Amino Acids and Peptides. Part 48.[1] Studies on the Structure of an Unexpected Reaction Product from Dipeptidyl Chloromethyl Ketone during Acid Hydrolysis†[2]

J. Chem. Research (S), 1997, 10–11
J. Chem. Research (M), 1997, 0171–0185

Toshio Yokoi, Hiroaki Taguchi, Yasuhiro Nishiyama, Kazuo Igarashi, Fumiyo Kasuya and Yoshio Okada*

Faculty of Pharmaceutical Sciences, Kobe-Gakuin University, Nishi-ku, Kobe 651-21, Japan

During the course of the synthesis of peptidyl chloromethyl ketones (CMKs), it was revealed that amino acid recovery after acid hydrolysis (6 mol dm^{-3} HCl, 110 °C, 20 h) of dipeptidyl chloromethyl ketones was markedly low (7.1–33.7%) owing to the formation of 5-methylpyrazin-2(1*H*)-one derivatives during the acid hydrolysis.

Previously, we reported that Boc-Phe-Leu-CH_2Cl gave a Phe residue in only a low yield (13.0%) on an amino acid analysis of its acid hydrolysate and, instead, a diethyl ether-soluble substance (62.3% in isolated yield) was obtained.[5] Its spectroscopic data showed it to be not an amino acid or peptide but an unexpected product. This paper deals with the structure elucidation of the unexpected product and the study of the reaction mechanism.

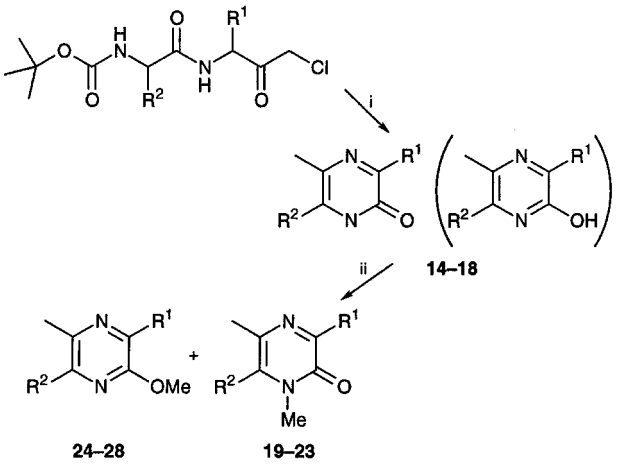
First, to examine whether or not other dipeptidyl CMKs show low recoveries of amino acid on amino acid analysis of their acid hydrolysates as stated above, we synthesized various dipeptidyl CMKs. The results of the amino acid analysis of acid hydrolysates of Boc-dipeptidyl CMKs exhibited markedly low (7.1–33.7%) recovery of the constituent amino acid for each dipeptidyl CMK. These results suggested that a similar, unexpected reaction occurred during acid hydrolysis of all the dipeptidyl CMKs so far examined.

To elucidate their structure, the unexpected products formed from dipeptidyl CMKs during acid hydrolysis were isolated in pure form. Their molecular formulae were deduced from their mass spectra and elemental analyses. The results suggest that, during the acid hydrolysis, removal of the Boc group, intramolecular dehydration and substitution of the chlorine atom with a hydrogen atom occur.

The ^{1}H NMR spectrum of **14** obtained from Boc-Phe-Leu-CH_2Cl shows the presence of isobutyl [δ 0.97 (6 H, d, J 6.6 Hz), 2.05 (1 H, m), 2.40 (2 H, d, J 7.4 Hz)], benzyl [δ 4.06 (2 H, s), 7.14–7.41 (5 H, m)], vinyl methyl [δ 2.28 (3 H, s)], and N-H [δ 13.3 (1 H, br s)]. No methine proton (α-proton to carbonyl) is present. In the ^{13}C NMR spectrum of **14**, five signals (δ 157.6, 154.1, 138.2, 135.1, 130.0) owing to quaternary sp^2 carbons are observed. One of them is ascribable to a benzene carbon, and the other four would be assigned to newly formed ring carbons. ^{1}H and ^{13}C NMR spectra of the other unexpected products show also the presence of signals corresponding to the side chains of the constituent amino acid in the corresponding Boc-dipeptidyl CMKs and the presence of newly formed ring carbons.

From these observations, the structures of the unexpected products are proposed as **14–18**.

To confirm further the structures of **14–18**, these compounds were treated with diazomethane. In diethyl ether the reaction gave two compounds from each starting material, presumably owing to the occurrence of both *N*- and *O*-methylation, as shown in Scheme 2.



14, 19, 24, R^1 = Bz, R^2 = Bu^i
15, 20, 25, R^1 = Bz, R^2 = Me
16, 21, 26, R^1 = Bz, R^2 = Bz
17, 22, 27, R^1 = H, R^2 = Bz
18, 23, 28, R^1 = Bz, R^2 = H

Scheme 2 *Reagents and conditions:* i, 6 mol dm^{-3} HCl, 110 °C, 20 h; ii, CH_2N_2–Et_2O, room temperature

The ^{1}H and ^{13}C NMR spectra of the *N*-methylated products, rather than the *O*-methylated ones, are similar to those of the parent compounds, indicating that the products exist preferentially in their keto forms. This fact is consistent with the conclusion reported by Cox and Bothner-By that 2-hydroxy-3-methylpyrazine exists in the keto form in neutral solution.[11,12]

Finally, to elucidate the mechanism of ring formation from dipeptidyl chloromethyl ketones during acid hydrolysis, the cyclization reaction was carried out in a deuterated solvent ($DCl–D_2O–CD_3OD$). In this experiment, deuteration of the methyl group at position 5 was observed by ^{1}H NMR. This result suggests that the substitution of the chlorine atom with deuterium from the deuterated solvent occurs during the ring formation reaction. On the other hand, when compound **14** was refluxed in a deuterated solvent for 2 h, the methyl group at position 5 was not deuterated. From these results, we were able to deduce the reaction mechanism for the formation of pyrazin-2(1*H*)-one derivatives from dipeptidyl chloromethyl ketones as shown in Fig. 3.

In conclusion, we have shown that recovery of amino acid on amino acid analysis after acid hydrolysis (6 mol dm^{-3} HCl, 110 °C, 20 h) of Boc-dipeptidyl CMKs is generally low owing to the occurrence of a side reaction during acid hydrolysis and the structure of the side reaction product was well elucidated. Furthermore, it can be emphasized that the treatment of dipeptidyl CMKs by hydrochloric acid would be a simple and convenient synthetic procedure for 2-hydroxypyrazine [or pyrazin-2(1*H*)-one] derivatives with various substituents at the 3 and 6 positions.

*To receive any correspondence (*e-mail:* okada@pharm.kobegakuin.ac.jp).

†The customary L indication for amino acid residues is omitted. Standard abbreviations for amino acids, peptides and their derivatives are those recommended by the IUPAC–IUB Commission on Biochemical Nomenclature: *Biochemistry*, 1966, **5**, 2485; 1967, **6**, 362; 1972, **11**, 1726. Other abbreviations used are: Boc, *tert*-butoxycarbonyl; Et_3N, triethylamine; NMM, *N*-methylmorpholine; DMF, *N*,*N*-dimethylformamide; AcOEt, ethyl acetate; THF, tetrahydrofuran; Bzl, benzyl.

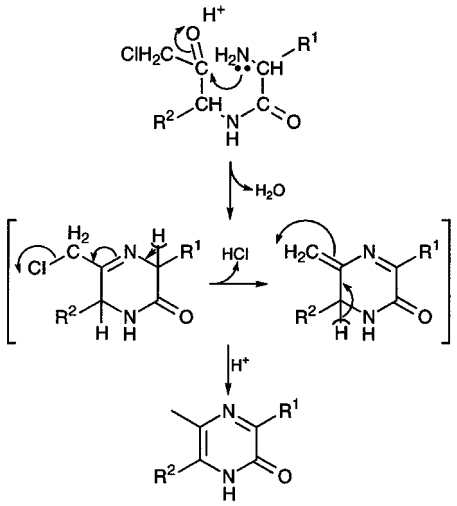


Fig. 3 Proposed reaction mechanism of pyrazin-2(1*H*)-one derivatives during acid hydrolysis

Techniques used: ^{1}H and ^{13}C NMR, MS, TLC, polarimetry, amino acid analysis

References: 20

Schemes: 2

Table 1: Amino acid recovery after acid hydrolysis of peptidyl CMKs

Table 2: ^{13}C NMR chemical shifts of *N*- or *O*-methylated pyrazines **19–28** and their parent pyrazinones **14–18**

Fig. 1: ^{1}H NMR spectra of Boc-Phe-Leu-CH_2Cl and the unexpected product **14**

Fig. 2: ^{1}H NMR spectra of **14** and its deuterated product

Received, 23rd September 1996; Accepted, 11th October 1996
Paper E/6/06548A

References cited in this synopsis

1 Part 47: Y. Okada, H. Taguchi and T. Yokoi, *Chem. Pharm. Bull*., 1996, **44**, 2259.
2 Preliminary communication, H. Taguchi, T. Yokoi, F. Kasuya, Y. Nishiyama, M. Fukui and Y. Okada, *J. Chem. Soc*., *Chem. Commun*., 1994, 247.
5 H. Taguchi, Y. Nishiyama, A. C. M. Camargo and Y. Okada, *Chem. Pharm. Bull*., 1993, **41**, 2038.
11 R. H. Cox and A. A. Bothner-By, *J. Phys. Chem*., 1968, **72**, 1642.
12 R. H. Cox and A. A. Bothner-By, *J. Phys. Chem*., 1968, **72**, 1646.