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PEA-SEEDLING DIAMINE OXIDASE : APPLICATIONS IN SYNTHESIS AND EVIDENCE RELATING TO ITS MECHANISM OF ACTION

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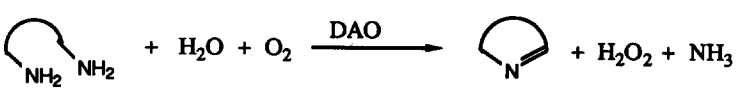
Summary: Diamines (6)-(8) and (11) act as acceptable substrates for diamine oxidase (DAO) and the novel phenacyl derivatives (2) are obtained in satisfactory preparative yield; no enantioselectivity is observed with (11) as an enzyme substrate. Remarkably, the triamines (9) and (13) are not oxidized and, moreover, (9) acts as a notable inhibitor for the DAO catalysed oxidation of (4) and (5). Kinetic evidence relevant to the mechanism of DAO action is presented, and is discussed in terms of a model for DAO-catalysed oxidation.

Diamine oxidases (DAO, EC 1.4.3.6) play an important role in the regulation of cellular levels of natural polyamines which are implicated in the growth and proliferation of living cells.[1] The reaction catalysed by DAO is shown in Scheme 1. In notable, recent work it has been proved[2] that, *inter alia*, the copper-containing DAO which is isolated from pea seedlings, is a quinoprotein, that is to say this enzyme belongs to a so-far small, but growing, group of oxidoreductases which use pyrroloquinoline quinone (PQQ) (21) as a coenzyme.[3,4]

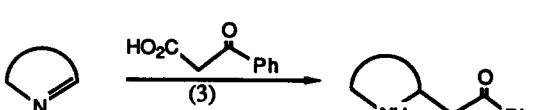
Pea-seedling DAO is easily isolated and handled.[5] The best substrates for the enzyme are putrescine (4) and cadaverine (5) but the enzyme shows a broad substrate tolerance.[6-9] We have used this DAO as a very convenient catalyst, lying off the beaten track of other enzymes used in synthesis,[10] in the preparation of key phenacyl intermediates [as (2)] for the chemical synthesis of a number of alkaloids.[11] We report here: (i) results which extend the range of amines acceptable to the enzyme to include, *inter alia*, the quite novel substrates (7) and (8); (ii) V_{max} and K_M data for (4)-(7), and K_I for inhibitor (9), in the presence of (4) and (5), which bear on the as-yet obscure mechanism of DAO catalysis.

With putrescine (4), cadaverine (5), and spermidine (14) serving as reference compounds (*cf.* ref. 6) the following diamines were found to act as satisfactory substrates for DAO-catalysed oxidation : 2,2′-



(1)

Scheme 1



(1) **Scheme 2** (2)

Table : Oxidation of Diamines Catalysed by DAO.

Diamine	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(13)	(14)
Expt 1[a]										
O_2 uptake[b]	7.3	8.4	2.3	2.2	5.7	0[h]	0.6	2.5	0[h]	2.6
Amine oxidation[c]	32.4	37.5	10.1	9.6	25.6	0[h]	2.8	11.2	0[h]	11.5
Yield of (2)%	85	78	48	39	14	-	-	48	-	-
Expt 2[d]										
V_{max}[e]	742	658	369	216						
K_M[f]	0.14[g]	0.04	0.05	0.3						

a: diamine, 3.3μmol ml^{-1} *i.e.* enzyme saturated with substrate; pH7; 30°C, catalase. b: μl min^{-1} enzyme $unit^{-1}$, results in duplicate. c: calc. from O_2 uptake, μmol min^{-1} $unit^{-1}$. d : peroxidase-coupled assay with *o*-dianisidine (see ref. 14); pH 7.0, 25°C. e : μmol mg^{-1} h^{-1}; plot of S/V vs. S, ± 4-8%; substrate conc. 0.06-0.2mM. f : mM. g: similar value to ref. 15, *cf.* ref. 8. h: no oxidation up to 3mM, 15 min.

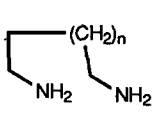
oxybis(ethylamine) (7)[12], bis(2-aminoethyl)sulphide (8)[13], 1,6-diaminohexane (6), and 1,5-diamino-3-methylpentane (11). Comparative rates of oxidation are shown in the Table (Expt 1) as are yields of the phenacyl derivatives (2) produced by *in situ* reaction with benzoylacetic acid (3) (Scheme 2) (*cf.* ref. 11). The novel phenacyl derivatives (18) and (19) were obtained by this means in satisfactory preparative yield from, respectively, (7) and (8). Similarly, (4)-(6) gave (15)-(17).

Which of the two amino groups in (12) is oxidized by pea-seedling DAO depends on the chirality of the substrate.[9] The absence of optical activity in the (20) derived from (11) indicates, by contrast, that the DAO does not distinguish between the enantiotopic $-CH_2CH_2NH_2$ groups of (11) (we have preliminary, unpublished evidence that a phenyl rather than a methyl substituent at C-3 of cadaverine does result in enantioselectivity). There is a fall in the rate of oxidation of (11) compared to (5) which is attributable to increased steric bulk.

The most interesting compound which we examined as a DAO substrate was diethylenetriamine (9) which, in contrast to the oxygen and sulphur analogues (7) and (8), was not oxidized. The amine (13) also failed to undergo oxidation and although some oxidation occurred of the *N*-methyl compound (10) no derivative (2) was obtained. Examination of the range of compounds tested (Table, Expt 1) reveals clearly that when two nitrogens are present and are separated by a chain of two carbon atoms little [with (10)] or no oxidation [with (9) and (13)] occurs; when the separation is by three or more carbon atoms then oxidation does occur. Moreover, in preliminary experiments (9) was found to inhibit the oxidation of (4).

There is a fair, working consensus[4], that enzymic (DAO) oxidation involving PQQ (21) proceeds *via* (22) and (23) as shown in Scheme 3. Formation of (24) would inhibit further enzymic reaction. This intermediate with a relatively stable six-membered ring could be formed with (9), (13) (which are not oxidized) and, to a

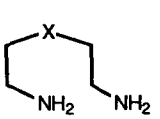
lesser extent, (10) but not with the other amines which are oxidized and which would all require formation of larger, much less stable rings. The results in the Table (Expt 1) are consistent with this hypothesis. Further evidence pertaining to this was obtained in Expt. 2. The results show that (13) is a potent and *competitive* inhibitor for the oxidation of each of the natural DAO substrates (4) and (5) [K_I = 12.37μM; general experimental details as in Expt. 2, substrate conc. 0.06-0.2mM as for data in Table; inhibitor conc. 5-20μM; Y intercept of S/V vs. S plotted against inhibitor conc. (4 values for each substrate); X intercept = K_I]. The competitive nature of the inhibition (K_M increases, V_M remains unchanged)[16] is consistent with the formation of (24) as being the way in which (9) inhibits the oxidation of (4) and (5).



(4) n = 1

(5) n = 2

(6) n = 3

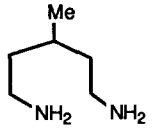


(7) X = O

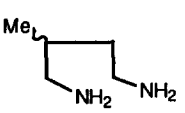
(8) X = S

(9) X = NH

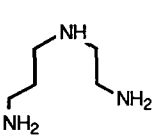
(10) X = NMe



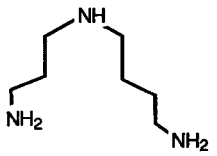
(11)



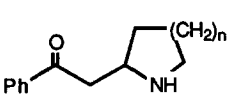
(12)



(13)



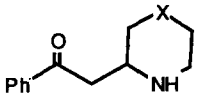
(14)



(15) n = 1

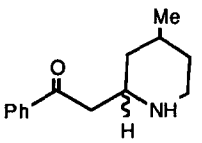
(16) n = 2

(17) n = 3

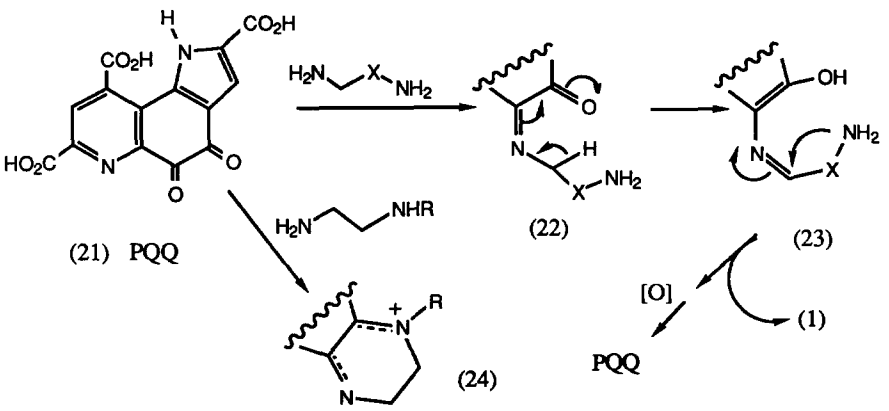


(18) X = O

(19) X = S



(20)



(21) PQQ

(22)

(23)

(24)

Scheme 3

Very recently, essentially the same model has been proposed for the competitive inhibition by ethylene diamine and *cis*-1,2-diaminohexane of the PQQ-containing enzyme lysyl oxidase.[17] In this case it was shown, in addition, that inhibition was irreversible. Further work is in hand on the inhibition of DAO as a contribution to understanding the mechanism of action of this quinoprotein. Also there is the possibility of useful inhibition of DAO action at the cellular level which has promising applicability.

It is notable that V_{max} for (6) is significantly lower than the corresponding values for (4) and (5). Release of oxidized diamine from (23) may reasonably be hypothesized to involve participation of the second amino group as shown and in any case is likely to be a slow step. Formation of a seven-membered ring in the case of (6) as against six- and five-membered rings in the case of (5) and (4), respectively, would be less favoured and the hypothesis that the second amino group participates in this way is thus far supported by the V_{max} data.

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