Tetrahedron Letters No.3, pp. 159-165, 1964. Pergamon Press Ltd.
Printed in Great Britain.

RACEMISATION DURING PEPTIDE SYNTHESIS VI.[+] ACTIVE PHENYL THIOLESTERS: THEIR RACEMISATION IN THE PRESENCE OF TRIETHYLAMINE

Bogdan Liberek and Zbigniew Grzonka

Department of Chemistry, School of Education,
Gdańsk-Poland, Sobieskiego 18

(Received 24 November 1963)

It is known that peptide bonds can be formed in aqueous media from thiophenyl esters, because they are stable to hydrolysis and yet react with amines albeit rather slowly.[1,2] Some years ago, Wieland discussing the stability to hydrolysis of phenyl thiolesters, suggested that little or no danger of racemisation would be here expected, because the sulphur atom seems to prevent the carbonyl carbon from the internal attack of the enol oxygen, thus not allowing the formation of the azlactone.[3]

We have previously reported that, in addition to the oxazolone theory, active derivatives of N-protected amino acids can also racemise by direct proton abstraction from the asymmetric centre followed by formation of, or simple resonating with, the contributing ionic structures in which the asymmetry at C-alpha is lost.[4,5,6]

The purpose of this communication is, first of all, to report that phenyl thiolesters show a special tendency to racemisation in alkali, secondly, to discuss some aspects of reactivity of thiophenyl esters with reference to enhanced lability of the α-hydrogen atom in them.

Racemisation runs with phenyl thiolesters of N-protected amino acids were made in acetone in the presence of a constant

[+]Part V, Zeszyty Naukowe Wyższej Szkoły Pedagogicznej, Gdańsk, Mat-Fiz-Chem., in press

amount of triethylamine. The loss of optical activity was followed polarimetrically and to about 90 per cent completion a relatively good first order behaviour was observed. "Relative half-periods of racemisation" /c=2.0/ have been calculated and are recorded in Table I. From all racemisation experiments over 90 per cent of crude racemic thiophenyl esters of N-protected amino acids were regenerated. For comparison analogous racemisation runs with corresponding p-nitrophenyl esters were made and approximate values of "relative half-periods of racemisation" are also included in Table I / the yields of regenerated racemic p-nitrophenyl esters were lower/.

T A B L E I

All racemisation experiments carried out at 22.5° ± 1° 2% acetone solution containing in 100 ml 1.2 ml of $N/Et/_3$ /c=2.0/

Compound	Relative half-period of racemisation	Relative half-period of racemisation for the corresponding p-nitrophenyl ester	Ratio of rates /phenyl thiolester : p-nitrophenyl ester/
Z-Ala(CN).SPh [a]	11 min.	28 min.	2.54 : 1
Z-Cys(SBz).SPh [b]	106 min.	206 min.	1.95 : 1
Z-Asp(OBz).SPh [b]	4.8 hrs	9.6 hrs	2 : 1
Z-Asp(SPh).SPh [b]	70 min.	180 min. [c]	2.57 : 1
Phth-Ala.SPh [b]	78 min.	148 min.	1.92 : 1
Phth-Phe.SPh [b]	16 hrs	3.35 hrs	0.21 : 1 [d]

Symbols according to Goodman and Kenner, Adv. Protein Chem., 12, 465 /1957/

For all new compounds /L and DL isomers/ satisfactory nitrogen analyses have been obtained.

a/ for experimental details see ref.[5]

b/ experimental details will be published in a subsequent paper

c/ for di-p-nitrophenyl ester

d/ perhaps some steric effects may be responsible for such a ratio of rates

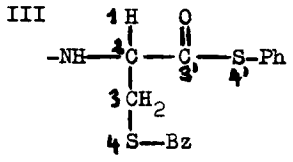
In all racemisation runs, with the exception of esters of phthaloyl-L-phenylalanine, the thiophenyl ester racemised faster than the corresponding p-nitrophenyl ester. Kenner and coll[7] studied the relative reactivities of active esters of carbobenzoxy glycine towards aminolysis reaction and found that the reactivity of p-nitrophenyl ester towards alanine was greater than that of the corresponding phenyl thiolester but less than that of the p-nitrophenyl thiolester / ratio of rates 16 : 1 : 140 /.

It is evident that there is a special tendency to base-catalysed racemisation in phenyl thiolesters.

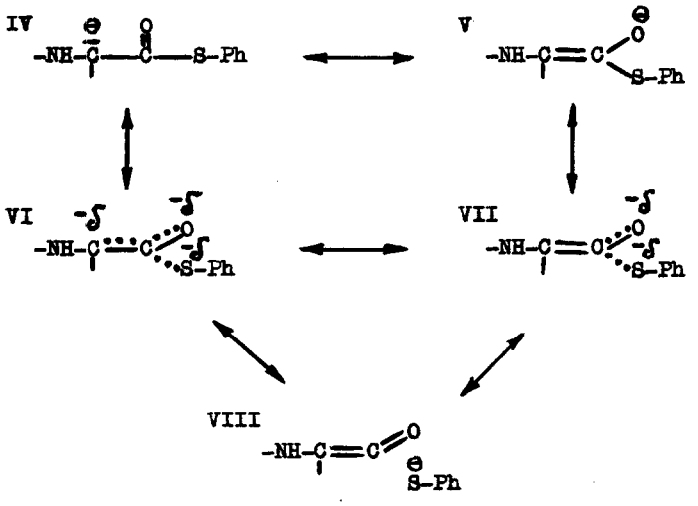
It is well known that active derivatives of N-protected S-benzyl-L-cysteine are readily racemised in alkali.[8,9,10] Young suggested that this tendency to racemisation may be explained in terms of lability of the α-hydrogen and stabilizing of anion / I / by resonance with the contributing form /II/.[9]

Swan explained the ease of base-catalysed racemisation in derivatives of S-benzyl-L-cysteine in terms of reversible elimination and readdition of benzyl mercaptan and supported this hypothesis by isolation of a comparatively large quantity of dibenzyl disulphide.[10]

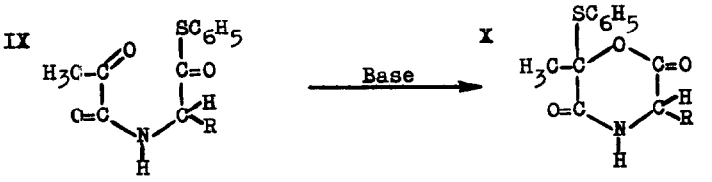
In phenyl thiolesters of N-protected amino acids and in derivatives of S-benzylcysteine the α-hydrogen atom and the sulphur atom are in an analogous position /III/. Thus in analogy to Young's suggestion[9] the tendency to base-catalysed racemisation in phenyl thiolesters may be explained by assuming that the initial proton abstraction from the asymmetric centre is followed by mere enolisation, because the enol



contributing form in the case of thiolesters may be additionally stabilized by conjugation effects involving the outer shell electrons of the sulphur atom /IV,V,VI,VII,VIII/.



We wish to reiterate the parallelism between derivatives of S-benzylcysteine and phenyl thiolesters. Swan succeeded in isolation of dibenzyl disulphide - the oxidation product of benzyl mercaptan eliminated in alkali from derivatives of S-benzylcysteine.[10] Wieland and coll. found that phenyl thiolesters of pyruvoyl amino acids rearrange in the presence of triethylamine or even natrium hydrogen carbonate to morpholine derivatives /IX,X/.[11,12]

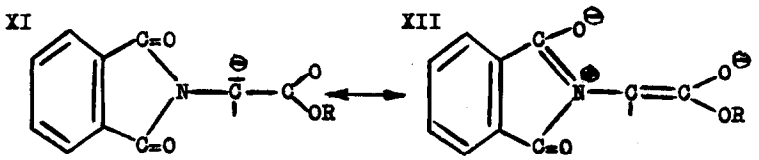


Wieland suggested an elimination mechanism involving only the aryl thiolester grouping and supported this proposal by the evidence of base-catalysed exchange of thiophenol

component in phenyl thiolester of carbobenzoxy-L-phenylalanine for ^{35}S-labelled thiophenol.[12] We have repeated this experiment /in tetrahydrofuran at 22.5°/ and found an 28 per cent loss of optical activity within 24 hours. Thus in recrystallisations to constant number of impulses fractionation may have occurred and that may have significantly affected the reported results.[12]

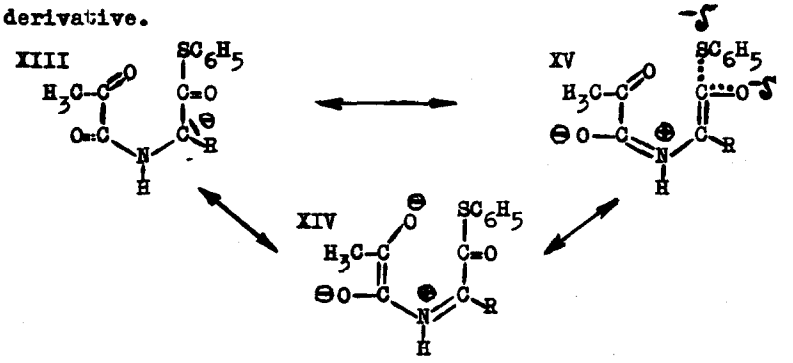
It may be noted that the course of racemisation suggested by Young for derivatives of S-benzyl-L-cysteine[9] and our proposal for explaining the tendency to α-carbon isomerization in phenyl thiolesters of N-protected amino acids are just the incipient phases of elimination reactions. Of course, the loss of optical activity may proceed by complete elimination and readdition of benzyl mercaptan or thiophenol, respectively. However, viewing our results in conjugation with those of Wieland,[11,12] Swan[10] and the suggestion of Young[9], it seems that the lability of the α-hydrogen is an important factor influencing the reactivity of aryl thiolesters.

It was previously reported that active derivatives of phthaloyl amino acids are very easily racemised in alkali.[6] This tendency to racemisation have been attributed to the lability of the α-hydrogen and stabilising of carbanion /XI/ by enol-like contributing species /XII/.[6]

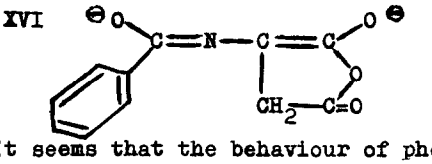


An analogous type of electron shift might be postutated for the stabilization of the carbanions of aryl thiolesters of pyruvoyl amino acids /XIII,XIV,XV/. This additional stabilization conferred by the pyruvoyl residue upon the enol contributing forms of aryl thiolester carbanions /IV,V,VI,VII, VIII/ makes the α-hydrogen more labile and will thus facilitate ionization and racemisation and will likely influence the elimination of thiophenol with rearrangement to morpholine

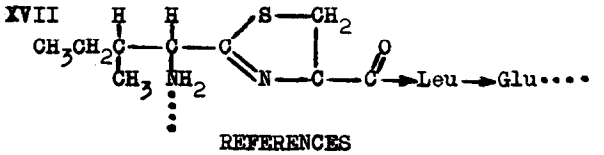
derivative.



It should be mentioned that somewhat similar type of stabilization was already postulated by Barker[13] to account for the greater ease of racemisation of benzoyl aspartic anhydride than acetyl aspartic anhydride /XVI/.



It seems that the behaviour of phenyl thiolesters should have a direct bearing on the interpretation of the ease of racemisation of the N-terminal isoleucine residue in bacitracine /XVII/.[14]



REFERENCES

1. Th.Wieland, W.Schäfer, E.Bokelmann, Ann., 573, 99 /1951/
2. Th.Wieland, W.Schäfer, ibid., 576, 104 /1952/
3. Th.Wieland, Proc.Symposium on Methods of Peptide Synthesis, Prague, 1958, Coll. Czechoslov. Communs, 24, Special Issue, p.46 /1959/
4. B.Liberek, Tetrahedron Letters, 1963, 925
   B.Liberek, A.Nowicka, Zb.Grzonka, ibid., 1963, 1479
5. B.Liberek, Bull.Acad.Pol.Sci.,Ser.Sci.Chim., in press
6. B.Liberek, Tetrahedron Letters, 1963, 1103

7. J.A.Farrington, P.J.Hextall, G.W.Kenner, J.M.Turner, J.Chem.Soc. 1407 (1957).

8. B.Iselin, M.Feurer, R.Schwyzer, Helv. Chim. Acta. 38, 1508 (1955).

9. G.T.Young, Proc.Symposium on Methods of Peptide Synthesis, Prague,(1958). Coll.Czechoslov.Communs. 24, Special Issue, 118 (1959).

10. J.A.Maclaren, W.E.Savige, J.M.Swan, Austral.J.Chem. 11, 345 (1958); J.W.Stapleton, J.M.Swan, ibid. 13, 416 (1960).

11. Th.Wieland, B.Heinke, K.H.Shin, Chem.Ber. 93, 3027 (1960).

12. Th.Wieland, J.Stark, ibid. 96, 2410 (1963)

13. C.C.Barker, J.Chem.Soc.453 (1953).

14. L.C.Craig, J.Polymer Sci. 49, 71 (1961).