Biochemical Systematics and Ecology 67 (2016) 103–109

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Pentacyclic triterpenes responsible for photoprotection of *Corema album* (L.) D.Don white berries

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ARTICLE INFO

Article history:
Received 6 April 2016
Received in revised form 13 May 2016
Accepted 14 May 2016
Available online 14 June 2016

Keywords:
Photoprotection
Berries
Corema album
Triterpene acids
Ultraviolet reflectance
Ursolic acid
Oleanolic acid

ABSTRACT

Screening-based photoprotection is a first-line defence of plants against potentially harmful solar radiation. In the Iberian Peninsula, most natural fleshy fruits are red, purple or black, although there do exist a few plants that naturally present white fruit, such as *Corema album* (L.) D.Don (Ericaceae). This is a wild shrub of Atlantic coastal areas, which presents white fleshy fruit that ripens throughout the summer. We analysed the reflectance of fully ripened fruit in the field, and laboratory (before and after hexane washing and pericarp removal). The aqueous and ethyl acetate extract was successively obtained from the crushed fruit and submitted to column chromatography on Silicagel. The fractions were analysed through gas chromatography/mass spectrometry, and two compounds were separated and identified as oleanolic and ursolic acids. The reflectance measurements were taken with the UniSpec-SC Spectral Analysis System. The fruit in the field and laboratory exhibited elevated UV reflectance. The ethyl acetate extract, fractions, and isolated compounds showed high reflectance in UV, which indicated that these triterpene acids could be responsible for reflectance in natural berries. Ursolic and oleanolic acids can play a photoprotective role in berries of *Corema album*. In sunny environments white fruit can be more visible to dispersers.

1. Introduction

Solar radiation is both a source of light for photosynthesis and ultraviolet (UV) radiation, which can induce photodamage in plants. Screening-based photoprotection is a first-line defence of plants against potentially harmful solar radiation: it is based on the synthesis and accumulation of the pigments that selectively absorb UV or the visible part of the spectrum (Solovchenko and Merzlyak, 2008). Furthermore, several of them directly scavenge active oxygen species (Chen et al., 2013).

Compounds that are perceived by humans to have colour are generally referred to as pigments. The screening pigments present in coloured fleshy fruits belong to three key groups of compounds differing in chemical structure and the biosynthetic

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http://dx.doi.org/10.1016/j.bse.2016.05.009
0305-1978/

pathways (Solovchenko, 2010). These compounds include Carotenoids (tetraterpenes), which can produce hues ranging from pale yellow to red. Flavonols and Anthocyanins (two types of phenolic compounds) produce hues in the yellow, red, purple, and blue range, and may even include black from high concentrations of pigments (Saewan and Jimtaisong, 2013). Betalains are nitrogen-compounds that produce colours ranging from red to violet, and sometimes produce blue and yellow colours. All three classes of pigments also act as visible signals to attract insects, birds and animals for pollination and seed dispersal (Tanaka et al., 2008).

In the Iberian Peninsula, most natural fleshy fruits are red, purple or black, although there do exist a few plants that naturally present white fruit, such as *Viscum album* L. and *Corema album* (L.) D.Don (Valdés et al., 1987). *Corema album* fruit production extends from July to October, a period of elevated temperatures, absence of rainfall and elevated irradiation, especially on the exposed areas of coastal dunes. We study the hypothesis that white fruit may contain compounds responsible for their white colour and that these compounds may act as a photoprotective screen against harmful solar radiation. The objectives of this study are: to analyse the optical properties of *C. album* fruit; to determine its chemical composition; and to discuss its photoprotective and ecological significance.

2. Material and methods

2.1. Fruit production characteristics of the study species

Corema album (Ericaceae) is an evergreen wild shrub that grows in coastal habitats along the Atlantic coast of the Iberian Peninsula. It is a wind-pollinated, animal-dispersed dioecious species that produces abundant terminal flowers in February–March. In summer, female plants produce hundreds to thousands of white, almost spherical berries throughout the periphery of the canopy. In our study area, we have recorded an average of 4000 fruits/m^2 of plant, and collected 15,000 fruits from one single plant (not published). Fruit length, in its peninsular range, varies between 5.73 and 11.91 mm, whereas fruit width varies between 6.31 and 12.99 mm (Larriaga and Guitián, 2016). Fruits are rich in water, which attains 80% of fresh weight (Zunzunegui et al., 2006). Each fruit contains three small seeds (exceptionally two or four): length 3.75 ± mm; width 2.81 ± 0.18 mm (Larriaga and Guitián, 2016).

2.2. Plant materials

Wild ripe *C. album* berries were harvested in September 2009, in Asperillo, Doñana Natural Park (Spain) 37° 04′10.15″ N–6° 41′15.45″ W, and were identified by Dr. Mari Cruz Diaz Barradas, from the Department of Plant Biology and Ecology, University of Seville.

2.3. Reagents

Standards of ursolic and oleanolic acids were purchased from Sigma Chemical Products (Madrid, Spain). Hexane and ethyl acetate were obtained from Panreac (Barcelona, Spain). Pyridine was purchased from AppliChem (Barcelona, Spain). Derivatisation reagents BSTFA (N,O bis (trimethylsilyl acetamide) and TMCS (trimethylchlorosilane) were purchased from Supelco (Madrid, Spain). All chemicals were of analytical reagent grade.

2.4. Extraction and isolation

We crushed the berries (250 g) and made successive extractions with water and ethyl acetate at 40 °C for 60 min in an ultrasound bath. After filtration, the solvent was removed using a rotary evaporator (45 °C) to obtain the ethyl acetate extract (0.7% yield). This extract (1.5 g) was fractionated by column chromatography on silica gel 60 (Merck) using *n*-hexane/ethyl acetate mixtures of increasing polarity, in order to yield 20 fractions (10 mL).

2.5. Gas chromatography/mass spectrometry (GC/MS) analysis

The identification of compounds was carried out by comparing the mass spectra and the retention times with derivatised standards. Both standards and samples of *C. album* were derivatised by adding 50 μL of the silylating mixture BSTFA + TMCS (in excess to ensure complete derivatisation) and heated in a closed minivial at 60 °C for 30 min. GC/MS analyses were performed on an Agilent 6890 N gas chromatograph, fitted with a DB-5MS (30 m × 0.25 mm × 0.25 μm) fused silica capillary column (Supelco, Madrid, Spain) and coupled with a mass detector Autospec-Q. Samples were injected using splitless injections; the injection volume was 1 μL. Helium was used as the carrier gas with an average flow of 1 mL/min. The mass scan range was set at m/z 45 to 650 in the 70 eV electron impact ionization (EI) mode.

2.6. Reflectance measurements of berries in the field

Reflectance of the berries was measured in the field in September 2012. Since the flowering period lasts up to 4 months, the start of fruit production is also variable, resulting in the presence of berries at different stages of development on the same plant.