Tetrahedron: Asymmetry Vol. 2, No. 1, pp. 39-42, 1991
Printed in Great Britain

0957-4166/91 $3.00+.00
Pergamon Press plc

Enantioselective Hydrolysis of Aryloxypropionic Esters by Bovine Serum Albumin: Enhancement in Selectivity by β-Cyclodextrin

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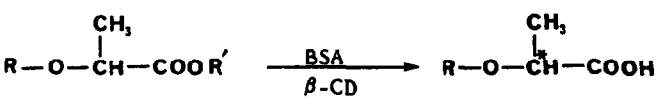
(*Received* 5 *December* 1990)

Abstract: Bovine serum albumin catalyzed hydrolysis of racemic aryloxypropionic esters affords appreciable enantioselectivity. The effect of β-cyclodextrin in this process of hydrolysis has been studied.

The biological importance of phenoxypropionic acids particularly chloro substituted ones is well established. These are presently being used as racemates due to their herbicidal[1] and hypocholesterolemic[2] as well as for their activation of microsomal stearoyl-Coa desaturase activity[3], whereas the biological property resides mainly in the R-enantiomers. A number of chemical and biological methods[4,5] have been described in the literature for the resolution of racemic phenoxypropionic acid derivatives.

There are numerous reports[6] on the chromatographic separation of enantiomers employing bovine serum albumin (BSA) as a stationary phase. Further, in recent years the role of cyclodextrins (CDs) in bioconversion or fermentation media has gained much importance[7]. In connection with our studies on biocatalytic processes[8], the present investigation illustrates a new application of BSA for the hydrolysis of esters **1a-l** in aqueous media, for the first time with appreciable enantioselectivity. The improvement in selectivity and efficiency is due to β-CD.



1a-l 2a-l

R = substituted aryl; R^1 = alkyl

As an example; **1a** (300 mg) was taken in 20 ml of 0.01 M phosphate buffer (pH 11.5) solution containing 0.03 M equivalents of BSA[9] and 0.2 M equivalents of β-CD was added. The mixture was stirred at 37°C. The course of the reaction was followed by HPLC on TSK ODS-120A, 5 μm column (4.6 x 250 mm, eluted with pH 6, 0.1 M phosphate buffer containing 40% methanol at 0.5 ml per min. flow rate and monitored at 275 nm wavelength. The λ_{max} and corresponding ε_{max} were found to be identical for the acids and their respective esters. The amount of hydrolysis was determined from the relative areas of the ester and acid peaks. The unhydrolyzed ester was extracted from the reaction mixture with hexane. The reaction mixture was then brought to pH 6.5 by acidification with 10% HCl solution. The enantiomeric excess was directly determined by HPLC employing LKB enantioPac, α_1-AGP 10 μm column (4.0 x 100 mm), mobile phase with 8 mM phosphate buffer (pH 6.9) containing 0.6% of N,N-dimethyloctyl amine at 0.3 ml per min. flow rate and monitored at 220 nm wavelength. The absolute configuration was determined by comparing the optical rotation of the acid obtained with the data from the literature[10].

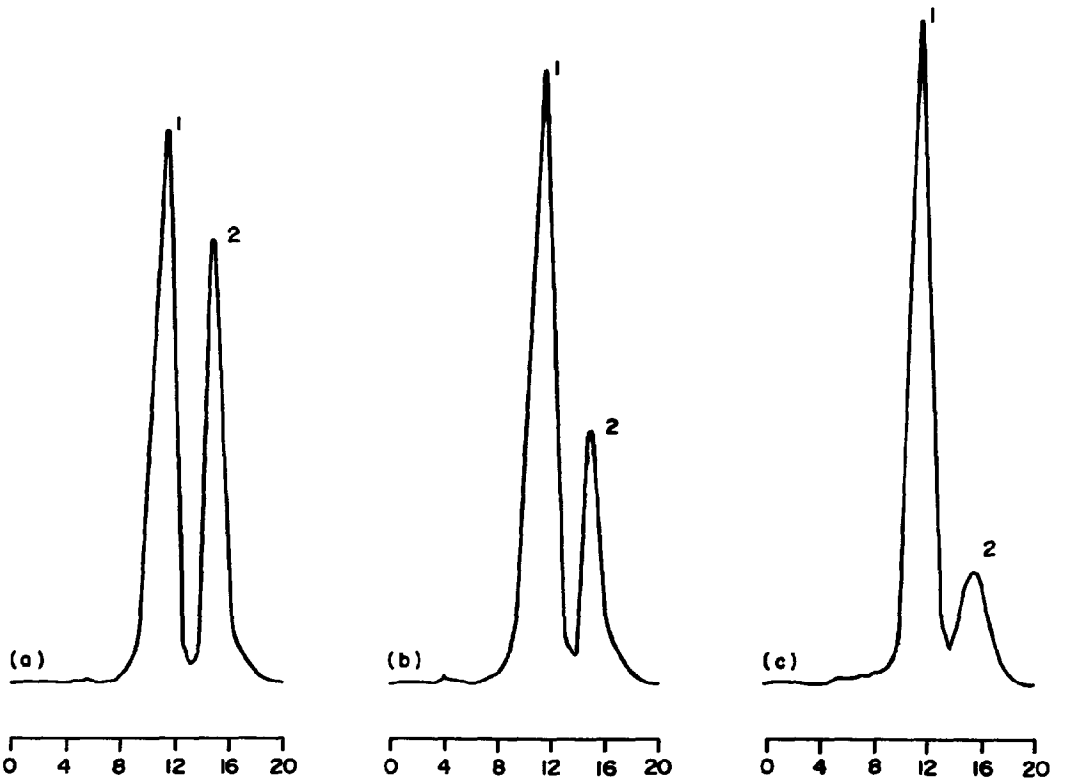
Table 1. Enantioselective hydrolysis of Aryloxypropionic esters 1a-1 by BSA and β-CD

	R	R^1	BSA[a]			BSA + β-CD		
			Time (h)	Conversion (%)[b]	ee (%)[b]	Time (h)	Conversion (%)[b]	ee (%)[b]
a	C_6H_5	CH_3	8	43	66	4	62	89
b	C_6H_5	C_2H_5	8	57	65	4	60	98
c	2'-ClC_6H_4	CH_3	6	36	57	2	47	87
d	2'-ClC_6H_4	C_2H_5	6	32	73	2	41	97
e	2'-ClC_6H_4	i-C_3H_7	5	30	68	3	38	86
f	4'-ClC_6H_4	CH_3	6	40	81	3	45	96
g	4'-ClC_6H_4	C_2H_5	8	41	75	2	46	96
h	4'-ClC_6H_4	i-C_3H_7	10	52	58	4	57	78
i	2'-CH_3, 4'-ClC_6H_3	CH_3	6	48	69	4	45	97
j	3'-CH_3, 4'-ClC_6H_3	CH_3	6	39	71	2	42	95
k	3'-$C_{15}H_{31}C_6H_4$	CH_3	8	46	49	3	45	87
l	α-naphthyl	CH_3	5	44	62	2	51	> 99

a) BSA alone was employed in this reaction in the absence of β-CD

b) Employing HPLC

Similarly, various substituted phenoxypropionic esters were hydrolyzed to the corresponding acids enantioselectively. Selected results are illustrated in Table 1 and Fig. 1. It was observed that the enantioselectivity of the hydrolysis of most substrates by BSA was appreciable. However, the addition of β-CD in this reaction not only enhanced the enantioselectivity but also accelerated the rate of the hydrolysis. Negligible hydrolysis was observed with β-CD alone confirming that β-CD enhances BSA action rather than participating in the reaction directly. Ester variations, such as methyl, ethyl or isopropyl did not significantly effect the selectivity pattern. For all the substrates, the R-enantiomer was preferentially hydrolyzed, as found in other enzymatic hydrolyses of phenoxypropionic acids[11] and by analogy with the α-chymotrypsin models[12].



a) Racemic 2a; peak 1 is R-enantiomer and peak 2 is S-enantiomer.
b) 2a obtained upon hydrolysis of 1a in presence of BSA.
c) 2a obtained upon hydrolysis of 1a in presence of BSA and β-CD.

In conclusion, this study provides a practical and convenient method for resolution of aryloxypropionic esters, employing BSA in conjunction with β-CD to catalyse enantioselective ester hydrolysis.

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IICT Communication No. 2730