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# Phenylacetyl pepstatin inhibitors of aspartyl proteases from *Streptomyces varsoviensis*

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## Abstract

A new pepstatin with a phenylacetyl group, pepstatin Pa (1), and its methyl ester (2) were isolated from *Streptomyces varsoviensis* DSM 40346. Their structures were determined by high-resolution mass spectrometry and nuclear magnetic resonance techniques. The absolute configuration was determined using the Marfey´s method. Both pentapeptide products are inhibitors of pepsin and cathepsin D. Interestingly, the bacterial genome contains no biosynthetic gene cluster for the new pepstatin, suggesting an extrachromosomal origin of the biosynthetic genes.

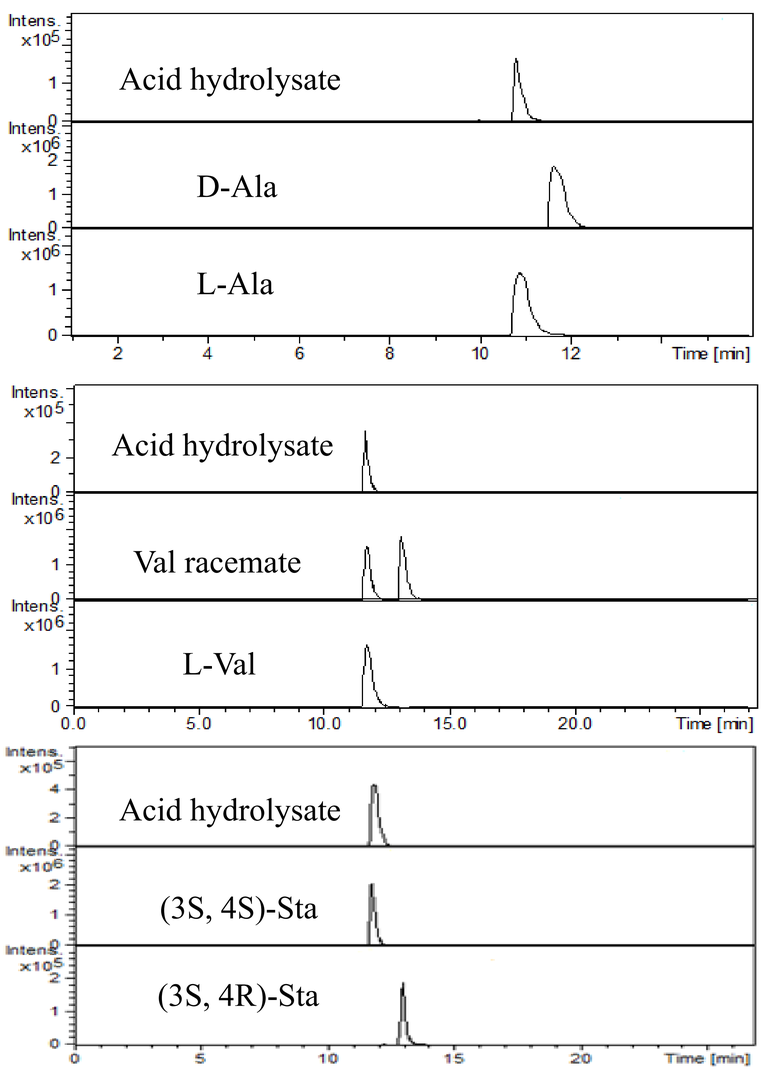
## Structure assignment

**Table 1.** The $^{1}$H and $^{13}$C NMR data for **1** and **2** in DMSO-$d_6$.

| | | Pepstatin Pa (1) | | Pepstatin Pa methyl ester (2) | |
|---|---|---|---|---|---|
| | | $\delta_C$, type | $\delta_H$ ($J$ in Hz) | $\delta_C$, type | $\delta_H$ ($J$ in Hz) |
| Acyl | CO | 170.1. C | | 170.1. C | |
| Benzyl | $CH_2$ | 42.1. $CH_2$ | $H_a$ 3.45. d (16.0) | 42.1. $CH_2$ | $H_a$ 3.45. d (16.0) |
| | | | $H_b$ 3.54. d (16.0) | | $H_b$ 3.54. d (16.0) |
| | 1 | 136.7. C | | 136.7. C | |
| | 2.6 | 128.1. CH | 7.27. m | 128.1. CH | 7.27. m |
| | 3.5 | 129.0. CH | 7.25. m | 129.0. CH | 7.25. m |
| | 4 | 126.2. CH | 7.19. m | 126.2. CH | 7.19. m |
| Val-1 | CO | 170.7. C | | 170.7. C | |
| | α | 57.7. CH | 4.21. dd (8.9. 7.1) | 57.7. CH | 4.21. dd (8.9. 7.1) |
| | ß | 30.5. CH | 1.93. m | 30.5. CH | 1.93. m |
| | γ | 18.2. $CH_3$ | 0.80. d[a] | 18.2. $CH_3$ | 0.79. d[a] |
| | γ' | 19.3. $CH_3$ | 0.81. d[a] | 19.3. $CH_3$ | 0.80. d[a] |
| | NH | | 8.12. d (8.8) | | 8.12. d (8.9) |
| Val-2 | CO | 170.7. C | | 170.7. C | |
| | α | 58.1. CH | 4.13. dd (9.0. 7.3) | 58.1. CH | 4.13. dd (9.0. 7.3) |
| | ß | 30.3. CH | 1.94. m | 30.3. CH | 1.94. m |
| | γ | 18.1. $CH_3$ | 0.80. d[a] | 18.1. $CH_3$ | 0.79. d[a] |
| | γ' | 19.2. $CH_3$ | 0.81. d[a] | 19.2. $CH_3$ | 0.80. d[a] |
| | NH | | 7.89. d (7.4) | | 7.90. d (7.4) |
| Sta-1 | CO | 171.0. C | | 171.0. C | |
| | α | 39.3. $CH_2$ | 2.12. dd (8.0.16.0) | 39.3. $CH_2$ | 2.12. dd (8.0. 16.0) |
| | ß | 68.6. CH | 3.84. m | 68.6. CH | 3.85. m |
| | γ | 50.7. CH | 3.81. m | 50.6. CH | 3.81. m |
| | δ | 38.6. $CH_2$ | $H_a$ 1.26. m | 38.3. $CH_2$ | $H_a$ 1.26. m |
| | | | $H_b$ 1.36.m | | $H_b$ 1.36. m |
| | ε | 24.2. CH | 1.53. m | 24.2. CH | 1.53. m |
| | ζ | 21.7. $CH_3$ | 0.82. d[a] | 21.6. $CH_3$ | 0.82. d[a] |
| | ζ ' | 23.2. $CH_3$ | 0.86. d[a] | 23.2. $CH_3$ | 0.85. d[a] |
| | NH | | 7.46. d (9.1) | | 7.46. d (8.8) |
| Ala | CO | 172.2. C | | 172.3. C | |
| | α | 48.3. CH | 4.24. m | 48.3. CH | 4.22. m |
| | ß | 18.5. $CH_3$ | 1.17. d (7.1) | 18.4. $CH_3$ | 1.17. d (7.1) |
| | NH | | 7.90. d (7.4) | | 7.91. d (7.4) |
| Sta-2 | CO | 173.0. C | | 171.9. C | |
| | $OCH_3$ | | | 51.2. $CH_3$ | 3.56. s |
| | α | 39.3. $CH_2$ | $H_a$ 2.12. dd (8.0. 16.0) | 39.0. $CH_2$ | $H_a$ 2.19. dd (8.0. 16.0) |
| | | | $H_b$ 2.25. dd (8.0. 16.0) | | $H_b$ 2.33. dd (8.0. 16.0) |
| | ß | 69.0. CH | 3.83. m | 69.0. CH | 3.86. m |
| | γ | 50.3. CH | 3.82. m | 50.2. CH | 3.82. m |
| | δ | 38.6. $CH_2$ | $H_a$ 1.26. m | 38.3. $CH_2$ | $H_a$ 1.26. m |
| | | | $H_b$ 1.36. m | | $H_b$ 1.36. m |
| | ε | 24.2. CH | 1.53. m | 24.2. CH | 1.53. m |
| | ζ | 22.0. $CH_3$ | 0.82. d[a] | 21.9. $CH_3$ | 0.82. d[a] |
| | ζ ' | 23.5. $CH_3$ | 0.86. d[a] | 23.4. $CH_3$ | 0.85. d[a] |
| | NH | | 7.35. d (9.4) | | 7.38. d (9.2) |

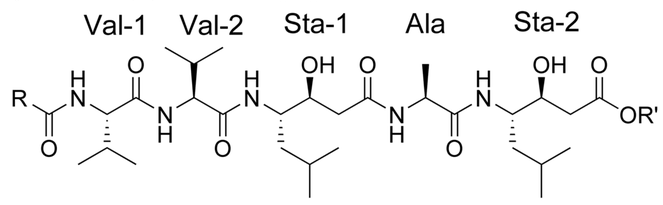
[a] $J$-value was not determined due to overlapped signals.

**Figure 2**. UPLC-MS analysis of the L-FDLA derivatives of the acid hydrolysate of pepstatin Pa (**1**).




## Structure

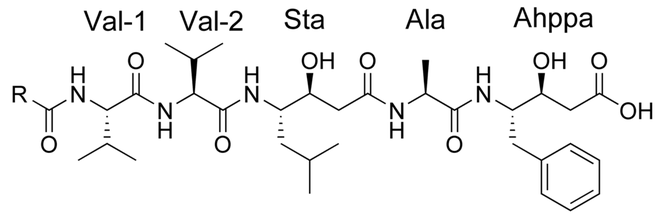
**Figure 1**. Structure of pepstatins and ahpatinins. Pepstain Pa (**1**) and its methyl ester (**2**) are new pepstatins with an aromatic acyl group.




**Pepstatin A:** R = $(CH_3)_2CHCH_2$; R' = H

**Pepstatin Pa (1):** R = $C_6H_5CH_2$; R' = H

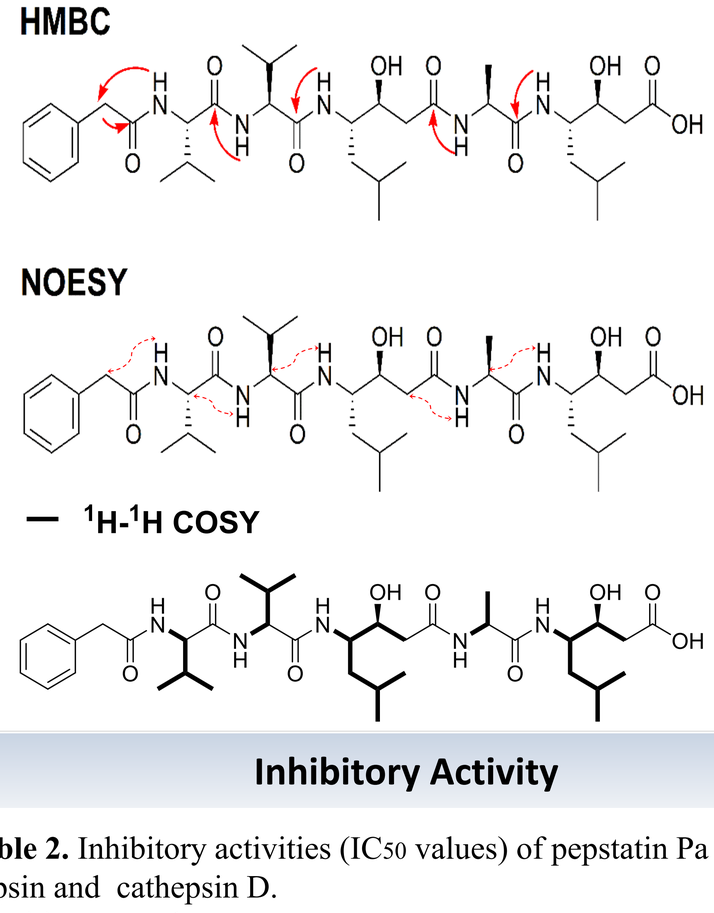
**Pepstatin Pa methyl ester (2):** R = $C_6H_5CH_2$; R' = $CH_3$




**Ahpatinins:** R = alkyl

## Structure determination

**Figure 3**. Inter-residue HMBC, NOESY and 1H-1H COSY correlations across the amide bonds in pepstatin Pa (**1**).




## 2D-NMR Spectrum of Pepstatin Pa(1)

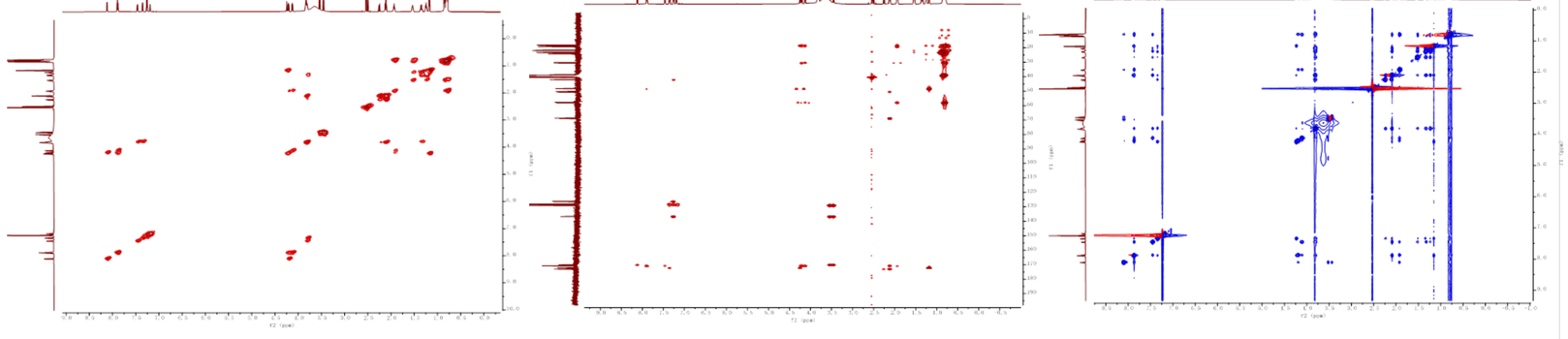


**Figure 4**. $^{1}$H-$^{1}$H COSY, HMBC and NOESY spectrum of pepstatin Pa (**1**) in DMSO-*d*6 (800 MHz)

## Inhibitory Activity

**Table 2.** Inhibitory activities ($IC_{50}$ values) of pepstatin Pa (**1**) against pepsin and cathepsin D.
*Positive control.

| | Pepstatin A* | Pepstatin Pa (1) | Pepstatin Pa methyl ester (2) |
|---|---|---|---|
| Pepsin (nM) | 41 ± 10 | 69 ± 23 | 49 ± 11 |
| Cathepsin D (μM) | <0.01 | 3.1 ± 0.1 | 2.6 ± 0.1 |

## Summary

In summary, a new pepstatin with an aromatic acyl group, pepstatin Pa (**1**), has been identified, purified and characterized from *Streptomyces varsoviensis* DSM 40346. Its carboxylic terminus is partially esterified in methanol, affording the methyl ester (**2**). The inhibitory activity of these new peptide products is little different from previously reported pepstatins. Importantly, an extrachromosomal origin is implicated for the biosynthetic genes of the pepstatin due to their absence in the bacterial genome.

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