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STEREOCHEMISTRY OF THE REACTIONS OF BIOPOLYMERS V.

Steric effect of chiral substituents in enzyme-catalyzed reactions

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The E_s constant in the Taft-Ingold relationship[1] /1/ defined by eq. /2/

$$\log \frac{k_x}{k_o} = \rho^* \sigma^* + \delta E_s \qquad /1/ \qquad E_s = /\log k_x/k_o/_A \qquad /2/$$

is the most widely used in order to estimate quantitatively the steric effect of R groups /R = alkyl, substituted alkyl, aralkyl/ in aliphatic reactions. However, the so-defined E_s constant of a composite substituent $CR^1R^2R^3$ /$R^1 \neq R^2 \neq R^3$/ has no application for reactions with chiral reagents. In this case, compounds containing $/CR^1R^2R^3/_{\underline{R}}$ and $/CR^1R^2R^3/_{\underline{S}}$ groups of absolute configuration R and S, respetively, react at different rates:

$$\log \frac{k/CR^1R^2R^3/_{\underline{R}}}{k/CR^1R^2R^3/_{\underline{S}}} \neq 0 \qquad /3/$$

Since $\sigma^*_{\underline{R}} = \sigma^*_{\underline{S}}$ it follows from /1/ that

$$/\ E_s/_{\underline{R}} \neq /\ E_s/_{\underline{S}} \qquad /4/$$

A quantitative description of /3/ is given by the Ugi-Ruch equation[2] which, however, gives a description of stereospecificity only and finds no application for achiral substituents.

We herewith present a relationship, which is derived from the Taft-Ingold equation and which describes, for the case of compounds of fixed conformation, the steric effect of both chiral and achiral substituents. Our considerations are based on the finding of Japanese authors[3] that the E_s constant of $CR^1R^2R^3$ groups is composed of three individual E_s constants:

$$E_s/CR^1R^2R^3/ = a.E_s1 + b.E_s2 + c.E_s3 + d \qquad /5/$$

In systems wherein the $CR^1R^2R^3$ group is fixed /e.g. in enzyme-substrate complexes/ one or two terms of /5/ will bear no significance. Thus in an enzyme-substrate complex shown schematically in Fig.1, the steric effect of group R^3 is negligible in a reaction between the catalytic site of the enzyme /A/ and the reaction center of the substrate /B/. Accordingly, in such a system the steric effect of substituent $CR^1R^2R^3$ is expressed by the following relationship:

$$E_s/CR^1R^2R^3/ = a.E_s1 + b.E_s2 + d \qquad /6/$$

Similarly, in a complex depicted in Fig.2, neither R^2 nor R^3 has considerable steric effect and, consequently, in this case the steric effect of the same group will be characterized merely by the steric constant of R^1:

$$E_s/CR^1R^2R^3/ = a.E_s1 + d \qquad /7/$$

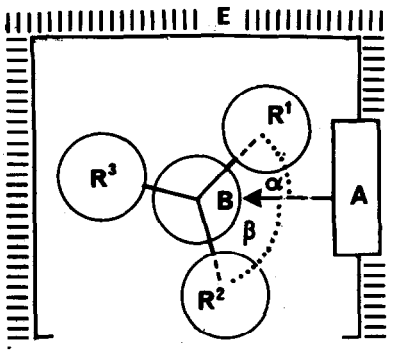

Figure 1

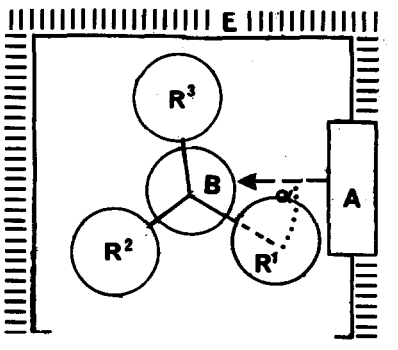

Figure 2

According to Charton[4], there is a good correlation between the E_s constant and the van der Waals radii of spherical X substituents within the series of groups CH_2X, CHX_2 and CX_3:

$$E_s = \psi \cdot r_v + h \qquad /8/$$

From /6/ and /8/ it follows that

$$E_s/CR^1R^2R^3/ = a.\psi_1.r_{v,R^1} + b.\psi_2.r_{v,R^2} + C \qquad /9/$$

where r_{v,R^1} and r_{v,R^2} are the van der Waals radii of groups R^1 and R^2. Similarly, /7/ and /8/ results /10/:

$$E_s/CR^1R^2R^3/ = a.\psi_1.r_{v,R^1} + C \qquad /10/$$

On basis of /9/ and /10/, the δE_s steric term of the Taft-Ingold relationship can be described by /11/ and /12/ respectively.

$$\log \frac{k/CR^1R^2R^3/}{k/CH_3/} - \rho^*\sigma^* = \delta E_s/CR^1R^2R^3/ = \varkappa_1 \cdot r_{v,R^1} + \varkappa_2 \cdot r_{v,R^2} + C' \qquad /11/$$

$$\log \frac{k/CR^1R^2R^3/}{k/CH_3/} - \rho^*\sigma^* = \delta E_s/CR^1R^2R^3/ = \varkappa_1 \cdot r_{v,R^1} + C' \qquad /12/$$

where $\varkappa_1 = \delta.a.\psi_1$ and $\varkappa_2 = \delta.b.\psi_2$.
The reaction constants $\varkappa_1$ and $\varkappa_2$ are functions of the angles between R^1 or R^1 and R^2 and the direction A-B / α and β in Fig.1 and α in Fig.2/, respectively. These are termed orientation constants.

The applicability of the relationship is demonstrated for two enzyme-catalyzed reactions in Figs. 3 and 4. Fig.3 shows the validity of /12/ for the case of hydrolysis of acyl- α -chymotrypsins

$$\text{E-CO-R} \xrightarrow{H_2O} \text{E-H} + \text{R-COOH}$$

/E-H = α -chymotrypsin/ while Fig.4 concerns the acylase-I catalyzed hydrolysis of acyl-amino acids:

$$\text{R-CH(NH-CO-R')-COOH} \xrightarrow[\text{acylase-I}]{H_2O} \text{R-CH(NH}_2\text{)-COOH} + \text{R'-COOH}$$

In both cases, the substrates contain both chiral /$R^1 \neq R^2 \neq R^3$/ and achiral /$R^1 = R^2 = R^3$ or $R^1 = R^2 \neq R^3$/ composite substituents $CR^1R^2R^3$.

For the hydrolysis of acyl- α -chymotrypsins, eq. /12/ offers a good correlation /r=0.97/, i.e., the reactivity depends on the van der Waals radius of only one substituent. Thus, the conformation of the acyl group within the compound E-CO-R corresponds to the case depicted in Fig.2. Accordingly, stereospecificity is interpreted in terms of the orientation of an H atom /r_v = 1.20 Å / and a CH_3 group /r_v = 1.97 Å / towards the active site A of the enzyme in cases of compounds containing an α-haloacyl group of R and S configuration, respectively. The value of $\varkappa/r_{v,CH_2} - r_{v,H}/$ gives the stereospecificity. The reactivity of the isobutyryl compound containing the $CH/CH_3/_2$ group, too, is correlated with the van der Waals radius of the H atom. The derivatives containing dihaloacetyl groups find good correlation with the van der Waals radius of the corresponding halogen atom, hence in this case a halogen atom appears to be oriented towards the active site of the enzyme /R^1 = Cl, Br/.

For acylase-I-catalyzed reactions, again relationship /12/ provides good correlation /r = 0.95/. In this reaction, too, only one of the substituents of CHR^1R^2 group exhibits steric hindrance. Substituent R^3 is always a H atom, as the r_v value of neither group is correlable with the r_v value of the H atom /except the CH_3 group/. One of the stereoisomers of the α -chloropropionyl derivatives gives good correlation with $r_{v,Cl}$/similarly as in the case of group $CHCl_2$/, and the other with r_{v,CH_3} /as in the case of the $CH/CH_3/_2$ group. Consequently, the R^1 and R^2 substituents of stereoisomeric α -chloropropionyl groups of R and S configuration are in interchanged positions.

Comparison of the $\varkappa$ values corresponding to these two reactions reveals a much more

moderate steric effect of the R^1 group in the hydrolysis of acyl- α-chymotrypsins /κ =1.10/ than in acylase-I-catalyzed reactions /κ = 4.05/. The use of $k_{cat.}/K_m$ values in the latter reaction is justified[5] by the mechanism of the reaction[6].

The purity of the substrates employed was supported by elementary analysis and NMR and mass spectra. The experimental values used for the Lineveawer-Burk equation[7] were obtained by spectrophotometric measurement of the p-nitrophenol released from pNO_2-phenyl esters in the α-chymotrypsin catalyzed reactions and ninhydrin-spectrophotometric measurement of norvaline released during acylase-I-catalyzed hydrolysis of N-acyl-L-norvalines.

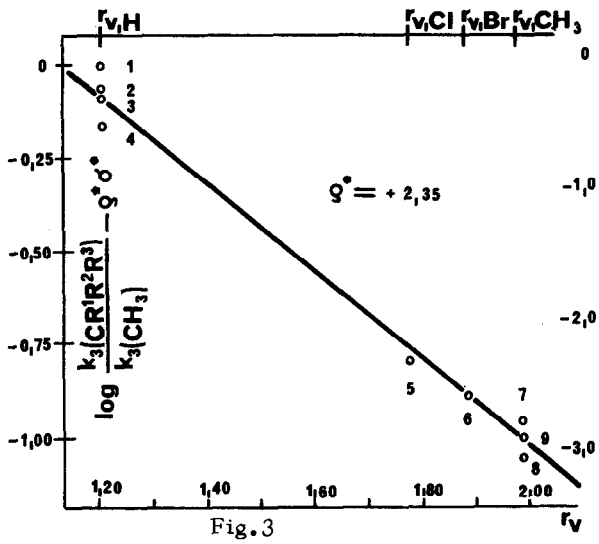

Fig.3

1. CH_3
2. $CH/CH_3/_2$
3. CH_3CHCl /R/
4. CH_3CHBr/R/
5. $CHCl_2$
6. $CHBr_2$
7. CH_3CHBr/S/
8. CH_3CHCl/S/
9. $C/CH_3/_3$

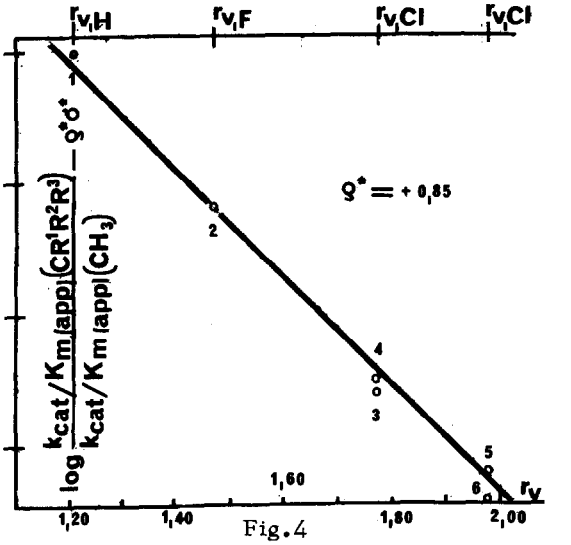

Fig.4

1. CH_3
2. CHF_2
3. $CHCl_2$
4. CH_3CHCl /S/
5. CH_3CHCl /R/
6. $CH/CH_3/_2$

The van der Waals radii used are those given by A.Bondi[8].

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