Phytochemistry xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Phytochemistry

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

Absolute configurations of phytotoxins seiricardine A and inuloxin A obtained by chiroptical studies

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ARTICLE INFO

Article history:
Received 25 November 2014
Received in revised form 1 March 2015
Accepted 3 March 2015
Available online xxxx

Keywords:
Phytopathogenic fungi
Phytotoxins
Absolute configuration
Optical rotation
Electronic Circular Dichroism
Vibrational Circular Dichroism

ABSTRACT

The absolute configuration (AC) of the plant phytotoxin inuloxin A, produced by *Inula viscosa*, and of the fungal phytotoxin seiricardine A, obtained from *Seiridium* fungi, pathogen for cypress, has been determined by experimental measurements and theoretical simulations of chiroptical properties of three related methods, namely, Optical Rotatory Dispersion (ORD), Electronic Circular Dichroism (ECD), and Vibrational Circular Dichroism (VCD). Computational prediction by Density Functional Theory (DFT) of VCD spectra and by Time-dependent DFT (TDDFT) of ORD and ECD spectra allowed to assign (7*R*,8*R*,10*S*) AC to naturally occurring (+)-inuloxin A. In the case of compound (−)-seiricardine A, which lacks useful for the analysis UV–Vis absorption, and thus provides a hardly detectable ECD spectrum and quite low ORD values, an introduction of a suitable chromophore by chemical derivatization was performed. The corresponding derivative, 2-*O*-*p*-bromobenzoate ester, gave rise to an intense ECD spectrum and higher ORD and VCD values. The comparison of computed spectra with the experimental ones allowed to assign (1*S*,2*R*,3a*S*,4*S*,5*R*,7a*S*) AC to (−)-2-*O*-*p*-bromobenzoate ester of seiricardine A and then to (−)-seiricardine A. This study further supports a recent trend of concerted application of more than a single chiroptical technique toward an unambiguous assignment of AC of flexible and complex natural products. Moreover, the use of chemical derivatization, with insertion of suitable chromophoric moieties has allowed to treat also UV–Vis transparent molecules by ECD and ORD spectroscopies.

1. Introduction

The relevance of absolute configuration (AC) of chiral compounds to their bioactivity is well known in naturally occurring metabolites, drugs, flavors, and fragrances (Holmstedt et al., 1990; Kurihara and Miyamoto, 1998; Baillie and Schultz, 1997; Collins et al., 1992; Brenna et al., 2003). This relationship has also been clearly established for natural bioactive compounds coming from plants or fungi (Evidente et al., 2011, 2013). Therefore, for a complete structural characterization of such chiral natural compounds the assignment of their absolute stereochemistry is mandatory, in particular, when the biological activity of the molecules is investigated.

Quite often natural products for structural studies are available in very small amounts and/or in non-crystalline form and also lack heavy atoms, features that prevent a direct assignment of AC by X-ray analysis. For these reasons other techniques which display a high sensitivity and allow measurements in solution are required. Fortunately, chiroptical spectroscopic methods provide such good opportunities for assignment of AC to natural products. Configurational studies can sometimes be carried out on a microscale using very dilute solutions (Berova et al., 2010). Moreover, the recent advance in the *ab initio* predictions of chiroptical properties has significantly increased the use of computational methodologies in the structural analysis (Polavarapu, 2012; Autschbach, 2009, 2012). It has been shown that the concerted use of more than

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http://dx.doi.org/10.1016/j.phytochem.2015.03.001
0031-9422/

one chiroptical method, such as Optical Rotatory Dispersion (ORD), Electronic Circular Dichroism (ECD), and Vibrational Circular Dichroism (VCD), can provide superior reliability in the treatment of structurally complex molecules, including those containing a few different functional groups and stereogenic centers, and possessing a high molecular flexibility, features often seen in natural products (Polavarapu, 2008; Polavarapu et al., 2011; Stephens et al., 2004; Mazzeo et al., 2013). Recent studies also revealed the usefulness of chemical modification approach where original UV-transparent molecules are transformed into suitable chromophoric derivatives often with reduced molecular flexibility as well (Tartaglia et al., 2008; Superchi et al., 2001, 2004, 2006, 2012).

Therefore the spirit of this study is thus a pursuit in these recent trends in configurational analysis of natural products. Specifically, the ACs of the plant phytotoxin inuloxin A (**1**, Fig. 1) and of the fungal phytotoxin seiricardine A (**2**, Fig. 1) have been herein established by a combined analysis of their ORD, ECD, and VCD properties and, in the case of **2**, by introducing a suitable chromophoric moiety through chemical transformations.

Metabolites from specific Mediterranean plant species have a great potential as "natural herbicides" for parasitic weed management programs. Among them there is *Inula viscosa* (L.) Aiton (syn *Cupularia viscosa* G. et G., *Dittrichia viscosa* Greuter), also known as sticky fleabane, a perennial weed native of the Mediterranean basin (Zermane et al., 2011). This plant belongs to the genus *Inula* (Asteraceae), widely spread throughout Europe, Africa and Asia, and previously reported as an herbal source for fungicidal preparation against foliar diseases caused by pathogenic fungi to some important agrarian crops, such as, cucumber, tomato, potato, wheat and sunflower (Wang et al., 2004). Many plants of this genus have long been used in folk medicine and are a rich source of sesquiterpenoids exhibiting a wide range of biological activities. Cytotoxic and anti-tumor activities of *Inula* sesquiterpenoids have been extensively studied since the 1970s (Wang et al., 2014). One promising sesquiterpene lactone from traditional herb *Inula japonica*, has displayed potent *in vitro* and *in vivo* anti-tumor activity against Burkitt's lymphoma. Other sequiterpenoids lactone from the same plant are capable of suppressing the abnormal vascular smooth muscle cell proliferation, with the induction of apoptosis *in vivo* and *in vitro* (Wang et al., 2014).

Compound **1** was recently isolated from the aerial part of *I. viscosa* together with three close related bi- and tri-cyclic sesquiterpenoids, named inuloxins B–D, and the well known α-costic acid, and characterized as a new bicyclic 3-oxo-germacra-4,11(13)-dien-8β-12-olide. Its relative stereochemistry was established as (4*E*,7*R**,8*R**,10*S**) (Andolfi et al., 2013). When assayed against broomrapes (*Orobanche crenata* and *Orobanche ramosa*) and dodder, **1** strongly inhibited the seed germination of both parasitic plants, suggesting its potential use as natural herbicide for biological control of very dangerous *Orobanche* sp. and *Cuscuta campestris*. Moreover, inuloxins A (**1**), C, and D demonstrated strong activity against *Leishmania donovani*, the protozoan parasite and causative agent for visceral leishmaniasis, with **1** being the most active (Avolio et al., 2014).

Seiricardine A (**2**) is produced, together with the close related seiricardines B and C, by three *Seiridium* species (*cardinale*, *cupressi* and *unicorne*), fungi associated with the canker disease of Italian cypress (*Cupressus sempervirens* L.) (Graniti, 1998). This destructive disease kills the trees and causes heavy losses in nursery industry and in cypress plantations, either ornamental or used for afforestation and wind-breaks (Evidente et al., 2010). The phytotoxin **2** was characterized by spectroscopic methods as a new octahydro-1-isoprenyl-2,5-dihydroxy-3a,4,5-trimethyl-1*H*-indene and its relative stereochemistry determined as (1*S**,2*R**,3a*S**,4*S**,5*R**,7a*S**) (Ballio et al., 1991; Evidente and Motta, 2001; Evidente et al., 2010). The phytotoxic and the antifungal activity of **2** was also tested. Its fungistatic activity observed against the three fungi tested (including phytopathogenic species as *Botrytis cinerea* and *Fusarium solani*) has allowed to hypothesize its possible use as natural fungicide against other fungi pathogenic of cypress plant as *Diplodia*, *Pestalotiopsis* and *Sphaeropsis* species (Evidente et al., 2010). Surprisingly, fungal phytotoxins also showed to possess potential medical applications as antimalarial, against *Aedes aegypti* L. (Diptera: Culicidae), the major vector of dengue fever, and anticancer (Bajsa et al., 2007; Cimmino et al., 2013; Evidente et al., 2014). In particular, the *in vitro* growth inhibitory activity of seiricardines B and C was tested in six cancer cell lines (Balde et al., 2010). The very promising biological activity of **1** and **2** therefore prompted this study toward the assignment of their absolute stereochemistry, so that future studies on the mechanism of action and development of their total enantioselective synthesis become possible. In fact, by taking into account the low yield obtained from natural sources, it is obvious that only a total synthesis could permit to produce these fungal phytotoxins on a large scale necessary for potential practical application.

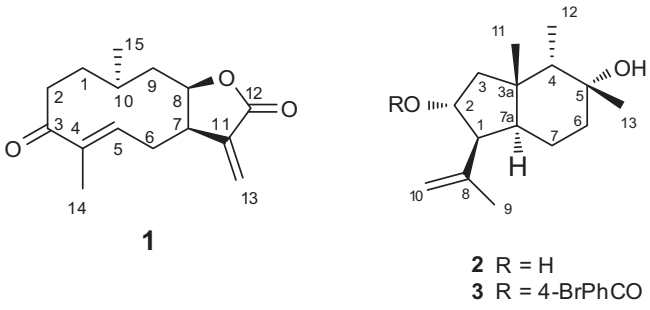

Fig. 1. Structure and assigned AC of inuloxin A (**1**), seiricardine A (**2**), and seiricardine A *p*-Br-benzoyl ester (**3**).

2. Results and discussion

2.1. Isolation of compounds *1* and *2*

Inuloxin A ((+)-**1**), was isolated as yellow oil (262 mg/kg) from the organic extract of *I. viscosa* as reported in details in the Experimental part and was characterized by its optical rotation (OR) and spectroscopic (UV, IR and ^{1}H and ^{13}C NMR) data (Andolfi et al., 2013). Seiricardin A ((−)-**2**) was isolated as white needles (3.1 mg/L) from the culture filtrates of *Seiridium cardinale* as reported in the Experimental and was identified on the basis of its OR and spectroscopic (UV, IR ^{1}H and ^{13}C NMR and MS) data, that were found to be identical to those reported in literature (Ballio et al., 1991). For the purpose of ORD, ECD, and VCD studies, (−)-**2** was converted into the corresponding *p*-Br-benzoyl ester (−)-**3** (Fig. 1), by reaction with *p*-bromo-benzoyl chloride. ^{1}H NMR of derivative **3** differed from that of **2** for the downfield shift of H-2 ($\Delta\delta$ 1.17) resonating as a triple doublet (J = 9.0 and 6.6 Hz) at δ 5.72, and for the presence of the signals typical of the *para*-substituted benzoyl residue appearing as two doublets (J = 8.4 Hz) at δ 7.87 and 7.56 for H-3′,5′ and H-2′,6′, respectively. Its ESI MS spectrum showed the sodium clusters at m/z 445 [M+2+Na]$^+$ and 443 [M+2+Na]$^+$.

2.2. Absolute configuration of compounds *1* and *2*

Taking into account the known relative configuration of **1** (Andolfi et al., 2013) conformational analysis was carried out on the randomly chosen (7*S*,8*S*,10*R*)-**1** stereoisomer. The conformational search at MM level with MMFF94s force field, provided