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Resolution of Racemic ε-Lactones

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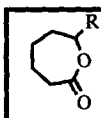
Abstract : Kinetic resolution of racemic ε-lactones by Pig Liver Esterase give optically active *R* (+) ε-lactones. When alkyl group is higher than propyl, Horse Liver Esterase leads to the destruction of the opposite enantiomer. Enantiomeric excess is easily evaluated by G.C. on a chiral stationary phase.

Besides playing a part in the organoleptic character of molecules, enantiomerism which is often related to a biological activity, can also affect their odour perception threshold [1]. It is therefore useful to resolve racemates and to assess the degree of purity of the enantiomers obtained. Rousseau and co-workers [2,3] have shown that the use of commercial HLE (Horse Liver Esterase - L 9627) and PLE (Pig Liver Esterase - L 8285) allows an enantioselective hydrolysis of lactones of different ring sizes. We have accordingly examined the behaviour of a series of racemic ε -lactones to study the part played by the alkyl group. Asymmetric destruction is followed by chiral gas chromatography. Such 7-ring lactones derived from menthone [4] , camphor [5], or limonoids [6, 7] have already been identified in natural extracts.

Results : A series of hydrolyses was carried out at 20°C, at pH (7.4) and a conversion rate of 60%. The ratio enzyme/substrate was constant throughout the series (c. 1 g. of weighted enzymatic extract to 4.10^{-3} mole of ε-lactone). The yield of optically active lactones obtained, ranged from 30 to 35% with respect to the racemic. The results for the asymmetric transformations using these two hydrolases are shown in Table 1.

Table 1

	P.L.E.		H.L.E.		
R =	time (hours)	e.e.(%) config. *R*	time (hours)	e.e.(%)	config.
Methyl	2,20	89	3,00	84	*R*
Ethyl	2,00	98	2,40	22	*R*
Propyl	2,00	62	2,00	4	*R*
Butyl	1,05	88	3,10	38	*S*
Pentyl	1,00	77	4,20	53	*S*
Hexyl	1,20	33	6,00	50	*S*
Heptyl	1,20	60	9,20	60	*S*
Octyl	2,15	65	1,00	63	*S*

The absolute configurations are deduced by comparing them either with authentic samples or with the published specific rotation [2, 3]. Confirmation for such attributions is obtained by measuring the chiral capillary gas chromatography retention times when, in the series concerned, enantiomer *S* is eluted first. Whatever the length of the alkyl chain, PLE hydrolyses the *S* -enantiomer preferentially. The antipodic isomer is collected with a reasonable enantiomeric excess (e.e), except in the case of the hexyl group (33%). The mean hydrolysis time is higher with HLE than with PLE. With a constant HLE concentration (1/30) significant variations in the reaction rate are observed for the 60% conversion rate which is obtained from 1 to 9 hours according to the alkyl chain length and enantioselectivity reversal from the butyl group onwards (fig.1) is also observed. In fact, in the case of methyl and ethyl groups, the *S* -enantiomer of the lactone is hydrolysed faster while from the butyl group onwards the opposite enantiomer is destroyed. Using a HLE concentration of 1/10 for the hydrolysis, Rousseau and co-workers isolated the (*R*)-6-Methyl ε-lactone [3], (e.e. 83%), while they collect (*S*) -6-Propyl ε-lactone [2] (e.e. 35%) at 1/20 concentration. In the case of the reduction by bakers' yeast of a series of homologous alkyl γ-chloroacetoacetates [8] a similar inversion process has already been observed which suggests that the increase in enzyme concentration generates the enantioselective destruction of a higher homolog. This behaviour may be accounted for by the presence of several kinds of hydrolases in the HLE extract and whose rates of reaction are different. This hypothesis seems to be in good agreement with the optical purity of the residual enantiomer whose enantiomeric excess does not exceed 63%. The enzymatic reaction is followed by chiral gas chromatography and we ensured that the phase used, Lipodex E (Octakis-(2,6-di-O-Pentyl-3-O-Butyryl) γ-cyclodextrins) allows the resolution of the racemates, throughout the series, without inverting the elution order.

To this end, using the retention time indices, we have been able to verify that there exists a linear relationship such as : I(*R*)= a. I(*S*) (fig. 2) which shows that the enantiomer interactions with respect to the phase remain proportional throughout the series and that no entropic effect likely to trigger any alteration in the elution order is to be found.

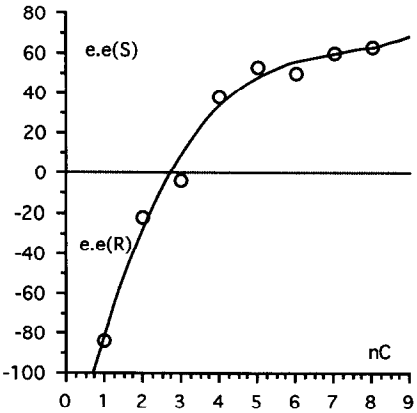


Figure 1 : Plot of (e.e.) vs.carbon atom number (nC) of the alkyl group of ε-lactones , in the case of hydrolysis with HLE.

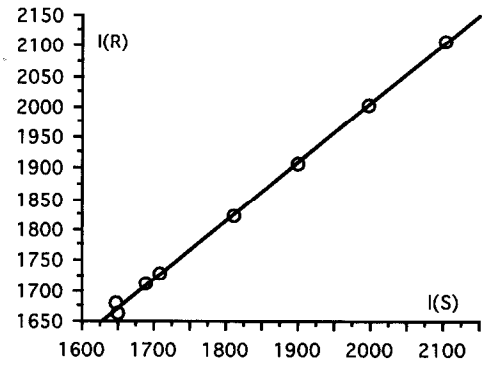
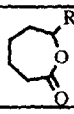


Figure 2 : I(R) = 1,008 I(S) correlation coefficient = 1,000 Plot of Indices of R-enantiomers vs. Indices of S-enantiomers

The various chromatographic data are recorded in Table 2 where the Van den Dool Indices [9]are calculated from the series of linear hydrocarbures C_6 to C_{24} as references.

Table 2

	Indices		capacity ratio		resolution	separation factor
R =	I (*S*)	I (*R*)	k' (*S*)	k' (*R*)	R (*R,S*)	α
Methyl	1651	1664	28,49	29,05	1,98	1,020
Ethyl	1647	1678	28,35	29,64	7,34	1,046
Propyl	1689	1711	30,14	31,05	6,16	1,030
Butyl	1709	1729	30,96	31,80	5,74	1,027
Pentyl	1812	1824	35,14	35,60	3,21	1,013
Hexyl	1899	1907	38,39	38,71	2,27	1,008
Heptyl	1997	2003	42,10	42,34	1,73	1,006
Octyl	2102	2107	46,49	46,75	1,19	1,005

The chromatographic approach enabled us to follow an enantiomer destruction kinetics and to determine the enantiomeric excess easily as can be seen in fig. 3 which exhibits chiral separations for R = nButyl to nOctyl; chromatogram 1 represents the resolution of the racemic mixture, chromatograms 2 and 3 evidence the most numerous enantiomers, *R* or *S*, according to the hydrolase used.

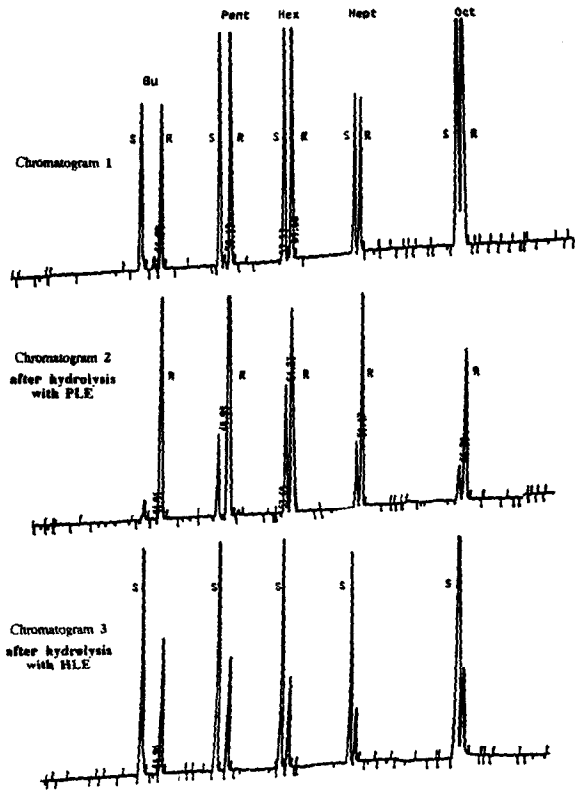
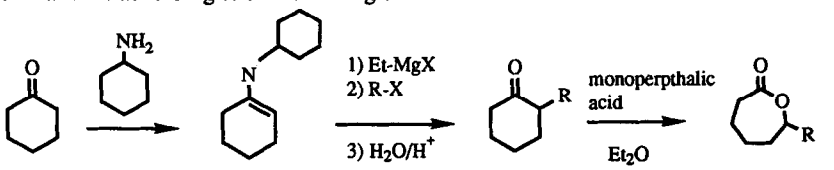


Figure 3

Conclusion : In the HLE extract the presence of various hydrolases is likely to revert the destruction rate of one of the enantiomers according to the length of the alkyl group. It is easy to follow such phenomena, however unexpected, by means of chiral gas chromatography.

Material and methods : * *ε-lactone synthesis* : ε-lactone synthesis is carried out by Baeyer-Villiger oxidation with monoperphtalic acid [10], from 2-alkylcyclohexanones [11], which are themselves obtained by the alkylation of cyclohexanones according to the following scheme:



Expected lactones are obtained regioselectively with a 40% yield. Only 6-methyl ε-lactone is accompanied by 5% of the lactone arising from the oxygen insertion on the least substituted carbon chain. The structures have been verified by the proton RMN (Brucker AC 200) and by GC/MS (Hewlett-Packard HP 5971 A). Mass spectra of the whole series reveal allow intensity molecular peak as well as charactacteristic fragments of the ε-lactone ring (m/e = 113, 85, 55 and 41)

* *enzymatic hydrolysis* : Hydrolysis carried out at 20 °C in a thermostat bath is placed into an Erlenmeyer where a 30 ml phosphate buffer his introduced as well as about 1 g of accurately weighted enzymatic extract. 4.10^{-3} mole of ε-lactone mole are added while keeping the pH at 7.4 by addition of sodium hydroxide 0.2N. As soon as the 60 % conversion rate is reached, the solute is filtered through celite and is extracted three times using 50 ml of dichloromethane. After drying, the optically active lactone is collected.

* *gas phase chromatography* : The chromatograph used is a HP 5890 model fitted with a Flame Ionisation Detectot (FID) and linked to an integrator-recorder HP 3392 A. A Lipodex E (Macherey Nagel) capillary column was used (fused silica, L = 25 m.: ϕ = 0.25 mm.). The carrier gas is nitrogen; column inlet pressure is 60 kPa; split 1/100, t° injector 230°C, t° detector 250°C. The test conditions range from 60°C to 180°C with a temperature programmed of 2°/mn. The samples are in a 10% solution in dichloromethane.

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