Kinetic Resolution in the Oxidation of Iminium Ion to Lactam Catalysed by Aldehyde Oxidase

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A rabbit liver enzyme preparation oxidised racemic iminium ions **1** and **2** to optically active lactams **3** and **4** with enantiomer ratios $E_{R/S}$ = 5.5 and 0.14, respectively.

The use of enzymatic reactions in the preparation of organic compounds in optically active form is a technique under rapid development and some types of enzymes have been used extensively.[1–4] On the other hand, the group of molybdenum-containing enzymes[5–7] has, to the best of our knowledge, not been studied with respect to chirality of substrates or products. We present here the first study of this kind.

Rabbit liver aldehyde oxidase (E.C. 1.2.3.1), in disagreement with its name, shows its highest affinity for unsaturated *N*-heterocycles,[8,9] including iminium ions.[10,11] Dioxygen is an efficient reoxidant for the enzyme. We used a crude rabbit liver enzyme preparation for the oxidation of racemic iminium ions **1** and **2** and obtained the lactams **3** and **4**, respectively, in optically active form (Fig. 1). The enantiomeric compositions were determined by chiral column GLC using (*S*)-**4** and optically impure (*R*)-**4** as references. The latter was prepared from (−)-4-amino-2-methylbutanoic acid,[12] the optical rotation of which indicated the presence of a 62:38 mixture of *R* and *S* forms. *N*-Benzylation and ring closure gave **4**, which on chiral column GLC showed two peaks in the ratio 61:39 which should be due to the *R* and *S* forms, respectively.

In the oxidation of (±)-**1** to **3**, which is a kinetic resolution, the enantiomeric excess of (*R*)-**3** varied with the extent of conversion, in agreement with a general equation given by Sih *et al.*[13] Calculation of $E_{R/S}$[13] (Fig. 2) gave the value 5.5 for the low-conversion points and possibly a somewhat lower value for the high-conversion points. Thus, in this competition between *R* and *S* forms, the "unnatural" reaction (*R*)-**1** → (*R*)-**3** is 5.5 times faster than the reaction (*S*)-**1** → (*S*)-**3**, which is the final step[10] in the metabolism of natural (*S*)-nicotine to (*S*)-cotinine [(*S*)-**3**]. This result

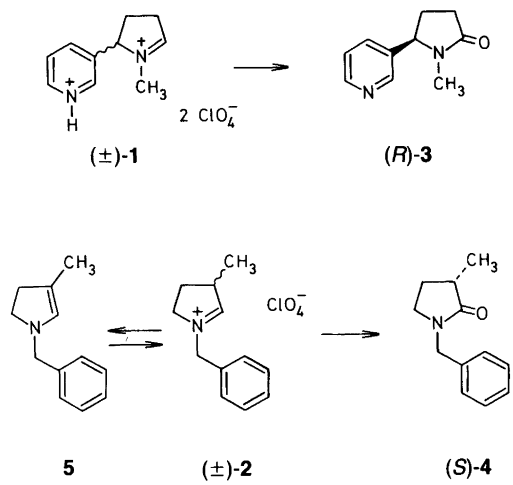

Fig. 1. Major enantiomers formed in the oxidation of racemic iminium ions **1** and **2** with the rabbit liver enzyme preparation.

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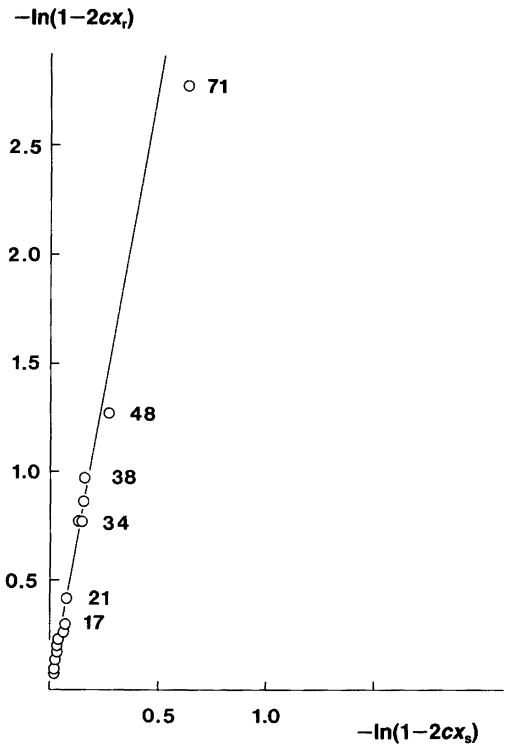

Fig. 2. Evaluation of the kinetic resolution **1** → **3** mediated by the rabbit liver preparation. An enantiomer ratio ($E_{R/S}$[13]) of 5.5 is obtained from the combined use of the plot and the equation $E_{R/S} = \ln(1-2cx_r)/\ln(1-2cx_s)$, in which c is % conversion/100 and x_r and x_s are the mole fractions of (*R*)-**3** and (*S*)-**3**, respectively. This equation is derived from the somewhat more complex eqn. 5 in Ref. 13 by using the relations $ee_R = 2x_r - 1$ and $x_s = 1 - x_r$. The figures in the plot are the chemical yields of **3**.

is remarkable but, in view of the broad substrate specificity of rabbit liver aldehyde oxidase, hardly surprising.

The enzymatic oxidation of (±)-**2** to **4** was performed at pH 8.1 in order to favour continuous base-catalyzed racemisation of the substrate via the achiral enamine **5**. Due to the low rate of oxidation and the instability of the enzyme, it was difficult to achieve a high conversion of **2**; our best yield of **4** was 46 %. Seven values of the ratio (*R*)-**4**/(*S*)-**4** were determined, corresponding to yields between 2 and 46 %, and were found to be randomly distributed within the range 0.14±0.04. $E_{R/S}$ must therefore equal this value.

Finally, it may be noted that the question of favoured/disfavoured side of substitution of the five-membered ring iminium ion varies with the position in the ring. To obtain the fastest reaction, the methyl group in the 4-position must be on the *si* side of the iminium carbon (C-5), whereas the pyridyl group in the 2-position must be on the opposite (*re*) side, as shown in Fig. 1.

Experimental

NMR spectra and pH values were recorded as described previously.[14] A Hewlett-Packard 5830A gas chromatograph equipped with a 25 m OV-101 fused silica capillary column and a 18850 A GC terminal ws used for the quantification of **3** and **4** using diphenylamine as internal standard (N_2 as carrier gas). A GLC–MS–SIR technique was also used for the quantification of **3** (**6** as internal standard). Enantiomeric compositions were determined using Chirasil–Val–D and Chirasil–Val–L columns (26 m, Chrompack) using helium as carrier gas; two samples of **3** were checked by measuring optical rotation (Perkin-Elmer 241 polarimeter).

Enzyme preparation. Frozen rabbit livers (ca. 200 g) were homogenized. Subsequent heat treatment, centrifugation and precipitation with ammonium sulfate were performed as described previously.[15] The precipitate was dissolved in phosphate buffer (pH 7.8, 100 ml, 0.05 M) containing EDTA (0.2 mM) and the solution was dialyzed against 10 l of the buffer. A typical enzyme activity determined by the standard assay[15] using 3-(aminocarbonyl)-1-methylpyridinium chloride was 1×10^{-8} mol sec^{-1} ml^{-1}.

Typical enzymatic oxidation. A solution of (±)-**1** (25 mg) in water (1 ml) and 6 ml of enzyme preparation were mixed with phosphate buffer (pH 7.4, 60 ml) containing EDTA (0.2 mM) and the mixture was stirred for 30 min (22 °C). An additional 6 ml aliquot of enzyme preparation was added and the stirring continued for 1 h. Sodium borohydride (1.0 g) in water (10 ml) and (±)-**6** picrate (2.50 mg) were added. After 30 min the mixture was acidified with 4 M hydrochloric acid, saturated with ammonium carbonate and extracted three times with ethyl acetate/light petroleum (9:1). The combined organic phases were back-extracted twice with 0.1 M hydrochloric acid, the

extracts made alkaline (pH 10), saturated with sodium chloride and extracted with methylene chloride. After drying (Na_2SO_4), GLC–MS–SIR analysis showed a yield of 17 %. If two more aliquots of enzyme preparation were added at 30 min intervals, the yield was 71 %.

Oxidations of **2** (0.3–10 mg) were carried out at pH 8.1 using cysteine as additive (10 mM). The two compounds which appeared at the retention times of authentic (*R,S*)-**4** on the chiral GLC column gave rise to mass spectra which were indistinguishable from those of (*R*)- and (*S*)-**4**, respectively.

3,4-Dihydro-1-methyl-2-(R,S)*-[3-pyridinyl]-2H-pyrrolium perchlorate hydroperchlorate* [(±)-**1**] was prepared from (±)-nicotine[16] via (±)-**3**, as described for the optically pure compound;[14] m.p. 231–235 °C (sealed tube).

1-([$^2H_3$]-methyl)-5-(R,S)*-[3-pyridinyl]-2-pyrrolidinone* (**6**) was prepared from nicotine-d_3[17] by the method used for the unlabelled compound [(*S*)-**3**].[14] The compound was purified and administered as its picrate, m.p. 123–125 °C.

1-Phenylmethyl-2-pyrrolidinone. A solution of 2-pyrrolidinone (10.0 g. 0.12 mol) in toluene (175 ml) was treated (30 °C, 30 min) with sodium hydride (0.12 mol) in the form of 55 % suspension (5.1 g) in mineral oil, and benzyl bromide (20.1 g, 0.12 mol) was then added. After heating at 90 °C (2 h), the mixture was cooled and water was added. Separation of the phases, extraction of the aqueous phase with methylene chloride, combination of the organic phases, drying (Na_2SO_4) and distillation (117–119 °C, 70 Pa) gave the title compound in 61 % yield; lit.[18] b.p. 122–123 °C (270 Pa); ^{1}H NMR: δ 7.23 (s, 5H), 4.40 (s, 2H), 3.20 (t, 2H), 2.6–1.6 (m, 4H).

(R,S)*-3-Methyl-1-phenylmethyl-2-pyrrolidinone* [(*R,S*)-**4**]. The above lactam (10.2 g, 67.5 mmol) was added to the stirred reaction mixture (−50 °C) obtained by dissolving sodium (1.55 g, 67.4 mmol) and $Fe(NO_3)_3 \cdot 9H_2O$ (20 mg) in liquid ammonia (100 ml). After stirring for 30 min, methyl iodide (19.0 g, 13.5 mmol) was added and, after 1 h, 50 ml of ether. The cooling bath was removed and after 16 h water was added. Extraction with ethyl acetate, drying (Na_2SO_4) and evaporation gave a crude mixture (10.0 g) which was separated on silica gel (10×15 cm) using light petroleum/ethyl acetate (3:1) as eluant. The dimethylated lactam was eluted first, then (*R,S*)-**4** (3.6 g, 33 %) and finally the starting lactam. ^{1}H NMR: δ 7.28 (s, 5H), 4.45 (s, 2H), 3.17 (dd, 2H, *J* 8.3 and 5.4 Hz), 2.5–1.5 (m, 3H), 1.24 (d, 3H, *J* 6.9 Hz). MS [*m/z* (relative abundance)]: 189 (56 %, M^+), 160 (11), 132 (13), 118 (11), 106 (19), 98 (31), 92 (14), 91 (100), 65 (25), 56 (11) and 55 (13).

(R,S)*-3,5-Dihydro-4-methyl-1-phenylmethyl-2H-pyrrolium perchlorate* [(*R,S*)-**2**] was prepared by the technique described[14] for (*S*)-**1**. On addition of perchloric acid in methylene chloride to the *N,S*-acetal, little or no crystalline iminium salt was obtained; the material was therefore purified on an alumina column, eluting first with ether and then (to give the salt) with ethanol. Repeated co-distillation with toluene and crystallization from ether/ethanol (3:2) containing anhydrous perchloric acid gave 2.0 g (43 %) of (*R,S*)-**2**; m.p. 45–47 °C (sealed tube); ^{1}H NMR: δ 8.58 (broad s, 1H), 7.42 (s, 5H), 5.16 (s, 2H), 4.16 (broad t, 2H), 3.9–3.7 (m, 1H), 2.8–1.6 (m, 2H), 1.44 (d, 3H), *J* 7.4 Hz).

Optically active **4** [(*R*)/(*S*) = 62/38]. 4-Amino-2-methylbutanoic acid showing $[\alpha]_D^{24}$ − 1.60° (*c* 7.8, water) was prepared.[12] Based on the literature value for the maximum rotation[12] of $[\alpha]_D^{24}$ − 6.7° (*c* 2.8, water), our material was 62 % *R*, 38 % *S*.

A solution of the above acid (0.20 g, 1.7 mmol) and benzaldehyde (0.19 g, 1.8 mmol) in methanol (20 ml) was stirred with 4Å molecular sieves (22 °C, 2 h). Sodium cyanoborohydride (0.27 g, 4.3 mmol) in methanol (5 ml) was added in portions over a period of 3 h; after 16 h, dilute hydrochloric acid was added to give pH circa 3. Filtration and evaporation of the solvent gave a residue which was dissolved in 10 ml of a 1 M solution of hydrogen chloride in methanol. Molecular sieves (4Å, 60 mg) were added and the mixture was heated under reflux (4 h). After filtration, hydrochloric acid (2 M, 10 ml) was added, the methanol was removed by evaporation and the remaining solution was washed with ether. Aqueous sodium hydroxide was added to give pH 10 and the solution was extracted three times with ether. Drying (Na_2SO_4) and evaporation of the solvent led to a mixture of amino ester and pyrrolidone. Heating the mixture under

reflux with methanol (16 h) gave a product (0.15 g), the purity of which was ca. 80 % (^{1}H NMR). Chromatography on a column of silica gel (1×15 cm), using ether/light petroleum (1:1) as eluant, afforded 90 mg (28 %) of pure **4**; $[\alpha]_D^{24}$ + 3.7° (*c* 7.2, $CDCl_3$). Gas chromatography on the Chirasil–Val–D column (120 °C) gave almost base line separation of the enantiomers, which were eluted after 23.76 min (61 % of the area) and 24.11 min (39 % of the area), respectively.

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References

1. Whiteside, G. M. and Wong, C.-H. *Angew. Chem. Int. Ed. Engl. 24* (1985) 617.
2. Simon, H., Bader, J., Günther, H., Neumann, S. and Thanos, J. *Angew. Chem. Int. Ed. Engl. 24* (1985) 539.
3. Sih, C. J. and Chen, C.-S. *Angew. Chem. Int. Ed. Engl. 23* (1984) 570.
4. Jones, J. B. *Tetrahedron 42* (1986) 3351.
5. Bray, R. C. *The Enzymes 12B* (1975) 299.
6. Coughlan, M. P. In: Coughlan, M. P., Ed., *Molybdenum and Molybdenum-Containing Enzymes,* Pergamon Press, Oxford 1980, p. 121.
7. Burgmayer, S. J. N. and Stiefel, E. I. *J. Chem. Educ. 62* (1985) 943.
8. Krenitsky, T. A., Neil, S. M., Elion, G. B. and Hitchings, G. H. *Arch. Biochem. Biophys. 150* (1972) 585.
9. Beedham, C. *Drug Metab. Revs. 16* (1985) 119.
10. Brandänge, S. and Lindblom, L. *Biochem. Biophys. Res. Commun. 91* (1979) 991.
11. Thomas, H. G. and Ruenitz, *J. Heterocycl. Chem. 21* (1984) 1057.
12. Adams, R. and Fles, D. *J. Am. Chem. Soc. 81* (1959) 4946.
13. Chen, C.-S., Fujimoto, Y., Girdaukas, G. and Sih, C. J. *J. A. Chem. Soc. 104* (1982) 7294.
14. Brandänge, S. and Lindblom, L. *Acta Chem. Scant., Ser. B33* (1979) 187.
15. Rajagopalan, K. V. and Handler, P. *Methods Enzymol. 9* (1966) 364.
16. Brandänge, S., Lindblom, L., Pilotti, Å. and Rodriguez, B. *Acta Chem. Scand., Ser. B37* (1983) 617.
17. Seeman, J. I., Secor, H. V. and Forrest, G. *J. Labelled Compd. Radiopharm. 16* (1979) 387.
18. Hanford, W. E. and Adams, R. *J. Am. Chem. Soc. 57* (1935) 921.

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