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STRUCTURAL AND OPTICAL SELECTIVITY OF HYDROXY ESTER HYDROLYSIS BY α-CHYMOTRYPSIN

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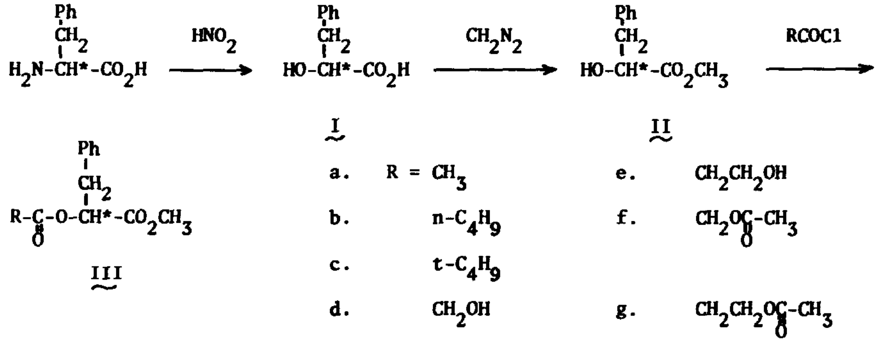
It is well known that an amino acid derivative having benzyl, p-hydroxybenzyl or indol-3-yl-methyl substituent on α-carbon atom, has special affinity toward α-chymotrypsin. Thus, N-acetyl-L-tryptophane p-nitrophenyl ester was hydrolyzed by chymotrypsin, and the rate of hydrolysis, 10^7 $sec^{-1}M^{-1}$ (acylation rate constant), was the fastest one ever reported for this enzyme[1]. In general, only L-amino acid derivatives are effectively hydrolyzed by the enzyme[2].

In a contrast to well investigated amino acids, only limitted works have been carried out for hydroxy acids.[3] In this article, we wish to report that derivatives of β-phenyllactic acid II, III have affinity toward α-chymotrypsin where catalytic constants and D,L-selectivity depend on the structure of modifying groups R.



I

II

III

a. R = CH_3
b. $n\text{-}C_4H_9$
c. $t\text{-}C_4H_9$
d. CH_2OH
e. CH_2CH_2OH
f. $CH_2OC(O)\text{-}CH_3$
g. $CH_2CH_2OC(O)\text{-}CH_3$

Optically active (D- or L-) β-phenyllactic acid was prepared from the corresponding

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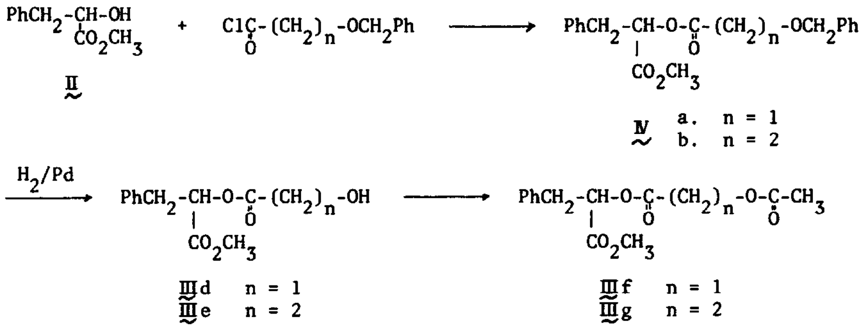
phenylalanine[3]. Methyl ester of I(II) was prepared by the treatment of I with diazomethane. Esterification of II was carried out with the corresponding acyl chloride in the presence of pyridine[4], where esters IIIa-c were obtained. Esters IIId-g were prepared according to Scheme I.

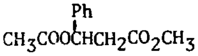


Scheme I[5]

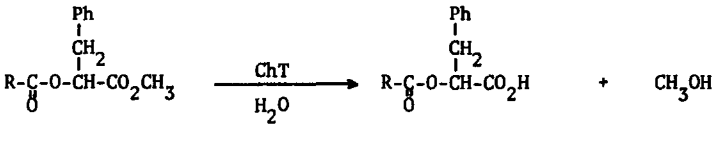
These esters (6.5 - 10.6 X 10^{-4}M) were hydrolyzed in 4.76 vol% aqueous acetonitrile in the presence or absence of α-chymotrypsin (4.6 X 10^{-6}M) at pH 8.0 at room temperature. The reaction rate was followed by pH stat procedure(to keep pH 8.0). In the absence of α-chymotrypsin, no reaction was practically observed. The results are summarized in Table 1.

Table 1 Effect of Acyloxyl Group in Hydrolysis of $PhCH_2\underset{X}{\underset{|}{C}}HCOOCH_3$ Catalyzed by α-ChT

X	rel "k_L"	rel "k_D"	"k_L/k_D"
CH_3COO	1.0	1.0	7.2
$HOCH_2COO$	1.8	0.8	16
$HOCH_2CH_2COO$	2.0	1.3	15
CH_3COOCH_2COO	5.3	1.5	26
$CH_3COOCH_2CH_2COO$	3.3	1.6	15
$n\text{-}C_4H_9COO$	3.1	1.4	15
$t\text{-}C_4H_9COO$	0.3	0.6	3.6
HO	3.7	1.7	16


$CH_3COO\overset{Ph}{\overset{|}{C}}HCH_2CO_2CH_3$ rel "k_L" < 0.01

All of the esters used, IIIa-g, were only affected methoxycarbonyl (not acyloxyl) group by chymotrypsin as shown in eq. (1), but for such a substrate having modifying group of



$$\text{R-}\underset{\text{O}}{\overset{\|}{\text{C}}}\text{-O-}\overset{\overset{\text{Ph}}{|}}{\overset{\text{CH}_2}{|}}{\text{CH}}\text{-CO}_2\text{CH}_3 \xrightarrow[\text{H}_2\text{O}]{\text{ChT}} \text{R-}\underset{\text{O}}{\overset{\|}{\text{C}}}\text{-O-}\overset{\overset{\text{Ph}}{|}}{\overset{\text{CH}_2}{|}}{\text{CH}}\text{-CO}_2\text{H} \quad + \quad \text{CH}_3\text{OH} \qquad (1)$$

specific affinity toward chymotrypsin as IV, the enzyme affected both of ester functions probably because of competitive binding of both benzyl into a hydrophobic pocket of the enzyme.

Analysis of the rate profile for a racemic mixture of a given ester indicated that the L-isomer was hydrolyzed much faster than the D-isomer. However, the rate of the hydrolysis of the D-isomer was still appreciable in the present condition[6]. The rate ratio was further ascertained for II, IIIa, IIIb and IIIc by using each optical isomer in separate rate measurements. Starting from a racemic ester, reaction was quenched at approximately 50 % conversion, then optical rotation of both of acid (hydrolysis product) and recovered ester was investigated. To a solution of 2.0 g of IIIa dissolved in 100 *ml* of acetonitrile, 1 *l* of Kolthoff's buffer solution was added. After 114 *mg* of α-chymotrypsin was added into the solution, the hydrolysis rate was followed by means of gas chromatography. When approximately 50 % of an ester was hydrolyzed (ca. 200 min), hydrochloric acid was added to make pH 4 or below to quench the reaction and the mixture was salted out and extracted with ether. The enzyme deposited during the treatment was carefully filtered off. Usual work up gave a recovered ester from ether layer and an acid from aqueous alkali solution, identified with α-acetoxy-β-phenyllactic acid V, which was further hydrolyzed to I (eq. 2). Optical

$$\text{IIIa} \longrightarrow \text{CH}_3\text{CO}_2\text{CH(CH}_2\text{Ph)CO}_2\text{H} \quad \text{V} \quad \xrightarrow[\text{OH}^-]{\text{H}_2\text{O}} \text{I} \qquad (2)$$

rotation of I, derived from IIIa and V, were from IIIa, $[\alpha]_D^{20}$ = +10.9 (*c* 1.51, EtOH)(63.5 %) (lit[7] +17.2); from V, $[\alpha]_D^{20}$ = -10.3 (*c* 1.31, EtOH)(61.6 %)(lit[7] -17.3), indicating that chymotrypsin catalyzed the hydrolysis of IIIa lead to 81 % L-acid together with 19 % D-acid.

$$\text{DL-}\underset{\sim}{\text{III}} \xrightarrow[H_2O]{ChT} \underset{\text{(mostly L)}}{RCO_2\overset{*}{C}H(CH_2Ph)CO_2H} + \underset{\text{(mostly D)}}{RCO_2\overset{*}{C}H(CH_2Ph)CO_2CH_3}$$

These data are in a marked contrast to the hydrolysis of D-α-acetoxyl-β-phenyllactic acid ethyl ester[3a], which was reported completely inactive. The present results lead to a conclusion that an appropriately modified ester of α-hydroxy acid can be preparatively resolved into D- and L- optical isomers.

As shown in Table 1, optical selectivity (k_L/k_D) observed was dependent on nature of the R of III. Presence of α-OH increased the selectivity (OAc vs. OH) and introduction of a carbonyl five atom apart from α-carbon also increased the selectivity. L-Reactivity (and selectivity) decreased on introduction of a bulky acyloxyl at α-carbon. Benzyl substituent seems to be very important to develop reactivity by comparing with phenyl substituent. These findings suggests that binding and transition states for hydroxy acid derivatives should closely resemble those for amino acid derivatives[8].

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