Biochemistry

sym-Homospermidine, a Naturally Occurring Polyamine*

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ABSTRACT: 1,9-Diamino-5-azanonane (1, $H_2N(CH_2)_4NH(CH_2)_4NH_2$), symmetrical "homospermidine," previously unreported in nature, has now been discovered in sandal (*Santalum album* L.), where it comprises 0.5–1.5% of the weight of the dried leaves. The new polyamine is conveniently isolated from an aqueous extract of the leaves by ion-exchange chromatography.

The proof of structure rested on nmr and mass spectrometry and on the comparison with a synthetic sample made available by a new and practical route from 4-aminobutyric acid. Biosynthetic and biochemical implications are discussed.

D uring a routine examination of the amino acids from an ethanolic extract of the leaves of the sandalwood tree, a prominent ninhydrin-positive spot (spot 5 in Figure 1), hitherto unrecognized, was observed in two-dimensional chromatograms. However, when an aqueous extract was examined, which had been desalted on a column of Dowex 50 (H^+ form) by elution with 1.0 N ammonia, this spot was absent in otherwise unchanged chromatograms. The unknown, strongly basic compound could be eluted with 1.0 M piperidine. Repetition of this procedure afforded a chromatographically homogeneous product whose characterization as sym-homospermidine forms the subject of this paper.

Materials

Reagents. 4-Aminobutyric acid and p-nitrophenol were Eastman reagent grade. Carbobenzyloxychloride was purchased from Cyclo Chemical Corp.; N,N'-dicyclohexylcarbodiimide, from Aldrich Chemical Co.; and a 1.0 M solution of borane in tetrahydrofuran, from Alfa Inorganics, Inc. The 10% palladium/carbon catalyst was a product of Englehard Industries, Inc. Tetrahydrofuran (Baker, reagent) was dried by distillation from excess lithium aluminum hydride (Metal Hydrides, Inc.) after standing overnight at room temperature. All solvents were of reagent grade. Silica gel (0.05–0.2 mm) for column chromatography and purified piperidine for ion-

exchange chromatography were obtained from E. Merck A. G. (Darmstadt). Dowex 50-X8 beads (reagent) were supplied by J. T. Baker.

Instruments. Infrared spectra (chloroform or KBr pellets) were measured with Perkin-Elmer spectrophotometers, Models 237 and 421, respectively. Mass spectra were obtained with the double-focusing Hitachi RMU-6E mass spectrometer. Proton magnetic resonance spectra (D_2O or $CDCl_3$) were measured on the Varian Associates Model A-60 spectrometer. Chemical shifts are reported as δ values (parts per million) relative to tetramethylsilane ($CDCl_3$) or 3-(trimethylsilyl)propanesulfonic acid, sodium salt (D_2O), as internal standards. Melting points were taken on a Kofler hot stage and are corrected. A Perkin-Elmer polarimeter, Model 141, with a sodium vapor source was used to measure the optical rotation.

Chromatography. Whatman No. 1 papers were employed for paper chromatography. Two-dimensional paper chromatography was carried out with buffered papers according to Subramanian and Rao (1955). Silica gel G and cellulose ("Avicel") thin-layer chromatographic plates (0.25 mm thick) were purchased from Analtech (Wilmington, Del.) and were used without further treatment. Solvent systems for paper chromatography were: 1-butanol–acetic acid–water (4:1:1, v/v) (A), collidine–lutidine–water–diethylamine (1:1:1:0.12, v/v) (B), and phenol–0.2 M KCl–HCl buffer, pH 1 (50:7, v/v) (C). The system for thin-layer chromatography on silica was toluene–ethyl formate–formic acid (5:3.5:1.5, v/v) (D). 2-Propanol–concentrated ammonia (7:3, v/v) (E) was used with thin-layer chromatography on cellulose. Spots on paper chromatograms were visualized with a 0.4% solution of ninhydrin in acetone containing 2% collidine. Thin-layer chromatography chromatograms were developed at room temperature with iodine vapor or a ninhydrin spray (0.1% in meth-
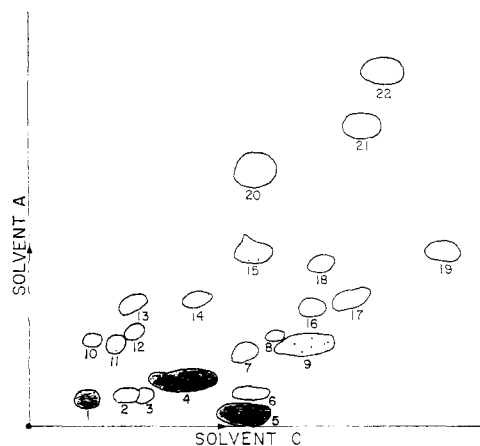
FIGURE 1: Free amino acids or amines in sandal leaves: (1) ornithine, (2) lysine, (3) histidine, (4) arginine, (5) *sym*-homospermidine, (6) putrescine (traces), (7) glutamine, (8) citrulline, (9) *allo*-hydroxyproline, (10) aspartic acid, (11) serine, (12) glycine (traces), (13) glutamic acid, (14) threonine, (15) alanine, (16) ethanolamine, (17) unidentified, (18) γ-aminobutyric acid, (19) proline, (20) tyrosine, (21) valine and methionine (?), and (22) leucine and isoleucine. (Two-dimensional paper chromatography was done according to Subramanian and Rao (1955)). Solvent C was used in the first development, solvent A for the second (R. K. and A. N. R., preliminary results).

anol–1-butanol–2.0 N acetic acid (20:10:1, v/v) followed by brief heating at 100°.

Methods

Isolation Procedure. EXTRACTION. Fresh sandal leaves (3 kg) were cut into small pieces, dried at 50° to constant weight (48 hr), powdered, and passed through a 40-mesh sieve. Batches of 250 g of the powder were stirred mechanically with 2 l. of water on a boiling-water bath for 3 hr, then allowed to settle. The supernatant was decanted and the residue was extracted twice with 1 l. of water as before. The pooled extracts were centrifuged (3000g), and the supernatant was acidified (HCl) to pH 5 and kept overnight with octanoic acid (0.1 ml/l.) added as a preservative. After removal of a fine precipitate by filtration, the aqueous extracts from three such batches were pooled (15-l. total).

SEPARATION. The above extract was passed through a 2.2 × 50 cm column (200-ml bed volume) of Dowex 50-X8 (H⁺ form) resin and the column was washed with 3 l. of water. Amino acids were eluted with 1 l. of 1.0 N ammonia, then 2 l. of 1.0 M piperdine and 200-ml fractions were collected. Paper chromatography in solvent system A and detection by a ninhydrin spray showed that the unknown (R_F 0.06) appeared in the second and third piperidine fractions. These were combined (400-ml total) and concentrated to a small volume *in vacuo*. Since the concentrate showed arginine to be present as well as traces of other amino acids, it was rechromatographed on a similar Dowex column, eluted with 2 l. each of 1.0 N ammonia and 1.0 M piperidine, and collected as 25-ml fractions. The fractions containing the unknown amine were pooled (250-ml total) and concentrated *in vacuo* to a small volume. After acidification to pH 1–2 with 6.0 N HCl, absolute ethanol was added to precipitate the hydrochloride of the unknown amine. After drying *in vacuo* 3.0 g of crude hydrochloride was obtained.

PURIFICATION. The crystalline residue was washed with ice-cold absolute ethanol to remove colored contaminants;

the remaining solid (2.7 g), dissolved in 100 ml of water, was decolorized by boiling for 15 min with 100 mg of acid-washed charcoal, and filtered. The charcoal treatment was repeated twice to obtain a colorless filtrate. After concentration, acetone was added to reprecipitate the hydrochloride (2.0 g). This material was suspended in boiling absolute ethanol and water added dropwise until solution was complete. The solution was allowed to come to room temperature. The hydrochloride crystallized on standing at 4°. Repetition of the procedure gave a yield of 1.5 g of fine granular crystals: mp 291–294° dec (heating rapidly); R_F (A) 0.06; (B) 0.12; (C) 0.42. An analytical sample was recrystallized from aqueous ethanol–2-propanol–ether.

QUANTITATION IN LEAVES. The abundance of the unknown amine in sandal leaves was estimated by standardization of small Dowex 50 columns and evaluation of the ninhydrin color of the chromatographically pure piperidine elutions with leucine as a standard by Rosen's procedure (1957); with several samples of leaves, the amount of the base was found to be in the range of 0.5–1.5% of the weight of the dried leaves.
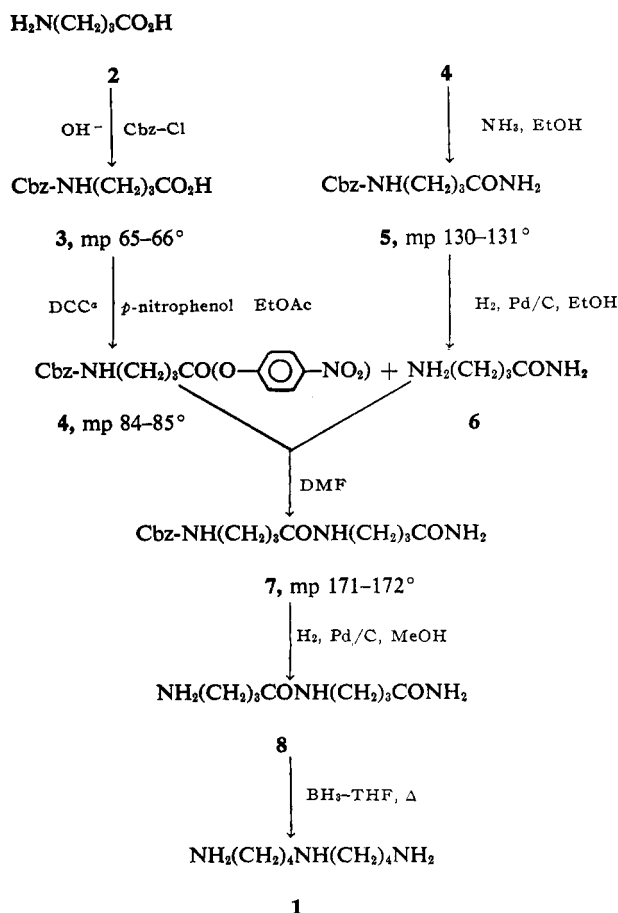
SYNTHESIS OF *sym*-HOMOSPERMIDINE (SCHEME I). The *p*-nitrophenyl ester of *N*-carbobenzyloxy-4-aminobutyric acid (4) and 4-aminobutyramide (6) were coupled in dimethylformamide by the procedure of Bodanszky and du Vigneaud (1959) to afford, after 2 hr at room temperature, a quantitative yield of the blocked diamide (7). The ester 4 was prepared in 86% yield from *N*-carbobenzyloxy-4-aminobutyric acid (3) and *p*-nitrophenol with dicyclohexylcarbodiimide in ethyl acetate at 0° (Elliot and Russel, 1957). The protected acid 3 was obtained in yields of 77–83% from 4-aminobutyric acid (2) and carbobenzyloxychloride in dilute alkali. The amide 6 resulted in 94% overall yield from ammonolysis of 2 in ethanol followed by hydrogenolysis of 5 with a 10% Pd/C catalyst in ethanol for 2 hr. Hydrogenation of 7 for 2 hr in methanol gave a quantitative yield of the aminodiamide 8. This was refluxed (Brown and Heim, 1964) with excess borane in dried tetrahydrofuran for 4 hr to give *sym*-homospermidine, 1, which was isolated in 60% yield as the hydrochloride (mp 283–285° dec) or in comparable yield as the sulfate (mp 190–195° dec). Details on this synthesis as well as a new synthesis of spermidine will be reported elsewhere.

BENZOYLATION. A sample of 34.6 mg of the hydrochloride from sandal leaves, dissolved in aqueous bicarbonate, could not be extracted into ethyl acetate or chloroform. After benzoylation with excess benzoyl chloride and additional alkali at room temperature overnight, extraction with ethyl acetate, drying of the extract (Na₂SO₄), and evaporation, 57 mg of oil was obtained, which failed to crystallize, even though homogeneous by silica thin-layer chromatography (R_F 0.36–0.39, D). Chromatography on a column (0.7 × 29.5 cm) of silica gel, with 10% methanol in chloroform and collection of 0.36-ml fractions, afforded 56.9 mg of a colorless, homogeneous oil which still failed to crystallize (R_F 0.44, CH₃OH–CHCl₃, 1:9, v/v). Evaporation of a sample of this oil in acetone afforded a thin film on a weighing boat. This was dried to constant weight for combustion analysis. A mass spectrum of the oily benzoyl derivative was obtained with the sample inlet of the spectrometer at 400° and 80-eV ionizing voltage. The infrared spectrum was measured in chloroform.

Likewise the *synthetic sym*-homospermidine was benzoylated with excess benzoyl chloride in 2.0 N NaOH overnight, at room temperature, and also gave an oil, which on prolonged drying *in vacuo* set into a glass.

COMPARISON AND IDENTIFICATION. The nuclear magnetic

SCHEME I: New and Improved Synthesis of sym-Homospermidine.

$H_2N(CH_2)_3CO_2H$

$\overset{\textbf{2}}{\big\downarrow} OH^- \mid Cbz\text{-}Cl$

$Cbz\text{-}NH(CH_2)_3CO_2H$

3, mp 65–66°

$\big\downarrow DCC^a \mid p\text{-nitrophenol} \quad EtOAc$

$Cbz\text{-}NH(CH_2)_3CO(O\text{-}\langle O \rangle\text{-}NO_2) + NH_2(CH_2)_3CONH_2$

4, mp 84–85°   **6**

$\overset{\textbf{4}}{\big\downarrow} NH_3, EtOH$

$Cbz\text{-}NH(CH_2)_3CONH_2$

5, mp 130–131°

$\big\downarrow H_2, Pd/C, EtOH$

$\big\downarrow DMF$

$Cbz\text{-}NH(CH_2)_3CONH(CH_2)_3CONH_2$

7, mp 171–172°

$\big\downarrow H_2, Pd/C, MeOH$

$NH_2(CH_2)_3CONH(CH_2)_3CONH_2$

8

$\big\downarrow BH_3\text{-}THF, \Delta$

$NH_2(CH_2)_4NH(CH_2)_4NH_2$

1

^{a} DCC = dicyclohexylcarbodiamide; DMF = dimethylformamide; THF, tetrahydrofuran.
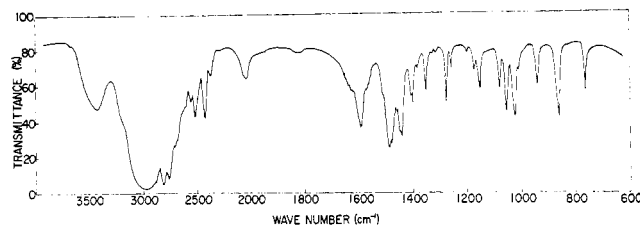

FIGURE 2: Infrared spectrum of sandal amine hydrochloride (1 mg/300 mg of KBr).

molecular ion, one mass unit less than the parent molecular weight, is not uncommon (Budzikiewicz et al., 1964). Other peaks corresponding to abundant ions and their probable relation to M^+ were: 350 ($M^+ - C_6H_5CONH_2$), 295, 245 ($M^+ - C_6H_5COC_6H_5CONH_2$), 205, 193, 174 (295 $- C_6H_5CONH_2$), 105 (C_6H_5CO, base peak), 84 (174 $- C_6H_5CONH_2$), 77 (C_6H_5COCO). The 295 peak may be related to M^+ by the loss of a $(CH_2)_4NHCOC_6H_5$ unit. Peak 174 was very intense and indicates a favored fragmentation pathway.

The mass spectrum together with the combustion analysis for the benzoyl derivative established the formula $C_{29}H_{33}N_3O_3$ (Anal. Calcd: C, 73.86; H, 7.05; N, 8.91. Found: C, 74.11; H, 7.06; N, 8.92) and indicated that *three* benzoyl groups were added to a triamine of the formula $C_8H_{21}N_3$. On this basis, the yield of the benzoylation of the natural triamine was 94%. The combustion analysis of the natural amine hydrochloride is in good agreement with a trihydrochloride: $C_8H_{21}N_3 \cdot 3HCl$ (Anal. Calcd: C, 35.76; H, 9.02; Cl, 39.59; N, 15.64. Found: C, 35.78; H, 8.91; Cl, 39.40 (ionic); N, 15.61). These formulae indicate the absence of rings or double bonds.

The absence of optical activity at 589 nm in the hydrochloride (c 2.06, H_2O), end absorption only in the ultraviolet region, and the simplicity of the nuclear magnetic resonance spectrum necessitate structure **1** for the natural amine from sandalwood.

The melting point of the hydrochloride, on rapid melting, reproducibly in the range 290–294°, agrees well with the literature values reported for sym-homospermidine, 290° dec (Dudley and Thorpe, 1925) and 287° (von Braun and Pinkernelle, 1937). The nuclear magnetic resonance signals and assignments reported by Potier et al. (1960) for spermidine (NCH_2, 100 Hz upfield from HOD; $CH_2CH_2CH_2$, 180 Hz upfield from HOD) match the chemical shifts observed for sym-homospermidine and our assignments. With respect to the HOD signal, the multiplets at δ 3.08 would fall 96 Hz upfield, those at δ 1.78, at 174 Hz upfield.

Finally, the natural amine hydrochloride was compared to synthetic sym-homospermidine hydrochloride. The infrared spectra (KBr) of the natural and synthetic materials were identical and the nuclear magnetic resonance spectrum of the synthetic hydrochloride (D_2O) (multiplets at 94 and 171 Hz upfield from HOD signal) was superimposable on that of the natural hydrochloride. In addition, their chromatographic behavior (cellulose thin-layer chromatography, R_F 0.67 (E)) was identical.

sym-Homospermidine can be separated nicely from spermidine and putrescine by two-dimensional paper chromatography (Figure 3).

The infrared spectra and R_F values (0.32 (D)) of the natural and synthetic tribenzoyl derivatives were also identical. Furthermore, the mass spectrum of the synthetic tribenzoyl derivative was identical in all respects with that of the natural product.
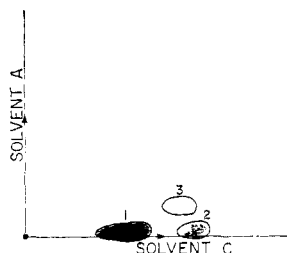
resonance spectrum of the natural amine hydrochloride (D_2O) revealed two multiplets of equal intensity centered at δ 3.08 and 1.78. After benzoylation, these multiplets were observed at δ 3.43 and 1.68 (CDCl$_3$). In addition, multiplets at δ 8.82 and 7.45 attributable to the benzoyl substituents were observed. The upfield multiplets in both compounds were tentatively assigned to methylene protons adjacent to nitrogen and situated between CH_2 groups, respectively.

The *infrared spectrum* (Figure 2) of the natural amine hydrochloride showed NH_3^+ stretching vibrations (2940 cm^{-1}, asym; 2800 cm^{-1}, sym), NH_2^+ stretching vibrations (2500 cm^{-1}, asym; 2420 cm^{-1}, sym), and bending vibrations at 1625–1590 cm^{-1} (NH_3^+ and NH_2^+. The broad absorption at 2020 cm^{-1} can also be ascribed to the NH_3^+ group. The 1485- and 1445-cm^{-1} absorptions may represent δ_{sym} NH_3^+ and δ CH_2, respectively.

The benzoyl derivative (CHCl$_3$) exhibited as prominent absorptions bands at 3480, amide N–H stretching vibration; 1660, secondary amide C=O stretching vibration; 1620, tertiary amide C=O stretching vibration; 1605 and 1585, phenyl; 1520, amide II band; and 1490 cm^{-1}, CH_2.

The *mass spectrum of the benzoylated amine* was particularly informative. The spectrum displayed a triplet of peaks at m/e 470, 471, and 472 (relative intensities, approximately 1:0.4:0.2) as the highest molecular ions. The next highest ion at m/e 366 must be related to the parent (M^+) by a loss of C_6H_5CO (105 mass units) and hence the true parent molecular ion is most probably at m/e 471. The observation of a

FIGURE 3: Separation of hydrochlorides of spermidine (1), sym-homospermidine (2), and putrescine (3). Solvent C was used in the first development where these components had the following R_F's: (1) 0.21, (2) 0.36, and (3) 0.32. In the second development with solvent A, these R_F's were observed: (1) 0.01, (2) 0.01, and (3), 0.04. Buffered Whatman No. 1 paper (pH 1.0) was used.

Discussion

sym-Homospermidine was first reported in 1925 by Dudley and Thorpe as the hydrolysis product of *N,N*-di-4-benzamido-butylamine (9), which was obtained in 10% yield as a by-

$$[C_6H_5CONH(CH_2)_4]_2NH$$

9
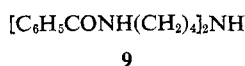
product during a synthesis of monobenzoylputrescine by ammonolysis of 4-benzamido-*n*-butyl iodide. In 1937, during the study of spermidine analogs, 9 was synthesized by von Braun and Pinkernelle in 50% yield from monobenzoylputrescine and 4-benzamido-*n*-butyl chloride. *sym*-Homospermidine resulted on acid hydrolysis. They commented pessimistically on the likelihood of finding spermidine analogs in nature: "In der belebten Natur ist man unter diesen Triaminen bisher nur dem Spermidine begegnet, und es ist unwahrscheinlich, dass man auf die anderen Triamine stossen wird."

However, in 1962, the discovery of a naturally occurring spermidine analog was claimed. Johnson and Markham (1962) reported the isolation of *sym*-norspermidine, *i.e.*, 1,7-diamino-4-azaheptane, from turnip yellow mosaic virus as well as several other purified *plant virus* species in which it appeared to be associated with the viral RNA (Johnson and Hills, 1963). The polyamine was absent from healthy plants (Johnson and Markham, 1962; Markham, 1968).[1] This polyamine, as well as spermine, stabilizes turnip yellow mosaic virus RNA toward heat denaturation (Mitra and Kaesberg, 1963).

Two British patents (Drewitt and Green, 1948; British Celanese Ltd., 1949) deal with the synthesis of *sym*-homospermidine from 4-phthalimido-*n*-butyl chloride with a 3-5 molar excess of putrescine, followed by hydrolysis. Copolymers of 1 with dicarboxylic acid derivatives have been reported (British Celanese Ltd., 1949).

For the synthesis of 1, we devised a route starting with readily available 4-aminobutyric acid. Although a number of steps were required, the yields were all excellent and no step involved monosubstitution of bifunctional reactants, as with all the other syntheses.

sym-Homospermidine was detected in both 75% alcohol and hot water extracts of sandal leaves, either fresh or dried, and is therefore most probably present in an unconjugated

form in the leaves and not as an alkaloid or peptide component. For example, spermidine is liberated on alkali fusion or hydrolysis of the alkaloids palustrine (Eugster *et al.*, 1960) and lunarine (Potier *et al.*, 1960), respectively, and has recently been discovered as the C-terminal portion of the peptide antibiotics, edeine A and B (Hettinger and Craig, 1970).

While putrescine, spermidine, and spermine occur widely in the animal world (Tabor *et al.*, 1961; Tabor and Tabor, 1964), at this time only putrescine can be considered a common product in the plant world (Tabor and Tabor, 1964). The higher polyamines have seldom been found in plants[2] and for this reason the discovery of *sym*-homospermidine in sandal leaves is remarkable.

Two tetramethyl, acylated derivatives of *sym*-homospermidine, *Solapalmitine* (10) and *Solapalmitenine* (11), have recently been discovered in *Solanum tripartitum*. These new alkaloids were reported to have significant tumor-inhibiting activity (Kupchan *et al.*, 1967, 1969). In an earlier and related report Israel and coworkers demonstrated (1964) antitumor activity in a series of synthetic spermidine and spermine analogs, though *sym*-homospermidine was not studied.

$$[(CH_3)_2N(CH_2)_4]_2NR$$
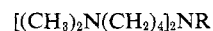
10, R = palmitoyl $(CH_3(CH_2)_{14}CO)$
11, R = α,β-(*trans*)-dehydropalmitoyl

The *biosynthesis* of *sym*-homospermidine is at present not known, although routes involving putrescine and either glutamic acid γ-semialdehyde (Vogel and Davis, 1952) (*via* 12) or 4-aminobutyraldehyde (*via* 13) would appear likely. Putrescine could arise either from arginine, *via* agmatine and *N*-carbamylputrescine (Smith, 1968), or by the decarboxylation of ornithine. 4-Aminobutyraldehyde, a known putrescine metabolite (Tabor, 1951; Mann and Smithies, 1955; Hasse and Maisack, 1955; Kim and Tchen, 1962), would seem particularly attractive. These possibilities are currently under investigation.

$$H_2N(CH_2)_4N=CH(CH_2)_2CHR$$
$$|$$
$$NH_2$$
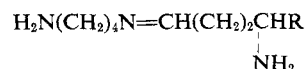
12, R = CO_2H
13, R = H

sym-Homospermidine functions, nearly as effectively as spermidine, in maintaining the growth of *Homophilus para-influenzae* (Herbst *et al.*, 1955). This bacterium degrades spermidine to 1,3-diaminopropane and *sym*-homospermidine to putrescine (Weaver and Herbst, 1958a). Putrescine and *sym*-norspermidine were also effective; however the latter, unlike *sym*-homospermidine, was toxic at higher concentrations. The relative rates of oxidation of spermidine and *sym*-homospermidine to these diamines were determined with the help of an enzyme system from another bacterium, *Neisseria perflava*, adapted to spermine oxidation, which degraded *sym*-homospermidine at 60% the rate of spermidine, while other

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[1] We are indebted to a referee for directing our attention to the recent work of Beer and Kosuge (1970) in which the results of Johnson and Markham are questioned. They report finding *only* spermidine and spermine in preparations of turnip yellow mosaic virus.

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[2] The occurrence of free spermidine in plants seems limited to the following: spermidine, putrescine, cadaverine, and spermine have been discovered in the germs of a number of cereal grains (Moruzzi and Caldarera, 1964; Bagni *et al.*, 1967). Spermidine and putrescine occur in Chinese cabbage (Tabor and Tabor, 1964) and in the alga *Chlorella ellipsoidea* (Kanazawa *et al.*, 1966). Spermidine is also found in tomato leaves (Tabor and Tabor, 1964).

spermidine analogs were oxidized much more slowly (Weaver and Herbst, 1958b).

It is tempting to speculate that *sym*-homospermidine might be more widespread in nature than its sole known occurrence in sandal would suggest and that organisms might exist, adapted to its metabolism. In this regard it may be significant that a *Pseudomonas* strain has recently been discovered lacking spermidine but containing, in addition to putrescine, a new polyamine, 2-hydroxyputrescine (Rosano and Hurwitz, 1969). This polyamine is bound to the ribosomes in a manner very similar to the binding of spermidine to the ribosomes of *Escherichia coli*.

Acknowledgment

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References

Bagni, N., Caldarera, C. M., and Moruzzi, G. (1967), *Experientia 23*, 139.

Beer, S. V., and Kosuge, T. (1970), *Virology 40*, 930.

Bodanszky, M., and du Vigneaud, V. (1959), *J. Amer. Chem. Soc. 81*, 5688.

British Patent to British Celanese, Ltd. (1949), 631,020; *Chem. Abstr. 44*, 5641.

Brown, H. C., and Heim, P. (1964), *J. Amer. Chem. Soc. 86*, 3566.

Budzikiewicz, H., Djerassi, C., and Williams, D. H. (1964), Interpretation of Mass Spectra of Organic Compounds, San Francisco, Calif., Holden-Day, Inc., pp 54, 64, 99, 100, 108, 112, 232, 239, 251.

Drewitt, J. G. N., and Green, M. B. (1948), British Patent 597,253; *Chem. Abstr. 42*, 4604.

Dudley, H. W., and Thorpe, W. V. (1925), *Biochem. J. 19*, 849.

Elliot, D. V., and Russel, D. W. (1957), *Biochem. J. 66*, 49P.

Eugster, C. H., Dietsche, W., and Baumann, C. G. (1960), *Angew. Chem. 73*, 371.

Hasse, K., and Maisack, H. (1955), *Biochem. Z. 327*, 296.

Herbst, E. J., Glinos, E. G., and Amundsen, L. H. (1955), *J. Biol. Chem. 214*, 175.

Hettinger, T. P., and Craig, L. C. (1970), *Biochemistry 9*, 1224.

Israel, M., Rosenfield, J. S., and Modest, E. J. (1964), *J. Med. Chem. 7*, 710.

Johnson, M. W., and Hills, G. J. (1963), *Virology 21*, 517.

Johnson, M. W., and Markham, R. (1962), *Virology 17*, 276.

Kanazawa, T., Yanagisawa, T., and Tamiya, H. (1966), *Z. Pflanzenphysiol. 54*, 57; *Chem. Abstr. 66*, 17419f.

Kim, K.-h., and Tchen, T. T. (1962), *Biochem. Biophys. Res. Commun. 9*, 99.

Kupchan, S. M., Davies, A. P., Barboutis, S. J., Schnoes, H. K., and Burlingame, A. L. (1967), *J. Amer. Chem. Soc. 89*, 5718.

Kupchan, S. M., Davies, A. P., Barboutis, S. J., Schnoes, H. K., and Burlingame, A. L. (1969), *J. Org. Chem. 34*, 3888.

Mann, P. J. G., and Smithies, W. R. (1955), *Biochem. J. 61*, 89.

Markham, R. (1968), *in* Recent Aspects of Nitrogen Metabolism in Plants, Hewitt, E. J., and Cutting, C. V., Ed., London, Academic Press, p 203.

Mitra, S., and Kaesberg, P. (1963), *Biochem. Biophys. Res. Commun. 11*, 146.

Moruzzi, G., and Caldarera, C. M. (1964), *Arch. Biochem. Biophys. 105*, 209.

Potier, P., Le Men, J., Janot, M.-M., and Bladon, P. (1960), *Tetrahedron Letters No. 18*, 36.

Rosano, C. L., and Hurwitz, C. (1969), *Biochem. Biophys. Res. Commun. 37*, 677.

Rosen, H. (1957), *Arch. Biochem. Biophys. 67*, 10.

Smith, T. A. (1968), *in* Recent Aspects of Nitrogen Metabolism in Plants, Hewitt, E. J., and Cutting, C. V., Ed., London, Academic Press, p 139.

Subramanian, N., and Rao, M. V. L. (1955), *J. Sci. Ind. Res. 14C*, 56.

Tabor, H. (1951), *J. Biol. Chem. 188*, 125.

Tabor, H., and Tabor, C. W. (1964), *Pharmacol. Rev. 16*, 245.

Tabor, H., Tabor, C. W., and Rosenthal, S. M. (1961), *Annu. Rev. Biochem. 30*, 579.

Vogel, H. J., and Davis, B. D. (1952), *J. Amer. Chem. Soc. 74*, 109.

von Braun, J., and Pinkernelle, W. (1937), *Ber. 70B*, 1230.

Weaver, R. H., and Herbst, E. J. (1958a), *J. Biol. Chem. 231*, 637.

Weaver, R. H., and Herbst, E. J. (1958b), *J. Biol. Chem. 231*, 647.