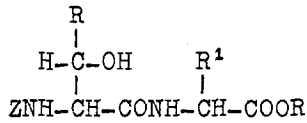
SELECTIVE FRAGMENTATION OF THREONYL AND SERYL DIPEPTIDES

Ferruccio D'Angeli, Virgilio Giormani, Fernando Filira
and Carlo Di Bello

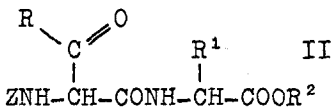
Istituto di Chimica Organica dell'Universita'
Sezione VIII del Centro Nazionale di Chimica
Macromolecolare del C.N.R.
Padova, Italy

Received August 3, 1967

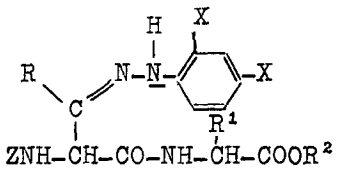
We reported recently that the oxidizing system composed of dimethyl sulfoxide - dicyclohexylcarbodiimide - phosphoric acid (Pfitzner and Moffat, 1963, 1965; Torssell, 1966) converts the threonyl derivatives (I) into β-keto-amides (II); these in turn react with phenyl hydrazine, producing cleavage of the adjacent peptide bond (D'Angeli, Scoffone, Filira and Giormani, 1966).



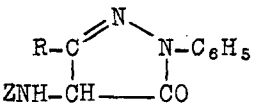
I



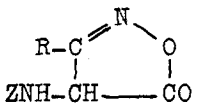
II



III



IV



V

I, II, III ... R = CH_3; I^1, II^1, III^1 ... R = H
a:R^1=$CH_2C_6H_5$, R^2=CH_3; b:R^1=CH_3, R^2=C_2H_5; c:R^1=H, R^2=C_2H_5

In the present research, we tested model seryl dipeptides (I^1) under the same conditions. Such compounds were converted by the oxidizing system into β-aldehydo--amides (II^1). When the latter were treated, in turn, with phenyl hydrazine, the cleavage of the peptide bond adjacent to the parent serine was practically negligible. This behaviour indicates a smaller tendency of the hypothetical intermediate (III^1, X = H) to undergo a ring closure with concurrent fragmentation; the chemical basis and potential usefulness of this behaviour are under current investigation. Meanwhile, we observed that hydroxylamine cleaves, under mild conditions, the peptide bonds adjacent to the carbonyl groups in both β-ketoamides (II) and β-aldehydo-amides (II^1), yielding the amine moiety and an iso-oxazolone derivative (V). Furthermore, at or below pH 7, the β-ketoamides are cleaved faster than the β-aldehydo-amides.

The consecutive use of phenyl hydrazine and hydroxylamine proved expedient in some experiments where the latter was caused to react with the intermediate product obtained from an aryl hydrazine and a β-aldehydo-amide.

Additional research is being carried out to ascertain if the above fragmentation can be applied to the elucidation of the primary structure of proteins; in particular, we are studying the behaviour of model polipeptides containing two or more units of threonine or serine, and other potentially reactive amino acids.

The fragmentation of the β-carbonyl-amides is reminiscent of the cleavage of the acetoacetyl group, recently proposed as a reversible protector in peptide synthesis and protein studies (D'Angeli et al., 1965, 1966; Marzotto, Pajetta and Scoffone, 1967).

EXPERIMENTAL

1) Oxidation

α-Benzyloxycarbonylamino-α-formyl-acetyl-DL-phenylalanine methyl ester (II^{1}a). A sample of N-benzyloxycarbonyl-DL-seryl-DL-phenylalanine methyl ester (I^{1}a) (0.82 g; 2 mmole) was dissolved in 1.5 ml anhydrous dimethyl sulphoxide (DMSO) and mixed with dicyclohexyl carbodiimide (DCC; 0.82 g; 4 mmoles) in 6.5 ml DMSO containing 0.32 ml of a 3.12 M ethereal solution of phosphoric acid (1 mmole). The resulting suspension was allowed to remain for two hours at room temperature. The excess DCC was decomposed by addition of 10 ml water containing 0.24 ml of acetic acid (4 mmoles) and the dicyclohexyl urea was filtered, washed with water and then with ethyl acetate, dried, weighed and discarded (0.84 g; 92%). The mother liquor and the washings were treated with 20 ml of ethyl acetate in a separatory funnel; the ethyl acetate layer was washed with water, dried and concentrated in vacuo. A crude oil was obtained that did not crystallize when dissolved in ethyl acetate and precipitated with petrol ether. It was then dissolved in warm ethanol; upon cooling, colourless needles of the aldehyde (II^{1}a) semiacetal were obtained, m.p. 113-5°. (Found %: C 62.39; H 6.35; N 6.26. Calc. for $C_{23}H_{28}N_2O_7$: C 62.15; H 6.35; N 6.30).

α-Benzyloxycarbonylamino-α-formyl-acetyl-DL-alanine ethyl ester (II^{1}b) was obtained as II^{1}a from N-benzyloxycarbonyl-DL-seryl-alanine ethyl ester (I^{1}b). For the purpose of identification, the oil was transformed into its 2,4-dinitrophenyl hydrazone (III^{1}b, X = NO_2) m.p. 174-6°C. (Found %: C 51.07; H 4.60; N 15.72; Calc. for $C_{22}H_{24}N_6O_9$: C 51.16; H 4.68; N 16.27).

In general, the yields in the oxidation step averaged 50-60%; further experiments are in progress to increase them.

2) Cleavage. Samples of either β-aldehydo amide (II^1), alone or in admixture with a β-keto-amide (II) were treated with phenyl hydrazine hydrochloride or with hydroxylamine hydrochloride in hydroalcoholic solution and the pH was eventually adjusted to the desired value by addition of acetic acid or N sodium hydroxide. The mixtures were kept at 20°, 40° or 70°C and the progress of the reaction was followed by thin-layer chromatography on Silica Gel G Merck with the ninhydrin test and isolation of some products; the liberated amino acids were assayed using an automatic amino acid analyzer.

A) Cleavage by hydroxylamine of the amino acids adjacent to threonine and serine. 0.7 mmoles (0.235 g) each of α-benzyloxycarbonylamino-acetoacetyl-glycine ethyl ester (IIc) and α-benzyloxycarbonylamino-α-formyl--acetyl-DL-alanine ethyl ester (II^1b) were dissolved in 24 ml of the solvent mixture acetic acid, water, methanol (1:1:2) and added with hydroxylamine hydrochloride (1 g; 1.4 mmoles). The resulting solution had a pH of 1.5; the release of both glycine and alanine at 70° reached a maximum within 20-30 minutes.

B) Selective cleavage by phenyl hydrazine of the amino acid adjacent to threonine. The mixture of 0.67 g. (2 mmole) of the β-ketoamide (IIc) and 0.81 g (2 mmole) of the β-aldehydo-amide (II^1a) was dissolved in 80 ml of the above solvent mixture and treated with 0.6 g (4 mmole) of phenyl hydrazine hydrochloride. The solution was left 14 h at room temperature, concentrated in vacuo until most of the acid was eliminated, and then taken up with 10 ml each of water and ethyl acetate. A solid

precipitated which was washed with ether and identified as 1-phenyl-3-methyl-4-benzyloxycarbonylamino-pyrazoline--5-one (IV, R = CH_3); m.p. 193-6° (dec.). Found %: C 66.06; H 5.27; N 12.66. Calc. for $C_{18}H_{17}N_3O_3$: C 66.86; H 5.30; N 13.00).

The aqueous phase was washed with ethyl acetate and concentrated to dryness, and crude glycine ethyl ester hydrochloride was obtained. It was recrystallized and identified by comparison with an authentic sample: yield 90%. No phenylalanine could be detected.

The ethyl acetate phase was gradually concentrated: an additional amount of compound (IV) was filtered off and a semi-solid mass was obtained. A sample of this crude product was reacted with hydroxylamine hydrochloride for 20 min. at 70°C in aqueous acetic acid as described in paragraph A. Phenylalanine ethyl ester became evident on the chromatograms. Another sample of the crude product was passed through a column of silica gel (Merck, 0.05--0.2 mm), yielding few solid fractions; one of them gave analytical values corresponding to a condensation product of phenyl hydrazine with the β-aldehydo-amide (II[1]a) and is still under study.

C) Example of consecutive fragmentation of a β-keto--amide and a β-formyl-amide by hydroxylamine. A sample of the mixture mentioned under paragraph B and consisting of 1 mmole of each β-carbonyl-amide, dissolved in 13 ml of ethanol-water (10:3), was added with 0.14 g (2 mmole) of hydroxylamine hydrochloride and kept 10 h at room temperature. The ethanol was removed under vacuum at 20°C, water was added to 10 ml and the solution was extracted with ethyl acetate. Chromatography of the aqueous phase revealed the formation of glycine ethyl ester with only trace amounts of phenylalanine; glycine ethyl ester was

obtained, upon concentration to dryness, in 50% yield. The organic phase (unreactive to ninhydrin) was evaporated to dryness, taken up with 30 ml of the mixture acetic acid, water, ethanol (1:1:1) and kept 30' at 70°. The working up of the mixture as above resulted in the formation of a solid containing about equal amounts of the hydrochlorides of glycine and phenylalanine esters.

Acknowledgement. We are indebted to Prof. Ernesto Scoffone for helpful interest during the investigation.

REFERENCES

D'Angeli,F., Scoffone,E., Filira,F., and Giormani,V., Tetrah. Lett., 2475 (1966).

D'Angeli,F., Filira,F., and Scoffone,E., Tetrah. Lett., 605 (1965).

D'Angeli,F., Filira,F., Giormani,V. and Di Bello,C., Ric. Sci. 36, 11 (1966).

Marzotto,A., Pajetta,P. and Scoffone,E., Biochem. Biophys. Res. Comm., 26, 517 (1967).

Pfitzner,K.E. and Moffat,J.C., J. Amer. Chem. Soc., 85, 3027 (1963); 87, 5661, 5670 (1965).

Torssell,K., Tetrah. Lett., 4445 (1966).