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SYNTHESIS AND GROWTH-STIMULATING ACTIVITY OF IONIC CARBOXYLIC ACID DERIVATIVES CONTAINING TRIMECAINE MOIETY

Abstract: *This research presents a synthesis, growth-regulating activity of ionic carboxylic acid derivatives containing trimecaine moiety. The novel ionic substances were synthesized by N-alkylation of trimecaine with iodine-containing carboxylic acids under ultrasound-assisted and microwave irradiation, with results compared to traditional thermal methods. Alternative synthesis methods have shown higher yields in a shorter time than traditional synthesis methods. The synthesized compounds were evaluated for their effects on germination energy and capacity across various varieties and hybrids of sweet sorghum seeds. Notably, promising results were observed in seeds stored for extended periods, where germination activity typically declines. The synthesized ionic compounds outperformed the control in terms of germination energy and capacity across various sorghum seed varieties and hybrids. Furthermore, diluted ionic compound solutions (10^{-3} wt. %) were more effective in promoting seed growth than more concentrated solutions (10^{-2} wt. %) and water. Additionally, certain ionic compounds provided better stimulation for specific sorghum seed types compared to the commercially available trimecaine hydrochloride.*

Key words: *Synthesis, trimecaine, ultrasound activation, microwave-assisted synthesis, germination, sweet sorghum seeds.*

Introduction

Today, the application of green synthesis methods is becoming more and more popular, which makes it more efficient and environmentally friendly. In many factories, green synthesis is used to produce new substances, polymers, and other materials. One of the effective methods of environmentally friendly synthesis is the microwave (MW) method, which is widely used in chemical synthesis [1]. A lot of organic molecules have been successfully synthesized through this rapid, efficient, and eco-friendly technique, which is becoming a widely used method in synthetic chemistry, greatly improving organic synthesis. MW belongs to the category of not ionised radiation, that not change the structure of compounds. The extent to which a molecula interacts with MW energy is determined by its dielectric constant. As a result, solvents such as dimethylformamide, methanol, acetone, and water heat up quickly under MW irradiation, whereas compounds like tetrachloromethane, methylbenzene, and hydrocarbons exhibit minimal heating. The energetic power that used in this irradiation quicky convert into heat, affecting to jump in kinetic energy between reacting reactants. Since MW irradiation fastly affects the reactants, it needs few energy for heating and eliminates the need to heat surrounding materials, unlike classical heaters. This targeted energy transfer enhances efficiency and reduces energy consumption [2]. As a result, samples heated by MW irradiation exhibit distinct temperature profiles compared to those heated by classical means, with MW heating generating a hotter core and a cooler outside. Finally, MW heating is nearly instantaneous due to the quick transformation of MW energy into heat and the

key advantage is its selectivity – since different reagents absorb MW energy to varying degrees, MW heating enables targeted activation of specific reaction sites rather than normaly heating the entire test.

Ultrasound activation is a solution-phase method, as cavitation occurs exclusively in liquids. This way is based on acoustic cavitation, a phenomenon in which microscopic bubbles in this environment expand and then collapse violently, producing extremely high localized pressures and temperatures. Although this intense process takes place within the liquid phase, it does not directly influence the vibrational energy of chemical bonds. However, it can effectively vibrate or rate up chemical reactions, making it a valuable tool in synthesis [3]. US reactions occuring in just liquid, liquid–liquid and solid–liquid enviroment. US activation is great tool to break the bonds between carbon and halogen in aromatic halogens [4]. US assisted synthesis suitable marchine in green chemistry area, because energy-efficient, do not need toxic sabstances [5].

The organization of United Nations Food and Agriculture (FAO) 2030 «Sustainable Development Goals» program focuses on promoting sustainable technologies to produce essential goods and adaptive to harsh farming methods. These types of methods are designed to boost yields and efficiency while preserving ecological stability. Additionally, the program emphasizes the gradual improvement of soil quality to support long-term agricultural sustainability [6]. The unclassical perennial wild sorghum (*Sorghum almum*) has been studied as a forage crop to various methods of feeding domestic animals. The research proves [7], some verbs show enough adaptability to different illnesses, pests and dry climate. These plants sustainable for variety moist state, producing good survive abilities, and exhibit rapid regrowth after mowing. Furthermore, it is well-suited for conventional crop cultivation technologies. Research conducted over several years has shown that Sorghum almum (perennial wild sorghum) can be cultivated on the same productivity for a long time with higher yields [8]. Nutrient analysis of Sorghum almum revealed the following composition: average moisture (74%), dry compozition (26%), gross fat (2.3%), crude protein (10.6%), and crude fiber (39%). The average nutritive value was 0.20 nutrient units per kilogram, with metabolizable energy reaching 8.54 MJ/kg of not wet materilas. Finally, the plant gives base minerals, for example phosphorus and calcium, simultaneously with a mean carotene [9]. A significant focus of sorghum research is the development of phytobiomes enriched with effective microorganisms to enhance the plant's green mass productivity and nutrient content. To maximize its potential, it is necessary to advance technologies for its cultivation, optimize fodder production processes, and develop improved methods for producing haylage from sorghum.

This report presents the synthesis of ionic compounds derived from trimecaine, utilizing MW and US activation alongside conventional thermal activation methods. The synthesized ionic compounds demonstrated effectiveness as growth regulators for sorghum seeds. Additionally, the biological activities of these newly synthesized complexes were thoroughly investigated.

Materials and Methods

The ionic substance's m.p. was investigated by using capillary tube OptiMelt (Stanford Research System). IR spectra were reconstructed using KBr probes on a detector known as the «Nicolet 5700 FT-IR». The NMR spectra of 13C- and 1H-NMR were recognized by NMReady 60 MHz spectrometer at 25 or 30^{o}C with $CDCl_3$ as a solvent. Thin layer chromatography on selective plates (Sigma Aldrich®, Germany) with appropriately developed vectors was used to test the product's purity. As eluent the mixtures of ethanol and diethyl ether (3:1 V/V and 6:1 V/V) were used. A direct current US generator (42 kHz, 100 W) and a conventional home microwave oven (70-110 W) was used for synthesis. Purification and isolation of the molecules were achieved by crystallization by selecting suitable solutions.

The original molecula, 2-diethylamino-N-(2,4,6-trimethylphenyl)acetamide (trimecaine base), was synthesized from commercially available hydrochloride (HUBEI KANGBAOTA FINE CHEMICAL CO., LTD) through neutralization, with the conditions and methods detailed in [10]. The general equation for via N-alkylation of trimecaine iodine-containing carboxylic acids is presented in Figure 1.

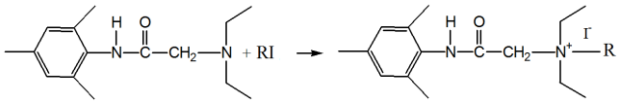


Figure 1 – Synthesis of trimecaine moiety compounds

The process under US and MW methods and using classical way was used to synthesize trimecaine iodine-containing carboxylic acids. Table 2 shows the reaction time of trimecaine base with iodine-containing carboxylic acids using ultrasonic and microwave activation as well as under standard circumstances (reflux in acetonitrile). Once the procedure was finished, the solution's volume was chilled and half of it evaporated. Crystallization was used to separate and purify the resultant isolated product, and TLC was used to verify the product's purity using a 5:1 diethyl ether and ethanol mixture.

The 0.01 mol of trimecaine base was dissolved in 15 ml of acetonitrile in a 100 ml flask. Following that, 0.011 mol of iodine-containing carboxylic acids was added, and the reactants was then heated using the traditional procedure (78-85 ^{o}C). A similar mixture of solutions was used in various methods. The reactants were added in a US reactor and its accelerated and reacted by US conditions, which included 42 kHz and 100 W at 25–35^{o}C. Meanwhile, the mixture was put in a microwave reactor and its contents reacted under 80–160 W of microwave radiation at 25-60^{o}C.

The N-(carboxymethyl)-N,N-diethyl-2-(mesitylamino)-2-oxoethan-1-aminium iodide, **1a**, was separated as white crystals after crystallization process. M.p. 182-184^{o}C. IR (KBr), cm^{-1}: 3176 (N-H) 1696 (C=O amide), 1483 (C_{sp2}=C_{sp2}). ^{13}C NMR ($CDCl_3$, 25 ^{o}C) δ, ppm: 161.15 (C=O); 138.87 (CH_3); 135.71 (C_{sp2}-NH); 128.88 (C_{sp2}); 68.9 (CO-CH_2-N+); 53.69 (N+-CH_2-CH_3); 21.03 (C_{sp2}-CH_3); 20.01 (C $_{sp2}$-CH_3); 8.19 (N+-CH_2-CH_3). ^{1}H NMR ($CDCl_3$, 25 ^{o}C) δ, ppm: 13.19 (s, N-H); 6.81 (s, $H_{aromatic}$); 3.93 (s, CO-CH_2-N+); 3.75 (q, (-CH_2-) N+-CH_2-CH_3); 2.54 (C_{sp2}-CH_3); 2.43 (C_{sp2}-CH_3); 1.57 (t, (-CH_3) N+-CH_2-CH_3).

The N-(3-carboxyethyl)-N,N,-diethyl-2-(mesitylamino)-2-oxoethan-1-aminium iodide, **1b**, was separated as white crystals after crystallization process. M.p. 160-163°C. IR (KBr), cm^{-1}: 3180 (N-H) 1699 (C=O amide), 1486 (C_{sp2}=C_{sp2}). ^{13}C NMR ($CDCl_3$, 25 ^{o}C) δ, ppm: 161.15 (C=O); 138.96 (CH_3); 135.09 (C_{sp2}-NH); 129.1 (C_{sp2}); 69.1 (CO-CH_2-N+); 53.9 (N+-CH_2-CH_3); 21.05 (C_{sp2}-CH_3); 20.09 (C $_{sp2}$-CH_3); 8.19 (N+-CH_2-CH_3). ^{1}H NMR ($CDCl_3$, 25 ^{o}C) δ, ppm: 13.15 (s, N-H); 6.85 (s, $H_{aromatic}$); 3.96 (s, CO-CH_2-N+); 3.77 (q, (-CH_2-) N+-CH_2-CH_3); 2.59 (C_{sp2}-CH_3); 2.45 (C_{sp2}-CH_3); 1.59 (t, (-CH_3) N+-CH_2-CH_3).

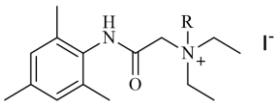
The N-(4-carboxypropyl)-N,N,-diethyl-2-(mesitylamino)-2-oxoethan-1-aminium iodide, **1c**, was separated as white crystals after crystallization process. M.p. 142-144°C. IR (KBr), cm^{-1}: 3185 (N-H) 1701 (C=O amide), 1490 (C_{sp2}=C_{sp2}). ^{13}C NMR ($CDCl_3$, 25 ^{o}C) δ, ppm: 161.21 (C=O); 139.01 (CH_3); 134.93 (C_{sp2}-NH); 129.1 (C_{sp2}); 69.2 (CO-CH_2-N+); 54.1 (N+-CH_2-CH_3); 21.06 (C_{sp2}-CH_3); 20.11 (C $_{sp2}$-CH_3); 8.20 (N+-CH_2-CH_3). ^{1}H NMR ($CDCl_3$, 25 ^{o}C) δ, ppm: 13.19 (s, N-H); 6.86 (s, $H_{aromatic}$); 4.01 (s, CO-CH_2-N+); 3.81 (q, (-CH_2-) N+-CH_2-CH_3); 2.61 (C_{sp2}-CH_3); 2.69 (C_{sp2}-CH_3); 1.71 (t, (-CH_3) N+-CH_2-CH_3).

The experiment's next step was to determine how the trimecaine iodine-containing carboxylic acids solution affected the germination vigor and ability of sorghum seed sorts, like Kaz-16 2017, Kiz-20 2017, Kiz-9 2015, Kiz-7 2017, and AC-76 2015, which are commercially available. The solutions with concentration 10^{-3} % and 10^{-2} % (by mass) using the newly synthesized products to accomplish this aim. Glassware used for chemicals, including utensils, was cleaned and dried in an oven set to 110°C for 50 minutes. After being sterilized with alcohol for five to seven minutes, all genotypes of sweet sorghum seeds were repeatedly cleaned with distilled water. The seeds were positioned carefully and spaced apart to prevent any contact with the dish's walls. For the filter paper was added water for the control and synthesized products solutions for stimulation prior to seeding. The prepared examples were kept at a temperature between 20 and 25°C in an opaque cabinet. The standard was followed while measuring germination and germination capacity. Two measurements of the root and shoot lengths of the seeds that germinated – four and nine days after sowing – were made.

Results and discussions

When used in synthetic formulations for organic chemicals and ionic liquids, MW or US activation provides excellent stimulation and efficacy. These techniques can increase reaction rates and yields while saving energy. The non-toxicity of MW radiation and US activation is crucial for the fields of organic chemistry, bioorganic chemistry, and the chemical industry. Synthesized and tested ionic compounds based on trimecaine are shown in Table 1.

Table 1 – Synthesized and tested compounds

Core structure	R, the substituent on the tertiary nitrogen	Code
	-CH_2COOH	**1a**
	- CH_2CH_2COOH	**1b**
	- $CH_2CH_2CH_2COOH$	**1c**

All reaction results were gathered and presented in Table 2.

Table 2 – The conditions and yields of *N*-alkylation reaction

Products	Reaction conditions	Time, [min]	Yield, [%]
1a	Classical	180	73
	US	60	71
	MW	20	84
1b	Classical	240	71
	US	80	69
	MW	25	82
1c	Classical	300	49
	US	100	63
	MW	40	80

In all approaches, initial compound trimecaine dissolved in acetonitrile and heated with iodine-containing carboxylic acids in conventional conditions as well as utilizing microwave (MW) radiation and ultrasound (US) assisted synthesis. The use of US and MW techniques proves to be more efficient in minimizing exposure time and enhancing chemical efficacy when compared to conventional syntheis. By incorporating ionic conductivity and dipolar polarization through a dielectric mechanism, it is possible to heat the reactants within an electromagnetic radiation frequency range of 0.3 to 300 GHz. This leads to a uniform temperature rise throughout the entire sample, in contrast to conventional conductive heating. In MW heating, the material's ability to absorb microwave energy and convert it into heat results in significantly more efficient and localized heating. Each molecule does not absorb the heat generated from ultrasound; instead, it can rotate or vibrate within a frequency range of 20 to 100 kHz, without altering the ultrasonic frequencies.

Certain derivatives of trimecaine have demonstrated growth-regulating properties, prompting an investigation into the effects of synthesized compounds on the germination of sweet sorghum seeds [11]. Prior to the field sowing of various sweet sorghum genotypes, their germination potential was assessed under laboratory conditions. The germination of sweet sorghum seeds was influenced by multiple factors, such as the parent plant's genotype, the length of seed storage, and the inoculation of seeds with various microorganisms. Experiments were conducted using solutions at concentrations of 10^{-3} % and 10^{-2} % to assess the effects of synthesized ionic compounds derived from trimecaine on the germination energy and capacity of sweet sorghum seeds. The results shown in Table 3 demonstrate the impact of trimecaine-based ionic compounds on the germination energy of sweet sorghum seeds and compared to a control solution water and trimecaine hydrochloride (**1·HCl**), as well as trimecaine ionic compounds **1a**, **1b**, and **1c** at a concentration of 0.001%, including standard deviation.

Table 3 – Germination energy of sweet sorghum seeds with solutions **1·HCl, 1a, 1b, 1c** (0.001%) including standard deviation. (rl – root length; sl – shoot length; cm).

		Control			1·HCl			1a			1b			1c		
№	Genotype	rl	sl	%	rl	sl	%	rl	sl	%	rl	sl	%	rl	sl	%
1	Kiz-9 (2015)	1.4±0.1	1.2±0.1	31±2	4.6±0.2	0.8±0.1	36±2	3.9±0.2	2.3±0.1	46±3	4.6±0.3	3.6±0.2	31±1	1.5±0.1	2.6±0.2	16±1
2	Kiz-20 (2017)	3.4±0.2	0.9±0.1	31±2	3.1±0.2	1.8±0.2	86±3	1.6±0.1	2.4±0.2	100	4.7±0.3	4.9±0.2	86±3	3.3±0.2	4.1±0.2	46±3
3	AC-76 (2015)	0.9±0.1	0.4±0.1	46±3	3.1±0.2	1.8±0.2	66±2	0.8±0.1	0.6±0.1	41±2	2.3±0.1	4.6±0.3	61±3	2.8±0.2	3.1±0.1	31±2
4	Kiz-7 (2017)	2.1±0.1	1.8±0.1	16±1	2.4±0.2	1.6±0.1	11±1	2.6±0.1	2.1±0.1	41±2	3.1±0.2	5.5±0.3	16±1	3.3±0.2	5.2±0.2	11±0.3
5	Kaz-16 (2017)	2.4±0.2	1.1±0.1	56±2	1.1±0.1	1.1±0.1	21±1	1.1±0.1	1.1±0.1	11±1	7.1±0.3	4.7±0.3	100	5.2±0.3	4.6±0.2	100

The study reveals that applying ionic compound solutions during pre-sowing treatments affects both seed germination and germination energy, thereby stimulating gemmogenesis (shoot formation) and enhancing rhizogenesis (root system development). Affecting with **1·HCl** gives a moderate rise in rhizogenesis in the genotypes AC-76 and Kiz-9 than control group. The longest growth activity of the root system was got in the varieties Kiz-20 and Kiz-7 by the effect of **1a**. The effect of growth regulants on the intensity of gemmogenesis in germinated sweet sorghum seeds, with treatment **1a**, revealed that the cultivar Kiz-20 exhibited the greatest values. The effect of germination energy of sweet sorghum seeds with control solutions (water and **1·HCl**) and **1a**, **1b**, and **1c** ionic compounds of trimecaine with a concentration of 0.01% (Table 4).

Table 4 – Germination energy of sweet sorghum seeds with solutions **1a**, **1b**, and **1c** (0.01%) including standard deviation. (rl – root length; sl – shoot length; cm).

		Control			1·HCl			1a			1b			1c		
№	Genotype	rl	sl	%	rl	sl	%	rl	sl	%	rl	sl	%	rl	sl	%
1	Kiz-9 (2015)	0.3±0.1	0.5±0.1	56±2	0.5±0.1	0.8±0.1	35±2	0.3±0.1	0.4±0.1	5±1	0.3±0.1	0.6±0.1	86±2	0.4±0.1	0.3±0.1	41±2
2	Kiz-20 (2017)	-	-	-	0.7±0.1	0.6±0.1	35±1	0.6±0.1	0.4±01	56±2	-	-	-	-	-	-
3	AC-76 (2015)	0.1	0.1	16±1	0.5±0.1	0.7±0.1	26±1	0.1	0.4±0.1	51±1	0.3±0.1	0.5±0.1	71±1	0.4±0.1	0.2±0.1	42±1
4	Kiz-7 (2017)	0.5±0.1	0.7±0.1	61±2	-	-	-	-	-	-	0.7±0.1	0.7±0.1	81±2	1.3±0.1	0.9±0.1	76±2

In our experiments evaluating different seed pretreatment ways, it was observed that in **1b**, the cultivars Kiz-9 and AC-76 achieved germination rates of 86% and 71%, respectively. In contrast, water gives smaller germination rates of only 56% and 16%. Additionally, these genotypes exhibited pronounced sensitivity to the **1·HCl** solution used for seed pretreatment, leading to a substantial improvement in root growth and overall development.

Conclusion

The results of our experiments suggest that US-assisted synthesis and MW irradiation techniques can serve as effective alternatives to classical synthesis methods. This study aligns with existing literature, indicating a rising trend in reaction efficiency, ranked as follows: classical methods, ultrasound activation, and microwave activation. In certain reactions, ultrasound activation yielded products that were marginally lower than those obtained under classical conditions; however, the synthesis duration was reduced by a factor of three. Seeds from genotype Kiz-9 (2015) treated with **1b** demonstrated 86% increase in germination compared to the control when treated with **1·HCl**. Notably, the Kiz-20 cultivar achieved a germination rate of 100%, while the control group recorded only 30%. The growth intensity of the root and shoot systems in **1b** was superior to both the control and **1·HCl** treatments across all sweet sorghum seed types. The ionic compound solutions were responsive to seed pretreatment agents, significantly enhancing germination rates. Most synthesized ionic compounds outperformed the control in terms of germination energy and capacity across various sorghum seed varieties and hybrids. Furthermore, diluted ionic compound solutions (10^{-3} wt. %) were more effective in promoting seed growth than more concentrated solutions (10^{-2} wt. %) and water. Additionally, certain ionic compounds provided better stimulation for specific sorghum seed types compared to the commercially available trimecaine hydrochloride.

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ТРИМЕКАИН БӨЛІГІ БАР ИОНДЫҚ КАРБОН ҚЫШҚЫЛЫ ТУЫНДЫЛАРЫНЫҢ СИНТЕЗІ ЖӘНЕ ӨСУІН ЫНТАЛАНДЫРАТЫН БЕЛСЕНДІЛІГІ

Бұл зерттеу құрамында тримекаин бөлігі бар иондық карбон қышқылы туындыларының синтезі мен өсуін реттейтін белсенділігін қарастырады. Жаңа иондық заттар ультрадыбыстық және микротолқынды сәулеленудің әсерінен құрамында йод бар карбон қышқылдары бар тримекаинді N-алкилдеу арқылы синтезделді, бұл дәстүрлі термиялық әдістермен салыстыруға болатын нәтижелерге қол жеткізді. Альтернативті синтез әдістері дәстүрлі синтез әдістеріне қарағанда қысқа уақыт ішінде жоғары өнімділікті көрсетті. Синтезделген қосылыстар олардың өну энергиясына және тәтті құмай тұқымдарының әртүрлі сорттары мен будандарында өну қабілетіне әсері үшін бағаланды. Бір қызығы, өну белсенділігі әдетте төмендейтін тұқымдарды ұзақ уақыт сақтау арқылы перспективалы нәтижелер алынды. Синтезделген иондық қосылыстар құмай тұқымының әртүрлі сорттары мен будандарында өну энергиясы мен өнімділігі бойынша бақылаудан асып түсті. Сонымен қатар, иондық қосылыстардың сұйылтылған ерітінділері (10^{-3} мас. %) концентрацияланған ерітінділерге қарағанда тұқымның өсуін ынталандыруда тиімдірек болды (10^{-2} мас. %) және су. Сонымен қатар, кейбір иондық қосылыстар тримекаин гидрохлоридімен салыстырғанда құмай тұқымының белгілі бір түрлеріне жақсы ынталандыруды қамтамасыз етті.

Түйін сөздер: *Синтез, тримекаин, ультрадыбыстық белсендіру, микротолқынды белсендіру, өну, тәтті құмай тұқымдары.*

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СИНТЕЗ И РОСТ-СТИМУЛИРУЮЩАЯ АКТИВНОСТЬ ПРОИЗВОДНЫХ ИОННЫХ КАРБОНОВЫХ КИСЛОТ, СОДЕРЖАЩИХ ТРИМЕКАИНОВЫЙ ФРАГМЕНТ

Это исследование посвящено синтезу и рост-регулирующей активности производных ионной карбоновой кислоты, содержащих тримекаиновую часть. Новые ионные вещества были синтезированы путем N-алкилирования тримекаина йодсодержащими карбоновыми кислотами под воздействием ультразвука и микроволнового излучения, что позволило получить результаты, сравнимые с традиционными термическими методами. Альтернативные методы синтеза показали более высокие выходы за более короткое время, чем традиционные методы синтеза. Синтезированные соединения были оценены на предмет их влияния на энергию прорастания и способность к прорастанию у различных сортов и гибридов семян сладкого сорго. Примечательно, что многообещающие результаты были получены при длительном хранении семян, при котором активность прорастания обычно снижается.

Ключевые слова: *Синтез, тримекаин, активация ультразвуком, микроволновая активация, проращивание, семян сладкого сорго.*

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