Structural Factors Affecting the Direct Exchange Racemisation of Benzyloxycarbonyl-*S*-benzyl-L-cysteine Pentachlorophenyl Ester and Related Compounds by Triethylamine in Chloroform [1]

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The rates of racemisation by triethylamine in chloroform of a series of acyl-amino- and -imino-acid pentachlorophenyl esters related to benzyloxycarbonyl-*S*-benzyl-L-cysteine pentachlorophenyl ester have been determined. It is shown that the optical lability of benzyloxycarbonyl-*S*-benzyl-L-cysteine pentachlorophenyl ester is not due to a simple electron-withdrawing effect exerted by the sulphur atom on the α-CH, but that an effect involving stabilisation of negative charge at the α-carbon by a bridging interaction with the sulphur atom across the β-methylene group operates. It is pointed out that the enhanced α-CH acidity of thiol esters, which has hitherto been explained by a resonance argument involving the carbonyl group, may be open to the same interpretation. It is also proposed that the relatively high optical stability of most acylimino-acid derivatives is due to steric interference with conjugation in the carbanion formed by ionisation at the α-carbon.

We have recently been interested [2] in the synthesis of peptides containing L-thiazolidine-4-carboxylic acid (Thz) by methods involving, *inter alia*, the active ester (1). The close structural analogy between (1) and the corresponding easily racemised [3] *S*-benzylcysteine derivative (2) led us to investigate the optical stability of the thiazolidine ester (1) in the presence of tertiary base. We were surprised to find that it is in fact not at all optically labile in chloroform containing triethylamine under conditions which cause rapid racemisation of (2) (see Figure 1).

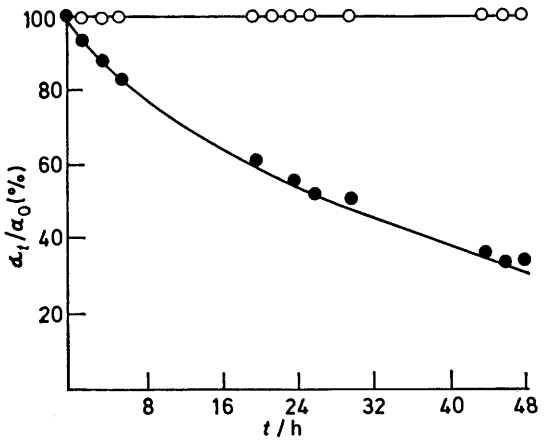


FIGURE 1 Comparison of the rates of racemisation of Z-Cys-(Bzl)-OPcp (●) and Z-Thz-OPcp (○) by equivalent amounts of triethylamine in 0.025M-solution in chloroform at 20 °C

Investigations of the racemisation of benzyloxycarbonylamino-acid active esters by triethylamine in organic solvents have encompassed derivatives of β-cyanoalanine [4], aspartic [4,5] and glutamic acids,[5] phenylalanine,[6] alanine,[7] and α-phenylglycine,[8] but *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteine active esters, especially (2),[3,6,8] have been at the focus of attention. Studies on (2) have established [3] that its triethylamine-catalysed racemisation in chloroform at ambient temperature does not proceed by reversible β-elimination (once the favoured [8] mechanism), as addition of ^{35}S-labelled toluene-thiol does not lead to incorporation of radioactivity. It has further been found that racemisation is much faster than deuterium exchange when (2) is treated with triethylamine in chloroform containing deuteriomethanol, which has been interpreted as indicating that the α-proton never becomes fully dissociated from the molecule but is carried from one side of the asymmetric centre to the other by the relative movement of the components of a tightly bound carbanion–triethylammonium cation pair, *i.e.* isoracemisation [9] is operating. However, these elegant experiments leave open the question of the nature of the role of the *S*-benzyl side-chain, and the effect of other *S*-blocking groups has not been investigated. Our results (Table 1) bear on both these matters.

The derivatives of alanine, methionine, phenylalanine, and *O*-benzylserine show a trend in optical lability *O*-benzylserine > phenylalanine, methionine > alanine

TABLE 1

Second-order rate constants for racemisation by equivalent amounts of triethylamine in 0.025M-solution in chloroform at 20 °C

Ester	$10^{-4}k_2$/l mol^{-1} s^{-1}
Z-Cys(Bzl)-OPcp	2.45
Z-Cys[Bzl(4-NO_2)]-OPcp	8.50
Z-Cys[Bzl(4-OMe)]-OPcp	1.86
Z-Cys[Bzl(4-Cl)]-OPcp	3.84
Z-Cys[Bzl(4-CN)]-OPcp	6.63
Z-Cys(Me)-OPcp	2.33
Z-Cys(Btm)-OPcp	4.06
Z-Cys(Dpm)-OPcp	3.10
Z-Cys(Trt)-OPcp	2.30
Z-Ser(Bzl)-OPcp	0.61
Z-Thm-OPcp (5)	< 0.05
Z-Thz-OPcp	< 0.05
Z-Phe-OPcp	0.19
Z-Ala-OPcp	< 0.05
Z-Met-OPcp	0.18
OCH-Pro-OPcp	< 0.05
OCH-Thz-OPcp	2.15
Ac-Thz-OPcp	*ca.* 0.05
Bz-Thz-OPcp	0.12
Z-Gly-Thz-OPcp	0.09
$MeOCH_2CO$-THz-OPcp	0.20
$ClCH_2CO$-Thz-OPcp	0.24
Cl_2CHCO-Thz-OPcp	0.45
CF_3CO-Thz-OPcp	1.59

which corresponds qualitatively with the trend in Taft substituent constants [10] for the side-chains (σ^* values for X in the side-chain $-CH_2X$ are alkoxy +0.66, phenyl +0.22, $-CH_2SCH_3$ +0.17, -H 0.00). These results are therefore consistent with the view that in these cases the lability is a consequence of the general electron-withdrawing effect of the side-chains. The *S*-benzylcysteine and *S*-methylcysteine derivatives, on the other hand, are much more easily racemised than one would expect on this basis. The σ^* value for alkylthio is substantially less (+0.42) than that for alkoxy (+0.66) but benzyloxycarbonyl-*S*-benzyl-L-cysteine pentachlorophenyl ester and the corresponding *S*-methyl compound are nevertheless racemised much more quickly than the analogous serine derivative. This suggests that the sulphur atom has some special capacity to stabilise negative charge at the cysteine α-carbon atom in the carbanion intermediate or carbanion-like critical transition state and this conclusion is reinforced by consideration of a Hammett correlation of the effect of varying the substituent in a series of 4-substituted *S*-benzyl-L-cysteine derivatives (see Figure 2). The ρ value of +0.62 reveals a sensitivity to substituent changes which

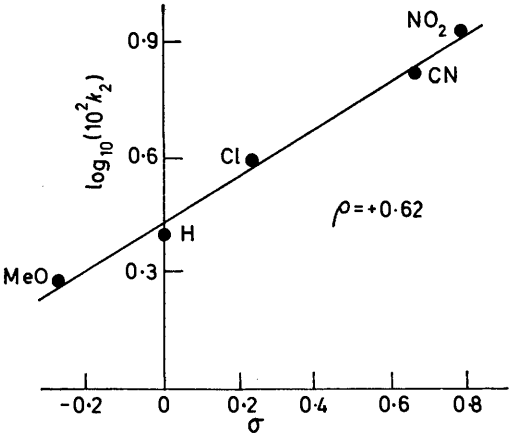
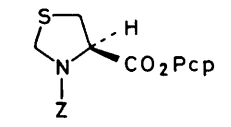


FIGURE 2 Hammett plot for the racemisation by triethylamine in chloroform at 20 °C of a series of derivatives of Z-Cys(Bzl)-OPcp with different *para*-substituents in the *S*-benzyl group

is greater than that to be expected if the operative effect were merely an inductive one transmitted from the aromatic ring through a three-atom chain to the α-carbon. Carbanion formation is generally associated with large positive ρ values; *e.g.* the methoxide-catalysed exchange rates of the 2-protons of a series of ring-substituted 2*H*-2-phenyl-hexafluoropropanes in dimethyl sulphoxide–methanol correlate with Hammett σ values giving $\rho = +4.0$.[11] We can take this example, which seems reasonably typical, as an analogy for the racemisation of (2) and correct for the attenuating effect of the $-CH_2SCH_2-$ group which separates the reaction site from the aromatic ring in (2) by using an inductive effect transmission factor of 0.4 [12] for each atom in the chain. This leads to the conclusion that ρ for the racemisation of (2) should be in the range +0.2 to +0.3 if the influence of a ring substituent at the cysteine α-carbon is expressed by the operation of inductive effects alone. The observed ρ value would perhaps be consistent with a β-elimination pathway but this has been ruled out,[3] so we have suggested [1] that the sulphur atom assists race-



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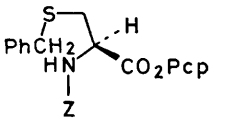

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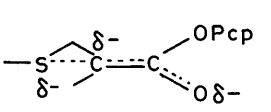

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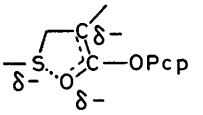

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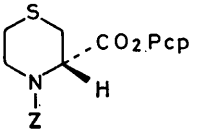
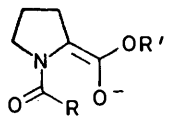


(5)

misation by stabilising the carbanion through acceptance of some negative charge into its *d* orbitals as conjectured in (3), although more recently Kovacs and his colleagues [13] have favoured the representation (4) in which the special effect of the sulphur operates in a five-membered ring. The proposal (3) has a distant analogy in the tentative suggestion of Yano and Oae [14] that the enhanced rate of base-catalysed dehydrobromination of γ-(phenylthio)propyl bromides may be due to the stabilisation of a carbanion-like transition state by sulphur in a similar fashion.

The resistance of (1) to racemisation—which is not a peculiarity of the five-membered ring, as the analogous six-membered ring derivative (5) behaves similarly—seems likely to be related to the special difficulty of bringing about direct exchange racemisation in a cyclic amino-acid residue. That there is such a special difficulty is apparent from, for example, the finding [15] that *N*-methylamino-acid ester residues suffer much more racemisation (presumably by direct exchange) than corresponding derivatives with unsubstituted *N*-H groups on saponification, but proline methyl ester residues can be saponified with complete retention of configuration. This has been regarded as curious,[15] but a possible explanation for it may lie in the fact that ionisation at the α-carbon presumably can only occur at all if the charge is delocalised in the transition state as in an enolate. This is only possible if there is some structural readjustment: in the α-ionisation of a proline ester all four conformations (6)—(9) of the carbanion in which conjugation is maximised have unfavourable intramolecular interactions, since they all require the ester

oxygens to lie in the same plane as the amide carbonyl group. It is suggested, therefore, that the greater optical stability of (1) than of (2) is due to the negation of the special sulphur effect by incorporation of the



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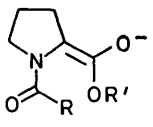

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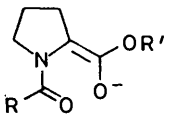

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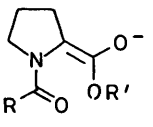


(9)

chiral centre in a ring in which the carbanion cannot easily be stabilised. Support for this interpretation comes from a study of the effect on racemisation rate of varying the acyl group in a series of acylthiazolidine-

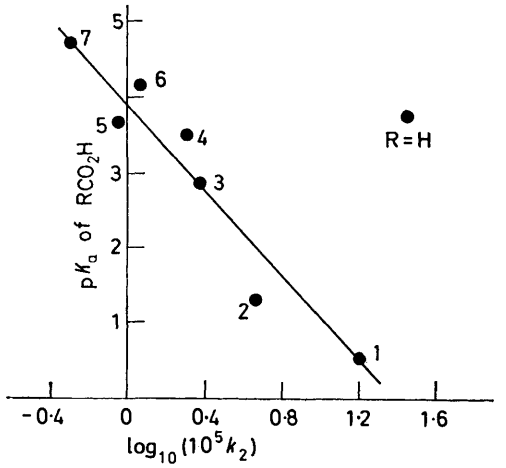
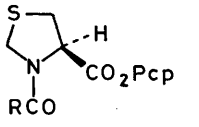


FIGURE 3 Correlation of the ease of racemisation by triethylamine in chloroform at 20 °C of a series of esters RCO-Thz-OPcp with the pK_a of RCO_2H; 1, R = CF_3; 2, R = Cl_2CH; 3, R = $ClCH_2$; 4, R = $MeOCH_2$; 5, R = $ZN{-}CH_2$; 6, R = Ph; 7, R = Me

4-carboxylic acid pentachlorophenyl esters (10) (Figure 3). It is seen that a very strongly electron-withdrawing acyl group is able to overcome the chirality-protecting factors associated with the ring, but that, of the derivatives with an acyl group derived from a weaker acid, only the formyl case (11) is racemised rapidly by tertiary base. In this case alone it is possible to draw and make reasonable models of a configuration (12) of the enolate in which conjugation is maximised without unreasonable steric compression. The fact that the formyl-thiazolidine derivative is optically labile solely by virtue of the presence of sulphur is shown by comparison with the analogous proline derivative, which is stable. In this case the sulphur effect can only operate as in (3), and it would seem reasonable to suppose that the effect operates similarly in all the cases studied.

The enhancement of acidity shown by α-protons in sulphides is well known, although its interpretation is a matter of current debate.[16] In contrast, the activation of protons which are β with respect to sulphur appears to have been recognised before only * in the biochemically important case of the thiol esters, which show enhanced kinetic [18] and thermodynamic [19] acidity. The widely accepted [20] explanation for this has been in terms of a resonance argument [21] involving the carbonyl oxygen which is between the sulphur and the carbon bearing the activated proton. The present work, however, demonstrates that sulphur can promote *C*–H acidity at a carbon which is β with respect to it by interaction through space across an intervening methylene group—a situation to which a resonance argument is obviously



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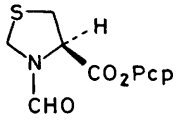

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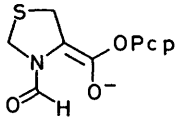

(12)

inapplicable. The interpretation of the elevated acidity of thiol esters and related structures therefore calls for re-examination.

Finally, the fact that the base-catalysed racemisation of active esters in non-polar solvents is a mechanistic curiosity should not be allowed to obscure the fact that there is some practical danger [3] of racemisation attending the use of cysteine active esters for coupling if any excess of tertiary base is present. Our results show that the danger is not confined to *S*-benzyl derivatives but extends to other protecting groups, and we can expect from the general trends we have observed that it will be greatest with those *S*-substituents which withdraw electrons most strongly from the sulphur atom.

EXPERIMENTAL

The pentachlorophenyl esters required were all prepared *via* the corresponding acids by standard methods, and no difficulties worthy of remark were encountered: the physical constants and elemental analyses for those which are new are listed in Table 2. All the esters used had the expected n.m.r. and i.r. spectra and were chromatographically pure. M.p.s were determined with a Kofler hot-stage apparatus. Optical rotations were measured on a Perkin-Elmer 241 automatic polarimeter in a 1-dm cell. The second-order rate constants for racemisation of the esters by triethylamine in chloroform at 20 °C were determined by measuring

* The high optical lability of *o*-nitrophenylsulphenyl-*N*-carboxy-anhydrides [17] may be a further case.

TABLE 2

Physical constants and elemental analyses for the new pentachlorophenyl esters prepared

Ester [a]	M.p. (°C)	Specific rotation (°) [b]	Formula	Required (%) C	H	Cl	N	S	Found (%) [c] C	H	Cl	N	S
Z-Cys[Bzl(4-NO_2)]-OPcp	139—141	−41.8	$C_{24}H_{17}Cl_5N_2O_6S$	45.1	2.7	27.8	4.4	5.0	45.4	2.7	28.0	4.3	5.1
Z-Cys[Bzl(4-OMe)]-OPcp	148—150	−45.1	$C_{25}H_{20}Cl_5NO_4S$	48.1	3.2	28.0	2.25	5.1	48.2	3.0	27.7	2.3	5.0
Z-Cys[Bzl(4-Cl)]-OPcp	150—151	−41.5	$C_{24}H_{17}Cl_6NO_4S$	46.0	2.9	34.0	2.2	5.1	46.2	3.1	33.7	2.3	5.2
Z-Cys[Bzl(4-CN)]-OPcp	143—147	−42.4	$C_{25}H_{17}Cl_5N_2O_4S$	48.5	2.8		4.5		48.6	2.8	n.d.	4.4	n.d.
Z-Cys(Me)-OPcp	139—140	−28.0	$C_{18}H_{14}Cl_5NO_4S$	41.7	2.7	34.3	2.7	6.2	42.0	2.5	34.6	2.6	6.3
Z-Cys(Btm)-OPcp	136—137	−46.2	$C_{25}H_{20}Cl_5NO_4S_2$	46.9	3.1	27.8	2.2	10.1	47.2	2.9	26.8	2.2	10.0
Z-Cys(Dpm)-OPcp	134—135	−22.1	$C_{30}H_{22}Cl_5NO_4S$	53.8	3.3	26.5	2.1	4.8	53.9	3.0	26.8	2.1	5.0
Z-Cys(Trt)-OPcp	178—179	+18.8	$C_{36}H_{25}Cl_5NO_4S$	58.0	3.4	23.8	1.9	4.3	58.3	3.7	23.9	2.0	4.3
Z-Ser(Bzl)-OPcp	125—126	+2.9	$C_{24}H_{18}Cl_5NO_5$	50.0	3.1	30.7	2.7		49.9	3.2	30.8	2.8	
Z-D-Thm-OPcp (5)	128—130	+50.8	$C_{19}H_{14}Cl_5NO_4S$	43.0	2.6	33.5	2.6		42.8	2.4	34.0	2.7	n.d.
Z-Thz-OPcp	123—125	−69.7	$C_{18}H_{12}Cl_5NO_4S$	41.9	2.3	34.4	2.7	6.2	41.7	2.4	34.6	2.5	6.5
OCH-Pro-OPcp	109—110	−64.5	$C_{12}H_6Cl_5NO_3$	36.8	2.1	45.3	3.6		37.0	2.2	45.5	3.8	
OCH-Thz-OPcp	157—158	−93.0	$C_{11}H_6Cl_5NO_3S$	32.2	1.5	43.3	3.4	7.8	32.3	1.55	43.5	3.2	7.8
Ac-Thz-OPcp	155	−67.0	$C_{12}H_8Cl_5NO_3S$	34.0	1.9	41.85	3.3	7.65	34.1	1.8	41.7	3.3	7.5
Bz-Thz-OPcp	89—90	−142	$C_{17}H_{10}Cl_5NO_3S$	42.05	2.1	36.5	2.9	6.6	42.3	2.1	36.1	3.0	6.2
Z-Gly-Thz-OPcp	147—148	−115	$C_{20}H_{15}Cl_5N_2O_5S$	41.95	2.6	30.95	4.9	5.6	41.8	2.7	30.75	4.9	5.8
CH_3OCH_2CO-Thz-OPcp	121—122.5	−53.0	$C_{13}H_{10}Cl_5NO_4S$	34.4	2.2	39.1	3.1	7.1	34.6	2.2	39.2	2.95	7.05
$ClCH_2CO$-Thz-OPcp	154—155	−65.0	$C_{12}H_7Cl_6NO_3S$	31.5	1.5	46.45	3.1	7.0	31.7	1.7	46.2	3.1	7.0
Cl_2CHCO-Thz-OPcp	166.5—167	−56.5	$C_{12}H_6Cl_7NO_3S$	29.3	1.2	50.4	2.9	6.5	29.5	1.3	50.6	2.9	6.65
CF_3CO-Thz-OPcp	146—148	−59.0	$C_{12}H_5Cl_5F_3NO_3S$	30.15	1.05	37.2	2.9	6.7	30.15	0.85	37.2	2.95	6.8

[a] With the exception indicated, all are L. [b] (*c* 1 in $CHCl_3$). [c] n.d. = not determined.

at intervals the optical rotations of solutions in chloroform (dried over $CaCl_2$) which were 0.025M with respect to both ester and triethylamine. Excellent and reproducible second-order plots were obtained.

We thank Dr. G. Lowe for a gift of the acid from which (5) was made, Dr. G. T. Young for discussions, and the S.R.C. for postgraduate studentships (to M. B. and M. J. W.).

[8/2113 *Received*, 8*th December*, 1978]

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