Unexpected Reaction of Dipeptidyl Chloromethyl Ketone during Acid Hydrolysis

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Amino acid recovery of dipeptidyl chloromethyl ketones is remarkably low owing to the formation of 2-hydroxy-3, 6-dialkyl-5-methylpyrazine during acid hydrolysis.

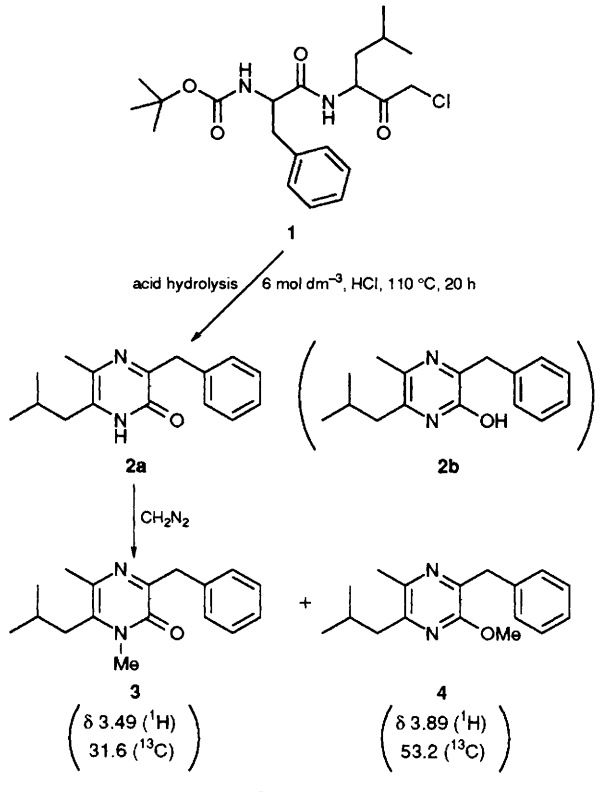
Peptidyl chloromethyl ketones (CMKs) are well known irreversible inhibitors for serine and thiol proteinases, and offer one of the useful tools to investigate properties of these enzymes.[1,2]

Previously, we reported that Boc-Phe-Leu-CH_2Cl gave the Phe-residue in low yield (13.0%) on amino acid analysis of its acid hydrolysate and a diethyl ether-soluble substance (62.3% in isolated yield),[3] although the structure of this product was not yet studied. Its spectroscopic data showed it to be an unexpected reaction product. This communication deals with the structural elucidation of the unexpected product.

First, we synthesized the various dipeptidyl CMKs, and examined whether these CMKs showed low recovery of amino acid as stated above. As shown in Table 1, amino acid recovery was remarkably low (11.8–33.7%) in every case of dipeptidyl CMK, while acid hydrolysate of tripeptidyl CMK gave normal recovery of constituent amino acids. These results suggested that a similar unexpected reaction occurred during acid hydrolysis (6 mol dm^{-3} HCl, 110 °C, 20 h) of all dipeptidyl CMKs.

Table 1 Amino acid recovery after acid hydrolysis of peptidyl CMKs

Compound	Recovery (amino acid) (% yield)
Boc-Phe-Leu-CH_2Cl	13.0 (Phe)
Boc-Phe-Gly-CH_2Cl	28.0 (Phe)
Boc-Phe-Ala-CH_2Cl	11.8 (Phe)
Boc-Phe-Phe-CH_2Cl	12.9 (Phe)
Boc-Gly-Leu-CH_2Cl	33.7 (Gly)
Boc-Gly-Ala-CH_2Cl	23.9 (Gly)
Boc-Gly-Phe-CH_2Cl	13.0 (Gly)
Boc-Gly-Gly-CH_2Cl	26.4 (Gly)
Boc-Ala-Ala-CH_2Cl	33.1 (Ala)
Boc-Gly-Phe-Leu-CH_2Cl	80.2 (Phe) [85.3 (Gly)]



Scheme 1

We examined the acid hydrolysate of Boc-Phe-Leu-CH_2Cl **1** because of its high yield of the unexpected reaction product. The diethyl ether-soluble reaction product **2** was obtained as colourless needles, mp 133–135 °C, $[\alpha]_D^{25}$ 0. Its mass spectrum [*m/z* 256 (M^+)] and elemental analyses indicated the molecular formula of $C_{16}H_{20}N_2O$.

^1H NMR spectrum of **2** shows the presence of isobutyl [δ 0.97 (6H, d, *J* 6.6 Hz), 2.05 (1H, m), 2.40 (2H, d, *J* 7.4 Hz)], benzyl [δ 4.06 (2H, s), 7.14–7.41 (5H)], vinyl methyl [δ 2.28 (3H, s)], and N–H [δ 13.3 (1H, br s)]. Methine proton (α-proton to carbonyl) is not present. In ^{13}C NMR spectrum of **2**, 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 four would be assigned to the newly-formed ring carbons.

From these observations, the structure of the unexpected product is proposed as **2a** or **2b** (a tautomer of **2a**).

The reaction of the product **2** with diazomethane in diethyl ether gave an *N*-methyl derivative **3** [δ 3.49 (^1H), 31.6 (^{13}C)] and an *O*-methyl derivative **4** [δ 3.89 (^1H), 53.2 (^{13}C)] in a ratio of 2:3 (Scheme 1). ^1H and ^{13}C NMR spectra of **2** are similar to those of **3**, indicating that the product exists in a keto form. This fact is consistent with the conclusion reported by Cox and Bothner-By[4] that 2-hydroxy-3-methylpyrazine exists in the keto form in neutral solution. Therefore the product is assigned to be **2a**.

The structures of major unexpected reaction products (yields 60–75%) of other dipeptidyl CMKs were also established to be 2-hydroxypyrazine derivatives by the spectroscopic method.

Thus, it was revealed that recovery of amino acid on amino acid analysis after acid hydrolysis (6 mol dm^{-3} HCl, 110 °C, 20 h) of dipeptidyl CMKs is generally low due to the occurrence of a side reaction during acid hydrolysis. Furthermore, it can be emphasized that the treatment of dipeptidyl CMKs by hydrochloric acid would be a simple and convenient synthetic method for 2-hydroxy-pyrazine (or 2-pyrazinone) derivatives with various substituents at 3 and 6 positions which might be key intermediates for acylating agents.[5,6]

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