# Peptide Sequencing with the 2-Pyridinecarboxaldehyde Schiff Base Peptide Degradation

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Received March 20, 1980

Dedicated to Professor John C. Sheehan on the occasion of his sixty-fifth birthday.

Peptides treated with 2-pyridinecarboxaldehyde are converted to Schiff bases, pyridoimidazoles, α-ketoacyl amino acids and peptide amides, and diketopiperazines. Nitrogen abstraction and condensation with 2-pyridinecarboxaldehyde gives heterocyclic products. The products are separable by gas chromatography and when analyzed by mass spectrometry afford information for sequence assignment. Thus, a nonvolatile peptide can be converted to a mixture of products in one step that by mass spectrometric analysis affords the sequence. Peptides of up to eight and nine amino acids have been analyzed using this degradation as the basis of a one-step procedure.

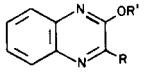
*J. Heterocyclic Chem.*, **17**, 1651 (1980).

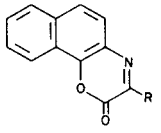
We report here the successful utilization of 2-pyridinecarboxaldehyde in the generation of gc separable compounds from peptides and peptide derivatives in one procedural step which itself reflects a set of reactions. The parent peptide amino acid composition and the mass spectra of these compounds provide sufficient information for the assignment of sequence of the parent peptide. The overlap information appears in the form of dipeptide derivatives generated by random cleavage. Higher molecular weight derivatives are formed but generally are not amenable to gc/ms analysis. Confirmation of reaction products was achieved by comparison with authentic samples and/or by generation of derivatives.

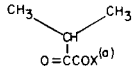
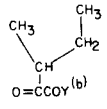
It had been previously reported that certain Schiff base derivatives of peptides gave a large portion of the total ion current in "internal fragments" which were described as fragments of peptide derivatives that had lost portions of both the *C*- and *N*-termini (1). It was subsequently reported that the internal fragments were in part cleavage products derived from reaction prior to electron impact (2) and were amenable to separation by gc. In this report we show the identity of the "internal fragments" and their use in sequence assignments.

Table 1

Derivatives Observed from the PSB Degradation of Val-Ile-Ala





| Cleavage Product | Quinoxalinol | | Methylated | | Trimethyl Silylated | | Oxazinone |
|---|---|---|---|---|---|---|---|
|  | R<br>$CH_3$ | R′<br>H | R<br>$CH_3$ | R′<br>$CH_3$ | R<br>$CH_3$ | R′<br>$Si(CH_3)_3$ | R<br>$CH_3$ |
|  | $C_3H_7$ | H | $C_3H_7$ | $CH_3$ | $C_3H_7$ | $Si(CH_3)_3$ | $C_3H_7$ |
|  | $C_4H_9$ | H | $C_4H_9$ | $CH_3$ | $C_4H_9$ | $Si(CH_3)_3$ | $C_4H_9$ |

(a) X = $NH_2$, $NHCH(C_4H_9)CONH_2$. (b) Y = $NH_2$, $NHC(CH_3)=C=O$.

0022-152X/80/081651-03$02.25

## EXPERIMENTAL

2-Pyridinecarboxaldehyde Schiff Base Treatment.

The peptides and peptide derivatives in glacial acetic acid (~0.1*M*) were treated with 0.1 equivalent up to an equimolar amount of 2-pyridinecarboxaldehyde (2-PC). Usual conditions required 10-30 min., 120°. Mild conditions, *e.g.*, 1 minute, 100°, were suitable for many peptides. *N*-Blocked peptides were treated with 2-PC and dimethylammonium acetate. The Schiff bases of peptide esters and amino acid esters were prepared according to Day, *et al* (3).

GC/MS.

An Hitachi-Perkin-Elmer RMU-7 mass spectrometer interfaced with a Varian Series 1740 gas chromatograph by way of an all-glass Watson-Biemann separator was used (4). For gc/ms runs, the mass spectrometer resolution was usually M/ΔM = 1000. The ionizing voltage was 70 eV.

Derivatives. Quinoxalinols by Procedure 1.

The derivatives were prepared by treatment of hydrolyzates of the 2-pyridinecarboxaldehyde Schiff base (PSB) reaction products with *o*-phenylenediamine (OPDA) in glacial acetic acid by heating a few minutes at 100°. The hydrolyzates were prepared by treatment with 6*N* hydrochloric acid at 105° for 16 hours in an evacuated, sealed tube. Reference compounds were prepared by reaction of appropriate α-keto-acids with OPDA by the procedure of Nielsen (5).

Quinoxalinols by Procedure 2.

The derivatives were generated directly from the PSB products by direct treatment with OPDA in hot (100-120°) glacial acetic acid for a few minutes.

Oxazinone Derivatives.

Treatment of the PSB products with 2-amino-1-naphthol (2-AN) in glacial acetic acid at ~100° for a few minutes gave the corresponding oxazinones.

Silyl Ethers of Quinoxalinols.

Silylation of the quinoxalinols was effected with "Tri-Sil" (6).

Permethylation.

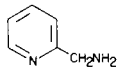
The procedure of MacGee and Allen was utilized (7).

Amino Acid Analyses.

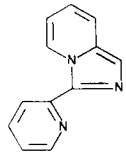
The procedure of Benson was used (8).

## Results.

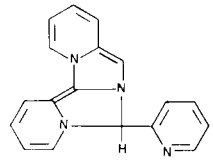
The peptide Val-Ile-Ala, when treated with the PSB reagent, gave cleavage products which could be separated and analyzed by gc/ms. The products from Val-Ile-Ala did not include significant amounts of diketopiperazines, but contained the α-ketoacyl derivatives shown in Table 1. The other major products were compounds **1**, **2**, and **3** below and the three pyridoimidazoles **4**, **5**, and **6**.



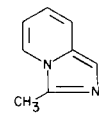
1



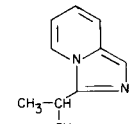
2



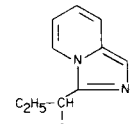
3



4



5



6

Peptides that were *N*-blocked with stable groups such as acetyl or benzoyl were not degraded by the 2-pyridinecarboxaldehyde unless an amine was added. Suitable amines were dimethyl and diethylamine which themselves did not give volatile Schiff base products. Enamine formation was not found to occur under our conditions.

Treatment of control Val-Ile-Ala itself with OPDA or 2-AN gave no detectable modification products. Treatment of the Val-Ile-Ala/PSB reaction products as well as the hydrolysis products with OPDA gave the three expected quinoxalinols (Table 1). The quinoxalinols and their derivatives were confirmed by mass spectral analysis and by comparison with reference compounds synthesized from α-ketoacids. The oxazinones (Table 1) were obtained by treatment of the Val-Ile-Ala/PSB reaction products with 2-AN and compared with samples prepared by condensation of 2-AN with available α-ketoacids.

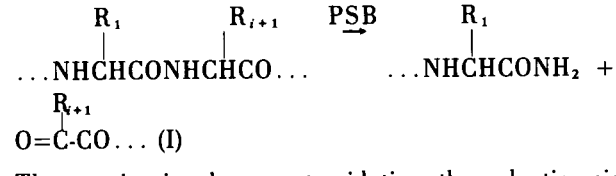
More complex peptides afford the same type of cleavage products. The oxidative deamidation products and DKP's from an octapeptide fragment from glucagon and from synthetic blocked oxytocin are found (Tables 2 and 3). The α-ketoacyl products of the PSB degradation were also confirmed by derivatizing to naphtho-oxazinones. A redundancy of overlap data are generated in both cases. In addition to the α-ketoacyl amino acid amides, some ketenes were noted. The ketenes appear to arise from the amides by processes described by Shemyakin, *et al.* (9).

The degradation also produces products from ammonia abstracted from the peptides principally by transamination. The *N*-terminal amino acid is degraded more rapidly than the other residues and contributes its nitrogen to three products (**1**, **2**, and **3**).

The amides were confirmed by mass spectral analysis and by ammonia analysis of the hydrolyzates of the PSB products.

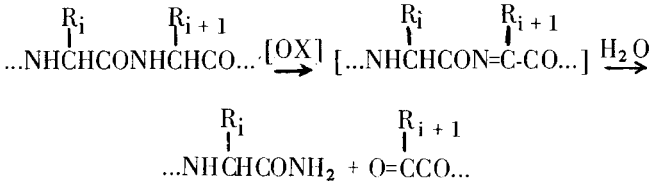
## Discussion.

The formation of α-ketoacyl derivatives by the PSB reagent occurs by a reaction not described before, namely, an oxidative deamidation (Reaction I).




The reaction involves a net oxidation; the reduction side-products identified to date are **1** and **3**. The stoichiometry

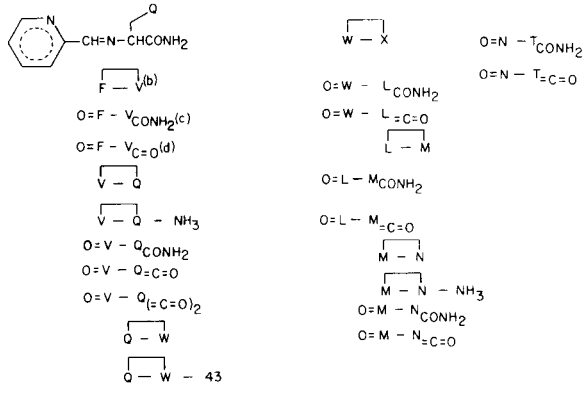
indicates that there are more **1** and **3** than necessary to account for the α-ketoacyl products seen in almost all cases examined. It is suggested that an oxidized intermediate may be formed and cleaved by water formed in Schiff base formation:



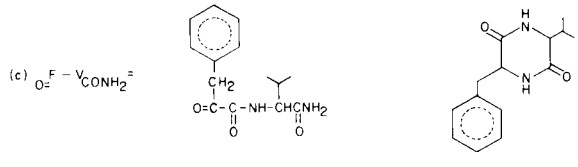
Diketopiperazine (DKP) formation appears to arise both during the PSB treatment and during the gc/ms analysis. Pyrolytic formation of DKP had been noted earlier (10). The sterically hindered peptides such as Val-Ile-Ala show little or no DKP formation whereas most peptides including the octapeptide and the nonapeptide reported here give significant amounts.

Table 2

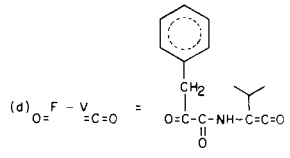
Glucagon Octapeptide Fragment.
Products from PSB Treatment Peptide Sequence.
FVQWLMNT (a) Observed Products



(a) One letter amino acid abbreviations used. (b) $\overbrace{\text{F-V}}$ = Diketopiperazine of Phe and Val. Other diketopiperazines are indicated analogously.



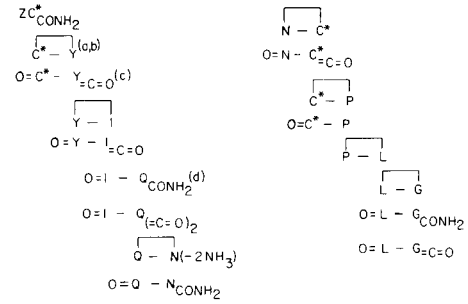
Other α-ketoacylamino acid amides are indicated analogously.



Other ketenes presumably arising from the corresponding amides or acids are indicated analogously. Q and N can form diketenes; only the diketene from Q was found.

Table 3

Blocked Oxytocin. Products from PSB Treatment
Peptide Sequence. Z-C*YIQNC*PLG-$NH_2$ (a)
Observed Proucts



(a) Z is Benzyloxycarbonyl; C* is S-benzylcysteyl; one letter amino acid abbreviations utilized. (b) Analogous to footnote (b), Table 2. (c) Analogous to footnote (d), Table 2. (d) Analogous to footnote (c), Table 2.

The pyridoimidazoles (**4, 5,** and **6**) contain no sequence information; however, the pyridoimidazoles observed in most cases are predominantly derived from the *N*-terminal residue. Their formation is analogous to the the oxidative decarboxylation of amino acids by ninhydrin treatment (11). The pyridoimidazole **2** was identical to that formed by the procedure of (12).

The PSB degradation provides a route to sequence assignment that in some cases requires less time than other procedures. Adequate data have been generated in less than one hour from reaction to gc/ms analysis that allow a sequence assignment to be made. The peptides shown here are of the size typically encountered in enzymatic digests of proteins or in conventional peptide syntheses and thus may be applicable to typical sequencing problems or verification of sequence.

Acknowledgement.

This work was supported in part by grants from NSF GP-8490 and NIH GM-23154. We are grateful to A. Mandelbaum for useful discussions concerning this work and suggesting the identities of compounds **2** and **3**.

REFERENCES AND NOTES

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