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The effect of the cyclopropyl group on the conformation of chemotactic formyl tripeptides

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Abstract

Certain formyl peptides are powerful chemoattractants towards neutrophils. In this study, several formyl tripeptides were synthesized and used to investigate the effects of different amino acid residues in position 1 on their ability to stimulate neutrophil chemotaxis. Pig neutrophil chemotaxis towards the formyl tripeptide, HCO–Ac_3C–Leu–Phe–OMe **1**, where Ac_3C represents 1-amino-1-cyclopropane carboxylic acid, was observed. Pig neutrophil chemotaxis towards a very similar formyl tripeptide, HCO–Aib–Leu–Phe–OMe **2**, where Aib represents α-amino isobutyric acid, was not observed. Compared to the isopropyl group, it was shown that the cyclopropyl group induces a greater percentage of the *E* conformation about the formamide functionality in these peptides. For **1** and **2**, the *E* isomer distributions in CDCl3 are 36 and 9%, respectively. Since a major difference between these two peptides is the *Z*/*E* isomeric distribution, one implication is that the peptide–receptor site interactions involving the *E* conformer are more effective than those of the *Z* conformer. No pig neutrophil chemotaxis towards the formyl tripeptides, HCO–Ala–Leu–Phe–OMe **3** and HCO–Gly–Leu–Phe–OMe **4** was observed. These formyl tripeptides exhibit a low percentage of the *E* isomer, similar to that of peptide **2**.

Keywords: Formyl peptides; Chemotaxis; Conformation; Pig neutrophils; Ab initio calculations; NMR spectroscopy

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1. Introduction

One category of by-products that results from bacterial protein degradation is formyl peptides, and tripeptides have been shown to induce greater chemotactic response [1] than dipeptides [2]. The formyl peptide, HCO–Met–Leu–Phe–OH was one of the first tripeptides shown to initiate chemotactic responses in animals and humans in the defense against acute inflammation [3]. Responses include: lysosomal enzyme release [4], superoxide formation [5], and cation redistribution [6]. In the body's defense mechanism against bacteria, neutrophils migrate to the site of an infection based on the concentration gradient of a chemoattractant. Specific interactions between the formyl peptide and the receptor site play a major role in the initiation of the different responses observed [7], and effective interactions are often dictated by a specific conformation of the formyl peptides [8].

Various formyl peptides have been shown to be chemoattractants for neutrophils [9], and there are structural features of formyl peptides that appear to influence their ability to initiate effective neutrophil chemotaxis. Formyl peptides that have either the carboxylic acid or an ester functionality have been shown to induce greater chemotactic activity, compared to formyl peptides without these functional groups [10]. In addition, the nature of the side chain of formyl peptides appears to play a role. The presence of methionine in the first position [11], and a hydrophobic residue, such as leucine in the second position also influence the effectiveness of formyl peptides [10].

In an attempt to develop a model to better understand the factors that contribute to the chemotactic responses in animals and humans, our research group is investigating the nature of the interactions that exist between chemoattractants and different types of neutrophils. In this study, pig neutrophils were used to determine the conformational requirements of formyl peptides for chemotaxis. Several tripeptides were synthesized and used to investigate the effect of different amino acid residues in position 1 on their ability to stimulate neutrophil chemotaxis. The tripeptides investigated include: HCO–Xaa–Leu–Phe–OMe, where Xaa represents 1-amino-1-cyclopropane carboxylic acid, α-amino isobutyric acid, alanine, and glycine. Formyl tripeptides that do not have bulky alkyl side-chains in position 1, similar to those of **3** and **4**, are known to induce only minimal lysosomal enzyme release from rabbit leukocytes [10]. This is the first study to analyze the effects that the cyclopropyl group in position 1 have on the conformation of chemotactic peptides.

2. Materials and methods

2.1. Chemical synthesis

The formyl peptides **1**, **2**, **3**, and **4** were synthesized by established procedures as outlined in the literature [12]. Melting points were determined on Electrothermal 9100 and are uncorrected. NMR spectra were obtained using a Varian Unity Inova 500 NMR spectrometer. IR spectra were obtained using an Analect RF-65 FTIR

spectrometer. Quantitative Technologies, New Jersey, carried out the elemental analysis.

HCO–Ac$_3$C–Leu–Phe–OMe ***1***. The crude product was chromatographed on silica gel with CH_2Cl_2–EtOAc (1:1) as eluent, then recrystallized from Et_2O–MeOH. Percent yield 22% and m.p. = 146–147 °C. ^{1}H NMR ($CDCl_3$) δ in ppm: 0.88–0.92 (m, 6H), 1.01–1.13 (m, 2H), 1.47–1.65 (m, 5H), 3.04–3.17 (m, 2H), 3.71 (s, 1.93H), 3.72 (s, 1.07H), 4.36–4.42 (m, 1H), 4.80–4.86 (m, 1H), 6.07 (s, 0.64H), 6.19 (d, 0.36H, $J = 11.5$ Hz), 6.39 (d, 0.37H, $J = 7.5$ Hz), 6.51 (d, 0.63H, $J = 7.5$ Hz), 6.66 (d, 0.62H, $J = 8$ Hz), 6.72 (d, 0.38H, $J = 8$ Hz), 7.09–7.13 (m, 2H), 7.24–7.31 (m, 3H), 8.18 (d, 0.36H, $J = 11$ Hz), 8.19 (d, 0.64H, $J = 1$ Hz). ^{13}C NMR ($CDCl_3$) δ in ppm: 17.15, 17.22, 17.24, 17.50, 21.95, 22.07, 22.75, 22.86, 24.67, 24.82, 34.27, 35.47, 37.78, 37.80, 40.73, 40.85, 52.18, 52.34, 52.37, 52.40, 53.08, 53.20, 127.03, 127.14, 128.57, 128.59, 129.24, 129.28, 135.67, 135.86, 170.55, 171.24, 171.60, 171.64, 171.66, 171.68. IR (NaCl): (3294.6, 3030.1, 2955.3, 2866.7, 1746.2, 1681.6, 1644.0, 1531.1, 1454.1, 1294.6, 1210.9, 1174.7, 1033.0, 967.6, 869.6) cm^{-1}. *Anal*. Calcd. for $C_{21}H_{29}N_3O_5$: C, 62.51; H, 7.24; N, 10.41. Found: C, 62.64; H, 7.11; N, 10.12.

HCO–Aib–Leu–Phe–OMe ***2***. The crude product was chromatographed on silica gel with CH_2Cl_2–EtOAc (1:1) as eluent, then recrystallized from Et_2O–MeOH. Percent yield 29% and m.p. = 197–198 °C. ^{1}H NMR ($CDCl_3$) δ in ppm: 0.89–0.92 (m, 6H), 1.45–1.72 (m, 9H), 3.07–3.11 (m, 1H), 3.16–3.19 (m, 1H), 3.72 (s, 2.72H), 3.74 (s, 0.28H), 4.37–4.42 (m, 1H), 4.80–4.84 (m, 1H), 6.12 (s, 1H), 6.57 (d, 1H, $J = 8.5$ Hz), 6.62 (d, 1H, $J = 8$ Hz), 7.12–7.13 (m, 2H), 7.21–7.30 (m, 3H), 8.09 (d, 0.91H, $J = 1.5$ Hz), 8.24 (d, 0.09H, $J = 12.5$ Hz). ^{13}C NMR ($CDCl_3$) δ in ppm: 21.74, 22.96, 24.86, 25.05, 25.22, 37.65, 40.73, 52.05, 52.33, 53.24, 57.23, 127.01, 128.53, 129.24, 136.02, 161.07, 171.44, 171.76, 173.53. IR (NaCl): (3289.4, 3062.8, 2955.5, 2866.7, 1752.6, 1644.0, 1547.9, 1434.4, 1391.1, 1207.8, 1167.5, 991.7, 869.9, 751.8, 703.6) cm^{-1}. *Anal*. Calcd. for $C_{21}H_{31}N_3O_5$: C, 62.20; H, 7.71; N, 10.36. Found: C, 62.24; H, 7.49; N, 10.21.

HCO–Ala–Leu–Phe–OMe ***3***. The crude product was recrystallized from Et_2O–MeOH. Percent yield 43% and m.p. = 186–187 °C. ^{1}H NMR ($CDCl_3$) δ in ppm: 0.89–0.92 (m, 6H), 1.34 (d, 3H, $J = 7$ Hz), 1.49–1.68 (m, 3H), 3.05–3.09 (m, 1H), 3.13–3.17 (m, 1H), 3.72 (s, 3H), 4.50–4.54 (m, 1H), 4.64–4.69 (m, 1H), 4.82–4.86 (m, 1H), 6.63–6.79 (m, 1H), 6.79–6.96 (m, 1H), 7.09–7.11 (m, 2H), 7.20–7.28 (m, 3H), 8.07 (d, 0.03H, $J = 12$ Hz), 8.12 (s, 0.97H). ^{13}C NMR ($CDCl_3$) δ in ppm: 18.80, 22.15, 22.71, 24.69, 37.75, 41.43, 47.35, 51.78, 52.35, 53.26, 127.10, 128.54, 129.19, 135.76, 106.73, 171.43, 171.71, 171.74. IR (NaCl): (3266.4, 3095.5, 2958.0, 1752.1, 1690.3, 1636.9, 1558.4, 1446.3, 1380.6, 1215.9, 1158.3, 1038.5, 699.4) cm^{-1}. *Anal*. Calcd. for $C_{20}H_{29}N_3O_5$: C, 61.36; H, 7.47; N, 10.73. Found: C, 61.14; H, 7.45; N, 10.56.

HCO–Gly–Leu–Phe–OMe ***4***. The crude product was chromatographed on silica gel with CH_2Cl_2, then EtOAc as eluent, then recrystallized from Et_2O–MeOH. Percent yield 38% and m.p. = 166–167 °C. ^{1}H NMR ($CDCl_3$) δ in ppm: 0.90 (t, 6H, $J = 7$ Hz), 1.48–1.64 (m, 3H), 3.04–3.08 (m, 1H), 3.13–3.17 (m, 1H), 3.73 (s, 3H), 3.93 (d, 2H, $J = 5.5$ Hz), 4.52–4.56 (m, 1H), 4.85–4.89 (m, 1H), 6.59 (d, 2H, $J = 8.5$ Hz), 6.79 (d, 1H, $J = 8.5$ Hz), 7.09–7.11 (m, 2H), 7.21–7.28 (m, 3H), 8.02

(d, 0.02H, $J = 12$ Hz), 8.19 (d, 0.98H, $J = 0.5$ Hz). ^{13}C NMR ($CDCl_3$) δ in ppm: 22.11, 22.77, 24.69, 37.85, 41.33, 41.49, 51.73, 52.40, 53.15, 127.14, 128.53, 129.24, 135.69, 161.34, 168.08, 171.39, 171.76. IR (NaCl): (3260.0, 3062.8, 2954.1, 2866.7, 1750.5, 1645.7, 1556.1, 1539.1, 1435.9, 1374.7, 1267.3, 1210.7, 1174.7, 1103.8, 1022.1, 989.4, 766.0, 701.8) cm^{-1}. *Anal.* Calcd. for $C_{19}H_{27}N_3O_5$: C, 60.46; H, 7.21; N, 11.13. Found: C, 60.28; H, 7.20; N, 10.88.

HCO–Ac$_3$C–OMe **5**. The crude product was chromatographed on silica gel with CH_2Cl_2–EtOAc (1:4) as eluent. The product was obtained as a viscous oil. Percent yield 53%. ^{1}H NMR ($CDCl_3$) δ in ppm: 1.14–1.20 (m, 2H), 1.50–1.56 (m, 2H), 3.62 (s, 1.98H), 3.66 (s, 1.02H), 6.73 (br s, 0.35H), 6.81 (br s, 0.65H), 8.08 (s, 0.66H), 8.14 (d, 0.34H, $J = 11.5$ Hz). ^{13}C NMR ($CDCl_3$) δ in ppm: 17.38, 17.42, 32.31, 34.32, 52.46, 52.77, 162.26, 167.10, 172.32, 172.94. IR (NaCl): (3292.7, 3022.5, 2955.7, 2858.7, 1735.1, 1685.8, 1518.1, 1440.0, 1416.4, 1390.8, 1342.2, 1298.9, 1202.9, 1161.8, 1040.8, 986.0, 932.9, 878.4, 827.1, 757.9) cm^{-1}. *Anal.* Calcd. for $C_6H_9NO_3$: C, 50.35; H, 6.34; N, 9.78. Found: C, 49.65; H, 6.93; N, 8.75.

HCO–Aib–OMe **6**. The crude product was chromatographed on silica gel with CH_2Cl_2–EtOAc (1:10) as eluent. The product was obtained as a viscous oil. Percent yield 63%. ^{1}H NMR ($CDCl_3$) δ in ppm: 1.59 (s, 0.94H), 1.62 (s, 5.06H), 3.78 (s, 2.46H), 3.79 (s, 0.54H), 6.26 (br s, 1H), 8.12 (d, 0.85H, $J = 1$ Hz), 8.30 (d, 0.15H, $J = 12$ Hz). ^{13}C NMR ($CDCl_3$) δ in ppm: 24.65, 27.08, 52.87, 53.09, 56.21, 56.53, 160.21, 162.82, 174.08, 174.87. IR (NaCl): (3306.6, 2997.4, 2953.8, 1739.3, 1667.5, 1531.7, 1455.3, 1384.7, 1365.4, 1289.3, 1240.1, 1191.0, 1153.4) cm^{-1}. *Anal.* Calcd. for $C_6H_{11}NO_3$: C, 49.65; H, 7.64; N, 9.65. Found: C, 48.61; H, 7.70; N, 9.24.

HCO–Ac$_3$C–Leu–OMe **7**. The crude product was chromatographed on silica gel with CH_2Cl_2–EtOAc (4:1, then 1:1), then recrystallized from Et_2O. Percent yield 32% and m.p. = 77–78 °C. ^{1}H NMR ($CDCl_3$) δ in ppm: 0.93–0.95 (m, 6H), 1.02–1.19 (m, 2H), 1.53–1.70 (m, 5H), 3.73 (s, 3H), 4.58–4.63 (m, 1H), 6.47 (s, 0.55H), 6.62 (d, 0.45H, $J = 11.5$ Hz), 6.92 (dd, 1H, $J = 8$, 23.5 Hz), 8.23 (s, 0.57H), 8.27 (d, 0.43H, $J = 11.5$ Hz). ^{13}C NMR ($CDCl_3$) δ in ppm: 16.92, 17.02, 17.28, 17.40, 21.79, 21.85, 22.77, 22.79, 24.75, 24.94, 34.13, 35.56, 41.17, 41.42, 51.18, 51.39, 52.33, 52.38, 162.25, 166.63, 170.65, 171.63, 173.32, 173.79. IR (NaCl): (3292.3, 2954.7, 2870.6, 1746.6, 1664.1, 1540.3, 1466.3, 1436.1, 1386.0, 1364.1, 1338.1, 1273.0, 1245.9, 1198.6, 1150.7, 1021.3) cm^{-1}. *Anal.* Calcd. for $C_{12}H_{20}N_2O_4$: C, 56.23; H, 7.87; N, 10.93. Found: C, 54.53; H, 7.70; N, 10.30.

HCO–Aib–Leu–OMe **8**. The crude product was chromatographed on silica gel with CH_2Cl_2–EtOAc (4:1, then 1:1), then recrystallized from Et_2O. Percent yield 13% and m.p. = 86–87 °C. ^{1}H NMR ($CDCl_3$) δ in ppm: 0.95 (d, 6H, $J = 6$ Hz), 1.56–1.69 (m, 9H), 3.74 (s, 3H), 4.57–4.61 (m, 1H), 6.31 (br s, 0.85H), 6.45 (br s, 0.15H), 6.74 (d, 1H, $J = 7.5$ Hz), 8.15 (d, 0.87H, $J = 2$ Hz), 8.30 (d, 0.13H, $J = 12.5$ Hz). ^{13}C NMR ($CDCl_3$) δ in ppm: 21.77, 21.87, 22.79, 24.89, 24.92, 25.02, 25.27, 27.33, 27.34, 41.14, 41.35, 51.08, 52.34, 52.45, 57.07, 57.29, 160.86, 163.47, 173.11, 173.29, 173.80, 173.84. IR (NaCl): (3299.9, 2954.5, 2871.6, 1748.8, 1660.4, 1540.2, 1523.4, 1456.6, 1436.2, 1387.4, 1364.1, 1246.5, 1197.4, 1147.4, 1020.4) cm^{-1}. *Anal.* Calcd. for $C_{12}H_{22}N_2O_4$: C, 55.80; H, 8.58; N, 10.85. Found: C, 55.99; H, 8.53; N, 10.68.

2.2. Ab initio calculations

Calculations were executed with Gaussian 94 using standard basis sets with no modification [13]. All calculations were performed on our Silicon Graphics Indigo computer and Digital Personal (433 au) Workstation. Conformations were optimized at each level of theory, and convergence was to the limits imposed internally by Gaussian 94. Vibrational frequencies were calculated at each level of theory and the results were used to determine the nature of the structure (minima, saddle points, or second-order saddle points). All conformations studied were minima at the level of theory examined.

2.3. Biological assays

Chemotactic activity measurements and neutrophil isolation from pig's blood were carried out by established procedures [14]. Separate experiments were performed in this study and all assayed within a few hours of blood collection. Ten milliliters of whole pig blood was centrifuged at 2000 rpm for 10 min at 25 °C. The buffy coat (WBC) and a few milliliters of red blood cells (RBC) obtained from the whole blood were mixed with 3 mL of Roswell Park Memorial Institute (RPMI) 1640 medium. The buffy coat–RPMI mixture was layered onto 4 mL of Histopaque 1077 and 4 mL of Histopaque 1119 (Sigma) and centrifuged at 2000 rpm for 30 min at 25 °C. The polymorphonuclear cells (PMN) interface including some RBC were aspirated and transferred to a clean, sterile conical centrifuge tube. Into the PMN cells was added 4 mL of RPMI and the PMN's–RPMI mixture was centrifuged at 2000 rpm for 10 min at 25 °C. After decanting RPMI, red blood cells were lysed using 9 mL of reverse osmosis water for 30 s and then 1 mL of 10× PBS was added to the tube to normalize the pH. PMN pellet was centrifuged at 2000 rpm for 10 min at 25 °C and supernatants were discarded. The lysing procedure was repeated 2–3 times until RBC's were no longer evident. The isolated PMN cells were resuspended in 1 mL of RPMI-10 and counted. The cell concentration was adjusted to 5×10^6 cells/mL. A 48-well Boyden chamber (Neuro Probe, Cabin John, MD) was used to measure the migration of neutrophils toward 10^{-12}–10^{-4} M of formyl tripeptides in RPMI-10. Five fields per well were counted at 1000× under oil immersion with a light microscope. The same experiment was repeated at least six times. Chemotaxis data were expressed in terms of chemotactic index (CI), which is the ratio: (migration toward test attractant − migration toward the negative control)/(migration toward the negative control) and the mean value of chemotactic index was calculated. Linear and quadratic regression coefficients were calculated using general linear model procedure with cell counts as the dependent variable and concentrations of formyl tripeptides as the independent factors.

3. Results and discussion

Fig. 1 shows the dose–response for HCO–Ac_3C–Leu–Phe–OMe **1**, HCO–Aib–Leu–Phe–OMe **2**, HCO–Ala–Leu–Phe–OMe **3**, and HCO–Gly–Leu–Phe–OMe **4**.

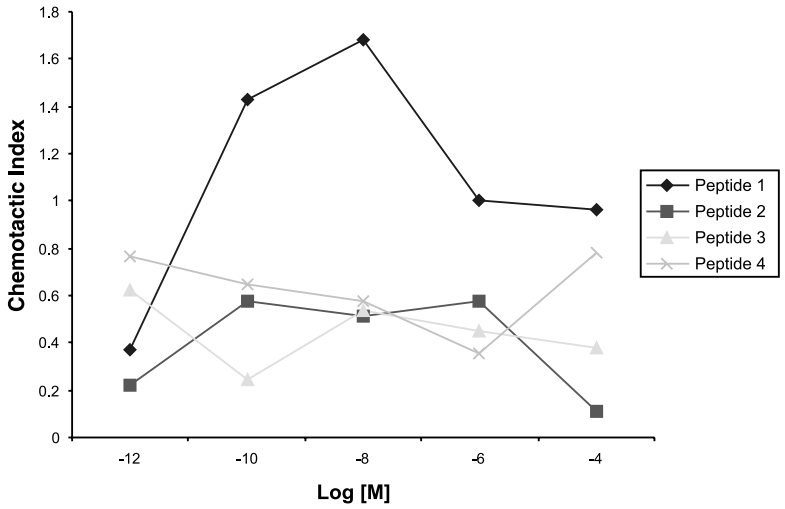


Fig. 1. Chemotactic index, determined at different peptide concentrations, for HCO–Ac_3C–Leu–Phe–OMe **1**, HCO–Aib–Leu–Phe–OMe **2**, HCO–Ala–Leu–Phe–OMe **3**, HCO–Gly–Leu–Phe–OMe **4** toward pig neutrophils. The points are the mean of five separate experiments done in duplicate.

Fig. 2 shows the chemotactic index of C5a, fMLP, peptides **1**, **2**, **3**, and **4** determined at the peak concentration of 10^{-8} M; C5a and fMLP were used as positive controls. It is obvious from Figs. 1 and 2 that neutrophils exhibit a much greater tendency to migrate towards tripeptide **1**, compared to the other tripeptides. Particularly interesting is the observation that neutrophil migration toward peptide **1** is greater than that

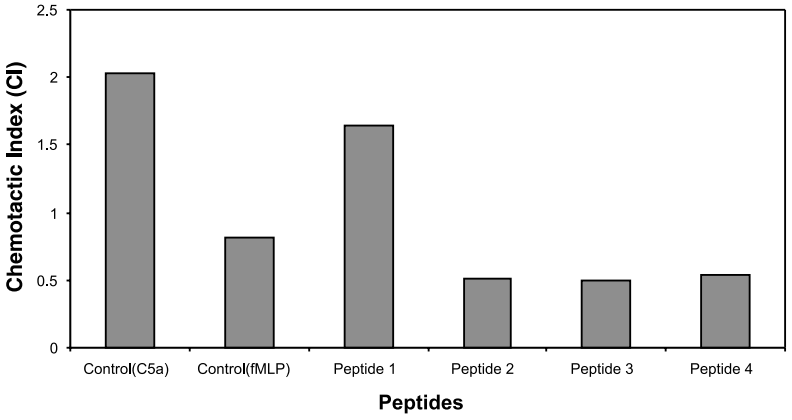


Fig. 2. Chemotactic index (CI), determined at 10^{-8} M peptide concentration, for HCO–Ac_3C–Leu–Phe–OMe **1**, HCO–Aib–Leu–Phe–OMe **2**, HCO–Ala–Leu–Phe–OMe **3**, HCO–Gly–Leu–Phe–OMe **4** relative to C5a and fMLP towards pig neutrophils. Standard errors are C5a (0.16), fMLP (0.35), peptides **1** (0.42), **2** (0.35), **3** (0.42), and **4** (0.42). CI values are determined at 10^{-8} M peptide concentration.

of peptide **2**, even though both peptides have similar structure. The NMR spectra, however, of these two compounds are different in $CDCl_3$. In the NMR spectra, the formyl proton for **1** appears as two doublets ($J = 11$ Hz and $J = 1$ Hz) in the ratio of 36:64; whereas, the formyl proton of **2** appears as two doublets ($J = 12.5$ Hz and $J = 1.5$ Hz), but in the ratio of 9:91. There are two geometric conformations about amide bonds, the Z and the E conformations, with splitting patterns similar to that observed for peptides **1** and **2** [15]. The implication of these results is that the E and Z isomers are present in solution, but their distributions differ drastically for these two peptides. The NMR spectra of tripeptides **3** and **4** were also investigated. The splitting patterns for **3** and **4** are similar to those of tripeptides **1** and **2**; the formyl proton of **3** appears as a doublet ($J = 12$ Hz) and a singlet in the ratio of 3:97, and the formyl proton of **4** appears as two doublets ($J = 12$ Hz and $J = 0.5$ Hz) in the ratio of 2:98 (Table 1).

To better evaluate the cause of the difference in the Z/E distribution for these peptides, simpler yet representative molecules, HCO–Ac_3C–OMe **5**, HCO–Aib–OMe **6**, HCO–Ac_3C–Leu–OMe **7**, and HCO–Aib–Leu–OMe **8** were synthesized and their NMR spectra also investigated. These compounds have the same functional groups as compounds **1** and **2**; except the Lue–Phe portion is absent on **5** and **6**, and the Phe is absent on **7** and **8**. For these compounds the splitting pattern of the formyl hydrogen is similar to that of tripeptides **1** and **2**. From Table 1, formyl peptides (and formyl amino acids) that contain the cyclopropyl group in position 1 (peptides **1** and **7** and formyl amino acid **5**) have a greater percentage of the E isomer, compared to peptides that do not have the cyclopropyl group in the same position. From these results, it is apparent that the cyclopropyl group adjacent to the formamide functionality has the effect of increasing the E isomer distribution, compared to hydrogen or regular alkyl groups in the same position.

If bulky groups are bonded to the nitrogen of amides, the distributions of the Z/E isomers are known to be different [16]; and hindered rotation about the C–N bond is experienced, which results in a greater percentage of the E isomer. The cyclopropyl group is known to be smaller than the isopropyl group [17]; thus, if steric effect was the only factor that affects the Z/E distribution, there should be a greater abundance of the Z isomer of peptide **1**, compared to that of peptide **2**. Since this expectation

Table 1
Distribution of isomers that have different Z/E arrangement about the formamide group for formyl peptides esters and formyl amino acid esters in $CDCl_3$ at 25 °C

Number	Peptide	Z (%)	E (%)
1	HCO–Ac_3C–Leu–Phe–OMe	64	36
2	HCO–Aib–Leu–Phe–OMe	91	9
3	HCO–Ala–Leu–Phe–OMe	97	3
4	HCO–Gly–Leu–Phe–OMe	98	2
5	HCO–Ac_3C–OMe	66	34
6	HCO–Aib–OMe	85	15
7	HCO–Ac_3C–Leu–OMe	57	43
8	HCO–Aib–Leu–OMe	87	13

contradicts the experimental observations, additional effects are operational. The Walsh model describes the carbons of cyclopropane as sp^2 hybridized [18] and as a result, an additional effect: inductive effect, is observed for molecules that have the cyclopropyl group, compared to molecules that have normal alkyl groups [19].

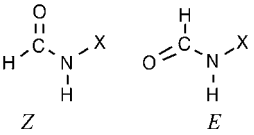
To determine whether inductive effect influences the Z/E isomeric distribution about the formamide bond, the relative energies of selected formamide conformers that have different substituents, including the cyclopropyl and the isopropyl groups, were determined by ab initio calculations, the results are shown in Table 2. The E isomer of N-cyclopropyl formamide is 3.1 kcal/mol more stable than the Z isomer; whereas, the Z isomer of N-isopropyl formamide is 2.2 kcal/mol more stable than the E isomer. The relative energies of N-methyl formamide and N-trifouoromethyl formamide were determined also because there is a very close isosteric relationship between fluorine and hydrogen [20]. From the results, the E isomer of N-trifluoromethyl formamide is more stable than the Z isomer by 1.4 kcal/mol, and the E isomer of N-methyl formamide is less stable than the Z isomer by 1.4 kcal/mol. These results clearly indicate that steric effect is not the only factor that contributes to the isomeric distribution of substituted formamides.

The experimental and theoretical results indicate that the E conformer of formyl peptides that have an electronegative group in position 1 exists in a greater abundance than the E conformer of formyl peptides that have an alkyl group in the same position. It is difficult to determine precisely which effect, inductive or steric, influences the distribution the most unless a complete quantitative structure–property relationship (QSPR) analysis is carried out on a wider spectrum of substituted formamides. Such an analysis is presently under investigation in our lab. Based on the results of this study, however, one possible explanation for the observation that

Table 2
MP2/6-31+G* relative energies and dipole moments for E and Z conformers of N-substituted formamides

X	Conformer	ΔE (kcal/mol)	Dipole moment (D)
i-C_3H_7	Z	0	4.3
	E	2.2	5.0
c-C_3H_7	E	0	4.8
	Z	3.1	4.3
CH_3	Z	0	4.5
	E	1.4	4.8
CF_3	E	0	1.4
	Z	1.4	4.8

Relative energy values are in kcal/mol.



peptide **1** initiates neutrophil migration, and peptides **2**, **3**, and **4** do not is due to the greater concentration of the *E* conformer. To further test this hypothesis, pig neutrophil chemotaxis towards formyl dipeptides **7** and **8** was determined, since the *Z*/*E* distributions of formyl dipeptides **7** and **8** are similar to those of peptides **1** and **2**, respectively (Table 1). The migration of neutrophils toward formyl dipeptide **7** is 25% greater than toward formyl dipeptide **8**.

In conclusion, a plausible explanation for the observation that **1** and **7** initiate neutrophil migration much better than the other formyl peptides analyzed is due to the greater abundance of the *E* isomer for formyl peptides that have the cyclopropyl group in position 1. Another factor that may contribute to the migration of neutrophils towards formyl peptides **1** and **7** better than the other peptides of this study is the conformation of the entire peptide, which is induced by the cyclopropyl group. It is known that certain groups force peptides into a C_7 conformation [21]; and the cyclopropyl amino acid moiety of 2,3-methanomethionine is known to impart a γ-turn to peptides [22]. These effects apparently result in a better receptor site–peptide fit for peptides **1** and **7**.

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