Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences
Tome 71, No 3, 2018

CHEMISTRY
Organic chemistry

TWO NEW GUAIANOLIDES FROM *INULA OCULUS-CHRISTI* L.

Antoaneta Trendafilova, Milka Todorova, Ina Aneva*

(*Submitted by Corresponding Member V. Bankova on February 19, 2018*)

Abstract

Two new guaianolides were isolated from the aerial parts of *Inula oculus-christi* L., growing in Bulgaria. Their structures were elucidated by spectral methods.

Key words: *Inula oculus-christi*, Asteraceae, sesquiterpene lactones, guaianolides

Introduction. The genus *Inula* L. (family Asteraceae) comprises more than 100 species, of which 19 are distributed in Europe [1]. Numerous *Inula* species have been used in the folk medicine of many countries all over the world since ancient times to treat a large number of different diseases. Thus, among the European taxa *I. britannica* has been reported to treat bronchitis, digestive disorders, inflammation, asthma, and as an expectorant, antibacterial, carminative, diuretic, stomachic, tonic rapid-healer, for hepatitis and tumours [2]. *I. helenium* is also an important medicinal plant, roots of which are used against tuberculotic enterorrhea, enterogastritis and bronchitis, lung disorders, tuberculosis, etc. [3]. Phytochemical investigations of *Inula* genus demonstrate a great variety of secondary metabolites, many of which exhibited biological activity [4–6]. Sesquiterpene lactones are the major group of isolated components, followed by phenolic compounds, and other terpenoids. Studies on biological activity of sesquiterpene lactones showed promising results for further investigations on their phytomedicinal application.

This work was supported by the National Science Fund, Ministry of Education and Science, Bulgaria, Project DN 09/11.
DOI:10.7546/CRABS.2018.03.05

Genus *Inula* is represented by ten species in Bulgaria and we have recently started their phytochemical investigation. Four new sesquiterpene lactones were isolated from the aerial parts of *Inula oculus-christi* L.: one eudesmanolide (4α, 15α-epoxypulchellin E) and three guaianolides (2α-acetoxy-4α,9β-dihydroxy-1β-guaia-11(13),10(14)-dien-12,8α-olide, 9β,10β-epoxy-gaillardin, 9α,10α-epoxy-2-*epi*-gaillardin), in addition to the known gaillardin, pulchellin E and pulchellin C [7]. In continuation of our research of this species, herein we report the isolation and identification of two new metabolites (**1** and **2**).

Materials and methods. ***Plant material.*** The aerial parts of *I. oculus-christi* were collected in full flowering stage in July 2016. The species was identified by Dr. Ina Aneva from the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences. A voucher specimen (SOM 1360) has been deposited in the Herbarium of the Institute of Biodiversity and Ecosystem Research, Sofia, Bulgaria.

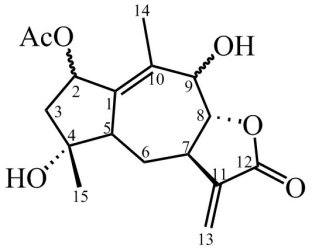
Extraction and isolation. The air-dried and powdered aerial parts (95 g) were extracted with $CHCl_3$ three times (each for 24 h) at room temperature. The combined extracts were concentrated under vacuum to afford 4.5 g crude extract. The latter was worked up as described previously [7]. In addition to the earlier found sesquiterpene lactones, compounds **1** (0.8 mg) and **2** (1.2 mg) were isolated from Fr-6/4 by prep. TLC on silica gel, cyclohexane/ethylacetate, 1:1 (four times eluting).

2α-Acetoxy-4α,9α-dihydroxy-guaia-1(10),11(13)-dien-12,8α-olide (**1**): Colourless oil; IR (film) ν_{max} 3465, 1765, 1735 cm^{-1}; HRESIMS m/z 323.14872 $[M+H]^+$ (calcd for $C_{17}H_{23}O_6$ 323.14884); ^1H NMR (600 MHz, $CDCl_3$, TMS, δ in ppm): 6.22 (d, J = 3.3 Hz, 1H, H-13), 5.66 (brt, J = 5.5 Hz, 1H, H-2), 5.53 (d, J = 3.2 Hz, 1H, H-13′), 4.52 (s, 1H, H-9), 4.00 (d, J = 10.5 Hz, 1H, H-8), 3.55 (m, 1H, H-7), 2.17 (dd, J = 14.5, 5.5 Hz, 1H, H-3), 2.16 (d, 12.0 Hz, 1H, H-5), 2.11 (m, H-6), 2.06 (s, 3H, OAc), 1.88 (s, 3H, H-14), 1.88 (dd, J = 14.5, 5.5 Hz, 1H, H-3′), 1.32 (s, 3H, H-15), 1.30 (m, H-6′).

2β-Acetoxy-4α,9β-dihydroxy-guaia-1(10),11(13)-dien-12,8α-olide (**2**): Colourless oil; IR (film) ν_{max} 3460, 1760, 1730 cm^{-1}; HRESIMS m/z 323.14887 $[M+H]^+$ (calcd for $C_{17}H_{23}O_6$ 323.14892); ^1H NMR (600 MHz, $CDCl_3$, TMS, δ in ppm): 6.20 (d, J = 3.1 Hz, 1H, H-13), 5.79 (brd, J = 6.7 Hz, 1H, H-2), 5.49 (d, J = 3.1 Hz, 1H, H-13′), 4.62 (d, J = 10.0 Hz, 1H, H-9), 3.69 (t, J = 10.0, 1H, H-8), 2.87 (m, 1H, H-7), 2.64 (brd, J = 12.6 Hz, 1H, H-5), 2.16 (dt, J = 12.6, 2.5 Hz, 1H, H-6), 2.08 (s, 3H, OAc), 2.04 (dd, J = 14.8, 6.7 Hz, 1H, H-3), 1.94 (dt, J = 14.8, 1.7 Hz, 1H, H-3′), 1.83 (s, 3H, H-14), 1.30 (s, 3H, H-15), 1.04 (m, 1H, H-6′).

Results and discussion. Reinvestigation of *I. oculus-christi* afforded two new guaianolides (**1** and **2**). Their structures are presented in Fig. 1.

Compound **1** was isolated as a colourless oil with a molecular formula of $C_{17}H_{23}O_6$ established by HR-ESI-MS ($[M+H]^+$, m/z 323.14872). Its IR spec-



1 2α-OAc, 9α-OH
2 2β-OAc, 9β-OH

Fig. 1. Structures of sesquiterpene lactones **1** and **2**

trum showed absorptions at 1765 (C=O, α-methylene-γ-lactone), 1735 (C=O, ester) and 3465 (OH) cm^{-1}. In the ^1H NMR spectrum of **1** two downfield signals were observed – at δ_H 4.52 and 5.66 each for one proton, which indicated the presence of two oxygen containing functional groups – hydroxyl and acetyl (δ_H 2.06, 3H), respectively. A broad doublet for H-8 oxymethine at δ_H 4.00, characteristic multiplet for H-7 at 3.55 ppm as well as two doublets at 5.53 and 6.22 ppm for two C-13 exomethylene protons corresponded to a lactone ring. A three-proton singlet at δ_H 1.88 and the lack of olefinic proton showed the presence of tetrasubstituted double bond. One more oxygen function (tertiary OH group) was confirmed by a downfield shifted singlet for a methyl group at δ_H 1.32. Further, COSY experiment confirmed the connectivity of H-5/H-6/H-7/H-8/H-9 as well as C-2 acetoxy and C-3 methylene groups. The places of the secondary OH group and the tetrasubstituted double bond can be only at C-9 and C-1/C-10, respectively. It should be noted that the signal for H-8 was a singlet, i.e. the vicinal $J_{8,9}$ was very small (< 1 Hz) but COSY interaction between H-8/H-9 was visible in the spectrum. Inspection of the Drieding model showed that the dihedral angle between H-8/H-9 is 90° when OH is α oriented and this is a reason for $J_{8,9} < 1$ or $J_{8,9} = 0$ Hz. *Trans*-fusion of the lactone ring was deduced from coupling constant ($J_{7,8} = 10.5$ Hz), while the relative stereochemistry at C-2 and C-4 was assumed by analogy with the previously isolated guaianolides [7]. Thus, **1** was identified as 2α-acetoxy-4α,9α-dihydroxy-guaia-1(10),11(13)-dien-12,8α-olide.

Compound **2** was obtained as yellowish oil and its IR spectrum showed characteristic absorption bands at 3460 (OH), 1730 (C=O, ester) and 1760 (C=O, lactone) cm^{-1}. The HR-ESI-MS mass spectrum exhibited a molecular ion peak $[M+H]^+$ at m/z 323.14887 establishing the molecular formula $C_{17}H_{23}O_6$. The ^1H NMR spectrum indicated the presence of an olefinic methyl group (δ_H 1.83 ppm), an acetyl ester function (δ_H 5.79, 1H, and 2.08, 3H) and a secondary OH group (δ_H 4.62). One more oxymethine proton, which appeared at δ_H 3.69 and two doublets for exomethylene protons at δ_H 5.49 and 6.20 confirmed the presence

of a lactone ring. The singlet for a methyl group which was observed at δ_H 1.30 obviously is deshielded by an oxygen function, in this case OH. The obtained molecular formula $C_{17}H_{23}O_6$ which required seven degrees of unsaturation as well as ^{1}H NMR spectral data and COSY experiment revealed a guaiane structure of the described compound, the same like that of **1**. The relative stereochemistry was determined on the base of vicinal coupling constants. Thus, *trans*-fusion of the lactone ring and β-orientation of H-9 was proven by large coupling constants $J_{7,8} = J_{8,9} = 10.0$ Hz. Further, the H-2 signal in **2** was a doublet, while in **1** it was a triplet. The only reason could be the different stereochemistry at C-2 in both components and we assumed β disposition of C-2 acetyl group in **2**. Finally, **2** was determined as 2,9-diastereoisomer of **1.** Therefore, compound **2** was shown to be 2β-acetoxy-4α,9β-dihydroxy-guaia-1(10),11(13)-dien-12,8α-olide.

REFERENCES

[1] Ball P. W., T. G. Tutin (1976) Title!, In: Flora Europaea, Vol. 4 (Eds T. G. Tutin, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine et al.), London, UK, Cambridge University Press, 133–136.

[2] Khan A. L., J. Hussain, M. Hamayun, S. A. Gilani, S. Ahmad et al. (2010) Secondary Metabolites from *Inula britannica* L. and their biological activities, Molecules, **15**(3), 1562–1577.

[3] Konishi T., Y. Shimada, T. Nagao, H. Okabe, T. Konoshima (2002) Antiproliferative sesquiterpene lactones from the roots of *Inula helenium*, Biol. Pharm. Bull., **25**(10), 1370–1372.

[4] Seca A. M. L., A. Grigore, D. C. G. A. Pinto, A. M. S. Silva (2014). The genus *Inula* and their metabolites: from ethnopharmacological to medicinal uses, J. Ethnopharmacol., **154**(2), 286–310.

[5] Seca A. M. L., C. G. A. Pinto, A. M. S. Silva (2015) Metabolomic Profile of the Genus *Inula*, Chemistry & Biodiversity, **12**(6), 859–906.

[6] Wang G. W., J. J. Qin, X. R. Cheng, Y. H. Shen, L. Shan et al. (2014) *Inula* sesquiterpenoids: structural diversity, cytotoxicity and anti-tumor activity, Expert Opin. Investig. Drugs, **23**(3), 318–345.

[7] Trendafilova A., V. Ivanova, M. Todorova, I. Aneva (2017) New sesquiterpene lactones from *Inula oculus-christi* L., Phytochem. Lett., **21**(4), 221–225.

Institute of Organic Chemistry
with Centre of Phytochemistry
Bulgarian Academy of Sciences
Acad. G. Bonchev St, Bl. 9
1113 Sofia, Bulgaria
e-mail: trendaf@orgchm.bas.bg

**Institute of Biodiversity*
and Ecosystem Research
Bulgarian Academy of Sciences
2 Gagarin St
1113 Sofia, Bulgaria