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α-MERCAPTO-α-AMINO ACIDS AND DEHYDRO AMINO ACIDS. - SYNTHESES, RELATIONSHIPS AND INTERCONVERSIONS

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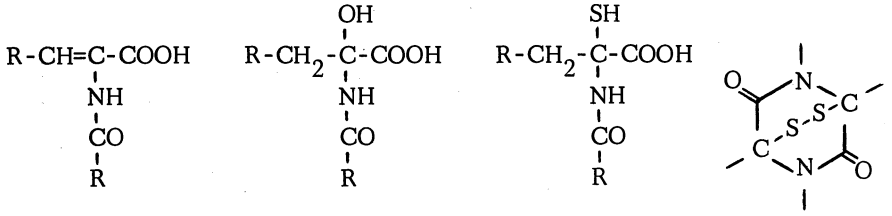
Abstract - Dehydro amino acids, α-methoxy-α-amino acids and α-mercapto-α-amino acids have been found to be components of naturally occuring peptides and mould metabolites. Biomimetic experiments in view of relationships between these compounds have been made.

INTRODUCTION

In recent years dehydro amino acids (1), α-hydroxy-α-amino acids (2) and α-mercapto-α-amino acids (3) have been shown to be characteristic units of various mould metabolites and relationships seem to exist between these compounds (Ref. 1). Indications that dehydrogenation of amino acids or peptides may occur in the metabolism of moulds have been found, offering a broad spectrum of subsequent transformations of dehydro amino acid units.



(1) (2) (3) (4)

SULFUR CONTAINING DIOXOPIPERAZINES (Ref. 2)

The cyclic dipeptide of two α-mercapto-α-amino acids in its disulfide form represents a unit often found as a characteristic component of mould metabolites. Examples of this structure (4) are the antibiotics of the aranotine group, the sporidesmines, gliotoxine and dehydrogliotoxine with remarkable antiviral action ensueing from the inhibition of the RNA synthesis. Besides these disulfides the epitrisulfides and the bis-thioethers have been found. The bis-thioethers are however biologically inactive and not toxic.

The nucleophilic introduction of sulfur functions into the cyclodipeptide nucleus was first described by Trown (Ref. 3). - Dibromo-sarcosyl-sarcosine anhydride - formed by direct bromination of the dioxopiperazine - was reacted with sodium thiolacetate. Hydrolysis of the reaction product formed the dimercapto compound, which was dehydrogenated to the epidisulfide.

A little later we were engaged in the study of the reaction of dibromo-sarcosyl-sarcosine anhydride with sodium disulfide intending the direct formation of the epidisulfide. Surprisingly only the epitetrasulfide could be isolated (Ref. 4). The enhanced stability of this epitetrasulfide, compared with that of the epidisulfide is understandable in view of the fine structure of this compound. The X-ray analysis made by I. Bernal (Ref. 5) proves all the interplanar angles of the sulfur-sulfur bonds to be exactly 90 degrees which is indeed the most stable conformation of an S-S-bond. In comparison the angle of the S-S-bond in

the epidisulfide is only about 20 degrees.

We selected L-prolyl-L-proline anhydride as a substrate for the study of electrophilic substitution of a cyclic dipeptide (Ref. 6). Alternate metalation and reaction with sulfur, reduction of the epipolysulfides formed and transformation of the cis-dithiol leads to the epidisulfide (Ref. 7), epitrisulfide and epitetrasulfide (Ref. 8) in more than 50% with respect to the L-prolyl-L-proline anhydride and with only 10% loss of the L-L-configuration.

Dehydrogliotoxine and the sporidesmines have been synthesized in an excellent work by Kishi (Ref. 9-11) : His synthesis starts with the anisaldehyde mercaptal of dimercapto-sarcosyl-sarcosine anhydride, which is successively metalated and alkylated to construct the carbon skeleton of the natural compounds. The S-S-bond is formed only in the final stages by oxidation of the mercaptal to the mono-S-oxide and elimination of anisaldehyde by boron trifluoride. In these syntheses the often critical step leading to the formation of the dimercaptan is omitted.

OCCURRENCE OF DEHYDRO AMINO ACIDS, α-MERCAPTO-α-AMINO ACIDS AND α-HYDROXY-α-AMINO ACIDS

Besides these sulfur substituted cyclodipeptides the analogous sulfur free but unsaturated compounds are often produced by the same strains (Ref. 12). It is not clear whether the sulfur compound is formed via the unsaturated product or the other way round. If the sulfur compound should be generated from the olefine, it must have been initiated by an α-addition of a sulfur-hydrogen compound to the acrylic double bond. But more common are nucleophilic β-additions to dehydro amino acids, for example in the cysteine biosynthesis by addition of a mercapto compound to pyridoxylideneamino-acrylic acid.

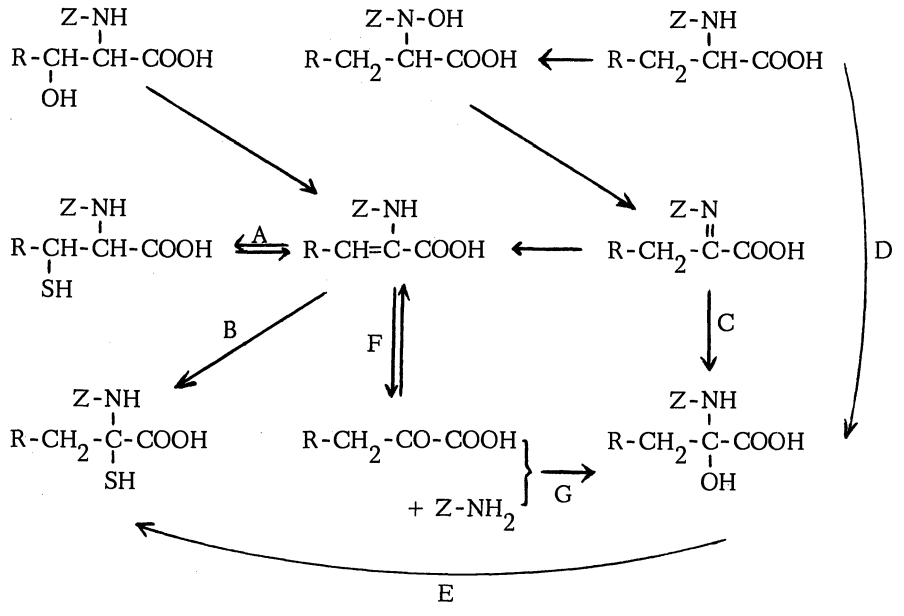
Dehydro amino acids as units of peptides particularly of mould origin have been found sporadically. Interesting examples are the antibiotics nisin and subtilin with dehydro-amino-butyric acid and dehydro alanine units (Ref. 13). The heterodetic peptide rings in nisin and subtilin contain D-amino-butyric acid or D-alanine in methyllanthionine or lanthionine units presumably formed by the addition of a cysteine sulfur to the parent dehydro amino acid.

Another example for dehydro amino acids as intermediates is given by the biosynthesis of the penicillins and cephalosporins according to the theory of Arnstein as modified by Cooper (Ref. 14). The thiazolidine nucleus of penicillin is proposed to arise by addition of a sulfur function to the double bond of dehydrovaline after the β-lactam ring has been formed.

The widespread group of peptide alkaloids represents ansa compounds (Ref. 15). Their 13 to 15 membered ring contains a β-phenoxyvaline or β-phenoxyleucine unit. It seems convincing to imagine the structure to have been formed by addition of phenolic hydroxyl to dehydrovaline. An analogous linear compound with free phenolic hydroxyl group and the dehydro amino acid unit has also been isolated from plant material (Ref. 16).

Finally an important α-methoxy-α-amino acid compound ought to be mentioned. The cephamycines isolated from the culture filtrate of streptomyces species are formed presumably by addition of methanol to the imino form of dehydrocephalosporine and attract attention because of their enhanced activity against gram negative bacteria (Ref. 17).

The scheme shows the possible relationship between dehydro amino acids, α-hydroxy-α-amino acids, α-mercapto-α-amino acids and α-keto acids. Dehydro amino acids are formed by β-elimination from serine or cysteine or perhaps by oxidation of amino acids to α-hydroxy-α-amino acids or α-hydroxylamino acids and splitting off water. Addition of water or sulfur compounds to enamino acids or the tautomeric imino acids gives α-mercapto-, β-mercapto and α-hydroxy-α-amino acids.



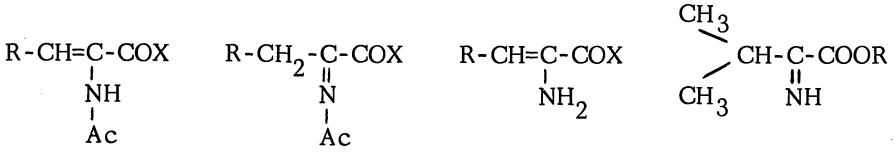
SYNTHESIS OF DEHYDRO AMINO ACIDS

N-acetyl dehydroalanine was synthesized by M. Bergmann from pyruvic acid nearly 45 years ago. For the synthesis of more complicated acylenamino acids (5) especially in peptides β-elimination reactions using serine tosylates and phosphates or sulfonium salts of cysteine are recommended.

We described a very simple route from N-acylamino acids to acylenamino acids (Ref. 1). The transformation is based on N-chlorination of N-acylamino acid derivatives followed by dehydrochlorination. Using tertiary amines acylimino acid compounds (6) are formed which are rearranged into the enamino acid compounds (5) with strong bases such as DBU. Reaction of the N-chloro compounds with sodium methylate in methanol affords by dehydrochlorination and subsequent addition of methanol the α-methoxy-α-amino acid derivative (path C). Acylenamino acid compounds are obtained by methanol elimination with HCl.

In this way acylenamino (5) and acylimino acids (6) are accessible. The tertiary butoxycarbonyl protecting group makes possible the synthesis of enamino acid esters (7) with free amino groups as the BOC-group is removed together with methanol by treatment of the aminal with HCl (Ref. 18).

Finally the imino acid esters as (8) can be formed very easily by chlorination and dehydrochlorination of amino acid esters (Ref. 1). There is no detectable equilibrium between imino and enamino compounds. If the enamino acids are thermodynamically more stable than the imino acids, the latter can be rearranged into the enamino acids via the hydrochlorides (Ref. 19).



(5) (6) (7) (8)

X = OR, NH_2

α-MERCAPTO-α-AMINO ACIDS FROM DEHYDRO AMINO ACIDS

Merdapto compounds add to imino acid esters such as (8) leading to an equilibrium (Ref. 20). The addition of acid chlorides to imino acids opens a simple way to sulfur substituted cyclic dipeptides. Dehydroproline amide adds pyruvoyl chloride. Subsequent reaction with methanol and ringclosure forms the methoxy hydroxy cyclodipeptide, the hydroxy and methoxy functions of which are exchanged by mercapto groups without difficulty (Ref. 21).

The addition of sulfur-hydrogen compounds to acylenamino (5) and acylimino acids (6) seems to be a more important route in biological systems. Hydrogensulfide adds to acyl-imino acid compounds (6) very quickly forming the moderately stable α-mercapto-α-acyl-amino acids (Ref. 20).

In the addition of SH-functions to acylenamino compounds two directions (paths A and B) can be observed :

In strong acid solution α-addition (path B) occurs after formation of an α-carbocation. This reaction - first performed by Olsen with acetylaminoacrylic acid (Ref. 22 & 23) - could not be satisfactorily applied to the addition of mercaptans or thiolacetic acid to unsaturated dioxopiperazines. This reaction - investigated by P. Sammes (Ref. 24) - gave mono and di-addition with bis-dehydro-prolyl-proline anhydride. Stereoselectivity could not be observed.

The nucleophilic β-addition of mercaptans and thiolacetic acid to acylenamino acids (path A) has been known for 35 years. - Bearing in mind the possibility of cysteine synthesis from dehydroalanine within a peptide chain we investigated the mercaptan addition to dipeptides containing dehydroalanine and found a remarkable stereoselectivity in the reaction of acyl-aminoacryl-L-proline N-methylamide (Ref. 25). The addition of methyl mercaptan forms the D-cysteinyl-L-proline peptide with a stereoselectivity of more than 85 % . The dependence on reaction conditions and structural features of the olefine is as follows :

The high stereoselectivity is only observed with strong alkaline catalysis; using piperidine, stereoselectivity decreases remarkably. In adding the mercaptan photochemically no steric preference can be observed. Alkylation of both nitrogens does not seem to have any influence. But in the reaction of the corresponding proline ester instead of the amide the stereoselectivity is virtually lost. All of these results can be explained by assuming a nucleophilic reaction and formation of a carbanion as intermediate which is fixed in space so that the proton can only approach from one direction. A hydrogen bridge is of no importance. We prefer to assume a fixation of the carbanion within a carbanion-immonium ion pair.

α-MERCAPTO-α-AMINO ACIDS VIA α-HYDROXY-α-AMINO ACIDS

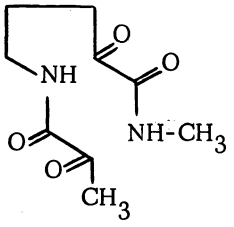
α-Hydroxy-α-amino acids representing hemi-aminals of α-keto acids can be transformed without difficulty into their thioaminals which are α-mercapto-α-amino acids (Ref. 26-28).

In cyclic dipeptides oxygen can be introduced into the α-position by radical reactions. Some amino acid derivatives, particularly dioxopiperazines and hydantoins are oxidized to hydroperoxides by irradiation in the presence of traces of benzophenone (Ref. 21). Reduction of these hydroperoxides and transformation of the hydroxides into mercaptans demonstrates a biologically feasible way to sulfur containing dioxopiperazines (paths D and E). Acetoxy groups can be introduced directly by reaction of dioxopiperazines with lead (IV) acetate (Ref. 27).

A further possibility of forming α-hydroxy-α-amino acid derivatives and α-mercapto-α-amino acids ensues from the fact that they are aminals and thioaminals of α-ketocarboxylic acids. The α-hydroxy-α-amino acids are intermediates in the hydrolysis of acylenamino acids to α-keto acids and amides (path F). But this reaction is reversible and α-hydroxy-α-amino acids can be formed from α-keto acids and amides (path G).

In 1974 this intramolecular reaction to a six membered ring was found and investigated with regard to reaction conditions and stereochemistry (Ref. 28). A twofold ringclosure of this type may be represented : δ -pyruvoylamino-α-diethoxy-valeric acid methylamide (9)

was obtained in a seven step synthesis. Careful and brief treatment with dilute HCl removes the blocking group in α-position and by longer reaction in the same medium the twofold ringclosure proceeds smoothly. The two hydroxyl groups can be replaced by mercapto groups. Only the cis-dithiol is formed in about 50% based on the open chain compound. It is oxidized to (10) (Ref. 30). It should be mentioned that the δ-amino-α-oxovaleric acid represents the α-keto acid corresponding to the amino acids arginine and ornithine.



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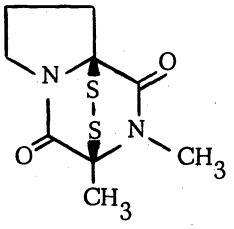

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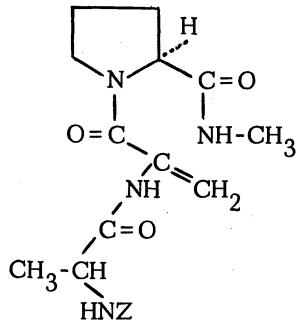
Finally two further transformations of dehydropeptides should be mentioned briefly. A peptide with a dehydroalanine unit can form an imidazolone or a cyclodipeptide (Ref. 31) depending on which of the two contiguous amide nitrogens is added to the double bond of the dehydroalanine. These two possibilities have been realised with model peptides by alkaline or acid catalysis (Ref. 32). A more complicated example illustrating the formation of the cyclodipeptide is given by the tripeptide Z-Ala-DHAla-Pro-$NHCH_3$ (11), which on treatment with DBU undergoes ringclosure forming (12). In this compound the amino side chain can be exchanged by a mercapto group.

This example demonstrates again the relationships between dehydro amino acids and mercapto amino acids. In the last years indeed clear indications have been found that dehydrogenation of amino acids or peptides may occur in the metabolism of moulds offering a broad spectrum of subsequent transformations. Some of these possibilities have been investigated here by biomimetic experiments.



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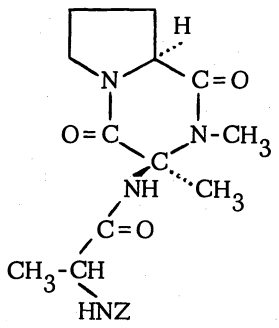

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REFERENCES

1. H. Poisel and U. Schmidt, Chem. Ber. 108, 2547 (1975), and literature cited therein.
2. P.G. Sammes in Fortschritte der Chemie Organischer Naturstoffe, 32, 87 ff., Springer Verlag Wien-New York (1975).
3. P.W. Trown, Biochem. Biophys. Res. Commun. 33, 402 (1968).
4. H. Poisel and U. Schmidt, Angew. Chem. 83, 114 (1971); Angew. Chem. Int. Ed. 10, 130 (1971).
5. B.R. Davis, I. Bernal and U. Schmidt, Angew. Chem. 84, 640 (1972); Angew. Chem. Int. Ed. 11, 632 (1972).
6. H. Poisel and U. Schmidt, Chem. Ber. 105, 625 (1972).
7. E. Öhler, H. Poisel, F. Tataruch and U. Schmidt, Chem. Ber. 105, 635 (1972).
8. E. Öhler, F. Tataruch and U. Schmidt, Chem. Ber. 105, 3658 (1972).
9. Y. Kishi, T. Fukuyama and S. Nakatsuka, J. Am. Chem. Soc. 95, 6490 (1973).
10. Y. Kishi, T. Fukuyama and S. Nakatsuka, J. Am. Chem. Soc. 95, 6492 (1973).
11. Y. Kishi, S. Nakatsuka, T. Fukuyama and M. Havel, J. Am. Chem. Soc. 95, 6493 (1973).
12. For example : M.S. Ali, J.S. Shannon and A. Taylor, J. Chem. Soc. (London) C, 2044 (1968).
13. E. Gross, H.H. Kiltz and E. Nebelin, Hoppe-Seyler's Z. Physiol. Chem. 354, 810 (1973).
14. R.D.G. Cooper, J. Am. Chem. Soc. 94, 1018 (1972).
15. E.W. Warnhoff in Fortschritte der Chemie Organischer Naturstoffe, 28, 162 ff., Springer Verlag Wien-New York (1970).
16. J. Marchand, M. Pais, X. Monseur and F.-Xavier Jarreau, Tetrahedron 25, 937 (1969).
17. R. Nagarajan, L.D. Boeck, M. Gorman, R.L. Hamill, C.E. Higgens, M.M. Hoehn, W.M. Stark and J.G. Whitney, J. Am. Chem. Soc. 93, 2308 (1971).
18. H. Poisel and U. Schmidt, Angew. Chem. 1976, in press.
19. E. Öhler and U. Schmidt, Angew. Chem. 1976, in press.
20. H. Poisel, Chem. Ber. 1976, in press.
21. H. Poisel and U. Schmidt, Chem. Ber. 108, 2917 (1975).
22. A.L. Love and R.K. Olsen, J. Org. Chem. 37, 3431 (1972).
23. S.M. Patel, J.O. Currie and R.K. Olsen, J. Org. Chem. 38, 126 (1973)
24. P.J. Machin and P.G. Sammes, J. Chem. Soc. Perkin Transactions I, 698 (1974).
25. U. Schmidt and E. Öhler, Angew. Chem. 88, 54 (1976); Angew. Chem. Int. Ed. 15, 42 (1976).
26. E. Öhler, F. Tataruch and U. Schmidt, Chem. Ber. 106, 165 (1973).
27. E. Öhler, F. Tataruch and U. Schmidt, Chem. Ber. 106, 396 (1973).
28. J. Häusler and U. Schmidt, Chem. Ber. 107, 2804 (1974).
29. U. Schmidt and J. Häusler, Angew. Chem. 1976, in press.
30. E. Öhler and U. Schmidt, Chem. Ber. 108, 2907 (1975).
31. U. Schmidt, A. Perco and E. Öhler, Chem. Ber. 107, 2816 (1974).
32. E. Öhler and U. Schmidt, Chem. Ber., in press