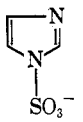
water to give products similar to those obtained from the reactions of II.

A sample of II was dissolved in water and brought to pH 11 by the addition of sodium hydroxide solution. Back-titration of the aqueous solution with 0.1 *N* hydrochloric acid gave results which indicated the presence of two ionizable groups in II with p*K* values of 5.12 and 6.86. The p*K* of 6.86 can best be assigned to the protonation of the imidazole which is present in II (lit.[9] p*K* = 6.91). The p*K* of 5.12 can then be assigned to the protonation of the anion IV. Protonation of IV would be expected to occur at the nitrogen at position 3 of the ring (see formula Ib). The p*K* value for the



IV

(9) The Merck Index of Chemicals and Drugs, 7th ed, Merck and Co., Inc., Rahway, N. J., 1960, p 551.

protonation of this nitrogen might have a lower value than that for the protonation of nitrogen in imidazole because of the influence of the partial positive charge on the sulfur attached to the nitrogen in position 1.[10]

Whether sulfate transfer in biological systems occurs by way of intermediates related to compounds I, II, and III is not established.[11] However, our results do show that the imidazole species can participate in high-yield sulfate transfer reactions with a variety of acceptors. Further studies on these systems are in progress.

Acknowledgment. The support of the National Science Foundation is gratefully acknowledged.

(10) A downfield deshielding shift in the nmr spectra for given types of protons was noted for all the compounds studied when one goes from the unsulfonated to sulfonated material, *i.e.*, imidazole to I, methanol to monomethyl sulfate, and hydroquinone to hydroquinone monosulfate. This effect can best be explained as due to the influence of the partial positive charge on the sulfur atoms in the sulfonated systems.

(11) Sulfate transfer from 3′-phosphoadenosine 5′-phosphosulfate to acceptor compounds is catalyzed by the sulfokinases. See F. Lipmann, *Science*, **128**, 575 (1958).

Stereospecificity in the Hydrolysis of Conformationally Homogeneous Substrates by α-Chymotrypsin[1]

Marc S. Silver and Tyo Sone

Contribution from the Department of Chemistry, Amherst College, Amherst, Massachusetts 01002. *Received April 26, 1968*

Abstract: The relative rates of hydrolysis by α-chymotrypsin of the *p*-nitrophenyl esters of *trans-dl-*, *cis-(R)-*, and *cis-(S)*-3-*t*-butylcyclohexanecarboxylic acids are 1:6.5:210. The enzyme here preferentially cleaves equatorial ester groups and, furthermore, exhibits considerable stereospecificity in the hydrolysis of the equatorial ester groups of the pair of *cis* enantiomers. These and other experiments generally support the view that the reactive conformation of D-3-carbomethoxydihydroisocarbostyril possesses an equatorial ester group.

A large number of rotational conformations are available to methyl acetyl-L-phenylalaninate (L-APME) and other specific substrates of α-chymotrypsin (ChT). Attempts[2-7] to use the less flexible substrate D-3-carbomethoxydihydroisocarbostyril (D-CDIC) as a model for defining that conformation of L-APME (the "reactive" conformation) most susceptible to hydrolysis by the enzyme require knowing whether D-CDIC undergoes hydrolysis by ChT with its ester group in the axial[8a] or equatorial[8b] position. In the place of direct evidence on this point, which is not readily obtainable, there have appeared conjectures based on studies of a second generation of model compounds, substances whose geometry is even less ambiguous than that of D-CDIC and whose behavior hopefully will answer the D-CDIC conformational question. The results of these studies have been interpreted as supporting either the axial[7] or the equatorial[4b,9,10] ester hypothesis.

The observation[2] that ChT hydrolyzes D-CDIC 200 (k_0) to 4000 (k_0/K_0) times more rapidly than L-CDIC places a further demand on the reactive conformation of D-CDIC, namely that it be sufficiently more reactive than both axial and equatorial L-CDIC. This is readily imagined for the axial ester hypothesis if the relative rates of hydrolysis of the various conformations of the enantiomers are equated to the relative spatial positions of the carbonyl carbon atoms of their ester groups when the aromatic and amide functions are superimposed upon each other.[2,3,11] In contrast, the requirement of the equatorial ester hypothesis that ChT distinguish

(1) Supported by Grant AM 08005 of the U. S. Public Health Service.

(2) G. Hein and C. Niemann: (a) *Proc. Natl. Acad. Sci. U. S.*, **47**, 1341 (1961); (b) *J. Am. Chem. Soc.*, **84**, 4487, 4495 (1962).

(3) E. S. Awad, H. Neurath, and B. S. Hartley, *J. Biol. Chem.*, **235**, PC35 (1960).

(4) (a) I. B. Wilson and B. F. Erlanger, *J. Am. Chem. Soc.*, **82**, 6422 (1960); (b) B. F. Erlanger, *Proc. Natl. Acad. Sci. U. S.*, **58**, 703 (1967).

(5) M. S. Silver and T. Sone, *J. Am. Chem. Soc.*, **89**, 457 (1967).

(6) S. C. Cohen and R. M. Schultz, *Proc. Natl. Acad. Sci. U. S.*, **57**, 243 (1967).

(7) W. B. Lawson, *J. Biol. Chem.*, **242**, 3397 (1967).

(8) The following shorthand notations have been used: (a) the conformation with an axial ester group = axial conformation, and the proposition that this is the reactive conformation = axial ester hypothesis; (b) the terms equatorial conformation and equatorial ester hypothesis are similarly defined.

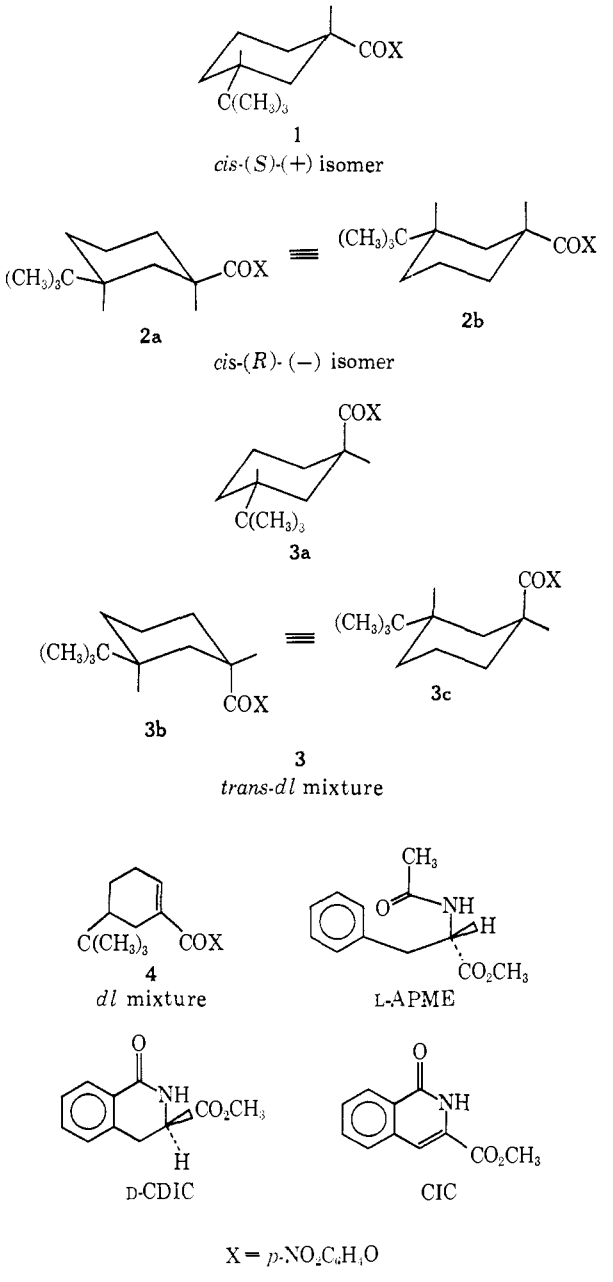
(9) M. S. Silver, *J. Am. Chem. Soc.*, **88**, 4247 (1966).

(10) S. G. Cohen, L. H. Klee, and S. Y. Weinstein, *ibid.*, **88**, 5302 (1966).

between the carbonyl carbon atoms of equatorial D- and L-CDIC to the necessary extent is most severe, since these atoms are only 1.5 Å apart.[11]

We have attempted to determine whether ChT can meet this condition by investigating the enzymatic hydrolysis of a pair of enantiomers, **1** and **2** (Chart I),

Chart I



which must possess equatorial ester groups. Comparison of the enzymatic reactivities of **1–4** also provides an additional test of the claim[9] that ChT preferentially hydrolyzes cyclohexanecarboxylic acid derivatives bearing an equatorial ester function.

Results

The parameters k_0 and K_0 defined by eq 1–4 were evaluated from a series of turnover ($K \simeq [S]_0 \gg [E]_0$, Table I) and/or acylation reactions ($K_m \gg [E]_0 > [S]_0$,

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} ES' + P_1 \xrightarrow{k_3} E + P_2 \quad (1)$$

$$v = k_0[E]_0[S]/(K_0 + [S]) \quad (2)$$

$$K_m = (k_{-1} + k_2)/k_1 \simeq k_{-1}/k_1 \quad (3)$$

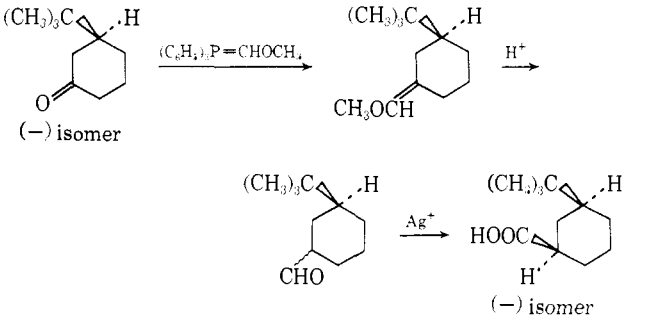
$$K_0 = (k_3/(k_2 + k_3))K_m \quad k_0 = k_2k_3/(k_2 + k_3) \quad (4)$$

Table II).[12] The degree of inhibition by indole in the hydrolysis of **1–4** is similar to that observed previously for related compounds under the same reaction conditions.[9] *p*-Nitrophenyl cyclohexanecarboxylate exhibits clean competitive inhibition, but accurate determination of the type of indole inhibition in the hydrolysis of the *t*-butyl derivatives has proved impossible (see ref 9 and Table I).

First-order plots of individual acylation reactions for *dl*-**3** and *dl*-**4** with $[E]_0 \simeq 5 \times 10^{-5}\ M >> [S]_0$ were carefully scrutinized through 95% reaction to see if a difference in the reactivity of the two enantiomers present could be detected. The slope of the plot for **3** had decreased by perhaps 10% by the end of the reaction, while with **4** no deviation from linearity was seen. ChT clearly exhibits much greater stereoselectivity in the hydrolysis of **1** and **2** than in the hydrolysis of **3** or **4**, a point of concern in our later discussion.

Conversion of (−)-3-*t*-butylcyclohexanone of known absolute configuration[13] to (−)-*cis*-3-*t*-butylcyclohexanecarboxylic acid according to Chart II established the absolute configurations of **1** and **2**.[12] Correct absolute configurations are shown throughout this paper.

Chart II



Discussion

Observation that **1** is hydrolyzed 30 times more rapidly than **2** by ChT (Table III) provides the first unequivocal demonstration of the ability of the enzyme to distinguish between equatorial ester groups of two enantiomers. A necessary condition for the validity of the equatorial ester hypothysis has been formally met.

In addition, the new data afford a second example[9] where ChT preferentially hydrolyzes an equatorial ester group. The specificity is somewhat greater than in the 4-*t*-butylcyclohexanecarboxylic acid family, for **1** is 200 times more reactive than **3**.

Definitive proof of the relevance of these model studies in ascertaining the reactive conformation of D-CDIC is impossible. Justification for the models rests

(11) In this situation the carbonyl carbon atoms of equatorial D- *vs.* axial L-CDIC, or axial D- *vs.* equatorial L-CDIC are ~2.5 Å apart, while those of axial D- and axial L-CDIC are very far apart. See ref 3 and 9.

(12) The Experimental Section provides details of the experimental methods, analysis of the kinetic data, synthesis of **1–4**, and reactions outlined in Chart II.

(13) C. Djerassi, E. J. Warawa, R. E. Wolff, and E. J. Eisenbraun, *J. Org. Chem.*, **25**, 917 (1960).

Table I. Kinetic Parameters for Some α-Chymotrypsin-Catalyzed Turnover Reactions[a]

Substrate	Runs[b]	$10^6[S]_0$, M	10^2k_0, sec^{-1}	10^6K_0, M	k_0/K_0, M^{-1} sec^{-1}
1[c]	14	1.98–7.53	3.29 ± 0.17	2.79 ± 0.35	11,800
2	15	1.95–7.40	1.28 ± 0.42	31.0 ± 10.4	413
D-CDIC[d]			2270	527	43,100
L-CDIC[d]			12.4	11,700	11
CIC[d]			13.4	1,410	95

[a] In 20% methanol–3% acetonitrile, pH 8.0 ± 0.05, 25.1 ± 0.5°, $[E]_0 = 5.50–5.60 \times 10^{-7}$ M. [b] Number of points in the Lineweaver–Burk plot. [c] For **1**, with [indole] = 8.13×10^{-3} M, $k_0 = 1.83 \pm 0.10 \times 10^{-2}$ sec^{-1}, $K_0 = 6.07 \pm 0.57 \times 10^{-6}$ M, and $K_i = 2.8 \times 10^{-3}$ M if $K_i = [I]_0/\{[(k_0/K_0, [I] = 0)/(k_0/K_0, [I] = [I]_0)] - 1\}$. [d] From ref 2b, in water, pH 7.9, 25.0°.

Table II. Kinetic Parameters for Some α-Chymotrypsin-Catalyzed Acylation Reactions[a,b]

Substrate	Runs[c]	$10^6[S]_0$, M	$10^6[E]_0$, M	$10^3[I]_0$, M	10^3K_i,[d] M	k_2', M^{-1} sec^{-1}
1	10	1.98–7.53	5.05–9.27	0		13800 ± 1000
	3	4.70–7.53	9.27	5.80	2.9	4570 ± 355
	6	3.77–7.53	9.27	8.13	2.5	3220 ± 101
2	14	1.95–6.17	5.14–56.6	0		443 ± 37
	6	3.70–7.41	9.43–56.6	5.80	2.7	140 ± 10
	6	3.70–7.41	9.43–56.6	8.13	2.3	96 ± 7
3	15	3.95–7.91	4.77–52.5	0		64.5 ± 3.3
	6	3.30–7.91	52.5	5.75	2.7	20.6 ± 1.2
	6	3.30–7.91	52.5	8.13	2.8	16.5 ± 1.5
4	16	4.03–8.06	4.60–50.6	0		48.4 ± 3.2
	6	3.36–8.06	50.6	5.80	2.5	14.6 ± 1.4
	6	3.36–8.06	50.6	8.13	2.2	10.8 ± 0.9

[a] Footnote *a*, Table I, except for $[E]_0$. [b] k_2' should equal k_0/K_0 of Table I (F. J. Kézdy and M. L. Bender, *Biochemistry*, **1**, 1097 (1962)); all uncertainties in k_2' are standard deviations. [c] Number of runs used for determining k_2'. [d] Calculated from the formula in footnote *c*, Table I.

Table III. Relative Reactivity of Several *p*-Nitrophenyl Esters toward α-Chymotrypsin and Hydroxide Ion[a]

p-Nitrophenyl ester of	Conformation of ester group	Relative reactivity toward ChT[b]	Relative reactivity toward OH^-
cis-4-*t*-Butylcyclohexanecarboxylic acid	Axial	1	1
dl-trans-3-*t*-Butylcyclohexanecarboxylic acid (**3**)	Axial	1.3	0.8
trans-4-*t*-Butylcyclohexanecarboxylic acid	Equatorial	68	10
cis-(*S*)-(+)-3-*t*-Butylcyclohexanecarboxylic acid (**1**)	Equatorial	270	9
cis-(*R*)-(−)-3-*t*-Butylcyclohexanecarboxylic acid (**2**)	Equatorial	8.5	9
Cyclohexanecarboxylic acid	Primarily equatorial	121	11
4-*t*-Butylbenzoic acid	Coplanar to ring	36	8
Benzoic acid	Coplanar to ring	13	18
dl-5-*t*-Butyl-1-cyclohexenecarboxylic acid (**4**)	Coplanar to ring	1	2
Acetic acid		16	52

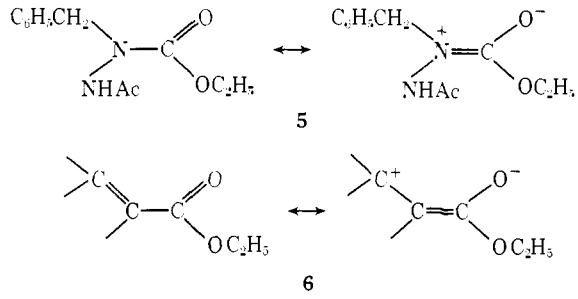
[a] Data from this paper or ref 9. [b] Based on second-order acylation constants except for entry 6, where k_0/K_0 from turnover experiments is used.

primarily on demonstrating similarities in the behavior of model and isocarbostyril substrates, and an earlier discussion[9] of the limitations of this approach is equally apt here. Subject to these caveats, two aspects of the data of Table III support the analogy we wish to draw between **1**–**4** and D- and L-CDIC.

(A) A similar effect is produced by introducing an sp^2-hybridized carbon atom at the position α to the reactive ester group for both model and CDIC families, since **1** is 270 times more reactive than **4** and D-CDIC is 450 times more reactive than CIC. The hybridization effect is a common one, since it is also encountered when esters of cyclohexanecarboxylic, *trans*-4-*t*-butylcyclohexanecarboxylic, 3,4-dihydro- or 1,2,3,4-tetrahydro-2-naphthoic, D-hydrocoumarilic, and L-α-acetaminohydrocinnamic (L-phenylalanine) acids are compared to esters of benzoic, 4-*t*-butylbenzoic (small effect), 2-naphthoic,[14] coumarilic,[15] and α-acetaminocinnamic[16] acids, respectively.[17] The report[18] that ethyl 1-acetyl-2-benzylcarbazate (**5**) is not a substrate for ChT suggests that a contributing factor to the hybridization phenomenon may be that the oxygen atoms of the ester groups of the planar compounds prefer a conformation coplanar with the α,β-double bond (**6**) which is not conducive to rapid enzymatic hydrolysis.[19]

(B) The 30-fold difference in reactivity between **1** and **2** supports both the analogy and the equatorial ester hypothesis. Obviously, to assert this when D-CDIC is 4000 times more reactive than L-CDIC is a matter of opinion rather than fact, but we believe this degree of stereospecificity in the hydrolysis of admittedly crude

(14) Rate diminutions of 5–500-fold are encountered. See ref 5.
(15) The effect is large. See ref 7.
(16) The rate depression appears to be at least 1000 times. See S. Kaufman and H. Neurath, *Arch. Biochem.*, **21**, 437 (1949).
(17) Substrates possessing this α-sp^2-hybridized carbon atom will be termed "planar" compounds.
(18) A. N. Kurtz and C. Niemann, *J. Am. Chem. Soc.*, **83**, 1879 (1961).
(19) The resonance energy (RE) associated with the interaction pictured in **6** may be about 2 kcal/mol, since the difference between the RE of benzene and styrene is 0.9 and that between benzene and methyl *trans*-cinnamate is 2.9 kcal/mol. See G. W. Wheland, "Resonance in Organic Chemistry," John Wiley & Sons, Inc., New York, N. Y., 1955, Chapter 3.



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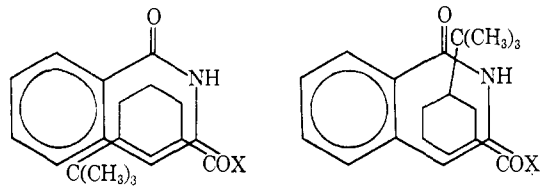
models **1** and **2** to be quite remarkable.[20] For example, the degree of stereospecificity (k_0/K_0) in the hydrolysis of methyl benzoylalaninate and methyl α-hydroxy-β-phenylpropionate is only 8 and 20, respectively.[2b,21]

Arguments A and B would be more persuasive if it could be clearly shown that stereospecificity in the hydrolysis of **1** and **2** arises directly from a difference in the positioning of the ester groups of the two enantiomers and not from some extraneous factor introduced by the presence of the *t*-butyl groups. The search for evidence of this nature gave no simple answers. To understand the arguments we must first consider how **1** and **2** should sit down at the enzymatic active site, relative to D-CDIC, for an ideal demonstration that it is the positioning of the ester groups which solely governs the specificity of the reaction of **1** and **2**. The orientations are illustrated as **1**, **2a**, and D-CDIC of Chart I, where the *t*-butyl groups of **1** and **2a** and the benzene ring of D-CDIC are located at about the same region of the active site of the enzyme because of some favorable interaction (schematically, as in **7**). The difference in reactivity between **1** and **2** would then be ascribed to the slightly different positioning of their ester groups in direct analogy to the comparison of equatorial D- and L-CDIC.[22] If this were all true, *d*- and *l*-**3** would be expected to show markedly different rates of hydrolysis, since when the *t*-butyl groups of the two enantiomers of **3** (**3a,b**) are aligned as in **7**, the axial ester groups are pointing in opposite directions. No such difference in reactivity was detected.



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An alternate approach to understanding the role of the *t*-butyl groups begins by assuming that the essentially identical rates of reaction of the two enantiomers of **3** requires that the ester groups are identically located, the *t*-butyl groups are not aligned (**3a** *vs.* **3c** or **7** *vs.* **8**), and the difference in the position of the *t*-butyl groups has no effect on reactivity. This in turn leads to the false prediction that **1** should have the same reactivity as **2**, since the ester groups of **1** and **2** are identically situated (**1** *vs.* **2b**) when the *t*-butyl groups are located as just postulated for the enantiomers of **3**.

(20) Furthermore the stereospecificity of ChT in the hydrolysis of the *p*-nitrophenyl esters of D- and L-3-carboxydihydroisocarbostyril is unknown.

(21) J. E. Snoke and H. Neurath, *J. Biol. Chem.*, 182, 577 (1950).

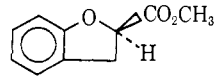
(22) Despite the appearance of Chart I, molecular models show that the ester group of either **1** or **2a** is easily made to correspond more closely to that of equatorial D-CDIC.

Therefore, no simple explanation for both the stereospecific hydrolysis of **1** and **2** and the nonstereospecific hydrolysis of **3** presents itself, and the precise role of the *t*-butyl groups remains undefined. This conclusion unfortunately makes futile any attempt to substantiate the analogy between **1** and **2** and D- and L-CDIC by correlating relative reactivities and absolute configurations of the four esters. All arguments of this kind require assuming some preferred orientation of the *t*-butyl groups.[22]

Other Evidence Relevant to the Equatorial Ester Hypothesis. Cohen and his coworkers[6,10] have interpreted their studies on esters of maleic, fumaric, 3,4-dihydroisocoumarin-3-carboxylic, and related acids as supporting the equatorial ester hypothesis and have developed a model for the reactive conformation of L-APME similar to ours.[5,9,23] Erlanger[4] has claimed that his model for the reactive conformation of L-APME, based on studies with inactivators and reactivators of ChT and very different from that developed by Cohen and ourselves, is also most compatible with the equatorial ester hypothesis. In our opinion the experimental evidence does not support Erlanger's model and consequently any conclusions based upon it are suspect.[23]

Lawson[7] has claimed that his observation that ChT stereospecifically and rapidly hydrolyzes D-**9** negates the equatorial ester hypothesis, since the quasiaxial carbomethoxy group of D-**9** is a close model for axial but not for equatorial D-CDIC (Chart III). We believe Lawson's analysis of the situation with regard to D- and L-**9** to be oversimplified. He assumes that equatorial conformations of nonrigid compounds (*e.g.*, D-CDIC) and planar substrates[17] (*e.g.*, CIC) should undergo enzymatic hydrolysis at approximately the same rate, apparently because the ester group of a planar substrate more or less resembles an equatorial conformation. If this assumption is correct, the equatorial ester hypothesis is hardly defensible. However, all the evidence of which we are aware suggests that Lawson's analogy is false and that factors (described earlier) other than spatial orientation contribute to the low reactivity of the planar compounds. The reactivity of planar **4** is similar to that of axial **3**, not equatorial **1** (or **2**). Planar **10** appears to be less reactive than either D- or L-**9**,[7] although its carbomethoxy group would be positioned between those of D- and L-**9** if all three substrates were similarly aligned. Finally, the ester group of CIC is farther from that of axial D-CDIC than is the ester group of equatorial L-CDIC when the amide and aromatic functions of the three substrates are made to coincide (see distances cited below). The axial ester hypothesis plus the assumption that spatial orientation alone determines the reactivity of planar compounds therefore incorrectly predict (ref 2 and Table I) CIC to be *less* reactive than L-CDIC.

Chart III



D isomer

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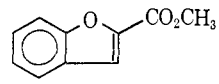

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(23) Detailed evaluation of the merits of the various formulations of the reactive conformation of L-APME will appear later.

A second objection to Lawson's arguments arises from study of molecular models of D-**9**. They clearly show that, because of the puckering of the amide ring of CDIC, D-**9**, with its quasiaxial ester group, is really a much better model for unreactive equatorial L-CDIC than for either axial or equatorial D-CDIC if the vertical distance from the carbonyl carbon atom of the carbomethoxy group to the plane determined by the aromatic ring is taken to be critical. Measurements on Dreiding models show this distance to be +1.1–1.2 Å for D-**9**, +2.2–2.3 Å for axial D-CDIC, −0.6 Å for equatorial D-CDIC, and +0.6 Å for equatorial L-CDIC, if + indicates above the plane and −, below the plane of the paper, when the esters are oriented as in Charts I and III.

We believe that neither the axial nor the equatorial ester hypothesis offers a simple explanation for the behavior of D- and L-**9**.[24]

Conclusion

Although studies of **1–4** appear to support the equatorial ester hypothesis, the exact relevance of the model studies to the CDIC problem remains unclear because the role of the *t*-butyl groups of **1–4** and related substrates has not been established. The inability to explain the hydrolysis of D- and L-**9** further emphasizes the need for careful evaluation of these model substrate experiments and illustrates the shortcomings of the approach. We hope that esters of the rigid *trans*-decalin-2-carboxylic acids will prove to be compounds whose behavior will settle the axial–equatorial question and thus define the reactive conformation of D-CDIC.

Experimental Section

Kinetic Procedures.[25] Worthington lot CDI 6150-51 of ChT was used. In a kinetic run, 3.0 ml of pH 8, 20% methanol buffer and 0.100 ml of aqueous enzyme solution were mixed in a cuvette placed in a thermostated cell holder in a Cary Model 14 recording spectrophotometer; the reaction was initiated by adding 0.100 ml of a solution of substrate in acetonitrile and the optical density recorded as a function of time. In runs with indole, either 0.100 ml of an acetonitrile solution containing both ester and indole or 0.050 ml of substrate solution plus 0.050 ml of indole solution, both in acetonitrile, were substituted for the substrate solution of the standard recipe. Reaction mixtures containing 3.0 ml of either pH 8 or 10.9 20% methanol buffer plus 0.100 ml of substrate solution provided the data for calculating the rate of the hydroxide ion promoted hydrolysis of the esters. Experiments at pH 8 permitted correction of the enzyme-catalyzed reaction rates for spontaneous hydrolysis while those at pH 10.9 gave the entries in the last column of Table III. The apparent pH's of typical reaction mixtures were determined with a Radiometer PHM 4C meter and were 7.94–8.02 for enzyme-catalyzed or blank reactions at "pH 8" and 10.93–10.94 for the "pH 10.9" runs.

Analysis of Data for Enzymatic Hydrolyses.[25] Esters **1–4** were studied under conditions with $K_m \gg [E]_0 > [S]_0$ (acylation reactions). That part of each run which gave a straight line when plotted according to the integrated form of the second-order rate law supplied the experimental second-order rate constant, k_2', of Table II. All runs gave such linear plots, at least for the initial points. Most runs followed the integrated form of the first-order kinetic law to a high percentage of reaction. The ratio $k_1'/[E]_0$, where k_1' is the experimental first-order rate constant, agreed with k_2' in all cases, as was observed previously.[9]

The initial velocities, v_0, of the individual turnover runs ($K_0 \simeq [S]_0 \gg [E]_0$) on which the data of Table I are based were obtained by three methods: visual estimate, slope of a fitted polynomial at zero time,[26] and the product $k_1''[S]_0$, where k_1'' is the experimental first-order rate constant for that part of each reaction which obeyed the integrated form of the first-order rate law. Thus three sets of v_0's were obtained, and each yielded a value for k_0 and K_0.[27,28] Agreement among the three methods of analysis was very good; the results of the polynomial procedure are entered in Table I.

All calculations were by the method of least squares and were carried out on an IBM 1130 computer.

Synthesis of Substrates.[29,30] The following sequence of reactions provided the necessary acid precursors: *p*-*t*-butylphenol → 2-hydroxy-5-*t*-butylbenzoic acid → → 5-*t*-butyl-1-cyclohexenecarboxylic acid → *cis*- and *trans*-3-*t*-butylcyclohexanecarboxylic acids. Carbonation of commercial *p*-*t*-butylphenol yielded crude 2-hydroxy-5-*t*-butylbenzoic acid,[31] mp 152–154° (lit.[32] mp 152°) after recrystallization from benzene–hexane.

5-*t*-Butyl-1-cyclohexenecarboxylic Acid. Catalytic reduction of 25 g of 2-hydroxy-5-*t*-butylbenzoic acid over 5 g of 5% rhodium on alumina (Englehand Industries, Inc., lot 8381) in 250 ml of acetic acid at room temperature and 50 psi of hydrogen in a Parr hydrogenator was complete in 6 hr. Removal of the catalyst by filtration and evaporation of the solvent at reduced pressure gave a solid residue to which a solution of 62.5 g of potassium hydroxide in 250 ml of ethylene glycol was added. The resultant solution was heated and distillation allowed to proceed until the temperature of the solution reached 200° (about 3 hr), and it was then heated under reflux for 6 hr more. A considerable amount of white solid collected in the condenser during this heating period. The solid had mp 80–82° and was probably *cis*- and/or *trans*-4-*t*-butylcyclohexanol.[33] The cooled solution was diluted with 750 ml of water, filtered, extracted with ether, and acidified. The resultant precipitate was collected and recrystallized from hexane to give 5-*t*-butyl-1-cyclohexenecarboxylic acid: total yield 48%, 6.8 g, mp 136–138°, and 4.5 g, mp 134–136° (lit.[34] mp 139.5–140°).

***trans*- and *cis*-3-*t*-Butylcyclohexanecarboxylic Acids.** The unsaturated acid was quantitatively hydrogenated over platinum oxide catalyst in acetic acid.[34] Fractional crystallization from heptane of the solid residue obtained by collecting the catalyst by filtration and removing the acetic acid at reduced pressure gave pure *trans*-3-*t*-butylcyclohexanecarboxylic acid, mp 117.5–118.5° (lit.[34] mp 114–115.5°). Heating the mixture of *cis*- and *trans*-3-*t*-butylcyclohexanecarboxylic acids remaining from these crystallizations under reflux in ethylene glycol–potassium hydroxide[35] solution produced pure *cis* acid, mp 93–95° (from heptane) (lit.[34] mp 94.5–95°). Dr. Tichy kindly sent us authentic samples of the *cis* and *trans* acids, mp 94–95 and 118–119°, respectively. Mixture melting points and infrared spectra established the identity of our preparations.

Resolution of *dl*-*cis*-3-*t*-Butylcyclohexanecarboxylic Acid. Commercial *l*-menthol was converted[36] to *l*-menthylamine hydrochloride, $[\alpha]^{20}D$ −37.2° (*c* 2, water) (lit.[35] $[\alpha]^{15}D$ −35.8°), *via* *l*-menthone and its oxime. The salt formed by the reaction of 25.8 g of racemic acid with 26.85 g of *l*-menthylamine hydrochloride precipitated from aqueous solution. It was collected, dried, and fractionally crystallized from acetone until a less soluble salt of constant melting point and rotation (mp 168–170°, $[\alpha]^{25}D$ −13.3° (*c* 1.6, absolute ethanol)) and a more soluble salt (mp 125–128°, $[\alpha]^{25}D$ −29.7° (*c* 1.6, absolute ethanol)) were obtained. Neutralization of the former with hydrochloric acid gave an acid which was recrystallized from heptane, 1.9 g, mp 93–94°, $[\alpha]^{25}D$ +21.1° (*c* 1.6, $CHCl_3$). Two recrystalliza-

(24) Dr. Lawson has informed us that the D:L rate ratio for the methyl hydrocoumarilates is appreciably less than for the CDIC's. Perhaps the five-membered ring introduces a complication which makes the former poorer models for the latter than one might have expected.

(25) Reference 9 provides a more detailed description of the chemicals, buffers, techniques, and kinetic analyses used.

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(29) Dr. Fred D. Lewis carried out preliminary studies on these syntheses.

(30) Typical runs are described in all instances. All melting points were taken on a Mel-Temp apparatus and are uncorrected.

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Table IV. Physical Properties of *p*-Nitrophenyl Esters

Compound	Mp, °C	Formula	Calcd, % C	Calcd, % H	Found, %[a] C	Found, %[a] H	$[\alpha]_D$,[b] deg
1[c,d]	44–45	$C_{17}H_{23}NO_4$	66.86	7.59	66.69	7.54	+26 ± 2
2[d,e]	44–46	$C_{17}H_{23}NO_4$	66.86	7.59	66.69	7.65	−26 ± 2
3	57.5–61	$C_{17}H_{23}NO_4$	66.86	7.59	66.56	7.73	
4	105.5–109	$C_{17}H_{21}NO_4$	67.31	6.98	67.33	7.03	

[a] Analyses by Micro-Tech Laboratories, Skokie, Ill. [b] Based on rotation of a 0.7% solution in $CHCl_3$ at room temperature. [c] From (+)-acid. [d] The *dl-cis* ester had mp 90–91°. [e] From (−)-acid.

tions from heptane of the acid obtained by acidification of the lower melting salt gave 0.7 g of acid, mp 91–93°, $[\alpha]^{25}D$ −19.1° (*c* 1.6, $CHCl_3$).

Preparation of the *p*-Nitrophenyl Esters. These were synthesized by the reaction of equimolar mounts of the appropriate acid, *p*-nitrophenol, and dicyclohexylcarbodiimide in ethyl acetate. Recrystallization of the esters from hexane provided the products listed in Table IV. The optical purity of **1** and **2** was probably greater than 90%, as indicated both by their physical properties and by the observation that the (+) isomer in some acylation reactions showed excellent first-order kinetics through 95% reaction. The presence of any appreciable amount of (−) isomer should have caused a marked diminution in these rates of reaction at high per cent reaction.

The Absolute Configuration of (+)- and (−)-*cis*-3-*t*-Butylcyclohexanecarboxylic Acids.[37] Optically active 3-*t*-butylcyclohexanone, $[\alpha]^{25}D$ −4.2° (*c* 4.5, $CHCl_3$), was prepared from commercially available racemic ketone *via* the brucine salt of *cis*-3-*t*-butylcyclohexyl acid phthalate.[13] The optically pure ketone has[13] $[\alpha]^{25}D$ −25°. Three grams of the partially resolved 3-*t*-butylcyclohexanone was treated[38] with methoxymethylenetriphenylphosphorane in ether, the ether was evaporated, and the residual oil was hydrolyzed with a saturated solution of perchloric acid in ether. The resultant solution, containing some 3-*t*-butylcyclohexanecarboxaldehyde, was washed with a small amount of sodium bicarbonate solution and dried, and the ether was removed by distillation. The residue was oxidized[39] with silver oxide in boiling ethanol–water solution. Dilution of the cooled reaction mixture with water, extraction with ether, acidification of the aqueous layer, extraction of that with ether, and evaporation of the second ethereal extract to dryness yielded an oily semisolid that was mostly *cis*-3-*t*-butylcyclohexanecarboxylic acid. Recrystallization of this material from hexane gave the results summarized in Table V. Racemic product tended to preferentially separate, but there is no doubt that the rotations observed in later fractions are all or mostly caused by *cis*-3-*t*-butylcyclohexanecarboxylic acid. The infrared spectrum of A was identical with that of genuine racemic *cis* acid, and vapor phase chromatography showed that fractions B–D contained only *cis* acid and less than 1% 3-*t*-butylcyclohexanone, while the oily residue may have had as much as 10% *trans* acid and about 1% ketone. The absolute configurations shown in Chart II and elsewhere in this paper are therefore correct.

Table V. Properties of *cis*-3-*t*-Butylcyclohexanecarboxylic Acid Prepared from (−)-3-*t*-Butylcyclohexanone

Fraction	Mp, °C	Wt, mg	$[\alpha]^{25}D$,[a] deg
A	93	270	0
B	93	29	
C	92.5–93.5	30	−0.75
D	90–92	82	
E	89.5–91.5	89	−1.7
Residue	Oil	240	−10.3

[a] All rotations taken by dissolving the entire fraction in 3 ml of $CHCl_3$.

(37) Many of these experiments were carried out by Mrs. Mai Stoddard.

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