EFFECT OF SOME NATURAL UV-ABSORBERS ON THE PHOTOSTABILIZATION OF ACTIVE INGREDIENTS IN GERMAN CHAMOMILLE FLORAL EXTRACTS. PART I.

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(Received: February 9, 2012 - Accepted: June 28, 2012)

ABSTRACT

The effect of some natural UV absorbers on the photostabilization of spiroketal enol ethers (en-yn-dicycloethers) present in German Chamomille extracts pre-coated on glass plates (solid phase) was examined. Plates were exposed to sunlight radiation from a UV-sun radiation lamp, in the presence and absence of gallic acid, cinnamic acid, caffeic acid, ferulic acid and aloin, all natural UV absorbers. At particular intervals the remaining concentration of the en-yn-dicycloethers was analyzed by HPLC. Using first-order kinetic equation, the dissipation half-life values (DT_{50}) for the degradation of the en-yn-dicycloethers under UV-sun radiation were obtained. The results showed that the addition of cumaric acid and caffeic acid provided moderate degree of photostabilization of the en-yn-dicycloethers and that addition of ferulic acid provided the best photostabilization among the UV absorbers studied. Photostabilization effect appears to be due to a competitive energy absorption of UV photons by the absorber molecules.

Keywords: spiroketal enol ethers, German Chamomille, photostabilization, UV absorbers

INTRODUCTION

The use of conventional broad-spectrum synthetic insecticides is rapidly declining due to public concern and regulatory demands for selective and environmentally benign pest control products. Consequently, in recent years, the focus of research has been increasingly toward the development of natural insecticides originating from plants (botanical pesticides), which are believed to be innocuous.^{1,2}

German Chamomille (*Matricaria recutita* L.), a plant belonging to the Asteraceae family, is considered by many as one of the most important of the medicinal plants, due to its anti-inflammatory, anti-espasmodic, anti-allergenic, anti-bacterial and fungicidal properties.³ Close to 120 secondary metabolites have been identified in Chamomille, including 28 terpenoids, 36 flavonoids and 52 additional components, all of them with potential pharmacological activity.⁴⁻⁷

One of the metabolites found in flowers heads of Chamomille is the en-yn-dicycloether (Fig. 1). This spiroketal enol ether was isolated and characterized from hexane extracts of Chamomille flowers, and found that they exist in the extract in two isomeric forms, *Z* and *E*.⁸ Bioassays performed on the fungus *Botrytis cinerea* have shown that the en-yn-dicycloether is one of the active principles responsible for the important fungicidal activity shown by the floral extracts of German Chamomille. This compound has also been found in floral extracts of the species *Chrysanthemum coronarium*, which have shown and important acaricidal and insecticidal activity.⁹

As most natural pesticides originating from plants, en-yn-dicycloethers are highly photolabile, either breaking down or isomerizing rapidly in the presence of sunlight, mainly due to the presence of sensitive functionalities like π -electrons in conjugated double and triple bonds. Degradation of these active ingredients in the sunlight is the major limitation encountered in its use in agriculture pest control, because this botanical insecticide should remain intact on the plants so as to provide the insects enough time to be in contact with the material causing its death. Therefore, for its effective use these Chamomille extracts should be photostabilized by the addition of UV absorbers.

UV light absorbers have been used to enhance the photostability of azadirachtin (Aza-A), one of the most promising biopesticide isolated from the Neem tree (*Azadirachta indica*, A. Juss) of Indian origin.¹⁰⁻¹³

In this study, five natural innocuous UV light absorbers, namely ferulic acid, gallic acid, caffeic acid, cumaric acid and aloin from botanic resource were selected. These natural UV light absorbers can strongly absorb broad spectrum UV light. The structures and UV spectra of the absorbers selected are shown in Fig. 1 and Fig. 2.

The objective of this study was to determine whether the presence of selected natural UV light absorbers can prevent the light-induced degradation of the en-yn-dicycloethers under controlled conditions.

Figure 1. Structure of en-yn-dicycloether and the natural UV light absorbers.

EXPERIMENTAL

Materials

Flowers of *Matricaria recutita* L., were collected during the months of September through December from an area 20 km north of Valparaíso. The plant was authenticated by Professor Otto Zöellner (Biology Institute, Pontificia Universidad Católica de Valparaíso). Flower buds were dried for 3 days in green houses provided by the Centro Experimental de Entomología La Cruz, then ground in a Moulinex mill, and stored in glass containers in a cold room (at 5°C). German chamomille extract was prepared according to the procedure developed by our group recently.⁸

The UV absorbers (ferulic acid, caffeic acid, cumaric acid, gallic acid and aloin A) were all reagent grade and purchased from Sigma-Aldrich and used without further purification. Solvents (acetonitrile, ethanol, acetic acid) were HPLC grade and purchased from Merck. Water used in the HPLC mobile phase was ultrapure (Millipore grade).

Standard Solutions

Solutions of Chamomille extract (5000 ppm) were prepared along with the five UV absorbers in the mole ratio of 1 : 1 (extract : UV absorber) and 1 : 0 (no UV absorber) in ethanol. The solutions were stored in amber-coloured bottles between 0-4 °C.

Irradiation experiments

Standard solutions (1 mL each) of Chamomille extract with and without UV absorbers were applied using a micropipette onto the surface of a glass petri dish (each of 5 cm diameter). Ethanol was evaporated at room temperature leaving behind a thin film of the extracts. Pre-coated petri dishes were irradiated from the top in a rotating system equipped with an OSRAM UV-sun radiation lamp Model Ultra-Vitalux, which emitted radiation simulating the spectral distribution of natural sunlight.¹⁴ The lamp is designed to provide radiation intensity equivalent to mid-day natural sunlight (1 KW/m² = 1.42 cal/cm²min) at the irradiated surface when the lamp is adjusted 50 cm above the surface. The lamp was adjusted at a distance of 12.5 cm above the irradiated discs and

provided radiation intensity equivalent to 16 times that of mid-day natural light. The samples were irradiated for 10, 20, 30, 40, 50 or 60 min to simulate exposure to mid-day sunlight for equivalent of 0.5, 1.0, 1.5, 2.0, 2.5 or 3.0 days, respectively. Pre-coated slides with and without any UV stabilizer were kept in the dark under similar experimental conditions which served as a control.

HPLC Analysis

The HPLC system used in this study was LaChrom Merck Hitachi equipped with a L-7100 quaternary pump, variable wavelength detector L-7420 and a Lichrosorb RP-18 column. The operating parameters were as follows: mobile phase consisting of acetonitrile, water and acetic acid (70 + 28 + 2, v:v), isocratic at 1 mL/min; UV detector wavelength, 324 nm.

Two glass dishes each with and without UV absorbers were removed at intervals of 10 min up to a total period of 60 min of exposure to the Osram UV-sun radiation lamp. These dishes were then rinsed with ethanol (2 x 1 mL) and the solutions were stored in amber-coloured flasks between 0-4 °C. Control samples were collected at the same intervals and extracted and analyzed similarly. The concentration of the *Z* and *E* en-yn-dicycloethers was determined at particular intervals by HPLC. Aliquots of 10 µL were injected

directly into the HPLC system and quantification of the en-yn-dicycloethers was based on external standard method.

RESULTS AND DISCUSSION

The effect of the five natural UV light absorbers on the rate of photodegradation of the en-yn-dicycloethers was examined. The percentage remaining of the en-yn-dicycloethers recovered at different intervals of time (in minutes) for the six ethanolic solutions from different pre-coated petri dishes exposed to sun radiation lamp with and without UV stabilizers are shown in Table 1.

In the presence of UV light absorbers such as ferulic acid, gallic acid, curamic acid, caffeic acid and aloin, the rate of photodegradation of en-yn-dicycloethers was found to be less in comparison to an extract without any stabilizer. From the graph one can see that ferulic acid provided the highest degree of photostabilization of the en-yn-dicycloethers under a sun radiation lamp.

Table 1. Degradation of en-yn-dicycloethers in the presence of different UV absorbers.

Time (min)	0	10	20	30	40	50	60
Treatment % recovered	%	% RSD (n=3)	% RSD (n=3)	% RSD (n=3)	% RSD (n=3)	% RSD (n=3)	% RSD (n=3)
Extract	100	90.7 1.14	53.0 6.74	28.2 4.00	22.0 2.76	18.1 8.21	8.2 8.21
Extract + ferulic acid	100	84.9 1.50	79.4 2.46	80.8 15.5	69.4 6.35	64.6 5.59	55.0 1.36
Extract + cumaric acid	100	77.6 2.43	70.5 2.42	65.5 2.42	55.0 2.18	43.1 2.41	35.6 2.41
Extract + aloin	100	68.6 3.85	56.2 4.48	41.0 3.94	35.2 3.85	26.1 3.85	18.9 3.18
Extract + gallic acid	100	90.6 2.76	74.8 2.65	64.9 2.22	57.6 4.79	34.7 6.40	28.3 6.30
Extract + caffeic acid	100	73.1 4.47	75.3 7.97	46.4 9.7	38.7 7.50	35.1 7.1	30.6 4.37

The rate constant values (in min⁻¹) and values of dissipation half-life (DT₅₀) (in min) are given in Table 2. The dissipation half-life values for the degradation of en-yn-dicycloethers under sun radiation lamp were obtained using first-order equation and they were found to be higher than that of the Chamomille extract alone. The DT₅₀ values of en-yn-dicycloethers in the presence of ferulic acid were found to increase more than two-fold as compared with the extract alone. The addition of cumaric acid and caffeic acid also showed a good protection of the active ingredients of German chamomile extracts, while aloin and gallic acid only showed a moderate protection.

Table 2. Rate constant values and dissipation half-life of en-yn-dicycloethers in the presence of different UV absorbers.

Treatment	Rate constant (k, min ⁻¹)	Dissipation half-life (DT ₅₀ , min) (DT ₅₀ , days)*
Extract	-0.027	26.7 1.3
Extract + ferulic acid	-0.013	55.4 2.8
Extract + cumaric acid	-0.016	43.3 2.2
Extract + aloin	-0.022	31.5 1.6
Extract + gallic acid	-0.022	31.5 1.6
Extract + caffeic acid	-0.018	38.2 1.9

*DT₅₀ extrapolated to outdoors conditions (mid-day natural light)

Fig. 2 (a-f) shows the absorption spectra of the en-yn-dicycloethers and five UV absorbers in ethanol. An examination of the UV spectra of the en-yn-dicycloethers and ferulic acid (Fig. 2a and 2b) shows that both molecules absorb strongly near 324 nm and 321 nm respectively. Better photostabilization of en-yn-dicycloethers by ferulic acid molecule appears to be due to competitive energy absorption by the absorber molecule of UV photons which cause degradation of the dicycloethers. The λ_{max} of cumaric acid and caffeic acid

(Fig. 2c and 2d) are similar to that of en-yn-dicycloethers, but the extinction coefficients at λ 324 nm for these absorbers are smaller than that of ferulic acid (see Fig. 2) and this probably causes relatively less efficient stabilization of en-yn-dicycloether molecules. Gallic acid and aloin showed only marginal effect in stabilizing the en-yn-dicycloethers as they showed λ_{max} at 220 and 273 nm for the first, and 297 and 358 for the last, with extinction coefficients at λ 324 much smaller than the other UV absorbers (Fig. 2e and 2f). Evidently their ability to capture the photons at 324 nm is low and hence there is a decrease in their capacity to stabilize UV-labile en-yn-dicycloethers.

Figure 2. UV-Visible spectra of en-yn-dicycloethers and five natural UV absorbers (in EtOH).

CONCLUSIONS

It can be concluded that the active ingredients in a German Chamomile flower extract, the en-yn-dicycloethers are highly photolabile in the presence of sunlight. Moreover, the data presented here on the percentage recovery of the dicycloethers after irradiation with a sun-radiation lamp in the presence of five natural UV stabilizers, suggests that the addition of Ferulic acid (in 1 : 1 mole ratio) can provide excellent photo-stabilization of the active molecules in German Chamomile extracts. Photostabilization of the en-yn-dicycloethers by different UV light absorbers appears to be due to a competitive energy absorption of UV photons by the absorbers molecules, which causes degradation of the en-yn-dicycloethers

ACKNOWLEDGMENTS

The authors thank FONDEF Project N° DO3I 1135 and Dirección de Investigación PUCV, Doctoral Thesis Grant N° 037.368/2011, for financial support of this work.

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