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Synthesis, characterization and antibacterial evaluation of palmitoylphenylalanine and palmitoyltryptophan

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Abstract
The synthesis, characterization and anti-bacterial evaluation of two palmitoyl amino acids is reported in this work. The reported antimicrobial activity of some fatty acid derivatives encouraged the investigation of the possible influence of an aromatic group substituent on a saturated fatty acid residue. The compounds were synthesized by the condensation of palmitoyl chloride and the respective aromatic amino acid; and they were unequivocally characterized by different spectroscopic techniques. The compounds were tested for possible antibacterial activity against clinical isolate of *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa,* and *Escherichia coli,* and were found to possess no activity against any of the tested organisms.

Keywords: Synthesis; Palmitoylphenylalanine; Palmitoyltryptophan; Antibacterial

INTRODUCTION

The increasing incidence of resistant strains of microorganisms to available drugs calls for renewed efforts toward the discovery of potent and safer molecules for the treatment of emerging infectious diseases. Research in this area has not been limited to bioactive molecules from microorganism and their derivatives alone but other sources of compounds are being considered for possible activities against a wide range of organisms (Altieri *et al.*, 2007). Fatty acids and their derivatives have been investigated for their possible antimicrobial activity and some of them have been found to show interesting inhibition against the growth of microorganisms (Altieri *et al.*, 2009). Recent research has also shown that 2-hexadecynoic acid, a 2-alkynoic fatty acid, has antibacterial activity against *Mycobacterium tuberculosis* (Carballeira, 2008) and that linoleic acid (18:2), a polyunsaturated fatty acid, has antifungal activity against several plant pathogenic fungi (Liu *et al.*, 2008). Fatty acids are organic acids characterized by the presence of a carboxyl group (-COOH) at one end and a methyl group (-CH_3) at the other end. Fatty acids are ubiquitous in nature and as such they belong to a physiologically important class of molecule involved in cell energy storage (e.g. adipose tissues), membrane structure (phospholipid bilayer) and in various signaling pathways (Liu *et al.*, 2008). Fatty acids vary in length and degree

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of saturation, with naturally occurring fatty acids having a chain length of 4 to 28 carbons which may be saturated or unsaturated (Sylvain *et al*., 2009). Saturated fatty acids are straight chains and consist of a carbon chain with single bonds, while unsaturated fatty acids contain one or more double carbon-carbon bonds (C=C) which introduces fixed bends into the carbon chain. Antimicrobial free fatty acids can be saturated or unsaturated and in general the antimicrobial efficiency of fatty acids increases with an increase in chain length (Sylvain *et al*., 2009). Hydrophobic groups of saturated fatty acids play an important role in bioactivity (Branen *et al*., 1980). Modification of the carboxylic group of a saturated fatty acid using two different aromatic amino acids was carried out and the resulting compounds were screened for antibacterial activity.

EXPERIMENTAL

The starting materials were purchased from commercial sources and used without further purification. Phenylalanine, tryptophan and palmitoyl chloride were obtained from Sigma Aldrich (Germany). Muller- Hinton agar was obtained from Oxoid (U.K). The pre-coated thin layer chromatography (TLC), silica gel 60 F_{254} plates used to monitor the reaction, was obtained from Merck (Darmstadt, Germany). Melting points were determined with an electrothermal melting point apparatus and were uncorrected. Infra red (IR) spectra were measured on a Buck scientific IR M500 instrument. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Varian Gemini 200 (250MHz). Chemical shifts in were reported in part per million (ppm) relative to tetramethylsilane (TMS). Mass spectra (MS) were recorded on a Varian MAT 44S mass spectrometer operating at 70eV. Elemental analysis agreed favourably with the calculated values.

Synthesis of palmitoylphenylalanine. To a stirring mixture of phenylalanine (0.6g, 3.64 mmol.) in 15 mL of 1, 4-dioxane in ice cold chamber was added palmitoylchloride (1.1 mL, 3.64 mmol.), followed by the addition of 1M NaOH (3.63mL). Stirring continued in ice cold water for 3hours. At the end of the reaction, the solution was acidified by the addition of 1N HCl, and diluted with 20 mL of water. It was left overnight and was then filtered under suction and washed with water. The product was air dried and recrystallized from methanol /water (1:1).

Yield: 1.14g (78%), **Melting point:** 63-65^{0}C,
IR (KBr) 3305cm^{-1} (OH), 2931cm^{-1}(CH), 1702cm^{-1} (C=O), 1645cm^{-1}, 1537, 1457, 1400, 1200 cm^{-1}.
^{1}H NMR (DMSO d_6) δ: 0.86 (t, J = 6.0 Hz, 3H, CH_3), 1.22 (brs 22H, $(CH_2)_{11}$), 1.33-1.39 (quint, J = 7.0 Hz, 2H, CH_2), 2.01-2.04 (quint, J = 7.5 Hz, 2H, CH_2), 2.13-2.19 (t, J = 7.25 Hz, 2H, CH_2), 4.40 (q, J = 5 Hz, 1H,CH),7.16-1.7.27 (brt, 4H, Ar-H) 8.00-8.04 (d, J = 8Hz, 1H, N-H), 12.07 (brs 1H,O-H),
^{13}C NMR (DMSO d_6) δ: 14, 25, 26,29,32, 34,35, 47, 54, 127 (Ar -C), 128 (Ar -C), 130 (Ar -C), 138 (Ar -C), 173 (C = O), 175 (C = O)
MS: 402.2 (M^+) (100%), 255.2 (12%)
Elemental analysis: Calculated; C: 74.40, H: 10.24, N: 3.47; Found; C: 74.20. H: 10.10, N: 3.35.

Synthesis of palmitoyltryptophan. To a stirring mixture of tryptophan (0.743g, 3.62 mmol.) in 15mL of 1, 4-dioxane in ice cold chamber was added palmitoylchloride (1.1mL, 3.64 mmol.), followed by the addition of 1M NaOH (3.7 mL). Stirring continued for 3 hours in ice cold water. At the end of the reaction, the solution was acidified by the addition of 1N HCl, and diluted with 20 mL of water. It was left overnight and was then filtered under suction and washed with water. The product was air dried and recrystallized from methanol /water (1:1).

Yield: 1.090g (68%), **Melting point:** 102-104^{0}C,
IR (KBr) 3416.60cm^{-1}(OH), 3351.40cm^{-1} (NH), 1717.97cm^{-1} (C=O), 1644.44cm^{-1} (C=O), 1525.23, 1452, 1405, 1207 cm^{-1}.
^{1}H NMR (DMSO d_6) δ: 0.81-0.86 (t, J = 6.5, 3H, CH_3), 1.17-1.22 (m, 22H, $(CH_2)_{11}$), 1.33-1.39 (quint, J = 7.0 Hz, 2H, CH_2), 2.01-2.04 (quint, J = 7.5 Hz, 2H, CH_2),

2.13-2.19 (t, J = 7.25 Hz, 2H, CH_2), 4.40 (q, J = 5 Hz, 1H,CH), 6.92-7.01(m, 4H, Ar-H), 7.30 (d, J = 7.0 Hz, 1H, CH), 7.50 (d, J = 7.75 Hz, 1H, N-H), 8.0 (d, J = 8 Hz, 1H, N-H), 10.80 (s,1H, COOH).

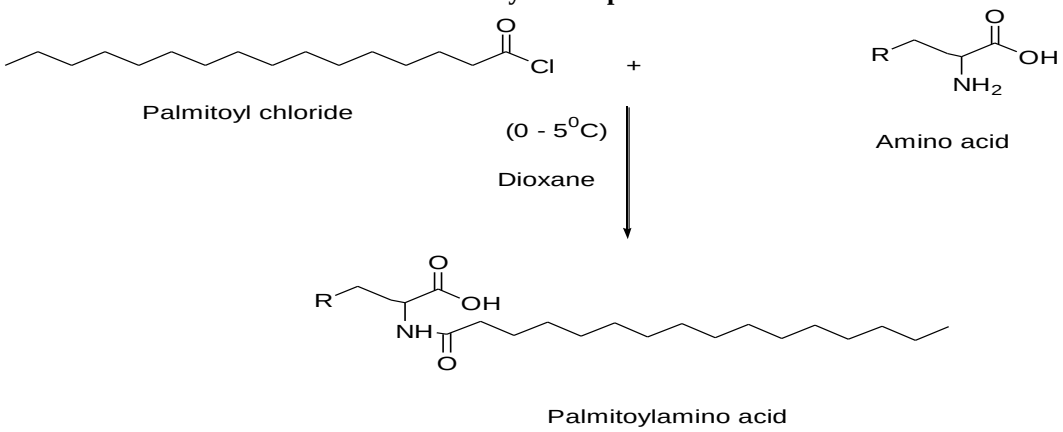
^{13}C NMR (DMSO d_6) δ: 14, 25, 26,29,32, 34,35, 47, 53, 111 (Ar-C), 112 (Ar-C),119 (Ar-C), 121 (Ar-C),124 (Ar-C), 128 (Ar-C), 137 (Ar-C), 173 (C=O), 175 (C=O)

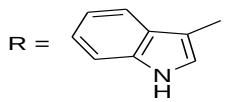
MS : 441.2 (M^+) (100%), 309, 255.2

Elemental analysis: Calculated; C: 73.39, H: 9.36, N: 6.34, Found; C : 73.39, H : 9.20, N : 6.30

Agar diffusion method (zone of inhibition measurement). Four clinical bacterial isolates (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*) were obtained from the microbial bank of department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy Niger Delta University, Wilberforce Island, Nigeria; for the antimicrobial screening of the synthesized compounds. The bacterial isolates were standardized using colony suspension method and matching the strain's suspension with 0.5 McFarland standard to give a resultant concentration of 1.5 x 10^8 cfu/ml. The antibiotic susceptibility testing was determined using the modified Kirby-Bauer diffusion technique by swabbing the Mueller–Hinton agar (MHA) (Oxoid U.K) plates with the resultant saline suspension of each strain and four wells were made in the agar with aid of cork borer (No. 4, the diameter of the borer is 6mm).

General synthetic plan





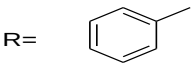


Table 1. Antibacterial activity

Organisms	The Diameter of Zone of Inhibition of the compounds								Genta-micin	Ciproflo-xacin
	Palmitoyl phenylalanine (conc. µg/ml)				Palmitoyl tryptophan (conc. µg/ml)					
	1000	5000	250	125	1000	500	250	125	10µg	5µg
B. subtilis	-	-	-	-	-	-	-	-	25	29
S. aureus	-	-	-	-	-	-	-	-	29	27
E. coli	-	-	-	-	-	-	-	-	27	35
P. aeruginosa	-	-	-	-	-	-	-	-	19	18

The wells were sealed at the bottom with molten sterilized agar, 0.1ml, 0.05ml, 0.025ml, 0.0125ml solutions of the synthesized compounds representing 1000µg/ml, 500µg/ml, 250µg/ml and 125µg/ml respectively were aseptically dispensed into the labeled wells while antibiotic discs (ciprofloxacin 5µg and gentamicin 10µg) used as control were placed on the agar aseptically. The plates were then incubated at 37°C for 24 hours. The zone diameters of inhibition produced by each concentration of the compounds and that of the antibiotic discs were measured and recorded (CLSI, 2008).

RESULTS AND DISCUSSION

The synthesis of the two compounds was accomplished by direct condensation of the amino acids with palmitoyl chloride. The compounds were unequivocally characterized using the combination of ^{1}H and ^{13}C NMR (nuclear magnetic resonance) and mass spectrometry. The result of the antimicrobial activity is shown in Table 1. Contrary to earlier report (Ouattara *et al.*, 1997), the result showed that the palmitoylphenylalanine and palmitoyltryptophan have no antibacterial activity against the tested strains of the microorganisms compared to the reference antibiotics used. This implies that the introduction of the aromatic group destroys the activity of the fatty acid instead of enhancing it. The reason for this could be that the aromatic group increases the bulkiness of the molecule resulting in decreased permeability of the bacterial cell membrane. It could also be inferred that the carboxylic group of fatty acid is essential in the antimicrobial activity of the compound. The minimum inhibitory concentration was not determined since there was no zone of inhibition.

Conclusion

It has been demonstrated that the introduction of aromatic group into the fatty acid moiety obliterated the activity of the resulting compounds. Further work utilizing the combinations of other saturated fatty acids other than palmitic chain could be carried out in order to have a comprehensive understanding of this group of compounds.

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REFERENCES

Altieri C., Bevilacqua A., Cardillo D. and Sinigaglia M. (2009). Antifungal activity of fatty acids and their monoglycerides against *Fusarium* spp. in a laboratory medium; *International Journal of Food Science and Technology*.; 44:242-245.

Altieri C., Cardillo D., Bevilacqua A. and Singaglia M (2007); Inhibition of *Aspergillus* spp. and *Penicillium* spp. by fatty acids and their monoglycerides; *Journal of Food Protection*.;70:1206-1212.

Branen A.L., Davidson P.M. and Katz B (1980); Antibacterial properties of phenolic antioxidants and lipids; *Food Technology*; 34:42-63.

Carballeira N.M. (2008). New advances in fatty acids as antimalarial, antimycobacterial and antifungal agents-a review. *Progress in Lipid Research.*;47:50-61.

Clinical and Laboratory Standard Institute (CLSI) Performance standards for Antimicrobial Susceptibility Testing Eighteenth informational supplement; M100-S18. 2008; 28(1): 34-52

Liu S., Weibin R., Jing L., Hua X., Jingan W., Yubao G. and Jingguo W. (2008); Biological control of phytopathogenic fungi by fatty acids; *Mycopathologia*; 166:93-102.

Ouattara B., Simard R.E., Holley R.A., Piette G.J-P. and Begin A. (1997). Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. *International Journal of Food Microbiology*; 37:155–162

Sylvain L.S., Lucia, V.M. and Elisabetta G. (2009); Effect of α-linolenic, capric and lauric acid on the fatty acid biosynthesis in *Staphylococcus aureus*. *International Journal of Food Microbiology*.; 129:288-294.