Predicting the Enantiomeric Selectivity of Chymotrypsin. Homologous Series of Ester Substrates

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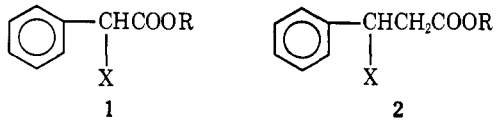
Abstract: Kinetic studies of the α-chymotrypsin-catalyzed hydrolyses of the enantiomers of methyl indan-1-carboxylate, methyl cyclohex-3-enecarboxylate, and their homologues, have confirmed that the enantiomeric selectivity of the enzyme always inverts when methylene groups are progressively inserted between the chiral center and the hydrolyzable ester function. The stereospecificity reversals occur when the aromatic or cyclohexene rings and ester group separation reaches, or exceeds, two methylene groups or their equivalent. This spacing corresponds to the aromatic ring–ester distance in good specific substrates of chymotrypsin such as *N*-acetyl-L-phenylalanyl methyl ester. From the data now available, it is clear that this type of stereospecificity inversion is a general phenomenon. This extends still further the value of chymotrypsin as a predictable chiral catalyst for resolution and absolute configuration assignment purposes.

Enzymes are well documented to be of considerable practical value for resolution and other asymmetric synthesis applications. α-Chymotrypsin (EC 3.4.21.1), with its enantiomeric specificity being well defined for a broad structural range of chiral ester and amide substrates,[2] is particularly valuable in this regard. Furthermore, the stereospecificity of the enzyme is largely predictable, with hydrolyses occurring in the same stereochemical sense for all substrates possessing grossly similar structural features. However, for the pairs of acetamido and hydroxy ester substrates, **1a, 2a,** and **1b, 2b,**



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a, X = $NHCOCH_3$; R = CH_3
b, X = OH; R = C_2H_5

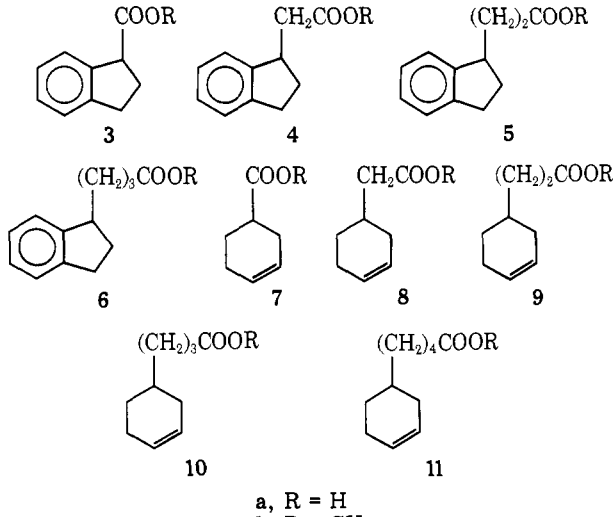
respectively, the basic L specificity of the chymotrypsin-catalyzed hydrolyses of **1a,b** changes to D for the homologues **2a,b**.[2,3] These reversals of enantioselectivity appear to be uniquely associated with homologation on the ester side of the chiral center. No changes in stereospecificity occur when up to four methylene groups are interposed between the phenyl rings and chiral centers of **1a,b**.[4] Analogous stereospecificity differences have also been reported for some homologous chiral inhibitors of the enzyme.[5,6]

These intriguing data prompted us to undertake a more thorough investigation of the effects of side-chain homologation on the enzyme's enantiomeric specificity. In this paper we report on the chymotrypsin-catalyzed hydrolysis of two structurally different homologous series of substrates, **3b–6b** and **7b–11b.** Clear reversals of enantiomeric preference are observed in both series, and the phenomenon is now seen to be a general and predictable one.

Results

The racemic indan esters **3b–6b** were prepared by literature methods, or by unexceptional variations thereof, and the racemic cyclohexenyl compounds from (±)-**7a** were prepared via successive Arndt–Eistert homologations. The enantiomers of the indan acids and esters, **3a,b–6a,b,** were obtained by resolution of the racemic acids as their α-phenethylamine salts.

In the cyclohexenyl series, the antipodes of **7a** were also isolated using α-phenethylamine salt resolution.[7] The enantiomers of the higher homologues were then prepared by subjecting (+)- and (−)-**7a** to successive Arndt–Eistert homologation, a procedure known to be stereoselective.[9] Owing



a, R = H
b, R = CH_3

to partial epimerization during the **7a** → **8b** steps, the **8b** enantiomers isolated did not retain their full optical integrity. However, the enantiomerically enriched acids (+)- and (−)-**8a** so obtained were easily rendered optically pure by two or three recrystallizations of their α-phenethylamine salts. No racemization occurred during the subsequent homologations of (+)- and (−)-**8a** to the enantiomers of **9b** and **10b**.[10]

The absolute configurations of the stereoisomers of the indan acids and esters **3a,b–6a,b** were assigned by taking (−)-**3a** of known 1*S* configuration[11] and subjecting it to repeated homologation by the Arndt–Estert procedure, for which the critical Wolff rearrangement step proceeds with retention of configuration.[9] The absolute configurations of the stereoisomers in the cyclohexenyl series were similarly assigned from the knowledge that the (+)- and (−)-**1a** starting materials for the Arndt–Eistert homologations were the (1*R*)-[8] and (1*S*)-cyclohex-3-enylcarboxylic acids, respectively. The absolute configuration relationships of all the substrates evaluated are recorded in Table I.

A qualitative examination of the substrate activities of the racemic esters showed that each of (±)-**3b–10b** was hydrolyzed at a significant rate in the presence of chymotrypsin but that the cyclohexenylpentanoic acid ester ((±-**11b**) was not a substrate. Detailed kinetic analyses were then performed on each individual substrate enantiomer. The results, which are summarized in Table I, show clear reversals of enantiomeric selectivity in both series. These occur between **3b** and **4b** for the indan esters and between **9b** and **10b** for the cyclohexenyl substrates.

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Table I. Kinetic Parameters for the Chymotrypsin-Catalyzed Hydrolysis of the Enantiomers of **3b–10b**[a]

substrate	abs configuration type[b]	k_{cat}, s^{-1}, $\times 10^3$	K_m, mM	k_{cat}/K_m, M^{-1} s^{-1}	stereospecificity ratio[c] (L/D)
(a) indan series[d]					
(+)-(1*R*)-**3b**	L	1.95 ± 1.28	52.3 ± 37.8	0.04	0.06
(−)-(1*S*)-**3b**	D	6.72 ± 0.57	11.2 ± 1.50	0.60	
(+)-(1′*S*)**4b**	L	7.50 ± 0.41	8.56 ± 0.60	0.88	16.2
(−)-(1′*R*)-**4b**	D	0.14 ± 0.01	2.65 ± 0.60	0.05	
(+)-(1′*S*)-**5b**	L	178 ± 7	0.97 ± 0.07	184	2300
(−)-(1′*R*)-**5b**	D	1.16 ± 0.50	14.5 ± 6.9	0.08	
(−)-(1′*R*)-**6b**	L	125 ± 32	4.36 ± 1.27	28.7	4.79
(+)-(1′*S*)-**6b**	D	92.3 ± 61.1	15.4 ± 10.8	5.99	
(b) cyclohexenyl series[e]					
(+)-(1*R*)-**7b**	D	5.93 ± 1.06	116 ± 24	0.05	0.04
(−)-(1*S*)-**7b**	L			0.002[f]	
(+)-(1′*R*)-**8b**	D	2.91 ± 0.26	11.9 ± 1.7	0.25	0.56
(−)-(1′*S*)-**8b**	L	3.49 ± 0.39	25.3 ± 3.7	0.14	
(+)-(1′*S*)-**9b**	D	34.6 ± 5.1	2.61 ± 0.77	13.3	0.89
(−)-(1′*R*)-**9b**	L	41.6 ± 2.8	3.54 ± 0.42	11.8	
(+)-(1′*R*)-**10b**	D	24.0 ± 1.0	0.92 ± 0.09	26	7.35
(−)-(1′*S*)-**10b**	L	394 ± 42	2.06 ± 0.32	191	

[a] Kinetics were performed at pH 7.8 and 25 °C on aqueous solutions containing 0.1 M KCl and 40% Me_2SO. [b] The D,L descriptors are based on the initial correlations made for **3a** in ref 11 and for **7a** in ref 8. [c] Taken as the ratio of the respective specificity constants k_{cat}/K_m. [d,e] (+) and (−) represent the signs of $[\alpha]_D$ determined in benzene[d] or in chloroform[e]. [f] Average value of $V/[E][S]$ from four runs.

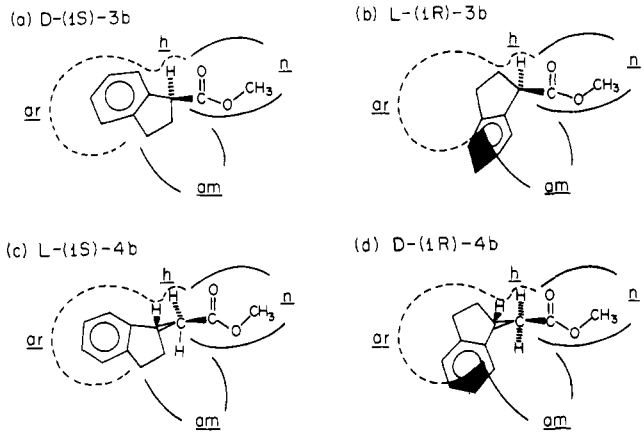

Figure 1. Schematic comparisons of the binding to chymotrypsin of the enantiomers of the homologous indan esters **3b** and **4b** using the active-site representation of Cohen.[17] For **3b**, only the D enantiomer (a) can be suitably positioned such that the aromatic, $COOCH_3$, and H groups occupy their complementary loci *ar*, *n*, and *h*, respectively. The L antipode cannot be similarly bound without exceeding the dimensions of one or more loci, thereby incurring adverse steric interactions as indicated by the solid areas in b. For **4b**, the antipodal preference is reversed, with the binding and orientation of the L enantiomer (c) being much favored over that of the D stereoisomer (d). The L preferences for the enantiomers of **5b** and **6b** are interpretable in an analogous manner. (A detailed discussion of the application of this analytical approach is provided in ref 2.)

Discussion

In order to provide as broad an evaluation as possible of the structural basis for stereospecificity reversal on homologation, substrate types differing markedly from the previously studied examples, **1b** and **2b**, were considered. The choice of the indan esters was further guided by the well-documented specificity of chymotrypsin toward fused bicyclic esters,[12] while the cyclohexenyl derivatives were taken as representative of chiral monocyclic aliphatic substrates.[13] The accessibility of these compounds and the ease with which their absolute configurations could be assigned were also major considerations.

Owing to solubility problems, it was necessary to carry out the kinetic studies on aqueous solutions contaning 40% aqueous dimethyl sulfoxide.[14] As indicated by the Table I data for the homologous series of indan substrates, there is a marked difference between the enantiomeric preference of chymotrypsin with respect to **3b** and the higher homologues **4b–6b**. For **3b**, hydrolysis of the D enantiomer is preferred, while for **4b–6b** the L antipodes are the more rapidly hydrolyzed stereoisomers in each case.

The D stereospecificity of the of the chymotrypsin-catalyzed hydrolysis of **3b** is in accord with the D enantiomeric preference observed with other bicyclic substrates.[2] This enantiomeric selectivity, and the opposite stereochemical preference of the enzyme for the higher indan ester homologues, are readily rationalizable when the preferred binding and orientation modes available to each pair of enantiomers of **3b–6b** at the active site are analyzed. This is illustrated in Figure 1 for the stereoisomers of **3b** and **4b**.

An analogous, but more gradual, inversion of stereospecificity is revealed by the kinetic data for the cyclohexenyl substrates **7b–10b** (Table I). Chymotrypsin exhibits a clear D-type preference toward **7b**. However, the enantiomeric specificity diminishes progressively with each homologation and is virtually absent in the **9b** hydrolyses. The ability of the enzyme to discriminate between enantiomers returns with (+)- and (−)-**10b** as substrates and is accompanied by an unambiguous preference for the L antipode.[18]

Cyclic ester substrates of chymotrypsin are usually hydrolyzed at a maximal rate when the ring(s) and the hydrolyzable function are separated by the two-methylene-equivalent spacing found in good specific aromatic amino acid substrates.[2] The data for the indan substrates are in accord with this generalization, with the best substrate of the series, (1*S*)-**5b**, being the one most closely resembling an L-tryptophan ester. However, despite its tryptophan-like structure (1*S*)-**5b** is evidently less than ideal as a substrate since its specificity constant is ~60-fold lower than that of methyl *N*-acetyl-L-tryptophanate.[4] In contrast, for the cyclohexenyl series the maximum rate is observed with (1*S*)-**10b**, for which the ring–COOMe spacing is $(CH_2)_3$ rather than the two CH_2 equivalents of the L-phenylalanine esters which are its nearest aromatic amino acid substrate analogues. While this rate maximum–methylene chain relationship is unusual, it does have literature analogy.[19] The fact that (±)-**11b** is not a substrate of chymotrypsin is the result of its exceeding the dimensions of the active site, a situation which is well documented for aliphatic substrates possessing extended polymethylene chains.[20]

Table II. Physical Properties of Enantiomers of **3a,b–10a,b** and Their Intermediate α-Phenethylamine (PEA) Salts

compound	mp, °C	bp, °C (mm)	$[\alpha]_D^{25}$, deg (*c*, solvent[a])	anal. (C, H)
(1*R*)-**3a**, (+)-PEA			+40.9 (2, A)	
(1*R*)-**3a**	44–45 (lit.[24] 45.7–46.6)		+43.6 (2.5, B) (lit.[24] +43.3 (2.6, B))	
(1*R*)-**3b**	27–28 (lit.[12] 26.5–27)		−0.5 (2.3, E) (lit.[12] −2.07 (2, E))[35]	
(1*S*)-**3a**, (−)-PEA			−40.7 (2, A)	
(1*S*)-**3a**	45–47 (lit.[11] 45.7–46.5)		−43.8 (2.5, B) (lit.[11] −43.4 (2.7, B)	
(1*S*)-**3b**	27–28 (lit.[12] 26.5–27)		+0.5 (2.5, E), −1.2 (2.2, B) (lit.[12] +2.07 (2.1, E))[35]	
(1′*R*)-**4a**, (+)-PEA			+40.2 (2, A)	
(1′*R*)-**4a**	79.5–80		−8.5 (2.6, B)	$C_{11}H_{12}O_2$
(1′*R*)-**4b**		94 (0.3)	−13.8 (2.5, B), +5.1 (2.7, E)	$C_{12}H_{14}O_2$
(1′*S*)-**4a**, (−)-PEA			−40.3 (2, A)	
(1′*S*)-**4a**	78–80		+8.6 (2.6, B)	$C_{11}H_{12}O_2$
(1′*S*)-**4b**		77–78 (0.1)	+13.5 (2.6, B), −5.2 (2.6, E)	$C_{12}H_{14}O_2$
(1′*R*)-**5a**, (−)-PEA			−34.0 (2, A)	
(1′*R*)-**5a**	52–53.5		−25.9 (2.6, B)	$C_{12}H_{14}O_2$
(1′*R*)-**5b**		92 (0.3)	−18.0 (3.3, B), −2.5 (2.6, E)	$C_{13}H_{16}O_2$
(1′*S*)-**5a**, (+)-PEA			+30.6 (2, A)	
(1′*S*)-**5a**	50–52		+25.0 (3, B)	$C_{12}H_{14}O_2$
(1′*S*)-**5b**		90 (0.1)	+17.3 (3.4, B), +2.7 (2.9, E)	$C_{13}H_{16}O_2$
(1′*R*)-**6a**, (−)-PEA			−42.3 (2, A)	
(1′*R*)-**6a**	54–55		+1.0 (10, B)	$C_{13}H_{16}O_2$
(1′*R*)-**6b**		110 (0.15)	−2.4 (2.7, B), −12.4 (3.5, E)	$C_{14}H_{18}O_2$
(1′*S*)-**6a**, (+)-PEA			+43.0 (2, A)	
(1′*S*)-**6a**	54–55		−1.0 (10, B)	$C_{13}H_{16}O_2$
(1′*S*)-**6b**		102 (0.15)	−2.5 (2.7, B), +12.3 (2.7, E)	$C_{14}H_{18}O_2$
(1*R*)-**7a**, (−)-PEA			+40.5 (1, M)	
(1*R*)-**7a**		66 (0.15)	+95.3 (1, C)	$C_7H_{10}O_2$
(1*R*)-**7b**		66 (4.7)	+86.5 (1, C)	$C_8H_{12}O_2$
(1*S*)-**7a**, (+)-PEA			−40.2 (1, M)	
(1*S*)-**7a**		66 (0.15)	−95.9 (1, C)	$C_7H_{10}O_2$
(1*S*)-**7b**		66 (4.7)	−86.3 (1, C)	$C_8H_{12}O_2$
(1′*R*)-**8a**, (−)-PEA			+31.9 (1, M)	
(1′*R*)-**8a**		88 (0.5)	+73.6 (1, C)	$C_8H_{12}O_2$
(1′*R*)-**8b**		60 (1.2)	+68.4 (1, C)	$C_9H_{14}O_2$
(1′*S*)-**8a**, (+)-PEA			−32.3 (1, M)	
(1′*S*)-**8a**		88 (0.5)	−71.0 (1, C)	$C_8H_{12}O_2$
(1′*S*)-**8b**		60 (1.2)	−71.0 (1, C)	$C_9H_{14}O_2$
(1′*R*)-**9a**, (−)-PEA			−31.9 (1, M)	
(1′*R*)-**9a**	23–24	120 (0.15)	−79.2 (1, C)	$C_9H_{14}O_2$
(1′*R*)-**9b**		58 (0.5)	−71.2 (1, C)	$C_{10}H_{16}O_2$
(1′*S*)-**9a**	23–24	120 (0.15)	+77.6 (1, C)	$C_9H_{14}O_2$
(1′*S*)-**9b**		58 (0.5)	+72.8 (1, C)	$C_{10}H_{16}O_2$
(1′*R*)-**10a**		98 (0.05)	+70.4 (1, C)	$C_{10}H_{16}O_2$
(1′*R*)-**10b**		86 (0.3)	+64.7 (1, C)	$C_{12}H_{18}O_3$
(1′*S*)-**10a**, (+)-PEA			−34.9 (1, M)	
(1′*S*)-**10a**		98 (0.05)	−72.3 (1, C)	$C_{10}H_{16}O_2$
(1′*S*)-**10b**		86 (0.3)	−65.8 (1, C)	$C_{12}H_{18}O_2$

[a] A, acetone; B, benzene; C, chloroform; E, ethanol; M, methanol.

From the total data now available, it is clear that chymotrypsin's stereospecificity toward chiral esters must be expected to reverse itself whenever methylene units are progressively interposed between the chiral center and the hydrolyzable function. In each homologous series studied so far, the change of antipodal preference occurs when the separation of the aromatic ring, or its equivalent, and the ester group reaches, or just exceeds, the two-methylene-equivalent spacing found in good specific aromatic amino acid substrates such as *N*-acetyl-L-phenylalanine methyl ester. While the effect is more dramatic within some homologous series than in others, it is evidently quite general and, most importantly, is predictable within narrow limits. It must therefore be taken into account whenever projections are being made regarding chymotrypsin's stereospecificity toward new substrates, particularly when the enzyme is being exploited for resolution[2] or absolute configuration determination purposes.[21]

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and

are uncorrected. IR spectra were recorded on a Perkin-Elmer 237 spectrophotometer, NMR spectra (Me_4Si internal standard) on a Varian T-60 instrument, mass spectral data on a Bell and Howell 21-490 spectrometer, and optical rotations on a Perkin-Elmer 141 polarimeter. GLC analyses were performed on an F&M 400 instrument (flame ionization detector) using 3 m × 10 mm columns of 3% QF1 on Chromosorb G. All substrates were purified until no GLC impurities were detectable. Chymotrypsin (3× crystallized) was purchased from Worthington Biochemical Corp.

Indan-1-carboxylic Acid ((±)-3a) and Its Methyl Ester (±)-3b. Indene-1-carboxylic acid[22] was hydrogenated in ethanol over 10% Pd/C at 50 psi for 8 h to give indan-1-carboxylic acid ((±)-**3a**, 89% yield). After recrystallization from *n*-hexane it had mp 55–57 °C (lit.[23] mp 56.5–57 °C).

The methyl ester (±)-**3b** was obtained in 65% yield by the general method of Brenner and Huber,[24] bp 70 °C (0.2 mm) (lit.[25] bp 130–133 °C (13 mm)).

(1-Indanyl)acetic Acid ((±)-4a) and Its Methyl Ester (±)-4b. Methyl (1-indenyl)acetate, prepared from 1-indanone using the procedure of Anderson and Wade,[26] was hydrogenated in ethanol over 10% Pd/C at 50 psi to give (±)-**4b** (95% yield), bp 68 °C (0.05 mm) (lit.[26] bp 140–143 °C (10 mm)).

Hydrolysis of (±)-**4b** with aqueous methanolic potassium hydroxide gave the acid (±)-**4a**, which was recrystallized from *n*-hexane (87% yield), mp 59–61 °C (lit.[26] mp 59–60 °C).

3-(1-Indanyl)propanoic Acid ((±)-5a) and Its Methyl Ester (±)-5b. Methyl 3-(3-indenyl)propanoate[27] was hydrogenated in the usual way to give (±)-**5b** in 71% yield. It had bp 118–120 °C (0.4 mm) (lit.[28] bp 144–154 °C (10 mm)).

The methyl ester (±)-**5b** was saponified as described above to give the acid (±)-**5a** (60% yield after recrystallization from *n*-hexane), mp 48–51 °C (lit.[22] 49.5–51 °C).

4-(1-Indanyl)butanoic Acid ((±)-6a) and Its Methyl Ester (±)-6b. Thionyl chloride (28.6 g, 0.24 mol) was added with stirring to 3-(1-indanyl)propanoic acid ((±)-**5a**, 38 g, 0.2 mol) in benzene (80 mL). The mixture was then refluxed for 2 h and evaporated, and the crude acid chloride was added at 20 °C to ethereal diazomethane (0.4 mol). After the excess diazomethane had been neutralized with anhydrous $MgSO_4$, the solution was filtered and evaporated. The diazo ketone so obtained was dissolved in methanol (400 mL) and silver benzoate (4.6 g, 20 mmol) in triethylamine (60 mL) was added dropwise to the refluxing solution.[29] After being heated under reflux for 1 h the mixture was filtered and evaporated, and the residue was dissolved in ether (200 mL), washed with 5% aqueous sodium bicarbonate, and finally washed with water. The dried ($MgSO_4$) ether solution was evaporated and distilled to give methyl 4-(1-indanyl)butanoate ((±)-**6b**, 29.6 g), bp 112–120 °C (0.2 mm). Saponification yielded the acid (±)-**6a**, 91% yield after recrystallization from *n*-hexane, mp 71–72.5 °C (lit.[30] mp 92 °C).

Preparations of the Cyclohexenyl Acids (±)7a–11a and Their Methyl Esters (±)-7b–11b. Successive applications of the Arndt–Eistert procedure described above were employed to prepare the esters (±)-**8b–11b** using cyclohex-3-enecarboxylic acid ((±)-**7a**, Aldrich) as the initial starting material. The acids (±)-**8a–11a** were obtained by saponification of the appropriate methyl ester and (±)-**7b** by treatment of (±)-**7a** with diazomethane. The compounds obtained had the following properties.

Methyl cyclohex-3-enecarboxylate ((±)-**7b**, from (±)-**7a** (0.07 mol), 65% yield): bp 48 °C (4 mm) (lit.[31] bp 73 °C (20 mm)).

Methyl cyclohex-3-enylacetate ((±)-**8b**, from (±)-**7a** (0.63 mol), 70% yield): bp 62 °C (4 mm) (lit.[32] bp 90–95 °C (20 mm)).

Cyclohex-3-enylacetic acid ((±)-**8a**, from (±)-**8b** (0.07 mol), 92% yield): bp 72 °C (0.1 mm) (lit.[32] bp 150–155 °C (25 mm)).

Methyl 3-(3-cyclohexenyl)propanoate ((±)-**9b** from (±)-**8a** (0.07 mol), 76% yield): bp 76 °C (4 mm); IR (film) 1724 and 1653 cm^{-1}; NMR (CCl_4) δ 1.0–2.5 (11 H, m), 3.66 (3 H, s), and 5.64 ppm (2 H, apparent s). Anal. ($C_{10}H_{16}O_2$) C, H.

3-(3-Cyclohexenyl)propanoic acid ((±)-**9a**, from (±)-**9b** (0.04 mol), 74% yield): bp 92 °C (0.2 mm) (lit.[33] bp 99–102 °C (2 mm)).

Methyl 4-(3-cyclohexenyl)butanoate ((±)-**10b**, from (±)-**9a** (0.03 mol), 23% yield): bp (spinning band) 95 °C (4 mm) (lit.[34] bp 67–70 °C (0.5 mm)).

4-(3-Cyclohexenyl)butanoic acid ((±)-**10a**, from (±)-**10b** (0.02 mol), 64% yield): bp 88 °C (0.05 mm) (lit.[34] 105–106 °C (0.2 mm)).

Methyl 5-(3-cyclohexenyl)pentanoate ((±)-**11b**, from (±)-**10a** (0.03 mol), 27% yield): bp (spinning band) 105 °C (4 mm); IR (film) 1748 and 1658 cm^{-1}; NMR (CCl_4) δ 0.8–2.5 (15 H, m), 3.61 (3 H, s), and 5.62 ppm (2 H, apparent s); MS *m/e* 196. Anal. ($C_{12}H_{20}O_2$) C, H.

5-(3-Cyclohexenyl)pentanoic acid ((±)-**11a**, from (±)-**11b** (0.01 mol), 42% yield): bp 98 °C (0.08 mm); IR ($CHCl_3$) 1715 and 1656 cm^{-1}; NMR (C^2HCl_3) δ 0.8–2.5 (15 H, m), 5.64 (2 H, apparent s), and 11.4 ppm (1 H, s); MS *m/e* 182. Anal. ($C_{11}H_{18}O_2$) C, H.

Preparation of the Enantiomers of the Indan Acids 3a–6a and Esters 3b–6b. This was achieved by resolutions of the racemic acids via ≥5 recrystallizations to constant rotation of their (+)- and (−)-α-phenethylamine (PEA) salts from acetone, followed by esterification[24] of the optically pure acids. The acids were recrystallized to constant rotation from *n*-hexane. IR and NMR spectra of the enantiomers were identical with those of the racemates. The physical properties of the individual enantiomers and salts are summarized in Table II.

Preparation of the Enantiomers of the Cyclohexenyl Acids 7a–10a and Esters 7b–10b. Resolution of cyclohex-3-enylcarboxylic acid ((±)-**7a**) was effected via ≥5 recrystallizations of the (+)- and (−)-α-phenethylamine salts. The enantiomers of **7b** were then obtained by esterification with diazomethane. The homologous pairs of enantiomers of **8a,b–10a,b** were obtained by successive Arndt–Eistert syntheses starting from (1*R*)-**7a** and (1*S*)-**7a**, respectively. Some racmization occurred during the preparations of (+)- and (−)-**8b**. The optical integrity of these esters was restored by subjecting the corresponding acids (+)- and (−)-**8a** to α-phenethylamine salt recrystallization procedures. The PEA salts of (1*R*)-**9a** and (1*S*)-**10a** were prepared merely to confirm that the homologation procedures leading to (+)- and (−)-**9b** and **10b** were completely stereospecific. IR and NMR spectra of each enantiomer were identical with those of the corresponding racemate. The physical properties of the compounds obtained are summarized in Table II.

Absolute Configuration Assignments. Absolute configurations in both series were assigned by carrying out stereoselective (with retention of configuration)[9] Arndt–Eistert homologations, beginning with the acids (1*R*)- and (1*S*)-**3a**[11] and (1*R*)- and (1*S*)-**7a**,[8] respectively. The total assignments, recorded in Table I, were based on the following results.

Indan series: (1*S*)-**3a** → (1′*R*)-**4b** (19% optically pure due to partial epimerization during workup); (1′*R*)-**4a** → (1′*R*)-**5b**; (1′*S*)-**5a** → (1′*R*)-**6b**.

Cyclohexenyl series: (1*R*)-**7a** → (1′*R*)-**8b** (50% optically pure only due to partial epimerization during workup); (1′*R*)-**8a** → (1′*S*)-**9b**; (1′*S*)-**9a** → (1′*R*)-**10b**.

Kinetic Studies. The rates of α-chymotrypsin-catalyzed hydrolyses were carried out at 25 °C under N_2 in 40% aqueous dimethyl sulfoxide 0.1 M in KCl using a Radiometer pH stat to maintain the apparent pH at 7.8. For each substrate 7–10 runs were carried out under steady-state conditions within the substrate concentration range 0.1–10 K_m and with enzyme concentrations of 1–20 × 10^{-5} M. The enzyme concentrations were determined spectrophotmetrically[36] and the data were analyzed by the Lineweaver–Burk method using least-squares regression analysis. The k_{cat} and K_m values obtained are recorded in Table I.

References and Notes

(1) (a) This work was supported by the National Research Council of Canada. (b) Abstracted from the M.Sc. Thesis of H.M.S., University of Toronto, 1975, and, in part, from the Ph.D. Thesis of P.W.M., University of Toronto, 1972. (c) Ontario Graduate Fellow, 1968–1971.
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Stoichiometric Determination of Chlorophyll a–Water Aggregates and Photosynthesis. Symbiotic Roles of the Magnesium Atom and the Ring V Cyclopentanone Group in the Structural and Photochemical Properties of Chlorophyll a Monohydrate and Dihydrate

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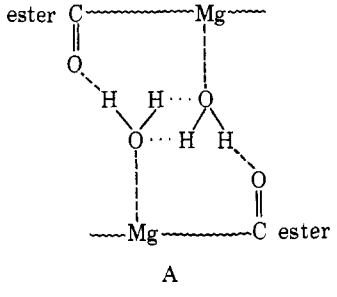
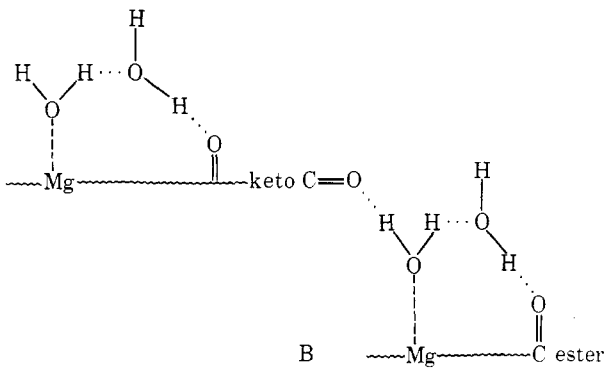
Abstract: X-ray photoelectron spectroscopy has been employed to probe the water content of aggregates of pheophytin a and chlorophyll a. The O 1s spectra of these compounds show a distinct shoulder at 533.7 eV when water is present, which is used to calculate the number of bound waters of hydration of the aggregate. We have been unable to observe anhydrous chlorophyll, but have characterized both Chl a·H_2O and Chl a·$2H_2O$. The latter occurs in a crystalline form of Chl a that absorbs light at 743 nm. For pheophytin a, however, water-free films are readily prepared. Since the Mg in chlorophyll a is replaced by two hydrogen atoms in pheophytin a, we conclude that the water in monohydrate Chl a is bound directly to the Mg atom. The probable role of the Chl a Mg atom in plant photosynthesis is discussed in terms of current and earlier considerations of the apparently symbiotic functions of Mg and the ring V cyclopentanone ring in the Chl a molecule.

The study of chlorophyll a–H_2O interactions is of current interest because of the probable role of Chl a–H_2O aggregates in photosynthesis[3-10] and in the photochemical splitting of water in in vitro solar conversion.[4k,l,11,12] There has been much research activity in probing the possibility that the in vivo P700 reaction center may be one of several plane-parallel dimers of Chl a, interlinked by C_2 symmetrical bonding interactions with H_2O molecules.[3-10] A comparison[4g,i,10] between the properties of the in vitro 700-nm absorbing Chl a dimers with those of the in vivo P700 aggregate and the delineation of the differences in the physical[4g] and photochemical[4i] properties of the C-10 C=O···H(H)O···Mg[3,4] linked and the C-9 C=O···H(H)O···Mg[4g,6,7,9] linked dimers led to the conclusion that the symmetrical dimer A of Chl a monohydrate provides a reasonable model for P700.[4g,i,13] Chlorophyll a is stable as the monohydrate[4c,d] at temperatures up to 120 °C,[4e] which accounts for the earlier observations that the water content of nonpolar solutions containing 10^{-6}–10^{-4} M Chl a cannot be reduced to a level lower than that of the order of the chlorophyll concentration.[14-17] The presence of excess water in nonpolar Chl a solutions results in the precipitation of the crystalline material which has a characteristic red absorption maximum at 743 nm (P743).[4h] It has been assumed[4c,d] that the structure of P743 is in accord with the x-ray diffraction-determined structure of the ethyl chlorophyllide dihydrate,[5] a dimeric segment B of which is given as:



A



B

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