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Microwave activation of isovaleric acid monoglyceride synthesis and its antimicrobial activity

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The article describes the microwave synthesis of isovaleric acid monoglyceride by the isopropylidene method using microwave-assisted synthesis. The yield of the target product was 94%. The resulting compound was characterized by ^{1}H NMR, ^{13}C NMR, IR spectroscopy and elemental analysis. The resulting product was studied for antibacterial and antifungal activity. It was found that isovaleric acid monoglyceride has pronounced antibacterial activity against *Pseudomonas aeruginosa* and moderate antifungal activity against *Candida albicans.*

Keywords: microwave-assisted synthesis, isopropylidene protection, isovaleric acid monoglyceride, antibacterial and antifungal activity.

INTRODUCTION

Carboxylic acid esters, representing a large and important class of organic compounds, are widely distributed in nature in the form of essential oils, fats, waxes, phosphatides, tannins and have a variety of applications as solvents, extractants, for preparation of lacquers, drying oils, etc. [1-3]. Many resins and synthetic fibres are produced based on polyesters of polyfunctional acids and polyatomic alcohols. Esters of short- and medium-chain carboxylic acids with short- and medium-chain alcohols, having a pleasant smell, are used as scented substances in perfumery, cosmetic products and sweet-smelling food essences [4,5].

Esters of polyatomic alcohols and carboxylic acids are widely distributed in nature and many of them have biological activity. All fats and oils of vegetable and animal origin almost entirely consist of glycerol esters (triglycerides). Another important class of natural substances present in plants are tannins which are partially a mixture of various esters of gallic acid and glucose [6-8].

Monoglycerides belong to those few nonionic surfactants that have numerous valuable properties. They are non-toxic, do not irritate the skin and mucous membranes, and show surface activity in a broad pH range. These features explaintheir use in the food, cosmetic, pharmaceutical industry and other national economy sectors as emulsifiers and stabilizers [9, 10].

Polyatomic alcohol esters are also used in the production of synthetic lubricants and applied in small quantities in the production of plasticizers and surfactants of the slag type. Synthetic lubricating oils (SLO) based on polyatomic alcohol esters are used when operating machines and mechanisms in extreme conditions: high or low temperatures, heavy mechanical loads, etc. As for the operational properties, polyatomic alcohol ester-based SLOs have increased thermal stability, increased detergent-dispersing and lubricating properties, lower volatility and viscosity, especially low-temperature, compared with conventional mineral-based lubricants. Despite the higher (3-6 times) cost compared to mineral oils, the use of SLO based on polyatomic alcohol esters provides significant economic benefits by reducing fuel consumption (up to 10%) and exhaust toxicity, reducing oil consumption (2-3 times compared to mineral oil consumption), increasing the life of internal combustion engines and the efficiency of machines and mechanisms [11-14].

There are well-known synthetic methods for the synthesis of esters. The most important of them are esterification, acylation of alcohols and phenols, alkylation of carboxylate anions, alcoholization and acidolysis of esters, transesterification of esters, alcoholization of nitriles, Bayer-Villiger oxidation of aldehydes (formic acid esters) and ketones, alkoxycarbonylationofthe nucleophilic carbon

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atom by carbonates and alkoxycarbonylation of the electrophilic carbon atom by oxide carbon and alcohols.

Esters of polyatomic alcohols and carboxylic acids are widely distributed in nature and many of them also have biological activity. All fats and oils of vegetable and animal origin consist almost entirely of glycerol esters (triglycerides). Monoglycerides of C8-C9 acids inhibit the growth of a wide range of bacteria and can be used in the food industry as preservatives since they prevent the formation of moulds [15-19].

There is a known method for producing isovaleric acid monoglyceride by the reaction of hydroalkoxycarbonylation of isobutylene with carbon monoxide and glycerin, but unlike the known method, the $Pd(Acac)_2$-PPh_3-TsOH system is used as a catalyst in a ratio of 1:7:12, and the process is carried out at a temperature of 100 °C and a pressure of 20 atm for 3 hours [20, 21].

A method for producing isovaleric acid monoglycerides by direct esterification of isovaleric acid with glycerin is known. Isovaleric acid monoglyceride can be also synthesized by the 'isopropylidene' method based on the acylation of α,β-isopropylideneglycerol followed by the removal of the protective isopropylidene group by acid hydrolysis [22].

Research in this direction is of great functional interest because esters of isovaleric acid have several useful properties.

Previously, we synthesized menthylisovalerate and α-bromoethylisovalerate by the reaction of hydroalkoxycarbonylation of isobutylene with menthol and ethanol in the presence of palladium phosphine complexes. These compounds have sedative-antispasmodic properties and are widely used as medicines [23].

It was found that cyclohexyl ether of isovaleric acid has high antibacterial and antifungal activity against pathogens [24].

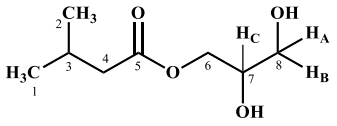
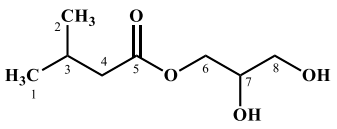
MATERIALS AND METHODS

The IR spectrum was registered on the Shimadzu IR Prestige-21 device (Japan), in the range of400–4000 cm^{-1}. ^{1}Hand^{13}CNMRspectrawere obtained on Bruker DPX 400 (Germany) device, with an operating frequency of 300 MHz. Tetramethylsilane was taken as a reference. The elemental analysis was performed on the CHNS-O elemental analyzer EuroEA3028-HT-OM device (EuroVector S.p.A., Milan, Italy). The physicochemical characteristics are given in Table 1.The IR spectrum of the synthesized isovaleric acid monoglyceride is characterized by typical absorption bands of esters: the carbonyl band at 1735$cm^{-1}$and the 'ether band' (valence vibrations of the C-O-C grouping of the ester bond) at 1188 cm^{-1}. There are absorption bands at 1051 and 1121 cm^{-1}, characteristic of primary and secondary alcohol hydroxyls, and a wide absorption band in the 3403-3466 cm^{-1}region of associated hydroxyl groups (Table 2).

Table 1. Physicochemical characteristics of isovaleric acid monoglyceride

Compound	T.boiling. °C/mmHg	n_d^{20}	Molecular formula	Elemental analysis	
				Calculated	Found
Isovaleric acid monoglyceride	187/30	1.4440	$C_8H_{16}O_4$	C – 54.53 H – 9.15	C – 53.60 H – 9.16

Table 2. ^{1}H and ^{13}C NMR of isovaleric acid monoglyceride

Compound	^{1}HNMR, δ, ppm (J, Hz)						
	CH_3	CH	$C\underline{H}_2OH$	CH_2O	CH_2OH	CHOH	OH
	0.86 d (6.5)	2.00 d	2.13 d (6.9)	4.02 d (5.4)	3.48 dd (11.8; 6.5) 3.57 dd (11.2; 3.2)	3.82 m	3.6 d 3.66 t
	^{13}C NMR, δ, ppm						
		CH_2O	CH_2OH	$\underline{C}H_3$	$\underline{C}H$	CH_2CO	CHO
	173.92	65.21	63.72	22.65	25.92	43.48	70.45

The table shows the ^{1}H and ^{13}C NMR spectra of the obtained isovaleric acid monoglyceride (Figures 1-2). Protons of the isovaleric acid monoglyceride residue resonate in the strong-field region of the ^{1}H NMR spectrum.Protonsofmethylgroups ($C^{1}H_3$ and $C^{2}H_3$) give a signal of doublets at 0.86 ppm (J=6.5Hz). Methine proton ($C^{3}H$) resonates as a multipletat 2.0 ppm, methylene protons ($C^{4}H_2$) manifest as a doublet at 2.13 ppm (J^{3}=6.9Hz). Protons of the glycerol residue resonate in the weak-field region of ^{1}H NMR. Methylene protons ($C^{6}H_2$) resonate at 4.02 ppm (J=5.4Hz). Methine proton ($C^{7}H$) displays as a multiplet at 3.82 ppm. Protons of hydroxyl groups at C^{7} and C^{8} carbon atoms manifest as an expanded and non-split doublet and triplet at 3.6 and 3.66 ppm. It should be noted that protons on H_A and H_B, due to geminal cleavage among themselves and vicinal cleavage from a neighboring proton ($C^{7}H$), have a doublet-doublet multiplet with chemical shifts of 3.48 ppm and 3.57 ppm.

In the ^{13}C NMR spectrum of isovaleric acid monoglyceride, a carbon atom of the carbonyl group C^{5}=Oresonates at 173.92 ppm in the form of a peak with weak intensity, which corresponds to a chemical shift of quaternary carbonyl carbon atoms (Figure 2). The strongest field peak of strong intensity at 22.65 ppm refers to the carbon atoms of the methyl groups C^{1} and C^{2} of the isovaleric acid. The peaks at 25.92 ppm and 43.48 ppm belong to the carbon atoms C^{3} and C^{4}. The remaining signals refer to the carbon atoms of the glycerol residue: C^{7} – 70.45 ppm, C^{6} – 65.21 ppm and C^{8} – 63.72 ppm.

The antimicrobial and antifungal activity of isovaleric acid monoglyceride was studied in bacterial strains *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and yeast fungus *Candida albicans* by diffusion into agar (wells). The test sample was dissolved in 96% ethyl alcohol at a concentration of 1 mg/ml.

The cultures were grown at a temperature of 37 °C in 18-24 hours. The grown crops were bred in 0.9% sodium chloride solution; bacteria, 1 ml each were introduced into cups with meat-peptone agar and sown according to the method of obtaining a 'continuous lawn'. Wells with a diameter of 6 mm were formed, where the drug and 96% ethyl alcohol were introduced as a control.

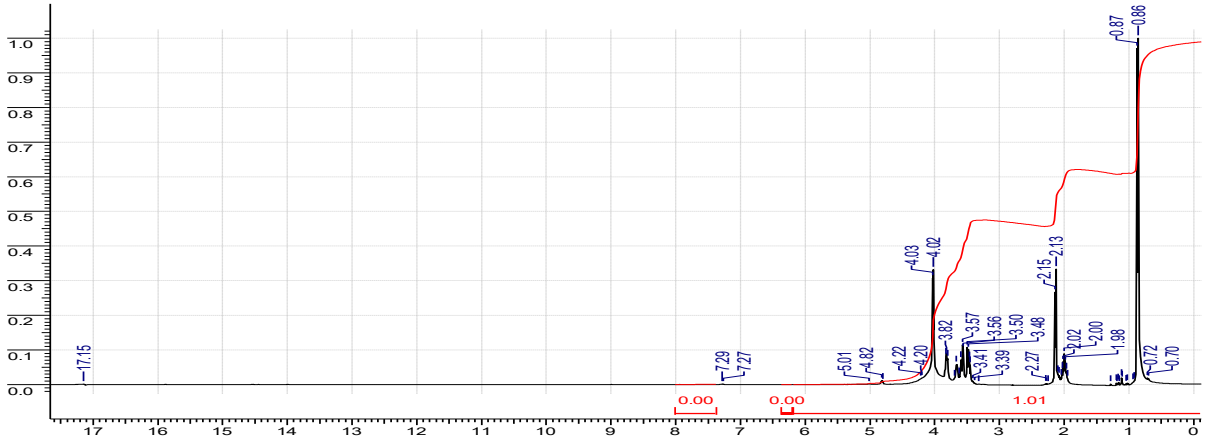


Figure 1. ^{1}H NMR spectrum of isovaleric acid monoglyceride

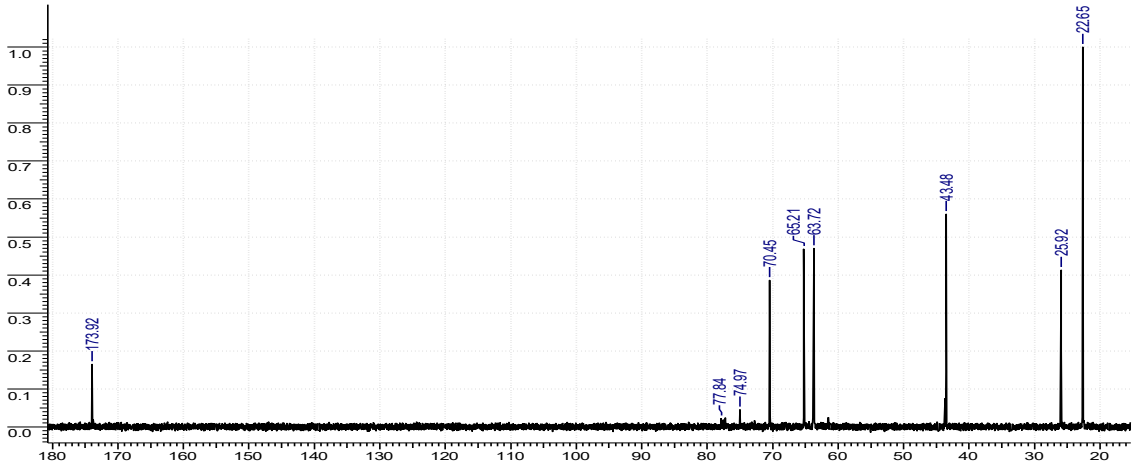
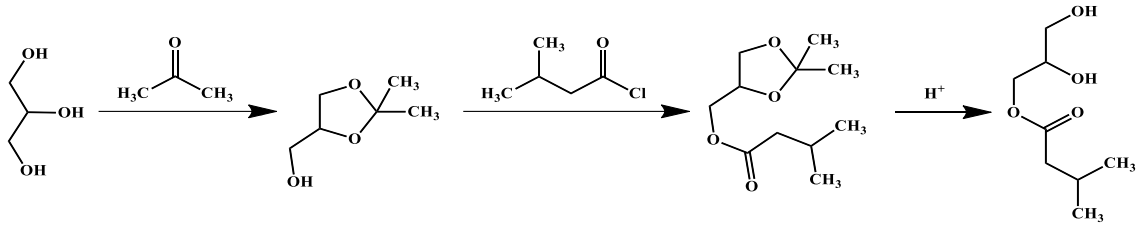


Figure 2. ^{13}C NMR spectrum of isovaleric acid monoglyceride



RESULTS AND DISCUSSION

Isovaleric acid monoglyceride was synthesized by the 'isopropylidene' method with a yield of 94%:0.1 mol of isovaleryl chloride was added to a stirred mixture of 0.1 mol of α,β-isopropylideneglycerol and 0.12 mol of dry pyridine in chloroform at -5-0°C. The reaction mixture was stirred at -5-0 °C for 5 h, and then subjected to ultrahigh-frequency irradiation with a frequency of 2450 ± 75 MHz and a power of 100 W for 20 min. The precipitate of pyridine hydrochloride was separated from the chloroform solution, which was further cooled to 0 °C and washed with cold water to reach pH=7. The chloroform solution was dried with magnesium sulfate, the solvent was driven away and the left-over was distilled in vacuum at 2 mm Hg. Isovalerate-α,β-isopropylideneglycerol was treated with a 10% aqueous solution of acetic acid under ultrahigh-frequency irradiation with a frequency of 2450 ± 75 MHz and a power of 100 W for 5 minutes until the formed emulsion disappeared. Acetic acid, acetone and water were distilled in vacuum at 40-45°C. α-Monoglyceride of isovaleric acid was isolated from the residue by column adsorption chromatography on largecoarse silica gel (KSK) (0.1-0.16 mm), using chloroform:methanol as an eluent (9:1). The yield of the target product was 94%.

The antimicrobial activity of the sample was estimated by the diameter of the growth retardation zones of the test strain (mm). The diameter of growth retardation zones less than 10 mm was estimated as absence of antimicrobial activity, 10-15 – weak activity, 15-20 – moderate, 20 mm and above – pronounced.

The results of the studies of antimicrobial activity (against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*) and antifungal activity (against *Candida albicans)* are shown in Table 3.As an outcome of the study pronounced antibacterial activity of isovaleric acid monoglyceride against *Pseudomonas aeruginosa* and moderate antifungal activity against *Candida albicans*was found.

Table 3. Antimicrobial and antifungal activity of isovaleric acid monoglyceride, mm.

Staphylo-coccus aureus	*Escherihia coli*	*Pseudomonas aeruginosa*	*Candida albicans*
8	11	20±0.1	15±0.1

CONCLUSION

Thus, we synthesized isovaleric acid monoglyceride by the isopropylidene method under microwave irradiation. It was found that the use of microwave irradiation shortens the duration of the process from 20 hours to 20 minutes and increases the yield of the target product from 82% to 94%. Isovaleric acid α-monoglyceride was isolated by column adsorption chromatography on KSK silica gel (0.1-0.16 mm), using chloroform:methanol (9:1) as an eluent. As a result of thestudies, it was found that isovaleric acid monoglyceride has pronounced antibacterial activity against *Pseudomonas aeruginosa* and moderate antifungal activity against *Candida albicans*.

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