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Synthesis and characterization of some polymer derivatives of carvacrol as drug delivery system

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Abstract

Carvacrol is one of the main components of the some essential oils in some Labiatae (Laminaceae) members like oregano, thyme and savory. Carvacrol has a lot of health benefits such as, antibacterial and antioxidant activity. We synthesized some silicon derivatives of carvacrol and characterized them by standard methods. The monomer of dimethylvinylsilyl carvacrol was also synthesized. The copolymer of the synthesized monomer with methacrylic acid (MAA) was prepared with different ratios. Some of them can be used as drug delivery systems in the pH = 1, and7.4.

Keywords: Carvacrol; oregano; silyl ethers; copolymer; drug delivery.

Introduction

Carvacrol (2-p-cymenol or 5-isopropyl-2-methylphenol) (Figure 1) is one of the main components of theEssential oils (EO) of some Labiatae (Laminaceae) members like oregano, thyme and savory, the content of which can reach up to 86%[1,2,25].

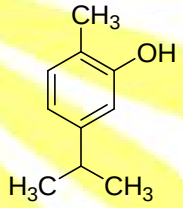

Figure 1. Carvacrol

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Actually, the EO of oregano is composed of carvacrol and/or thymol as dominant components, which are followed by γ-terpinene, pcymene, linalool, terpinen-4-ol and sabinene hydrate [3,25].

It has been indicated that the antioxidant activity of the EO of the above mentioned herbs is due to the carvacrol, its isomer thymol and some other phenols [1,4]. They also possess antibacterial activity and therefore could be applied in oral diseases treatment [5].Their antifungal activity has been examined against phytopathogenic fungi [6]. Additionally, these EOs exhibit analgesic activity which is also related to the carvacrol content [7].Antiviral activity has also been observed for whole EOs containing carvacrol as the major component [8,9], but only very low antiviral activity has been observed for carvacrol alone [10].

Despite extensive research on carvacrol in recent years, not much is known about the mechanism of action of carvacrol against bacteria. The antibacterial activity of carvacrol has been attributed to its hydrophobic nature, the presence of a free hydroxylgroup and a delocalized electron system with considerable effects on the structural and functional properties of the membrane. Carvacrol, which acts on the cytoplasmic membrane, becomes increasingly permeable to protons and ions and loses its integrity. Carvacrol has also been shown to inhibit ATPase in bacteria [11,25].

Silyl ethers have proved to be versatile substrates for a wide variety of organic reactions. They could be prepared *via* the reaction of alcohol and silicon halide in basic media such as trimethylamine in stoichiometric quantity [13-21,25].

Carvacrol, due to its hydroxyl groups shows hydrophilic property. Replacement of the hydroxylgroups with silicon groups increases its lipophilic character. Whereas, body cell membranes are made of phospholipid and havelipophilic properties, so, silyl derivatives crosses cell membranes, hydrolyzed intracellular. In this way, carvacrol releases and can be passed from lipid barrier simply.

Silicones are biologically and toxicologically inertgroups [22]. This article discusses the chemical properties of polymers containing carvacrol and organosilicon systems, which could be used in drug-delivery systems.

Experimental

All silylation reactions were carried out under dry argon gas to exclude oxygen and moisture from the system because chlorosilanes are highly moisture sensitive

reagents. The solvents and regents were purchased from Merck Company. All the solvents were distilled and stored over a drying agen. t-Butyl, Me_2SiCl, $Me_2VinylSiCl$, and Me_3SiCl were used in pure form. The initiator, 2,2'-azobisisobutyronitrile (AIBN), was purified by recrystallization from methanol.

^1H NMR spectra were recorded on a Bruker 400 AC spectrometer in $CDCl_3$. The IR spectra were recorded on a Shimadzu FT-IR-408 spectrophotometer. The DSC curves were obtained on a TGA/SDTA 851 calorimeter at heating and cooling rates of 10 ^oC/min in the air. TLC was performed by the use of Merck's silica gel.

Synthesis of trimethylsilyl derivative of carvacrol

In a round bottom flask, carvacrol (2 mL, 13 mmol), trimethylchlorosilane(1.65 mL, 13 mmol), and triethylamine (1.81 mL, 13 mmol) in dried THF (10 mL) under dried argon gas at room temperature, were stirred for 24 h. Themixture was filtered, and the pure roduct (Figure 2) was purified by column chromatography by n-hexane-EtOAc (6:1) as eluent.

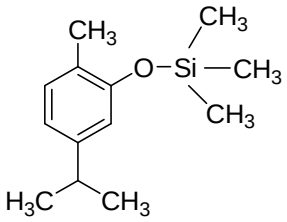

Figure 2. Trimethylsilyl carvacrol

IR (neat, cm^{-1}): 1613 (stretching C=C Aromatic), 1272 and 1253 (stretching Si-C)and 1057 (stretching Si-O) (Figure 3).

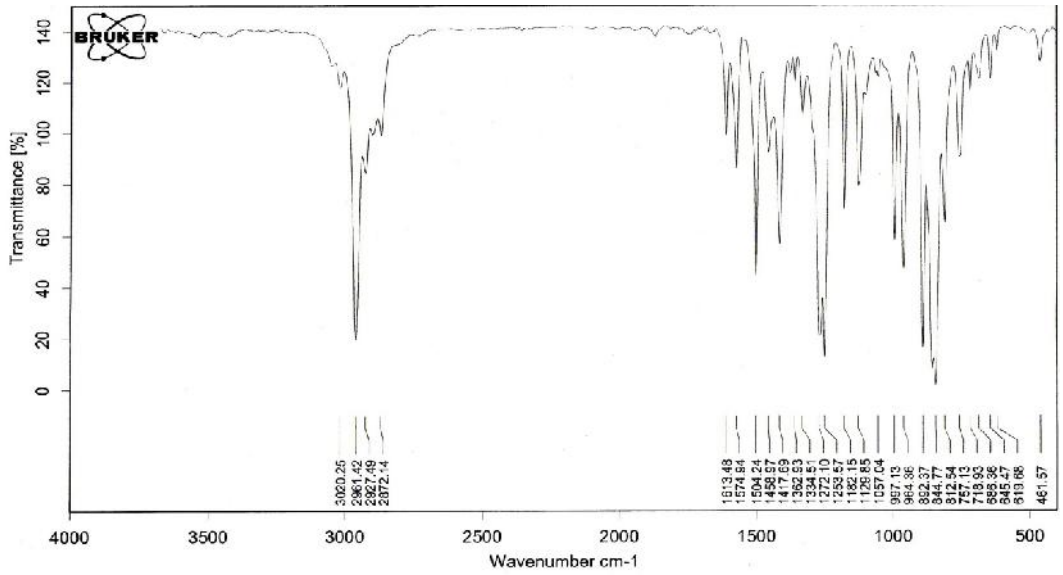

Figure 3. FT-IR of trimethylsilyl carvacrol

The trimethylsilyl derivative of carvacrol is very unstable due to its low spatial exclusion, and decomposed after a short period of time. Steps of hydrolysis of trimethylsilyl carvacrol were recorded by FT-IRspectra (Figure 4).

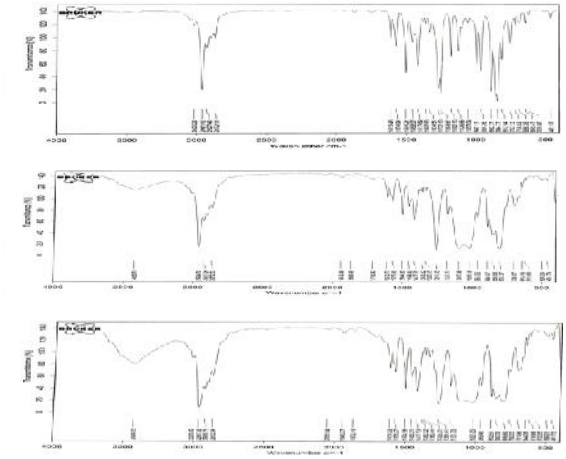

Figure 4. FT-IR study of trimethylsilyl carvacrol hydrolysis

Synthesis of t-butyldimethylsilyl derivative of carvacrol

In a round bottom flask, carvacrol (2 mL, 13 mmol), trimethylchlorosilane (1.96 mL, 13 mmol), and triethylamine (1.81mL, 13 mmol) in dried THF (10 mL) under dried argon gas at room temperature were stirred for 24 h. The mixture was filtered and the pure product (Figure 5) was purified by column chromatography by n-hexane-EtOAc (8:1) as eluent.

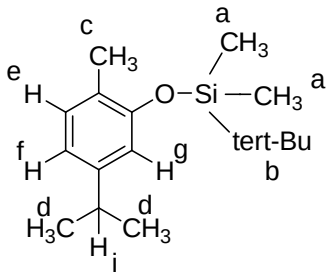

Figure 5. T-butyldimethylsilyl carvacrol

IR (neat, cm^{-1}): 1612 (stretching C=C aromatic), 1262 (stretching Si-C) and 1022 (stretching Si-O).^{1}H-NMR (400 MHz, $CDCl_3$): δ; 0.22 (s, $6H_a$), 1.00 (s, $9H_b$), 1.25 (d, $6H_d$), 2.2 (s, $3H_c$), 2.8 (m, $1H_i$), 6.63 (s, $1H_g$), 6.73 (d, $1H_e$), 7.03 (d, $1H_f$).

Synthesis of vinyldimethylsilyl derivative of carvacrol

In a round bottom flask, carvacrol (2 mL, 13 mmol), trimethylchlorosilane (1.79 mL, 13 mmol), and triethylamine (1.81mL, 13 mmol) in dried THF (10 mL) under dried argon gas at room temperature was also stirred for 24 h. The mixture was filtered and the pure product (Figure 6) was purified by column chromatography by n-hexane-EtOAc (10:1) as eluent.

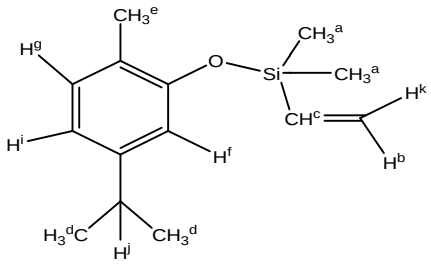

Figure 6. Vinyl dimethyl silyl carvacrol

IR (neat, cm-1): 1613 (stretching C=C Aromatic), 1262 (stretching Si-C) and 1056 (stretching Si-O).^{1}H-NMR (400 MHz, $CDCl_3$): δ; 0.5 (s, $6H_a$), 1.35 (d, $6H_d$), 2.3 (s, $3H_e$), 2.9 (m, $1H_j$), 6.02 (m, $1H_c$), 6.22 (m, $1H_k$), 6.42 (m, $1H_b$), 6.73 (s, $1H_f$), 6.89 (m, $1H_i$), 7.19 (m, $1H_g$).

Copolymerization

Copolymer of vinyldimethylsilyl carvacrol monomer andmethacrylic acid (MAA) was synthesized with different ratios.

In a pyrex glass ampoule a mixture of vinyldimethylsilylcarvacrol (1 mL, 6.5 mmol) and MAA, with various ratio (Table 1) in the presence of AIBN as initiator ([I] = 0.01 M), were frozen and degassed under vacuum. The freezing and degassing procedure were repeated three times, and the ampoules were sealed. The solution was polymerized at 70 °C for 72 h. Then the crystal solution was poured from the ampoule in to cold methanol, the precipitate was collected and washed several times with methanol, and dried under vacuum at room temperature to give the pure product (Figure 7).

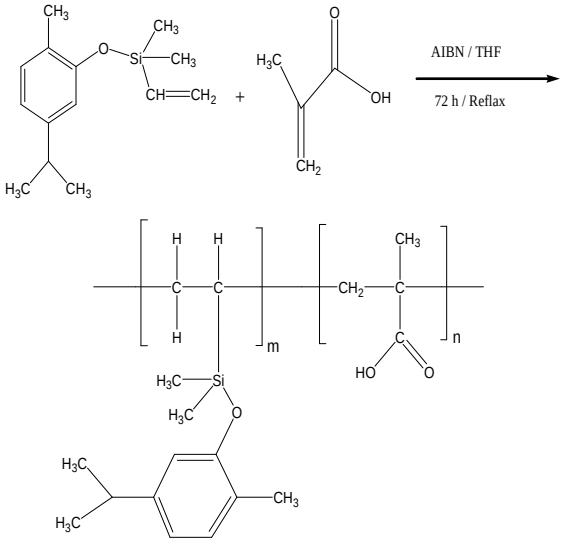

Figure 7. The structure of copolymer

The copolymer characterization

The copolymers with ratio 1:1, 1:3, and 1:5 was performed according to above mentioned procedure and has been characterized by FT-IR, and TGA methods. The TGA of all copolymers were showed in the Table 1.

IR (neat, cm-1): 2400-3400 (stretching OH), 1271 (stretching Si-C), 1703 (stretching C=O).

Table 1. The TGA of various molar ratios of copolymers

Monomers/ratios	VinyldimethylSilyl carvacrol	Methacrylic acid (MAA)	TGA of copolymers
1:1	1	1	**385**
1:3	1	3	**390**
1:5	1	5	**395**

In vitro release studies

The copolymers (10 mg) were poured into 3 mL of aqueous buffer solution (SGF: pH 1 orSIF: pH 7.4). The mixture was introduced into a cellophane membrane dialysis bag. The bags were closed and transferred to a flask containing 20 mL of the same solution maintained at 37 ˚C. The external solution was continuously stirred, and 3 mL samples were removed at selected intervals. The volume removed was replaced with SGF or SIF. The samples were analyzed by UV spectroscopy [24].

Results and discussion

Silyl ethers are a class of chemicals which contain a silicon atom covalently bonded to an alkoxy group. The general structure is $R_1R_2R_3Si{-}O{-}R_4$ where R_4 is an alkyl or an aryl group. Silyl ethers are usually used as protecting groups for alcohols in organic synthesis. One of the main features of the silicon-containing materials is a dramatic increase in their lipophilic properties. The cell membrane is made of phospholipids and has lipophilic properties. Silicon compounds can easily pass through the cell membrane and hydrolyzed to the original state of the drugs that have pharmacological properties [33,34].

Prodrugs are considered inactive molecules prior to administration, but after exposure to certain physiological conditions they are triggered to metabolize or spontaneously break down into an active therapeutic[26].Common physiological conditions used to degrade prodrugs include acidic milieus, reducing environments and elevated enzymatic levels [27]. Frequently, the acidic conditions known to exist in the endocytic pathway in cancer cells in areas of inflammation and within tumor tissue are exploited to catalyze the degradation of prodrugs. Consequently, a high payload of drug can be deposited in these areas; therefore, smart affection of drug on the target tissues can be raised. Previously, acid sensitive prodrugs have been assembled using a number of specialized chemicals including hydrazine, trityls, aconityls, vinyl ethers, poly(ketals), acetals, poly(ortho esters), and thiopropionates but these strategies lack tunability, produce toxic byproducts, or necessitate exhaustive multi-step syntheses [28,29].

Silyl ethers are among the most widely used protecting groups for the alcohol functionally because the rate of deprotection can be modulated by simply altering the substituents on the silicon atom. As a result, the synthesis of small-molecule silyl ether prodrugs have been explored using a variety of acid sensitive silane attachments including trimethylsilyl ether (TMS), triethylsilyl ether

(TES), and triisopropylsilyl ether (TIPS) (Figure 8).

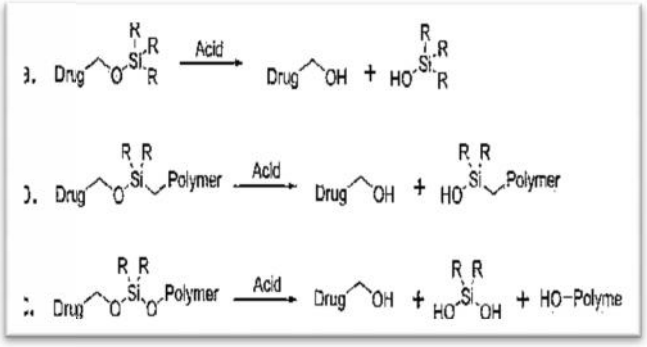

Figure 8. Three types of silyl ether prodrugs a) small molecule monofunctionalsilyl ether, b) polymeric monofunctional silyl ether prodrug, and c) polymeric asymmetric bifunctional silyl ether prodrug

Results from the two methods, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and Ferric reducing antioxidant power (FRAP) showed antioxidant activity of carvacrol which is stronger when compared with its derivatives. We can say that the antioxidant activity of carvacrol is owed to the hydroxyl functional group. The antibacterial activity of carvacrol largely depends on its hydroxyl group and the synthesized silicon compounds showed little antibacterial activity. A variety of gram-positive and gram-negative bacteria showed sensitivity to carvacrol. Considering these data and the results reported in antibacterial activity of carvacrol, it is quite evident that hydroxyl functional group of carvacrol is essential for action. Carvacrol impact on bacteria through the effectiveness of the external membrane of bacteria and bacterial cell membrane is impaired [25].

The carvacrol interacted with $^{t}BuMe_2SiCl$, $Me_3SiCl$and $Me_2VinylSiCl$ and the hindrance effects of siliconhalides on the stability of resulting compounds were investigated. All reactions were carried out with high yields approximately above 90 percent.

Calibration diagram

With the preparation of known concentrations of carvacrol solution and the absorbance read at 274 nm wave length, absorption calibration curves were plotted. Results were shown in Table.2 and Figure 9.

Table 2. Absorption of carvacrol standards

Concentration (mg/mL)	Absorption
5	0.04
20	0.179
40	0.378
60	0.653
80	1.00
100	1.21

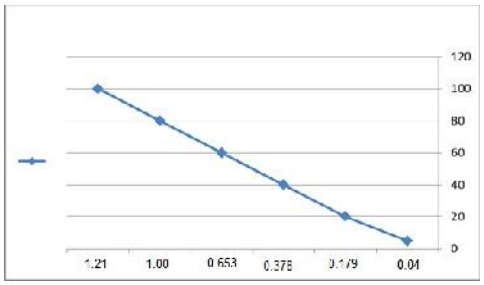

Figure 9. Calibration diagramming

The release of copolymer

The release of copolymers was carried out according to the agenda. The results in a concentration-time curve were plotted.

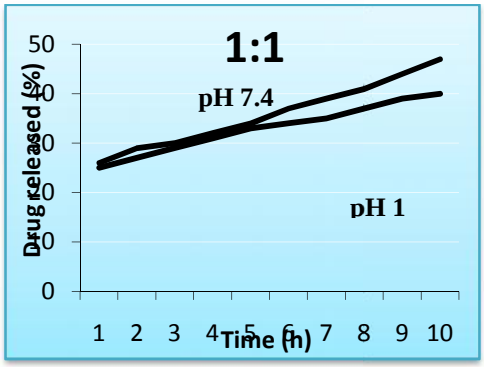

Figure 10. Concentration-time curve of copolymers

From the study of charts, the remarkable thing is that the release of these copolymer in PH=1 startin almost 30% .Connection of vinyl dimethylsilyl carvacrol with methacrylic acid *via* the chemical bond and release carvacrol is also necessary to break the chemical bond between oxygen and silicon. When the copolymers are in exposure to acidic conditions (pH = 1), Si-O bonds rapidly cleave followed by hydrolysis of the siloxane groups. Therefore, primary hydrolysis of copolymers starts from 30%; consequently, sensitivity to pH in such copolymers cannot be observed.

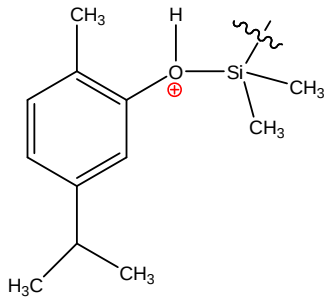

Figure 11. Protonated form of the copolymer at pH = 1

Conclusion

Investigation of the behavior of the copolymer at pH = 7.4 in the two ratio of 1: 1 and 1:3 copolymer did not show the sensitivity to pH. But the ratio of 1:5 shows sensitivity to pH. This can be attributed to the presence of 5 acidic groups and hydroxyl group of carvacrol. The acidic groups in the

pH =7.4 are COO^- and the released proton into the environment and water molecules in the environment come in the form of hydrogen ions and also these ions can easily protonate the O-Si bond and causes to hydrolyze it.

Acknowledgements

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