Tetrahedron Letters No.59, pp. 5143-5146, 1969. Pergamon Press. Printed in Great Britain.

NMR STUDY OF CONFORMATIONALLY RESTRICTED SUBSTRATES OF α-CHYMOTRYPSIN.

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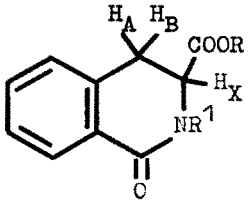
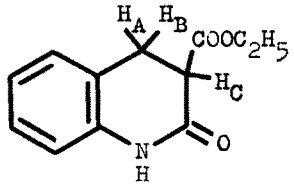
(Received in UK 14 November 1969; accepted for publication 19 November 1969)

In topographical studies of the active center of α-chymotrypsin, use has been being made for some time of conformationally restricted substrates, in particular, D-3-carbomethoxy-3,4-dihydroisocarbostyril (Ia) (1). Naturally, in order that the substrate properties of these substances could serve as basis for inferences about the spatial arrangements of the various loci in the active center of the enzyme, one must know the orientation of the ester group with respect to the tetrahydroisoquinoline ring. However, surmises as to the conformation of this group have been made only on the basis of indirect evidence, resulting in conflicting opinions, arguments being advanced in favor of both its axial (2,3) and equatorial (4,5) orientation.



Ia: R=Me, R^1=H
Ib: R=Et, R^1=H
II: R=Me, R^1=Me



III

More direct data could be obtained by NMR and the present paper describes the results of a study by this method of the conformations of compounds (Ib), its N-methyl derivative (II) and the ethyl ester (III). The conformations were determined from analysis of the vicinal ^3J spin-spin coupling constants of the atoms H_A, H_B, $H_{X(C)}$ and NH (see Table). These constants were found to be practically temperature- and solvent-independent for compounds

(II) and (III). With compound (Ib), while $^3J_{AX}$ remains constant as before, $^3J_{BX}$ changes markedly with temperature in all solvents, except $CDCl_3$, and decreases in value with increase in solvent polarity.

Table. Vicinal Coupling Constants

Compound	Ib			II		III	
Solvent	J_{AX}	J_{BX}	J_{NH-CH}	J_{AX}	J_{BX}	J_{AC}	J_{BC}
$CDCl_3$	5.9	9.2	1.7	6.2	2.6	9.0	6.2
$(CD_3)_2CO$	6.0	6.0	3.3	6.5	2.4	8.3	7.3
CD_3OD	6.0	5.5	-	6.2	2.4	8.0	7.0
$(CD_3)_2SO$	6.4	3.8	4.2	6.6	2.3	8.2	7.6

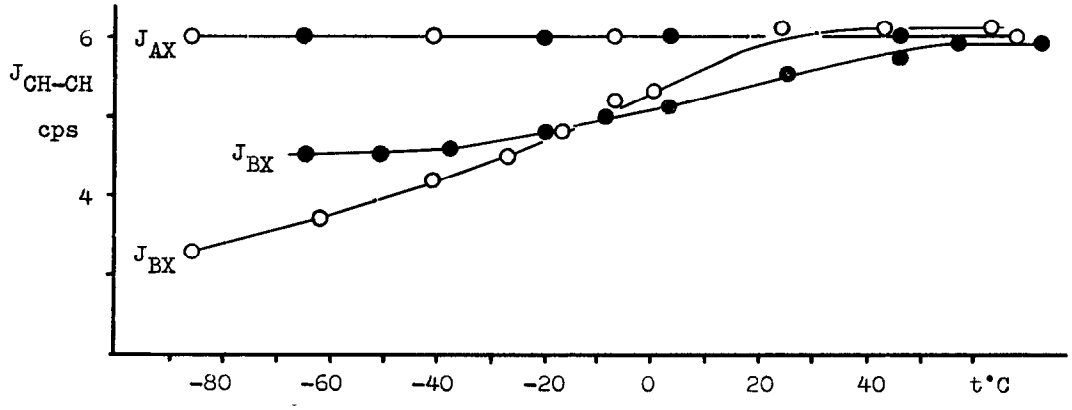
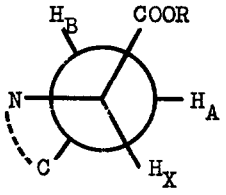


Fig. The temperature dependence of the vicinal coupling constants J_{AX} and J_{BX} for Ib in $(CD_3)_2CO$ (○) and CD_3OD (●) solutions.

Evidently, this must be due to conformational rigidity of compounds (II) and (III) and flexibility of compound (Ib). From the angular dependence of the vicinal constants (6) and their comparative analysis, the conclusion was drawn that Ib can possess two conformations (A) and (B) about the bond C_3-C_4. Since the dihedral angles H_X-C_3-C_4-H_A are approximately equal in both conformations, this explains the temperature and solvent independence of the $^3J_{AX}$ constant for this compound. In the weakly polar solvent $CDCl_3$, conforma-

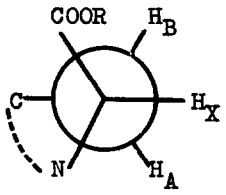
tion (A) with quasi-equatorial orientation of the COOR substituent is the preferable conformation over the entire temperature range investigated (see Fig.).



$^3J_{AX} \simeq 6.2$ cps

$^3J_{BX} \simeq 9.2$ cps

A

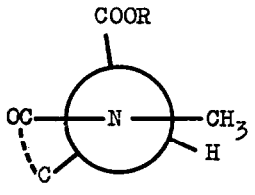


$^3J_{AX} \simeq 6.2$ cps

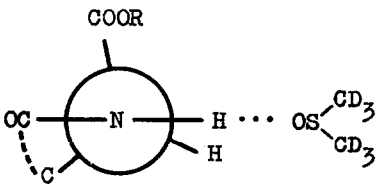
$^3J_{BX} \simeq 2.4$ cps

B

In the solvents CD_3OD and $(CD_3)_2CO$ at room temperature Ib is in the form of 1:1 equilibrium mixture of the two conformers, the value of the constant $^3J_{BX}$ being the average for the two constants: $(^3J_{BX}^{(A)}+{}^3J_{BX}^{(B)})/2$. At low temperature the equilibrium is shifted in the direction of conformer (B) with quasi-axial orientation of COOR. Conformation B is also the prefered conformation for compound Ib in the strongly polar solvent $(CD_3)_2SO$, and for compound II under all the conditions studied. The preference for this conformation in compound II is apparently due to steric repulsion between its N-Me and COOR groups (C). In the case of compound Ib, in place of the N-Me group the stabilizing factor is apparently the hydrogen bonded solvent molecule (D).



C



D

This conclusion is supported by the value for the $^3J_{NH-CH}$ constant of isocarbostyril (Ib) which is small when the substituents are in quasi-equatorial conformation for which the dihedral angle NH-CH approaches 90° ; in $(CD_3)_2SO$ in which the preferable conformation is (D), the dihedral angle for NH-CH is close to 20° with consequent increase in value of the constant (7,8).

From the above said follows that compound (III) must under all conditions exist preferably in the conformation with quasi-equatorial COOR.

If one now bears in mind that the active center of α-chymotrypsin is a hydrophobic cavity which should therefore be analogous to a non-polar solvent (9) and that the amide group does not form hydrogen bonds with the corresponding locus in the enzyme, that is does not undergo "solvation" by the enzyme (10) there are good grounds to assume that the preferable conformation of the grouping in question for Ib attached to the active center of the enzyme is quasi-equatorial.

From the data on the alkaline and enzymatic hydrolysis of compounds (Ia) (k^{OH} 31.6 $M^{-1}\cdot sec^{-1}$, k_o 22.7 sec^{-1}, K_m 5.3 mM at pH 7.9 (1)), II (k^{OH} 0.83 $M^{-1}\cdot sec^{-1}$, no appreciable hydrolysis at pH 7.2 and E_o $1\cdot10^{-5}$) and III (k^{OH} 0.41 $M^{-1}\cdot sec^{-1}$, k_o 0.33 sec^{-1}, K_m 1.71 mM at pH 7.2)* it is obvious that quasi-axial (II) does not possess properties of a substrate. However, the difference in the substrate properties between (Ia) and (III) cannot be due to differences in conformation of the ester groupings, since it is the same for both compounds. A possible explanation could be anchimeric assistance of the NH group in the enzymatic hydrolysis as had been proposed earlier (11).

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* Experimental details will be published elsewhere.