2, 5-Diketopiperazines (Cyclic Dipeptides) in Beef: Identification, Synthesis, and Sensory Evaluation

M.Z. Chen, M.L. Dewis, K. Kraut, D. Merritt, L. Reiber, L. Trinnaman, and N.C. Da Costa

ABSTRACT: Stewed beef and grilled dry aged beef were analyzed as part of an in-depth analytical program, with the aim of creating new flavors incorporating only compounds identified in the target foods and identifying new synthesis targets. In-house GC-MS analyses of several types of cooked beef have identified over 1000 volatile and semivolatile components; many for the 1st time. Among the semivolatiles detected were ten 2, 5-diketopiperazines (cyclic dipeptides) previously unreported in beef. These cyclic dipeptides are *cis*-cyclo(L-Ile-L-Pro), *cis*-cyclo(L-Leu-L-Pro), *cis*-cyclo(L-Pro-L-Pro), *cis*-cyclo(L-Pro-L-Val), *cis*-cyclo(L-Ala-L-Pro), cyclo(Gly-L-Pro), cyclo(Gly-L-Leu), *cis*-cyclo(L-Met-L-Pro), *cis*-cyclo(L-Phe-L-Pro), and *cis*-cyclo(L-Phe-L-Val). All 10 cyclic dipeptides were synthesized and evaluated organoleptically. Among them *cis*-cyclo(L-Leu-L-Pro), *cis*-cyclo(L-Met-L-Pro), and *cis*-cyclo(L-Phe-L-Pro) were found to be of particular organoleptic interest.

Keywords: 2,5-diketopiperazines, beef, flavor, identification, synthesis

Introduction

2, 5-Diketopiperazines, also called cyclic dipeptides, have been found in various foods. The bitter flavor of many foods has been attributed partially to diketopiperazines. In 1997, Gautshi and others identified 7 proline-based diketopiperazines in beer. When evaluated at concentrations from 10 to 50 ppm, these compounds were described as having flavor characteristics described as bitter, mouth coating, and astringent. Ginz and Engelhardt (2000, 2001) reported the presence of 10 diketopiperazines in roasted coffee although no sensory evaluation was reported for those compounds. Using a 2-step mass spectrometric approach, Chen and others (2004) tentatively identified 20 diketopiperazines in chicken essence. Some of the diketopiperazines were quantified before and after thermal treatment of chicken essence and it was found that the concentrations of the diketopiperazines were determined not only by the relative ease of formation, but also by the type of process. Most recently, Stark and Hofmann (2005) and Stark and others (2006) identified 25 diketopiperazines in roasted cocoa nibs. In addition, the taste recognition thresholds of the compounds were reported. The data revealed that among the 25 diketopiperazines, 5 compounds, namely, *cis*-cyclo(L-Ile-L-Pro), *cis*-cyclo(L-Pro-L-Val), *cis*-cyclo(L-Leu-L-Val), *cis*-cyclo(L-Ala-L-Ile), and *cis*-cyclo(L-Ala-L-Leu) were present above their individual bitter taste threshold concentrations and therefore contributed to the cocoa taste. Furthermore, judging by the overdose threshold factor, *cis*-cyclo(L-Pro-L-Val) was the most important compound contributing to the bitter taste.

In-depth beef analytical projects have been conducted over the last 10 years. Different beef cuts prepared in different ways were subjected to various analyses. Volatile profiles for each beef sample were produced utilizing at least 3 different extraction techniques, including steam distillation, liquid/liquid extraction followed by fractionation and headspace trapping. Of particular use in finding and identifying diketopiperazines were the liquid/liquid extracts followed by solvent fractionation. In this process, 10 diketopiperazines have been identified which were not previously reported in beef, namely, *cis*-cyclo(L-Ile-L-Pro), *cis*-cyclo(L-Leu-L-Pro), *cis*-cyclo(L-Pro-L-Pro), *cis*-cyclo(L-Pro-L-Val), *cis*-cyclo(L-Ala-L-Pro), cyclo(Gly-L-Pro), cyclo(Gly-L-Leu), *cis*-cyclo(L-Met-L-Pro), *cis*-cyclo(L-Phe-L-Pro), and cis-cyclo(L-Phe-L-Val) (Figure 1). All of them were synthesized and sensorily evaluated.

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Materials and Methods

Chemicals

The following reagents are commercially available: *O*-(benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate (HBTU), 1-hydroxy-benzotriazole (HOBt), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC), diisopropylethylamine (DIPEA), trifluoroacetic acid (TFA), acetic acid, acetonitrile (ACN), N,N-dimethylformamide (DMF), toluene, methanol (MeOH), dichloromethane (DCM), ethyl acetate (EtOAc), triethylamine (TEA), Merrifield resin, potassium fluoride (KF), and N-Boc protected amino acids. Note that all amino acids used started in the L-configuration.

Sample preparation

Dry aged beef: Sirloin steak is aged by hanging in a cool, dry environment for 10 to 28 d. Moisture evaporates from muscle creating more flavor and taste. Natural enzymes break down tissue making it tenderer within 10 to 14 d of drying. The aged beef was grilled for 3 to 4 min each side then cubed. Stewed beef: topside beef was cubed and seared in a little fat. Minced and cooked in beef stock/veal bones that had been simmered with water and reduced over 6 h. Steam distillation-extraction was performed using a Likens–Nickerson apparatus. Approximately, 150 g of minced dry aged beef or stewed beef were added to 2.5 L of water and distilled into 150 mL of DCM over 3 h. The procedure was repeated and the combined extracts dried over anhydrous sodium sulfate, filtered, and reduced to 1 mL using a solvent evaporator. For the liquid/liquid extraction 200 g of minced dry aged beef or stewed beef were steeped overnight in 200 mL of DCM. The mixture was filtered and the liquid extract dried over anhydrous sodium sulfate. Further

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filtration was followed by reduction to 10 mL under rotary evaporation. This extract was uniformly mixed with 20 g of silica gel (Purasil 60 Å, 70 to 230 mesh, Whatman, Brentford, London, U.K.) and the slurry poured over a Buchner funnel of 200 g of silica gel. The extract was washed through with 500 mL of hexane, and concentrated to 1 mL under rotary evaporation with reduced pressure. The silica gel slurry was washed further with 500 mL of MeOH and the extracted dried and concentrated to 1 mL as previously described.

Gas chromatography

The liquid/liquid extract fractions and steam distillation extracts were analyzed using an HP6890 GC with a 50-m nonpolar OV-1 capillary column with a split ratio of 40:1, flow rate of 1.0 mL/min, temperature program was 40 to 270 °C at 2 °C/min with a hold at 270 °C of 10 min. Polar phase, a 50-m CPwax capillary column with a split ratio of 40:1, flow rate of 1.0 mL/min, temperature program was 60 °C held for 10 min then 60 to 220 °C at 2 °C/min and held for 20 min.

GC-mass spectrometry and NMR

The chromatographic conditions were the same as described for GC analysis. Micromass Autospec® high resolution, double-focusing, magnetic sector spectrometer (Waters, Milford, Mass., U.S.A.), scanning from m/z 450 to 33 at 0.3 s per decade. MS library identification was achieved using in-house and commercial libraries such as Wiley 7 and NIST. Standard retention data was used calibrated with ethyl esters. Compounds were confirmed using a 500 MHz Bruker 500 Avance NMR spectrometer (Bruker, Billerica, Mass., U.S.A.). Proton and carbon-13 experiments were run to confirm structures.

Synthesis of 2, 5-diketopiperazines

Synthesis was achieved by following a synthesis route reported by Kowalski and Lipton (1996) with slight modifications. The synthesis of *cis*-cyclo(L-Ala-L-Pro) (**1**; Figure 1) is used here as an example to illustrate the details.

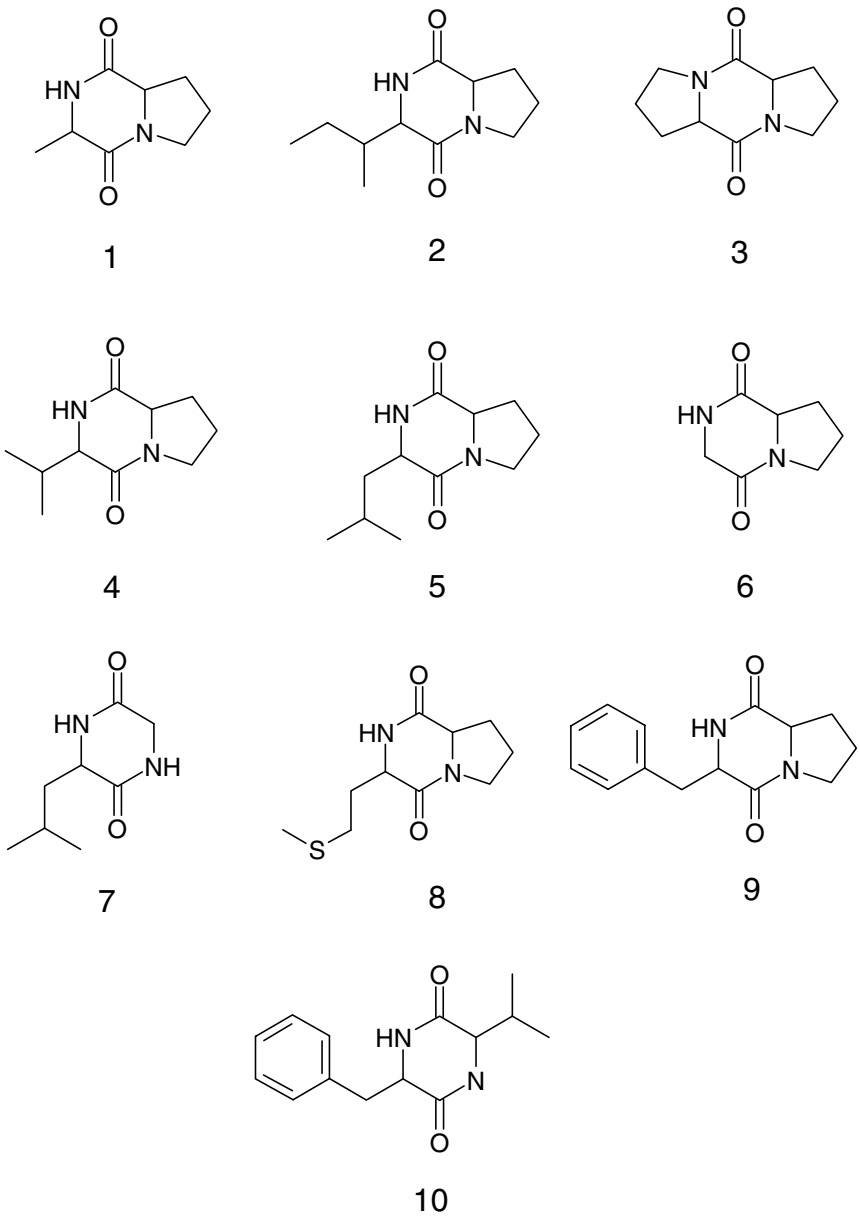

Figure 1 — Structures of diketopiperazines found in cooked beef.

1. Loading Boc-Ala-OH onto Merrifield resin. Forty grams of Merrifield resin (substitution 1.2 mmol/g) were added to a 3-neck round bottom flask equipped with a mechanical stirrer, also added were 18.2 g of Boc-Ala-OH (96 mmol), 11.1 g of KF (192 mmol), and 300 mL of DMF. With heating and stirring, the reaction was kept at 85 °C for 10 h. After cooling down to room temperature, the reaction mixture was filtered to remove the solvent and resin was washed successively with DMF, DMF/H_2O (1/1), H_2O, and MeOH. After drying overnight under vacuum, 47.6 g of resin were obtained. The substitution rate was calculated as 1.05 mmol/g based on the weight increase.

2. Coupling the 2nd amino acid Boc-Pro-OH to the resin. The synthesis followed the standard Boc peptide solid phase synthesis procedure. Ten grams of Boc-Ala-Merrified resin were added to a solid phase peptide synthesis vessel. After de-Boc (50% TFA/DCM), neutralization (10% TEA/DCM), and DMF washing, a solution of 6.5 g of Boc-Pro-OH (30 mmol), 4.05 g of HOBt (30 mmol), 11.4 g of HBTU (30 mmol), and 10.4 mL of DIPEA in 100 mL of DMF was added to the reaction vessel. After stirring for 2.5 h at room temperature, the solvent was removed and the resin was washed successively with DMF, MeOH, and DCM. De-Boc again with 50% TFA/DCM and after washing with DCM, MeOH and drying under vacuum overnight, 11.7 g of TFA-NH_2-Pro-Ala-Merrifield resin were obtained.

3. Cyclization to form the *cis*-cyclo(L-Ala-L-Pro) (**1**). 11.7g of TFA-NH_2-Pro-Ala-Merrifield resin, 120 mL of toluene, and 12 mL of acetic acid were added to a 3-neck round bottom flask. The reaction mixture was refluxed for 2 h then filtered to remove the resin. The solution was concentrated under vacuum to give a white solid residue of 1.4 g. Product (0.9 g) was obtained after recrystalization from EtOAc.

cis-Cyclo(L-Ala-L-Pro), (**1**): $C_8H_{12}N_2O_2$ (MW 168.1), ^{1}H NMR ($CDCl_3$) δ: 1.48 (3H, d, J = 6.84 Hz), 1.85 to 1.96 (1H, m), 1.99 to 2.07 (1H, m), 2.09 to 2.18 (1H, m), 2.32 to 2.39 (1H, m), 3.51 to 3.64 (2H, m), 4.11 to 4.15 (2H, m), 6.05 (1H, s); EI-MS *m/z* 168, M^+(58), 140 (10), 125 (34), 112 (9), 97 (49), 70 (100), 44 (57), 42 (23), 41 (34), 28 (18).

cis-Cyclo(L-Ile-L-Pro), (**2**): $C_{11}H_{18}N_2O_2$ (MW 210.1), ^{1}H NMR ($CDCl_3$) δ: 0.93 (3H, t, J = 7.41 Hz), 1.07 (3H, d, J = 7.20 Hz), 1.12 to 1.26 (1H, m), 1.36 to 1.49 (1H, m), 1.83 to 1.96 (1H, m), 1.97 to 2.11 (2H, m), 2.27 to 2.41 (2H, m), 3.51 to 3.57 (1H, m), 3.60 to 3.67 (1H, m), 3.97 (1H, s), 4.05 to 4.09 (1H, m), 6.48 (1H, s); EI-MS *m/z* 210, M^+(1), 154 (100), 125 (14), 98 (4), 86 (26), 70 (67), 55 (8), 41(23).

cis-Cyclo(L-Pro-L-Pro), (**3**): $C_{10}H_{14}N_2O_2$ (MW 194.1), ^{1}H NMR (d^6-DMSO) δ: 1.77 to 1.92 (4H, m), 1.93 to 1.99 (2H, m), 2.08 to 2.16 (2H, m), 3.34 to 3.37 (4H, m), 4.28 (2H, t, J = 8.03 Hz); EI-MS *m/z* 194, M^+(32), 166 (5), 153 (1), 138 (11), 124 (8), 110 (9), 96 (18), 83 (4), 70 (100), 55 (9), 41 (29).

cis-Cyclo(L-Pro-L-Val), (**4**): $C_{10}H_{16}N_2O_2$ (MW 196.1), ^{1}H NMR ($CDCl_3$) δ: 0.92 (3H, t, J = 6.81 Hz), 1.07 (3H, d, J = 7.24 Hz), 1.85 to 1.96 (1H, m), 2.00 to 2.10 (2H, m), 2.33 to 2.41 (1H, m), 2.58 to 2.68 (1H, m), 3.51 to 3.58 (1H, m), 3.61 to 3.68 (1H, m), 3.94 (1H, s), 4.06 to 4.10 (1H, m), 6.01 (1H, s); EI-MS *m/z* 196, M^+(4), 154 (100), 138 (5), 125 (40), 110 (7), 98 (6), 72 (33), 70 (83), 55 (10) 41 (25).

cis-Cyclo(L-Leu-L-Pro), (**5**): $C_{11}H_{18}N_2O_2$ (MW 210.1), ^{1}H NMR ($CDCl_3$) δ: 0.96 (3H, d, J = 6.37 Hz), 1.00 (3H, d, J = 6.58 Hz), 1.49 to 1.56 (1H, m), 1.72 to 1.80 (1H, m), 1.87 to 1.95 (1H, m), 1.99 to 2.18 (3H, m), 2.32 to 2.39 (1H, m), 3.51 to 3.64 (2H, m), 4.02 (1H, d, J = 9.43 Hz), 4.10 to 4.14 (1H, m), 6.00 (1H, s); EI-MS *m/z* 210, M^+(1), 195 (4), 167 (5), 154 (100), 139 (7), 125 (10), 86 (26), 70 (80), 55 (8), 41 (20).

Cyclo(Gly-L-Pro), (**6**): $C_7H_{10}N_2O_2$ (MW 154.1), ^{1}H NMR ($CDCl_3$) δ: 1.86 to 1.97 (1H, m), 2.01 to 2.13 (2H, m), 2.34 to 2.42 (1H, m), 3.53 to 3.59 (1H, m), 3.62 to 3.68 (1H, m), 3.87 to 3.92 (1H, m), 4.08 (1H, s), 4.11 (1H, s), 6.45 (1H, s); EI-MS *m/z* 154, M^+(92), 126 (11), 111 (100), 98 (31), 83 (98), 70 (77), 69 (58), 55 (43), 41 (75).

Cyclo(Gly-L-Leu), (**7**): $C_8H_{14}N_2O_2$ (MW 170), ^{1}H NMR ($CDCl_3$) δ: δ: 0.95 (3H, d, J = 6.41 Hz), 0.98 (3H, d, J = 6.39 Hz), 1.62 to 1.69 (1H, m), 1.73 to 1.88 (2H, m), 3.87 to 4.00 (3H, m), 7.17 (1H, s), 7.20 (1H, s); EI-MS *m/z* 170, M^+(2), 155 (4), 127 (32), 114 (100), 99 (22), 85 (60), 70 (14), 56 (38), 43 (64), 30 (81).

cis-Cyclo(L-Met-L-Pro), (**8**): $C_{10}H_{16}N_2O_2S_1$ (MW 228), ^{1}H NMR ($CDCl_3$) δ: 1.85 to 2.12 (4H, m), 2.13 (3H, s), 2.32 to 2.45 (2H, m), 2.69 (2H, t, J = 7.02 Hz), 3.51 to 3.65 (2H, m), 4.11 (1H, t, J = 8.2 Hz), 4.21 (1H, t, J = 5.64 Hz), 6.78 (1H, s); EI-MS *m/z* 228, M^+(41), 181 (2), 167 (31), 154 (100), 139 (16), 125 (4), 70 (50), 61 (10), 56 (8), 41 (12).

cis-Cyclo(L-Phe-L-Pro), (**9**): $C_{14}H_{16}N_2O_2$ (MW 244.1) ^{1}H NMR ($CDCl_3$) δ: 1.87 to 1.95 (1H, m), 1.96 to 2.05 (2H, m), 2.30 to 2.36 (1H, m), 2.79 (1H, d), 3.53 to 3.69 (3H, m), 4.05 to 4.09 (1H, m), 4.28 (1H, d, J = 10.45 Hz), 5.68 (1H, s), 7.23 (2H, d, J = 7.12 Hz), 7.29 (1H, t, J = 7.31 Hz), 7.34 (2H, t, J = 7.55 Hz); EI-MS *m/z* 244, M^+(49), 153 (45), 125 (100), 91 (53), 70 (48), 41 (20), 28 (13).

cis-Cyclo(L-Phe-L-Val), (**10**): $C_{14}H_{18}N_2O_2$ (MW 246.1) ^{1}H NMR (d^6-DMSO) δ: 0.28 (3H, d, J = 6.80 Hz), 0.65 (3H, d, J = 7.07 Hz), 1,68 (1H, m), 2.88 (1H, d, J = 13.5 Hz), 3.14 (1H, d, J = 13.5 Hz), 3.53 (1H, d, J = 1.52 Hz), 4.20 (1H, s), 7.16 to 7.19 (3H, m), 7.23 (2H, d, J = 6.74 Hz), 7.88 (1H, s), 8.07 (1H, s); EI-MS *m/z* 246, M^+(95), 229 (3), 204 (12), 175 (5), 155 (18), 127 (57), 113 (18), 91 (100), 85 (23), 72 (12).

Sensory evaluations

The cyclic dipeptides were prepared for evaluation in a 0.30% salt–water solution as the tasting media. Each sample was set up for taste evaluations at various levels ranging from 10 to 1000 ppm depending on the taste profile of the peptide. The samples were evaluated by a trained technical panel, typically consisting of 5 flavorists, using standard flavor descriptors. Note that wording such as "slightly" denotes a nuance to the overall flavor not a primary flavor descriptor. The evaluations began with tasting the weaker dilutions first and working up to the stronger dilutions. The group comments were recorded and a final rating given. Note that all cyclic dipeptides were made up initially at the lower concentrations and only the range where taste effects were observed are reported in the table. Extreme ends of the scale either gave no flavor or just continued bitterness.

Results and Discussion

Cyclic dipeptides have been found in steam distillation extracts; however, for the dry aged grilled beef and stewed beef the majority of this class of compounds were detected in the methanolic fraction of the liquid/liquid extract (Figure 3). Their elution occurs late on a 2 h OV1 nonpolar GC program and consequently the chromatographic resolution becomes less sensitive. However, many cyclic dipeptides give GC-MS spectra and can be assigned standard retention data on a nonpolar capillary column (Table 1). Note that the hexane fraction contained largely nonpolar components such as hydrocarbons and no cyclic dipeptides.

Another point of interest between the stewed beef and dry aged grilled beef is that longer cooking times appear to increase the number the diketopiperazines generated and their concentrations (Table 1). Both beef preparations used similar top quality cuts

Table 1—Cyclic dipeptides detected in cooked beef and their concentrations in cooked beef and retention indices.

Dry aged beef (short cooking time)	Conc. in dry aged beef (ppm)	Stewed beef (long cooking time)	Conc. in stewed beef (ppm)	Retention indices (Kovats)
		cis-Cyclo(L-Ala-L-Pro)	11.3	1562
		Cyclo(Gly-Pro)	2.0	1650
		Cyclo(Gly-Leu)	2.7	1669
cis-Cyclo(L-Pro-L-Val)	2.0	*cis*-Cyclo(L-Pro-L-Val)	24.0	1748
cis-Cyclo(L-Pro-L-Pro)	2.7	*cis*-Cyclo(L-Pro-L-Pro)	54.7	1846
cis-Cyclo(L-Ile-L-Pro)	2.0	*cis*-Cyclo(L-Ile-L-Pro)	12.7	1852
cis-Cyclo(L-Leu-L-Pro)	3.3	*cis*-Cyclo(L-Leu-L-Pro)	20.6	1854
		cis-Cyclo(L-Phe-L-Val)	2.0	2140
		cis-Cyclo(L-Met-L-Pro)	3.3	2146
		cis-Cyclo(L-Phe-L-Pro)	36.0	2212

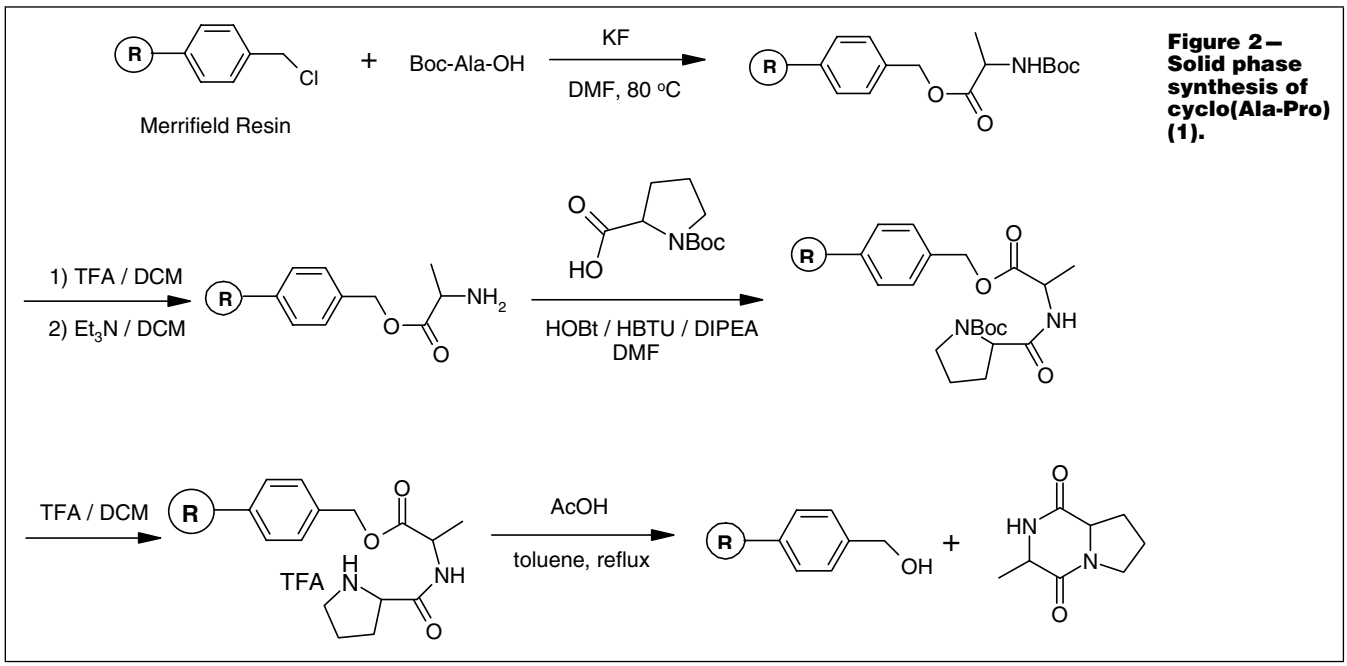

Figure 2—Solid phase synthesis of cyclo(Ala-Pro) (1).

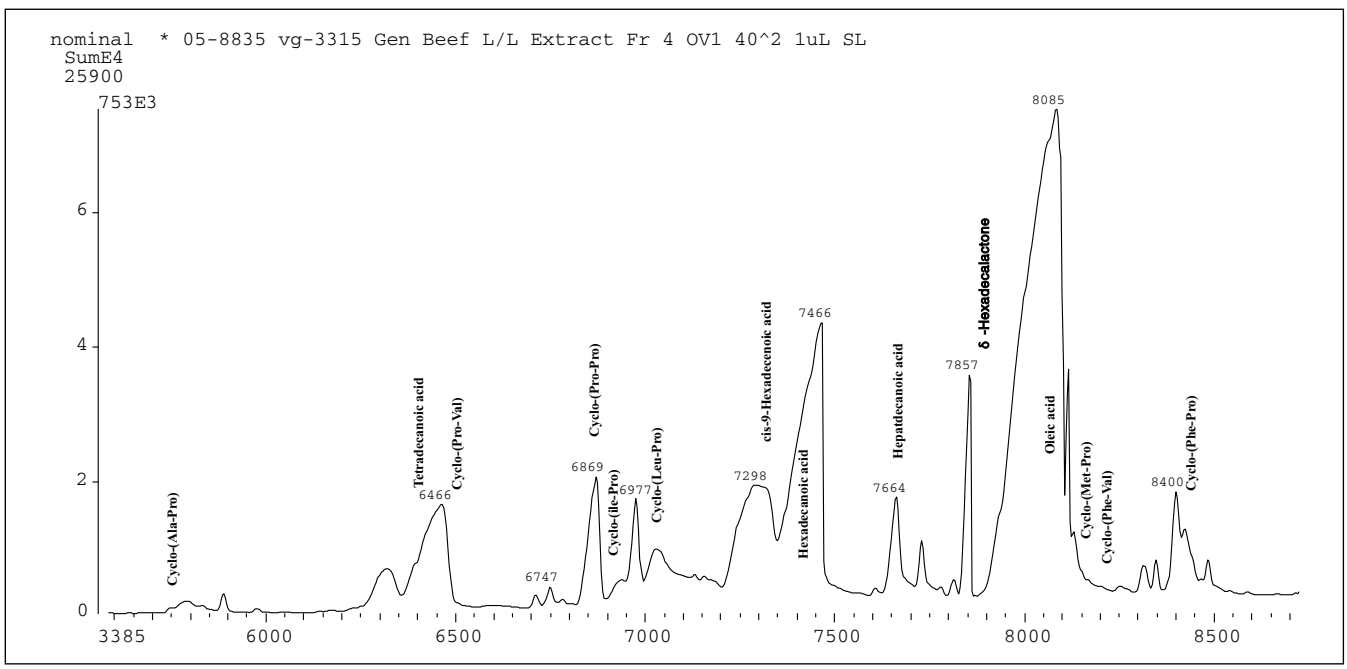

Figure 3—Non-polar total ion chromatogram of the methanol fraction of stewed beef (in scan numbers).

of muscle rich meat. The stewed beef was heated over 6 h and yielded 10 cyclic dipeptides at higher concentrations, whereas the grilled beef was cooked for under 10 min yielded only 4 cyclic dipeptides and at relatively lower concentrations. One possible mechanism of diketopiperazine formation is the intramolecular cyclization of linear peptide precursors (Pickenhagen and others 1975). The cyclic dipeptide structures were either initially proposed by external MS library matches and/or by interpretation of the mass spectrum. Synthesis of these diketopiperazines was undertaken after their initial identification so that they could be evaluated sensorily and also to use them as standards to confirm identification by standard retention data using ethyl esters and MS data.

There are many reports about the synthesis of diketopiperazine and their derivatives available (Kowalski and Lipton 1996; Szardenings and others 1997; Bianco and others 2000; Jeedigunta and others 2000; Lambert and others 2001). Cyclic dipeptides have 2 possible structures: *cis*- and *trans*-. In this study, starting from L-amino acid derivatives, stereospecific synthesis was carried out and only *cis*-isomers were made. Initially, we tried both solution phase and solid phase synthesis, the purpose was to compare the 2 methods and pick up the better one for the majority of syntheses. The solid phase synthesis of *cis*-cyclo(L-Ala-L-Pro) (**1**) is outlined in Figure 2. The 1st step is to attach the Boc-protected alanine to Merrifield resin. Using KF as base and DMF as solvent, the nucleophilic substitution reaction worked well. The following treatment with TFA/DCM removed the Boc group and neutralization with base TEA provided the free amine. Then the 2nd amino acid, Boc-protected proline was coupled to the resin to form the linear dipeptide. Three equivalents of coupling reagents were used to drive the reaction to completion. Again, TFA/DCM was used to remove the Boc group. In the last step, cyclization happened in the presence of AcOH (Gisin and Merrifield 1972) to form the desired *cis*-cyclo(L-Ala-L-Pro). The crude product had a purity higher than 90% and one simple recrystalization afforded the pure product. The solution phase synthesis of *cis*-cyclo(L-Ile-L-Pro) is outlined in Figure 4. In the 1st step, methyl ester of proline was coupled with Boc-Ile-OH; in the following step, TFA was used to remove the Boc group; in the 3rd and last step, under basic conditions, cyclization occurred to form the *cis*-cyclo(L-Ile-L-Pro) (**2**). The HPLC purity of the crude material was about 70% and preparative HPLC was required to purify the product. In comparing the 2 methods, the following observations were made. The solid phase method has advantages such as an easy work up and purer product; however, since this method uses a solid support and requires using excess of reagents in the coupling step, it is more expensive. On the other hand, the solution phase method uses less reagents, but usually a more tedious purification is necessary. Our initial focus is on preparing the compounds quickly and cost is not a factor at this stage and so it was decided to use the solid phase method to conduct the synthesis of compounds **2 to 10**.

It is not clear why in some cases *cis*-isomers are dominant (Stark and Hofmann 2005), and in some cases both *cis*- and *trans*-isomers are present (Ginz and Engelhardt 2000, 2001). Our sterospecific synthesis produced the *cis*-cyclic dipeptides only and their stereochemical purities were confirmed by GC, HPLC, and NMR. In addition, by comparing the synthesized compounds' chromatography retention values with those obtained from analysis, we concluded that only *cis*-cyclic dipeptides were present in beef.

Sensory evaluation results of compounds **1 to 10** are listed in Table 2. Most of them possess flavor characteristics described as bitter, mouth coating, and metallic. However, among them *cis*-cyclo(L-Leu-L-Pro), *cis*-cyclo(L-Met-L-Pro), and *cis*-cyclo(L-Phe-L-Pro) were found to be of organoleptic interest.

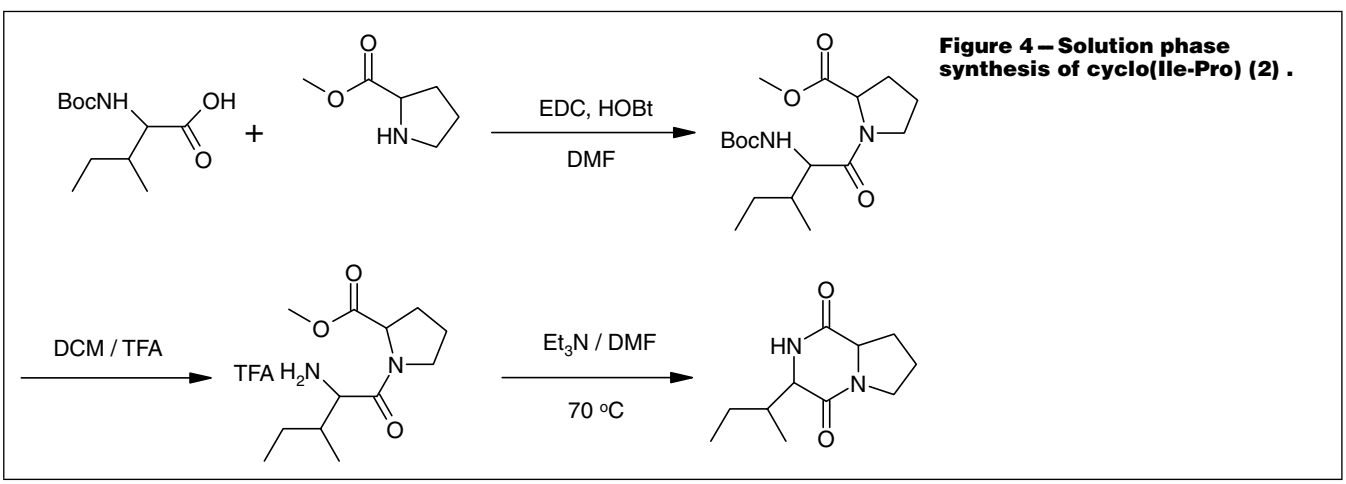

Figure 4—Solution phase synthesis of cyclo(Ile-Pro) (2) .

Table 2—Sensory taste evaluations of beef cyclic dipeptides.

Cyclic dipeptide	Sensory evaluation
cis-Cyclo(L-Ala-L-Pro)	100 ppm-nothing, 200 ppm-beefy, slightly bloody, 400 ppm-bitter
cis-Cyclo(L-Ile-L-Pro)	500 ppm-bitter, 1000 ppm-bitter, potato
cis-Cyclo(L-Pro-L-Pro)	10 ppm-nothing, 100 ppm-nothing, 200 ppm-slightly brothy, metallic, green, 1000 ppm-brothy, metallic, green
cis-Cyclo(L-Pro-L-Val)	10 ppm-nothing, 200 ppm-mouthfeel, nothing, 500 ppm-bitter
cis-Cyclo(L-Leu-L-Pro)	10 ppm-ethyl acrylate, glue, pineapple, 100 ppm-vegatative, green, green beans, rare beef
Cyclo(Gly-L-Pro)	20 ppm-slight tea note, cooling
Cyclo(Gly-L-Leu)	250 ppm-chalky, 500 ppm-chalky, dirty, 1000 ppm-dirty, asparagus, isopropanol
cis-Cyclo(L-Met-L-Pro)	10 ppm-faint, sauerkraut, cheese, 50 ppm-brussel sprouts, cabbage, alliacious, rotten, cheddar sauce, other cheese type sauces
cis-Cyclo(L-Phe-L-Pro)	10 ppm-nothing, 200 ppm-fishy, bitter, meaty, savory
cis-Cyclo(L-Phe-L-Val)	10 ppm-mouthfeel, nothing, 100 ppm-phenolic, band-aid, sweaty, formaldehyde, 200 ppm-phenolic, band-aid

Conclusions

Stewed beef and grilled dry aged beef were analyzed as part of an in-depth analytical program. GC-MS analysis identified ten 2, 5-diketopeperazines (cyclic dipeptides) previously unreported in beef. These cyclic dipeptides were *cis*-cyclo(L-Ile-L-Pro), *cis*-cyclo(L-Leu-L-Pro), *cis*-cyclo(L-Pro-L-Pro), *cis*-cyclo(L-Pro-L-Val), *cis*-cyclo(L-Ala-L-Pro), cyclo(Gly-L-Pro), cyclo(Gly-L-Leu), *cis*-cyclo(L-Met-L-Pro), *cis*-cyclo(L-Phe-L-Pro), and *cis*-cyclo(L-Phe-L-Val). Each was successfully synthesized and evaluated organoleptically in house. The sensory evaluations indicate that although not strong, they possess different taste effects at different concentrations. These compounds are generally reported as weakly bitter and slightly astringent, but the sensory work presented here indicates that concentration is key to other taste effects they display. Almost all of the cyclic dipeptides were found to occur in the cooked beef at concentrations lower than that could be detected sensorily (Table 1 and 2). The only exception was *cis*-cyclo(L-Leu-L-Pro), whose concentration of 20.6 ppm in stewed beef is higher than its taste threshold value (about 10 ppm). As a result, *cis*-cyclo(L-Leu-L-Pro) contributes to the overall beef flavor. Though several of these compounds have been reported in other foods *cis*-cyclo(L-Met-L-Pro) had not been previously reported until very recently (Da Costa and others 2008).

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