# The Crystal Structure of the Stable Isomer of α-Benzamidocinnamic acid: the Influence of *cis–trans*-Isomerism on the Kinetics of the Hydrolysis of the Products of Interaction of α-Chymotrypsin with the Isomeric 4-Benzylidene-2-phenyl-Δ²-oxazolin-5-ones

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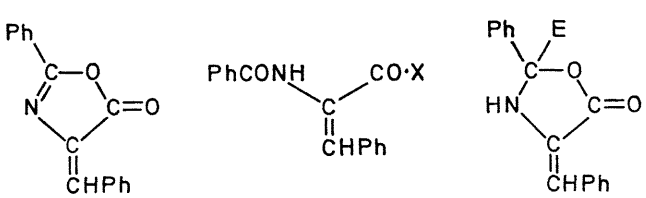
*Summary* *X*-Ray analysis of the stable isomer of α-benzamidocinnamic acid has shown it to have the *trans*-configuration, a result which contradicts most previous assignments of configuration and which bears upon the properties of the products of reaction of α-chymotrypsin with the isomeric 4-benzylidene-2-phenyl-Δ²-oxazolin-5-ones.

DURING recent years the use of the stable isomer of 4-benzylidene-2-phenyl-Δ²-oxazolin-5-one (I) and its derived methyl ester, methyl α-benzamidocinnamate (II) as substrates for α-chymotrypsin has been examined.[1–3] Although the configurations of the geometrical isomers of (I) and of their derived esters *e.g.* (II), acids (III), acylenzymes (IV), and other possible enzyme derivatives (V) had not been definitely established and had been the subject of some discussion,[2–7] it had been assumed by most workers that the stable isomer of (I) possessed the *cis*-configuration. Since it is probable[5,8] that configurational integrity is retained in the solvolysis of oxazolinones such as (I) the configurations of the stable isomers of (II)—(V) should be the same as that of the stable isomer of (I). It became the more important to determine unequivocally the configurations of the geometrical isomers of (I)—(V) when we found that the kinetic characteristics of the hydrolysis of the product of interaction of α-chymotrypsin and the stable isomer of (I) differ markedly from those of the product of interaction of α-chymotrypsin and the labile isomer of (I).




(I) X (V)

(II) OMe
(III) OH
(IV) E(α-chymotrypsin)

The stable isomer of (III) was chosen for crystallographic study because of the ease of its crystallization. Colourless, needle-shaped crystals of the stable isomer of (III) (m.p. 199°) were obtained from aqueous methanol by slow evaporation and used for the *X*-ray analysis. *Crystal data:* $C_{16}H_{13}NO_3$: $M = 267$; monoclinic; $a = 10{\cdot}043(3)$, $b = 15{\cdot}62(2)$, $c = 9{\cdot}823(4)$ Å; $\beta = 112{\cdot}57(2)°$; $U = 1418{\cdot}8$ Å$^3$; $D_m = 1{\cdot}25$; $Z = 4$; $D_c = 1{\cdot}246$ g cm$^{-3}$; space group $P2_1/c$. Visual intensity measurements, made on Weissenberg films recorded with Cu-$K_\alpha$ radiation, yielded 1942 independent non-zero intensities from a possible 2932. The structure was solved by application of Sayre's equation[9] using Long's[10] program for generation of multiple sign sets, the correct set being selected on the basis of criteria also recommended by Long.[10] Least-squares refinement of the positional and isotropic temperature factors has reduced the conventional *R*-factor to 18·6% for all observed reflexions. Further refinement of the structure is in progress. The Figure

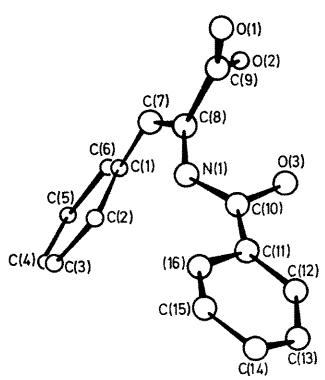



FIGURE. *α-Benzamido-*trans*-cinnamic acid: molecular configuration viewed approximately down the* a*-axis.*

shows a schematic drawing of the molecule.

The main feature of the structure of the stable isomer of (III) is that the cinnamic acid moiety has the *trans*-configuration. This contrasts with most previous tentative assignments of configuration (see above) but confirms the assignment made by Morgenstern, Schutij, and Nauta[7] on the basis of an n.m.r. study of some related α-benzamidocinnamates and their corresponding oxazolinones. There are significant deviations from coplanarity in the cinnamic acid moiety. The carboxy-group is rotated about the C(8)–C(9) bond and the benzamido-group is rotated about the N(1)–C(8) bond in such a way that they are not coplanar with the rest of the molecule. In the crystal, molecules appear to be linked by interactions between neighbouring amide groups, giving a structure which resembles the β-pleated sheet found in polypeptides. Furthermore, the molecules appear to be held together by interactions between neighbouring carboxy-groups lying across a centre of symmetry.

The long wavelength u.v. absorption bands of both the

*cis*- (labile) and *trans*- (stable) isomers of (I) (*cis*, $\lambda_{max}$ 361 nm, $\epsilon_{max}$ $3{\cdot}6 \times 10^4$, *trans*, $\lambda_{max}$ 361 nm, $\epsilon_{max}$ $3{\cdot}9 \times 10^4$, solvent MeCN) are destroyed by admixture of solutions of the oxazolinones in acetonitrile with a molar excess of α-chymotrypsin in buffer In acetate buffer pH 5·5, containing 10% acetonitrile, the 361 nm bands of the oxazolinones are replaced by absorption bands at shorter wavelengths characteristic of the substituted cinnamoyl chromophore of (IV) or (V) (*cis*, $\lambda_{max}$ 308 nm, $\epsilon_{max}$ $2{\cdot}1 \times 10^4$, *trans*, $\lambda_{max}$ 302 nm, $\epsilon_{max}$ $1{\cdot}75 \times 10^4$, difference spectra *versus* α-chymotrypsin) At higher pH's, the subsequent first-order decay of the absorbance at 310 nm permits comparative measurements of the rates of hydrolysis ($k_3$) of these cinnamoyl derivatives of α-chymotrypsin The $k_3$–pH profile in the pH range *ca* 6—10 for the derivative of the *trans*-oxazolinone is qualitatively that expected for the deacylation of an acyl-α-chymotrypsin (IV) catalysed by the enzyme's "electronic relay system" (see ref 11) (p$K$ 7 8, $\bar{k}_3 = 159 \times 10^{-3}$ s$^{-1}$, *cf* the $k_3$–pH profile for *trans*-cinnamoyl-α-chymotrypsin,[12] p$K$ 7 15, $\bar{k}_3 = 12\,5 \times 10^{-3}$ s$^{-1}$) On the other hand, the $k_3$–pH profile for the α-chymotrypsin derivative of the *cis*-oxazolinone is more complex Thus, in the pH range 7—8·5, $k_3(trans)/k_3(cis)$ is *ca* 125, but, at higher pH's where $k_3(trans)$ is becoming pH independent, the $k_3(cis)$–pH profile becomes steeper and the log $k_3(cis)$–pH plot approaches a slope of 1, suggesting that the hydrolysis of this derivative may be subject to catalysis by hydroxide ion The rate of this hydrolysis in the high pH region [$k_3(cis)$ *ca* 0 01 s$^{-1}$ at pH 10 0] is more than two orders of magnitude higher than would be expected for the alkaline hydrolysis of a simple cinnamoyl ester [12,13] Possible interpretations of this high alkaline rate are under investigation *e g* (i) a strained acyl-serine, (ii) an acyl-histidine, and (iii) reaction of the enzyme at the carbon atom of the incipient α-benzamido-group of the oxazolinone to provide (V) (see ref 3) followed by hydrolysis of this adduct

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[1] K Brocklehurst and K Williamson, *Chem Comm*, 1966, 462, K Brocklehurst and K Williamson, *Biochem Biophys Res Comm*, 1967, **26**, 175, K. Brocklehurst and K Williamson, *Chem Comm*, 1967, 666, J de Jersey and B Zerner, *Biochem Biophys Res Comm*, 1967, **28**, 173
[2] J de Jersey and B Zerner, *Biochemistry*, 1969, **8**, 1975
[3] K Brocklehurst, *F E.B S Letters*, 1969, **5**, 63
[4] R Buckles, R Filler, and L Hilfman, *J Org Chem*, 1952, **17**, 233, G Stefanovic and M Stefanovic, *ibid*, 1956, **21**, 161, N K. Kochetkov, E I Budovskii, R M Khomutov, M Ya Karpeiskii, and E S Severin, *J Gen Chem (U S S R)*, 1960, **30**, 2557
[5] R Filler, *Adv Heterocyclic Chem*, 1965, **4**, 75
[6] K Brocklehurst, H S Price, and K Williamson, *Chem Comm*, 1968, 884
[7] A P Morgenstern, C Schutij, and W Th Nauta, *Chem Comm*, 1969, 321
[8] H E Carter and W C Risser, *J Biol Chem*, 1941, **139**, 255
[9] D Savre, *Acta Cryst*, 1952, **5**, 60
[10] R E Long, Ph D Thesis, U C L A, 1965
[11] D M Blow and T A Steitz, *Ann Rev Biochem*, 1970, **39**, 63
[12] M I Bender, G R Schonbaum, and B Zerner, *J Amer Chem. Soc*, 1962, **84**, 2540.
[13] K Brocklehurst, unpublished results