Degradation of ($\pm$)-Synephrine by *Arthrobacter synephrinum*

OXIDATION OF 3,4-DIHYDROXYPHENYLACETATE TO 2-HYDROXY-5-CARBOXYMETHYLMUCONATE SEMIALDEHYDE

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1. Cell-free extracts of *Arthrobacter synephrinum* catalyse the oxidation of 3,4-dihydroxyphenylacetate. 2. The product of oxidation was characterized as 2-hydroxy-5-carboxymethylmuconate semialdehyde from its chemical behaviour as well as from nuclear-magnetic-resonance spectra. 3. A 3,4-dihydroxyphenylacetate 2,3-dioxygenase (EC 1.13.11.15) was partially purified from *A. synephrinum*. 4. The enzyme had a K_m of 25 μ M towards its substrate and exhibited typical Michaelis–Menten kinetics. 5. The enzyme also catalysed the oxidation of 3,4-dihydroxymandelate and 3,4-dihydroxyphenylpropionate, at reaction rates of 0.5 and 0.04 respectively of that for 3,4-dihydroxyphenylacetate. 6. The enzyme was sensitive to treatment with thiol-specific reagents. 7. The molecular weight of the enzyme as determined by Sephadex G-200 chromatography was approx. 282000.

Arthrobacter synephrinum, isolated from citrus-garden soils, degrades ($\pm$)-synephrine [($\pm$)-*p*-hydroxy- α -(methylaminomethyl)benzyl alcohol] by a pathway involving *p*-hydroxyphenylacetaldehyde, *p*-hydroxyphenylacetate and 3,4-dihydroxyphenylacetate as intermediates (Abitha Devi *et al.*, 1975). The enzyme catalysing the conversion of ($\pm$)-synephrine into *p*-hydroxyphenylacetaldehyde has been demonstrated in a cell-free system (Veeraswamy *et al.*, 1976). Although 3,4-dihydroxyphenylacetate is metabolized in *A. synephrinum* by a *meta* fission, the cleavage could occur between C-2 and C-3 (Adachi *et al.*, 1964; Blakley *et al.*, 1967) or between C-4 and C-5 of the benzene ring (Dagley & Wood, 1965). The present paper describes the isolation and identification of the product of oxidation of 3,4-dihydroxyphenylacetate formed in the presence of cell-free extracts of *A. synephrinum* as 2-hydroxy-5-carboxymethylmuconate semialdehyde. The partial purification and properties of 3,4-dihydroxyphenylacetate 2,3-dioxygenase (EC 1.13.11.15) catalysing this reaction are also reported.

Organism and culture conditions

A. synephrinum was grown as described by Veeraswamy *et al.* (1976) and stored at -20°C .

Isolation of the product of oxidation of 3,4-dihydroxyphenylacetate

Frozen cells of *A. synephrinum* (500 mg) were crushed in a chilled mortar with an equal amount of glass powder and extracted with 10 ml of 50 mM-potassium phosphate buffer, pH 7.2. The slurry was centrifuged at 20000g for 10 min and the clear supernatant was heated at 50°C for 30 min. The clear supernatant obtained on centrifugation was used for the isolation of the product of oxidation of 3,4-dihydroxyphenylacetate.

A mixture of potassium phosphate buffer, pH 7.2 (2 mmol), 3,4-dihydroxyphenylacetate (50 μ mol) and heat-treated cell-free extract (5 ml), total volume of 10 ml, was incubated in a 50 ml conical flask at 30°C in a metabolic shaker. The reaction was allowed to proceed until 3,4-dihydroxyphenylacetate had completely disappeared, as tested by the method of Arnov (1937). The contents of ten such flasks were pooled and treated with 10% (w/v) metaphosphoric acid. The precipitated proteins were removed by centrifugation and the supernatant was continuously extracted with peroxide-free diethyl ether. The ether layer was shaken with anhydrous Na_2SO_4 and evaporated to dryness. The yellow residue was recrystallized from ethyl acetate by the addition of a suitable amount of light petroleum (b.p. $60\text{--}80^{\circ}\text{C}$).

Experimental

Chemicals

($\pm$)-Synephrine, 3,4-dihydroxyphenylacetate, 3,4-dihydroxymandelate, lysozyme, urease (type VI), alcohol dehydrogenase (yeast), catalase (C-100) and peroxidase (type VI) were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. DEAE-cellulose was from H. Reeve Angel and Co., London E.C.4, U.K. All other chemicals were of analytical-reagent grade available commercially.

Conversion of the oxidation product into pyridine-dicarboxylate

The oxidation product of 3,4-dihydroxyphenylacetate was cyclized with ammonia (Blakley *et al.*, 1967), followed by oxidation with alkaline KMnO_4 to yield the corresponding pyridinedicarboxylate (Adachi *et al.*, 1964).

Enzyme assay

The reaction mixture consisted of potassium phosphate buffer (250 μmol), pH 7.2, 3,4-dihydroxyphenylacetate (1.0 μmol) and enzyme preparation (6 μg) in a total volume of 3.0 ml. Increase in A_{380} was recorded in a Beckman DB recording spectrophotometer at 25°C after starting the reaction by the addition of the substrate. The amount of the product formed was calculated from the increase in A_{380}/min , by using a molar extinction coefficient of 31000 litre $\cdot$ mol $^{-1}$ $\cdot$ cm $^{-1}$, a value obtained in the present studies for the product of oxidation of 3,4-dihydroxyphenylacetate. A unit of activity is the amount of enzyme required for the formation of 1 μmol of the product/min at 25°C.

Determination of protein

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Purification of enzyme

Frozen cells of *A. synephrinum* (30 g) dispersed in 200 ml of 50 mM-potassium phosphate buffer, pH 7.2, were incubated with 200 mg of lysozyme at 37°C in a metabolic shaker after distribution among several 50 ml conical flasks. The contents were pooled after 3 h and centrifuged at 20000g for 20 min. The supernatant was treated with 10 ml of aq. 3% (w/v) protamine sulphate and centrifuged at 20000g for 20 min. The residue was extracted with 80 ml of 200 mM-potassium phosphate buffer, pH 8.0, for 1 h and the supernatant obtained by centrifugation at 20000g for 20 min was subjected to $(\text{NH}_4)_2\text{SO}_4$ fractionation. The precipitate obtained between 30 and 60% saturation of $(\text{NH}_4)_2\text{SO}_4$ was dissolved in a minimum amount of 50 mM-potassium phosphate buffer, pH 7.2. This solution (8 ml) was fractionated with acetone at -15°C, and the fraction obtained between 40 and 80% (v/v) of acetone was dissolved in 11 ml of 50 mM-potassium phosphate buffer, pH 7.2, and centrifuged at 20000g for 20 min. The supernatant was adjusted to 60% saturation with $(\text{NH}_4)_2\text{SO}_4$ and centrifuged at 20000g for 20 min. The resulting precipitate was dissolved in the minimum amount of 50 mM-potassium phosphate buffer, pH 7.2 (3.1 ml), and loaded on a column (70 cm $\times$ 1.5 cm) of Bio-Gel

P-300 pre-equilibrated with the same buffer and eluted at an average flow rate of 20 ml/h. Fractions (5 ml) were collected, and those showing enzyme activity were pooled and loaded on a DEAE-cellulose column (15 cm $\times$ 1 cm) equilibrated with 50 mM-potassium phosphate buffer, pH 7.2. The enzyme was eluted with a linear gradient (total volume 200 ml) of 0–600 mM-NaCl in the same buffer. The active fractions were pooled and desalted by dialysis against 5 mM-potassium phosphate buffer, pH 7.2. The enzyme was stored at -20°C.

Electrophoresis

Polyacrylamide-disc-gel electrophoresis was carried out by the method of Davis (1964) in 7% (w/v) gels. Coomassie Brilliant Blue R was used for staining.

Determination of molecular weight

The molecular weight of the enzyme was determined by the procedure of Andrews (1965) on a column (135 cm $\times$ 1.4 cm) of Sephadex G-200 standardized with the following protein markers: urease, mol. wt. 483000 (Sehgal *et al.*, 1965); catalase, mol. wt. 244000 (Samejima & Shibata, 1961); yeast alcohol dehydrogenase, mol. wt. 151000 (Kagi & Vallee, 1960); peroxidase, mol. wt. 40200 (Maehly, 1955). The column was pre-equilibrated with 50 mM-potassium phosphate buffer, pH 7.2; 1 ml fractions were collected and each marker was followed by its activity. Urease was assayed by Nesslerization (Yellin & Wriston, 1966), and standard procedures were used to determine the activities of alcohol dehydrogenase (Vallee & Hoch, 1955), catalase (Beers & Sizor, 1952) and peroxidase (Maehly & Chance, 1954).

Results

Identification of the product of oxidation of 3,4-dihydroxyphenylacetate

The reaction product obtained as described in the Experimental section closely resembled 2-hydroxy-5-carboxymethylmuconate semialdehyde in its chemical properties (Table 1). The compound produced 4 mol of CO_2 on treatment with acidic KMnO_4 (Long, 1942) and 1 mol of CO_2 on treatment with acidic $\text{Ce}(\text{SO}_4)_2$ (Meister, 1952). The melting point (268°C) and u.v.-absorption spectrum of the pyridinedicarboxylate derived from this compound were identical with those of pyridine-2,5-dicarboxylate obtained from 2-hydroxy-5-carboxymethylmuconate semialdehyde by Adachi *et al.* (1964).

The i.r. spectrum of the isolated compound in Nujol mull indicated absorption frequencies characteristic of 2-hydroxy-5-carboxymethylmuconate semi-

Table 1. Comparison of the properties of the product of oxidation of 3,4-dihydroxyphenylacetate by *A. synephrinum* with those reported for 2-hydroxy-5-carboxymethylmuconate semialdehyde

Property	Isolated compound	2-Hydroxy-5-carboxymethylmuconate semialdehyde
Colour of the product	Yellow	Yellow (Adachi <i>et al.</i> , 1964; Blakley <i>et al.</i> , 1967)
Reaction with acidic KMnO ₄ solution	Decolorized	Decolorized (Adachi <i>et al.</i> , 1964)
Reaction with neutral FeCl ₃ solution	Brown colour	Brown colour (Adachi <i>et al.</i> , 1964)
Reaction with 2,4-dinitrophenylhydrazine	Reddish 2,4-dinitrophenylhydrazone (m.p. 175°C)	Reddish 2,4-nitrophenylhydrazone (m.p. 172–175°C) (Blakley <i>et al.</i> , 1967)
Number of mol of CO ₂ produced from 1 mol of the compound on treatment with:		
(a) Acidic KMnO ₄	Four	Four (Blakley <i>et al.</i> , 1967)
(b) Acidic Ce(SO ₄) ₂	One	One (Blakley <i>et al.</i> , 1967)
Effect of pH on the absorption maximum at 380nm	Increased with increase in pH	Increased with increase in pH (Blakley <i>et al.</i> , 1967)
M.p. of pyridinedicarboxylate derived from the oxidation product of 3,4-dihydroxyphenylacetate	268°C	268°C (Adachi <i>et al.</i> , 1964)

aldehyde. An absorption band at 3350cm^{-1} indicated a hydroxyl group (hydrogen-bonded), and another at 2865cm^{-1} indicated aldehyde C–H stretching. A broad absorption band obtained between 2450 and 2500cm^{-1} accounted for the hydroxyl stretching of the carboxyl groups. A strong absorption band obtained between 1695 and 1725cm^{-1} could be assigned to C=O stretching attributed to the carboxyl groups and the aldehyde carbonyl group and also to the ketone resulting from the keto–enol tautomerism of the compound. C=C stretching was indicated by the absorption peaks at 1620 and 1595cm^{-1} .

Further confirmation of the structure of the isolated compound was obtained by studying the n.m.r. (nuclear-magnetic-resonance) spectrum of the compound, which has not been reported hitherto in the literature. Fig. 1(a) represents the n.m.r. spectrum of the compound in Polysol-d and Fig. 1(b) represents that after the addition of $^2\text{H}_2\text{O}$. The n.m.r. spectrum, (Fig. 1a) shows a singlet at δ 9.0 (1H), an AB quartet (2H, δH_a 7.67, δH_b 6.50, J_{AB} 12 Hz), a singlet at δ 3.3 (2H) and a broad signal centred at δ 5.7 p.p.m. The last signal is attributed to the exchangeable hydrogen atoms of CO₂H and OH groups and the solvent Polysol-d. This contention is justified by the observation that this signal collapses on addition of $^2\text{H}_2\text{O}$ to a sharp singlet at δ 4.3 p.p.m. (Fig. 1b), the resonance position of H₂O in $^2\text{H}_2\text{O}$. Of the two structures to be considered (Fig. 2) only structure (I) could give rise to the spectrum shown in Fig. 1(a), with the following

assignments: CHO, δ 9.0; H_a, δ 6.50; H_b, δ 7.67 (AB quartet, vicinal coupling J_{AB} 12 Hz); CH₂, δ 3.3 p.p.m. Structure (II) would give rise to a doublet for CHO, a doublet for H_a (coupling with CHO) and a singlet for H_b. Though the pattern could apparently be present, the δ values do not permit the assignment of structure (II).

Justification for our assignment of signals to atoms H_a and H_b as well as for the whole molecule also comes from the behaviour in the presence of added $^2\text{H}_2\text{O}$ (Fig. 1b). This leaves a singlet at δ 9.0 (1 H atom, CHO), a singlet at δ 7.6 (1 H) and a singlet at δ 3.3 p.p.m. (2 H atoms, CH₂). In structure (I) keto–enol tautomerism is possible (Fig. 2) and this could cause the exchange of H_b with ^2H . Hence the signal for atom H_b is lost, accompanied by the loss of coupling, leading to a singlet for H_a. This sort of behaviour could only be possible with structure (I). In structure (II), the same keto–enol tautomerism can also be envisaged, but would lead to the loss of the singlet for atom H_b, with the AB quartet of CHO and H_a remaining unchanged, on exchange with $^2\text{H}_2\text{O}$. Hence the structure of the isolated compound is 2-hydroxy-5-carboxymethylmuconate semialdehyde. (structure I in Fig. 2).

General properties of the enzyme

The summary of purification of the enzyme catalysing the oxidation of 3,4-dihydroxyphenylacetate is given in Table 2. Two major protein bands were

Fig. 1. N.m.r. spectra of the oxidation product of 3,4-dihydroxyphenylacetate in Polysol-d (a) and in Polysol-d after the addition of $^2\text{H}_2\text{O}$ (b)

The n.m.r. spectra were recorded in Polysol-d (Stohler Isotopic Chemicals, Waltham, MA, U.S.A.) with a Varian T-60 spectrometer by using tetramethylsilane (Me_4Si) as internal standard. The resonances are given in δ -scale.

Fig. 2. Structures of 2-hydroxy-5-carboxymethylmuconate semialdehyde (I) and 2-hydroxy-4-carboxymethylmuconate semialdehyde (II)

Table 2. Summary of purification of 3,4-dihydroxyphenylacetate 2,3-dioxygenase from *A. synephrinum*

Details are given in the Experimental section. One unit is the amount of enzyme that will catalyse the formation of 1 μ mol of 2-hydroxy-5-carboxymethylmuconate semialdehyde/min.

Step	Total protein (mg)	Total activity (units)	Specific activity (munits/mg)	Recovery (%)	Purification (fold)
Crude extract	460	60	130	100	1.0
Protamine sulphate precipitation and re-extraction	221	49	220	82	1.7
30–60%-satd. $(\text{NH}_4)_2\text{SO}_4$	131	43	320	72	2.5
40–80% acetone	51	31	610	52	4.7
0–60%-satd. $(\text{NH}_4)_2\text{SO}_4$	34	30	900	50	6.9
Bio-Gel P-300	20	23	1150	38	8.8
DEAE-cellulose	4.2	10	2510	17	19.3

obtained on polyacrylamide-gel electrophoresis at pH 8.3 (Fig. 3). Incubation of a duplicate gel with 3,4-dihydroxyphenylacetate (5 μ mol) in 50 mM-potassium phosphate buffer, pH 7.2 (5 ml), resulted in the formation of a yellow activity band corresponding to the upper protein band. The purified enzyme was fully active for at least 1 month, and partial loss of activity on prolonged storage was not prevented by the addition of acetone (10%, v/v) or thiol compounds (5 mM) such as reduced glutathione or 2-mercaptoethanol. In the presence of the enzyme, 1 mol of 3,4-dihydroxyphenylacetate was oxidized to 1 mol of 2-hydroxy-5-carboxymethylmuconate semialdehyde, with the consumption of 1 mol of oxygen (Table 3). The optimum activity of the enzyme was observed at approx. pH 7.2. The activity of the oxygenase was greatest at 50°C and showed good stability

towards temperature increase. Full activity was retained even after keeping the enzyme at 50°C for 30 min. The reaction was linear with respect to time. The enzyme on preincubation for 15 min with HgCl_2 (0.05 mM) or CuSO_4 (0.05 mM) lost 90 or 70% of its activity respectively. Metal-chelating agents such as *o*-phenanthroline, 8-hydroxyquinoline, EDTA and 1,1'-bipyridine at a final concentration of 1 mM did not inhibit the dioxygenase activity. Full activity was retained even after preincubation of the enzyme with still higher concentration of *o*-phenanthroline (up to 10 mM) or on extensive dialysis of the enzyme preparation against this compound.

As shown in Table 4, thiol-specific reagents such as *p*-chloromercuribenzoate and 5,5'-dithiobis-(2-nitrobenzoate) markedly inhibited the dioxygenase activity, but iodoacetate had no effect. Reduced

Fig. 3. Polyacrylamide-gel electrophoresis of 3,4-dihydroxyphenylacetate 2,3-dioxygenase from *A. synephrinum* at pH 8.3

Details are given in the Experimental section.

Table 3. Stoichiometry of the reaction catalysed by 3,4-dihydroxyphenylacetate 2,3-dioxygenase from *A. synephrinum*

Oxygen uptake was measured in Warburg manometers at 25°C containing 0.2 ml of KOH (40%) in the central well and with air as the gas phase. Each flask contained potassium phosphate buffer, pH 7.2 (250 μ mol), and the purified enzyme (30 μ g) in a total volume of 2.8 ml in the main compartment. The reaction was started by tipping 0.2 ml of a solution containing the indicated amount of 3,4-dihydroxyphenylacetate and allowed to proceed until no further change in the manometer reading was observed. The reaction mixture, when tested by the method of Arnov (1937), showed complete disappearance of 3,4-dihydroxyphenylacetate. 2-Hydroxy-5-carboxymethylmuconate semialdehyde formed was measured spectrophotometrically at 380 nm.

3,4-Dihydroxyphenylacetate loss (μ mol)	Oxygen consumed (μ mol)	2-Hydroxy-5-carboxymethylmuconate semialdehyde formed (μ mol)
1.15	1.09	1.01
2.28	2.15	2.29

glutathione, dithiothreitol and L-cysteine, which are well-known thiol compounds, did not have any effect on the dioxygenase activity. Even preincubation of the enzyme, which was partially inactivated on prolonged storage, with each of these compounds, either alone or in combination with FeSO_4 (1 mM), did not restore the complete activity. Treatment with 6M-guanidine hydrochloride resulted in considerable inactivation of the enzyme, but other denaturing agents (8M-urea and 2.5% sodium dodecyl sulphate) had no effect.

Substrate specificity

The activity of the dioxygenase towards various analogues of 3,4-dihydroxyphenylacetate was determined by measuring the oxygen uptake by each of these compounds (1 μ mol) under the conditions

Table 4. Effect of thiol-specific reagents and thiol compounds on 3,4-dihydroxyphenylacetate 2,3-dioxygenase from *A. synephrinum*

The enzyme activity was measured as described in the Experimental section except for the preincubation with the test compound (1 mM) before the addition of the substrate. The activity is expressed as a percentage of the control.

Compound	Activity (%)
<i>p</i> -Chloromercuribenzoate	41
5,5'-Dithiobis-(2-nitrobenzoate)	0
Iodoacetate	100
L-Cysteine	98
2-Mercaptoethanol	100
Reduced glutathione	100
Dithiothreitol	99

described in Table 3. Apart from 3,4-dihydroxyphenylacetate, the enzyme catalysed the oxidation of 3,4-dihydroxymandelate and 3,4-dihydroxyphenylpropionate at relative rates of 0.5 and 0.04 respectively. Compounds such as catechol (1,2-dihydroxybenzene), 3,4-dihydroxybenzoate, 2,5-dihydroxybenzoate, 3,4,5-trihydroxybenzoate, 2,5-dihydroxyphenylacetate, 4-hydroxy-3-methoxyphenylacetate, 4-hydroxy-3-methoxymandelate and caffeate [3-(3,4-dihydroxyphenyl)prop-2-enoate] did not show any detectable activity when they were incubated with 30–200 μ g of the purified dioxygenase.

Effect of substrate analogues

Structural analogues of 3,4-dihydroxyphenylacetate, such as catechol, 3,4-dihydroxybenzoate, 2,5-dihydroxybenzoate, phenylacetate, 3-hydroxyphenylacetate, 4-hydroxyphenylacetate, 2,5-dihydroxyphenylacetate, 4-hydroxy-3-methoxyphenylacetate, 4-hydroxy-3-methoxymandelate and 3,4-dihydroxyphenylalanine, did not inhibit the dioxygenase activity even at a final concentration of 5 mM.

Reaction kinetics

Double-reciprocal plots of initial velocities of the enzyme reaction against the concentration of 3,4-dihydroxyphenylacetate were linear, indicating that the enzyme followed Michaelis-Menten kinetics. The K_m for 3,4-dihydroxyphenylacetate was 25 μ M, and that for 3,4-dihydroxymandelate was 0.55 mM.

Molecular weight

The molecular weight of the enzyme as determined by Sephadex G-200 column chromatography was 282000.

Discussion

Compounds such as 3,4-dihydroxyphenylacetate, having two adjacent hydroxyl groups on the benzene ring, are attacked by two classes of enzymes known as intradiol and extradiol dioxygenases. 3-Carboxymethylmuconolactone has been shown to be formed in *Tilletiopsis washingtonensis* subsequent to the intradiol fission of the benzene ring between C-3 and C-4 with the initial formation of β -carboxymethyl-*cis,cis*-muconate (Subba Rao *et al.*, 1971). Extradiol or *meta* fission of 3,4-dihydroxyphenylacetate results in the formation of a substituted muconate semialdehyde. Formation of 2-hydroxy-5-carboxymethylmuconate semialdehyde as a result of the cleavage of the benzene ring between C-2 and C-3 has been reported in *Pseudomonas* (Adachi *et al.*, 1964; Blakley *et al.*, 1967) and *Acinetobacter* (Sparnins *et al.*, 1974). The isomeric 2-hydroxy-4-carboxymethylmuconate semialdehyde is formed from 3,4-dihydroxyphenylacetate in a *Pseudomonas* through the cleavage of the benzene ring between C-4 and C-5 (Dagley & Wood, 1965). The properties of the two extradiol products resemble each other closely and even the pyridinedicarboxylates derived from these compounds are similar in chemical behaviour. In the present studies the structure of the oxidation product of 3,4-dihydroxyphenylacetate formed in the presence of cell-free extracts of *A. synephrinum* has been unequivocally established as 2-hydroxy-5-carboxymethylmuconate semialdehyde on the basis of its n.m.r. spectra.

The enzyme isolated from *A. synephrinum* resembles the 3,4-dihydroxyphenylacetate 2,3-dioxygenase isolated by Kita *et al.* (1965) from *p*-hydroxyphenylacetate-adapted cells of *Pseudomonas ovalis* in the mode of action on the substrate (Kita, 1965). The enzyme could be effectively solubilized from ($\pm$)-synephrine-grown cells by incubation with lysozyme. This procedure resulted in a better degree of extraction of the enzyme than preparation of acetone-dried bacterial cells followed by buffer extraction, treatment with butanol or toluene, sonication or maceration with glass powder. In addition to the conventional methods, such as $(\text{NH}_4)_2\text{SO}_4$ precipitation, acetone fractionation, Bio-Gel filtration and DEAE-cellulose chromatography, the purification procedure includes a variation in the treatment with protamine sulphate. Generally, protamine sulphate is added to remove nucleic acid complexes from the cell-free extracts. In the present case 3,4-dihydroxyphenylacetate 2,3-dioxygenase was found to react with protamine sulphate, and hence an attempt was made to precipitate the enzyme and then re-extract it from the protamine sulphate complexes. A similar procedure was successfully used earlier in our laboratory for the purification of L-phenylalanine ammonia-lyase from *Rhizoctonia solani* by Kalghatgi & Subba Rao (1975).

The 3,4-dihydroxyphenylacetate 2,3-dioxygenase of *Arthrobacter* resembles the enzyme isolated by Kita *et al.* (1965) from *Pseudomonas* in many of its properties, including its behaviour towards metal-chelating agents and thiol-specific reagents (Kita, 1965; Ono-Kamimoto, 1973; Ono-Kamimoto & Senoh, 1974). There is striking similarity in the specificity of both the enzymes, although there are differences in their relative activities towards various substrates (Ono-Kamimoto, 1973). A marked difference is observed in the molecular weights of these enzymes. Whereas a molecular weight of 282000 has been obtained for the *Arthrobacter* enzyme, the *Pseudomonas* enzyme, which has four identical subunits, has a molecular weight of 140000 (Ono-Kamimoto & Senoh, 1974).

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