Research Article

Studies on some essential amino acids: Synthesis of methyl esters and antifungal evaluation of their quaternary ammonium bromides

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Abstract
The quaternization of amino group of some amino acid methyl ester derived from L-leucine, L-phenylalanine and L-methionine, with bromoacetic acid were prepared using the *N*-alkyl reaction. The structures of newly synthesized compounds were confirmed by IR, ^{1}H and ^{13}C NMR. The compounds investigated were tested against three phytopathogenic fungal strains namely *Fusarium oxysporum*, *Fusarium commune* and *Fusarium rodelens* and showed remarkable activity.

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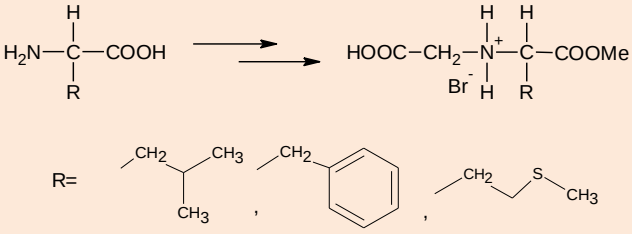


Keywords: Quaternary ammonium salts, amino acid methyl ester, antifungal activity.

Introduction

Of the 22 standard amino acids, 10 are called essential amino acids because the human body cannot synthesize them from other compounds at the level needed for normal growth, so they must be obtained from food. Between them are L-phenylalanine, L-methionine and L-tyrosine that is produced from phenylalanine, while L-leucine cannot produced internally. These acids possess similar polarity and neutrality with slight different structural features [1]. The amine and carboxylic acid functional groups found in amino acids allow them to have amphiprotic"amphoteric" properties. Carboxylic acid groups ($-CO_2H$) can be deprotonated to become negative carboxylates ($-CO_2^-$), and α-amino groups (NH_2-) can be protonated to become positive α-ammonium groups ($^+NH_3-$) a molecular state which is known as a zwitterion [2].

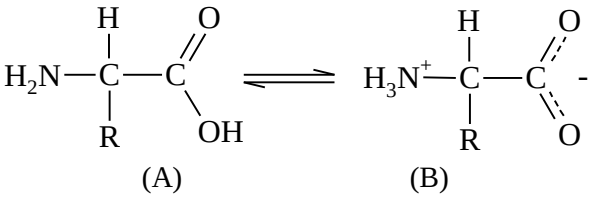


Figure 1 An un-ionized amino acid (A) and Zwitter ionic form (B).

Esterification of acids is capable to stop this phenomenon and to free the true amino group, that becomes capable to form quaternary ammonium salts. Amino acid methyl esters are important intermediates in organic synthesis [3]. Quaternary ammonium salts (QAS) are one of the most used classes of disinfectants[4] with a large applicability. They are used as bactericides [5-6], fungicides [5-8], antimalarial [9], and corrosion inhibitor [10]. QAS are positively charged cations, hence, their mode of action is related to their attraction to negatively charged materials such as bacterial and fungal proteins [11].The structural point of view quaternary ammonium halides containing COOH group can be considered as bi-functional compounds. The cohesion forces in the crystals of these compounds are dominated by $COOH\cdots X^-$ hydrogen bonds, $N^+\cdots X^-$ and $N^+\cdots O$ electrostatic interactions, and $C\text{-}H\cdots X$ contacts [12].

The structure-activity study presented herein was conceived with the aim of combining the antifungal activity of the quaternary ammonium compounds with the properties of the bromo acetic acid.

Experimental

Materials and Reagents

All the chemicals and reagents were obtained from Sigma Aldrich and Biochem. Melting points were measured using BUCHI 540 apparatus and are uncorrected, IR spectra were recorded as potassium bromide pellets on a Shimadzu 8300 spectrophotometer ($\bar{\upsilon}$ max in cm^{-1}), The ^{1}H and ^{13}C NMR spectra were recorded in D_2O on a BrukerAM , NMR spectrometer (300 MHz) using TMS as internal standard (δ in ppm). Proceeding of reactions and checking the purity of the compounds were made by TLC on silica gel supplied by MERCK, iodine was used for visualization.

General procedure for the preparation of amino acid methyl esters 2a-2c

Into a solution of the corresponding amino acids**1a-1c** (1.00 g, approximately 0.0075 mole.) in absolute methanol (50 mL) and concentrated sulfuric acid (2 mL) was heated at 80°C in an oil bath. The completion of the reaction was checked with TLC to obtain the desired compound. The excess of acid was neutralized with sodium bicarbonate then the solvent was evaporated and the product was collected. Results summarized in Table 1.

Table 1 Physical properties and analytical data of the synthesized compounds 2a-c and 3a-c.

Comp.	%Yield	M. P. ^{o}C	Rf Values	Solvent system
2a	72	129-130	0.23	$CHCl_3$
2b	84	164-165	0.8	$CHCl_3$
2c	67	234 -235	0.66	$CHCl_3/CH_3OH$ 4/ 1
3a	81	hygroscopic	0.46	$CHCl_3$
3b	93	hygroscopic	0.65	$CHCl_3$
3c	74	hygroscopic	0.50	$CHCl_3$/ CH_3OH 4/1

General procedure for the synthesis of quaternary ammonium bromides 3a-3c

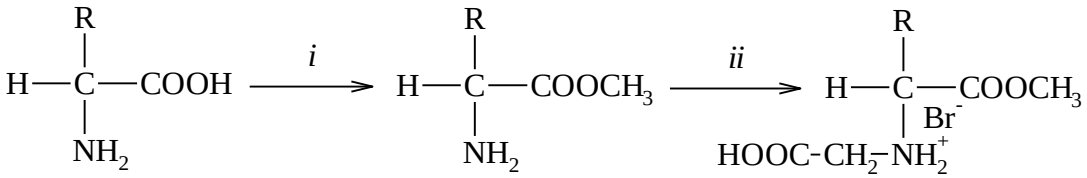
The corresponding amino acid methyl ester **2a-2c** (0.50 g, approximately 0.003 mole.) with an equivalent amount of bromoacetic acid in dry acetone. The mixture was refluxed on a water bath for 7-8 h. After the completion of reaction, the reaction mixture was cooled. The solid was separated by filtration, the filtrate was washed with diethyl ether, and the excess solvent was removed by vacuum evaporation. Physical properties, analytical data, IR, ^{1}H and ^{13}C NMR were stated earlier.

Biology

The antifungal activity was determined by the radial growth method [13]. The fungal cultures were incubated at 37°C for 4 days.Finally, the zones of inhibition were carefully measured. In this technique, sterilized hot PDA nutrient medium (composition: potato (200 g), dextrose (20g), agar (15g) and distilled water 1000 mL) and 4 mm diameter hole punch were used, on the PDA. After solidification of media, respective fungal spore suspensions were transferred to petri plates. Each test compound was dissolved in water and then diluted at the desired concentration. The fungal cultures were incubated at 37°C for 4 days.Finally, the zones of inhibition were carefully measured.

Results and Discussion

Esterification of amino acids L-leucine, L-phenylalanine and L-methionine with methanol and H_2SO_4, obtained according to literature [14] (see Table 1, experimental section). Results were characterized by IR spectroscopy and are summarized in Table 1.The quaternary ammonium salts **3a-3c** were prepared starting from the corresponding amino acid methyl ester **2a-2c** with an equivalent amount of bromoacetic acid in dry acetone (see scheme 1).



1 **2** **3**

a, R=-$CH_2CH(CH_3)_2$; **b**, R=-CH_2-Ph ; **c**, R=-$(CH_2)_2$-S-CH_3.

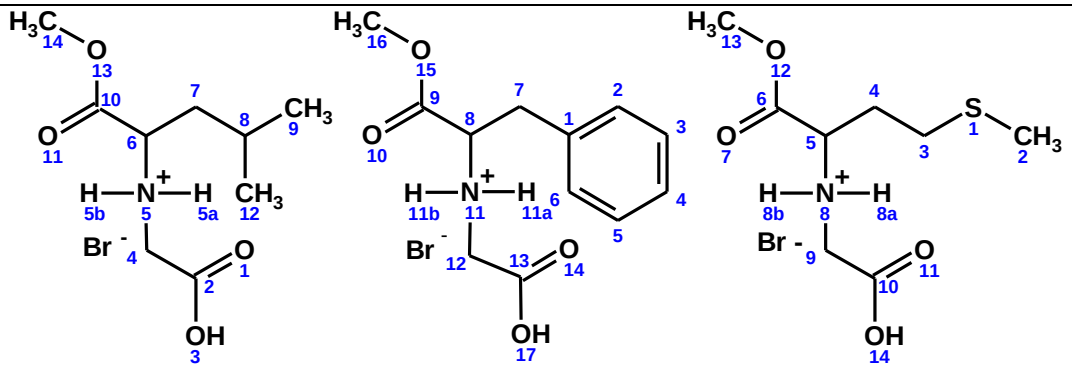
i: CH_3OH , H_2SO_4 ; ***ii*** :Br-CH_2-COOH.

Scheme 1 Global synthesis of esters **2a-c** and QASs **3a-c**.

QASs **3a-3c**, characterized by IR as shown in Table 2 and by ^1H and ^{13}C NMR spectra as illustrated in Figure 2.

Table 2 The characteristic infrared absorptions in $\bar{\upsilon}$ cm^{-1} of the synthesized compounds 2a-c and 3a-c.

Comp.	OH	N-H	=C-H	C=O	C=C	C-N	C-O-C	C-S-C
2a	-	3421.5	-	1745.4	-	1230.5	1006.8	-
2b	-	3485.1	3030	1747.4	1407.9	1240.1	1008.7	-
2c	-	3413.8	-	1743.5	-	1153.4	1016.4	802.3
3a	3425.3	-	-	1733.3	-	1211.2	1064.6	-
3b	3454.3	-	3004.9	1739.7	1488.9	1199.6	1055	-
3c	3427.3	-	-	1724.2	-	1290.3	1010.6	898.8



3a **3b3c**

Figure 2 Schematic drawings of structures **3a, 3b** and **3c** for ^1H and ^{13}C NMR purposes.

^1H and^{13}C NMR (300MHz, D_2O), δ (ppm) of 3a: 8.091 (1H, 3, 5a, 5b);4.709 (1H, 6);4.071 (3H, 14); 3.892 (2H, 4);2.085 (2H, 7);1.606 (1H, 8);0.824 (3H, 12, 9); 177.301 (C10), 171.258 (C2),59.238(C6), 53.433 (C14);38.718 (C4), 26.970 (C7), 23.835 (C7), 21.419 (C8), 23.025 (C9, C12).

^1H and^{13}C NMR (300MHz, D_2O), δ (ppm) of 3b: 8.090 (1H, 17, 11a, 11b);7.286 (1H, 5); 7.279 (1H, 3);7.200 (1H, 6);4.707 (1H, 8);4.073 (2H, 12);3.893 (3H, 16); 3.473 (2H, 7) **,** 176.250 (C9),172.689 (C13),133.644(C1),129.352 (C2,C6);129.181 (C3,C5),128.038 (C4),58.234 (C8), 53.162 (C16), 26.899 (C12) ,23.751 (C7).

^1H and^{13}C NMR (300MHz, D_2O), δ (ppm) of 3c: 8.454 (1H, 14, 8a, 8b);4.708 (1H, 5);4.160 (2H, 9);3.896(3H, 13); 3.604 (2H, 3);3.001 (2H, 4);2.089 (3H, 2),174.267 (C6),171.807 (C10),55.382(C5),54.039 (C13);30.705 (C9),30.249 (C4),28.919 (C3),26.901 (C2).

Antifungal activities tests:

The antifungal activities of the synthesized compounds **2a-c** and **3a-c** were studied in different concentrations (50, 100, 250 and 500µg/ml) against three phytopathogenic fungal strains namely *Fusarium oxysporum*, *Fusarium*

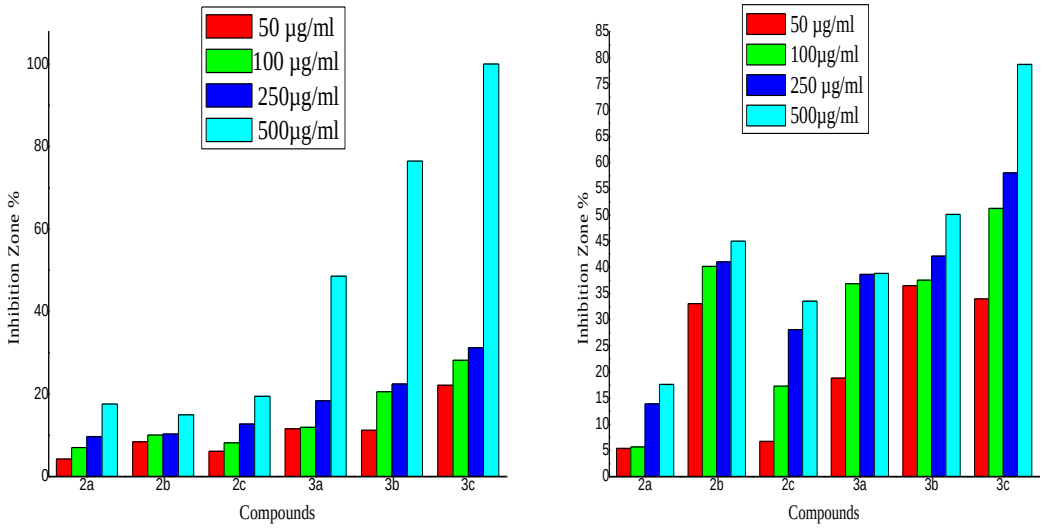
commune and *Fusarium rodelens*. The antifungal activity was determined by the radial growth method [14]. The fungal cultures were incubated at 37°C for 4 days.Finally the zones of inhibition were carefully measured and activity data are listed in Table 3.

Table 3 Preliminary *in vitro* antifungal activity for 4 days of the synthesized compounds.

Comp.	Inhibition Zone %											
	Fusarium oxysporum				*Fusarium commune*				*Fusarium rodelens*			
	Concentration in µg/ml											
	50	100	250	500	50	100	250	500	50	100	250	500
2a	5.39	5.68	13.92	17.61	4.23	7.06	9.73	17.58	0.58	11.56	12.15	12.74
2b	33.05	40.19	41.02	45.01	8.41	10.09	10.28	14.95	2.91	4.44	9.50	14.41
2c	6.75	17.29	28.10	33.51	6.12	8.16	12.71	19.46	0.98	7.64	9.80	17.05
3a	18.83	36.89	38.64	38.83	11.60	11.96	18.39	48.57	3.36	4.20	13.27	56.13
3b	36.47	37.52	42.13	50.10	11.21	20.56	22 .45	76.44	0.59	2.03	28.81	69.32
3c	33.98	51.26	58 .05	78.76	22.14	28.21	31.25	100	2.37	13.05	69.83	100

As indicated in Table 3, at concentration of 500µg/ml, all of the tested compounds exhibited moderate to good inhibitory effects against all the tested fungi.

Histograms (Figures 2-4) shows clearly that quaternary ammonium of amino acid metyl ester showed high antifungal activity due to presence of quaternary ammonium and carboxylic group in the compounds **3a-c**.While amino acid methyl ester **2a-c** showed moderate activity against the tested fungi.



Fusarium oxysporum *Fusarium commune*

Figure 3 Histograms representation of compounds **2a-c** and **3a-c** against *Fusarium oxysporum* and *Fusarium commune*.

Conclusions

In conclusion, new compounds containing multiple active moieties based on amino acid methyl esters with attached secondary ammonium bromide group derived from three essential amino acids were successfully synthesized and characterized and their physical and *antifungal* properties were studied. The synthesized compounds showed promising antifungal potential against the phytopathogenic test fungi.

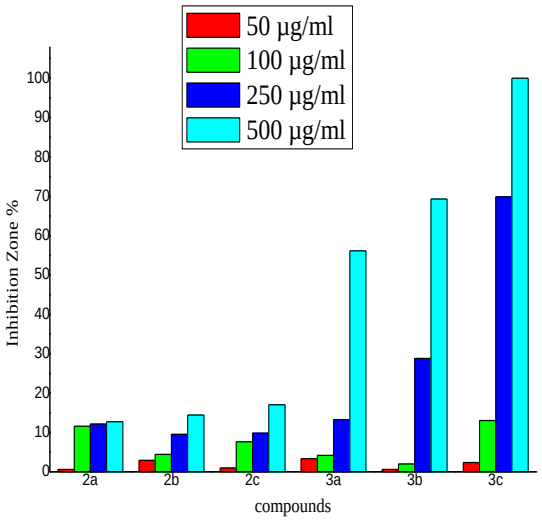


Figure 4 Histogram representation of compounds **2a-2c** and **3a-3c** against *Fusarium rodelens.*

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