The C_2-Ketone Rule in Horse Liver Alcohol Dehydrogenase (HLADH)-catalysed Oxidation and Reduction

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Summary The enantiomer selectivity of HLADH with respect to the racemic C_2-ketone substrates (**1**), (**3**), (**5**), and (**7**) has been examined, showing that the enzyme exhibits a remarkable selectivity which is opposite to that indicated by the microbial C_2-ketone rule for *Curvularia lunata* and *Rhodotorula rubra*.

Our previous papers[1] reported a marked enantiomer selectivity of *C. lunata* and *R. rubra* towards various C_2-ketones,† and we summarized the results in a C_2-ketone rule which states that these microbes preferentially reduce P-C_2-ketones enantiomers having the larger parts of the molecule in upper-right (UR) and lower-left (LL) quadrants in the quadrant orientation (Figure). This finding prompted us to examine the expected enantiomer slectivity of horse liver alcohol dehydrogenase (HLADH), which study led us to another C_2-ketone rule opposite to that for the microbial reduction.

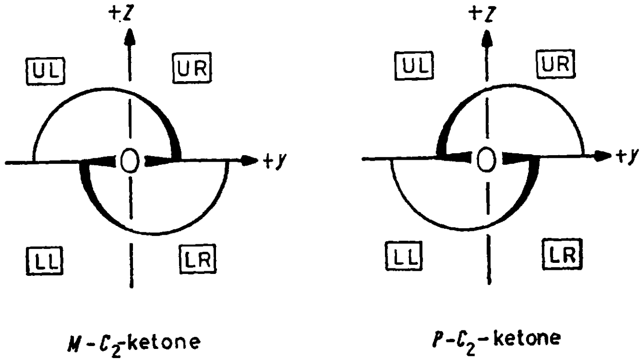
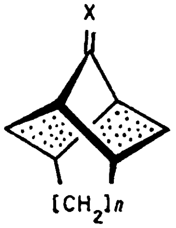
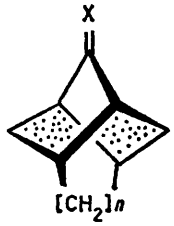


Figure. Two quadrant orientations for the enantiomers of C_2-ketones with M and P helicity.

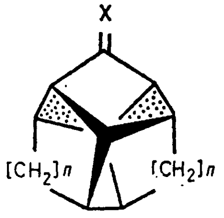
Our results are summarized in the Table. In the reduction experiments (i—iv), 500 ml of 1/15 N Sørensen phosphate buffer solution (pH 7·0) containing the racemic substrate C_2-ketone‡ (±)-bisnoradamantanone (**1**), (±)-9-twistbrendanone (**3**), (±)-D_3-trishomocubanone (**5**), and (±)-C_2-methanoditwistanone (**7**) respectively (50 mg), NADH (1 mol. equiv. with respect to the substrate), and HLADH (5 mg)§ were incubated at 20 °C, and the ether-extracted metabolites were analysed by g.l.c. and separated by t.l.c. (silica gel).

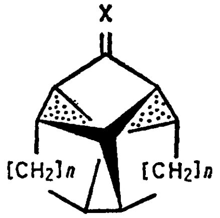
The oxidation experiment (v) was carried out by incubating a mixture of the racemic alcohol (**2**) (50 mg), 1/20 N glycine–NaOH buffer solution (pH 9·0) (250 ml), NAD$^+$ (1 mol. equiv. with respect to the substrate), and HLADH (5 mg).





(+)-(**1**)	$n = 1$, $X = O$	(−)-(**1**)
(−)-(**2**)	$n = 1$, $X = <^{H}_{OH}$	(+)-(**2**)
(−)-(**3**)	$n = 2$, $X = O$	(+)-(**3**)
(−)-(**4**)	$n = 2$, $X = <^{H}_{OH}$	(+)-(**4**)





(−)-(**5**)	$n = 1$, $X = O$	(+)-(**5**)
(−)-(**6**)	$n = 1$, $X = <^{H}_{OH}$	(+)-(**6**)
(−)-(**7**)	$n = 2$, $X = O$	(+)-(**7**)
(−)-(**8**)	$n = 2$, $X = <^{H}_{OH}$	(+)-(**8**)

In the recycling experiments (vi) and (vii), the amount of the oxidative coenzyme NAD$^+$ was reduced to 0·12 mol. equiv. with respect to the substrate, with addition of 2 mol. equiv. of FMN.[2]

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† Proposed name for ketones which belong to the C_2 point group and have the C_2 axis coincident with the carbonyl axis.

‡ All structural formulae in this communication are presented in their absolute configurations.

§ Purchased from Boeringer (Mannheim).

Table. Enantiomer selectivity of HLADH toward C_2-ketones.

				Products[a]			
	Substrate.	Incubation	Ratio of	Alcohol		Ketone	
Expt.	All (±)	period/h	alcohol : ketone	Config.	Opt. purity/%	Config.	Opt. purity/%
(i)	(**1**)[b]	122	16 : 84	(−)-(**2**)	51	(−)-(**1**)	11
(ii)	(**3**)[b]	6	47 : 53	(−)-(**4**)	73	(+)-(**3**)	90
(iii)	(**5**)[b]	2	52 : 48	(−)-(**6**)	76	(+)-(**5**)	93
(iv)	(**7**)[c]	70	43 : 57	(−)-(**8**)	96	(+)-(**7**)	81
(v)	(**2**)[b]	48	47 : 53	(+)-(**2**)	51	(+)-(**1**)	57
(vi)	(**4**)[b]	15	39 : 61	(+)-(**4**)	50	(−)-(**3**)	32
(vii)	(**6**)[b]	52	68 : 32	(+)-(**6**)	23	(−)-(**5**)	63

[a] All isolated metabolites were found to show satisfactory spectroscopic data and elemental analyses. [b] For the absolute configurations and the absolute rotations, see ref. 1. [c] The absolute configurations, the absolute rotation, and the microbial reduction of (**7**) have been reported by M. Nakazaki, H. Chikamatsu, K. Naemura, and M. Iwasaki, The 41th Annual Meeting of the Chemical Society of Japan, Osaka, April 1980, Abstracts, vol. II, p. 1009.

All these experiments unambiguously demonstrate that HLADH preferentially reduces the cage-shaped C_2-ketones (+)-(**1**), (−)-(**3**), (−)-(**5**), and (−)-(**7**), containing the bicyclo[2·2·1]heptan-7-one unit and with the (+90°, −20°, −55°)$_2$[3] twist-boat cyclohexane (dotted part) as a common structural feature, and preferentially oxidizes the corresponding alcohols. From the quadrant projection (Figure), these ketones are classified as *M*-C_2-ketones[1] and the demonstrated enantiomer selectivity of HLADH is completely opposite to that found in the microbial process which selectively reduces the *P*-C_2-ketones.

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[1] M. Nakazaki, H. Chikamatsu, K. Naemura, M. Nishino, H. Murakami, and M. Asao, *J. Chem. Soc., Chem. Commun.*, 1978, 667; *J. Org. Chem.*, 1979, **44**, 4588.

[2] J. B. Jones and K. E. Taylor, *Can. J. Chem.*, 1976, **54**, 2969.

[3] W. Klyne and V. Prelog, *Experentia*, 1960, **16**, 521.