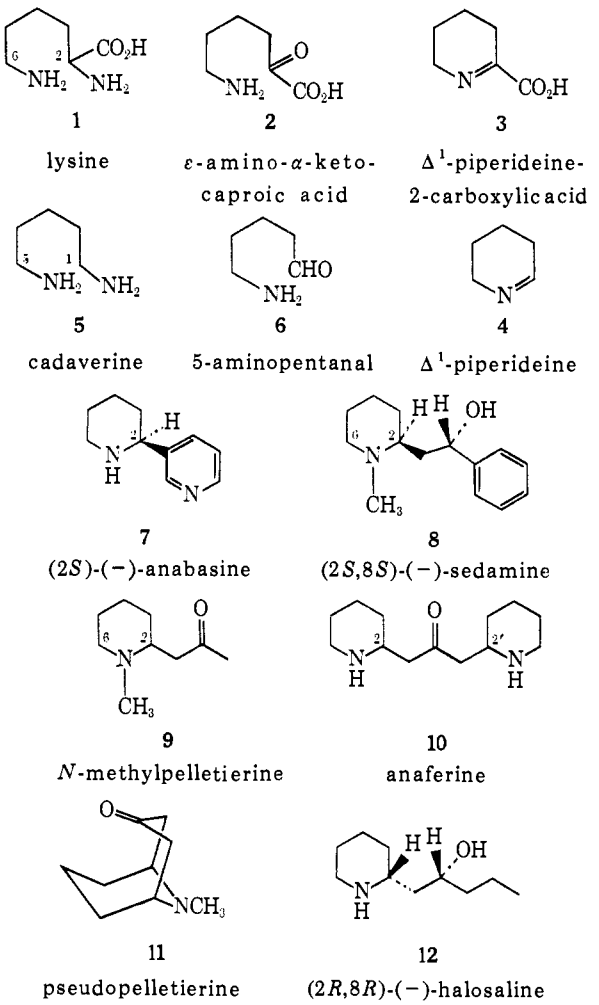
Before a clearer insight into the biosynthesis of the piperidine nucleus of alkaloids such as *N*-methylpelletierine (**9**), sedamine (**8**), and anabasine (**7**) can be gained, a paradox, posed by the current state of knowledge of the origin of these compounds, must be resolved.

The paradox arises from inferences based on the results of tracer experiments designed to determine the mode of incorporation of lysine and to identify intermediates between lysine and the alkaloids. It would appear from these results that none of the most obvious contenders for that role, cadaverine (**5**), ϵ-amino-α-ketocaproic acid (**2**), and α-aminoadipic-δ-semialdehyde, can be implicated.

Label from DL-[2-^{14}C]lysine (**1**) was shown to be confined to C-2 of the piperidine nucleus of anabasine (**7**),[3-6] sedamine (**8**),[7] *N*-methylpelletierine (**9**),[8] and to C-2 of one or both of the piperidine nuclei of anaferine (**10**).[8] Label from DL-[6-^{14}C]lysine was found to be restricted to C-6 of the nucleus of sedamine (**8**),[7] *N*-methylpelletierine (**9**),[9] and halosaline (**12**).[10] Label from DL-[4,5-$^3H_2$,6-^{14}C]lysine was incorporated into *N*-methylpelletierine (**9**)[9] and sedamine (**8**)[11] without change in the ^{3}H:^{14}C ratio. It follows that an intact C_5 unit, derived from the C_5 chain (C-2 to C-6) of lysine, enters the piperidine nucleus in a nonsymmetrical manner.

It was inferred from these results that a symmetrical compound such as cadaverine (**5**) could not be an intermediate between lysine and the alkaloids.[5,8,9,12,13] Yet label from [1,5-^{14}C]cadaverine (**5**) entered anabasine (**7**)[12] and pseudopelletierine (**11**)[14] nonrandomly and was incorporated also into *N*-methylpelletierine (**9**).[14] While intermediacy of enzyme-bound rather than free cadaverine was recognized as compatible with the mode of incorporation of lysine and cadaverine,[12] the utilization of cadaverine was considered to be an aberrant process.[13]

The observations that ^{15}N entered the piperidine ring of anabasine (**7**) when DL-[ϵ-^{15}N,2-^{14}C]lysine served as the substrate but that ^{15}N was almost entirely lost, relative to ^{14}C, when DL-[α-^{15}N,2-^{14}C]lysine was administered to *Nicotiana glauca*,[4] and that the ^{3}H:^{14}C ratio of DL-[6-^{3}H,6-^{14}C]lysine was maintained in *N*-



(1) Presented as part of a symposium lecture at the 23rd International Congress of Pure and Applied Chemistry, IUPAC., Symposium O-13, Boston, Mass., July 1971, Abstract No. 91, p 36.
(2) On leave of absence from the Department of Plant Physiology, Ruhr-Universität, Bochum, Federal German Republic.
(3) E. Leete, *J. Amer. Chem. Soc.*, 78, 3520 (1956).
(4) E. Leete, E. G. Gros, and T. J. Gilbertson, *ibid.*, 86, 3907 (1964).
(5) T. Griffith and G. D. Griffith, *Phytochemistry*, 5, 1175 (1966).
(6) E. Leete and M. R. Chedekel, *ibid.*, 11, 2751 (1972).
(7) R. N. Gupta and I. D. Spenser, *Can. J. Chem.*, 45, 1275 (1967).
(8) M. F. Keogh and D. G. O'Donovan, *J. Chem. Soc. C*, 1792 (1970).
(9) R. N. Gupta and I. D. Spenser, *Phytochemistry*, 8, 1937 (1969).
(10) D. G. O'Donovan and P. B. Creedon, *Tetrahedron Lett.*, 1341 (1971).
(11) R. N. Gupta and I. D. Spenser, *J. Biol. Chem.*, 244, 88 (1969).
(12) E. Leete, *J. Amer. Chem. Soc.*, 80, 4393 (1958).
(13) E. Leete, *ibid.*, 91, 1697 (1969).
(14) H. W. Liebisch, N. Marekov, and H. R. Schütte, *Z. Naturforsch. B*, 23, 1116 (1968).

methylpelletierine (**9**)[9] and sedamine (**8**)[11] demonstrated that a lysine-derived C_5N unit enters the piperidine nucleus and that its nitrogen atom is derived from the ε-amino group of lysine.

It was inferred from these results that the ε-deamination product of lysine, α-aminoadipic-δ-semialdehyde, was not implicated in the pathway, whereas the α-deamination product of lysine, 6-amino-2-oxohexanoic acid (ε-amino-α-ketocaproic acid) (**2**) or its cyclodehydration product, Δ^1-piperideine-2-carboxylic acid (**3**), was an intermediate.[3-5,7-11,13]

The immediate precursor of the piperidine nucleus, Δ^1-piperideine (**4**),[12] whose nonrandom incorporation into anabasine has been demonstrated,[13] was inferred to arise normally by decarboxylation of Δ^1-piperideine-2-carboxylic acid (**3**)[8,9,13] (but might, under "aberrant" conditions, also originate from cadaverine (**5**) by oxidative deamination,[13] a transformation which is known to occur in plant tissues[15-19]).

The inference that ε-amino-α-ketocaproic and Δ^1-piperideine-2-carboxylic acid were intermediates on the route to the piperidine nucleus was tested experimentally and found to be untenable.[20] It was shown that the $^3H:^{14}C$ ratio was maintained in sedamine (**8**) when DL-[2-^{3}H,6-^{14}C]lysine served as the substrate. Intermediacy of ε-amino-α-ketocaproic (**2**) and Δ^1-piperideine-2-carboxylic acid (**3**) demands complete loss of tritium relative to ^{14}C.

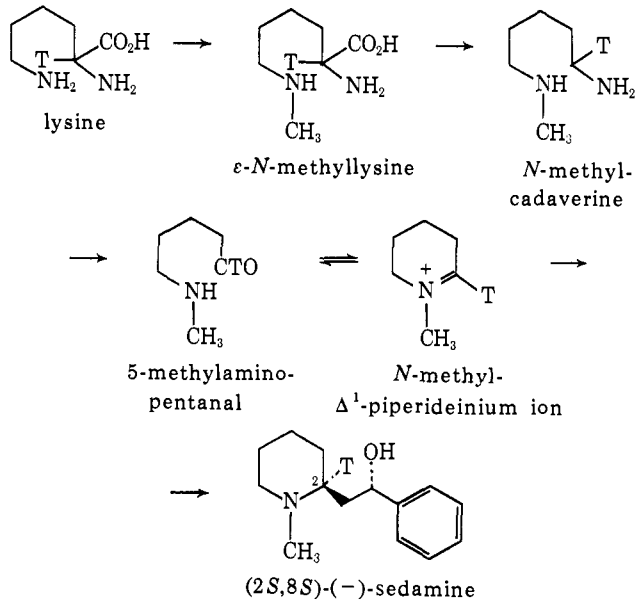
The tracer evidence bearing on the origin of the piperidine ring of the piperidine alkaloids thus requires reinterpretation. In particular, the question of the identity of the intermediates between lysine and Δ^1-piperideine (**4**) must be reopened, since the three obvious contenders appear to be eliminated by the results of the tracer experiments which have been performed. Intermediacy of cadaverine (**5**), the decarboxylation product of lysine, is contraindicated by the mode of incorporation of lysine, which excludes a symmetrical intermediate. α-Aminoadipic-δ-semialdehyde, the ε-deamination product of lysine, is excluded on the basis of the incorporation of the ε-amino nitrogen but not of the α-amino nitrogen of lysine and also on the basis of the retention within the products of the hydrogen atoms at C-6 of lysine. ε-Amino-α-ketocaproic acid (**2**), the α-deamination product of lysine, is eliminated on the basis of the retention within the product of the hydrogen atom at C-2 of lysine.

The aim of the present study was to test alternative models for the route from lysine into the alkaloids, consistent with the accumulated tracer evidence.

Discussion

The first model to be tested (Scheme I)[20] invoked protection of the ε-amino group of lysine (*e.g.*, by a methyl substituent), followed by decarboxylation of the product to yield a mono-N-substituted and therefore nonsymmetrical derivative of cadaverine. Removal of the primary amino group, *i.e.*, of the α-amino group of the original lysine, then leads to an aminoaldehyde whose cyclic derivative, an N-substituted Δ^1-piperideinium ion, serves as the immediate precursor of the piperidine nucleus of the piperidine bases. Retention of tritium during the removal of the primary amino group of the nonsymmetrical derivative of cadaverine, when this is derived from [2-^{3}H]-lysine, might be due to a very large primary hydrogen–tritium isotope effect, or more probably, to stereospecificity in the deamination reaction. Complete retention of tritium in the course of such a stereospecific deamination is possible only if the *N*-methylcadaverine were chirally tritiated. This, in turn, requires that decarboxylation of the [2-^{3}H]-*N*-methyllysine takes place in a stereospecific manner. This might be achieved most readily if only one enantiomer of lysine or of ε-*N*-methyllysine entered the pathway, *i. e.*, if only one enantiomer were utilized as a precursor of the alkaloids.

Scheme I. The "ε-*N*-Methyl" Model of the Route from Lysine to the Piperidine Alkaloids[20] (Now Disproved)



Several requirements of this model have been tested experimentally. The first of these requirements is that either L-lysine or D-lysine, but not both, is utilized in the biosynthesis of the alkaloids. It was indeed found that L-lysine serves as a specific precursor of anabasine (**7**), sedamine (**8**), and *N*-methylpelletierine (**9**), whereas D-lysine was not incorporated into the alkaloids.[1,21] This was demonstrated by a new tracer method designed to establish stereospecificity in a biosynthetic precursor–product relationship.[1,21]

Another requirement of the model is that ε-*N*-methyllysine and mono-*N*-methylcadaverine are formed within the plant. We have now confirmed the presence of ε-*N*-methyllysine, identical with an authentic sample,[22] in *Sedum acre* and have demonstrated its formation from methionine and lysine in *S. acre* and *S. sarmentosum* by isolation of labeled samples of ε-

(15) P. J. G. Mann and W. R. Smithies, *Biochem. J.*, **61**, 89 (1955).
(16) K. Hasse and H. Maisack, *Biochem. Z.*, 327, 296 (1955).
(17) E. Werle, I. Trautschold, and D. Aures, *Z. Physiol. Chem.*, 326, 200 (1961).
(18) H. R. Schütte, D. Knöfel, and O. Heyer, *Z. Pflanzenphysiol.*, **55**, 110 (1966).
(19) K. Hasse, O. T. Ratych, and J. Salnikow, *Z. Physiol. Chem.*, 348, 843 (1967).
(20) R. N. Gupta and I. D. Spenser, *Phytochemistry*, **9**, 2329 (1970).
(21) E. Leistner, R. N. Gupta, and I. D. Spenser, *J. Amer. Chem. Soc.*, **95**, 4040 (1973).
(22) We thank Dr. L. Benoiton, University of Ottawa, for a generous sample of synthetic ε-*N*-methyl-L-lysine (L. Benoiton, *Can. J. Chem.*, 42, 2043 (1964)).

Table I. Incorporation of Lysine

		$^3H:^{14}C$ ratio	
	(i) *S. sarmentosum*		
Precursor			
L-[*methyl*-^{14}C]Methionine plus DL-[6-^{3}H]lysine		5.5 ± 0.1	
Products			
N-Methylpelletierine		0.58 ± 0.04	
ε-*N*-Methyllysine		7.2 ± 0.1	
	Specific activity, dpm/mmol		Relative specific activity, %
	(ii) *S. acre*		
Precursor			
DL-[6-^{14}C]Lysine + inactive cadaverine			
Products			
Cadaverine (picrate)	1.92 (±0.01) × 10^6		
Sedamine	0.95 (±0.02) × 10^4		
Degradation of sedamine			
Sedamine (diluted)	0.28 (±0.01) × 10^4		100 ± 2
N-Methyl-β-alanine (DNP deriv)	0.27 (±0.01) × 10^4		97 ± 2
	Specific activity with respect to ^{14}C, dpm/mmol	$^3H:^{14}C$ ratio	% retention of ^{3}H relative to ^{14}C
	(iii) *S. acre*		
Precursor			
DL-[4,5-$^3H_2$,6-^{14}C]lysine		7.36 ± 0.28	100 ± 4
Products			
Cadaverine (*N*,*N'*-dibenzoyl deriv)	8.70 (±0.02) × 10^5	7.57 ± 0.02	103 ± 4
Sedamine	1.31 (±0.03) × 10^5	7.61 ± 0.08	103 ± 4

N-methyllysine from plants to which [*methyl*-^{14}C]-methionine and [6-^{3}H]lysine had been administered. Contrary to expectation, however, attempts to demonstrate the formation of *N*-methylcadaverine in either of the two *Sedum* species proved unsuccessful. Thus, the *N*-methylcadaverine, which was reisolated from plants of each of the two species to which [*methyl*-^{14}C]methionine had been administered in admixture with unlabeled *N*-methylcadaverine, was entirely inactive. Similarly, when [*methyl*-^{14}C]methionine was administered to *Nicotiana glauca* in admixture with unlabeled *N*-methylanabasine at a time of development of the plant when radioactivity from [^{14}C]lysine is incorporated into anabasine, the *N*-methylanabasine which was reisolated was totally inactive. Since the ε-N-methylation model demands the intermediacy of *N*-methylcadaverine in the biosynthesis of each of the three alkaloids (**7**–**9**) and predicts the demethylation of *N*-methylanabasine as a step of the route to anabasine (**7**), these negative results threw doubt on the validity of the ε-*N*-methyl hypothesis,[23] notwithstanding the demonstration that ε-*N*-methyllysine was formed within the plant.

Formation of a compound from substrates which are specifically incorporated into a given product at a stage of development when the product is generated is a necessary condition for the intermediacy of the compound on the route to the product but cannot, under any circumstances, be taken as proof of such intermediacy. Thus, the formation of ε-*N*-methyllysine in *S. acre* from lysine and methionine, known precursors of *N*-methylpelletierine[9] and sedamine,[7] at a time when alkaloid formation takes place, does not prove that ε-*N*-methyllysine is an intermediate in the formation of sedamine and *N*-methylpelletierine. A further experiment proved that it cannot be an intermediate.

[*methyl*-^{14}C]Methionine, which supplied the *N*-methyl group of *N*-methylpelletierine,[14] and [6-^{3}H]-lysine, which enters the product without loss of tritium, relative to [6-^{14}C]lysine,[9] were administered in admixture to cuttings of *S. sarmentosum*. Radioactive ε-*N*-methyllysine and *N*-methylpelletierine were then isolated from the plant material. If ε-*N*-methyllysine served as an intermediate between lysine and *N*-methylpelletierine and supplied an N-methylated C_5N unit to be transformed intact into the *N*-methylpiperidine nucleus of the alkaloid, then the $^3H:^{14}C$ ratio within the samples of ε-*N*-methyllysine and *N*-methylpelletierine, isolated from this experiment, would be expected to be identical. Conversely, if the $^3H:^{14}C$ ratios of the two compounds were to differ, ε-*N*-methyllysine could not be a direct precursor of *N*-methylpelletierine or an intermediate between lysine and the alkaloid. The results of the experiment are presented in Table I (expt i). The $^3H:^{14}C$ ratio of the ε-*N*-methyllysine (7.2 ± 0.1) was found to be entirely different from that of *N*-methylpelletierine (0.58 ± 0.04). ε-*N*-Methyllysine, even though present in the plant, is not an intermediate. The ε-N-methylation model must thus be rejected.

The second model[1,6] (Scheme II) for the route from lysine into the alkaloids is based on a concerted oxidative decarboxylation of lysine in the manner of the Strecker degradation of α-amino acids, a reaction which yields the corresponding lower aldehyde with

(23) It has now been clearly demonstrated that *N*-methylanabasine is not normally present in *N. glauca*, even though the plant is capable of sustaining the synthesis of this compound under abnormal conditions.[6]

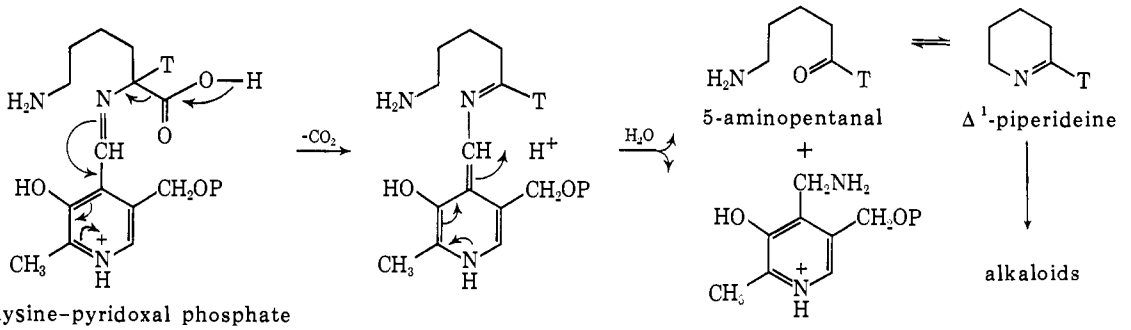
Table II. Incorporation of Cadaverine

	Specific activity, dpm/mmol	Relative specific activity, %
(i) *S. sarmentosum*		
Precursor		
[1-^{14}C]Cadaverine		
Products		
N-Methylpelletierine	$6.23 (\pm 0.10) \times 10^4$	100 ± 2
N-Methyl-β-alanine (DNP deriv) (by degradation of *N*-methylpelletierine)	$2.68 (\pm 0.07) \times 10^4$	43 ± 1
(ii) *S. acre*		
Precursor		
[1-^{14}C]Cadaverine		
Products		
Sedamine	$5.59 (\pm 0.07) \times 10^3$	100 ± 1
N-Methyl-β-alanine (DNP deriv) (by degradation of sedamine)	$2.64 (\pm 0.06) \times 10^3$	47 ± 1

Experiments with Enantiotopically Labeled Cadaverine
(iii) *S. sarmentosum*

	Precursor: [1A-^{3}H,1-^{14}C]Cadaverine		Precursor: [1B-^{3}H,1-^{14}C]Cadaverine	
	^{3}H:^{14}C ratio	% retention of ^{3}H relative to ^{14}C	^{3}H:^{14}C ratio	% retention of ^{3}H relative to ^{14}C
Precursor	2.37 ± 0.05	100 ± 2	18.88 ± 0.43	100 ± 2
Products				
N-Methylallosedridine	2.42 ± 0.09	102 ± 4	10.45 ± 0.09	55 ± 1
N-Methylpelletierine	2.34 ± 0.03	99 ± 2	10.06 ± 0.29	53 ± 2
N-Methyl-β-alanine (by degradation of *N*-methylpelletierine)	2.05 ± 0.16	87 ± 7	19.29 ± 0.04	102 ± 2

Scheme II. The "Streckerase" Model of the Route from Lysine to the Piperidine Alkaloids[1-6] (Now Disproved)



retention of the α-hydrogen atom.[24-26] This model,[27] like the first one, can account for the nonsymmetrical incorporation of a C_5N chain derived from C-2 to C-6 and N_ϵ of lysine and is consistent with the complete retention of hydrogen from C-2 and C-6 of lysine within the alkaloids. The model cannot account for the entry of cadaverine into the pathway, except in the role of an abnormal "aberrant" substrate which is not normally present but is convertible into 5-aminopentanal if presented to the biosynthesizing system. Examination of the position of cadaverine within the system can serve as a critical test of the validity of this model. This follows from the following reasoning. The model is based on the premise that activity from lysine enters the piperidine nucleus of the alkaloids in a specific fashion, such that a symmetrical molecule, *e.g.*, cadaverine, is excluded as a normal intermediate of the pathway.[5,8,9,12,13] Since label enters the alkaloids nonrandomly when radioactive cadaverine is presented to the plant,[4,12,14] and cadaverine thus serves as a precursor when present, it follows that the model implies that cadaverine cannot normally be present in the system or that, if it were, it cannot be derived from lysine.

It follows that if cadaverine were a normal constituent of the plant and if it could be shown to originate from lysine at a time when lysine is incorporated into the alkaloids, the Strecker model would be invalidated.

The presence of cadaverine in *S. acre* is now demonstrated. The base was isolated by ion-exchange chromatography of the basic fraction of the plant extract and identified by gas–liquid chromatography. Its specific formation from lysine, concurrent with sedamine biosynthesis, is demonstrated by the isolation of doubly labeled cadaverine as well as of doubly labeled sedamine, each with a ^{3}H:^{14}C ratio identical with that of the ad-

(24) I. D. Spenser, J. C. Crawhall, and D. G. Smyth, *Chem. Ind.*, 796 (1956).
(25) J. C. Crawhall and D. G. Smyth, *Biochem. J.*, **69**, 280 (1958).
(26) J. G. Kay and F. S. Rowland, *J. Org. Chem.*, **24**, 1800 (1959).
(27) It should be noted in passing that the "Strecker" model proposes an enzyme-catalyzed concerted oxidative decarboxylation of an α-amino acid to the corresponding lower aldehyde, with pyridoxol as coenzyme. Such a concerted conversion of an α-amino acid to the lower aldehyde is unknown as an enzymic process.

ministered DL-[4,5-$^3H_2$,6-^{14}C]lysine (Table I, expt iii).

Sedamine derived from [6-^{14}C]lysine contains all activity at C-6.[7] The nonsymmetric manner of incorporation of the C_5 chain of lysine is unaffected by the presence of an overload of cadaverine. This is shown by partial degradation of the sedamine obtained from *S. acre* plants to which [6-^{14}C]lysine had been administered, together with inactive cadaverine. *N*-Methyl-β-alanine (C-6 to C-4), obtained by degradation of the sedamine, retained all activity of the alkaloid (Table I, expt ii). This should be contrasted with the observation that when [1-^{14}C]cadaverine served as the precursor, the *N*-methyl-β-alanine obtained by degradation of the radioactive sedamine contained only one-half of the activity of the alkaloid (Table II, expt ii). Similarly, incorporation of cadaverine into *N*-methylpelletierine (Table II, expt i) was also shown to be specific.

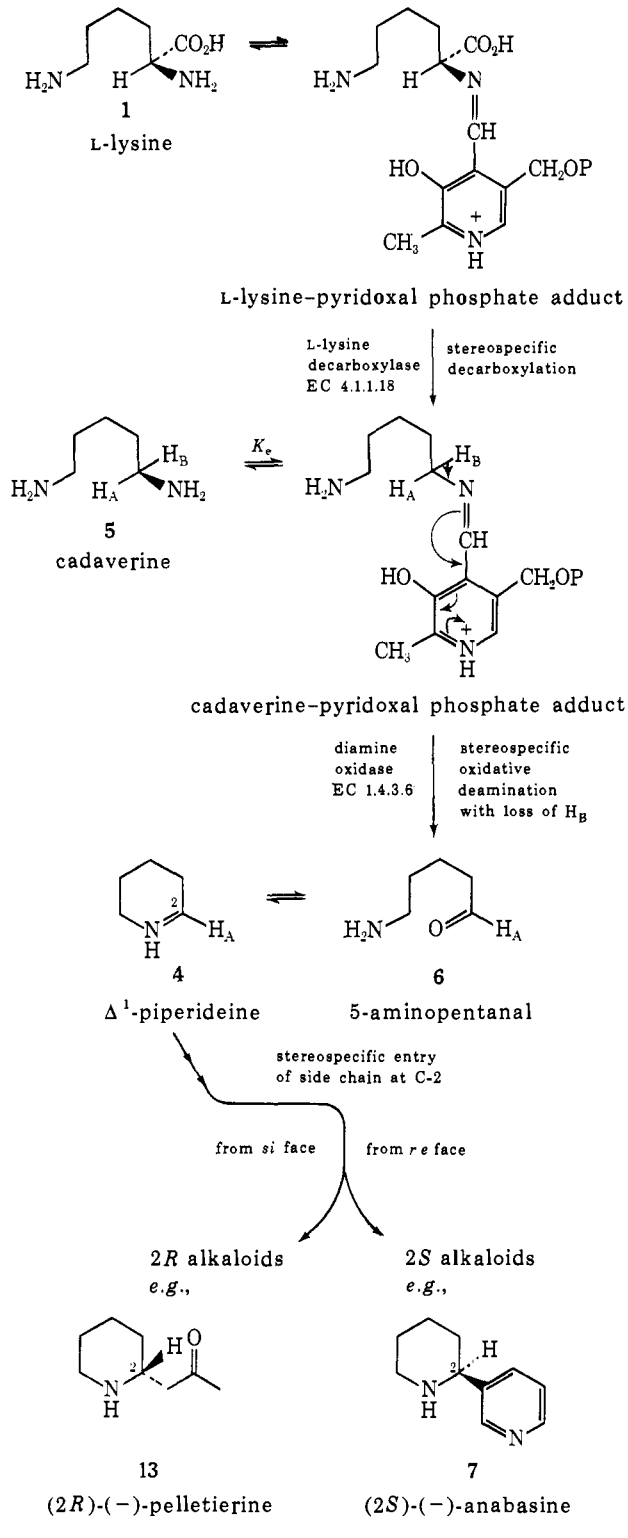
It is clear from these results that cadaverine is a normal component of *S. acre*, that it serves as a specific precursor of sedamine, and that it is formed from lysine at a time when lysine is incorporated into sedamine. It follows that the "Strecker" model,[27] or any other biogenetic hypothesis which cannot accommodate cadaverine as a normal component of the system, must be regarded as untenable.

The evidence here presented clearly demands that a normal position must be assigned to cadaverine in any biogenetic scheme for the conversion of lysine into the piperidine alkaloids. Yet the older evidence precludes the intermediacy of free cadaverine in the pathway. A biogenetic model which accommodates these conditions and is also consistent with all other tracer evidence is presented in Scheme III. The only new assumption which must be made is that the cadaverine which serves as an intermediate of the pathway is bound and that the equilibrium constant between this bound species and free cadaverine favors the former (*i.e.*, K_e = [bound cadaverine]/[free cadaverine] $\gg$ 1). Since L-lysine decarboxylase[28] (EC 4.1.1.18, L-lysine carboxylyase) and diamine oxidase[29] (EC 1.4.3.6, diamine:oxygen oxidoreductase (deaminating)), the two enzymes postulated to participate in the conversion of lysine into Δ^1-piperideine, are both pyridoxal phosphate enzymes,[30] it is attractive to postulate a Schiff's base of cadaverine and pyridoxal phosphate as the bound species of cadaverine.

The model accounts for the nonsymmetrical incorporation of lysine as well as for the entry of cadaverine into the alkaloids. It remains to examine whether the model is consistent with the retention within the alkaloids of tritium from DL-[2-^3H]lysine, as was observed in the case of sedamine.[20]

If activity from DL-[2-^3H,6-^{14}C]lysine entered sedamine by the route outlined in Scheme III, the observed retention of tritium, relative to ^{14}C, is explicable only if two conditions were fulfilled: (1) that the decarboxylation of the lysine were to take place stereospecifically, yielding chirally tritiated [1-^3H,5-^{14}C]cadaverine; and (2) that this chirally tritiated cadaverine were to undergo stereospecific oxidative deamination

Scheme III. The "Chiral" Model of the Route from Lysine to the Piperidine Alkaloids[1] (Consistent with All Available Evidence)

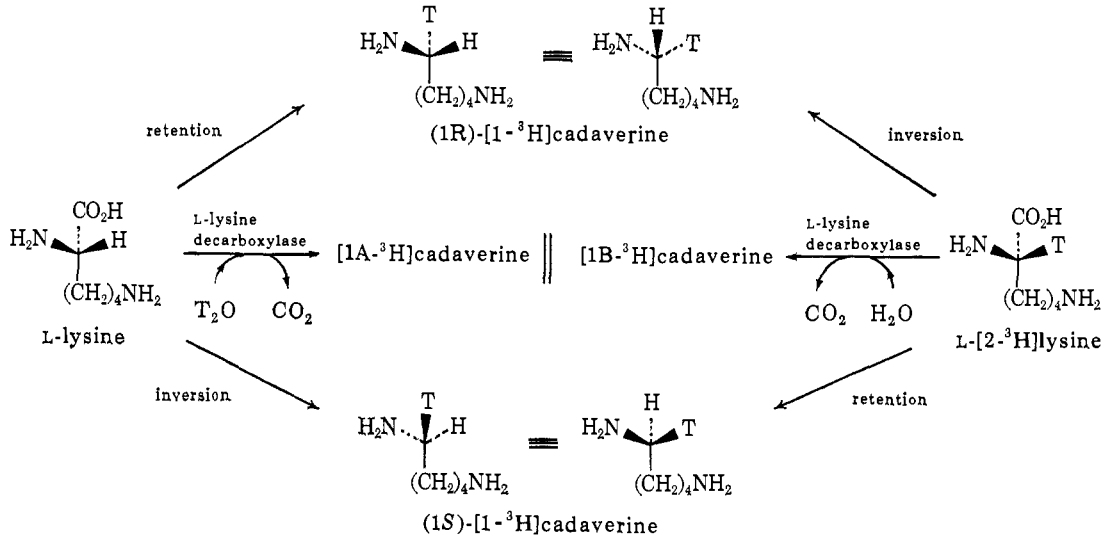


to [1-^3H,5-^{14}C]-5-aminopentanal, followed by ring closure to [2-^3H,6-^{14}C]-Δ^1-piperideine.

The decarboxylation of L-lysine, catalyzed by L-lysine decarboxylase (EC 4.1.1.18) of *Bacillus cadaveris*, is known to take place with stereochemical specificity,[31] but it is not known whether the process takes place with retention or inversion of configuration. The analogous decarboxylation of L-tyrosine, catalyzed

(28) K. Soda and M. Moriguchi, *Methods Enzymol.*, 17B, 677 (1971).
(29) E. A. Zeller, *Enzymes*, 8, 313 (1963).
(30) M. Dixon and E. C. Webb, "The Enzymes," 2nd ed, Longmans, London, 1964, pp 688, 762.
(31) S. Mandeles, R. Koppelman, and M. E. Hanke, *J. Biol. Chem.*, 209, 327 (1954).

Scheme IV. Derivation of Enantiotopically Labeled [1-^{3}H]Cadaverine



by L-tyrosine decarboxylase (EC 4.1.1.25, L-tyrosine carboxylyase), has been shown to take place with retention of configuration.[32]

Since it has been shown[21] that L-lysine but not D-lysine serves as a precursor of the piperidine nucleus, and since it can be assumed that the L-lysine decarboxylase of plants[19] like that of bacteria catalyzes a stereospecific reaction, it follows that the cadaverine generated from DL-[2-^{3}H,6-^{14}C]lysine must be chirally tritiated [1-^{3}H,5-^{14}C]cadaverine. Existing evidence thus favors the view that the first of the required conditions ((1), above), is fulfilled. While the D component of the doubly labeled lysine remains unchanged, decarboxylation of the L component of DL-[2-^{3}H,6-^{14}C]lysine with retention would yield (1*S*)-[1-^{3}H,5-^{14}C]cadaverine, whereas decarboxylation with inversion would yield (1*R*)-[1-^{3}H,5-^{14}C]cadaverine (Scheme IV).

The oxidative deamination of cadaverine and other diamines is catalyzed by diamine oxidase (EC 1.4.3.6). The stereochemistry of this reaction has not been investigated. A related reaction, the oxidative deamination of tyramine, catalyzed by monoamine oxidase (EC 1.4.3.4, monoamine:oxygen oxidoreductase (deaminating)) has been shown to take place in a sterically controlled manner. The *pro-R* proton is detached in the course of the deamination[33] and the aldehyde which is generated as the product of this reaction retains the *pro-S* proton from C-1 of the substrate.

In an attempt to test the second condition ((2), above), which must hold if the model outlined in Scheme III were to be validated, the two enantiomeric chirally tritiated [1-^{3}H]cadaverines were prepared and their incorporation into *N*-methylpelletierine and *N*-methylallosedridine was investigated. One of the samples of [1-^{3}H]cadaverine was obtained from DL-[2-^{3}H]lysine by decarboxylation catalyzed by L-lysine decarboxylase (EC 4.1.1.18) from *B. cadaveris*. The other sample was prepared similarly by decarboxylation of L-lysine in the presence of tritiated water. Since it has not been determined whether the decarboxylation of lysine, catalyzed by this enzyme, takes place with retention or inversion, the absolute configuration of the two enantiomeric samples of [1-^{3}H]cadaverine is unknown. By analogy with precedent[34] the two samples will be referred to as [1A-^{3}H]- and [1B-^{3}H]cadaverine. The designation [1A-^{3}H]cadaverine will be arbitrarily assigned to the sample obtained from L-lysine by decarboxylation in tritiated water, while the designation [1B-^{3}H]cadaverine will be given to the sample obtained by decarboxylation of L-[2-^{3}H]lysine (or, strictly speaking, of DL-[2-^{3}H]lysine). If decarboxylation of L-lysine occurred with retention, the absolute configuration of [1A-^{3}H]- would be (1*R*)-[1-^{3}H]-, and that of [1B-^{3}H]- would be (1*S*)-[1-^{3}H]cadaverine. If decarboxylation of L-lysine took place with inversion, [1A-^{3}H]- would be (1*S*)-[1-^{3}H]-, and [1B-^{3}H]- would be (1*R*)-[1-^{3}H]cadaverine (Scheme IV).

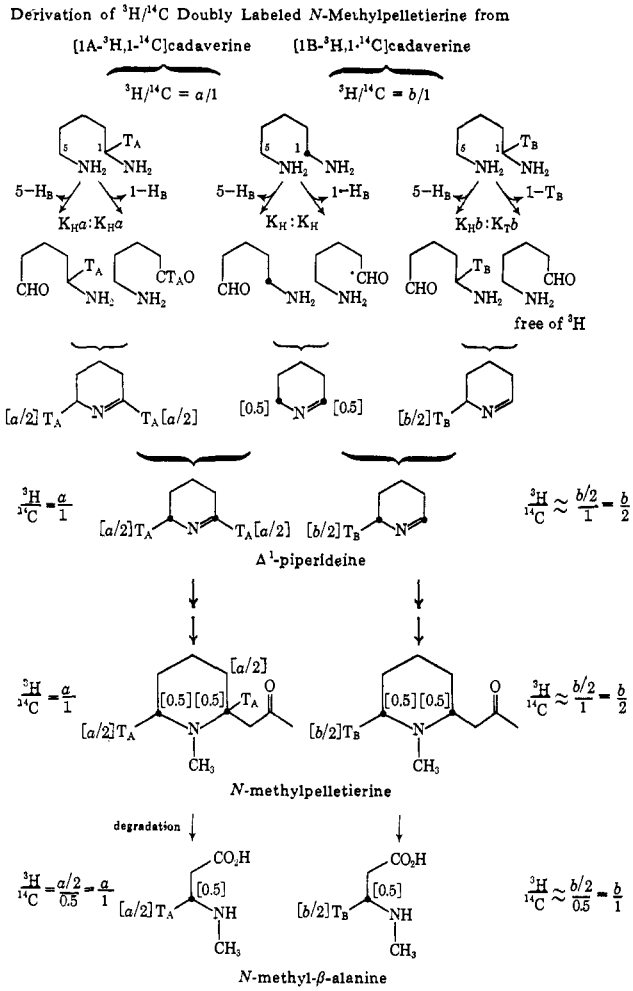
Each of the two samples of chiral [1-^{3}H]cadaverine was mixed with [1-^{14}C]cadaverine and administered to cuttings of *S. sarmentosum* in separate experiments. *N*-Methylpelletierine and *N*-methylallosedridine were isolated from the plants.

The alkaloid samples derived from [1A-^{3}H,1-^{14}C]-cadaverine retained the ^{3}H:^{14}C ratio of the doubly labeled substrate (Table II, expt iii). It is known that half of the label from [1-^{14}C]cadaverine is associated with C-6 of the alkaloids (as shown by degradation to *N*-methyl-β-alanine) (Table II, expt i and ii) and the other half is associated with C-2.[12] Since the ^{3}H:^{14}C ratio of the *N*-methyl-β-alanine (containing C-6 but not C-2 of the piperidine nucleus), obtained by degradation of the *N*-methylpelletierine derived from [1A-^{3}H,1-^{14}C]-cadaverine, was similar to that of the precursor, ^{3}H from 1-H_A of the substrate was also equally divided between C-6 and C-2. It follows that the hydrogen at C-2 of *N*-methylpelletierine was derived from 1-H_A of cadaverine (Scheme V).

The alkaloid samples derived from [1B-^{3}H,1-^{14}C]-cadaverine had lost half the tritium, relative to ^{14}C (Table II, expt iii). Since *N*-methyl-β-alanine contains one-half of the ^{14}C of the intact *N*-methylpelletierine (C-6) (Table II, expt i), and since the ^{3}H:^{14}C ratio at this site corresponds to that of the substrate (Table

(32) B. Belleau and J. Burba, *J. Amer. Chem. Soc.*, 82, 5751 (1960).
(33) B. Belleau, M. Fang, J. Burba, and J. Moran, *J. Amer. Chem. Soc.*, 82, 5752 (1960).
(34) J. W. Cornforth, R. H. Cornforth, C. Donninger, G. Popjak, G. Ryback, and G. J. Schroepfer, Jr., *Proc. Roy. Soc. Ser. B*, 163, 436 (1966).

Scheme V. Incorporation of Enantiotopically Labeled [1-^{3}H]Cadaverine (in Admixture with [1-^{14}C]Cadaverine) into *N*-Methylpelletierine. Predicted ^{3}H:^{14}C Ratios of Products Formed with Loss of the *pro-B* Hydrogen from C-1(5) of Cadaverine



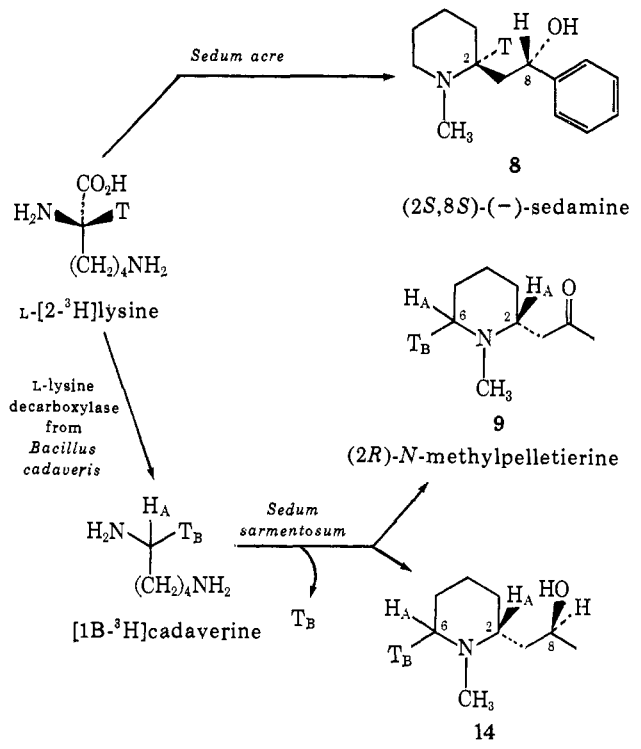
II, expt iii), it follows that all the tritium of the alkaloid derived from [1B-^{3}H,1-^{14}C]cadaverine resides at the site C-6. It then follows further that the site C-2 is free of tritium, and that 1-H_B of cadaverine was lost in the course of the sequence which converts C-1 of cadaverine into C-2 of *N*-methylpelletierine (Scheme V).[35]

It is thus clearly demonstrated that the oxidative deamination of cadaverine in *S. sarmentosum* is a stereospecific process, as required by the hypothesis for the derivation of the piperidine alkaloids from lysine, outlined in Scheme III.

This hypothesis is consistent with all available evidence derived from the incorporation of labeled substrates into the piperidine alkaloids, whose nucleus is derived from lysine in a nonsymmetrical manner. The hypothesis outlined in Scheme III thus supersedes the earlier disproven suggestions (Schemes I and II). The hypothesis accommodates the nonsymmetrical

(35) When H_B at C-1 of cadaverine is tritiated, the process leading to loss of T_B from C-1 of cadaverine is likely to be slower than the process leading to loss of H_B from C-5 of cadaverine due to a primary hydrogen–tritium isotope effect. It follows that somewhat less than one-half of the tritium, relative to ^{14}C, should be lost in the course of the incorporation of the doubly labeled cadaverine into the alkaloids. This is indeed observed (found: % retention of tritium, relative to ^{14}C, in *N*-methylpelletierine, 53 ± 2%, in *N*-methylallosedridine, 55 ± 1% (Table II, expt iii)).

Scheme VI. Two Steric Courses of the Decarboxylation of Lysine



incorporation into the alkaloids of a C_5N unit derived from L-lysine by way of bound cadaverine and of Δ^1-piperideine. It assigns a normal position to free cadaverine, and can account for the maintenance of the ^{3}H:^{14}C ratio of DL-[2-^{3}H,6-^{14}C]- and DL-[6-^{3}H,6-^{14}C]lysine and for the mode of incorporation of chirally labeled cadaverine[36] in stereochemical terms.

The same hypothesis can account also for the origin of those piperidine alkaloids into which a lysine-derived C_5N unit is incorporated in a symmetrical fashion (*e.g.*, decodine,[37] cernuine,[38] lycopodine,[39] lupine alkaloids[40]). If the equilibrium constant K_e, controlling the dissociation of bound cadaverine, favors free cadaverine (*i.e.*, K_e = [bound cadaverine]/[free cadaverine] ≤ 1), symmetrical incorporation is assured.

While the hypothesis outlined in Scheme III offers a satisfactory interpretation of the tracer evidence, a number of stereochemical problems remain to be solved.

(36) The change in the ^{3}H:^{14}C ratio observed when nonchirally tritiated [1-^{3}H,1-^{14}C]cadaverine served as a precursor of *N*-methylpelletierine (**9**)[14] can now also be explained. It was found that approximately 25% tritium was lost, relative to ^{14}C, in the course of the incorporation. This is exactly what is predicted when incorporation takes place according to Scheme V, with loss of T_B from the cadaverine carbon which generates C-2 of the alkaloid, but with retention of the other three tritium atoms. These are maintained at C-2 (T_A) and at C-6 (T_A and T_B). The loss of only 33% ^{3}H, relative to ^{14}C, rather than 50% in the conversion of [1-^{3}H,1-^{14}C]cadaverine into pseudopelletierine (**11**),[14] can be rationalized only in terms of a large primary hydrogen–tritium isotope effect in the conversion[8] of *N*-methylpelletierine into pseudopelletierine.

(37) S. H. Koo, R. N. Gupta, I. D. Spenser, and J. T. Wrobel, *Chem. Commun.*, 396 (1970).

(38) Y. K. Ho, R. N. Gupta, D. B. MacLean, and I. D. Spenser, *Can. J. Chem.*, **49**, 3352 (1971).

(39) M. Castillo, R. N. Gupta, D. B. MacLean, and I. D. Spenser, *Can. J. Chem.*, **48**, 1893 (1970).

(40) H. R. Schütte in "Biosynthese der Alkaloide," K. Mothes and H. R. Schütte, Ed., VEB Deutscher Verlag der Wissenschaften, Berlin, 1969, p 324.

The first of these concerns an apparent anomaly in the stereospecificity of the decarboxylation of lysine, catalyzed by L-lysine decarboxylase of *B. cadaveris* on the one hand and in *S. acre* plants on the other. Decarboxylation of DL-[2-^{3}H]lysine by the bacterial enzyme yielded [1B-^{3}H]cadaverine which, when administered to *S. sarmentosum*, lost tritium in the course of conversion into the alkaloids (Table II, expt iii). Yet, when the same DL-[2-^{3}H]lysine was administered to *S. acre* in admixture with DL-[6-^{14}C]lysine, all tritium, relative to ^{14}C, was recovered in the sedamine,[20] to which the L component of the doubly labeled tracer had been converted[21] (Scheme VI).

It follows either that the decarboxylation of lysine catalyzed by L-lysine decarboxylase of *B. cadaveris* takes the opposite steric course (retention or inversion) to that catalyzed by the L-lysine decarboxylase of *S. acre* or that, if both reactions take the same steric course, yielding [1B-^{3}H]cadaverine, the oxidative deamination of cadaverine in *S. acre* dislodges H_A, whereas that in *S. sarmentosum* removes H_B of cadaverine.

This problem will be resolved with the determination of the absolute stereochemistry of [1A-^{3}H]- and [1B-^{3}H]-cadaverine. This work is in hand.

A second problem which remains concerns the stereochemistry of the alkaloids. Whereas naturally occurring (−)-anabasine (**7**),[41] (−)-sedamine (**8**),[42] and (+)-sedridine[43] have been shown to possess the 2*S* configuration, naturally occurring (−)-pelletierine (**13**)[44] (from *Punica granatum*), (+)-*N*-methylallosedridine (**14**) (from *S. sarmentosum*),[45] and (−)-halosaline (**12**)[46] have the 2*R* configuration.

If Δ^1-piperideine is the common intermediate in the pathway to all these bases and acts as the acceptor of the side chain, as seems likely, the side chain precursor must enter the *si* face to yield the 2*R* alkaloids and the *re* face to give the 2*S* alkaloids (Scheme III).

The configuration of the pelletierine of *S. acre* or of the *N*-methylpelletierine of *S. sarmentosum* has not been determined. If the chirality of these compounds corresponded to that of the (2*R*)-(−)-pelletierine (**13**) of *P. granatum*,[44] then a stereospecific enzymic reduction of (2*R*)-*N*-methylpelletierine (**9**) would account for the origin of (2*R*)-(+)-*N*-methylallosedridine (**14**)[45] in *S. sarmentosum;* a simple conversion of this type cannot, however, lead from (2*R*)-(−)-pelletierine to the (2*S*)-(+)-sedridine[43] of *S. acre*.

Experimental Section

ε-*N*-Methyllysine. Extraction of Basic Amino Acids from *S. acre*. Fresh plant material (250 g) was homogenized with a small amount of water. The mixture was poured into a glass column (40 × 5 cm) and the aqueous extract was collected. The column was percolated in turn with 200 ml of methanol, 200 ml of methanol containing 2% ammonia (0.880), and again with 100 ml of methanol. To the combined eluates a small excess of hydrochloric acid was added and the mixture was evaporated until 30 ml of solvent remained. Water (20 ml) was added, and the solution was acidified by addition of hydrochloric acid (5%) to pH 2 and extracted with ether (3 × 60 ml). The pH of the aqueous layer was then adjusted to pH 9 with ammonia and the solution was extracted with dichloromethane (4 × 50 ml). The aqueous layer was then acidified (pH 6) by addition of hydrochloric acid and extracted continuously with ether for 7 hr. The aqueous solution was evaporated to dryness and the residue was repeatedly triturated with methanol. The methanol was evaporated, and the residue was dissolved in water and passed over a column (25 × 1 cm) of Amberlite IRC 50.

Acidic and neutral amino acids were eluted with water (200 ml) and basic amino acids were then eluted with ammonia (4%, 100 ml). The eluate was evaporated and the residue was dissolved in water (5 ml). A 200-μl portion was applied to the automatic amino acid analyzer (Beckmann 120 C).[47] The length of the column was modified to 16 cm. Otherwise standard conditions[48] for elution of basic amino acids (0.35 *N* citrate buffer, pH 5.28) were employed. The presence of lysine (retention time, 64 min) and ε-*N*-methyllysine (retention time, 70 min), identical with that of an authentic specimen,[22] was demonstrated.

Isolation of Labeled ε-*N*-Methyllysine from *S. sarmentosum* after Administration of L-[*methyl*-^{14}C]Methionine. Cuttings of *S. sarmentosum* (total fresh weight, 45 g) were distributed among five 30-ml beakers. L-[*methyl*-^{14}C]Methionine (nominal total activity, 100 μCi; nominal specific activity, 60 mCi/mmol, Radiochemical Centre) dissolved in glass distilled water (2.5 ml) was distributed among the beakers. After 2 hr, ε-*N*-methyl-L-lysine monohydrochloride[22] (4 mg) was added to each beaker. The cuttings were kept in contact with tracer for 24 hr.

The plant material was homogenized in a little water, the homogenate was refluxed 15 min, the mixture was poured into a glass column (40 × 5 cm), and the solid was percolated in turn with hot water (500 ml), aqueous ethanol (50% v/v, 500 ml), and ethanol (95%, 500 ml). The combined extracts were concentrated to a volume of 50 ml, the precipitate was centrifuged off, and the pellet was reextracted with hot water. The combined supernatant liquids were extracted with toluene to remove chlorophyll and the aqueous solution was concentrated and applied to a column (30 × 2 cm) of Dowex 50W-X4 (H^+ form, mesh 50–100). The column was washed with water (300 ml) and amino acids were eluted with ammonia (1 *M*). The eluate was evaporated and the residue, dissolved in a little water, was applied to Whatman No. 1 paper. The chromatogram was developed with isopropyl alcohol–0.880 ammonia–water (8:1:1). The area corresponding to ε-*N*-methyllysine (R_f 0.3) was eluted, the product was applied to a plate coated with Silica Gel G, and the thick-layer chromatogram was developed with isopropyl alcohol–0.880 ammonia (7:3) (R_f 0.2). Chromatography on Whatman No. 1 was then repeated (*n*-butyl alcohol–acetic acid–water (2:1:1)), yielding ε-*N*-methyllysine, R_f 0.3. The product was eluted with water, inactive ε-*N*-methyl-L-lysine monohydrochloride[22] (10 mg) was added as carrier, the solution was applied to a column (10 × 0.5 cm) of Dowex 50W-X4 (H^+ form, mesh 50–100), and the product was eluted with ammonia (1 *M*). The eluate was evaporated, the residue was dissolved in methanol, and ε-*N*-methyllysine was precipitated with ether, yield 6 mg; specific activity 7.70 (±0.06) × 10^5 dpm/mmol.

The product was rechromatographed (Whatman No. 1, *tert*-butyl alcohol–methyl ethyl ketone–0.880 ammonia–water (4:3:1:2)) and on radioscanning showed a single radioactive peak, R_f 0.27. In the same solvent system methionine shows R_f 0.52.

Isolation of Labeled ε-*N*-Methyllysine from *S. acre* after Administration of L-[*methyl*-^{14}C]Methionine. Cuttings of *S. acre* (total fresh weight, 40 g) were distributed among five 30-ml beakers. L-[*methyl*-^{14}C]Methionine (nominal total activity, 100 μCi; nominal specific activity, 60 mCi/mmol, Radiochemical Centre) and inactive ε-*N*-methyl-L-lysine monohydrochloride were distributed among the beakers, and the experiment continued as described in the corresponding experiment with *S. sarmentosum*. Isolation of active ε-

(41) R. Lukeš, A. A. Arojan, J. Kovář, and K. Bláha, *Collect. Czech. Chem. Commun.*, 27, 751 (1962).
(42) H. C. Beyerman, J. Eenshuistra, and W. Eveleens, *Recl. Trav. Chim.*, 76, 415 (1957); C. Schöpf, G. Dummer, and W. Wüst, *Justus Liebigs Ann. Chem.*, 626, 134 (1959).
(43) H. C. Beyerman, L. Maat, J. P. Visser, J. C. Craig, R. P. K. Chan, and S. K. Roy, *Recl. Trav. Chim.*, 88, 1012 (1969); C. Schöpf, E. Gams, F. Koppernock, R. Rausch, and R. Walbe, *Justus Liebigs Ann. Chem.*, 732, 181 (1970).
(44) H. C. Beyerman and L. Maat, *Recl. Trav. Chim.*, 82, 1033 (1963); H. C. Beyerman, L. Maat, A. van Veen, A. Zweistra, and W. von Philipsborn, *ibid.*, 84, 1367 (1965); H. C. Beyerman, L. Maat, and J. P. Visser, *ibid.*, 86, 80 (1967).
(45) H. C. Beyerman, B. S. L. Bordes, L. Maat, and F. M. Warnaar, *ibid.*, 91, 1441 (1972).
(46) K.-H. Michel, F. Sandberg, F. Haglid, T. Norin, R. P. K. Chan, and J. C. Craig, *Acta Chem. Scand.*, 23, 3479 (1969).
(47) We are indebted to Dr. W. W. Chan, Department of Biochemistry, McMaster University, for carrying out this analysis.
(48) S. Moore, D. H. Spackman, and W. H. Stein, *Anal. Chem.*, 30, 1185 (1958); D. H. Spackman, W. H. Stein, and S. Moore, *ibid.*, 30, 1190 (1958).

N-methyllysine was carried out in the same way as described above: yield of ε-*N*-methyllysine, 5 mg; specific activity, 3.34 (±0.09) × 10^4 dpm/mmol.

Isolation of Doubly Labeled ε-*N*-Methyllysine and *N*-Methylpelletierine from *S. sarmentosum* after Administration of a Mixture of L-[*methyl*-^{14}C]Methionine and DL-[6-^{3}H]Lysine (Table I, Expt i). Cuttings of *S. sarmentosum* (fresh weight 50 g) were distributed among five 30-ml beakers. A solution containing L-[*methyl*-^{14}C]-methionine (nominal total activity, 100 μCi; nominal specific activity, 60 mCi/mmol, Radiochemical Centre) and DL-[6-^{3}H]lysine (nominal total activity, 500 μCi; nominal specific activity, 5500 mCi/mmol, New England Nuclear) (total volume 2.5 ml, 0.5 ml per beaker, ^{3}H:^{14}C ratio 5.5 ± 0.1) was added. After 2 hr, ε-*N*-methyl-L-lysine monohydrochloride (total weight 20 mg, 4 mg per beaker) was added. The cuttings were kept in contact with tracer solution for 24 hr. The plants were homogenized in a little water and the homogenate was refluxed for 15 min. The material was poured into a glass column and the column percolated in turn with hot water (500 ml), aqueous ethanol (50% v/v, 500 ml), and ethanol (95%, 500 ml). Concentrated hydrochloric acid (2 ml) was added to the filtrate and the solution was concentrated to 50 ml. The precipitate was centrifuged off; the pellet was suspended in hot water and again centrifuged off. The supernatant liquids were combined, chlorophyll was extracted into toluene, and the pH of the aqueous layer was adjusted to pH 7 with ammonia. The precipitate was again centrifuged off, the pellet was washed, and the combined supernatant liquids were passed over a column (30 × 2 cm) of Amberlite IRC 50 (acetate form) which had been prepared[49] by washing, in turn, with 4% sodium hydroxide, with distilled water, with acetate buffer (1 *M*, pH 7, 200 ml), and finally with distilled water. The plant extract (at pH 7) was applied to this column. Neutral and acidic amino acids were eluted from the column with water (100 ml). The basic amino acids and most of the *N*-methylpelletierine were eluted with ammonia (0.5 *M*, 400 ml). The alkaloid was extracted from the ammoniacal eluate into dichloromethane. ε-*N*-Methyllysine was isolated from the aqueous layer (*vide infra*).

Isolation of *N*-Methylpelletierine. The dried dichloromethane extract was flushed with hydrogen chloride gas and then evaporated to dryness. Methanolic sodium hydroxide (2% w/v, 0.5 ml) was added and a portion of the solution was analyzed by radiogas chromatography (*vide infra*).

Radioactivity was associated mainly with *N*-methylpelletierine (retention time, 18.3 min) but also with *N*-methylallosedridine[50] (retention time, 19.8 min). The *N*-methylpelletierine fraction was trapped, authentic *N*-methylpelletierine[9] (0.1 ml) was added, and the product was converted into the hydrochloride salt which was purified by crystallization from methanol–ether, followed by high-vacuum sublimation at 10^{-3} mm and 125–130° and crystallization from ethyl acetate: mp 158–159° (lit.[9] mp 158–159°[9]); ^{3}H:^{14}C ratio, 0.58 ± 0.04.

Isolation of ε-*N*-Methyllysine. The ammoniacal eluate was evaporated under reduced pressure and the residue subjected to paper chromatography (Whatman No. 1 ascending; isopropyl alcohol–ammonia–water (9:1:1); ε-*N*-methyllysine, R_f 0.3). The amino acid was eluted and rechromatographed on silica gel G (isopropyl alcohol–ammonia (7:3); R_f 0.2) and again on paper in two solvent systems (Whatman No. 1, descending without overrun; *m*-cresol (redistilled) saturated with 1% ammonia; *N*-methyllysine, R_f 0.9; lysine, R_f 0.6; and Whatman No. 1, ascending; butanol–acetic acid–water (2:1:1); ε-*N*-methyllysine, R_f 0.3). In every case the amino acid was eluted with water. An authentic sample of ε-*N*-methyl-L-lysine monohydrochloride[22] (10 mg) was added to the final eluate and the solution was passed over a column (10 × 0.5 cm) of Dowex 50W-X4 (H^+). ε-*N*-Methyllysine was eluted with ammonia (1 *M*), the eluate was evaporated, and the residue was dissolved in methanol and precipitated with ether: yield, 6 mg; ^{3}H:^{14}C ratio, 7.2 ± 0.1.

Cadaverine. Large-Scale Isolation of Cadaverine[51] from *S. acre*. Fresh cuttings of *S. acre* (4 kg) were homogenized in water, the water was filtered off, and the plant material was extracted with aqueous ethanol (80% v/v) in the Soxhlet extractor for 24 hr. The extracts were combined and clarified by centrifugation, and the supernatant was acidified (pH 4) with hydrochloric acid (6 *M*). The extract was concentrated to 50 ml, some EDTA was added, and the solution was basified with ammonia and continuously extracted with benzene. The benzene layer was dried (Na_2SO_4), hydrogen chloride was passed, the mixture was evaporated to dryness, the residue dissolved in water and neutralized with ammonia, and the solution was applied to a column of Amberlite IRC 50 which had been prepared as already described (*vide supra*) The column was washed with water (400 ml), 1 *M* ammonia (400 ml), and saturated ammonium carbonate solution (400 ml). The aqueous and ammoniacal eluates were discarded. The carbonate solution was placed in a 1-l. beaker and carefully evaporated on a heating plate until 15 ml was left. The solution was allowed to stand overnight when ammonium carbonate had crystallized. The crystals were repeatedly washed with a total of 60 ml of 95% ethanol. Filtrate and washings were combined and allowed to stand until further ammonium carbonate crystallized. The supernatant was decanted, a little EDTA and a small excess of hydrochloric acid (6 *M*) were added, and the solution was evaporated. To part of the residue methanolic potassium hydroxide (2%, 0.5 ml) was added and the mixture was analyzed by vapor phase chromatography (*vide infra*). A peak with retention time of 11.9 min was detected which coincided with that of an authentic sample of cadaverine, chromatographed separately and on cochromatography.

Isolation of Labeled Cadaverine and Labeled Sedamine from *S. acre* after Administration of DL-[6-^{14}C]Lysine in the Presence of Inactive Cadaverine (Table I, Expt ii). *Sedum acre* cuttings (total fresh weight, 50 g) were distributed among five 30-ml beakers. A solution containing DL-[6-^{14}C]lysine dihydrochloride (nominal total activity, 100 μCi; nominal specific activity, 48 mCi/mmol, Commissariat à l'Énergie Atomique, France) and unlabeled cadaverine dihydrochloride (20 mg) in 1 ml of water was distributed among the beakers, and the plants were kept in contact with the substrate mixture for 24 hr.

The plant material was homogenized in a little water and poured into a glass column (40 × 5 cm), and the column percolated in turn with warm aqueous ethanol (50% v/v, 500 ml) and warm ethanol (95%, 500 ml). Concentrated hydrochloric acid (0.5 ml) was added to the combined eluates, which were then concentrated *in vacuo* to 30 ml. The pH was adjusted to 7 with ammonia, the precipitate was removed by centrifugation, the pellet was resuspended and recentrifuged, and the supernatant liquids were combined and extracted three times with toluene. The aqueous layer was applied to a column of Amberlite IRC 50 (30 × 2 cm) which had been prepared as already described. The column was eluted by the solvent sequence listed above and the cadaverine-containing fraction obtained as already described. A sample of this fraction was injected into the radiogas chromatography system (*vide infra*). A peak with retention time of 11.9 min indicated recovery of administered carrier cadaverine. Radioactivity was associated with this cadaverine peak.

Unlabeled cadaverine dihydrochloride (100 mg) and a little methanolic hydrochloric acid were added to the fraction containing cadaverine. The solution was evaporated and the residue sublimed at 10^{-3} mm. The sublimate was dissolved in methanol and product reprecipitated by addition of ether. Crystals of cadaverine dihydrochloride were collected and precipitation from methanol–ether was repeated twice. A portion was converted into the dipicrate, mp 236–237° dec (lit.[52] mp 237° dec). Another portion was converted into *N*,*N*′-dibenzoylcadaverine, mp 129–131° (lit. mp 129–131°,[53] 135°[54]).

The ammoniacal eluate of the Amberlite column was extracted repeatedly with methylene chloride. The extract was washed with water, dried (Na_2SO_4), and evaporated. Unlabeled sedamine (100 mg) was added to the residue, and the mixture was subjected to sublimation at 10^{-3} mm. The sedamine which sublimed was recrystallized from hexane and then chemically degraded, as described below.

Isolation of Doubly Labeled Cadaverine and Sedamine from *S. acre* after Administration of Doubly [^{3}H,^{14}C]-Labeled DL-Lysine (Table I, Expt iii). A mixture of DL-[4,5-$^{3}H_2$]lysine (nominal total activity, 1 mCi; nominal specific activity, 18.8 Ci/mmol, Radiochemical Centre) and DL-[6-^{14}C]lysine (nominal total activity, 0.1 mCi; nominal specific activity, 48 mCi/mmol, Commissariat à l'Énergie Atomique, France) was administered to cuttings of *S. acre* (total fresh weight, 40 g). The cuttings were kept in contact with tracer for 24 hr.

(49) J. C. Winters and R. Kunin, *Ind. Eng. Chem.*, 41, 460 (1949).
(50) We are indebted to Dr. L. Maat, Technische Hogeschool, Delft, Netherlands, for a sample of synthetic (±)-*N*-methylallosedridine.
(51) T. A. Smith, *Anal. Biochem.*, 33, 10 (1970).
(52) R. Brown and W. E. Jones, *J. Chem. Soc.*, 781 (1946).
(53) A. Ellinger, *Ber.*, 32, 3542 (1899).
(54) J. v. Braun, *ibid.*, 37, 3583 (1904).

The plant material was homogenized with a little water. Unlabeled cadaverine dihydrochloride (20 mg) was added to the homogenate from which cadaverine and sedamine were then isolated as described in the preceding experiment.

Specific Incorporation of [1-^{14}C]Cadaverine into Sedamine in *S. acre* **(Table II, Expt ii) and into *N*-Methylpelletierine in *S. sarmentosum* (Table II, Expt** i). *S. acre*. Cuttings (total fresh weight 40 g) were distributed among five 30-ml beakers and allowed to remain in contact for 24 hr with [1-^{14}C]cadaverine dihydrochloride (nominal total activity, 50 μCi; nominal specific activity, 3.2 mCi/mmol, New England Nuclear) in glass distilled water (2.5 ml). Sedamine was isolated as described in an earlier paper.[11]

S. sarmentosum. Cuttings (total fresh weight 50 g) were kept in contact with [1-^{14}C]cadaverine dihydrochloride (50 μCi) (New England Nuclear, as above) for 48 hr. *N*-Methylpelletierine was isolated by the method described in an earlier paper.[9]

The method of extraction employed in these experiments is not suitable for isolating cadaverine.[49]

Degradation of the sedamine and *N*-methylpelletierine obtained from these experiments was performed as described below. In each case *N*-methyl-β-alanine was obtained as a degradation product and isolated as the *N*-dinitrophenyl (DNP) derivative.

Preparation of Stereospecifically Labeled Cadaverines. [1A-^{3}H]Cadaverine by Decarboxylation of L-Lysine in Tritiated Water. Potassium dihydrogen phosphate (22.5 mg) and disodium hydrogen phosphate (7.9 mg) were dissolved in tritiated water (HTO, 1.1 ml, nominal total activity, 5 Ci, Amersham/Searle), and L-lysine decarboxylase (3 mg) was added. The solution was equilibrated 15 min at room temperature. L-Lysine monohydrochloride (2.1 mg) was then added. The flask was shaken at 36° in a water bath for 30 min. The solution was flushed with gaseous sulfur trioxide until the pH was 3. The solution was lyophilized and the residue dissolved in methanolic potassium hydroxide (2% w/v, 0.5 ml) and applied to chromatography paper (Whatman No. 1). The chromatogram was developed in *n*-butyl alcohol–acetic acid–water (2:1:1) (R_f values: cadaverine 0.5; lysine 0.12), dried at room temperature in the fume hood for 4 days, and cadaverine was eluted with methanol (8 ml). Radioactivity in a fraction of the methanolic solution was assayed by liquid scintillation counting. Residual tritiated water was removed as follows.

Inactive cadaverine dihydrochloride (20 mg) was added, together with a little methanolic hydrochloric acid. The cadaverine dihydrochloride was precipitated with ether. The crystals were collected and dissolved in water. The water was evaporated and the crystals were redissolved in water. Dissolving and evaporating were repeated four times until the total radioactivity in the flask remained constant (3.6 μCi).

A solution of [1-^{14}C]cadaverine dihydrochloride (nominal total activity, 1.3 μCi; nominal specific activity, 3.2 mCi/mmol) was added. The ^{3}H:^{14}C ratio of the [1A-^{3}H,1-^{14}C]cadaverine, so obtained, was 2.37 ± 0.05.

[1B-^{3}H]Cadaverine by Decarboxylation of DL-[2-^{3}H]Lysine. L-Lysine decarboxylase ("Type II," ex *B. cadaveris*, Sigma Chemical Co.) (10 mg) was dissolved in phosphate buffer (0.2 *M*, pH 6.0, 2.5 ml) and a solution of DL-[2-^{3}H]lysine (nominal total activity, 400 μCi; nominal specific activity, 35 mCi/mmol, Commissariat à l'Energie Atomique, France[20]) in water (2 ml) was added. The mixture was agitated at 36° for 20 min. L-Lysine monohydrochloride (2.5 mg) was added and incubation continued for 40 min. A radiochromatoscan of a sample of the mixture (Whatman No. 1, isopropyl alcohol–0.880 ammonia–water (8:1:1)) indicated peaks of equal area at R_f 0.1 (lysine, presumably D-lysine) and R_f 0.5 (cadaverine).

The reaction mixture was flushed with hydrogen chloride and lyophilized. The residue was suspended in methanolic potassium hydroxide (2%, 0.5 ml) and the solution applied to Whatman No. 1 paper, which was developed as before. Cadaverine (R_f 0.5) was eluted with methanol, a drop of hydrochloric acid in methanol was added, and the solution was mixed with a solution of [1-^{14}C]cadaverine dihydrochloride (nominal total activity, 5 μCi; nominal specific activity, 3.2 mCi/mmol, New England Nuclear) and the solvent was evaporated.

The residue was dissolved in water. The ^{3}H:^{14}C ratio of the [1B-^{3}H,1-^{14}C]cadaverine, so obtained, was 18.88 ± 0.43.

Administration of Chirally Labeled Cadaverines to *S. sarmentosum* (Table II, Expt iii). In each of two feeding experiments, cuttings of *S. sarmentosum* (total fresh weight 90–100 g) were distributed among ten 30-ml beakers, and the solutions of [1A-^{3}H,1-^{14}C]- and of [1B-^{3}H,1-^{14}C]cadaverine were distributed among the beakers. The plants were kept in contact with tracer solution for 48 hr.

The plant material was homogenized and the pH adjusted to 11 by addition of aqueous ammonia. The homogenate was transferred to a glass column (40 × 5 cm) and percolated first with ether saturated with water (500 ml) and then with ether, until 1 l. of eluate had been collected. The ether layer of the eluate was separated from the aqueous layer and washed with water.

The ether layer was dried (Na_2SO_4), the solvent evaporated, and the residue dissolved in a little methanol. A portion of the methanol solution (5 μl) was injected into the radiogas chromatography system described below. Two major radioactive peaks were identified as *N*-methylpelletierine and *N*-methylallosedridine.

The fractions, retention times 18.3 and 19.8 min, which emerged after passage through the 20% Carbowax column (*vide infra*) in the glc system and through the proportional counter, showed radioactivity and were trapped individually in methanol and their ^{3}H:^{14}C ratios were determined by liquid scintillation counting.

It was shown that the shorter retention time matched that of an authentic sample of *N*-methylpelletierine[9] and the longer retention time corresponded to that of *N*-methylallosedridine[50] under the same conditions. Accordingly, inactive *N*-methylpelletierine hydrochloride (100 mg) was added to the methanol solution of the column fraction, retention time 18.3 min, to which a little hydrogen chloride had been added. The resulting *N*-methylpelletierine hydrochloride was precipitated by addition of ether and recrystallized from ethyl acetate, mp 156–157°. The product which was radioactive showed the same ^{3}H:^{14}C ratio as the original column fraction, retention time 18.3 min.

The column fraction, retention time 19.8 min, showed the same ^{3}H:^{14}C ratio. This fraction had the same retention time as one of two components, presumably *N*-methylsedridine and *N*-methylallosedridine, obtained by sodium borohydride reduction of (±)-*N*-methylpelletierine. One of these had retention time 19.8 min, the other retention time 20.8 min. The two compounds were separated by glc. The mass spectrum of the isomer, retention time 19.8 min (major peaks, *m/e* 139, base peak, 98), corresponded to that of a sample of identical retention obtained by glc of the basic fraction from *S. sarmentosum* (100 g fresh weight). These two compounds also showed identical retention times (18.3 min) on a column of 5% QF-1 on Chromosorb W HMDS 60/70 mesh (4 ft × 0.125 in. o.d., stainless steel). That the material, retention time 19.8 min, is the naturally occurring enantiomer of *N*-methylallosedridine was confirmed by direct comparison (glc, mass spectrum) with a synthetic sample of (±)-*N*-methylallosedridine.[50] The two samples were found to be identical.

Degradation of Sedamine and of *N*-Methylpelletierine Hydrochloride. *N*-Methylpelletierine hydrochloride (60 mg) or sedamine (80 mg), respectively, was dissolved in a hot solution (2 ml) of chromium trioxide (510 mg) in concentrated sulfuric acid (7.6 ml) and refluxed for 7 hr. In the course of this period, ten 1-ml portions of the same chromic acid solution were added. The solution was cooled, diluted with water (10 ml), and transferred to a 1-l. beaker which was then heated on the steam bath while a hot aqueous solution of barium hydroxide (6% w/v, 500 ml) was added until the solution was slightly basic. Heating was continued for 1 hr, and the pH was then adjusted to 7 by addition of dilute sulfuric acid. The precipitate of barium sulfate was centrifuged off, the pellet was resuspended in hot water and recentrifuged, and the supernatant was filtered through a sintered glass filter to remove residual barium sulfate, and the filtrate was evaporated.

The oxidation mixture derived from sedamine, containing ω-amino acids, was treated directly with 2,4-dinitrofluorobenzene. The oxidation mixture derived from *N*-methylpelletierine was further purified as follows. The evaporated filtrate containing amino acids was passed over a column (20 × 1.3 cm) of Dowex 50W-X4 (H^+ form, mesh 100–200). The amino acids were eluted with 1 *M* ammonia. The eluate was evaporated and applied to Whatman No. 3MM chromatography paper. The chromatogram was developed in isopropyl alcohol–formic acid–water (20:1:5). The ninhydrin positive zone at R_f 0.6, corresponding to authentic *N*-methyl-β-alanine, was eluted with water.

The dinitrophenyl (DNP) derivatives of the mixed ω-amino acids from the sedamine degradation and of a portion of the *N*-methyl-β-alanine fraction from the *N*-methylpelletierine degradation were prepared as described in an earlier paper.[55]

The mixture of DNP derivatives was separated on preparative silica plates by development in the solvent system benzene–pyridine–acetic acid (40:10:1). Three bands, R_f 0.7 (unidentified), R_f 0.6

(55) R. N. Gupta and I. D. Spenser, *Can. J. Chem.*, **49**, 384 (1971).

(corresponding to that of an authentic sample of dinitrophenyl-*N*-methyl-γ-aminobutyric acid), and R_f 0.45 (corresponding to that of an authentic sample of dinitrophenyl-*N*-methyl-β-alanine (most abundant)), appeared. The yellow zone, R_f 0.45, was scraped off the plate, the DNP derivative eluted with ethanol, the solution evaporated, the residue dissolved in dilute hydrochloric acid, and the DNP derivative extracted into ether. The dried ether extract was evaporated and the residue sublimed at 160–170° and 10^{-3} mm, yielding dinitrophenyl-*N*-methyl-β-alanine (8 mg, 8%), mp 140–142° (lit.[55] mp 142–143°), mass spectrum *m/e* 269.

Radiogas Chromatography. A sample of the mixed bases dissolved in methanol (5 μl) was injected into a gas chromatograph (Varian Aerograph, 1840-1), equipped with a column (4 ft × 0.125 in. o.d., stainless steel) of 20% Carbowax 20M coated with 5% KOH on Chromosorb HMDS (mesh 60/70). Helium (40 ml/min) was passed through the column. The injector and detector were kept at 220°. The column was kept at 60° for the first 3 min after injection. The temperature was then raised at a rate of 6°/min to a maximum temperature of 220°. The effluent of the column was passed through a stream-splitter which channelled 10% of the effluent into a flame ionization detector and 90% through a heated (200°) tube into a radiogas chromatography counting system (Model 4998, Nuclear Chicago, connected to a Model 8731 single-channel rate meter, Nuclear Chicago). The counting system contained a model 461 gas flow detector (85 ml), which was kept at a temperature of 200°. The high voltage of the counting chamber with 4π geometry was set at 2000 to 2400 V. Before the effluent from the gas chromatograph had entered the counting chamber it was mixed with preheated butane gas, passing at a rate of 40 ml/min. The radioactivity was recorded on a radioactivity recorder (Model 8416, Nuclear Chicago). The time lag between mass recording and radioactivity recording depended on the gas flow rates and was 8 sec for the above rates. Column fractions emerging from the outlet of the counting chamber were trapped in methanol or methanolic hydrochloric acid. Radioactivity (or $^3H:^{14}C$ ratios) of the solutions containing the trapped column fractions was determined by liquid scintillation counting (Mark 1 liquid scintillation computer, Model 6860, Nuclear Chicago). For isolation of the labeled product, inactive carrier was added and the alkaloid purified by sublimation and recrystallization.

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Studies on Transfer Ribonucleic Acids and Related Compounds. VI.[1] Synthesis of Yeast Alanine Transfer Ribonucleic Acid 3′-Terminal Nonanucleotides and 5′-Terminal Hexanucleotides

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Abstract: The nonanucleotide, CpGpUpCpCpApCpCpA, was synthesized from the protected hexanucleotide, C^{Bz}(OBz)-p-C^{Bz}(OBz)-p-A^{Bz}(OBz)-p-C^{Bz}(OBz)-p-C^{Bz}(OBz)-p-$A^{Bz}2(OBz)_2$, in two different ways: (1) by condensation with the trinucleotide MMTr-C^{Bz}(OBz)-p-G^{iBu}(OiBu)-p-U(OBz)-p and (2) by two successive condensations involving the protected mononucleotide MMTr-U(OBz)-p and the dinucleotide MMTr-C^{Bz}(OBz)-p-G^{iBu}(OiBu)-p. In both condensations arylsulfonyl chloride was the activating reagent. The product was separated first by gel filtration on Sephadex LH-20 in the protected form, and the unprotected nonanucleotide was isolated by DEAE-cellulose chromatography in 7 *M* urea. The nonanucleotide was identified by tlc and base composition. The hexanucleotide GpGpGpCpGpU was synthesized by condensation of the protected ribodinucleotide GpGp and protected GpCpGpU using triisopropylbenzenesulfonyl chloride as the condensing reagent. The dinucleotide R-G^{iBu}(OiBu)-p-G^{iBu}(OiBu)-p was synthesized by polymerization of the mononucleotide, and the tetranucleotide G^{iBu}(OiBu)-p-C^{Bz}(OBz)-p-G^{iBu}(OiBu)-p-U(OBz) was prepared by stepwise condensation of mononucleotides. These synthetic oligonucleotides constitute double helical amino acid acceptor ends of the yeast alanine tRNA.

The synthesis of tRNA segments is important in its own right and may aid studies on the recognition of tRNA by enzymes.

Previously we have reported the synthesis of the hexanucleotide of the yeast alanine tRNA 3′ end by condensation of the trinucleotides.[2] The partially protected hexanucleotide bearing the free 5′-hydroxyl group (**1**)[3] (Chart I) was intended to be an intermediate for further elongation of the chain. The present paper describes the synthesis of the nonanucleotide having the sequence of the 3′ end of the yeast alanine tRNA (**3**) by condensation of this hexanucleotide **1** with properly protected mono- or oligonucleotides and also reports the synthesis of the ribooligonucleotide GpGpGpCpGpU (**9**) which corresponds to the 5′-terminal hexanucleotide of the same tRNA.

As shown in Chart II, the hexanucleotide **9** was synthesized by condensation of the dinucleotide R-G^{iBu}-(OiBu)-p-G^{iBu}(OiBu)-p (**7**) and the tetranucleotide G^{iBu}(OiBu)-p-C^{Bz}(OBz)-p-G^{iBu}(OiBu)-p-U$(OBz)_2$ (**8**). The homologous dinucleotide **7** was obtained by poly-

(1) Part V of this series: E. Ohtsuka, S. Morioka, and M. Ikehara, *J. Amer. Chem. Soc.*, **94**, 3229 (1972).

(2) E. Ohtsuka, M. Ubasawa, and M. Ikehara, *ibid.*, **93**, 2296 (1971).

(3) Abbreviations are as suggested by IUPAC–IUB combined commission, *J. Biol. Chem.*, **241**, 531 (1966). For the protected ribonucleotide, see ref 2.