Fitoterapia 99 (2014) 198–203

Contents lists available at ScienceDirect

Fitoterapia

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

A new acetophenone derivative from flowers of *Helichrysum italicum* (Roth) Don ssp. *italicum*

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ARTICLE INFO

Article history:
Received 24 June 2014
Accepted in revised form 24 September 2014
Accepted 25 September 2014
Available online 5 October 2014

Keywords:
Acetophenones
Antioxidant
Asteraceae
Helichrysum italicum
Inflammation
Reactive oxygen species

ABSTRACT

A new acetophenone derivative named gnaphaliol 9-*O*-propanoate (**1**) was isolated from the chloroform fraction of EtOH extract of *Helichrysum italicum* ssp. *italicum* flowers along with the five known acetophenones 12-acetoxytremetone (**2**), 13-(2-methylpropanoyloxy)toxol (**3**), [2,3-dihydro-2-[1-(hydroxymethyl)ethenyl]-5-benzofuranyl]-ethanone (**4**), 1-[2-[1-[(acetyloxy) methyl]ethenyl]-2,3-dihydro-3-hydroxy-5-benzofuranyl]-ethanone (**5**) and gnaphaliol (**6**). The structures of compounds **1**–**6** were elucidated by extensive spectroscopic methods including 1D- (^{1}H and ^{13}C) and 2D-NMR (DQF-COSY, HSQC, HMBC, TOCSY and ROESY) experiments as well as ESIMS analysis. The isolated compounds were investigated for their cytotoxicity, anti-inflammatory and antioxidant properties. Biological assays on human colonic epithelial cells showed that compound **2** possessed antioxidant effects reducing reactive oxygen species (ROS) production.

1. Introduction

The genus *Helichrysum* (family Asteraceae, tribe Inuleae) consists of a few hundred species widespread throughout the world, but particularly distributed in the Mediterranean region [1], where plays an important role in traditional medicine. Particularly, its flowers and leaves are the most used parts in the treatment of health disorders such as allergies, colds, cough, skin, liver and gallbladder disorders, inflammation, infections and sleeplessness [2]. One of the species with more reported traditional uses is *Helichrysum italicum* (Roth) G. Don, a species rich in active compounds such as acetophenones, flavonoids and phloroglucinol derivatives [2]. Extracts obtained from *H. italicum* have been reported to possess remarkable anti-inflammatory and antioxidant properties [2–4]. These activities have been attributed to the presence of flavonoids such as 4,2′,4′,6′-tetrahydroxychalcone-2′-glucoside, kaempferol-3-glucoside, naringenin-glycoside (three glycosyl-flavonoids), gnaphaliin (a methoxyflavone), pinocembrin (a flavanone) and tiliroside (a flavonol acyl-glucoside) which have been found active in assays of antioxidant and anti-inflammatory activities [5,6]. *H. italicum* also contains non-flavonoid phenolics (pyrones, phloroglucinols, acetophenones) that are involved in its biological activities; indeed, arzanol, a pyrone-phloroglucinol etherodimer isolated from the aerial parts of *H. italicum* (ssp. *microphyllum*) has been demonstrated to exert a potent antioxidant activity protecting animal cells from lipid peroxidation [7,8].

In order to further identify the chemical compounds responsible for the above mentioned properties of *H. italicum*, and as a continuation of our previous research on this important Mediterranean medicinal plant [9], we performed a phytochemical analysis on the flowers of *H. italicum* ssp. *italicum* collected in Southern Italy, which resulted in the identification of six

Abbreviations: BHT, 2,6-Di-*tert*-butyl-4-methylphenol; DCFH-DA, 2-7-dichlorofluorescein diacetate; FBS, fetal bovine serum; LPS, lipopolysaccharides; NR, neutral red; PBS, phosphate buffered saline; ROS, reactive oxygen species.

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http://dx.doi.org/10.1016/j.fitote.2014.09.019
0367-326X/

acetophenones, including one with a new chemical structure. The biological profile of some of these compounds has been investigated in assays of cytotoxicity, inflammation and inhibition of oxidative stress in cell culture.

2. Experimental

2.1. General experimental procedures

Optical rotations were determined on a Jasco P-1010 digital polarimeter. UV spectra were obtained on a Jasco 7800 UV–Vis spectrophotometer. IR measurements were obtained on a Bruker IFS-48 spectrometer. NMR experiments were performed on a Bruker DRX-600 spectrometer (BrukerBioSpinGmBH, Rheinstetten, Germany) equipped with a Bruker 5 mm TCI Cryo Probe at 300 K. All 2D-NMR spectra were acquired in CD_3OD (99.95%, Sigma Aldrich) and standard pulse sequences and phase cycling were used for DQF-COSY, HSQC, and HMBC spectra. The NMR data were processed using UXNMR software. Exact masses were measured by a Voyager DE mass spectrometer. Samples were analyzed by matrix-assisted laser desorption ionization time-of-flight (MALDITOF) mass spectrometry. A mixture of analyte solution and α-cyano-4-hydroxycinnamic acid (Sigma) was applied to the metallic sample plate and dried. Mass calibration was performed with the ions from ACTH (fragment 18–39) at 2465.1989 Da and angiotensin III at 931.5154 Da as internal standard. ESIMS was obtained on Applied Biosystem API-2000 mass spectrometer. Merck Silica gel (70–230 mesh), deactivated with 15% H_2O, was used for column chromatography. Normal-phase HPLC was performed with a TSP SpectraSeries P100 instrument equipped with rheodyne injector and a refractive index detector, using a hypersil silica column (Thermo, 250 × 4.6 mm, flow rate 1.5 mL/min). Thin-layer chromatography (TLC) was performed on plates coated with silica gel 60 F_{254} Merck, 0.25 mm.

2.2. Chemicals

Hydrogen peroxide (H_2O_2), iron(II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), 3-amino-7-dimethylamino-2-methylphenazine hydrochloride (neutral red, NR), 2-7-dichlorofluorescein diacetate (DCFH-DA), lipopolysaccharides (LPS) from *Escherichia coli* serotype O111:B4, 2,6-Di-*tert*-butyl-4-methylphenol (BHT), dexamethasone, dimethyl sulfoxide (DMSO) and phosphate buffered saline (PBS) tablets were purchased from Sigma-Aldrich (Milan, Italy). All reagents for cell culture were obtained from Sigma (Milan, Italy), Bio-Rad Laboratories (Milan, Italy) and Microtech Srl (Naples, Italy). All solvents (analytical, deuterated and HPLC grade) were obtained from Carlo Erba Reagenti (Milan, Italy).

2.3. Plant material

Flowers of *H. italicum* ssp. *italicum* were collected in July 2007 in San Potito Sannitico loc. Sardarulo (CE, Southern Italy). A voucher specimen (N° NAP# 23/06) has been deposited at the Herbarium Neapolitanum (NAP), Department of Biological Sciences of University "Federico II" of Naples (Italy).

2.4. Extraction and isolation

Air-dried and powdered flowers of *H. italicum* (235 g) were soaked with EtOH (3500 mL) three times at room temperature for 12 h. The ethanol extracts, concentrated under vacuum, afforded 18 g of a glassy material and were subjected to a Kupchan's partition procedure [10] modified as follows. The ethanol extract was dissolved in 10% aqueous methanol and partitioned against *n*-hexane. The water content (% v/v) of the MeOH was adjusted to 20 and 40% and partitioned against $CHCl_3$. The aqueous phase was concentrated to remove MeOH and then extracted with *n*-butanol (*n*-BuOH). Three extracts were obtained as follows: *n*-hexane (0.85 g), $CHCl_3$ (2.45 g) and *n*-BuOH (5.15 g). All the extracts were submitted for biological testing (data not shown). The chloroform extract, which was shown to be the most active, was further purified to yield compounds **1**–**6** (Fig. 1). Particularly, the $CHCl_3$ extract was dissolved in $CHCl_3$ and then submitted to column chromatography on silica gel 60 Merck (70–230 mesh, deactivated with 15% H_2O), eluting with $CHCl_3$/MeOH (from 100:0 to 0:100 gradient), to afford 15 fractions of 250 mL each. The fractions 1–15, estimated by thin-layer chromatography (TLC) (eluent system petrol/EtOAc (1:1 vv), spray reagent $Ce(SO_4)_2$ in H_2SO_4), were opportunely gathered on the basis of their similar TLC

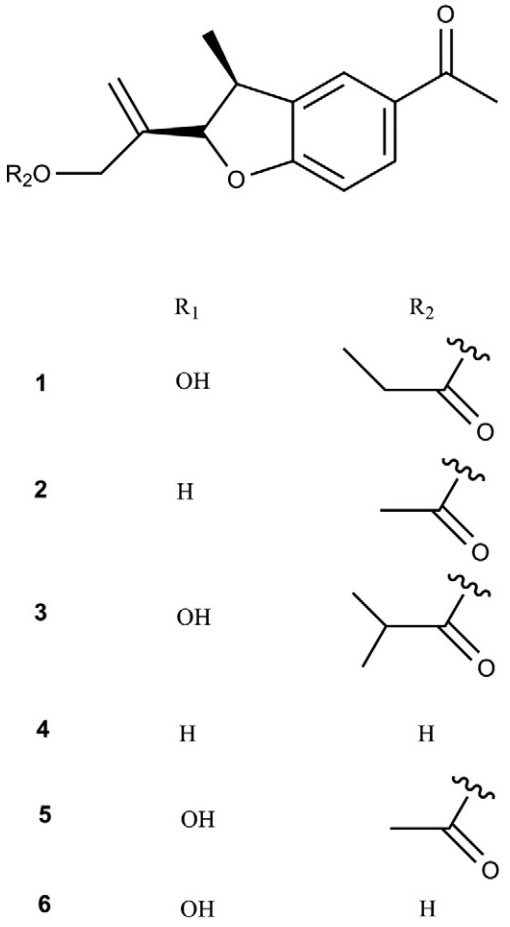

Fig. 1. Compounds isolated from *Helichrysum italicum* ssp. *italicum* flowers.