Mechanistic Aspects of Oxidation of *N*-Substituted 5*H*-Dibenz[*c*,*e*]azepines by Aldehyde Oxidase

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5*H*-Dibenz[*c*,*e*]azepine (**2**) and its *N*-ethyl and *N*-(2-ethoxyethyl) analogues **3** and **4** were prepared and evaluated as substrates for aldehyde oxidase. Quaternization of **2** with ethyl iodide furnished **3**, while **4** was prepared by lithium aluminum hydride reduction of *N*-(2-ethoxy)ethyldiphenimide followed by mercuric acetate oxidation of the resultant amine **6**. The rates of oxidation of **2** and **3** were similar, suggesting a lack of selectivity by the enzyme for the respective imine and iminium functional groups in these compounds. The rate of oxidation of **3** decreased with increasing *p*H while the extent of "hydration" of this substrate increased over a similar *p*H range, signifying a preference by the enzyme for **3** over its carbinolamine equilibrium partner. Experiments with deuterium labelled analogues of **2** and **3** indicated that azomethine hydrogen loss from these substrates during enzymatic oxidation was not rate determining. Thus 5*H*-dibenz[*c*,*e*]azepine-5,5,7-d_3 (**7**), prepared by lithium aluminum deuteride reduction of diphenimide (**5**), and its *N*-ethyl analogue **8**, had respective enzymatic oxidation rates which did not differ from those of their non-deuterated counterparts.

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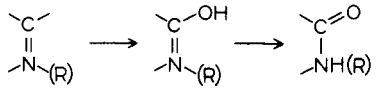
Introduction.

Aldehyde oxidase (AO) catalyzes the conversion of a wide variety of aromatic heterocycles containing aldimine or aldiminium moieties to the corresponding α-hydroxylated derivatives, many of which revert to lactams (Scheme I) [2-4].

Scheme I

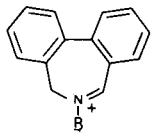


As its name implies, AO also catalyzes the oxidation of aldehydes under *in vitro* conditions [5]. However, aldehydes do not seem to be important substrates for this enzyme *in vivo*, since there are other enzymes, such as NADH-dependent aldehyde dehydrogenase, which have greater activity toward aldehydes than does aldehyde oxidase [6,7].

Examples of aldimine containing substrates are quinoline and related aromatic heterocycles including chemotherapeutic drugs quinine and methotrexate [2,8,9]. Examples of substrates containing the aldiminium ion moiety are *N*-ethyl quinolinium [8], 1-methylnicotinamide (1-MN) [10,11], and dehydronicotine, an intermediate in the conversion of nicotine to its lactam metabolite, cotinine [12,13].

The *in vivo* conversion of numerous other chemicals containing *N*-alkylpyrrolidine and homologous rings to the corresponding lactams suggests the involvement of AO [14-17]. Furthermore, *in vitro* studies of the electrophilic nature of putative cyclic iminium ion intermediate metabolites of some pyrrolidines and piperidines [18-20] implies a possible role of AO in deactivating these potentially toxic metabolites [21]. However, few studies with iminium ions derived from compounds bearing these ring systems have been carried out, due to their high degree of chemical instability. Thus, dehydronicotine was found to undergo undesirable reactions in solution, including isomerization to the corresponding enamine followed by irreversible dimerization, and hydration followed by ring opening [12].

Like nicotine, the tricyclic antihypertensive drug azapetine was found to undergo oxidation to a lactam *via* an aldiminium ion intermediate, dehydroazapetine (**1**) [22]. This intermediate underwent characteristic iminium ion addition reactions such as hydration and cyanide addition, and was readily oxidized to a lactam metabolite by a soluble liver enzyme later determined in unpublished experiments to be aldehyde oxidase. However, **1** was incapable of enamine isomerization and did not undergo detectable ring opening.



	R
1.	$CH_2CH{=}CH_2$
2.	$CH_2CH{=}CH_2$
3.	CH_2CH_3
4.	$CH_2CH_2OCH_2CH_3$

We thus envisioned the use of **1** to investigate several mechanistic aspects of catalytic iminium ion oxidation. First, since these ions can undergo hydration to carbinolamines, it was of interest to determine whether AO had a preference for the latter, as has been suggested in studies with 1-MN [23]. Also, we wanted to determine whether H-transfer from the methine adjacent to the nitrogen in the bound substrate was the rate determining step in the oxidation. Finally, we wanted to investigate the effect of removal of the R group on the rate of oxidation. Similar removal of *N*-alkyl groups from 1-MN and *N*-ethylquinolin-

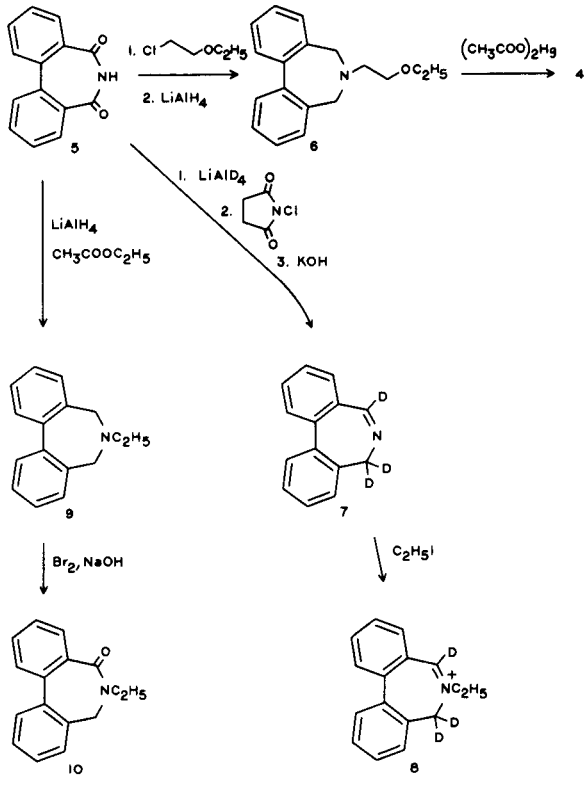
ium caused respective 98% and 68% decreases in catalytic oxidation rates [8].

N-Allyliminium ions related to **1** are known to be light sensitive [24]. Thus, we chose to commence these studies with side chain saturated analogues of 5*H*-dibenz[*c,e*]azepine (**2**), namely, 6-ethyl-5*H*-dibenz[*c,e*]azepine (**3**) and the 2-ethoxy analogue of this compound **4**.

Results.

Reduction of diphenimide (**5**) with lithium aluminum hydride followed by *N*-chlorination and dehydrohalogenation [22] afforded **2**. This was quaternized with ethyl iodide in ether, or more efficiently using benzene as solvent, to give **3**. Base assisted hydrogen-deuterium exchange of the aldimine methine proton in **3**, a procedure initially reported for similar exchange in 16(17)-dehydrolupanine [25] and later for 1′(5′)-dehydronicotine [26], was not successful. We thus prepared the trideuterated compounds **7** and **8**, as summarized in Scheme II, for kinetic isotope effect studies. Compound **2** could not be quaternized by treatment with 2-chloroethyl ethyl ether under a variety of reaction conditions, thus **4** was prepared by mercuric acetate oxidation of **6**. Preparation of an authentic sample of lactam **10**, the putative product of enzymatic oxidation of **3**, was accomplished by reduction of **5** with excess lithium aluminum hydride followed by addition of dry ethyl acetate, followed by oxidation of resultant tertiary amine **9** with bromine in a two-phase solvent system containing aqueous base [27].

Scheme 2



Preliminary experiments showed that both **3** and **4** were substrates for AO. Thus, thin layer chromatograms of extracts from separate 60 minute incubations of these compounds with AO indicated the presence of single products, with R_f values of 0.66 and 0.79, respectively. Qualitatively, these appeared to predominate over the amounts of substrates recovered, which in turn had R_f values of 0.26 and 0.41. The products were not seen in extracts from incubations to which boiled enzyme was added. That seen in the incubation of **3** was purified by preparative tlc and found to have infrared and mass spectral features identical to those of lactam **10**.

Since **3** had a uv maximum at 335 nm (ϵ = 2900 M^{-1} cm^{-1}) which was not present in its metabolite, the decrease in absorbance at this wavelength in the presence of AO was used initially to measure its oxidation rate. Indirect determination, by following spectrally the rate of reduction of dichloroindophenol, gave quantitatively similar results and afforded a seven-fold increase in sensitivity over the direct method. This method was thus adopted for all enzymatic oxidation rate studies.

Rates of oxidation of **2** and its *N*-alkyl analogues are shown in Table I, in comparison with that of 1-MN. The oxidation rates of **2** and **3** were similar, but were less than that of the standard substrate. As shown in Figure 1, increasing the *p*H of the incubation medium resulted in an increase in the rate of 1-MN oxidation, but caused a decrease in that of oxidation of **3**. The rate of oxidation of **4** was much lower than that of **3** at *p*H 9 or less.

Table I

Comparative Oxidation Rates of 1-Methylnicotinamide (1-MN) and **2-4**

Compound [a]	Oxidation Rate nmoles/mg of protein/minute
1-MN	10.5
2 [b]	4.8
3	4.7
4	1.8
7 [b]	4.8
8	4.8

[a] All compounds except **2** and **7** were used as chloride salts. [b] Due to poor solubility, this compound was added to cuvettes in 10 $\mu\ell$ of ethanol. This concentration of ethanol (0.33%) had no effect on measurements.

Table II

Kinetics of Oxidation of 1-MN, **3**, and **4**

Compound	Km, mM	v max, nmoles/mg of protein/minute
1-MN	0.20	12.8
3	0.20	5.5
4	0.44	2.3

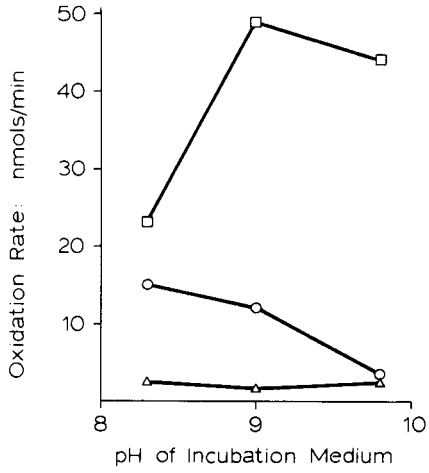


Figure 1. Effect of Incubation Mixture *p*H on Rate of Oxidation of: 1-MN (□), **3** (O), and **4** (Δ).

Incubation mixtures contained, in a volume of 3.0 ml, 15 μmoles of substrate, 0.6 μmole of dichloroindophenol, 150 μmoles of glycine buffer of the appropriate *p*H, and 3 mg of enzyme. Note: since addition of enzyme (in a volume of 0.3 ml) lowered the *p*H by about one unit in the *p*H 9.0 and 9.8 incubation mixtures, the *p*H of these was adjusted one *p*H unit above the final desired values by addition of 5% aqueous sodium hydroxide prior to adding the enzyme solution.

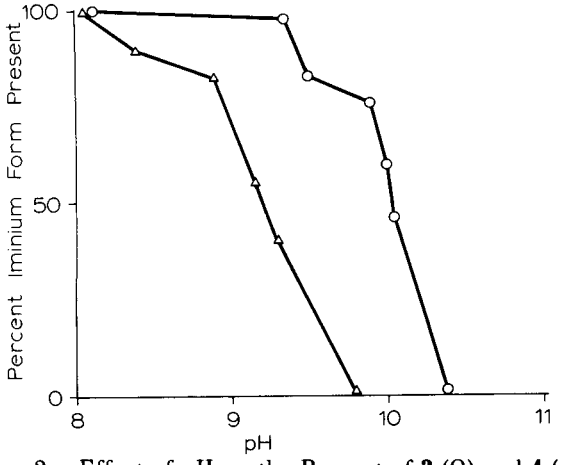


Figure 2. Effect of *p*H on the Percent of **3** (O) and **4** (Δ) in the Ionic (Iminium) Form in Aqueous Solution as Determined by nmr Spectral Analysis.

The chloride salt of each compound was dissolved in 0.5 ml of deuterium oxide to give a concentration of about 0.5 *M*. Solution *p*D was adjusted by addition of 10% sodium deuteroxide in deuterium oxide. After each addition, the *p*D was determined potentiometrically and the integrated intensities of the aldiminium CH singlet at 8.8 ppm, and the CCH_3 quartet at *ca.* 1.3 ppm were determined. The percent iminium ion present was calculated by multiplying the ratio of the above intensities by 300. The *p*D was converted to *p*H using the equation *p*D = *p*H + 0.40 [28].

The nmr spectra of **3** and **4** in deuterium oxide showed no evidence of *aldehyde* methine protons at any of the *p*H values chosen, indicating the absence of ring-opened products. However, as shown in Figure 2, the amount of respective iminium ion forms decreased with increasing *p*H, an effect which commenced at lower *p*H with **4** than with **3**. At solution *p*H values above 10, the integrated intensities of *all* signals in the spectra of both compounds was decreased over those found in solutions of lower *p*H. Also, solutions of *p*H above 10 had to be clarified by centrifugation due to formation of precipitates. Analysis of the precipitate from one such solution of **3** was carried out. Its nmr spectrum (deuteriochloroform) featured no peaks between 8.5-10 ppm, but rather two singlets of about equal intensity at 4.6 and 4.9 ppm, the region in which carbinolamine methine protons are found. And the other aliphatic protons of the ring system of **3** appeared as dual sets of signals of about equal integrated intensity. This, plus elemental analysis data, suggested the product to be an approximately equal mixture of the carbinolamine, formed by addition of hydroxide to the iminium moiety of **3**, and a dimer, formed by reaction of the carbinolamine with a second equivalent of **3**. Treatment of this product with aqueous ethanolic perchloric acid afforded a perchlorate salt which had an nmr spectrum nearly identical to that of **3**.

Further evidence that **3** and **4** underwent progressive conversion to their respective carbinolamine counterparts as a function of increasing *p*H was obtained from uv spectral studies. In neutral aqueous solutions, these compounds each had maxima at about 285 nm and 335 nm

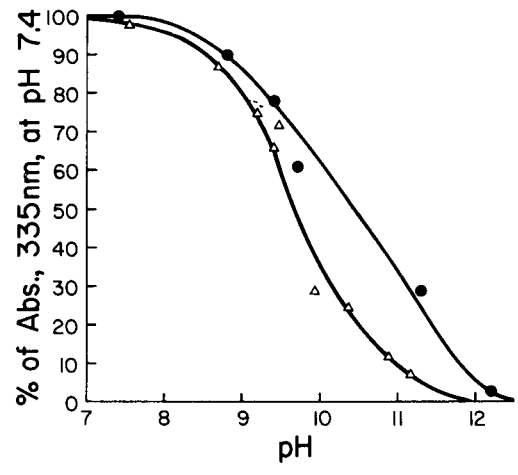


Figure 3. Effect of Solution *p*H on the Absorption at 335 nm of **3** (●) and **4** (Δ).

To 1.0 ml aliquots of the compounds in water (0.2 μmoles) were added varying amounts of 10% aqueous sodium hydroxide. Volume was adjusted to 2.0 ml and *p*H was determined potentiometrically. Ultraviolet spectra were recorded from 250-400 nm.

due to the presence of conjugated iminium species. (In contrast **2**, which has a non-ionized conjugated imine chromophore, has no such maxima at neutral *p*H but does in 0.1 *N* hydrochloric acid due to protonation.) Absorbance of **3** and **4** at 280 nm is greatly decreased, and that at 335 nm is absent in highly alkaline solutions. Thus, quantitation of the progressive decrease in A_{335} with increased solution *p*H was used to estimate the amounts of respective iminium species present as a function of *p*H. Results are shown in Figure 3. In addition, the constituent spectra obtained between *p*H 8 and 12 for each compound exhibited an isosbestic point at 267 nm.

Incubation of **7** or **8** with AO under conditions described in Table I resulted in no change in observed oxidation rates in comparison with those of their respective nondeuterated counterparts, **2** and **3**.

Discussion.

In a previous study it was shown that substrates bearing an aldiminium group underwent more facile oxidation than did the corresponding non-quaternized aldimines [8]. With the present compounds this wasn't the case, since **3** was oxidized at the same rate as was **2**. That **2** was in the aldimine state, rather than in the aldiminium state due to protonation, was evident in its uv spectrum, in which the C=N chromophore, centered at 335 nm in strongly acidic solutions of **2** was absent at the *p*H used for measuring enzymatic oxidation rates.

No kinetic isotope effect on the rates of oxidation of either **3** or **4** was seen. In contrast, an isotope effect (k_H/k_D) of 2.2 was observed in the oxidation of the pyrrolidine rings of tremorine in the presence of unwashed rat liver microsomes [29]. This was suggested to be due to rate limiting C-H bond breakage during initial mixed function oxidase mediated hydroxylation, rather than to a rate limiting step associated with the conversion of the intermediate oxidation product to a lactam.

Compounds **3** and **4** are examples of uniquely stabilized aldiminium ions. They differ from other aromatic iminium ions such as *N*-ethyl quinolinium and 1-MN in the sense that generation of an alternative iminium ion resonance structure is not possible. And they differ from aliphatic aldiminium ions since (a) enamine isomerization is not possible, (b) ring opening does not occur, as indicated in our nmr and uv studies. Rather, these studies suggested a *single* type of chemical instability: reversible conversion to the corresponding carbinolamines under mildly alkaline conditions. Constituent uv spectra of each compound between *p*H 8 and 12 featured an isosbestic point, considered to be indicative of binary mixtures [30]. Inspection of nmr spectra of more concentrated solutions of varying alkalinity suggested that hydration was accompanied by dimerization due to reaction of an equivalent of carbinolamine with a second equivalent of iminium ion. Such reactivity was previously seen in 2-dehydro-3-alkyl-3-azoniabicyclo-[3.3.1]nonanes, which after exposure to alkaline conditions were isolated as dimeric ether bases [31]. Thus, each incremental increase in solution *p*H caused about a two-fold greater decrease in amounts of iminium species present than was seen in the uv study in which analytes were present in much lower concentrations (*cf.* Figures 2 and 3).

In studies with **3**, increasing *p*H caused a decrease in the iminium ion-carbinolamine ratio which was paralleled by a decrease in the rate of substrate oxidation (Figure 1). This suggested that **3** rather than its carbinolamine counterpart was the form of the substrate preferred by AO. Similarly, results of an inhibition kinetic study using 1'(5')-dehydronicotine suggested this form, rather than the corresponding carbinolamine or one of its decomposition products [32], to interact most strongly with AO [12]. And results of a study of the conversion of 2-methylisoquinolines to their 1-oxo metabolites in the presence of rat liver enzymes suggested the involvement of iminium ions as substrates [33]. These findings are in accord with a proposed catalytic mechanism for AO in which oxidation of substrate is initiated by attack of the persulfide group of the enzyme active site with the iminium moiety of the substrate [34].

A study with a carbinolamine metabolite of a pyrrolidine ring-containing drug further suggested the importance of iminium ion character in substrates of AO. This metabolite was stabilized by intramolecular OH...N hydrogen bonding involving an adjacent ring nitrogen atom [35,36]. This metabolite failed to react with cyanide, a reaction requiring conversion of carbinolamine to iminium ion. And it was evidently not a substrate for AO since none of its requisite lactam metabolite was found in extracts from incubations with liver 9 S fraction [35].

The relatively slow oxidation of **4** (Figure 1) initially suggested to us that it, in analogy with the above pyrrolidine carbinolamine metabolite, might revert largely to its carbinolamine in the *p*H range studied due to intramolecular OH...O hydrogen bonding involving the side chain ether oxygen. While the data in Figures 2 and 3 indicate that it undergoes reversion at a somewhat lower *p*H than does **3**, the difference is not of such magnitude as to account for observed differences in oxidation rates at *p*H 9. Rather, the rate differences at these *p*H values may be due to the increased size of the side chain in **4**, which results in increased K_m and decreased V_{max} compared to those of **3** (Table II). In a related experiment, enzymatic conversion of 1-*n*-propylnicotinamide *via* oxidation at C-6 was decreased over that seen with 1-MN as substrate [23].

Other reports have suggested the involvement of carbinolamines as substrates in the metabolism of 1-MN [23] and non-quaternary (imine) compounds [3] by AO. The chemical oxidation of iminium ions to lactams probably involves

prior conversion to the respective carbinolamines since such oxidations do not occur under anhydrous conditions [27,37,38]. Unequivocal resolution of the question of the relative involvement of iminium ions and their carbinolamine counterparts as substrates of AO will require further refinements of mechanistic studies, with a wider variety of stabilized iminium ion substrates.

EXPERIMENTAL

Reagents and solvents were of analytical reagent grade or better. Ether was dried over sodium wire prior to use. Diphenimide (**5**) was prepared as previously reported [39], as was 5*H*-dibenz[*c*,*e*]azepine (**2**) [22]. Analytical and preparative thin layer chromatography (tlc) was done using, in turn, 5 × 20 cm (0.25 mm thickness) and 20 × 20 cm (1 mm thickness) silica gel GF 254 glass backed plates (Analtech, Inc., Newark, DE). Unless otherwise indicated, the solvent for tlc was chloroform-methanol-28% aqueous ammonia (95-5-0.5, v/v). Melting points (uncorrected) were obtained on a Thomas-Hoover capillary melting point apparatus. Ultraviolet (uv) spectrometry was done using a Bausch and Lomb spectronic 2000 instrument. Infrared spectra (ir) were obtained using a Perkin-Elmer 467 infrared spectrometer. Proton nuclear magnetic resonance (nmr) and electron ionization mass spectra (ms) were obtained using JEOL JNM/MX-90Q (I) and Finnigan 4000 quadrupole spectrometer systems, respectively.

6-Ethyl-5*H*-dibenz[*c*,*e*]azepine Chloride (**3**).

To 1.0 g (5.2 mmoles) of **2** in 25 ml of stirred benzene was added 9.75 g (63 mmoles) of ethyl iodide in one portion. After 12 hours, the mixture was concentrated *in vacuo*. The resulting amorphous solid separated from ethanol-ether as yellow crystals, 0.65 g (36%), mp 192-194°.

Anal. Calcd. for $C_{16}H_{16}IN$: C, 55.03; H, 4.62; I, 36.34; N, 4.01. Found: C, 55.10; H, 4.65; I, 36.15; N, 4.02.

This was converted to the chloride salt by passage of a methanolic solution through a column of BioRad AG 2-X8 (chloride form). Concentration of the eluent afforded a light yellow powder; ir (potassium bromide): ν 1650 cm^{-1} ($C=N^+$); nmr (deuterium oxide): δ 1.38 (t, J = 7 Hz, 3H, CCH_3), 4.08 (q, J = 7 Hz, 2H, CH_2CH_3), 4.30 (s, 2H, ring NCH_2), 7.10-7.90 ppm (m, 8H, aromatic H), 8.8 ppm (s, 1H, $HC=N^+$); uv (water): λ 285, 335 nm (conjugated $C=N^+$).

5*H*-Dibenz[*c*,*e*]azepine-5,5,7-d_3 (**7**).

This was prepared by the same procedure used to prepare **2**, beginning with the reduction of **5** with lithium aluminum deuteride. Treatment of an ethereal solution of the product with excess aqueous ethanolic perchloric acid afforded a white precipitate which was collected and dried; nmr (deuterioacetonitrile): δ 5.02 (s, 1H, NH), 7.50-8.10 ppm (m, 8H, aromatic H); ms: (70 eV) m/e 196 (M).

6-Ethyl-5*H*-dibenz[*c*,*e*]azepine-5,5,7-d_3 (**8**).

A mixture of 53 mg (0.27 mmole) of **7**, 5 ml of benzene and 1 ml of ethyl iodide was stirred for 72 hours. The mixture was filtered and the filtrate was washed with benzene and dried to yield 63 mg (66%) of **8**. This was dissolved in water and eluted through a column of 25 g of Bio Rad AG 2-X8 (chloride form). The eluent was lyophilized to afford a yellow powder; ir (potassium bromide): ν 1635 cm^{-1} ($C=N^+$); nmr (deuterium oxide): δ 1.35 (t, J = 7 Hz, 3H, CCH_3), 4.06 (q, J = 7 Hz, 2H, CH_2), 7.12-7.90 ppm (m, 8H, aromatic H); ms: m/e (70 eV) 225 (M).

6-(2-Ethoxy)ethyl-6,7-dihydro-5*H*-dibenz[*c*,*e*]azepine (**6**).

To a stirred solution of 11.7 g (45 mmoles) of **5** (potassium salt) in 100 ml of *N*,*N*-dimethylformamide was added a solution of 7.65 g (50 mmoles) of 2-bromoethyl ethyl ether in 10 ml of *N*,*N*-dimethylformamide. The mixture was heated for 5 hours, then cooled and poured into 300 ml of stirred water. The resulting precipitate (7.25 g, 55%) was collected and dried *in vacuo*, mp 76-78°. This product (5 g, 17 mmoles) was reduced with a mixture of 9 g of lithium aluminum hydride in 600 ml of ether in a Soxhlet extractor [40]. After 48 hours, the reaction mixture was allowed to cool and excess hydride was destroyed by slow addition of 9 ml of water, 9 ml of 15% aqueous ammonium chloride, and 5 ml of water. The mixture was filtered, and the filtrate was dried (sodium sulfate) and treated with excess 35% perchloric acid in aqueous ethanol. The resulting precipitate was filtered, air dried, and crystallized from ethanol, mp 178-180°; nmr (deuteriochloroform): δ 1.25 (t, J = 7 Hz, 3H, CCH_3), 2.75 (t, J = 7 Hz, 2H, NCH_2CH_2), 3.40 (s, 4H, ring NCH_2), 3.30-3.80 (m, 4H, CH_2OCH_2), 7.20-7.60 ppm (m, 8H, aromatic H).

Anal. Calcd. for $C_{18}H_{22}ClNO_5$: C, 58.78; H, 6.03; N, 3.81. Found: C, 58.65; H, 6.05; N, 3.78.

6-(2-Ethoxy)ethyl-5*H*-dibenz[*c*,*e*]azepine Chloride (**4**).

To 2.0 g (7.6 mmoles) of **6** (prepared by equilibration of **6** perchlorate between ether and 20% aqueous sodium hydroxide, followed by concentration of the ether phase) was added 100 ml of 5% aqueous acetic acid and 9.55 g (30 mmoles) of mercuric acetate. The mixture was stirred and refluxed for 36 hours, then cooled, saturated with hydrogen sulfide, and centrifuged. The supernatant was made basic by addition of 40% aqueous sodium hydroxide, and extracted three times with equal volumes of ether. The combined extracts were dried (sodium sulfate), filtered, and excess 35% perchloric acid in aqueous ethanol was added. The precipitate was filtered and dried to afford 1.85 g (67%) of crude **4** perchlorate. This was crystallized from methanol-ether, mp 115-117°; ir (potassium bromide): ν 1660 cm^{-1} ($C=N^+$).

Anal. Calcd. for $C_{18}H_{20}ClNO_5$: C, 59.09; H, 5.51; N, 3.83. Found: C, 59.04; H, 5.54; N, 3.84.

This was converted to the chloride salt by passage of a methanolic solution through a column of BioRad AG 2-X8 (chloride form). Concentration of the eluent afforded a white powder, uv (water): λ 288, 342 nm (conjugated $C=N^+$); nmr (deuteriochloroform): δ 0.87 (t, J = 7 Hz, 3H, CCH_3), 3.25 (q, J = 7 Hz, 2H, CH_2CH_3), 3.84 (t, J = 5 Hz, 2H, OCH_2CH_2), 4.60 (t, J = 5 Hz, 2H, $^+NCH_2CH_2$), 4.85 (broad peak, 2H, ring ^+N-CH_2), 7.50-8.00 (m, 7H, arom H), 8.14 (d, J = 7 Hz, 1H, arom H ortho to $C=N^+$), 9.10 ppm (s, 1H, $HC=N^+$).

6-Ethyl-6,7-dihydro-5*H*-dibenz[*c*,*e*]azepine (**9**).

A mixture of 9 g (237 mmoles) of lithium aluminum hydride in 600 ml of dry ether was used to reduce 5.58 g (25 mmoles) of diphenimide by the standard procedure [40]. To the cooled reaction mixture was slowly added 54 g (60 ml, 620 mmoles) of ethyl acetate. Enough water was added to destroy residual hydride. Then the mixture was filtered. The precipitated residue was washed with ether. The filtrate was treated with excess ethereal hydrogen chloride. After cooling, the supernatant was decanted. The residual oil was dissolved in water and treated with a slight excess of 70% aqueous perchloric acid. The precipitate was filtered and washed with cold water. Recrystallization from water gave 5.9 g of white crystals, mp 199-201°.

Anal. Calcd. for $C_{16}H_{18}ClNO_4$: C, 59.35; H, 5.60; N, 4.33. Found: C, 59.17; H, 5.74; N, 4.19.

The free base was liberated by equilibration of this salt between ether and 10% aqueous sodium hydroxide followed by concentration of the upper phase: nmr (deuteriochloroform): δ 1.20 (t, J = 7 Hz, 3H, NCH_2CH_3), 2.58 (q, J = 7 Hz, 2H, NCH_2CH_3), 3.40 (s, 4H, benzylic CH_2), 7.25-7.55 ppm (m, 8H, arom H).

6-Ethyl-6,7-dihydrodibenz[*c*,*e*]azepine-5-one (**10**).

To 0.23 g (1 mmole) of **9** in a stirred mixture of 15 ml of methylene chloride and 12.5 ml of 5% aqueous sodium hydroxide was added dropwise over a period of 0.5 hours a solution of 0.38 g (2.4 mmoles) of bromine in 12 ml of methylene chloride. After stirring for 24 hours at room temperature, the organic layer was separated, and the aqueous layer was extracted with an equal volume of dichloromethane. The combined organic extracts were concentrated. The residue was dissolved in 20 ml of ether and the solution was washed with three 5-ml portions of 1% aqueous hydrochloric acid. The organic layer was dried (sodium sulfate) and concentrated to give 0.12 g (51%) of a viscous yellow oil, nmr (deuteriochloroform): δ 1.19 (t, J = 7 Hz, 3H, CH_3), 3.60 (q, J = 7 Hz, 1H, 0.5 CH_2), 3.68 (q, J = 7 Hz, 1H, 0.5 CH_2), 3.94 and 4.33 (d, J = 14 Hz, 2H, benzylic CH_2), 7.50 (m, 7H, arom H), 8.05 ppm (m, 1H, arom H ortho to

C=O). Purification of 50 mg of the product by preparative tlc (benzene-ether-chloroform 4-1-1) gave 40 mg of a white solid; mp 86-88°; ir (carbon tetrachloride): ν 1640 cm^{-1} (C=O); ms: (70 eV) m/e 237 (M).

Anal. Calcd. for $C_{16}H_{15}NO$: C, 80.98; H, 6.37; N, 5.90. Found: C, 80.88; H, 6.44; N, 5.82.

Experiments with Aldehyde Oxidase.

Modification of a previously reported procedure [41] was used for partial purification. Livers from mature rabbits were obtained from Pel-Freez Biologicals, Rogers, AR. To 100 g of minced liver was added 300 ml of 50 *mM* potassium phosphate buffer *p*H 6.8 containing 0.1 *mM* tetrasodium EDTA (PE buffer, *p*H 6.8). The mixture was homogenized in a Waring Blendor and heated at 55° for 10 minutes with vigorous stirring. The mixture was cooled in an ice bath and all remaining procedures were carried out at 0-5°. The mixture was centrifuged at 13,000 g for 20 minutes, and the supernatant was filtered through glass wool. In this was dissolved ammonium sulfate (0.38 g/ml of supernatant). After 30 minutes, the mixture was centrifuged at 13,000 g for 20 minutes. The precipitate was dissolved in 30 ml of PE buffer, *p*H 7.8. To the stirred solution of this was added 20 ml of cold (0°) acetone over a period of 2 minutes. The mixture was centrifuged at 3000 g for 5 minutes. To the stirred supernatant was added 10 ml of cold (0°) acetone over a period of 1 minute. The mixture was centrifuged at 3000 g for 5 minutes. The precipitate was dissolved in 10 ml of PE buffer, *p*H 7.8. If necessary, the solution was clarified by centrifugation at 13,000 g for 15 minutes. Protein concentration was determined by uv spectrometery [42]. Enzyme activity was checked by uv spectral measurement of the rate of 1-MN oxidation [23]. The solution was freeze dried. The reconstituted preparation retained full activity for at least three months.

Iodide and perchlorate salts of the iminium ions obtained for this study were converted to the corresponding chloride salts prior to use, in order to obviate (a) potential inhibitory effects of these anions on AO, such as have been observed with other enzymes [43], (b) possible charge-transfer complex formation in concentrated stock solutions, a property of analogous iminium iodides [38].

Oxidation rates of **2-4**, **7**, and **8** were determined in uv cuvettes. To each of two cuvettes was added 3 μmoles of substrate in 0.05 ml of 0.05 *M* potassium phosphate buffer, *p*H 7.8, 0.03 μmole of dichloroindophenol in 0.05 ml of the same buffer, and 2.8 ml of buffer. Reaction was started by addition of 0.1 ml of enzyme solution (*ca.* 1 mg of protein); 0.1 ml of buffer was added to the reference cuvette. The decrease in absorbance at 600 nm was measured. The K_m and V_{max} were determined using final substrate concentrations of 0.25, 0.5, 1.0, 2.0, and 4.0 *mM*. Reaction times of 1 minute were used.

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