UNUSUAL ENAMIDE CLEAVAGE OF FRANGUFOLINE UNDER MILD ACIDIC CONDITION[1]

Dae-Yeon Suh, Yong Chul Kim, Yong Nam Han, and Byung Hoon Han*

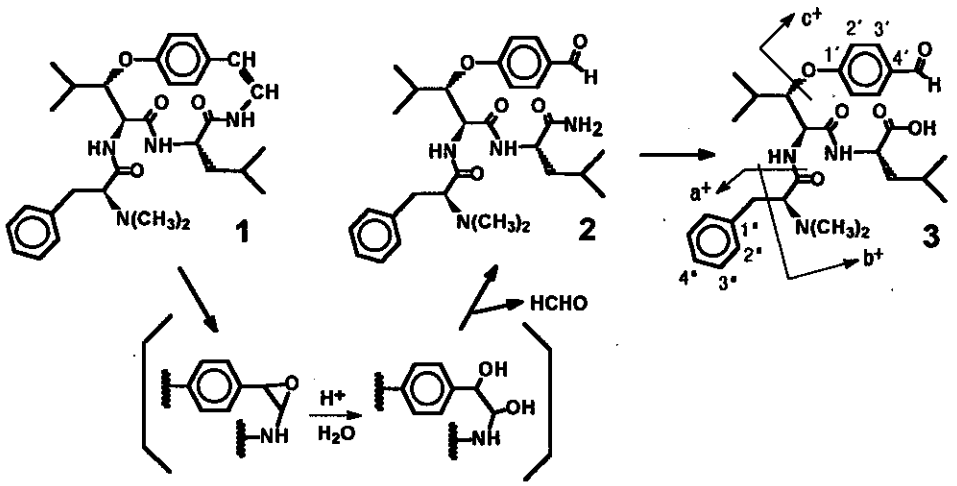
Natural Products Research Institute, Seoul National University,
28 Yunkeun-dong, Chongno-ku, Seoul 110-460, Republic of Korea

Abstract - In mild acidic condition (2N HCl, 55 °C), frangufoline, a frangulanine type 14-membered cyclopeptide alkaloid was first converted via unusual enamide cleavage to sanjoinine-G2, which was further hydrolyzed to a linear compound, (*S*)-[(*N'*,*N'*)-dimethylphenylalanyl]-(2*S*,3*S*)-3-(*p*-formylphenoxyleucyl)-(*S*)-leucine. From the extensive acid hydrolysates, 4-hydroxybenzaldehyde was also isolated. Air oxidation of the vinylic double bond followed by the liberation of formaldehyde is proposed for a possible mechanism for the ring cleavage.

Several sedative frangulanine type 14-membered cyclopeptide alkaloids including frangufoline (**1**) have been isolated from the seeds of Zizyphus vulgaris var. spinosus L., an important sedative in Oriental medicine.[2] Frangulanine type cyclopeptide alkaloids are characterized by strained 14-membered ring system and an aryl ether resulting from *p*-hydroxystyrylamine and β-hydroxyleucine.[3] SAR study of a series of cyclopeptide alkaloids revealed that the styrylamine unit is important for the in vivo activity.[4] Reduction or hydration of the double bond resulted in significant loss in activity. In the course of metabolic study on **1**, we found that **1** was rapidly converted to a metabolite in rodents in vivo and in vitro and that the metabolite was identical based on hplc with a compound (**3**) that was produced by acid treatment of **1**. Herein, the structure of **3** is described and a possible mechanism for the ring cleavage is discussed.

A soln of **1** (30 mg) in 2N HCl was heated at 55 °C for 10 h in open air. The reaction was monitored by hplc analysis.[5] Following neutralization by 1N NaOH, the reaction mixture was extracted with BuOH, and the BuOH extract was divided into two fractions (A and B) by

column chromatography on silicagel eluting with a stepwise gradient of $CHCl_3$-MeOH (50 : 1, 20 : 1). Fraction A was subjected to column chromatography on silica gel ($CHCl_3$-MeOH, 50 : 1) to yield 4-hydroxybenzaldehyde (1 mg) and crude **2**. **2** was further purified by rechromatography on silica gel (3.2 mg, $CHCl_3$-MeOH-HOAc, 30: 1 : 0.5). Fraction B was chromatographed on silica gel (Benzene-EtOH, 5 : 1) to give **3** (10 mg).



A time course study using hplc revealed that **1** was first converted to **2**, which was subsequently converted to **3** (data not shown). It was first thought that **2** would be formed by ring cleavage via usual enamide hydrolysis of **1**, thus having para-substituted phenylacetaldehyde and leucinamide moieties at the open ends. However, the results obtained in this study proved that this is not the case.

Uv spectra of **3**, which exhiited λ_{max} 277 nm (logε 5.0 in CH_3OH) indicated that **3** is a linear molecule, as 14-membered cyclopeptide alkaloids generally show only end absorption due to the strained ring system.[2,3] Its ^1H nmr spectrum was similar to that of **1**, but two olefinic proton signals and the amide proton signal of styrylamide unit of **1** were absent in **3**. Instead, a formyl proton at δ 9.70 (1H, *s*) was observed.[6] More importantly, two doublet peaks at δ 7.00 and 7.71 (each 2H, J = 8.8 Hz) attributable to 1,4-disubstituted phenyl protons correspond well with the aromatic protons of 4-hydroxybenzaldehyde, which, in fact, was isolated from the acid hydrolysates of **1**.[7] These two proton signals were also indicative of the ring cleavage, since the corresponding aromatic protons should give four distinctive signals (each 1H) in 14-membered cyclopeptide ring systems.[8] An additional support for the attachment of -CHO other than $-CH_2CHO$ on the *p*-phenoxy group in **3** was provided by Elmass spectrum of **3**.[9] By an aromatic McLafferty rearrangement[10] the fragment originating

from the aromatic nucleus gave the molecular ion peak (c^+) at m/z 121. The presence of a carboxyl group at the other end of **3** was confirmed by methylation with CH_2N_2.[11] The CImass with NH_3 as the chemical ionization source of **3** exhibited a $[M+H]^+$ ion at m/z 540 with the molecular formula of $C_{30}H_{41}N_3O_6$, which was established by HREImass spectrometry.[12] The above findings together with the analysis of nmr spectra (^1H-^1H COSY (DQF), ^1H-^{13}C COSY (HMQC) and DEPT) enabled the structure of **3** to be determined as (*S*)-[(*N'*,*N'*)-dimethylphenylalanyl]-(2*S*,3*S*)-3-(*p*-formylphenoxyleucyl)-(*S*)-leucine. Since epimerization was not expected to occur under the reaction conditions employed, the stereochemistry of **3** was assigned referring to that of **1**, which has been established by means of gc analyses of diastereomeric derivatives of individual amino acid units in the acid hydrolysates and NOE.[13]

Compound **(2)** was readily assigned as sanjoinine-G2 ((*S*)-[(*N'*,*N'*)-dimethylphenylalanyl]-(2*S*,3*S*)-3-(*p*-formylphenoxyleucyl)-(*S*)-leucinamide) by direct comparison with the authentic sample.[2,14]

Having established the structures of **2** and **3**, it became clear that **3** was produced by acid hydrolysis of the leucinamide of **2**. However, **2** should have been produced from **1** via an unusual enamide cleavage. The finding that conversion of **1** to **2** went at negligible rate under otherwise identical N_2 atmosphere indicated that molecular oxygen plays a role in the cleavage reaction. Based on this and the structures of **1** and **2**, it was proposed that the enamide bond in **1** is not cleaved via usual acid-catalyzed enamide hydrolysis probably due to the ring strain, rather **1** is first oxidized on the vinylic double bond, possibly to an epoxide, which subsequently undergoes hydrolysis and a retro-aldol type rearrangement to produce **2** with liberation of HCHO. In fact, HCHO formed from the degradation was detected by the reaction with dimedone to yield methylenebisdimedone.[15] Although **2** has previously been obtained from **1** by ozonolysis[16] or by OsO_4-$NaIO_4$ and alkali,[2] this is the first report that a frangulanine type cyclopeptide alkaloid undergoes unusual enamide cleavage, in mild acidic conditions, to be converted to a linear compound with one less carbon unit.

REFERENCES AND NOTES

1. Supported by a grant (G7 project, 4-4-4) from the Ministry of Science and Technology, R.O.K.
2. B. H. Han, M. H. Park, and Y. N. Han, *Phytochemistry*, 1990, **29**, 3315; B. H. Han, M.

H. Park, and J. H. Park, *Pure & Appl. Chem.*, 1989, **61**, 443.

3. R. Tschesche and E. U. Kaußmann, *The Alkaloids. Chemistry and Pharmacology*, Vol. XV, ed. by R. H. F. Manske, Academic Press, New York, 1975, pp. 165-205; K. L. Bhat and M. M. Joullie, *J. Chem. Ed.*, 1987, **64**, 21.
4. B. H. Han, M. H. Park, and Y. N. Han, *Yakhak Hoeji*, 1993, **37**, 143.
5. Column: RPC_{18}, 5 μm, 4.6×150 mm; solvent: MeCN-H_2O (1 : 2.5, pH 3 with H_3PO_4); flow rate: 1 ml/min; detection: uv 230 or 280 nm; R_t: **1**, 12.7; **2**, 9.2; **3**, 17.2; 4-hydroxybenzaldehyde, 4.4 min.
6. Crystalline solid ($CHCl_3$), mp 207-208 ° (uncorr.), $[\alpha]^{22}_D$ -80 ° (MeOH; c 0.350); Ir (cm^{-1}, KBr): 3440 (NH, COOH), 1682 - 1655 (amide, aldehyde), 1255 (al-C-O-C-ar) cm^{-1}. ^1H Nmr (500 MHz, CD_3OD, TMS): 0.79, 0.82 (each 3H, d, *J* = 6.0 Hz, 2×Leu-Me), 0.83, 0.91 (each 3H, d, *J* = 6.8 Hz, 2×hyLeu-Me), 1.43 (2H, m, β-H of Leu), 1.46 (1H, m, γ-H of Leu), 1.83 (1H, m, γ-H of hyLeu), 2.86 (6H, s, *N,N*-Me_2), 3.06, 3.25 (each 1H, dd, *J* = 5.2, 11 Hz, β-H of *N,N*-Me_2Phe), 4.04 (1H, t, *J* = 5.2 Hz, α-H of *N,N*-Me_2Phe), 4.08 (1H, dt, *J* = 6.3, 8.8 Hz, α-H of Leu), 4.60 (1H, m, β-H of hyLeu), 4.68 (1H, m, α-H of hyLeu), 6.84 (1H, d, *J* = 8.7 Hz, NH-Leu), 7.00 (2H, d, *J* = 8.8 Hz, H-2′), 7.14 (2H, dd, *J* = 1.1, 7.5 Hz, H-3″), 7.18 (1H, d, *J* = 7.4 Hz, H-4″), 7.21 (2H, d, *J* = 7.6 Hz, H-2″), 7.23 (1H, d, *J* = 6.8 Hz, NH-hyLeu), 7.71 (2H, d, *J* = 8.8 Hz, H-3′), 9.70 (1H, s, CHO); ^{13}C nmr (125 MHz, CD_3OD): 17.2, 20.9 (hyLeu-Me), 22.7, 23.4 (Leu-Me), 26.2 (γ-C of Leu), 31.4 (γ-C of hyLeu), 36.0 (β-C of *N,N*-Me_2Phe), 42.4 (β-C of Leu), 43.0 (*N,N*-Me_2), 52.5 (α-C of Leu), 56.4 (α-C of hyLeu), 70.8 (α-C of *N,N*-Me_2Phe), 82.7 (β-C of hyLeu), 116.9 (C-2′), 129.2 (C-4″), 130.4 (C-2″), 130.6 (C-3″), 132.9 (C-3′), 136.7 (C-4′), 145.9 (C-1″), 167.5 (C-1′), 170.3 (Leu-COOH), 175.2 (hyLeu-CO), 178.6 (*N,N*-Me_2Phe-CO), 193.5 (CHO).
7. 4-Hydroxybenzaldehyde. ^1H Nmr (300 MHz, $CDCl_3$, TMS): 5.85 (1H, s, OH), 6.96 (2H, dd, *J* = 1.8, 6.9 Hz), 7.82 (2H, dd, *J* = 1.8, 6.9 Hz), 9.88 (1H, s, CHO).
8. B. H. Han, Y. C. Kim, M. K. Park, J. H. Park, H. J. Go, H. O. Yang, D.-Y. Suh, and Y.-H. Kang, *Heterocycles*, 1995, **41**, 1909.
9. Elms (70 eV) m/z (rel. int): 539 $[M]^+$ (0.01), 462 (2), 448 (b^+, 10), 326 (11), 195 (11), 167 (5), 148 (a^+, 100), 121 (c^+, 53).
10. E. Zbiral, E. L. Menard, and J. M. Muller, *Helv. Chim. Acta*, 1965, **48**, 404; B. K. Cassels, G. Eckhardt, E. U. Kaußmann, and R. Tschesche, *Tetrahedron*, 1974, **30**, 2461.
11. Methyl ester of **3**, amorph., ^1H nmr (500 MHz, $CDCl_3$, TMS): 3.43 (3H, s, -$COOCH_3$).

12. HRms : calculated for $C_{30}H_{41}N_3O_6$: 539.2995. Found : 539.2975

13. B. H. Han and M. H. Park, *Arch. Pharm. Res.*, 1987, **10**, 203.

14. ^{1}H Nmr (300 MHz, $CDCl_3$, TMS): 0.82, 0.86 (each 3H, *d*, *J* = 6.0 Hz, 2×Leu-Me), 0.94, 1.02 (each 3H, *d*, *J* = 6.9 Hz, 2×hyLeu-Me), 1.49 (2H, *m*, β-H of Leu), 1.66 (1H, *m*, γ-H of Leu), 1.92 (1H, *m*, γ-H of hyLeu), 2.28 (6H, *s*, *N,N*-Me_2), 2.90, 3.12 (each 1H, *dd*, *J* = 5.7, 14 Hz, β-H of *N,N*-Me_2Phe), 3.33 (1H, *t*, *J* = 5.7 Hz, α-H of *N,N*-Me_2Phe), 4.30 (1H, *m*, α-H of Leu), 4.66 (1H, *m*, β-H of hyLeu), 4.72 (1H, *m*, α-H of hyLeu), 5.07, 5.86 (each 1H, *s*, amide NH_2), 6.40 (1H, *d*, *J* = 8.1 Hz, NH-Leu), 7.06 (2H, *d*, *J* = 8.7 Hz, H-2'), 7.19-7.26 (5H, aromatic), 7.57 (1H, *d*, *J* = 8.7 Hz, NH-hyLeu), 7.80 (2H, *d*, *J* = 9.0 Hz, H-3'), 9.86 (1H, *s*, CHO).

15. At the end of the reaction at 55°C in 2N HCl, the pH was adjusted to 4 with NH_4OH and 3 eq. of dimedone (5,5-dimethyl-1,3-cyclohexanedione) was added to the reaction mixture. After standing for 24 h at 25°C in a sealed vial, methylenebisdimedone was detected from the butanol extract by direct comparison with the reaction product of dimedone and HCHO on tlc and hplc. Tlc: Merck silica gel 60F, $CHCl_3$-MeOH-HOAc, 200 : 1 : 0.1, R_f = 0.53, uv detection. Hplc: RPC_{18}, 5 μm, 4.6×150 mm; solvent: MeCN-H_2O (1 : 2.5, pH 3 with H_3PO_4); flow rate: 1 ml/min; detection: uv 250 nm; R_t: 22.0 min.

16. F. K. Klein and H. Rapoport, *J. Am. Chem. Soc.*, 1968, **90**, 3576.

Received, 24th July, 1996