Bioactive epoxides and hydroperoxides derived from naturally monoterpenic geranyl acetate

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A B S T R A C T
Geranyl acetate (1) was oxidized thermally and photochemically using (mcpba, H 2O 2) respectively to obtain (E)-5-(3, 3-dimethyloxiran-2-yl)-3-methylpent-2-enyl acetate (2) and 3-(2-(3, 3-dimethyloxiran-2-yl) ethyl)-3-methylxiran-2-yl) methyl acetate (3). On the other hand, photooxygennation of 1 with tetraphenyl porphin (TPP) as a photo sensitizer gave corresponding acitic acid 2,6-bis-hydroperoxy-7-methyl-3-methylene-oct-7-enyl-ester (4), acitic acid 7-hydroperoxy-3,7-dimethyl-octa-2,5-dienyl ester (5) and Acitic acid 3-hydroperoxy-7-methyl-3,7-dimethyl-octa-1,6-dienyl ester (6). Antifungal studies were carried out on geranyl acetate and its derivatives. Studies on the antifungal activity especially Microsporum gypsum, Trichophyton vercossum and Candida tropicalis showed that geranly acetate, its epoxide and hydroperoxide derivatives have good antifungal action.

1. Introduction

In the last years, there has been a rise in the usage of aromatic medicinal plants and their essential oils in technical research and industrial applications including nutritional therapeutical, and cosmetic uses (Kebele & Hayelom 2008; Kejlová et al., 2010; Nisaet et al., 2011; Ekor 2013; Ramadan 2015; Emmanuel et al., 2016). The therapeutic properties of certain medicinal plants generally related to their content of secondary metabolites, such as polyphenols, terpenes, phytosteroids, and alkaloids, which produced in considerable amounts and variable proportions (Emam et al., 2009). The essential oils of the plants are the principle of their fragrane. They are called also ethereal oils, or volatile oils because they vaporize rapidly when exposed to the air at ordinary temper-atures. In general, the essential oils consist of many mixtures including different sorts of molecules. These chemical constituents divided into two broad classes: terpenes and phenylpropanoids. Nevertheless, most essential oils consist mainly of monoterpenes, which are the main chemical constituents of the essential oils of these plants that found as mixtures of odoriferous components and can be obtained by steam distillation or solvent extraction from a large variety of aromatic plants. They are found in edible as well as in medicinal plants with a therapeutic properties (Dudai et al., 2005; Sousa et al., 2006; Quintans-Junior et al., 2008; Nerio et al., 2010; Reinaldo et al., 2011).

On the other hand, the chemistry of singlet molecular oxygen (1O 2), (which prepared through photooxidation reaction) has amazing consideration by chemists because of its environmental and biomedical importance beside its interesting mechanistic and synthetic aspects (Stratakis and Orfanopoulos, 2000; Khayyat 2011) Moreover, unsaturated terpenes are adept of trapping activated oxygen species in vivo to give intermediate epoxides which can alkylate DNAs, proteins, and other biological species (Richter, 2003; Elgendy and Khayyat, 2008; Khayyat and Saddiq, 2015). Geranyl acetate (1) is a monoterpane extant in the volatile oils of many plant species, such as Cypress, Origanum, and Eucalyptus oils (Alianni et al., 2001; Delaquis et al., 2002) (see Fig. 1). Taking into account significant therapeutic value of monoterpane especially geranyl acetate and the important applications of its epoxides and hydroperoxide derivatives, in this study, we investigated some oxidation reaction of geranyl acetate and the biological activities of its products.
2. Materials and methods

2.1. Chemistry

$^1$H-NMR spectra were obtained in CDCl$_3$ solution with a Bruker AVANCE D.P.X. 600 MHz apparatus. A sodium lamp (Phillips G/5812 SON) was used for photo-irradiation reactions. Thin layer chromatography (TLC) and preparative layer chromatography (PLC): Polygram SIL G/W 254, Mecherey-Nagel.

2.2. General epoxidation procedures of geranyl acetate (1)

2.2.1. Method A: Photochemical epoxidation using hydrogen peroxide

A solution of H$_2$O$_2$ (2.5 mL, 50%) was added cautiously drop wise over 5 min to a stirred solution of 1 (5 mmol) in C$_2$H$_5$OH (25 mL) at 0 °C. The reaction mixture was washed using sodium lamp in an atmosphere of nitrogen. The reaction mixture evaporated and purified through column chromatography by petroleum ether 60–80 °C and ether (8:2) as elution to give the epoxide derivatives as viscous oils (Elgendy and Khayyat, 2008) (Table 1). One gram of 1 was taken in place in test tube, mixed with CHCl$_3$ and TPP as a sensitizer, then exposed to sodium lamp at ~20 °C. Stream of dry oxygen gas was passed into the mixture of reaction through-out the irradiation. The solvent was evaporated at 20 °C. Then the crude product was purified by column chromatography on silica gel as adsorbent and a mixture of petroleum ether and ethyl acetate as elution to give the hydroperoxides products 4, 5 and 6, which were successfully separated in pure form in the yields (Elgendy and Khayyat, 2008) (Table 2).

2.4. Spectroscopic data

2.4.1. Acetic acid 3,7-dimethyl-octa-2,6-dienyl ester (1)

Colorless oil, C$_{12}$H$_{20}$O$_2$ (M 212.29). IR (thin film): ν: 1021, 1227, 1365, 1738.5 (COO), 2914.9 (CH str.) cm$^{-1}$. $^1$H NMR (CDCl$_3$): δ: 1.72 (s, 3H, 10CH$_3$), 1.8 (s, 6H, 8,9CH$_2$), 2.0 (s, 3H, CH$_3$, H-11), 2.5 (Comp. pat., 4H, H-4, 5), 4.6 (d, 2H, J = 8 Hz, H-1), 5.06 (dd, 1H, J = 11 Hz, H-6), 5.33 (dd, 1H, J = 11 Hz, H-2). $^{13}$C NMR spectrum, δ ppm: 15.8 (10C), 17.4 (8,9C), 20.3 (OCH$_3$), 25.4 (6C), 25.9 (C), 39.1 (6C), 60.7 (C), 118.0 (C), 123.3 (C), 131.1 (7C), 141.4 (5C), 170.3 (CO).

2.4.2. (E)-5-(3,3-dimethyloxiran-2-yl)-3-methylpent-2-enyl acetate (2)

Colorless oil, C$_{12}$H$_{20}$O$_3$ (M 212.29). $^1$H NMR (CDCl$_3$): δ: 1.27 (s, 3H, CH$_3$, H-8), 1.30 (s, 3H, CH$_3$, H-9), 1.73 (s, 3H, CH$_3$, H-10), 2.2 (Comp. pat., 4H, H-4, 5), 2.61 (dd, 1H, J = 8 Hz, H-6), 4.5 (d, 2H, J = 8 Hz, H-1), 5.30 (dd, 1H, J = 11 Hz, H-2). $^{13}$C NMR spectrum, δ ppm: 16.1 (10C), 20.7 (OCH$_3$), 24.2 (8,9C), 35.2 (C), 26.1 (C), 42.2 (6C), 61.2 (C), 118.6 (C), 140.5 (C), 170.2 (CO).

2.4.3. 3-(2-(3,3-dimethyloxiran-2-yl)-3-methylxiran-2-yl) methyl acetate (3)

Colorless liquid, C$_{12}$H$_{20}$O$_4$ (M 228.29). $^1$H NMR (CDCl$_3$): δ: 1.22 (s, 3H, 10CH$_3$), 1.32 (s, 3H, 8,9CH$_3$), 1.34 (s, 3H, 10CH$_3$), 1.81 (Comp. pat., 4H, H-4, 5), 2.10 (s, 3H, COCH$_3$), 2.61 (dd, 1H, J = 8 Hz, H-6), 2.71 (Comp. pat., 1H, H-2), 4.08 (Comp. pat., 1H, H-1), 4.30 (Comp. pat., 1H, H-6). $^{13}$C NMR spectrum, δ ppm: 15.66 (10C), 20.56 (CH$_3$-CO), 24.1 (6C), 24.4 (6C), 35.1 (C), 58.1 (C), 58.9 (C), 59.98 (6C), 63.01 (7C), 117.6 (C), 170.5 (CO).

2.4.4. Acetic acid 2,6-bis-hydroperoxy-7-methyl-3-methylene-oct-7- enyl-ester (4)

Colorless liquid, C$_{12}$H$_{20}$O$_5$ (M 260.28). $^1$H NMR (CDCl$_3$): δ: 1.72 (bs, 2H, H-5), 2.06 (bs, 2H, H-4), 2.19 (s, 3H, COCH$_3$), 4.26 (bs, 2H, H-16), 4.57 (bs, 2H, H-12), 4.97 (bs, 1H, H-8), 5.07 (bs, 1H, H-10), 5.33 (bs, 1H, H-8), 5.66 (bs, 1H, H-10), 8.95 (s, 1H, OOH), 9.46 (s, 1H, OOH). $^{13}$C NMR spectrum, δ ppm: 17.4 (10C), 20.7 (OCH$_3$), 24.2 (6C), 26.6 (6C), 61.2 (C), 81.5 (C), 88.4 (C), 114.0 (10C), 118.8 (2C), 142.0 (C), 143.8 (6C), 172.4 (C).

2.4.5. Acetic acid 7-hydroperoxy-3,7-dimethyl-octa-2,5- dienyl ester (5)

Colorless liquid, C$_{12}$H$_{20}$O$_6$ (M 228.14). $^1$H NMR (CDCl$_3$): δ: 1.3 (s, 6H, 8,9CH$_2$), 1.7 (s, 3H, 10CH$_3$), 2.1 (s, 3H, 11CH$_3$), 2.86 (Comp. Pat., 2H, H-4), 4.5 (bs, 1H, H-1), 5.53 (dd, 1H, H-6), 6.1 (Comp. Pat., 1H, H-5), 7.3 (s, 1H, OOH). $^{13}$C NMR spectrum, δ ppm: 17.3 (10C), 24.2 (8,9C), 45.0 (C), 60.4 (C), 82.0 (7C), 125.5 (C), 128.5 (C), 136.2 (C), 142.5 (C), 174.9 (CO).

2.4.6. Acetic acid 3-hydroperoxy-7-methyl-3,7-dimethyl-octa-1,6- dienyl ester (6)

Colorless liquid, C$_{12}$H$_{20}$O$_7$ (M 228.14). $^1$H NMR (CDCl$_3$): δ: 1.27 (s, 5H, H-4,10), 1.5–1.9 (Comp. pat., 8H, H-5,8,9), 2.21 (s, 3H, CH$_3$-CO), 24.7 (8,9C), 35.1 (C), 58.1 (C), 59.98 (6C), 63.01 (7C), 117.6 (C), 170.5 (CO).

2.4.7. Photooxidation reaction of Geranyl acetate

Table 1 Thermal and photo epoxidation of Geranyl acetate.

| Comp. No. | Epoxidation | Solvent | Yield | Epoxid. Prod. |
|-----------|-------------|---------|-------|--------------|
| 1         | Thermally (mcpba) | CHCl$_3$ | 0.80% | 2: 60% 3: 20% |
| 1         | Photochemically (H$_2$O$_2$) | C$_2$H$_5$OH | 0.55% | 3: 55% |
2.5. Biological activity

The, Microsporum gypseum, Trichophyton verrucosum and Candida tropicalis isolates were obtained from King Faisal Specialist Hospital & Research Centre-Jeddah, Saudi Arabia. Culture medium was the sabouraud dextrose agar (Oxoid CM 41), it used to the growth of fungi and yeast (Khayyat and AlKattan, 2017).

2.6. Antifungal activity

Well-cut diffusion technique was carried out to assay antifungal activity (EL-Masry et al., 2000). The Sabaroud dextrose agar media was inoculated with 1 mL from tested spore suspension, then wells were cut from the plate using a sterile 1 cm cork borer. About 1.0 mL of the tested compounds were added into each well. All plates were incubated at 4 °C for 2 h to slow fungal growth and gives suitable time for the antidermatophytic agent to diffuse. The plates were later incubated at 28 °C for a week (Mtolera and Semesi, 1996). After incubation, the diameter of the growth inhibition zone was measured in mm (Attaie et al., 1987).

3. Results and discussion

3.1. Chemistry

Medicinal and aromatic plants have demonstrated its contribution to the treatment of diseases such as HIV/AIDS, and microbial infections (Khayyat & Alzahrani, 2014). Geranyl acetate (1) presented pharmacological properties related to inflammation and pain-related processes, and antioxidant effects (Quintans-Júnior et al., 2013). The chemical structure of 1 was confirmed by spectral measurements. $^1$H NMR spectrum of 1 showed doublet of doublet at $\delta$ 5.33 ppm of proton of C2, and doublet of doublet at $\delta$ 5.06 ppm of protons of C6. $^{13}$C NMR spectrum of 1, the C2 and C3 signals were located at $\delta c$ 118.0 and 141.4 ppm, respectively. Signal at $\delta c$

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Scheme 1. Epoxidation and photooxygenation of Geranyl acetate.
Thermal epoxidation of 1 using mcpba in chloroform gave 80% mixture of Geranyl epoxides 2 & 3 (Scheme 1). On the other hand, Photochemical epoxidation of 1 with hydrogen peroxide (H$_2$O$_2$, 30% by volume) in ethanolic medium under irradiation with sodium light (irradiation time 15 h) gave only 55% of 3-(2-(3,3-dimethyloxiran-2-yl) ethyl)-3-methyloxiran-2-yl) methyl acetate (3) (Scheme 1). The structures of epoxidation products 2 & 3 were established by spectral measurements. $^1$H NMR spectrum of 2 showed doublet of doublet at δ 5.30 from protons H-2. The other proton spectra as doublet of doublet at δ 2.61 ppm from H-6 in oxiran ring. Compound 3 displayed in $^1$H NMR two complex patterns at δ 2.61 ppm and δ 2.71 ppm for two protons of H-6 and one proton of H-2 in oxiran ring respectively. $^{13}$C NMR spectrum of 2 showed signals of the oxirane carbon atoms at δ 42.2 (C6) and δ 61.2 ppm (C7). Whereas compound 3 showed signals at δ 58.9, 76.9 of oxiran carbon atoms at (C2 & C3) respectively, and δ 58.1 ppm and 63.01 of (C6 & C7) of the other oxirane carbon atoms.

Interesting is the photooxygenation reaction of 1, in the presence of tetraphenyl porphin (TPP) as singlet oxygen sensitizer to give a mixture of acetic acid 2,6-bis-hydroperoxy-7-methyl-3-methylene-oct-7-enyl-ester (4), Acetic acid 7-hydroperoxy-3,7-dimethyl-octa-2,5-dienyl ester (5) and Acetic acid 3-hydroperoxy-7-methyl-3,7-dimethyl-octa-1,6-dienyl ester (6) which were separated in pure case successfully (Scheme 1).

The chemical structures of 4, 5 and 6 were supported by spectral studies. $^1$H NMR spectrum of 4 showed two singlet signals at 170.3 ppm for carbonyl group.
δ 8.95 ppm & δ 9.46 ppm for two protons of OOH groups and showed shift of protons signals 2 & 6 to δ 4.57 ppm & δ 4.26 ppm respectively. Whereas, the 1H NMR spectrum of 5 showed one singlet at δ 7.3 ppm for OOH group and compound 6 showed the signal of OOH group at δ 7.3 ppm. 13C NMR spectrum of 4 showed δ 81.5 and δ 88.4 ppm for (C2 and C6) respectively, and showed two signals at δ 114.0 and δ 118.2 for (C10 and C8) respectively. Compound 5 showed signals at δ 128.5 ppm and δ 136.2 ppm for (C5 & C8) respectively. Whereas compound 6 showed signals of methylene group at δ 136.2 ppm and 136.2 ppm for (C1 & C2) and signal at δ 81.4 for (C3).

The epoxidation of 1 using mcpba thermally under carefully controlled gave the compounds 2 & 3. Whereas the photoepoxidation of 1 using H2O2 in ethanol gave one compound 3 (Scheme 1).

The probable mechanism for production of epoxide derivatives 2 & 3 is believed to be through the formation of the oxirane intermediates via elimination of H2O or mcpba (depending on the epoxidizing agents, which were used (H2O2 or mcpba) (Scheme 2).

The formation of hydroperoxides 4, 5 and 6 may be assumed to proceed via peroxirane transition state, which have been done through three probable pathways. (Scheme 3).

3.2. Antifungal activity

It is known that some monoterpenes and their derivatives have antimicrobial activity (Saddiq & Khayyat, 2010). Therefore, a comparative test was taken of 1, its epoxide and hydroperoxide derivatives against Microsporum gypseum, Trichophyton verrucosum and

![Fig. 2. Effect of the tested compounds (c) control, Geranyl acetate (1), geranyl epoxides (2,3), Geranyl hydroperoxide (4,5,6) on the radial growth of Microsporum gypseum grown on the solid media.](image)
Candida tropicalis. These fungi were obtained from American type culture collection (ATCC; Rockville, MD, USA). The antifungal activities (zone inhibition are summarized in Table 3, Fig. 2, all the compounds were at 1000 µg/ml concentrations. The results were showed that geranyl acetate and its derivatives inhibited Microsporum gypsum Trichophyton verrucosum and Candida tropicalis growth, specially epoxide 3 was more effective than (4, 2, 6, 5, 1) respectively. The maximum antifungal activity was against Microsporum gypsum. These results promoted us to believe that compounds are beneficial to human health, and have the potential to be used for medical purposes.

4. Conclusion

The present study described the thermal and photo epoxidation of Geranyl acetate (1) to produce mono and diepoxy derivatives 2 & 3, whereas photooxygenation of 1 gave mono and dihydroperoxide derivatives 4, 5 & 6. Geranyl acetate (1) and its epoxide and hydroperoxide derivatives showed significant activities against Microsporum gypsum more than Trichophyton verrucosum and Candida tropicalis respectively. The maximum inhibition zones was 40 mm of diepoxide 3 against Microsporum gypsum.

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