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OXIDATION OF PUTRESCINE AND CADAVERINE DERIVATIVES BY DIAMINE OXIDASES

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Abstract: A range of putrescine and cadaverine derivatives has been synthesized and assayed as substrates for the diamine oxidases from pea seedlings and pig kidney. K_M and V_{max} data are reported, mainly for the pea enzyme. *N*-Alkylputrescines and *C*-alkylcadaverines are generally poorer substrates than the parent compounds with up to 4500-fold reduction in V_{max}. There is significantly less variation in K_M values, indicating that the binding site of the pea enzyme is relatively non-specific and that enzymic specificity lies at the catalytic stage. This suggests that poor substrates might act as convenient, short-lived inhibitors of diamine oxidases.

Diamine oxidases (DAO, EC 1.4.3.6) form a ubiquitous class of enzymes central to polyamine metabolism in most organisms.[1-3] They catalyse the oxidative deamination of a range of primary diamines (indicated in Scheme 1) in a reaction for which there are no generally reliable or convenient non-enzymatic methods available. DAO is a key enzyme in the metabolism of polyamines which are essential for the growth and replication of all living cells.[4-6] Inhibitors of DAO are known to possess antimalarial, antitrypanosomal, antibacterial, and antifungal activities, together with possible roles in cancer chemotherapy. This dual role as an enzyme system for possible use in industrial biotransformation processes and as a potential target for specifically designed drugs and inhibitors makes DAO a prime candidate for combined chemical and biophysical study. We report here on a study of the substrate activities of pea seedling and pig kidney DAO towards a series of putrescine and cadaverine derivatives, using a kinetic assay procedure[7] suitable for systematic characterisation of the molecular recognition process by these enzymes.

Table 1. Oxidation rates of *N*-alkylputrescines relative to putrescine, catalysed by pea seedling and pig kidney diamine oxidase (DAO) at 37°C and pH 7.4[a]

Substrate	Pea seedling DAO		Pig kidney DAO	
	Activity (nmol/30min)	Relative rate (%)	Activity (nmol/30min)	Relative rate (%)
(1) Putrescine	122	100	106	100
(2) *N*-methylputrescine	119	98	90	85
(3) *N*-ethylputrescine	83	68	79	74
(4) *N*-propylputrescine	70	57	63	53

[a]bis-hydrazone method (ref.8).

Table 2. K_M and V_{max} values for oxidation of putrescine and *N*-alkylputrescines catalysed by diamine oxidase from pea seedlings and pig kidney at 25°C and pH 6.3[a]

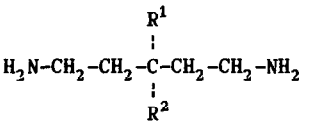
Substrate	Pea seedling DAO		Pig kidney DAO	
	K_M (mM)	V_{max} (μmol $mg^{-1}h^{-1}$)	K_M (mM)	V_{max} (μmol $mg^{-1}h^{-1}$)
(1) Putrescine	1.18(±.37)	1170(±220)	0.43(±.17)	0.030(±.005)
(2) *N*-methylputrescine	1.12(±.14)	211(±24)	1.34(±.28)	0.011(±.002)
(3) *N*-ethylputrescine	1.17(±.40)	3.1(±.3)	-	<0.002
(4) *N*-propylputrescine	0.68(±.22)	0.26(±.06)	-	<0.002

[a]Peroxidase-coupled assay (ref.7), standard deviations in parentheses.

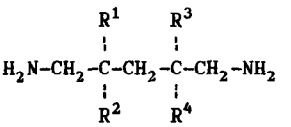
$$RNH(CH_2)_nNH_2 + H_2O + O_2 \xrightarrow{DAO} RNH(CH_2)_3CHO + H_2O_2 + NH_3$$

n=4 (putrescines):- (1): R = H (3): R = C_2H_5
(2): R = CH_3 (4): R = n-C_3H_7 Scheme 1

n=5 (cadaverines):-



(5) $R^1 = R^2 = H$ (cadaverine)
(6) $R^1 = H$, $R^2 = Me$ (3-methylcadaverine)
(7) $R^1 = R^2 = Me$ (3,3-dimethylcadaverine)
(8) $R^1 = OH$, $R^2 = Me$ (3-hydroxy-3-methylcadaverine)



(9) $R^1 = R^2 = Me$, $R^3 = R^4 = H$ (2,2-dimethylcadaverine)
(10) $R^1 = R^3 = Me$, $R^2 = R^4 = H$ (2,4-dimethylcadaverine)
(11) $R^1 = Me$, $R^2 = R^3 = R^4 = H$ (2-methylcadaverine)

Results and Discussion

The *N*-alkylputrescines (2)-(4) were prepared following published procedures.[9] 3,3-Dimethylcadaverine (7) was prepared from 2,2-dimethylpropane-1,3-diol *via* the dimesylate and dinitrile. The remaining cadaverine derivatives were made from substituted glutaric diacids or anhydrides after reduction to the corresponding diols using borane in tetrahydrofuran. Conversion of the diols into the diamines was carried out by the procedure of Golding and co-workers *via* the diazides.[10] All compounds were tested as substrates for pea-seedling and pig kidney DAO by a variety of techniques. Preliminary analysis using the bis-hydrazone[8] assay method indicated that DAO-catalysed oxidation is observable for all the putrescine (Table 1) and cadaverine (Table 3) derivatives investigated here, including *N*-methylputrescine (2) which has previously been reported to lack such activity.[8] However, although this procedure is convenient for routine screening of potential DAO substrates, it is unsuitable for detailed kinetic studies[13] and the relative rates are of qualitative value only. Measurement of initial reaction rates over a

Table 3. Oxidation rates of substituted cadaverines relative to cadaverine, catalysed by pea seedling and pig kidney DAO at 37°C and pH 7.4[a]

Substrate	Pea seedling DAO		Pig kidney DAO	
	Activity (nmol/30min)	Relative rate (%)	Activity (nmol/30min)	Relative rate (%)
(5) Cadaverine	165	100	114	100
(6) 3-Methylcadaverine	73	44	18	15
(7) 3,3-Dimethylcadaverine	2	1	18	15
(8) 3-Hydroxy-3-methylcadaverine	142	86	98	86
(9) 2,2-Dimethylcadaverine	15	9	16	14
(10)*meso*-2,4-Dimethylcadaverine	18	11	41	36

[a]bis-hydrazone method (ref.8).

Table 4. K_M and V_{MAX} values for oxidation of cadaverine and substituted cadaverines catalysed by DAO from pea seedlings and pig kidney at 25°C and pH 6.3[a]

Substrate	Pea seedling DAO		Pig kidney DAO	
	K_M (mM)	V_{max} (μmol $mg^{-1}h^{-1}$)	K_M (mM)	V_{max} (μmol $mg^{-1}h^{-1}$)
(5) Cadaverine	0.24(±.07)	2680(±410)	0.91(±.35)	0.18(±.07)
(11)2-Methylcadaverine	0.26(±.03)	1250(±130)	-	<0.008
(6) 3-Methylcadaverine	0.60(±.02)	260(±70)	-	<0.008
(7) 3,3-Dimethylcadaverine	0.61(±.06)	38(±4)	-	<0.004
(8) 3-Hydroxy-3-methylcadaverine	0.23(±.03)	770(±150)	-	-
(10)*meso*-2,4-Dimethylcadaverine	0.30(±.05)	300(±30)	-	<0.004

[a]Peroxidase-coupled assay (ref.7), standard deviations in parentheses.

much shorter time period, using the peroxidase-coupled assay,[7] confirmed the general trend of substrate activities and yielded Michaelis-Menten parameters for each enzyme/substrate pair (Tables 2 & 4), although only a few substrate systems could be investigated for the pig enzyme because of the poor specific activity of the commercial product. Values quoted here are reproducible for each batch of enzyme and, with cadaverine (5), are comparable to published data.[15] However, with putrescine (1) as substrate, literature estimates of K_M for pea seedling DAO show some lack of agreement, with K_M values ranging from 0.1 mM, or less,[16] up to about 1 mM,[17] consistent with results reported here (Table 2). Some of this variation undoubtedly arises from differences in experimental conditions and the different assay procedures employed, together with the natural species and isozyme distribution patterns to be expected. However, during the preliminary stages of this work we found that kinetic experiments using a limited range of substrate concentrations gave misleadingly low estimates of K_M values. Although there is a natural temptation to conserve scarce synthetic products, valid kinetic data can only be guaranteed from initial rate data spanning a concentration range from below K_M to approaching saturation kinetics (*ca.* 3 x K_M).

For pea-seedling DAO there is a progressive decrease in V_{max} with increasing size of the *N*-alkyl substituent for putrescine derivatives, (2)-(4) (see Table 2), together with similar effects for substituents at central positions in cadaverine derivatives, (6)-(7), (10), (11), which might be partially alleviated by inclusion of a more polar group, as in (8). These trends are reflected for both enzymes using the less accurate assay procedure of Tables 1 and 3. Although the range of substrate analogues investigated here is still too small to attempt, with confidence, any mechanistic interpretation of DAO selectivity, one intriguing feature of the data is that despite up to 4500-fold differences in saturation velocities (V_{max}), the K_M values show no significant variations. This suggests that the DAO active site is relatively non-specific as regards substrate binding, and that the source of enzyme selectivity lies in the detailed mechanism of the catalysis process which might be sensitive to steric hindrance and/or changes in pK/polarity of the substrate molecule.

One interesting consequence of this apparent lack of binding site specificity is that compounds which are relatively poor substrates might act as partially competitive inhibitors for DAO, since their binding affinities (K_M values) are of similar magnitude. We have confirmed this for a range of poor substrates using the quinazolium cation assay[8] which, when present at 1 mM concentration, reduce the apparent rates of DAO-catalysed putrescine and cadaverine oxidation by 50% or more. More detailed studies using the peroxide coupled procedure give data consistent with mixed competitive inhibition[14] (Figure 1) that one would expect for a molecule which competes for the same site on the enzyme as the substrate (putrescine or cadaverine), yet reacts at much slower rates. The inhibition constants (K_I) obtained from such experiments are comparable to the K_M values

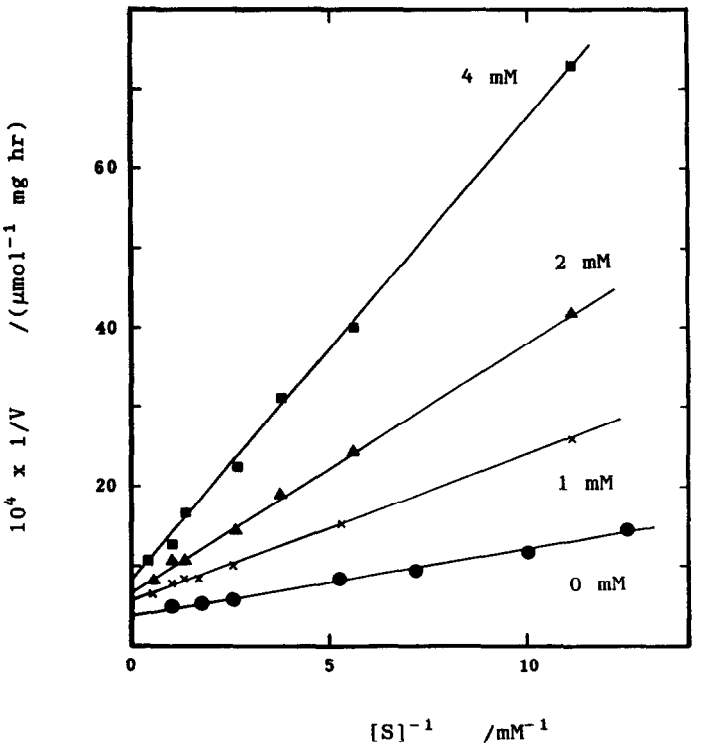


Figure 1: Lineweaver-Burk plot showing examples of the inhibition of pea seedling DAO catalysis of oxidation of cadaverine (5) by increasing concentrations (0, 1, 2, and 4 mM) of the less active substrate, 3,3-dimethylcadaverine (7). V is the initial total oxidation rate (measured as H_2O_2 using the peroxidase coupled assay[7]), [S] is the initial cadaverine concentration. The lack of a common intercept, indicating mixed inhibition,[14] was consistently observed and was statistically significant in least-squares analysis.

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of these compounds when assayed as substrates. For example, with cadaverine (5) as substrate and 3,3-dimethylcadaverine (7) as inhibitor (Fig.1), the K_I for (7) is 0.7(±0.1) mM, compared to the K_M value of 0.61(±0.06) mM for the same molecule when acting as a substrate (Table 4). With putrescine (1) as substrate, the K_I for (7) is about 0.3 mM. The mixture of competitive and non-competitive inhibition, indicated by kinetic data such as illustrated in Fig.1 (and confirmed by Eadie-Hofstee plots), suggests that different molecules might be capable of binding simultaneously within a rather extended active site region of the enzyme, thereby interfering with the catalytic mechanism. This would be consistent with the poor binding specificity indicated by the K_M values discussed above. Finally, it occurs to us that one technical advantage in the use of such poor substrate molecules as inhibitors of DAO activity *in vivo* might be that their residual susceptibility to DAO oxidation will limit their active lifetime in the biological system and avoid any potentially harmful build up of these reagents in sensitive tissues.

Experimental

M.p.s were measured with a Kofler hot-stage apparatus and are uncorrected. N.m.r. spectra were recorded for solutions in deuteriochloroform with tetramethylsilane as internal standard unless otherwise stated on a Perkin-Elmer R32 spectrometer operating at 90 MHz or on a Bruker WP-200SY spectrometer operating at 200 MHz for ^1H. Mass spectra were obtained with AEI MS12 or 902 spectrometers. T.l.c. of the diamine dihydrochlorides was carried out on Kieselgel G plates of 0.25 mm thickness, developed with isopropanol-conc.ammonia, 5:3, and the diamines were located with ninhydrin.

Pig kidney diamine oxidase, purchased from Sigma, had a nominal activity of 0.06 units per mg solid.[11] Pea seedling diamine oxidase was extracted from 10 day old pea seedlings as described by Hill and purified up to Step 4.[1] Enzyme activity was 1200 units per mg solid (at 25 °C), with a yield of ca. 30 mg per kg seedlings. SDS gel electrophoresis showed that the commercial pig enzyme is a heterogeneous mixture of many polypeptide species whereas the pea preparation, while not homogeneous, comprises just two major protein components. Protein concentrations were determined by the Coomassie Blue method of Sedmak and Grossberg.[12]

The kinetics of DAO-catalyzed oxidation of putative substrates were determined by the procedure of Stoner.[7] This involves a peroxidase-coupled assay (horseradish peroxidase, EC 1.11.1.7, from Sigma) to monitor continuously the hydrogen peroxide released during diamine oxidation (Scheme 1) at 25 °C, 70 mM phosphate buffer, pH 6.3, in the presence of 3-methyl-2-benzothiazolinone hydrazone (MBTH) and 3-(dimethylamino)benzoic acid (DMAB).[7] Oxidative coupling generates stoichiometric quantities of an indamine dye with a characteristic absorbance maximum at 595 nm, and rates of reaction can be determined directly in the spectrophotometer. A typical reaction mixture in a 1 cm pathlength cuvette comprised 3 ml of buffer containing peroxidase (6 μg/ml), MBTH (20 μM), DMAB (1 mM), and DAO (0.03-0.06 mg/ml for the pea-seedling enzyme, 2 mg/ml for pig kidney DAO). Substrate concentrations ranged up to 3 mM. The reaction was calibrated using standard hydrogen peroxide solutions and control experiments showed that, at the low concentrations used in this assay, bis-hydrazone formation by reaction with MBTH was negligible. Reaction was initiated by addition of standard enzyme solution to the thermally equilibrated reaction mixture, followed immediately by substrate addition, thereby minimizing the possibly inhibiting effects of extensive preincubation of DAO with the chromogenic agents.[7] Initial rates were determined over a range of substrate concentrations from the linear absorbance changes during the first minute of reaction, and Michaelis-Menten kinetic behaviour was observed in all cases. Rate data were analysed for K_M and V_{max} by least-squares fitting of Eadie-Hofstee (V *vs* $V/[S]$) and Lineweaver-Burk ($1/V$ *vs* $1/[S]$) plots.[14] Experiments using

histamine as substrate gave K_M and V_{max} comparable to literature values.[7] No special precautions were taken to control oxygen concentrations in the reaction mixtures, but control experiments using partially degassed buffers showed that reduction of O_2 to as low as 25% saturation (oxygen electrode) had no effect on measured rates, thus confirming that the reaction kinetics are not limited by O_2 levels under normal circumstances. Data quoted are means of at least three determinations. (Complete kinetic data for pig kidney DAO were unobtainable for several derivatives because of the very low activity of this enzyme preparation with these substrates.) Inhibition studies were performed in the same way by incorporating various concentrations of putative inhibitor/co-substrate into the initial reaction mixture. In a few instances, as a check on the validity of this assay procedure, reaction rates were measured under the same conditions by monitoring O_2 uptake as a function of time in a thermostatted reaction vessel fitted with a calibrated oxygen electrode. Similar rate data were obtained.

Substrate activity was also investigated by two less accurate procedures, following Frydman *et al.*[8] The first measures the extent of oxidation of putrescine and the *N*-alkylputrescines with diamine oxidase during a 30 minute incubation (37 °C, 50 mM Tris buffer, pH 7.2) by quenching and trapping the aminoaldehydes with 3-methyl-2-benzothiazolinone hydrazone and measuring the absorbance of the bis-hydrazone cation at 660 nm. The second involves detection of the quinazolium salt of reaction products formed in the presence of *o*-aminobenzaldehyde, measured at 435 nm, pH 7.4, phosphate buffer, 37 °C.[8]

Synthesis of Diamine Dihydrochlorides

The *N*-alkylputrescines, (2)-(4), were prepared as described[9], and purified by recrystallisation of the dihydrochlorides.[9] *N*-Methylputrescine (2) dihydrochloride, m.p. 175 °C (lit.[9] 176 °C). *N*-Ethylputrescine (3) dihydrochloride, m.p. 224 °C (lit.[9] 220-221 °C). *N*-Propyl-putrescine (4) dihydrochloride, m.p. 269 °C (lit.[9] 272 °C)

3,3-Dimethylcadaverine (7) dihydrochloride

2,2-Dimethylpropane-1,3-diol (2.71 g, 26 mmol) was dissolved in anhydrous THF (50 ml) and the solution was cooled to -78°C. Methanesulphonyl chloride (4.0 ml, *d* 1.48, 52 mmol) was added with stirring and then triethylamine (5.25 g, 52 mmol) was added slowly. The mixture was allowed to reach room temperature overnight, then was poured into ice/water (100 ml) and extracted with methylene chloride (3 x 75 ml). The combined extracts were dried (Na_2SO_4), filtered and concentrated to a yellow oil, which crystallised from diethyl ether to give 2,2-dimethylpropane-1,3-diol dimethanesulphate (5.66 g, 92%), m.p. 73-75 °C; ν_{max} (KBr disc) 3020, 1350, 1175, 957 and 950 cm^{-1}; δ_H (90 MHz) 4.03 (4H, s), 3.03 (6H, s) and 1.06 p.p.m. (6H, s); *m/z* 205, 175, 151, 97, 85, 79, 55 (100) and 41. (Found: C, 32.5; H, 6.20; S, 24.71. $C_5H_{16}S_2O_6$ requires C, 32.29; H, 6.20; S, 24.63%).

2,2-Dimethylpropane-1,3-diol dimethanesulphonate (5.03 g, 19.3 mmol) in dry DMSO (12 ml) was added by syringe to a stirred solution of NaCN (2.08 g, 42.8 mmol) in DMSO (60 ml) at 85-90 °C under a dry argon atmosphere. The reaction mixture was stirred at this temperature for one week, then allowed to cool. The solution was diluted with methylene chloride (250 ml) and the mixture was washed thoroughly with brine (5 x 100 ml). The organic solution was dried (Na_2SO_4), filtered, and concentrated to afford 3,3-dimethylglutaronitrile as a yellow oil (1.01 g, 43%), b.p. 185-190°C at 1 mm Hg; ν_{max} (thin film) 2970, 2940, 2241, 1355, 1175 and 1060 cm^{-1}; δ_H (90 MHz) 2.42 (4H, s) and 1.25 p.p.m. (6H, s); *m/z* 123, 107, 82 (100), 55 and 41.

3,3-Dimethylglutaronitrile (1.0 g, 8.2 mmol) was dissolved in anhydrous THF (15 ml) which was heated to reflux under a dry argon atmosphere. Borane in THF (1M, 18.5 ml, 18.5 mmol) was added dropwise by syringe. Heating at reflux was continued for 15 min and then the solution was allowed to cool to room temperature. Methanolic hydrogen chloride (1M, 20 ml) was added with vigorous evolution of hydrogen being observed after each addition and after a short induction period. When the addition was complete, the apparatus was rearranged for distillation. The boron residues azeotroped as methyl borate with the THF at 60 °C. Final traces of methyl borate were removed by addition of a few ml of methanol and evaporation under reduced pressure. The crude solid product was then dried *in vacuo* over P_2O_5 for 24 h. 3,3-Dimethylcadaverine (7) dihydrochloride was recrystallised from absolute ethanol, water and acetone (1.06 g, 64%), m.p. 247-249 °C; ν_{max} (KBr disc) 3010, 2920, 1610, 1515 and 1410 cm^{-1}; δ_H (90 MHz) (D_2O) 4.93 (HOD), 3.33 (4H, m), 1.89 (4H, m) and 1.24 p.p.m. (6H, s); *m/z* 167, 129, 112, 69, 55 and 36 (100). (Found: C, 32.68; H, 9.72; N, 16.38. $C_7H_{20}N_2Cl_2.NH_4Cl$ requires C, 32.75; H, 9.36; N, 16.37%).

General Procedure for synthesis of cadaverine derivatives from substituted glutaric acids or anhydrides.

The substituted glutaric acid or anhydride (1 mmol) dissolved in THF (20 ml) was brought to reflux under a dry argon atmosphere. Borane in THF (1M, 2 mmol) was added dropwise *via* syringe. Heating at reflux was continued for *ca.* 18 h. The reaction mixture was allowed to cool, then water (20 ml) was added *via* syringe. The aqueous layer was then saturated with K_2CO_3 (*ca.* 4 g). Diethyl ether (10 ml) was then added to the reaction mixture. The reaction mixture was extracted using diethyl ether (4 x 20 ml); the organic layer was dried (Na_2SO_4), filtered, and concentrated to leave the product as a yellow oil, which was then distilled.

A solution of hydrazoic acid in benzene (1.0M, 2.4 mmol) was added to the substituted diol (1 mmol) in THF (20 ml). A solution of di-isopropyl azodicarboxylate (2.2 mmol) in THF was then added with stirring. To this mixture was added triphenylphosphine (4.4 mmol) in THF

(60 ml). The reaction temperature depends on the rate of addition, which was maintained at 40 °C. The reaction mixture was stirred for 1 h at room temperature, then was heated at 50 °C for 3 h. Water (2 ml) was added, and the solution was stirred at 50 °C for a further 3 h. The solvents were removed *in vacuo* and the residue was partitioned between 1M HCl (80 ml) and CH_2Cl_2 (80 ml). The aqueous layer was further extracted with CH_2Cl_2 (2 x 80 ml). The aqueous layer was evaporated *in vacuo* leaving the diamine dihydrochloride which was recrystallised from aqueous ethanol/acetone (1:1).

Preparation of Hydrazoic Acid

A paste was prepared from equal weights of water and sodium azide (32.5 g) in a 500 ml three-necked flask. To this paste was added benzene (200 ml) and the mixture was cooled to *ca.* 7 °C. Conc. sulphuric acid (13.3 ml, 0.25 mol) was added slowly to the flask with continued stirring and cooling, keeping the temperature below 10 °C. The mixture was stirred for 1 h after the acid had been added, then was cooled to 0 °C. The organic layer was decanted and dried over Na_2SO_4. The hydrazoic acid concentration was determined by titration against standard NaOH solution.

3-Methylcadaverine (6) dihydrochloride

Using 3-methylglutaric acid, 3-methylpentane-1,5-diol was obtained as a yellow oil (75% yield), b.p. 160 °C at 1mm Hg; ν_{max} (thin film) 3360, 2960, 2930, 1455, 1380, 1070 and 1060 cm^{-1}; δ_H (90 MHz) 3.67 (4H, t, *J* 6Hz), 3.23 (2H, br s), 1.62-1.32 (5H, m) and 0.90 p.p.m. (3H, d, *J* 6Hz); *m/z* 116 (M^+-2H), 100 (M^+-H_2O), 88, 82, 70, 67, 55, 41 (100), 39, 31 and 29.

3-Methylcadaverine dihydrochloride was obtained as a white crystalline solid (24% yield), m.p. 267-268 °C; R_f 0.29; ν_{max} (KBr disc) 3420, 3000, 1595, 1475 and 1400 cm^{-1}; δ_H (90 MHz) (D_2O) 4.93 (HOD), 3.23 (4H, t, *J* 7Hz), 1.80 (5H, br s) and 1.12 p.p.m. (3H, d, *J* 5Hz); *m/z* 117, 99, 70, 56, 45, 38, 36, and 30 (100). (Found: C, 38.10; H, 9.55; N, 14.80; Cl, 37.69. $C_6H_{18}N_2Cl_2$ requires C, 38.10; H, 9.52; N, 14.81; Cl, 37.57%).

2,2-Dimethylcadaverine (9) dihydrochloride

Treatment of 2,2-dimethylglutaric acid as described in the general procedure afforded 2,2-dimethylpentane-1,5-diol as a yellow oil (88% yield); b.p. 180 °C at 1 mm Hg; ν_{max} (CCl_4) 3350, 2950, 2865, 1470, 1360 and 1050 cm^{-1}; δ_H (90 MHz) 3.45 (4H, d, *J* 6Hz), 3.30 (2H, br s), 1.69 (4H, m), 0.94 (3H, s) and 0.87 p.p.m. (3H, s); *m/z* 114, 97, 83, 69, 55, and 41 (100).

2,2-Dimethylcadaverine dihydrochloride was prepared in 4% yield, m.p. 158-160 °C; R_f 0.47; ν_{max} (KBr disc) 3440, 3020, 2960, 1595, 1500, 1400 and 1375 cm^{-1}; δ_H (90 MHz) (D_2O) 4.80 (HOD), 3.14 (2H, t, *J* 8Hz), 3.00 (2H, s), 2.00-1.30 (4H, m), 1.10 (6H, s); *m/z* 115, 101, 83, 70, 55, 45, and 30 (100). (Found: C, 41.31; H, 9.79; N, 13.53. $C_7H_{20}N_2Cl_2$ requires C, 41.38; H, 9.85; N, 13.79%).

meso-2,4-Dimethylcadaverine (10) dihydrochloride

When *meso*-2,4-dimethylglutaric anhydride was treated using the standard procedure, 2,4-dimethylpentane-1,5-diol was produced in 75% yield; b.p. 165 °C at 1 mm Hg; ν_{max} (CCl_4) 3350, 2955, 2920, 1410, 1360, 1340 and 1075 cm^{-1}; δ_H (90 MHz) 3.47 (4H, d, *J* 8Hz), 2.55 (2H, br s), 1.68 (4H, s) and 0.90 p.p.m. (6H, d, *J* 4Hz); *m/z* 114 (M^+-H_2O), 102, 84, 69, 55 (100), 41, and 31.

Using 2,4-dimethylpentane-1,5-diol, *meso*-2.4-dimethylcadaverine dihydrochloride was obtained as a white crystalline solid (22% yield); m.p. 223-224 °C; R_f 0.64; ν_{max} (KBr disc) 3400, 3020, 2960, 1600, 1565, 1495, 1470, 1180, 1120 and 1010 cm^{-1}; δ_H (90 MHz) (D_2O) 4.83 (HOD), 3.28-2.78 (4H, m), 2.20-1.90 (2H, m), 1.55-1.33 (2H, m), 1.15 (6H, d, *J* 7Hz); *m/z* 131, 113, 101, 98, 70, 59, 42, 36, and 30 (100). (Found: C, 41.27; H, 9.83; N, 13.80; Cl, 34.79. $C_7H_{20}N_2Cl_2$ requires C, 41.38; H, 9.85; N, 13.79; Cl, 34.98%).

3-Hydroxy-3-methylcadaverine (8) dihydrochloride

To a stirred solution of 3-hydroxy-3-methylglutaric acid (5 g, 31 mmol) in methanol (250 ml) at 0 °C was added dropwise thionyl chloride (8.81 g, 74 mmol). The solution was then evaporated *in vacuo* to give the dimethyl ester as a yellow oil, which was distilled *in vacuo* (5.32 g, 91%), b.p. 70 °C at 0.5 mm Hg; ν_{max} (CCl_4) 3510, 2970, 2940, 1730, 1430, 1195 and 1170 cm^{-1}; δ_H (90 MHz) 4.70 (1H, br s), 3.70 (6H, s), 2.69 (4H, s) and 1.36 p.p.m. (3H, s); *m/z* 175, 117, 85 and 43 (100).

3-Hydroxy-3-methylpentane-1,5-diol was prepared from the dimethyl ester using the standard method in 88% yield; b.p. 165 °C at 0.6 mm Hg; ν_{max} (CCl_4) 3420, 2950, 2920 and 1015 cm^{-1}; δ_H (90 MHz) 3.68 (4H, t, *J* 7Hz), 1.68 (2H, br s), 1.50 (4H, m) and 0.95 p.p.m. (3H, s); *m/z* 131, 89, 71, 60, 55 and 43(100).

3-Hydroxy-3-methylcadaverine (8) dihydrochloride was synthesised from 3-hydroxy-3-methylpentane-1,5-diol in 4% yield; m.p. 249 °C (dec); R_f 0.15; ν_{max} (KBr disc) 3420, 2980, 2920, 1450, 1280 and 1110 cm^{-1}; δ_H (90 MHz) (D_2O) 5.06 (HOD), 3.20 (4H, m), 1.90 (4H, m) and 1.15 p.p.m. (3H, s); *m/z* 116, 76, 59, and 43 (100).

2-Methylcadaverine (11) dihydrochloride was prepared from 2-methylcadaverine (Aldrich), m.p. 165-167°C. (Found: C, 38.01; H, 9.49; N, 14.73. $C_6H_{18}N_2Cl_2$ requires C, 38.10; H, 9.52; N, 14.81%).

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References and Notes

1. Hill, J.M. *Methods in Enzymology*, 1971, **17B**, 730.
2. Malmström,B.G.; Andreassson,L.-E. and Reinhammar,B. in *The Enzymes*, Vol.XII(B), ed.P.D.Boyer, 1975, p.507.
3. Smith,T.A. *Biochem.Soc.Trans.*, 1985, **13**, 319.
4. Bachrach,U. Function of Naturally Occurring Polyamines (Academic Press, 1973).
5. Tabor,C.W. and Tabor,H. *Ann.Rev.Biochem.*, 1984, **53**, 749-790.
6. Walters,D. *Biologist*, 1987, **34**, 73.
7. Stoner,P. *Agents and Actions*, 1985, **17**, 5.
8. Frydman,R.B.; Ruiz,O.; Kreisel,M. and Bachrach,U. *FEBS Lett.*, 1987, **219**, 380.
9. Alonso Garrido,D.O.; Buldain,G. and Frydman,B. *J.Org.Chem.*, 1984, **49**, 2021.
10. Fabiano,E.; Golding,B.T. and Sadeghi,M.M. *Synthesis*, 1987, 190.
11. One standard unit will oxidise 1.0 μmol of putrescine (1) per hour at 37°C and pH 7.2
12. Sedmak,J.J. and Grossberg,S.E. *Anal.Biochem.*, 1977, **79**, 544.
13. Cooper,A.; Equi,A.M.; Ner,S.K.; Watson.A.B. and Robins,D.J. *Tetrahedron Lett.*, 1989, **30**, 5167.
14. Fersht,A.R. Enzyme Structure and Mechanism (2nd. edition, W.H.Freeman and Co., 1985).
15. Costa,M.T.; Rotilio,G.; Agro,.A.F.; Vailogini,M.P. and Mondovi,B. *Arch.Biochem. Biophys.*, 1971, **147**, 8.
16. Srivastava,S.K. and Prakash,V. *Phytochemistry*, 1977, **16**, 189.
17. Haviger,A. and Pec,P. *Acta Univ.Palack.Olomuc.,Fac.rerum nat.*, 1986, **85**, Chem.25, 191.