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BIOSYNTHESIS OF *N*-METHYLPELLETIERINE[1]

R. N. GUPTA and IAN D. SPENSER

Department of Chemistry, McMaster University, Hamilton, Ontario, Canada

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Abstract—The biosynthesis of *N*-methylpelletierine (VII) was studied in excised shoots of *Sedum sarmentosum*. Consistent with classical biogenetic concepts, the piperidine nucleus of the alkaloid is generated from lysine, which is incorporated by way of nonsymmetrical intermediates, very probably ϵ-amino-α-ketocaproic acid and Δ^1-piperideine-2-carboxylic acid. The propanone side-chain originates from acetate. Direct incorporation into the side-chain of an intact three-carbon unit derived from acetoacetate could not be demonstrated.

INTRODUCTION

ACCORDING to classical biogenetic concepts[2,3] the skeleton of pelletierine[4] (VI), one of the simple piperidine alkaloids, is derivable from lysine (I) and acetoacetate. Successful model syntheses[5–16] based on the reaction sequence (V)→(VI) have lent verisimilitude to this view.

It has been shown by biosynthetic tracer experiments that the nucleus of several naturally occurring 2-substituted piperidine derivatives (pipecolic acid[17] (III), sedamine[18] (X), anabasine[19–21] (IX)) is indeed derived from lysine. It is the ϵ-amino group of lysine which supplies the nitrogen atom of the piperidine ring,[17,20] and it is the α-carbon atom of lysine which is transformed into C-2 of the piperidine nucleus.[18–21] It is evident from these observations that lysine is incorporated into the piperidine nucleus in a nonsymmetrical manner, and it can be inferred that the nonsymmetrical intermediate is Δ^1-piperideine-2-carboxylic acid (II), arising by α-deamination of lysine and spontaneous cyclization of the resulting ϵ-amino-α-ketocaproic acid. Reduction of Δ^1-piperideine-2-carboxylic acid (II) yields pipecolic acid[17] (III). Decarboxylation of Δ^1-piperideine-2-carboxylic acid (II) gives Δ^1-piperideine (V), a

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[16] R. N. GUPTA and I. D. SPENSER, *Can. J. Chem.* **47**, 445 (1969).
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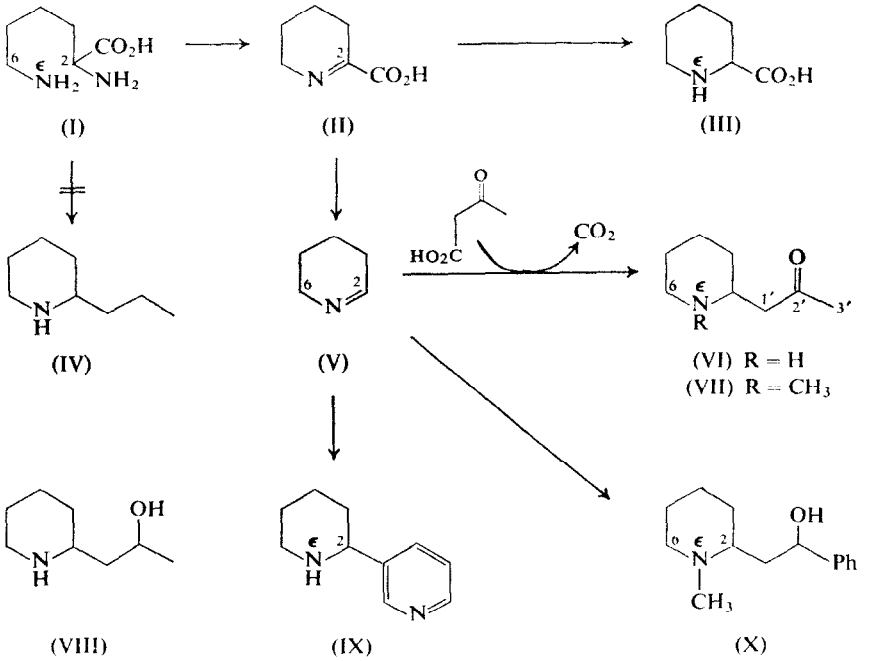
precursor of the piperidine nucleus of anabasine[22] (IX) and probably also of that of sedamine[18] (X). Since incorporation of lysine (I) and of Δ^1-piperideine (V) takes place in a nonsymmetrical manner, a symmetrical compound such as cadaverine (1,5-diaminopentane) cannot be a normal intermediate. Nevertheless, cadaverine was found to serve as a precursor of anabasine,[23] presumably because it is convertible into Δ^1-piperideine when supplied to the system.



Another group of 2-substituted piperidine derivatives, the alkaloids of hemlock (e.g. coniine (IV)), are not derived from lysine. Radioactivity from U-^{14}C-lysine,[24,25] U-^{14}C-Δ^1-piperideine-2-carboxylic acid[25] and U-^{14}C-Δ^1-piperideine[25] enters the hemlock bases. Incorporation of this activity cannot be direct, however, since it has been established by unequivocal degradation of coniine and conhydrine, isolated from hemlock plants after administration of 1-^{14}C-acetate, that the carbon skeleton of these alkaloids is polyketide in origin.[26]

We have investigated the biosynthesis of *N*-methylpelletierine (VII) in *Sedum sarmentosum*. The carbon skeleton of this compound corresponds to that of coniine (IV) and might be assumed, on structural grounds, to be of similar, polyketide, origin. The structural analogy of the pelletierine skeleton with that of sedamine (X) is equally obvious, and derivation from lysine, consistent with classical concepts, equally plausible. The results here reported demonstrate that the piperidine ring of *N*-methylpelletierine, like that of sedamine and anabasine, but unlike that of coniine, is derived from lysine.

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RESULTS AND DISCUSSION

Origin of the Nucleus of N-*Methylpelletierine*

Radioactive *N*-methylpelletierine was obtained from excised shoots of *Sedum sarmentosum* which had been kept in contact with 6-^{14}C-DL-lysine. Degradation of the product showed (Table 1) that all activity was confined to C-6 of the piperidine nucleus, demonstrating non-random and non-symmetrical incorporation of label.

TABLE 1. DEGRADATION OF LABELLED *N*-METHYLPELLETIERINE OBTAINED FROM LYSINE AND FROM β-HYDROXYBUTYRATE

Precursor	6-^{14}C-DL-Lysine		Sodium 3-^{14}C-DL-β-hydroxybutyrate		Sodium 4-^{14}C-DL-β-hydroxbutyrate	
Compound	SA*	RSA†	SA	RSA	SA	RSA
N-Methylpelletierine	6·58±0·17	100±3	0·93±0·04	100±4	2·31±0·06	100±2
Formaldehyde (C-6) (as dimedone derivative)	6·36±0·19	97±4	—	—	—	—
N-Methylpipecolic acid (nucleus+C-1′)	—	—	0·17±0·01	18±1	1·27±0·04	55±2
Acetic acid (C-2′+C-3′) (as α-naphthylamide)	—	—	0·73±0·02	79±4	—	—
Benzoic acid (C-2′)	—	—	—	—	0·07±0·01	3±1
Methylamine (C-3′) (as picrate)	—	—	—	—	0·78±0·02	34±1

* SA = Specific activity (cpm m mole^{-1}) × 10^{-4}.
† RSA = Relative specific activity (per cent) (*N*-methylpelletierine = 100).

Two further experiments strengthen the evidence that an intact C_5N chain, derived from lysine, supplies the skeleton of the piperidine nucleus of the product, and that it is the ε-amino group of lysine which provides its nitrogen atom. *N*-Methylpelletierine, isolated from shoots to which doubly labelled 4,5-$^3H_2$,6-^{14}C-DL-lysine had been administered, showed a $^3H/^{14}C$ ratio identical with that of the precursor (Table 2). Since the catabolic degradation of lysine is accompanied by complete removal of hydrogen from C-4,[27] preservation of the $^3H/^{14}C$

TABLE 2. INCORPORATION OF DOUBLY LABELLED LYSINES INTO *N*-METHYLPELLETIERINE AND PIPECOLIC ACID

	$^3H/^{14}C$ ratio		
	Of precursor	Of products	
Precursor		*N*-Methylpelletierine	Pipecolic acid
4,5-$^3H_2$,6-^{14}C-DL-Lysine	7·4±0·1	7·5±0·2	—
6-^{3}H,6-^{14}C-DL-Lysine	18·3±0·4	18·5±0·2	18·3±0·2

[27] A. MEISTER, *Biochemistry of the Amino Acids*, Vol. 2, Chapter 6P, 2nd edition, Academic Press, New York (1965).

ratio in this experiment indicates preservation of the integrity of at least three of the carbon atoms of lysine. When 6-^{3}H,6-^{14}C-DL-lysine served as the precursor of *N*-methylpelletierine, the $^3H/^{14}C$ ratio was again maintained. Furthermore, an identical $^3H/^{14}C$ ratio was found also in pipecolic acid, obtained from the same experiment (Table 2). Maintenance of the $^3H/^{14}C$ ratio is consistent with an α-deamination or α-transamination of lysine to ϵ-amino-α-ketocaproic acid and Δ^1-piperideine-2-carboxylic acid, on route to pipecolic acid and also to *N*-methylpelletierine. On the other hand, ϵ-deamination of lysine, with the formation of α-aminoadipic-δ-semialdehyde and Δ^1-piperideine-6-carboxylic acid, should have been accompanied by loss of tritium relative to carbon, and would have been reflected in a lowering* of the $^3H/^{14}C$ ratio in the product.[17]

The finding that label from 6-^{14}C-lysine enters C-6 of the piperidine nucleus, exclusively, reinforces the view that Δ^1-piperideine-2-carboxylic acid, rather than Δ^1-piperideine-6-carboxylic acid, is the biosynthetic intermediate. Decarboxylation of either of these compounds yields Δ^1-piperideine. The position of the double bond in Δ^1-piperideine is now known to be fixed.[16, 22] It follows that conversion of 6-^{14}C-lysine into Δ^1-piperideine (V) via Δ^1-piperideine-2-carboxylic acid (II) must yield 6-^{14}C-Δ^1-piperideine, whereas conversion via Δ^1-piperideine-6-carboxylic acid must lead to 2-^{14}C-Δ^1-piperideine.

It is likely that attachment of the side-chain occurs at C-2, the electrophilic center. It is this carbon which has been shown to be the sole site of activity in sedamine[18] (X) and in anabasine[19–21] (IX), derived from 2-^{14}C-lysine. It is C-6 which is labelled exclusively in *N*-methylpelletierine (VII) and in sedamine[18] (X), when 6-^{14}C-lysine serves as the precursor. These results are consistent with the intermediacy of Δ^1-piperideine-2-carboxylic acid, i.e. with α-deamination of lysine.

The observations that, in *S. sarmentosum*, activity from 6-^{14}C-DL-lysine enters *N*-methylpelletierine (VII) non-randomly, and that doubly labelled lysine yields *N*-methylpelletierine and pipecolic acid (III) without change in $^3H/^{14}C$ ratio, parallels our earlier findings[17, 18] on the incorporation of singly and doubly labelled lysine into sedamine (X) and pipecolic acid (III) in *S. acre*. They show that the piperidine nucleus of *N*-methylpelletierine in *S. sarmentosum*, like that of sedamine[17, 18] in *S. acre* and that of anabasine[19, 20] in *Nicotiana glauca*,is generated from lysine by way of nonsymmetrical intermediates, very probably ϵ-amino-α-ketoadipic acid, Δ^1-piperideine-2-carboxylic acid (II) and Δ^1-piperideine (V),[17, 22] in which the ϵ-amino group of lysine is preserved.

Cadaverine, a symmetrical molecule, cannot be a normal intermediate. However, incorporation of label from 1,5-^{14}C-cadaverine into *N*-methylpelletierine in *Punica granatum* has recently been reported.[28] Activity from 2-^{14}C-lysine enters the nucleus of the alkaloid.[29] The status of cadaverine in the biosynthesis of *N*-methylpelletierine in *P. granatum* remains to be clarified. If it should be established by degradation that activity from 2-^{14}C-lysine is confined to C-2 of the nucleus and that incorporation of lysine into *N*-methylpelletierine is nonsymmetric in *P. granatum*, as it is in *S. sarmentosum*, the position of cadaverine in *N*-methylpelletierine biosynthesis,[28] similar to its status in anabasine biosynthesis,[23] must be that of a compound which is not normally implicated in the pathway, but can yield a normal intermediate (presumably Δ^1-piperideine) when presented to and metabolized by the tissue.

* The possibility that maintenance of the $^3H/^{14}C$ ratio is the fortuitous result of a combination of isotope effects, accompanying the ϵ-deamination of lysine and the further reaction of the products, cannot be completely exluded, but is regarded as remote (cf. Ref. 17).

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Origin of the Side-chain of N-*Methylpelletierine*

Acetoacetate has long been regarded as the hypothetical precursor of the propanone side-chain of pelletierine[2,3] and serves as its source in model syntheses.[5–16] These are based on Mannich condensation of Δ^1-piperideine with the β-keto acid, which spontaneously decarboxylates in the course of the reaction.

Specific incorporation of radioactivity from ^{14}C-labelled acetate, a potential precursor of acetoacetate, into the propanone side-chain of *N*-methylpelletierine has been demonstrated in *P. granatum*.[28,29] Over 90 per cent of the activity of *N*-methylpelletierine, derived from 1-^{14}C-acetate, was located at the carbonyl carbon atom (C-2′).[29] The *C*-methyl group of the side-chain (C-3′) contained 44 per cent of the activity of the alkaloid, obtained from 2-^{14}C-acetate.[28] These results are consistent with the notion that the propanone side-chain represents a three-carbon unit generated from acetoacetate by decarboxylation. Since acetate can supply carbon for the biosynthesis of lysine,[27] it is not surprising that approximately 10 per cent of label from 1-^{14}C-acetate, and approximately 12 per cent of activity from 2-^{14}C-acetate (assuming equal labelling at C-3′ and C-1′) should enter the lysine-derived piperidine nucleus.

It was of interest to test whether the propanone side-chain of *N*-methylpelletierine was indeed derived from an intact three-carbon unit related to acetoacetate. Incorporation of label from 3-^{14}C- and from 4-^{14}C-DL-β-hydroxybutyrate was therefore tested.* Partial degradation of the labelled products (Table 1) indicated that in each case activity from these substrates had entered the alkaloid indirectly, since label was not confined to the predicted site (C-2′ from 3-^{14}C-β-hydroxybutyrate and C-3′ from 4-^{14}C-β-hydroxybutyrate). The observed distribution of activity is very similar to that found when 1-^{14}C-acetate[29] and 2-^{14}C-acetate,[28] respectively, served as precursors of *N*-methylpelletierine. The incorporation pattern is consistent with the view that, prior to incorporation, 3-^{14}C-β-hydroxybutyrate had undergone biological degradation to 1-^{14}C-acetate, and 4-^{14}C-β-hydroxybutyrate to 2-^{14}C-acetate (or to the correspondingly labelled acetyl-CoA), presumably via acetoacetate and acetoacetyl-CoA.

The mode of incorporation of acetate into the side-chain thus remains an open question. The observations do not disprove the mechanism postulated by the classical hypothesis of biogenesis, but point to a rapid equilibration of acetoacetyl-CoA and acetyl-CoA by the β-ketothiolase reaction, whose equilibrium constant is known[30] to favour acetyl-CoA.

The side-chain of sedamine (X) is not of polyketide origin but is specifically derived from phenylalanine.[18] It was conceivable that the side-chain of pelletierine might originate from an analogous precursor. When labelled α-aminobutyric acid was administered to *S. sarmentosum*, the *N*-methylpelletierine which was isolated contained negligible radioactivity. Structural analogy thus does not indicate analogy in biosynthetic origins.

Alkaloids of Sedum acre

For the investigation of the biosynthesis of a Sedum alkaloid with a skeleton analogous to that of coniine, it had been our original intention to use *S. acre*, the species we had employed

* This compound rather than acetoacetate was chosen, since it was less likely to undergo chemical degradation as a result of experimental manipulation. A metabolic relationship of β-hydroxybutyrate and acetoacetate in *Sedum*, as in other tissues, is a plausible assumption.

[30] L. JAENICKE and F. LYNEN, in *The Enzymes* (edited by P. D. BOYER, H. LARDY and K. MYRBACK), Vol. 3, p. 3, Academic Press, New York (1960); F. LYNEN and M. TADA, *Angew. Chem.* **73**, 513 (1961); F. LYNEN, *Pure Appl. Chem.* **14**, 137 (1967).

in our earlier work on the origin of sedamine[18] (X). In addition to sedamine,[31–34] this species is reported to contain pelletierine[33] (VI), and the corresponding secondary alcohol sedridine[32–36] (VIII), as well as two isomeric α,α′-disubstituted piperidine derivatives, $C_{17}H_{25}NO_2$, sedinine and sedinone.[33, 34, 37] It has been reported that *S. acre* of Dutch[35] and of German[36] origin does not contain sedamine (but cf. Refs. 32–34), whereas *S. acre* collected in Canada yields sedamine as the sole alkaloid.[38] We were unable to detect pelletierine or sedridine in *S. acre*, collected in the Royal Botanical Gardens, Hamilton, and propagated in the greenhouse. Carrier pelletierine and sedridine was added to separate portions of the basic fraction obtained by methanol extraction of shoots of *S. acre* which had been kept in contact with 6-^{14}C-DL-lysine.[18] The samples of pelletierine and sedridine which were re-isolated were inactive, even though the basic fraction was highly radioactive and yielded radioactive sedamine (X) whose label was localized at C-6 of the piperidine nucleus.[18]

Gas–liquid chromatography of the basic fraction, isolated from *S. acre*, showed sedamine to be the major component. Two other components were detected which differed in retention time from pelletierine or sedridine. Mass spectrometry of the basic fraction revealed signals *m/e* 219, 273 and 275 (cf. sedamine, molecular weight 219; sedinine and sedinone, molecular weight 275). Signals corresponding to the molecular ions of pelletierine, sedridine and nicotine[31] (molecular weight 141, 143, and 162 respectively) were not observed. Since the variety of *S. acre* at our disposal did not contain pelletierine or sedridine, *S. sarmentosum*, from which *N*-methylpelletierine had been isolated as the major alkaloid,[39] was used for our investigation.

EXPERIMENTAL

(±)-N-*Methylpelletierine*

(±)-*N*-Methylpelletierine was prepared (cf. Refs. 5, 7, 12) by condensation of acetoacetic acid with the $LiAlH_4$ reduction product of *N*-methyl-α-piperidone.[6]

$LiAlH_4$ (1·9 g, 0·05 mole), suspended in anhydrous ether (100 ml), was added in small portions, over a period of 30 mins, to a stirred, refluxing, solution of *N*-methyl-α-piperidone (11·3 g, 0·1 mole) in anhydrous ether (100 ml). Refluxing and stirring was continued for a further 30 mins. The mixture was cooled and excess hydride was decomposed by dropwise addition of cold water. The ether layer was decanted, the residue was washed with ether (2 × 20 ml), and the washings added to the ether layer, which was then mixed with an aqueous solution of sodium acetoacetate (prepared from ethyl acetoacetate (32·5 g, 0·25 mole) by hydrolysis with 500 ml 0·5 M NaOH). 0·5 M HCl was added in portions and the mixture was shaken until the pH of the aqueous phase was 5. Ether was now removed and the aqueous solution kept at room temperature for 48 hr. KOH (10% w/v) was added and the solution was extracted with ether. The ether extract was washed with water, and was then extracted with 0·1 M HCl. The acid solution was made alkaline, and the product was finally extracted into ether. The ether solution was dried (Na_2SO_4), the solvent was evaporated and the residue distilled at 0·1 mm and 110–120° when (±)-*N*-methylpelletierine (4·5 g, 30 per cent yield) was obtained. A portion of the product was converted to the hydrochloride, m.p. 158–159°, after recrystallization from methanol–ether mixture and sublimation at 10^{-3} mm and 120–130°. Reported m.p. 157–158°;[6] 158–159°.[7] Another portion was converted to the picrate, m.p. 156–157° after recrystallization from methanol. Reported m.p. 155°;[5] 157–158°;[6] 160°.[39]

[31] L. MARION, *Can. J. Research* **23B**, 165 (1945); L. MARION, R. LAVIGNE and L. LEMAY, *Can. J. Chem.* **29**, 347 (1951).
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[39] L. MARION and M. CHAPUT, *Can. J. Research* **27B**, 215 (1949).

Degradation of N-*Methylpelletierine*

Oxidation to N-*Methylpipecolic Acid and Acetic Acid*

CrO_3 (200 mg) in water (0·2 ml) was added to a solution of (±)-*N*-methylpelletierine hydrochloride (200 mg) in H_2SO_4 (1 ml, 20% v/v), and the mixture kept at 85–90° for 45 min. Steam was then passed through the reaction mixture until about 150 ml distillate had been collected. *N*-Methylpipecolic acid, in 80 per cent yield, was isolated from the reaction mixture, as already described.[18] The distillate, containing HOAc, was titrated to pH 9 with NaOH (0·1 M), and the solution evaporated. The resulting NaOAc was either converted to the α-naphthylamide[40] or subjected to the Schmidt reaction[41] to yield CH_3NH_2, which was isolated as the picrate.

Formaldehyde from Carbon-6

A solution of (±)-*N*-methylpelletierine picrate (1 g) in methanol was mixed with a suspension of Rexyn AG1 (OH^- form) (20 g) in water (20 ml). The mixture was shaken 6 hr, was filtered through glass wool and the residue was washed with methanol. Filtrate and washings were concentrated *in vacuo* and the oily residue dissolved in methanol (5 ml). $NaBH_4$ (0·5 g) was added in small portions and the mixture was kept overnight. Solvent was evaporated, and the residue was decomposed with ice-cold HCl (5 M). The solution was made alkaline with NaOH (10% w/v) and extracted with $CHCl_3$. The extract was dried, evaporated, and the residue was distilled at 0·1 mm and 120–130°. The oily distillate, presumably 1-(2-*N*-methylpiperidyl)-propan-2-ol (cf. Ref. 39) was dissolved in dry ether (10 ml), MeI (1 ml) was added, and the mixture was kept 24 hr at room temp. The resulting methiodide was degraded as previously reported.[16] Hofmann degradation and hydrogenation gave 8-dimethylaminooctan-2-ol, which on further Hofmann degradation, followed by ozonolysis, yielded formaldehyde, which was isolated as the dimedone derivative.

Benzoic Acid from the Carbonyl Carbon

(±)-*N*-Methylpelletierine hydrochloride (200 mg) was suspended in anhydrous ether. PhLi in ether–benzene (70/30) (1·9 M, 5 ml) was added, the mixture was refluxed 1 hr in N_2, and was then decomposed by addition of excess 1 M HCl. The aqueous phase was separated, washed with ether, made alkaline and the product extracted into ether. The ether extract was dried and evaporated. The residue was distilled at 0·1 mm and 110–130°. The product, a viscous liquid, was dissolved in cold conc. H_2SO_4 (1 ml) and the solution kept 1 hr at 75–80°. CrO_3 (250 mg) in water (0·5 ml) was then added and the mixture was heated for 1 hr. Water was added, the solution was extracted with ether, the ether layer was washed with water and was then extracted with NaOH (1 M, 2 × 1 ml). The ether layer contained traces of acetophenone, in insufficient quantity for isolation. The basic extract was acidified and extracted with ether, the ether was evaporated and the residue sublimed at 0·1 mm and 80–90°, yielding benzoic acid, m.p. 119–120°. Yield 20 mg (15 per cent).

Administration of Labelled Compounds to Sedum sarmentosum

Sedum sarmentosum was propagated in the greenhouse. Shoots, 3 to 4 in. in length, were cut and packed with cut surfaces downward into 100-ml beakers, each beaker containing 15–18 g of fresh plant material (six to nine beakers were used in each experiment). Each radioactive compound was dissolved in 10 ml glass-distilled water. The solution containing the radioactive compound was distributed equally among the beakers. Distilled water (1 ml) was added to each beaker after 24 hr, when most of the original tracer solution had been absorbed, and the experiment was continued for a further 24 hr. The following ^{14}C-labelled compounds (nominal total activity 0·1 mc in each case) were administered in individual experiments: 6-^{14}C-DL-lysine, nominal specific activity 9·2 mc per m mole, Commissariat a l'Energie Atomique, France; sodium 3-^{14}C-DL-β-hydroxybutyrate, 11·8 mc per m mole, Radiochemical Centre; sodium 4-^{14}C-DL-β-hydroxybutyrate, 8·6 mc per m mole, Radiochemical Centre; 3-^{14}C-DL-α-aminobutyric acid, 10 mc per m mole, Commissariat a l'Energie Atomique, France. The samples of lysine, multiply labelled with tritium acid ^{14}C, were prepared as follows.

4,5-$^3H_2$,6-^{14}C-*DL-Lysine*. This intermolecularly doubly labelled lysine was a mixture of 6-^{14}C-DL-lysine (nominal total activity 0·1 mc, nominal specific activity 9·2 mc per m mole, Commissariat a l'Energie Atomique, France) and of 4,5-$^3H_2$-DL-lysine[17] (nominal total activity 1 mc, nominal specific activity 3·0 c per m mole, New England Nuclear). Radiochemical purity of each of the two samples was confirmed by radiochromatography (1-butanol–acetic acid–water, 2:1:1) and radioscanning (model 7201, Radiochromatogram scanner, Packard). Each of the samples showed a single radioactive peak, R_f 0·29. The two samples were dissolved in glass-distilled water, the solutions were mixed and the total volume adjusted to 10 ml. The $^3H/^{14}C$ ratio of this solution of doubly labelled lysine was found to be 7·4 ± 0·1.

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6-^{3}H,6-^{14}C-*DL-Lysine*. This intermolecularly doubly labelled lysine was a mixture of 6-^{14}C-DL-lysine (nominal total activity 0·1 mc, nominal specific activity 9·2 mc per m mole, Commissariat a l'Energie Atomique, France) and 6-^{3}H-DL-lysine[17] (nominal total activity 1·5 mc, nominal specific activity 5·5 c per m mole, New England Nuclear). The radiochemical purity of each of the samples was confirmed by radiochromatography as described above. The two samples were dissolved in glass-distilled water, the solutions mixed and the total volume adjusted to 10 ml. The ^{3}H/^{14}C ratio of this solution of doubly labelled lysine was found to be 18·3 ±0·4.

Isolation of Labelled N-*Methylpelletierine*

After 48 hr in contact with tracer solution the shoots were homogenized, ammonia was added, and the homogenate was transferred into a glass tube (24×3 in.), plugged with cotton wool, and allowed to drain. The eluate was then repeatedly extracted with $CHCl_3$ (1 l.). $CHCl_3$ (2 l.) was also percolated through the solid now packed into the glass tube. The $CHCl_3$ extracts were combined and evaporated. (±)-Methylpelletierine (600 mg) was added to the residue as carrier, the mixture was dissolved in ether (10 ml), and the solution was extracted with HCl (0·5 M, 5×5 ml). The acid solution was washed with ether, was made alkaline and was then extracted with $CHCl_3$. The $CHCl_3$ extract was dried, evaporated and the residue was distilled at 0·1 mm and 110–120°, yielding *N*-methylpelletierine as a colourless oil. It was converted into the hydrochloride or into the picrate (*vide supra*), which were purified to constant activity. Labelled samples obtained in individual tracer experiments were degraded, by the reactions described above, to establish the distribution of label (Table 1).

Isolation of Pipecolic Acid

Pipecolic acid was isolated, as described previously,[17] from cuttings of *S. sarmentosum* which had been kept in contact with intermolecularly labelled 6-^{3}H,6-^{14}C-DL-lysine (^{3}H/^{14}C ratio 18·3±0·4) (*vide supra*).

Determination of Radioactivity

The specific activity of ^{14}C-labelled compounds was assayed on samples of finite thickness on aluminium planchets, using a Nuclear Chicago Corp. gas flow system (Model 4342). The usual corrections for background and self-absorption were applied. Limits shown in the tables are standard deviation of the mean. For plating a 1% solution of collodion in dimethylformamide was used as the solvent.

^{3}H/^{14}C ratios of doubly labelled samples were determined by liquid scintillation counting (Mark 1, Liquid Scintillation Computer, Model 6860, Nuclear Chicago Corp.). Activity due to ^{3}H and ^{14}C was determined simultaneously, by external standardization counting using ^{133}Barium. Samples were dispersed with the aid of methanol in a solution of "LIQUIFLUOR^{R}" (Nuclear Chicago Corp.) diluted 25× with toluene. Duplicate samples of each compound were counted under comparable conditions of quenching. Confidence limits shown in the results are standard deviation of the mean. For highly quenched samples the confidence limits of the quench correction curves were ±5 per cent.

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