Phytochemistry, 1974. Vol. 13, pp. 435 to 437. Pergamon Press. Printed in England.

BIOSYNTHESIS OF THE PIPERIDINE NUCLEUS METABOLISM OF DL-ε-*N*-METHYL-^{3}H-LYSINE-2-^{14}C BY *SEDUM ACRE*

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(*Received* 27 *February* 1973. *Accepted* 2 *August* 1973)

Key Word Index—*Sedum acre*; Crassulaceae; piperidine alkaloids; ε-*N*-methyllysine; sedamine; biosynthesis. biosynthesis.

Abstract—DL-ε-*N*-Methyl-^{3}H-lysine-2-^{14}C was prepared and administered to excised shoots of *Sedum acre* from which DL-sedamine was isolated. The ε-*N*-methyllysine was not incorporated without degradation and, therefore, it is probably not a direct precursor of the piperidine ring of sedamine.

INTRODUCTION

RECENT experiments[1,2] suggested that ε-*N*-methyllysine might be a precursor of those piperidine alkaloids which incorporate lysine in an unsymmetrical manner. In order to investigate this question, we prepared DL-ε-*N*-methyllysine-2-^{14}C by methylation of DL-α-*N*-benzoyl-ε-*N*-p-toluenesulfonyllysine-2-^{14}C with dimethylsulfate and hydrolysis of the product. The DL-ε-*N*-methyl-^{3}H-lysine was prepared in the same manner using ^{3}H-dimethylsulfate. A mixture of ε-*N*-methyl-^{3}H-lysine and ε-*N*-methyllysine-2-^{14}C with a ratio of ^{3}H:^{14}C of 6·4 was fed to excised shoots of *Sedum acre*.

RESULTS

ε-*N*-Methyllysine was not readily taken up by the shoots; when the feeding was terminated after 5 days, only 67% had been taken up. Incorporation into sedamine was only 0·006% based on tritium. The ratio of ^{3}H:^{14}C in the alkaloid was 4·2; degradation showed that 91% of the tritium and 0% of the ^{14}C was in the methyl group of sedamine.

DISCUSSION

Spenser's hypothesis[1] that ε-*N*-methylysine is metabolized to an unsymmetrical precursor of sedamine and similar piperidine alkaloids has been examined. The change in the ratio of ^{3}H:^{14}C indicates that ε-*N*-methyllysine is not incorporated intact, but is degraded to a methyl derivative and lysine which are incorporated independently.

These results are in agreement with recent experiments of Spenser[3] and Leete.[4] Spenser's group fed methyl-^{14}C-methionine and ^{3}H-lysine to *S. acre*. They isolated ε-*N*-methyllysine and sedamine. The ^{3}H:^{14}C ratios of the two compounds were quite different. This indicated that ε-*N*-methyllysine was probably not a direct precursor.

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Leete's group fed *N*-methyl-Δ^1-piperideinium chloride-2-^{14}C to *Nicotiana glauca*. They isolated *N*-methylanabasine and anabasine. The *N*-methyl anabasine had the same specific activity as the *N*-methyl-Δ^1-piperideinium chloride-2-^{14}C. Therefore, *N*-methyl-Δ^1-piperideinium chloride is an aberrant precursor.

These combined results show that ϵ-*N*-methyllysine is probably not a precursor of those piperidine alkaloids which incorporate lysine in an unsymmetrical fashion.

The actual biosynthetic route is probably that suggested by Leete and Chedekel[4] in which L-lysine is decarboxylated and deaminated in the same step to 5-aminopentanal. The 5-aminopentanal cyclizes to Δ^1-piperideine. The Δ^1-piperideine condenses with benzoylacetic acid to form norsedamine. The norsedamine is methylated to sedamine.

EXPERIMENTAL

General methods. The m.ps were determined on a Fisher-Johns block. The IR spectra were obtained with a Beckman IR-33. The NMR spectra were obtained with a Varian A-60A. MS were obtained with a Finigan 3000. The radioactivity was determined with a Packard 3375 liquid scintillation counter using the external standard method of determining the efficiency.[13]

Labelled compounds. DL-lysine-2-^{14}C and ^{3}H-dimethylsulfate were purchased from International Chemical and Nuclear Corporation. The DL-ϵ-*N*-methyllysine was prepared by the method of Benoiton[5] except that all of the intermediates were isolated.

DL-ϵ-N-p-*Toluenesulfonyllysine*-2-^{14}C. DL-Lysine-2-^{14}C.HCl (1·028 g, 5·63 mM, 138 μCi) was dissolved in H_2O (65 ml) and heated to reflux. $CuCO_3.Cu(OH)_2$ (1·670 g, 7·56 mM) was added in small portions. After the addition was complete, reflux was continued for 2 hr and the hot suspension filtered. The residue was washed with 50 ml hot H_2O. The filtrate and wash were combined and cooled. *p*-Toluenesulfonylchloride (1·58 g, 9·11 mM) in 65 ml acetone and $NaHCO_3$ (1·750 g, 20·63 mM) was added and the mixture stirred at room temp. for 10 hr. The copper complex was filtered, washed with 5 ml cold H_2O, 5 ml acetone and 5 ml Et_2O and dried (1·65 g, 88%). The finely ground copper complex was suspended in 25 ml H_2O and heated to reflux: H_2S was bubbled in for 15 min with stirring. The hot soln was acidified (0·65 ml 6 N HCl) and Cu_2S was filtered and the pH adjusted to 6 with 5 N NaOH. DL-ϵ-*N*-*p*-Toluenesulfonyllysine crystallized out. The yield (recrystallized) was 0·9441 g (55·8%), m.p. 235–240° (lit.[6] 237–238°).

DL-α-N-*Benzoyl*-ϵ-N-p-*toluenesulfonyllysine*-2-^{14}C. DL-ϵ-*N*-*p*-toluenesulfonyllysine-2-^{14}C (0·944 g, 3·14 mM) was added to ice-cold NaOH (0·251 g, 6·28 mM) in 5 ml H_2O. Benzoyl chloride (0·524 ml, 4·54 mM) in 1 ml Et_2O was added and the mixture stirred for 1 hr. It was acidified (HCl) and the aq. phase was decanted from an oil which was dissolved in H_2O with heating. The product crystallized out in the cold. The yield was 0·778 g (61·3%), m.p. 136–139° (lit. 140°)[7]. IR: (KBr) 3369, 3289, 1720, 1628 and 1155 cm^{-1}. MS: (70 eV) (M^+) 404.

DL-α-N-*Benzoyl*-ϵ-*N*-*p*-*toluenesulfonyl*-ϵ-N-*methyllysine*-2-^{14}C. DL-α-Benzoyl-ϵ-*N*-*p*-toluenesulfonyllysine-2-^{14}C (0·779 g, 1·93 mM) was added to ice-cold NaOH (0·154 g, 3·85 mM) in 10 ml H_2O. Dimethyl sulfate (0·44 ml, 4·68 mM) was added and the soln was stirred until cloudy. Then 10 ml 2·5 N NaOH was added and the reaction continued for 1 hr. The cold soln was acidified (HCl) and on sitting in the cold the product crystallized. It was dried by dissolving in 30 ml acetone adding 30 ml C_6H_6 and evaporating at reduced pressure × 3. The product crystallized and was filtered and washed with Et_2O. The yield of DL-α-*N*-benzoyl-ϵ-*N*-*p*-toluenesulfonyl-ϵ-*N*-methyllysine-2-^{14}C was 0·436 g (54·2%), m.p. 90–92°. IR: (KBr) 3309, 1725, 1630 and 1150 cm^{-1}. NMR: (DMSO-d_6); 2·54 δ (N–Me). MS: (70 eV) (M^+) 418.

DL-ϵ-N-*Methyllysine*-2-^{14}C.*HCl*. DL-α-*N*-benzoyl-ϵ-*N*-*p*-toluenesulfonyl-ϵ-*N*-methyllysine-2-^{14}C (0·436 g, 1·04 mM) was refluxed in 25 ml of 40% HBr for 2 hr. H_2O (25 ml) was added and the mixture cooled and filtered. The filtrate was evaporated. The semicrystalline residue was dissolved in H_2O (10 ml) and applied to a column of AG-50-8x (20 × 1·5 cm). The column was eluted with (50 ml) H_2O, (50 ml) 1 N HCl, (50 ml) H_2O, and (100 ml) NH_4OH and 10 ml fractions were collected. The ninhydrin positive fractions were combined and evaporated to dryness. The residue was dissolved in 20 ml 6 N HCl and the solvent evaporated. The residue was dried by dissolving in EtOH (2 × 15 ml) and evaporating. The dry residue was taken up in EtOH (5 ml). Pyridine was added to form the ϵ-*N*-methyllysine.HCl which was precipitated by the addition of Et_2O. It was recrystallized to constant specific activity from EtOH and Et_2O, m.p. 224–226°. It gave an IR (KBr) typical of amino acid hydrochlorides. The NMR (D_2O) had a peak at 2·70 δ assigned to N–Me. The yield was 0·0531 g (25·9%). The sp. act. was 5·24 × 10^7 dpm/mM. Previous runs had afforded yields as high as 77%.

DL-ϵ-N-*Methyl*-^{3}H-*lysine*.*HCl*. The DL-ϵ-*N*-methyl-^{3}H-lysine.HCl was prepared in the same manner as the DL-ϵ-*N*-methyllysine-2-^{14}C using ^{3}H-dimethyl sulfate. The sp. act. was 6·57 × 10^7 dpm/mM.

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DL-*Sedamine.* DL-Sedamine was prepared by the method of Beyerman *et al.*[8] m.p. 89–90° (lit. 89–90°).[8] The IR (KBr) was identical to that in the lit.[9]

Administration of labelled DL-ε-N-*methyllysine to* Sedum acre. The *S. acre* plants were grown from seed in soil for 7 months. The labelled DL-ε-*N*-methyllysine was fed to excised shoots from these plants as described by Gupta and Spenser.[10] Because the uptake of tracer was slow, 2 ml nutrient soln[11] was added in addition to the tracer solution and this maintained the shoots in a healthy condition for 5 days.

Isolation of DL-*sedamine.* The excised shoots (45 g) were rinsed with H_2O, and blended in $CHCl_3$ (250 ml) and NH_4OH (50 ml). Carrier sedamine (150 mg) was added and the mixture stood overnight. The $CHCl_3$ layer was washed (H_2O, 2 × 50 ml) and extracted with 5% v/v HCl (4 × 25 ml). The acid layer was washed with Et_2O (3 × 50 ml). The acid layer was made alkaline with NH_4OH and extracted with Et_2O (3 × 50 ml). The Et_2O soln was dried (Na_2SO_4), and evaporated. The residue was taken up in hexane (40 ml). The hexane was reduced to 5 ml and the sedamine crystallized in the cold. The sedamine was purified to constant sp. act. by sublimation and recrystallization from hexane, m.p. 89–90°. The yield was 52·3 mg. The sp. act. was $1{\cdot}04 \times 10^4$ dpm/mM of ^3H and $2{\cdot}49 \times 10^3$ dpm/mM of ^{14}C.

Demethylation of DL-*sedamine.* DL-Sedamine (36·6 mg, 0·167 mM) was added to a flask containing freshly dist. HI (4 ml), NH_4I (100 mg), and $AuCl_3$ (one crystal). The flask was heated to 360° and the MeI produced was swept by N_2 through a trap containing 5% $CdSO_4$ and 5% $Na_2S_2O_3$ (1:1), into a cold trap containing EtOH (15 ml) and Et_3N (3 ml). After 1 hr, the trap was evaporated and the methyltriethyl-NH_4I was recrystallized from EtOH and Et_2O. The yield was 10·36 mg (26%) m.p. 295–300° (lit.[12] 297°).

Acknowledgements—The authors thank the National Institutes of Health for support through Research Grant 1 R01 HE13613 MCHA.

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