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SYNTHESIS AND GROWTH-STIMULATING ACTIVITY OF TRIMECAINE ETHYL IODOETHANOATE

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Abstract: *Introduction.*Novel activation methods such as ultrasonic activation and microwave irradiation are becoming increasingly popular in the green synthesis of ionic liquids and ionic compounds. The use of ionic liquids and ionic compounds is becoming more and more interesting for scientists precisely because of their growth-stimulating activity.*The purpose*.Synthesisof trimecaine ethyl iodoethanoatewascarried out via N-alkylation in classical conditions and using ultrasonicirradiation and microwave-assisted synthesis. *Methodology*.Thestructure and functional groups of novel synthesized substanceidentifiedby^{1}H,^{13}C NMRandIR,and growth-stimulating activity tested fortengenotypes of sweet sorghum seeds.Thesynthesis of trimecaine ethyliodoethanoate was carried out via N-alkylation in classical conditions and using microwave radiation and ultrasonic activation. *Results.*The synthesized ionic compoundswere characterized by IR, ^{1}H and ^{13}C NMR, growth-stimulating activity was tested on ten varieties and hybrids of sweet sorghum seeds. Alternative methods for the synthesis of ultrasonic and MW activation showed a good result in the synthesis of N,N-diethyl-N-ethylethanoate-N-(2-(mesitylamino)-2-oxoethyl)aminium iodide with a higher isolation yield in a shorter time compared to classical conditions. *Conclusion.*The product with the highest yield is formed under MW irradiation in a short time, while under classical conditions the yield was lower, and the reaction time was longer. The synthesized ionic compound had a better effect on the energy and germination of all types of sweet sorghum seeds compared to the control.

Key words: microwave-assisted synthesis, ultrasonicirradiation, trimecaine, germination capacity, germination energy, sweet sorghum

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1. Introduction

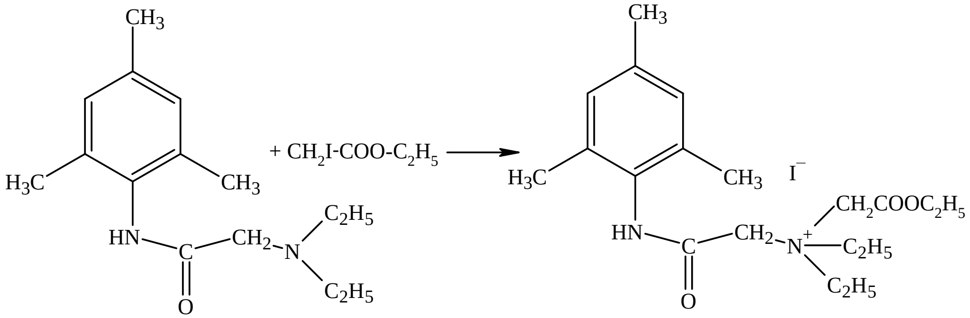
Alternative activation methods such as ultrasonic activation and microwave irradiation are getting more popular in green synthesis methodologies of organic and inorganic substances[1]. The use of ultrasound (US) activation in chemicalsynthesis in a liquid phase, can generate various physico-chemical effects [2]. During ultrasonic activations, intense thermal, mechanical,and chemical effects occur, leading to the phenomenon of cavitation, throughtheformation, growth, and collapse of gaseousmicrobubbles in the liquid phase[3,4].Electromagneticradiation with a frequency in the range of 0.3–300 GHz, microwave (MW) band, can heat matter through a dielectricmechanism, which may include dipolar polarization and ionicconductivity. This is the ability of a material to absorb microwaveenergy and convert it into heat, causing volumetric heating [5]. MW and US activation has been used to speed up a lot of organic reactions with shorter times and higher yields[6].

One of the important applications of ionic liquids and ionic compounds, of great interest to scientists, is their growth-stimulating activity. Plant growth stimulants are substances that can speed up the metabolism and stimulate the set of green mass in representatives of the flora; active compounds of the physiological class, which in small quantities cause tangible changes of a positive nature in the process of plant growth[7-8]. They can be of natural or synthetic origin. The use of growth stimulants allows you to get a more lush, branched, and voluminous plant, which in the end will bring a rich harvest[9-10]. They may also include metabolic stimulants, since they speed up the metabolism, without which it is simply impossible to achieve rapid growth.Various growth regulators such as gibberellic acid, ethephon, and chlormequat are used to promote germination and yield in sorghum[11].This report presents the synthesis and growth-stimulating activity of a new ionic compound obtained by N-alkylation based on trimecaine.

2. Experimental part

1. Materials and methods. The m. p. of ionic substance was measuredinopencapillarytubeonanOptiMelt (StanfordResearchSystem). The 1H- and ^{13}C-NMR spectrawererecordedusing a NMReady 60 MHz spectrometerat 25 or 30 °C by using $CDCl_3$ as a solvent. IR spectra was recordedon a spectrometer «Nicolet 5700 FT-IR» using KBr pellets. Thepurityofproductweretested by thin layer chromatography onsilicaplates (SigmaAldrich®, Germany) withiodinevaporsdevelopment. Thediethylether:ethanolmixtures (4:1 V/V and 5:1V/V) wereusedaseluents. The TLC spotsonthedevelopedplateswereobservedin UV light (λ = 254 nm). AllthereactantsandsolventsfromSigmaAldrich®. AnultrasonicprobefromColeParmer (42 kHz, 100 W) and a domesticmicrowavegenerator (80 W) wereusedforthereaction. Theseparationandpurificationofsubstanceswascarriedout by crystallizationfromappropriatesolvents.

Trimecainebase - wassynthesizedfromcommerciallyavailablehydrochloride by neutralization, conditions and methods discussed in [12]. The equation of synthesis trimecaine ethyl iodoethanoate from trimecaine base is given in Scheme1.



Scheme 1 – SynthesisofN,N-diethyl-N-ethylethanoate-N-(2-(mesitylamino)-2-oxoethyl)aminium iodide.

Thesynthesis of the trimecaine ethyl iodoethanoate had been carriedout under conventional method and using MW and US activation. The reaction time of trimecaine base with ethyl iodoethanoate under normal conditions (reflux in acetonitrile) and using ultrasonic and microwave activation is presented in Table 1. After completion of the process, the volume of the solution was evaporated by half and cooled. The resulting isolated product was separated and purified by crystallization, and the purity of the product was checked by TLC using a mixture of diethyl ether and ethanol (5:1).

2. Spectral and other data for the N,N-diethyl-N-ethylethanoate-N-(2-(mesitylamino)-2-oxoethyl)aminium iodide. 15 ml of acetonitrile was added to a 100 ml flask and 0.01 mol of trimecaine base was dissolved. After that, a solution of 0.011 mol of ethyl iodoethanoate was added and the resulting solution was boiled according to the classical method (75-82 °C). The same mixture of

solutions was used in alternative methods, and the reaction mixture was placed in a US reactor, and the contents were reacted under US conditions, parameters: 42 kHz, 100 W at 25–35 °C, while the solution of mixture wasplaced in a microwave reactor, and the contents reacted under microwave irradition at 80-160 W at 25–60 °C.

The N,N-diethyl-N-ethylethanoate-N-(2-(mesitylamino)-2-oxoethyl)aminium iodide was separated as pale-yellow crystals after crystallization process. M.p. 125-128°C. IR (KBr), cm^{-1}: 3179 (N-H), 1699 (C=Oamide), 1481 (C_{sp2}=C_{sp2}). ^{1}H NMR ($CDCl_3$, 25 °C) δ, ppm: 13.11 (N-H); 6.79 ($C_{sp2}H$); 3.91 (CO-CH_2-N^+); 3.71 (q, (-CH_2-)N^+-C$\underline{H_2}$-CH_3); 2.55 (C_{sp2}-CH_3); 2.42 (C_{sp2}-CH_3); 1.56(t, -CH_3)N^+-CH_2-C$\underline{H_3}$). ^{13}C NMR ($CDCl_3$, 25 °C) δ, ppm: 161.11 (C=O); 134.89 (C_{sp2}-NH); 128.9 (C_{sp2}); 68.8 (CO-$\underline{C}H_2$-N^+); 53.7 (N^+-$\underline{C}H_2$-CH_3); 21.01 (C_{sp2}-$\underline{C}H_3$); 20.07 (C_{sp2}-$\underline{C}H_3$); 8.17 (N^+-CH_2-$\underline{C}H_3$).

The next stage of the experiment was to find out how the trimecaine ethyl iodoethanoate solution affects the vigor of germination and the ability of genotypes of sweet sorghum seeds, like Kiz-9 2015, Kaz-20 2015, UNL 3016 2014, Black top 2015, Kaz-8 2013, Victoria-4 2013, Baikadam 2019, Rio 2014, Topper 76 2015, Kaz-16 2017. To achieve this goal, 0.01 and 0.001 % (by mass) solutions were made with the new synthesized substance. Utensils and other chemical glasswares were cleaned and dried in oven at 110°C for 50 min. All genotypes of sweet sorghum seeds were sterilized with alcohol for 5–7 min and washed several times with distilled water. For every sample, 10 Petri dishes were used for standard and 50 dishes (20 seeds) for one solution of trimecaine ethyl iodoethanoate. Seeds were added and arranged far from to each other without touching the wall of dish. Before planting the seeds, the filter paper wetted with water for control and solutions of trimecaine ethyl iodoethanoate for the stimulating. The prepared examples were placed in an opaque cabinet with a temperature of 22 to 25°C. Germination and and germination capacity were measured according to the standard. The root length and shoot length of germinated seeds were measured twice, i.e., 4 and 9 days after sowing. The number of germinated seeds counted from every batch, which had 100 seeds. If thefinalresultsofthegerminationofindividualbatchees didn't exceedthe SD, as a result, the arithmetic mean was determined with an exactly of one percent, the batches were considered comparable.

3. Results and discussion

The rightimplementation of US and MW in the field of green chemistrydepends on the scalability of the excellentlaboratory research results for industrial use. Some preliminaryresults were encouraging on a continuous or experimental scale, but it is important to demonstrate the possibleUS and MW contributions that open doors for industrialapplications. Compared with the classical method, the use of US and MW is a good method in organic synthesis, to obtaining products with higher yields in a shorter time. The results of theN-alkylation reaction with the different type of condition and an average yield of productgiven in Table 1.

Table 1–Thereaction time and yieldofN,N-diethyl-N-ethylethanoate-N-(2-(mesitylamino)-2-oxoethyl)aminium iodide

Synthesis / reaction conditions	Time, min	Yield, %
Classical method (thermal activation)	90-120	82.8
US activation	30-40	79.4
MW activation	15-20	92.2

The greatest productivity is obtained with microwave-assisted synthesisinthe shorter time, while in classical conditions the reaction time was 6 times longer at a poor yield. Ultrasonic irradiation had almost the same yield results as the classical method, but the reaction speed was faster.To study the growth-stimulating activity of trimecaine ethyl iodoethanoatetowards the sweet sorghum seeds, experimentshave been carried outwith a solution (concentration 0.001 and 0.01% by mass).Theaveragenumber of the resultsoftrimecaine ethyl iodoethanoateon the effect of germination energy and capacity ofsweet sorghum seeds with control (water), includingstandard deviation (SD)is given in Table 2.

Table 2–The results of N,N-diethyl-N-ethylethanoate-N-(2-(mesitylamino)-2-oxoethyl)aminium iodideeffect on germination energy and capacity ofsweet sorghum seeds (concentration of solutions are 0.01 and 0.001 %; lengths of shoot and root in cm)

№	Genotype	Germination energy, %			Germination capacity, %		
		Length of root (SD ±0.05)	Length of shoot (SD ±0.05)	% (SD ±3)	Length of root (SD ±0.05)	Length of shoot (SD ±0.1)	% (SD ±3)
Control(water)							
1	Kiz-9 2015	0.7	1.2	10	0.4	1.4	10
2	Kaz-20 2015	0	0	0	0	0	0
3	UNL3016 2014	0	0	0	0	0	0
4	Black top 2015	0	0.3	5	0	0	0
5	Kaz-8 2013	0.2	0.4	10	0.7	1.4	10
6	Victoria-4 2013	0	0	0	0	0	0
7	Baikadam 2019	0.4	0.6	45	0.8	1.7	55
8	Rio 2014	0	0	0	0	0	0
9	Topper 762015	0.9	1.6	35	0.9	2.4	25
10	Kaz-16 2017	0.3	0.5	45	0.5	1.2	50
Tr.CH_2I-COO-C_2H_5(0.01%)							
1	Kiz-9 2015	2.1	0.8	5	3.9	4.5	10
2	Kaz-20 2015	1.9	1.2	15	3.7	4.9	20
3	UNL3016 2014	1.2	1.7	5	1.7	3.4	15
4	Black top 2015	0.2	0.5	5	2.8	4.8	15
5	Kaz-8 2013	0	0	0	0	0.3	5
6	Victoria-4 2013	0	0	0	0	0	0
7	Baikadam 2019	0.8	2.1	55	2.7	4.8	75
8	Rio 2014	0.7	1.2	30	1.6	2.7	35
9	Topper 76 2015	0.7	1.7	45	1.4	4.8	50
10	Kaz-16 2017	2.0	2,4	75	3.7	2.9	95

Tr.CH$_2$I-COO-C$_2$H$_5$(0.001%)							
1	Kiz-9 2015	0.2	0.4	10	1.9	2.9	35
2	Kaz-20 2015	0.3	0.4	5	0.4	0.6	20
3	UNL3016 2014	0	0	0	0	0	0
4	Black top 2015	0.2	0.3	5	0.7	2.3	25
5	Kaz-8 2013	1.2	0.5	15	5.7	5.2	20
6	Victoria-4 2013	0	0	0	0	0	0
7	Baikadam 2019	0.9	2.2	80	3.2	5.7	80
8	Rio 2014	2.3	2.1	25	4.3	5.3	30
9	Topper 76 2015	3.2	1.7	70	1.7	4.7	55
10	Kaz-16 2017	2.7	2.9	80	3.6	5.8	100

The germination capacity in ethalon(water)was 55 %, in ionic substance solution (0.01%) 75 % and in dilute solution (0.001%) 80 % in the genotype of *Baikadam 2019*. Studies have shown that the solution of an ionic substancecan improve the germination of seeds, and the energy of germination, increasing the processes of gemmogenesis (the process of shoot growth) and the intensity of rhizogenesis (the process of formation of the root system).Solutionof ionic compoundwith different concentrationsaffected better to intensity of rhizogenesis in genotypes *Topper 76 2015*and *Kaz-16 2017*than standard solution. The germination ability and energy, including the intensity of rhizogenesis and gemmogenesis, were higher when using a solution of trimecaine ethyl iodoethanoatein all varieties of sweet sorghum seedthan standard solution (control).Effect of a solution of ionic compound on gemmogenesis of*Baikadam 2019*, *Rio 2014*, *Topper 76 2015*, and *Kaz-16 2017* presented in Figure.

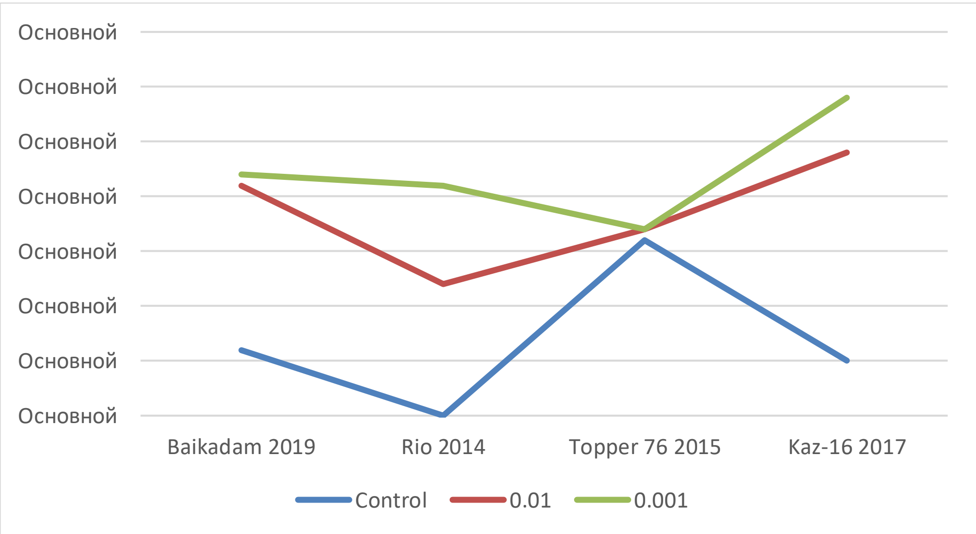


Figure – The effect of growth regulatoron intensity of gemmogenesis.

The germinated sweet sorghum seed of *Baikadam 2019*root length wastwice longer in synthesized ionic compound than in control, as well as in genotype *Kiz-9 2015* root length in control was 0.7 cm when it was 2.1 cm in 10^{-2} % solution. The dilute solution of ionic compoundshowed the highest intensity to thegemmogenesisand rhizogenesis of *Kaz-16 2017*.

4. Conclusion

Alternative methods of synthesis US and MW activationshowed greater result in synthesis N,N-diethyl-N-ethylethanoate-N-(2-(mesitylamino)-2-oxoethyl)aminium iodidewith a higher isolated yield in shorter time compared with classical condition. The greatest mass of product is formed under microwave-assisted synthesis, in a shorter time, while in classical conditiontheproductivity was poor and reactionrate wasgreater. The synthesized trimecaine ethyl iodoethanoatebetter influencedthe germination capacity and energyfor all genotypes of sweet sorghum seeds compared with thecontrol. The germinationenergy in control was 45 % while in ionic compound solution(0.01%) it was75 % and in dilute solution (0.001%)showed80%germination energyfor genotype of Kaz-16 2017. Thedilute solution of ionic compoundstimulates sweet sorghum seeds better than water and concentrated solution.

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Conflict of Interest: The authors declare that they have no competing interests.

ТРИМЕКАИН ЭТИЛ ЙОДЭТАНОАТЫНЫҢ СИНТЕЗІ ЖӘНЕ ӨСУ- СТИМУЛЯТОРЛЫҚ БЕЛСЕНДІЛІГІ

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Түйіндеме. *Кіріспе.*Иондық сұйықтықтар мен иондық қосылыстардың жасыл синтезінде ультрадыбыстық белсендіру және микротолқынды сәулелендіру сияқты жаңа белсендіру әдістері барған сайын танымал болып келеді. Иондық сұйықтықтар мен иондық қосылыстарды қолдану олардың өсуді ынталандыратын белсенділігіне байланысты ғалымдар үшін барған сайын қызықты болып келеді.*Мақсаты.*Тримекаин этилйодэтанатының синтезі классикалық жағдайда және микротолқынды сәулеленуді және ультрадыбыстық белсендіруді қолдану арқылы N-алкилдеу арқылы жүзеге асырылды. *Методологиясы.*Синтезделген иондық қосылыс ИҚ, ^{1}H және ^{13}C ЯМР-мен сипатталды, өсуді ынталандырушы белсенділік тәтті құмай тұқымдарының он сорттары мен будандарында сыналды. Ультрадыбыстық және микротолқындықбелсендіру балама әдістері N,N-диэтил-N-этилэтанат-N-(2-(меситиламин)-2-оксоэтил)амин иодидін синтездеуде жақсы нәтиже көрсетті. Микротолқынды сәулелендіру әдісіең жоғары шығымды, қысқа мерзімде түзсе, ал классикалық жағдайларда өнімділік төмен және реакция уақыты ұзағырақ болды.

*Нәтижесі.*Дәстүрлі әдіспен салыстырғанда, ультрадыбыстық (УД) синтездеуде өнім шығымы бірдей болғанмен, реакция уақыты УД синтездеуде әлде қайда төмен болды. Синтезделген иондық қосылыс бақылаумен (сумен) салыстырғанда тәтті құмай тұқымдарының барлық түрлері мен генотиптерінің энергиясы мен өнгіштігіне жақсы әсер етті. Kaz-16 2017 генотипіне,бақылаудағы өну энергиясы 45% болса, иондық қосылыс ерітіндісінде (10^{-2}%) 75%, ал концентрациясы төмен ерітіндіде (10^{-3}%) өну энергиясы 80% көрсетті.*Қорытынды.* Қарапайым дәстүрлі әдістен қарағанда МТ және УД белсендіру әдістері әлде қайда жоғары нәтиже көрсетті. Иондық қосылыстың сұйытылған ерітіндісі тәтті құмай тұқымдарының барлық түрлерінің өну күші мен өнгіштігіне, бақылау мен концентірлі ерітіндісіне қарағанда жақсы әсер етті.

Түйінді сөздер: тримекаин, иондық қосылыстар, микротолқынды және ультрадыбысты активтендіру, тәтті құмай, өсу белсенділі

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СИНТЕЗ И РОСТСТИМУЛИРУЮЩАЯ АКТИВНОСТЬ ЭТИЛ ЙОДЭТАНОАТА ТРИМЕКАИНА

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Резюме. *Введение.* Современные методы активации, такие как ультразвуковая активация и микроволновое облучение, становятся все более популярными в зеленом синтезе ионных жидкостей и ионных соединений. Применение ионных жидкостей и ионных соединений становится все более интересным для ученых именно их ростостимулирующей активностью.*Цель.*Синтез этилйодоэтаноата тримекаина осуществляли путем N-алкилирования в классических условиях и с использованием микроволнового излучения и ультразвуковой активации. *Методология.* Синтезированное ионное соединение охарактеризовано с помощью ИК, ^{1}H и ^{13}C ЯМР, рост-стимулирующая активность проверена на десяти сортах и гибридах семян сладкого сорго. Альтернативные методы синтеза УЗ и МВ активации показали высокий результат в синтезе N,N-диэтил-N-этилэтаноат-N-(2-(мезитиламино)-2-оксоэтил)аминия йодида с более высоким выходом выделения за более короткое время по сравнению с классическими условиями. *Результаты.*Продукт с наибольшим выходом образуется при МВ облучении за короткое время, тогда как в классических условиях выход был ниже, а время реакции больше. Синтезированное ионное соединение лучше влияло на энергию и всхожесть всех видов семян сладкого сорго по сравнению с контролем. Энергия прорастания в контроле составила 45 %, тогда как в растворе ионного соединения (10^{-2}%) она составила 75 %, а раствор с меньшей концентрацией (10^{-3} %) показал 80 % энергии прорастания для генотипа Каз-16 2017. *Заключение.*Методы активации МВ и УЗ показали гораздо более высокие результаты, чем классические методы синтеза.Синтезированное ионное соединение лучше влияло на энергию прорастания и всхожесть ко всем видам семян сладкого сорго по сравнению с контролем.

Ключевые слова: тримекаин, ионное соединение, микроволновое излучение, активация ультразвуком, сладкое сорго

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