On the Origin of Carbon 1 in Tetrahydroisoquinoline Alkaloids[1a,b]

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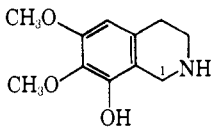
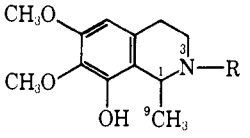
Abstract: *N*-Acetyl-*1'*-^{14}C-3-demethylmescaline-8-^{14}C was prepared and administered to peyote cactus. Anhalamine (I) and anhalonidine (II) isolated 2 weeks later were both radioactive. A systematic degradation of II established that essentially all its radioactivity was located at C-3. These results indicated that the administered *N*-acetyl compound was being deacetylated to 3-demethylmescaline prior to its incorporation into alkaloids I and II. Peyoxylic (XXVII) and peyoruvic acids (XXVI), the glyoxylic and pyruvic acid condensation products of 3-demethylmescaline, were identified in the amino acid fraction of peyote by glc–mass spectrometry of their TMS derivatives. Administration of [1,9-^{14}C]peyoxylic acid and [1-^{14}C]peyoruvic acid to peyote led to the specific incorporation of the label in C-1 of I and II, respectively, as proven by isolation and degradation studies. Facile decarboxylation of carboxyl-labeled acids XXVI and XXVII was also observed when incubated with fresh peyote slices. Work-up of decarboxylation mixture in the case of [9-^{14}C]peyoruvic acid resulted in the isolation of radioactive dihydroisoquinoline XXVIII with essentially all its activity located at C-9 as shown by degradation and suggested its intermediacy in the biosynthesis of anhalonidine. These results present the first biological support to Hahn's hypothesis regarding the origin of tetrahydroisoquinoline alkaloids in plants.

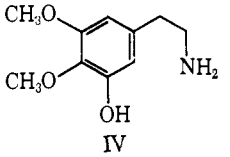
It is a matter of general agreement that the tetrahydroisoquinoline alkaloids such as anhalamine (I), anhalonidine (II), and pellotine (III) found in peyote (*Lophophora williamsii*) are derived from phenylalanine, tyrosine, or dopamine.[3,4] Recently, 3,4,5-trihydroxy-β-phenethylamine and its methyl ethers have been tested



I



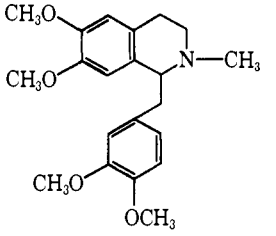
II, R = H
III, R = CH_3



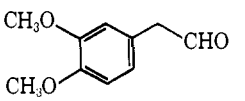
IV

as precursors of the tetrahydroisoquinoline alkaloids of peyote.[5–8] In particular, 3-hydroxy-4,5-dimethoxy-β-phenethylamine (IV, 3-demethylmescaline) served as an excellent precursor of I and II.[7,8] 3-Demethylmescaline (IV) has also been identified in the peyote cactus.[9,10]

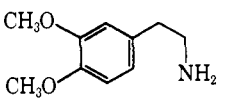
The process by which the phenethylamines cyclize to tetrahydroisoquinolines has been a matter of speculation since 1911 when Pictet and Spengler,[11] on the basis of the facile condensation of β-phenethylamine with methylal in concentrated hydrochloric acid, proposed that an analogous condensation leads to tetrahydroisoquinoline alkaloids in plants. Winterstein and Trier[12] put forward a similar proposal for the biosynthesis of benzylisoquinoline alkaloids, *e.g.*, laudanosine (V) from dimethoxyphenylacetaldehyde (VI) and dimethoxyphenethylamine (VII). This biosynthetic proposal involving condensation of appropriate phenethylamines and aldehydes was extended to the peyote alkaloids by Späth[13] in 1921. Schöpf and Bayerle,[14]



V



VI



VII

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(1) (a) Part X of the series on peyote alkaloids. Part IX: G. J. Kapadia, Y. N. Vaishnav, and M. B. E. Fayez, *J. Pharm. Sci.*, **58**, 1157 (1969); supported by research Grant No. MH-15573 from the National Institute of Mental Health. Preliminary results were presented at the 116th Annual Meeting of the American Pharmaceutical Association, Montreal, Canada, May 1969. (b) Contribution No. 103 from the Natural Products Laboratory, University of Minnesota; supported by research Grant No. GM-13246 from the U. S. Public Health Service.

(2) (a) Howard University; (b) National Heart and Lung Institute; (c) University of Minnesota; (d) M. B. E. F. on leave from the National Research Center, Cairo, U. A. R., wishes to thank the U. S. National Science Foundation for a Senior Foreign Scientist Fellowship.

(3) E. Leete, *J. Amer. Chem. Soc.*, **88**, 4218 (1966).

(4) A. R. Battersby, R. Binks, and R. Huxtable, *Tetrahedron Lett.*, 563 (1967).

(5) A. R. Battersby, R. Binks, and R. Huxtable, *ibid.*, 6111 (1968).

(6) J. Lundström and S. Agurell, *ibid.*, 4437 (1968).

(7) J. Lundström and S. Agurell, *ibid.*, 3371 (1969).

(8) A. G. Paul, K. L. Khanna, H. Rosenberg, and M. Takido, *Chem. Commun.*, 838 (1969).

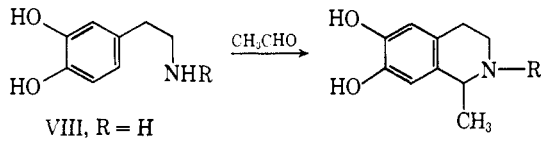
(9) S. Agurell and J. Lundström, *ibid.*, 1638 (1968).

(10) G. J. Kapadia, Y. N. Vaishnav, and M. B. E. Fayez, *J. Pharm. Sci.*, **58**, 1157 (1969).

(11) A. Pictet and T. Spengler, *Ber.*, **44**, 2030 (1911).

(12) E. Winterstein and G. Trier, "Die Alkaloide," Borntraeger, Berlin, 1910, p 307.

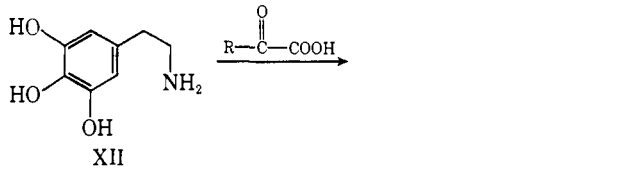
in 1934, achieved the condensation of acetaldehyde with 3,4-dihydroxyphenethylamine (VIII) and its *N*-methyl derivative (IX) under physiological conditions of pH, temperature, and concentration to produce the tetrahydroisoquinolines X and XI in excellent yields.



VIII, R = H
IX, R = CH_3

X, R = H
XI, R = CH_3

On the other hand, Hahn[15] regarded the carbonyl group of α-keto acids, such as pyruvic acid, as the source of C-1 in tetrahydroisoquinolines found in nature rather than the more reactive aldehydes indicated in the earlier



XII

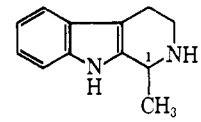
XIII

XIV

proposals. However, Whaley and Govindachari,[16] considered α-keto acids to be unlikely precursors because of their lower reactivity and the fact that the resulting condensation products (XIII) do not decarboxylate under "mild" laboratory conditions. Such reactions occur easily in the presence of plant enzymes and this criticism does not seem valid today.

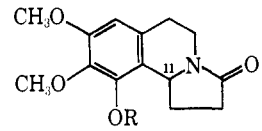
In recent years the origin of C-1 and its substituent in tetrahydroisoquinoline alkaloids has been investigated in many laboratories. Battersby, Binks, and Huxtable[4] showed that formate and *both* carbon atoms of acetate can contribute to the C-1,C-9 two-carbon unit in pellotine (III). On the other hand, O'Donovan and Kenneally[17] demonstrated that C-1 in eleagnine (XV)[18] arises almost exclusively from the carbonyl carbon of acetate in *Eleagnus angustifolia* presumably through intact incorporation. To confuse the issue further, Lundström and Agurell[6] found that methionine also serves as a source of C-1 in anhalamine (I). No simple biosynthetic scheme has appeared reconciling these facts.

The recent identification[19] of peyoglutam (XVI) and mescalotam (XVII), compounds evidently containing



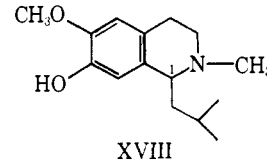
XV

the α-ketoglutaric acid moiety, in peyote, appeared to support Hahn's proposals. Further, the fact that XVI



XVI, R = H
XVII, R = CH_3

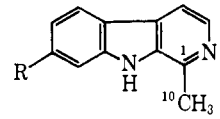
(and its C-11 carboxy precursor) formed spontaneously on admixture of 3-demethylmescaline (IV) and α-ketoglutaric acid further substantiated this theory. Based on these observations, Kapadia and Fales[19] suggested glyoxylate as the source of the C-1 methylene in anhalamine (I) and pyruvate as the source of C-1 and its attached methyl group in anhalonidine (II) and pellotine (III). Battersby, *et al.*,[4] had also suggested that pyruvate (alanine), glycine, or serine be tested as precursors of the C-1,C-9 two-carbon unit in III. In the case of lophocerine (XVIII)[20,21] and its trimer, pilocerine,[21] mevalonic acid (and to a lesser extent leucine) has been found to be incorporated specifically into the C-1 isobutyl groups of these tetrahydroisoquinolines in the cacti *Lophocereus schottii* and *Pachycereus margin-*



XVIII

atus. The intermediacy of 3-methylbutyraldehyde, which may arise from either mevalonic acid or leucine, and its condensation with an appropriately hydroxylated phenethylamine (such as VIII) have been proposed in the biosynthesis of these alkaloids.

Leete and Braunstein[22] independently considered pyruvate in the case of anhalonidine (II) and showed specific incorporation of 3-^{14}C-pyruvate into the C-9 methyl of II. Apparent support for pyruvate as the immediate precursor was forthcoming from Stolle and Gröger[23] who demonstrated that the corresponding C-1, C-10 two-carbon unit in harmine (XIX) originates from pyruvate rather than acetate in *Peganum harmala*.



XIX, R = OCH_3
XX, R = H

However, in this case it appears that pyruvate as such was not involved in the cyclization since Slaytor and

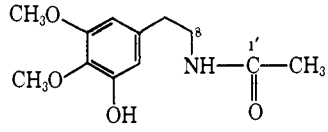
(13) E. Späth, *Monatsh.*, 42, 97 (1921).
(14) C. Shöpf and H. Bayerle, *Justus Liebigs Ann. Chem.*, 513, 190 (1934).
(15) G. Hahn, L. Barnard, O. Schales, and H. Werner, *ibid.*, 520, 107 (1935); G. Hahn and H. Werner, *ibid.*, 520, 123 (1935); G. Hahn and K. Stiehl, *Ber.*, 69, 2627 (1936); G. Hahn and F. Rumpf, *ibid.*, 71, 2141 (1938).
(16) W. H. Whaley and T. R. Govindachari, *Org. React.*, 6, 155 (1951).
(17) D. G. O'Donovan and M. F. Kenneally, *J. Chem. Soc. C*, 1110 (1967).
(18) Analogous to the tetrahydroisoquinolines, this β-carboline alkaloid is hypothetically derivable from tryptophane and acetaldehyde. An efficient synthesis of XV involving condensation of tryptamine with acetaldehyde substantiates this hypothesis: G. Hahn and H. Ludewig, *Ber.*, 67, 2031 (1934).
(19) G. J. Kapadia and H. M. Fales, *Chem. Commun.*, 1688 (1968).
(20) D. G. O'Donovan and H. Horan, *J. Chem. Soc. C*, 2791 (1968); *ibid.*, 1737 (1969).
(21) H. R. Schütte and G. Seelig, *Justus Liebigs Ann. Chem.*, 730, 186 (1969).
(22) E. Leete and J. D. Braunstein, *Tetrahedron Lett.*, 451 (1969).
(23) K. Stolle and D. Gröger, *Arch. Pharm.*, 301, 561 (1968).

Table I. Incorporation of Labeled Precursors in Peyote Alkaloids

Precursor	Spec activity, dpm/mmol	Alkaloid isolated	Spec activity, dpm/mmol	% incorporation	Carbon atom	Estimated as[a]	Spec activity, dpm/mmol	% of total activity
N-Acetyl-*1'*-^{14}C-3-demethylmescaline-*8*-^{14}C (XXI)	4.20×10^8	Anhalonidine Anhalamine[b]	2.20×10^6 2.00×10^6	0.52 0.48	C-3	Formaldehyde	2.10×10^6	95.5
[1-^{14}C]Peyoruvic acid (XXVI)	1.00×10^8	Anhalonidine	6.01×10^6	6.0	C-1	CO_2	5.93×10^6	98.7
[1,9-^{14}C]Peyoxlic acid (XXVII)	1.31×10^9	Anhalamine	8.92×10^7	6.8	C-1	CO_2	8.79×10^7	98.5
[9-^{14}C]Peyoruvic acid (XXVI)[c]	2.20×10^6	Schiff's base XXVIII	1.06×10^6	48.0	C-9	CO_2	1.05×10^6	99.1

[a] See Scheme IV and Experimental Section for details. [b] In addition to anhalonidine and anhalamine, radioactive mescaline was also isolated having an activity of $<0.01 \times 10^6$ dpm/mmol (<0.005% incorporation). [c] This experiment was performed with peyote slices.

McFarlane[24] found that *N*-acetyltryptamine serves as a direct precursor of harman (XX) in *Passiflora edulis*. It was therefore suggested by Leete and Braunstein[22] that the same process may be involved in the formation of tetrahydroisoquinoline alkaloids carrying a methyl group at C-1, especially since the requisite *N*-acetyl phenethylamine (XXI) had been detected[19] in the nonbasic fractions of peyote. The failure of acetate to be incorporated directly into the tetrahydroisoquinoline



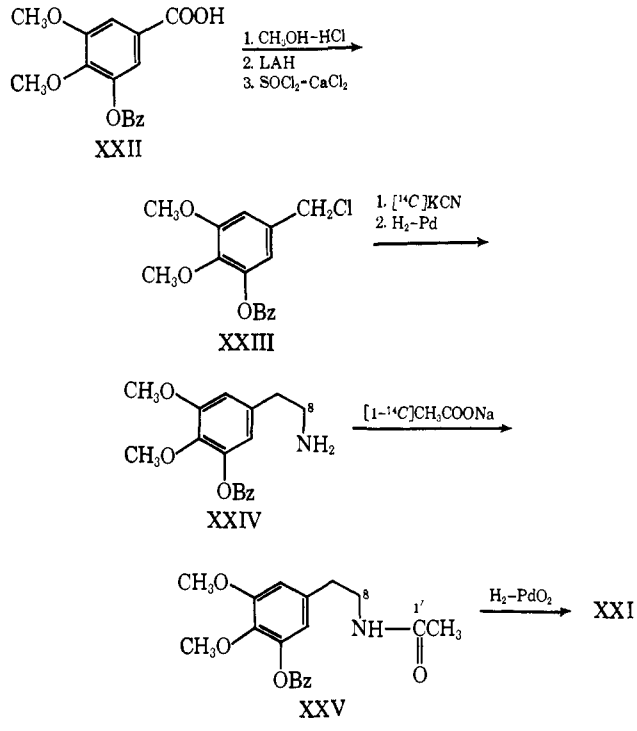
XXI

alkaloids in peyote[4] was rationalized by these same authors who suggested that the plant lacks enzymes capable of utilizing acetic acid directly for the formation of such an acetyl derivative, and a biosynthetic scheme from pyruvate → acetyl coenzyme A → XXI was proposed. Thus, the immediate progenitor of anhalonidine (II) would be *N*-acetyl-3-demethylmescaline (XXI).

This amide (XXI) has now been prepared, doubly labeled with ^{14}C at C-8 and on the *N*-acetyl group at C-1', and tested as a precursor of anhalonidine (II) in peyote. 3-Demethylmescaline (IV) has previously been synthesized by several methods.[10,25] However, the labeled compound (XXI) was prepared from 3-benzyloxy-4,5-dimethoxybenzoic acid (XXII) by a modification of the procedure of Späth and Röder[26] as outlined in Scheme I. The ratio of activity on the acetyl group to that at C-8 was 4.6.

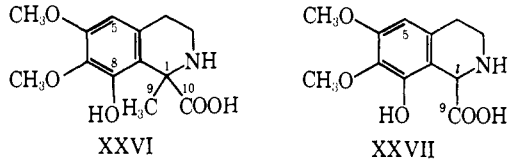
The amide XXI was injected into the bulbous stem of the cactus and 2 weeks later harvested and the alkaloids isolated without dilution.[27] High levels of activity were found in anhalamine (I) and anhalonidine (II), but negligible activity was found in mescaline.[28] A systematic degradation of II as previously described[3] indicated that more than 95% of the activity was located at C-3 (Table I). If the doubly labeled *N*-acetyl-3-demethylmescaline (XXI) had been incorporated intact, the expected activity would have been 18% (0.81/4.6). The *N*-acetyl derivative is thus apparently undergoing

Scheme I



deacetylation to 3-demethylmescaline (IV) in the cactus prior to incorporation into II.

The possibility of direct involvement of pyruvate in the cyclization of 3-demethylmescaline was suggested by the fact that IV reacts readily with pyruvic acid at pH 4.0–4.5 and room temperature to form the amino acid XXVI in nearly quantitative yield. Similar treatment of IV with glyoxylic acid gave the cyclization product XXVII. Spectral data (ir, nmr, and mass spectra)



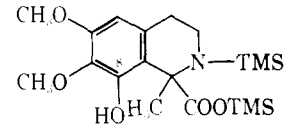
XXVI XXVII

supported the structures shown. In particular, the cyclization proceeded ortho rather than para to the phenolic hydroxyl as proved by the characteristic nmr shift of the C-5 aromatic proton in alkali.[19,29]

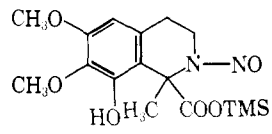
The trimethylsilyl (TMS) derivatives of acids XXVI and XXVII, used in their identification in peyote (see below), were unique in that XXVI formed a di-TMS

(24) M. Slaytor and I. J. McFarlane, *Phytochemistry*, 7, 605 (1968).
(25) J. Ratcliff and P. Smith, *Chem. Ind. (London)*, 925 (1959).
(26) E. Späth and H. Röder, *Monatsh.*, 43, 93 (1922).
(27) J. Lundström and S. Agurell, *J. Chromatogr.*, 30, 271 (1967).
(28) The low level of activity in mescaline isolated is consistent with the previous observations that 3-demethylmescaline (IV) is a poor precursor of mescaline: see ref 7 and K. L. Khanna, H. Rosenberg, and A. G. Paul, *Chem. Commun.*, 315 (1969).
(29) R. J. Highet and P. F. Highet, *J. Org. Chem.*, 30, 902 (1965).

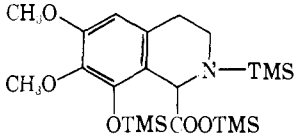
derivative (XXVIa) while XXVII afforded a tri-TMS derivative (XXVIIa). This marked effect on silylation is undoubtedly related to the steric hindrance of the



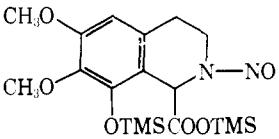
XXVIa



XXVIc



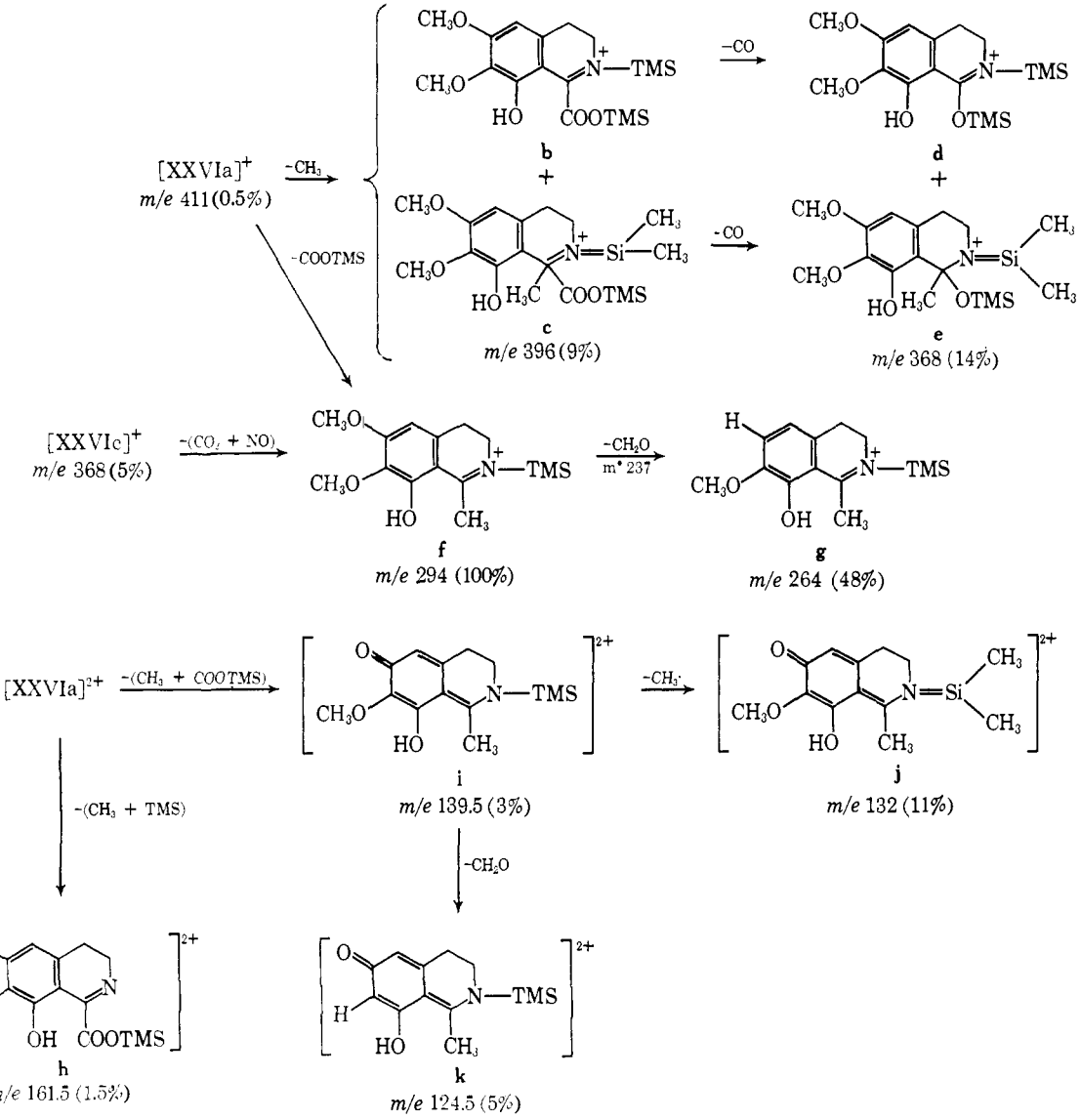
XXVIIa



XXVIIc

(*m/e* 294, **f**) in the mass spectra of XXVIa and XXVIc corresponds to the loss of –COOTMS[30] and (CO_2 + NO) groups, respectively, from the parent ions. A similar expulsion of –COOTMS and (CO_2 + NO) groups leads to the analogous base peak (*m/e* 352, **p**) in the spectra of XXVIIa and XXVIIc. The next predominant fragmentation of ion **p** (containing *N,O*-di-TMS) appears to be the loss of $(CH_3)_2$—Si=CH_2 from the *N*-TMS group with the transfer of a proton presumably to the nitrogen. On the other hand, the major pathway for the collapse of ion **f** (containing *N*-TMS) is the expulsion of CH_2O (formaldehyde) from the C-6 methoxyl group meta to the phenolic hydroxyl[31] at C-8. In the mass spectra of these TMS derivatives it is consistently observed that the loss of CH_2O is a significant event

Scheme II

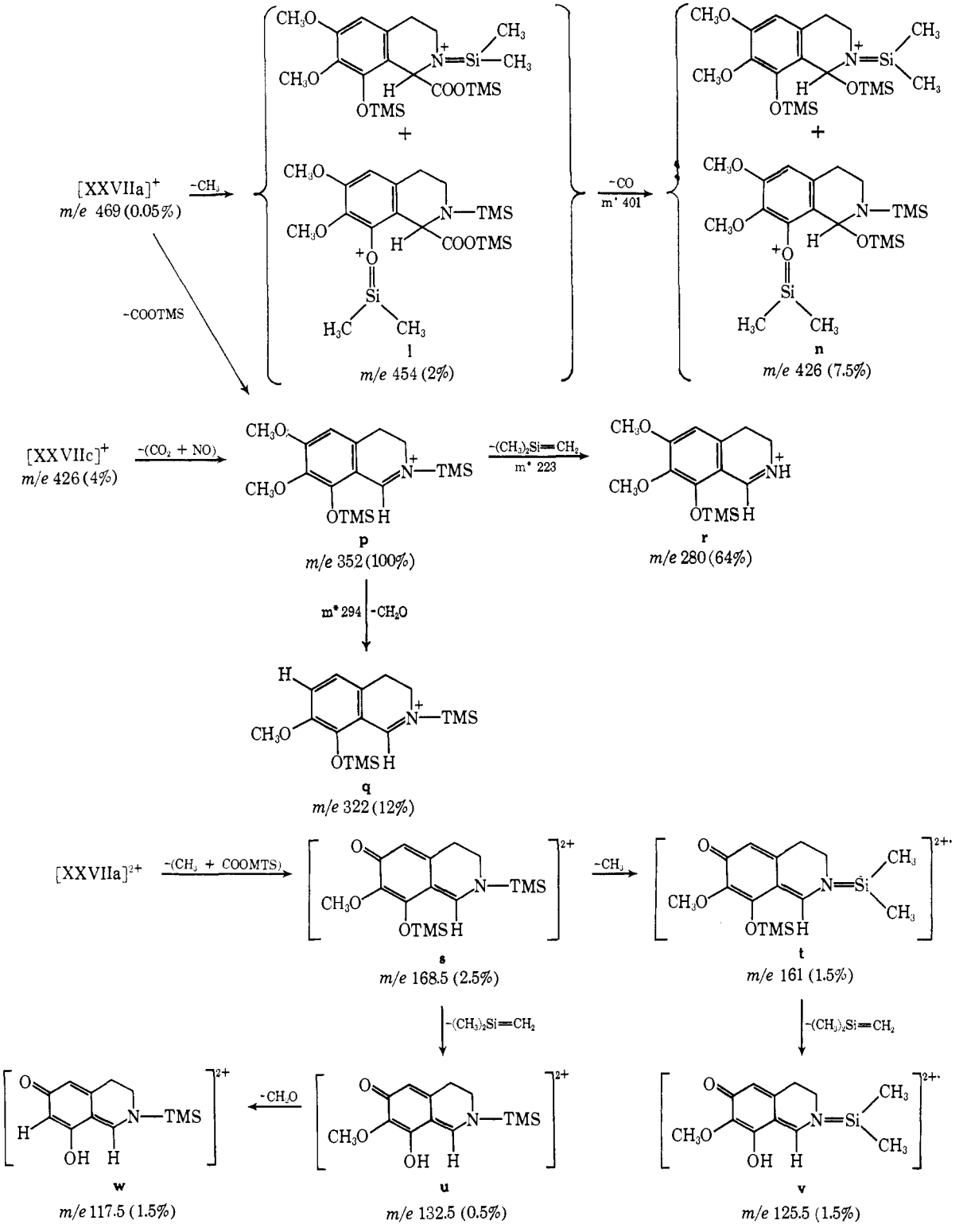


C-8 phenolic hydroxyl group in XXVI. To investigate this point further, silylation of the *N*-nitroso derivatives XXVIb and XXVIIb of the two amino acids was carried out. As expected XXVIb gave a mono-TMS derivative (XXVIc) while XXVIIb formed a di-TMS derivative (XXVIIc). Glc–mass spectrometry was employed in the characterization of these TMS derivatives of the amino acids (see Schemes II and III and the Experimental Section for the mass spectra). The base peak when the free phenolic hydroxyl group is present at C-8 as in the formulations **b**–**f**. The loss of CH_2O (12%) from the ion **p** which contains the *O*-TMS group (compared to the 64% expulsion of $(CH_3)_2$—Si=(CH_2))

(30) T. J. Sprinkle, A. H. Porter, M. Greer, and C. M. Williams, *Clin. Chim. Acta*, **25**, 409 (1969).

(31) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Mass Spectrometry of Organic Compounds," Holden-Day, San Francisco, Calif., 1967, p 246.

Scheme III



is probably due to the migration of the TMS group from the phenolic hydroxyl to C-1. The doubly charged species **h**–**k** and **s**–**w** are readily derivable from $[XXVIa]^{2+}$ and $[XXVIIa]^{2+}$, respectively, by the loss of appropriate fragments indicated in Schemes II and III.

A systematic search was then made for the presence of XXVI and XXVII in the amino acid fraction of peyote. Paper chromatography indicated their presence both by R_f values and by color reactions. Both compounds XXVI and XXVII and the amino acid fraction were converted to silyl derivatives with bis(trimethylsilyl)trifluoroacetamide (BTFSA) and subjected to glc–mass spectrometry with the results shown in Figure 1. Retention times and mass spectra correlated well, thus confirming the presence of XXVI and XXVII, now designated peyoruvic and peyoxylic acids, in peyote.

Condensation of 3-demethylmescaline (IV) with appropriately labeled pyruvic and glyoxylic acids gave the radioactive amino acids XXVI and XXVII. As pointed out by Whaley and Govindachari,[16] the 1-carboxyl-1,2,-3,4-tetrahydroisoquinolines XXVI and XXVII were found to be resistant to decarboxylation under laboratory conditions, although both underwent partial decarboxylation on glc to give products with retention times and glc–mass spectra identical with II and I, respectively. According to Hahn's hypothesis, *in vivo* decarboxylation of XXVI and XXVII would also yield anhalonidine (II) and anhalamine (I), respectively. To verify this, the radioactive acids XXVI and XXVII were

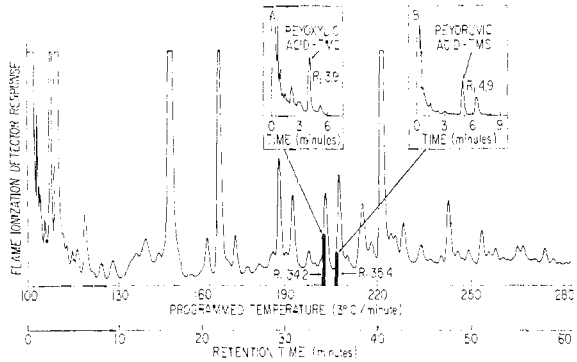
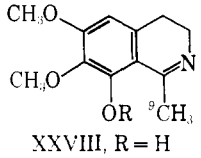


Figure 1. Glc of the silylated peyote amino acid fractions (column: 6 ft, glass, containing 1% OV-17 on 80–100 mesh Supelcoport; helium flow rate: 15 ml/min). Fractions A and B (inserts), obtained by preparative paper chromatography, were chromatographed on the same column at 190°. The unmarked peaks were not investigated further.

tested as possible precursors of alkaloids II and I in peyote cactus.

A simple test for the presence of enzymes capable of decarboxylating XXVI and XXVII was performed by incubating carboxyl-labeled peyoruvic acid (XXVI) (2.4×10^6 dpm/mmol) and peyoxylic acid (XXVII) (1.4×10^9 dpm/mmol) with fresh slices of peyote for a period of 10 hr. This led to the evolution of 47 and 31%, respectively, of the label as [^{14}C]carbon dioxide [collected in 1 *M* *p*-(diisobutylcresoxyethoxyethyl)-dimethylbenzeneammonium hydroxide (Packard Instrument Co., "Hydroxide of Hyamine 10X")]. It seems clear that this effect was not due to microbial contamination because [^{14}C]carbon dioxide was evolved rapidly at first, leveling off at ~50% after 10 hr. Further, the rate was unchanged when the slices were wet with 0.1% *p*-chlorophenol. An attempt was made to characterize the decarboxylation product in the case of [9-^{14}C]peyoruvic acid (XXVI) (2.2×10^6 dpm/mmol). Work-up of the decarboxylation mixture led to the isolation of the Schiff's base (XXVIII) with 48% of the total activity of the original acid. The Schiff's base (XXVIII) was identified by comparison of nmr and mass spectra with an authentic sample obtained by

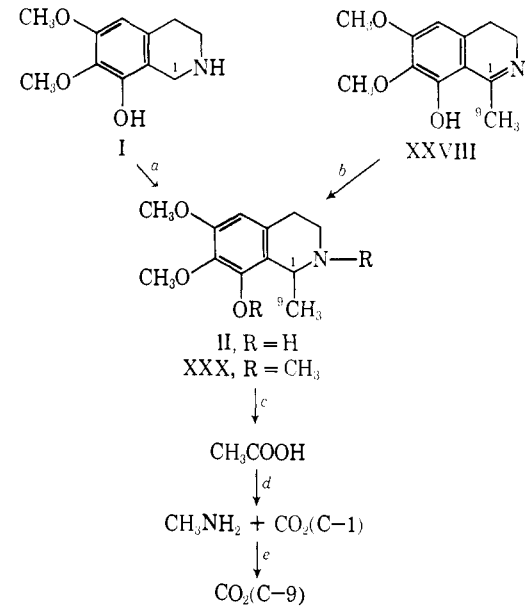


XXVIII, R = H
XXIX, R = Ac

hydrolysis of the *O*-acetyl derivative XXIX.[32] Degradation experiments (see Scheme IV) after dilution with authentic XXVIII established that essentially all the activity (Table I) resided in the predicted C-9. In all the slice experiments described here, the optically active radioactive acids used were not resolved and the yields (~50%) suggest that the decarboxylation is stereospecific.

Isolation of the Schiff's base XXVIII instead of the expected anhalonidine (II), might be taken to indicate that this substance is the direct precursor to II (*via* a stereospecific reduction by NADPH) and isolated here because of the short period of the experiment. On the other hand, some interference with the normal pathway in intact plants may have been responsible. To investigate this point further, [1-^{14}C]peyoruvic acid

Scheme VI



[a] Conversion of I to XXX: ref 6. [b] Reduction with $NaBH_4$. [c] Kuhn–Roth oxidation: E. Weissenberger, *Microchim. Acta*, **33**, 51 (1948). [d] Schmidt degradation: R. O. Brady, R. M. Bradley, and E. G. Trams, *J. Biol. Chem.*, **235**, 3093 (1960). [e] Oxidation with $KMnO_4$: ref 20.

(XXVI) (1.0×10^8 dpm/mmol) and [1,9-^{14}C]peyoxlic acid (XXVII) (1.3×10^9 dpm/mmol) were administered in separate experiments to intact peyote cacti by injection into the bulbous portions. The cacti were harvested after 3 weeks and radioactive anhalonidine (II) and anhalamine (I) were isolated without dilution and showed incorporations of 6.0 and 6.8%, respectively, of the original activity[33] from XXVI and XXVII. Degradation experiments (see Scheme IV) confirmed the location of essentially all activity in the predicted C-1 atom of radioactive I and II (Table I).

These results suggest that C-1 in anhalamine (I) originates from glyoxylate rather than formate although the latter may be incorporated also *via* a one-carbon pool. The C-1,C-9 two-carbon unit in anhalonidine (II) probably originates from pyruvate instead of ace-

(32) E. Späth, *Monatsh.*, 43, 477 (1922); E. Späth and J. Passl, *Ber.*, 65, 1778 (1932).

(33) Further work-up of the [1-^{14}C]peyoruvic acid fed plant resulted in the isolation of a water-soluble basic substance possessing about 90% of the administered radioactivity. From preliminary nmr and mass spectral data on the acetate derivative of the isolated base (mol wt 651), the unknown appears to be anhalonidine (or a derivative) attached to a polar moiety. Efforts to characterize this base are in progress.

tate; the latter may, of course, also become labeled through the same one-carbon pool. This work therefore constitutes the first biological evidence for Hahn's hypothesis regarding the origin in nature of C-1 and its substituent in tetrahydroisoquinoline alkaloids. It seems reasonable to suppose that many other α-keto acids are involved in similar condensations in the biosynthesis of alkaloids from other amino acids related to phenylalanine such as tyrosine, dihydroxyphenylalanine, as well as tryptophan and histidine.

Experimental Section

General Remarks. Melting points were determined on a hot-stage microscope and are corrected. Spectra were recorded on Perkin-Elmer Model 21 infrared, Varian HA-100 nmr, and LKB 9000 gc–mass spectrometers. Mass spectra of compounds XXI and XXIII were determined by Mr. Adrian Swanson on a Hitachi-Perkin-Elmer Model RMU mass spectrometer. Mass spectra were determined at 70 eV and only the parent and predominant peaks are reported. The abbreviation P refers to the parent ion. Nmr chemical shifts are given in parts per million downfield from tetramethylsilane as an internal standard. The abbreviations s and m refer to singlet and multiplet, respectively. Radioactivity measurements were made either on a Packard Tri-Carb Model 314-DC or a Nuclear Chicago Model 724 liquid scintillation spectrometer with the aid of the usual scintillators in toluene or dioxane.[34] All labeled compounds were recrystallized to constant activity. Analytical and preparative tlc were performed with Merck Silica Gel F-254 precoated plates, adsorbent thickness 0.25 and 2.0 mm, respectively. Elemental analyses and Kuhn–Roth oxidations were carried out by Mr. J. F. Alicino, Metuchen, N. J. Elemental analyses of compounds XXI, XXIII, and XXV were performed by Mr. Henry Isaacson at the University of Minnesota.

Peyote plants employed in this investigation were generously provided by Dr. Norman Foster, Chief Chemist, Food and Drug Administration, Dallas, Texas. [1-^{14}C]- and [U-^{14}C]glyoxylic acids and [1-^{14}C]-, [2-^{14}C]-, and [3-^{14}C]pyruvic acids utilized in the synthesis of labeled acids XXVI and XXVII were purchased from Amersham/Searle Corp., Arlington Heights, Ill. Potassium cyanide-^{14}C (New England Nuclear Corp., Boston, Mass.) and sodium acetate-*1*-^{14}C (Tracerlab, Waltham, Mass.) were employed in the synthesis of the labeled amide XXI.

3-Benzyloxy-4,5-dimethoxybenzyl Chloride (XXIII). 3-Benzoyloxy-4,5-dimethoxybenzoic acid (XXII), mp 176–177°, lit.[26] mp 170–172° (4.0 g) was dissolved in methanol (50 ml) and the solution saturated with hydrogen chloride. After refluxing for 10 hr the solution was evaporated and the residue was washed with potassium bicarbonate solution and then extracted with ether. Evaporation of the dried ($MgSO_4$) extract yielded methyl 3-benzyloxy-4,5-dimethoxybenzoate as a colorless viscous oil (4.0 g). This ester was refluxed in ether with lithium aluminum hydride (0.5 g) for 3 hr. Aqueous sodium hydroxide was then added and the ether layer separated and dried by azeotroping with a mixture of ethanol and benzene. The residual benzyl alcohol obtained on evaporation was dissolved in benzene (20 ml) and stirred with thionyl chloride (2 ml) in the presence of powdered calcium chloride (0.5 g) at room temperature for 12 hr. The mixture was then added to ice, neutralized with sodium bicarbonate, and extracted with ether. Evaporation of the dried ($MgSO_4$) extract yielded a pale yellow solid, which on crystallization from petroleum ether afforded colorless needles of 3-benzyloxy-4,5-dimethoxybenzyl chloride (1.7 g); mp 67–68°; mass spectrum *m/e* 292 and 294 (parent ions due to the Cl isotopes). *Anal.* Calcd for $C_{16}H_{17}O_3Cl$ (292): C, 65.64; H, 5.85. Found: C, 65.58; H, 5.86.

3-Benzyloxy-4,5-dimethoxyphenethylamine-*8*-^{14}C Hydrochloride (XXIV). The previously described benzyl chloride XXIII (292 mg, 1 mmol) was dissolved in dry dimethyl sulfoxide (3 ml) and stirred with potassium cyanide-^{14}C (65 mg, 1 mmol, 0.5 mCi) and potassium iodide (10 mg) for 6 hr at 100–110°. The cooled reaction mixture was then diluted with water and extracted with ether. Evaporation of the dried extract yielded the nitrile as a colorless oil which was dissolved in ethanol (50 ml) containing concentrated hydrochloric acid (0.2 ml) and hydrogenated in the presence of Adam's catalyst (0.1 g) at 3 atm pressure for 16 hr. Evaporation of the filtered reaction mixture gave a solid, which on crystallization from a mixture of ethanol and ethyl acetate afforded colorless needles of 3-benzyloxy-4,5-dimethoxyphenethylamine-*8*-^{14}C hydrochloride (116 mg); mp 147–148° (lit.[10] 150–152°); specific activity 8.1×10^8 dpm/mmol.

N-Acetyl-*1'*-^{14}C-3-benzyloxy-4,5-dimethoxyphenethylamine-*8*-^{14}C (XXV). The previously described amine XXIV hydrochloride (87 mg, 0.1 mCi) was dissolved in water (3 ml) together with sodium acetate-*1*-^{14}C (40 mg, 0.5 mCi) and ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride[35] (400 mg). After stirring at room temperature for 3 hr the reaction mixture, which contained crystals of the amide, was extracted with methylene chloride. The residue obtained on evaporation of the dried ($MgSO_4$) extract was crystallized from a mixture of benzene and petroleum ether affording colorless needles of *N*-acetyl-3-benzyloxy-4,5-dimethoxylphenethylamine (61 mg); mp 90–91°; specific activity of 4.5×10^9 dpm/mmol. *Anal.* Calcd for $C_{19}H_{23}NO_4$: C, 69.28; H, 7.04; N, 4.25. Found: C, 69.09; H, 7.12; N, 4.27.

N-Acetyl-*1'*-^{14}C-3-hydroxy-4,5-dimethoxyphenethylamine-*8*-^{14}C (XXI) (*N*-Acetyl-3-demethylmescaline). The previously described benzyl derivative XXV (43.3 mg) was dissolved in ethanol (30 ml) and hydrogenated in the presence of palladous oxide (30 mg) at 3 atm pressure for 16 hr. The filtered reaction mixture was evaporated and the residue sublimed (180°, 0.001 mm). Crystallization of the sublimate from a mixture of benzene and petroleum ether afforded colorless needles of *N*-acetyl-3-demethylmescaline (27 mg): mp 101–102°; mass spectrum *m/e* 239 (P); specific activity 4.55×10^9 dpm/mmol; the ratio of activity at the C=O group to that at C-8 = 3.74/0.81 = 4.6. *Anal.* Calcd for $C_{12}H_{17}NO_4$ (239): C, 60.24; H, 7.16; N, 5.85. Found: C, 59.96; H, 7.16; N, 5.59.

Administration[36] of N-Acetyl-*1'*-^{14}C-3-demethylmescaline-*8*-^{14}C (XXI) to Peyote Cactus and Isolation of the Radioactive Alkaloids. The labeled *N*-acetyl-3-demethylmescaline XXI (22.1 mg, 4.2×10^8 dpm) was dissolved in ethanol (0.1 ml). A drop of Tween 80 and then water (0.9 ml) was added and the resultant emulsion injected directly into the top of a peyote cactus (2 years old) by means of a hypodermic syringe. After 2 weeks, the cactus (fresh wt 69 g) was macerated in a Waring blendor with a mixture of chloroform (400 ml) and 15 *N* ammonia (10 ml). The filtered mixture was separated into an aqueous layer (1.8×10^8 dpm) and a chloroform layer (9.4×10^7 dpm). The evaporated chloroform layer was then fractionated yielding the phenolic and nonphenolic alkaloids by the method of Lundström and Agurell.[27] Preparative tlc of the nonphenolic fraction yielded mescaline isolated as its hydrochloride (21.5 mg) having an activity of $<0.01 \times 10^6$ dpm/mmol. The phenolic alkaloid fraction afforded anhalonidine hydrochloride (26.5 mg, 2.2×10^6 dpm/mmol) and anhalamine hydrochloride (8.7 mg, 2.0×10^6 dpm/mmol).

Degradation of Anhalonidine (II) derived from N-Acetyl-*1'*-^{14}C-3-demethylmescaline-*8*-^{14}C (XXI). The anhalonidine hydrochloride was degraded as previously described.[3] Methylation afforded *O,N*-dimethylanhalonidine methiodide (2.3×10^6 dpm/mmol), which on Emde reduction yielded 2-ethyl-3,4,5-trimethoxyphenethyl-*N,N*-dimethylamine, isolated as its methiodide (2.2×10^6 dpm/mmol). A Hoffmann degradation of this methiodide yielded 2-ethyl-3,4,5-trimethoxystyrene. This styrene was cleaved by treatment with osmium tetroxide and sodium metaperiodate yielding formaldehyde, collected as its dimedone derivative (2.1×10^6 dpm/mmol) and 2-ethyl-3,4,5-trimethoxybenzaldehyde, isolated as its oxime (0.1×10^6 dpm/mmol).

6,7-Dimethoxy-8-hydroxy-1-methyl-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid (Peyoruvic Acid, XXVI). A mixture of 100 mg of 3-demethylmescaline (IV) hydrochloride[10] (mp 178–179°) and 58 mg of sodium pyruvate in 0.5 ml of water was adjusted to pH 4.0–4.5 with ammonium hydroxide and left at room temperature for 48 hr. The crystalline deposit (114 mg) was collected by filtration and recrystallized from methanol to yield fine needles of XXVI: mp 233–234° dec; ir (KBr) 1640, 1600, 1565 cm^{-1} (carboxylate anion, hydrogen bonded, and free); nmr (D_2O) δ 1.87 (s, 3, C_1–CH_3), 2.90–3.14 (m, 2, C_4–H_2), 3.20–3.60 (m, 2, C_3–H_2), 3.79 (s, 3) and 3.85 (s, 3) (C_6, C_7-di-OCH_3), 6.57 (s, 1, aromatic C_5–*H*); nmr (D_2O + NaOD) δ 5.98 (s, 1, aromatic C_5–*H*) shifted upfield by 0.59 ppm. *Anal.* Calcd for $C_{13}H_{17}NO_5$: C, 58.42; H, 6.41; N, 5.24. Found: C, 58.25; H, 6.42; N, 5.25.

(34) A. R. Friedman and E. Leete, *J. Amer. Chem. Soc.*, **85**, 2141 (1963).

(35) J. C. Sheehan, P. A. Cruickshank, and G. L. Boshart, *J. Org. Chem.*, **26**, 2525 (1961).

(36) This feeding experiment was carried out at the University of Minnesota.

6,7-Dimethoxy-8-hydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic Acid (Peyoxylic Acid, XXVII). A solution of 100 mg of 3-demethylmescaline (IV) hydrochloride in water (0.5 ml) was treated with glyoxylic acid monohydrate (50 mg) and left at room temperature for 20 hr after adjusting the pH to about 4.5 with ammonium hydroxide. The crystalline product (106 mg) was recrystallized from acetone–methanol to give XXVII as fine needles: mp 237–238° dec; ir (KBr) 1625, 1600, 1575 cm^{-1} (carboxylate anion, hydrogen bonded, and free); nmr (D_2O) δ 2.86–3.10 (m, 2, C_4–H_2), 3.40–3.62 (m, 2, C_3–H_2), 3.82 (s, 3) and 3.88 (s, 3) (C_8, C_7–di-OCH_3), 5.09 (s, 1, C_1–H), 6.60 (s, 1, aromatic C_5–H); nmr (D_2O + NaOD) δ 6.03 (s, 1, aromatic C_5–H) shifted upfield by 0.57 ppm. *Anal.* Calcd for $C_{12}H_{15}NO_5$: C, 56.91; H, 5.97; N, 5.53. Found: C, 56.87; H, 5.89; N, 5.51.

Isolation of the Peyote Amino Acid Fraction. Dry powdered peyote (73 g) was defatted successively with petroleum ether (bp 30–60°) and benzene and subsequently extracted with methanol (4 l.) and methanol containing 2% ammonia (4 l.). The residues remaining from the latter two extracts (12.4 and 4.0 g, respectively), were separately dissolved in 150 ml of water, each basified with ammonium hydroxide, and extracted exhaustively with 200-ml portions of chloroform. The aqueous layers after chloroform extraction were evaporated, and the residues combined (10.15 g), dissolved in 100 ml of water, and passed through a column (110 ml) of a weak cation exchange resin [Amberlite IRC 50 (H+)].[37] The column was washed with water (1.5 l.) and the combined effluents passed through another column (110 ml) of a strong cation exchange resin (Amberlite IR 120 (H+)].[37] The material exchanged on the second column was recovered by elution with ammonia solution (5%, 400 ml, then 1%, 700 ml). A portion (0.40 g) of the residue (0.65 g) (see Figure 1 for glc of this fraction after silylation) from the eluates was fractionated by preparative paper chromatography (descending, S & S paper, *n*-butyl alcohol–acetic acid–water, 4:1:5) which indicated the presence of at least 12 products responding to the ninhydrin and Fast Blue salt B[38] spray reagents. These included proline (R_f 0.34) and two products (R_f 0.51 and 0.62) which were identical with synthetic peyoxylic (XXVII) and peyoruvic (XXVI) acids, respectively (colors: with ninhydrin XXVII (yellow) and XXVI (none); with Fast Blue salt B, both pink). Elution of the zones corresponding to the R_f values of compounds XXVI and XXVII from the paper chromatograms afforded the fractions A (38.5 mg) and B (32.0 mg) containing peyoxylic and peyoruvic acids, respectively. (See inserts A and B in Figure 1 for glc of these two fractions after silylation.)

Glc–Mass Spectrometry of the TMS Derivatives of the Amino Acids. Silylation of XXVI, XXVII, and the peyote amino acid fractions was carried out in tetrahydrofuran by the usual treatment with BTFSA.[39] The *N*-nitroso derivatives XXVIb and XXVIIb were obtained by treating the amino acids with sodium nitrite and glacial acetic acid, and the reaction products after drying under vacuum were derivatized as above. The reaction mixtures were directly injected into the glc column (see Figure 1 for details) and the mass spectra recorded as the appropriate TMS derivatives began eluting at the following retention times: XXVIIa, 3.9; XXVIa, 4.9; XXVIIc, 10.0; and XXVIc, 12.1 min. The mass spectra exhibited the following major peaks: *m/e* (relative intensity) XXVIa, 411 (0.5, P) 410 (1.5, P − 1), 396 (9, **b**, **c**), 368 (14, **d**, **e**), 366 (2, 396 − CH_2O), 338 (2.5, 368 − CH_2O), 294 (100, **f**), 264 (48, **g**), 161.5 (1.5, **h**), 139.5 (3, **i**), 132 (11, **j**), 124.5 (5, **k**); XXVIc, 368 (5, P), 294 (100, **f**), 264 (21, **g**); XXVIIa, 469 (0.05 P), 468 (0.1, P − 1), 426 (7.5 **n**), 352 (100, **p**), 322 (12, **q**), 280 (64, **r**), 168.5 (2.5, **s**), 161 (1.5, **t**), 132.5 (0.5, **u**), 125 (1.5, **v**), 117.5 (1.5, **w**); XXVIIc, 426 (4, P), 425 (12, P − 1), 352 (100, **p**), 322 (11, **q**), 280 (16, **r**). (See Schemes II and III for the structures of formulations **b**–**w**.)

6,7-Dimethoxy-8-hydroxy-1-methyl-3,4-dihydroisoquinoline (the Schiff's Base XXVIII). Treatment of 3-demethylmescaline (IV) hydrochloride[10] (0.2 g) with acetic anhydride (1 ml) and sodium acetate (1.0 g) gave 0.21 g of amorphous[32] *N*-acetyl-3-*O*-acetyl-4,5-dimethoxyphenethylamine. This *N*,*O*-diacetyl derivative (0.21 g) was cyclized by refluxing with toluene (3 ml) and phosphorous pentoxide (1 g) for 1 hr to yield 0.15 g of 3-*O*-acetyl-6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline (XXIX), mp 105–106° (lit.[32] mp 105–107°). Compound XXIX was hydrolyzed in base and the product crystallized from benzene–chloroform to afford 0.11 g of the Schiff's base XXVIII: mp 173–175°; nmr ($CDCl_3$) δ 2.83 (s, 3, C_1–CH_3), 2.49–2.90 (m, 2, C_4–H_2), 3.50–3.72 (m, 2, C_3–H), 3.80 (s, 3) and 3.88 (s, 3) (C_6, C_7–di-OCH_3), and 6.02 (s, 1, aromatic C_5–H); mass spectrum *m/e* (relative intensity) 221 (100, P) and 206 (60, P − CH_3).

[10-^{14}C]Peyoruvic Acid (XXVI). A mixture of 25 mg of 3-demethylmescaline (IV) hydrochloride and 13.5 mg of sodium pyruvate-*1*-^{14}C (0.2 μCi) in 1.5 ml of water was adjusted to pH 4.0–4.5 with ammonium hydroxide and allowed to stand at room temperature for 48 hr. The crystalline product formed was separated by centrifugation and recrystallized to constant radioactivity from acetone–methanol to yield 21.5 mg of [10-^{14}C]peyoruvic acid; mp 233–234° dec; specific activity 2.4×10^6 dpm/mmol.

[9-^{14}C]Peyoruvic Acid (XXVI). Admixture of 25 mg of IV with 13.5 mg of sodium pyruvate-*3*-^{14}C (0.2 μCi) as described above and recrystallization of the resulting product gave 20.3 mg of [9-^{14}C]-peyoruvic acid; mp 233–234° dec; specific activity 2.2×10^6 dpm/mmol.

[1-^{14}C]Peyoruvic Acid (XXVI). A 25-mg quantity of IV was mixed with 13.5 mg of sodium pyruvate-*2*-^{14}C (8.0 μCi) and the condensation product was isolated as described above and recrystallized to constant radioactivity from acetone–methanol to afford 20.9 mg of [1-^{14}C]peyoruvic acid; mp 233–234° dec; specific activity 1.0×10^8 dpm/mmol.

[9-^{14}C]Peyoxylic Acid (XXVII). A mixture of 25 mg of 3-demethylmescaline (IV) and 12.5 mg of sodium glyoxylate-1-^{14}C (25 μCi) in 1.9 ml of water was brought to pH 4.0–4.5 by the dropwise addition of ammonium hydroxide and let stand at room temperature for 12 hr. The crystalline condensation product formed was separated by centrifugation and recrystallized to constant radioactivity from methanol to yield 20.6 mg of [9-^{14}C]peyoxylic acid; mp 237–238° dec; specific activity 1.4×10^9 dpm/mmol.

[1,9-^{14}C]Peyoxylic Acid (XVII). A 35-mg quantity of IV was treated with 17.5 mg of sodium glyoxylate-U-^{14}C (50 μCi) as described above and the product recrystallized to constant radioactivity from methanol to give 31.4 mg of [1,9-^{14}C]peyoxylic acid; mp 237–238° dec; specific activity 1.31×10^9 dpm/mmol.

Incubation of [10-^{14}C]Peyoruvic Acid (XXVI) with Peyote Slices. A peyote cactus was cut into 4 circular slices of about 0.2-cm thickness and the slices were placed at the bottom of a 250-ml Erlenmeyer flask. With a Hamilton microsyringe, 50 μl of an aqueous solution containing 2.5 mg of [10-^{14}C]peyoruvic acid (specific activity 2.4×10^6 dpm/mmol) was carefully spread over the open tissues of the cactus. The evolving [^{14}C]carbon dioxide was swept into a trap containing 1 ml of 1 *M* Hyamine Hydroxide 10X (Packard) in 10 ml of scintillator solution [4.0 g of Omnifluor (New England Nuclear Corp.) in 1.0 l. of toluene] by bubbling a slow stream of nitrogen. At the end of a 10-hr incubation period, the scintillator solution was transferred to a counting vial and the radioactivity measured. Forty-seven per cent of the radioactivity from the incubated carboxyl-labeled XXVI was found to be trapped as [^{14}C]carbon dioxide.

Incubation of [9-^{14}C]Peyoxylic Acid (XXVII) with Peyote Slices. A 3.1-mg quantity of [9-^{14}C]peyoxylic acid (specific activity 1.4×10^9 dpm/mmol) was incubated with fresh peyote slices as described above and 31% of the label was accounted for as [^{14}C]carbon dioxide evolved during a 10-hr period.

Incubation of [9-^{14}C]Peyoruvic Acid (XXVI) with Peyote Slices and Isolation of [9-^{14}C]Schiff's Base XXVIII. A 4.8-mg quantity of [9-^{14}C]peyoruvic acid (specific activity 2.2×10^6 dpm/mmol) was incubated with peyote slices for a period of 10 hr as described above. The slices were then transferred to a Waring blendor and macerated with 250 ml of methanol for 5 min. The filtered methanol extract was evaporated to dryness *in vacuo* and the residue extracted with 100 ml of 1% hydrochloric acid. The acidic extract (3.6×10^4 dpm) was extracted with 300 ml of chloroform (6.0×10^3 dpm). The acidic layer was then basified with ammonium hydroxide and extracted with chloroform (5×60 ml). Negligible radioactivity was found in the chloroform extract. The aqueous basic layer was concentrated to a small volume and subjected to preparative paper chromatography (solvent system *n*-butyl alcohol saturated with water). The zone (R_f 0.75) in the chromatogram corresponding to the maximum radioactivity was cut and extracted with methanol to yield 3.7 mg of a yellow residue (1.3×10^4 dpm). Crystallization of this residue from benzene–chloroform afforded 2.3 mg of [9-^{14}C]-Schiff's base XXVIII; mp 173–175°; specific activity 1.06×10^6 dpm/mmol. The nmr and mass spectra of the isolated base were identical with that of an authentic material (above).

Administration of [1-^{14}C]Peyoruvic Acid (XXVI) to Peyote Cactus and Isolation of Radioactive Anhalonidine (II). A solution of 14.0 mg of [1-^{14}C]peyoruvic acid (1.0×10^8 dpm/mmol) in 0.1 ml of water containing a few drops of methanol was injected into the

(37) Rohm and Haas Co., Philadelphia, Pa.
(38) E. Merck, Darmstadt, W. Germany.
(39) Regisil, Regis Chemical Co., Chicago, Ill.

bulbous part of a peyote cactus with a Hamilton microliter syringe. Three weeks later the cactus was macerated in a Waring blendor with 300 ml of methanol. The methanol extract was filtered and evaporated to dryness, and the residue was extracted with 100 ml of 1% hydrochloric acid. The aqueous acidic extract (4.9×10^6 dpm) was basified with ammonium hydroxide and extracted with chloroform (6×50 ml). The pooled chloroform extract (5.1×10^5 dpm) was fractionated[27] into the phenolic and nonphenolic alkaloids, and the radioactive anhalonidine (19.8 mg, 6.01×10^6 dpm) was isolated by preparative tlc as its hydrochloride from the phenolic fraction.

Administration of [1,9-^{14}C]Peyoxylic Acid (XXVII) to Peyote Cactus and Isolation of Radioactive Anhalamine (I). A 15.5-mg quantity of [1,9-^{14}C]peyoxylic acid (1.32×10^9 dpm/mmol) was administered to a peyote cactus as described above. The plant was harvested after 3 weeks and the isolation of anhalamine hydrochloride (6.8 mg, 8.92×10^7 dpm/mmol) from the phenolic alkaloid fraction was carried out as in the case of II (above).

Degradation of Anhalonidine (II) Derived from [1-^{14}C]Peyoruvic Acid (XXVI). Kuhn–Roth oxidation of anhalonidine (19.8 mg, 6.01×10^6 dpm/mmol) gave acetic acid which was collected as sodium acetate (5.95×10^6 dpm/mmol). Schmidt[40] degradation of acetic acid gave carbon dioxide having an activity of 5.93×10^6 dpm/mmol.

Degradation of Anhalamine (I) Derived from [1,9-^{14}C]Peyoxylic Acid (XXVII). The radioactive anhalamine (6.8 mg, 8.92×10^7 dpm/mmol) was diluted with 35 mg of cold anhalamine and converted to *O*-methyl pellotine (XXX, 8.81×10^7 dpm/mmol) according to the procedure of Lundström and Agurell.[6] Kuhn–Roth oxidation of XXX gave acetic acid collected as sodium acetate (8.80×10^7 dpm/mmol) which on Schmidt degradation afforded carbon dioxide possessing an activity of 8.79×10^7 dpm/mmol.

Degradation of the Schiff's Base XXVIII Derived from [9-^{14}C]-Peyoruvic Acid (XXVI). The radioactive Schiff's base (2.3 mg, 1.06×10^6 dpm/mmol) was mixed with 25 mg of cold XXVIII and reduced with sodium borohydride to yield anhalonidine (II), specific activity 1.058×10^6 dpm/mmol. Kuhn–Roth oxidation of II afforded acetic acid collected as sodium acetate (1.051×10^6 dpm/mmol). Schmidt degradation of acetic acid liberated carbon dioxide (nonradioactive) and methylamine, which on oxidation with potassium permanganate,[20] yielded carbon dioxide having an activity of 1.05×10^6 dpm/mmol.

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(40) See Scheme IV, footnote *d*.

(41) *Cf.* A. Brossi, F. Schenker, and W. Leimgruber, *Helv. Chim. Acta*, 47, 2089 (1964), for the synthesis of these alkaloids.

Methylchlorocarbene[1]

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Abstract: Methylchlorocarbene, CH_3CCl, was generated by photolysis of methylchlorodiazirine and added to tetramethylethylene, trimethylethylene, isobutene, *cis*-butene, and *trans*-butene to give the anticipated cyclopropanes. Vinyl chloride was also formed by rearrangement of CH_3CCl. The relative reactivities (25–30°) of the above olefins toward CH_3CCl were 3.87, 2.44, 1.00, 0.74, and 0.52, respectively. In additions to trimethylethylene and *cis*-butene, CH_3CCl added so as to give mainly that cyclopropane isomer in which Cl was syn to the larger number of methyl groups. Syn:anti ratios were 1.45:1 and 2.84:1, respectively. Additions of CH_3CCl to *cis*-butene and *trans*-butene were stereospecific. Discussion of the data correlates the reactivity and stereoselectivity of several carbenes of structure XCCl.

Although there have been many quantitative studies of selectivity in carbene–olefin addition reactions, the carbenic substituents have been largely restricted to hydrogen, halogen, aryl, and carboalkoxy.[3] Those few cases in which the substituents have been "alkyl" have generally been concerned with highly unsaturated species,[4] in which intramolecular rearrangements of the carbene in solution either cannot occur, or are of rate comparable to intermolecular reaction of the carbene with an olefin.

By way of contrast, the facile and manifold rearrangements of simple alkylcarbenes[5] have precluded study of their addition to olefins, thus all but preventing assessment of the influence of simple alkyl substituents on carbene selectivity.

Methylcarbene has been successfully added to propene, but not to higher alkenes.[6] Phenylmethylcarbene, in which resonance interaction of the carbenic p orbital[7] and the phenyl group presumably slows the intramolecular 1,2-hydride shift, does add to alkenes at rates competitive with its rearrangement to styrene.[8]

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(1) Stereoselectivity of Carbene Intermediates. VII.[2]

(2) Part VI: R. A. Moss, J. R. Whittle, and P. Freidenreich, *J. Org. Chem.*, 34, 2220 (1969).

(3) R. A. Moss, in preparation.

(4) (a) M. S. Newman and T. B. Patrick, *J. Amer. Chem. Soc.*, 91, 6461 (1969); (b) H. D. Hartzler, *J. Org. Chem.*, 29, 1311 (1964), and references therein; (c) R. A. Moss, *ibid.*, 31, 3296 (1966); (d) M. Jones, Jr., A. M. Harrison, and K. R. Rettig, *J. Amer. Chem. Soc.*, 91, 7462 (1969); (e) W. M. Jones and C. L. Ennis, *ibid.*, 91, 6391 (1969), and references therein; (f) W. M. Jones, M. E. Stowe, E. E. Wells, Jr., and E. W. Lester, *ibid.*, 90, 1849 (1968), and references therein; (g) P. S. Skell and R. R. Engel, *ibid.*, 88, 3749 (1966); 87, 2493 (1965); (h) W. M. Jones, M. H. Grasley, and W. S. Brey, Jr., *ibid.*, 85, 2754 (1963).

(5) For a brief, recent review see: R. A. Moss, *Chem. Eng. News*, 47, 60 (June 16, 1969); 50 (June 30, 1969). We here omit discussion of alkylcarbenoids.

(6) (a) H. M. Frey, *J. Chem. Soc.*, 2293 (1962); *Chem. Ind. (London)*, 218 (1962). (b) See, however, H. M. Frey and I. D. R. Stevens, *J. Chem. Soc.*, 1700 (1965).

(7) We assume sp^2 hybridization for a singlet carbene.

(8) (a) G. L. Closs and J. J. Coyle, *J. Org. Chem.*, 31, 2759 (1966); (b)