Safranal epoxide – A potential source for diverse therapeutic applications

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Abstract
Safranal is an organic compound isolated from saffron oil. Photo epoxidation and thermal reactions of safranal can be a significant tool for the design of drugs to act as anticancer agents and potent chemoprevention. Safranal was subjected to oxidation reactions either thermally using m-chloroperbenzoic acid or photochemically with hydrogen peroxide. Photochemically and thermally oxidation reaction gave the corresponding monoepoxy together with diepoxyl derivatives. The primary tested of epoxide derivatives showed a moderate degree of DNA alkylation. Studies on the antimicrobial, especially Methicillin resistant Staphylococcus aureus (MRSA), showed high activity of safranal diepoxide (3) against the growth of bacteria Methicillin resistant Staphylococcus aureus (MRSA) more than safranal (1) and monoepoxide (2) respectively. The epoxidation products were tested against bacterium Methicillin resistant Staphylococcus aureus (MRSA).

1. Introduction
Saffron has been used since early times as a medicinal plant and a cooking spice (Rios et al., 1996; Malathi et al., 2014; Khayyat, 2017). A monoterpene glycoside precursor of safranal is the main component of volatile oil which is responsible for the aroma (Lozano et al., 2000; Carmona et al., 2007; Maggi et al., 2011). Aqueous extract of saffron could inhibit cyclophosphamide, cisplatin, mitomycin-C, and urethane induced alterations in lipid peroxidation (Samarghandian and Borji, 2014; Kou and Abraham 2017). Several studies showed that saffron has an antioxidant activity and there are many analytical studies that have been conducted to study the characteristics of many potential biologically active compounds that are found in saffron (Martinez et al., 2001; Ali et al., 2002; Ochiai et al., 2004; Premkumar et al., 2003; Sadeghnia et al., 2005; Boroushaki et al., 2007). Natural deglycosylation of picrocrocin will yield safranal. In order to preserve safron, dehydration is not the only important process as it is actually important to release the safranal from picrocrocin via enzymatic activity. The reaction yields safranal and D-glucose (John et al., 2010). Various methods of extraction have been used to extract the essential oils from plant material such as microsimultaneous hydrodistillation extraction (Kanakis et al., 2004), vacuum headspace (Tarantilis and Polissiou, 1997). Many chromatographic analytical techniques such as gas liquid chromatography have been used to quantify safranal (Kanakis et al., 2004; Maggi et al., 2009). It has been proven that Safranal has a high antioxidant potential (Assimopoulou et al., 2005; Kanakis et al., 2004), as well as cytotoxicity towards certain cancer cells in vitro (Escribano et al., 1996). Safranal is a protective agent against gentamicin-induced nephrotoxicity in rat (Boroushaki, and Sadeghnia, 2009) and was able to protect kidney against hexachlorobutadiene-induced nephron toxicity and ischemia/reperfusion injury in rat (Sadeghnia et al., 2005; Boroushaki et al., 2007). A number of dietary, monoterpene were shown to act in chemoprevention and chemotherapy of different types of cancers in animals effectively, at cellular level, and in human clinical trials (Crowell, 1999; Carnesecchi, 2001). However, plant monoterpene are subjected to oxidation when they are exposed to air. Oxidation is increased by irradiation (Saddig and Khayyat, 2010), chemical catalysts (Meou et al., 1999) or heat (Elgendy and Khayyat, 2008). The photo
binding and intercalation activities of monoterpenes to DNA were intensively investigated (Knobler et al., 1988; Niccolai et al., 1990), nevertheless, although epoxides can be efficient DNA-alkylating agents (Elgendy and Fadaly, 2002; Khayyat, 2012). Little is known about such activity for the naturally occurring safranal epoxides. In view of the potential epoxy-biological interest in monoterpenes, this work investigated in detail the epoxidation reactions of safranal and evaluated the antimicrobial activity of the products.

2. Methods and materials

2.1. Chemistry

Safranal was purchased from Fluka. IR spectra were performed on a NICOLET IS50 FT-IR spectrophotometer. 1H NMR & 13C NMR spectrum were obtained in CDCl3 solution with a Bruker Ascend TM 850 MHz apparatus. Thin layer chromatography (TLC) and preparative layer chromatography (PLC): Polygram Sil G/W 254, Merckey-Nagel. A sodium lamp (Phillips G/5812 SON) was used for photo-irradiation reactions. Gas chromatography-mass spectrometry GC-MS was performed using a Hewlett-Packard 5890 series II chromatograph equipped with a 5972 series mass selective detector (MSD) in the electron impact mode (70 eV). Evaporation of final product solutions was done by rotary evaporator (at 20 °C/15 torr).

2.2. Thermally epoxidations with m-chloroperbenzoic acid

A solution of 10 mmol of 80% m-chloroperbenzoic acid was carefully added in a dropwise manner over a period of 15 min to a solution of 5 mmol of compound 1 in 25 ml of chloroform at 0 °C. The solution mixture was stirred at room temperature under nitrogen, the progress of the reaction being monitored by TLC. The reaction mixture was stirred at room temperature under nitrogen. The mixture was then carefully washed with a saturated aqueous solution of NaHCO3 (3 ml) and distilled water (3 ml) and peroxide test with a 10% solution of KI. The mixture was then reduced pressure at room temperature, and the residue was separated, dried over anhydrous Na2SO4, and evaporated under reduced pressure at room temperature, and the residue was subjected to column chromatography on silica gel using petroleum ether (bp 60–80 °C)/ethyl acetate (9:2) as eluent to isolate: 0.16 g of mixture 2 and 3 with ratio = 65:35, overall yield (70%), which can be separated in pure form (Elgendy and Khayyat, 2008).

2.3. Photochemical epoxidation with hydrogen peroxide

A 30% solution of hydrogen peroxide, 2.5 ml, was carefully added dropwise over a period of 5 min to a solution of 5 mmol of safranal (1) in 25 ml of ethanol under stirring at 0 °C. The mixture was irradiated with a sodium lamp for 50 h under nitrogen. The mixture was then evaporated under reduced pressure at room temperature, the yellow residue was treated with 25 ml of chloroform, and the extract was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was subjected to column chromatography on silica gel using petroleum ether (bp 60–80 °C)/ethyl acetate (9:2) as eluent to isolate: 0.16 g of mixture 2 and 3 with ratio = 65:35, overall yield (50%), which can be separated in pure form as a viscous oils (Khayyat and Elgendy, 2007).

2.4. Spectroscopic data

2,6,6-Trimethylcyclohexa-1,3-diene-1-carbaldehyde (1): Colorless oil, C10H14O (M 150.21). 1H NMR spectrum, δ ppm: 1.19 s (6H, C2H5), 2.15 d.d (2H, 5-H, J = 11, 14 Hz), 2.17 s (3H, C3H3), 5.93 d (1H, 3-H, J = 10 Hz), 6.16 d.t (1H, 4-H, J = 5, 10 Hz), 10.14 s (1H, CHO). 13C NMR spectrum, δC ppm: 17.4 (C9), 25.9 (C7a), 32.3 (C6), 40.7 (C5), 129.6 (C4), 134.1 (C3), 137.1 (C2), 146.6 (C1), 191.3 (CO). GC-MS data: retention time 13.2 min; m/z (Irel, %): 150 (70) [M]+, 135 (10) [M+CH3], 121 (80) [M+2CH3], 107 (100) [M+4CH3OH], 91 (90) [M+8CH3OH], 79 (15) [M+12CH3OH], 41 (7) [C3H3]+.

2,2,6-Trimethyl-7-oxabicyclo[4.1.0]-hept-4-ene-1-carbaldehyde (2): Colorless oil, C10H16O2 (M 166.21). 1H NMR & 13C NMR spectrum were obtained in CDCl3 solution with a Bruker Ascend TM 850 MHz apparatus. Thin layer chromatography (TLC) and preparative layer chromatography (PLC): Polygram Sil G/W 254, Merckey-Nagel. A sodium lamp (Phillips G/5812 SON) was used for photo-irradiation reactions. Gas chromatography-mass spectrometry GC-MS was performed using a Hewlett-Packard 5890 series II chromatograph equipped with a 5972 series mass selective detector (MSD) in the electron impact mode (70 eV). Evaporation of final product solutions was done by rotary evaporator (at 20 °C/15 torr).

2,5,5-Trimethyl-3,8-dioxy-tricyclo[5.1.0.02,4]octane-4-carbaldehyde (3): Colorless oil, C10H16O2 (M 182.21). 1H NMR spectrum, δ ppm: 1.00 s (3H, C1H3), 1.01 s (3H, C2H3), 1.21 s (3H, C3H3), 1.31 d (1H, 3-H, J = 2.8 Hz), 1.60 d (1H, 4-H, J = 2.8 Hz), 2.26 m (1H, 5-H, J = 8 Hz), 9.62 s (1H, CHO). 13C NMR spectrum, δC ppm: 24.7 (C1), 24.9 (C2), 26.1 (C3), 30.9 (C4), 39.6 (C5), 45.1 (C6), 67.4 (C7), 67.5 (C8), 90.9 (C9), 203.0 (CO). GC–MS data: retention time: min; 16:45–16:85; m/z (Irel, %): 183 (10) [M]+, 182 (6) [M]+, 166 (5) [M+O], 150 (3) [M+2O2], 137 (15) [M+CO2], 121 (15) [M+2CO2], 109 (30) [M+C3H3O], 82 (20) [C6H9O]+, 43 (100) [C3H3]+.

2.5. Antibacterial activity

The agar well diffusion method was employed for the determination of antibacterial activities of the tested compounds on bacteria (MRSA) (Collins et al., 1989). The result of antibacterial is reported in Table 1 and Fig. 1.

3. Result and discussion

3.1. Chemistry

Crocus sativus L. is the botanical name of saffron which belongs to the family Iridaceae. The order of the saffron is Asparagales and it belongs to the class Liliopsida and division Magnoliophyta (Caballeró-Ortega et al., 2007; John et al., 2010).

Safranal (1) is the main component of saffron oil was epoxidized using m-chloroperbenzoic acid (mcpba) at room temperature to give a mixture of 2,2,6-trimethyl-7-oxabicyclo[4.1.0]-hept-4-ene-1-carbaldehyde (2) and diepoxy derivative (3), in the yields of 65 and 35% respectively. This mixture can be separated in pure forms (Scheme 1). On the other hand, 1 was epoxidized with hydrogen peroxide in presence of sodium lamp at room temperature to give 2,2,6-trimethyl-7-oxabicyclo[4.1.0]-hept-4-ene-1-carbaldehyde (2) and diepoxy derivative (2,5,5-Trimethyl-3,8-dioxy-tricyclo[5.1.0.02,4]octane-4-carbaldehyde (3), in the yields of 65 and 35% respectively. This mixture can be separated in pure forms (Scheme 2).

Table 1

| No. | Treatment  | Inhibition zone diameter (mm) |
|-----|------------|------------------------------|
| a   | Control    | 20                           |
| b   | Safranal (1)| 27.00                        |
| c   | Epoxide (2) | 33.00                        |
| d   | Epoxide (3) | 35.00                        |
The chemical structure of Safranal (1) was confirmed by spectral measurements. $^1$H NMR spectrum of 1 showed three singlet signals for nine-protons at $\delta$ 1.19, 1.19 and 2.17 for three methyl groups 7, 8 and 9 respectively. Double doublet signals at $\delta$ 2.15 of two protons at position 5. Doublet signals at $\delta$ 5.93 for proton 3, double triplet signals at $\delta$ 6.16 of proton 4 and singlet signal at $\delta$ 10.14 of aldehydic proton. $^{13}$C NMR spectrum of 1, signals of the hexadiene carbon atoms were present at $\delta$ 32.3, 40.7, 129.6, 134.1, 137.1, 146.6, 191.3 for (C₆), (C₅), (C₄), (C₃), (C₂), (C) and (CO) respectively. Compound 1 was characterized by a retention time of 13.2 min. (GC-MS), and its mass spectrum contained the molecular ion peak with $m/z$ 150.21

The chemical structures of epoxidation products 2 and 3 were confirmed by spectral measurements. $^1$H NMR spectrum of 2 showed three singlet signals for nine-protons at $\delta$ 1, 1.01 and 1.5 for three methyl groups 7, 8 and 9, respectively. Three double doublet signals at $\delta$ 1.84, 2 and 5.6 of protons 3, 3 and 4 respectively. Doublet signals at $\delta$ 5.7 of proton 5 and singlet signal at $\delta$ 9.61 of aldehydic proton.

$^{13}$C NMR spectrum of 2, signals of the hexene carbon atoms were present at $\delta$ 39 39.5, 45, 95, 125.8, 132, 200 for (C₂), (C₃), (C₄), (C₅), (C₆), (C₇), (C₈), (C₉) and (CO), respectively. Compound 2 was characterized by a retention time of 13.93 min. (GC-MS), and its mass spectrum contained the molecular ion peak with $m/z$ 166.21.

The structure of epoxidation product 3 was established by spectral measurements. $^1$H NMR spectrum showed three singlet signals for nine – protons at $\delta$ 1, 1.01 and 1.21 for three methyl groups 7, 8 and 9 respectively. Two double doublet signals at $\delta$ 1.31 and 1.6 of
protons 3 and 3 respectively. A complex pattern at δ 2.62 of proton 4 and doublet signal at δ 3.2 of proton 5 and singlet signal at δ 9.62 of aldehydic proton.

13C NMR spectrum of 3, signals of the hexane carbon atoms were present at δ 26.1, 39.6, 45.1, 67.4, 67.5, 90.9, 203 for (C2 to C6), (C1) and (CO) respectively. Compound 3 was characterized by a retention time of 16.65 min. (GC-MS), and its mass spectrum contained the molecular ion peak with m/z 182.21.

A probable mechanism for the formation of epoxidation products 2 and 3 is believed to involve one oxirane intermediate (A) and elimination of m-chlorobenzoic acid molecule or water to form product 2 or two oxirane intermediate to form product 3.

3.2. Antibacterial activity

3.2.1. Bacterial pathogenic: Methicillin Resistant Staphylococcus aureus (MRSA) from Laboratory of Jeddah Hospital in the kingdom of Saudi Arabia. It was cultured on Mueller Hinton media (Oxioid CM 41) at 37 °C.

3.2.2. The agar disc diffusion method was employed for the determination of antibacterial activities of the safranal and its epoxide derivatives in questions (Hasenekog˘lu, 1990). Suspension of the tested microorganisms (106 CFU/μl) was spread on Mueller Hinton Agar (Oxioid) for bacteria. Each test solutions were prepared in CHCl3. filter paper discs (6 mm in diameter) were soaked with 20 μl of the stock solutions and placed on the inoculated plates. After keeping at 2 °C for 2 h, they were incubated at 37 °C for 24 h. The diameter of the inhibition zones were measured in millimeters.

Medicinal and aromatic plants have demonstrated its contribution to the treatment of diseases such as HIV/AIDS, and microbial infections (Khayyat and Alzahrani, 2014).

The data in (Table 1) revealed that there were antibacterial activity of the tested compounds (1, 2 & 3) on Methicillin resistant Staphylococcus aureus (MRSA). The maximum inhibition zones 33 and 35 mm by mono epoxide 2 and diepoxide 3 (Photo c and d), while 27 mm for Safranal 1 (against MRSA) (Photo 4).

4. Conclusion

The present study demonstrates that Photo chemically and thermally oxidation reaction of safranal gave the corresponding monoepoxy together with diepoxy derivatives, safranal and its epoxidation derivatives showed significant activity against Staphylococcus aureus (MRSA). The maximum inhibition zones was 35 mm diepoxide 3, while 33, 27 mm for safranal monoepoxide 2 and Safranal 1 (against MRSA).

References

Ali, B., Al-Qarawi, A., Mousa, H., 2002. The effect of calcium load and the calcium channel blocker verapamil on gentamicin nephrotoxicity in rats. Food Chem. Toxicol. 40, 1843–1847.
Assimopoulou, A., Sinalos, Z., Papageorgiou, V., 2005. Radical scavenging activity of Crocus sativus L. extract and its bioactive constituents. Phytother. Res. 19, 957–1000.
Boroushaki, M., Mofidpouir, H., Sadeghnia, H., 2007. Protective effect of safranal against hexachlorobutadiene-induced nephrotoxicity in rat. Iran J. Med. Sci. 32 (3), 173–176.
Boroushaki, M., Sadeghnia, H., 2009. Protective effect of safranal against gentamicin-induced nephrotoxicity in rat. Iran J. Med. Sci. 34 (4), 285–288.
Caballer-o-Ortega, H., Pereda-Miranda, A., 2007. HPLC quantification of major active components from 11 different saffron (Crocus sativus L.) sources. Food Chem. 100, 1126–1131.
Carnevecchi, S., Schneider, Y., Ceraline, J., Duranton, B., Goss, F., Seller, N., Raul, F., 2001. Geraniol, a component of plant essential oils inhibit growth and polyamine synthesis in human colon cancer cells. J. Pharm. Exp. Ther. 298 (1), 197–200.
Carmona, M., Zalacain, A., Salinas, M., Alonso, G., 2007. A new approach to saffron aroma. Crit. Rev. Food Sci. Nutr. 47, 145–159.
Collins, C.H., Lyne, P.M., Grange, J.M., 1989. Microbial Meth. 6, 410.
Crowell, P., 1999. Prevention and therapy of cancer by dietary monoterpene. J. Nutr. 129 (3), 775s–778s.
Escribano, J., Alonso, G., Coca-Prados, M., Fernandez, J., 1996. Crocin, safranal and picrocrocin from saffron (Crocus sativus L.) inhibit the growth of human cancer cells in vitro. Cancer Lett. 100, 23–30.
Elgendi, S., Khayyat, S., 2008. Oxidation studies on some natural monoterpene: citral, pulegone and camphene. Russ. J. Organ. Chem. 44 (6), 814–822.
Elgendi, E., Ramadan, A., Fadaly, W., Hammouda, M., 2002. Epoxidation of some natural furoucarumins and furuchromones using gamma-ray. Bollettino chimico farmaceutico 141 (3), 188–191.
Hasenekog˘lu, 1990. Laboratory Techniques for Micro fungi. Atatürk University, Erzurum, Turkey, p. 66.
John, P., Melnyk, S., Wang, M., Marcone, F., 2010. Chemical and biological properties of the world’s most expensive spice: Saffron. Food Res. Int. 43, 1981–1989.
Kalakis, C., Dafnera, D., Tarantolis, P., Polissiou, M., 2004. Qualitative determination of volatile compounds and quantitative evaluation of safranal and 4-hydroxy-2, 6, 6-trimethyl-1-cyclohexene-1-carboxaldehyde (HTCC) in Greek saffron. J. Agri. Food Chem. 52, 4515–4521.
Khayyat, S., 2017. Phytochemical and antimicrobial studies of Saffron and its essential oil. J. Comput. Theor. Nanosci. 14 (5), 1–8.
Khayyat, S., Al-Zahrani, S., 2014. Thermal, photosynthesis and antibacterial studies of bioactive safrole derivative as precursor for natural flavor and fragrance. Arab. J. Chem. 7, 800–804.
Khayyat, S., 2012. Epoxidation of curcumin belongs to curcuminoid. Adv. Sci. Lett. 5, 1–5.

Khayyat, S., Elgendy, E., 2017. Methods of killing bacteria or treating bacterial infection with oxidation products of safranal and methods of synthesizing safranal epoxides, July 18, 2017, US 9707203B1.

Knobler, R., Honigsmann, H., Edelson, R., 1988. Psoralen DNA photobiology. In: Gasparro, F.P. (Ed.), CRC Press Inc., Boca Raton, Florida. 2,117.

Koul, A., Abraham, S., 2017. Intake of saffron reduces γ-radiation-induced genotoxicity and oxidative stress in mice. Toxicol. Mech. Meth. 27 (5), 428–434.

Lozano, P., Delgado, D., Gomez, D., Rubio, M., Iborra, J., 2000. A non-destructive method to determine the safranal content of saffron (Crocus sativus L.) by supercritical carbon dioxide extraction combined with high-performance liquid chromatography and gas chromatography. J. Biochem. Biophys. Methods. 43 (1–3), 367–378.

Maggi, L., Sanchez, A., Carmona, M., Charalabos, D., Anastasaki, E., Polissiou, B., Gonzalo, M., Alonso, L., 2011. Rapid determination of safranal in the quality control of saffron spice (Crocus sativus L.). Food Chem. 127, 369–373.

Maggi, L., Carmona, M., Del Campo, C., Kanakis, C., Anastasaki, E., Tarantilis, P., 2009. Worldwide market screening of saffron volatile composition. J. Sci. Food Agric. 89, 1950–1954.

Malathi, M., Ramya, D., Vedha, H., 2014. Crocus sativus Linn – a potential source for diverse therapeutic applications. Int. J. Pharm. Sci. Res. 26, 2. 51, 299-305.

Martinez-Tomé, M., Jiménez, A., Ruggieri, S., 2001. Antioxidant properties of Mediterranean spices compared with common food additives. J. Food Prot. 64, 1412–1419.

Ochiai, T., Ohno, S., Soeda, S., 2004. Crocin prevents the death of rat pheochromyctoma (PC-12) cells by its antioxidant effects stronger than those of alphatocopherol. Neurosci. Lett. 1362, 61–64.

Pramkumar, K., Abraham, S., Santhiya, S., Ramesh, A., 2003. Protective effects of saffron (Crocus sativus Linn.) on genotoxinsinduced oxidative stress in Swiss albino mice. Phytother. Res. 17, 614–617.

Rios, J., Recio, M., Giner, R., Manez, S., 1996. A update review of saffron and its active constituents. Phytother. Res. 10, 189–193.

Sadeghnia, H., Boroushaki, M., Mofidpour, H., 2005. Effect of safranal, a constituent of saffron (Crocus Sativus L.), on lipid peroxidation level during renal Ischemia-reperfusion injury in rats. Iran. J. basic Med. Sci. 8, 179–185.

Samarghandian, S., Borji, A., 2014. Anticarcinogenic effect of saffron (Crocus sativus L.) and its ingredients. Pharmacog. Res. 6 (2), 99–107.

Tarantilis, P., Polissiou, M., 1997. Isolation and identification of the aroma components from saffron (Crocus sativus L.). J. Agric. Food Chem. 45, 459–462.