The Oxidation of Lysine and Oxalysine by *Mytilus edulis*

IDENTIFICATION OF THE PRODUCTS FORMED IN THE PRESENCE AND THE ABSENCE OF CATALASE

By D. B. HOPE AND K. C. HORNCastle
Department of Pharmacology, University of Oxford

(Received 20 June 1966)

1. *O*-(2-Aminoethyl)serine (oxalysine) was shown to be a substrate of the L-amino acid oxidase of the digestive gland of the common mussel, *Mytilus edulis*.
2. Three atoms of oxygen were consumed per mole of oxalysine oxidized in the presence of catalase; L-lysine under the same conditions consumed only one atom.
3. The products of oxidation of oxalysine in the presence and the absence of catalase were: ethanolamine, *N*-oxalylethanolamine and 3-morpholone (the oxygen analogue of 2-piperidone). After acid hydrolysis 70% of the oxalysine oxidized was recovered as ethanolamine.
4. In the absence of catalase 2-aminoethoxyacetic acid was also detected.
5. The products identified account quantitatively for the oxalysine oxidized and for the oxygen uptake.
6. *N*-Oxalylethanolamine and 2-aminoethoxyacetic acid have been synthesized.
7. Treatment of extracts of the digestive gland at pH 3.0 completely inactivated the catalase, leaving the L-amino acid oxidase unaffected.
8. The major product of the oxidation of lysine in the absence of catalase was 2-piperidone.

Recent observations in this Laboratory have shown that thialysine, *S*-(2-aminoethyl)-L-cysteine, is oxidized by the L-amino acid oxidase of *Mytilus edulis*. The oxygen consumption is three times as great with lysine as substrate (Hope, 1965). The mechanism of further oxidation has not been established and the possibility that this may be oxidation of the thio ether to a sulphoxide or sulphone led us to study the corresponding oxygen analogue, oxalysine (Tesser & Nefkens, 1959). We now wish to report the results of a study of the oxidation of *O*-(2-aminoethyl)serine (oxalysine) by the enzyme. This substance (Fig. 1) is oxidized less readily than thialysine but the final oxygen uptake is similar. The products of oxidation have been identified as ethanolamine, *N*-oxalylethanolamine, 3-morpholone and 2-aminoethoxyacetic acid. A short account of this work has been given (Horncastle & Hope, 1965).

MATERIALS AND METHODS

Chemicals. Ethanolamine, diethyl oxalate, ethylchloroacetate, *o*-tolidine, 2,4-dinitrofluorobenzene, triethylamine and 8-hydroxyquinoline were reagent-grade materials; potassium iodide, acetic acid, butan-1-ol, barium hydroxide octahydrate and ninhydrin were AnalaR reagents obtained from British Drug Houses Ltd. (Poole, Dorset). L-Lysine monohydrochloride, L-alanine and L-arginine hydrochloride were obtained from Koch-Light Laboratories Ltd. (Coln-

brook, Bucks.); the arginine salt was once recrystallized from water. DL-Oxalysine dihydrochloride was the gift of Dr G. I. Tesser of Nijmegen University. The material was analytically pure (Found: N, 12.55; Cl, 32.12, Calc. for $C_5H_{14}N_2O_3Cl_2$ N, 12.67; Cl, 32.07%). 2-Piperidone and δ -aminovaleric acid were purchased from Aldrich Chemical Co. Inc. (Milwaukee, Wis., U.S.A.).

Preparation of L-amino acid oxidase free from catalase. The common mussels, *Mytilus edulis*, were supplied by the Marine Biological Laboratory, Plymouth. On arrival the digestive glands, including a portion of the intestine, were dissected out and frozen. An acetone-dried powder of the

Fig. 1. Lysine and its oxygen and sulphur-containing analogues.

material (250g.) was prepared by homogenization in 1.5l. of acetone precooled to -20° (yield: 62g.).

A portion (30g.) of the acetone-dried powder was suspended in 300ml. of 0.067M-sodium dihydrogen phosphate solution acidified to pH 3.0 with N-hydrochloric acid and dialysed for 48hr. against a total of 5l. of this solution. The suspension was centrifuged at 750g for 45 min., resuspended in 300ml. of 0.067M-disodium hydrogen phosphate buffer, pH 7.15, and again centrifuged at 750g for 45 min. The sediment was resuspended in 300ml. of the same buffer and dialysed against a total of 6l. of the buffer with six changes over a period of 48hr. The final volume was 270ml. This preparation contained the equivalent of 0.45g. wet wt. of tissue/ml. and consisted of a suspension of insoluble particles. The enzymic activity was associated with the particulate material.

Manometric experiments. The total oxygen consumption when oxalysine, lysine and arginine were oxidized was measured manometrically at 25° . The manometer flasks contained 1ml. of enzyme suspension and 0.6ml. of either water or water containing 2 units of crystalline bovine catalase (Worthington Biochemical Corp.). The side bulbs contained 10 μ moles of substrate as 0.4ml. of aqueous solution: the final substrate concentration was thus 5mM, which gave maximal rates with L-arginine. The central wells contained filter paper and 0.3ml. of N-KOH.

Chromatography. Descending paper-partition chromatography was done on Whatman no. 4 paper run with the grain. The solvent system was butan-1-ol-acetic acid-water (4:1:5, upper phase) as described by Partridge (1948). This procedure was used for the detection of ninhydrin- and Sakaguchi-positive substances. The reagents, used as dips, were (1) 0.2% (w/v) ninhydrin in acetone-water (19:1), (2) 0.1% (w/v) 8-hydroxyquinoline in acetone followed by 0.5N-NaOH containing 0.2ml. of bromine/100ml. (Jepson & Smith, 1953). The mobilities of the substances are expressed relative to the distance moved by alanine as R_A values.

Thin-layer chromatography was carried out on Kieselgel G (E. Merck A.-G., Darmstadt, Germany), 0.25mm. thick, in four different solvent systems (chloroform-methanol, 9:1; chloroform-methanol, 4:1; methanol; ethanol) with 15cm. migration. The atmosphere in the chromatography jars was saturated by inserting filter paper into the jar. Multiple development was used for slow-running substances. The presence of ionic substances proved troublesome for the identification of N-oxalylethanolamine and these were removed on a column (0.6cm. $\times$ 5.0cm.) of Dowex 50 (X8; H⁺ form; 200-400 mesh). The eluate was concentrated *in vacuo* before chromatography. 2,4-Dinitrophenyl derivatives acted as self indicators. Other substances were detected by the chlorine-o-tolidine-potassium iodide reagent of Pataki (1963). Small amounts of amides gave a blue-violet colour and larger amounts a yellow colour. Amines also reacted but the intensity of colour was less.

Detection and analysis of ninhydrin-positive substances

Preparation of samples. After the oxidation of L-lysine (10 μ M) and of DL-oxalysine (20 μ M) the reaction mixture (2ml.) was removed from the manometer flask and centrifuged at 20000g-min. The supernatant was used for

analysis. For paper chromatography 5-20 μ l. was used. For amino acid analysis, before hydrolysis, 0.5ml. of supernatant was diluted to 5ml. with citrate buffer, pH 2.2 (Moore & Stein, 1954). A portion (2ml.) of this solution (corresponding to 1 μ mole of L-amino acid substrate) was applied to the 50cm. column of the amino acid analyser. For amino acid analysis after hydrolysis samples were hydrolysed in acid by a method based on that of Crestfield, Moore & Stein (1963). Supernatant (0.5ml.) and 2.5ml. of constant-boiling hydrochloric acid were sealed into a hydrolysis tube *in vacuo*. After hydrolysis at 110° for 17hr. the contents of the tube were removed and transferred to a small beaker with washings and evaporated *in vacuo* over calcium chloride and sodium hydroxide pellets. The residue was dissolved in 5ml. of citrate buffer, pH 2.2, and 2ml. was applied to the 50cm. column of the amino acid analyser. In control experiments known amounts of ethanolamine and of oxalysine were recovered quantitatively after this procedure. Analytical figures for ammonia are given after correction for the amount present in the hydrolysate of the enzyme blank. The elution volumes and colour yields of the ninhydrin-positive substances analysed on the 50cm. column at 30° were: 2-aminoethoxyacetic acid, 55ml., 15.20; lysine, 267ml., 24.06; oxalysine, 204ml., 23.44; ethanolamine, 233ml., 17.15; ammonia, 246ml., 22.33. On the same column at 50° δ -aminovaleric acid was eluted at 280ml. with a colour yield 17.61. The colour yields for lysine, ethanolamine and ammonia are somewhat less than those reported by Spackman, Stein & Moore (1958) because the diameter of the photometer tube of the amino acid analyser was less.

Elementary analyses. Microanalyses were by Weiler and Strauss, Oxford, and Dr H. Bieler, Organisch-Chemisches Institut der Universität Wien.

Melting points. A Kofler block with a Reichert microscope and crossed Nicols was employed. The melting points are recorded uncorrected.

Characterization of ethanolamine as N-DNP-ethanolamine. The presence of ethanolamine amongst the products of oxalysine oxidation was confirmed by conversion into the DNP derivative by a method based on that described by Grassman, Hörmann & Endres (1953). The insoluble enzyme was removed by centrifugation at 55000g for 30min. and the supernatant was made alkaline with sodium hydrogen carbonate (0.1g./ml.). 1-Fluoro-2,4-dinitrobenzene (0.05g.) dissolved in 4ml. of ethanol was added. The mixture was shaken at room temperature overnight and glycine (12mg.) was then added with further shaking for 2hr. to remove excess of reagent. DNP-ethanolamine was extracted with 20ml. of chloroform (Axelrod, Reichenthal & Brodie, 1953) and the extract was concentrated to 1ml.

For comparison an authentic sample of DNP-ethanolamine was prepared by the method of Grassman *et al.* (1953).

Preparation of reference substances

Barium N-(β -hydroxyethyl)-oxamate. Diethyl oxalate (67.5ml.; 0.5mole) was mixed with 600ml. of acetone at 3° . Ethanolamine (30.5ml.; 0.5mole) in 60ml. of acetone was added dropwise over a period of 4.5hr. The reaction mixture was left overnight at room temperature, cooled to 0° and the colourless crystalline solid was collected. This material (4.24g.) was shown by its melting point ($168-170^{\circ}$)

and its infrared spectrum to be *NN*-(di- β -hydroxyethyl)-oxamide (see below). The filtrate was concentrated *in vacuo* and the residue was dissolved in 100 ml. of water and cooled to 4°. A solution of 78.8 g. of barium hydroxide octahydrate in 1 l. of water was added dropwise with stirring. The pH of the solution was not allowed to rise above 9.0 until the final stage when it rose to 10. A heavy white precipitate (13.6 g.) was removed by centrifugation. This was shown by titration with potassium permanganate to be barium oxalate. The filtrate was evaporated *in vacuo* at 40°. The residue was powdered and extracted with methanol. The insoluble white solid (61 g.) was dissolved in 60 ml. of boiling water from which it crystallized on cooling. It was washed with water and dried in air at room temperature. The product (46 g.) had m.p. 156–157° (Found: C, 21.9; H, 3.3; N, 6.2; $C_8H_{12}N_2O_8Ba \cdot 2H_2O$ requires C, 22.0; H, 3.7; N, 6.4%).

NN'-(Di- β -hydroxyethyl)oxamide. This compound was prepared to identify the first by-product isolated in the preceding experiment. Diethyl oxalate (3.37 ml.) was added dropwise in 10 min. to 3.04 ml. of ethanolamine at 0°. A vigorous reaction occurred and the white solid which separated was recrystallized from 110 ml. of hot methanol. The product (2.95 g.) crystallized in long colourless needles, m.p. 168–170° (Found: C, 41.3; H, 7.0; N, 16.0; $C_8H_{12}N_2O_4$ requires C, 40.9; H, 6.9; N, 15.9%).

N-(β -Hydroxyethyl)oxamic acid (N'-oxalylethanolamine). Barium *N*-(β -hydroxyethyl)oxamate (5.0 g.) was dissolved in 50 ml. of water and 22.9 ml. of *N*-sulphuric acid was added slowly with stirring. Barium sulphate was removed by centrifugation and the clear supernatant was concentrated *in vacuo* at 30°. The semi-crystalline residue was crystallized from 16 ml. of methanol. The product (1.6 g.) separated in colourless rhomboids, m.p. 133–134°. The crystals began to sublime above 120°; however, the sublimate and the original material melted simultaneously (Found: C, 35.8; H, 5.1; N, 10.3; $C_4H_7NO_4$ requires C, 36.1; H, 5.3; N, 10.5%).

3-Morpholine. This substance was prepared by the method described by Vièles & Séguin (1953). The material was twice recrystallized from toluene and had m.p. 104–105.5°; Vièles & Séguin (1953) give m.p. 105° (Found: N, 13.9. Calc. for $C_4H_7NO_2$: N, 13.9%). The yield was lower than had been claimed.

2-Aminoethoxyacetic acid. 3-Morpholine (0.94 g.) was dissolved in 20 ml. of 6*N*-hydrochloric acid. Hydrolysis of the 3-morpholine was brought about by heating the solution at 110° for 6 hr. The hydrolysate was concentrated *in vacuo* at 30° and the oily residue was dissolved in 50 ml. of ethanol. Triethylamine (2 ml.) was added to precipitate the amino acid and the crude material was recrystallized from 20 ml. of methanol. The product (0.30 g.) showed m.p. 184.5–186° (Found: C, 40.3; H, 7.8; N, 11.6; $C_4H_9NO_3$ requires C, 40.3; H, 7.6; N, 11.8%). On the amino acid analyser the elution volume was 55 ml. and the colour yield was 15.20.

N-2,4-Dinitrophenylethanolamine. 2,4-Dinitrofluorobenzene (1.0 g.) was shaken with a solution of 1 ml. of ethanolamine in 5 ml. of water. Sufficient ethanolamine was used to act as a base. The reaction mixture was cooled to room temperature and a yellow crystalline compound rapidly separated (1.1 g.). This was recrystallized from ethanol-water (5:1) at 60°. The product (0.92 g.) showed m.p. 90–90.5°; Axelrod *et al.* (1953) give m.p. 90°.

RESULTS

In preliminary experiments with a crude suspension of the digestive glands of *Mytilus edulis* (Blaschko & Hope, 1956), DL-oxalysine was found to be oxidized. The rate was less than that with the parent amino acid, L-lysine, but the total oxygen consumption was considerably greater. In these experiments an appreciable amount of oxygen was consumed by the suspension alone. This difficulty was overcome by acidifying the enzyme preparation to pH 3.0 for a period of 48 hr. The activity of the L-amino acid oxidase towards L-arginine was unaffected by this treatment but catalase activity was completely destroyed. Thus the final oxygen uptake with L-arginine was doubled after acid treatment of the crude suspension and this effect was reversed upon addition of 2 units of crystalline bovine catalase.

For all other experiments described in this paper we have used the catalase-free preparation of L-amino acid oxidase (see the Materials and Methods section) both in the presence and the absence of crystalline catalase.

Oxygen uptake by L-arginine, L-lysine and DL-oxalysine. The rates of oxygen uptake by the three substrates relative to that of L-arginine are shown in Table 1. The rate with L-lysine was some 20% greater than with L-arginine, in agreement with earlier results (Blaschko & Hope, 1956). The rate of oxygen uptake with DL-oxalysine was approximately half of that with L-lysine. In the absence of catalase the rate of oxygen uptake with L-arginine and with L-lysine were approximately doubled (Fig. 2). With DL-oxalysine there was also some increase in the rate of oxygen uptake but the change in rate was less marked than with lysine.

Table 1. *Rates of oxidation of basic amino acids by the L-amino acid oxidase of the digestive gland of Mytilus edulis*

The manometric procedure is described in the Materials and Methods section. The rate of oxygen uptake was measured for 20 min. and is expressed as a percentage of the rate of oxygen uptake with L-arginine in the presence of catalase. The rate of oxygen uptake with L-arginine in the presence of catalase was 79 μ l. (64–94) in 20 min. The oxygen uptake in the 'enzyme blank' was zero during this period. The initial substrate concentration was 5 mM.

Substrate	Relative rate of uptake of O ₂	
	With 2 units of catalase	Without catalase
L-Arginine	(100)	175
L-Lysine	117	226
DL-Oxalysine	63	86

The theoretical total oxygen uptake for the oxidation of 10 μ moles of L-amino acid to the corresponding α -oxo acid in the presence of

Fig. 2. Oxidation of L-lysine and of DL-oxalysine by L-amino acid oxidase. ○, 10 μ moles of L-lysine oxidized in the presence of catalase. △, 10 μ moles of L-lysine oxidized in the absence of catalase. ●, 10 μ moles of L-oxalysine oxidized in the presence of catalase. ▲, 10 μ moles of L-oxalysine oxidized in the absence of catalase.

Table 2. Final oxygen uptake for the oxidation of L-amino acid by L-amino acid oxidase

The manometric procedure is described in the Materials and Methods section. The results are expressed as the mean of eight experiments together with the s.e.m. The oxidation of 10 μ moles of oxalysine was complete in 3 hr. In the same period the oxygen uptake in the 'enzyme blank' was 8 μ l. (4–16 μ l.).

Substrate	O ₂ consumed (μ l./ μ mole of amino acid)	
	With 2 units of catalase	Without catalase
L-Arginine	111 $\pm$ 8	224 $\pm$ 21
L-Lysine	115 $\pm$ 8	231 $\pm$ 18
L-Oxalysine	323 $\pm$ 25	393 $\pm$ 43

Table 3. R_f values of products obtained from oxalysine in thin-layer chromatography on Kieselgel G

Solvent systems: (1) chloroform-methanol (9:1); (2) chloroform-methanol (4:1); (3) methanol; (4) ethanol.				
Solvent system.....	(1)	(2)	(3)	(4)
DNP-Ethanolamine	0.56	—	0.66	0.59
3-Morpholone	0.44	0.57	0.59	0.33
N-Oxalylethanolamine	—	—	0.11	0.04
2-Piperidone	0.52	0.65	0.60	0.39

catalase is 112 μ l. The results with L-lysine and L-arginine were in close agreement with this figure whereas the oxygen consumption with 20 μ moles of DL-oxalysine was almost three times as great (Table 2). At the end of the experiment with oxalysine an amino acid analysis on the contents of the flask showed that half of the substrate, presumably D-oxalysine, remained unchanged. This can be understood since it is known that the digestive gland of *Mytilus edulis* is free from D-amino acid oxidase (Blaschko & Hope, 1956).

Oxidation products of L-lysine. As already mentioned, the oxygen uptake by lysine was doubled in the absence of catalase. Attempts to detect δ -aminovaleric acid in the oxidation products of 10 μ moles of lysine by paper chromatography were unsuccessful. However, when the products were analysed on the 50 cm. column of the amino acid analyser at 50° small amounts of δ -aminovaleric acid were detected corresponding to 0.08 μ mole/ μ mole of lysine oxidized. Meister (1954), using paper chromatography, had shown earlier that the oxidation of ϵ -amino- α -oxocaproic acid (Δ' -piperidine-2-carboxylic acid) with hydrogen peroxide under a variety of conditions gave δ -aminovaleric acid and he concluded that the latter was the major product. Under our conditions the main oxidation product appeared to be an unknown substance detected by thin-layer chromatography. Boulanger & Osteux (1956), using L-amino acid oxidase of turkey liver, also failed to find δ -aminovaleric acid, but they established that the product in the absence of catalase was 2-piperidone. When an authentic sample of this substance was compared by thin-layer chromatography with our unknown material the R_f values were identical in four solvent systems (Table 3). The presence of 2-piperidone in the oxidation product was confirmed by acid hydrolysis. The oxidation products of 1 μ mole of lysine contained 0.08 μ mole of δ -aminovaleric acid before and 0.86 μ mole after hydrolysis. The increase is due to the hydrolysis of 2-piperidone.

Oxidation products of DL-oxalysine. In a chromatographic investigation of the reaction products from a manometer flask by the amino acid analyser the theoretical amount of ammonia (10 μ moles) was

recovered and, in addition, an unknown substance close to the ammonia peak. With the 50 cm. column of the amino acid analyser the unknown peak appeared in the position of ethanolamine, 0.4 μ mole being detected for each μ mole of L-oxalysine oxidized. The identification was confirmed by conversion into the *N*-dinitrophenyl derivative and comparison with authentic DNP-ethanolamine by thin-layer chromatography in three solvent systems (Table 3).

Ethanolamine is known to undergo oxidation by hydrogen peroxide, in the presence of catalase (Keilin & Hartree, 1945). The possibility that some ethanolamine had been destroyed in this way was tested by the addition of ethanolamine in a range of concentrations (2–50 mm) to manometer flasks in which L-arginine was oxidized in the presence of catalase. No increase in oxygen consumption was detected. The amount of ethanolamine formed was less (0.18 μ mole) when oxalysine was oxidized by catalase-free enzyme, and the analysis of the products on the 50 cm. column showed a second unknown peak with a small elution volume. The oxidation mixture from a similar experiment was investigated by thin-layer chromatography in chloroform-methanol (4:1). Two spots were detected by Pataki's reagent, one at the origin and one with R_F 0.57 corresponding to a lipophilic substance. As a control the products from a manometric experiment in which lysine had been oxidized by catalase-free enzyme were similarly investigated: again a lipophilic substance, 2-piperidone (R_F 0.65), was detected.

The results obtained with L-lysine suggested that the lipophilic substance formed during oxidation of oxalysine was 3-morpholone. Thin-layer chromatography of a synthetic sample in four solvent systems gave R_F values identical with those of the

unknown. Acid hydrolysis of 3-morpholone gave a new ω -amino acid 2-aminoethoxyacetic acid which on the amino acid analyser gave a peak at the same position as that of the substance formed when oxalysine was oxidized in the absence of catalase. The amount of this substance was increased after hydrolysis of the oxidation product owing to ring cleavage of 3-morpholone. The amount of 3-morpholone formed (Table 4) was independent of the presence of catalase. After hydrolysis the amount of ethanolamine was increased, which suggested the presence of an acid-labile derivative of ethanolamine. This increase was approximately the same whether or not catalase was present (Table 4). The ethanolamine and 2-aminoethoxyacetic acid present in hydrolysates together fully accounted for the oxalysine oxidized.

The nature of the substance responsible for the increase in ethanolamine on hydrolysis was also established by thin-layer chromatography in methanol. In addition to 3-morpholone (R_F 0.59) a slower-running substance was detected (R_F 0.11). The latter was identified as *N*-oxalylethanolamine by comparison with synthetic material by chromatography on thin-layer plates with methanol and ethanol as solvents. This substance could have arisen by oxidation of the α -oxo acid at the β -carbon atom followed by oxidative decarboxylation by hydrogen peroxide.

DISCUSSION

When the γ -methylene group of lysine is substituted by an oxygen atom, the rate of oxidation by the L-amino acid oxidase of *Mytilus edulis* is reduced but the final oxygen uptake is markedly increased. In this respect L-oxalysine resembles the

Table 4. Quantitative estimation of the products from the oxidation of DL-oxalysine

Unchanged D-oxalysine, ammonia, ethanolamine and 2-aminoethoxyacetic acid were estimated on the 50 cm. column of the EEL amino acid analyser at 30° before and after hydrolysis with 6N-HCl. The amounts of *N*-oxalylethanolamine and 3-morpholone are obtained from the difference in ethanolamine and 2-aminoethoxyacetic acid respectively before and after hydrolysis.

Ninhydrin-positive compounds	Catalase present		Catalase absent	
	Before hydrolysis	After hydrolysis	Before hydrolysis	After hydrolysis
D-Oxalysine	0.95	0.98	1.01	0.96
Ammonia	0.98	0.99	1.00	0.98
Ethanolamine	0.40	0.68	0.18	0.50
2-Aminoethoxyacetic acid	0.00	0.30	0.10	0.42
By difference on hydrolysis				
<i>N</i> -Oxalylethanolamine	0.28		0.32	
3-Morpholone	0.30		0.32

Fig. 3. Autoxidation of a cyclic Schiff base to a hydroperoxide. For comparison the cyclic, anhydro- α -oxo acids derived from lysine and oxalysine are given.

corresponding sulphur analogue L-thialysine (Hope, 1965). In the presence of catalase L-lysine consumes one atom of oxygen whereas L-oxalysine consumes three atoms per molecule of substrate. The formation of ethanolamine demonstrates that the consumption of the additional oxygen is accompanied by a cleavage of the oxygen- β -carbon linkage. The secondary oxidation is likely to be an autoxidation reaction at the β -carbon atom of the cyclic form of the α -oxo acid.

The α -oxo acid of lysine cyclizes with loss of one molecule of water to give the Schiff base Δ^1 -piperidine-2-carboxylic acid (Meister, 1954; Schweet, Holden & Lowy, 1955). In the oxygen analogue, Δ^3 -morpholine-3-carboxylic acid, electron withdrawal by the oxygen atom coupled with that of the aza-methine bond may be responsible for the autoxidation. A similar example of autoxidation has been reported by Parcell & Hauck (1963) (see Fig. 3) where electron withdrawal by the phenyl group parallels that of the oxygen atom in the ring of Δ^3 -morpholine-3-carboxylic acid. The hydroperoxide of 2-methyl-3-phenyl-3,4,5,6-tetrahydropyridine is a relatively stable crystalline solid. A hypothetical hydroperoxide of Δ^3 -morpholine-3-carboxylic acid, as a peroxy-hemiacetal, would be expected to undergo hydrolysis to give ethanolamine. The formation of a δ -lactone, by elimination of water, followed by the specific oxidative decarboxylation by hydrogen peroxide would result in morpholine-2,3-dione. Hydrolysis of the latter could explain the formation of N-oxalylethanolamine. The formation of 3-morpholone from oxalysine with the enzyme both in the absence and presence of catalase shows that Δ^3 -morpholine-3-carboxylic acid is more susceptible to oxidation by hydrogen peroxide to the cyclic amide than the parent compound, Δ^1 -piperidine-2-carboxylic acid.

Fig. 4. Relationship between the substances identified in the products resulting from the oxidation of L-oxalysine by L-amino oxidase from digestive glands of *Mytilus edulis*.

The results in Table 4 suggest that Δ^3 -morpholine-3-carboxylic acid is susceptible to two competing reactions: autoxidation at the β -carbon atom and oxidation at the α -carbon atom by hydrogen peroxide. A summary of the oxidation reactions undergone by oxalysine is given in Fig. 4.

Further work will be necessary to establish whether the α -oxo acid of thialysine undergoes oxidation in the same way. It is known that administration of thialysine to rats results in the excretion of some cystamine although α -N-acetyl thialysine appears to be the major metabolite. It is possible that cysteamine could be formed from the α -oxo acid of thialysine in a similar way to the formation of ethanolamine from oxalysine.

The authors thank Dr G. I. Tesser for samples of DL-oxalysine dihydrochloride. They also thank Mrs Christina J. Walker for the amino acid and amine analyses. This work was supported by a research grant from the Medical Research Council.

REFERENCES

- Axelrod, J., Reichenthal, J. & Brodie, B. B. (1953). *J. biol. Chem.* **204**, 903.
- Blaschko, H. & Hope, D. B. (1956). *Biochem. J.* **62**, 335.
- Boulanger, P. & Osteux, R. (1956). *Biochim. biophys. Acta*, **21**, 552.
- Crestfield, A. M., Moore, S. & Stein, W. H. (1963). *J. biol. Chem.* **238**, 622.

- Grassman, W., Hörmann, H. & Endres, H. (1953). *Ber. dtsh. chem. Ges.* **86**, 1477.
- Hope, D. B. (1965). *Ciba Found. Study Grp.* **19**, 88.
- Horncastle, K. C. & Hope, D. B. (1965). *Biochem. J.* **96**, 80P.
- Jepson, J. B. & Smith, J. (1953). *Nature, Lond.*, **172**, 1100.
- Keilin, D. & Hartree, E. F. (1945). *Biochem. J.* **39**, 393.
- Meister, A. (1954). *J. biol. Chem.* **206**, 577.
- Moore, S. & Stein, W. H. (1954). *J. biol. Chem.* **211**, 893.
- Parcell, R. F. & Hauck, F. P. (1963). *J. org. Chem.* **28**, 3468.
- Partridge, S. M. (1948). *Biochem. J.* **42**, 238.
- Pataki, G. (1963). *J. Chromat.* **12**, 541.
- Schweet, R. S., Holden, J. T. & Lowy, P. H. (1955). In *Symposium on Amino Acid Metabolism*, pp. 496-506. Ed. by McElroy, W. D. & Glass, H. B. Baltimore: The Johns Hopkins Press.
- Spackman, D. H., Stein, W. H. & Moore, S. (1958). *Analyt. Chem.* **30**, 1190.
- Tesser, G. I. & Neffkens, G. H. L. (1959). *Rec. Trav. chim. Pays-Bas*, **78**, 404.
- Vièles, P. & Séguin, J. (1953). *Bull. Soc. Chim. Fr.* **20**, 287.